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**ON THE CAPABILITY OF BIOSURFACTANTS FOR THE REMOVAL  
OF HEAVY METALS FROM SOIL AND SEDIMENTS**

**by**

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requirements of the degree of Doctor of Philosophy**

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## **ABSTRACT**

Batch soil washing experiments were used to evaluate the feasibility of using biosurfactants for the removal of heavy metals from a contaminated soil and sediments. Surfactin from *Bacillus subtilis*, rhamnolipids from *Pseudomonas aeruginosa* and sophorolipid from *Torulopsis bombicola* were evaluated using a soil contaminated with hydrocarbon and metals (890 mg/kg zinc, 420 mg/kg copper, 12.6% oil and grease) and metal contaminated sediments (110 mg/kg copper, 3300 mg/kg zinc).

Although water alone removed insignificant levels of metals, results showed that the biosurfactants could remove 5% of the zinc (with 12% rhamnolipid) and 19.5% of the zinc (with 4% sophorolipid with 0.7% HCl). Copper could also be removed and was most efficiently extracted (greater than 25%) with 12% rhamnolipid or with 2% rhamnolipid with 1% NaOH. 1% NaOH alone removed only 5% of the copper and 2% zinc. After a series of five batch washes, 90% of the copper could be removed by 0.1% surfactin with 1% NaOH while 4% sophorolipid with 0.7% HCl was able to remove 100% of the zinc. From the sediment, a single washing with 0.5% rhamnolipid removed 65% of the copper and 18% of the zinc whereas 4% sophorolipid removed 25% of the copper and 60% of the zinc.

Sequential extraction procedures were used on the soil and sediments. For both matrices, the carbonate and the oxide fractions accounted for over 90% of the zinc present in the soil. The organic fraction constituted over 70% of the copper in the soil and sediments. Sequential extraction of the soil and sediments after washing with the various surfactants indicated that the biosurfactants, rhamnolipid or surfactin with NaOH, could remove the organically-bound copper and that the sophorolipid with acid could remove the

carbonate and oxide bound zinc and cadmium.

Concerning the mechanism for metal removal by the surfactants, the techniques of octanol-water partitioning, ultrafiltration and zeta potential measurements indicated that the surfactants removed the metals first by sorption at the soil interphase, followed by desorption of the metal through interfacial tension lowering and fluid forces and then solubilization of the metal within the micelle.

## RÉSUMÉ

Des expériences de lavage en mode batch ont été menées afin d'évaluer la faisabilité d'utiliser des agents tensio-actifs biologiques pour l'enlèvement des métaux lourds des sols et sédiments contaminés. Le surfactin produit par la souche *Bacillus subtilis* de même que les rhamnolipides produits par *Pseudomonas aeruginosa* et les sophorolipides produits par *Torulopsis bombicola* ont été évalués dans un sol contaminé par les hydrocarbures et métaux lourds (890 mg/kg zinc, 420 mg/kg cuivre, 12,6% huiles et graisses) et des sédiments contaminés par les métaux (110 mg/kg cuivre et 3 300 mg/kg zinc).

L'eau toute seule a enlevé des niveaux négligeables de métaux (solubles). Les résultats ont démontré que l'avantage des agents tensio-actifs biologiques est qu'ils peuvent enlever le zinc dans des proportions de 5% (avec 12% de rhamnolipides) et de 19,5% (avec 4% de sophorolipides et 0,7% de HCl). Le cuivre peut aussi être enlevé dans une proportion de plus de 25% avec 12% de rhamnolipides ou 2% de rhamnolipides et 1% de NaOH. Le NaOH, dans une proportion de 1%, peut enlever 5% de cuivre et 2% de zinc. Après une série de cinq lavages, en mode batch, 90% de cuivre peut être enlevé par 0,1% de surfactin et 1% de NaOH. Cependant, 4% de sophorolipides et 0,7% HCl ont pu enlever 100% de zinc. Quant aux sédiments, un seul lavage avec 0,5% de rhamnolipides a enlevé 65% de cuivre et 18% de zinc, et les sophorolipides ont enlevé 25% de cuivre et 60% de zinc.

Un protocole d'extraction séquentielle a été appliqué pour le sol et les sédiments. Pour les deux matrices, les fractions de carbonate et d'oxyde représentaient plus de 90% du zinc dans le sol, alors que la fraction organique représentait plus de 70% du cuivre dans le sol et les sédiments. L'extraction

séquentielle des métaux du sol et des sédiments après lavage avec divers agents tensio-actifs a démontré que les biosurfactants, les rhamnolipides et le surfactin avec le NaOH, peuvent enlever le cuivre de la fraction organique et que les sophorolides peuvent enlever le zinc et le cadmium associés aux fractions carbonate et oxyde.

Afin d'étudier les mécanismes d'enlèvement des métaux lourds par les agents tensio-actifs, cet étude a utilisé les techniques de partition octanol-eau, d'ultrafiltration et les mesures de potentialité zêta. Ceux-ci ont indiqué que les agents tensio-actifs ont enlevé les métaux premièrement par l'adsorption des agents tensio-actifs au sol, suivie par la désorption du métal du à une diminution des tensions des interfaces et des forces dynamiques du fluide et ensuite par la solubilisation du métal dans la micelle.

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## **PUBLICATIONS**

The following papers were presented during the course of this research project:

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C.N. Mulligan, R.N. Yong and B.F. Gibbs (1997) The use of biosurfactants for the removal of metals from oil-contaminated soil. Presented at the *Air & Waste Management Association's 90th Annual Meeting & Exhibition*, June 8-13, Toronto, Ontario.

C.N. Mulligan and R.N. Yong (1997). The use of biosurfactants in the removal of metals from contaminated soil. Presented at *Geoenvironmental Conference. Contaminated Ground: Fate of Pollutants and Remediation*, September 16-18, Cardiff, Wales.

C.N. Mulligan, R.N. Yong and B.F. Gibbs (1998) On the use of biosurfactants for the removal of heavy metals from oil-contaminated soil. *Environmental Progress* (in press).

C.N. Mulligan, R.N. Yong and B.F. Gibbs (1998) Removal of heavy metals from contaminated soil and sediments using the biosurfactant surfactin. Submitted to *Journal of Soil Contamination*.

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## LIST OF SYMBOLS AND ABBREVIATIONS

CEC	Cation exchange capacity
COD	Chemical oxygen demand
CMC	Critical micelle concentration
DCE	Dichloroethylene
DNAPL	Dense non-aqueous phase liquids
EDTA	Ethylenediaminetetraacetic acid
IFT	Interfacial tension
$K_m$	Micelle-water partition coefficient
$K_{ow}$	Octanol-water coefficient
LC	Lachine Canal
LNAPL	Light non-aqueous phase liquids
MEF	Ministère de l'environnement et de la faune du Québec
MSR	Molar solubilization ratio
NAPL	Non-aqueous phase liquids
PAH	Polycyclic aromatic hydrocarbons
Rhamn	Rhamnolipid
SDS	Sodium dodecyl sulfate
Soph	Sophorolipid
Surf	Surfactin
SSE	Selective sequential extraction
TCE	Trichloroethylene
THC	Toronto Harbour Commission
TPH	Total petroleum hydrocarbon

## I. INTRODUCTION

### 1.1 General background

Over the past decades, the contamination of the environment through the water, land and air has occurred due to the generation of waste by agricultural enterprises, industrial plants and through the extraction and treatment of natural resources. There are approximately 1000 of these types of enterprises in North America of which approximately half are situated along the St. Lawrence River (Québec économique, 1991). Contaminants include oil and grease, sludges, solvents, acids, cyanides and metals. In Quebec in 1994, it was estimated that 52 tonnes of copper, 2352 tonnes of zinc and 421 tonnes of lead were released to the soil (Environnement Canada, 1996). Some examples of the toxic effluents and their compositions are shown in Table 1.1. Some of their toxic effects are shown in Table 1.2.

**Table 1.1 Sources and composition of toxic effluents (Yong et al., 1992b)**

INDUSTRY	Waste streams
Laboratories	acids, bases, heavy metals, inorganics, ignitable wastes, solvents
Flexography	acids, bases, heavy metals, inorganics, solvents, ink sludges, spent plating
Pesticides	metals, inorganics, pesticides, solvents
Construction	acids, bases, ignitable wastes, solvents
Metal finishing	acids, bases, reactives, heavy metals, inorganics, ignitable wastes, solvents
Formulators	acids, bases, reactives, heavy metals, inorganics, ignitable wastes, solvents, pesticides
Chemical manufacture	acids, bases, reactives, heavy metals, inorganics, ignitable wastes, solvents
Dry cleaning	dry clean filtration residue, solvents

**Table 1.2 Acute effects of some hazardous wastes on human health**

Type of waste	Nervous system	Gastric system	Neural system	Respiratory system	Skin	Death
Halogenated organic pesticide	X		X	X		X
Methyl bromide			X			
Halogenated organic phenoxy herbicide					X	
Organophosphorous pesticide	X		X	X		X
Organonitrogen herbicide		X				X
Carbamate insecticide	X		X	X		X
Dimethyldithiocarbonate fungicide				X		
Aluminum phosphide		X				
Polychlorinated biphenyls		X			X	
Cyanide wastes	X		X	X		X
Halogenated organics	X		X		X	
Non-halogenated volatile organics			X	X		
Zn, Cu, Se, Cr, Ni		X		X	X	
As		X			X	X
Organic lead compounds	X	X	X			
Hg	X	X	X			X
Cd		X		X		X

Source: Governor's Office of Appropriate Technology, Toxic Waste Assessment Group, Calif. 1981

Surfactants are surface-active compounds which can assist in the solubilization, dispersal and desorption of hydrophobic compounds and heavy metals due to their hydrophobic and hydrophilic portions. In soil decontamination projects, surfactants can be added to washing water for contact with excavated soils in a washing unit for subsequent return of the cleaned soils to their original site. Another possible application is during pump and treat procedures where the groundwater is recovered for ground level treatment after *in situ* flushing of the contaminated soil with a surfactant-containing solution. Several synthetic surfactants have been evaluated in soil decontamination tests (Ellis et al., 1985; Texas Research Institute, 1985; Nash et al., 1987).

Excavation of contaminated soils was once the chosen solution. However, due to the high cost of incineration (\$600 to 1000/m<sup>3</sup>) and for disposal (approximately \$150/m<sup>3</sup>) at a hazardous wastes landfill, in addition to the lack of available landfill sites, these disposal methods are becoming increasingly less popular (Clarke et al., 1991). In the United States, 1200 sites are on the National Priority (Superfund) List (NPL) for the treatment of contaminated soils, indicating the extent of this problem. Approximately 25% of the underground petroleum tanks are leaking thus releasing considerable amounts of petroleum into the soil and groundwater (USEPA, 1988). Approximately 63% of the sites on the NPL include contamination from toxic heavy metals (Hazardous Waste Consultant, 1996). For example, lead was found at 15% of the sites, followed by chromium, cadmium and copper at 11, 8 and 7 % of the sites respectively. Therefore, oil and metal contamination is a major problem as emphasized in Figure 1.1.

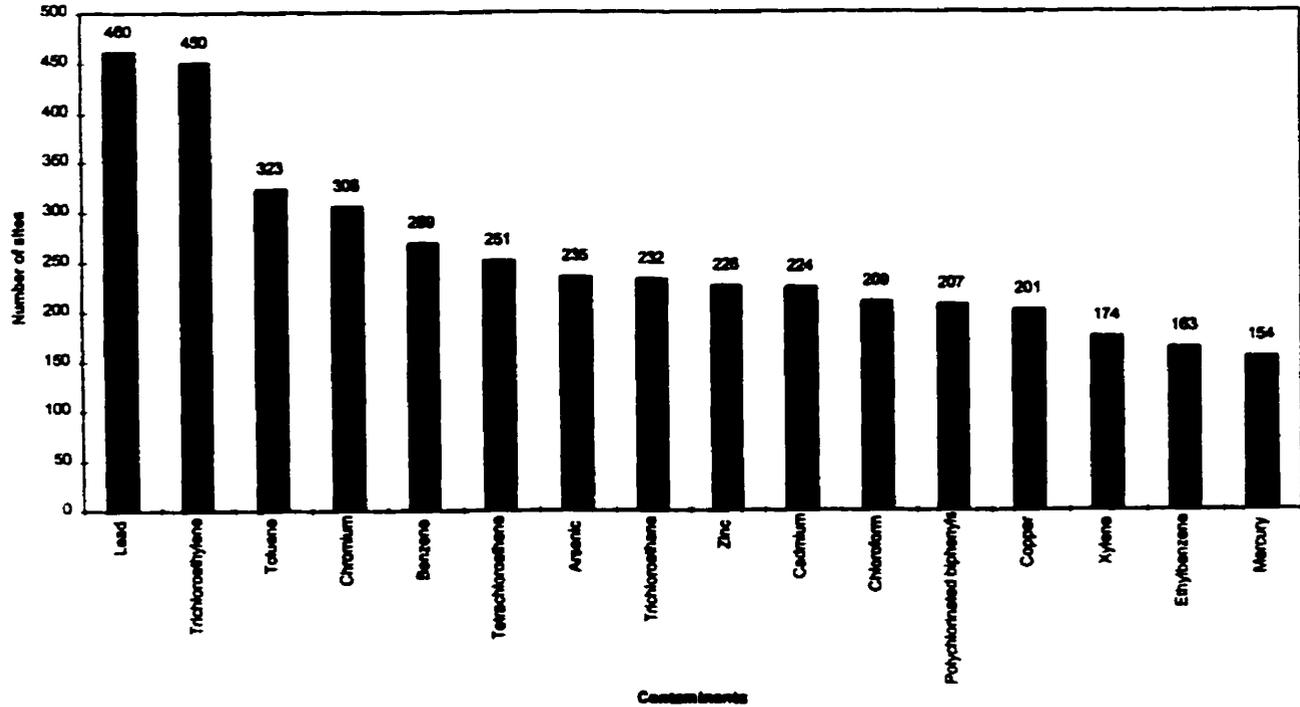


Figure 1.1. Most common contamination at National Priority List Sites (US EPA, 1997)

## **1.2 Problem statement**

To decrease costs, various technologies are now being developed and implemented for the remediation of soils and sediments contaminated with hydrocarbons and heavy metals. However, the removal of hydrophobic compounds with low volatility and metals bound to the soil (typical contaminants of weathered contaminated soils) is still a major problem. Soil venting techniques can be applied for highly volatile hydrocarbons such as benzene, toluene, ethylbenzene and xylene. Traditional pump and treat techniques can only efficiently remove highly soluble contaminants. Biological treatment is efficient for biodegradable compounds but is unsuitable for compounds with low solubilities since the transportation across microbial cell membranes is limited.

Significant losses of surfactants can occur during soil washing due to precipitation and sorption onto the soil (Rouse et al., 1993). These surfactants must, therefore, be non-toxic and biodegradable so that they do not contribute to further pollution problems. The addition of biodegradable surfactants such as biosurfactants to solubilize and disperse contaminants as a soil washing technique is a potential solution for heavy metal and hydrocarbon removal. In addition, they can be produced from readily available and renewable substrates such as sugars and food grade oils. Other advantages include effectiveness at extreme temperatures, pH and salt concentrations, low critical micelle concentration (CMC) values and high degrees of effectiveness in lowering the surface tension (Mulligan and Gibbs, 1993).

Although the biosurfactants are negatively charged, their ability to enhance the removal of the positively charged metal from soil has not been investigated. As the surfactant encounters the bound metals on the soil, the surfactant/metal bond will have to overcome the various types of metal-soil

bindings to remove the metals from the soil. Several types of soil-metal associations are represented in Figure 1.2. The strength and nature of the surfactant-metal complexes are unknown (whether they are van der Waals forces, electrostatic or complexation) and will have to be investigated, in addition to determining the factors affecting the binding.

This thesis will focus on the investigation of the mechanisms of metal removal by selected biosurfactants to understand their role in the enhancement of soil washing techniques before it can be considered as a viable remediation technique.

### **1.3 Objectives**

The objectives of this research are as follows:

- To determine the feasibility of using three different types of biosurfactants to enhance the removal of heavy metals (lead, zinc and copper) for soil and sediments and the factors influencing their behaviour.
- To determine the mechanisms of heavy metal removal by biosurfactants from soils and sediments.

### **1.4 Scope of work**

To meet the objectives described in the previous section, the scope of work detailed in Figure 1.3 was performed. Step 1 includes the work to achieve the first objective and step 2 was required for the second objective.

### **1.5 Organization of the thesis**

The thesis is divided into ten chapters and three appendices. The contents are as follows:

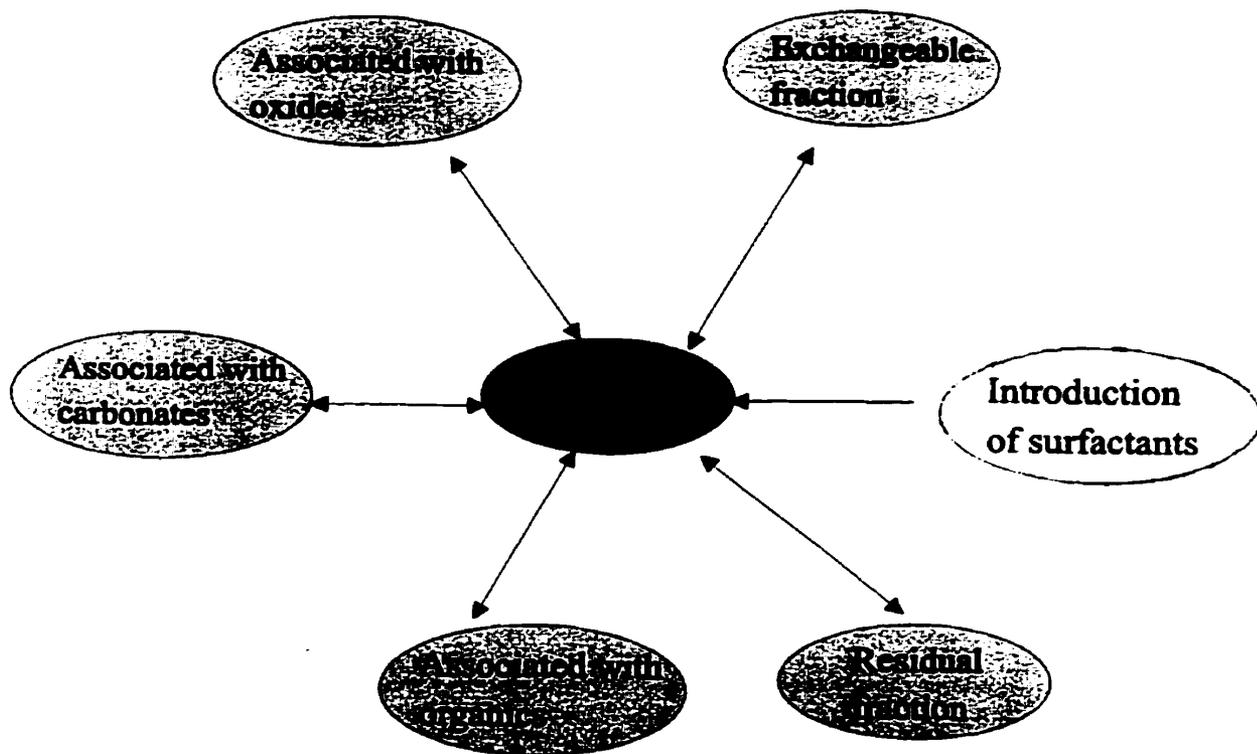


Figure 1.2. Effect of surfactants on heavy metal retention mechanisms

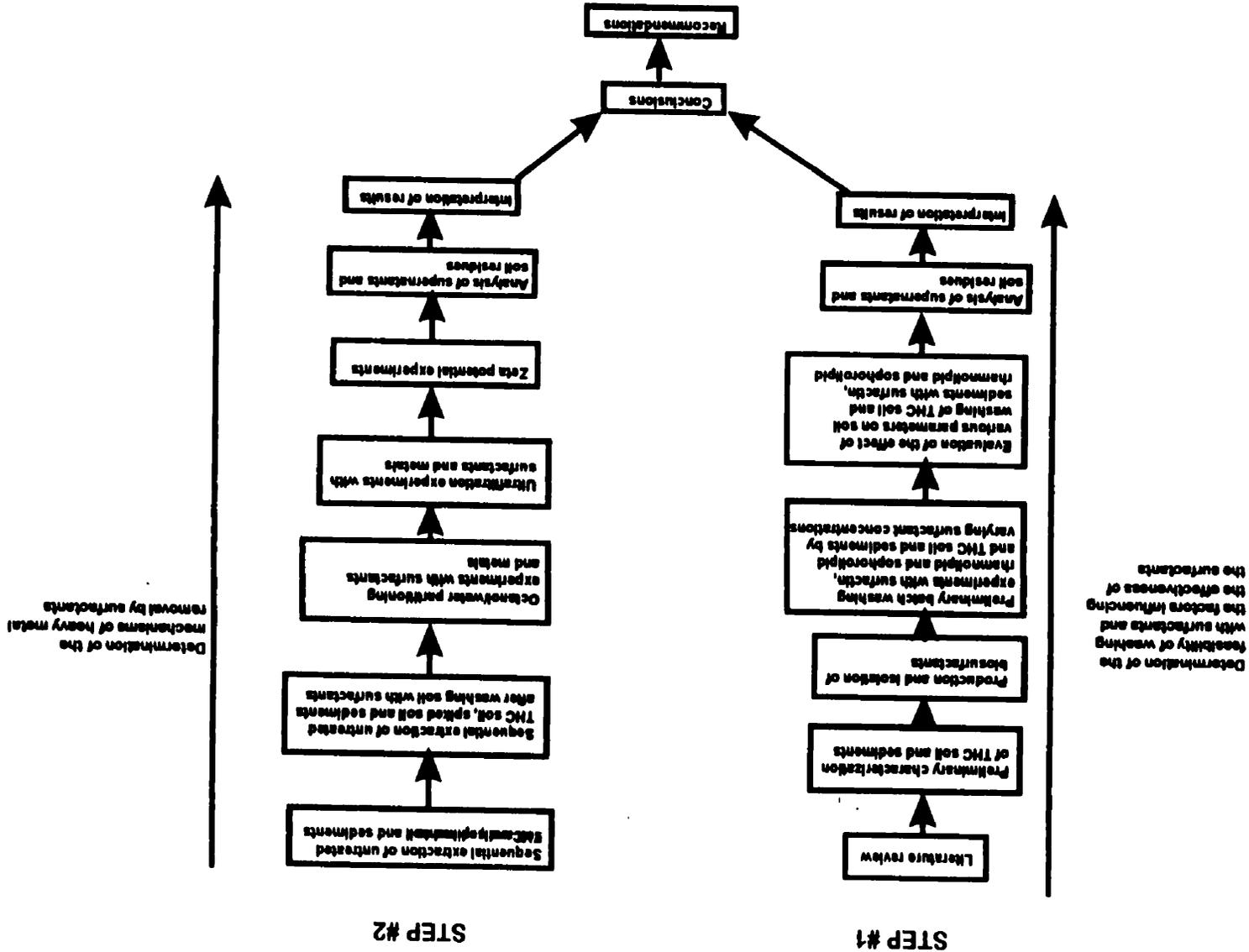


Figure 1.3 Diagram indicating scope of work

- 
- Chapter 1 includes the extent, context and description of the problem and the objectives and organization of this particular study;
  - Chapter 2 gives background information for the various concepts covered in the thesis and a literature review for similar studies;
  - Chapter 3 describes the materials, methods and procedures used in the experiments;
  - Chapters 4, 5, 6, 7 and 8 shows the results of the experiments for soil and surfactant characterization, soil washing, sequential extraction and metal removal mechanism studies, respectively;
  - Chapter 9 discusses and interprets the results presented in the previous chapters and relates the results to other previously performed studies;
  - Chapter 10 includes the conclusions, recommendations for further work and its scientific contribution
  - Appendix A: Photographs of the fermentation setup and the batch soil washing supernatants
  - Appendix B shows the results for X-ray diffractogram and private laboratory results
  - Appendix C includes the fitting of the results to models for the soil washing experiments.

## **II. LITERATURE REVIEW**

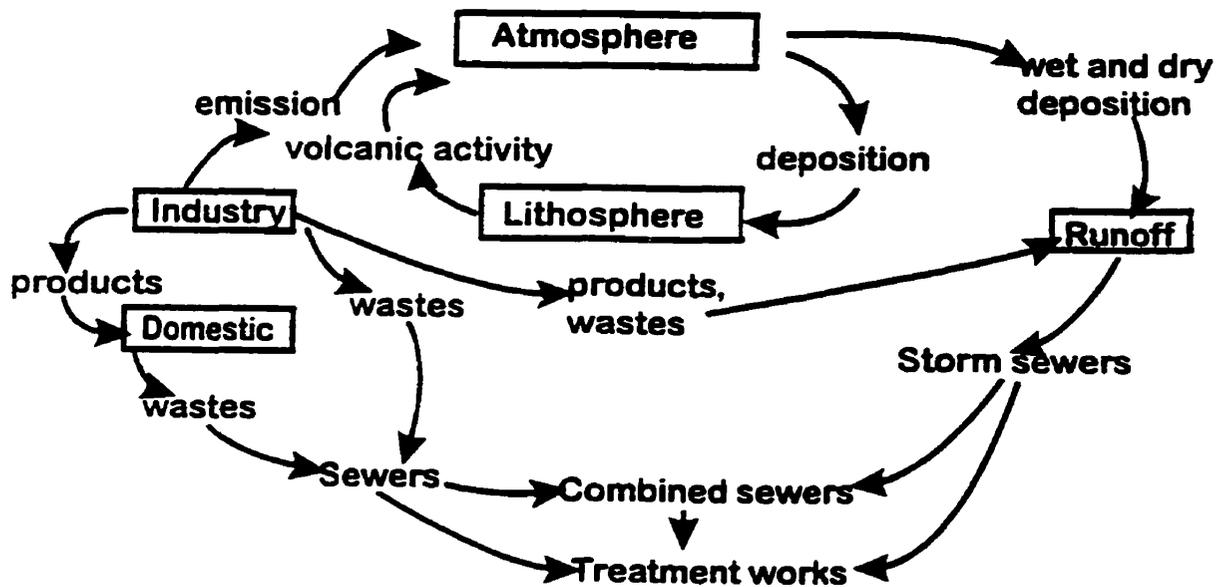
### **II.1 Effects of heavy metals**

Heavy metals are those which have a density above 5 g/mL in their elemental form (Mathews, 1984). From the periodic table, these metals are included in Group IIA, IIIB, IVB, VB and VIB. Although there are thirty-eight such elements, there are twelve common ones are cadmium, chromium, cobalt, copper, iron, mercury, manganese, molybdenum, nickel, lead, tin and zinc (Cameron, 1992).

Chemically, the metals are classified according to their ability to accept or donate electrons in terms of a Lewis acid or base. Some metals such as copper, zinc, and selenium are beneficial at low doses but toxic at high levels. Most are toxic at levels from 1 to 2 mg/kg tissue (Truhart, 1979). The term, heavy metal, is thus usually used to describe the toxicity and pollution capability of these elements.

Cadmium, copper, lead, mercury, nickel and zinc are considered to be the most hazardous and are included on the EPA's list of priority pollutants (Cameron, 1992). Sources of metals include, domestic and industrial effluents, the atmosphere, runoff and lithosphere. Once metals are allowed to pass through the municipal waste treatment facility, the heavy metals return to the environment where they are persistent, cannot be biodegraded and can thus follow a number of different pathways as is demonstrated in Figure 2.1.

Exposure to the heavy metals through drinking water (particularly where water is reused) and foods can lead to accumulation in both animals, plants and humans. Metal accumulation can lead to extinction or alteration of plants and animals. Levels of metals can accumulate in the following order, first in the



**Figure 2.1. Pathways for heavy metal transport (Stephenson, 1989)**

river sediments, then in bacteria, tubicids and fish, and finally in man when he eats these fish.

Over the past years, use of metals such as copper, cadmium and zinc have increased substantially (Table 2.1). Copper is produced more than any other metal, whereas more zinc reaches the soil than any other metal. Lead use has decreased due to toxicity concerns. In Canada, according to the National Pollutant Release Inventory, approximately 13,300 tonnes of copper, 9,500 tonnes of zinc, 1,300 tonnes of lead and 33 tonnes of cadmium were released to the air, water and soil (NPRI, 1995).

Sources of metallic wastes (Tables 2.2 and 2.3) include: industrial and urban aerosols from fuel combustion, ore refining, incineration, liquid and solid wastes from humans and animals, mining operations and industrial and agricultural chemicals.

**Table 2.1 Global production of metals and the rate of metals reaching the soil (10<sup>3</sup> t/year)**

Metal	1975	1980	1985	1990	Emissions to the soil in 1980s
Cd	15.2	18.2	19.1	20.2	22
Cu	6739.0	7204.0	7870.0	8814.0	954
Pb	3432.2	3448.2	3431.2	3367.2	796
Zn	3975.4	4030.3	4723.1	5570.9	1372

Adapted from World Resources Institute (1992/1993) and Nriagu and Pacyna (1988)

### II.1.1 Cadmium

In its natural form, cadmium is relatively rare and concentrated in argillaceous and shale deposits as greenockite (CdS) or otavite (CdCO<sub>3</sub>) and is usually associated with zinc, lead or copper in sulfide form (Cameron, 1992). It is a bluish-white soft metal or grayish powder. It is more mobile, though, than zinc at low pH values, particularly at pH values between 4.5 and 5.5. Above pH 7.5, cadmium is not very mobile. Its divalent form is soluble but it can also complex with organics and oxides. Volcanoes release cadmium into the air. It is only in the last twenty years that cadmium has become a concern due to the extensive extraction for use in steel plating, pigment stabilization and nickel-cadmium batteries (Fasset, 1980). Sources of cadmium include alloys, polyvinyl chloride plastic manufacturing (PVC), solders, fungicides, enamels,

**Table 2.2 Domestic and industrial sources of heavy metals**

Source	Cd	Cu	Pb	Zn
Automotive products				X
Caulking products			X	X
Cleaners		X	X	X
Cosmetics		X		X
Disinfectants	X		X	
Driers				
Fillers				X
Fire extinguishers				X
Fuels		X	X	
Pesticides	X	X	X	X
Inks		X		X
Lubricants			X	X
Medicine		X		X
Oils		X	X	X
Ointments		X		X
Paints			X	X
Photography			X	
Pigments	X	X	X	X
Polish		X		X
Powders				X
Preservatives			X	X
Suppositories				X
Water treatment		X		X

Adapted from Atkins and Hawley, 1978

**Table 2.3. Heavy metals from different industries**

Industry	Cd	Cu	Pb	Zn
<b>Metal industries</b>				
<b>Power plants</b>				
<b>Founderies (ferrous)</b>	X	X	X	X
<b>Founderies (non-ferrous)</b>	X	X	X	X
<b>Plating</b>	X	X		X
<b>Chemical industries</b>				
<b>Cement and glass</b>				
<b>Organic and petroleum</b>	X		X	X
<b>Inorganic chemicals</b>	X		X	X
<b>Fertilizers</b>	X	X	X	X
<b>Oil refining</b>	X	X	X	
<b>Others</b>				
<b>Paper</b>		X	X	
<b>Leather</b>				
<b>Textiles</b>				X
<b>Electronics</b>		X	X	X

Adapted from Dean et al., 1972.

motor oil, textile manufacturing, electroplating and rubber, sewage sludges and phosphate fertilizers (Matthews, 1984). It enters the environment through industrial effluents and landfill leaching, spills and leaks at hazardous waste sites, mining and household wastes. In wastes, it is found in the form of cadmium oxide, cadmium chloride, and cadmium sulfate or sulfide.

Cadmium is very hazardous since it is retained strongly in humans (Friberg et al., 1974), particularly in the liver (half-life of 5 to 10 years) and kidney (half-life of 10 to 40 years). This can lead to vomiting, diarrhea, colitis, renal dysfunction, etc. (Vivoli et al., 1983). It is also a probable carcinogen. Plants can accumulate cadmium up to levels as high as 5 to 30 mg/kg have been found (normal range is 0.005 to 0.02 mg/kg) (Cameron, 1992).

### **II.1.2 Copper**

Copper is found naturally in sandstones and in minerals such as malachite and chalcopyrite. It is a reddish-brown metal that binds strongly to organic matter and clay minerals, thus decreasing its mobility. The organic matter, however, can be degraded through anaerobic or aerobic means releasing the copper in its monovalent or divalent states, respectively. The average content of copper in soils is 2 to 100 mg/kg. Plants can accumulate copper. Average contents are in the range of 5 to 30 mg/kg. Toxic levels in plants are in the range of 20 to 100 mg/kg. Increased levels of copper are due to uses in fertilizers, building materials, rayon manufacture, pesticide sprays, agricultural and municipal wastes and industrial emissions (Cameron, 1992).

The human requirement of copper is 0.03 mg/kg for adults. Once copper is absorbed into the gastrointestinal tract, it enters the blood and muscle, liver and brain. Renal damage, hepatic necrosis, jaundice and ulcers are all symptoms of copper toxicity (Sandstead, 1974). Copper can cause "pink

disease" in infants when 0.8 mg/L in water was consumed and is carcinogenic to animals (National Academy of Sciences, 1977). The toxicity of copper is influenced by pH, alkalinity and water hardness.

### **II.1.3 Lead**

Lead is found naturally in soils. It is most commonly found in the ore galena (PbS) and in smaller quantities in cerussite (PbCO<sub>3</sub>), anglesite (PbSO<sub>4</sub>) and crocoite (PbCrO<sub>4</sub>). It is a bluish-white, silvery or grey metal with a high density of 11.4 g/cm<sup>3</sup>. Lead can be found in soils at the surface and organic matter in higher quantities. Sources of lead include lead-zinc smelters, ammunition, solder, glass, piping, insecticides, paints and batteries (Jaworsky, 1978). The divalent form is the most common and is capable of replacing calcium, strontium, barium and potassium in soils. In general, levels less than 10 mg/kg are found in natural soil and mobility of lead in soils is low. Organic matter can adsorb substantial quantities of lead. Lead is released into the air from burning of wastes and fossil fuels and subsequently lands onto the soil. It also reaches the soil from landfills and paints.

Lead enters the body through drinking water or food and can accumulate in the bones. Lead poisoning is characterized by anemia, vomiting, renal damage and encephalopathy. In children, irritability, appetite loss, vomiting, abdominal pain and constipation can occur (Yule and Lansdown, 1983). It is also toxic to embryos and is teratogenic (Wilson, 1966). Animals ingest lead via crops and grasses grown in contaminated soil (levels in plants usually range from 0.5 to 3 mg/kg while lichens have been shown to contain up to 2,400 mg/kg of lead (Cameron, 1992).

### **II.1.4 Zinc**

Although not as toxic as cadmium, zinc is quite often associated with this

metal. Zinc is a soft, white metal with bluish tinge. Soil texture, pH, nature of the parent rocks and organic content all affect the natural content of zinc in the soil. Under acidic conditions, zinc is usually divalent and quite mobile. At high pH, zinc is bioavailable due to the solubility of its organic and mineral colloids. Zinc hydrolyses at pH 7.0 to 7.5, forming  $Zn(OH)_2$  at pH values higher than 8. Under anoxic conditions,  $ZnS$  can form upon precipitation, whereas the unprecipitated zinc can form  $ZnOH^+$ ,  $ZnCO_3$  and  $ZnCl^+$ . Natural levels of zinc in soils are 30 to 150 mg/kg. Levels of 10 to 150 mg/kg are normal while 400 ppm in plants are toxic. Sources of zinc include brass and bronze alloys, galvanized products, rubber, copying paper, cosmetics, pharmaceuticals, batteries, televisions, tires, metal coatings, glass, paints and zinc-based alloys (Cameron, 1992). It can enter the environment from galvanizing plant effluents, coal and waste burning, leachates from galvanized structures, natural ores and municipal waste treatment plant discharge. Zinc is commonly found in wastes as zinc chloride, zinc oxide, zinc sulfate and zinc sulfide (Agency for Toxic Substances and Disease Registry, 1995).

Zinc is required in the human diet in amounts of 10 to 20 mg daily for protein and nucleic acid metabolism, and for cell division. Levels of 600 to 2,000 mg/L in water lead to the symptoms of zinc poisoning including vomiting, dehydration, fever and anemia but its contribution to cancer is unclear. (Smith et al., 1976).

## **II.2 Surfactants**

Surfactants are amphiphilic compounds (containing hydrophobic and hydrophilic portions) that reduce the free energy of the system by replacing the bulk molecules of higher energy at an interface. They contain a hydrophobic portion with little affinity for the bulk medium and a hydrophilic group that is attracted to the bulk medium.

Surfactants have been used industrially as adhesives, flocculating, wetting and foaming agents, de-emulsifiers and penetrants (Mulligan and Gibbs, 1993). Petroleum users have traditionally been the major users, as in enhanced oil removal applications. In this application, surfactants increase the solubility of petroleum components (Falatko, 1991). They have also been used for mineral flotation and in the pharmaceutical industries. Typical desirable properties include solubility enhancement, surface tension reduction, the critical micelle concentrations, wettability and foaming capacity (Table 2.4).

### II.2.1 Types of surfactants

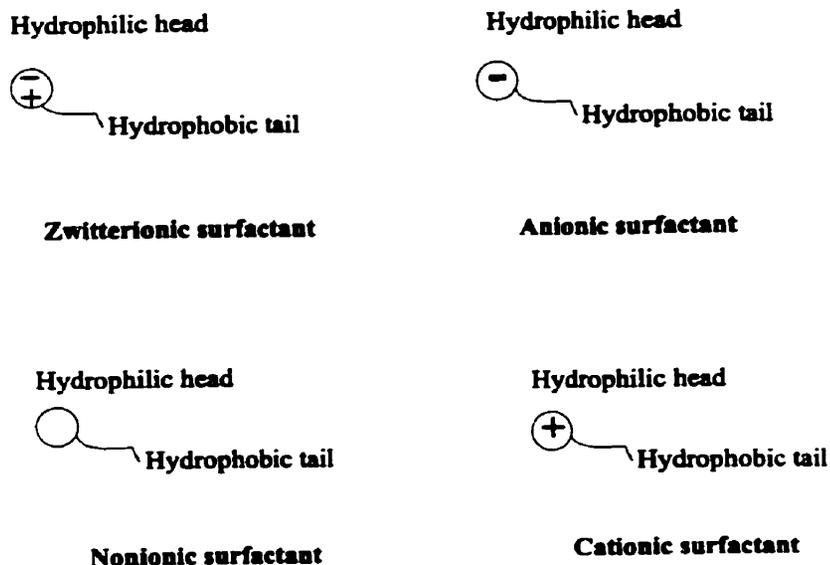
Surfactants are classified as cationic, anionic, zwitterionic and nonionic (Fig. 2.2) and they can be made synthetically from hydrocarbons, lignosulfonates or triglycerides. Total sales in the the United States annually is approximately \$1.7 billion with a 3.5% growth rate (Lemezis, 1994). Major types of synthetic surfactants include linear alkyl benzenesulfonates, alcohol sulphates, alcohol ether sulphates, alcohol glyceryl ether sulfonates,  $\alpha$ -olefin sulfonates, alcohol ethoxylates and alkylphenol ethoxylates (Layman, 1985). Surfactants are potentially useful in every industry dealing with multiphase systems. Sodium dodecyl sulphate (SDS,  $C_{12}H_{25}-SO_4^- Na^+$ ) is an example of a widely used anionic surfactant. A recent study indicated that sodium dodecyl sulfate, probably the most well known surfactant, was inhibitory to anaerobic bacteria at concentrations above 500 mg/L (Monroy-Hermosillo et al., 1994). It contains a straight chain aliphatic hydrocarbon with a sulfate group.

The effectiveness of a surfactant is determined by its ability to lower the surface tension which is a measure of the surface free energy per unit area or the work required to bring a molecule from the bulk phase to the surface

**Table 2.4 Characteristics of surfactants required for various applications**

Application	Characteristics
Detergency	Low CMC, good salt and pH stability, biodegradability, food foaming characteristics
Emulsification	Proper HLB, environmental safety
Lubrication	Chemical stability, surface adsorption
Mineral flotation	Adsorption on specific ores
Petroleum recovery	Wetting of oil-bearing formations, microemulsion formation and solubilization, ease of emulsion breaking after recovery
Pharmaceuticals	Biocompatibility, low toxicity

Adapted from Myers, 1988.

**Figure 2.2 Representation of anionic, cationic, zwitterionic and nonionic surfactants**

(Rosen, 1978). These amphiphilic compounds known as surfactants concentrate at interfaces (solid-liquid, liquid-liquid or vapour-liquid). An interfacial boundary exists between two immiscible phases. The hydrophobic portion concentrates at the surface while the hydrophilic is oriented towards the solution. When a surfactant is present, the surface tension is reduced. For example, a good surfactant can lower the surface tension of water from 72 to 35 mN/m and the interfacial tension (tension between non-polar and polar liquids) for water against n-hexadecane from 40 to 1 mN/m. The surface tension correlates with the concentration of the surface-active compound until the critical micelle concentration (CMC) is reached (Figure 2.3). Efficient surfactants have low critical micelle concentration (i.e. less surfactant is necessary to decrease the surface tension). The CMC is defined as the minimum concentration necessary to initiate micelle formation (Becher, 1965). In practice, the CMC is also the maximum concentration of surfactant monomers in water and is influenced by pH, temperature and ionic strength. Figure 2.4 shows how other parameters vary as a function of surfactant concentration.

An emulsion is defined as a "heterogeneous system, consisting of at least one immiscible liquid dispersed in another in the form of droplets, whose diameters, in general, exceed 0.1  $\mu\text{m}$ . Such systems possess a minimal stability, which may be accentuated by such additives as surfactants, finely defined solids, etc." (Becher, 1965). It is necessary to specify water-in-oil (w/o) or oil-in-water (o/w) phases. The term hydrophilic-lipophilic balance (HLB) is used to classify which type of emulsion the emulsifier will favour. Emulsifiers and de-emulsifiers stabilize or destabilize the emulsion, respectively. Many of these products are polymeric and do not change the surface tension.

The choice of surfactant is primarily based on product cost (Mulligan and

Gibbs, 1993). In general, surfactants are used to save energy and consequently energy costs (such as the energy required for pumping in pump and treat techniques). Charge-type, physicochemical behaviour, solubility and adsorption behaviour are some of the most important selection criteria for surfactants. New markets are currently being developed for use in the bioremediation of contaminated land sites (Oberbremer et al., 1990; Samson

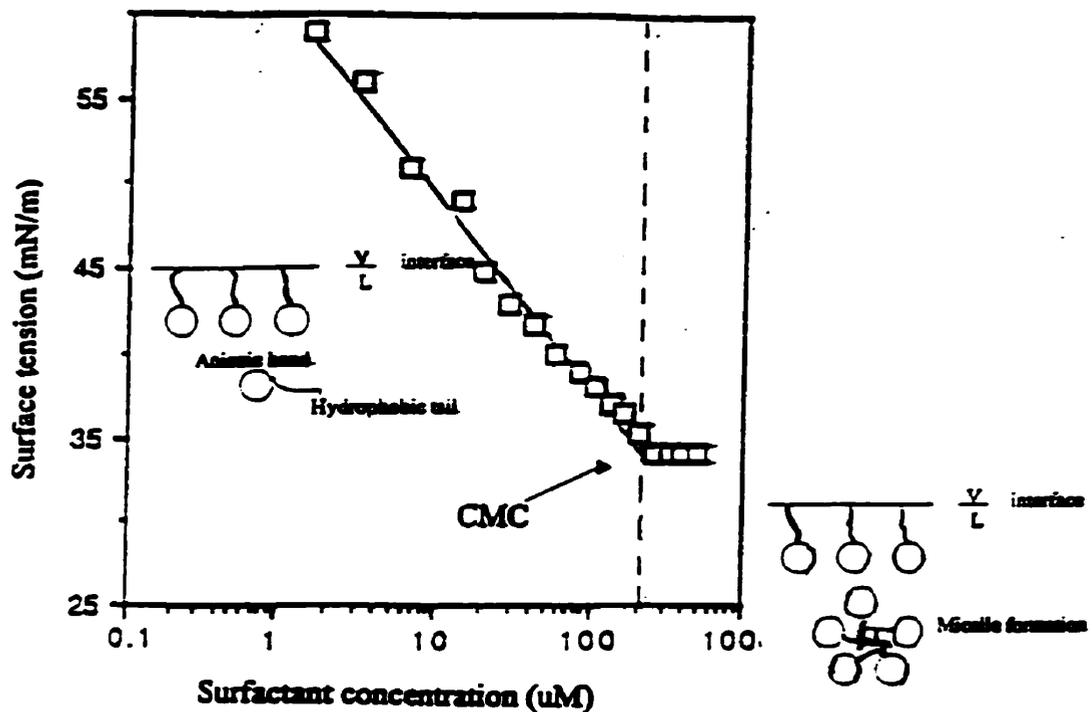


Figure 2.3. Surface tension as a function of surfactant concentration and surfactant orientation. Surfactant accumulates at the vapour/liquid (V/L) interface. As the concentration of surfactant increases, the surface tension decreases no further and micelle formation is initiated in the bulk solution at the CMC.

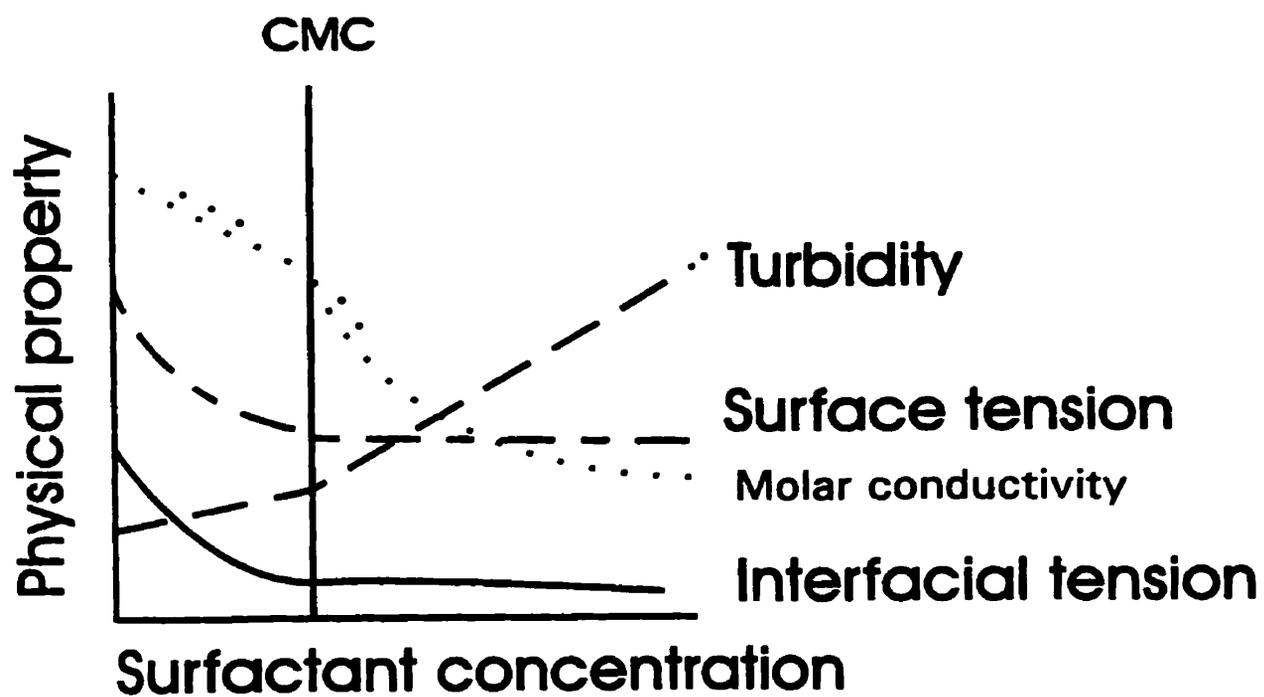


Figure 2.4 Changes in physical properties at the CMC (Myers, 1988).

et al., 1990). Surfactants in addition to organic solvents, chelating agents, acids and bases have been used to enhance metal removal (Holden et al., 1989).

### **II.2.2 Biosurfactants**

Some surfactants, known as biosurfactants, are biologically produced from yeast or bacteria from various substrates including sugars, oils, alkanes and wastes (Lin, 1996). For example, *Pseudomonas aeruginosa* can produce rhamnolipids from substrates including C<sub>11</sub> and C<sub>12</sub> alkanes, succinate, pyruvate, citrate, fructose, glycerol, olive oil, glucose and mannitol (Robert et al., 1989). They are synthesized as metabolic by-products. Composition and yields depend on the fermentor design, pH, nutrient composition, substrate and temperature used (Mulligan and Gibbs, 1993). They can be potentially as effective with some distinct advantages over the highly used synthetic surfactants including high specificity, biodegradability and biocompatibility (Cooper, 1986). For example, glycolipids from *Rhodococcus species 413A* were 50% less toxic in naphthalene solubilization tests than Tween 80 (Kanga et al., 1997).

Biosurfactants are grouped as glycolipids, lipopeptides, phospholipids, fatty acids and neutral lipids (Bierman et al., 1987). Most of these compounds are either anionic or neutral. Only a few are cationic such as those containing amine functions. The hydrophobic part of the molecule is based on long-chain fatty acids, hydroxy fatty acids or  $\alpha$ -alkyl- $\beta$ -hydroxy fatty acids. The hydrophilic portion can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol. Their CMCs generally range from 1 to 200 mg/L and their molecular weights from 500 to 1500 amu. (Lang, 1987). A wide variety of microorganisms can produce these compounds (Table 2.5).

**Table 2.5. Classification and microbial origin of biosurfactants**

Surfactant class	Microorganism
Trehalose lipids	<i>Arthrobacter paraffineus</i> <i>Corynebacterium spp.</i> <i>Mycobacterium spp.</i> <i>Rhodococcus erythropolis</i>
Rhamnolipids	<i>Pseudomonas aeruginosa</i>
Sophorose lipids	<i>Candida apicola</i> <i>Candida bombicola</i> <i>Candida lipolytica</i>
Glucose-,fructose-, saccharose lipids	<i>Arthrobacter spp.</i> <i>Corynebacterium spp.</i> <i>R. erythropolis</i>
Cellobiose lipids	<i>Ustilago maydis</i>
Polyol lipids	<i>Rhodotorula glutinus</i> <i>Rhodotorula graminus</i>
Diglycosyl diglycerides	<i>Lactobacillus fermentii</i>
Lipopolysaccharides	<i>Acinetobacter calcoaceticus (RAG1)</i> <i>Pseudomonas spp.</i> <i>Candida lipolytica</i>
Lipopeptides	<i>Arthrobacter sp.</i> <i>Bacillus pumilis</i> <i>Bacillus subtilis</i> <i>Bacillus licheniformis</i> <i>Pseudomonas fluorescens</i>
Ornithine, lysine peptides	<i>Thiobacillus thiooxidans</i> <i>Streptomyces sioyaensis</i> <i>Gluconobacter cerinus</i>
Phospholipids	<i>T. thiooxidans</i> <i>Corynebacterium alkanolyticum</i>
Sulfonylipids	<i>Capnocytophaga spp.</i>
Fatty acids (corynomycolic acids, spiculisporic acids, etc.)	<i>Penicillium spiculisporum</i> <i>Corynebacterium lepus</i> <i>Arthrobacter paraffineus</i> <i>Talaromyces trachyspermus</i>

Adapted from Mulligan and Gibbs (1993) and Banat (1995)

Biosurfactants have been tested in enhanced oil recovery and the transportation of crude oils (Hayes et al., 1986). They were demonstrated to be effective in the reduction of the interfacial tension of oil and water *in situ* and the viscosity of the oil, the removal of water from the emulsions prior to processing and in the release of bitumen from tar sands. Emulsan® has been commercialized for this purpose (Anonymous, 1984). It contains a polysaccharide with fatty acids and proteins attached. Although most biosurfactant-producing organisms are aerobic, a few examples of anaerobic producers exist. *Bacillus licheniformis* JF-2 is an example which would be well suited for *in situ* studies for enhanced oil recovery or soil decontamination (Javaheri et al., 1985). A 70% bioremediation and bioreclamation rate of a slop oil-contaminated soil was achieved with oil degrading cultures (Dave et al., 1994).

Most biosurfactants are produced on hydrocarbons (Syldatk and Wagner, 1987). Production is most often growth associated (i.e., highest production occurs as microbial growth is highest). In this case, they can either use the emulsification of the substrate (extracellular) or facilitate the passage of the substrate through the membrane (cell wall associated). Biosurfactants, however, are also produced from carbohydrates which are soluble in water. They are usually secondary metabolites, produced during the late logarithmic and stationary growth phases. One of the biosurfactants that has been studied extensively is the rhamnolipids from *Pseudomonas aeruginosa* (Hitsatsuka et al., 1971; Guerra-Santos et al., 1984). They produce two types of rhamnolipids containing two rhamnoses attached to  $\beta$ -hydroxydecanoic acid or one rhamnose connected to the identical fatty acid (Figure 2.5) from both glucose and hydrocarbon substrates. Pilot studies have been performed on their production with final concentrations reaching 2.0 g/L. Surface tensions of 29 mN/m are characteristic of these compounds.

Rhamnolipid surfactants have been tested and found to release three times as much oil as water alone from the beaches in Alaska after the Exxon Valdez tanker spill (Harvey et al., 1990). Scheibenbogen et al. (1984) found that the rhamnolipids from *P. aeruginosa* UG2 were able to effectively remove a hydrocarbon mixture from a sandy loam soil and that the degree of removal was dependent on the type of hydrocarbon removed and the concentration of the surfactant used. Van Dyke et al., (1993) previously found that the same strain could remove at a concentration of 5 g/L, approximately 10% more hydrocarbon from a sandy loam soil than a silt loam soil and that sodium dodecyl sulfate (SDS) was less effective than the biosurfactants in removing hydrocarbons. Recently, Deschênes et al. (1994) showed that the rhamnolipids from the same strain in a bioslurry could enhance the solubilization of four-ring PAHs more significantly than three-ring PAHs and that the biosurfactants were five times more effective than SDS. The benefit of the addition of the biological and synthetic surfactants in assisting the biodegradation process was less evident. Research with another strain, *Pseudomonas marginalis*, also indicated that the biosurfactants produced solubilized PAHs such as phenanthrene and enhance biodegradation (Burd and Ward, 1996).

*Torulopsis bombicola* is one of the few yeasts known to produce biosurfactants (Cooper and Paddock, 1984). High yields of a sophorolipid from soybean oil and glucose of 0.35 g/g of substrate or 67 g/L have been reported. Other substrates such as animal fat (Despande and Daniels, 1995) and lactose and canola oil (Zhou and Kosaric, 1995) have recently been evaluated as alternative carbon sources. Sophorose lipids (Figure 2.6) can lower the surface tension to 33 mN/m. Recently, the technique of self-cycling fermentation has been used to produce sophorolipids (McCaffrey and Cooper, 1995). In the cosmetic industry, this biosurfactant has been derivatized and used as a

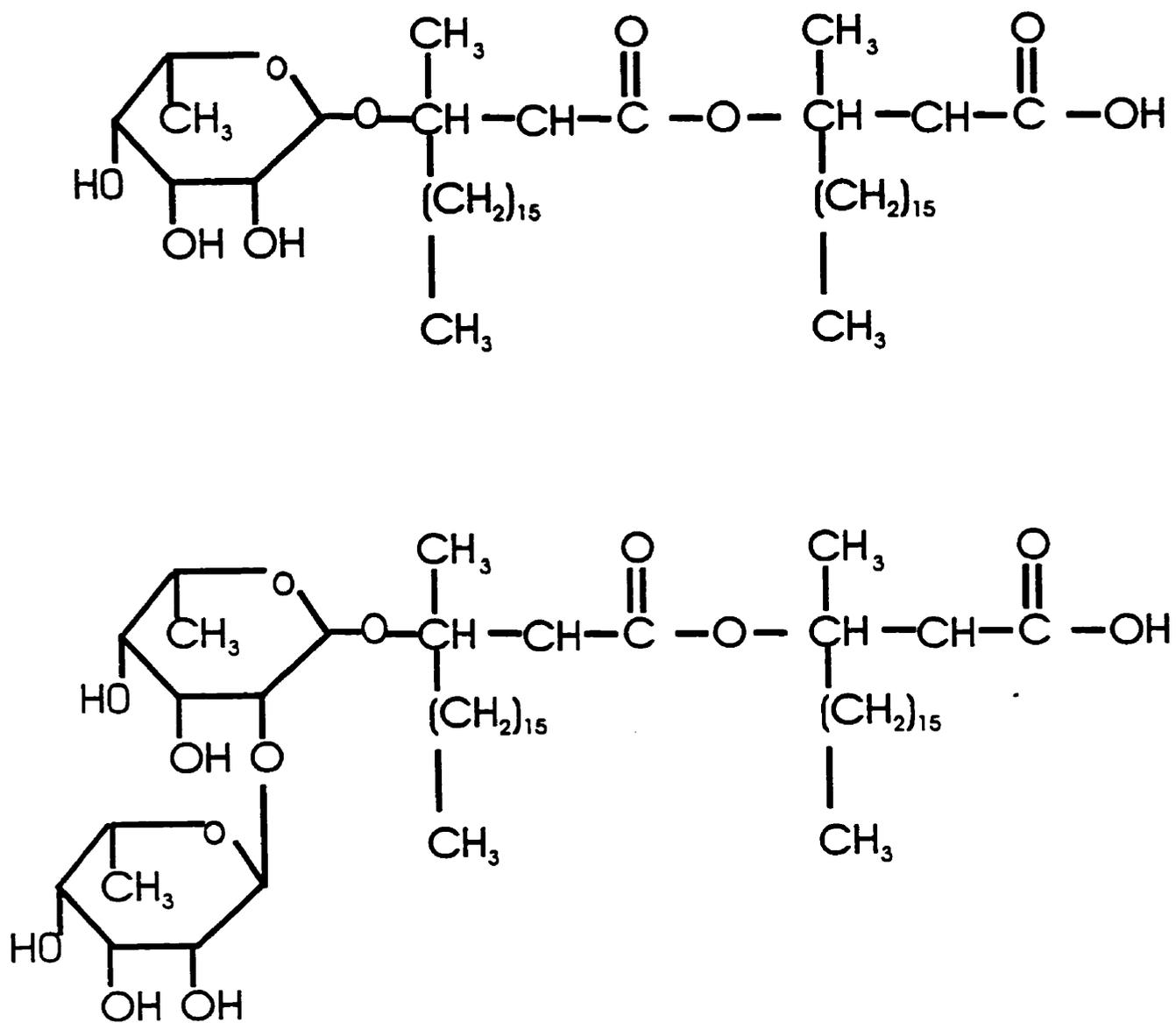


Figure 2.5. Structure of rhamnolipids from *P. aeruginosa*

protective substance (Inoue, 1988). Although the sophorolipid has been used to release bitumen from tar sands (Cooper and Paddock, 1984), no applications have been reported so far concerning its potential to enhance the remediation of contaminated soils.

*Bacillus subtilis* produces a lipopeptide called surfactin containing seven amino acids bonded to the carboxyl and hydroxyl groups of a 14-carbon acid (Kakinuma et al., 1969). The surfactant is very effective (27 mN/m at concentrations of as low as 0.005% can be used) but low yields during production are obtained (0.02 g/g glucose) (de Roubin et al., 1989). Its foaming characteristics at a concentration of 0.05 mg/L are comparable to SDS and bovine serum albumin (BSA) (Razafindralambo et al., 1996). This compound has been shown to improve the mechanical dewatering of peat by greater than 50% by changing the flow characteristics of the trapped water within the peat waxes on the particle surfaces (Cooper et al., 1986) at very low concentrations (0.0013 g/g wet peat). Enhanced surfactin production can be determined by blood agar plate screening (Mulligan et al., 1984) and genetic manipulation (Mulligan et al., 1989). It is used in biochemical research for its ability to inhibit blood coagulation and protein denaturation and to accelerate fibrinolysis. It has recently been demonstrated to have antimyoplasmic properties (Vollenbroic, 1997). It can bind to metals (magnesium, manganese, calcium and barium) due to the presence of two negative charges, one on the aspartate and the other on the glutamate residues of surfactin (Thimon et al., 1992).

Unlike *B. subtilis* and *T. bombicola*, *P. aeruginosa* has been studied extensively in terms of its properties for enhancement of soil washing techniques and biodegradation studies with regards to hydrocarbon contamination. However, due to the presence of metals in hydrocarbon

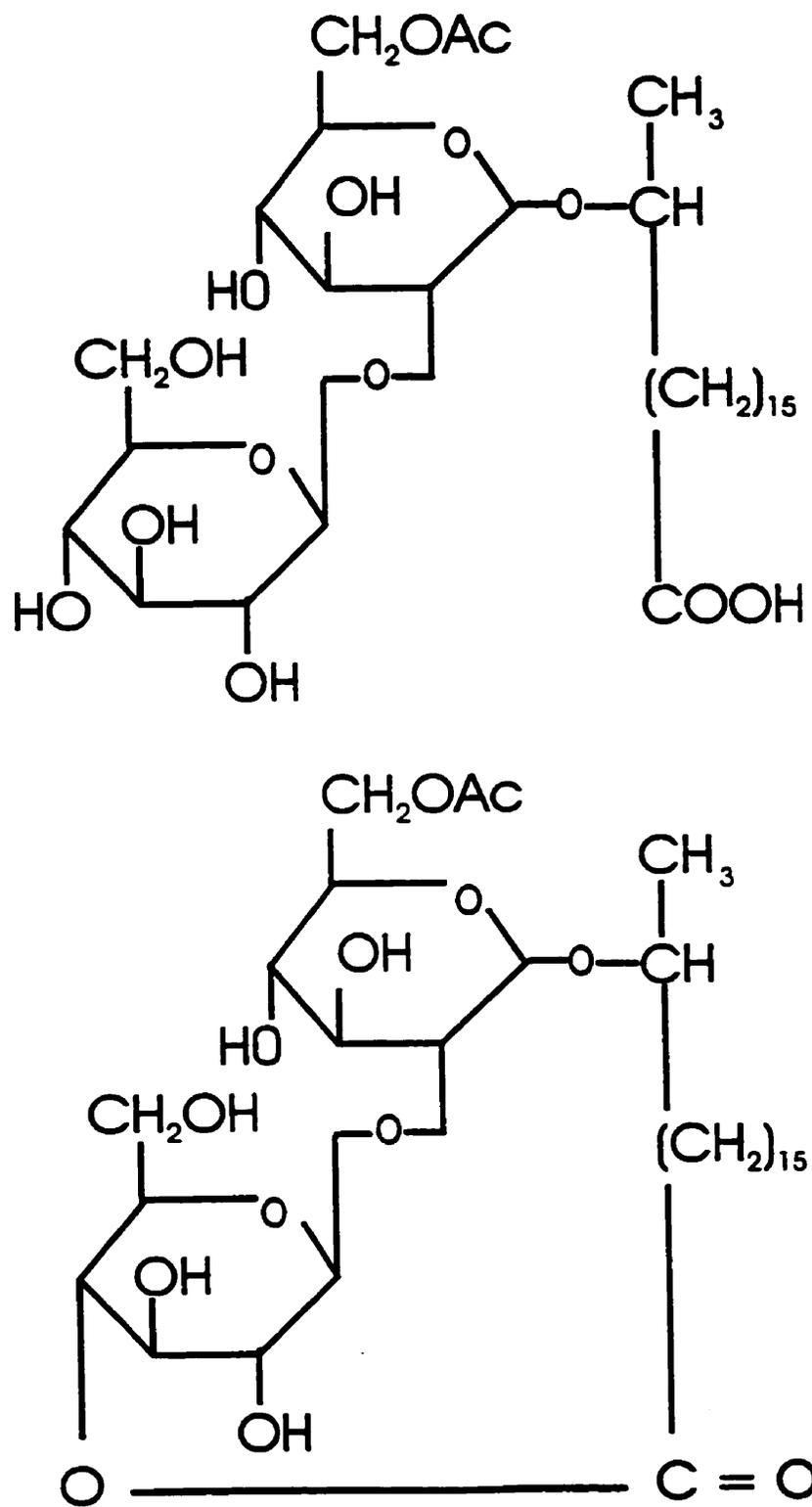


Figure 2.6 Structure of sophorolipid from *T. bombicola*



contaminated soils and the anionic nature of the biosurfactants, an examination of their potential to bind metals is required (Miller, 1996).

### II.3 Interaction of soils and sediments with contaminants

The interaction of contaminants, organic or inorganic, in their liquid phase with soil is a very complex matter since numerous factors are involved. Contaminants can be metals (cadmium, lead, zinc, copper, etc.), organics (PCBs, pesticides, alcohols, phenols, etc.) or anionic salts (chlorides, nitrates, etc.). The composition of the soil is one of the most important factors influencing the retention of contaminants in the soil (Yong et al., 1992b). The main components can be divided into soil organics and inorganics (amorphous and crystalline) which is shown in Figure 2.8. The proportion of each of these components affects the retention of contaminants due to their cation exchange capacity, surface area and functional groups. Each group will be discussed with respect to its origin, occurrence and contribution to the retention of contaminants. In addition, the various types of interaction which include ionic exchange, hydrogen bonding, van der Waals forces, adsorption, precipitation and complexation can be related to contaminant retention by the different soil components.

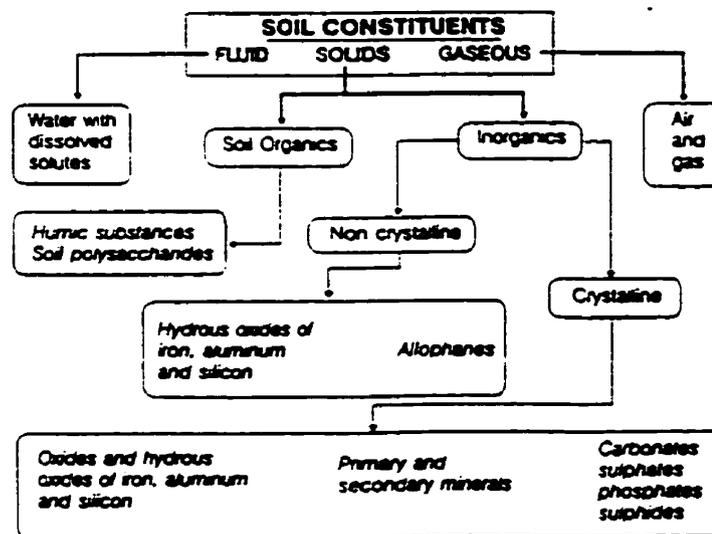


Figure 2.8. The main soil components (Yong et al., 1992b)

### **II.3.1 Components of soil**

Soil is heterogeneous and consists of air, water and mineral and organic solids. The minerals are either primary (rock fragments) or secondary. Primary minerals have not been weathered and are not changed from the parent material (Yong et al., 1992b). They make up a large proportion of sands and silts and are generally of large particle size. They include quartz, feldspar and others such as micas and amphiboles. These do not have a very great influence on contaminant retention since they have large particle sizes and low specific surface areas (Yong et al., 1992b).

Secondary minerals are formed as products of physical, chemical or biological weathering (Yong et al., 1992b). They include the layer silicates, kaolinites, chlorites, micas, montmorillonites and vermiculites (the silicates are the most abundant). Others are amorphous or non-crystalline and include allophanes, imogolites, amorphous silica, iron and alumina hydroxides and oxides. Sulfates and carbonates are precipitates.

#### Clay minerals

Clay minerals occur in nature in sedimentary rocks, in continental and marine sediments and are formed as a result of weathering and are essential components of most soils. They are also formed as a result of hydrothermal activity and are associated with volcanic activity and ore deposits. They provide the characteristics and properties of clay such as plasticity, refractoriness, colour and colloidal properties (Sposito, 1983).

All clay minerals, with possibly the exception of halloysite, have been formed from mixtures of oxides or hydroxides at moderately low temperatures and pressures but can be formed at ordinary conditions if the oxides are diluted together very slowly. Kaolinite will form in alumina-silica processes without

the presence of alkalies or alkaline soil. Illite is formed upon the addition of potassium while smectite and chlorite require magnesium. The formation of the clay minerals depends on the parent rock, climate, topography, vegetation and time period. During the transportation of clay minerals from freshwater to marine environments, mica degradation occurs. Illite and chlorite form smectite due to the adsorption of potassium and magnesium from the sea water. During hydrothermal processes such as geysers and hot springs, alkalies and alkaline earths can influence the formation of clay mineral (Simpson, 1993).

Kaolinite is a dominant component in many soils such as those which are rich in ash and organic matter. Illite and smectite occur in prairie soils and predominate in soil of arid regions and in black, fertile soils. Illite and chlorite soils are well suited to agriculture due to their ion-exchange capacities and ability to hold plant nutrients. Smectite has a very high water-holding capacity (Yong et al., 1992b).

Illite and chlorite are dominant in marine sediments. Smectite is found in substantial amounts in the Gulf of Mexico and in other regions. Sediment from nonmarine sedimentation may also contain illite, chlorite and attapulgite in desert areas (Sposito, 1983).

All clay minerals with the exception of attapulgite have been found in areas associated with hydrothermal processes such as geysers, hot spring and aureoles (Simpson, 1993). Mica and kaolinite are found frequently close to the source whereas chlorite and smectite are further away. The clay minerals dominate the content in soils and thus play a dominant role in contaminant retention. Therefore, their structures are very important.

In general, clay minerals consist of two units. The first is composed of two sheets of oxygen or hydroxyls with individual aluminum, iron or magnesium atoms in between the sheets. Each metal atom is in the middle and equidistant from the oxygens in an octahedral structure. When aluminum dominates, it is called the gibbsite structure and has the formula  $\text{Al}_2(\text{OH})_6$ . When magnesium dominates, it is named brucite and the structural formula is  $\text{Mg}_2(\text{OH})_6$ . In the second unit, a silica atom is in the middle of four oxygen or hydroxyls in a tetrahedral form. The sheet has the formula  $\text{SiO}_4\text{O}_6(\text{OH})_4$ .

Kaolinite contains a tetrahedral and octahedral sheet. The anionic octahedral layer and tips of silica tetrahedra form a common layer. Silica and aluminum form two-thirds of the anions which become oxy instead of hydroxy. The structure is  $(\text{OH})_8\text{Si}_4\text{Al}_4\text{O}_{10}$ . The specific surface area is small at 5 to 40  $\text{m}^2/\text{g}$ . Halloysite has two forms  $(\text{OH})_8\text{Si}_4\text{Al}_4\text{O}_{10}$  and the other with four water molecules. The spacing for the hydrated form is 10.1 Å and for the dehydrated 7.2 Å. The difference is due to the water thickness.

Illite minerals have an alumina sheet in the middle of two silica layers units which are bonded with potassium ions fitted into hexagonal holes in the silica sheets. The specific surface area is 100 to 200  $\text{m}^2/\text{g}$ . Smectite contains two silica tetrahedral sheets with a central alumina octahedral group. The smectite group is  $(\text{OH})_4\text{Si}_8\text{Al}_4\text{O}_{20} \cdot n\text{H}_2\text{O}$ . Aluminum or phosphorous may substitute for silicon. Magnesium, iron, zinc, nickel, lithium and others may substitute for the aluminum in the octahedral sheet. Their interlayer bonding is weak, thus allowing water to pass through, The specific surface area is larger than illite and kaolinite (700 to 800  $\text{m}^2/\text{g}$ ). Vermiculites have specific surface areas of 300 to 500  $\text{m}^2/\text{g}$  (White, 1987).

In summary, the siloxane and oxide/hydrous oxide predominate in clay minerals

and play very important roles in the behaviour of clays (Yong et al. 1992b). The siloxane surfaces dominate in micas, smectites and vermiculites while the oxide/hydrous oxide surfaces are exhibited by 1:1, 2:1 and 2:1:1 layer silicates. These are the functional groups which can interact with contaminants. Clay minerals usually occur with humic colloids and hydrous oxides.

#### Oxides of iron, manganese and aluminum

Secondary minerals can contain silica, iron, manganese, titanium and alumina and can exist as hydroxides, oxyhydroxides or oxides (Yong et al., 1992b). They are formed by the weathering of feldspar and other silicate minerals. Gibbsite is formed from the decomposition of kaolinite or halloysite (Yong et al., 1992b). Some examples of secondary minerals are shown in Table 2.6. Others include iron and aluminum hydroxides,  $\text{Al(OH)}_3$  and  $\text{Fe(OH)}_3$ .

**Table 2.6. Minerals found in soils (Simpson, 1983; Fuller and Warrick, 1985)**

Oxides/hydroxides	
Si-oxide	Quartz, tridymite
Fe-oxides/hydroxides	Goethite ( $\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$ ), hematite, limonite ( $\text{Fe}_2\text{O}_3 \cdot x\text{H}_2\text{O}$ )
Al-oxides/hydroxides	Gibbsite ( $\text{Al}_2\text{O}_3 \cdot 3\text{H}_2\text{O}$ ), boehmite, diaspore
Carbonates	Calcite ( $\text{CaCO}_3$ ), dolomite ( $\text{MgCa}(\text{CO}_3)_2$ )
Halides	Halite ( $\text{NaCl}$ ), sylvite ( $\text{KCl}$ ), carnallite ( $\text{KMgCl}_3 \cdot 6\text{H}_2\text{O}$ ), ( $\text{CaCl}_2 \cdot n\text{H}_2\text{O}$ )
Nitrates	Soda-nitre ( $\text{NaNO}_3$ ), nitre ( $\text{KNO}_3$ ), calcium nitrate ( $\text{Ca}(\text{NO}_3)_2$ )
Phosphates	Apatite ( $\text{Ca}_5(\text{F, Cl, OH})(\text{PO}_4)_3$ ), vivianite ( $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ )
Sulfates	Gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ )

Hydrous oxides or sesquioxides are the oxides of aluminum, iron and

manganese. In heavily weathered soils in the tropics, they can be more abundant than the clay minerals with which they are mixed (Wild, 1988). They can have several forms including soil particle coatings, void fillings and concentric nodules. The most common form is hydrous iron oxide. Iron precipitates to form ferrihydrite and then dehydrates to goethite (O'Neill, 1985). Aluminum hydroxide is commonly found as gibbsite and manganese oxide as birnssite, hollandite and lithiophorite (Brown, 1954).

Other components such as carbonates, halides, nitrates, phosphates and sulfates are also shown according to the chemical formulas in Table 2.6. The distribution of minerals is not uniform throughout the earth as demonstrated by the availability of plant nutrients such as phosphorous which is concentrated in deposits of mining quality in some areas but deficient for agricultural purposes in others (Simpson, 1983). Since minerals do not weather at the same rate, soils differ according the original components. Geological events such as volcanos, resulted in many variations throughout the world and yielded igneous, sedimentary and metamorphic rocks which lead to the various minerals varying in composition and rate of decomposition.

Carbonates such as calcite are found in arid and semi-arid regions, in addition to soils from calcium based parent material such as limestone or lime based soils. Calcium carbonate minerals can be found as particles or as coatings (Yong et al., 1992b). Carbonates can significantly increase the pH of the soil and can form precipitates with contaminants such as metals. Sulfates such as gypsum are also found in arid and semi-arid regions. Along with oxides, hydroxides, oxyhydroxides, and halide minerals, sulfates are restricted to these arid regions where leaching is limited and evaporation occurs due to their high solubilities. Leaching determines the profile of components within the soil. More soluble components are leached to a greater depth. All chlorides and

sodium, potassium and magnesium sulfates are more soluble than gypsum which is more soluble than calcium and magnesium bicarbonates (Simpson, 1983).

The properties of amorphous materials are important since they make up 10 to 30% of total soil material by themselves or as coatings of such minerals as kaolinite. Amorphous materials are noncrystalline (Yong et al., 1992b). Some examples include allophanes, imogolites, amorphous silica, amorphous iron and alumina. Amorphous alumina silicates are weathering products of volcanic ash or intense leaching (Yong et al., 1992b). Allophanes are the most common and consist of silica tetrahedra and ions such as alumina in a octahedral arrangement. Oxides of iron, titanium, phosphorous, calcium, magnesium, potassium and sodium (2-3%) can be present.

#### Organic matter

Although the content of organic matter in soils is generally small (0.5 to 5%, (Yong et al., 1992b) except for organic soils such as peat which is 100% organic) it contributes quite significantly to contaminant retention. It is originally plant material. Animals attack plant material and are therefore a secondary source of organic matter. Shrubs, grasses and trees will be decomposed by soil organisms. Animals contribute their waste materials and leave their bodies for decomposition. The general components are carbohydrates, fats, waxes, tannins, lignins and proteins (Buckman and Brady, 1969). Organic matter contains 58 to 60% organic carbon (Alloway, 1995).

As organic tissue is incorporated into the soil, it is converted by soil organisms into simpler products (Buckman and Brady, 1969). Oils, fats, and waxes are very resistant to attack. New compounds are formed by the organisms such as polysaccharides, organic acids and polyuronides. These two groups of

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compounds form the basis of organic material in soils.

The more decomposed organic material of animal and plant origin is called humus. It is usually black or brown and colloidal in nature. The substances making up humus are modified from the original tissues or synthesized by soil organisms. Its properties are thus very different from the parent and is amorphous (Buckman and Brady, 1969).

Humus has a much higher capacity to hold water and nutrients than clay (Buckman and Brady, 1969). It is also composed of carbon, hydrogen and oxygen rather than aluminum, silicon and oxygen. Humus is not as stable as clay but can degrade more easily and is not considered crystalline.

The major sources of negative charges in humus are carboxylic (-COOH) and phenolic (>-OH) groups. The charge is, however, pH dependent. Under acid conditions, the hydrogen cannot be replaced by other cations such as Ca, Mg and others. This is not the case at higher pH where substitution of the hydrogen with other cations is possible (Buckman and Brady, 1969).

Humus is classified according to its solubility in acids and bases (Yong et al., 1992b; Buckman and Brady, 1969).

- (i) Fulvic acid is light in colour, soluble in acid and base and low in molecular weight.
  - (ii) Humic acid is soluble in alkali only, precipitates in acids and is darker and of higher molecular weight.
  - (iii) Humin is the darkest and heaviest and insoluble in both acid and bases.
- These three groups exhibit similar cation adsorption and nutrient release properties.

The type of vegetation determines the type of humus formed. For example, grasses produce humic acid rich humus, whereas conifers yield a humus with a high fulvic acid content (Yong et al., 1992b).

The formation of humic materials has been actively disputed. Four hypotheses have been cited by Schnitzer and Khan (1978) including: formation through plant alteration, formation through chemical polymerization of degraded animal and plant material, production by autolysis of cellular materials, and synthesis by microbes.

Humic substances have very high molecular weights from hundreds to tens of thousands which in turn influence their ability to interact with other constituents of the soil. The most common functional groups are hydroxyls, carboxyls, phenolics and amines (Figure 2.9). They can gain and lose electrons depending on the pH, much like the hydroxyl groups of clay minerals. Carboxyls and phenolic groups are the most important for CEC and chelating abilities (Yong et al., 1992). The carbon and oxygen content, molecular weight, acidity and CEC decrease in the following order: humin > humic acid > fulvic acid (Hayes and Swift, 1978).

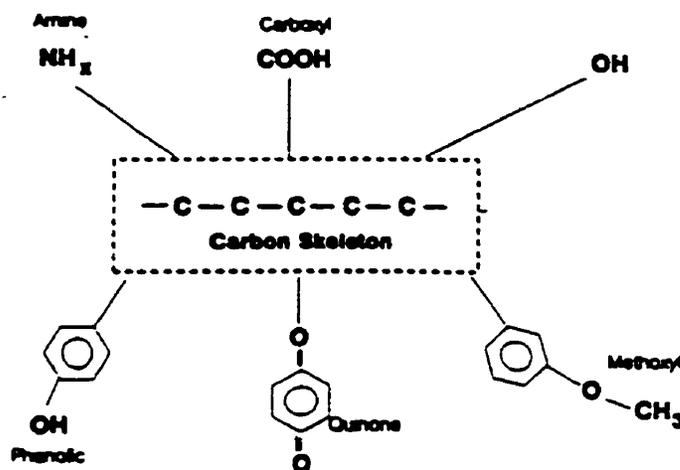


Figure 2.9. The functional groups of humus (Yong et al., 1992b)

Humic acids have molecular weights from 20,000 to 100,000 and contain 50-60% carbon, 30-40% oxygen, 3-6% hydrogen, 0.5-6% nitrogen and 0-2% sulfur (Yong et al. 1992b). Carboxyl groups are the most abundant functional group in humic acids (3-10%), followed by phenolic (2-5%), alcoholic (0-2%), carbonyls (0.5-4%), quinone (1-2%) and ketonic (1-4%).

Fulvic acids (Yong et al. 1992b) are higher in oxygen and sulfur contents than humic acids but lower in carbon, hydrogen and nitrogen (40-50% carbon, 40-50% oxygen, 4-7% hydrogen, 1-3% nitrogen, and 1-4% sulfur). Carboxyl (1.5-6%) and phenolic (2-6%) functional groups are approximately equal in abundance. Other include alcoholic (2.5-4%), quinones (2-2.5%), ketones (1.5-2%) and carbonyls (0.3-5%). Humins are not as well understood but are known to be comprised of carbon with smaller contents of nitrogen and polysaccharides (Yong et al., 1992b).

The functional groups can protonate or deprotonate depending on the pH of the soil in a water environment. The cation exchange of a soil varies with depth. The upper layers (A) contain higher contents of organic material compared to the lower horizons. In the B and C regions, clay content is mainly responsible for the cation exchange capacity (Heiling et al., 1964).

The hydrophobic effect of organic molecules occurs at the hydrophobic surfaces of organic matter. Non-polar molecules are preferred over water by aliphatic side chains and lignin groups in humic substances (Senesi and Chen, 1989).

### **II.3.2 Contaminant retention mechanisms**

Cation exchange capacity (CEC) for the various types of clay minerals is shown in Table 2.7. It is defined as the exchange of counter-ions at the solid

surface with the ions in solutions (Brown, 1954). In general, the CEC increases as the surface area increases. The specific surface area of kaolinite is 10 to 20 m<sup>2</sup>/g, illite is 80 m<sup>2</sup>/g, chlorite is 70-150 m<sup>2</sup>/g and montmorillonite is 600-800 m<sup>2</sup>/g. Ion exchange is an important mechanism for the retention of cations in clay minerals due to their particle size, crystallinity, and the nature of the broken bonds at the surface which leads to unsatisfied charges (Fitzpatrick, 1980). The higher the ion valency, the less likely it will be replaced.

**Table 2.7 Cation exchange capacities of common exchange materials in soils (Hausenbuiller, 1985)**

Exchange material	Cation exchange capacity meq/100 g
Organic matter	100-300
Vermiculite	100-150
Allophane	100-150
Montmorillonite	60-100
Chlorite	20-40
Illite	20-40
Kaolinite	2-16
Hydrous oxides	0

Organic matter can contribute quite extensively to the cation exchange capacity of soil as calculated by the following equation (Foth, 1981):

$$\text{CEC soil} = (\% \text{ organic matter} \times \text{CEC organic matter} + \% \text{ clay} \times \text{CEC clay matter}) \times 0.01$$

Amorphous iron has a specific surface area of 700 m<sup>2</sup>/g, CEC of 30 meq/100g and a high water holding capacity. Due to their hydroxyl functional group, they can form water bridges with contaminants. High specific surface areas (300-700 m<sup>2</sup>/g) and cation exchange capacities (150 meq/100g) are characteristic of allophanes which are found in some clays. Its composition is variable consisting of significant amounts of sulfate and phosphate molecules. The structure of allophane is approximated by Al<sub>2</sub>O<sub>3</sub>·2SiO<sub>2</sub>·H<sub>2</sub>O (Sposito, 1983). The oxides are functional groups in allophane which is mostly found in soils originating from volcanic ash and has high anion exchange capacities due to the pH dependent charge because of exposed hydroxyl groups. At low pH, H<sup>+</sup> ions are attracted resulting in a positive charge. Anions such as chlorides and nitrates can then be adsorbed.

Ion exchange in soils is highly variable (Hausenbuiller, 1985). There are both exchangeable and nonexchangeable ions. The former type are weakly associated, allowing easy replacement with other ions whereas the latter are difficult to displace. They are either held very strongly or are in a difficult place to remove. For example, potassium is held within the platelets of illite clay or mica and cannot be removed unless extensive weathering or organic matter decomposition takes place.

Ions can be bound to the soil by a combination of forces ranging from electrostatic to covalent with corresponding increases in bonding energy. When covalent bonding dominates, specific adsorption can be observed for certain cations and anions and the ion exchange is irreversible. The mechanisms and rates of reaction are dependent upon the type and amounts of clay, hydrous oxide and organic matter as well as the solute composition and concentration, pH and oxidation-reduction status (Keeney and Wildung, 1977).

The cation exchange capacity of a soil varies with pH. This is due to the presence of -OH groups in clay platelet matter, organic matter and amorphous clay. The negative charge is the result of hydrogen displacement in soil organic matter and allophane. They have neither cation exchange or a negative charge at low pH values. Both increase as the pH increases. The effect of pH dependency is less for clay kaolinite since it has a fixed charge.

Cation exchange rates are highest for organic matter, intermediate for expanding clays and allophane and lowest for nonexpanding clays and hydrous oxides (Table 2.7). Hydrous oxides have no charge and do not develop pH dependent charges due to ionic substitution. Kaolinite has only a small charge. Allophane, although close to kaolinite in structure, has tetrahedra and octahedra which are not well layered. The octahedra, however, have a high content of OH<sup>-</sup> ions and are able to produce a negative charge at higher pH. Illite and chlorite have high total internal charges but contain K<sup>+</sup> in the interlayers in illite and a positively charged interlayer in chlorite. Sand and silt have unsatisfied negative bonds (Foth, 1984) but have low specific surface areas and thus contribute little to cation exchange.

The influence of amorphous complexes on clay materials has been studied recently (Yong et al., 1992a). They influence the water holding capacity and bonding ability of clays due to the effect of pH on charge. At high pH, they develop hydroxylated negative charges and at low pH, they exhibit positive charges due to the adsorption of protons as shown below. The form would influence the formation of -OH bonds (hydrogen bonding) with other components. M represents the amorphous ion.



Metals can also precipitate as hydrous oxides or hydroxides on clay surface or adsorb as complexes (Mortland, 1976).  $\text{Al}^{3+}$ ,  $\text{Mg}^{2+}$  and  $\text{Fe}^{3+}$  can form hydroxy and oxide-hydroxy polymers. Ions such as  $\text{Li}^+$  can penetrate into the octahedral sheets of smectite. Organics can be adsorbed onto the surface of clays and form complexes. Hydrogen bonding can be another type of interaction and can take the form: (1) Hydrogen bonding between water molecules and the functional groups of the organic such as the carbonyl moiety. (2) Hydrogen bonding between the alcohol or amino groups on the organic and the exposed oxygens on the clay surface. (3) Hydrogen bonding between two organic molecules on the clay surface.

The oxides will assume various hydrated forms in water and will form colloidal-size particles. Hydrous oxides are very reactive with pollutants such as heavy metals in aqueous metal waste streams since they will form metal oxides (Fuller and Warrick, 1985). They can partake in the following reactions:



where Me is a metal and SOH is the hydrous oxide. These minerals are formed as highly weathered soils such as laterites and bauxites (Yong et al., 1992a). Unlike clay minerals which form layers, these surfaces of the crystalline oxides are very irregular due to broken bonds. The charge properties are pH dependent. Zinc, copper, chromium and others can coprecipitate as Fe and Mn oxides. The charge of the oxides is generally negative in alkaline conditions and positive in acidic conditions. The point of zero charge (PZC) is from 7 to 10 for iron oxides, 8 to 9.4 for aluminum oxides and 1.5 to 4.6 for manganese oxides (Alloway, 1995).

The hydrous oxides are not as sticky, plastic and cohesive as silicates (Fuller and Warrick, 1985) and soils containing significant amounts of these minerals exhibit high anion adsorption capacities. The hydroxyl groups are the functional groups in these compounds.

The binding capacity of organic soils for contaminants consists of several mechanisms: complexation, adsorption, and chelation (Yong al., 1992b). Van der Waals forces can be quite significant for large molecules due to the additive nature of these forces and the presence of hydrogen bonds.

Metals can form hydroxy complexes that are adsorbed depending on the pK values of the reaction  $Me^{2+} + H_2O \rightarrow MeOH^+ + H^+$  and the ionic size. Ions such as lead are adsorbed more strongly than copper since it has a larger ionic size. In decreasing order of adsorption according to Brummer (1986) are: Hg (pK = 3.4) > Pb (pK = 7.7) > Cu (pK = 7.7) >> Zn (pK = 9.0) > Co (pK = 9.7) > Ni (pK = 9.9) > Cd (pK = 10.1). Metals can also precipitate with secondary minerals in the soil as shown in Table 2.8.

**Table 2.8 Trace metals normally found co-precipitated with secondary minerals in soils (Sposito, 1983)**

Mineral	Co-precipitated trace metal
Iron oxide	Cu, Ni, Mo, Ni, V, Zn
Manganese oxide	Co, Ni, Pb, Zn
Calcium carbonate	Co, Cd, V
Clay mineral	Cu, Cr, Ni, Pb, Ti, Zn

Organic matter is an important factor in the sorption of metals (Ellis, 1973; Hodgson, 1963). Humic and fulvic acids are known chelaters. There is high correlation between metal sorption and organic content in soils. Also there is an increase in the extractability of metals once the soil has been treated with hydrogen peroxide which destroys organic matter.

The presence of the functional groups in organic matter such as carboxylates, phenols, alkyl amino and mercaptides (Keeney and Wildung, 1977) have the greatest potential for forming metal complexes. Esters, ether, amide and thioethers, and carbonyls will also participate in ion metal complexing but to a lesser extent.

Chelation (Keeney and Wildung, 1977) is a type of complexation that occurs between a metal ion and an organic ligand resulting in the formation of two or more bonds between the two in a heterocyclic ring (Figure 2.10). Humic and fulvic acids have high charge density due to their acidic functional groups (carboxyl, hydroxyl, phenolic, carbonyl and enolic). This characteristic leads to a strong affinity for cations. The amount of carboxyl groups is particularly important in determining the quantity of chelated ions. Fulvic and humic acids account for most of the metal immobilization in soil organic matter. The chelates of decreasing order of stability are:  $\text{Cu} > \text{Fe} = \text{Al} > \text{Mn} = \text{Co} > \text{Zn}$  (Alloway, 1985).

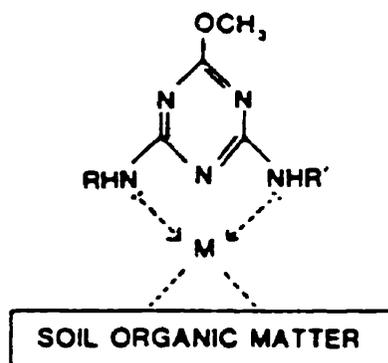


Figure 2.10 Binding of a metal by organic matter ligands (Yong et al., 1992b)

Sediments are very similar to soils. In general, they have higher water and organic matter contents. The higher content of organic matter can thus increase the likelihood of heavy metal complexation. Also since water is an oxygen carrier, it is more likely that oxide precipitation will increase. Metals tend to accumulate in the sediments and there is the potential for metal release via diffusion into the water above (Farrell, 1993).

### **II.3.3 Sequential extraction techniques**

The term speciation is related to the distribution of an element among chemical forms or species. Heavy metals can occur in several forms in water and soils. Interest has increased in these techniques to relate the degree of mobility with risk assessment, (i.e., the more mobile the metal is, the more risk associated with it (Bourg, 1995)). Not only is total metal concentration of interest, but it is now accepted that understanding the environmental behaviour by determining its speciation is of paramount importance.

To determine the speciation of metals in soils, specific extractants are used. The different extractants solubilize different phases of metals as shown in Fig. 2.11. By sequentially extracting with solutions of increasing strengths, a more precise evaluation of the different fractions can be obtained (Tessier et al. 1984). A soil or sediment sample is shaken over time with a weak extractant, centrifuged and the supernatant is removed by decantation. The pellet is washed in water and the supernatant removed and combined with the previous supernatant. A sequence of reagents are used following the same procedure until finally, mineral acid is used to extract the residual fraction. Heavy metal concentrations are then determined in the various extracts by atomic absorption or other means. Numerous techniques and reagents have been developed and have been applied to soils (Shuman, 1985), sediments (Lum and Edgar, 1983), sludge-treated soils (Petrozelli et al., 1983) and sludges

**FRACTION**

Reagent	Soluble	Exchangeable	Organic	Oxide	Residual
Distilled water	▬				
0.1M NaNO <sub>3</sub>	▬				
0.05 M CaCl <sub>2</sub>	▬				
0.01 M HNO <sub>3</sub>	▬				
1 M NH <sub>4</sub> OAc	▬				
0.1 M Ca(NO <sub>3</sub> ) <sub>2</sub>	▬				
0.5M HOAc	▬	▬			
0.005M DTPA	▬	▬			
1 M HNO <sub>3</sub>	▬	▬	▬		
0.05 M EDTA	▬	▬	▬		
HNO <sub>3</sub> /HF	▬	▬	▬	▬	

Denotes partially extracted  
 Denotes extraction

**Figure 2.11** Different extractants used for the different metal fractions (Jones et al., 1984)

(Lakanen and Ervio, 1972). These methods are not standardized and even the results can vary with the same reagents, pH, temperature, extractant strength and solid to volume of extractant ratio. None of the extractions is completely specific, however the extractants chosen attempt to minimize solubilization of other fractions.

To extract the exchangeable fraction, ammonium acetate, barium chloride or magnesium chloride at pH 7.0 is generally used (Lake, 1987). They cause the displacement of the ions in the soil or sediment matrix bound by electrostatic attraction. Pickering (1986) showed that magnesium chloride leached low quantities of other sulfides, organic matter, aluminum and silicon.

The carbonate phase (calcite and dolomite) is extracted at pH. 5.0 with sodium

acetate acidified with acetic acid. This solubilizes the carbonates, releasing carbonate-entrapped metals. Organic matter, oxides or clay components are not solubilized.

The reducible phase (iron and manganese oxides) are extracted with hydroxylamine hydrochloride with acetic acid at pH 2.0. The hydroxylamine hydrochloride reduces the ferrous and manganese hydroxides to soluble forms. Other components such as organic matter and clay components are not solubilized to any great extent (Tessier et al., 1979).

Hot hydrogen peroxide in nitric acid is used to oxidize the organic matter. The oxidized organic matter then releases metals that are complexed, adsorbed and chelated. These agents are used so that the silicates are not affected by this treatment.

In the final step, the silicates and other materials are dissolved by strong acids at high temperatures. This fraction is usually used to complete the mass balances for the metals.

Kabata-pendias (1992) demonstrated that the speciation of trace metals in natural soils depends on the physical and chemical characteristics of the soil. Soil pH, redox, organic, carbonate, clay and oxide contents all influence metal speciation and mobility. Simple and complex cations are the most mobile, exchangeable cations in organic and inorganic complexes are of medium mobility and, chelated cations are slightly mobile. Metals in organic or mineral particles are only mobile after decomposition or weathering and precipitated metals are mobile under dissolution conditions (e.g., change in pH). Kabata-pendias (1992) also showed the speciation of trace metals such as zinc, copper, cadmium and lead. Zinc and cadmium are mostly organically bound,

exchangeable and water soluble. Copper is mainly organically bound and exchangeable. Whereas, lead is slightly mobile and bound to the residual fraction. Chlopekca (1992) showed however, that the cadmium and zinc speciation of the soils depended significantly on the application of sewage sludge on the soil. Fertilizer addition, water and air pollution can also effect speciation.

Recently, sequential extraction techniques have been studied as a tool in various applications. Yong et al., (1993) examined sequential extraction to obtain a better appreciation of the ability of clay soil barriers to contain contaminants in landfill barriers. The effect of soil pH, constituents and heavy metal types were evaluated. In a study by Ramos et al. (1994), sequential extraction techniques were used to evaluate the mobility of cadmium, zinc, lead and copper in contaminated soil in a national park. Cadmium was found to be the most mobile and would likely be the most bioavailable.

Ravishankar et al. (1994) evaluated several sludges for Al, Cu, Fe, Mn and Zn speciation to predict bioleaching processes. They concluded that more stabilized sludges contained higher contents of organically bound metals and that sludges vary considerably making bioleaching prediction difficult.

A potential method to determine if the heavy metals can be removed by remediation techniques or predict removal efficiencies is to determine speciation with selective extractive techniques. It is believed that exchangeable, carbonate and reducible oxide fraction may be amenable to soil washing techniques (Li et al., 1995). Removal of organically and residually bound may not be economical to recover or necessary due to lack of bioavailability. Gombert et al. (1994) used sequential extraction to determine if cesium, cobalt and chromium could be removed by soil washing. Since less

than 20% was extracted after dissolving 20% of the soil mass, soil washing was abandoned as an option. Clearly, more work is needed in this field.

## **II.4 Soil and sediment remediation**

### **II.4.1 Various remediation techniques**

The different soil and sediment remediation methods depend on the concentrations and types of pollutants to be removed. Volatility, solubility and biodegradability of the contaminants are also very important. The main groups include non-volatile hydrocarbons, volatile hydrocarbons, polycyclic aromatic hydrocarbons, organic pesticides, polychlorinated benzenes (PCBs), heavy metals, and free and complex cyanides.

Contaminants can be isolated and contained to prevent further movement, to reduce the permeability of the waste to less than  $1 \times 10^{-6}$  m/sec and to increase the strength or bearing capacity of the waste (USEPA, 1994). Physical barriers made of steel, cement, bentonite and grout walls can be used. Another method is encapsulation which contains the contaminants not the area. This process is also called solidification or stabilization. Liquid monomers that polymerize and cement are injected to encapsulate the soils. Leaching of the contaminants must, however, be carefully monitored as is the case for vitrification, the formation of a glassy solid. Toxic gases can also be produced during vitrification.

Soils can be treated in situ or after excavation. The main types of remediation technologies include biodegradation, desorption, phase separation such as froth flotation and hydrocyclones, chemical or thermal destruction. Containment and treatment methods are summarized in Table 2.9.

Contaminated sediments differ from contaminated soils since the sediments

contain large amounts of water, their granular diameter is smaller, contaminants are insoluble in water and only a few types of contaminants are present.

### Thermal treatment

Temperatures of 200 to 700°C are used to evaporate contaminants. Organic pollutants and organic soil components such as humic acids can also be transformed. Higher temperatures (900 to 1100°C) can be used to oxidize the components and prevent dioxin emissions in a separate incineration step (van de Leur, 1990). Petrol, diesel oil, polycyclic aromatics, iron cyanides and halogenated organic compounds can all be removed. In terms of heavy metal treatment, mercury, arsenic and cadmium and its compounds can be evaporated at 800°C with the appropriate air pollution control system. Some of the metals remain in the solid residues which will have to be properly disposed.

### Mechanical separation

The aim of size selection processes is to remove the larger, cleaner particles from the smaller more polluted ones. To accomplish this, several processes are used. They include: hydrocyclones which separate the larger particles greater than 10 to 20 microns by centrifugal force from the smaller particles, fluidized bed separation which remove smaller particles at the top (less than 50 microns) in the countercurrent overflow in a vertical column by gravimetric settling and flotation which is based on the different surface characteristics of contaminated particles. Addition of special chemicals and aeration in the latter case causes these contaminated particles to float.

### Electrokinetics

Electrokinetic processes involve passing a low intensity electric current

**Table 2.9. Summary of remedial technologies (CCME, 1994)**

Technology	Description	Applicability
<b>Contaminant and isolation</b>		
Physical	Prevent movement by preventing fluid flow	Landfill covers and slurry walls
Encapsulation	Creation of an inert waste	Injection of solidifying chemicals
Vitrification	Application of electrical energy to vitrify contaminate	Shallow metal-contaminated soil
<b>Ex situ treatment</b>		
Groundwater extraction	Pump groundwaters for surface treatment	For contaminated groundwaters
Soil venting	Pumping of contaminated vapours	For volatile vapours
Soil washing	Addition of water to leach contaminants	For water soluble contaminants
Enhanced solubilization	Addition of surfactants and other additives to solubilize	For sorbed and NAPL residuals
Excavation	Removal of contaminated soils	Shallow, highly-contaminated soils
Landfarming	Biodegradation of soils or wastes spread on soil	Aerobic degradation of organics
Slurry bioreactor	Soil slurry within bioreactors	Aerobic degradation of organics
<b>In situ</b>		
Bioventing	Encouragement of biodegradation by venting	Aerobic degradation of organics (e.g., gasoline)
Bioremediation	Biodegradation of contaminants	Aerobic degradation of BTEX, and other organics
Reactive barriers	Creation of a permeable barrier	Sorption or degradation of contaminants in barrier
Soil flushing	Water flushing to leach contaminants	For soluble contaminants

between a cathode and an anode imbedded in the contaminated soil (Figure 2.12). Ions and small charged particles, in addition to water, are transported between the electrodes. Anions move towards the positive electrode and cations towards the negative. The electric gradient initiates movement by electromigration (charged chemicals movement), electro-osmosis (movement of fluid), electrophoresis (charged particle movement) and electrolysis (chemical reactions due to electric field) (Rodsand and Acar, 1995). The process can be used in situ or with excavated soil. Metals as soluble ions and bound to soils as oxides, hydroxides and carbonates are removed by this method. Other non-ionic components can also be transported due to the flow. Large metal objects can interfere with the process. Unlike soil washing, this process is effective with clay soils. Demonstrations of this technology have been performed but are limited. In Europe, this technology is used for copper, zinc, lead, arsenic, cadmium, chromium and nickel. Electrode duration and spacing is site specific.

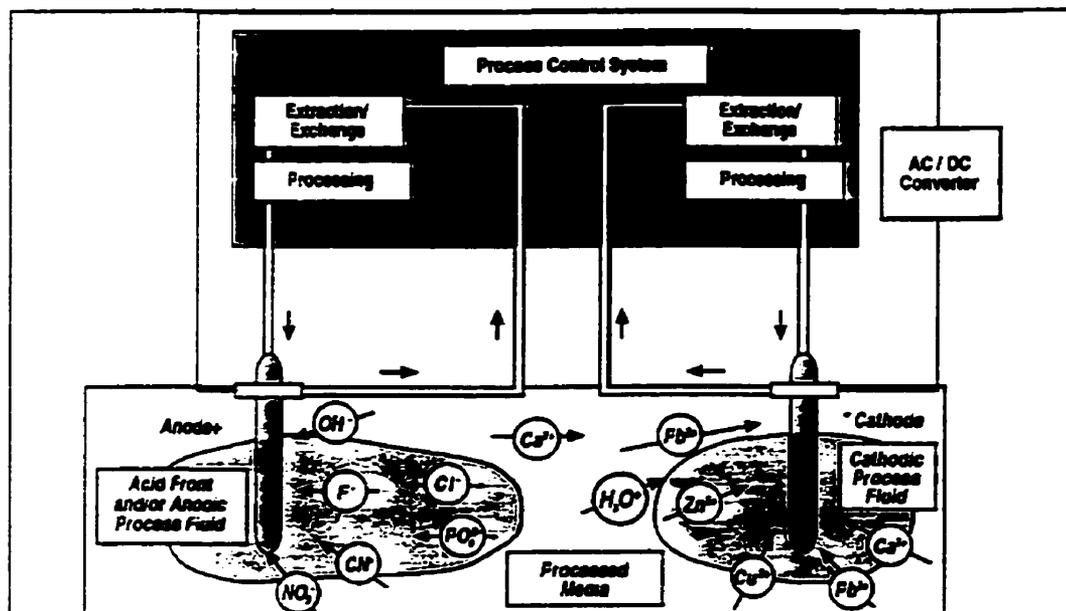


Figure 2.12. Schematic of electrokinetic remediation technology (USEPA, 1997)

### Vapour extraction

Volatile components (Figure 2.13) are removed by air injection and vacuum extraction (US EPA, 1991b). The contaminated air is then treated by activated carbon, biofiltration or catalytic incineration (Spuy, 1990). Soil type, structure, and contaminant type and concentrations determine the process operating conditions. An alternative is to inject steam which creates higher temperatures to increase volatility.

Trichloroethylene, perchloroethylene, benzene, toluene and other solvents have been remediated with vapour extraction at large scale. Soil permeability must be high enough to allow the air to pass through the soil. Therefore, this method cannot be applied to clay soils or to soils contaminated many years ago. Mercury and arsenic could potentially be removed but this has not been demonstrated. Air injection can also enhance the biodegradation of the non-volatile organics.

### Biodegradation of contaminants

Biodegradation involves the use of microorganisms to convert organics to carbon dioxide and water. For soil treatment, moisture and nutrient content, pollutant concentration and availability to the microorganisms, pH and redox potential are all important parameters affecting the rate of biodegradation. Oxygen is required for aerobic organisms. Degradation, however, can also occur by anaerobic means, particularly in the case of recalcitrant compounds (Soczo and Visscher, 1991).

Landfarming, bioreactors and *in situ* are the main types of processes employed. Landfarming involves spreading the contaminated soil in a thickness of 0.5 to 1.5m. Water, nutrients and trace elements may be added

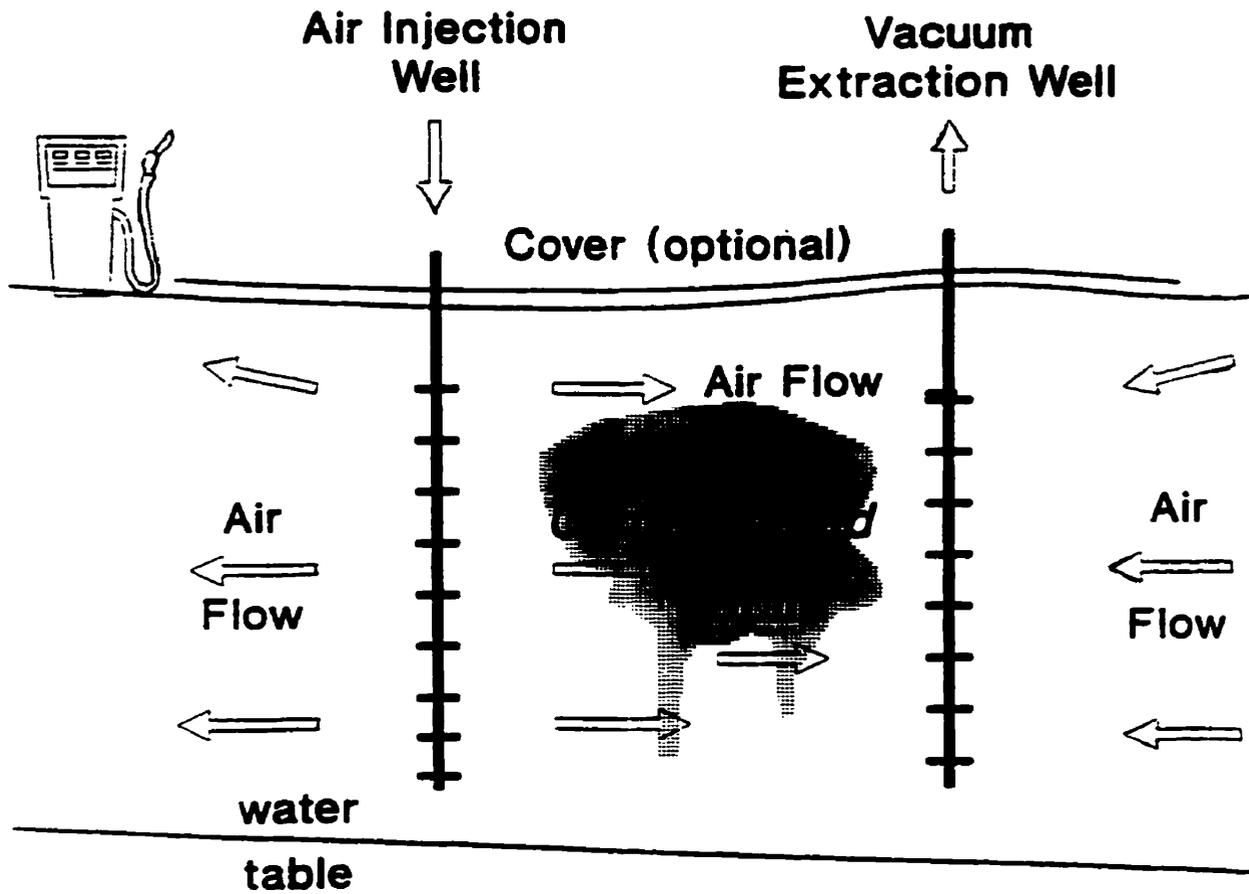


Figure 2.13 Schematic of soil venting techniques (CCME, 1994)

and the soil is mixed to promote aeration. Leachate can be recirculated over the soil. This method is fairly simple and requires, in general, one to three years for acceptable remediation (Harmsen, 1991). Cold temperatures limit efficiency.

Bioreactors are used to treat excavated soil in a slurry. Oxygen and nutrients are added to the soil slurry which is mixed intensively. Residence times are in the order of hours or days due to the better biodegradation rates that are achieved. The equipment, however, is more expensive (Kleijntjens et al., 1990).

*In situ* bioremediation (Figure 2.14) is comprised of adding nutrients, water, oxygen and microorganisms (if necessary) to the soil without excavation. Although, this process is much less expensive, the time required for treatment can take weeks, months or even years. In addition, the permeability of the soil must be sufficient to allow circulation of water and oxygen. Therefore, only sandy soils are suitable.

Presently, low molecular weight compounds and gasoline are the most effectively biodegraded. The presence of heavy metals can severely inhibit biodegradation. Larger molecular weight, less soluble compounds are less bioavailable to microorganisms and thus more difficult to treat. The addition of surfactants can increase the solubility of these compounds and therefore increase biodegradation rates (Scheibenbogen et al., 1994 ).

#### Biological treatment of metals

Techniques for the extraction of metals by biological means are rather limited at this point. The main methods include bioleaching and phytoextraction.

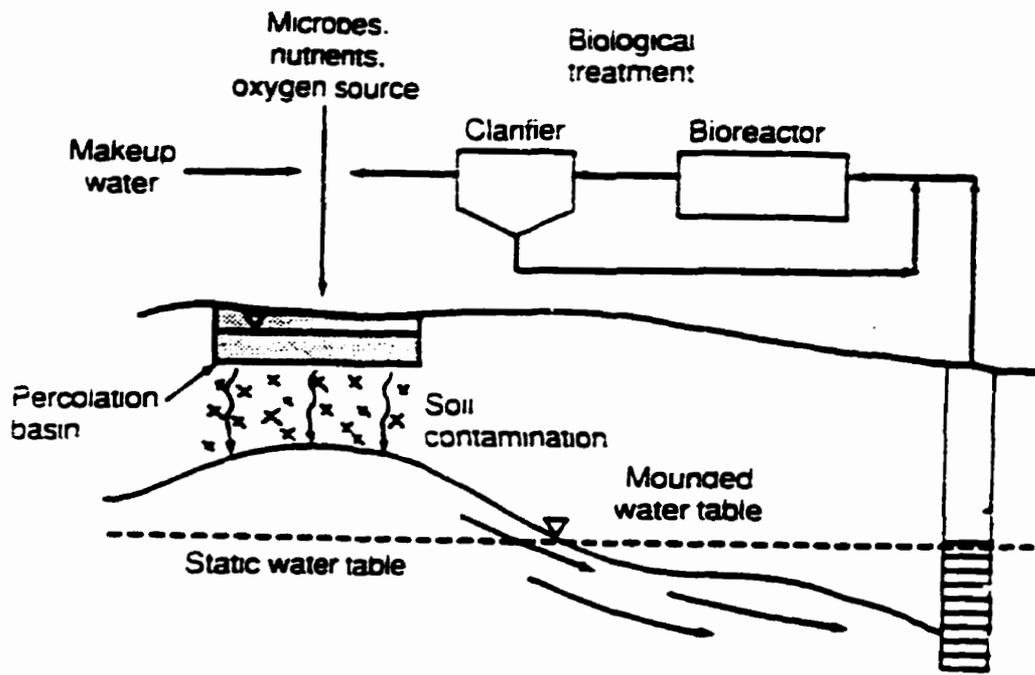


Figure 2.14 Diagram of *in situ* biodegradation  
(Bourquin, 1990)

Bioleaching involves *Thiobacillus sp.* bacteria which can oxidize sulphur species such as sulphide or pyrite ions under aerobic and acidic conditions (pH 4) at temperatures between 15 and 55°C. Leaching can be performed by indirect means, acidification of sulfur compounds to produce sulfuric acid which then can desorb the metals on the soil by substitution of protons. Direct leaching solubilizes metal sulfides by oxidation to metal sulfates.

Several options are available for bioleaching including heap leaching, bioslurry reactors and in situ. Anoxic sediments are more suitable for treatment since the bacteria can solubilize the metal compounds without substantially decreasing the pH. Soils require lower pH values to extract the metals since they have already been exposed to oxidizing conditions. For both heap leaching and reactors, the bacteria and sulfur compounds are added. In the reactor, mixing is used and pH can be controlled more easily, Leachate is recycled during heap leaching. Copper, zinc, uranium and gold have been removed by *Thiobacillus sp.* in biohydrometallurgical processes (Karavaiko et al, 1988). Several feasibility studies have indicated that contaminated soils can be remediated (Tichy et al., 1992). Sludges from anaerobic processes that contain metal sulfides could be treated in this manner (Blais et al., 1992). Biosorption is a biological treatment method which involves the adsorption of metals into biomass such as algae. There is currently strong competition from ion exchange resins. If large scale inexpensive production techniques for the biomass are developed, this heavy metal treatment is promising (Hazardous Waste Consultant, 1996).

#### Phytoremediation

Plants such as *Thlaspi*, *Urtica*, *Chenopodium*, and *Polygonum sachalase* have the capability to accumulate cadmium, copper, lead, nickel and zinc and can be therefore be considered as an indirect method of treating

contaminated soils (Baker et al., 1991). This method is limited to shallow depths of contamination. Phytoextraction involves uptake of metals by trees, herbs, grasses and crops. Phytostabilization is a process to excrete components from the plants to decrease the soil pH and form metal complexes. The plants will have to be isolated from wildlife and agricultural lands. The climatic conditions and bioavailability of the metals must be taken into consideration when using this method. Once contaminated, the plants will have to be disposed in an appropriate fashion. Some techniques include drying, incineration, gasification, pyrolysis, acid extractions, anaerobic digestion, extraction of the oil, chlorophyll fibers from the plants (Bolenz et al., 1990) or disposal since plants are easier to dispose of than soil.

In summary, the only techniques that can be used for metal removal are extraction/classification, electrokinetics and in-situ extraction. Phytoremediation and bioleaching can also be used but are not as well developed.

#### Treatment of sediments

Since sediments contain large quantities of water, dewatering is frequently necessary after dredging to enable treatment. Methods include draining of the water in lagoons with or without coagulants and flocculants or using presses or centrifuges. Treatment methods are similar to those used for soil such as hydrocyclone pretreatment, biological decontamination (landfarming for organic contaminants in particular) and chemical extraction (acids). Very few of these techniques have been used commercially in comparison to soil treatment.

#### Soil washing (chemical leaching)

Heavy metals can be removed from soils using various agents added to the soil. This can be done in reactors or as heap leaching. These agents are:

inorganic acids such as sulfuric and hydrochloric acids with pH less than 2, organic acids including acetic and citric acids (pH not less than 4), chelating agents such as ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetate (NTA), and various combinations of the above (USEPA, 1991a). The cleaned soil can then be returned to the original site. Soils with less than 10 to 20% clay and organic content (i.e., sandy soils) are most effectively remediated with these extractants. Both organics and metals are removed. However, modifications to the process which is commercially used have to be made for each type of soil (Hinsenveld et al., 1990). In general, soils with low contents of cyanide, fluoride and sulfide, CEC of 50 to 100 meq/kg and particle sizes of 0.25 to 2 mm, with contaminant solubility in water of greater than 1,000 mg/L, can be most effectively cleaned by soil washing (Hazardous Waste Consultant, 1996).

#### In situ treatment (soil flushing)

Extracting solutions are infiltrated into soil using surface trenches, horizontal drains or vertical drains. Water with or without additives are employed to solubilize contaminants. The efficiency of the extraction depends on the hydraulic conductivity of the soil. High permeability gives better results (greater than  $1 \times 10^{-3}$  cm/s). The solubility of the pollutants in water can affect removal efficiencies. Prior mechanical mixing of the soil can disturb the infiltration of the extractant. Understanding the chemistry of the binding of the contaminant and the hydrogeology of the site are very important (USEPA, 1987).

Since water solubility is the controlling removing mechanism, additives are used to enhance efficiencies. The research in this area is still quite limited, particularly where metal removal is concerning (USEPA, 1987). They include organic and inorganic acids, sodium hydroxide which can dissolve organic soil

matter, water soluble solvents such as methanol, displacement of toxic cations with nontoxic cations, complexing agents such as EDTA, acids in combination with complexation agents or oxidizing/reducing agents. Soil pH, soil type, cation exchange capacity (CEC), particle size, permeabilities and contaminants all affect removal efficiencies. High clay and organic matter contents are particularly detrimental.

Once the water is pumped from the soil, it must be extracted and then treated to remove the metals. Several technologies exist such as sodium hydroxide or sodium sulfide precipitation, ion exchange, activated carbon adsorption, ultrafiltration, reverse osmosis, electrolysis/electrodialysis and biological means (Patterson, 1985).

Large-scale treatment has been done mostly for organic removal. A 30,000 m<sup>3</sup> volume has been successfully treated in the Netherlands to decrease the cadmium content in 90% of the soil from 10 mg/kg to less than 1 mg/kg with dilute hydrochloric acid (pH 3 (Urlings, 1990)). Another full scale process was used at a electroplating shop with high chromium levels. Two infiltration basins were used to flush the low permeability soils. Treatment is still ongoing (USEPA, 1996). Chromium concentrations have decreased from 2,000 mg/L to 18 mg/L.

#### **II.4.2 Surfactant soil flushing and washing**

Cationic, anionic and nonionic surfactants can be used for soil washing (Fig. 2.15). Numerous batch and column studies have indicated that surfactants enhance recoveries of NAPL (Ellis et al., 1985; Abdul, et al., 1990; Kan et al, 1992; Soerens et al., 1992). There have also been indications that

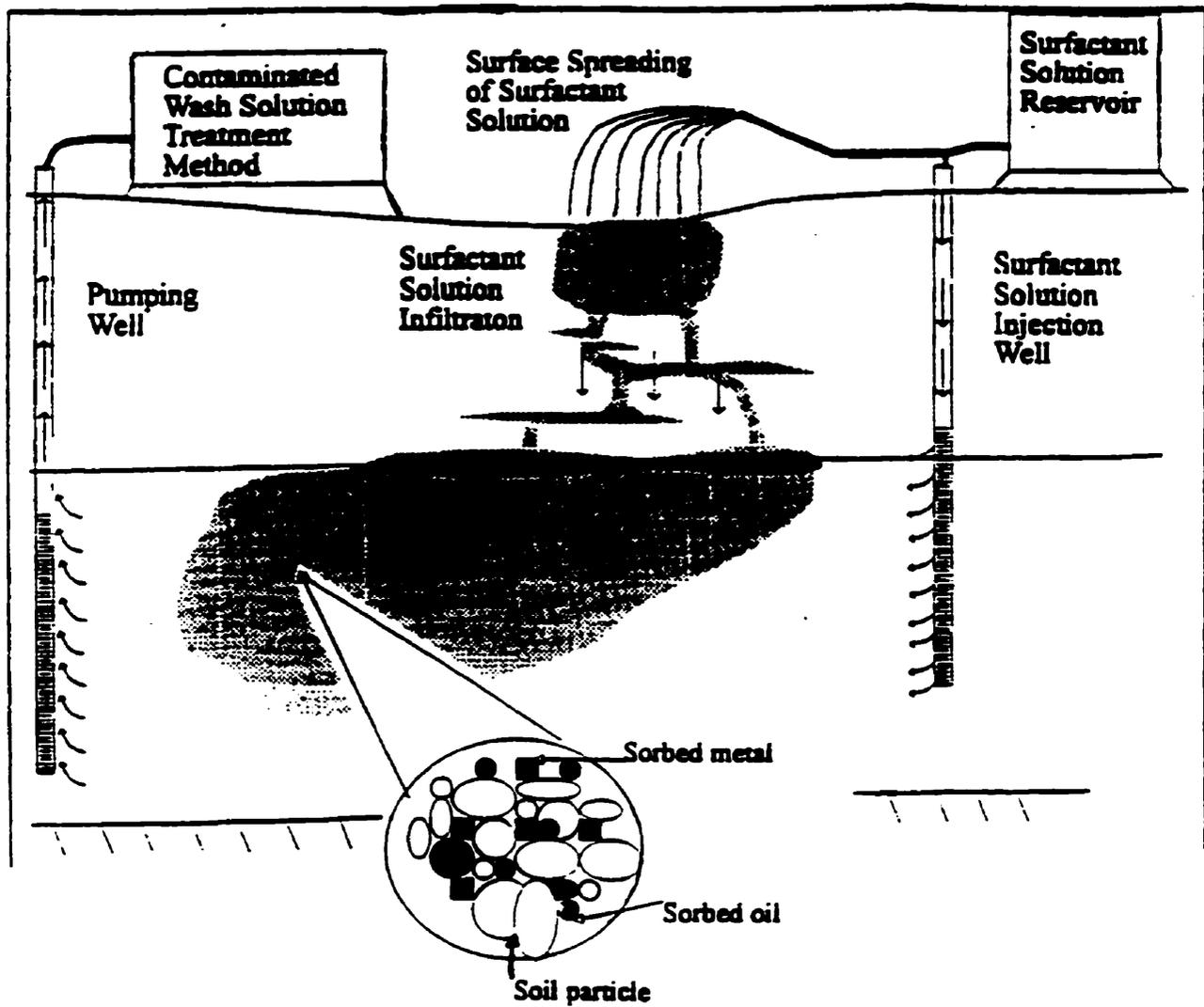


Figure 2.15. Schematic of soil flushing process.

pretreatment of a soil with surfactant washing (Igepal CA-720) to solubilize PAHs enhanced biodegradation of these contaminants (Joshi and Lee, 1995).

A few field studies have also been performed with surfactant *in situ* flushing. In 1988, a wood treating site was used to test surfactant washing (Sale, 1989). Two blends were tested, one as a prewash and the other to lower oil levels even further. Blend 1 consisted of 1% Polystep A, 0.7% NaHCO<sub>3</sub>, 0.1% Na<sub>2</sub>CO<sub>3</sub> and 1,000 mg/L xanthan gum and second blend was comprised of 1.4% Makon-10 surfactant, 0.7% NaHCO<sub>3</sub>, 0.8% Na<sub>2</sub>CO<sub>3</sub> and 1,000 mg/L xanthan gum. 95% of the oil was reduced and 99% of the surfactants were recovered. The combination of surfactants and sodium hydroxide has been used in enhanced oil recovery to reduce interfacial tensions. Alkaline agents can also react with hydrocarbons to form surfactants. The combination of these surfactants and the added surfactants can effectively reduce interfacial tension and enhance oil recovery (Sale and Pitts, 1989). This same mechanism then can be responsible for removal of hydrocarbons from a contaminated soil. The addition of polymer enhances the mobility ratio (i.e., enables the contaminant to be pushed instead of flow passing around it).

At a Canadian Forces Base Borden in Ontario, a test was performed in 1990-1991. The hydraulic conductivity at the site was  $1 \times 10^{-4}$  cm/sec with a low CEC and organic matter content. Based on the data at this site, approximately 21 pore volumes of 1% surfactant solution would be needed to remove trichloroethylene (TCE) over a 4 year period, whereas pumping and treating groundwater would require 2,000 pore volumes over 400 years to decontaminate the site (AAEE, 1993). Other examples are shown in Table 2.10.

Table 2.10. Summary of surfactant soil washing technologies

Group	Process	Contaminant removed	Reference
SUNY-Buffalo with Dupont at Corpus Christi Site	Addition of 1% surfactant (approved in food preparation)	73 gal carbon tetrachloride successfully removed in comparison to pump and treat	Fountain (1993)
Canadian Air Forces Base at Borden (SUNY)	2% mixture of surfactant added to sandy aquifer	80% of PCE recovered	Fountain and Hodges (1992)
General Motors NAO Research and Development Center	Surfactant washing with recovery	10% of PCBs, oils removed in first test and 14% in second, thereby exceeding expectations	Ang and Abdul (1991)
University of Michigan	Injection of 4% Witconol and Tween 80 and modelling	Removal of 10% dodecane required 0.7L surfactant and 130,000L water	Abriola (1993)
Eckenfelder, Inc	Injection of 2.5% SDS and recycle/reuse of surfactant	Injection of 7.7 pore volumes of 2.5% SDS removed as much PCB as 20 to 40 pore volumes of water	Underwood et al. (1993)

Abdul, et al. (1992) examined treatment of PCB-contaminated soils. The hydraulic conductivity was  $1 \times 10^{-3}$  cm/sec. A 0.75% solution of Witconol was applied to the surface. During the test, 1.6 kg of PCBs and 16.9 kg of carrier oil were recovered.

Anionic and nonionic surfactants are less likely to be absorbed to the soil. Cationic surfactants have been used to lower aquifer permeabilities by sorption on to the aquifer materials (Westall et al, 1992) . Surfactant soil washing was originally developed in petroleum recovery operations. Surfactants have potential for use in aquifer remediation of DNAPLs.

Several factors can influence the efficiency of soil flushing with surfactants, Groundwater that is too hard may be detrimental to the effectiveness of a surfactant (AEEE, 1993). Surfactants can adsorb onto clay fractions, reducing their availability. Too quick biodegradation can inactivate the surfactant while some degradability is required to avoid accumulation. Removal of the surfactant from the recovered water from flushing can be difficult and lead to high consumption rates. In summary, desirable surfactant characteristics include biodegradability, low toxicity, solubility at groundwater temperatures, low adsorption to soil, effective at concentrations lower than 3%, low soil dispersion, low surface tensions and low CMC (Kimball, 1992). Anionic surfactants may precipitate. However, co-injection with a nonionic surfactant can reduce precipitation and also CMC values (Sabatini et al., 1995). Biosurfactants may be more biodegradable, more tolerant to pH, salt and temperature variation and in some cases less expensive to use (West and Harwell, 1992).

To reduce risk, food grade surfactants (T-MAZ 28, T-MAZ 20 and T-MAZ 60) which have been approved by the Food and Drug administration have been

examined (Shiau et al., 1995b). These surfactants were able to remove one to two orders of magnitude more chlorinated organics such as PCE, TCE and 1,2 DCE than water alone by formation of microemulsions. Other surfactants with indirect food additive status such as alkyl diphenyl disulfonate (DOWFAX) indicated lower levels of losses via sorption and precipitation while substantially solubilizing naphthalene and other PAHs. Recently, a plant-based surfactant from the fruit pericarp of *Sapindus mukurossi*, a plant from the tropical regions of Asia has shown potential for the removal of hexachlorobenzene (Roy et al., 1997).

Mechanisms to remove hydrocarbons from soil include, micellar solubilization, lowering of interfacial tension, formation of emulsions and displacement of NAPL by increasing viscosity.

#### Solubilization

In the presence of surfactants, organic contaminants can be solubilized by incorporation into the interior of the hydrophobic portion of the micelle. The extent of incorporation into the micelle is related to the octanol-water coefficient ( $K_{ow}$ ) of the solute. The greater the  $K_{ow}$  the higher the rate of solubilization into the micelle. This phenomenon is due to the preference of the organic for the micelle compared to pure water (Smith et al., 1987). The solubility of TCE was increased by SDS from 980 mg/L to 19,600 mg/L (West and Harwell, 1992)

#### Mobilization

Surfactant remediation is also due to the reduction in interfacial tension. Surfactant-enhanced oil recovery is based on this mechanism. When the interfacial tension is high between the oil and water (30 to 50 mN/m), it is difficult to push an oil drop out of a pore space (Wilson, 1990). Reduction

of the interfacial tension requires less energy to push the drop and mobilization occurs more easily. Interfacial tensions less than  $1 \times 10^{-3}$  mN/m are preferable when designing a surfactant system.

#### Sorption and penetration of surfactant molecules

In weathered soils, the components of coal tar such as phenanthrene and asphaltene remain and have low water solubilities which are very difficult to remove. Surfactants are able to enhance the mobility which can increase biodegradation rates (Aronstein, 1995). Many of the studies concerning partitioning of hydrocarbons into micelles have been performed with artificially contaminated soils (Edwards et al., 1994). It has recently been postulated that for weathered soils, the surfactants can assist the removal of PAHs by increased concentration at the soil-water interface and by sorption on to the soil which in turn causes swelling of the soil-tar matrix and increases the diffusion of the PAHs (Yeom et al., 1996).

#### Emulsion formation

The transport of DNAPL contaminants by the formation of macroemulsions (Okuda et al., 1996) is another aspect that has not received much attention but is a potential mechanism of decontamination by surfactants. They found that approximately 30% of PCE removal could be accounted for by this approach. Surfactants which solubilize hydrocarbons can also form emulsions. These emulsions can affect the permeability of the soil and since they are mobile, their movement must be controlled during flushing with surfactants.

The efficiency of biosurfactants in enhancing the biodegradation of compounds and enhanced soil washing has not been extensively studied (Zhang and Miller, 1992; Scheibenbogen et al., 1994). The mechanisms of solubilization,

dispersal and displacement for the removal of hydrophobic compounds (Ang and Abdul, 1991; Clarke et al., 1991) using biosurfactants have been postulated but not thoroughly investigated as has been done for synthetic surfactants (Coles et al., 1991).

The mechanisms of soil cleaning of metals and hydrocarbons by surfactants would be slightly different (Figure 2.16). In general, the process involves detachment of the contaminant from the soil followed by isolation in the wash solution. The means of detachment involve lowering of the contaminant/soil interfacial tension (i.e. capillary forces trapping the contaminant onto the soil) followed by binding of the contaminant to the surfactant at the interface. For hydrophobic oil contaminants, the binding takes place through solubilization of the hydrophobic portion of the surfactant. The positively charged metal would bind to the hydrophilic anionic head of the surfactant monomer. Isolation of the contaminant (oil or metal) would then take place by the incorporation of the contaminant/surfactant complexes into micelles. There could be some competition for space between the metals and organics within the micelles. This could mean that more surfactant would be necessary.

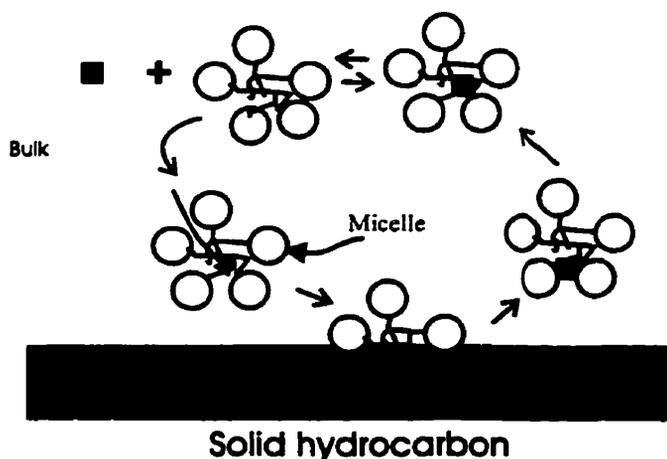


Figure 2.16 Micellar dissolution of solid hydrocarbon (Grimberg et al., 1996)

### III. MATERIALS AND METHODS

#### III.1 Experimental approach

The following experimental procedure was carried out. Three types of biological surfactants were produced and evaluated including surfactin from *Bacillus subtilis* ATCC 21332, rhamnolipids by *Pseudomonas aeruginosa* ATCC 9027 and sophorolipids from *Torulopsis bombicola* ATCC 22214. This required the development of the necessary experimental setup and procedures for sterilization of growth media, cultivation of the microorganisms, production and isolation of the biosurfactants (see top box in Fig. 3.1) These biosurfactants were selected for their effectiveness and because they can be produced from less expensive carbon sources (i.e., carbohydrates). *T. bombicola* was selected on the basis of the high surfactant yield, increasing its economic potential. Several commercially available synthetic nonionic surfactants were also chosen. Anionic surfactants, when compared to nonionic and cationic ones, should be the least sorbed due to the repellancy of soils which are mostly negatively charged.

Two contaminated soil matrices were chosen for study. The first is a hydrocarbon and metal contaminated soil from the Toronto Harbour area (Toronto, Ont.) run by the Toronto Harbour Commission (THC). This site contained numerous leaking underground storage tanks from refineries, oil terminals and coal storage and processing industries which released fly ash with elevated concentrations of lead, zinc and copper and those involved in metal finishing (Richardson and Ehrenreich, 1994). Therefore both hydrocarbon and metal contamination are present. The other matrix, sediments from the Lachine Canal (Lachine, Que.), have been heavily contaminated with metals and some hydrocarbons by many years of industrial discharges into the canal. The experimental scheme is shown in Fig. 3.1.

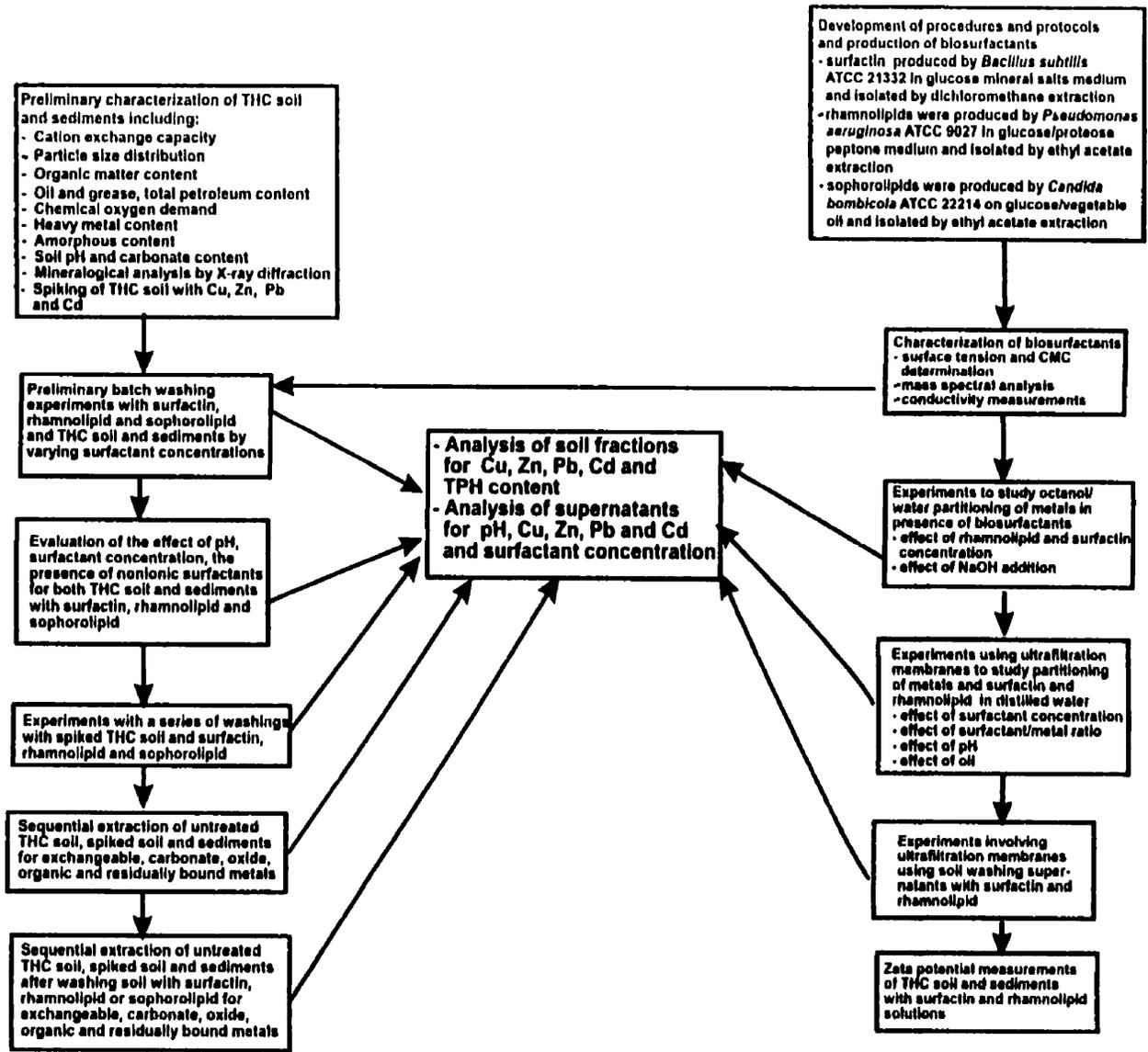


Figure 3.1 Schematic of experimental protocol

## **III.2 Soil and sediment characterization**

### **III.2.1 Cation exchange capacity**

In the modified procedure of Chhabra et al. (1975), 15 g of thiourea and 0.1 M ammonium acetate were dissolved in 0.5 L of water which was slowly added to silver nitrate (0.01M). pH was adjusted with acetic acid or sodium hydroxide to the desired value between 3 and 8. Approximately, 1 g of soil or sediment was added to 25 mL of solution for 4 h on an reciprocating shaker. The supernatant was then separated from the soil by centrifugation at 5,000 x g for 10 min analyzed for silver by atomic absorption spectrophotometry. The cation exchange capacity can then be expressed as meq/100 g soil.

### **III.2.2 Particle size distribution**

Grain size distribution was performed according to the sieve and hydrometer ASTM D422 method (ASTM, 1970). Approximately, 300 g of soil or sediment were dried at 100°C for 24 h and then cooled to room temperature. Standard brass sieves with mesh numbers from 1 to 200 were used. After placing the sieve column in the shaker for 15 min, dry weight retained by each sieve was determined.

### **III.2.3 Organic matter content**

Organic matter content was determined by two methods. Approximately 3 g of air-dried soil were weighed in pre-weighed porcelain dishes. The sample were then heated to 550°C for 1.5 h. After cooling the samples overnight in a dessicator, they were weighed. The difference in weight before and after ignition divided by the initial weight x 100% gave the organic matter %. The other method involved digestion with hydrogen peroxide. Aliquots of 10 mL hydrogen peroxide (30%) were added to 10 g of soil until bubbling was not longer observed. Soil was allowed to settle, the supernatant liquid poured off and the soil was then air-dried. Differences in the soil weight x 100% divided

by the original soil weight gave the organic matter content.

#### **III.2.4 Oil and grease and total petroleum hydrocarbon contents**

The Soxhlet extraction method was employed to determine the oil and grease fraction (APHA/AWWA/WPCF, 1995). Approximately 10 g of soil were added to a pre-weighed cellulose extraction thimble. 150 mL of clean freon were added to the Soxhlet apparatus and the heater was turned on. After repeating the extraction cycle until the solvent in the condenser was clear, the heater was turned off, the sample was removed for drying and subsequent weighing. The oil and grease content was then calculated from the difference in weight of the soil sample before and after the extract compared to the original weight  $\times 100\%$ .

Total petroleum hydrocarbon was determined by a modification of standard methods (APHA/AWWA/WPCF, 1995) by adding 0.3 g of soil to a test tube with approximately 30 ml of hexane. The tube was then placed in an ultrasonic bath for 30 min. The sample was agitated by hand every 5 min. After the ultrasonic treatment the soil was allowed to settle, the hexane was poured off and the soil sample was allowed to dry. The soil sample was weighed and the total petroleum content was determined by the difference in the soil weight divided by the initial soil weight  $\times 100\%$ .

The extraction and analysis of polycyclic aromatic hydrocarbons (PAHs) was performed by standard methods at an accredited private chemical analysis laboratory (ECO Laboratories, Pointe-Claire, Quebec).

#### **III.2.5 Chemical oxygen demand**

Chemical oxygen demand (COD) analyses were performed according to standard methods (APHA/AWWA/WPCF, 1995). A small amount (0.01 g) was

weighed into borosilicate culture tubes to which 2 mL of distilled water is added. Digestion solution (1.2 ml of  $K_2Cr_2O_7$  solution which included 17 g mercuric sulfate, 10.2 g potassium dichromate, 167 mL of sulfuric acid in 500 mL of distilled water) was added followed by 2.8 mL of sulfuric acid containing silver sulfate (22 g of silver sulfate in 4.1 kg of sulfuric acid). The tubes were capped with TFE-lined screw caps and mixed end to end. The samples were heated for 2 h at 150°C in a Hach COD reactor. The samples were cooled and mixed. Transmittance was read with a Canlab Pye Unicam SP6-350 visible spectrophotometer (600 nm). Potassium hydrogen phthalate (0.85 g per liter corresponds to 1000 mg/L of COD) at concentrations from 0 to 1000 mg/L was used to make the standard curve.

### **III.2.6 Heavy metal content**

Soil and sediment samples (0.5 g) were digested at 440°C in a Hach Digesdahl with 7 mL of concentrated sulfuric acid. After approximately 15 min, 12 mL of 30% hydrogen peroxide were gradually added. The digestion was allowed to proceed another 15 min before the sample was removed from the heat for cooling. Once the mixture was cooled, distilled water was added so that the total volume was 100 mL. Initial experiments were analysed using a GBC 902 double beam atomic absorption spectrophotometer. Final analyses were performed using the Perkin Elmer Model 3110 atomic absorption spectrometer. Copper (324.8 nm), zinc (213.9 nm), lead (283.3 nm) and cadmium (228.0 nm) were analysed after preparing the standards, blanks and samples according to standard methods (APHA/AWWA/WPCF, 1995).

### **III.2.7 Amorphous content**

Amorphous iron ( $Fe_2O_3$ ), aluminum, ( $Al_2O_3$ ) and silicon ( $SiO_2$ ) were extracted from the soil and sediment by acid and base washings according to Segalen (1968). After the washings, Al and Fe were determined in the base washes

while Si and Al were determined in the acid washes by atomic absorption spectrometry. Al, Fe and Si concentrations were then added and converted to the above oxide forms.

### **III.2.8 Soil pH and carbonate content**

Soil pH was determined by using a 1:10 soil to water ratio using a Beckman f12pH pH meter. Total carbonate content was analysed using the method of Hesse (1971). In addition, total carbon (TC) and total organic carbon (TOC) of the soil and sediment were analysed by an accredited laboratory (ECO Laboratories). Total inorganic carbon was then calculated from the difference in TC and TOC and then converted to carbonate (CO<sub>3</sub>).

### **III.2.9 Soil moisture content**

A 5 g sample of soil or sediment was placed in a pre-weighed dish and dried at 105°C overnight. It was then placed in a dessicator for cooling and weighed. The difference in the sample weight before and after drying divided by the original weight x 100% gave the moisture content.

### **III.2.10 Mineralogical analysis by X-ray diffraction**

X-ray diffraction was used to identify the crystalline components of the oil-contaminated soil and the sediment. X-rays of wavelengths of 0.01 to 100 Å allow the differentiation of crystalline structures of each mineral due to their distinct inter-atomic distances within their planes. A Siemens D-500 X-ray diffractometer with a Hewlett Packard D-500 AT system and a DACO-MD interface system was employed. The analyses were performed according to the procedure of Starkey et al. (1984). Approximately 10 to 15 g of soil and sediment were used for the analysis. The oil was removed from the soil by washing repeatedly until the solvent was clear with 10 mL of hexane or dichloromethane. Organic matter was destroyed by adding 10 to 15 mL of

hydrogen peroxide (30%) with gentle heating. Samples were washed with distilled water and allowed to air dry. A 20 to 30% soil suspension was prepared and 6 mL were added to a glass slide. The slide was then scanned at 0 to 65  $2\theta$  degrees, using Cu-K alpha radiation, 40 kV and 20 mA on 1° beam slit and 0.5° detector slit at room temperature. The scans were obtained at 0.02  $2\theta$  degrees/min. The rate meter was set at  $2 \times 10^3$  cps with a time constant of 1.2 sec. The semi-quantitative analysis was calculated by using the counts/sec for each peak over the total counts obtained for the scan according to the data from JCPDS (1988).

### III.3 Soil spiking procedure

Soil from the Toronto Harbour was spiked with a solution containing 1 mM of each  $\text{CuSO}_4$ ,  $\text{CdCl}_2$ ,  $\text{Pb}(\text{NO}_3)_2$  and  $\text{ZnSO}_4$ . A ratio of 1 g of soil per 16 mL of solution was used. The soil was shaken for three days on the rotary shaker then removed by centrifugation (5,000 x g, 20 min) and air-dried.

### III.4 Surfactants

Lecithin was purchased from a local health food store. Sodium dodecyl sulfate, Triton X-100, deoxycholic acid, cetyl pyridine, CHAPS, taurodeoxycholic acid, taurocholic acid were purchased from Sigma Chemical (St. Louis, MO). Samples of POE (80) sorbitan monolaurate (TMAZ 28), POE (20) sorbitan monolaurate (TMAZ 28), octylphenol ethoxylate Igepal CO-630 and Igepal CO-720 were obtained from Aldrich (Milwaukee, WI). Samples of rhamnolipid and surfactin were supplied by Prof. O. Kappeli (Switzerland) and Dr. Kakinuma (Japan), respectively.

### III.5 Biosurfactant production, isolation and characterization

#### III.5.1 Microorganisms

*Bacillus subtilis* ATCC 21332 was maintained on nutrient agar (Difco),

*Pseudomonas aeruginosa* ATCC 9027 on Pseudomonas agar P (Difco) and *Torulopsis bombicola* ATCC 22214 on YM broth agar (Difco) at 4°C.

### III.5.2 Cultivation conditions

After one day of growth of *B. subtilis* in 100 mL of 4% glucose and mineral salt medium (0.4 %  $\text{NH}_4\text{NO}_3$ , 0.41%  $\text{KH}_2\text{PO}_4$ , 0.57%  $\text{Na}_2\text{HPO}_4$ , 0.02%  $\text{MgSO}_4$ ) supplemented with 0.0089%  $\text{FeSO}_4$  in a shake flask shaken at 150 rpm (37°C), 70 mL was transferred to the 700 mL of similar medium in a pH controlled (pH 6.7), aerated fermentor (photograph in Appendix A). Foam was collected and collapsed in a flask on the air exhaust line. *P. aeruginosa* was grown in a similar manner in proteose peptone medium (0.11%  $\text{NH}_4\text{Cl}$ , 0.05% peptone, 0.15% KCl, 1.45% Tris, adjusted to pH 7 with HCl), supplemented with 0.033%  $\text{MgSO}_4$ , 0.5% glucose (Mulligan et al., 1989).

*T. bombicola* was grown in 0.1%  $\text{KH}_2\text{PO}_4$ , 0.5%  $\text{MgSO}_4$ , 0.01%  $\text{CaCl}_2$  and 0.01% NaCl, 0.5% yeast extract (Difco) with 10% glucose and 10% vegetable oil (Cooper and Paddock, 1984) in 100 mL medium in 500 mL shake flasks (30°C). Glucose was added first until substantial growth was apparent. Vegetable oil (purchased at a local grocery store) was added at this point and growth proceeded until the vegetable oil was no longer visible.

### III.5.3 Isolation of biosurfactants

All surfactant broths were centrifuged to remove cells (5,000 x g, 20 min). Surfactin was isolated by adding concentrated hydrochloric acid to the collapsed foam (Mulligan and Gibbs, 1990). Dichloromethane (1:1, v/v) was added to the suspension in a separatory funnel and shaken vigorously. The aqueous (bottom) layer was removed and extracted twice more as described above. The organic layer was pooled and evaporated. The residue was redissolved in water (pH 8) and filtered through Whatman No. 1 paper to

remove undissolved impurities. Concentrated HCl was again added and extracted with dichloromethane (1:1. v/v) three times and evaporated. Rhamnolipids from *P. aeruginosa* were isolated in a similar manner with the exception that ethyl acetate was substituted for dichloromethane as the extracting solvent. Sophorolipids from *T. bombicola* were isolated by extracting three times with ethyl acetate, filtration through Whatman no. 1 filter paper and allowing the solvent to evaporate. Ethyl acetate was again added to the residue three times for further extraction and allowed to evaporate. In each of the above cases, the residue remaining after evaporation of the solvents was the residue.

#### **III.5.4 Surface tension and critical micelle concentration**

Surfactant concentration was determined by measuring the surface tension at various dilutions (Mulligan and Gibbs, 1990). A Fisher Tensiomat Model 21 was used to measure surface tension and interfacial tensions by the duNouy method (ASTM D971, 1995). Another apparatus (Fisher Cat. No. 14-818) which was based on the capillary tube method was used on a routine basis. The CMC was determined by measuring the surface tension at various dilutions (Cooper et al., 1979). The CMC was taken as the point at which the surface tension abruptly increased. The reciprocal of CMC was used as an indication of relative concentrations. Interfacial tensions were measured by the tensiostat by submerging the ring in the surfactant solution and then adding a 1 cm depth of oil extracted with hexane from the Toronto Harbour soil. The ring was then pulled through the oil/water interphase until the ring broke the surface (ASTM D971 method).

#### **III.5.5 Surfactin concentration**

In the case of surfactin, a specific method for determining concentration was used which is based on amino acid analysis (de Roubin et al., 1989). A 10 mL

aliquot was dried and acid hydrolysed for 2.5 h at 150°C in a PICO-TAG amino acid analysis system. The residue was redissolved in 200 mL of sodium buffer and injected on a Beckman System 6300 high performance analyser equipped with a Beckman Model 7000 data station. All buffers and ninhydrin reagents were purchased from Beckman. The concentration of surfactin was calculated by multiplying the lipopeptide concentration by the molecular weight (1036 amu).

### **III.5.6 Rhamnolipid concentration**

A method described by Pruthi and Cameotra (1995) was used to determine the concentration of rhamnolipid in the soil washing supernatants. This method involved the addition of three volumes of chilled acetone per volume of supernatant. The precipitate (rhamnolipid) was collected, dried and weighed.

### **III.5.7 Mass spectral analysis**

Mass spectra were obtained in the positive ion mode on a triple stage mass spectrometer Model API-III (Sciex, Toronto, Canada). Briefly, the samples (concentration range of 10 to 100 mM) were dissolved in 10% acetic acid and infused through a stainless steel capillary (100 µm ID). A stream of air (pneumatic nebulization) was introduced to assist in the formation of submicron droplets (Covey et al., 1988). These droplets were evaporated at the interface by nitrogen gas producing highly charged ions which were detected by the analyzer.

The system's calibration was performed with hen's egg white lysozyme where the molecular weight is calculated at 14,305 amu  $\pm$  3 amu. Mass charges were used throughout the range of the instrument (0-2470 amu). Instrument tuning, data acquisition and processing are controlled by a Macintosh II computer with all software provided by the manufacturer.

### III.5.8 Conductivity measurements

The conductivity of surfactant solutions was measured using a Cardy Pocket Conductivity Meter Model C-173. The meter was calibrated with 0.1 N KCl.

### III.6 Ultrafiltration procedures

Ultrafiltration experiments were performed using a 50 ml Amicon magnetically stirred ultrafiltration cell, containing either a YM 10 membrane for the rhamnolipid studies or an XM 50 for the surfactin experiments as described in Mulligan and Gibbs (1990). Copper (10 mg/L), lead (0.3 mg/L), cadmium (20 mg/L) and zinc (10 mg/L) were added in the same form as the soil spiking studies to the various concentrations of surfactant, prior to the ultrafiltration procedure. Volumes of 25 mL or 50 mL of solution were added to the cell and pressurized to 400 kPa. The retentate and permeates were collected, the volumes of each recorded and then subjected to metal analysis. The results are shown as rejection ratio % (R) which is defined as:

$$R = \frac{\ln C_f / C_o}{\ln V_f / V_o} \times 100\%$$

where  $C_f$  is the final metal concentration in the retentate,  $C_o$  is the initial metal concentration,  $V_o$  is the initial solution volume and  $V_f$  is the final volume of the retentate.

### III.7 Octanol/water partitioning studies

Octanol studies were performed using a modified procedure of Friedel et al. (1994) by adding 3 mL of octanol per 10 mL of metal/surfactant solution in centrifuge tubes which were shaken overnight. The metal (in the same form as in the spiking studies) concentrations used were copper (5 mg/L), zinc (4.8 mg/L) and lead (0.6 mg/L) and cadmium (11.0 mg/L). The octanol and water fractions were separated by centrifugation, followed by atomic adsorption

spectrophotometric analysis of the water soluble fraction.

### III.8 Zeta potential measurements

Zeta potential was measured with the aid of a Zeta-meter Model ZM-75. Approximately 0.02 g (pre-seived through a 200 mesh sieve) of soil or sediment were added to 25 ml of solution prior to measurement. The zeta potential ( $\zeta$ ) was calculated from the electrophoretic mobility,  $Be = u_r/E$ , which is the ratio of the migration velocity of the particles to the field intensity applied, according to the equation:  $\zeta = u_r/E \times \eta/\epsilon_0\epsilon_r$ , where  $\epsilon_r$  is the absolute dielectric constant,  $\epsilon_0$  is the influence constant and  $\eta$  is the viscosity.

### III.9 Procedure for batch soil washing studies

Batch soil washing studies (Figure 3.2) were performed by varying surfactant concentrations and pH values in centrifuge tubes containing 10:1 wt/wt solution to soil ratios (15 mL/1.5 g) (Ellis et al., 1988). Samples were taken

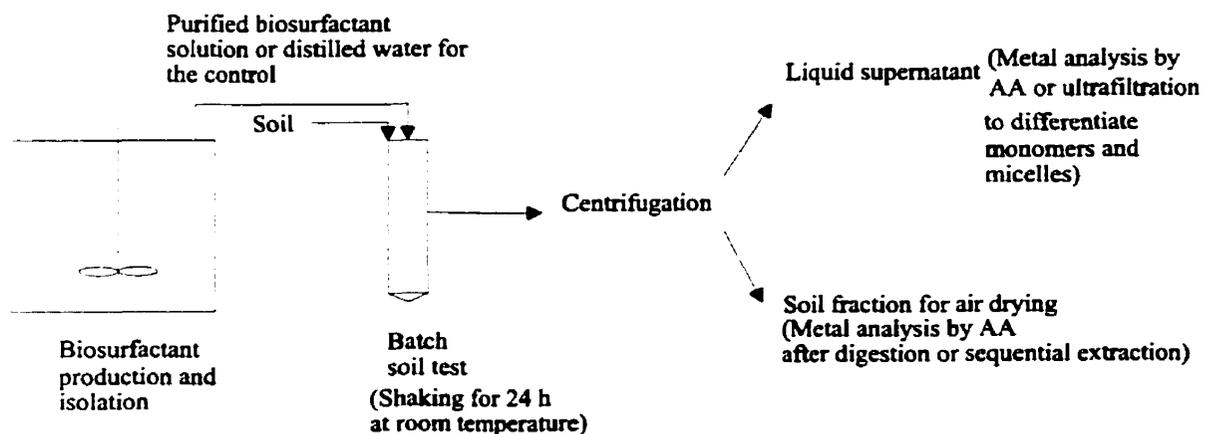


Figure 3.2 Batch soil washing procedure

after 24 h shaking to ensure that equilibrium has been reached and then centrifuged. The supernatant was then analyzed for metal concentration by atomic adsorption spectrophotometry. The percentage metal removal was determined by the equations:

(1)

$$mg\ metal_{supernatant} = \left( \frac{mg\ metal_{supernatant}}{L} \right) \times \left( \frac{1L}{1000\ mL} \right) \times 15mL$$

(2)

$$mg\ metal_{initial} = \left( \frac{mg\ metal_{initial}}{kg\ soil} \right) \times \left( \frac{kg}{1000\ g} \right) \times 1.5g$$

(3)

$$\% metal\ removal = \frac{mg\ metal_{supernatant}}{mg\ metal_{initial}} \times 100\%$$

The three biosurfactants were evaluated to determine their capabilities in removing heavy metal from the soil. The use of distilled water alone was used to account for removal of contaminants by physical mixing. Controls included the same additives as for the biosurfactant studies without the presence of the biosurfactant. All results are the average of duplicate experiments and are presented as % metal removal (equation 3).

### III.10 Sequential extraction procedures

The procedure used for sequential extraction was similar to that of Yong et al. (1993) as shown in Table 3.1. Soil or sediment samples (1.5 g) were washed

with the surfactant solutions and controls and then subsequently dried prior to sequential extraction. Each of the fractions were collected and the concentrations of heavy metals were determined in each of the fractions by atomic absorption spectrometry. The amounts of metals extracted from the soil by each of the extractants were then calculated.

**Table 3.1. Sequential extraction procedure (Yong et al., 1993)**

Order of sequence	Chemical reagents	
1	Extraction of metals by surfactants and controls overnight with 15 mL of solution	Soluble
2	Extraction of metals with 8 mL of 1 M MgCl <sub>2</sub> (pH 7) for 1 h	Exchangeable
3	Extraction of metals with 8 mL of 1 M NaOAc adjusted to pH 5 with acetic acid for 5 h	Carbonates
4	Extraction of metals with 20 mL of 0.04 M NH <sub>2</sub> OH.HCl in 25% (v/v) acetic acid (pH 2.5) at 96°C for 6 h	Oxides and hydroxides
5	Extraction with 3 mL of 0.02M HNO <sub>3</sub> and 5 ml of 30% H <sub>2</sub> O <sub>2</sub> (pH 2) for 2 h at 85°C, followed by 3 mL of 30% H <sub>2</sub> O <sub>2</sub> (pH 2) at 85°C for 3 h and then 5 mL of 3.2 M NH <sub>4</sub> OAc in 20% (v/v) HNO <sub>3</sub> diluted to 20 mL at room temperature for 30 min.	Organic matter
6	Digestion at 90°C with 25 mL of dilute aqua regia (50 mL HCl, 200 mL HNO <sub>3</sub> and 750 mL water) for 3 h	Residual fraction

#### **IV CHARACTERIZATION OF THE THC SOIL AND LC SEDIMENTS**

##### **IV.1 Organic characterization of the THC soil and Lachine Canal sediments**

Characterization of the organic content was performed on the untreated THC soil and sediment samples (Table 4.1). The PAH, COD, oil and grease and organic matter contents were determined. COD levels of the sediments were approximately 25 g/kg, considerably less than the THC soil. The COD gives an indication of the organic carbon and cannot differentiate between oil and grease and organic matter. As indicated, the THC soil contains substantially higher levels of petroleum contamination than the sediment. The levels of organic matter in the sediment originated mainly from non-petroleum sources such as algae. The high level of PAHs in the oil would also indicate that the oil is highly weathered and that the non-volatile high molecular weight compounds are those that remain. According to the MEF criteria, total PAH of 1 mg/kg is considered the background level and 20 mg/kg (level B) is considered contaminated. The individual levels are shown in Table 4.1. In regards to oil and grease, 100 mg/kg is considered background and 1,000 mg/kg is contaminated.

##### **IV.2 Inorganic characterization of the THC soil and sediments**

Table 4.2 contains the results of the analyses for inorganic components of the soil and sediment samples. The air-dried moisture content of the samples was approximately the same. The carbonate content of the sediment was approximately 20 times higher than in the soil. Although potassium levels were comparable for both matrices, calcium and sodium contents were 10 times higher and magnesium twice as high in the soil sample. The soil contained significant levels of lead, copper and zinc. In the sediment, concentration of copper was lower, zinc was substantially higher and lead was comparable.

**Table 4.1 Analysis of organic components in soil and sediment samples**

Parameter	MEF Level A Criteria (mg/kg)	MEF Level B Criteria (mg/kg)	MEF Level C Criteria (mg/kg)	Conc. in soil (mg/kg)	Conc. in sed. (mg/kg)
Naphthalene	<0.1	5	50	11	0.25
Acenaphthylene	<0.1	10	100	8.0	0.23
Acenaphthene	<0.1	10	100	26	0.24
Fluorene	<0.1	10	100	75	0.30
Phenanthrene	<0.1	5	50	90	1.3
Anthracene	<0.1	10	100	11	0.82
Fluoranthene	<0.1	10	100	17	2.5
Pyrene	<0.1	10	100	52	2.2
7,12-Dimethylbenzo(a)anthracene	<0.1	1	10	< 2	< 0.2
Benzo(ghi)perylene	<0.1	1	10	7.5	1.3
Benzo(c)phenanthrene	<0.1	1	10	6.2	0.50
Chrysene	<0.1	1	10	16	1.6
Benzo(a)anthracene	<0.1	1	10	10	1.6
Benzo(b + j + k)fluoranthene	<0.1	1	10	15	3.4
Benzo(a)pyrene	<0.1	1	10	6.1	1.4
3-Methylcholanthrene	<0.1	1	10	< 2	< 0.2
Indeno(1,2,3,-cd)pyrene	<0.1	1	10	5.6	1.2
Dibenzo(ah)anthracene	<0.1	1	10	6.7	0.90
Dibenzo(al)pyrene	<0.1	1	10	< 2	1.1
Dibenzo(ai)pyrene	<0.1	1	10	< 2	< 0.2
Dibenzo(ah)pyrene	<0.1	1	10	< 2	< 0.2
Total PAHs	1	20	200	363	21
Total hydrocarbon (C10-C50)	< 100	700	3500	170,000	1,700
Total oil and grease (%)				12.6	1.4
COD (g/kg)				360	25
Total carbon (%)				17.5	7.3
Total organic carbon content (%)				17.45	6.2
Total organic matter, ignition(%)				30.3	20.0
Organic content (hydrogen peroxide) (%)				20.7	13.4

**Table 4.2. Analysis of inorganic components of soil and sediment samples**

Parameter	MEF Level A criteria (mg/kg)	MEF Level B criteria (mg/kg)	MEF Level C criteria (mg/kg)	Conc. in soil (mg/kg)	Conc. in sediment (mg/kg)
Moisture content (%)	--	--	--	6.6	7.0
Inorganic carbon as carbonate (%)	--	--	--	0.25	5.5
Al <sub>2</sub> O <sub>3</sub> (%)	--	--	--	1.8	4.0
Boron	--	--	--	12	8
Cadmium	1.5	5	20	3	5
Calcium (%)	--	--	--	9.6	1.1
Chromium	75	250	800	844	100
Copper	50	100	500	414	109
Fe <sub>2</sub> O <sub>3</sub> (%)	--	--	--	9.0	4.9
Magnesium	--	--	--	8000	10,000
Manganese	--	--	--	534	456
Molybdenum	2	10	40	8	< 2
Nickel	50	100	500	71	49
Lead	50	500	1000	320	410
Potassium	--	--	--	1650	3700
SiO <sub>2</sub> (%)	--	--	--	25.1	48.8
Sodium	--	--	--	5900	500
Zinc	100	500	1500	870	3300

Other heavy metals such as chromium, boron and nickel were found in concentrations less than 10 mg/kg soil. Subsequently, the THC soil was used as an oil and metal contaminated matrix and the Lachine Canal sediments for its heavy metal contamination. Zinc and copper were retained for further study for both samples as they were considered contamination levels for both the soil and the sediment.

#### **IV.3 Amorphous content**

The contents of amorphous iron, silicon and aluminum were determined for the soil and sediment. The total amorphous content was 13.3% for the soil (1.8%  $\text{Al}_2\text{O}_3$ , 9.0 %  $\text{Fe}_2\text{O}_3$  and 2.5%  $\text{SiO}_2$ ) and 12.4% (4.0%  $\text{Al}_2\text{O}_3$ , 4.9%  $\text{Fe}_2\text{O}_3$  and 3.5%  $\text{SiO}_2$ ) for the sediment.

#### **IV.4 Particle size distribution**

The grain size distribution (Figure 4.1) of the THC soil indicates a sandy soil. Approximately 74.5% was finer than 2 mm and only 5% passed through a 0.075 mm sieve (10% silt and 90% sand). However, this distribution reflects agglomerated soil particles due to the hydrocarbon contamination which is not the natural distribution. In contrast, approximately 85% of the sediment particles passed through the 0.075 mm sieve (10% sand, 70% silt and 20% clay). Based on the Sheppard classification, the sediments are classified as fine grain clayey, silt or a silt loam via the soil triangle method (Cameron, 1992). As in the case of the soil, the organic and the carbonate contents can interfere with the sedimentology test and were removed according to ASTM (1984) procedures. The values obtained thus, represent the original mineralogical content.

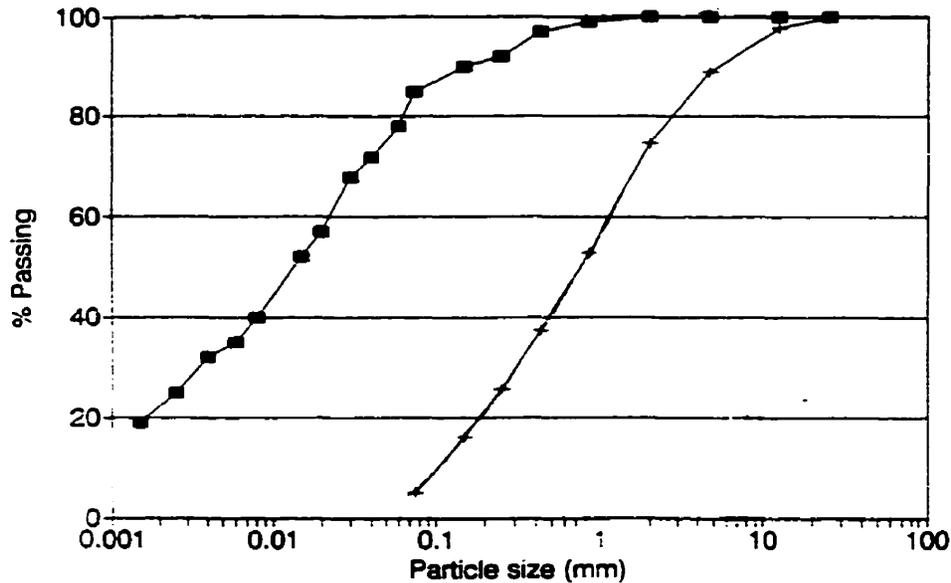


Figure 4.1. Particle size distribution for soil (■) and sediment (+) samples

#### IV.5 Cation exchange capacity determination

The curves of cation exchange capacity (CEC) for the Toronto Harbour soil and the Lachine Canal sediments are shown in Figures 4.2 and 4.3. In both cases, the CEC increases with the pH. CEC values above pH 8 could not be determined due to precipitation of the reagents. pH-dependent charged surfaces such as kaolinite, amorphous and organic matter are the cause of the variation of pH with CEC (Duquette and Hendershot, 1993). They also postulated that the inflection points are due to various surface reactions.

Variation with pH was not as significant for the THC soil compared to the sediment particularly below pH 5. This could be due to the fact that the final pH was significantly different for the THC soil compared to the sediments where the initial pH was only approximately 0.2 lower than the final pH whereas the final pH values were all in the range of 5.8 to 7.8 for all THC samples even the initial values ranged from 2 to 9. The buffering capacity of the THC soil was not as good as the sediments due to the lower carbonate content.

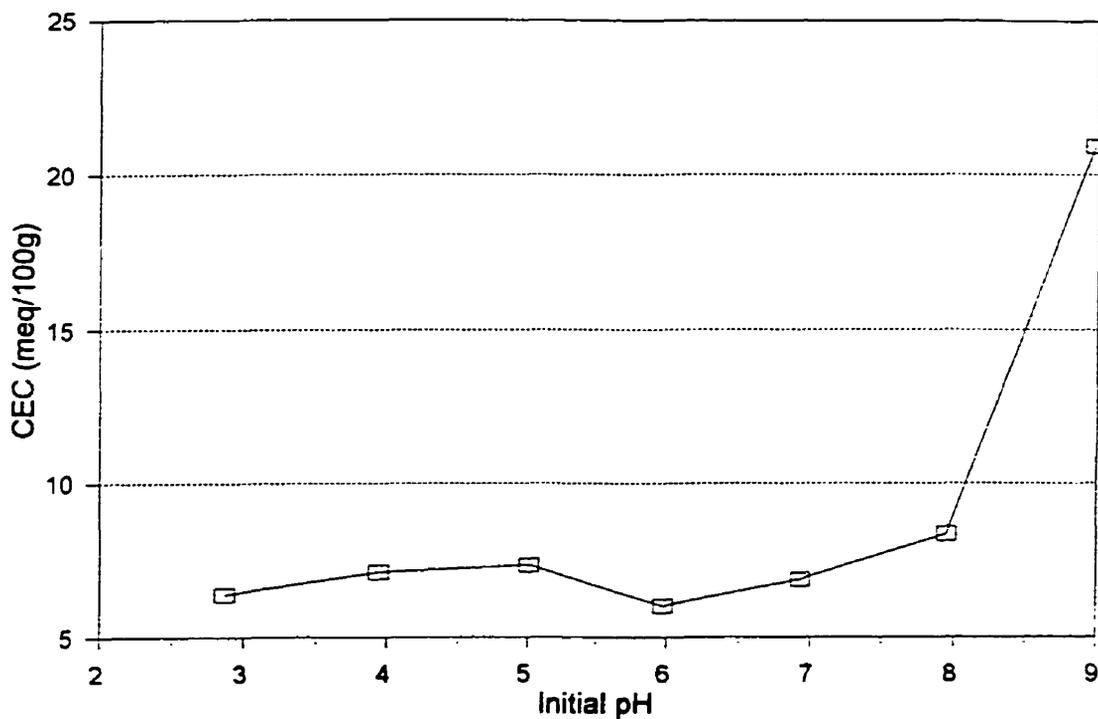


Figure 4.2. Cation exchange capacity versus pH for THC soil

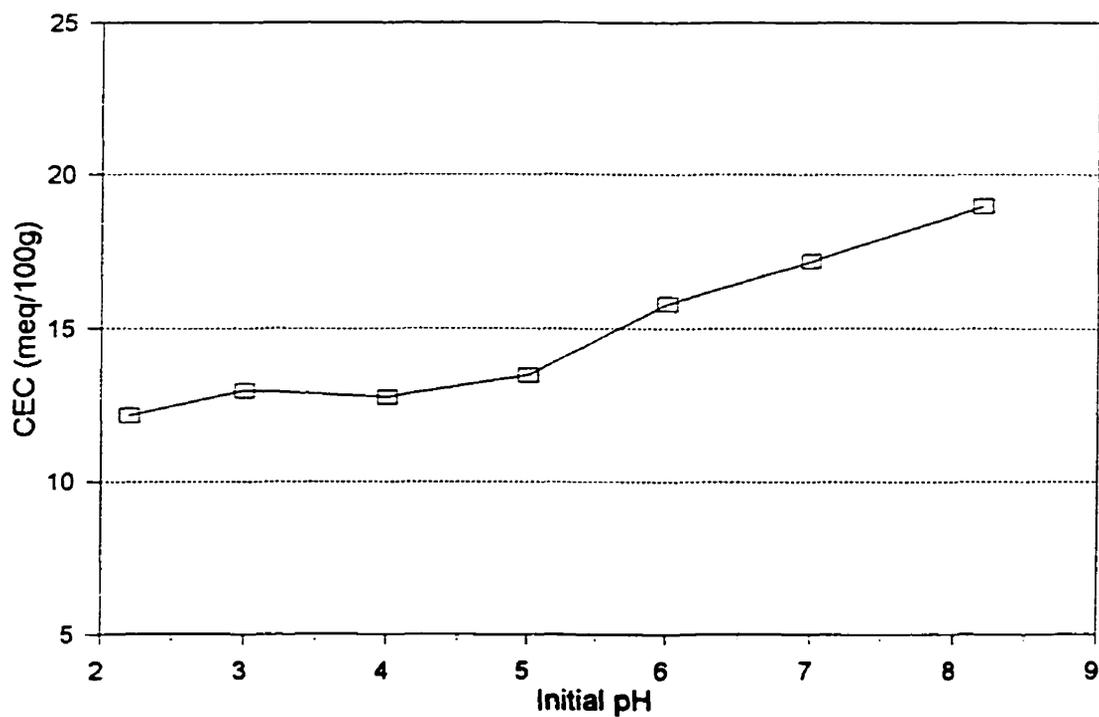


Figure 4.3. Cation exchange capacity versus pH for sediments

At pH 7, the CEC was lower for the THC soil compared to the sediment. According to Table 2.7, the CEC of both samples is in the region typical for kaolinite. The CEC of the sediments is slightly higher most likely due to the amorphous and organic matter content. Overall, soils of lower CEC bind metals less tightly and are considered more likely candidates for soil washing (American Academy of Environmental Engineers, 1993).

#### **IV.6 X-ray diffraction mineralogical analysis**

The X-ray diffraction results are summarized in Table 4.3. Since the oil contamination interfered with the analysis for the THC soil, the oil was removed by two different solvents, hexane and dichloromethane. These two scans (A and B) and the scan for the sediments (C) are included in Figure 4.4. The main primary minerals in all scans were quartz ( $\text{SiO}_2$ ) and feldspar ( $\text{KAlSi}_3\text{O}_8$ ). Quartz was indicated at 4.25, 3.33 and 2.28 Å and feldspar at 3.68, 3.18 and 3.02 Å. The hexane is the more efficient extractant of oil since more peaks can be seen which were hidden when dichloromethane was used. The quantified values for quartz agree fairly closely with the total  $\text{SiO}_2$  analyses in Table 4.2. The carbonate contents for both the soil and sediment in Table 4.2 also agree with values determined by the X-ray scans.

Kaolinite ( $\text{Al}_2\text{Si}_4\text{O}_{10}(\text{OH})_8$ ) was the main clay mineral found in the sediment and soil samples. Peaks were found at 4.46, 2.10 and 1.81 Å. Illite ( $(\text{Na},\text{K})\text{Al}_{12}(\text{Si}_3\text{AlO}_{10})$ ) was also found in the hexane-extracted soil and the sediment at peaks 2.59, 1.99 and 1.98 Å. Small amounts of chlorite ( $(\text{Mg},\text{Fe},\text{Al}) (\text{Al},\text{Si})_4\text{O}_{10}(\text{OH})_8$ ) were also seen in addition to mica.

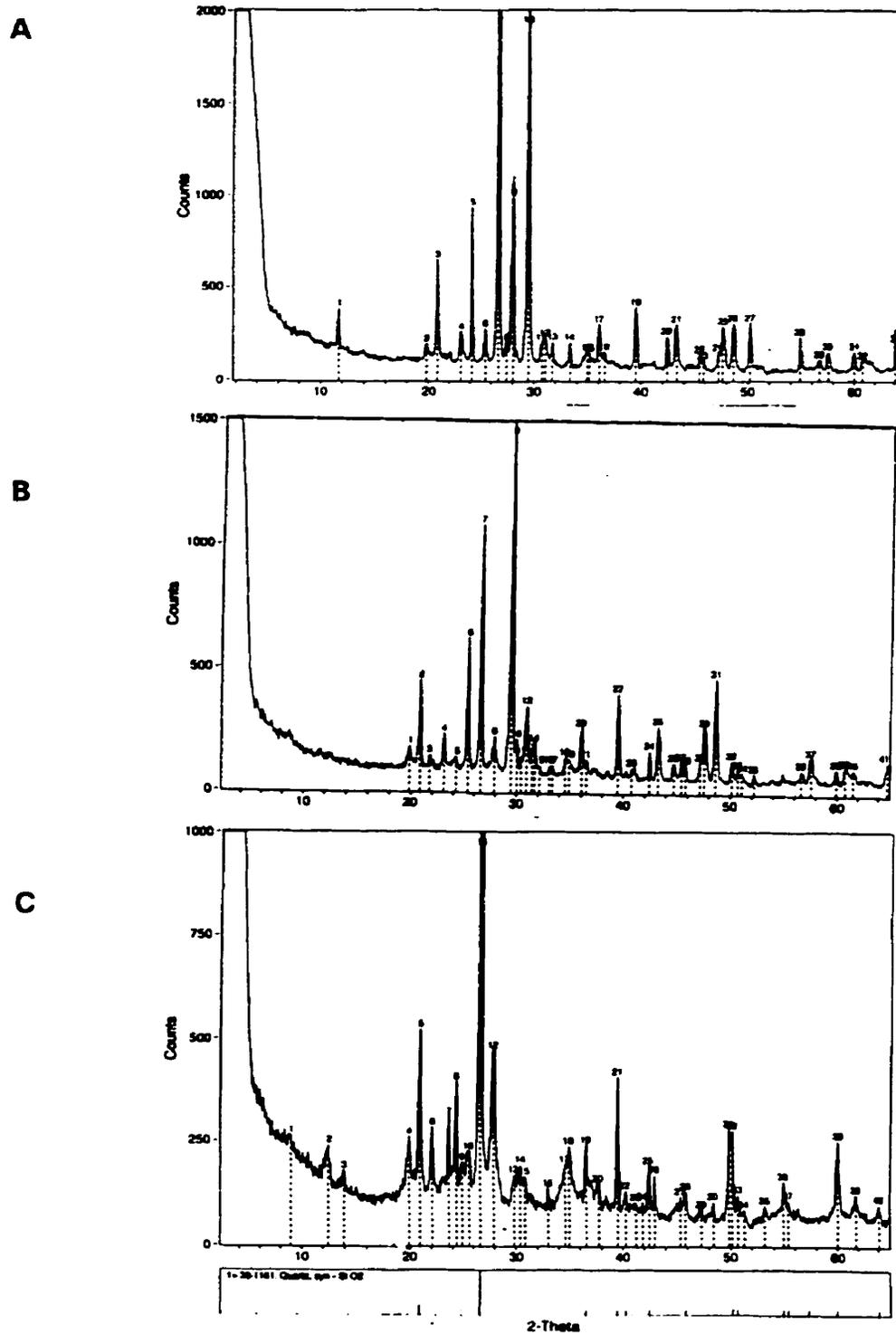


Figure 4.4 X-ray diffractogram for (A) hexane-extracted soil, (B) dichloromethane-extracted soil and (C) sediments.

**Table 4.3 Mineralogical composition of soil and sediment**

Component	Soil (hexane- extracted) concentration (%)	Soil (dichloromethane- extracted) concentration (%)	Sediment concentration (%)
Quartz	30	45	56
Feldspar	36	36	17
Illite	2	ND	7
Kaolinite	27	14	13
Chlorite	3	3	ND
Carbonate	0.5	ND	3
Others	1.7	2	4

ND- Not detectable

#### **IV.7 Heavy metal concentration as a function of THC soil particle size**

Various fractions of soils were analyzed for metal content. The soil was passed through various sieve sizes as noted in Table 4.4. The soil designated as untreated soil is the original THC soil. The copper content was of greater concentration for the soil which passed through sieve 75, whereas the zinc was slightly higher for all sieved fractions obtained. Lead and cadmium showed no particular preference for any particle size.

#### **IV.8 Spiking of the THC soil**

A sample of THC soil was spiked with heavy metals. The before and after spiking analyses are shown (Table 4.5). Clearly, cadmium was absorbed to the

**Table 4.4. Characterization of different soil fractions treatments**

Soil type	Metal content (mg/kg)			
	Zn	Pb	Cu	Cd
Soil passed through sieve 75	972	219	780	10
Soil passed through sieve 149	1100	172	518	15
Soil passed through sieve 355	1120	192	480	20
Untreated soil	870	116	460	20

**Table 4.5 Spiking of THC soil**

Metal	Content before spiking (mg/kg)	Content after spiking (mg/kg)
Pb	116	290
Cu	460	550
Zn	870	1200
Cd	20	2000

greatest extent followed by zinc, copper and lead, in decreasing order. It was decided to use this soil to obtain information on cadmium removal.

#### **IV.1.9 Summary and preliminary discussion**

The purpose of characterizing the soil and the sediment samples was to show the type and extent of contamination, the soil chemistry, and the physical properties of the soil. The values for contaminant concentrations for the soil and sediment were compared with MEF guidelines to show the extent of contamination.

According to these guidelines, Level A signifies the background values of the soil. No decontamination is necessary. Level B signifies that the soil is contaminated and remediation is necessary if the land is to be used for residential purposes. Level C indicates that the soil is contaminated and that it cannot be used for any purposes including industrial. Remediation in all cases is necessary and with little delay. Therefore, according to these criteria, values exceeding the B level may require decontamination while values exceeding level C are highly contaminated and must always be remediated. On this basis, for the individual PAHs, level B was exceeded whereas level C was exceeded for the total PAHs and the oil and grease contents for the soil. The sediment has a slightly higher content than level B of benzo(a)anthracene, chrysene, indeno (1,2,3-cd)pyrene, dibenzo(al)pyrene, benzo(b+j+k)fluoranthene, benzo(a)pyrene, oil and grease and total PAHs. In conclusion, in terms of these criteria, the THC soil was heavily contaminated with PAHs and oil and grease while the sediments are slightly contaminated.

The MEF criteria exist also for metals. It is not applicable to salts such as potassium, sodium and the constituents of the soil such as silicon. Compared to the level A and level B criteria the soil was heavily contaminated with chromium, and exceeds the B criteria for lead, copper and zinc. The sediment was found to be slightly above the B criteria for copper and lead and was significantly higher than the C criteria for zinc.

When examining potentially effective ways of remediating soil and sediments, the various techniques must be evaluated. Thermal extraction and vapour extraction would not be effective due to age of the contamination (i.e., all volatiles may be already removed to a large extent). Although biodegradation may be effective in removing the oil component of these soils, it will not be effective in removing the metals which are not biodegradable. Other means of

soil and treatment must be examined. Soil washing seems to be the only alternative which will enable removal of both the hydrocarbon and metal fraction together.

Particle size distribution is widely used to determine if soil washing is a viable alternative for soil remediation. According to the EPA (1990), particles of 0.063 mm to 0.25 mm are limited candidates for soil washing and particles of less than 0.063 mm are difficult to soil wash. The soil falls mainly in the first category whereas the sediment falls in to the second category. The American Academy of Environmental Engineers (1993) also determined a criteria which shows that the sediment would be difficult to wash, whereas the soil would be washable with washing fluids instead of water alone. On this basis, the conclusion may be drawn that the soil may be washable in the presence of additives such as surfactants but that the sediment may not be.

Cation exchange capacity is also used to indicate how tightly bound the pollutants are to the soil. Soils with high cation exchange capacity tend to bind pollutants more tightly. The CEC was highly dependent on pH for both the soil and sediments. This was most likely due to the presence of the organic matter and oxides in the soil and sediments. Organic matter, illite and kaolinite will all contribute to the CEC. Soils with high contents of illite, montmorillonite and organics (Table 2.7) have CEC values higher than 20 to 40 meq/100 g which is considered high (Cameron , 1992). CEC values of 12 to 20 meq/100 g are considered medium and below 12, low. The oxides do not contribute to the CEC. The higher value for the sediment compared to the soil would also indicate that the sediment is harder to remove the contaminants. However, both should be good candidates since their CEC values are fairly low.

As seen from section II.3.1, clay minerals, the oxides, carbonates and organic

matter all can potentially contribute to metal retention. Therefore, an analysis for all of these components was performed for both the soil and sediment samples. The presence of the clay minerals, kaolinite and illite was shown. Organic matter content determination is useful since it is an indication of the humic content of the soil or sediment. Humus is known to bind metals due to their carboxylic and phenolic groups. The oxides in the form of silica, alumina and iron were all found.

As seen in II.3.1, carbonates can increase the soil pH and enhance soil buffering. They can also form precipitates with metals. The pH of the sediments was indeed higher than that of the soil sample caused by the higher content of carbonate. The buffering capacity of the sediment was also higher.

In summary, based on the preliminary characterization data, the analysis of the oxides, carbonates and organic matter only indicate their presence in the soil and sediment sample but little information can be obtained concerning the role of each in the metal retention and if it is possible to remove these metals from the soil and sediments using surfactants. CEC and particle size determination cannot be used to a great extent to determine what soils can be remediated from heavy metal contamination.

## V CHARACTERIZATION OF BIOSURFACTANTS

### V.1 Surfactin

Initial experiments were performed using the isolated surfactin. However, a purified powder was used for the majority of the experiments. The purity of this powder was 62%. In addition, the ratio of 4:1:1:1 of leucine: aspartic acid: valine: glutamic acid was confirmed by amino acid analysis (Figure 5.1). Its molecular weight was also confirmed to be 1036 by mass spectrometry (Mulligan and Gibbs, 1990).

The surface active properties of surfactin are shown in Table 5.1. The surface tension is slightly higher than the value of 27 mN/m previously reported (Cooper et al., 1979). The CMC value is between the values of 0.011 g/L (Mulligan and Gibbs, 1990) and 0.025 g/L previously reported (Cooper et al., 1979). The surface tension and conductivity as a function of surfactin concentration is shown in Figure 5.2A. Addition of 1% NaOH to the surfactin

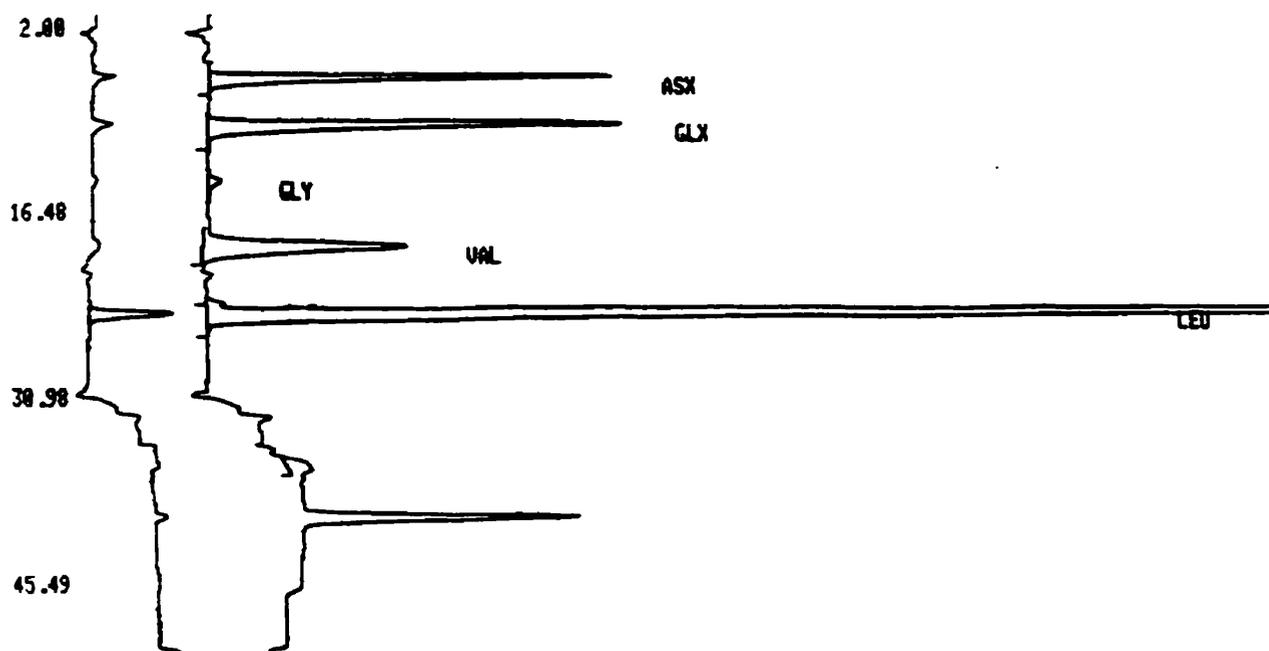


Figure 5.1 Amino acid analysis of surfactin. The amino acids shown are ASX: aspartic acid, GLX: glutamic acid, GLY: glycine, VAL: valine and LEU: leucine

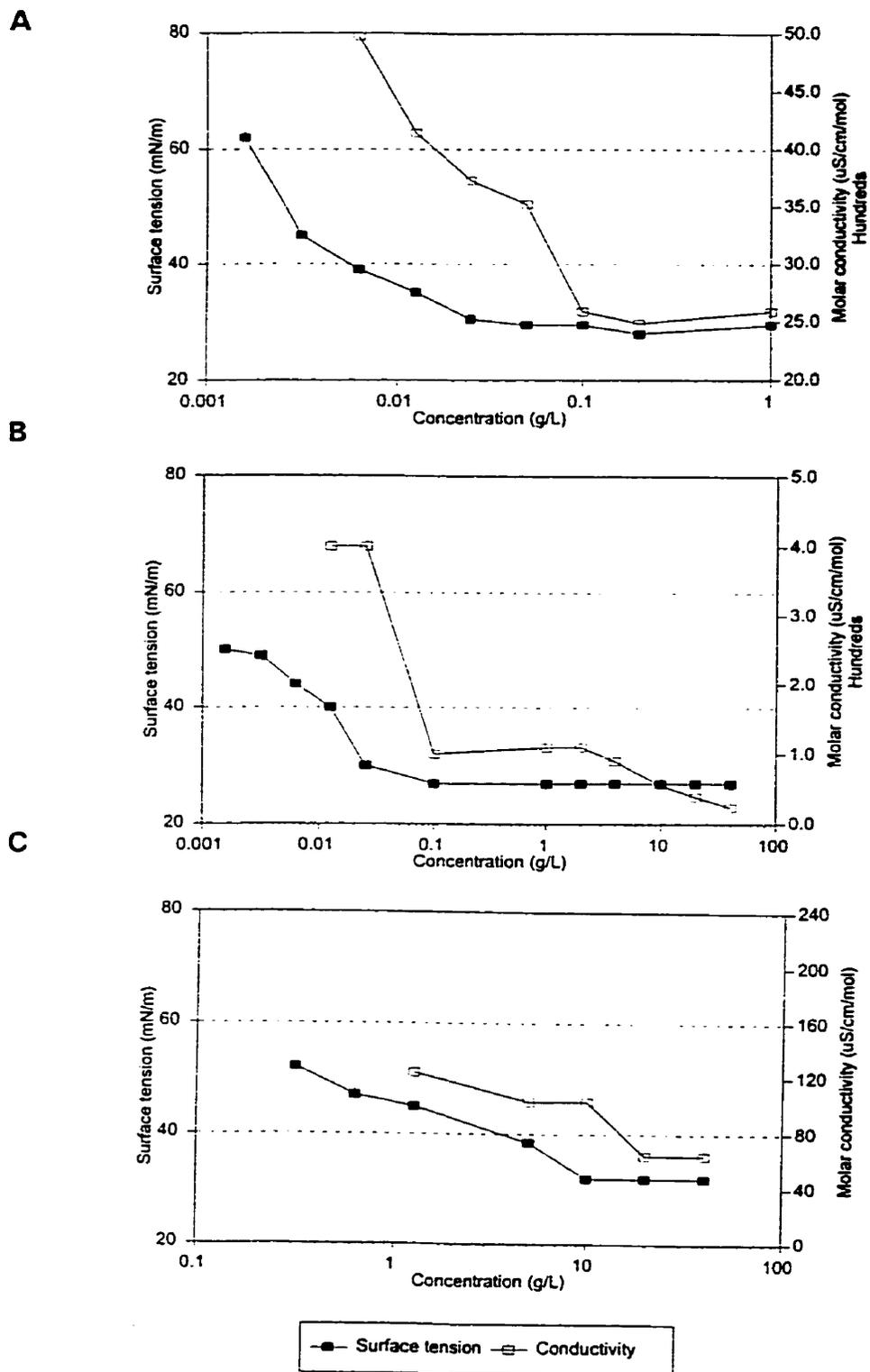


Figure 5.2. Surface tension and conductivity versus concentration for (A) surfactin, (B) rhamnolipid and (C) sophorolipid.

**Table 5.1. Characterization of biosurfactants**

Surfactant	Surface tension (mN/m)	CMC (g/L)	IFT against oil (mN/m)
Water	70	--	10
Sophorolipid	34	0.08	0.5
Triton X-100	33	0.4	<1
2:1 (wt:wt) Sophorolipid: Triton X-100	32	0.3	<1
1:1 (wt:wt) Sophorolipid: Triton X-100	32	0.25	<1
Surfactin	31	0.02	2
Surfactin + 1% NaOH	38	0.02	--
Rhamnolipid	26	0.02	0.5
Rhamnolipid + 1% NaOH	26	0.2	--

solution increased the surface tension but did not affect the CMC significantly. The IFT value of 2 mN/m is generally considered high for oil removal purposes since values of less than 0.001 mN/m are ideal (Wilson, 1990).

## V.2 Rhamnolipid

Like surfactin, although some initial experiments were performed with an isolated rhamnolipid, the majority were performed with a 60% pure sample of rhamnolipid. In general, rhamnolipids are a mixture of up to four different rhamnolipids. However, no attempt was made to separate and identify these.

Characterization was performed on the mixture since this was used in the soil washing tests.

The surface tension and CMC are shown in Table 5.1. The surface tension is in the same region of the 33.5 mN/m found by Thangamani and Shreve (1994) and the 30 mN/m determined by Reiling et al. (1986). The CMC of the rhamnolipids is comparable to the 0.018 g/L determined by Thangamani and Shreve (1994) and the 0.06 g/L (Hisatsuka et al., 1981). The CMC from Figure 5.2B is rather indistinct since this biosurfactant is a mixture of rhamnolipids each with their own CMC values. The IFT is less than 1 mN/m, indicating that this surfactant could be useful for oil removal. Lower values than this cannot be determined using the ring method. The addition of 1% NaOH to the rhamnolipid solution did not change the surface tension or CMC. The surface tension curve (not shown) is similar to that in Figure 5.2B. Surface tension and molar conductivity (using average molecular weight of 504 amu) measurements versus rhamnolipid concentration are shown in Figure 5.2B.

### V.3 Sophorolipid

The sophorolipid used in the studies was isolated after production by *Torulopsis bombicola*. The molecular weight was confirmed to be 646 by mass spectrometry. The surface tension (Table 5.1) was also in the range of 31 to 34 mN/m reported by Cooper et al. (1984). Using the CMC of 82 mg/L (Cooper et al., 1984), the purity of the sophorolipid was calculated to be 1%. The surface tension and molar conductivity as a function of sophorolipid concentration is shown in Figure 5.3.

### V.4 Summary and preliminary discussion

Characterization of the biological surfactants that were produced was performed to determine if these surfactants were as expected and to

determine the purity of the biosurfactants. Surface tension, critical micelle concentrations and molecular weight determination (structures are shown in Figures 2.5, 2.6 and 2.7) were all used for surfactin, rhamnolipid and sophorolipid since these values have all been previously determined and are thus well known. Amino acid analyses confirmed the structure of the surfactin in terms of leucine, glutamic acid, aspartic acid and valine ratio (Figure 2.7). These biosurfactants were chosen on the basis of their low surface tensions and CMC values.

All of these biosurfactants lower the surface tension of water from 72 mN/m to below 35 mN/m and have CMC values below 100 mg/L as previously determined by Guerra-Santos et al. (1984), McCaffrey and Cooper (1995) and Kakinuma et al. (1960) for rhamnolipid, sophorolipid and surfactin, respectively. The CMC from the surface tension curve for the rhamnolipid is less distinct and more difficult to determine than the others since this biosurfactant is actually a mixture of rhamnolipids. These CMC values have been reported to be 5, 40, 200 and 200 mg/L for the rhamnolipids RL1, RL2, RL3 and RL4 (Wagner et al., 1984). The effect of the addition of sodium hydroxide was not previously known, however. Conductivity measurements were also taken and divided by the molecular weight of the surfactants to obtain the molar conductivity to confirm the CMC values determined by surface tension measurements. Like the surface tension, the slopes of the molar conductivity curves changes at the CMC value as shown in Figure 2.4. This phenomenon was noted for all three biosurfactants. The conductivity measurements also confirm the anionic character of these surfactants. Based on the CMC values obtained and amino acid analysis for surfactin, the purity of surfactin and the rhamnolipid were 60% and the sophorolipid was 1% pure.

Interfacial tension measurements were made to determine if these surfactants could potentially be useful for oil removal and to determine if they agree with previously reported values which had been published for surfactin (1.8 mN/m) and the sophorolipid (1.5 mN/m) (Banat, 1995). Some values have been determined for the rhamnolipid. Interfacial tensions were determined to 1 mN/m and 0.25 mN/m by Guerra-Santos et al. (1986) and Robert et al. (1989). The measurements in this study are within the range of those previously determined using hexadecane. However, these are more useful since the oil extracted from the soil was used to measure the interfacial tension.

Therefore, since these biosurfactants are effective at lowering the surface and interfacial tensions at low surfactant concentrations and are produced from sugars and vegetable oil, they should be good candidates as non-toxic soil washing additives. This will be determined in the next step.

## VI SOIL WASHING EXPERIMENTS

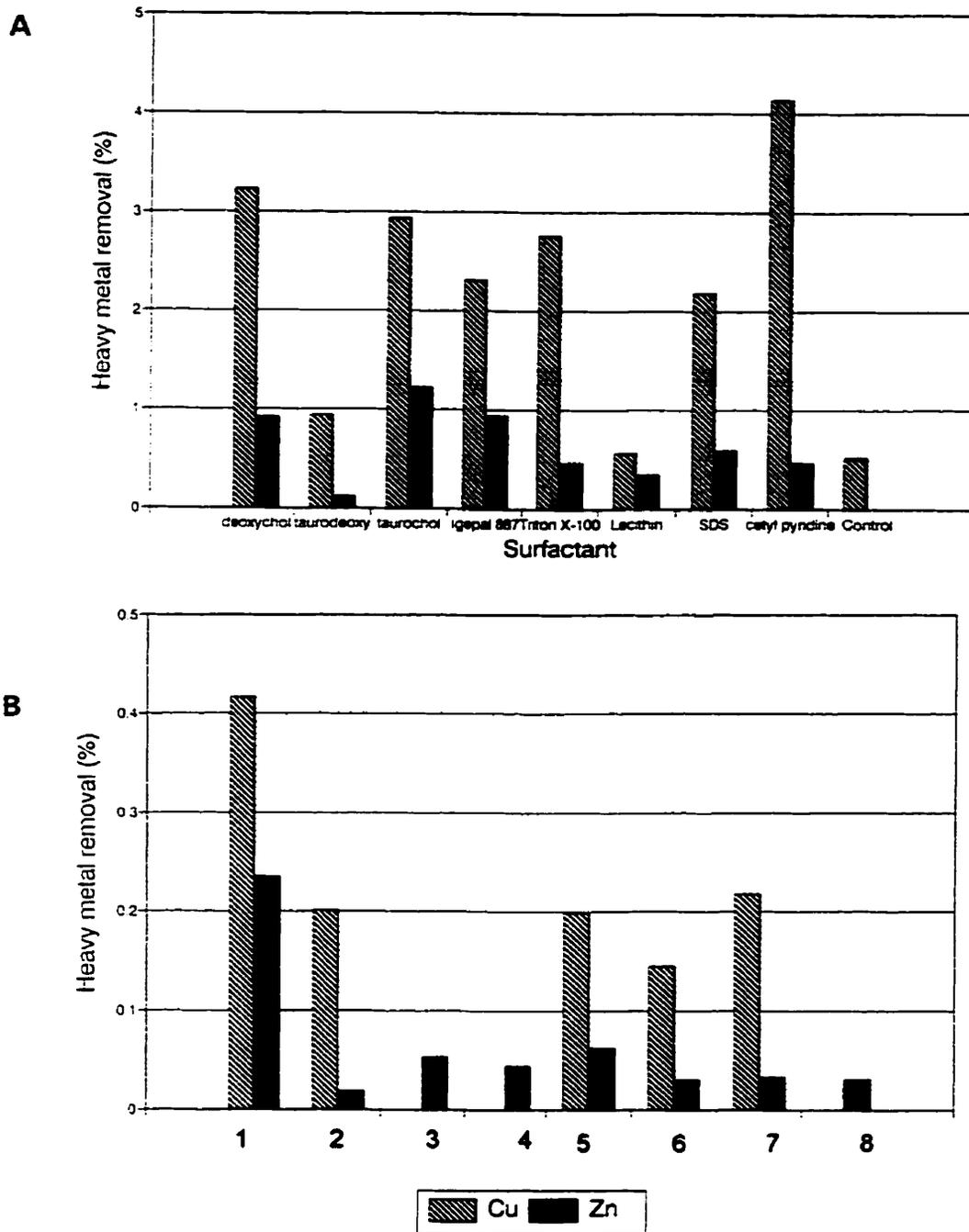
The results of the various soil washing experiments are presented in sections VI.1 to VI.5, with some preliminary discussion given at the end of the Chapter (in section VI.6) The objective of this work was to evaluate the capability of biosurfactants in enhancing the removal of metals and hydrocarbons and to determine what parameters affect their behaviour. This chapter has been organized in this fashion to provide a more complete picture of the efficiency of the washing process as a function of the various surfactants and additives used. The soil washing efficiency is presented throughout these sections as percent heavy metal removal which is defined as shown in section III.9 as:

$$\% \text{ metal removal} = \frac{\text{mg metal}_{\text{supernatant}}}{\text{mg metal}_{\text{original soil}}} \times 100\%$$

In sections VI.2 to VI.4, the results for a single washing of the THC soil are presented, followed by those for the spiked THC soil and the results for the sediments. The efficiency of the series of multiple washings for the spiked THC soil is then shown in each of these sections. Oil removal is shown in section VI.5. A more detailed discussion of all results is given in Section IX.

### VI.1 Various surfactants

A variety of surfactants was tested at the same concentration (1% w/v) for their abilities to remove metals from the soil (A) and sediment (B) (Figure 6.1). Heavy metal removal is shown as a function of the surfactant type which included a cationic (cetylpyridinium chloride, initial pH 4.5), anionic (SDS (initial pH 6.5) deoxycholic acid (initial pH 7.4) taurocholic (initial pH 4.5) and taurodeoxycholic acid (initial pH 5.7)), two zwitterionic (CHAPS (initial pH 8.0) and lecithin (initial pH 5.0) and nonionic (Igepal 887 (initial pH 7.5) and Triton X-100 (initial pH 7.5)). The control was water (pH adjusted to 4.5 or 7.5) to determine how much of the metals could be removed without additives.

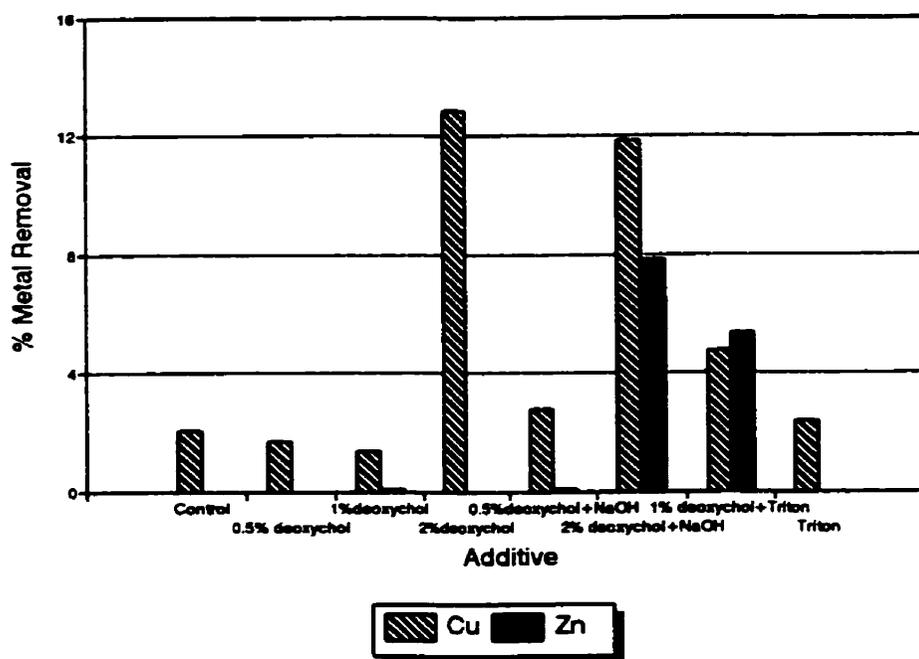


**Figure 6.1 Comparison of the ability of different surfactants to remove metals from the THC soil (A) and the sediment (B). They include 1: deoxychol or deoxycholic acid; 2: taurodeoxy or taurodeoxycholic acid; 3: taurochol or taurocholic acid; 4: Igepal 887; 5: Triton X-100; 6: CHAPS; 7: cetylpyridium chloride and 8: water (pH 4.5).**

Since little difference was shown for the water at the two different pH values only the control at pH 4.5 is shown. For the soil (A), copper removal was less than 0.5% and zinc removal was insignificant for water alone. The addition of surfactants enhanced copper removal as was shown for deoxycholic and taurocholic acids (anionic surfactants), and cetylpyridium chloride (a cationic surfactant). Zinc removal for all surfactants was approximately 1% or less which is at the limit of reliable detection for the soil (1.1%, assuming a limit of detection of 1 mg/L in the supernatant). Copper and zinc removal from the sediments (B) was very poor (less than 0.5% and) for all surfactants and the control. Therefore, a comparison between the surfactants is very difficult. In the case of a couple of the surfactants (taurocholic acid and Igepal 887) and the control, copper removal was insignificant.

Further experiments were performed with SDS (pH 6.5) using different concentrations with the THC soil. Increasing the concentration up to 4% did not increase the amount of zinc removed. Significant amounts of lead (37, 27, 10 and 24%) were removed with 0.1, 0.5, 1.0 and 2.0 % of SDS, respectively, compared to insignificant removal by the control (water at the same pH). Copper removal only became significant at a concentration of 4% when 7.8% of the initial soil metal content was brought into solution.

Deoxycholic acid was also tested for its ability to enhance the removal of copper and zinc as shown in Figure 6.2. This surfactant was chosen since it had been previously examined by Huang et al. (1994) in wastewater studies and since it is a natural surfactant derived from cholesterol. In this study, concentrations of 0.5, 1.0 and 2.0% were evaluated. The final pH values were 7.5, 7.6 and 7.8, respectively. The pH of the control was at 7.5. Zinc removal was negligible at all concentrations. Copper removal, however, increased as the concentration of deoxycholic acid used increased. Co-



**Figure 6.2** Removal of metals from THC soil by washing with various concentrations of deoxycholic acid (shown as deoxychol) with and without co-additives including 1% Triton X-100 or 1% NaOH.

addition of both NaOH and Triton X-100 increased the amount of zinc removed. While there was a noticeable amount of surfactant precipitated onto the soil particularly at the higher deoxycholic acid concentrations, the addition of NaOH and Triton X-100 seemed to decrease this phenomenon.

## VI.2 Surfactin

Initial metal removal experiments were accomplished with surfactin by producing it via the fermentation of *Bacillus subtilis*. A yellowish crude surfactin was obtained by acid precipitation and dichloromethane extraction with a purity of 30%. Consequently, a more purified white powder (62%)

was received from Dr. Kakinuma of Japan and was thus used for further studies. To solubilize this powder, the pH must be increased to 8.0 by addition of NaOH. The effect of surfactin concentration on metal removal efficiency was determined for between 0.13 and 4.0% surfactin. Without any other additives, only 2% and 4% surfactin showed any removal of copper (0.8 and 4.1% respectively) or zinc (1.3 and 2.9%, respectively). However, the effect of the addition of another 1% NaOH to surfactin at concentrations between 0.13% and 4% (pH 10 after addition) on copper and zinc removal was also determined (Figure 6.3). Copper removal significantly increased (Figure 6.3) in comparison to 1% NaOH alone (where no copper removal was noted) even though the pH was the same. Copper removal increased as the surfactin concentration increased to 0.25%, decreased until 1% concentration and then increased again as the concentration increased to 4%. Zinc removal followed the same trend with the exception of a decrease at 4% surfactin compared to the experiment with 2%.

The effect of co-addition of nonionic surfactants (0.5%) and NaCl (1.5%) to surfactin (0.5%) was also investigated (Figure 6.4) to determine if metal removal efficiency could be increased compared to surfactin alone (pH 8) which removed insignificant amounts of copper and zinc (less than 0.1%). In particular, zinc removal became significant in the presence of 0.5% T-MAZ 20 and surfactin (pH 8.0) but copper removal was not significant even though copper removal was shown when T-MAZ 20 was added alone. Zinc removal efficiencies were similar between the combination of surfactin and 0.5% T-MAZ 28 (pH 7.7) and 0.5% T-MAZ 28 (pH 7.6) alone whereas copper removal was twice that for the former compared to the latter. A concentration of 1.5% NaCl was added to the surfactin to determine if the addition of sodium (same molar concentration as 1% NaOH) would enhance metal removal compared to

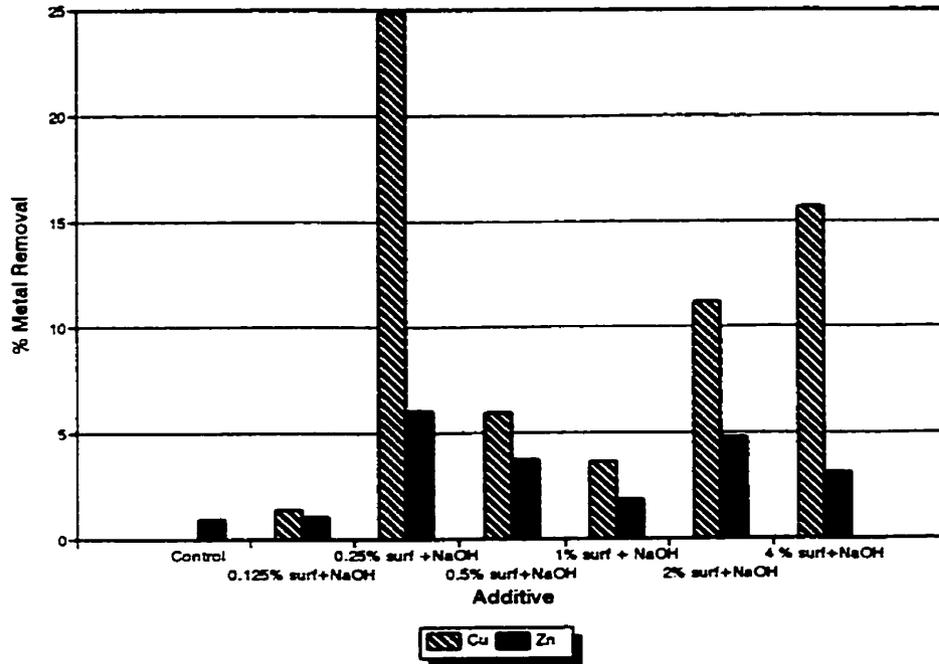


Figure 6.3 Metal removal efficiency for surfactin (with 1% NaOH) as a function of concentration (0.12 to 4%) for THC soil. Control was 1% NaOH.

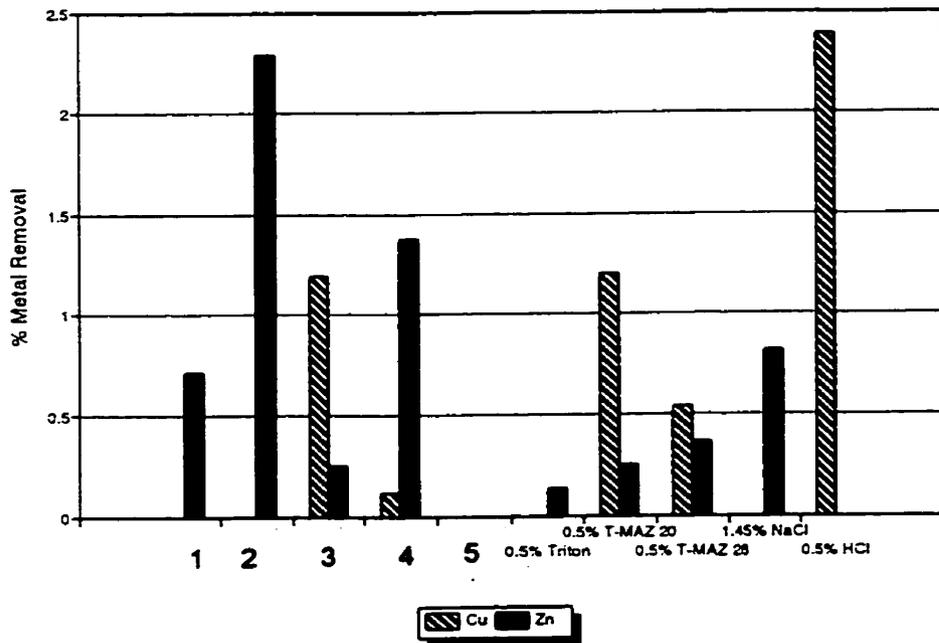


Figure 6.4 Effect of nonionic surfactants (1: 0.5% Triton X-100, 2: 0.5% T-MAZ 20, 3: 0.5% T-MAZ 28), 4: 1.45% NaCl and 5: 0.5% HCl on the removal of metals from the THC soil by 0.5% surfactin. The same additives without surfactin are shown on the right of the graph.

surfactin alone. It did not, however, since copper removal was very poor (less than 0.1%). Acid addition (0.5% HCl) to the surfactin solution was also evaluated. It decreased the pH to 6.5 from 8.0 and initiated precipitation of the surfactin which was highly visible. The addition of surfactin and HCl together showed lower levels of zinc removal than the acid alone.

Surfactin concentrations were determined in the supernatant after the soil washing experiments as shown in Table 6.1. Several trends seem evident. At higher concentrations, the losses by precipitation or sorption of surfactin are less. Also, the co-addition of the nonionic surfactant or NaOH decreased the losses of the surfactin at the lower concentrations. Addition of NaCl or HCl was not beneficial to metal removal by 0.5% surfactin since none of the surfactin was still in solution after soil washing.

Soil washing experiments were performed at two surfactin concentrations with and without NaOH for two other matrices, spiked THC soil (Figure 6.5) and sediment (Figure 6.6) to determine if surfactin behaved in a similar manner for these matrices as compared the THC soil (Figure 6.3). For the spiked soil (Figure 6.5), 2% surfactin and NaOH provided the most copper removal followed by 0.25% surfactin with NaOH which is different than the THC soil where 0.25% surfactin was superior to the higher surfactin concentration. Comparisons with 1% NaOH (pH 10.0) alone showed that the combination of surfactin/NaOH was superior where copper removal was concerned. In contrast, the 2% surfactin alone (pH 8.0) removed the highest percentage of cadmium, lead and zinc. It appears in this case that at the lower pH (8.0 compared to 10.0 in the presence of NaOH) was beneficial for removal of these metals. For the sediment (Figure 6.6), significant zinc removal was obtained at 2% surfactin (pH 8.0) while 2% surfactin with NaOH (pH 10.0) and 0.25% surfactin with NaOH (pH 10.0) removed equal amounts of copper.

**Table 6.1 Concentration of surfactin in the supernatant before and after soil washing of the THC soil**

Additive	Initial surfactin concentration (%)	Final surfactin concentration (%)
Surfactin	4.0	3.0
	2.0	1.5
	1.0	0.5
	0.5	ND
	0.25	0.15
	0.13	ND
Surfactin and 1% NaOH	4.0	3.5
	2.0	1.0
	1.0	0.3
	0.5	0.2
	0.25	0.12
	0.13	<0.1
Surfactin and Triton X-100	0.5	0.4
Surfactin and T-MAZ 20	0.5	0.3
Surfactin and T-MAZ 28	0.5	0.1
Surfactin and NaCl	0.5	ND
Surfactin and HCl	0.5	ND

ND- not detectable

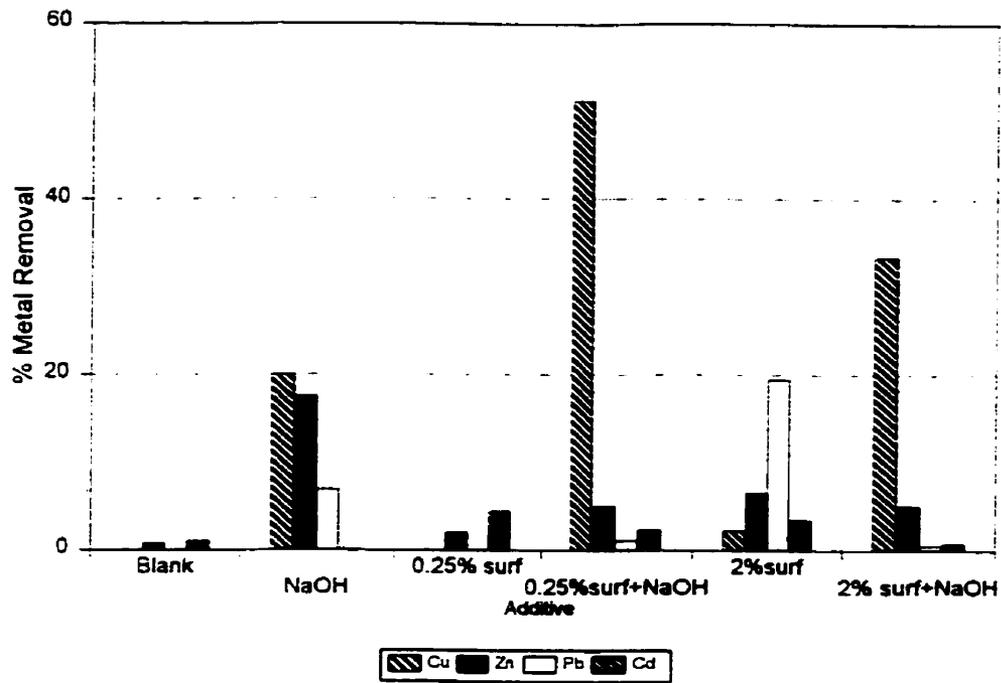


Figure 6.5 Metal removal from the spiked THC soil by surfactin at concentrations of 0.25 and 2.0% surfactin with (pH 10.0) and without 1% NaOH

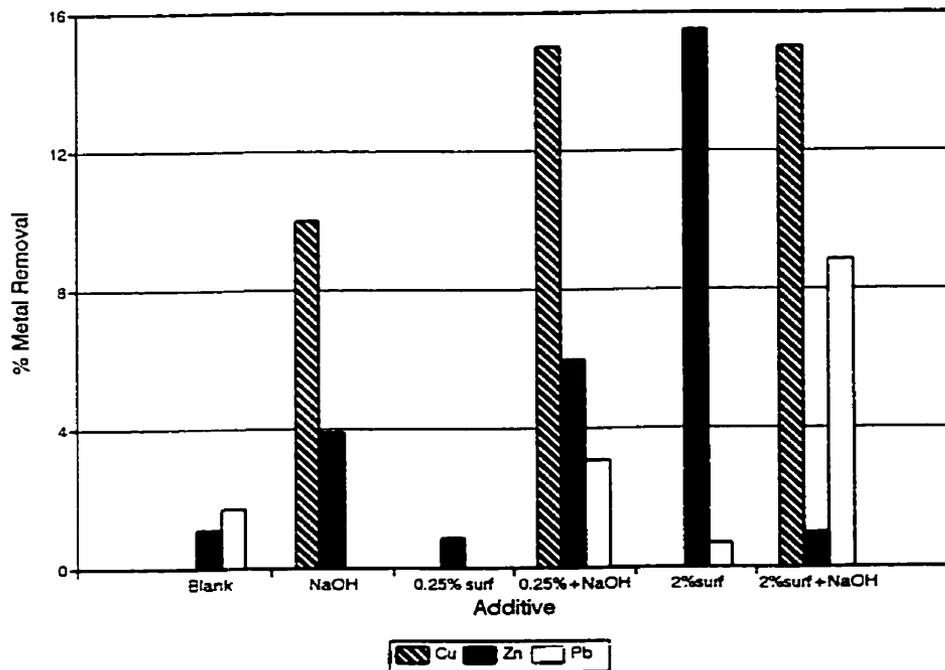
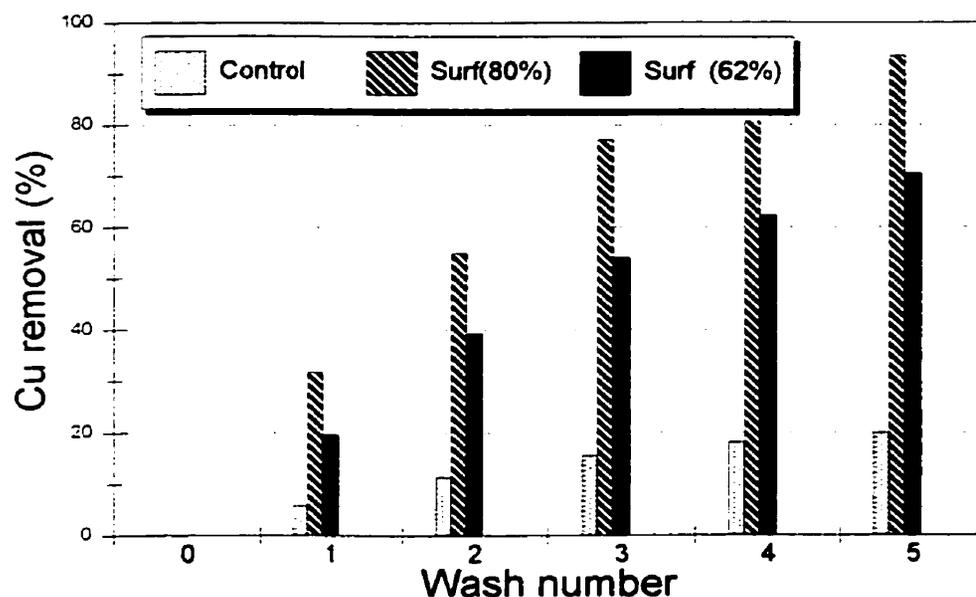


Figure 6.6 Removal of metals from the sediments by surfactin at concentrations of 0.25 and 2.0% surfactin with (pH 10.0) and without 1% NaOH (pH 8.0)

Lead removal was quite significant at 2% surfactin with NaOH. The soil residues from these experiments were used for subsequent sequential extraction studies.

A series of washings was performed on the spiked THC soil using 0.25% surfactin and 1% NaOH by removing the washing solution each day and replacing it with a fresh solution. Copper (Figure 6.7), zinc (Figure 6.8) and cadmium (Figure 6.9) removals were followed each day for 5 days. The control was 1% NaOH. For copper, the control showed a final cumulative removal of 20% while approximately 70% was removed by the surfactin. A purer form of surfactin was also evaluated and showed 93% total removal, indicating that some of the impurities in the surfactin could be decreasing its efficiency for metal removal. For zinc, removal rates were lower than for copper as



**Figure 6.7 Series of washings for copper by surfactin (with 1% NaOH) using the spiked THC soil using two purities of surfactin, (80%) and (62%). Supernatants were removed each day and replaced with a fresh solution. The control was 1% NaOH.**

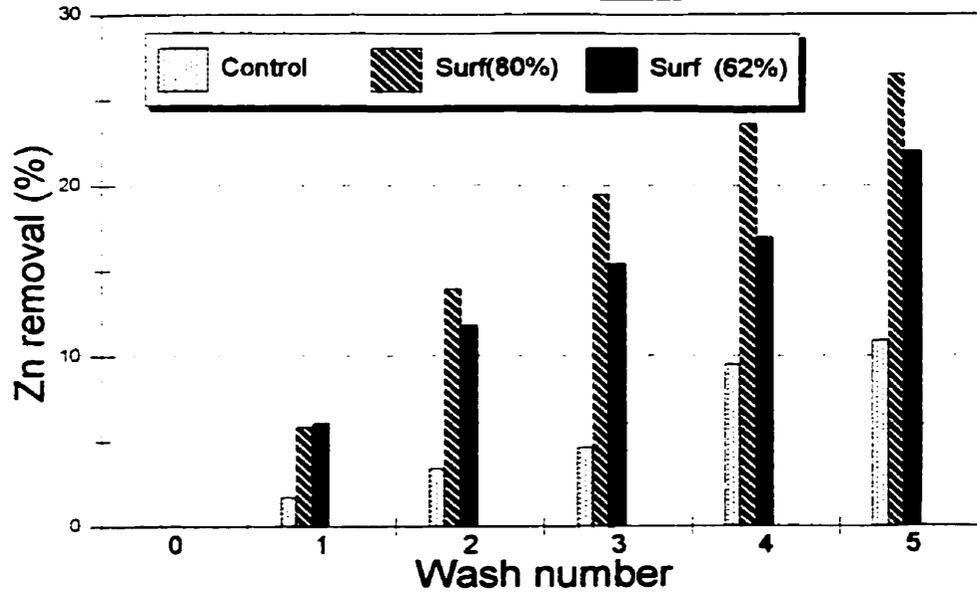


Figure 6.8 Series of washings for zinc with surfactin (with 1% NaOH) using the spiked THC soil using two purities of surfactin, (80%) and (62%). Supernatants were removed each day and replaced with a fresh solution. The control was 1% NaOH.

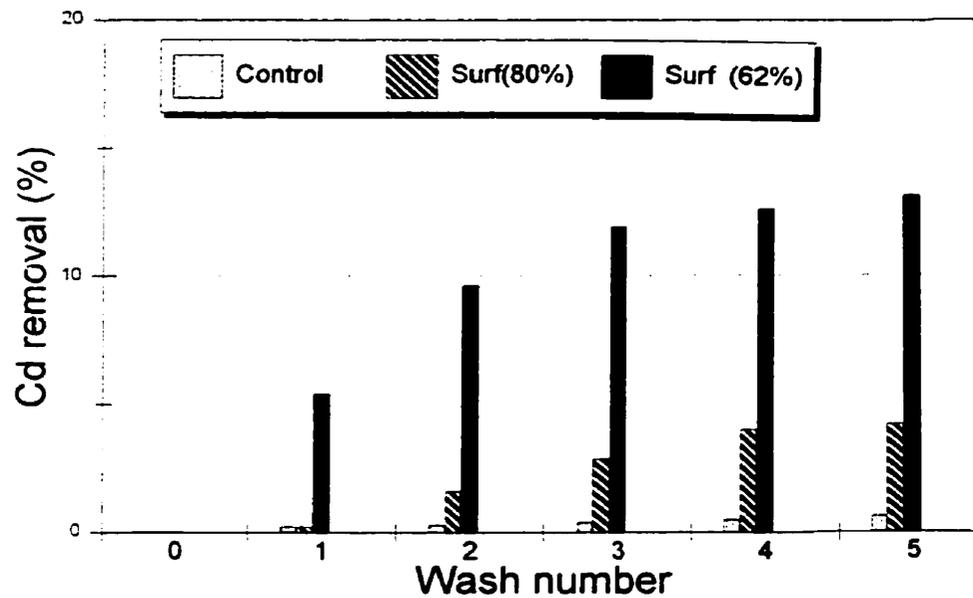


Figure 6.9 Series of washings for cadmium with surfactin (with 1% NaOH) using the spiked THC soil using two purities of surfactin, (80%) and (62%). Supernatants were removed each day and replaced with a fresh solution. The control was 1% NaOH.

shown previously, 10% for the control and 25% for surfactin (both purities). The control showed minimal cadmium removal, while surfactin removed approximately a total of 15%. For all metals, the rates of removal were the highest up to three days. Therefore, multiple washings with surfactin (with 1% NaOH) was highly beneficial for copper, with less benefit for zinc and cadmium.

### VI.3 Rhamnolipid

After performing a few initial experiments with a crude rhamnolipid produced and isolated in the lab from the fermentation broth of *Pseudomonas aeruginosa*, a rhamnolipid powder of approximately 60% purity was obtained from Dr. O. Käppeli of Switzerland. It was soluble in distilled water and gave a pH of 6.5. No NaOH or HCl addition was therefore necessary to solubilize the rhamnolipid.

Soil washing experiments (Figure 6.10) were then performed by varying the concentration of the rhamnolipid up to 12% (at which point the solution

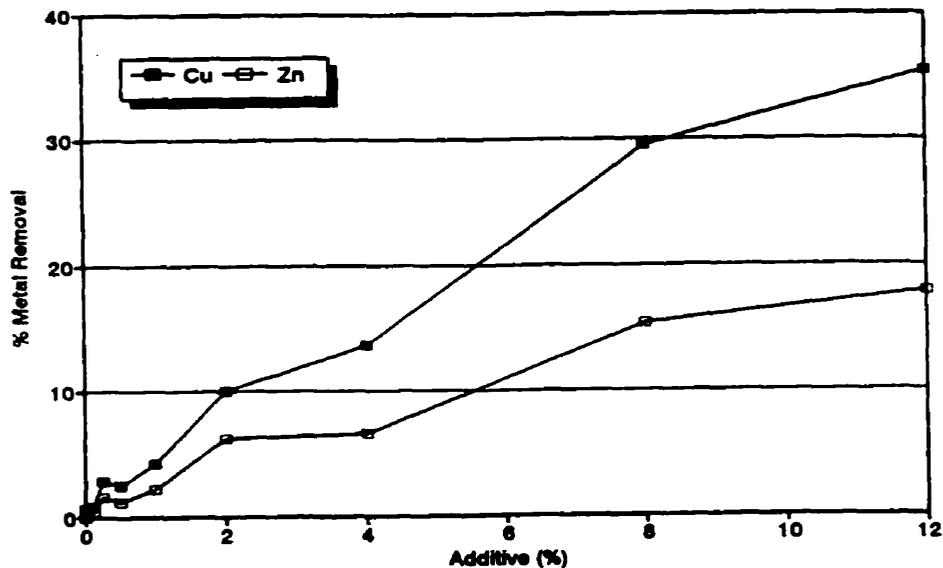
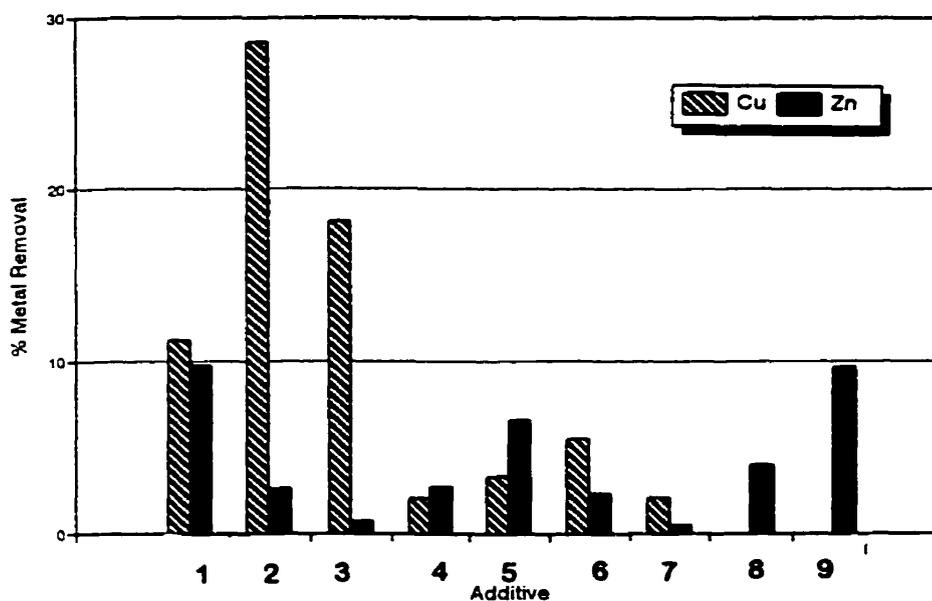


Figure 6.10 Effect of rhamnolipid concentration (pH 6.5) on metal removal (%) from the THC soil

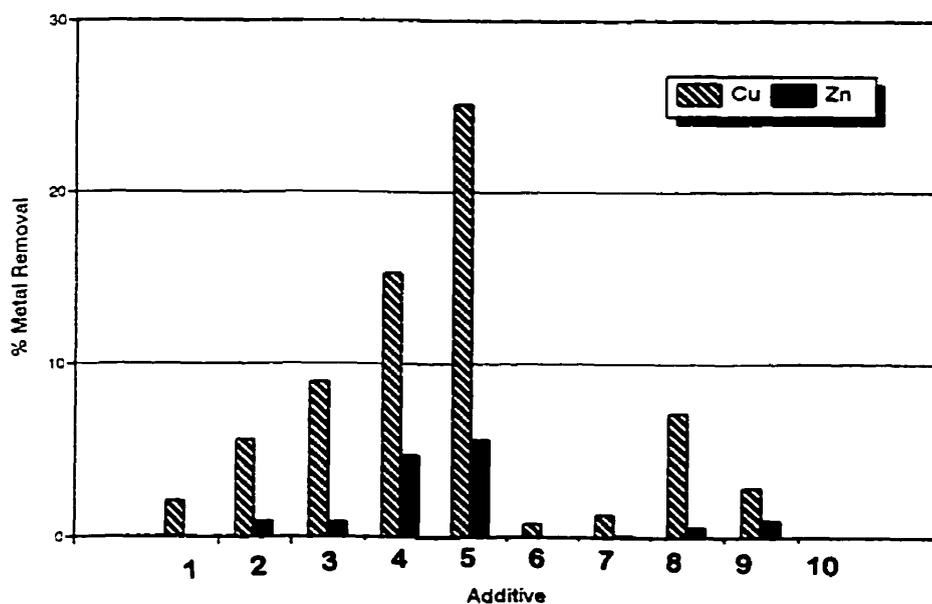
becomes very viscous). Copper and zinc removal increased as the concentration of rhamnolipid increased.

At 2% rhamnolipid concentration (pH 6.5), HCl, NaOH or Na<sub>2</sub>CO<sub>3</sub> were added to the washing solutions (Figure 6.11) to determine their effects on metal removal by the rhamnolipid. The addition of 1% NaOH (pH 10.0) to the surfactant significantly increased the copper removal efficiency but decreased that of zinc. Equal concentrations of sodium carbonate (pH 9.0) and sodium hydroxide (pH 10.0) were compared. Sodium carbonate with and without the rhamnolipid did not remove as much copper and zinc as the sodium hydroxide, possibly due to the lower pH of the carbonate. The addition of HCl caused an immediate precipitation of the surfactant by decreasing the pH to 6.2 (0.25% HCl) and 5.8 (0.5% HCl), making it unavailable for the metal removal as shown by the decreased removal of copper and zinc for the surfactant alone (pH 6.5). Zinc removal was better for HCl alone than in combination with the rhamnolipid at concentrations of 0.2 and 0.5%.

Another series of experiments was performed with 0.5% rhamnolipid. When the concentration of NaOH added with the surfactant was increased to 1.0% (pH 10.0), it enhanced both copper and zinc removal as compared to the lower concentrations of 0.5% (pH 9.7) and 0.1% NaOH (pH 9.5). In fact, 0.5% rhamnolipid and 1% NaOH was able to remove over twice as much copper removal as 2% rhamnolipid alone (Figure 6.12). Therefore, the addition of the NaOH can reduce the amount of rhamnolipid required with better results. When NaCl was added to the surfactant to give the same molar concentration of sodium as NaOH as performed for surfactin, its presence did not enhance copper removal as NaOH does. Therefore, it is not the sodium concentration that enhances metal removal.



**Figure 6.11** Effect of additives to 2% rhamnolipid. They include 1: rhamnolipid; 2: rhamnolipid+1% NaOH; 3: rhamnolipid+1% Na<sub>2</sub>CO<sub>3</sub>; 4: rhamnolipid+0.25% HCl; 5: rhamnolipid+0.5% HCl; 6: 1% NaOH; 7: 1% Na<sub>2</sub>CO<sub>3</sub>; 8: 0.25% HCl; 9: 0.5% HCl.



**Figure 6.12** Effect of additives to 0.5% rhamnolipid. They include: 1: water; 2: rhamnolipid; 3: rhamnolipid+0.1% NaOH; 4: rhamnolipid+0.5% NaOH; 5: rhamnolipid+1% NaOH; 6: 0.1% NaOH; 7: 0.5% NaOH; 8: 1.0% NaOH; 9: rhamnolipid +1.45%NaCl; 10: 1.45% NaCl.

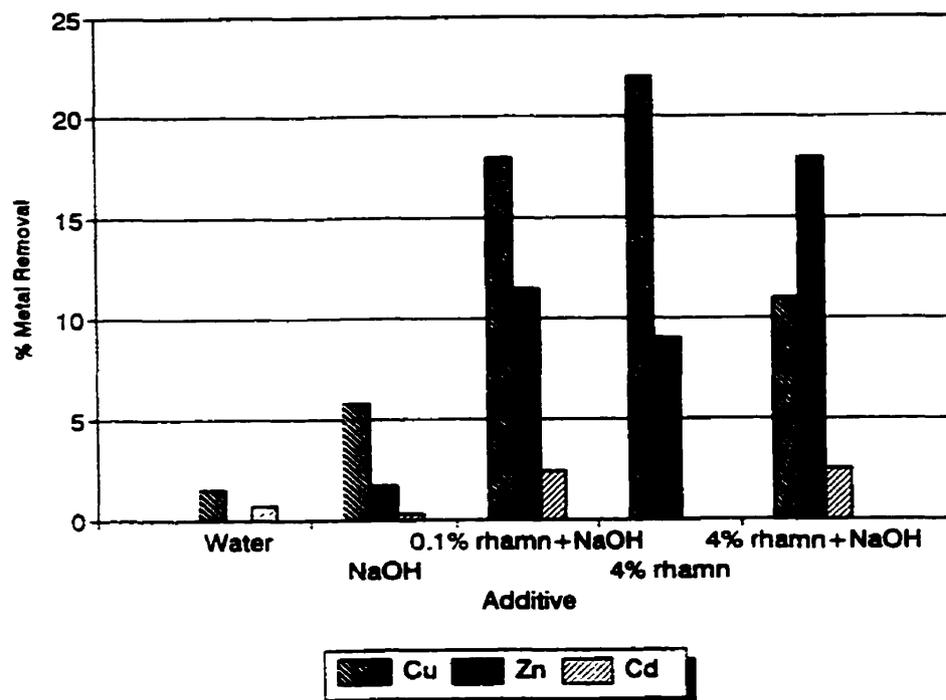
The effect of a nonionic surfactant on metal removal by rhamnolipid was tested (Table 6.2). For 0.5% rhamnolipid, Triton X-100 addition (same concentration as the rhamnolipid was beneficial for both zinc and copper removal for the soil. A small increase of pH was noted (pH 6.7). Another nonionic surfactant, T-MAZ 20 (pH 7.0), was also added to the rhamnolipid, (Table 6.2). Insignificant results were obtained through the addition of T-MAZ 20 alone, whereas addition with the rhamnolipid enhanced its ability to remove zinc but not copper.

Spiked THC soil was washed with two different concentrations of rhamnolipid (0.1 and 4.0%). Comparisons were also made for 4% rhamnolipid in the presence and absence of 1% NaOH. Figure 6.13 shows the results. The lower rhamnolipid concentration (0.1%) showed superior results for copper removal, slightly inferior results for zinc and approximately the same results for cadmium. For the 4% rhamnolipid, the presence of NaOH enhanced the removal of cadmium and zinc.

**Table 6.2 Effect of addition of T-MAZ 20 and Triton X-100 on metal removal from THC soil by 0.5% rhamnolipid**

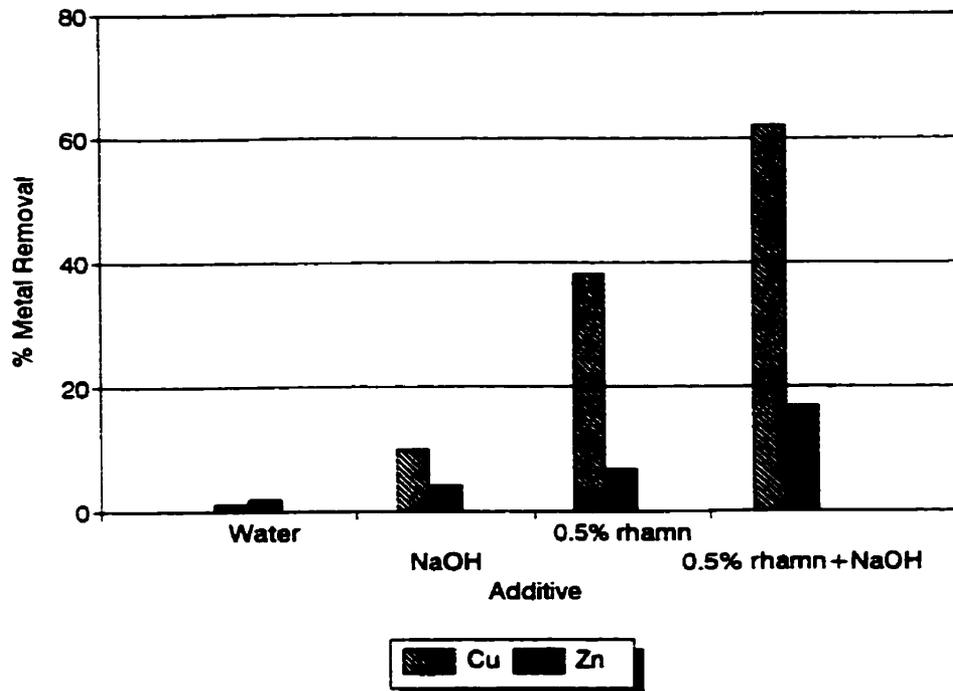
Additive	Zn removal (%)	Cu removal (%)
0.5% rhamnolipid	0.6	5.6
0.5% rhamnolipid + 0.5% T-MAZ 20	4.4	2.1
0.5% rhamnolipid + 0.5% Triton X-100	3.0	6.0
0.5% T-MAZ 20	ND	ND
0.5% Triton X-100	ND	1.8

ND- Not detectable



**Figure 6.13** Washing experiments were performed with water (pH 6.5), 1% NaOH (pH 10.0) 4% rhamnolipid (pH 6.5) alone, 0.1% rhamnolipid with (pH 10.0) and without 1% NaOH (pH 6.5) using spiked THC soil

Another matrix, sediments, was washed with 0.5% rhamnolipid (pH 6.5) with and without NaOH (Figure 6.14). Clearly, the presence of NaOH with rhamnolipid (pH 10.0) significantly enhanced the removal copper and zinc. Over four times more zinc and six times more copper were removed by the combination of rhamnolipid and NaOH compared to the NaOH alone (pH 10.0). Compared to water alone (pH 6.5), 38 times more copper and 4 times more zinc was removed by the rhamnolipid. Therefore, the rhamnolipid can enhance metal removal from the sediment as well as the oil-contaminated THC soil.



**Figure 6.14 Metal removal from sediments by rhamnolipid (0.5%) with and without 1% NaOH)**

A series of washings on spiked THC soil was performed using 0.1% rhamnolipid and 1% NaOH, in a similar manner to the surfactin experiments. The control consisted of 1% NaOH. Copper (Figure 6.15), zinc (Figure 6.16) and cadmium (Figure 6.17) removals were followed for five days. Approximately, 38% of the copper was removed after the five washings compared to 20% for the control. After two washings, the rate of removal decreased somewhat. Total zinc removal after the five washings was 15% compared to the 11% for the control. Cadmium removal was approximately eight times as much for the rhamnolipid as compared to the control. In all cases, the levels of removal continued to increase with each washing. This would indicate that more heavy metals could be removed after further washings with the rhamnolipid.

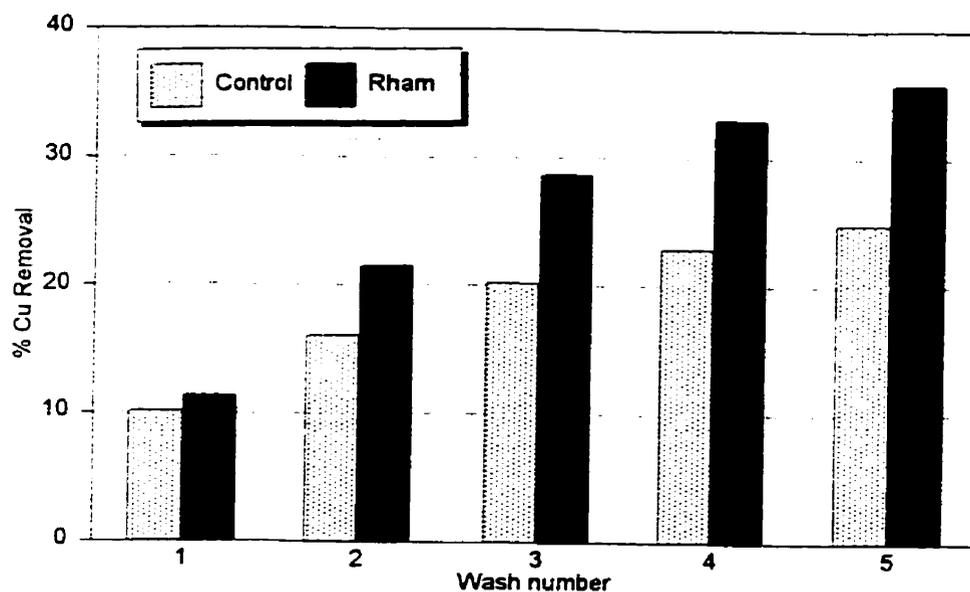


Figure 6.15 Accumulated copper removal after a series of five washings of spiked THC soil with 0.1% rhamnolipid / 1% NaOH and the control of 1% NaOH.

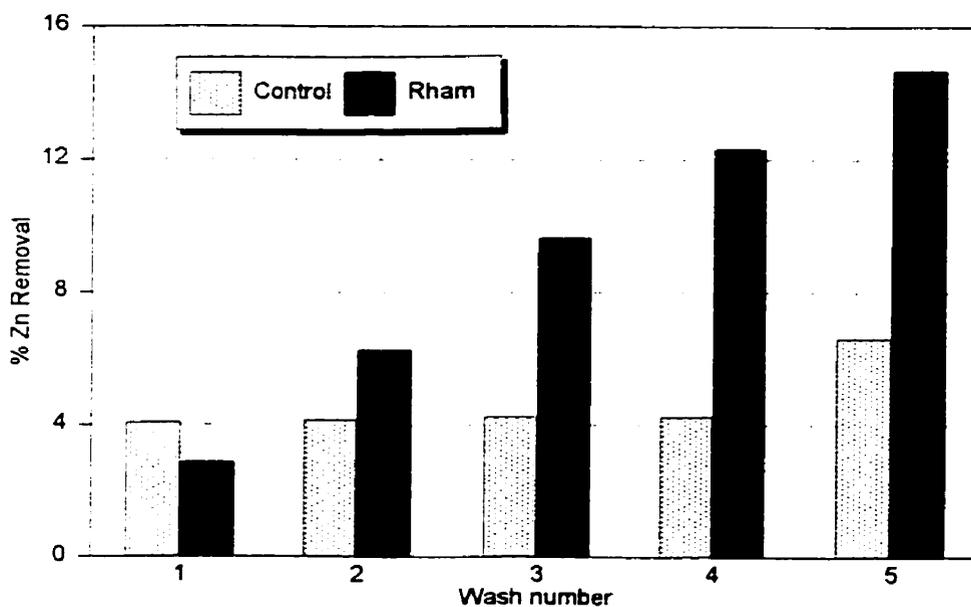
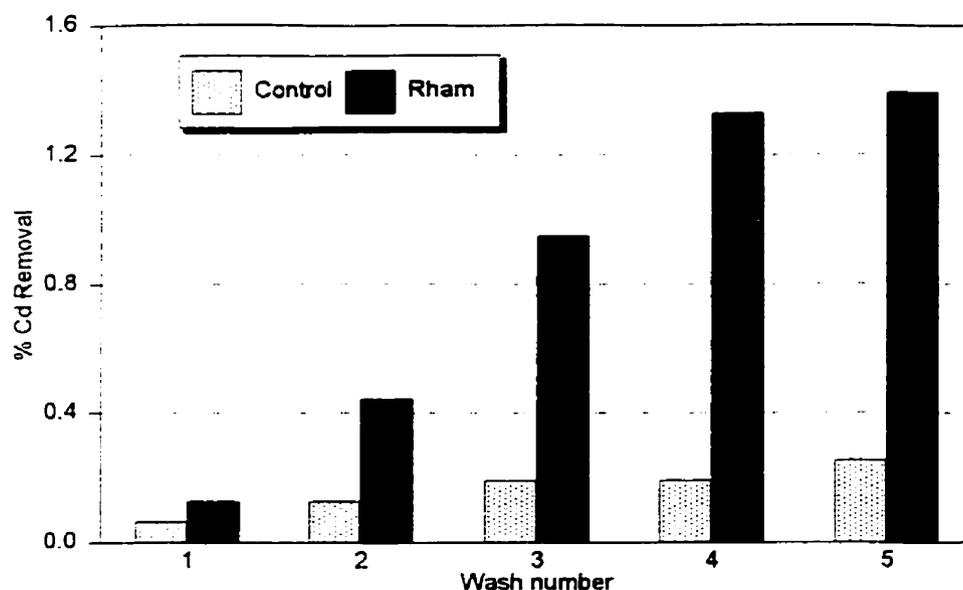


Figure 6.16 Accumulated zinc removal after a series of five washings of spiked THC soil with 0.1% rhamnolipid/1% NaOH and the control (1% NaOH)



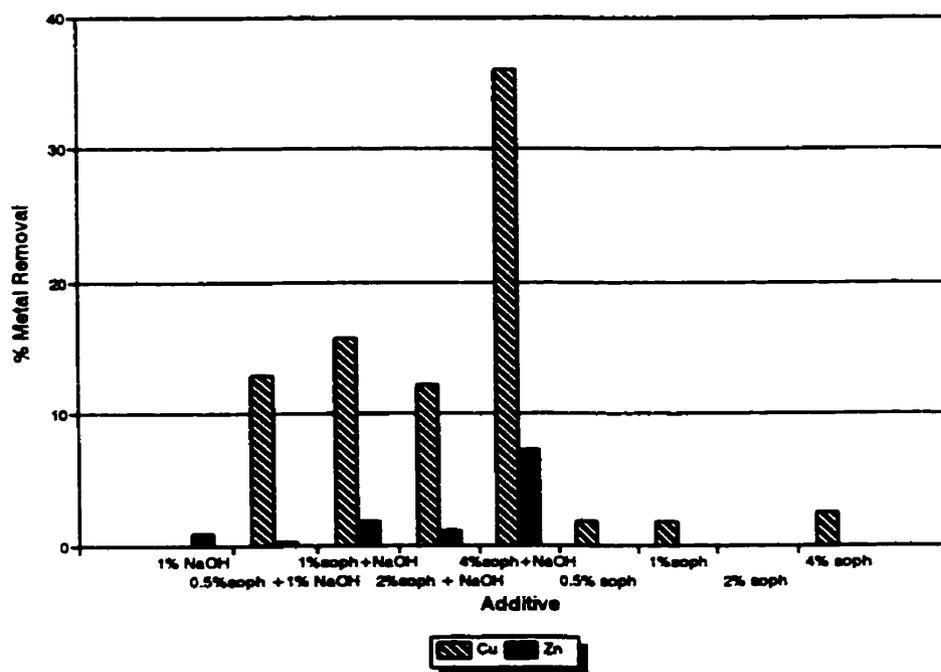
**Figure 6.17 Accumulated cadmium removal after a series of five washings of spiked THC soil with 0.1% rhamnolipid/1% NaOH and the control (1% NaOH)**

#### **VI.4 Sophorolipid**

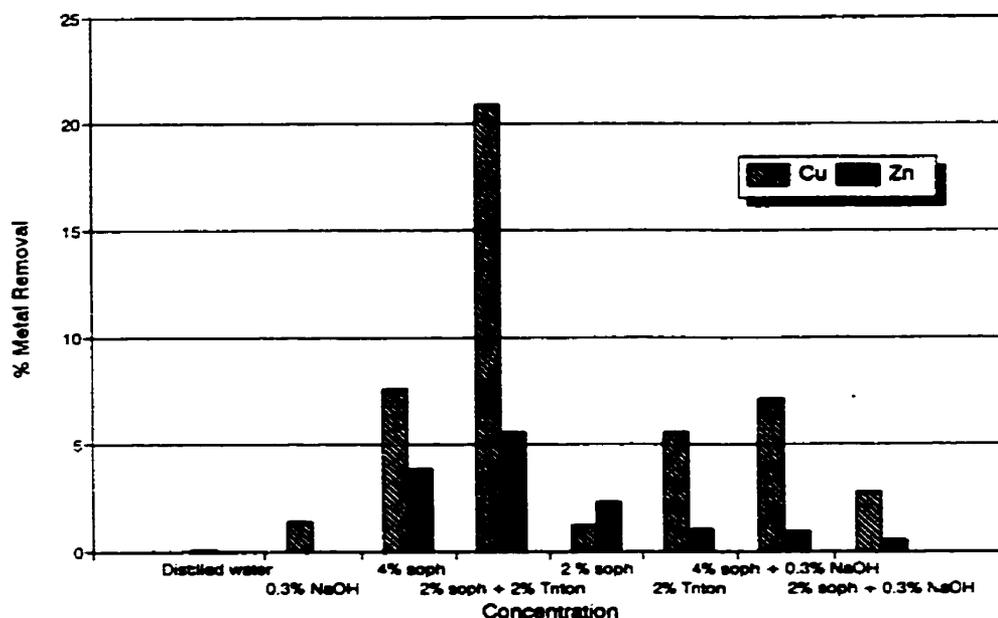
Several experiments were performed using the sophorolipid produced by the yeast *Torulopsis bombicola*. It was isolated from the fermentation medium by solvent extraction with ethyl acetate. The residue that remains is an oily substance and was used in the soil washing experiments as is. Its molecular weight has been confirmed by mass spectrometric analysis. It has also the following characteristics. When it is added to water, a pH of approximately 4.5 to 5.0 is obtained. If the pH is not adjusted, a pale yellow oily layer forms on the top of the water. If the pH is increased to above pH 7, the solution becomes a milky white suspension.

For the soil washing experiments, concentrations of up to 4% (pH 5.0) were used. Metal removal occurred as the concentration increased (Figure 6.18). Insignificant results were obtained at levels below 1%. A dark oily phase on top of the soil washing supernatant was always seen. The addition of 1.5% NaCl (same sodium molarity as 1% NaOH) with 4% sophorolipid decreased copper and zinc removal by 85.5% and 47.8%, respectively which was similar to the results for the other biosurfactants. The addition of Triton X-100 (pH 6.8) in an equal weight ratio to the sophorolipid (2%:2%), however, increased the amount of copper and zinc removed as compared to the sophorolipid alone.

The influence of NaOH addition to the surfactant solution was also investigated (Figure 6.19). The addition of 0.3% NaOH (pH 9.5) slightly increased copper removal with 2% sophorolipid present. Adding the same



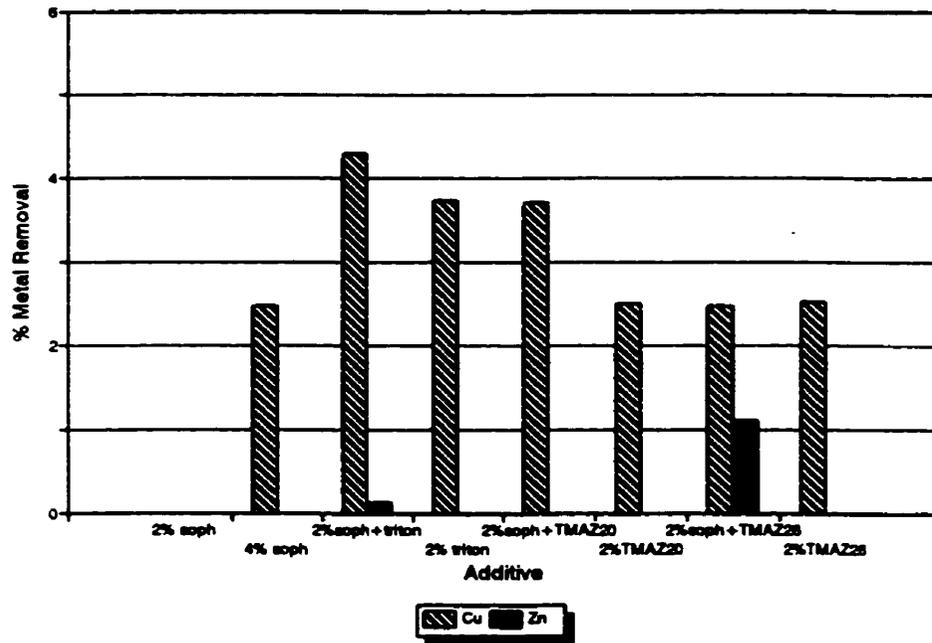
**Figure 6.18 Metal removal from THC soil by 0.5, 1.0 and 4.0% sophorolipid with and without NaOH (1%)**



**Figure 6.19 Effect of NaOH (0.3%) and Triton X-100 (2%) addition on metal removal (%) by sophorolipids (2 and 4%) from THC soil**

amount to 4% sophorolipid was not beneficial. In contrast, the effect of 1% NaOH (pH 10.0) was clearly beneficial at all concentrations of sophorolipid and that the effect between the NaOH and the sophorolipid is additive and superior to even the combination of the sophorolipid and the nonionic surfactant.

Since Triton X-100 seemed to assist metal removal when combined with the sophorolipid, other nonionics were examined including POE(20)sorbitan monolaurate (T-MAZ 20) (pH 6.9) and POE(80) sorbitan monooleate (T-MAZ 28) (pH 6.9) (Figure 6.20). Both are edible FDA food status surfactants (Shiau et. al., 1995) which could potentially be used safely in soil washing projects. There was only a slight additive effect for the sophorolipid and T-MAZ 20 on copper removal but none for zinc. The combination of T-MAZ 28 and the sophorolipid showed some zinc removal which was not evident when each was used alone.



**Figure 6.20 Effect of nonionic surfactant (Triton X-100, T-MAZ 20 and T-MAZ 28) addition on metal removal from THC soil by sophorolipids (2%)**

The effect of HCl addition with and without 4% sophorolipid is shown in Figure 6.21. Even lead removal was noteworthy at 0.5% HCl but was maximal at 0.7% HCl. Copper removal also increased as the acid concentration increased, reaching values of over 35% (sophorolipid + 0.7% HCl). For zinc, although the amount removed increased as the acid concentration increased, co-addition with the surfactant did not enhance removal. The final pH values are shown in Table 6.3. The pH for the combined sophorolipid and acid was very similar to the acid alone. Therefore, the improved removal was due to the action of the sophorolipid surfactant and not the effect of pH.

To determine if a pretreatment step which reduces the soil pH could enhance metal removal, an experiment was performed using two steps (Figure 6.22). A first wash was done for three samples using 0.5% HCl for 24 h which reduced the soil pH to 5.6. The supernatant was then removed in each case

and the second wash was performed with distilled water (pH 6.5), 1% sophorolipid (pH 6.5) or 4% sophorolipid (pH 6.5). With the higher concentration of surfactant, both copper and zinc removals were highest. Compared to the one step procedure (0.5% HCl), both copper and zinc removal were improved. Therefore, the two-step procedure was beneficial.

Several experiments (Figure 6.23) were performed by varying the ratio of sophorolipid to Triton X-100 since this nonionic surfactant influenced the behaviour of the anionic surfactant. Addition of 2% Triton to 2% sophorolipid (pH 6.9) enhanced the ability of the 2% sophorolipid (pH 6.5) to remove copper and zinc. To the 4% sophorolipid, 1, 2 and 4% Triton X-100 were added. The best results were obtained using the combination of 4% sophorolipid with 2% Triton X-100 (Figure 6.23).

**Table 6.3 Effect on pH of the supernatant from THC soil by increasing HCl concentrations with and without 4% sophorolipid**

Additive	Final pH
Control	7.02
0.2% HCl	5.86
0.5% HCl	5.75
0.7% HCl	5.62
4% sophorolipid	6.49
4% sophorolipid + 0.2% HCl	6.16
4% sophorolipid + 0.5% HCl	5.74
4% sophorolipid + 0.7% HCl	5.43

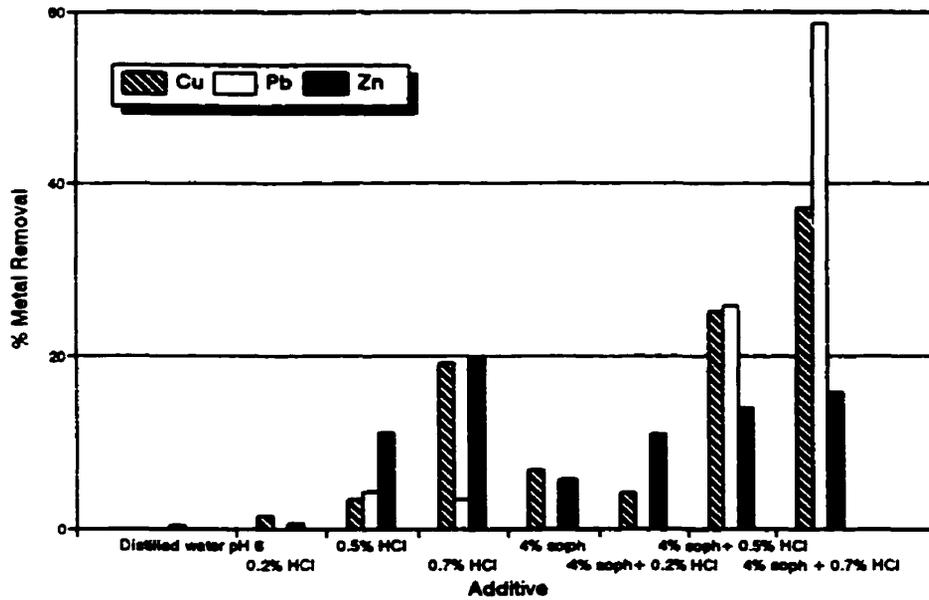


Figure 6.21 Effect of increasing acid addition from 0 to 0.7% HCl on metal removal by 4% sophorolipid from THC soil.

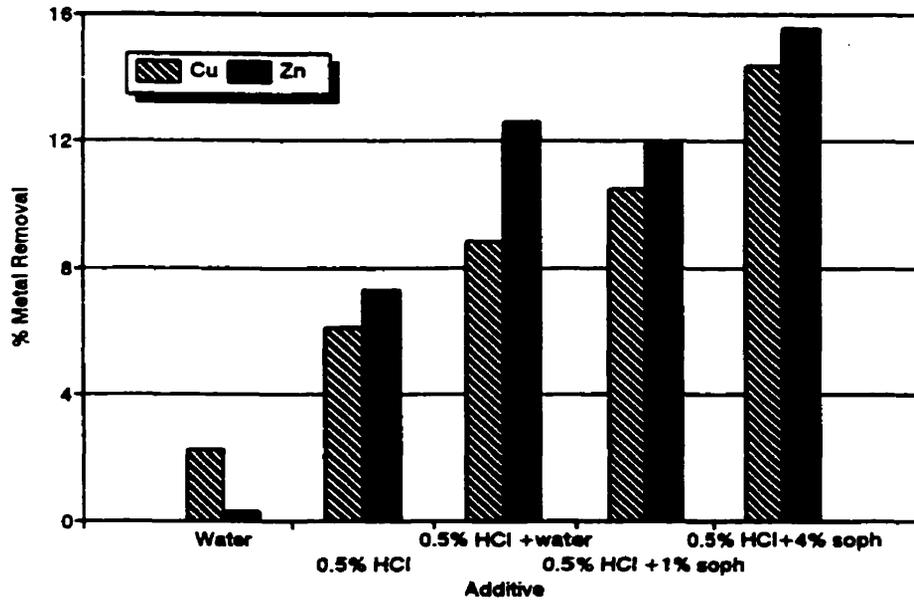
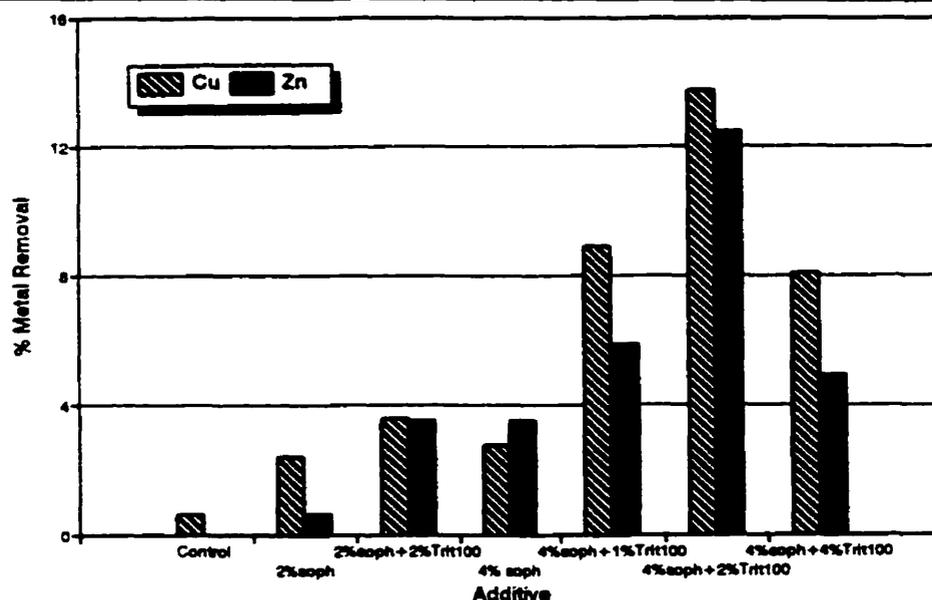


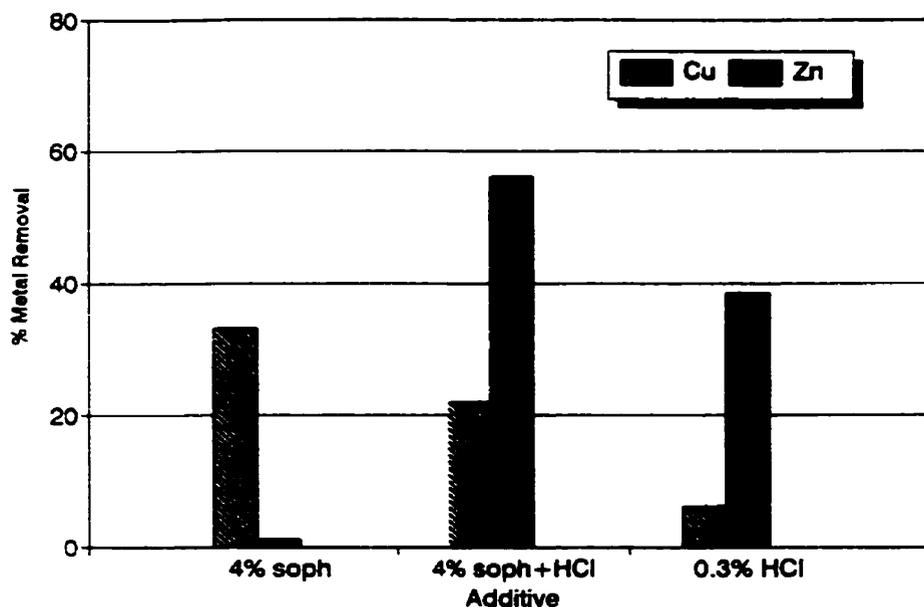
Figure 6.22 Two-step washing of THC soil. The soil was washed for 24 h with 0.5% HCl. The mixture was centrifuged and another solution (distilled water, 1% sophorolipid or 4% sophorolipid (pH 6.5)) was added for another 24 h. Total removal (%) results are shown in the figure.



**Figure 6.23** Effect of Triton X-100 (shown as Trit)/sophorolipid ratio on metal removal from THC soil. 2% Triton was added to 2% sophorolipid. 1, 2 and 4% Triton X-100 were added to 4% sophorolipid.

A 4% sophorolipid solution with 0.7% HCl (pH 5.4) was added to the other sediments to determine its capability of this biosurfactant in removing metals from this matrix in comparison to the acid alone. 100% of the copper and zinc were removed with and without the sophorolipid since the pH dropped to 2 for both cases after washing. Therefore, another experiment was run using a lower acid concentration (0.3% HCl) with and without 4% sophorolipid to evaluate the biosurfactant. The results (Figure 6.24) show that both copper and zinc removal improved in the presence of the surfactant compared to the acid alone. Copper removal decreased when the acid was added to the surfactant compared to the biosurfactant alone.

Figures 6.25, 6.26 and 6.27 show the removal of copper, zinc and cadmium after a series of washings of spiked THC soil for 5 days. For copper, the combinations of 4% sophorolipid with 2% Triton X-100 and 4% sophorolipid with 0.7% HCl showed similar trends and total removals of 50%. In addition, clearly the acid and Triton X-100 alone showed inferior results. The total



**Figure 6.24 Removal of copper and zinc from the sediments using 4% sophorolipid in combination with and without 0.3% HCl. 0.3% HCl was also tested alone.**

copper removal for the acid was 40% but this did not occur until the fifth wash. By this time enough acid had contacted the soil to destroy the structure of the soil and solubilize copper. Zinc removal was highest for the acid and acid with sophorolipid combination. The latter reached 100% removal after 4 washes compared to 5 washes for the acid alone. The sophorolipid with Triton X-100 showed total zinc removal of over 42%. Cadmium removal behaved in a similar manner to zinc. Again the sophorolipid/acid combination performed the best followed by the acid alone and the sophorolipid/Triton combination. Total removals were 90%, 70% and 55%, respectively. Triton X-100 alone gave insignificant removal rates, indicating that the combination of the anionic and nonionic surfactants was required for optimal metal removal.

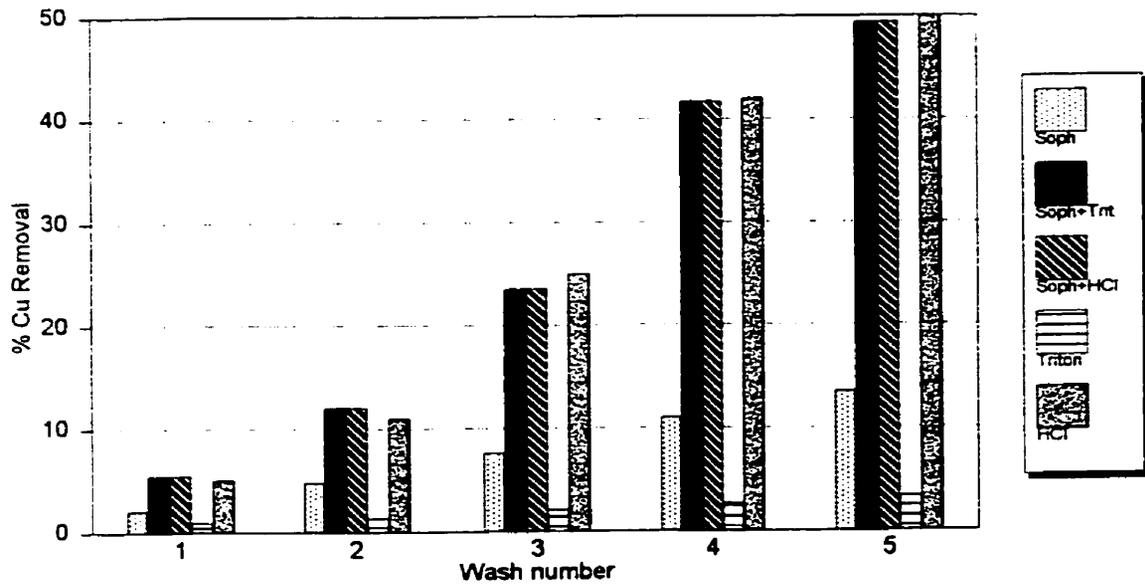


Figure 6.25 Accumulative copper removal from spiked THC soil after a series of five washings with sophorolipid. Sophorolipid (4%), sophorolipid (4%) and Triton X-100 (2%), sophorolipid (4%) with 0.7% HCl, 2% Triton X-100 and 0.7% HCl were evaluated.

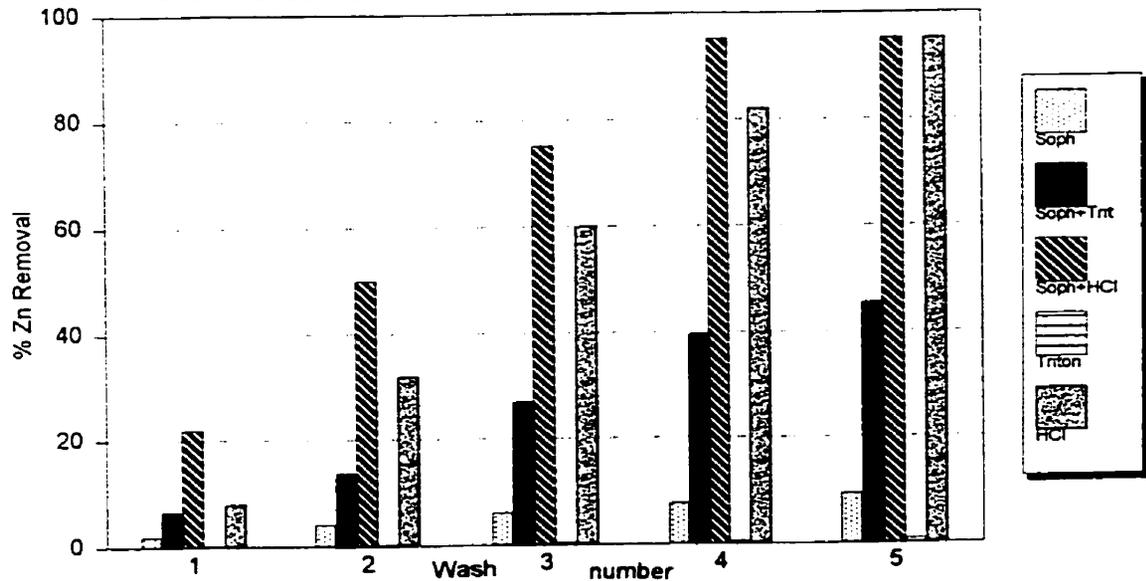
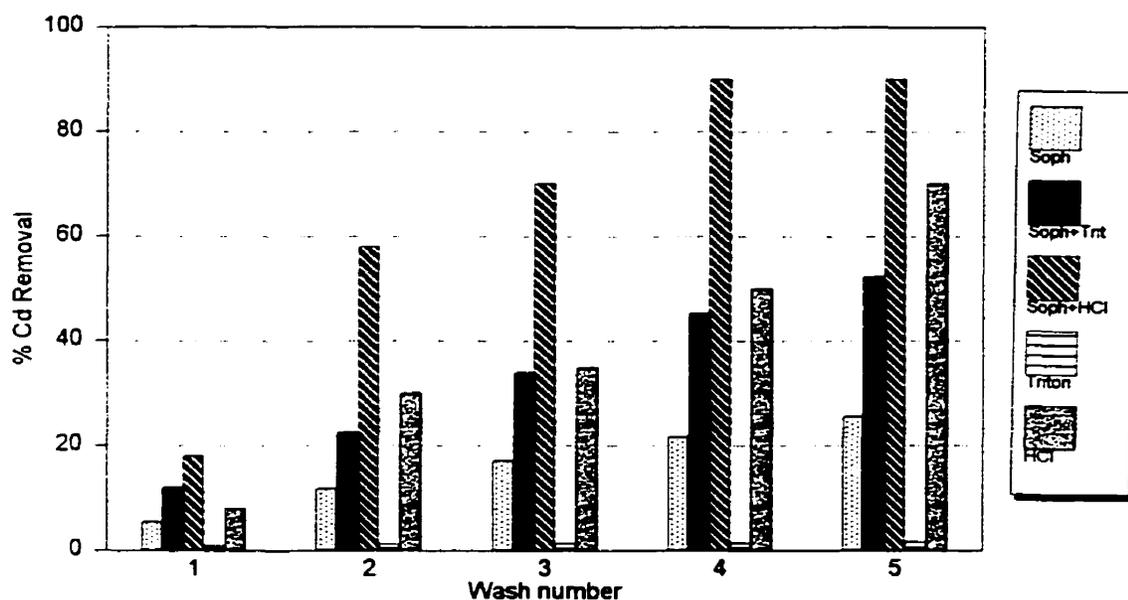


Figure 6.26 Accumulative zinc removal from spiked THC soil after a series of five washings with sophorolipid. Sophorolipid (4%), sophorolipid (4%) and Triton X-100 (2%), sophorolipid (4%) with 0.7% HCl, 2% Triton X-100 and 0.7% HCl were evaluated.



**Figure 6.27** Accumulative cadmium removal from spiked THC soil after a series of five washings with sophorolipid. Sophorolipid (4%), sophorolipid (4%) and Triton X-100 (2%), sophorolipid (4%) with 0.7% HCl, 2% Triton X-100 and 0.7% HCl were evaluated.

#### VI.5 Removal of oil during batch soil washing experiments

Initial analyses to determine oil removal during washing experiments were by COD determinations of the soil before and after washing. However, since sorption or precipitation of the surfactant onto the soil can contribute to the COD of the soil, correcting for the amounts of surfactin on the soil was necessary. By this method oil removal by surfactin washing was found to be 35% at 0.5% surfactin which was fairly close to the value of 30% by extracting with hexane (Table 6.4). This solvent was then adopted for all biosurfactant washings for oil determination since the three biosurfactants used in this study are not soluble in hexane and any precipitated or sorbed biosurfactant on the soil would not interfere with the analysis. The analysis of the untreated soil for TPH was determined to be 14%. This is only slightly different from the value of 12.6% by the traditional Soxhlet extraction method.

In general, the removal of oil by the surfactin increased as the concentration of surfactin increased. This is an indication that the oil is incorporated into the micelles since the concentration of surfactin is much higher than the CMC (0.02 g/L). The addition of NaOH was particularly beneficial at the lower surfactin concentrations for oil removal since there was still surfactin precipitation was minimized.

The rhamnolipid from *Pseudomonas aeruginosa* also showed the same trend of higher concentrations of rhamnolipid were more efficient in oil removal. There was a decrease in removal at 8 and 12% concentrations where the surfactant solutions became very viscous. Nonionic addition, Triton X-100 or T-MAZ 20 also enhanced removal.

Sophorolipid was also able to remove oil (Table 6.4). Unlike the rhamnolipid and surfactin, oil removal was shown by a floating dark layer of oil on top of the solution (photograph is shown in the Appendix A). The highest removal was shown with 4% sophorolipid. Since the sophorolipid reduces the interfacial tension to below 1 mN/m, it would be expected to be able to enhance oil removal.

Several experiments were also performed with multiple washings (Table 6.4). In all cases, surfactin, rhamnolipid and sophorolipid enhanced oil removal compared to the single washings at the same concentration.

Table 6.4 Oil removal from THC soil by biosurfactants and other additives

Biosurfactant (additive)	TPH removal (%)
<b>Single washing</b>	
Distilled water	25
4% surfactin	58
2% surfactin	46
1% surfactin	21
0.5% surfactin	23
4% surfactin (1% NaOH)	24
2% surfactin (1% NaOH)	46
1% surfactin (1% NaOH)	52
0.5% surfactin (1% NaOH)	46
0.25% surfactin (1% NaOH)	17
0.5% surfactin (0.5% T-MAZ 28)	70
0.5% TMAZ 28	60
12% rhamnolipid	84
8% rhamnolipid	89
4% rhamnolipid	100
2% rhamnolipid	70
2% rhamnolipid (2% Triton X-100)	91
1% rhamnolipid	40
0.5% rhamnolipid	43
0.5% rhamnolipid (1% NaOH)	46
0.5% rhamnolipid (0.5% T-MAZ 28)	90
0.25% rhamnolipid	44
4% sophorolipid	53
2% sophorolipid	31
2% sophorolipid (2% Triton X-100)	44
4% sophorolipid (4% Triton X-100)	13
4% sophorolipid (2% Triton X-100)	41
4% sophorolipid (1% Triton X-100)	49
4% sophorolipid (1% NaOH)	44
1% NaOH	30
<b>Multiple washings</b>	
Distilled water	28
1% NaOH	35
0.25% surfactin (1% NaOH)	46
0.1% rhamnolipid (1% NaOH)	56
4% sophorolipid	64
4% sophorolipid (2% Triton X-100)	61

## **VI.6 Summary and preliminary discussion**

Initial experiments with distilled water (Figure 6.1) indicated that additives were required since only minimal amounts of metals could be removed. Thus experiments were initiated with various additives (Figure 6.1).

Since heavy metal removal with surfactants has not been examined, this entire chapter contains data that has not been previously reported. Only surfactin has been previously shown to bind to various metals such as magnesium (Thimon et al., 1992). Miller (1996) wrote that rhamnolipid should be examined for its potential to remove metals from soils. Furthermore, it has never been shown that surfactants, whether synthetic or biological, are capable of removing both hydrocarbons and metal simultaneously.

It would be expected that the anionic and cationic surfactants would give the best results. The cationic metals would have an affinity for the negatively charged surfactants. The cationic surfactants, on the other hand, would act as cation exchange agents to displace the cations (Westall, 1992). However, the loss of these surfactants on to the soil would be quite high and thus not practical.

With the chemical surfactants, initial experiments showed that nonionic surfactants were not useful for metal removal but that the anionic and cationic surfactants showed some potential. Experiments with cationic surfactants were not continued since they would bind to the negatively-charged soils and losses would be high. Deoxycholic acid (a natural anionic surfactant) was investigated in some experiments (Figure 6.2) since it has been shown to assist metal removal in water (Huang et al., 1994). However, precipitation onto the soil was noted and studies were not continued.

Metal removal with surfactin was only significant when sodium hydroxide was added (Figure 6.3). Acid addition to the surfactin solution caused precipitation of the biosurfactant (Figure 6.4), making it unavailable for metal removal. This was expected since it has been shown by Cooper et al. (1986) and in this study (Table 6.1) that as the pH decreases below 5.5, surfactin precipitation occurs. The addition of the 1% sodium hydroxide ensures that the pH remains above 8. Therefore, no precipitation of the surfactin can occur.

It was unexpected, however, that surfactin would be more effective at 0.25% than at 2%. This is a reproducible phenomena, however. This experiment was repeated three times and the results varied by 5%. It was also shown that multiple washings of the soil and sediment were very effective and that copper removal was easier than either zinc or cadmium. In addition, the results with surfactin of higher purity (80% compared to 62%) showed that surfactin was responsible for the metal removal and not the contaminants (Figures 6.7 to 6.9). In fact, some of the contaminants in the surfactin itself might be interfering with the metal removal.

The results with the rhamnolipid were similar to surfactin (Figures 6.11 and 6.12). Acid addition caused precipitation of the rhamnolipid by decreasing pH to below 6. This phenomenon decreased metal removal efficiencies. Sodium hydroxide addition and multiple washings, however, increased copper removal significantly. Metal removal by rhamnolipids with no sodium hydroxide addition was more effective than for surfactin since the rhamnolipid is soluble at pH 6 to 6.5 compared to a pH over 8 for surfactin. Zinc and cadmium removal was significant with no addition of sodium hydroxide (Figure 6.13) which could be expected since zinc and cadmium become more mobile below pH 7 as discussed in Sections II.1.1 and II.1.4 (Cameron, 1992). Also unlike surfactin, higher concentrations of rhamnolipid showed higher metal removal

rates than lower concentrations (Figure 6.10).

The sophorolipid behaves differently than surfactin and rhamnolipid since it does not precipitate at low pH values (Copper and Paddock, 1984). Thus, acid addition with this surfactin was possible and was beneficial for lead, zinc and cadmium removal (Figure 6.21). When acid was added to the sophorolipid, the pH ranged from 5.4 to 6.5 (Table 6.3). This pH is slightly lower than for the rhamnolipid and is more suitable for metal removal such as cadmium which is highly mobile in the range between pH 4.5 and 5.5 (section II.1.1, Cameron, 1992). However, since the pH for the sophorolipid is in the same pH range and for the acid alone, the surfactant must be contributing in another manner (e.g., due to its surface active properties).

Addition of the nonionic surfactant (Triton X-100) increased the solubility of this surfactant and enhanced metal removal significantly, particularly after multiple washings (Figures 6.25 to 6.27). The pH of the sophorolipid/Triton X-100 mixture was close to pH 7, which is not supposed to be the ideal range for metal removal. Other mechanisms must be enhancing the metal removal in this case. The addition of Triton X-100 alone was clearly ineffective for metal removal so its only benefit was in enhancing the solubility of the sophorolipid which no longer floated on the surface of the washing solution.

All soil washing experiments were duplicated and the results were reproducible within 10%. Full confidence can therefore be placed in the results regarding all trends that were noted and discussed previously. This is due to the fact that the all efforts were employed to use as uniform soil and sediments samples as possible. All samples were obtained from the same container which was well mixed prior to use and presieved to remove large particles.

All three biosurfactants were capable of removing hydrocarbons and metals simultaneously as shown in Sections VI.2, VI.3 and VI.4 and VI.5. Oil removal has only been previously shown for the rhamnolipids (Harvey et al., 1990; Scheibenbogen et al., 1984 and others as discussed in II.2.2). Experiments were performed with samples which were contaminated previously. This leads to contamination that is more difficult to remove since the contaminants have been there for a long time and any that are easy to remove would have been removed already (eg., by rain or volatilization). Therefore, these compounds would be useful for remediating soils and sediments with mixed contamination which is a significant problem as shown in section I.1.

However, why certain conditions are more favourable than others is not known at this point. For example, why does the surfactant with sodium hydroxide addition assist copper removal. Why does acid addition with the surfactant assist removal of zinc and cadmium? Why are the multiple washings effective? What is the mechanism for metal removal by the surfactants? Is it similar to the one for hydrocarbon removal as discussed in section II.4.2?

Surfactants have been shown to desorb hydrocarbons from soil organic matter and to enhance hydrocarbon partitioning into the polar phase (Abdul et al., 1990) and as summarized in Table 2.10 in the literature review. They are also useful in enhancing the solubility of hydrocarbons (Fountain et al., 1991).

Alkalis such as sodium hydroxide and sodium carbonate have been used in *in situ* soil flushing for a number of reasons. They include formation of natural surfactants by reaction with the NAPL, alteration of the water salinity, and reduction of surfactant adsorption among others (Schmelling, 1994). The acidic components such as carboxylic acids, phenolics and asphaltene can hydrolyse by alkalis to form surfactants (de Zabala et al., 1982). These formed

surfactants are also negatively charged (de Zabala, et al., 1982) and thus must also be responsible for metal removal as shown earlier. In fact, a 1% NaOH solution was contacted with the oil isolated from the contaminated soil, a whitish solution is formed below the oil layer. When this was separated from the oil, the surface tension was measured and determined to be 34.5 mN/m, indicating the formation of surfactants.

The nonionic surfactant, T-MAZ 28, also minimizes surfactin precipitation and enhances oil removal. Some preliminary work has shown that precipitation and sorption can be reduced by combining nonionic with anionic surfactants (Yuan and Jafvert, 1995). This is the first time, though, that oil removal by surfactin has been shown. Other lipopeptides such as that produced by *Bacillus licheniformis* JF-2 have been isolated from oil field injection water and can potentially be useful for enhanced oil recovery (Javaheri et al., 1985).

Since interfacial tensions of rhamnolipids were found to be below 1 mN/m and 0.25 mN/m in others (Guerra-Santos et al., 1986 and Robert et al., 1989), it is expected that the rhamnolipid would be able to remove oil. The microbial surfactant (0.1%) was able to remove 2 to 3 times more oil from the gravel in Alaska after the Valdez spill (Harvey et al., 1990). Although other rhamnolipid solubilization studies have been performed, they have concentrated on model compounds such as hexadecane and octadecane (Zhang and Miller, 1995) or an aliphatic and aromatic hydrocarbon mixture (Scheibenbogen et al., 1994) which are not naturally contaminated soils.

Previous work on oil removal by sophorolipid is not extensively documented. Prior to this work, the only other known report (Cooper and Paddock, 1984) of oil removal by the sophorolipid showed that 1 g/L of sophorolipid could remove 8 times more bitumen from a tar sands sample than water alone.

## **VII SEQUENTIAL EXTRACTION EXPERIMENTS**

### **VII.1 Sequential extraction of soil, spiked soil and sediments**

Sequential extractions were then performed on the THC soil (pH 7.0), spiked THC soil (pH 7.0) and the sediment (pH 6.5) without washing or pH adjustment prior to the procedure. As can be seen from Figure 7.1 for the THC soil, the exchangeable fractions of copper, zinc and lead were very low. The carbonate fraction was significant only for zinc, whereas the oxide fractions accounted for over 50 and 70% of the lead and zinc present in the soil, respectively. The organic fraction constituted over 70% of the copper. Residual amounts made up about 20% of the lead and copper and approximately 10% of the zinc.

In the spiked THC soil (Figure 7.2), exchangeable lead and cadmium are present. The carbonate fraction increased slightly for copper and zinc. More than 30% of the cadmium was present in the carbonate form while the remaining amount was mostly in the oxide form. Less than 10% of the cadmium was in the organic phase and insignificant amounts (less than 1%) in the residual.

In the sediment (Figure 7.3), copper was found mainly in the organic fraction. For zinc, approximately 5% was exchangeable, 20% was carbonate, 20% organic and 5% residual with the largest amount being in the oxide fraction. Lead was similar to zinc with smaller exchangeable and carbonate fractions.

The results are summarized in Table 7.1. Despite the fact, that the sediment and the soil are quite different in composition and that the organic content of the THC soil is in the form of oil whereas the sediment is decomposed organic matter, the affinities for the various fractions are very similar.

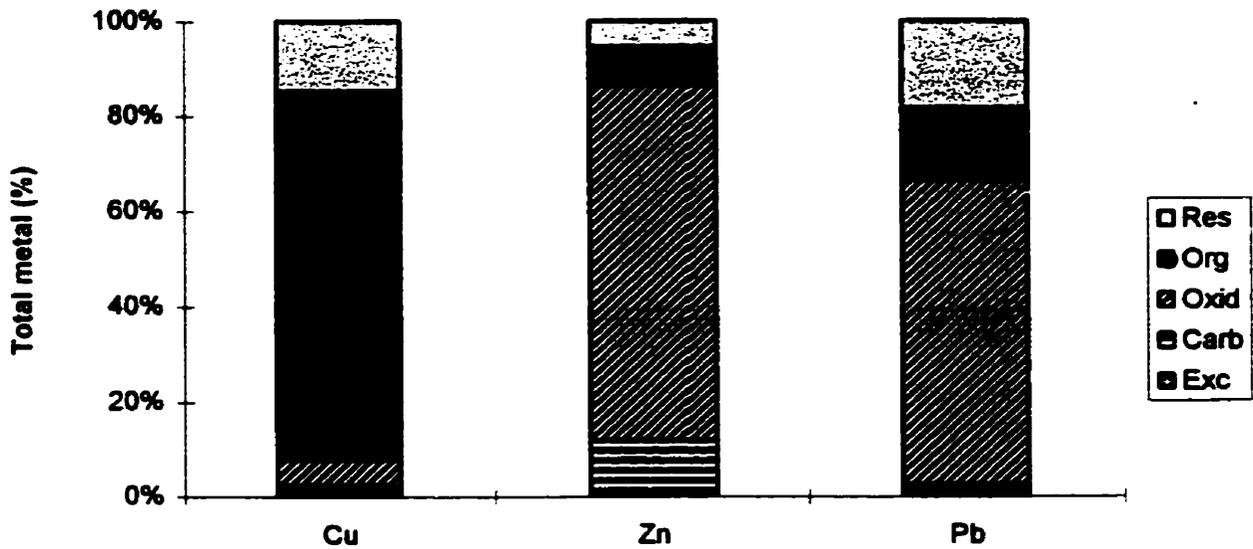


Figure 7.1 Sequential extraction of THC soil. The following are represented: Exc stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.

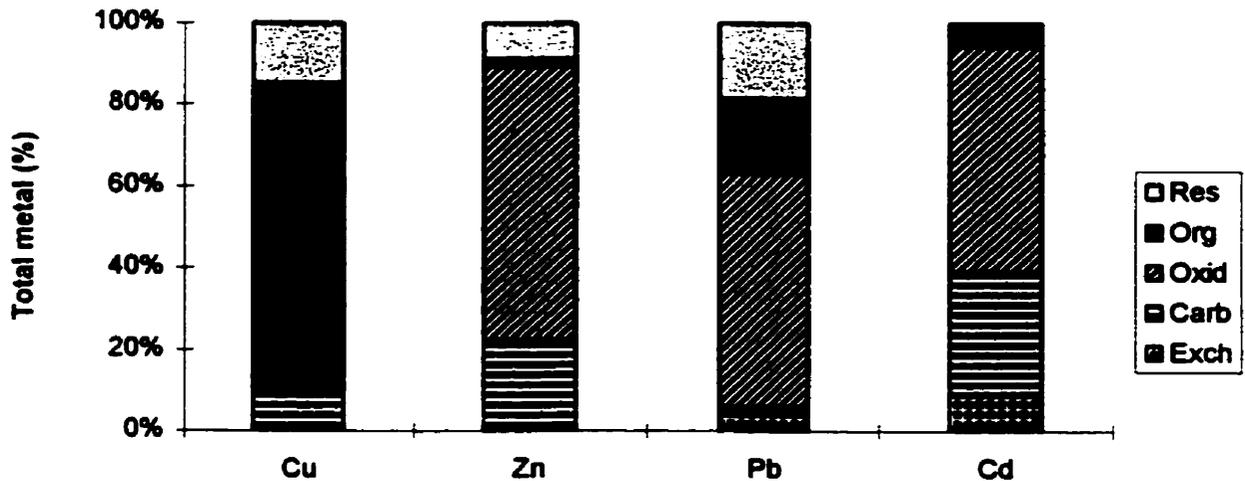


Figure 7.2 Sequential extraction of spiked THC soil. The following are represented: Exc stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.

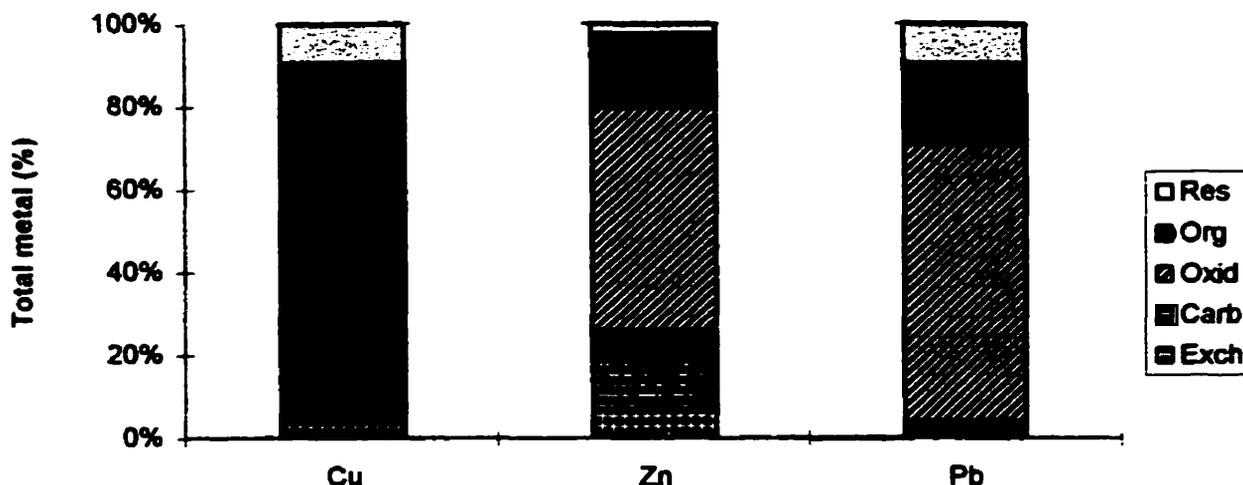


Figure 7.3 Sequential extraction of sediments. The following are represented: Exc stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.

Table 7.1 Heavy metal affinity for various fractions in the THC soil, spiked THC soil and sediments (pH of each matrix was not adjusted before SSE)

Heavy metal	Affinity for each fraction
<b>THC soil</b> Copper Zinc Lead	Organic > residual > oxide > carbonate > exchangeable Oxide > carbonate > organic > residual > exchangeable Oxide > residual > organic > carbonate > exchangeable
<b>THC spiked</b> Copper Zinc Lead Cadmium	Organic > residual > oxide > carbonate > exchangeable Oxide > carbonate > residual > organic > exchangeable Oxide > residual > organic > exchangeable > carbonate Oxide > carbonate > exchangeable > organic > residual
<b>Sediment</b> Copper Zinc Lead	Organic > residual > oxide > carbonate > exchangeable Oxide > carbonate > organic > exchangeable > residual Oxide > organic > residual > exchangeable > carbonate

## **VII.2 Sequential extraction in combination with surfactant washing**

Sequential extraction experiments were performed on the soil and sediment residues following soil washing of selected samples to determine which fractions were removed by the surfactants and other additives. The pH of the soil or sediment was not adjusted prior to sequential extraction. The fraction removed by the surfactant or control is designated as the soluble fraction. Soil or sediment residues from several soil washing experiments that showed significant metal removal results were chosen for study.

### **VII.2.1 Surfactin**

Experiments that were performed with the spiked THC soil and the sediments were used for sequential extraction after washing with surfactin (Figures 6.5 to 6.9). Figure 7.4 shows the sequential extraction of copper with 2% surfactin with 1% NaOH after one washing and for the 0.25% surfactin with 1% NaOH after one, three and five washes. The controls (1% NaOH) are also shown after one and five washes. The oxide, carbonate and exchangeable fractions were fairly consistent until the fifth washing where the oxide fraction was finally removed. The organic fraction, however, decreases with increasing copper removal. For zinc (Figure 7.5), the carbonate and organic fractions decreased insignificantly. Removal was mainly from the oxide fraction. Since cadmium removal by 1% NaOH was minimal, the sequential extraction showed similar characteristics to the untreated soil (Figure 7.6). Upon the addition of one wash of surfactin (0.25% with NaOH), some removal occurred in the exchangeable fraction. Further washings indicated removal from the carbonate fraction. The others (organic and oxide fractions) did not change appreciably. The wash with 2% surfactin showed mainly removal from the exchangeable fraction.

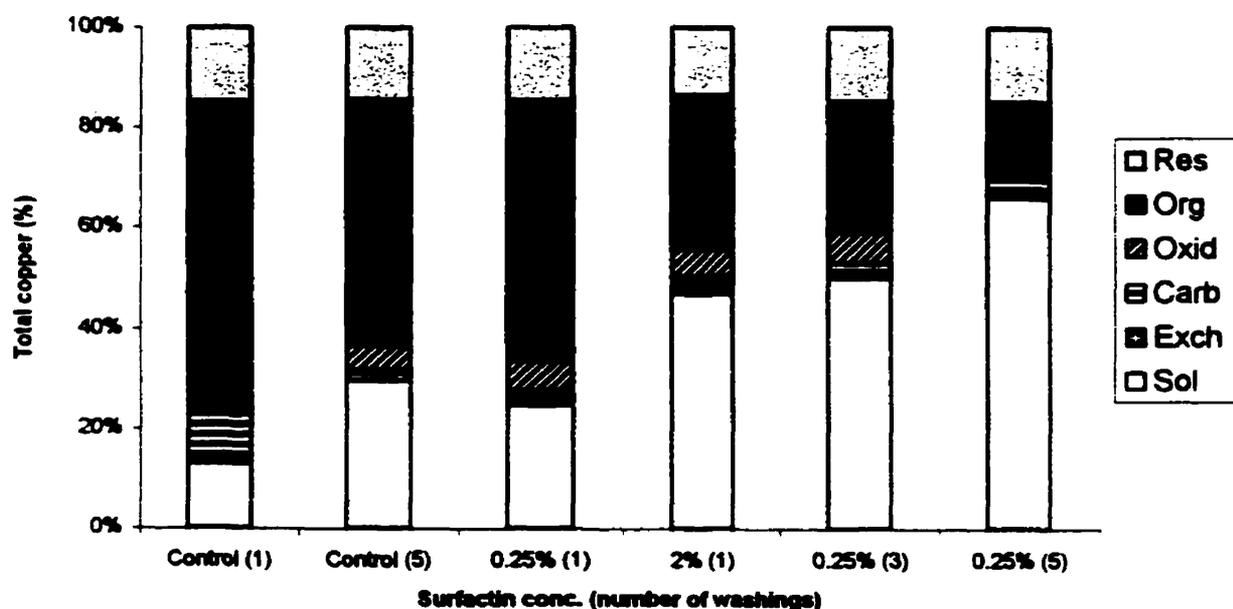


Figure 7.4 Sequential extraction of copper after surfactin washing of THC spiked soil. The following are represented: Sol for the soluble fraction, Exc stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.

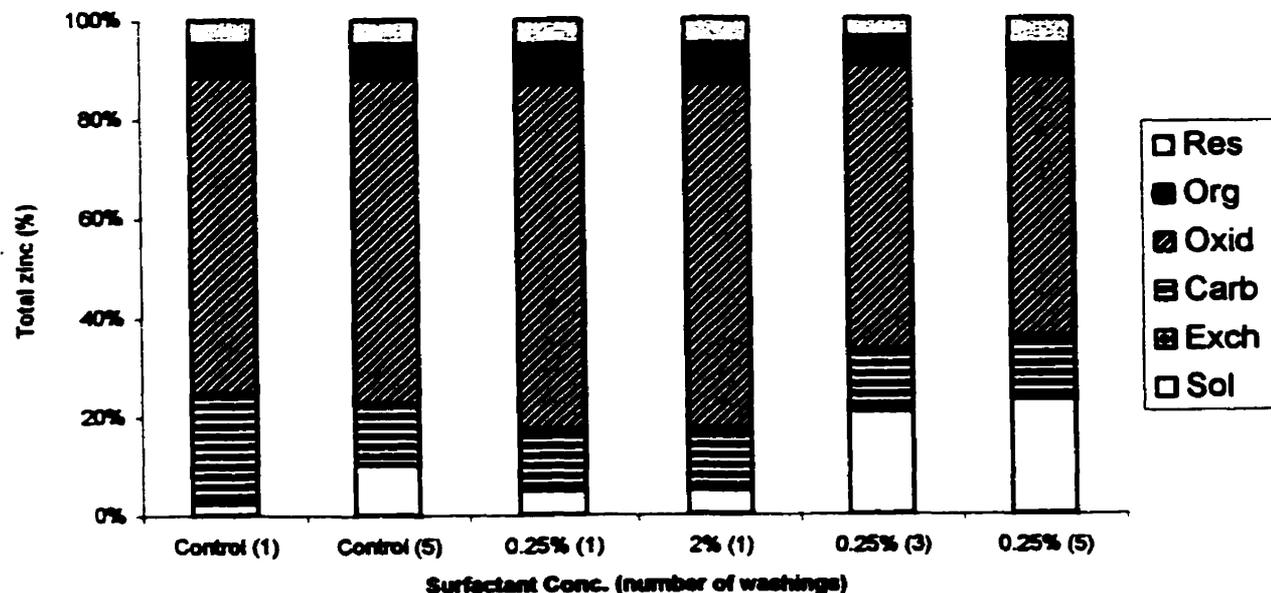
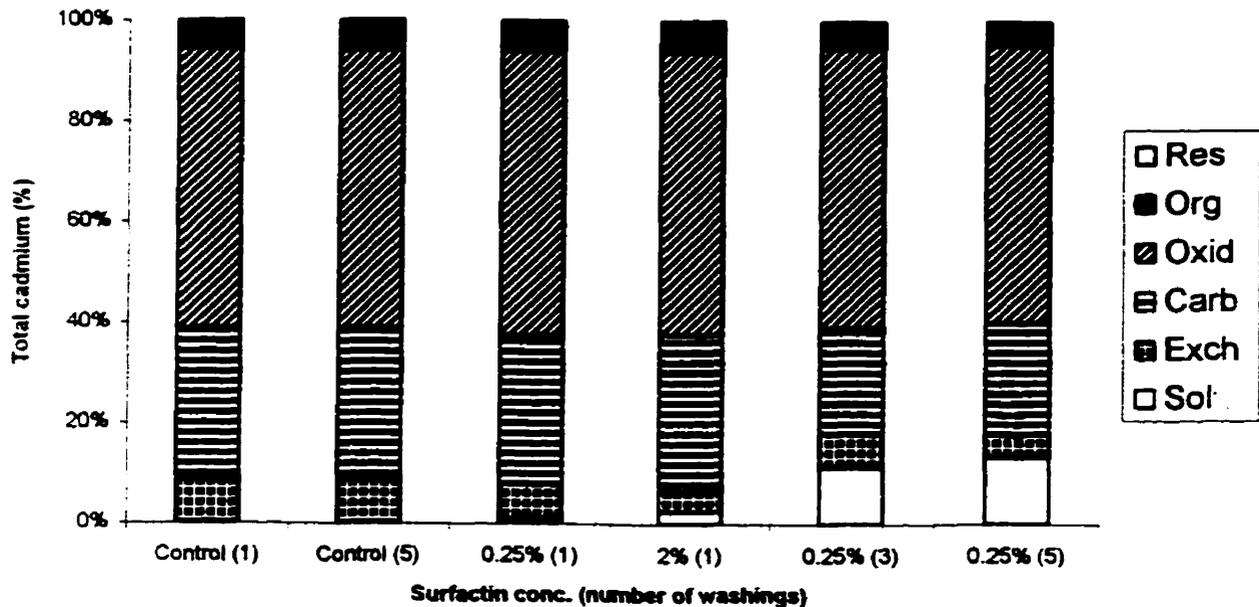


Figure 7.5 Sequential extraction of zinc after surfactin washing of THC spiked soil. The following are represented: Sol for the soluble fraction, Exc stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.



**Figure 7.6** Sequential extraction of cadmium after surfactin washing using THC spiked soil. The following are represented: Sol for the soluble fraction, Exc stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.

### VII.2.2 Rhamnolipid

Other sequential extractions were performed with the residue after a single washing with 4% rhamnolipid (with and without 1% NaOH) and multiple washings with 0.1% rhamnolipid (with NaOH) using the spiked soil (Figures 6.13 to 6.17). Figure 7.7 shows the removal extraction of copper from the various fractions. The combination of 4% rhamnolipid decreased the copper in the organic phase more than the rhamnolipid or NaOH alone. There was also some reduction in the carbonate fraction. After washing five times with the NaOH alone or 0.1% rhamnolipid with NaOH, there was a substantial reduction in the copper in the organic fraction. In the presence of the biosurfactant, additional removal was seen in the carbonate and oxide fractions.

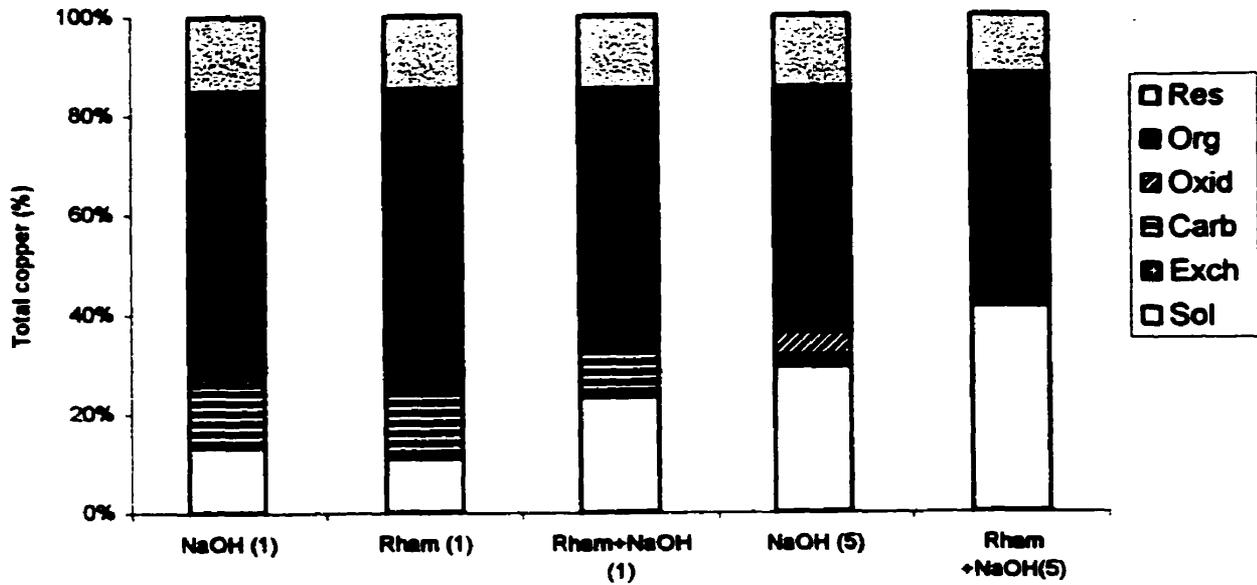


Figure 7.7 Sequential extraction of copper from spiked THC soil after rhamnolipid washing. The following are represented: Sol for the soluble fraction, Exc stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.

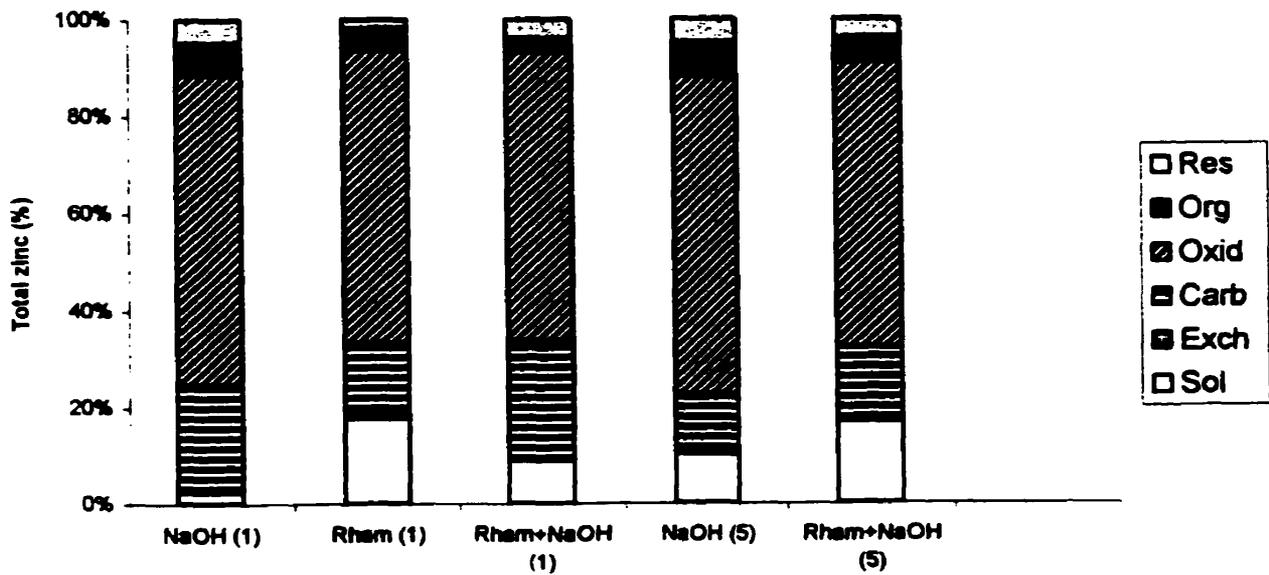


Figure 7.8 Sequential extraction of THC spiked soil for zinc after rhamnolipid washing. The following are represented: Sol for the soluble fraction, Exc stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.

For zinc (Figure 7.8), significant solubilization for the single washing with 4% rhamnolipid and rhamnolipid with NaOH. Some reduction occurred for the zinc in the organic fraction with rhamnolipid and NaOH. The oxide fraction decreased in all cases. Reduction of the carbonate fraction was also noted for the 0.1% rhamnolipid with NaOH and the NaOH alone after the five washings. Removals of the various cadmium fractions with rhamnolipids and copper with the sophorolipid were not examined since washing with these surfactants did not give significant results.

### **VII.2.3 Sophorolipid**

Figure 7.9 shows the results of the sequential extraction after one and five washings with 0.7% HCl or 4% sophorolipid with 0.7% HCl (Figures 6.23 to 6.27). For the single wash, the addition of the sophorolipid removed more zinc from the carbonate fraction than the acid. The oxide fraction decreased by the same amount in both cases from the initial 70% to 60%. Whereas washing five times with the acid decreased the carbonate and oxide fractions, the combination of the surfactant and the acid almost completely eliminated both of these fractions.

Figure 7.10 shows the sequential extraction of cadmium using the same combinations as Figure 7.9. The single wash of acid alone substantially decreased the exchangeable and the oxide fractions. Five washes with the acid decreased mainly the carbonate and some of the oxide. The single wash with the sophorolipid decreased the oxide fraction first and the exchangeable and carbonate. The results indicated that the removal by the sophorolipid/acid was mainly from oxide with small amounts from the organic fraction.

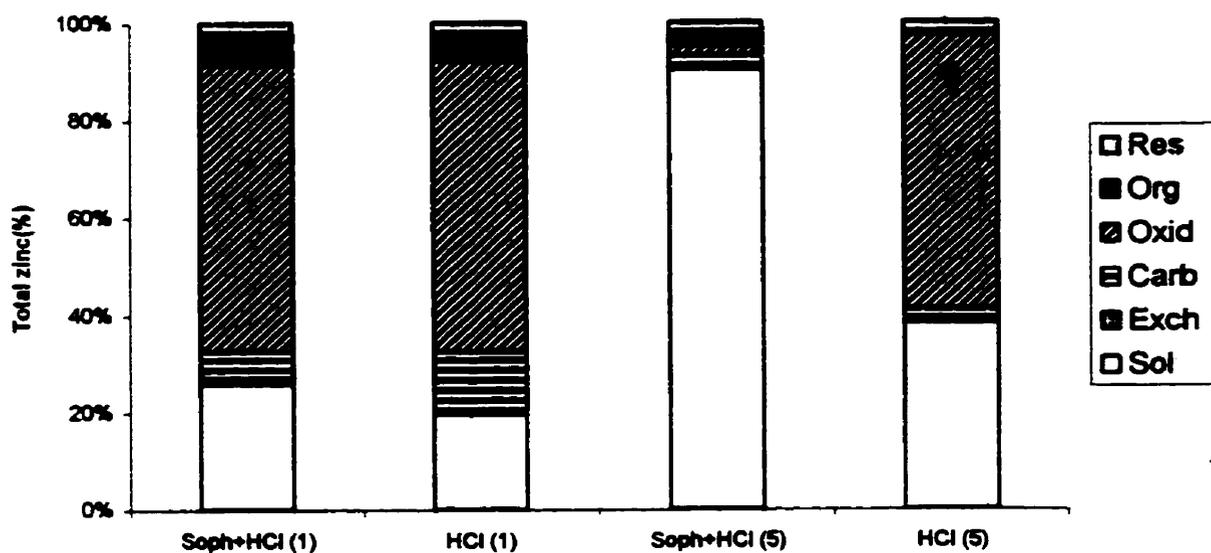


Figure 7.9 Sequential extraction of THC spiked soil for zinc after sophorolipid washing. The following are represented: Sol for the soluble fraction Exch stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.

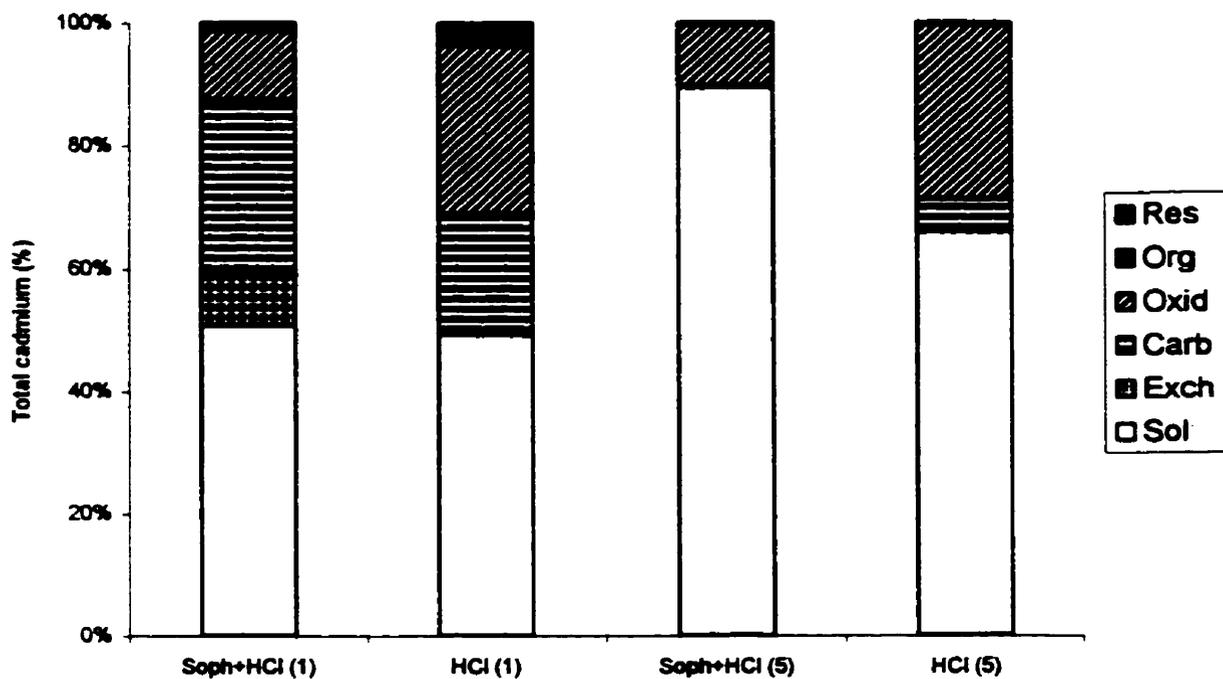
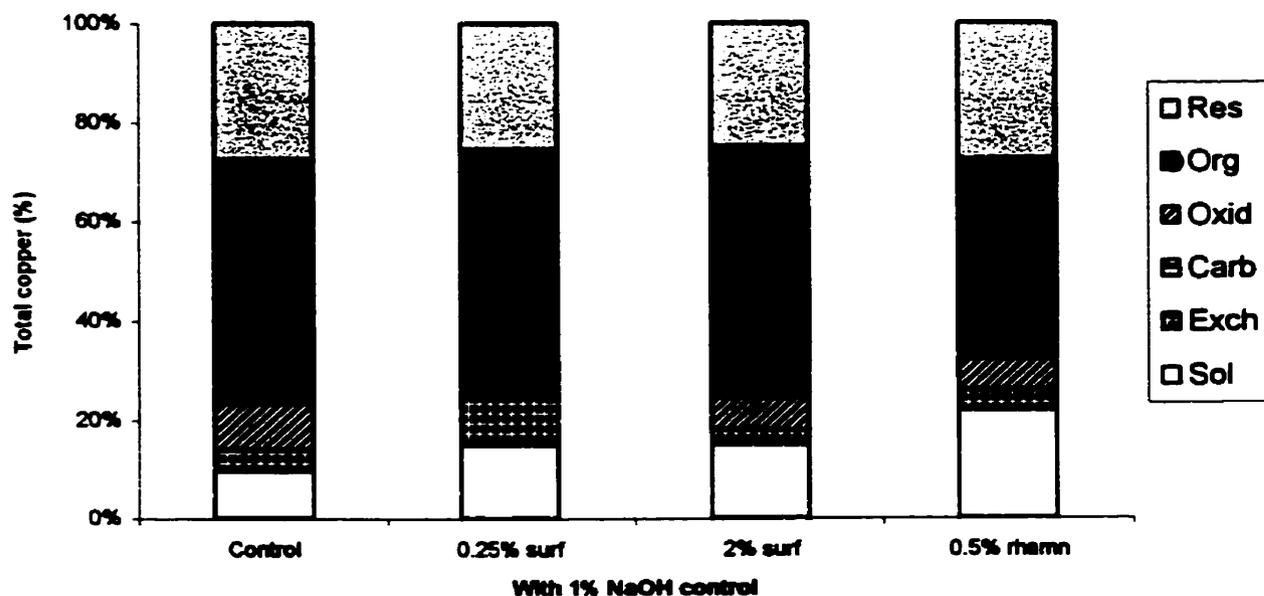


Figure 7.10 Sequential extraction of cadmium after sophorolipid washing of THC spiked soil. The following are represented: Sol for the soluble fraction, Exch stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.

A few experiments were also run with the sediment to examine washing of the sediments with the three biosurfactants. For the experiments with 1% NaOH (Figure 7.11), all showed a decrease in the copper in the organic fraction while the carbonate and oxide fractions were not significantly different. In Figure 7.12, without the addition of NaOH, only 0.5% rhamnolipid showed significant removal which was from the organic phase.

For zinc, the experiments with 1% NaOH (Figure 7.13) showed a decrease in the oxide phase after washing with 0.25% surfactin while there was little difference with the control for the 2% surfactin. The rhamnolipid (0.5%) soil residue showed lower oxide and carbonate contents. Using the surfactants with water (Figure 7.14), decreases of zinc in the carbonate fraction were noted for the 0.5% rhamnolipid and 2% surfactin. The sophorolipid (4%) did not show significantly different results from the control.



**Figure 7.11** Sequential extraction of copper after washing of the sediments with the two biosurfactants. The following are represented: Sol for the soluble fraction, Exc stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.

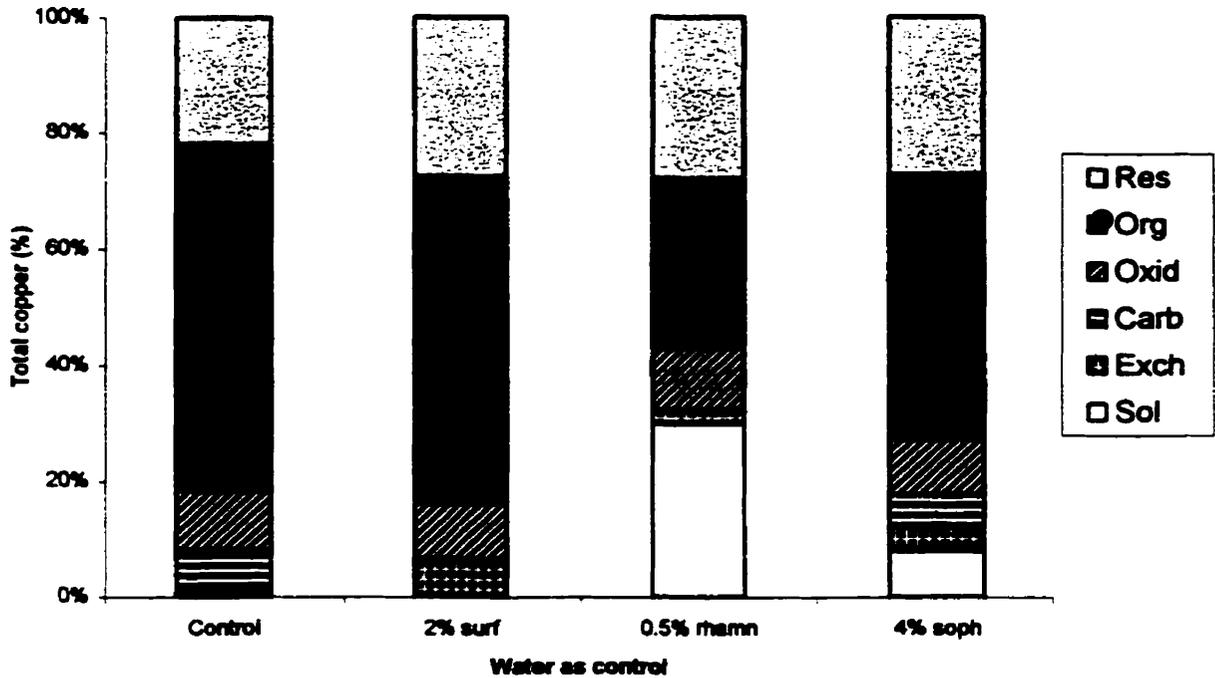


Figure 7.12 Sequential extraction of copper after washing of the sediments with the three biosurfactants. The following are represented: Sol for the soluble fraction, Exc stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.

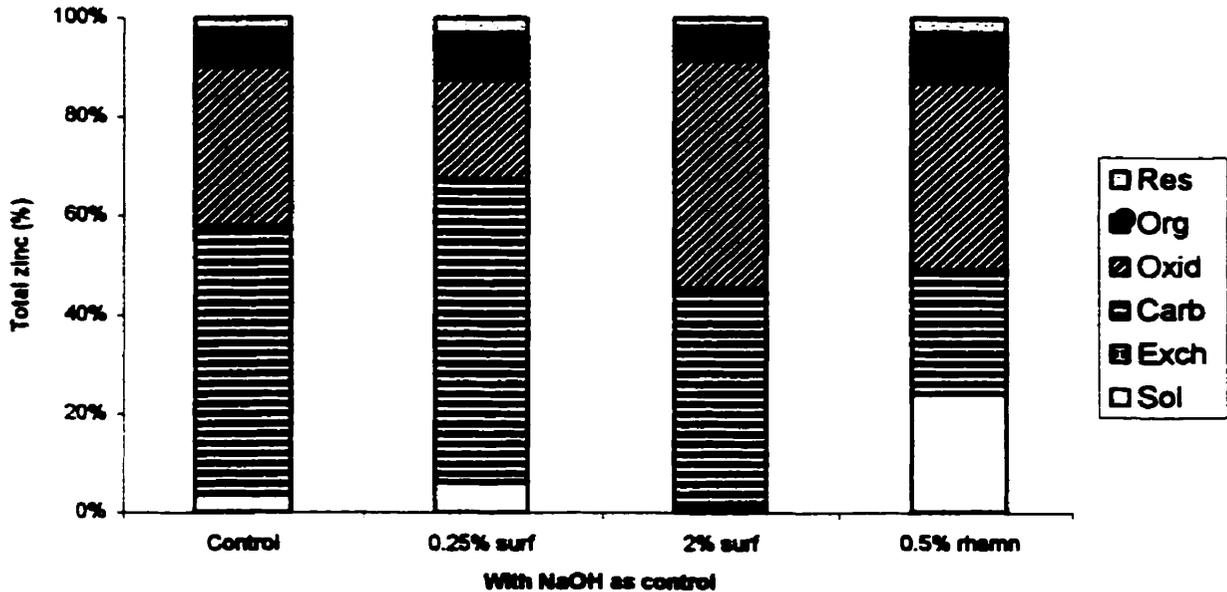
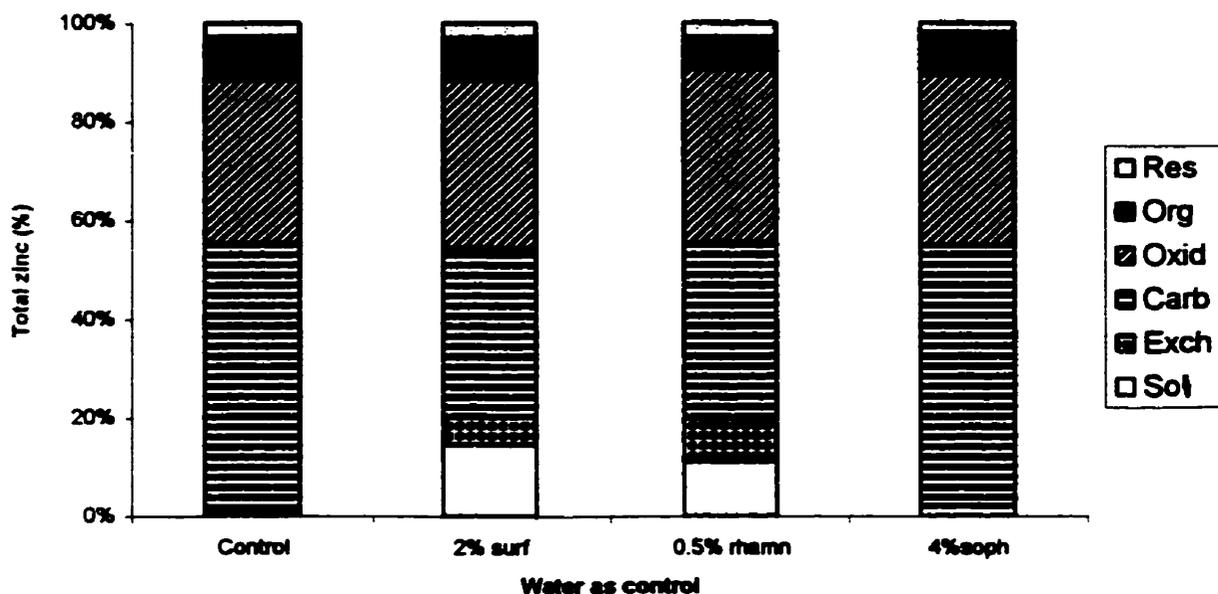


Figure 7.13 Sequential extraction of zinc after washing of the sediments with the two biosurfactants. The following are represented: Sol for the soluble fraction, Exc stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, Org for organic fraction and Res for residual fraction.



**Figure 7.14** Sequential extraction of zinc after washing of the sediments with the three biosurfactants. The following are represented: Sol for the soluble fraction, Exc stands for exchangeable, Carb for carbonate fraction, Oxid for oxide fraction, org for organic fraction and Res for residual fraction.

### VII.3 Summary and preliminary discussion

The purpose of the sequential extraction studies was to determine where the metals are present in the soil and sediments among the exchangeable, oxide, carbonate, organic and residual fractions. The harsher the chemicals required, the more difficult it is to remove the metals.

Few studies have been performed on the sequential extraction of soils and sediments contaminated with copper, zinc, lead and zinc. Cesium, cobalt and uranium fractions were examined by Gombert (1994). Others such as the study by Yong et al. (1993) have determined the distribution of zinc, lead and copper in kaolinite, illite, montmorillonite and clay soils which have organic contents of less than 2%. Despite the high organic contents (greater than 10%) of the soil and sediment used in this study comparisons can be made

with illite and natural clay soil (pH 7) for zinc, lead and cadmium since the organic phase does not play a large role in the retention of these metals. For both the THC soil and the sediment, the results for zinc, were similar, indicating, preference for the oxide phase followed by the carbonate. Both the natural clay and the illite, showed equal contributions of the oxide and carbonate fractions like the spiked soil for cadmium. The exchangeable fraction, however, was higher. For lead, a significant carbonate fraction was indicated for the illite and natural clay. In this study, the carbonate content of both the sediments and the soil was much lower (5 and 0.5%, respectively) compared to the 15 and 10% for the illite and natural clay soil.

In a study by Ramos et al. (1994), copper, lead, cadmium and zinc were sequentially extracted from soils with organic contents of approximately 15%. Similar to this research, they found that copper was associated with the organic matter or residual fractions, lead and zinc were primarily found in the oxide fraction. Cadmium, however, was found mainly in the exchangeable and carbonate fractions. Contrary to our findings however, they determined that a significant amount of cadmium was present in the residual fraction. This was most likely due to the fact that their soil was contaminated for a much longer period of time compared to the three day spiking procedure used in this study.

The purpose of the sequential extraction studies on soils that have been previously washed with the surfactants was to determine from what fraction these surfactants are removing the metals. This information can then be used to determine if soil washing is useful and if surfactant use is potentially effective and what surfactants might be useful and under what conditions. For example, if the metals are retained mainly in the residual fraction, it may be very difficult, if not impossible to use any of these surfactants to remove these metals.

Few attempts have been made to correlate sequential extraction results with soil washing results. Gombert II (1994) examined the sequential extraction of cesium, cobalt and chromium to determine if soil washing could be used to treat the soil. In this study, it was determined that any fraction other than the residual could be removed by chemically enhanced soil washing. Therefore, cesium could not be removed since it occurred mainly in the residual phase (80%). However, no detail is given for what types of additives can be used for effective soil washing from the different phases.

In this research project, only the residual was untouched during soil washing procedures. All fractions under different conditions could be decreased. For example, under acidic conditions, the oxide phase containing the zinc or cadmium would be released. However, under basic conditions (co-addition of the surfactants with NaOH) copper could be released since it was found mainly in the organic phase.

Another study by Li et al., (1995) examined the use of the sequential extraction procedure with EDTA extraction of metals. They concluded that the organic phase was very stable since metals in this phase could not be removed by the EDTA and should not be considered in soil washing processes since it would be uneconomical to treat. As seen here however, the organic phase associated metals can be easily removed using the appropriate conditions.

In addition, these results show that the organic phase-associated metals can be removed by either the surfactin or rhamnolipid with sodium hydroxide. Acidic conditions with sophorolipid addition were effective for removing the zinc and cadmium in the oxide and carbonate phases. Multiple washings are effective since they can remove the easier to remove phases first and then the more difficult (such as the oxide and then carbonate). Residual fractions, the

most difficult to remove, were not affected during the surfactant washing studies. This information is important in designing the appropriate conditions for soil washing.

## VIII STUDIES TO DETERMINE THE METAL REMOVAL MECHANISMS

### VIII.1 Octanol-water partitioning experiments

Octanol-water partitioning experiments were performed for the three biosurfactants to determine if the surfactant could transfer the metals from the aqueous to the octanol phase. Solutions of lead,  $(\text{PbNO}_3)_2$ , copper,  $(\text{CuSO}_4)_2$ , zinc,  $\text{Zn}(\text{SO}_4)_2$ , and cadmium,  $\text{CdCl}_2$  were studied using different concentrations of surfactant.

The behaviour of surfactin (pH 8) is shown in Figure 8.1. Even at low concentrations (above 0.12% surfactin), the  $K_{ow}$  for cadmium, copper, and lead were over 150. The  $K_{ow}$  for zinc was above 150 for a concentration of 0.5% only. However, upon the addition of sodium hydroxide (pH 10), the coefficient values decreased substantially at a surfactin concentration of 0.5% while the difference was not as significant at 2% surfactin. There was only a slight difference between 2% surfactin at pH 10 (with 1% NaOH) and pH 6.2 where zinc and copper were higher.

The  $K_{ow}$  values for rhamnolipid are shown in Figure 8.2. As in the case of surfactin, lower concentrations of rhamnolipid (0.12 and 0.25%) showed higher values (pH 6.5). At the higher concentrations of rhamnolipid, emulsions formed which decreased the ability of the surfactant to transfer the metals from the water to the octanol phase. The addition of 1% NaOH (pH 10) also decreased values significantly for 0.12% rhamnolipid. The difference was less when 0.5% rhamnolipid was used.

More lead and copper were transferred to the octanol phase by the control than any of the sophorolipid combinations tried as shown by the  $K_{ow}$  values in Figure 8.3. Zinc and cadmium were transferred at higher levels than the control but did not vary according to the sophorolipid concentrations tested.

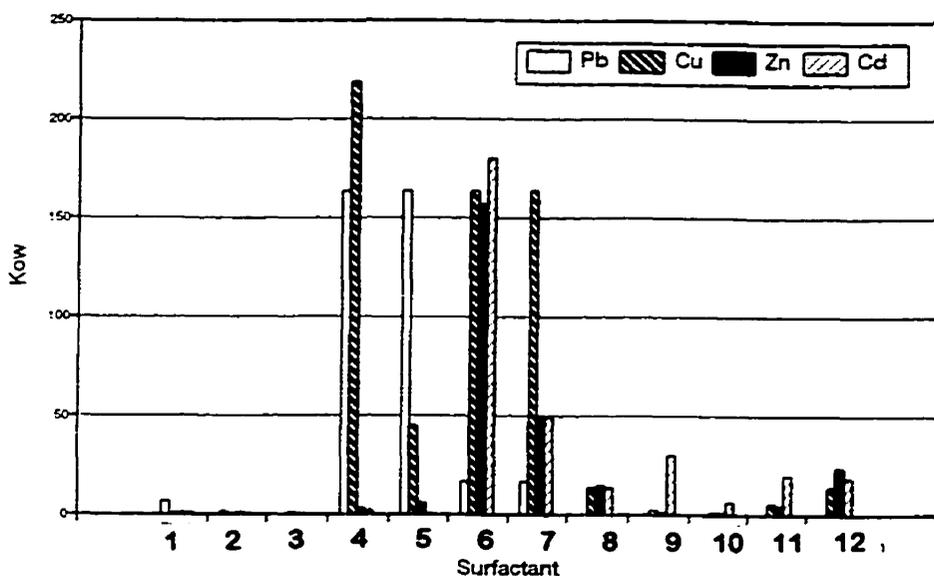


Figure 8.1  $K_{ow}$  values for surfactin. Concentrations are 1: Blank (pH 8); 2: 0.001%; 3: 0.01%; 4: 0.12%; 5: 0.25%; 6: 0.5%; 7: 1%; 8: 2% (pH 8). Other tests included, 9: 1% NaOH alone (pH 10); 10: 0.5% surfactin+1% NaOH; 11: 2% surfactin +1% NaOH (pH 10); 12: 2% surfactin (pH 6.2).

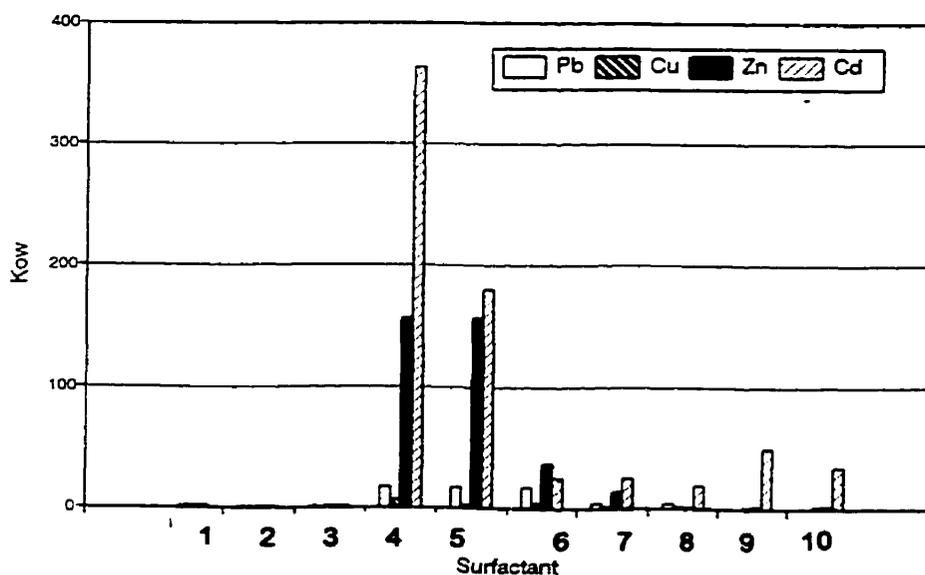


Figure 8.2  $K_{ow}$  values for rhamnolipid. Concentrations are, 1: Blank (pH 6.5); 2: 0.001%; 3: 0.01%; 4: 0.12%; 5: 0.25%; 6: 0.5% and 7: 1% (pH 6.5). Other tests included, 8: 1% NaOH alone (pH 10); 9: 0.12% rhamn+1%NaOH; 10: 0.5% rhamn with 1%NaOH (pH 10).

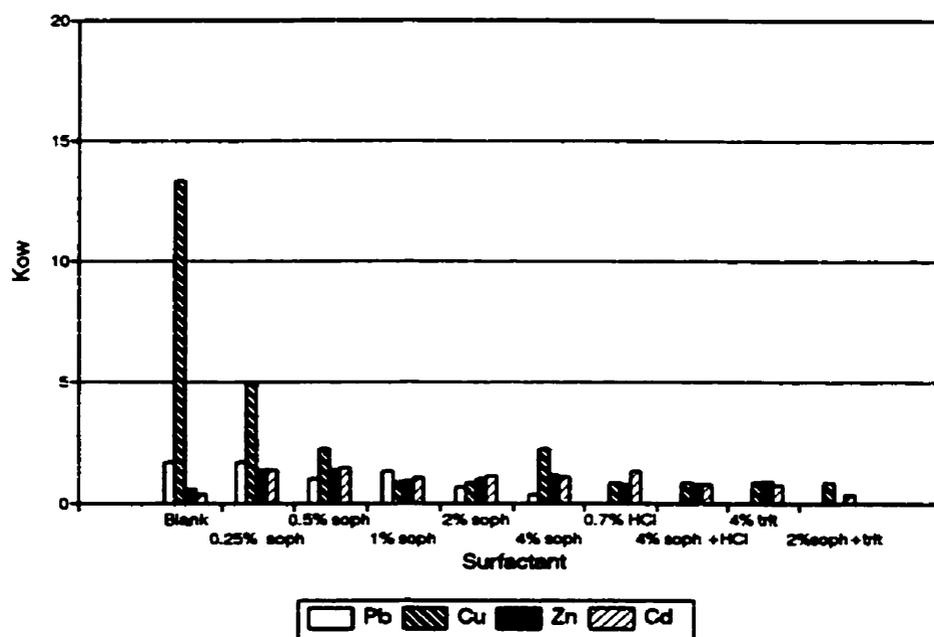


Figure 8.3  $K_{ow}$  values for sophorolipid for concentrations of 0.25% to 4%. 0.7% HCl, 4% sophorolipid with 0.7% HCl, 4% Triton X-100 and 2% sophorolipid with 2% Triton X-100 were evaluated (shown as trit).

### VIII.2 Ultrafiltration experiments

To examine if the surfactant micelles were solubilizing the metals or if the surfactant monomers were attaching to the metals, experiments were performed with ultrafiltration membranes. The membranes used were chosen based on the molecular weight cutoffs suitable for retention of the surfactant micelles (10,000 for the rhamnolipid and 50,000 for surfactin, Mulligan and Gibbs, 1990). If the metals are associated with the micelles they will be retained by the membranes. If they are attached to the surfactant monomer or are unattached they will pass through since the molecular weights of the surfactants and metals are much lower than 10,000. Since the sophorolipid forms an oily layer, it was not studied. Solutions of metals were mixed with the surfactant solutions at various concentrations near and above the critical micelle concentrations (CMC) which is the lowest concentration that micelles

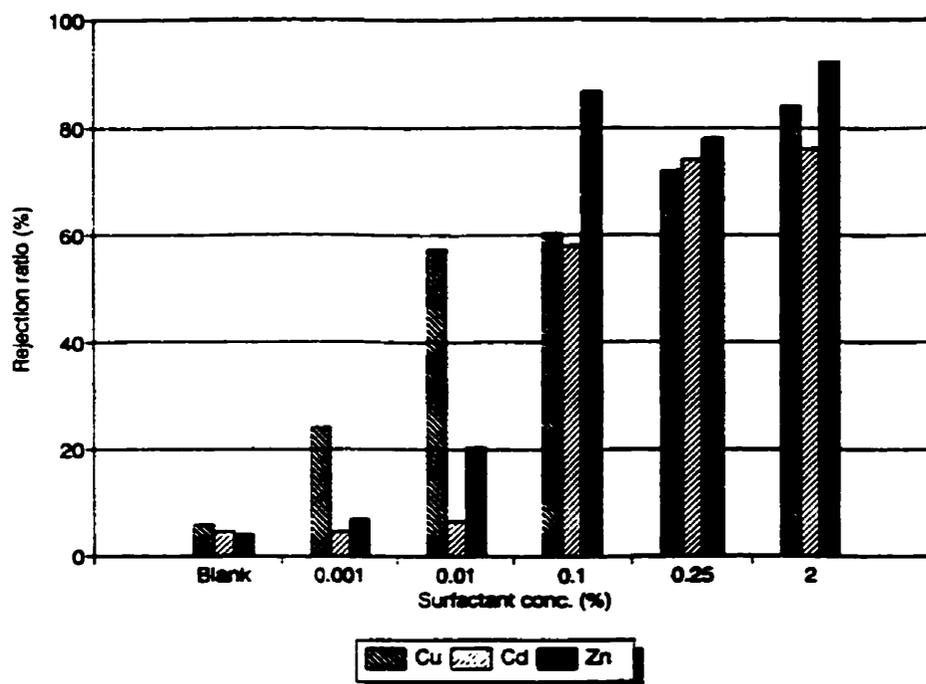
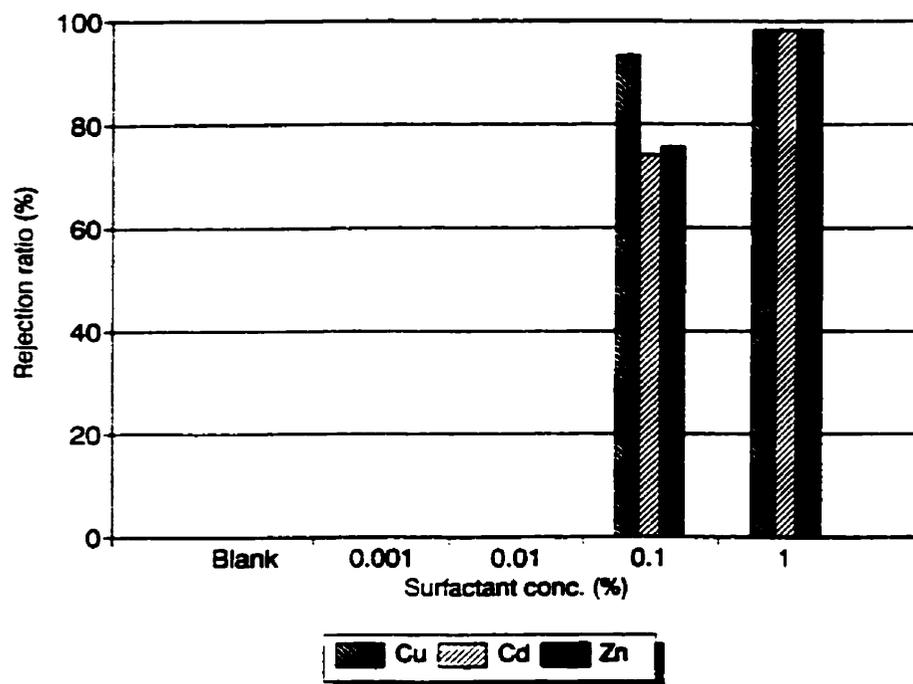


Figure 8.4 Ultrafiltration of metal/surfactin solutions

Table 8.1 Concentration of metals in the permeate at various surfactin concentrations

Initial surfactin conc. (%)	Final surfactin conc. in retentate (%)	Final surfactin conc. in permeate (%)	Metal concentration in permeate (mg/L)			
			Cu	Cd	Zn	Pb
Blank	--	--	9.7	19.7	9.7	0.3
0.001	0.2	0.001	6.6	17.7	9.0	0.1
0.01	0.1	0.001	1.5	13.4	7.7	<0.1
0.1	1.5	0.005	0.5	0.6	0.3	<0.1
0.25	1.8	0.03	0.7	0.4	0.4	<0.1
2.0	37.0	0.04	0.3	0.1	0.1	<0.1



**Figure 8.5 Ultrafiltration of metal/rhamnolipid solutions**

are formed (in the order of 0.002% for surfactin and 0.0025% for rhamnolipid). For both types of surfactants (Figures 8.4 and 8.5), more metals are retained in the retentate and less in the permeate (Table 8.1) as the concentration is increased above the CMC.

The effect of pH was examined for the surfactin and rhamnolipid at 0.1% concentrations (Figures 8.6 and 8.7). At pH below 7, some precipitation of surfactin can occur which will not occur at pH 8.3 and 11. For the rhamnolipid, pH 6.7 seemed to be optimal. Cadmium retention decreased at pH 11. Figure 8.7 also shows the effect of oil addition (0.4%) for the rhamnolipid. Although cadmium and copper retention was good, however, copper and zinc retention decreased somewhat. A supernatant (pH 11) with approximately 1.5% rhamnolipid was also examined. As compared to 1% rhamnolipid, copper, cadmium and zinc retention decreased by 40%.

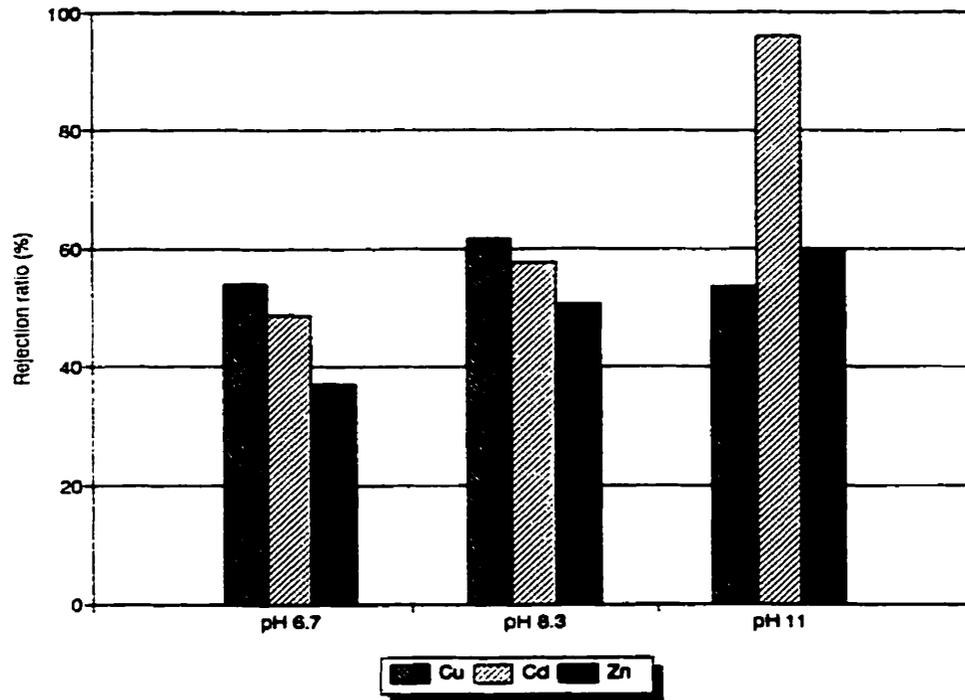


Figure 8.6 Ultrafiltration experiments with surfactin (0.1%) at various pH values

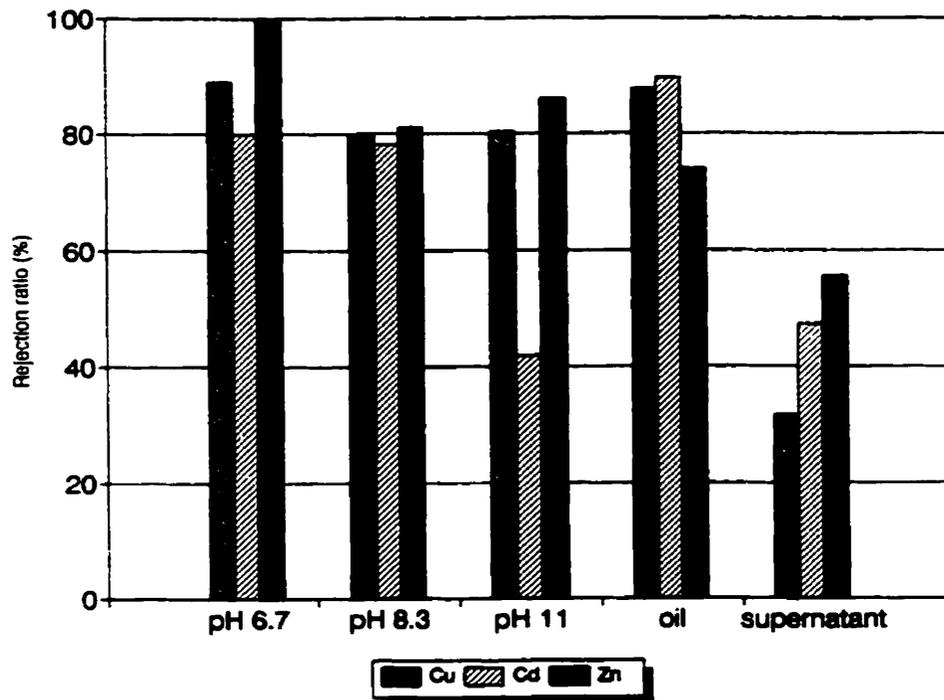
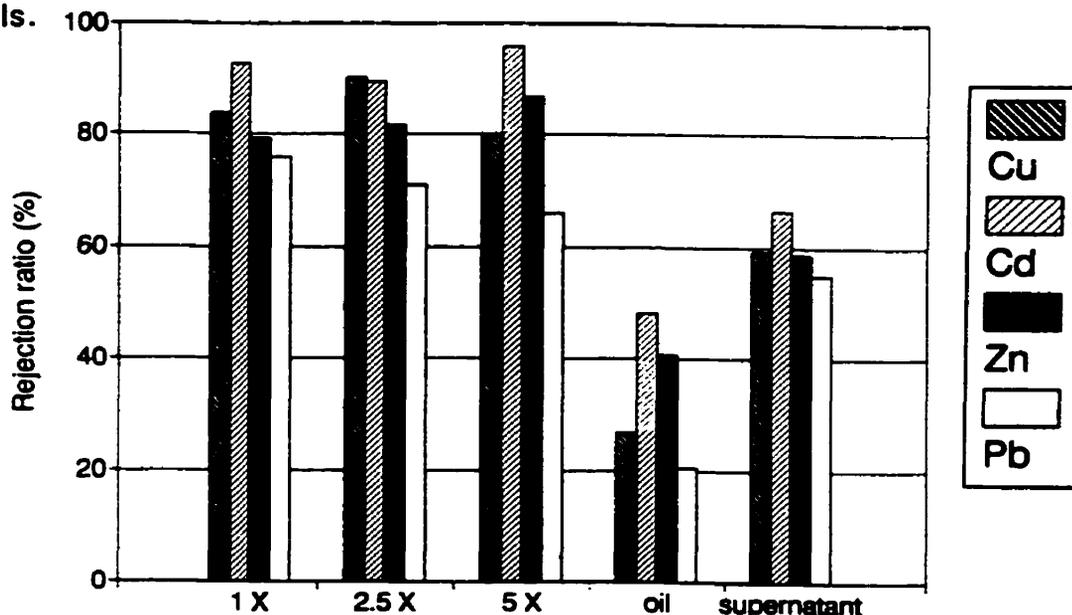


Figure 8.7 Ultrafiltration experiments with rhamnolipid (0.1%) at various pH values

Figure 8.8 shows the results with varying surfactin/metal ratios. A constant concentration (1 mM) of surfactin was used. The concentration of 1X represents the same concentration of metal (1 mM) used throughout the experiments. The metal concentration was increased to 2.5 and 5 mM (2.5 and 5 times). A ratio of surfactin/metal of 1:5 could still retain substantial amounts of metal in the retentate. However, the amounts of copper and cadmium started to decrease. As shown in Figure 8.4, the surfactin ratio was increased from 2:1 at 0.25% surfactin to 20:1 at 2% surfactin.

The supernatants (pH 11) from surfactin soil washing experiments were also examined (Figure 8.8). The surfactin concentrations were approximately 0.1%. In the supernatant, other components such as oil are present (0.4%). The results for copper and cadmium removal were similar to the results for 0.1%. Due to competition for space within the micelle, however, copper was rejected into the permeate. The results for 0.4% oil addition to a metal/surfactin (0.1%) solution are also shown. In this case, there was a slight decrease in all three metals.



**Figure 8.8** Ultrafiltration of surfactin (0.1%) with different metal concentrations

### VIII.3 Zeta potential measurements with rhamnolipid and surfactin

Zeta potential was used to determine the interaction of the surfactant and the solid interphase. The zeta potential of both the soil and the sediment is negative as seen in Table 8.2. Measurements were then made on the soil and sediment in the presence of the biosurfactants at a concentration above the critical micelle concentration for surfactin and rhamnolipid. In both cases there was a significant decrease in the zeta potential when the biosurfactant was added.

**Table 8.2 Zeta potential measurements of soil and sediment**

Surfactant solution used	Zeta potential of soil (mV)	Zeta potential of sediment (mV)
Distilled water (pH 6.5)	-42.5	-28.4
Distilled water (pH 9, adjusted with NaOH)	-46.6	-32.1
0.1% rhamnolipid (pH 6.5)	-89.1	-62.5
0.25% surfactin (pH 9, adjusted with NaOH)	-76.1	-48.1

### VIII.4 Summary and preliminary discussion

Since metal removal has not been investigated previously, it is not known then by what mechanisms metal removal by the surfactants occur. As discussed in section II.4.2, the mechanisms of solubilization, mobilization, sorption, and emulsion formation have been postulated for hydrocarbon removal but it is not known whether these are applicable for metals.

To determine the mechanisms, three different techniques were used to investigate surfactant/metal interactions. They include octanol/water

partitioning, partitioning between monomers and micelles using ultrafiltration, and the use of zeta potential to study the soil/surfactant interactions. Octanol-water partitioning is usually used to study the hydrophobicity of hydrocarbons (Schmelling, 1994). Ultrafiltration is usually used for separation of various components. The difference in the pore size of the surfactant monomers and micelles is exploited to determine if metals are associated with the monomers or micelles. Zeta potential is usually used to study agglomeration of particles.

When values of  $\log K_{ow}$  greater than 1 are obtained, this indicates that there is a preference for the organic phase or hydrophobicity (Schmelling, 1994). The lower values indicate hydrophilicity (preference for water). The addition of the surfactants (rhamnolipid or surfactin in particular) were able to change the preference of the metals from the water to the organic phase. Until now, this phenomenon has been noted for organic contaminants and is described as solubilization. Friedel et al. (1994) indicated that there could be formation of metal/lipid complexes (lipid portion of the surfactant). They also showed that increased concentrations of sodium and pH could substantially decrease this phenomenon. They found that a pH of 5 to 6 gave the highest  $K_{ow}$  values but did not show how much of a decrease there was at higher pH values. At high pH values, they postulated that hydroxy complexes were formed which led to decreased  $K_{ow}$  values. Increased sodium (NaCl) increased the ionic strength of the solution and  $K_{ow}$  significantly.

In studies with non-polar compounds, Jafvert et al. (1995) postulated that micelles can be considered as a separate phase where partitioning can occur. Large  $K_{ow}$  values mean that there is a greater tendency of the contaminants to go into the micellar phase. By this reasoning then, the significant  $K_{ow}$  values for the rhamnolipid and surfactin for the metals mean that they have excellent potential for solubilizing metals into the micelles.

To determine if the metals were indeed incorporated into the micelles, ultrafiltration studies were initiated. At concentrations of 0.01% surfactin or rhamnolipid, 99.8% of the surfactants was in the form of micelles and 0.2% are as monomers, the micelles are retained and only the surfactant monomers passed through the membrane as indicated by the surface tension which are above the minimum at the CMC. The metals, therefore must be solubilized in the micelles since the metals remain in the retentate with the micelles. The retention of a substantial amount of the surfactants by the membranes would enable the surfactants to be recovered for further reuse (Ang and Abdul, 1995). The metals and surfactants can be separated by pH adjustments. The surfactants precipitate below pH 5 and the metals at higher pH values. Solubilization in the micelles thus must be taking place.

Zeta potential was used to determine if the surfactant is concentrating at the soil-water interface. This is one of the mechanisms that was proposed for removal of hydrocarbons. Surfactants were shown to increase the removal of PAHs (Yeom et al., 1996) by concentrating at the soil interface and increasing diffusion of the PAHs into solution. The surface charge of both the soil and the sediment was confirmed as negative by the zeta potential measurements. This is in agreement with values of -3 to -60 mV which have previously been found for soil and clay minerals (Bohn, 1979) and those measured by Raatz and Hartel (1996) for kaolinite (-32 mV) and for illite (-20 mV).

Raatz and Hartel (1996) also showed that complete removal of oil content on the surface of the soil led to a decrease in the zeta potential of -4 mV. The addition of both the rhamnolipid and the surfactin, however, decreased the zeta potential substantially. Both of these surfactants are negatively-charged and subsequent adsorption of these surfactants on the surface caused the further decrease in surface charge. Therefore, sorption of these surfactants

is indeed occurring.

In summary, removal of the metals by the surfactin and rhamnolipid occurs through sorption on to the soil surface, removal of the metal from the soil into the soil solution and hence incorporation into surfactant micelles. These mechanisms are similar to those for hydrocarbon removal.

## **IX DISCUSSION**

### **IX.1 Soil washing with surfactants**

According to the criteria of the MEF and that established during the testing of the demonstration for the trials at THC (Richardson and Ehrenreich, 1994) which was based on the guidelines from the Ontario Ministry of the Environment (Table 9.1), a single washing with or without surfactant under the various conditions tested would not meet the objectives. Both the THC soil and the sediment were extremely difficult to treat. Water alone could only remove 2% of the copper, 1% cadmium and none of the zinc even after five washings. This would rule out the use of high power jet sprayers to remediate these soils. Clearly, additives to the water are required. The trials at THC were done with soils of low metal content (51 mg/kg copper, 277 mg/kg zinc, and 100 mg/kg lead) which was below the cleanup criteria, making comparison difficult (Richardson and Ehrenreich, 1994).

The use of multiple washings with surfactants, however, was beneficial. Although high concentrations of rhamnolipid could not remove more than 32% of the copper and 18% of zinc, five washings with 0.1% rhamnolipid removed 38% copper and 16% zinc. It would also be expected, however, that increasing the number of washings would not substantially increase heavy metal removal. To determine if this was a possibility, the data from Figures 6.14, 6.15 and 6.16 (details in Appendix C) were fitted to a natural log curve. Values of 47% copper removal and 19% zinc removal could be expected after 10 washings.

In the case of washing with surfactin (0.1% with 1% NaOH), the THC criteria copper would have been met but not that of the MEF which would probably require another two or three washings. Zinc removal would still be lower than the requirement (approximately 30%) and a maximum cadmium removal of

20% would be expected. These curves were not validated and were used only as an estimate.

The combination of the sophorolipid (4%) and Triton X-100 (2%) could possibly achieve both criteria for zinc removal (greater than 58%) with 10 washings. 63% copper would be achieved for the same amount and 68% for cadmium removal. Increasing the number of washings to 20 would probably be necessary. The addition of acid with the sophorolipid was similar to the results with the sophorolipid and Triton X-100. The THC criteria for copper removal could be met after 10 washings while the MEF criteria could not be met. Only three washings were required to meet both guidelines for zinc. In the case of cadmium, 90% removal was obtained after 5 washings. Another two or three washings would be necessary to achieve the 99% removal required by MEF.

**Table 9.1 Metal removal objectives**

	Required removal according to MEF B criteria (%)	Required removal according to THC criteria (Richardson and Ehrenreich, 1994)
<b>THC soil</b>		
Cu	77	47
Zn	42	32
<b>Spiked THC soil</b>		
Cu	81	59
Zn	58	50
Cd	99	UN
<b>Sediment</b>		
Cu	8	UN
Zn	85	UN

UN-Unknown

## **IX.2 Sequential extraction studies**

The benefit of sequential extraction studies is that they can assist in designing a soil washing procedure. The results can show if the heavy metals are exchangeable cations, associated with oxides and hydroxides, or carbonates and organic matter or considered within the lattice of the residual fraction. Based on these results, an appropriate washing fluid can be chosen. For exchangeable ions, solutions of neutral salts such as  $\text{MgCl}_2$  or  $\text{NaNO}_3$  can be used.

Metals within the residual fractions are generally considered stable and can only be removed with very harsh conditions such as strong acids at high temperatures. This fraction should be considered as background values when developing criteria for cleanup as it is not affected by any of the treatments used in this project. In the THC soil, this fraction included 79 mg/kg copper, 44 mg/kg zinc and 40 mg/kg lead. In the sediment, 10 mg/kg copper, 99 mg/kg zinc and 41 mg/kg lead were found in the residual fraction. These levels are comparable to normal levels of copper, zinc, and lead are 20 mg/kg, 10 to 300 mg/kg and 20 mg/kg, respectively (Cameron, 1992). Although lead is slightly high, feldspar and shales/clays have shown to have levels of 50 mg/kg (Richard and Bourg, 1991).

Both surfactin and rhamnolipid were able to remove copper more easily than the other heavy metals. As seen from the sequential extraction studies, the copper was found mainly in the organic fraction in the soil and sediment samples. Usually oxidative conditions are required to release metals in this form. Copper has been found to bind strongly to organic matter by forming complexes (Cameron, 1992). The use of a surfactant and hydroxide is a combination that has been used for enhanced oil recovery known as alkaline flooding. The presence of acidic components in the petroleum or organic

matter such as carboxylic acid, phenolics and asphaltene can hydrolyze under basic conditions to form surfactant products (de Zabala et al. 1982; Peru and Lorenz, 1990). These surfactants are thus negatively charged and have been postulated to be responsible for enhanced oil recovery and probably contribute to metal removal. They lower the interfacial tension between the oil and the rock as shown in Figure 9.1. These surfactants could also enable metal removal due to their negative charge and the interfacial surface tension lowering (Surkalo, 1990). It has been shown that the chemical reactions of the NaOH with the oil forms surfactants that lower the IFT between water and oil from 18 mN/m to 2 mN/m. The addition of surfactants with the NaOH can further reduce the IFT to 0.020 mN/m in the case of petroleum sulfonate (Lin et al., 1987).

The combination of the added surfactants and the base (whether it is NaOH or sodium bicarbonate) in this study has been shown to enhance copper removal and is therefore a good strategy to remediate soils containing copper in the organic fraction. Zinc also forms complexes with organic matter (Cameron, 1992) and some removal from the organic fraction was also noted using the biosurfactants with NaOH. The initial content of zinc in the organic matter in the soil and sediments was significantly less (10 and 15% for zinc compared to 80 and 85% for copper in soil and sediments, respectively).

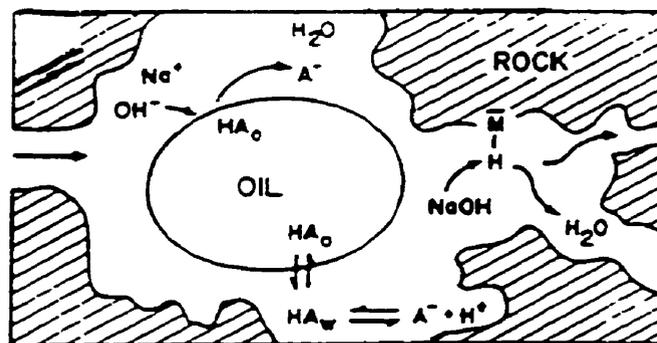


Figure 9.1 Effect of NaOH on oil (de Zabala et al., 1992)

Organic matter is an important factor in the sorption of metals (Ellis, 1973; Hodgson, 1963). Humic acid, one of the components of organic matter, is soluble in alkali only, precipitates in acids and is darker and of higher molecular weight, has been used for its surfactant ability and is a known chelater. Chelation (Keeney and Wildung, 1977) is a type of complexation that occurs between a metal ion and an organic ligand resulting in the formation of two or more bonds between the two in a heterocyclic ring. Humic acids have high charge density due to their acidic functional groups (carboxyl, hydroxyl, phenolic, carbonyl and enolic). This characteristic leads to a strong affinity for cations. They account for most of the metal immobilization in soil organic matter. With the present sequential extraction procedure, however, it is impossible to distinguish between the contribution of organic matter and petroleum components to metal retention in soils since oxidation is used to destroy both these fractions.

Zinc was associated with carbonates in the soil and the sediments while the spiked cadmium was found in the same fraction in the spiked THC soil sample. Illite, a component shown in the X-ray diffractograms of the soil and sediment, contains carbonates (Yong et al., 1992). It was able to retain lead and cadmium in this fraction. As the pH decreases from 7, the amount in the carbonate phase is dissolved (particularly at pH below 5). In this study, addition of 0.7% HCl enhanced the dissolution since the pH was lowered to 5.5. Addition of sophorolipid with acid removed more zinc from the carbonate was noted even though there was not a significant difference in pH. The sophorolipid most likely enhanced the solubility of this fraction. Multiple washings were required to totally remove the zinc and cadmium associated with this fraction which indicates that this fraction is not that easy to remove. Stover et al. (1976) indicated that cadmium and zinc carbonates are difficult to dissolve. Surfactin (pH 9) and rhamnolipid (pH 6.5) seem to enhance the

solubility of the carbonate phase of zinc from the sediments. High pH conditions after multiple washings can also decrease copper in the carbonate fraction.

The oxide fraction was particularly important for zinc and cadmium retention for the soil and sediment. Cameron (1992) indicated that zinc is often associated with iron and manganese oxides. The sequential extraction procedure cannot distinguish between oxides, oxyhydroxides or hydroxides but the latter two forms are the most probable species (Felmy et al., 1983). Yong et al. (1993) indicated that cadmium and zinc were retained in the oxide fraction at pH above 5. Solubilities of lead, copper, zinc and cadmium are shown in Figure 9.2 (Radha Krishnan, 1993). Minimum solubilities for zinc and cadmium are 9.0 and 11.2, respectively. This is why high pH conditions removed small amounts of cadmium and zinc even after five washings

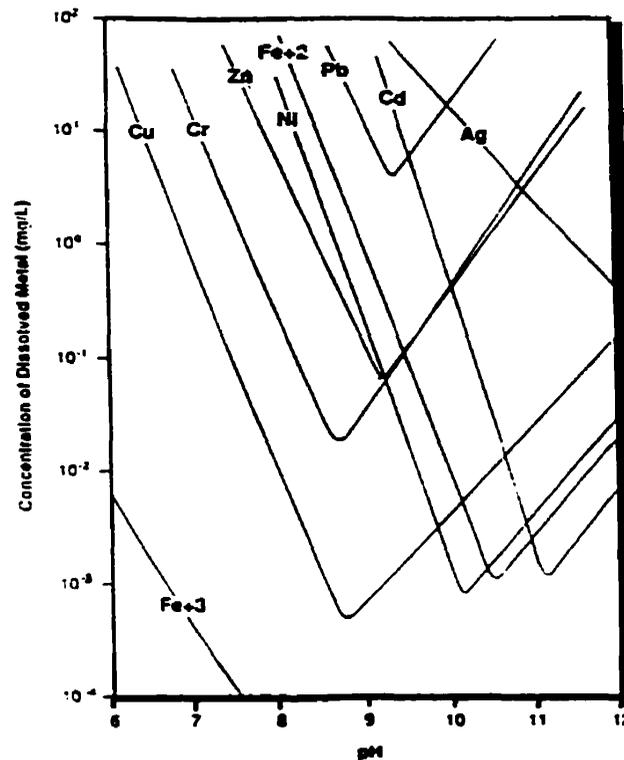


Figure 9.2 Solubility of metal hydroxides versus pH (Radha Krishnan, 1993)

(ie., in the presence of 1% NaOH). There was some evidence though that the presence of surfactin with 1% NaOH was able to decrease the zinc removal from the oxide phase through enhanced solubilization. Addition of acid with sophorolipid was clearly beneficial for removal of both zinc and cadmium from the oxide phase (pH 5.5). Programs such as MINTEQA2 provide a probable percentage of dissolved and precipitated species of metals but cannot predict the effect of surfactants on the solubility of the various species and how they affect the thermodynamics of the system due to a lack of information (Felmy et al., 1983).

The benefit of using sequential extractions is that it can be used to design a strategy for soil washing procedures. The addition of surfactant and hydroxide is a good strategy to use for copper removal which was bound mainly in the organic fraction. In contrast, the addition of the acid plus the surfactant is a good approach for removal of zinc and cadmium in the oxide and carbonate phases. A combination of these procedures (a series of washings with different additives) could then be used to remediate the soils and sediments to adequate levels which contain high levels of copper, zinc and cadmium.

### **IX.3 Mechanism of metal removal by surfactants**

Since the biosurfactants are negatively charged and the metals are positively charged, ion exchange mechanisms are not likely in this case but could apply to positively charged surfactants. It is also unlikely that the counter ions associated with the surfactants are responsible for the metal removal since from the sequential extraction experiments (Figures 7.4 to 7.6), it was determined that the fraction of metals retained as ion exchangeable for both the THC soil and the sediments was negligible. The metals were retained due to some form of complexation with the oxides and organics in the soils and sediments. It would, therefore be unlikely that the surfactants removed

the metals by ion exchange mechanisms (Sabatini and Knox, 1992).

According to double layer theory, a negatively charged soil will have a layer of anions and cations near its surface (a double layer). The distribution of positive charges forms the diffuse double layer as postulated by Guoy and Chapman in 1910 (Yong et al., 1992b). The Gouy-Chapman theory called the diffuse double layer (DDL) theory is a theoretically derived equation of the form:

$$\frac{n^-}{n_o} = \left( \coth\left(\frac{x}{2}\right) \frac{\sqrt{8\Pi^2 z^2 c_o N}}{\epsilon KT} \right)^2$$

is derived by assuming that the clay particle is an electrical plate. The electrical field as a function of distance from the particle can be computed. The position of the cations  $n^+$  or anions  $n^-$  as a function of distance  $x$  from the particle has been derived in the Boltzman equation. The valence ( $z$ ), the temperature ( $T$ ), and the concentration of ions in solution  $C_o$  are taken into account in this equation. This phenomenon is shown in Figure 5.3A. It can be seen that the number of cations decreases the further away from the particle which is negatively charged. The  $k$  value is the Boltzman constant which has been theoretically derived =  $1.38048 \times 10^{-23}$  joule/deg and originates from the Boltzman equation. Both the distribution of cations and anions have been theorized. The following assumptions have been made concerning the diffuse double layer (DDL) theory (Mitchell, 1976):

- (1) The ions in the double layer are only point charges and cannot interact with each other.
- (2) The charge is evenly distributed over the particle surface.
- (3) The particle surface is flat as a plate and much larger than the double layer thickness.

Zeta potential is a measurement of the electrophoretic mobility and is

determined by measuring the potential within the double layer which is where the absorbed divalent cations such as copper and zinc are located on the soil particle (Figure 9.3A). The addition of the anionic surfactants to a soil solution most likely leads to the formation of complexes with the cations in the double layer of cations which increases the electrokinetic potential (Figure 9.3B). Both the surfactin and rhamnolipid (Table 8.2) decreased the measured zeta potential of the soil and sediment. Since the surfactant is of anionic character, it is adsorbed through hydrophobic interaction with the soil. When the surfactant is added to the soil suspension, the surfactant will line up with the hydrophobic portion on the soil, and the hydrophilic part towards the water. From the  $K_{ow}$  measurement, it can be concluded that there is also an affinity of the hydrophobic portion of the surfactant for the metal.

When attractive forces dominate, as in the case of a positive ion on a negatively-charged surface, the removal of the metals by the surfactant would be as follows (Figure 9.4). The first step (A) after introduction of the surfactant involves the separation of the metal from the soil by the washing solution by a distance,  $h$ , which is so small that attractive forces will still dominate. The amount of energy required to accomplish this task is  $W_1$ . The next step is the removal of the metal completely from the soil. This is done through the use of shear forces ( $W_2$ ) during the mixing of the soil and washing solution. The metal is now carried away from the soil surface far enough that the attractive forces are now negligible. This is in agreement with the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory which says that the attractive forces between particles decreases significantly as the separation distance,  $h$ , increases.

In summary then, the total work required ( $W_t$ ) to overcome the adhesion of the metal to the soil surface is the summation of the two forces mentioned earlier,

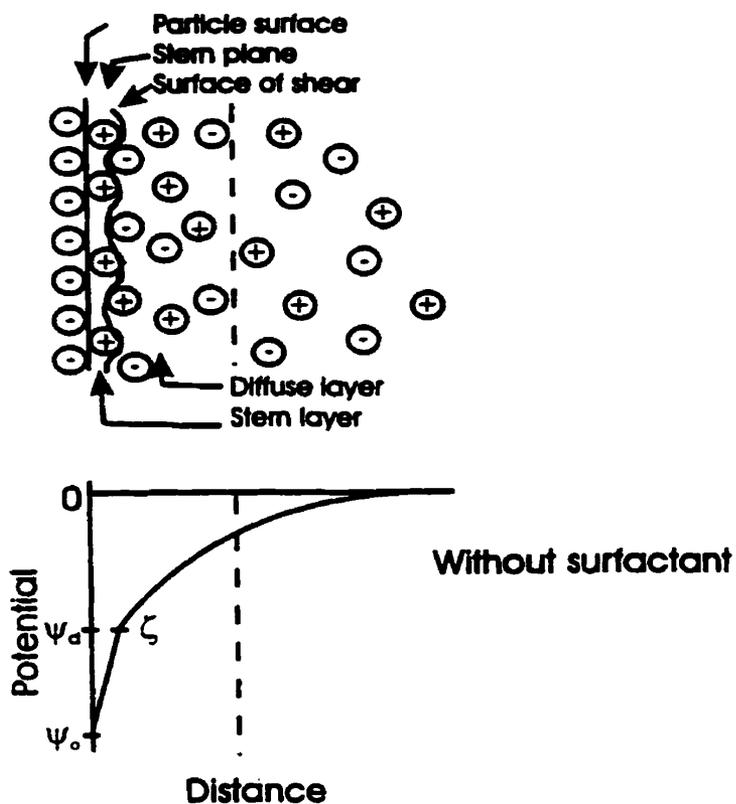


Figure 9.3A The distribution of cations and anions as a function of distance from the particle surface (Shaw, 1980).

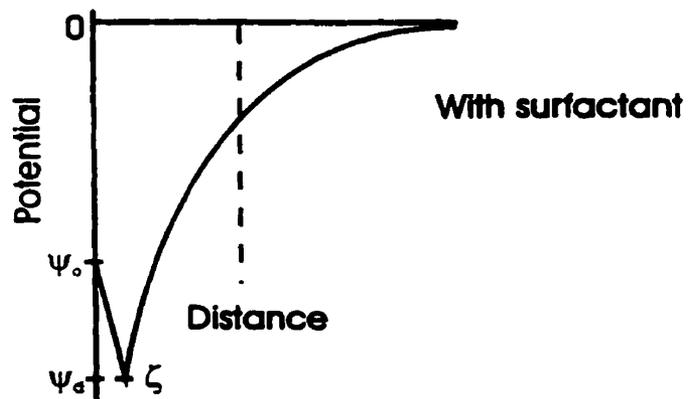
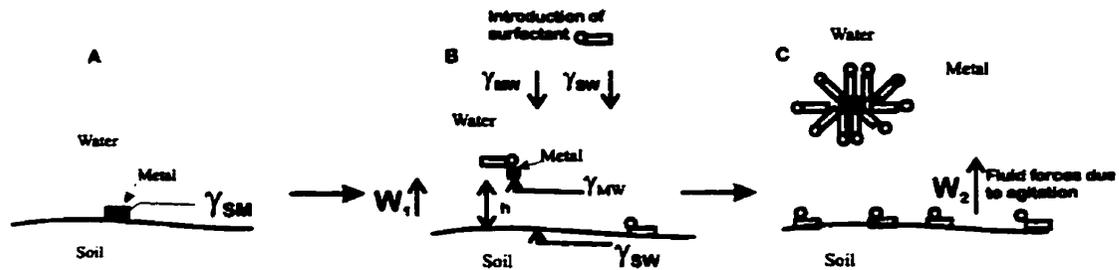


Figure 9.3B Influence of surfactant adsorption on zeta potential



**Figure 9.4 Lowering of the interfacial tensions at the soil/water ( $\gamma_{SW}$ ) and metal/water interfaces ( $\gamma_{MW}$ ) by the surfactant and work to remove the metal from the soil surface as shown in steps A, B and C.**

$W_1 + W_2$ , less the summation of the change in surface-interfacial energies:

$$W_t = W_1 + W_2 - \sum \Delta\gamma \quad (\text{Stokes and Evans, 1997})$$

In the numerous soil washing experiments that were performed, water by itself (eg., as shown by the control in Figure 4.7 A and B) was not able to dislodge the metals from the soil due to the high surface tension and inefficient wetting. By adsorption onto the metals and soil, the surfactants lower the interfacial tensions between the metal and water ( $\gamma_{MW}$ ) and soil and water ( $\gamma_{SW}$ ) enabling them to remove the metal into solution by reducing the work ( $W_t$ ) necessary to remove the metal from the surface (Figure 9.4). The larger the decrease in interfacial tensions by the surfactant addition, the easier it is to remove the metals. These metals can be in the form of carbonates, hydroxides or tied to organic matter as shown previously by the sequential extraction studies.

At solid surfaces,  $\gamma$  cannot be directly measured. However, the change in interfacial tension can be related to a change in surface excess of solute,  $\Gamma$ . The surface excess is defined as the number of moles in a sample at the surface minus the number of moles of solute in the sample in the bulk phase divided by the surface area. From the Gibbs equation below, it can be seen that a positive surface excess means that absorption is taking place and that there is a lowering of the interfacial surface tension.  $R$  is the gas constant,  $c$  is the concentration at the surface and  $T$  is the temperature (Heimenz and Rajagopalan, 1997).

$$\Gamma = -\frac{1}{RT} \frac{d\gamma}{dc}$$

Surfactants have been shown to increase the zeta potential of textiles during the detergency process (Coons et al., 1986). Surfactants which adsorb to the solid-water interface, and dirt-water interface are better detergents. According to Raatz et al. (1996), surfactant adsorption is essential for removal of soil contaminants. They used a nonionic surfactant (without charge) which adsorbed on the surface of kaolinite by hydrophobic interaction and increased the zeta potential from -32 mV to -7 mV. Popov et al. (1997) showed that chelating agents such as EDTA could also decrease the zeta potential of soil from -9 to -42 mV. They postulated that EDTA most likely forms negatively charged complex compounds with the cation contaminants which adsorb on to the surface and influence the electrokinetic potential. This could likely occur in the case of surfactin and rhamnolipid.

Following the desorption from soil, the metals are then incorporated into the micelles as seen by the ultrafiltration experiments (Figures 8.1 to 8.8) and demonstrated in Figure 9.5. The micelles are retained by the ultrafiltration

membrane since they are larger (containing 50 to 100 molecules of surfactant (Mulligan and Gibbs, 1990)) and only the surfactant monomers pass through the membrane.

The metals must therefore be solubilized in the micelles since the metals remain in the retentate with the micelles. This step prevents the metal from re-adsorbing onto the soil. The partitioning of the heavy metals between the micelle and aqueous phases can then be determined based on the results from the ultrafiltration studies. In addition, the results of the  $K_{ow}$  experiments indicate that the metals have an affinity for the hydrophobic portion of the surfactant and would likely be retained in the interior of the micelles which contains the hydrophobic portion of the surfactants. As mentioned in section II.4.2, solubilization is one of the mechanisms of remediation of organic contaminants. Smith et al. (1987) postulated that the higher rate of solubilization into the micelle is indicated by higher  $K_{ow}$  values. The hydrophilic portion of the surfactant would be facing towards the aqueous solution.

### Adsorbed metal

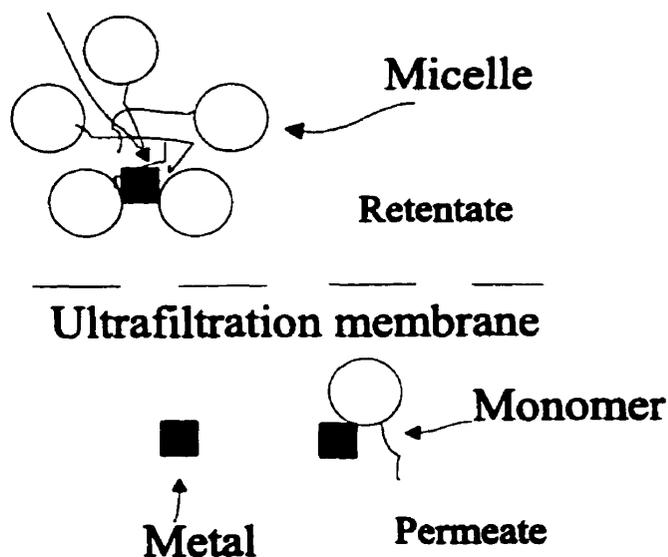
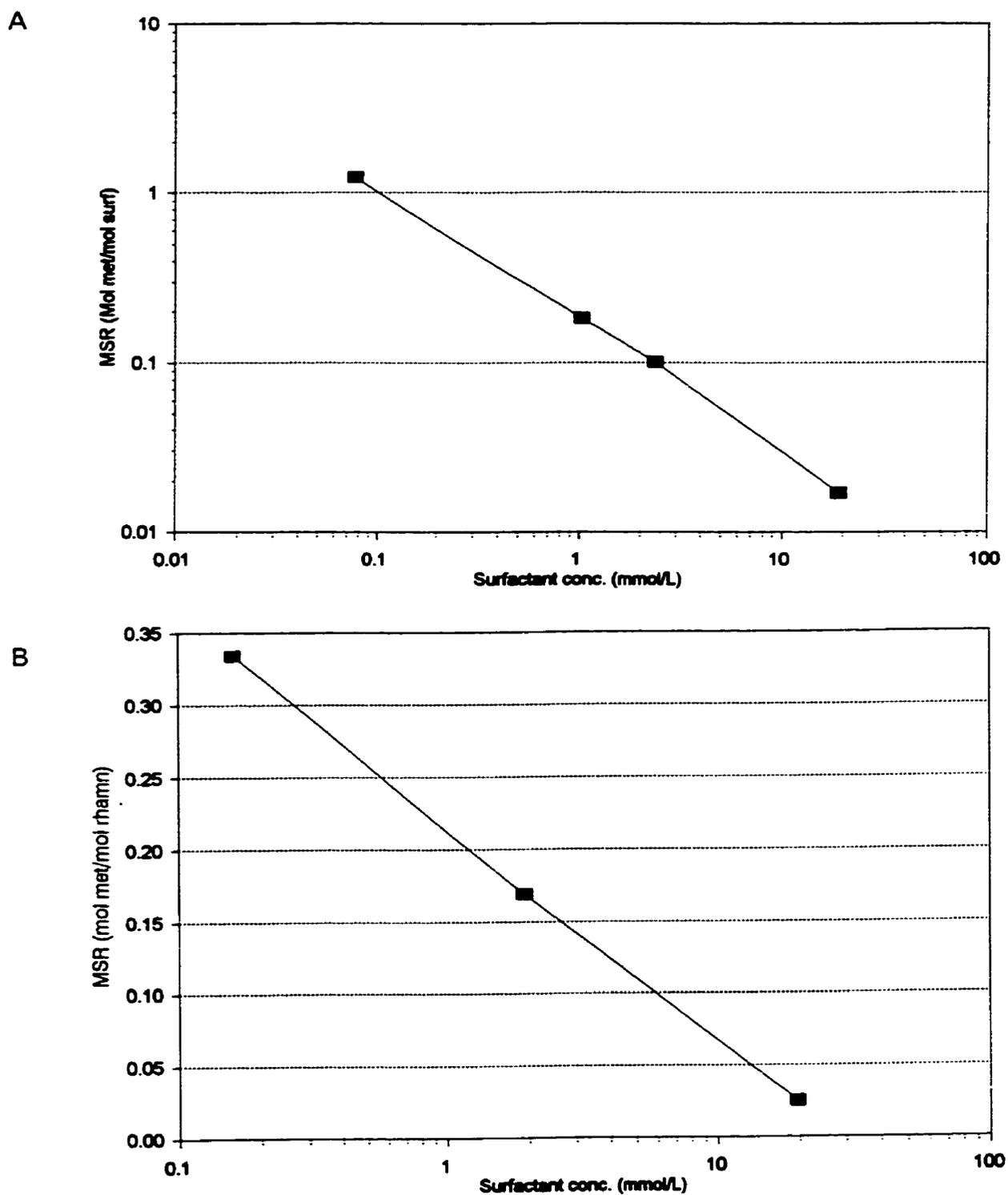


Figure 9.5 Retention of metals within the surfactant micelles

One way of approaching solubilization is to determine the amount of total moles of metal solubilized per mole of surfactant. This term is called the molar solubilization rate (MSR) and is defined as the moles of metal in the micelle per mole of surfactant in micellular form (Edwards et al., 1991). The term is shown as a function of surfactant concentration for both surfactin and rhamnolipid in Figure 9.6. The ratio decreases as the surfactant concentration increases in a linear relationship for both surfactants. Surfactin MSR values were higher at a concentration of 0.01 % compared to that for the rhamnolipid but were comparable at the other concentrations. In the case of the surfactin, the ratio is a log-log relationship whereas for the rhamnolipid the relationship is a log for the concentration only. This relationship is different as compared to the organic contaminants in the studies by Edwards et al. (1991 and 1994). They found that the organic solubilization was constant as the surfactant concentration increased.

For surfactin, the ratio at 0.1 % concentration (with 1 % NaOH) is 1.22 mol of metal mixture: 1 mole of surfactin. This value is only slightly lower than the maximum theoretical value of 2 mol of metal: 1 mol of surfactin due to the two charges on the surfactin molecule (aspartic and glutamic acids as shown in Chapter II). At higher concentrations, in the actual soil washing studies, the molar ratio of 0.15 mol metal per mol of initial surfactin concentration for multiple extractions at 0.1 % surfactin after five washings was seen (Figures 6.7 to 6.9). These lower amounts are due to competition of other components such as the oil and other organic components for space within the micelle and sorption of the surfactant onto the soil.

For rhamnolipid (assuming an average molecular weight of 504) the ratio of 0.33 mole metal: 1 mole of surfactant was found (at a concentration of rhamnolipid concentration of 0.01 %). In the soil washing experiments of



**Figure 9.6** Molar solubilization ratios as a function of surfactant concentration for (A) surfactin and (B) rhamnolipid.

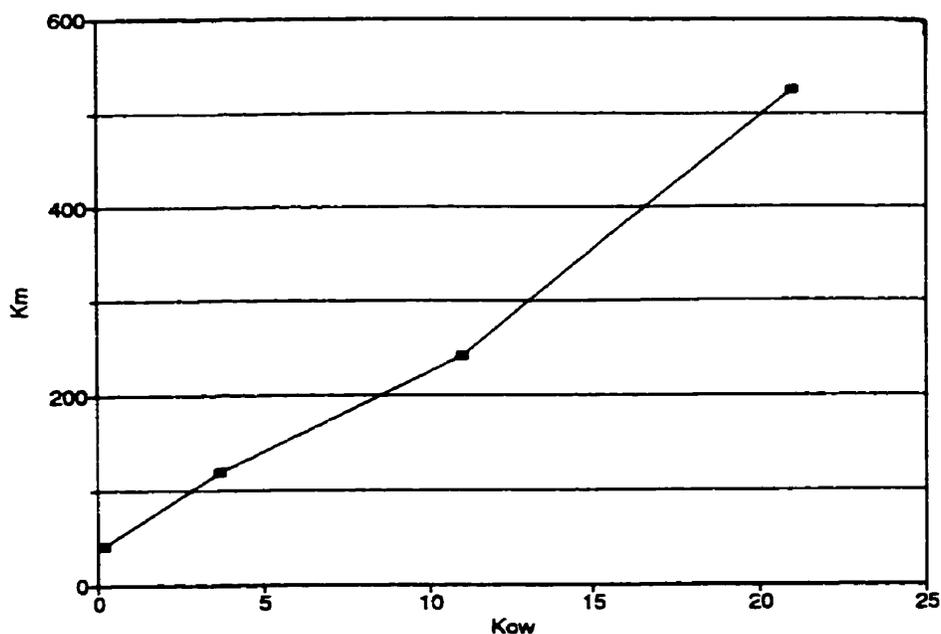
rhamnolipid versus concentration (Figure 6.10), 0.025 to 0.037 mole metal solubilized per mole of surfactant. The addition of NaOH at 0.5% rhamnolipid was similar at 0.025:1. However, the ratio increased to 0.083 mol metal per mol of surfactant when using 0.1% rhamnolipid with 1% NaOH after five extractions which is closer to the ultrafiltration values (Figures 6.14 to 6.16).

Another way to examine solubilization is by relating the micelle phase to the aqueous phase by the term,  $K_m$ , which is represented by the following equation:

$$K_m = \frac{X_{mic}}{X_{aq}}$$

where  $K_m$  = micelle-water partitioning coefficient which includes,  $X_{mic}$  = MSR / (1 + MSR) which is the mole fraction of total metal in the micelle and where MSR = Mol of metal in the micelle / mol of surfactant or molar solubilization ratio, and  $X_{aq}$  is the mole fraction in the aqueous phase (ie., the concentration of the metal in the permeate times the molar volume of water, 0.01805 L/mol) (Edwards et al., 1994).

These values are shown for surfactin as a function of  $K_{ow}$  in Figure 9.7. As shown in this figure, a higher fraction of metals is found in the micelles than the permeate as the  $K_{ow}$  increases. Edwards et al., (1991) showed a linear relationship between the log  $K_m$  versus log  $K_{ow}$  for Triton X-100, a nonionic surfactant for pyrene, phenanthrene, naphthalene and 1,2,3-trichlorobenzene. These  $K_m$  values are lower than those which have been determined for various surfactants with a variety of organic compounds (Jafvert, et al., 1995). For Triton X-100, the Log  $K_m$  were 4.55, 4.09 and 3.05 for pyrene, phenanthrene and naphthalene, respectively.



**Figure 9.7. Relationship between  $K_m$  and  $K_{ow}$  for surfactin**

From Table 9.2, it can be seen for the rhamnolipid that at lower concentrations, the ratio of metal in the micelle to the permeate is lower. In addition to the  $K_{ow}$  values which follows the same trend as surfactin. However, the  $K_{ow}$  at the highest concentration (1%) is lower than at 0.1%. This is probably due to the  $K_{ow}$  test itself since an emulsion was formed during the test which makes an accurate determination difficult.

Knowledge of the relationship of  $K_m$ - $K_{ow}$  is an essential prerequisite for modelling of the behaviour of surfactants and metal contaminants in washing systems. Metals appear to have a higher affinity for the hydrophobic portions of the surfactants and the micelles at low surfactant concentrations which agrees with the solubilization value trends. This may indicate why the multiple soil washings at low concentrations were so successful for metal removal.

**Table 9.2 Relationship between  $K_m$  and  $K_{ow}$  values for rhamnolipid**

Surfactant (concentration in %)	$K_{ow}$ value for total metal	$K_m$
Rhamnolipid (0.01)	9	33.9
Rhamnolipid (0.1)	30	85.4
Rhamnolipid (1.0)	7	105.4

## **X CONCLUSIONS AND SUMMARY**

### **X.1 Concluding remarks**

The purpose of Chapters IV and V was to characterize the soil and sediments, in addition to the biosurfactants that were to be used for the soil washing studies. Soil washing was determined to be the most viable alternative since both hydrocarbon and metal contamination were present. The extent of the contamination and other soil components were shown in Table 4.1 and 4.2. Preliminary indications from the CEC and particle size distribution data were that both the soil and the sediments could be difficult to wash but this does not give any indication what would be a good soil washing strategy. The presence of clay minerals, oxides, carbonates and organic was confirmed, all of which can contribute to metal retention. This data, however, was insufficient to determine the role of each soil constituent and the requirements for the removal of the metals from each of these fractions.

The results of the surface tension, interfacial tension and CMC determinations for the three biosurfactants were shown in Table 5.1. It was also concluded that the biosurfactants were indeed, surfactin, rhamnolipid and sophorolipid from their surface-active and chemical properties. It was also postulated after the characterization that these biosurfactants could be useful non-toxic soil washing additives.

The following conclusions for the soil washing studies can be made from Chapter VI. The maximal conditions for metal removal can be seen in Table 10.1. It seems that copper is easier to remove by the biosurfactants than zinc. In addition, the addition of hydroxide also assists copper removal significantly. This could be for several reasons as was discussed in Section IX.1.

**Table 10.1 Summary of conditions giving optimal results for each soil type**

Surfactant (other additives)	Cu removal		Zn removal	
	%	mg/kg	%	mg/kg
<b>Single washing of THC soil</b>				
4% sophorolipid (0.7% HCl)	37	155	16	139
4% sophorolipid (1% NaOH)	36	151	7	61
12% rhamnolipid	35	166	20	174
2% rhamnolipid (1% NaOH)	28	161	3	26
0.5% rhamnolipid (1% NaOH)	25	115	6	52
0.25% surfactin (1% NaOH)	25	115	6	52
<b>Multiple washings of spiked soil</b>				
0.1% surfactin (80% purity/1%NaOH)	90	495	25	300
0.1% rhamnolipid (1% NaOH)	38	209	17	204
4% sophorolipid (0.7% HCl)	50	275	90	1080
4% sophorolipid (2% Triton X-100)	50	275	50	600
<b>Sediment</b>				
0.25% surfactin (1% NaOH)	15	16	6	198
0.5% rhamnolipid	65	71	18	594
4% sophorolipid (0.3% HCl)	25	27	60	1980

The addition of acid with the biosurfactants, surfactin (Figure 6.4) and rhamnolipid (Figure 6.11), was not beneficial since these biosurfactants precipitate from solution significantly at pH below 5. Only the sophorolipid which is an oily substance could be used at low pH (Figures 6.21 and 6.24). Zinc removal seems to be enhanced at pH values below 7. Lead removal was also very significant at low pH values. Low pH would be expected to increase the solubility of the metals and enhance the metal removal. However, since the biosurfactant in most cases is removed from solution at low pH, it is therefore not effective and only the addition of the acid that leads to metal removal. The addition of a nonionic such as Triton X-100 was also beneficial (Figure 6.23). This probably has to do with the fact that the addition of nonionics with an anionic surfactant enhance the solubility of the anionic.

It was also shown that low concentrations of the three biosurfactants could remove significant amounts of both copper and zinc after multiple washings (Figures 6.7 to 6.9 for surfactin, Figures 6.14 to 6.16 for rhamnolipid and Figures 6.25 to 6.27 for sophorolipid and summarized in Table 10.1). Surfactin and sodium hydroxide removed 90% of the copper while 4% sophorolipid with acid removed 100% of the zinc and 90% of the cadmium. The combination of 4% sophorolipid and 2% Triton X-100 also enhanced metal removal significantly (50% of the copper, zinc and cadmium).

Regarding the sequential extraction studies in Chapter VII, the following conclusions can be drawn. The exchangeable fractions of copper, zinc and lead were very low in the three types of soils that were examined (THC soil (Figure 7.1), spiked soil (Figure 7.2) and sediments (Figure 7.3)) which is typical of soils and sediments that were contaminated long ago. The carbonate fraction was significant only for zinc, whereas the oxide fractions accounted for over 50 and 70% of the lead and zinc present in the THC soil and 60% of the lead and zinc in the sediments. In the sediments, however, 20% of the zinc and lead was found in the organic phase. The cadmium in the spiked THC soil was found mostly in the oxide fraction with significant amounts in the exchangeable and carbonate fractions. The organic fraction constituted over 70% of the copper in the THC soil and 90% in the sediments.

Sequential extraction was used to determine the effect of surfactants on metal removal (Section VII.2 and Figures 7.10 to 7.14). Removal of copper by the rhamnolipid and surfactin with NaOH was mainly from the organic phase, whereas zinc removal was from the oxide fraction of THC soil. For cadmium, the exchangeable and carbonate fractions decreased after washing with surfactin with NaOH. Removal of zinc by the acid/sophorolipid was from the carbonate fraction and the oxide after multiple washings from the spiked THC

soil. Removal of cadmium was mainly from the oxide fraction with some from the organic phase by the sophorolipid/acid from the spiked THC soil. In the sediments, as in the THC soil, copper was removed from the organic phase by the rhamnolipid. Copper removal from the carbonate phase was also significant and zinc removal by surfactin and rhamnolipid with and without NaOH was mainly from the carbonate fraction. Multiple washings were effective since they can remove the easier to remove phases first and the more difficult (such as oxide and carbonate). Residual fractions, the most difficult to remove, were not affected by any of the washing procedures.

Since metal removal was not previously investigated to any extent, their removal mechanisms also were not. The purpose of Chapter XIII was to investigate by what mechanisms metal removal by the surfactant occurs. It was determined in Table 8.2 that the surfactants, surfactin and rhamnolipid, adsorb onto the soil as indicated by the more negative values of zeta potential for both the sediment and soil and Table 6.1 which shows the difference in surfactin concentration in the washing medium before and after washing. The surfactants must, therefore, be forming complexes with the sorbed metal contaminants before detachment from the soil when the surfactant changes the surface properties through lowering of the interfacial tension which cannot be measured. The lowering of the interfacial tensions enables the lifting of the metal from the soil surface. Water alone cannot do this. The attractive forces between the metal and the soil are not overcome immediately. The resulting forces from the agitated fluid carries the metal away from the soil surface so that the attractive forces no longer come into play (which is consistent with the DLVO theory).

Once the surfactant/metal is in solution and since metal removal increases with surfactant concentrations above the CMC, and due to the results of the

ultrafiltration studies, solubilization of the metals within the micelles is occurring (Figures 8.4 to 8.8). The surfactant micelles of the rhamnolipid and the surfactin are solubilizing the copper, lead, cadmium and zinc. Other experiments showed that there is most likely competition for space within the micelle when oil and metals are solubilized together (Figures 8.7 and 8.8). In addition, the octanol/water partitioning studies indicated that surfactin and rhamnolipid can form lipid/metal complexes (Figures 8.1 to 8.3). In summary, the mechanism for metal removal can be illustrated in Figure 10.1.

In addition, it was also shown that the molar solubilization ratio decreased as surfactant concentration increased for both surfactin and rhamnolipid. A linear relationship was found between  $K_m$  and  $K_{ow}$  for surfactin. This trend is less evident due to emulsification problems in determining the  $K_{ow}$  at high rhamnolipid concentrations. This type of relationship is necessary to initiate modelling of partitioning in surfactant systems used to solubilize contaminants.

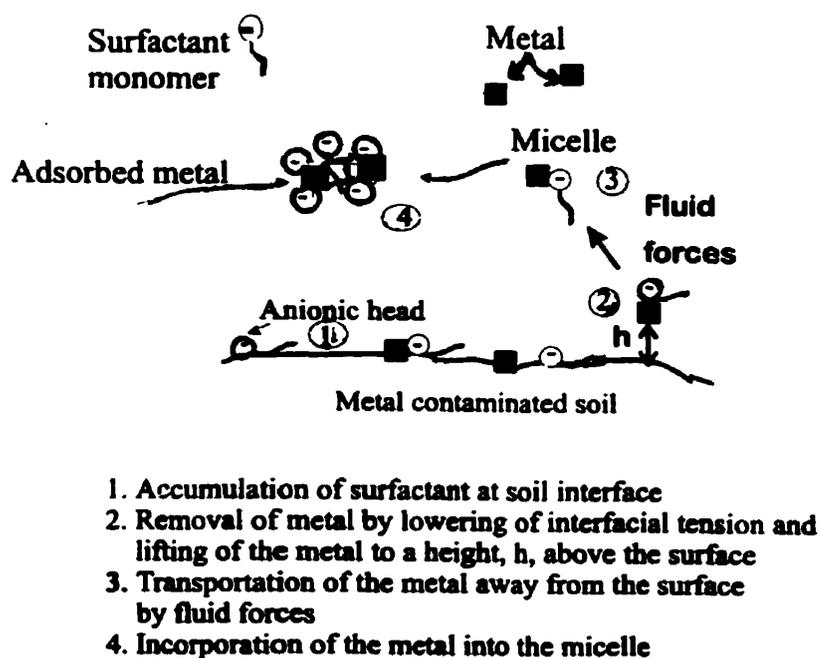


Figure 10.1 Suggested mechanism for metal removal by surfactants

## **X.2 Suggestions for future studies**

Future studies should include:

- Performing column studies to simulate *in situ* flushing with surfactants for metal removal
- Investigation of the potential for these biosurfactants to remove other heavy metals such as nickel, chromium and higher concentrations of lead and in other types of soils
- Further investigation into soil-surfactant interactions to minimize precipitation/sorption while maintaining metal removal efficiency
- Differentiation of the contribution of natural organic matter and petroleum contaminants to metal retention and removal
- Evaluation of other biosurfactants or natural surfactants for metal removal from soil
- Investigation into the effect of temperature on metal removal
- Determination of the efficiency of multiple washings using different additives to remediate soils to criteria levels for all metals
- Evaluation of *in situ* production of biosurfactants to enhance metal removal from contaminated soils
- Examination of the recovery of the biosurfactants by ultrafiltration and subsequent separation from the heavy metals in the retentate.

## **X.3 Contributions to knowledge**

The study has contributed to scientific knowledge in the following aspects:

- Evaluation of the potential of three different biologically-produced surfactants of low toxicity (surfactin, rhamnolipid and sophorolipids) to enhance the removal of heavy metals including copper, zinc, cadmium and lead in combination with other additives such acids, bases and nonionic surfactants from a contaminated soil and sediment. This study indicates that the surfactants can be used to remove oil and metal

contamination at the same time.

- Investigation and identification of the mechanisms for metal removal by anionic surfactants by using such techniques as ultrafiltration, octanol-water partitioning and zeta potential measurements.
- Correlation of soil washing procedures with sequential extraction studies. For example, if a soil is shown by sequential extraction techniques to contain copper mainly in the organic fraction, a surfactant with hydroxide would be the best procedure for its removal.
- Characterization of partitioning in surfactant-metal systems.
- Evaluation of ultrafiltration as a method to recover the surfactants and metals from the supernatant of the soil washing experiments.

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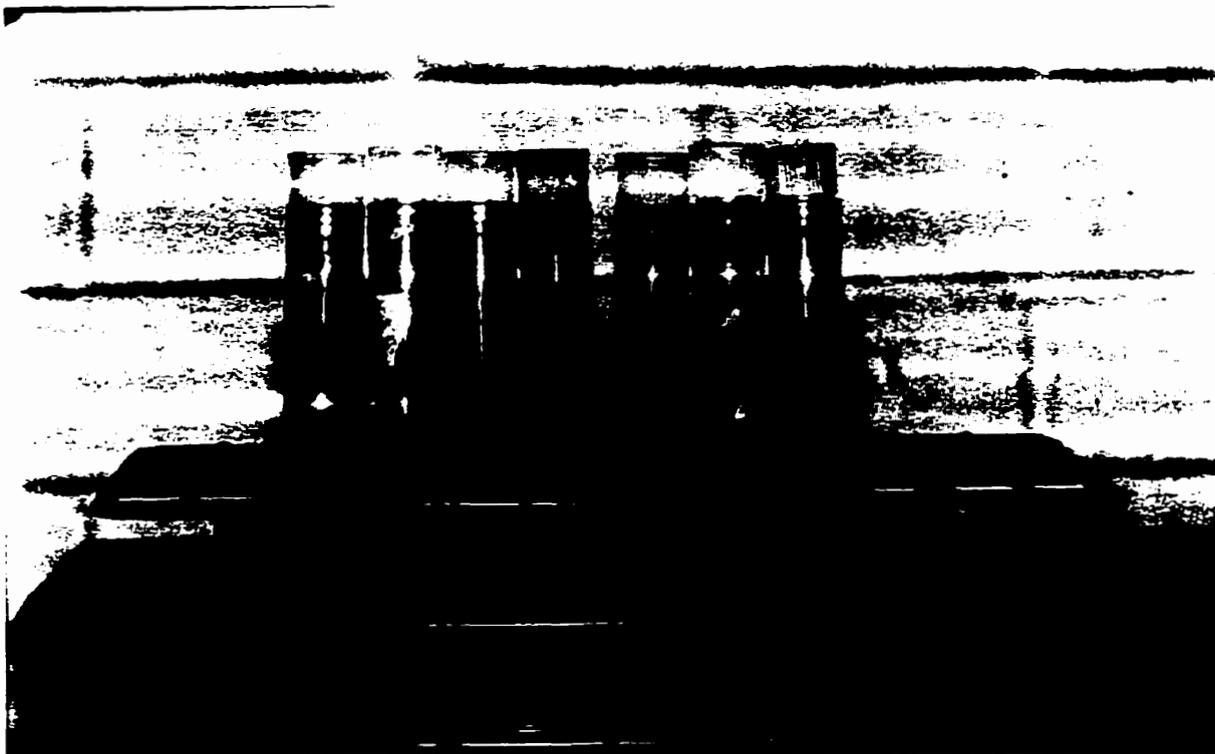
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**APPENDIX A:**

**Photographs of experimental equipment and soil washing experiments**



**Experimental setup for surfactant production including from left to right, pH controller with pump for NaOH addition, magnetic stirrer and temperature controller with the 700 ml stainless steel fermentor vessel and flask for foam collection on the exhaust air line. Air is sparged through a line with glass-wool filter and into the top of the fermentor through a submerged perforated ring.**



Supernatants of soil washing experiments after washing with water, 1% NaOH, 0.1% rhamnolipid with 1% NaOH, 4% sophorolipid, 4% sophorolipid and 2% Triton X-100, 2% Triton, 0.25% surfactin with 1% NaOH, as shown from left to right, respectively.



**Supernatants from 4% sophorolipid after 1, 2, 3, 4  
and 5 washes as shown from left to right**

**APPENDIX B:**  
**X-ray diffractogram and laboratory results**

# Hexane-extracted THC soil

```

===== Universite Laval , Quebec =====
Jade: Peak Listing                               Mon Jul 08 1996 @2:49pm
=====
File: JFD-CM1.MDI> CM 1 poudre 40Kv 20Ma P=1 JF Dube No:4815    <09:48 hr>
  
```

```

----- Scan Parameters: ----- Search Parameters: -----
Radiation = CU_1.54059           || Filter length(pts) = 25
Scan Range = 2- 65              || Noise level(sigmas) = 6.0
Step Size = .02                 || Intensity cutoff(%) = 1-100
Count Time = 1.2 sec.          || 2-Theta Zero (degs) = -.28
-----
  
```

#	Peak-Position		Centroid-Position		Peak & Area are without Bkgrd					
	2Theta	d	2Theta	d	Bkgrd	Peak	I%	Area	I%	FWHM*
1:	11.660	7.5831	11.658	7.5847	154	233	6.0	38	5.2	0.147
2:	19.798	4.4808	19.802	4.4800	104	92	2.4	21	2.9	0.205
3:	20.861	4.2548	20.861	4.2547	99	558	14.3	117	15.9	0.189
4:	23.120	3.8439	23.120	3.8440	97	170	4.4	42	5.7	0.222
5:	24.178	3.6780	24.181	3.6776	96	843	21.6	112	15.3	0.120
6:	25.479	3.4931	25.473	3.4939	106	175	4.5	28	3.8	0.144
7:	26.679	3.3386	26.676	3.3391	97	3896	100.0	734	100.0	0.170
8:	27.463	3.2452	27.469	3.2444	102	107	2.7	25	3.4	0.210
9:	28.025	3.1812	28.030	3.1807	102	889	22.8	194	26.4	0.196
10:	29.481	3.0274	29.485	3.0270	97	1917	49.2	476	64.9	0.223
11:	30.742	2.9060	30.744	2.9059	92	113	2.9	32	4.4	0.255
12:	31.019	2.8808	30.986	2.8837	88	139	3.6	60	8.2	0.388
13:	31.702	2.8202	31.702	2.8202	86	119	3.1	22	3.0	0.166
14:	33.381	2.6821	33.384	2.6819	68	136	3.5	20	2.7	0.132
15:	34.921	2.5672	34.923	2.5671	76	69	1.8	22	3.0	0.287
16:	35.122	2.5530	35.119	2.5532	76	66	1.7	11	1.5	0.150
17:	36.080	2.4874	36.080	2.4874	71	233	6.0	66	9.0	0.255
18:	36.578	2.4547	36.577	2.4547	84	66	1.7	8	1.1	0.109
19:	39.520	2.2785	39.526	2.2781	60	336	8.6	92	12.5	0.246
20:	42.441	2.1282	42.454	2.1275	54	183	4.7	31	4.2	0.152
21:	43.319	2.0870	43.310	2.0874	53	250	6.4	83	11.3	0.299
22:	45.461	1.9935	45.464	1.9934	51	82	2.1	14	1.9	0.154
23:	45.840	1.9780	45.840	1.9779	51	54	1.4	11	1.5	0.183
24:	47.223	1.9232	47.239	1.9226	52	96	2.5	25	3.4	0.234
25:	47.600	1.9088	47.617	1.9082	50	240	6.2	115	15.7	0.431
26:	48.620	1.8711	48.640	1.8704	48	260	6.7	84	11.4	0.291
27:	50.178	1.8166	50.190	1.8162	43	264	6.8	62	8.4	0.211
28:	54.917	1.6705	54.919	1.6705	52	176	4.5	28	3.8	0.143
29:	56.680	1.6227	56.685	1.6226	47	62	1.6	16	2.2	0.232
30:	57.560	1.6000	57.564	1.5999	52	97	2.5	26	3.5	0.241
31:	59.980	1.5411	59.978	1.5411	51	100	2.6	27	3.7	0.243
32:	60.779	1.5227	60.785	1.5226	46	59	1.5	12	1.6	0.183
33:	64.076	1.4521	64.076	1.4521	45	156	4.0	13	1.8	0.075

\* Intensity values are based on total raw counts.

# Dichloromethane-extracted THC soil

\*\*\*\*\* Universite Laval , Quebec \*\*\*\*\*  
 Jade: Peak Listing Mon Jul 08 1996 @2:10pm  
 \*\*\*\*\*  
 File: JFD-CM2.MDI> CM 2 poudre 40Kv 20Ma F=1 No:4816 <12:23 hr>

```

----- Scan Parameters: ----- Search Parameters: -----
Radiation = CU_1.54059           ||| Filter length(pts) = 25
Scan Range = 2- 65              ||| Noise level(sigmas) = 6.0
Step Size = .02                 ||| Intensity cutoff(%) = 1-100
Count Time = 1.2 sec.          ||| 2-Theta Zero (degs) = .2
-----
    
```

#	Peak-Position		Centroid-Position		Peak & Area are without Bkgrd					
	2Theta	d	2Theta	d	Bkgrd	Peak	I%	Area	I%	FWHM*
1:	19.820	4.4759	19.816	4.4766	93	88	6.1	13	3.1	0.133
2:	20.841	4.2589	20.830	4.2610	89	366	25.6	64	15.5	0.157
3:	21.723	4.0879	21.738	4.0852	94	52	3.6	7	1.7	0.121
4:	23.080	3.8504	23.079	3.8507	89	147	10.3	34	8.2	0.208
5:	24.257	3.6663	24.220	3.6718	90	51	3.6	14	3.4	0.247
6:	25.400	3.5039	25.392	3.5049	93	536	37.5	98	23.7	0.165
7:	26.640	3.3434	26.628	3.3450	92	998	69.7	212	51.2	0.191
8:	27.902	3.1951	27.898	3.1955	90	135	9.4	31	7.5	0.207
9:	29.440	3.0315	29.444	3.0311	92	1431	100.0	414	100.0	0.260
10:	30.039	2.9724	30.032	2.9731	97	117	8.2	18	4.3	0.138
11:	30.461	2.9322	30.474	2.9310	77	68	4.8	16	3.9	0.212
12:	30.940	2.8879	30.928	2.8890	91	260	18.2	81	19.6	0.280
13:	31.438	2.8432	31.440	2.8431	82	96	6.7	28	6.8	0.262
14:	31.680	2.8221	31.669	2.8231	79	122	8.5	28	6.8	0.207
15:	32.124	2.7841	32.124	2.7841	90	18	1.3	1	0.2	0.050
16:	33.039	2.7091	33.056	2.7077	68	39	2.7	8	1.9	0.185
17:	33.349	2.6846	33.349	2.6846	68	40	2.8	11	2.7	0.247
18:	34.541	2.5947	34.543	2.5945	74	67	4.7	8	1.9	0.107
19:	34.939	2.5660	34.939	2.5660	71	61	4.3	8	1.9	0.118
20:	36.040	2.4900	36.029	2.4908	67	197	13.8	56	13.5	0.256
21:	36.500	2.4597	36.500	2.4597	74	58	4.1	6	1.4	0.093
22:	39.460	2.2818	39.473	2.2810	55	349	24.4	90	21.7	0.232
23:	40.778	2.2110	40.778	2.2110	52	49	3.4	5	1.2	0.092
24:	42.400	2.1301	42.406	2.1298	51	115	8.0	16	3.9	0.125
25:	43.241	2.0906	43.260	2.0898	53	218	15.2	62	15.0	0.256
26:	44.699	2.0257	44.704	2.0255	53	71	5.0	16	3.9	0.203
27:	45.419	1.9953	45.421	1.9952	51	78	5.5	13	3.1	0.150
28:	45.795	1.9798	45.795	1.9798	50	65	4.5	4	1.0	0.055
29:	47.162	1.9255	47.172	1.9251	55	73	5.1	14	3.4	0.173
30:	47.580	1.9096	47.589	1.9092	49	219	15.3	97	23.4	0.399
31:	48.580	1.8726	48.599	1.8719	48	421	29.4	128	30.9	0.274
32:	50.120	1.8186	50.125	1.8184	43	88	6.1	21	5.1	0.215
33:	50.679	1.7998	50.677	1.7999	41	53	3.7	6	1.4	0.102
34:	51.058	1.7874	51.055	1.7875	39	48	3.4	8	1.9	0.150
35:	52.197	1.7510	52.197	1.7510	36	48	3.4	4	1.0	0.075
36:	56.621	1.6242	56.652	1.6234	45	49	3.4	21	5.1	0.386
37:	57.541	1.6005	57.546	1.6003	43	104	7.3	41	9.9	0.355
38:	59.959	1.5415	59.957	1.5416	44	57	4.0	9	2.2	0.142
39:	60.758	1.5232	60.759	1.5232	48	58	4.1	11	2.7	0.171
40:	61.540	1.5057	61.539	1.5057	45	54	3.8	6	1.4	0.100
41:	64.945	1.4347	64.941	1.4348	84	47	3.3	6	1.4	0.115

\* Intensity values are based on total raw counts.

# LC Sediments

\*\*\*\*\* Universite Laval , Quebec \*\*\*\*\*  
 Jade: Peak Listing Mon Jul 08 1996 @2:06pm  
 \*\*\*\*\*  
 File: JDP-CM3.MDI> CM 3 poudre 40Kv 20ma F=1 No:4817 <13:33 hr>

```
----- Scan Parameters: ----- Search Parameters: -----
Radiation = CU_1.54059           ||| Filter length(pts) = 25
Scan Range = 2- 65              ||| Noise level(sigmas) = 6.0
Step Size = .02                 ||| Intensity cutoff(%) = 1-100
Count Time = 1.2 sec.          ||| 2-Theta Zero (dms) = .2
-----
```

Peak-Position		Centroid-Position		Peak & Area are without Bkgrd						
#	2Theta	d	2Theta	d	Bkgrd	Peak	I%	Area	I%	FWHM*
1:	8.938	9.8855	8.939	9.8849	234	<del>33</del>	1.4	4	0.8	0.109
2:	12.478	7.0881	12.478	7.0881	155	87	3.8	5	0.9	0.052
3:	13.898	6.3668	13.896	6.3677	129	53	2.3	11	2.1	0.187
4:	19.880	4.4624	19.873	4.4641	118	145	6.3	45	8.4	0.279
5:	20.880	4.2510	20.878	4.2514	125	400	17.4	97	18.2	0.218
6:	22.040	4.0298	22.041	4.0296	130	157	6.8	34	6.4	0.195
7:	23.641	3.7603	23.636	3.7612	138	176	7.6	34	6.4	0.174
8:	24.360	3.6509	24.354	3.6518	137	261	11.3	49	9.2	0.169
9:	24.920	3.5701	24.919	3.5704	144	57	2.5	15	2.8	0.237
10:	25.581	3.4794	25.578	3.4799	143	85	3.7	15	2.8	0.159
11:	26.660	3.3410	26.653	3.3418	138	2302	100.0	533	100.0	0.208
12:	27.901	3.1952	27.894	3.1959	121	352	15.3	184	34.5	0.470
13:	29.860	2.9898	29.862	2.9896	94	76	3.3	12	2.3	0.142
14:	30.439	2.9342	30.432	2.9350	92	102	4.4	36	6.8	0.318
15:	30.859	2.8953	30.854	2.8958	92	72	3.1	20	3.8	0.250
16:	33.017	2.7108	33.023	2.7104	86	53	2.3	11	2.1	0.187
17:	34.620	2.5889	34.650	2.5867	95	100	4.3	49	9.2	0.441
18:	35.001	2.5616	34.992	2.5622	98	143	6.2	64	12.0	0.403
19:	36.581	2.4545	36.581	2.4545	103	140	6.1	33	6.2	0.212
20:	37.765	2.3802	37.716	2.3832	93	55	2.4	19	3.6	0.311
21:	39.541	2.2773	39.538	2.2774	83	326	14.2	68	12.8	0.188
22:	40.283	2.2371	40.278	2.2373	82	50	2.2	14	2.6	0.252
23:	41.217	2.1885	41.210	2.1888	82	<del>22</del>	1.0	3	0.6	0.123
24:	41.822	2.1582	41.819	2.1584	79	<del>26</del>	1.1	4	0.8	0.138
25:	42.461	2.1272	42.458	2.1273	76	116	5.0	28	5.3	0.217
26:	42.980	2.1027	42.980	2.1027	72	97	4.2	10	1.9	0.093
27:	45.360	1.9977	45.374	1.9972	61	59	2.6	10	1.9	0.153
28:	45.801	1.9795	45.795	1.9798	60	70	3.0	18	3.4	0.231
29:	47.326	1.9192	47.301	1.9202	58	<del>28</del>	1.2	11	2.1	0.354
30:	48.380	1.8799	48.377	1.8800	58	47	2.0	9	1.7	0.172
31:	49.860	1.8275	49.900	1.8261	53	230	10.0	75	14.1	0.293
32:	50.140	1.8179	50.110	1.8189	52	227	9.9	83	15.6	0.329
33:	50.680	1.7998	50.672	1.8001	50	71	3.1	13	2.4	0.165
34:	51.259	1.7808	51.235	1.7816	49	38	1.7	17	3.2	0.403
35:	53.161	1.7215	53.172	1.7212	60	38	1.7	10	1.9	0.237
36:	54.900	1.6710	54.906	1.6708	61	94	4.1	22	4.1	0.211
37:	55.359	1.6582	55.317	1.6594	62	45	2.0	34	6.4	0.680
38:	60.018	1.5402	60.013	1.5403	65	188	8.2	60	11.3	0.287
39:	61.796	1.5001	61.776	1.5005	66	56	2.4	27	5.1	0.434
40:	64.059	1.4524	64.052	1.4526	53	47	2.0	17	3.2	0.326

\* Intensity values are based on total raw counts.



Laboratoire **ECO** Laboratories

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**Certificat d'analyse • Certificate of Analysis**

Page 1 de 2

À l'attention de Cathy Mulligan  
 Client The SNC Research Corporation  
 2, Place Félix-Martin  
 Montréal, Qc  
 H2Z 1Z3

No de certificat 97-14397  
 Date d'émission 19/02/1997  
 Date de réception 12/02/1997  
 No. projet ---  
 Son de commande ---

Paramètre:

hydrocarbures Aromatiques Polycycliques	Méthode: EC-13 311 85		Instrument: CG-8M
Identification	TIC A	B AC	
Identification (suite)	Soil	Soil	Soil
Méthode	Soil	Soil	
Date de prélèvement	4/02/97	4/02/97	
Lieu du prélèvement	---	---	
Prélevé par	---	---	
Date de préparation	14/02/97	14/02/97	
Date d'analyse	14/02/97	14/02/97	
No de laboratoire	72380	72381	
	mg/kg	mg/kg	
Naphthalène	11	0.26	
Acénaphthylène	8.0	0.23	
Acénaphthène	26	0.24	
Fluorène	76	0.30	
Phénanthrène	90	1.3	
Anthracène	11	0.82	
Fluoranthène	17	2.5	
Pyrène	52	2.2	
7,12-Diméthylbenzo(a)anthracène	< 2	< 0.2	
Benzo(g)hépérylène	7.5	1.3	
Benzo(c)phénanthrène	6.2	0.60	
Chrysène	16	1.6	
Benzo(a)anthracène	10	1.6	
Benzo(b)fluoranthène	16	3.4	
Benzo(a)pyrène	6.1	1.4	
3-Méthylcholanthrène	< 2	< 0.2	
Indène(1,2,3-cd)pyrène	6.6	1.2	
Dibenzo(a,h)anthracène	6.7	0.90	
Dibenzo(a,h)pyrène	< 2	1.1	
Dibenzo(a,i)pyrène	< 2	< 0.2	
Dibenzo(a,j)pyrène	< 2	< 0.2	
Total	363	21	
Ajust de recouvrement (%) D10-Pyrène	N.D.	98	



Vérifié Sylvain Laporte  
 Sylvain Laporte

Chimiste Martin Brunel  
 Martin Brunel

Note: Ces résultats ne se rapportent qu'aux échantillons soumis pour analyse.

Ce certificat ne doit pas être reproduit, sinon en entier, sans l'autorisation écrite du laboratoire. Les échantillons mentionnés dans tout autre document pendant 30 jours à compter de la date de délivrance à compter d'aujourd'hui.

**APPENDIX C:**  
**Curve fitting of soil washing experiments**

### DATA CURVE FITTING RESULTS

X-VALUES:  
Y-VALUES:

INPUT DATA:	Extraction	% Cu removal
	(X)	(Y)
DATA POINT # 1 :	0	0
DATA POINT # 2 :	1	5.8
DATA POINT # 3 :	2	11.4
DATA POINT # 4 :	3	15.5
DATA POINT # 5 :	4	18.1
DATA POINT # 6 :	5	19.9

**RESULTS:**

	EQUATION	AVERAGE % DEVIATION	MAXIMUM % DEVIATION
EXPONENTIAL:	NOT APPLICABLE (NEGATIVE OR (0) Y-VALUES)	---	---
SQUARE ROOT:	$Y = -1.2531 + 9.331368 \cdot \text{SQRT}(X)$	8.85	39.28
→ LOGARITHMIC:	$Y = 5.631822 + 8.885841 \cdot \text{LN}(X)$	1.60	3.42
POWER:	NOT APPLICABLE (NEG. OR (0) X OR Y-VALUES)	---	---
INVERSE:	$Y = 21.85946 + -16.90392 / X$	7.76	17.60
LINEAR:	$Y = 1.747621 + 4.014285 \cdot X$	6.20	14.24
POLYNOMIAL:	CONSTANT COEFFICIENT: -.1571448 1 DEGREE COEFFICIENT: 6.871435 2 DEGREE COEFFICIENT: -.57143	1.42	5.91

Multiple extractions with 1% NaOH

**DATA CURVE FITTING RESULTS**

X-VALUES:  
Y-VALUES:

INPUT DATA:	Extraction	% Zn removal
	(X)	(Y)
DATA POINT # 1 :	0	0
DATA POINT # 2 :	1	1.7
DATA POINT # 3 :	2	3.4
DATA POINT # 4 :	3	4.6
DATA POINT # 5 :	4	9.5
DATA POINT # 6 :	5	10.9

**RESULTS:**

	EQUATION	AVERAGE % DEVIATION	MAXIMUM % DEVIATION
EXPONENTIAL:	NOT APPLICABLE (NEGATIVE OR (0) Y-VALUES)	---	---
SQUARE ROOT:	$Y = -1.692738 + 4.802532 \cdot \text{SQRT}(X)$	35.11	82.92
→ LOGARITHMIC:	$Y = .5065449 + 5.758188 \cdot \text{LN}(X)$	34.40	70.20
POWER:	NOT APPLICABLE (NEG. OR (0) X OR Y-VALUES)	---	---
INVERSE:	$Y = 10.63923 + -10.11511 / X$	37.82	69.16
LINEAR:	$Y = -.6333333 + 2.260001 \cdot X$	10.98	33.62
POLYNOMIAL:	CONSTANT COEFFICIENT: 3.928363E-02 1 DEGREE COEFFICIENT: 1.251074 2 DEGREE COEFFICIENT: .2017852	8.77	21.92

Multiple extractions with 1% NaOH

## DATA CURVE FITTING RESULTS

X-VALUES:  
Y-VALUES:

INPUT DATA:	Extraction	% Cu removal
	(X)	(Y)
DATA POINT # 1 :	0	0
DATA POINT # 2 :	1	31.8
DATA POINT # 3 :	2	54.9
DATA POINT # 4 :	3	77.1
DATA POINT # 5 :	4	88.5
DATA POINT # 6 :	5	93.4

RESULTS:

	EQUATION	AVERAGE % DEVIATION	MAXIMUM % DEVIATION
EXPONENTIAL:	NOT APPLICABLE (NEGATIVE OR (0) Y-VALUES)	---	---
SQUARE ROOT:	$Y = -4.335342 + 44.3447 * \text{SQRT}(X)$	7.39	25.81
→ LOGARITHMIC:	$Y = 30.75789 + 40.08582 * \text{LN}(X)$	3.47	6.63
POWER:	NOT APPLICABLE (NEG. OR (0) X OR Y-VALUES)	---	---
INVERSE:	$Y = 103.9846 + -76.30209 / X$	7.30	19.91
LINEAR:	$Y = 10.52379 + 18.83715 * X$	8.00	13.05
POLYNOMIAL:	CONSTANT COEFFICIENT: -.2678814 1 DEGREE COEFFICIENT: 35.02467 2 DEGREE COEFFICIENT: -3.237506	1.22	3.51

Multiple extractions with 0.25% surfactin + 1% NaOH  
(80% purity)

### DATA CURVE FITTING RESULTS

X-VALUES:  
Y-VALUES:

INPUT DATA:	Extraction	% Cu removal
	(X)	(Y)
DATA POINT # 1 :	0	0
DATA POINT # 2 :	1	19.6
DATA POINT # 3 :	2	38.3
DATA POINT # 4 :	3	53.9
DATA POINT # 5 :	4	62.2
DATA POINT # 6 :	5	70.4

RESULTS:

	EQUATION	AVERAGE % DEVIATION	MAXIMUM % DEVIATION
EXPONENTIAL:	NOT APPLICABLE (NEGATIVE OR (0) Y-VALUES)	---	---
SQUARE ROOT:	$Y = -4.92356 + 32.6808 * \text{SQRT}(X)$	9.93	41.61
→ LOGARITHMIC:	$Y = 18.48516 + 31.74401 * \text{LN}(X)$	2.81	5.71
POWER:	NOT APPLICABLE (NEG. OR (0) X OR Y-VALUES)	---	---
INVERSE:	$Y = 76.29014 + -60.0222 / X$	8.74	20.83
LINEAR:	$Y = 5.347619 + 14.15429 * X$	5.40	12.12
POLYNOMIAL:	CONSTANT COEFFICIENT: -.5214162 1 DEGREE COEFFICIENT: 22.95784 2 DEGREE COEFFICIENT: -1.76071	1.66	5.48

Multiple extractions with 0.25% surfactin + 1% NaOH

### DATA CURVE FITTING RESULTS

X-VALUES:  
Y-VALUES:

INPUT DATA:	Extraction	% Zn removal
	(X)	(Y)
DATA POINT # 1 :	0	0
DATA POINT # 2 :	1	5.8
DATA POINT # 3 :	2	13.9
DATA POINT # 4 :	3	19.5
DATA POINT # 5 :	4	23.6
DATA POINT # 6 :	5	26.5

RESULTS:

	EQUATION	AVERAGE % DEVIATION	MAXIMUM % DEVIATION
EXPONENTIAL:	NOT APPLICABLE (NEGATIVE OR (0) Y-VALUES)	---	---
SQUARE ROOT:	$Y = -2.500696 + 12.44333 * \text{SQRT}(X)$	15.32	71.42
→ LOGARITHMIC:	$Y = 5.460904 + 12.94947 * \text{LN}(X)$	2.44	5.84
POWER:	NOT APPLICABLE (NEG. OR (0) X OR Y-VALUES)	---	---
INVERSE:	$Y = 29.06848 + -24.54411 / X$	10.27	21.99
LINEAR:	$Y = 1.204762 + 5.471428 * X$	7.88	15.10
POLYNOMIAL:	CONSTANT COEFFICIENT: -.6107109 1 DEGREE COEFFICIENT: 8.194641 2 DEGREE COEFFICIENT: -.5446426	4.55	21.36

Multiple extractions with 0.25% surfactin + 1% NaOH

### DATA CURVE FITTING RESULTS

X-VALUES:  
Y-VALUES:

INPUT DATA:	Extraction	% Cd removal
	(X)	(Y)
DATA POINT # 1 :	0	0
DATA POINT # 2 :	1	5.4
DATA POINT # 3 :	2	9.6
DATA POINT # 4 :	3	11.9
DATA POINT # 5 :	4	12.6
DATA POINT # 6 :	5	13.1

**RESULTS:**

	EQUATION	AVERAGE % DEVIATION	MAXIMUM % DEVIATION
EXPONENTIAL:	NOT APPLICABLE (NEGATIVE OR (0) Y-VALUES)	---	---
SQUARE ROOT:	$Y = 1.961574E-02 + 6.261061 * \text{SQRT}(X)$	6.68	16.30
→ LOGARITHMIC:	$Y = 5.829247 + 4.898967 * \text{LN}(X)$	4.50	7.94
POWER:	NOT APPLICABLE (NEG. OR (0) X OR Y-VALUES)	---	---
INVERSE:	$Y = 14.95117 + -9.703277 / X$	1.81	5.20
LINEAR:	$Y = 2.380952 + 2.554285 * X$	10.31	21.98
POLYNOMIAL:	CONSTANT COEFFICIENT: 8.928728E-02 1 DEGREE COEFFICIENT: 5.991784 2 DEGREE COEFFICIENT: -.6874997	1.44	3.62

Multiple extractions with 0.25% surfactin + 1% NaOH

### DATA CURVE FITTING RESULTS

X-VALUES:  
Y-VALUES:

INPUT DATA:	Extraction	% Cu removal
	(X)	(Y)
DATA POINT # 1 :	0	0
DATA POINT # 2 :	1	18
DATA POINT # 3 :	2	25.1
DATA POINT # 4 :	3	27.4
DATA POINT # 5 :	4	34.6
DATA POINT # 6 :	5	40.5

RESULTS:

	EQUATION	AVERAGE % DEVIATION	MAXIMUM % DEVIATION
EXPONENTIAL:	NOT APPLICABLE (NEGATIVE OR (0) Y-VALUES)	---	---
SQUARE ROOT:	$Y = -7.122651E-02 + 17.42085 \cdot \text{SQRT}(X)$	3.35	9.86
→ LOGARITHMIC:	$Y = 16.53332 + 13.14538 \cdot \text{LN}(X)$	6.15	13.04
POWER:	NOT APPLICABLE (NEG. OR (0) X OR Y-VALUES)	---	---
INVERSE:	$Y = 40.03228 + -23.89548 / X$	8.97	17.03
LINEAR:	$Y = 6.080957 + 7.274284 \cdot X$	8.66	25.80
POLYNOMIAL:	CONSTANT COEFFICIENT: 2.503583 1 DEGREE COEFFICIENT: 12.64035 2 DEGREE COEFFICIENT: -1.073213	8.05	21.82

Multiple extractions with 0.1% rhamnolipid + 1% NaOH

### DATA CURVE FITTING RESULTS

X-VALUES:  
Y-VALUES:

INPUT DATA:	Extraction	% Zn removal
	(X)	(Y)
DATA POINT # 1 :	0	0
DATA POINT # 2 :	1	11.5
DATA POINT # 3 :	2	13.2
DATA POINT # 4 :	3	14.8
DATA POINT # 5 :	4	15.7
DATA POINT # 6 :	5	16.7

RESULTS:

	EQUATION	AVERAGE % DEVIATION	MAXIMUM % DEVIATION
EXPONENTIAL:	NOT APPLICABLE (NEGATIVE OR (0) Y-VALUES)	---	---
SQUARE ROOT:	$Y = 1.749453 + 7.325318 * \text{SQRT}(X)$	7.47	21.08
→ LOGARITHMIC:	$Y = 11.29364 + 3.223363 * \text{LN}(X)$	1.24	2.48
POWER:	NOT APPLICABLE (NEG. OR (0) X OR Y-VALUES)	---	---
INVERSE:	$Y = 17.13158 + -6.025357 / X$	2.95	6.96
LINEAR:	$Y = 5.004762 + 2.79143 * X$	13.02	32.20
POLYNOMIAL:		8.10	26.44
	CONSTANT COEFFICIENT: 1.689282 1 DEGREE COEFFICIENT: 7.764648 2 DEGREE COEFFICIENT: -.9946438		

Multiple extractions with 0.1% rhamnolipid + 1% NaOH

## DATA CURVE FITTING RESULTS

Rhamnolipid		
Concentration (%)	(X)	% Cu removal
	(X)	(Y)
DATA POINT # 1 :	0	0
DATA POINT # 2 :	1	4
DATA POINT # 3 :	2	10
DATA POINT # 4 :	4	14
DATA POINT # 5 :	8	30
DATA POINT # 6 :	12	35

**RESULTS:**

	EQUATION	AVERAGE % DEVIATION	MAXIMUM % DEVIATION
EXPONENTIAL:	NOT APPLICABLE (NEGATIVE OR (0) Y-VALUES)	---	---
SQUARE ROOT:	$Y = -4.004009 + 10.92994 * \text{SQRT}(X)$	21.46	73.14
LOGARITHMIC:	$Y = 1.597046 + 12.79613 * \text{LN}(X)$	22.68	60.07
POWER:	NOT APPLICABLE (NEG. OR (0) X OR Y-VALUES)	---	---
INVERSE:	$Y = 30.49939 + -30.38142 / X$	40.80	97.05
LINEAR:	$Y = 2.041861 + 2.990698 * X$	11.24	25.81
POLYNOMIAL:	CONSTANT COEFFICIENT: -.2484689 1 DEGREE COEFFICIENT: 4.751346 2 DEGREE COEFFICIENT: -.1475787	7.74	17.11

## DATA CURVE FITTING RESULTS

	Rhamnolipid Concentration (%)	% Zn removal
	(X)	(Y)
DATA POINT # 1 :	0	0
DATA POINT # 2 :	1	2
DATA POINT # 3 :	2	6
DATA POINT # 4 :	4	8
DATA POINT # 5 :	8	15
DATA POINT # 6 :	12	18

**RESULTS:**

	EQUATION	AVERAGE % DEVIATION	MAXIMUM % DEVIATION
EXPONENTIAL:	NOT APPLICABLE (NEGATIVE OR (0) Y-VALUES)	---	---
SQUARE ROOT:	$Y = -1.740206 + 5.551759 * \text{SQRT}(X)$	19.87	90.57
LOGARITHMIC:	$Y = 1.298523 + 6.398063 * \text{LN}(X)$	14.75	35.07
POWER:	NOT APPLICABLE (NEG. OR (0) X OR Y-VALUES)	---	---
INVERSE:	$Y = 15.87897 + -15.52078 / X$	32.24	82.09
LINEAR:	$Y = 1.406202 + 1.502326 * X$	16.28	45.42
POLYNOMIAL:	CONSTANT COEFFICIENT: 8.074725E-02 1 DEGREE COEFFICIENT: 2.521245 2 DEGREE COEFFICIENT: -8.540651E-02	9.63	25.82