Phytoremediation of Soil containing Mixed Contaminants

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ABSTRACT

Phytoremediation of Soil containing Mixed Contaminants

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This study investigated the application of surfactants and chelates to enhance the removal of mixed contaminants [Cd (II), Pb (II) and used engine oil] from a sandy soil cultivated with Indian mustard plants. For chelate additives, EDTA (Ethylenediamine tetraacetic acid) was found to be more efficient than EDDS (Ethylenediamine disuccinic acid) in increasing the accumulation of metal contaminants Pb (II) in the plants. EDTA was also more capable of removing the used engine oil through rhizodegradation than EDDS. EDTA caused a sharper decrease in basal soil respiration (BSR) than EDDS, indicating that the former was much more toxic to the microbes.

For surfactant additives, the results showed that Triton X-100 and Tween 80 at concentrations higher than their critical micellar concentration enhanced phytostabilization of Pb (II). The application of Tween 80 resulted in an increase in phytoremediation of Pb (II). At the same concentrations, Tween 80 was more effective than Triton X-100 in facilitating rhizodegradation of the used engine oil. Soil basal microbiological respiration tests showed that the application of Tween 80 resulted in an increase in BSR. These tests indicated that the lower concentration of Triton X-100 had a slightly positive effect on BSR, whereas at higher concentrations, it was inhibitory to the microbes.

Empirical phytoremediation models linked to the removal of the heavy metals from the soil were formulated in the study. The two first order kinetic models were able to describe the leaching process for both Cd (II) and Pb (II). The models also revealed that the uptake of Pb (II) and Cd (II) were well described by the Freundlich type model, in the presence of surfactants. On the other hand, in the presence of chelates the uptake of Pb (II) and Cd (II) was found to follow the Langmuir type model. According to the leachability index (LI) determined in the tests, all surfactants tested can be considered as safe additives for enhancing phytoremediation. Compared to Triton X-100, Tween 80 resulted in lower diffusivity of metals tested and higher values of LI indicating that this surfactant was also safer from the point of view of reducing ground water pollution. Compared to EDTA, EDDS resulted in higher values of LI, which is desirable.

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List of Symbols and Abbreviations

ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
BAF	Bioaccumulation Factor
BSR	Basal Soil Respiration
b	Langmuir constant
C	Final concentration of metal in the soluble form in soil
CAS	Chemical Abstracts Service
CEC	Cation Exchange Capacity
CMC	Critical Micelle Concentration
C_o	Metal concentration in soil before adding additives
C_i	Computed value
C_f	Metal concentration in the soil applied to the fast leaching compartment
C_L	Metal leached
C_{rf}	Metal concentration in the soil applied to the relatively fast leaching compartment
C_s	Metal concentration in the soil applied to the slow leaching compartment
DM	Dry Matter
D_{obs}	Observed diffusivity
EDDHA	2-Hydroxyphenylacetic Acid
EDDS	Ethylenediamine Disuccinic Acid
EDTA	Ethylenediamine Tetraacetic Acid
FAAS	Flame Atomic Adsorption Spectrometer

HOCs	Hydrophobic Organic Compounds
HMW	High Molecular Weight
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
k	Leaching rate
k_f	Fast leaching rate
K_f	Sorption capacity of Freundlich Model
K_H	Hydraulic conductivity
K_L	Sorption capacity of Langmuir Model
K_{ow}	Octanol-water partition coefficient
k_{rf}	Relatively fast leaching rate
k_s	Slow leaching rate
LI	Leachability Index
LMW	Low Molecular Weight
MEFQ	Ministère de l'Envirormement et de la Faune du Québec
MTBE	Methyl Tert-Butyl Ether
M_I	Initial mass of metal in the soil
M_T	Total mass of the metal in leachate
NPL	National Priority List
n	Freundlich constant
OC	Organic Content
O_i	Observed value
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls

q	Metal concentration in the plant
TPH	Total Petroleum Hydrocarbons
W_B	Weight of the burned soil
W_D	Weight of the dry Soil
W_{OM}	Weight of organic matter
U.S.EPA	United States Environment Protection Agency

Chapter 1

Introduction

1.1 Problem Statement

Heavy metals and hydrophobic organic compounds (HOCs) such as used engine oil are two important soil contaminants. About 9.6 % of the National Priority List (NPL) of sites was contaminated with heavy metals alone, while as high as 67.7 % was polluted by heavy metals and HOCs (U.S.EPA, 2003a). Roane et al. (2001) reported that approximately 55% of all hazardous waste sites contained mixed contaminants formed by heavy metals and HOCs. Soil, water and air contamination and exposure to heavy metals such as arsenic, mercury, cadmium and lead are serious growing concerns throughout the world. There are hundreds of sources of heavy metal pollution that include industries linked to coal, natural gas, paper, and chlor-alkali compounds (Alloway, 1995). There are more than 20 heavy metals, but four are of particular concern to health: As (II), Cd (II), Hg (II) and Pb (II). They are four of the top six hazardous materials present in toxic waste sites. These are highly toxic and can cause damaging effects even at very low concentrations. Also, they tend to get transported into the food chain and hence can be stored in tissues.

Soil contamination by Pb (II) and Cd (II) is of great concern because both of them are toxic to humans (U.S.EPA, 1998). Lead and cadmium are used in many industrial, urban, and agricultural applications. Pb (II) has low mobility in soil compared to Cd (II). Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants that are found in high concentration at sites linked to former manufactured gas plants and wood treatment activity (Cornelia, 1992).

PAHs are divided into two categories: low molecular weight (LMW) and high molecular weight (HMW). LMW PAHs are composed of fewer than four rings and HMW PAHs are composed of at least four rings. Solubility and volatility of PAHs directly depend on the number of rings. HMW PAHs are commonly distributed in the environment and because of their persistence and carcinogenic potential are main pollutants in the soil water system. HMW PAHs sorb to soils and sediments and will generally take weeks or months to break down in the environment. Microorganisms in soils and sediments are the main cause of this breakdown. These PAHs are carcinogenic to animals and humans. In the United States, approximately 800 million gallons of used motor oil are recycled annually (U.S. EPA, 2001). However, significant volumes of oil continue to be discharged improperly into local lands.

Used engine oil is a common and toxic environmental contaminant, and successful technologies for its remediation vary. Used engine oil is a petroleum hydrocarbon (PHC) representing a complex mixture of individual chemical constituents. Used engine oil contain hundreds to thousands of hydrocarbon compounds, including a substantial fraction of nitrogen- and sulfur-containing compounds (Koma et al. 2001). The existence of mixed contaminants (PAHs and heavy metals) in soils causes a great challenge for remediation. Although most sites require remediation for both contaminant groups (U.S. EPA, 2004), research has continued to focus on the remediation of either PAHs or heavy metals from soil. Very few conventional techniques are found to address the remediation of mixed contaminant sites (Sharma and Reddy, 2004). Effective remediation processes for mixed contaminants is a present need. However, few techniques have been investigated for remediation of these persistent contaminants in soils. In the next chapter these factors are discussed in more detail.

1.2 Objectives of the Research

The main objective of this study was to investigate the effects of soil additives on phytoremediation of soil polluted with mixed contaminants (heavy metals and organic pollutants) which are at low level concentrations. The greenhouse tests were conducted in two phases separately. In phase I, experiments were performed to assess the ability of surfactants for the removal of contaminants by the phytoremediation system. In phase II, similar to phase I, the tests were extended to study the ability of chelates for the removal of contaminants by the phytoremediation system.

The research aims to realize the following goals:

1. Determine the ability of the Indian mustard (Brassica juncea) plant to remove the mixed contaminant formed by Pb (II), Cd (II) and used engine oil present in a sandy soil through phytoremediation.
2. Examine the effects of soil additives (surfactants and chelates) on the uptake of heavy metals Pb (II), Cd (II) by the Indian mustard plant.
3. Investigate the effects of additives on rhizodegradation of used engine oil present in the mixed contaminants.
4. Study the effects of additives on soil microbial basal respiration which is an indicator of microbial activity.
5. Develop and assess the ability of a few empirical models to predict plant uptake of Cd (II) and Pb (II) from the soil media.

6. Evaluate the effects of additives on the leaching of Pb (II) and Cd (II) during the phytoremediation of soil containing mixed contaminants.

1.3 Organization of the Thesis

This thesis is composed of eight chapters.

Chapter 1 deals with the problem statement, research objectives and thesis organization.

Chapter 2 deals with literature review, including a discussion of phytoremediation systems for contaminated soils. This chapter also deals briefly with relevant facts related to phytoremediation systems.

Chapter 3 introduces the materials used and describes the experimental methods employed, as well as the analytical methods applied.

Chapter 4 discusses the effectiveness of chelates in remediating the contaminated soil through phytostabilization and phytoextraction of metals, as well as rhizodegradation of used engine oil.

Chapter 5 focuses on the effects of different surfactants on the removal of Cd (II), Pb (II) and used engine oil from the contaminated soil in which Indian mustard was grown.

Chapter 6 develops a few empirical models related to uptake of Cd (II) and Pb (II).

Chapter 7 assesses the leaching behavior of Cd (II) and Pb (II) in test pots.

Chapter 8 deals with conclusions and contributions.

In chapter 2, existing information related to phytoremediation are presented.

Chapter 2

Literature Review

2.1 Introduction

In Canada, the provinces and territories are responsible for developing site-specific clean up approaches. In Quebec, the provincial government, through the Ministère de l'Envirormement et de la Faune du Québec (MEFQ) has introduced guidelines for soil rehabilitation. The MEFQ has established controls to preserve the health of humans and protect the environment (MEFQ, 1999). Table 2.1 shows the level of generic criteria (A, B and C) for soils given by the MEFQ. The maximum concentration for each type of land used is indicated by these levels. The levels (A, B and C) may be defined as follows:

•Level A

At this level, the soil is slightly contaminated. There is no need to decontaminate the soil. However, one should know the sources of contamination and verify if new sources of contaminants exist. The soil can be used for residential purposes.

• Level B

This level defines the maximum acceptable levels for residential, recreational and institutional sites (hospitals, schools and daycare centers), including commercial sites located in residential districts.

• Level C

This level denotes the maximum acceptable limit for industrial sites and for commercial sites not located in a residential area. At this level, it is necessary to take action to decontaminate the soil.

Level higher than C

At this level, the soil is entirely contaminated and it will be necessary to carry out a detailed study and restoration process, before allowing any use of the soil. This soil cannot be used for any purpose.

Table 2.1 Criteria for soils: contamination level (mg/kg)

Element	A	B	C
Cadmium (Cd)	**1.5**	**5**	**20**
Cobalt (Co)	15	50	300
Copper (Cu)	40	100	500
Manganese (Mn)	770	1000	2200
Mercury (Hg)	0.2	2	10
Molybdenum (Mo)	2	10	40
Nickel (Ni)	50	100	500
Lead (Pb)	**50**	**500**	**1000**
Selenium (Se)	1	3	10
Zinc (Zn)	110	500	1500

(MEFQ, 1999)

2.2 Bioremediation

Bioremediation is defined as any process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition. Bioremediation allows natural processes to clean up harmful chemicals in the environment. Microscopic “bugs” or microbes that live in soil and groundwater consume certain harmful chemicals such as those found in gasoline and oil spills. Bioremediation has been used to successfully clean many polluted sites and is being used at 50 superfund sites across the United States of America. Superfund is related to the environmental program established to address selected abandoned hazardous waste sites (U.S. EPA, 2001). Bioremediation processes can be broadly categorized into two groups: ex situ and in situ. Ex situ bioremediation technologies contain bioreactors, biofilters, land farming and some composting methods. In situ bioremediation technologies include bioventing, biosparging. In situ treatments tend to be more attractive to vendors and responsible parties because they require less equipment, generally have a lower cost and generate fewer disturbances to the environment. However, the difficulties associated with implementing in situ processes have limited their application in the field (U.S. EPA, 2001).

2.3 Soil remediation and phytoremediation

Soil has a critical role in sustaining human welfare and agricultural productivity as well as environmental sustainability. Technologies to remediate contaminated soil can be classified as follows (U.S. EPA, 1998).

I) In-situ which is always done on-site

II) Ex-situ which can be done on or off-site

In the ex-situ remediation, the contaminant soil is excavated, and this is followed by treatment on-site or transported to a different location for treatment and disposal. The main advantage of the in situ treatment is that it allows soil to be treated without being excavated, transported. This results in significant cost savings (Table 2.2). In the in-situ treatment, the site can be treated without removing soil from the ground. Containment wells that create hydraulic barriers are shown to reduce the leakage of contaminants out of the boundaries of a target site (Vo et al, 2008). Phytoremediation is a bioremediation system in which use of green plants for in situ removal and degradation of contamination from soil, sludge, sediments, and ground water. Growing and, in some cases, harvesting plants on a contaminated site is a remediation method. It is effective to clean up sites with shallow, low to moderate levels of contamination (U.S. EPA, 1998).

Table 2.2 Estimated cost savings using phytoremediation vs. conventional treatment

Contaminant & matrix	Application	Cost per acre	Conventional treatment	Cost (annual running)	Projected savings
Lead in soil	Planting, irrigation water ,harvesting & disposal	$150-250k	Excavate & landfill	$500k	50-65%
Solvents in groundwater	Degradation and hydraulic control installation & maintenance	$100k	Pump & treat (earlier method)	$350k	15%
TPH in soil	Degradation	$50-100k	Excavate & landfill or incinerate	$500k	80%

(U.S. EPA, 2003a)

These include removal of contaminants such as PCBs (Polychlorinated biphenyls) by plants from soil and water, reduction of heavy metals like lead from brownfield sites and the removal of uranium from water by rhizofiltration.

Phytoremediation in Etobioke (Ontario)

A pilot-scale field trial of phytoextraction of PCBs provides insight into the practical application of phytoremediation, using pumpkin plant and sedge grass. The in situ remediation was carried out in Etobioke (Ontario) in 2006. The soil was contaminated by approximately 9000 tons of PCBs (<50 mg/kg).The site was cleaned and covered by an asphalt cap. Results were expressed in terms of shoot bioaccumulation factors (BAF = PCBs shoot / PCBs soil). BAF of 0.29 was achieved in sedge grass while pumpkin plants produced shoot BAFs of only 0.15. All two plant species are viable for PCBs phytoextraction (Melissa et al. 2007).

Phytoremediation in Careswell golf (Texas)

In 1996, the U.S. Air Force planted 662 eastern cottonwood trees to attenuate a TCE (Trichloroethylene) plume in groundwater that was migrating beneath the Carswell Golf (Texas). Results showed that the use of a phytoremediation system intercepted and removed part of the TCE plume. The technology used both hydraulic influence and in-situ biologically mediated reductive dechlorination. Hydraulic influence involves the interception and usage of contaminated groundwater by the trees. Biologically-mediated reductive dechlorination involved the generation of subsurface biodegradable organic matter by the tree root systems. The observed reduction in the mass flux of TCE across the down gradient end of the demonstration site was 11 percent (U.S. EPA, 2003a).

Phytoremediation in Trenton (New Jersey)

Successful reduction of lead contamination phytoextraction was demonstrated at a site in Trenton (New Jersey) that had been used for the manufacture of lead acid batteries. In 1999, phytoextraction using Indian mustard (Brassica juncea) was able to reduce the average surface lead concentration by 13 percent after six weeks of cultivation (U.S. EPA, 2001).

Rhizofiltration in Ashtabula (Ohio)

Dushenkov et al. (1997) tested the ability of sunflower to remove uranium (U) from an Ashtabula, Ohio, site with a U concentration of 56 mg / L. Sunflower removed more than

95% of the U from solution in 24 hours. Almost all of U removed from water in a laboratory experiment was concentrated in the roots. The bioaccumulation factor (BAF) based on the ratio of U concentration in the roots to U concentration in the water reached 30,000. Shoot U concentration was < 5mg /kg and root concentration was > 15,000 mg / kg. The suitable pH for U removal using rhizofiltration was found to be 5.5.

2.4 Fates of pollutants during phytoremediation

Phytostabilization

Phytostabilization is the immobilization of a contaminant in soil through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants (Fig 2.1), and the use of plant roots to decrease contaminant migration through leaching, and soil dispersion.

Phytostimulation

Plants can facilitate biodegradation of organic pollutants by microbes in their rhizosphere (Fig 2.1). This is called phytostimulation or rhizodegradation (Pilon-Smits, 2005). Phytostimulation works well for hydrophobic organics (PCBs, PAHs) that cannot be taken up by plants.

Phytodegradation

Plants can degrade organic pollutants directly through their own enzymatic activities, a process called phytodegradation (McCutcheon et al. 2003). Phytodegradation is used for organics that are mobile in plants such as herbicides, TNT (Trinitrotoluene), MTBE (Methyl tert-butyl ether) and TCE (Trichloroethylene). Phytodegradation of TCE by poplar tree has been the most popular and efficient species so far to degrade these pollutants.

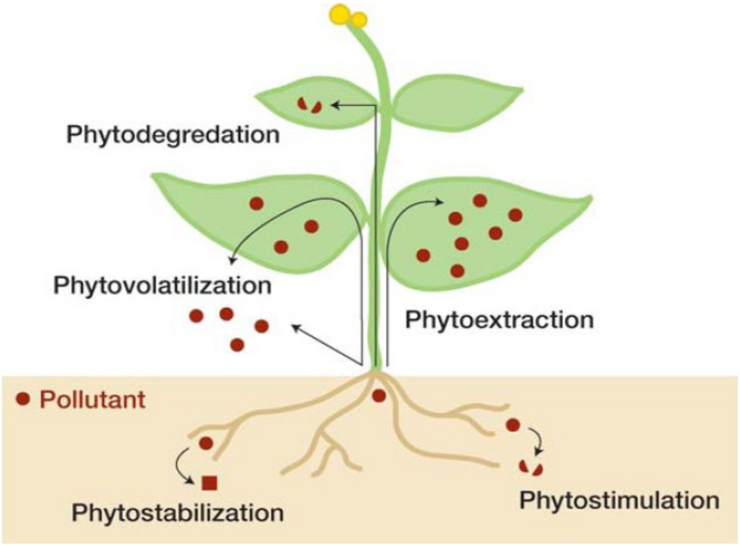

Fig 2.1 Possible fates of pollutants during phytoremediation
(Pilon-Smits, 2005)

Phytoextraction

Phytoextraction, also called phytoaccumulation refers to the uptake by plant roots of contaminants from the soil water system and translocation into plant parts, preferably shoots of the plant. Phytoextraction is usually associated with metal contaminants. Plants called hyper accumulators absorb large amounts of metals in comparison to other plants. A single plant species or a combination of plant species is selected, based on the type of metals present and/or other site conditions, and planted at the site. Plants for phytoextraction metal removal from soil should have the following characteristics (Garbisu, 2002).

I) Tolerance to high levels of contamination (metals and organics)

II) Ability to accumulate high levels of contaminants

III) Possess rapid growth rate

IV) Ability to produce high biomass though the development of profuse root system and an accumulation of a large range of heavy metals in their above-ground parts.

Phytovolatilization

Phytovolatilization occurs as plants take up water containing organic contaminants and release the contaminants into the air through their leaves. Plants can also break down organic contaminants and release breakdown products into air through leaves. Phytovolatilization generally is applied to groundwater but can also be applied to soluble soil contaminants (U.S.EPA, 2006). Phytovolatilization has been applied to both organic and inorganic contaminants (Table 2.3), but it must be reiterated that simply volatilizing a contaminant may not be an acceptable alternative (U.S.EPA, 2006). It also indicates the depth range in which each of the species is most effective.

2.5 Phytoremediation of metals

Phytoremediation of metals is a cost-effective green technology based on the use of specially selected metal-accumulating plants to remove metals from soil and water. The plants need not only macronutrients (N, P, K, S, Ca, and Mg), but also essential micronutrients such as Fe, Zn, Mn, Ni, Cu, and Mo. Many metals such as Zn, Mn, Ni and Cu are essential micronutrients. In common non accumulator plants, accumulation of these micronutrients does not exceed their metabolic needs (<10ppm) (U.S.EPA, 2001). Typical root depths of four plants commonly used in phytoremediation is shown in Fig 2.2. The figure illustrates the potential application of phytoremediation to generally shallow soils (U.S.EPA, 2000).

Table 2.3 Phytoremediation plants

Application	Media	Typical contaminants	Typical plants
Phytodegradation	Soil, groundwater landfill leachate	Herbicides, TCE	Salix family, including poplar, legumes (clover, alfalfa, cowpeas)
Phytostimulation	Soil, sediments, confined disposal facilities	Biodegradable organics (TPH, PAHs, PCBs pesticides)	Grasses with fibrous roots (Bermuda, fescue, rye)
Phytostabilization	Soil	Heavy metals, hydrophobic organics that are not biodegradable	Phreatophytic trees for hydraulic control; grasses with fibrous roots for erosion control
Phytoextraction	Soil and sediments	Metals (Pb, Cd, Zn, Ni, Cu)	Indian mustard (*Brassica juncea*)& sunflowers (*Helianthus* spp.)
Phytovolatilization	Soil and sediments	Selenium, arsenic, mercury & volatile organic compounds MTBE	Indian mustard & trees for groundwater capture

(Annette and Schnoo, 2001)

Hyper accumulator plants tolerate particularly high amounts of toxic substances, usually a metal or metalloid in their shoots during normal growth (Reeves, 1992; Baker and Whiting, 2002). The metal concentration that must be accumulated by the plant before it is designated a “hyper accumulator” depends on the particular metal.

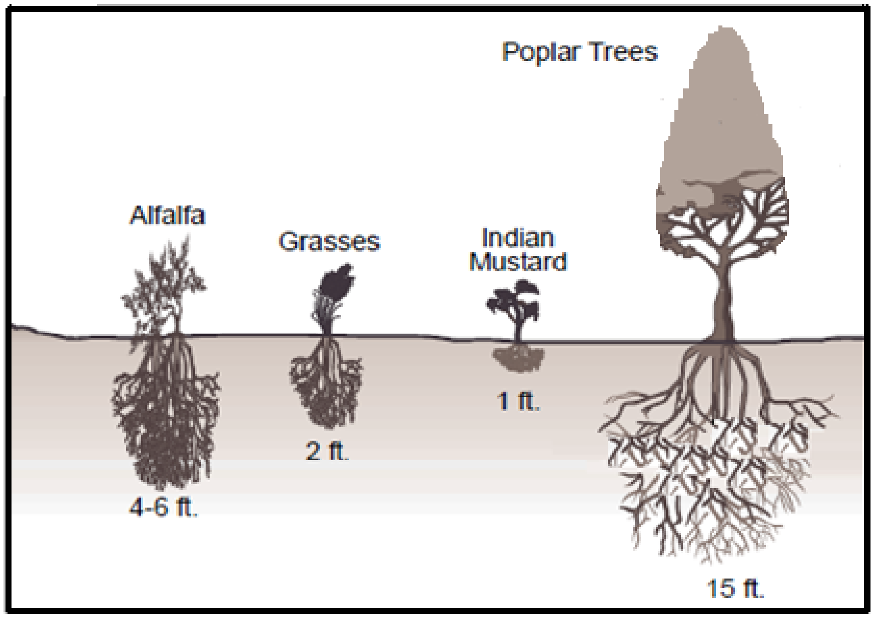

Fig 2.2 Typical root depths of four plants commonly used in phytoremediation (U.S.EPA, 2000)

Brooks (1977) stated that nickel (Ni) hyper accumulators as those accumulating greater than 1000 mg Ni kg^{-1} (0.1%) dry weight in their leaves. Baker and Whiting (2002) defined threshold concentrations for other metals hyper accumulated in plants as 100 mg kg^{-1} (0.01%) dry weight for Cd, 1,000 mg kg^{-1} (0.1%) dry weight for Ni, Cu, Co, Pb, and 10,000 mg kg^{-1} (1%) dry weight for Zn and Mn. Heavy metals themselves exhibit varying affinities for soil surfaces. For example, Pb (II) and Cu (II) are strongly sorbed to soil surfaces, while Cd (II) and Zn (II) generally have lower affinities for sorption. There is the decreased sorption of Cd (II), Cu (II), Pb (II), and Zn (II) when these metals are added together compared to when they are added alone to soils. Apparently due to chemical characteristics, Pb (II) is sorbed in preference to Cd (II) or Ca (II) regardless of the order in which it entered the soil systems (Kabata-Pendias, 2001).

Table 2.4 Approximate concentration of heavy metals in leaf tissue for plants (mg/kg)

Metal	Deficient	Normal	Toxic
Cd	----	0.05 – 0.2	5 – 30
Cu	2-5	5 – 30	50 – 100
Ni	----	0.1 – 5	10 – 100
Pb	----	5 – 10	30 – 300
Zn	10 – 20	27 -100	150- 400

(Kabata-Pendias, 2001)

Kabata-Pendias (2001) quantified the concentration of heavy metals in leaf tissue for plants (Table 2.4). The content of Cd (II) accumulated the fastest in plant tissues with increased Cd (II) concentration in soil solution, compared to Zn (II) and Cu (II). Mobility of metals in plant tissues and their total content in plants do not correspond to the metals content in soil solution and their changes.

2.6 Phytoremediation of organic pollutants

Organic pollutants are usually anthropogenic. There are no transporters for these compounds in plant membranes. Therefore organic pollutants tend to move into and within plant tissues driven by simple diffusion, depending on their chemical properties. Because the movement of organics into and through plants is a physical rather than a biological process, it is fairly predictable across plant species and lends itself well to modeling (Davis, 2003). The octanol-water partition coefficient K_{ow} is defined as the ratio of the chemical concentration in the octanol phase to its concentration in the aqueous phase. Chemicals that are quite water soluble (log K_{ow} < 1.0) are not sufficiently sorbed to roots nor actively transported through plant membranes (Helmond, 1999). Hydrophobic chemicals with log K_{ow} higher than 3.5 are bound strongly to the surface of roots and soil. Hence, they cannot be translocated easily within the plant. As such, hydrophobic chemicals with log K_{ow} > 3.5 are candidates for biodegradation in the root zone of the plant. The success of phytoremediation of hydrocarbon-contaminated soil is connected to plant capacity to enhance microbial activity in the rhizosphere. Plants adapted to contamination provide favorable conditions (such as additional supply of oxygen, nitrogen, and a carbon source through plant-root zone) for degradation by microbes (Anderson et al. 1993; Günther et al. 1996). The intense microbial activity in the rhizosphere has been utilized to biodegrade relatively recalcitrant compounds, such poly-aromatic hydrocarbons. Degradation rates in the rhizosphere are often higher, and acclimation periods are shorter in rhizosphere soil as compared to non rhizosphere soil (Eweis et al. 1998). Based on laboratory and pot experiments, Joner et al. (2002) and Chen et al. (2003) reported that plants enhance the dissipation of poly-aromatic hydrocarbons (PAHs) such as used engine oil. Willow plant (Salix viminalis) has been evaluated for the dissipation of mineral oil and PAHs in dredged sediment (Vervaeke et al. 2003). Mineral oil concentration decreased 57% after 1.5 years in the willow-planted treatment compared to 15% in unplanted controls. Dmitrieva et al. (2008) compared the results of oil-sludge degradation in the root zone of alfalfa plant with rye plant. The estimation of oil-sludge degradation in the root zone of the tested plants showed that rye plant accelerated cleanup most effectively, degrading all of the main contaminant fractions in the oil sludge by a total of 52%.

2.7 Phytoremediation of mixed contaminants

Only very limited studies linked to mixed contaminants (heavy metals and organic pollutants) have been conducted in the area of phytoremediation. For instance, in earlier study, a phytoremediation system composed of metal-tolerant plants inoculated with hydrocarbon-degrading or plant growth promoting bacteria was suggested as a technique for remediation of sites polluted by mixed contaminants such as hydrocarbons and heavy metals (Duxbury et al. 2000; Duxbury, 2000). Roy (2005) studied phytoremediation by willow plants grown in soil contaminated with 150 mg/kg of PAHs, 1760 mg/kg of Cu (II) and 3560 of Zn (II) during 2 weeks. The plant was able to remove 80 % of PAHs, 10 % of Cu (II) and 12% of Zn (II). In a recent study related to phytoremediation of marine sediments, Almeida et al. (2008) showed that PAHs can alter the Cu (II) sorption by plants or modify the Cu (II) solubility.

2.8 Surfactants and phytoremediation

Surfactants are organic compounds that are amphiphilic, which have both hydrophobic tail groups and hydrophilic head groups. They are soluble in organic solvents and water (Myers, 2006). Surfactants reduce the surface tension of water by adsorbing at the liquid-gas interface. They decrease the interfacial tension between oil and water by adsorbing at the liquid-liquid interface. Critical micelle concentration (CMC) is defined as the concentration of surfactant above which micelles are spontaneously formed. CMC is different for each surfactant. The formation of surfactant micelles is affected by temperature (Fig 2.3).

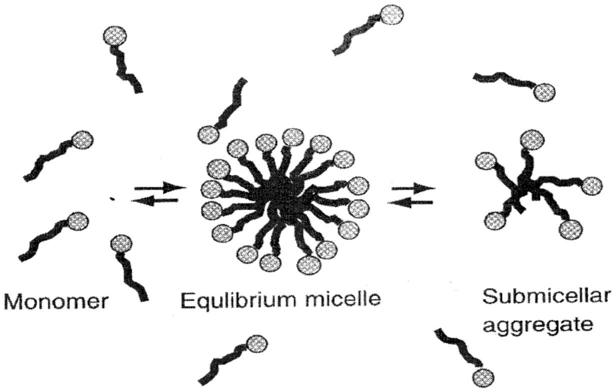

Fig 2.3 Surfactant formation (Myers, 2006)

The common classification of surfactant is based on the nature of the hydrophilic group. The following is a summary of the characterization of surfactant -classification (Myers, 2006).

1) Anionic: The hydrophilic group carries a negative charge. Typically it contains one or more of the following head groups: carboxylates, sulphates, suphonates, and phosphates.

2) Cationic: The hydrophilic has a positive charge. Cationic surfactant is used in fabric softener and other household products. They are generally compatible with most inorganic ions and hard water.

3) Nonionic: The hydrophilic group has no charge. It owes its water solubility to the highly polar groups.

4) Amphoteric group has both negative positive charges on the principal chain.

The nonionic surfactants are better solubilizing agents than ionic surfactants in a dilute solution because of lower CMC. In general, the order of solubilizing power of hydrocarbons and polar compounds with the same hydrophobic chain length are: nonionics>cationics>anionics. Santanu (2008) reported that surfactants retard the degradation rate when the surfactants are toxic to the bacteria or referential utilization of surfactants by hydrocarbon degraders as a nutrient.

Lipe et al. (1996) states that compared to cationic and nonionic surfactants, anionic surfactants are usually chosen for soil flushing procedures because of their lower degree of adsorption on soil particles. Also they are more easily recoverable after use. Desorption of HOCs (Hydrophobic organic compounds) from soil using surfactants is greatly influenced by the adsorption of surfactants on soil. Surfactants are more effective in enhancing HOCs desorption from the contaminated soil with relatively lower clay content and higher organic carbon content. The surfactants enhance the rate of hydrocarbon biodegradation by either increasing solubilization in the aqueous phase or by changing the cell affinity between the microbial cell and hydrocarbons by increasing cell surface hydrophobicity. There is no general rule for the effect of surfactants on hydrocarbon biodegradation. Almeida et al. (2008) have reported that the surfactant Triton X-100 could enhance Cu (II) sorption by salt marsh plants. The level of Cu (II) in the plant roots exposed to 0.25 mM added Triton X-100, in the soil was about two times higher than those found in the absence of the surfactant.

2.9 Chelates and phytoremediation

Nowak (2002) stated that chemically enhanced phytoremediation with the addition of some artificially produced chelates, such as EDTA and EDDHA (2-hydroxyphenylacetic acid) have been suggested as efficient additives for the cleaning up of soils contaminated with heavy metals. Metals such as Pb (II) are largely immobile in soil and their extraction rate is limited by solubility and diffusion at the plant root surface. Chemically enhanced phytoextraction can overcome these problems. Chelating agents are chemicals that form soluble, complex molecules with certain metal ions inactivating the ions so that they cannot normally react with other elements or ions to produce precipitates. They increase solubility of heavy metals for plant uptake during phytoremediation. Epelde (2008) reported that EDTA was much more efficient than EDDS for the enhancement of root Pb (II) uptake and root-to-shoot Pb (II) translocation. Because of the toxic effects, it is recommended that chelates be applied only after the plant has approached maturity. Lestan et al. (2003) describe the influence of chelates which are biodegradable (EDDS) and non-degradable (EDTA) on Pb (II) phytoextraction and leaching. They showed that addition of EDDS improves soil biological and physicochemical properties. Andrade et al. (2007) optimized the washing solution molarity and liquid to solid weight ratio addition to use smaller amounts of highly soluble $(NH_4)_2EDTA$ inputs to limit the amount of leachate.

2.10 Soil biological indicators

The goal of any soil remediation process must not only be to remove the contaminant from the polluted site but to restore soil quality. It is important that the soil continues to perform according to its full potential (Hernández-Allica et al. 2006). Biological indicators of soil quality are valuable monitoring tools (a) to assess the efficiency of a phytoextraction process and (b) to determine additives induced toxic effects on the soil microbial community. Biological traits are indicated by enzyme activities, microbial biomass, respiration, mineralizable nitrogen. These indicators are increasingly used to know the subtle changes in the soil as well as to their capacity to provide information that integrates many environmental factors (Alkorta et al. 2003). Measurement of the soil respiration rate is a widely used as biological activity indicator in environmental studies. It can be performed either in situ measuring the cumulative contribution of all organisms involved in the CO_2 release. The soil respiration rate also called basal soil respiration (BSR) gives an estimate of total microbial activity in the soil (Vanhala and Tamminen, 2005). Akerblom et al. (2007) observed a reduction in microbial activity in terms of soil respiration in forest soils containing metals. Tween 80 is shown to be effective in the rhizodegradation of oil under aerobic conditions and impart a positive effect on the soil microbial population in terms of BSR (Memarian and Ramamurthy, 2012).

2.11 Summary

This chapter primarily focused on the several different types of phytoremediation mechanisms with emphasis on the remediation target. However, a major drawback of most previous studies is that only a few of the studies dealt with phytoremediation efficiency in the sites contaminated with mixed contaminants, which are extremely important and yet complex to understand. For sites with mixed contaminants, more than one phytoremediation procedure may be required. No significant study has been performed on the effects of soil additives such as surfactants or chelates on phytoremediation of soils containing mixed contaminants. Since a majority of pervious research has been primarily focused on the effects of chelates on phytoremediation of soil containing metals, it is important to investigate the ability of plants to remove mixed contaminant through enhanced phytoremediation using surfactants and chelates.

In the next section (chapter 3), the materials used in the tests and test procedures are described.

Chapter 3

Materials and Methods

3.1 Introduction

To setup a phytoremediation experiment, understanding soil - plant - chemical interactions is essential. These interactions generate mass fluxes between soil and plants and affect both the plant morphology and the soil properties. It is important to stress the complexity of these interactions in enhancing contaminant removal from soil. From an environmental perspective, the soil chemical interaction is important as much as the plant chemical interactions than effect on phytoremediation directly.

3.2 Materials

The main materials used in this study can be divided into heavy metals, used engine oil, soil, plant, and additives (chelates and surfactants). All chemicals purchased were of reagent grade.

3.2.1 Heavy metals

Soil contamination by Pb (II) and Cd (II) is of great concern because both these heavy metals are toxic to humans (U.S.EPA, 1998). Lead and Cadmium are used in many industrial, urban, and agricultural applications. Pb (II) has low mobility in soil compared to Cd (II). In order to understand the efficiency of phytoremediation through uptake by both high and low sorption metals, Pb (II) and Cd (II) were selected. Some metals such as Cd, Pb have no known biological functions, while others such as Zn, Cu, Mn, and Ni are micronutrients necessary for plant growth. $PbCl_2$ and $CdCl_2$ were purchased from Fisher Scientific, Canada.

3.2.2 Used engine oil as an organic pollutant

Used engine oil applied in the experiments was supplied from the Petro Canada Refinery. It is categorized under CAS (Chemical Abstracts Service) number 8002-05-0. Used engine oil is a common and toxic environmental contaminant, and successful technologies for its remediation vary. Used engine oil is a petroleum hydrocarbon (PHC) representing a complex mixture of individual chemical constituents (Table 3.1). The car-based oils contain hundreds to thousands of hydrocarbon compounds, including a substantial fraction of nitrogen- and sulfur-containing compounds.

Table 3.1 Chemical composition of car engine base oil

Component	**(%)**
Saturated fraction	*90.9*
Normal Paraffin	15.5
Cyclic paraffin	75.4
Aromatic fraction	*9.1*
Naphthalene	1.7
Fluorene	1.2
Benzene	1.1
Dibenzofuran	1.0
Dinaphthenebenzene	0.8
Dibenzanthracene	0.6
Naphthobenzothiophene	0.3
Perylene	0.2
Benzothiophene	0.2
Chrysene	0.1
Unknown	1.9

(Koma et al. 2001)

The following procedure was to identify fractions of used engine oil:

1. Water content

The standard method D95 for determine water in Petroleum Products of the American Society for Testing and Materials (ASTM), was used for finding the water content in used engine oil (ASTM, 2005). The oil was heated with the solvent benzene. Condensed solvent and water were separated in a trap, and water settled as the bottom layer. The amount of water in the bottom layer was used for calculating the water content of the sample.

2. Volatile hydrocarbon content

To determine the amount of volatile hydrocarbons of the oil, a sample of known oil mass was placed in an oven at 105 °C for 24 h. The reduction in mass corresponded to the moisture and volatile hydrocarbon content in the sample. As the water content was measured previously, the volatile hydrocarbon content (in wt. %) was calculated as follows:

Volatile hydrocarbon% = [(reduced mass in g)/ (mass of tested sample in g)] × 100% – (water content in wt. %).

3. Solids content

The dried (105° C) oil sample was placed in a furnace at 550 °C for 30 min. The residue showed the solids content of the sample as a weight fraction (%):

Solids = [(residue remaining after burning in g) / (mass of tested sample in g)] × 100%

4. Nonvolatile hydrocarbon content

The nonvolatile hydrocarbon content of the used engine oil was determined in weight percent as follows:

Nonvolatile hydrocarbon = 100% – (volatile hydrocarbon in wt. % + solids in wt. % + water content in wt. %)

Mean characteristics of used engine oil was shown in Table 3.2.

Table 3.2 Mean characteristics of used engine oil (weight fraction %)

Water	Volatile hydrocarbon	Solid	Nonvolatile hydrocarbon
1.4	0.3	1.2	97.1

3.2.3 Soil

The soil used in this study contained 94 % of Ottawa sand and 6% vermiculite clay by weight. Sand was obtained from Geneq Inc., Canada and vermiculate clay was purchased from Sigma Aldrich (Table 3.3 shows physical - chemical characteristics of sand and clay). To 1 kg of the test soil, 50 g of peat was added and well mixed. The artificial soil was used because controlled tests can be made with a soil that has limited number of significant variables that affect phytoremediation. Natural soils contain a large number of components that may interfere with the soil- chemical interactions. The soil properties and initial concentration of contaminants are given in Table 3.4.

Table 3.3 Physical - chemical characteristics of sand and clay

Mineral components	Cation exchange capacity (CEC) (meq/100 g)	Particle size (mm)	pH in water	Specific surface area (m^2/g)
Sand	0	0.33 [a]	7.6	0.007[b]
Vermiculite clay	64[c]	< 1	6.8	22-38[d]

[a] from Tarnawski et al.2009

[b] from Lee et al.2001

[c,d] from Maqueda,2001

Table 3.4 Physical - chemical characteristics of the soil

Properties	Contents
Particle distribution	
Vermiculate clay (%)	6
Sand (%)	94
CEC (meq/100 g)	22.6
Organic contents (g/kg)	50
Hydraulic conductivity (cm/s)	2.7×10^{-4}
pH	6.6
Metals concentration	
Pb (mg/kg)	496.5
Cd (mg/kg)	49.7
Used engine oil (mg/kg)	495.1

Soil pH was measured using a soil to water ratio of 1:10.In this procedure about 20 g of soil was placed in a 400 ml beaker and 200 ml of distilled water was added. The solution was placed on an orbital shaker for 30 min and left for one hour to ensure equilibrium was reached prior to measurements.

3.2.4 Soil spiking procedures

To begin with, 1 kg of soil was prepared by thoroughly mixing sand (94%), vermiculate (6%). To this mixture, peat was added and well mixed as described in the last section**.** 300 mL of distilled water were added to 1 kg of soil mixture. This was contaminated with finely powdered $PbCl_2$ (500 mg) and $CdCl_2$ (50 mg). The concentrations of Cd (II) and Pb (II) were selected using the data of MEFQ contamination level as a guide (Table 2.1). The spiked soil was dried (24°- 35°c) and allowed to age for 1 month in the greenhouse. 500 mg of used engine oil which was dissolved in 200 ml hexane was added to 1 kg of dried soil and mixing thoroughly in large beaker. This procedure was repeated to form the soil containing the mixed contaminants.

3.2.5 Homogeneity test

In the test for homogeneity of contaminant distribution in the soil, a statistical study was performed using the analysis of variance (ANOVA) described by Berthouex (2002). It showed that the deviation of the contaminants in the soil was generally limited to 3% in the soil contained in the test pots. It showed that the soil was homogenous with 49.7 mg kg $^{-1}$ of Cd (II), 496.5 mg kg^{-1} of Pb (II) and 495.1 mg kg $^{-1}$ of used engine oil.

3.2.6 Plant

The ideal plant species to remediate a contaminated site should provide a high yielding crop that can both tolerate and accumulate the target contaminants. Indian mustard plant was well-known as specie that can take up and accumulate metals in its root (phytostabilization) and its shoot (phytoextraction) [Blaylock et al. 1997. Di Gregorio et al. 2006; Haag-Kerwer et al. 1999; Hamlin and Barker, 2006. Kumar et al. 1995]. Also, this plant has been shown to facilitate the degradation of organic pollutants in its rhizosphere zone (Roy, 2005). The certificated seeds of Indian mustard were obtained from S&S Seeds, Inc (California).

3.2.7 Additives

3.2.7.1 Surfactants

Successful implementation of surfactant-enhanced remediation requires careful consideration of surfactant properties. For surfactant-enhanced phytoremediation, anionic and cationic surfactants are not useful because of their phytotoxicity (Rosen, 1989; Lee et al. 2001). As mentioned above, non-ionic surfactants Tween 80 and Triton X-100 were selected for the present study. All additives were supplied by Fisher Scientific. The HLB (hydrophilic-lipophilic balance) and CMC (critical micelle concentration) of two nonionic surfactants used in this study are summarized in Table 3.5. HLB is a value defining the affinity of a surfactant for water or oil.

Table 3.5 Characteristics of Tween 80 and Triton X-100

Trade name	Chemical	Molecular weight (g/mol)	HLB	CMC (mg/L)
Tween 80	Polyoxyethylene (20) sorbitan monooleate	1309	15.0	35
Triton X-100	Polyoxyethylene (10) isooctylphenyl ether	646	13.5	136

(Soon and Min, 2006)

3.2.7.2 Chelates

Application of Ethylenediamine tetra acetic acid (EDTA) to Pb (II) contaminated soils has been shown to increase the uptake of Pb (II) by plants (Huang and Cunningham, 1996; Blaylock et al. 1997; Huang et al. 1997). Also, Ethylenediamine disuccinic acid (EDDS) is effective in enhancing the phytoremediation of soils contaminated with Pb (II), Zn (II), Cu (II) and Cd (II) (Tandy et al. 2006; Letsan, 2003).

3.3 Experiment methods

The experiments were carried out in the greenhouse facility (Hall building) of Concordia University. Essential nutrients are needed for plant growth (Epestein, 1972). To meet this requirement, for 1 kg of the soil, basal fertilizer (KH_2PO_4) was applied to provide 80 mg of P and 100 mg of K. Further, ammonium nitrate (NH_4NO_3) was added to the soil (1 kg) to provide 150 mg of N. 2 kg of the soil was placed in plastic pots and Indian mustard seeds were directly planted in the pots. After seedling emerged, the pots were thinned to one plant per test pot and grown in a greenhouse under natural light and temperature conditions (average day, 32^{O}C; night, 21^{O}C) for 50 days. After the initial 30 days (Fig 3.1), when the plants were mature (Safwan et al. 2008), Tween 80 and Triton X-100 at different concentrations (0.5, 1 and 2 CMC) and [S,S] EDDS and Na_2EDTA at three level concentrations (0.5, 1 and 2 mmol/kg) were individually applied to the test pots. Irrigation water was provided at 3-day intervals using distilled water. The total leachates, after each irrigation event, were collected from each test pot during the tests and were analyzed for Cd (II) and Pb (II).

Two control tests were conducted in this study. For control 1, the removal of used engine oil from the soil system subject to natural attenuation was studied in the absence of additives and plants. For control 2, soil with plants but without additives was used to know the effects of roots on the removal of used engine oil from the system. The soil solution pH values were not adjusted. They varied from 6.5 to 6.7, following the application of additives at various concentrations. It was also noted that the addition of additives did not change the soil pH. The plants were harvested 20 days after the application of additives. Each treatment included three replications. The Student's *t*-test was used to compare two treatment means at the 0.05 significance level. Fig 3.2 shows the visual symptom (light patches) toxicity in the plant leaves treated with Triton X- 100.

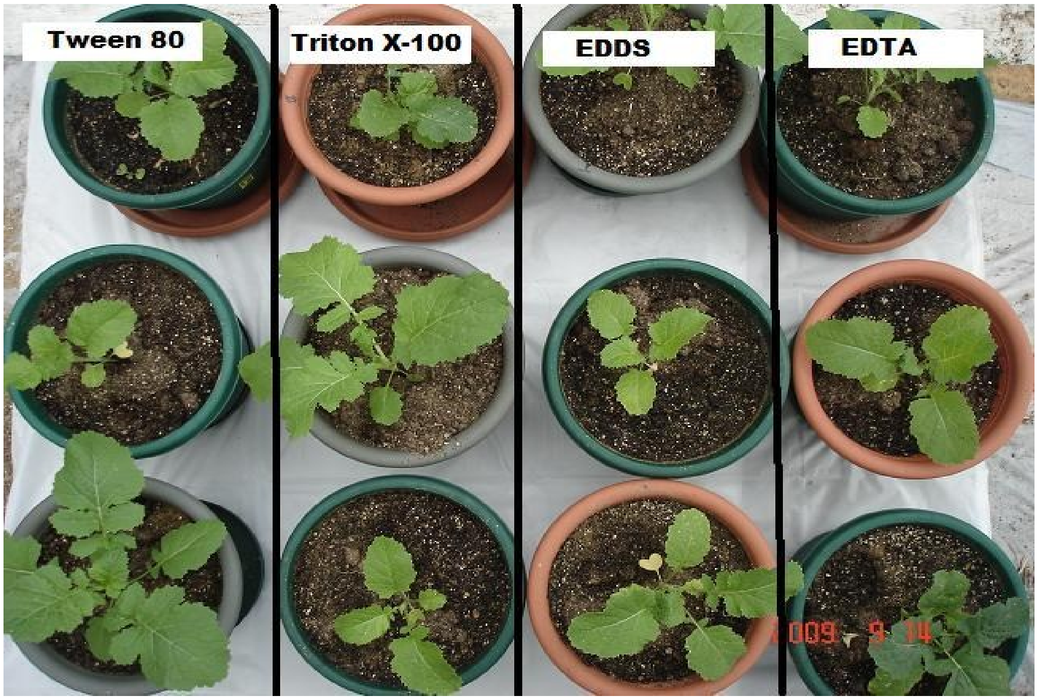

Fig 3.1 Treatments after 30 days of growing the plants

Fig 3.2 The visuals symptoms (light patches) due to Triton X- 100 toxicity

3.4 Samples analysis

3.4.1 Soil organic matter determination

This test was performed to determine the organic content (OC) of the soil (sand and vermiculate clay) according to ASTM D 2974 (ASTM, 2006).

1. Weigh out the empty, clean, and dry porcelain dish (W_1).

2. Place 40 g of the oven-dried soil in the porcelain dish and Weigh out the dish and soil (W_2).

3. Place the dish in a furnace. Gradually increase the temperature in the furnace to 440^{o}C and leave the soil in the furnace for 24hrs.

4. Remove the porcelain dish using the tongs and allow it to cool to room temperature. Determine and record the mass of the burned soil (W_3).

The weight of the dry soil $W_D = W_2 - W_1$

The weight of the burned soil $W_B = W_3 - W_1$

The weight of organic matter $W_O = W_D - W_B$

Percent of the organic contents (Eq. 2.1)

$$OC = W_O / W_D \times 100 \qquad (2.1)$$

3.4.2 Soil cation-exchange capacity (CEC)

This test was performed to measure the cation-exchange capacity (CEC) of the soil according to U.S. EPA method 9081.

1. Weigh out 4 g of the soil transfer the sample to a 50-mL centrifuge tube

2. Add 33 mL of 1.0 N NaOAc (Sodium acetate) solution, shake it in the shaker for 5 min, and centrifuge it until the supernatant liquid is clear.

3. Repeat the procedure described in paragraph 2 three more times.

4. Add 33 mL of 99% C_3H_8O (Isopropyl alcohol) than shake it in the shaker for 5 min, and centrifuge it until the supernatant liquid is clear.

5. Repeat the procedure described in paragraph 4 two more times.

6. Add 33 mL of NH_4OAc (Ammonium acetate) solution, shake it in a mechanical shaker for 5 min, and centrifuge it until the supernatant liquid is clear. Transfer the washing into the 100-mL volumetric flask.

7. Repeat the procedure described in paragraph 6 two more times.

8. Dilute the combined washing to the 100-mL with ammonium acetate solution and determine the sodium concentration by flame atomic adsorption spectrometer (FAAS).

3.4.3 Soil hydraulic conductivity

Hydraulic conductivity (K_H) defines the rate of movement of water through a soil. It is the constant of proportionality in Darcy's Law (Eq.2.2) and as such is defined as the flow volume per unit cross-sectional area of porous medium under the influence of a unit hydraulic gradient. It is measured by Darcy's law.

$$Q = - K_H A \, dh/dL \qquad (2.2)$$

Q = flow (cm^3/s)

K_H = hydraulic conductivity (cm/s)

A = cross-sectional area (cm^2)

dh/dL = hydraulic gradient

3.4.4 Heavy metal concentration in samples

1. The concentrations of Cd (II) and Pb (II) in the soil and leachate samples were determined by aqua regia digestion followed by analysis using FAAS and inductively coupled plasma mass spectrometry (ICP-MS). The analyzer could detect metal concentration to the nearest 0.1 mg/kg. At the end of the experiment, plants were harvested by removing them from the soil. The plants were washed with deionized water to remove the soil particles. To determine the amount of Pb (II) and Cd (II) in plant tissues, the roots and shoots were further separated and dried in an oven at 70°C for 48 h (McQuaker et al. 1979). Following this, the plant material was dissolved in 20 mL of 1 M HCl and diluted to 50 mL with deionized water. The concentrations of Pb (II) and Cd (II) in the extracts were determined by FAAS.

2. The concentrations of water-soluble Cd (II) and Pb (II) in the soil were determined by equilibrating 5 g of soil with 25 mL of 0.01 M KNO_3 for 2 h (Blaylock et al. 1997). The suspensions were centrifuged, and the supernatant solution was analyzed for soluble Cd (II) and Pb (II) by FAAS and ICP-MS.

3.4.5 Used engine oil content in the soil

The oil content in the soil of the test pots was determined by solvent extraction using n-hexane (Duffield et al. 2003). For 5 g of soil sample, 10 mL of n-hexane was added and shaken for 30 min. The extracted oil in n-hexane was collected in a standard volumetric flask. To this, n-hexane was added to bring the solution volume to 50 mL. Following this, the flask contents were transfer to a vial with little headspace. The vial was centrifuged for 30 min at a speed of 3,000 rev/min. The concentration of used engine oil in the supernatant was determined using an ultraviolet (UV) spectrophotometer.

3.4.6 Basal soil respiration (BSR)

Basal soil respiration (BSR) as an indicator of microbiological activity was determined according to ISO 16072 (2002). For analysis of BSR, soils were sieved to < 2 mm and stored fresh at 4º C until analysis.

1. 40 g of soil samples was placed in airtight jars and moistened to 60% WHC (water holding capacity).

Calculate the WHC (Eq.2.3) using the following equation:

$$WHC = [(W_s - W_{t)} / (W_t - W_b)] \times 100 \qquad (2.3)$$

W_s = Weight of beaker containing water saturated soil (g)

W_t = Weight of beaker containing oven-dried soil (g)

W_b = Weight of beaker (g)

2. The soil was incubated for 2 days at laboratory temperature (25° to 28°C). The vial containing 5 mL of 1M NaOH was placed on the soil sample to absorb the CO_2 evolved during the incubation period.

3. The CO_2 was calculated by using the data related to titration of the unused NaOH (not reacted with CO_2) with 0.1 M HCl. BSR is measured by below equation (Eq. 2.4):

$$BSR = MW_C \times (B - V) \times [M] \times 1000 / (DW \times T \times 2) \qquad (2.4)$$

Here, BSR is the basal soil respiration (μg C g^{-1} soil h^{-1}), MW_C is the molecular weight of C (12.01 g), B is the volume of HCl for blank titration, V is the volume of HCl for sample titration, M is the concentration of HCl (0.1 M), DW is the dry weight of the soil

(40 g), T is the incubation time (48 h) and 2 is the factor that accounts for two OH are consumed by one CO_2.

In the next section (chapter 4), the effect of chelates (EDTA and EDDS) on phytoremediation of the soil polluted by mixed contaminates (heavy metals and engine oil) are described.

Chapter 4

The effect of chelates on phytoremediation

4.1 Introduction

In phytoremediation practice, several conditions must be met for successful phytoremediation. The bioavailability of the contaminant in the soil is a main factor for plant uptake. Usually, little Pb (II) is taken up by plants principally due to its high sorption on the soil particles. The key to chelate-induced phytoremediation is to maintain an increased bioavailability of the target metal long enough for plants to take it up (Thayalakumaran et al. 2003). Previous research on the role of chelates such as EDDS and EDTA on remediation had targeted heavy metals, with very little attention to mixed contaminants. The aim of this part of the study focuses on the effect of the different low concentrations of EDDS (Ethylenediaminedisuccinic acid) and EDTA (Ethylenediaminetetraacetic acid) on the remediation of Cd (II) and Pb (II) and used engine oil from the soil cultivated with Indian mustard plant.

4.2 Effect of chelates on plant growth

Both root and shoot DMs were lower for the treatment with chelates than for the control tests (Table 4.1). The chelate effect was more pronounced on the shoot. The plant DM decreased with increasing chelate concentration rates for both chelates. The application of 2 mmol/kg EDTA and EDDS considerably depressed the growth of plants and decreased the plant DM. The maximum reduction in shoot DM observed was 8.3 g/plant for the plant for which 2 mmol/kg EDDS was applied to the soil. The corresponding value for EDTA was 8.4 g/plant. No significant difference was observed between influences of EDTA and EDDS on the DM of the plant. Vassil et al. (1998) stated that exposure of

Indian mustard to high concentrations of Pb (II) and EDTA caused a reduction in both the transpiration rate and the shoot water content and finally a reduction in plant DM.

Table 4.1 The influence of EDDS and EDTA on DM yield (g/plant)

Additive		Root	Shoot
Control (C 2)		5.1 ± 1.2	17.8 ± 2.8
EDDS (mmol/kg)	0.5	3.4 ± 1.6	9.6 ± 2.5
	1	3.4 ± 1.3	8.8 ± 2.2
	2	3.1 ± 1.5	8.3 ± 1.3
EDTA (mmol/kg)	0.5	3.6 ± 1.7	10.5 ± 2.5
	1	3.5 ± 1.2	9.2 ± 4.2
	2	3.3 ± 0.5	8.4 ± 2.7

4.3 Effect of chelates on phytoremediation of Cd (II)

An analysis of plant tissues for metals demonstrated that Cd (II) uptake was considerably enhanced by both EDDS and EDTA (Table 4.2). Shoot Cd (II) concentration in the control was 189 mg kg $^{-1}$. This increased to 363.5 and 400.6 mg kg $^{-1}$ for treatments with 2mmol/kg of EDDS and EDTA respectively. Also, the application of chelates particularly enhanced root Cd (II) concentration from 173.2 mg kg $^{-1}$ in control to 352.4 and 392.7 mg kg $^{-1}$ for treatments with 2mmol/kg of EDDS and EDTA respectively. The difference in enhanced uptake of Cd (II) by EDDS and EDTA can be traced to their respective formation constants (log K) of the metal-chelate complex. The greater log K indicates greater rate of metal chelate formation. Compared to Cd-EDDS (log K = 12.7), Cd-EDTA (log K = 16.5) would be preferentially formed (Tandy et al. 2006). Even at the small concentration of chelate (0.5 mmol/kg), a significant increase in Cd (II) uptake was noted (Table 4.2).

Table 4.2 Effect of EDDS and EDTA on Cd (II) uptake (mg/kg)

Additive		Root	Shoot
Control (C 2)		173.2 ± 11.2	189.1 ± 12.4
EDDS (mmol/kg)	0.5	294.1 ± 4.2	312.5 ± 14.7
	1	335.3 ± 31.2	354.4 ± 36.5
	2	352.4 ± 24.6	363 ± 22.8
EDTA (mmol/kg)	0.5	322.2 ± 11.8	330.1 ± 41.6
	1	357.1 ± 43.8	376.5 ± 11.8
	2	392.7 ± 11.8	400.6 ± 11.8

4.4 Effect of chelates on phytoremediation of Pb (II)

Pb (II) concentration in the plant (root and shoot) was higher than Cd (II) concentration in the plant (Table 4.2 and 4.3). The differences between plant uptake of Pb (II) and Cd (II) are linked to their initial concentrations in the soil and also to their phytotoxicity. John et al. (2009) reported that Cd (II) had a higher impact than Pb (II) in hampering Indian mustard growth. Also, Grčman et al. (2001) studied the effect of EDTA on the accumulation of Pb (II), Cd (II) and Zn (II) in the Brassica rapa plant. Compared to Cd (II) and Zn (II), they found that the accumulation of Pb (II) in the plant was much more.

EDTA was more effective for the uptake of Pb (II) than EDDS. As stated earlier, the difference in the uptake of Pb (II) by chelates can be attributed to their formation constants (log K) of the Pb-chelate complexes. Pb-EDTA with log K= 18.0 is a stronger complex compared to Pb-EDDS for which log K= 12.8 (Martell et al. 2004). Treatments with 2 mmol/kg of EDDS and 2 mmol/kg of EDTA augmented the shoot Pb (II) concentrations respectively to 14.96 and 15.73 (10^3mg/kg). Also, the Pb (II) concentration in roots increased from 5.72 in control to 16.3 and 18.16 (10^3mg/kg) respectively for treatments with 2 mmol/kg of EDDS and EDTA.

Table 4.3 Effect of EDDS and EDTA on Pb (II) uptake (10^3mg/kg)

Additive		Root	Shoot
Control (C2)		5.72 ± 1.2	5.41 ± 2.2
EDDS (mmol/kg)	0.5	11.73 ± 3.6	10.25 ± 3.6
	1	15.00 ± 2.8	13.74 ± 2.2
	2	16.3 ± 2.4	14.96 ± 3.7
EDTA (mmol/kg)	0.5	15.70 ± 4.4	14.10 ± 5.2
	1	17.50 ± 4.2	15.33 ± 2.8
	2	18.16 ± 3.5	15.73 ± 7.3

4.5 Effect of chelates on Cd (II) and Pb (II) leaching

In control tests without chelate application, the concentration of Cd (II) in the leachates was 0.7 mg kg^{-1}. This is about 1.4 % of the initial Cd (II) concentration in the soil .The concentration of Cd (II) in leachates increased with increasing concentration of chelates (Fig 4.1). At the same concentration, EDDS mobilized a smaller proportion of Cd (II). The difference in leaching of Cd (II) by EDDS and EDTA can be attributed to their formation constants of the Cd-chelating agents and their biodegradability. Low biodegradability of EDTA and high solubility of EDTA- metal complexes in soil water system result in a risk of environmental impact due to metal mobilization and long persistence (Alkorta et al. 2004). In this study, the rate of chelate degradation in the soil was not determined. EDTA has been reported to degrade very slowly in the soil. Five months after EDTA application, EDTA– metal complexes were still present in the soil pore water (Lombi et al. 2001).

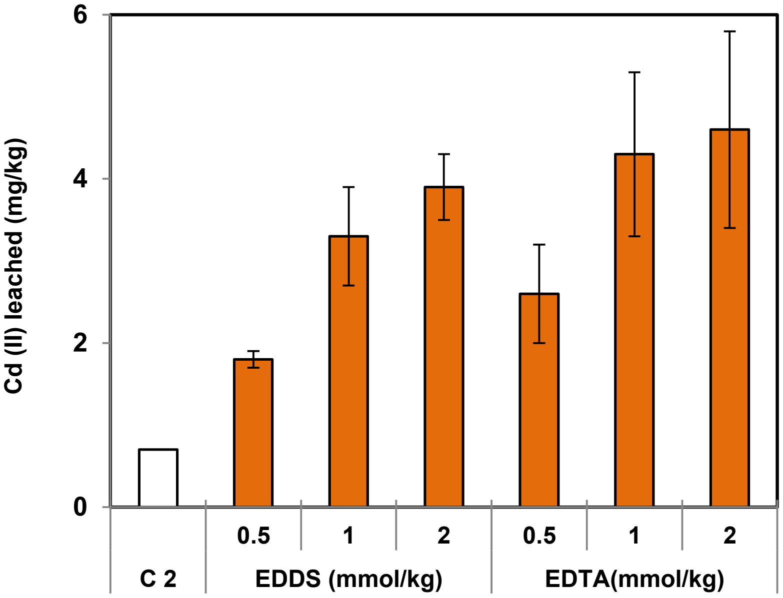

Fig 4.1 Effect of chelates on Cd (II) leachate

Epelde et al. (2008) obtained a degradation half-life of 24 hours for EDDS in a soil polluted with 5000 Pb (II) mg kg^{-1}. Jaworska et al. (1999) reported a calculated half-life of 2.5 days for EDDS in sludge amended soils.

For soils treated with 0.5, 1, and 2 mmol/kg EDDS, the leached Cd (II) was 3.6, 6.7 and 7.9 % of the initial soil Cd (II), respectively. The corresponding leachate values for EDTA were 5.2, 8.9 and 9.3 % of the initial soil Cd (II). In control tests without chelate addition, no Pb (II) was detected in the leachate (Fig 4.2). The concentration of Pb (II) in leachates increased with increasing concentrations of added chelates (Fig 4.2). At the similar concentration, EDDS mobilized a smaller proportion of Pb (II). For soils with 0.5, 1, and 2 mmol/kg EDDS, the leached Pb (II) was 1, 1.4 and 4% of the initial soil Pb (II), respectively. The corresponding leachate values for EDTA were 4.5, 7.6 and 11.4 % of the initial soil Pb (II).

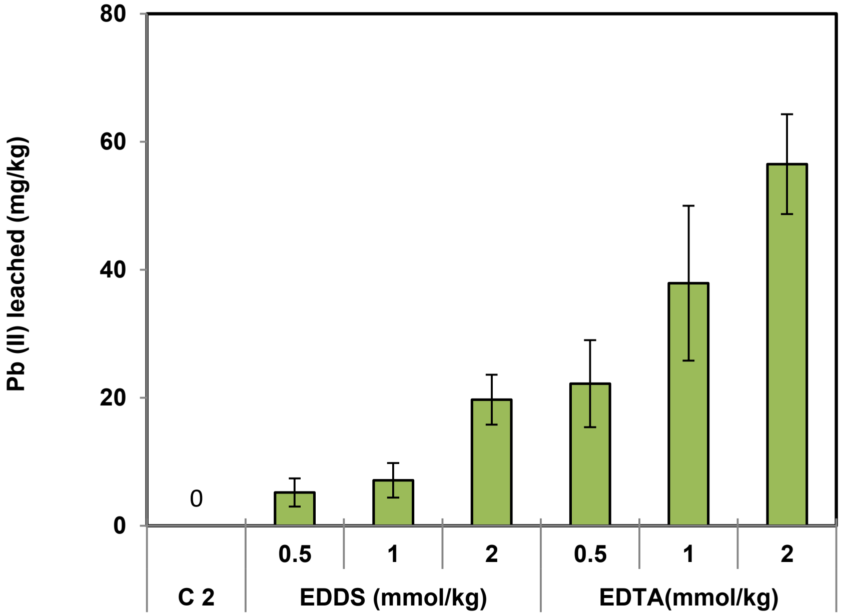

Fig 4.2 Effect of chelates on Pb (II) leachate

Also, the difference in leaching of Pb (II) by EDDS and EDTA can be attributed to the formation constants of the chelates and their biodegradability. The amount of leachates was determined from each leaching event. The mass of leachates was 1992 ± 46 g.

4.6 Cd (II) and Pb (II) mass balance for chelates application

For all chelates additive, the mass balance approach yielded the amount of the final values of Cd (II) and Pb (II) concentrations in the soil. For this, the amount of Pb (II) and Cd (II) accumulated in shoots (phytoextraction) and roots (phytostabilization) of the mustard plant and the quantity of Cd (II) and Pb (II) present in leachates were deducted from their initial concentrations in the soil. With the application of chelates, no significant change in the phytoremediation of Cd (II) was noted. However, using chelates led to increased phytoextraction of Pb (II).

Table 4.4 Cd (II) and Pb (II) mass balance for chelates application

Additive	Heavy metal	Initial Value (mg)	Final value (mg)						Recovery (%)
		Initial soil heavy metal	Heavy metal remaining in soil	Phytoextraction	Phytostabilization	Phytoremediation	Leachate	Total	
No chelate (C 2)	Pb(II)	993	860.0	96.3	29.2	125.5	0	985.5	99
	Cd(II)	99.4	91.9	3.4	0.9	4.3	1.4	97.6	98
EDDS (0.5mmol/kg)	Pb(II)	993	839.7	98.4	39.9	138.3	10.4	988.4	99
	Cd(II)	99.4	91.3	3	1	4	3.6	98.9	99
EDDS (1mmol/kg)	Pb(II)	993	799.2	120.1	51	171.1	14.2	984.5	99
	Cd(II)	99.4	85.6	3.1	1.14	4.24	6.6	96.4	97
EDDS (2mmol/kg)	Pb(II)	993	761.8	124.2	50.5	174.7	39.4	975.9	98
	Cd(II)	99.4	82.4	3	1.1	4.1	7.8	94.3	95
EDTA (0.5mmol/kg)	Pb(II)	993	725.5	148	56.5	204.5	44.4	974.4	98
	Cd(II)	99.4	84.5	3.46	1.16	5.62	5.2	95.3	96
EDTA (1mmol/kg)	Pb(II)	993	679.4	141	61.2	202.2	75.8	957.4	96
	Cd(II)	99.4	84.8	3.45	1.25	4.7	8.6	98.1	99
EDTA (2mmol/kg)	Pb(II)	993	670.3	132	60	192	113	975.3	98
	Cd(II)	99.4	83.2	3.4	1.3	4.7	9.2	97.1	98

Compared to EDTA, EDDS was less effective on phytoremediation of Pb (II), but EDDS caused a decrease in leaching of Pb (II) **(**Table 4.4). With the application of 2 mmol/kg

EDTA, the maximum phytoextraction (132 mg) of Pb (II) occurred. On the other hand, this additive resulted in a larger amount (113 mg) of Pb (II) in the leachate. For treatment with 2 mmol/kg EDTA, phytostabilization of Pb (II) was 60 mg.

4.7 Effect of chelates on rhizodegradation of used engine oil

The effect of the presence of EDDS and EDTA on the rhizodegradation of the oil is shown in Fig 4.3. Following the test period of 50 days, 38.3% of the oil in soil was removed by control (with plant and without chelates). The addition of 0.5 mmol /kg of EDTA increased the oil removal from 38.3 % to 47 % and the highest oil removal efficiency of 70 % could be achieved by EDTA at 2 mmol/kg while the corresponding value for EDDS was 54.2 %.

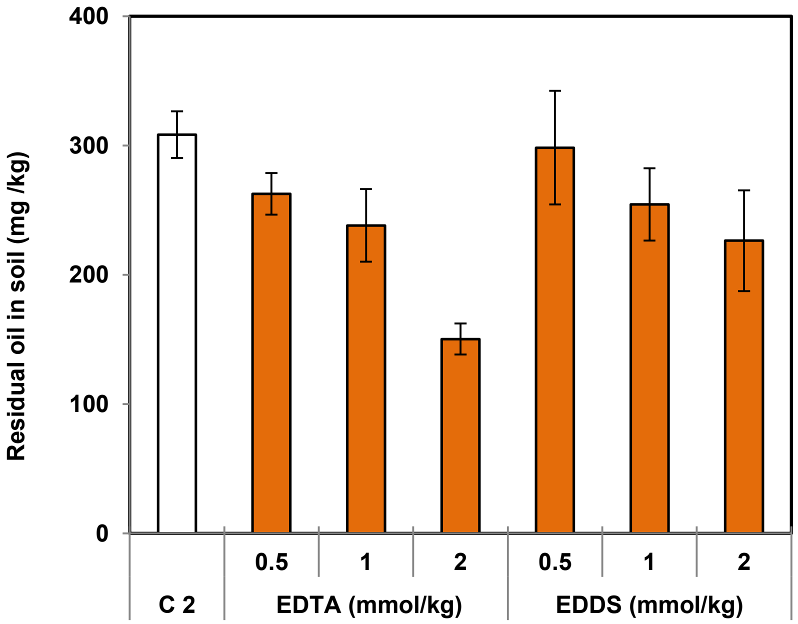

Fig 4.3 Effect of chelates on rhizodegradation of the oil

The results showed that EDTA was more effective than EDDS in increasing the rhizodegradation of the oil. The increase of rhizodegradation of the oil in soil by EDDS and EDTA can be attributed to their ability to remove Cd (II) and Pb (II) from the soil water system. The reduction of Cd (II) and Pb (II) in the soil decreases the toxicity of the metals to the microorganisms during rhizodegradation of the oil. Sandrin and Maier (2003) reported that chelating agents can be applied to reduce metal toxicity to microorganisms that degrade organics. In addition, EDTA amendments could also have a positive impact on some microbial populations of the Indian mustard rhizosphere (Roy, 2005).

4.8 Effect of chelates on basal soil respiration (BSR)

The addition of EDTA and EDDS at the similar concentrations exposed major differences in basal soil respiration. Regarding the initial and final values of basal soil respiration (BSR), EDDS treatments had no significant negative effect on microbe population. The application of EDTA in different concentrations caused a sharp decrease in BSR, indicating that EDTA was much more toxic to the microbes. The highest value of BSR (1.54 μg C g $^{-1}$ DW soil h $^{-1}$) was found for treatment with 0.5 mmol/kg of EDDS. The addition of EDDS resulted in a slight decrease in BSR. However, an increase in EDTA concentration resulted in a severely reduced activity of microbes in terms of BSR. The maximum decrease of BSR, (0.39 μg C g $^{-1}$ DW soil h $^{-1}$) was observed at 2 mmol/kg of EDTA (Fig 4.4).

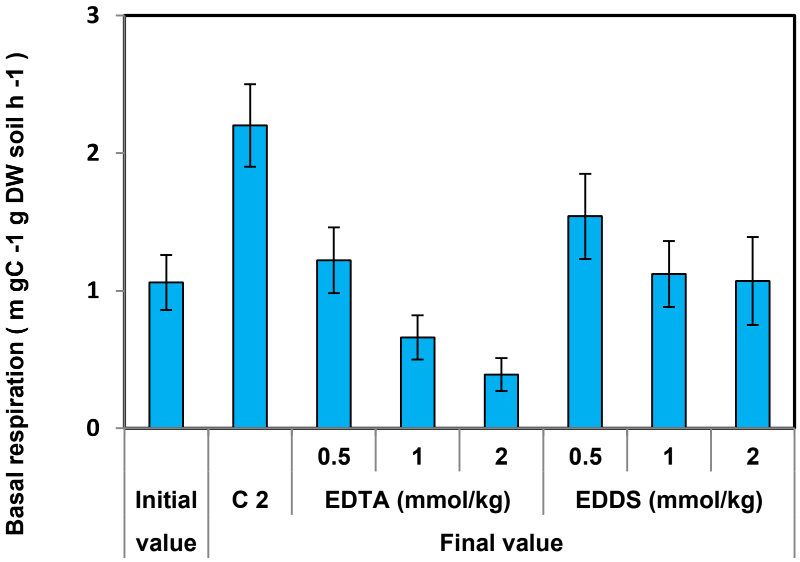

Fig 4.4 Influence of chelates on basal soil respiration

4.9 Summary

The application of EDTA to the soil had a higher effect on increasing phytoextraction of Cd (II) and Pb (II) than for the application of EDDS to the soil. This may be due to the difference in the corresponding formation constants (log K) of EDTA and EDDS. EDDS treatment resulted in a lower amount of Pb (II) in the leachate. The chelate effect on the Pb (II) uptake was noticeably more than that on Cd (II) uptake. Further, the rhizodegradation of the oil was increased with the increase of chelate concentration. It seems that the rhizodegradation of oil was affected by the presence of heavy metals [Cd (II) and Pb (II)]. The results suggested that enhanced phytoextraction of Cd (II) and Pb (II) by chelates promote the rhizodegradation of the oil. Compared to EDTA, the addition of EDDS caused a slight decrease in BSR. Also, an increase in EDTA concentration resulted in a significant drop in BSR. The effect of surfactants (Triton X-100 and Tween 80) on phytoremediation is presented in the chapter 5.

Chapter 5

The effect of surfactants on phytoremediation

5.1 Introduction

In phytoremediation processes, the mobility and bioavailability of the contaminant in the soil, particularly at the rhizosphere zone where root uptake is a critical factor affecting the success of phytoremediation. Developing new methods to enhance phytoremediation, the bioavailability of contaminants in the rhizosphere could significantly improve the efficiency of remediation techniques. Surfactants are used to enhance biodegradation and desorption of organic contaminants from the soil-water system (Wu et al. 2004). Besides many earlier studies, a few recent studies have also reported that surfactants can improve desorption of metals from soils (Almeida et al. 2009; Ramamurthy et al. 2008). The aim of this part of the study was to focus on the effect of different nonionic surfactants applied individually to remove Cd (II), Pb (II) and used engine oil from a soil containing a mixed contaminant by phytoremediation using Indian mustard. This technique has been adapted in this study as an engineering approach to solubilize contaminants from the soil and increase their diffusion into the rhizosphere zone (Ramamurthy and Memarian, 2011).

5.2 Effect of surfactants on plant growth

The effects of surfactants on dry matter (DM) yield of plant shoots and roots are shown in Table 5.1. Both root and shoot DM yields were lower for the treatment with Triton X-100 than for Tween 80 treatment. One notes that this effect was more pronounced on the shoots at the high surfactant concentrations. The results showed that the application of

2CMC Triton X- 100 severely depressed the growth of mustard plant and decreased the plant DM yield, especially the shoot DM yield.

Table 5.1 The influence of Triton X-100 and Tween 80 on DM yield (g/plant)

Additive		Root	Shoot
Control (C 2)		5.1 ± 1.2	17.8 ± 2.8
Triton X-100 (CMC)	0.5	4.9 ± 1.4	17.4 ± 2.1
	1	4.2 ± 1.5	15.7 ± 2.2
	2	2.8 ± 1.5	6.8 ± 1.3
Tween 80 (CMC)	0.5	5.1 ± 1.2	17.8 ± 2.2
	1	4.9 ± 1.2	17.4 ± 3.8
	2	4.7 ± 2.1	17.2 ± 2.7

5.3 Effect of surfactants on phytoremediation of Cd (II)

Table 5.2 shows Cd (II) concentrations in shoots and roots of mustard plants in the test soil that contained surfactants (0.5 to 2 CMC). Increased concentration of surfactants appears to increase the uptake of Cd (II) at higher concentrations. Since for both the surfactants, Cd (II) uptake is not significantly different at 1 and 2 CMCs, the use of 1 CMC for the surfactant additive of the soil is preferred. Uptake of Cd (II) was significantly enhanced at 1 and 2 CMCs. This demonstrates that micelle formation is probably responsible for the solubilization of Cd (II) and rendering it to be bioavailable to the roots in the rhizosphere zone. Further, the application of surfactants significantly increased Cd (II) concentration in the shoots for the control (no plant case) from 189.1 mg kg $^{-1}$ to 327.3 and 269.4 mg kg $^{-1}$ for 2 CMC Triton X-100 and 2 CMC Tween 80 respectively. At 2 CMC, the removal of Cd (II) by Triton X-100 is much more than that due to Tween 80. This difference may be due to the larger solution concentration (136 mg/L) of Triton X-100 compared to the solution concentration (35 mg/L) of Tween 80 (Soon et al. 2006).

Table 5.2 Effect of Triton X-100 and Tween 80 on Cd (II) uptake (mg/kg)

Additive		Root	Shoot
Control (C 2)		173.2 ± 11.2	189.1 ± 12.4
Triton X-100 (CMC)	0.5	173.8 ± 15.4	189.7 ± 21.5
	1	265.4 ± 19.5	309.8 ± 26.2
	2	289.6 ± 24.5	327.3 ± 14.7
Tween 80 (CMC)	0.5	173.6 ± 10.4	189.4 ± 32.8
	1	221.7 ± 31.4	255.1 ± 2.2
	2	241.5 ± 11.6	269.4 ± 10.3

5.4 Effect of surfactants on phytoremediation of Pb (II)

In the control tests related to phytoextraction**,** the uptake of Pb (II) in the shoots was 5.41 (10^3mg/kg). No significant change in the shoots uptake of Pb (II) was observed with the surfactant application. However, the root uptake of Pb (II) was enhanced through the addition of the surfactant to the soil (Fig 5.1). Triton X-100 was more effective than

Tween 80 in enhancing uptake of Pb (II) from the soil and makes it more bioavailable for accumulation in the roots.

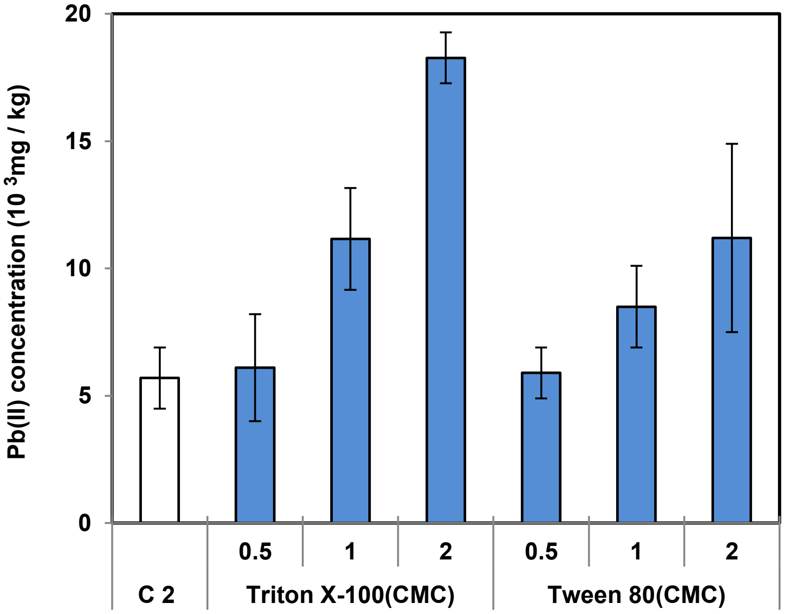

Fig 5.1 Effect of surfactants on root Pb (II) in plants

5.5 Effect of surfactants on Cd (II) and Pb (II) leaching

After watering the plants, the leachates were collected and analyzed for Cd (II) and Pb (II) concentration. In control tests without surfactant, the concentration of Cd (II) in the leachates was 0.7 mg kg^{-1}. This is less than 2.0 % of the initial Cd (II) concentration in the soil. The concentration of Cd (II) in lactates increased with increasing concentrations of surfactant (Fig 5.2). At the same surfactant concentration, compared to Triton X-100, Tween 80 mobilized a smaller proportion of Cd (II). For soils with the application of 0.5, 1, and 2 CMC Tween 80, the leached Cd (II) was 1.4, 1.8, and 2.2% of the initial soil Cd (II), respectively. The corresponding leachate values for Triton X-100 were 1.6, 2.4, and 5.1 % of the initial soil Cd (II).

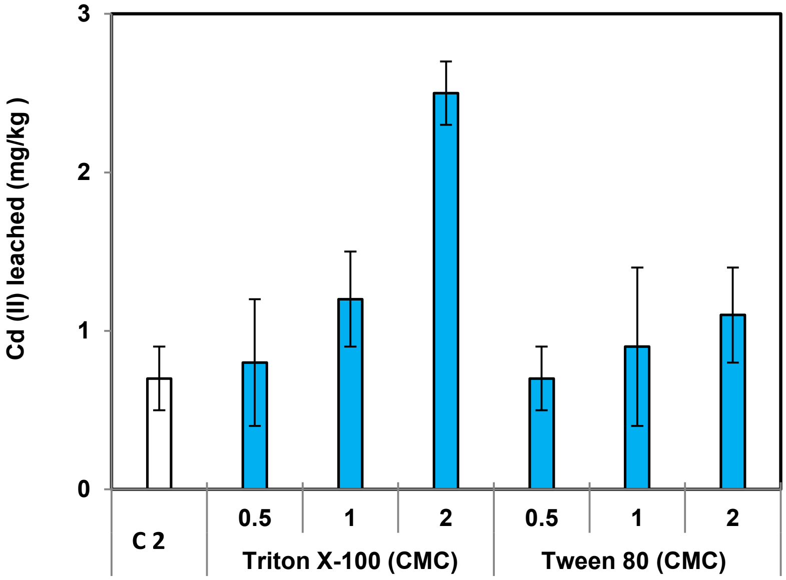

Fig 5.2 Effect of surfactants on Cd (II) leachate

In control test pots, without surfactant addition to the soil, no Pb (II) was detected in the leachate (Fig 5.3). Also, in the leachates from the test pots with both surfactants, at 0.5 CMC no detectable amount of Pb (II) was found. The application of 1 CMC of Triton X-100 and Tween 80 led to 5.4 and 3.8 mg kg^{-1} of Pb (II) in the leachate respectively. These correspond to 1.1% and 0.76% of the initial Pb (II) concentration. When the amount of Tween 80 was 2 CMC, the amounts of Pb (II) in the leachates increased to 5.1 mg kg^{-1}, whereas the corresponding value for Triton X-100 was 12.75 mg kg^{-1}. The weight of leachates was determined from each leaching event. They weighed 1992 ± 46 g.

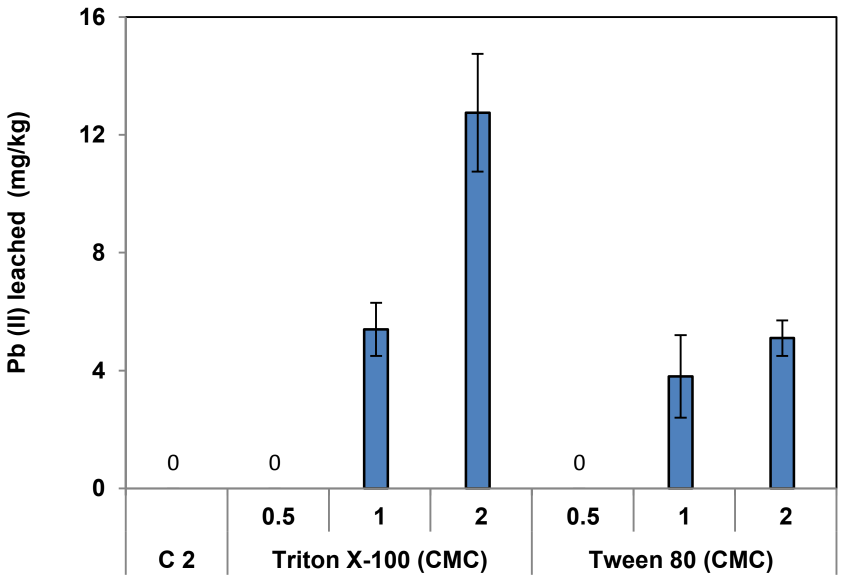

Fig 5.3 Effect of surfactants on Pb (II) leachate

5.6 Cd (II) and Pb (II) mass balance for surfactants application

For surfactants additive to the soil, the mass balance approach similar to the one described in section 4.6, yielded the amount of final Cd (II) and Pb (II) concentrations present in the soil. Phytoremediation is denoted in Table 5.3 as the sum of phytostabilization and phytoextraction. When the concentration of surfactants were below the CMC, enhanced phytoremediation (phytostabilization and phytoextraction) of Cd (II) and also Pb (II) were not considerable (Table 5.3). No significant change in the phytoextraction of Pb (II) was noted with surfactant application, except for concentrations of 1 and 2 CMC Triton X-100. At these concentrations of Triton X-100, plant growth appears to be inhibited due to toxicity which severely reduces the biomass (Table 5.1). This in turn causes a large reduction in the phytoremediation of Pb (II) compared to the control group. With the application of Tween 80 at 2 CMC, maximum phytostabilization (52.2 mg) of Pb (II) was realised. Also, with the application of Triton

X-100 at 2 CMC, maximum phytostabilization of Pb (II) was 52.7 and the leachate contained 25.5 mg of Pb (II). This amount corresponds to 2.6 % of initial concentration of Pb (II).

As in the phytoremediation of Pb (II), the application of 2 CMC Triton X-100 significantly caused a decrease in the removal of Cd (II) and it also resulted in leaching a considerable amount (4.4 mg) of Cd (II). The leached amount of Cd (II) corresponds to 4.5 % of its initial concentration. The increase in concentrations of Tween 80 enhanced phytoextraction of Cd (II) slightly. For all surfactant additives, no significant change in the phytostabilization of Cd (II) occurred (Table 5.3).

5.7 Effect of surfactants on rhizodegradation of used engine oil

Following the test period of 50 days, for control 2 (C2), it was noted that 38.3% of the oil in soil was removed while 27.5% of the oil was removed due to natural attenuation from the contaminated soil in the control 1 (C1) that did not have the plant. For Triton X-100 at concentration of 0.5 CMC, rhizodegradation of the oil increased to 51% (Fig 5.4). Even at 0.5 CMC, due to the reduction of interfacial tension, the surfactants increase the mobility of the oil in soil leading to higher bioavailability through rhizodegradation of the oil. These results also agree with previous studies which inferred that surfactants, even below CMC can enhance the bioavailability of hydrophobic organic pollutants due to the formation of hemimicelles (Klumpp et al. 1991 and Edwards et al. 1994).

Table 5.3 Mass balance for Pb (II) and Cd (II) for surfactants application

Additive	Heavy metal	Initial Value (mg)	Final value (mg)						Recovery (%)
		Soil heavy metal	Heavy metal remaining in soil	Phytoextraction	Phytostabilization	Phytoremediation	Leachate	Total	
No surfactant (C 2)	Pb(II)	993	860.0	96.3	29.2	125.5	0	985.5	99
	Cd(II)	99.4	91.9	3.4	0.9	4.3	1.4	97.6	98
TX-100 (0.5CMC)	Pb(II)	993	864.3	93.9	30.1	124.0	0	988.3	99
	Cd(II)	99.4	93.3	3.3	0.9	4.2	1.6	99.1	100
T X-100 (1CMC)	Pb(II)	993	839.6	86.3	46.9	133.2	10.8	983.6	99
	Cd(II)	99.4	88.8	4.9	1.1	6.0	2.4	97.2	98
T X-100 (2CMC)	Pb(II)	993	874.7	38.1	52.7	90.8	25.5	991	100
	Cd(II)	99.4	90.5	2.2	0.8	3.0	4.9	96.2	99
Tw 80 (0.5CMC)	Pb(II)	993	852.5	99.6	30.1	129.7	0	982.2	99
	Cd(II)	99.4	90.2	3.4	0.9	4.3	1.2	95.9	96
Tw 80 (1CMC)	Pb(II)	993	819.6	95.7	41.8	137.5	7.2	964.3	97
	Cd(II)	99.4	88.8	4.4	1.1	5.5	2.2	96.2	100
Tw 80 (2CMC)	Pb(II)	993	825.8	93.0	52.2	145.2	10.2	981.2	99
	Cd(II)	99.4	88.0	4.7	1.2	5.9	3.2	97.1	98

At concentrations higher than CMC of Triton X-100, rhizodegradation rate of the oil was noted to be even lower than the value for control 2 tests which had plants in the soil without surfactant (Fig 5.4). This may possibly be due to the damage to microorganism cell membranes and enzymes caused directly by Triton X-100 or the toxic effects linked to Cd (II) and Pb (II). Further, non-ionic surfactants can injure phospholipid membranes of microbial cells at concentrations above CMC (Cserhati, 1995) and consequently reduce rhizodegradation of the oil.

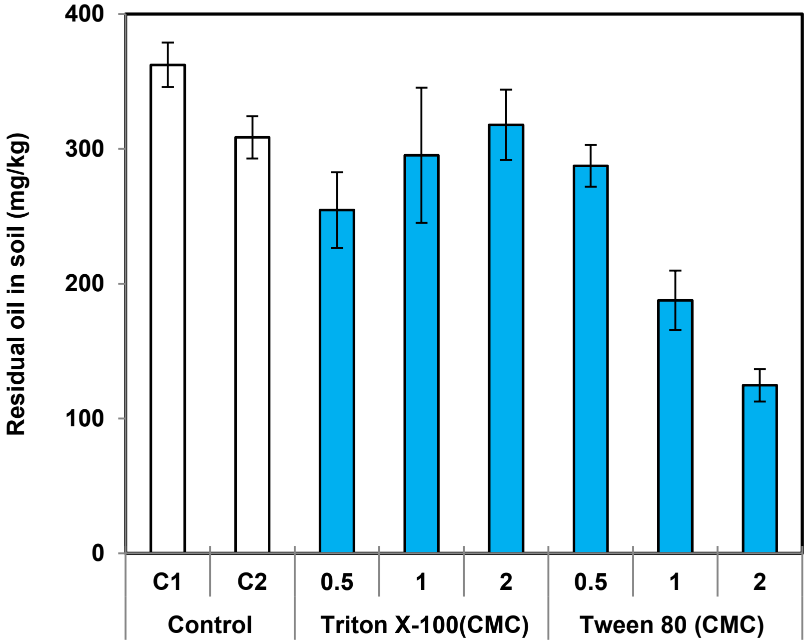

Fig 5.4 Effect of surfactants on rhizodegradation of oil in the soil

Non-ionic surfactant Tween 80 showed no apparent phytotoxicity for the plant growth (Table 5.1). Rhizodegradation rates of the oil and surfactant concentrations are positively correlated for all three concentrations of Tween 80 tested (Fig 5.4). Tween 80 increased the mobility of the oil by decreasing the interfacial tension between the oil and the soil-water phase. This leads to faster diffusion of oil from the soil particles and facilitates the utilization of oil by microorganisms. Gao et al. (2004) observed reduced residual of pyrene in rhizosphere zone in the presence of Tween 80 and Brij35, in contaminated soils. The maximum removal of 75.1% oil was observed in test pots remediated with 2 CMC of Tween 80. However, the removal of 51 % oil was noted at 0.5 CMC for Triton X-100.

5.8 Effect of surfactants on basal soil respiration (BSR)

Regarding the initial and final values of basal soil respiration (BSR), considerable improvements were observed in the soil with all surfactants except in the case of 2 CMC Triton X-100 (Fig 5.5). At the end of the test, the highest value of BSR (2.47 μg C g $^{-1}$ DW soil h $^{-1}$) was found for 2 CMC of Tween 80 application.

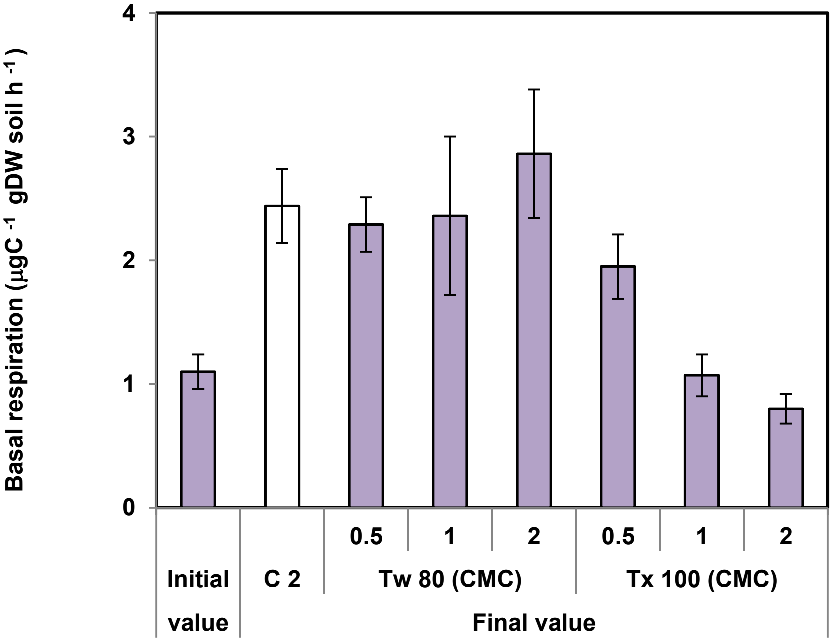

Fig 5.5 Influence of surfactants on basal soil respiration

The addition of Tween 80 resulted in a slight increase of BSR. Conversely, an increase in Triton X-100 concentration resulted in a severely reduced activity of microbe in terms of

BSR. The maximum decrease in the rate of BSR, (0.79 μg C g $^{-1}$ DW soil h $^{-1}$) was observed for 2 CMC Triton X-100 application. This may be attributed to the high Pb (II) remaining in soil. Pb (II) can inhibit the soil microbial activity. Further, the toxic effect of Triton X-100 has been confirmed by the glucose mineralization tests done by Willumsen et al. (1998). They reported that Triton X-100 was more inhibitory than Tween 80, for all the bacteria tested.

5.9 Summary

Although simultaneous removal of mixed contaminants formed by heavy metals and used engine oil from the soil is hard to achieve by phytoremediation, the application of non-ionic surfactant Tween 80 to the soil promoted the removal of mixed contaminants by the Indian mustard plant. Considering the leaching risk of Cd (II) and Pb (II) caused by the surfactants, one seeks additives that avoid high levels of Cd (II) and Pb (II) in the leachate. Basal soil respiration test showed that the application of Tween 80 resulted in an increase in BSR. This test indicated that the lower concentration of Triton X-100 had a slightly positive effect on BSR whereas at higher concentrations, it was inhibitory to the microbes. Tween 80 at 2 CMC was effective for enhancing phytoremediation of Pb (II) and Cd (II) from the soil containing the mixed contaminant. The results suggested that the application of Tween 80 not only led to enhance the removal of oil, Pb (II) and Cd (II) from the soil by Indian mustard but also resulted in an increase in BSR simultaneously during phytoremediation.

In the next section (chapter 6), the transfer mechanism of heavy metals from soil media to plants is described.

Chapter 6

Heavy metal transfer from soil media to plants

6.1 Introduction

The transport of heavy metals from the soil to the plant is affected by their physicochemical properties of the soil and the plant. During phytoremediation, the soil additive can influence concentration of heavy metals in the soil solution through sorption-desorption reaction, and their transport through leaching. Although the uptake relationship between soil and plants is possibly valid for narrow ranges of chemical concentration in the moderately nontoxic range (Jiang and Singh, 1994), some evidence demonstrates that plant uptake is dependent on metal concentration in the soil. Baes et al. (1984) reported that the uptake factors for Cd (II) and Zn (II) were inversely correlated with the soil concentration. The objective of the present study is to compare the empirical model approaches used to establish the partitioning of the observed Cd (II) and Pb (II) between the soil and the Indian mustard plant in chapters four and five.

6.2 Freundlich and Langmuir models

The transport of metals from soil to plant is a sorption process. Freundlich model is considered to be suitable for describing both multilayer sorption and sorption on heterogeneous surfaces (Ho et al. 2002). The Freundlich type function (Eq. 6.1) is defined as follows:

$$q = K_f C^{1/n} \qquad (6.1)$$

q = Contaminant concentration in plant (mg/kg)

C = Final concentration of contaminant in the soluble form in soil (mg/kg)

In the Freundlich model, K_f is a measure of the sorption capacity (larger K_f indicates a larger capacity), whereas the parameter $1/n$ is a measure of the strength of sorption (Tsotsis et al. 2005). Here, larger value of *n* indicates a higher sorption affinity. The Freundlich model is generally applied in an empirical way. It can be of theoretical interest in terms of adsorption onto an energetically heterogeneous surface (Davis et al. 2003; Adamson, 1990). The linear model is a special case of Freundlich model for $n = 1$. The observed data may be represented by the Langmuir equation (Eq. 6.2):

$$q = K_L bC/ (1+ bC) \qquad (6.2)$$

Here, K_L and b are Langmuir constants related to sorption capacity and energy of sorption, respectively (Langmuir, 1918). The model assumes a monolayer adsorption process on a homogeneous surface and would be most applicable in cases where all binding sites exhibit uniform behavior towards the sorbate (Papageorgiou et al. 2006). The linear form of the Langmuir equation (Eq. 6.3) model is usually described as (Weber et al, 1990):

$$\frac{1}{q} = \frac{1}{K_L.b}\frac{1}{C} + \frac{1}{K_L} \qquad (6.3)$$

The transformed linear models have some advantages in that the linearized form can be used to obtain starting values for iterative computation of the parameter estimates (Gordon, 2002). Model parameters were determined from experimental data by a non-linear regression algorithm (Newton method) using the Microsoft Excel solver. The least squares regression seeks to find the parameter combination that minimizes the sum of squares (Eq. 6.4). Each term in the summation is the difference between the observed value O_i and the computed value C_i at the corresponding value of the independent variable q_i:

$$\text{Min } S = \sum_{i=1}^{n}(O_i - C_i)^2 \tag{6.4}$$

6.3 Model evaluation

According to Loague and Green (1990), Michel et al. (2007) and Wehrhan et al. (2007), the modelling efficiency (EF) was used to test the fit between measured and modeled data using the following equation (Eq. 6.5) and it is defined as:

$$\text{EF} = \frac{\sum_{i=1}^{n}(x_i-\bar{x})^2 - \sum_{i=1}^{n}(y_i-x_i)^2}{\sum_{i=1}^{n}(x_i-\bar{x})^2} \tag{6.5}$$

Where x_i and y_i denote the measured and modeled values of the element i, $\bar{x}$ is the mean of measured values and n is the number of values. If modeled values perfectly match the measured ones then EF is equal to 1. Also, the student t-test with 95% confidence level was used to compare whether a significant difference existed between two observed and simulated values.

6.4 Predicting chelates induced metal uptake by the plant

At the end of the experiment, soil samples were analyzed to determine the soluble form of Cd (II) and Pb (II) as described in section 3.4.4. Total Cd (II) and Pb (II) in the solubilized form [column (1) of table 6.2] in the soil were calculated by adding their concentration in plant tissues (both root and shoot), the soluble form in the soil-water system and leachate. The effect of chelates on Cd (II) and Pb (II) in the soil is given in Table 6.1. The solubilized form of Cd (II) and Pb (II) and their uptake in the presence of chelate are shown in Table 6.2.

Table 6.1 Soluble Cd (II) and Pb (II) [mg/kg] in the soil containing chelate

Additive		Cd(II)	Pb(II)
EDDS (mmol/kg)	0.5	6.4	42.2
	1	8.5	59.7
	2	9.9	66
EDTA (mmol/kg)	0.5	7.2	53
	1	10.3	77.8
	2	11.5	105.5

Table 6.2 Solubilized form of Cd (II) and Pb (II) and their uptake in the presence of chelate

Additive		Cd (II)		Pb (II)	
		Solubilized form (mg/kg) (1)	Plant uptake (mg/kg) (2)	Solubilized form (mg/kg) (1)	Plant uptake (mg/kg) (2)
EDDS (mmol/kg)	0.5	10.2	307.7	116.6	10639.6
	1	13.9	349.1	152.3	14089.3
	2	15.9	360.1	173.2	15327.4
EDTA (mmol/kg)	0.5	12.1	328.1	177.4	14435.9
	1	16.9	371.2	217	15827
	2	18.4	398.4	258	16412.6

A positive relationship was found between the Cd (II) and Pb (II) concentrations in the plant and the total soluble Cd (II) and Pb (II) concentrations in the soil (Table 6.2). These results indicate that increased amounts of chelate resulted in increased levels of soluble Cd (II) and Pb (II) in the soil solution. This in turn augments the uptake of Cd (II) and Pb

(II) by the plant. The t-test indicated that there were no significant differences between observed and simulated values for the nonlinear models of Cd (II) uptake (Table 6.3). They are much less than the critical value of 2.447 at the 95 % confidence level (Harris, 2003). This indicated that there was a significant difference between observed and simulated values for the linear models of Cd (II) uptake. Also, the modeling efficiency EF for the linear model of Cd (II) biosorption was considerably lower than the EF obtained for the Langmuir type and the Freundlich type models (Table 6.3). Fig 6.1 shows that the linear model for chelate enhanced Cd (II) uptake. The Freundlich type and the Langmuir type models for chelate enhanced Cd (II) uptake are shown in Fig 6.2.

Table 6.3 Models for predicating chelate enhanced Cd (II) uptake

Models / Parameters	Linear	Freundlich	Langmuir
$t_{calculated}$	3.58	0.004	0.006
EF	- 0.88	0.96	0.98
Empirical constants	K = 23.73	K_F = 121	K_L = 586.12
		n = 2.47	b = 0.109

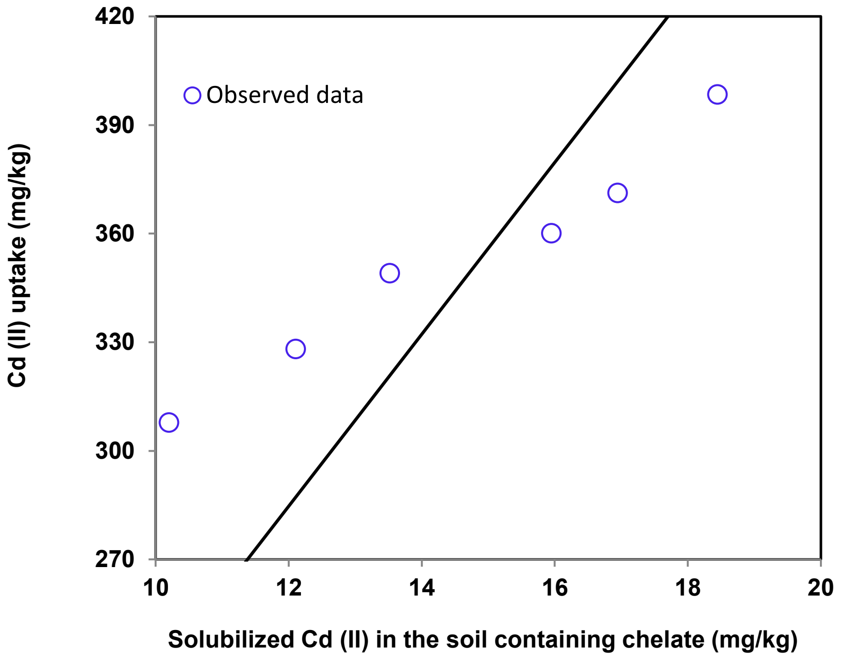

Fig 6.1 Linear model (n=1) for chelate enhanced Cd (II) uptake

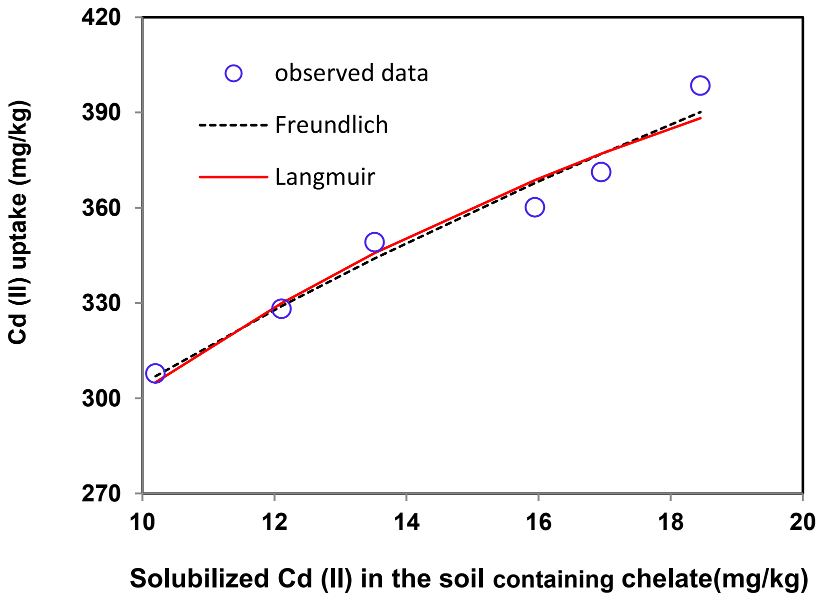

Fig 6.2 Freundlich type and Langmuir type models for chelate enhanced Cd (II) uptake

Compared to the Freundlich model, the EF and t- test data indicated that predictions of Cd (II) were slightly more consistent with simulation results using the Langmuir type model (Fig 6.2) in the presence of chelate. The t-test indicated that there were no significant differences between observed and simulated values for the nonlinear models of Pb (II) uptake in the presence of chelate (Table 6.4). Compared to the Freundlich type model, the Langmuir type model is slightly appropriate for transport of Pb (II). For Pb (II) uptake, there was reasonable agreement between observed and simulated values for the nonlinear models studied (Fig 6.4). Similar to the case of for Cd (II), the linear model fails to describe the observed Pb (II) uptake (Fig 6.3).

Table 6.4 Models for predicating chelate enhanced Pb (II) uptake

Models / Parameters	Linear	Freundlich	Langmuir
$t_{\text{calculated}}$	2.24	0.014	0.14
EF	0.2	0.81	0.85
Empirical constants	K=76.95	K_F = 1306.9	K_L = 32259.6
		n = 2.16	b = 0.0046

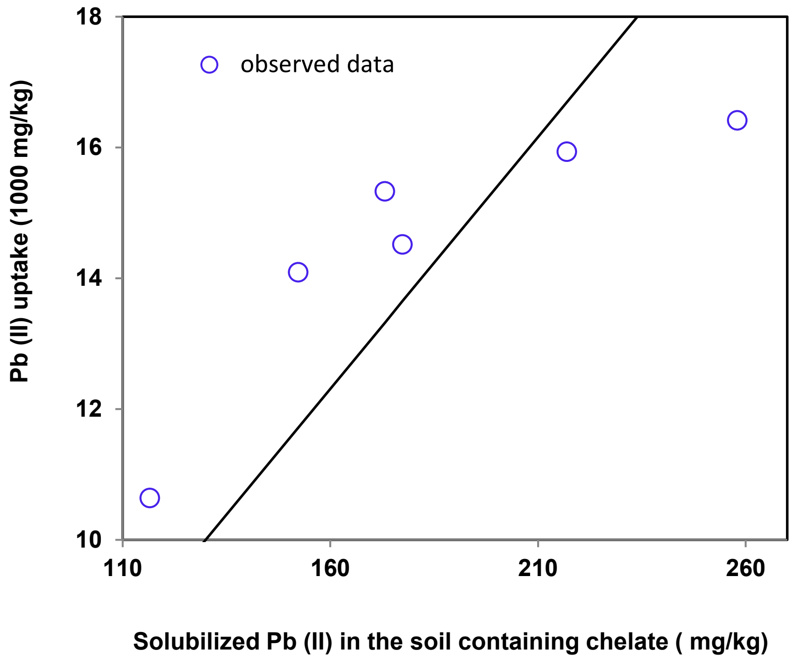

Fig 6.3 Linear model (n=1) for chelate enhanced Pb (II) uptake

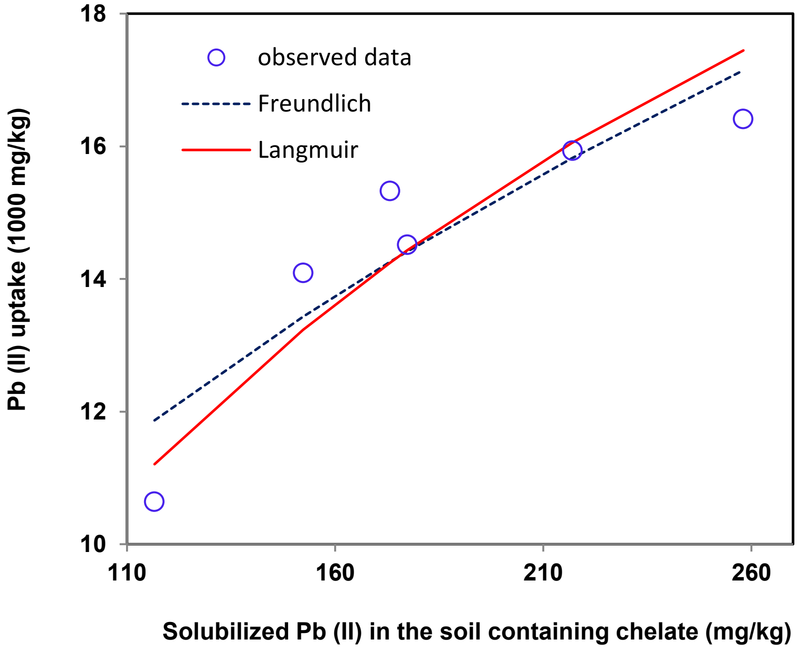

Fig 6.4 Freundlich type and Langmuir type models for chelate enhanced Pb (II) uptake

6.5 Predicting surfactants induced metal uptake by the plant

Similar to section 6.4 the linear, the Freundlich type and the Langmuir type models were used to describe the relation between the solubilized form of Cd (II) and Pb (II) and their uptake in the presence of surfactant**.** Table 6.5 shows the effect of surfactant on soluble Cd (II) and Pb (II) in the soil (section 3.4.4).

Table 6.5 Soluble Cd (II) and Pb (II) [mg/kg] in the soil containing surfactant

Additive		Cd(II)	Pb(II)
Triton X-100 (CMC)	0.5	4.1	21.8
	1	7.7	23.6
	2	9.5	39.7
Tween 80 (CMC)	0.5	6.9	20.6
	1	8.5	20.9
	2	7.8	23.1

The solubilized form of Cd (II) and Pb (II) and their uptake in the presence of surfactant are shown in Table 6.6. Similar to the effect observed for chelate in section 6.4, a positive relationship was present between the Cd (II) and Pb (II) uptake and the total soluble Cd (II) and Pb (II) concentrations in the presence of surfactant (Fig 6.5).

Table 6.6 Solubilized form of Cd (II) and Pb (II) and their uptake in the presence of surfactant

Additive		Cd (II)		Pb (II)	
		Solubilized form (mg/kg)	Plant uptake (mg/kg)	Solubilized form (mg/kg)	Plant uptake (mg/kg)
		(1)	(2)	(1)	(2)
Triton X-100 (CMC)	0.5	7	186.2	83.7	5553.8
	1	11.9	300.4	94.8	6615.7
	2	13.5	316.2	78.2	5362.5
Tween 80 (CMC)	0.5	9.7	185.9	85.5	5666.8
	1	12.2	247.7	93.4	6159.2
	2	11.7	263.4	102.1	6717.7

For all Cd (II) models tested, the $t_{calculated}$ values (Table 6.7) are much less than the critical value of 2.447 at the 95 % confidence level. Compared to the Langmuir type model, the Freundlich type model exhibits a slightly better fit for the Cd (II) uptake in the presence of the surfactant (Fig 6.6).

Compared to Cd (II) uptake, a higher biosorption capacity (K_f) was found for Pb (II) uptake (Tables 6.7 and 6.8). This may be linked to the relatively very high initial concentration of Pb (II) in the soil. Initial soil concentration of Pb (II) was 10 times more than that of Cd (II). The Freundlich type model indicates that K_f (biosorption capacity) of Pb (II) was about 2.6 times more than K_f for Cd (II). This denotes a higher biosorption of Pb (II) for the Indian mustard plant, in the presence of surfactant.

Table 6.7 Models for predicating surfactant enhanced Cd (II) uptake

Models / Parameters	Linear	Freundlich	Langmuir
$t_{\text{calculated}}$	0.11	0.02	0.03
EF	0.79	0.83	0.81
Empirical constants	K=30	$K_F = 33.8$	$K_L = 2629.8$
		n = 1.2	b = 0.0097

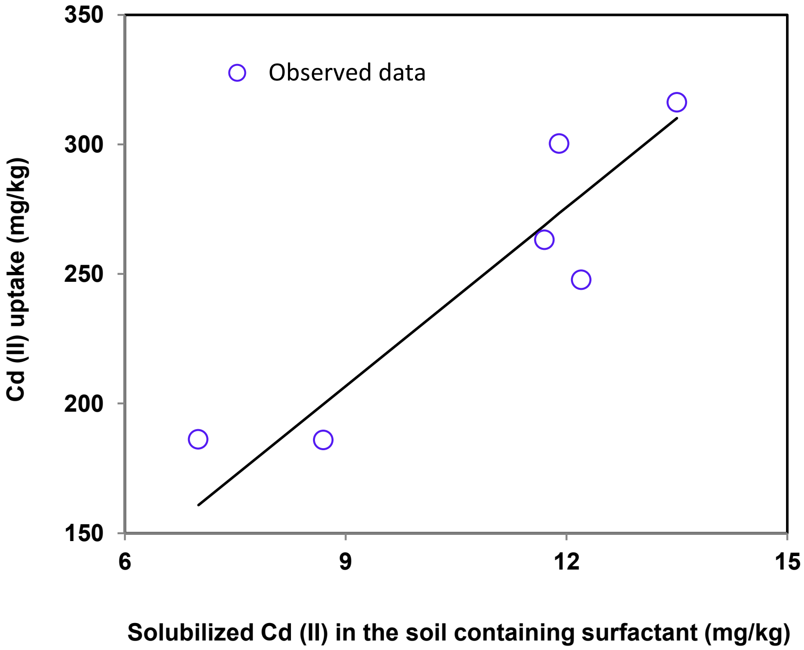

Fig 6.5 Linear model (n = 1) for surfactant enhanced Cd (II) uptake

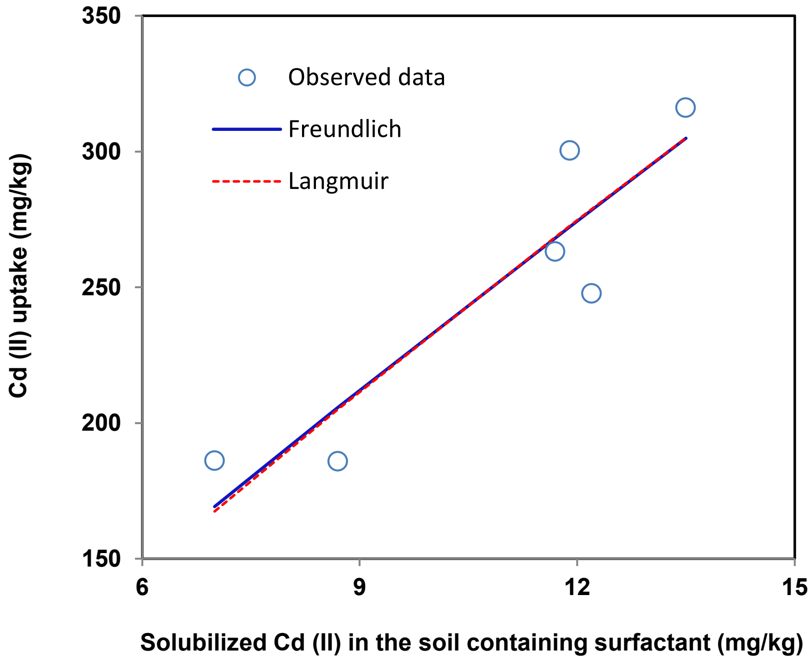

Fig 6.6 Freundlich type and Langmuir type models for surfactant enhanced Cd (II) uptake

According to EF values (Table 6.8), the linear model proves to be less effective in illustrating the biosorption process for Pb (II). In contrast, the Freundlich type and the Langmuir type exhibited a good fit for and Pb (II) with higher EF values. In particular, compared to the Langmuir type model, the Freundlich type model displays an excellent fit for Pb (II) in the presence of surfactant (Fig 6.8).

Table 6.8 Models for predicating surfactant enhanced Pb (II) uptake

Models / Parameters	Linear	Freundlich	Langmuir
$t_{calculated}$	0.05	0.0002	0.02
EF	0.78	0.94	0.90
Empirical constants	K= 67.08	K_F = 87.44	$K_L = 10^5$
		n = 1.066	$b = 7.143 \times 10^{-4}$

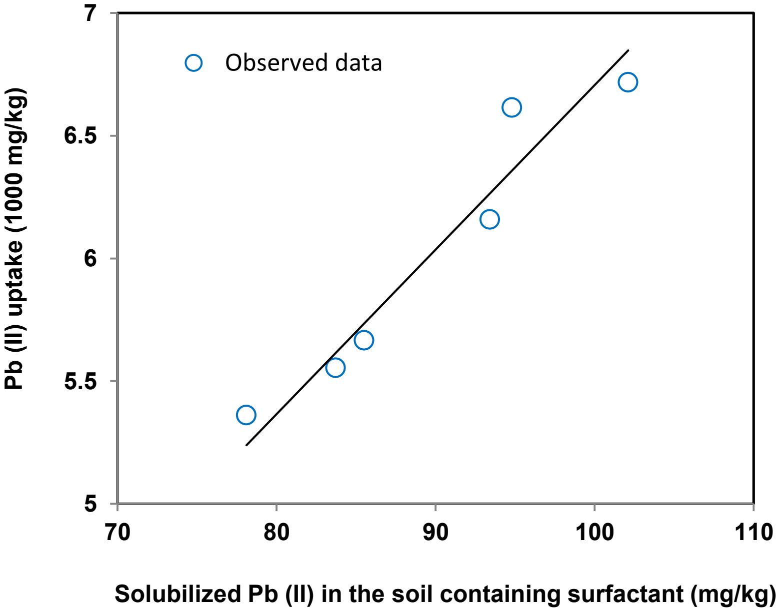

Fig 6.7 Linear model (n = 1) for surfactant enhanced Pb (II) uptake

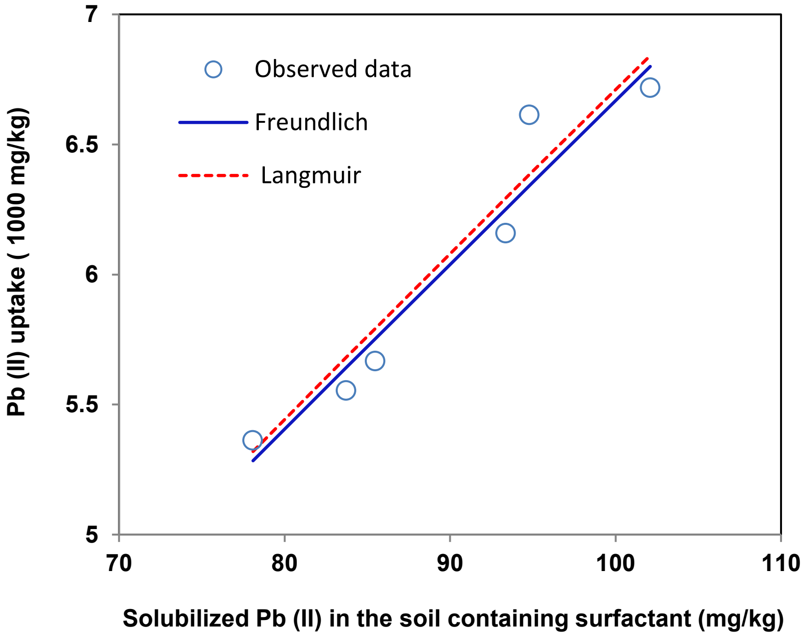

Fig 6.8 Freundlich type and Langmuir type models for surfactant enhanced Pb (II) uptake

6.6 Summary

Both Cd (II) and Pb (II) uptake by plants were correlated with their concentrations in the soil solution as a result of higher mobilization induced by soil additives. The Freundlich type and the Langmuir type models were suitable to describe the transfer of Cd (II) and Pb (II) to the plants from the soil in the presence of soil additives.

For chelate application, the uptake of both Cd (II) and Pb (II) were well described with the Langmuir type model. Compared to Cd (II) uptake, the Langmuir model indicated that the biosorption capacity of Pb (II) was about 5.5 times higher in the presence of chelate. For surfactant application, the uptake of Pb (II) and Cd (II) was well described by the Freundlich type and the Langmuir type models respectively. The Freundlich model indicated that the biosorption capacity for Pb (II) was about 2.6 times more than that for Cd (II), in the presence of surfactant. For the range of metal concentrations studied, the empirical models showed that the enhanced uptake of Cd (II) and Pb (II) in the presence of chelate was greater than their uptake with the surfactant application.

In the next chapter, leaching study model is presented. It permits one to compute the amount of Cd (II) and Pb (II) leachates during enhanced phytoremediation by chelates and surfactants.

Chapter 7

Leaching study

7.1 Introduction

Leachate release from a contaminated soil is a challenging problem due to their toxic impact. In general, leaching studies can be classified into the following categories (Environment Canada, 1991): (a) contaminant release tests under a specific environmental condition, (b) sequential chemical extraction tests, or (c) fundamental leaching parameter tests. Many of these tests are related to the environmental conditions under which leaching of the tested soil may actually occur. The aim of present leaching study is to quantify the mobility of Cd (II) and Pb (II) from the soil water system to leachates, during enhanced phytoremediation.

7.2 Leachability index

According to Environment Canada (1991), Leachability index (LI) value can be used as a performance criterion for the utilization and disposal of stabilized/solidified (S/S) contaminated soil. When LI values are higher than 9, a treatment process can be considered effective and S/S contaminated soil could be used in "controlled utilization". When LI values are lower than 8, contaminated soil is not considered appropriate for disposal. It must be treated until LI values are higher than 9. Although, this study is not related to stabilized/solidified contaminated soil, LI is used as the criteria to evaluate the risk of the leachate of Pb (II) and Cd (II) caused by the application of chelates and surfactants for phytoremediation.

Leachability index (Eq. 7.1) is equal to the negative logarithm of the observed diffusivity (D_{obs}) as defined below (Kosson et al. 1993):

$LI = -\log (D_{obs})$ (7.1)

7.3 Observed diffusivity

In a soil, the total Fickian transport coefficient of a contaminant is the sum of the mechanical dispersion coefficient and the chemical effective diffusion coefficient. Gillham and Cherry (1982) state that chemical effective diffusion is the dominant transport in the soil and that the water velocity in the soil is less than about 1.6×10^{-10} m/s. The observed diffusivity (Eq. 7.2) represents both chemical and physical aspects of leaching and can be calculated by the following equation (Godbee et al. 1980):

$D_{obs} = (\pi/4t)(V/S)^2 (M_T/M_I)^2$ (7.2)

Here,

D_{obs} = Observed diffusivity (cm^2/s)

t = Leaching time period = 20 days

V and S are respectively soil volume (cm^3) and surface area (cm^2) of soil in test pot.

M_T = Total mass of metal in leachate (mg)

M_I = Initial mass of metal in soil (mg)

7.4 Study of kinetics

For the single first-order (SFO) kinetics (Eq.7.3), the time for a decrease in the concentration by a fixed percentage is constant. Thus,

$C_L = C_o \exp(-kt)$ (7.3)

Here,

C_L = Metal leached at time t (mg/kg)

C_o = Metal concentration in soil before adding additive (mg/kg)

k = Leaching rate (1/ day)

Sometimes, the leaching process cannot be modeled by the above SFO kinetics. A fast initial decrease in a concentration is often followed by a slower reduction in concentration. According to Pignatello (2000) the available fraction often decreases with time due to slow diffusion processes. He also abbreviated the bi-exponential model (Eq. 7.4) as two-first-order (TWFO) kinetics. Metals are assumed to be sited into two independent compartments the fast and the slow leaching compartment respectively (Eq. 7.5).

$$C_L = C_f \exp(-k_f t) + C_s \exp(-k_s t) \qquad (7.4)$$

$$C_o = C_f + C_s \qquad (7.5)$$

Here,

C_f = Metal concentration in soil applied to the fast leaching compartment (mg/ kg)

C_s = Metal concentration in soil applied to the slow leaching compartment (mg/ kg)

k_f = Fast leaching rate (1/ day)

k_s= Slow leaching rate (1/ day)

In the present study, a three-exponential model (Eq. 7.6) is proposed and abbreviated as the three-first-order (THFO) kinetics model. Here, the metals are assumed to be placed in three independent compartments the fast, relatively fast and slow leaching compartment respectively.

$$C_L = C_f \exp(-k_f t) + C_{rf} \exp(-k_{rf} t) + C_s \exp(-k_s t) \qquad (7.6)$$

Here,

C_f, C_{rf} and C_s are metal concentration in soil applied to the fast, relatively fast and slow leaching compartments respectively. Also, k_f, k_{rf} and k_s are the rates for the fast, relatively fast and slow leaching compartments respectively (Eq. 7.7).

$$C_o = C_f + C_{rf} + C_s \qquad (7.7)$$

7.5 Observed diffusivity and leachability index of Cd (II) and Pb (II) for soil in the presence of chelate

The maximum leachability index for both metals was found for the application of EDDS at 0.5 mmol/kg to the soil (Table 7.1). These index values indicate that this additive generally caused the minimum amount of metal leaching (section 7.2). According to LI values, 0.5 mmol/kg of EDDS can be considered as the safest additive among of all chelates tested. Further, the LI values indicated that the chelates EDDS (2 mmol/kg), EDTA (1 mmol/kg) and EDTA (2 mmol/kg) cannot be considered as safe additives.

Table 7.1 Observed diffusivity and leachability index of Cd (II) and Pb (II) due to the application of chelates

Additive	Metal	Total leachate (mg)	Observed diffusivity (cm^2/s)	Leachability index
Control	Cd(II)	1.4	4.3E-10	9.4
	Pb(II)	-	-	-
EDDS (0.5 mmol/kg)	Cd(II)	3.6	2.9E-09	8.5
	Pb(II)	10.4	2.4E-10	9.6
EDDS (1 mmol/kg)	Cd(II)	6.6	9.6E-09	8
	Pb(II)	14.2	4.5E-10	9.3
EDDS (2 mmol/kg)	Cd(II)	7.8	1.3E-08	7.9*
	Pb(II)	39.4	3.4E-09	8.5
EDTA (0.5 mmol/kg)	Cd(II)	5.2	6E-09	8.2
	Pb(II)	44.4	4.4E-09	8.4
EDTA (1 mmol/kg)	Cd(II)	8.6	1.6E-08	7.8*
	Pb(II)	75.8	1.3E-08	7.9*
EDTA (2 mmol/kg)	Cd(II)	9.2	1.9E-08	7.7*
	Pb(II)	113	2.8E-08	7.5*

* Inappropriate additive

7.6 Observed diffusivity and leachability index of Cd (II) and Pb (II) for soil in the presence of surfactant

The maximum leachability index for both metals was found in the soil with the application of Tween 80 at 0.5 CMC. This indicates that this additive resulted in the minimum amount of leaching of Cd (II) and Pb (II). According to LI values, all surfactants tested can be considered as appropriate additives. Compared to Triton X-100, Tween 80 lowered the diffusivity and resulted in a higher value of LI indicating that this surfactant was safer (Table 7.2).

Table 7.2 Observed diffusivity and leachability index of Cd (II) and Pb (II) due to the application of surfactants

Additive	Metal	Total leachate (mg)	Observed diffusivity (cm^2/s)	Leachability index
Control	Cd(II)	1.4	4.3 E -10	9.4
	Pb(II)	0	-	-
Triton X-100 (0.5 CMC)	Cd(II)	1.6	5.7 E -10	9.2
	Pb(II)	0	-	-
Triton X-100 (1 CMC)	Cd(II)	2.4	1.3 E -9	8.9
	Pb(II)	10.8	2.6 E -10	9.6
Triton X-100 (2 CMC)	Cd(II)	4.9	5.3 E -10	8.3
	Pb(II)	25.5	1.4 E -9	8.8
Tween 80 (0.5 CMC)	Cd(II)	1.2	3.2 E -10	9.5
	Pb(II)	0	-	-
Tween 80 (1 CMC)	Cd(II)	2.2	1.1 E -9	9
	Pb(II)	7.2	1.2E -9	9.9
Tween 80 (2 CMC)	Cd(II)	3.2	2.3 E -9	8.6
	Pb(II)	10.2	2.3E -10	9.6

7.7 Leaching kinetics

Kinetics of the leaching process of Cd (II) and Pb (II) in the test pot soil with application of chelates was studied as a function of their initial concentration in the soil before adding chelates. In each leaching event, for both surfactants tested as well as the control treatment, Cd (II) and Pb (II) concentrations were too small for measurement. As described in chapter 3, after each irrigation event at 3-day intervals, the leachate collected from each test pot soil subjected to chelate application contained measurable Cd (II) and Pb (II). The observed data (Table 7.3) were fitted with the three models (SFO, TWFO and THFO). The fitted equations and their modeling efficiency (EF) values are shown in

Table 7.4 and Table 7.5. Fig 7.1 shows the fitted kinetic curves for an experimental result of Cd (II) leaching by EDDS and EDTA for the concentrations tested. Similar results as those shown in Fig 7.2 were obtained for the data sets of Pb (II). The TWFO model provided a good fit for the experimental data. However, the t-test indicated that there were significant differences between observed and simulated values for the SFO models of Pb (II) leaching in the presence of EDTA.

Table 7.3 Observed data of Cd (II) and Pb (II) leached (mg/kg) due to application of chelates

Chelate	Metal	Leaching intervals (day)							
		30	33	36	39	42	45	48	50
EDDS (0.5mmol/kg)	Cd (II)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Pb (II)	1.5	1.1	0.9	0.7	0.3	0.2	0.1	0.1
EDDS (1mmol/kg)	Cd (II)	1	0.7	0.6	0.4	0.2	0.2	0.1	0.1
	Pb (II)	2.2	1.4	1	0.8	0.5	0.4	0.4	0.4
EDDS (2mmol/kg)	Cd (II)	1.2	0.9	0.5	0.4	0.4	0.2	0.2	1.2
	Pb (II)	4.2	3.7	3.2	2.5	1.8	1.5	1.4	1.4
EDTA (0.5mmol/kg)	Cd (II)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Pb (II)	3.6	3.2	3	2.9	2.6	2.5	2.2	2.2
EDTA (1mmol/kg)	Cd (II)	1.2	0.9	0.6	0.5	0.3	0.3	0.2	0.2
	Pb (II)	6.6	5.7	5.2	4.6	4.3	4	3.8	3.7
EDTA (2mmol/kg)	Cd (II)	1.2	1	0.6	0.5	0.4	0.4	0.3	0.2
	Pb (II)	12.3	9.6	7.7	6.5	5.5	5.2	5	4.7

N.S. = Not Significant

Table 7.4 Kinetics equations of the leaching process for heavy metals in soil with EDDS

Chelate	Metal	Kinetic	Equation	EF
EDDS (0.5 mmol/kg)	Cd(II)	---	------------	---
	Pb(II)	SFO	446.4 exp (–0.184t)	0.82
		TWFO	420 exp (–0.76 t) + 26.4exp (–0.1t)	0.96
		THFO	397.7 exp (–0.63 t) + 25.3exp (–0.115t) + 23.4exp (–0.114t)	0.96
EDDS (1 mmol/kg)	Cd(II)	SFO	48.6 exp (–0.127t)	0.96
		TWFO	20 exp (–0.67 t) + 28.6exp (–0.11t)	0.98
		THFO	------------	---
	Pb(II)	SFO	446.4 exp (–0.174t)	0.83
		TWFO	438.5 exp (–0.2 t) + 7.9 exp (–0.07t)	0.98
		THFO	438.3exp (–0.19 t) + 7.15 exp (–0.16t) + 0.95exp (–0.022t)	0.98
EDDS (2 mmol/kg)	Cd(II)	SFO	48.6 exp (–0.1258t)	0.97
		TWFO	48.47 exp (–0.125 t) + 0.13exp (–0.02t)	0.98
		THFO	41.82exp (–0.125 t) + 6.7 exp (–0.125t) + 0.08exp (–0.012t)	0.98
	Pb(II)	SFO	446.4 exp (–0.16t)	0.15
		TWFO	417.7 exp (–0.55 t) + 28.7 exp (–0.063t)	0.97
		THFO	-----------------	---

Table 7.5 Kinetics equations of the leaching process for heavy metals in soil with EDTA

Chelate	Metal	Kinetic	Equation	EF
EDTA (0.5 mmol/kg)	Cd(II)	----	-----------------	---
	Pb(II)	SFO	446.4 exp (–0.147t)	- 10.52
		TWFO	439.6 exp (–0.27 t) + 6.8 exp (–0.023t)	0.98
		THFO	-----------------	---
EDTA (1 mmol/kg)	Cd(II)	SFO	48.6 exp (–0.121t)	0.97
		TWFO	48exp (–0.129 t) + 0.6exp (–0.034t)	0.99
		THFO	--------------	---
	Pb(II)	SFO	446.4 exp (–0.129t)	- 4.6
		TWFO	437.7 exp (–0.19 t) + 8.7 exp (–0.017t)	1
		THFO	434.7 exp (–0.19 t) + 8.4 exp (–0.04t) + 3.3 exp (–0.006t)	1
EDTA (2 mmol/kg)	Cd(II)	SFO	48.6 exp (–0.12t)	0.91
		TWFO	47.94exp (–0.13 t) + 0.66exp (–0.0264t)	0.98
		THFO	--------------------	---
	Pb(II)	SFO	446.4 exp (–0.114t)	0.26
		TWFO	443.1 exp (–0.132 t) + 3.3 exp (–0.004t)	1
		THFO	401.7 exp (–0.141 t) + 43.9 exp (–0.07t) + 0.8 exp (–0.03t)	1

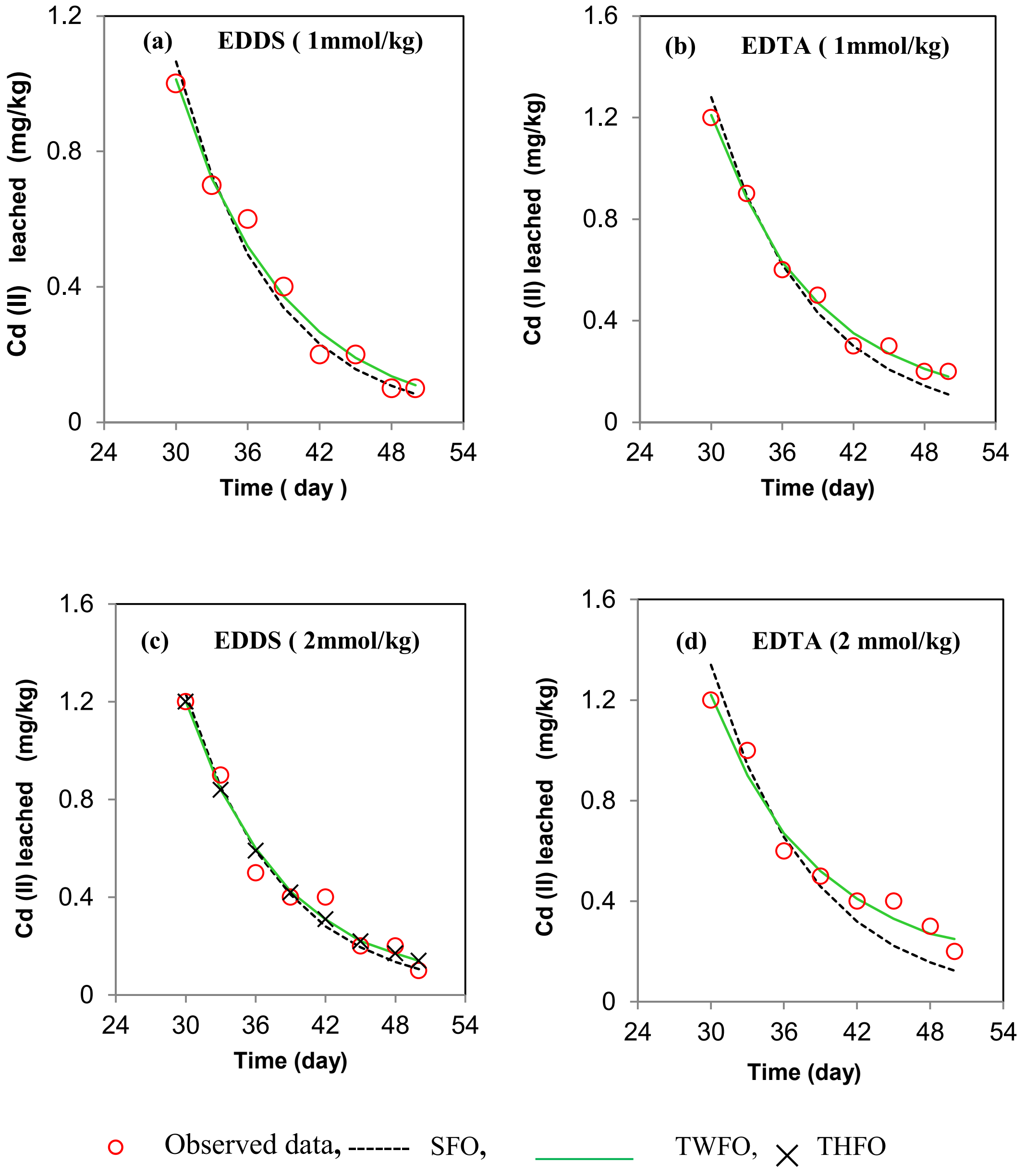

Fig 7.1 SFO, TWFO and THFO kinetics for the leaching process of Cd (II) due to EDDS and EDTA application

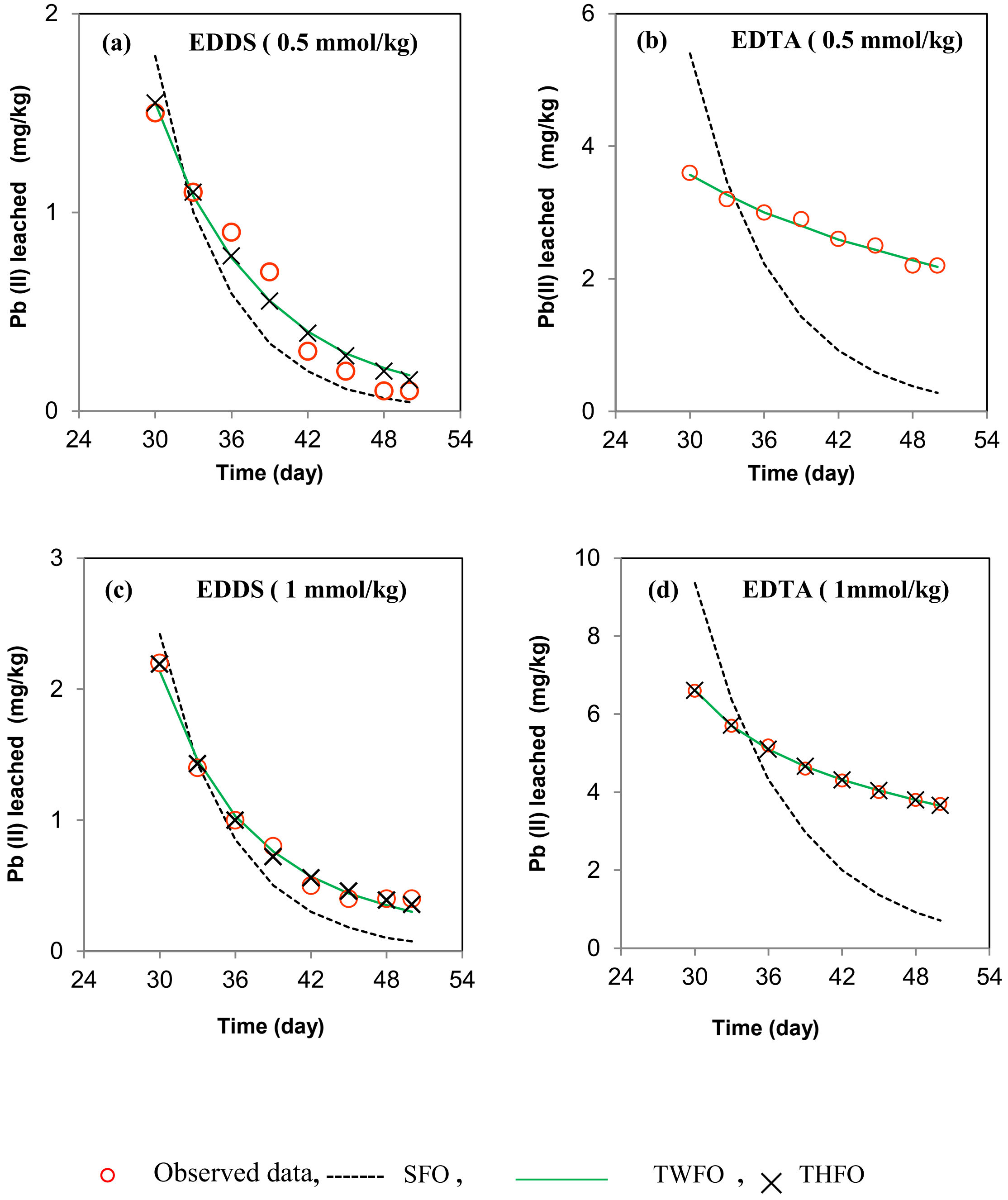

Fig 7.2 **SFO, TWFO and THFO kinetics for the leaching process of Pb (II) due to EDDS and EDTA application**

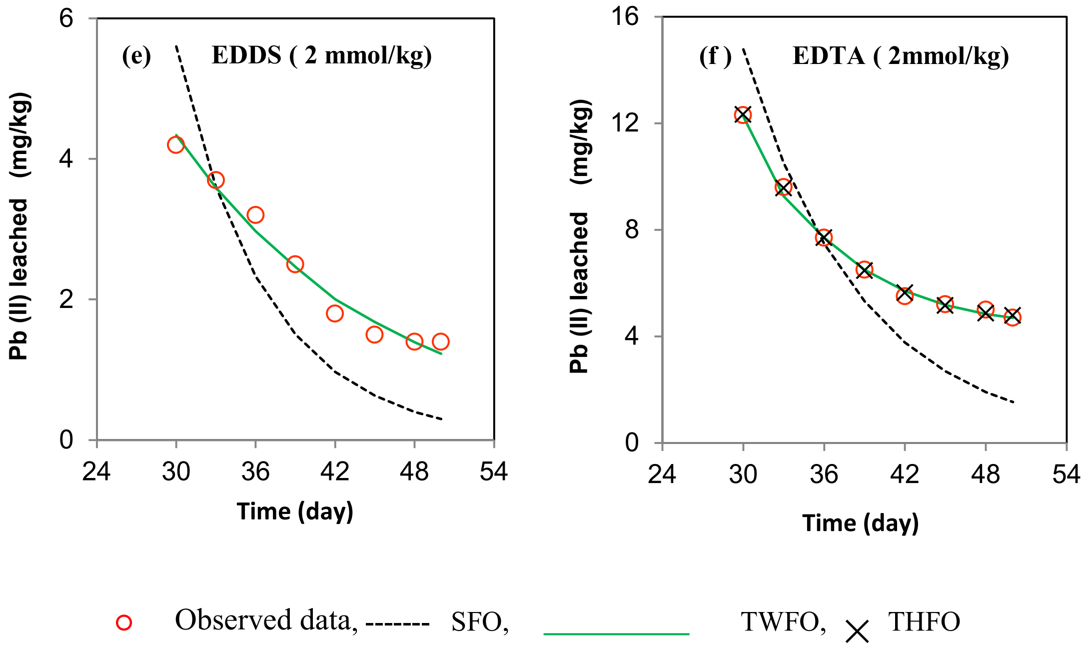

Fig 7.2 Continued

7.8 Summary

In order to assess the long-term leaching behavior of Cd (II) and Pb (II) in test pots treated with additives, the leachability index (LI) of the leached samples was determined based on the observed diffusivity. LI value revealed that 0.5 mmol/kg of EDDS can be considered as the safest additive among all chelates tested. Surfactant additives were very effective in reducing diffusivity of Cd (II) and Pb (II) than chelate additives. According to LI values, all surfactants tested can be considered as safe additives. Compared to Triton X-100, Tween 80 resulted in a lower diffusivity and a higher value of LI. This indicates that this surfactant was safer from the point of view of reducing ground water pollution.

The EF value for the SFO kinetic was considerably lower than the EF value obtained for TWFO and THFO kinetics. The SFO kinetics proves to be unsuitable to define the leaching process for Cd (II) and Pb (II). The EF indicated that there were no significant differences between TWFO and THFO kinetics for the predictions of Cd (II) and Pb (II) leaching. The TWFO kinetics exhibited an excellent fit for the metals leaching behavior.

The final section (chapter 8) deals with the conclusions and contributions related to the study.

Chapter 8

Summary and conclusions, contributions

8.1 Summary and conclusions

The present study evaluated the potential of enhanced phytoremediation technique for the remediation of soils contaminated with mixed contaminants composed of both heavy metals and a hydrocarbon (used engine oil).

Indian mustard plant alone removed 7.5 % of Cd (II), 13.4 % of Pb (II) by uptake and 38 % of used engine oil through rhizodegradation from the soil without additives (no surfactant or chelate). Compared to the plant uptake by the control (no soil additives), the ratio of the increase of Pb (II) uptake by the plant due to chelate additives ranged from 1.7 to 5. The corresponding ratio of the increase of Cd (II) uptake by the plant due to chelates as additives was in the range of 1.4 to 1.6. At same concentration, EDTA resulted in more phytoremediation of Cd (II) and Pb (II) than EDDS. Further, EDTA was also more effective in facilitating rhizodegradation of the used engine oil. On the other hand, EDDS treatment led to a lower amount of Pb (II) in the leachate. However, compared to EDTA, the addition of EDDS resulted in a slight decrease in BSR (basal soil respiration). Further, an increase in EDTA concentration resulted in a significant drop in BSR. Briefly, among the two chelates studied, EDDS proved to be the safer treatment from the point of view of reducing groundwater contamination as well as restoring soil quality in terms of BSR.

At the same concentration surfactants, treating the soil with Tween 80 enhanced phytoremediation of Pb (II). Triton X-100 was more effective in terms of uptake of Cd (II) and Pb (II). However, the plant biomass associated with Tween 80 additive was much higher than the biomass associated with triton X-100. The tests showed that the application of Tween 80 resulted in an increase in BSR. Briefly, the results indicated that the lower concentration of TritonX-100 had a slightly positive effect on BSR, whereas it was inhibitory to the microbes at higher concentrations.

Modeling studies indicated that the uptake of both Cd (II) and Pb (II) can be properly represented by the Langmuir type model for chelate (EDDS and EDTA) additives. Considering their initial soil concentrations [496.5 mg/kg of Pb (II) and 49.7 mg/kg of Cd (II)], the Langmuir type model indicates that the uptake capacity of Pb (II) was about 5 times more than Cd (II) in the presence of chelates. For surfactants (Triton X-100 and Tween 80) additives, the uptake of Pb (II) and Cd (II) was well described by the Freundlich type model. The Freundlich type model indicated that the uptake capacity of Pb (II) was about 2.6 times more than Cd (II) in the presence of surfactants. This signifies that compare to the plant uptake of Cd (II), Indian mustard plant has higher uptake capacity for Pb (II) in the presence of surfactants. The TWFO kinetic was able to describe the leaching process for both Cd (II) and Pb (II).

Based on LI (leachability index) of the samples established on the observed diffusivity model, leaching studies indicated that both surfactants tested are safe additives. Compared to Triton X-100, Tween 80 resulted in a lower values of diffusivity and higher values of LI, indicating that this surfactant was safer from the point of view of reduced ground water pollution. LI values revealed that 0.5 mmol/kg of EDDS can be considered as the safest additive among all chelates tested to limit ground water pollution.

8.2 Contributions

The present study indicates that chelates (EDDS and EDTA) and surfactant (Tween 80) can enhance phytoremediation of soils polluted by mixed contaminants (heavy metals and used engine oil). In particular, for phytoremediation using Indian mustard plant, chelate EDDS is a safer soil additive from the point of view of ground water pollution.

The results suggested that the application of Tween 80 also resulted in an increase in BSR during phytoremediation and this is desirable as it indicates improve soil quality.

The Freundlich type and the Langmuir type models are shown to describe the transfer of Cd (II) and Pb (II) to the plants from the soil in the presence of soil additives.

The two single first order (TWFO) kinetic model provided an excellent fit for the metal leaching behavior.

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Appendix

The following section included tabulated data related to the graphs in chapters 4 and 5.

Table A.1 Effect of chelates on Cd (II) and Pb (II) leachate (mg/kg)

Additives		Cd (II)	Pb (II)
Control (C 2)		0.7 ± 0.03	0
EDDS (mmol/kg)	0.5	1.8 ± 0.1	5.2 ± 1.5
	1	3.3 ± 0.4	7.1 ± 4.3
	2	3.9 ± 1.1	19.7 ± 5.5
EDTA (mmol/kg)	0.5	2.6 ± 0.6	22.2 ± 3.6
	1	4.3 ± 1.1	37.9 ± 5.3
	2	4.6 ± 1.2	56.5 ± 8.6

Table A.2 Effect of chelates on rhizodegradation of the oil (mg/kg)

Additives		Residual oil in soil
Control (C 2)		308.5 ± 16.5
EDDS (mmol/kg)	0.5	262.7 ± 16.6
	1	238.3 ± 28.2
	2	150.4 ± 12
EDTA (mmol/kg)	0.5	298.4 ± 44.3
	1	254.6 ± 28.2
	2	226.5 ± 39.5

Table A.3 Influence of chelates on basal soil respiration (μg C g^{-1} DW soil h^{-1})

Additives			BSR
Initial value			1.1 ± 0.14
Final value	Control (C 2)		2.44 ± 0.25
	EDDS (mmol/kg)	0.5	1.22 ± 0.24
		1	0.66 ± 0.16
		2	0.39 ± 12
	EDTA (mmol/kg)	0.5	1.54 ± 0.3
		1	1.12 ± 0.24
		2	1.07 ± 0.32

Table A.4 Effect of surfactants on root Pb (II) in plants (10^3mg/kg)

Additives		Pb (II) in root
Control (C 2)		5.72 ± 1.2
Triton X-100 (CMC)	0.5	6.10 ± 2.2
	1	11.16 ± 2.1
	2	18.27 ± 1.5
Tween 80 (CMC)	0.5	5.90 ± 1.4
	1	8.50 ± 2.4
	2	11.2 ± 4.7

Table A.5 Effect of surfactants on Cd (II) and Pb (II) leachate (mg/kg)

Additives		Cd (II)	Pb (II)
Control (C 2)		0.7 ± 0.03	0
Triton X-100 (CMC)	0.5	0.8 ± 0.4	0
	1	1.2 ± 0.29	5.4 ± 1.1
	2	2.5 ± 0.1	12.75 ± 2.1
Tween 80 (CMC)	0.5	0.6 ± 0.24	0
	1	1.1 ± 0.68	3.8 ± 1.7
	2	1.6 ± 0.24	5.1 ± 0.9

Table A.6 Effect of surfactants on rhizodegradation of the oil (mg/kg)

Additives		Residual oil in soil
Control (C 1)		362.2 ± 17.6
Control (C 2)		308.5 ± 16.5
Triton X-100 (CMC)	0.5	254.5 ± 23.2
	1	295.2 ± 47.2
	2	317.7 ± 24.2
Tween 80 (CMC)	0.5	287.3 ± 14.6
	1	187.5 ± 18.5
	2	124.6 ± 11.7

Table A.7 Influence of surfactants on basal soil respiration (μg C g $^{-1}$ DW soil h $^{-1}$)

Additives			BSR
Initial value			1.1 ± 0.14
Final value	Control (C 2)		2.44 ± 0.25
	Triton X-100 (CMC)	0.5	2.29 ± 0.22
		1	2.36 ± 0.66
		2	2.86 ± 0.42
	Tween 80 (CMC)	0.5	1.95 ± 0.23
		1	1.07 ± 0.15
		2	0.8 ± 0.12