REMEDIATION OF TRACE ELEMENT-CONTAMINATED GROUNDWATER AND SOILS USING REDOX-SORPTION AND PHYTOEXTRACTION TECHNIQUES

by

Alison Patricia Murata

A Thesis submitted to the Faculty of Graduate Studies of
The University of Manitoba
in partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

Department of Soil Science
University of Manitoba
Winnipeg, Manitoba

ABSTRACT

Murata, Alison Patricia. M.Sc., The University of Manitoba, February, 2013.
Remediation of Groundwater and Soils Contaminated with Trace Elements using Redox-Sorption and Phytoextraction Techniques. Major Professor: Francis Zvomuya.

Remediation of trace element-contaminated sites must consider both the nature of the contaminants and environmental surroundings. This thesis examined treatments for two contamination scenarios. The first study characterized chromium dynamics during the redox-sorption treatment of aqueous hexavalent chromium with the reducing agent sodium dithionite and two iron oxides. Results showed that chromium was successfully removed from solution by precipitation and sorption. The iron oxide derived from ferric chloride had a greater sorption capacity for hexavalent chromium than the oxide derived from ferrous chloride. The second study examined the phytoextraction treatment of soils contaminated with multiple trace elements. *Deschampsia caespitosa* plants had better early growth in the contaminated high-organic matter soil than three *Brassica* species. However, *D. caespitosa* plants did not take up sufficient amounts of trace elements during the study to be considered useful for short-term phytoextraction. These findings are applicable to the development of effective trace element remediation methods.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to: Dr. Francis Zvomuya for his guidance, support, and feedback as my M.Sc. advisor; Drs. Tee Boon Goh, Sylvie Renault, and Muhammad Tahir for their research contributions and service on my M.Sc. committee; Dr. Yonfeng Hu for his expertise and technical assistance with X-ray absorption spectroscopy; Messrs. Rob Ellis and Tim Stem for technical assistance with laboratory and growth room experiments; Soil and Landscape Management Section, Agri-Environment Knowledge Centre, Manitoba Agriculture, Food and Rural Initiatives for their laboratory service; the National Science and Engineering Research Council of Canada, Manitoba Graduate Scholarship, and University of Manitoba Graduate Fellowship for financial assistance; and my family and friends for their encouragement and support.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

1. INTRODUCTION

1.1 Contamination

Environmental contamination is a widespread and serious problem typically associated with industrial activity. Global estimates of contaminated sites are in the millions (Zvomuya and Murata 2012). According to Singh et al. (2009), the international market value for the remediation sector is in the range of US$30-35 billion. Remediation in the U.S.A. is thought to account for about 30% or US$12 billion of this total market value (Singh et al. 2009). According to Canada's Federal Contaminated Sites Inventory, there are over 22,000 contaminated or suspected contaminated sites in Canada that are either under the custodianship of federal departments, agencies, or consolidated Crown corporations or for which the federal government has taken at least partial financial responsibility (TBS 2011). These sites have been identified according to the federal government's definition of a contaminated site as one which contains substances above guideline levels or above normally-occurring background levels and at concentrations which likely pose short- or long-term danger to environmental and human health (TBS 2011). The number of contaminated sites under the responsibility of enterprise Crown corporations, private individuals, firms, and other levels of government would push Canada's total inventory higher. Singh et al. (2009) have estimated a total of 30,000

contaminated sites in Canada and with a soil remediation value of about $250-500 million.

The term "contaminant" can refer to a variety of substances, including but not limited to petroleum hydrocarbons, trace elements, pesticides, and radionuclides (Knox et al. 1999; Zvomuya and Murata 2012). All of these contaminants have the potential to cause harm to environmental and human health when present in sufficiently high concentrations. The focus of this thesis is trace element contamination, one of the most prevalent types of contamination. Trace elements are naturally occurring components of the Earth's crust, although they are usually present in very small concentrations. The concentration-based definition of trace elements is somewhat arbitrary and varies among sources. Hooda (2010) describes trace elements as existing at concentrations less than 100 mg kg^{-1} in the environment while Kabata-Pendias (2011) uses the threshold concentration of 1000 mg kg^{-1}. Most trace elements are metals although some belong to the metalloid, nonmetal, actinoid, or halogen groups (Hooda 2010). Some trace elements have biological functions and are considered essential micronutrients for living organisms. Trace elements required for plant growth include boron (B), cobalt (Co), copper (Cu), manganese (Mn), molybdenum (Mo), and Zinc (Zn) (Hooda 2010; Kabata-Pendias 2011; Zovko and Romic 2011). When trace element concentrations become elevated, toxicity concerns arise due to the impedance or disruption of metabolic reactions (Kabata-Pendias 2011). Contaminant transfer pathways between abiotic and biotic components of the environment can be grouped into two general categories: uptake by plants from contaminated media and subsequent transfer through the food chain and direct ingestion

and inhalation of contaminated abiotic media by humans and wildlife (Iskander and Kirkham 2001; Zovko and Romic 2011).

Since trace elements are naturally occurring, environmental levels may become elevated due to natural processes such as weathering and erosion, volcanic eruptions, and forest fires (Hooda 2010; Kabata-Pendias 2011). Natural trace element loading is typically limited in areal extent (Zvomuya and Murata 2012). Most trace element contamination problems occur as a result of mobilization or chemical alteration due to anthropogenic activity (Zvomuya and Murata 2012). These activities are usually related to energy and mineral consumption (Kabata-Pendias 2011). More specific anthropogenic sources of trace element loading in the environment include fossil fuel combustion, mining and smelting, manufacturing, sewage sludge application, industrial waste disposal, and agrochemical usage (Knox et al. 1999; Hooda 2010; Kabata-Pendias 2011). Trace elements from anthropogenic sources are typically considered to be more bioavailable than those of pedogenic origin (Kabata-Pendias 2011). Trace elements frequently present at contaminated sites and considered to be of high environmental concern include arsenic (As), cadmium (Cd), chromium (Cr), Cu, lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), and Zn (Kabata-Pendias 2011; Zvomuya and Murata 2012).

1.2 Contaminant Fate

Once a contaminant enters a soil system, it is subject to a variety of physical, chemical, and biological processes. The fate of any given soil contaminant depends heavily upon its properties as well as those of the soil in which it is found (Zovko and Romic 2011). In

general, a contaminant will be involved in one or more of the following processes: sorption, leaching or runoff, plant uptake, chemical or biological reaction, and volatilization (Brady and Weil 2008).

Contaminants can be transferred to and retained on solid soil surfaces through the process of sorption. The term sorption refers to several retention mechanisms, namely adsorption, surface precipitation, and polymerization (Sparks 2003). Soil does not sorb the total amount of a given contaminant present in the environment; rather, the contaminant will reach an equilibrium between the sorbed and dissolved states (Yong et al. 1992). The amount of contaminant which can be sorbed and the strength with which it is held depends on the sorbent (the soil) and the sorbate (the contaminant). Soil texture is an important soil property influencing sorption; fine-textured soils have a greater ability to sorb contaminants than do coarse-textured soils. This sorptive ability arises from the high specific surface area and net negative charge inherent to small clay particles (Brady and Weil 2008). The presence of colloidal organic matter, oxides, and hydroxides also promotes sorption by providing large surface areas (Zovko and Romic 2011).

The main characteristics of contaminants which affect sorption are charge and functional groups; in general, contaminants with more charged sites and functional groups are more easily sorbed (Brady and Weil 2008). Oxidation state of contaminants is also important. Many trace elements such as As, Cr, and Se can exist naturally in multiple oxidation states, depending on environmental conditions (Palmer and Fish 1991; Knox et al. 1999). Oxidation state may affect the form and charge of the contaminant, therefore influencing its tendency to be sorbed (Palmer and Fish 1991; Zovko and Romic 2011). Because of

the connection between charge and sorption, soil pH is another important factor in contaminant sorption. For inorganic contaminants, sorption is maximized at low pH for anions and at high pH for cations (Palmer and Fish 1991; Zovko and Romic 2011). For organic contaminants, the abundance of H^+ ions in acidic soils causes the protonation of functional groups which may cause a net positive charge and increase sorption on negatively charged clay particles (Brady and Weil 2008). Sorption is an important process because it can prevent contaminants from moving within or out of a given volume of soil. Therefore, contaminants which are strongly sorbed are at lower risk of being transported via leaching or runoff.

When contaminants are not sorbed to the soil, they are available to be lost with water movement. Leaching, the movement of water downward through the soil profile, and runoff, the movement over the soil surface, are potentially dangerous as they may spread contamination to groundwater or surface water bodies used by humans or wildlife. The tendency for a contaminant to be transported by water depends on its own properties as well as those of the soil matrix in which it is found. Contaminants which are highly water-soluble are more susceptible to loss through leaching and runoff (Brady and Weil 2008). Coarse-textured soils are particularly prone to leaching and the contamination of groundwater due to their high permeability caused by large pore spaces through which water and solutes can easily move (Knox et al. 1999; Brady and Weil 2008). Sandy soils are also low in sorption-promoting clays. In contrast, the small pores associated with fine-textured soils hamper rapid leaching. However, the presence of large vertical cracks or macropores in clayey soils can allow contaminants to move down the soil profile

extremely quickly via preferential flow without passing through the soil matrix (Brady and Weil 2008). When considering runoff, infiltrability is a highly influential soil property. Runoff occurs whenever the rate at which water is being added to the soil via precipitation or irrigation exceeds the rate at which the water is able to penetrate into the soil. Fine-textured soils are more susceptible to runoff since they typically have lower infiltration rates than sandy soils (Brady and Weil 2008). This runoff may carry soluble contaminants with it and may also cause erosion and the transport of contaminants sorbed to soil particles.

Both organic and inorganic contaminants can be absorbed by higher plants. Once a contaminant is taken up from the soil through a plant's root system, it may remain unchanged or it may be broken down (Brady and Weil 2008). Some plant species are known to take up exceptionally high concentrations of contaminants and are called hyperaccumulators. *Brassica juncea* (L.) Czern. (Indian mustard) for example, is known to accumulate large amounts of Pb, Zn, Ni, Cu, Cd, Cr, U, and Mn in its tissues (Environment Canada 2003). Contaminants may also undergo a variety of chemical reactions, including oxidation-reduction, hydrolysis, and photodecomposition. Some reactions are very slow while others can take place in a matter of hours (Henson 1991; Brady and Weil 2008). Contaminants are affected by biological reactions involving microbes within the soil as well. Biological reactions may result in the complete mineralization of a compound or partial degradation (Henson 1991). Some compounds are easily decomposed while others are very persistent (Brady and Weil 2008). Soil conditions are important to both chemical and biological reactions. For example, pH may

affect the solubility of a contaminant, soil components may catalyze reactions, and temperature and moisture content may affect microbial activity (Henson 1991). Although chemical and biological reactions decrease the amount of contaminant present at an impacted site, it is important to consider the breakdown products from these reactions which may be more toxic than the original contaminant (Henson 1991; Brady and Weil 2008). The tendency of a contaminant to evaporate from an impacted site depends heavily upon the contaminant's volatility (Yong et al. 1992). More volatile contaminants, such as low-molecular weight organic compounds, are more easily lost to the atmosphere.

1.3 Remediation

Remediation is generally defined as the process of treating a contaminated site to prevent, minimize, or mitigate damage to human or environmental health (Zvomuya and Murata 2012). Remediation strategies generally involve some or all of the following elements: removal, degradation, immobilization, containment, and monitoring (Zvomuya and Murata 2012). Since contamination may occur under a wide variety of circumstances, remediation techniques must also be applicable to many different situations. In some cases, combinations of remediation methods, or treatment trains, may be used to treat contamination at a given site. In general, remediation techniques can be classified as either in situ or ex situ. In situ methods involve the treatment of the contaminated material without any excavation or pumping. Ex situ methods involve the removal of

contaminated material prior to its treatment. Ex situ methods often require transportation of contaminated materials which can be very expensive.

Remediation techniques may also be categorized as physical, chemical, biological, or thermal. Physical remediation methods include soil washing, the use of water or surfactants combined with scrubbing to clean soils; soil flushing, the use of an extraction solution to flood and clean soils; and soil vapour extraction, the use of vacuum pumping to remove gases or volatile contaminants from soils (Sims and Sims 1991; Khan et al. 2004; Singh et al. 2009). Chemical remediation techniques include the addition of acids or solvents to extract contaminants; the addition of oxidizing or reducing agents to react with contaminants; and stabilization, the solidification of contaminated soil to prevent contaminant transport (Sims and Sims 1991; Khan et al. 2004; Singh et al. 2009). Biological remediation may utilize methods such as bioremediation, the addition of water, nutrients, and oxygen to encourage biodegradation of contaminants by microbes, or phytoremediation, the use of plants to degrade, immobilize, or remove soil contaminants (Sims and Sims 1991; Knox et al. 1999; Khan et al. 2004; Singh et al. 2009). Thermal remediation includes incineration, the heating of soil to degrade or detoxify contaminants; thermal desorption, the heating of soil to evaporate and then collect volatile contaminants; and vitrification, the heating of soil to form a solid glassy material in which contaminants are immobilized (Khan et al. 2004; Singh et al. 2009). Consideration of contaminant concentration, behaviour, and extent, hydrogeological conditions, and treatment cost must all be included in developing and selecting remediation strategies for different scenarios (Knox et al. 1999).

1.4 General Objective

The overall objective of this thesis was to examine potential remediation methods for two types of environmental contamination. In the first study, described in Chapter 2, laboratory experiments were conducted to investigate Cr dynamics during a chemical reduction-sorption treatment of a hexavalent Cr solution. Findings from the study were used to evaluate the applicability of the treatment as a remediation technique for hexavalent Cr-contaminated groundwater. In the second study, described in Chapter 3, growth room experiments were carried out to examine the use of different plant species for the phytoextraction of soils contaminated with multiple trace elements. More specific objectives are outlined in the appropriate chapters. The results of both of these studies are important for building knowledge regarding remediation methods which could be used to ensure that contaminated sites are cleaned up effectively and efficiently. This is vital to preventing or reducing the negative impact of contamination on human and environmental health.

1.5 References

Brady, N.C. and Weil, R.R. 2008. Soils and chemical pollution. In: Anthony, V.R., editor, The nature and properties of soils. 14th ed. Pearson Prentice Hall, Upper Saddle River, NJ, U.S.A. p. 794-839.

Environment Canada. 2003. Phytorem: Potential green solutions for metal contaminated sites. Environment Canada, Ottawa, ON.

Henson, M.J. 1991. Characterization of subsurface degradation processes. In: Site characterization for subsurface remediation. Seminar publication EPA/625/4-91/026. U.S. Environmental Protection Agency, Washington, DC, U.S.A. p. 193-201.

Hooda, P.S., editor. 2010. Trace elements in soils. John Wiley and Sons, Chichester, U.K.

Iskander, I.K. and Kirkham, M.B. 2001. Trace elements in soil. CRC Press, Boca Raton, FL, U.S.A.

Kabata-Pendias, A. 2011. Trace elements in soils and plants. 4th ed. CRC Press, Boca Raton, FL, U.S.A.

Khan, F.I., Husain, T., and Hejazi, R. 2004. An overview and analysis of site remediation technologies. J. Environ. Manage. 71:95-122.

Knox, A.S., Gamerdinger, A.P., Adriano, D.C., Kolka, R.K., and Kaplan, D.I. 1999. Sources and practices contributing to soil contamination. In: Adriano, D.C., Bollag, J.M., Frankenberger, Jr., W.T., and Sims, R.C., editors, Bioremediation of contaminated soils. Agron. monogr. 37. ASA, Madison, WI, U.S.A. p. 53-87.

Palmer, C.D. and Fish, W. 1991. Physiochemical processes: Inorganic contaminants. In: Site characterization for subsurface remediation. Seminar publication EPA/625/4-91/026. U.S. Environmental Protection Agency, Washington, DC, U.S.A. p. 179-192.

Sims, R.C. and Sims J.L. 1991. Remediation techniques for contaminated soils. In: Site characterization for subsurface remediation. Seminar publication EPA/625/4-91/026. U.S. Environmental Protection Agency, Washington, DC, U.S.A. p. 215-241.

Singh, A., Kuhad, R.C., and Ward, O.P. 2009. Biological remediation of soil: An overview of global market and available technologies. In: Singh, A., Kuhad, R.C., and Ward, O.P., editors, Advances in applied bioremediation. Soil biology 17. Springer, Dordrecht, Netherlands. p. 1-20.

Sparks, D.L. 2003. Environmental soil chemistry. 2nd ed. Academic Press, Amsterdam, Netherlands.

Treasury Board of Canada Secretariat (TBS). 2011. Federal contaminated sites inventory. http://www.tbs-sct.gc.ca/fcsi-rscf/home-accueil-eng.aspx (accessed August 21 2012).

Yong, R.N., Mohamed, A.M.O., and Warkentin, B.P. 1992. Principles of contaminant transport in soils. Elsevier, Amsterdam, Netherlands.

Zovko, M. and Romic, M. 2011. Soil contamination by trace metals: Geochemical behaviour as an element of risk assessment. In: Dar, I.A. and Dar, M.A., editors, Earth and environmental sciences. InTech, Rijeka, Croatia. p. 437-456.

Zvomuya, F. and Murata, A.P. 2012. Soil contamination and remediation. In: El-Shaarawi, A.H. and Piegorsch, W.W., editors, Encyclopedia of environmetrics. 2nd ed. John Wiley and Sons, Chichester, U.K. (in press).

2. CHROMIUM DYNAMICS DURING REDOX-SORPTION TREATMENT OF HEXAVALENT CHROMIUM-CONTAMINATED WATER

2.1 Abstract

Chromium (Cr) is commonly found at contaminated sites due to its extensive use in industrial activities such as leather tanning, wood treatment, and metal plating. The behaviour of Cr in the environment is highly dependent upon its speciation. Hexavalent chromium [Cr(VI)] is highly mobile and toxic while trivalent chromium [Cr(III)] is much less mobile and considered to be an essential micronutrient for humans. For this reason, many remediation techniques for Cr(VI)-contaminated groundwater focus on the reduction of Cr(VI) to Cr(III) followed by immobilization. This study examined the use of sodium dithionite ($Na_2S_2O_4$), in conjunction with iron (Fe) oxides derived from either ferric chloride or ferrous chloride to remediate water artificially contaminated with Cr(VI). Chromium speciation in the aqueous and sorbed phases was determined using colorimetry, atomic absorption spectroscopy, and x-ray absorption near edge structure (XANES) spectroscopy. Sodium dithionite was effective in reducing Cr(VI) to Cr(III). The acid-consuming reduction reaction increased solution pH enough to cause the removal of aqueous Cr(III) through formation of insoluble precipitates. The ferric-derived oxide was much more effective in removing aqueous Cr from solution than the ferrous-derived oxide. This was especially evident in treatments with low $Na_2S_2O_4$ concentrations where a greater proportion of Cr(VI) was present and less Cr(III) had been

removed by precipitation. Sorption isotherms showed that the ferric-derived oxide had a greater Cr(VI) sorption capacity than the ferrous-derived oxide. Overall, the remediation technique was successful and could be applied to sites with Cr(VI)-contaminated groundwater. In some situations, groundwater treatment with the ferric-derived oxide alone could remove sufficient quantities of Cr. The addition of 3-6 mM $Na_2S_2O_4$ to the treatment would be useful in achieving higher Cr removal rates.

2.2 Introduction

2.2.1 Chromium Applications and Sources

Chromium (Cr) is a trace metal which occurs naturally in the environment. Chromium levels in Earth's crust average around 100 mg kg^{-1} but concentrations from 200 to over 3000 mg kg^{-1} can be found in ultramafic rocks and serpentine group minerals (Oze et al. 2007; Kabata-Pendias 2011). About 95% of global Cr resources are located in Kazakhstan and Southern Africa (USDI and USGS 2012). In Canada, chromite ($FeCr_2O_4$) ore deposits can be found in Quebec, Ontario, British Columbia, and Newfoundland (CCME 1999*a*; CCME 1999*b*). These deposits, which are considered low- and medium-grade, have been commercially mined in the past but are no longer being exploited (CCME 1999*a*; CCME 1999*b*). Chromium is used extensively for industrial purposes and is considered among the top five most valuable metals in terms of economic significance (Bartlett and James 1996). An estimated 24 Mt of Cr was produced worldwide in 2011 (USDI and USGS 2012). In 1991, Statistics Canada reported that Canada imported approximately 74,000 Mg of Cr-containing materials,

41,000 Mg of which were Cr ferroalloys used in the production of steel (CEPA 1994). Chromium is also used for a wide variety of other industrial applications, such as leather tanning, pigment and dye production, and wood treatment (Palmer and Wittbrodt 1991; CEPA 1994). Its resistance to corrosion makes it common in metal industries such as metal plating and finishing (Palmer and Wittbrodt 1991; CEPA 1994).

2.2.2 Chromium Contamination and Behaviour

Chromium concentrations in the environment are generally low. Background dissolved and particulate Cr concentrations in uncontaminated surface and marine waters, for example, are typically less than 1 mg L^{-1} (CEPA 1994). Environmental Cr levels may become elevated due to natural events or processes as well as anthropogenic activities (Palmer and Wittbrodt 1991; CCME 1999*a*). Since Cr exists naturally in the earth's crust, weathering and erosion, volcanic activity, and wind-blown dust can potentially cause elevated Cr concentrations in the environment (CCME 1999*a*; CCME 1999*b*; Oze et al. 2007). Soils derived from Cr-rich parent materials may have Cr concentrations as high as 100,000 mg kg^{-1} (Kabata-Pendias 2011). Forest fires, vegetative debris, and marine aerosols also contribute to Cr loading in the environment (CCME 1999*a*; CCME 1999b). The main source of Cr contamination, however, is anthropogenic activity (Bartlett and James 1996). In Canada, it is estimated that Cr loading occurs at rates of 84 Mg yr^{-1} into the atmosphere, over 27 Mg yr^{-1} into water, and over 5000 Mg yr^{-1} onto land (CEPA 1994). Major pathways by which Cr may enter the environment include emissions, effluent, sludge, and spills from the numerous industries in which Cr is used as well as emissions from fossil fuel combustion, leachate from waste disposal sites,

discharge from sewage treatment plants, and urban runoff (Palmer and Wittbrodt 1991; Kabata-Pendias 2011). A study conducted by the Ontario Ministry of the Environment (1998) found that Cr concentrations in raw sewage were as high as 820 mg L^{-1} and averaged 51 mg L^{-1}. The same study measured up to 140 mg L^{-1} Cr in treated water (Ontario Ministry of the Environment 1998). These concentrations greatly exceed the Canadian drinking water guideline of 0.05 mg L^{-1} (Pawlisz et al. 1997; CCME 1999*a*).

The behaviour of Cr in the environment is highly dependent upon it speciation. Chromium can exist in any of nine oxidation states ranging from -2 to 6, with Cr(III) and Cr(VI) being the predominant species (Kabata-Pendias 2011). Trivalent chromium [Cr(III)] is the main form in the earth's crust and therefore also the main form in areas contaminated with Cr due to natural processes (CEPA 1994). It is also the dominant species in the atmosphere, soils, anaerobic sediments, and biological tissue (CEPA 1994). Hexavalent chromium [Cr(VI)], on the other hand, is typically found dissolved in aerobic surface waters and groundwater (CEPA 1994). The difference in behaviour between Cr(III) and Cr(VI) is due to their basic chemical properties. Trivalent chromium exists as the chromic cation (Cr^{3+}), which is strongly sorbed by negatively charged clays and iron (Fe) and aluminium (Al) oxides found in the soil (Bartlett and James 1996). Trivalent chromium also readily precipitates out of solution as hydroxides, such as $Cr(OH)_3$, $Cr(OH)_2^+$, and $Cr(OH)_4^-$ (CCME 1999*a*; Kabata-Pendias 2011). The sorption and precipitation of Cr(III) reduces its mobility and bioavailability. An exception may occur when Cr(III) forms stable complexes with dissolved ligands (Bartlett and James 1996; CEPA 1994; Kabata-Pendias 2011). Since Cr(III) is typically associated with relatively

inert solid particles, it can accumulate and persist in the environment (CEPA 1994). Nevertheless, Cr(III) toxicity to humans is of low concern. In fact, small amounts of Cr(III) may be necessary in the activation of insulin during glucose metabolism (Bartlett and James 1996).

The behaviour and toxicity of Cr(VI) are opposite to those of Cr(III). Hexavalent chromium generally exists as anions such as chromate ($Cr_2O_4^{2-}$) and dichromate ($Cr_2O_7^{4-}$) (Palmer and Wittbrodt 1991; Bartlett and James 1996). These compounds are weakly sorbed by net negative soil surfaces, making Cr(VI) highly mobile and bioavailable (Bartlett and James 1996; Taylor et al. 2000). Hexavalent chromium is also much more toxic than Cr(III) and can cause harmful effects in both plants and animals at low concentrations (Bartlett and Kimble 1976*b*). This is because Cr(VI) can penetrate cell membranes relatively easily (CCME 1999*a*). Up to 10% of the total Cr(VI) ingested may be absorbed from the gastrointestinal tract compared to only up to 3% of Cr(III) (CEPA 1994). Toxic effects of Cr(VI) exposure to animals include gene mutation and damage to the liver, kidney and DNA (CEPA 1994). Epidemiological studies have also established that inhaled Cr(VI) is a human carcinogen which increases the risk of lung cancer (USEPA 1999).

The chemical reduction of Cr(VI) to Cr(III) may be carried out by many reducing agents (USEPA 2000). In contrast, the transformation of Cr(III) to Cr(VI) is considered rare because oxidizing agents capable of the chemical reaction are uncommon and the reaction kinetics are very slow (CEPA 1994). Recently however, it has been determined

that birnessite, a common manganese-containing mineral, is capable of oxidizing Cr(III) to Cr(VI) (Oze et al. 2007).

2.2.3 Remediation of Hexavalent Chromium-Contaminated Groundwater

Since Cr(VI) commonly found in contaminated groundwater is highly mobile, bioavailable, and toxic, effective remediation techniques are needed to protect human and environmental health. The conventional remediation strategy for Cr(VI)-contaminated groundwater is pump-and-treat (Palmer and Wittbrodt 1991; Blowes 1997; USEPA 2000). Pump-and-treat involves extracting Cr(VI)-contaminated groundwater in order to contain the plume (Keely 1989; USEPA 2000). One of the major drawbacks to this method is that, while Cr(VI) concentrations in extracted water are initially high, concentrations decrease quickly to a persistent tail which may remain above guideline levels (Palmer and Wittbrodt 1991; USEPA 2000). Therefore, pump-and-treat remediation may extend over a long time frame. The tail effect occurs for two main reasons. First, while extraction wells can pull water through highly permeable geologic layers very quickly, Cr(VI)-contaminated water which has migrated to less permeable layers is removed much more slowly by diffusion into the groundwater during the flushing process (Palmer and Wittbrodt 1991; USEPA 2000). Secondly, some Cr(VI) may have become associated with the solid phase over time and becomes released slowly back into solution during pumping (Palmer and Wittbrodt 1991; USEPA 2000). Pump-and-treat is also an expensive remediation technique (Powell et al. 1995; Blowes 1997).

In order to improve the effectiveness and efficiency of Cr(VI)-contaminated groundwater remediation, in situ methods may be used. Since Cr(III) is a far lesser environmental concern than Cr(VI), many in situ remediation strategies involve the chemical reduction of Cr(VI) to Cr(III) followed by immobilization (Blowes 1997; Han et al. 2000; USEPA 2000; Kabata-Pendias 2011). Reducing agents which may be used to transform Cr(VI) into Cr(III) include structural and dissolved ferrous iron [Fe(II)], zerovalent iron (Fe^0), sodium dithionite ($Na_2S_2O_4$), calcium polysulfide (CaS_x), and organic matter (Powell et al. 1995; Taylor et al. 2000; Graham et al. 2005; Niu et al. 2005; Graham et al. 2006). Immobilization may be carried out via fixation of Cr to solid surfaces of the aquifer, called geological fixation, or via fixation of Cr to reactive media added to the system (USEPA 2000). The latter case is referred to as the permeable reactive barrier (PRB) method. In the PRB method, a portion of the contaminated aquifer is removed and replaced with a reactive material (Blowes 1997). The reactive material may be positioned in the path of groundwater flow either horizontally as a treatment layer or vertically as a treatment wall (Blowes 1997; Blowes et al. 2000). Contaminated groundwater may be allowed to flow passively through the reactive material or assisted with pumping wells (Blowes 1997; Wilkin et al. 2005). Both the geological fixation and permeable reactive barrier methods prevent the spread of Cr(VI) contamination along the groundwater flow gradient and the uptake of Cr(VI) with groundwater usage.

2.2.4 Objectives

The overall objective of this study was to examine Cr dynamics during reduction-sorption treatment of aqueous Cr(VI). Sodium dithionite was used as the reducing agent and two different types of Fe oxide were used as sorbents. Specific objectives were to:

1. Determine the speciation of aqueous and precipitated Cr following reduction with varying amounts of $Na_2S_2O_4$;
2. Determine the speciation of aqueous and sorbed Cr following treatment with the two Fe oxides;
3. Model Cr sorption by the Fe oxides; and
4. Evaluate the effectiveness of the reduction-sorption treatment as a potential remediation strategy for groundwater contaminated with Cr(VI).

2.3 Materials and Methods

2.3.1 Iron Oxides

Two types of synthetic Fe oxide were prepared for this study according to a method adapted from Goh et al. (1987). The first oxide (ferric-derived oxide) was prepared by titrating 3.2 L of a 0.05 M ferric chloride ($FeCl_3$) solution with 0.5 M sodium hydroxide (NaOH) to pH 7 under constant stirring. The second oxide (ferrous-derived oxide) was prepared by titrating 3.2 L of a 0.05 M ferrous chloride ($FeCl_2$) solution with 0.5 M NaOH to pH 7 under constant stirring. Both titrations were done by adding the NaOH dropwise at room temperature (~21 ± 2°C). After titration, the volumes of the Fe oxide suspensions were adjusted to 4.5 L with reverse osmosis (RO) water. Three 4.5 L

batches of each type of Fe oxide were prepared. Each batch was stored in a plastic carboy to age for 7 wk at room temperature.

After aging, the carboys were agitated to resuspend the Fe oxides. The suspensions were then filtered through 0.45 μm Millipore filter membranes under a pressure of approximately 275 kPa. Five 600-mL aliquots of RO water were flushed through the filtration system to wash each Fe oxide sample. The oxide samples were then transferred from the filter membranes into glass beakers and oven-dried at 80°C for 24 h. This temperature was chosen to speed up the drying process while avoiding mineralogical changes (Goh 2011, personal communication). Subsamples of each dry Fe oxide sample were further oven-dried for 24 h at 105°C. Moisture content was calculated as the difference between the subsample mass after drying at 80°C and the subsample mass after drying at 105°C relative to the subsample mass after drying at 105°C.

The ferric- and ferrous-derived oxides were taken to the Canadian Light Source (CLS) synchrotron in Saskatoon, SK for Fe analysis by X-ray absorption near edge structure (XANES) spectroscopy. In this technique, X-ray radiation is applied to samples and spectra showing X-ray absorption as a function of energy are analyzed. At the specific energies required to excite core electrons of a given element, photoelectrons are produced and sharp increases in X-ray absorption can be seen (Schulze and Bertsch 1995; Penner-Hahn 2004). The location of these absorption edges, as well as spectral features near the edges can be used to gain information about the samples including elemental composition, bonding, and oxidation state (Schulze and Bertsch 1995; Penner-Hahn 2004). Statistical analysis of the XANES data was done using Athena software version

0.8.054 (Bruce Ravel, Chicago, IL). Counts from the total electron yield detector were normalized against the upstream slit current and plotted against the scanning energy. Scans were corrected with a +2.4 eV energy shift when necessary. Scans for the same oxide were merged into single spectra and compared to reference spectra.

2.3.2 Hexavalent Chromium Reduction

This experiment was conducted to examine Cr dynamics during reduction of Cr(VI) to Cr(III) by different concentrations of $Na_2S_2O_4$. The methodology was based on a study by Taylor et al. (2000). A 0.02 M Cr(VI) stock solution was prepared using potassium chromate (K_2CrO_4). Ten millilitres of the stock solution were added to each of six 50-mL plastic centrifuge tubes. A 0.1 M hydrochloric acid (HCl) solution was prepared and 2.1 mL were added to each centrifuge tube to bring the pH to ≤ 5 to prevent precipitation of Cr hydroxides, which occurs above pH 5.5 (Bartlett and Kimble, 1976*a*; Kabata-Pendias 2011). A stock solution of 0.25 M $Na_2S_2O_4$ was prepared and 0, 0.24, 0.48, 0.76, 0.96, and 1.2 mL were added to the centrifuge tubes. The $Na_2S_2O_4$ concentrations were chosen so that the highest $[S_2O_4^{2-}]$:[Cr(VI)] ratio was 3:2. This ratio was based on that used by Murata (2010) and the proposed redox reaction equation

$$2CrO_4^{2-} + 3S_2O_4^{2-} + 4H^+ \rightleftharpoons 2Cr^{3+} + 6SO_3^{2-} + 2H_2O \qquad \textbf{[1]}$$

which shows that three moles of dithionite react with every two moles of Cr(VI). The volume of each centrifuge tube was adjusted to 20 mL with RO water to give final treatment concentrations of 10 mM Cr(VI) and 0, 3, 6, 9.5, 12, and 15 mM $Na_2S_2O_4$.

The solutions were allowed to react at room temperature for 24 h before colorimetric determination of Cr speciation. Hexavalent chromium concentration was determined by the s-diphenyl carbazide method (Bartlett and Kimble 1976*a*). In this method, the s-diphenyl carbazide reagent reacts with Cr(VI) in solution, reducing it to Cr(III) and forming a reddish violet diphenylcarbazone complex (Bartlett and James 1996). Trivalent chromium originally in the solution does not interfere with this method and the limit of quantitation is approximately 0.3 μmol L^{-1}, which was acceptable for this study (Bartlett and James 1996). The s-diphenyl carbazide reagent was prepared by dissolving 200 mg of s-diphenyl carbazide in 100 mL of 95% ethanol and adding 120 mL of 85% phosphoric acid (H_3PO_4) in 280 mL of water. A small amount (approximately 10-15 crystals) of potassium permanganate ($KMnO_4$) was added until the solution turned pink and then the solution was heated to 60°C until the pink colour disappeared and the solution turned orange. The reagent was stored under refrigeration at 4°C.

In order to determine the concentration of Cr(VI) in a given sample, 1 mL of s-diphenyl carbazide reagent was added to 7 mL of RO water and 1 mL of sample and allowed to stand for 5 min. Absorbance was read at 540 nm using a UV-vis spectrophotometer (Ultrospec 2100 Pro, GE Healthcare, Mississauga, ON). When necessary, the 1 mL of sample was diluted to fall within the linear calibration range of the UV-vis spectrophotometer.

The samples were then filtered through 0.45 μm Millipore filter membranes under vacuum suction to remove any precipitate and analyzed for total dissolved Cr concentration by atomic absorption spectroscopy. The concentration of Cr(III) in each

sample was calculated as the difference between the concentration of total Cr and the concentration of Cr(VI).

The reduction study was carried out in duplicate and then repeated with the same concentrations but volumes an order of magnitude larger in order to facilitate the recovery of any precipitate formed. The precipitates were rinsed with three 20-mL aliquots of a 0.1 mM sodium chloride (NaCl) solution and three 20-mL aliquots of RO water. The precipitates were taken to the CLS synchrotron for Cr analysis by XANES spectroscopy. Statistical analysis of the XANES data was done using Athena software. Counts from the silicon drift detector were normalized against the beam intensity and plotted against the scanning energy. Scans were corrected with a +2.0 eV energy shift when necessary. Scans for the same treatments were merged into single spectra. The resulting precipitate spectra were fitted to the reference spectra using the linear combination fit function.

2.3.3 Reduction-Sorption

The methodology for the reduction-sorption study was based on work by Cheslock-Fitzgerald (1990) and Taylor et al. (2000). In this study, Cr(VI) solutions were reduced to Cr(III) to varying degrees using $Na_2S_2O_4$ as described above. After allowing the solutions to react for 24 h, 260 mg (dry wt.) of each oxide (ferric-derived and ferrous-derived) were added to the centrifuge tubes. The study was carried out in triplicate for each type of Fe oxide. The centrifuge tubes were placed on a mechanical shaker set to 120 excursions per minute for another 24-h reaction period. Agitating the samples

ensured that the solid oxides remained in suspension and had the maximum surface area exposed to the Cr solution and available for sorption. The samples were filtered through 0.45 μm Millipore filter membranes under vacuum suction to remove and recover the Fe oxides and any precipitate that had formed. The filtrates were analyzed for total Cr concentration by atomic absorption spectroscopy and for Cr(VI) concentration by the s-diphenyl carbazide method. Trivalent chromium concentration was calculated as the difference between total Cr and Cr(VI) concentrations. Recovered Fe oxides and precipitates were rinsed with three 20-mL aliquots of a 0.1 mM NaCl solution and three 20-mL aliquots of RO water. They were then taken to the CLS synchrotron for Cr analysis by XANES spectroscopy.

The amount of aqueous total Cr removed from solution due to each reduction-sorption treatment was calculated as the difference between the pre-treatment concentration of Cr (10 mM) and the final concentration of Cr in the filtrate. Analysis of variance (ANOVA) was performed using PROC MIXED in SAS version 9.2 (SAS Institute, 2008) to determine the effects of $Na_2S_2O_4$ addition and oxide type on aqueous total Cr removal.

The amount of Cr(VI) and Cr(III) sorbed by each treatment (q_i, mmol g^{-1} of Fe oxide) was found using the equation

$$q_i = (C_i - C_f)*V/m \quad \textbf{[2]}$$

where C_i is the aqueous concentration (mM) of Cr(VI) or Cr(III) after reaction with $Na_2S_2O_4$, C_f is the aqueous concentration of Cr(VI) or Cr(III) after sorption (mM), V is the volume of solution (L), and m is the dry mass of Fe oxide used (g). The amount of each Cr species sorbed was plotted against its equilibrium (post-treatment) concentration

in solution. Langmuir and Freundlich models were fitted to the sorption data using PROC NLIN in SAS. When applicable, the fits of the Langmuir and Freundlich models were compared using the Akaike's Information Criterion (AIC). In this method, AIC is calculated for each model using the equation

$$AIC = n*\ln(SSE/n) + 2k + (2k(k+1))/(n-k-1) \quad \textbf{[3]}$$

where n is sample size, SSE is the sum of squares for the residuals, and k is the number of parameters in the model (Motulsky and Christopolous 2003). The probability of the model with the lower AIC being more correct was then calculated using the equation

$$probability = (e^{-0.5\Delta AIC})/(1+ e^{-0.5\Delta AIC}) \quad \textbf{[4]}$$

where ΔAIC is the lower AIC score minus the higher AIC score (Motulsky and Christopolous 2003).

Statistical analyses of the XANES spectroscopy data for the recovered Fe oxides and precipitates were performed using Athena software as described previously. The derivative option of the linear combination fit function was used because fitting using the mean option resulted in an underestimated significance of the Cr(VI) pre-edge peak.

2.4 Results and Discussion

2.4.1 Iron Oxides

The $FeCl_3$ began as an orange solution and gradually became dark reddish brown when titrated with NaOH. On average, 1.13 L of 0.5 M NaOH were required to adjust the pH of 0.05 M $FeCl_3$ to 7. This was greater than the 810 mL of NaOH required for the titration of $FeCl_3$ in the study by Murata (2010) because a lower concentration of $FeCl_3$ (0.03 M) was used in that experiment. As the samples aged in plastic carboys, the dark

reddish brown Fe oxide settled to the bottom and the supernatant became clear and colourless. After the Fe oxide samples were recovered via filtration, dried at 80°C, and combined, the ferric-derived oxide appeared reddish-brown. The total dry mass of ferric-derived oxide made from the three titrations was 50.1 g. Its moisture content was 321 g kg^{-1}. This was greater than the 83 g kg^{-1} moisture content measured for the ferric-derived oxide prepared by Murata (2010).

The $FeCl_2$ began as an orange solution and gradually became dark greenish black when titrated with NaOH. The average amount of 0.5 M NaOH added to the 0.05 M $FeCl_2$ to reach pH 7 was 333 mL. This volume of NaOH was very similar to the volume used in the preparation of ferrous-derived oxide by Murata (2010). When the suspensions were aged in plastic carboys, the dark brown Fe oxide settled to the bottom and the supernatant lost its dark greenish colour and became brownish orange. After the ferrous-derived oxide was recovered via filtration, dried at 80°C, and combined into one composite sample, it appeared dark brown in colour. The total mass of ferrous-derived oxide prepared from the three titrations was 16.2 g. Its moisture content was 116 g kg^{-1}. This was greater than the 19 g kg^{-1} moisture content measured for the ferrous-derived oxide prepared by Murata (2010).

It is possible that the higher moisture contents determined in this study compared to those reported by Murata (2010) occurred because, in the earlier study, the oxides were air-dried and ground with a mortar and pestle before being oven-dried at 80°C. Grinding the oxides could have allowed more water to evaporate during the oven-drying process by breaking up oxide aggregates. Despite the differences in the moisture content

measurements, the ferric-derived oxide was found to have the greater moisture content in both this study and the study by Murata (2010).

Results from XANES spectroscopy showed that the two Fe oxides differed from their starting materials ($FeCl_2$ and $FeCl_3$) and each other (Figure 2.1).

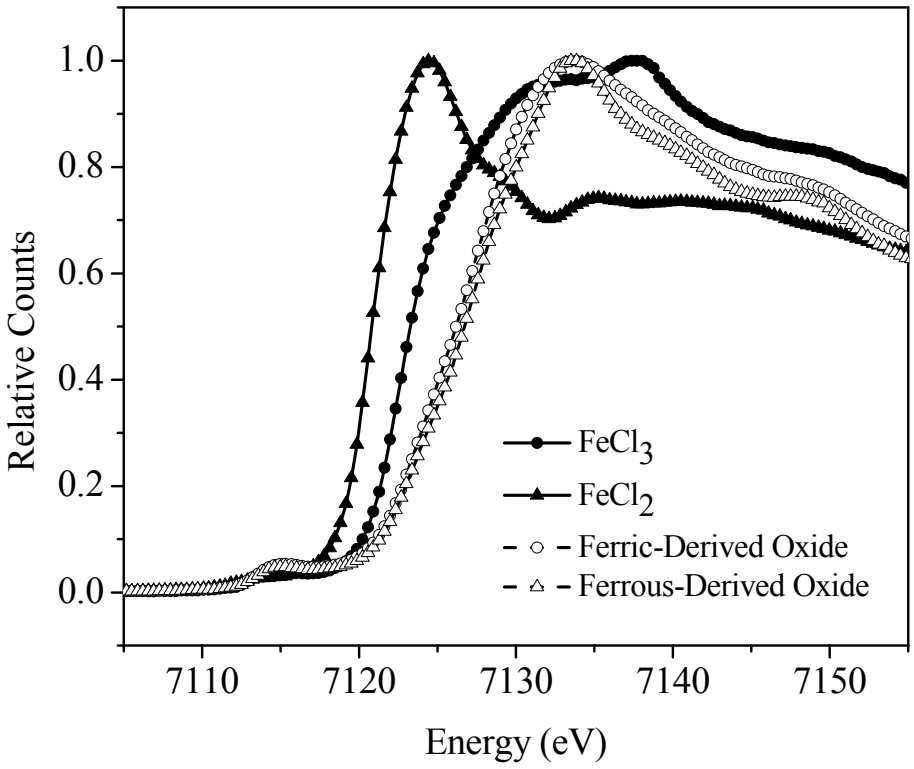

Figure 2.1 X-ray absorption near edge structure (XANES) spectroscopy spectra showing iron (Fe) peaks for the synthetic Fe oxides and their starting materials.

The difference in the peak energy of $FeCl_2$ compared to that of $FeCl_3$ was due to oxidation state. The difference in the peak energies of $FeCl_2$ and $FeCl_3$ compared to those of the Fe oxides was due to the presence of oxygen bonds. Small differences between the spectra for the ferric- and ferrous-derived oxides are indicative of underlying

structural or compositional dissimilarities which were also suggested by differing NaOH titration volumes, colours, and moisture contents.

2.4.2 Hexavalent Chromium Reduction

The K_2CrO_4-contaminated water was initially bright yellow and became increasingly green with the addition of $Na_2S_2O_4$. After 24 h, a precipitate was observed in all treatments where $Na_2S_2O_4$ was added. The precipitate appeared yellowish brown at lower concentrations of $Na_2S_2O_4$ ($\leq$6 mM) and green at higher concentrations of $Na_2S_2O_4$ ($\geq$9.5 mM).

Results from the aqueous Cr speciation analysis showed that, as expected, the $Na_2S_2O_4$ was successful in reducing Cr(VI). Figure 2.2 shows that the concentration of aqueous Cr(VI) decreased as more $Na_2S_2O_4$ was added. The concentration of aqueous total Cr also decreased as more $Na_2S_2O_4$ was added, likely due to precipitation of Cr(III) formed following reduction of Cr(VI).

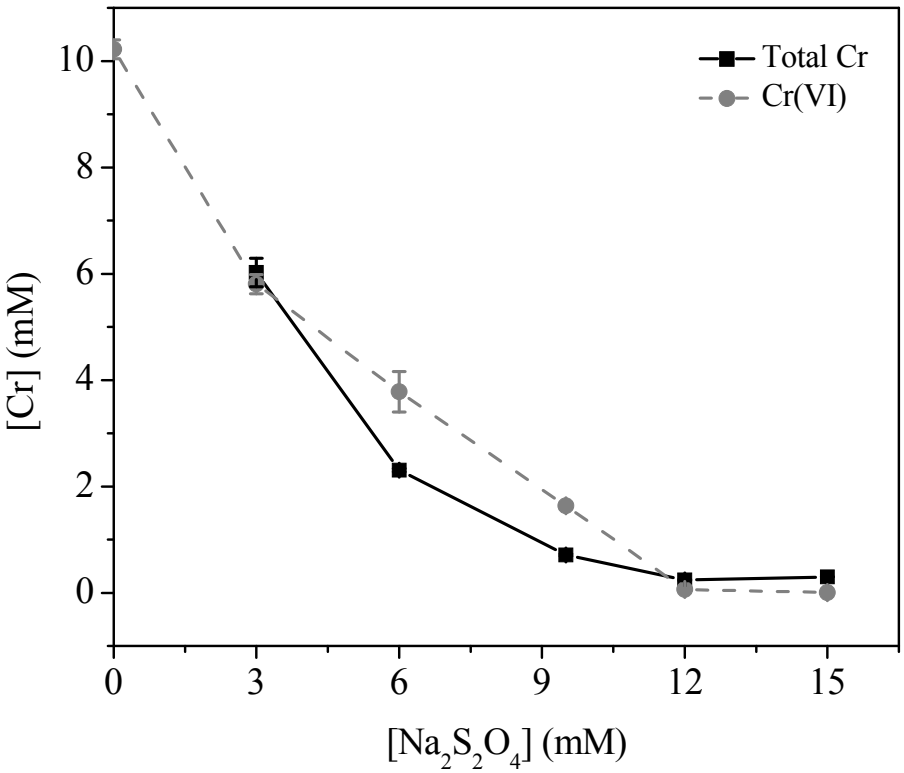

Figure 2.2 Speciation of aqueous chromium (Cr) following addition of various concentrations of sodium dithionite ($Na_2S_2O_4$). Vertical bars represent standard deviation.

Hexavalent chromium concentrations appear to exceed total Cr concentrations for the 6 mM and 9 mM $Na_2S_2O_4$ treatments. This may have been caused by continued Cr(VI) reduction and Cr(III) precipitation during the time following analysis by colorimetry but prior to atomic absorption spectroscopy.

These Cr reduction results differed from those from a study by Murata (2010) where the formation of a precipitate was not observed. This was likely due to the fact that Murata (2010) reduced the pH of the solutions to 5 or lower as the final step in the laboratory method rather than one of the first steps. Since the reduction of Cr(VI) to Cr(III) is an

acid-consuming reaction (Equation 1), the methodology used in this study would allow the pH to increase above the desired level, resulting in the precipitation of Cr(III).

X-ray absorption near edge spectroscopy was successful in differentiating between Cr(III) and Cr(VI) in the solid precipitate. In Figure 2.3, the principal Cr peak can be observed around 6112 eV for all treatment spectra. A smaller pre-edge peak can be observed around 5995 eV for low-$Na_2S_2O_4$ (≤6 mM) treatment scans. This peak is only observed when Cr(VI) is present.

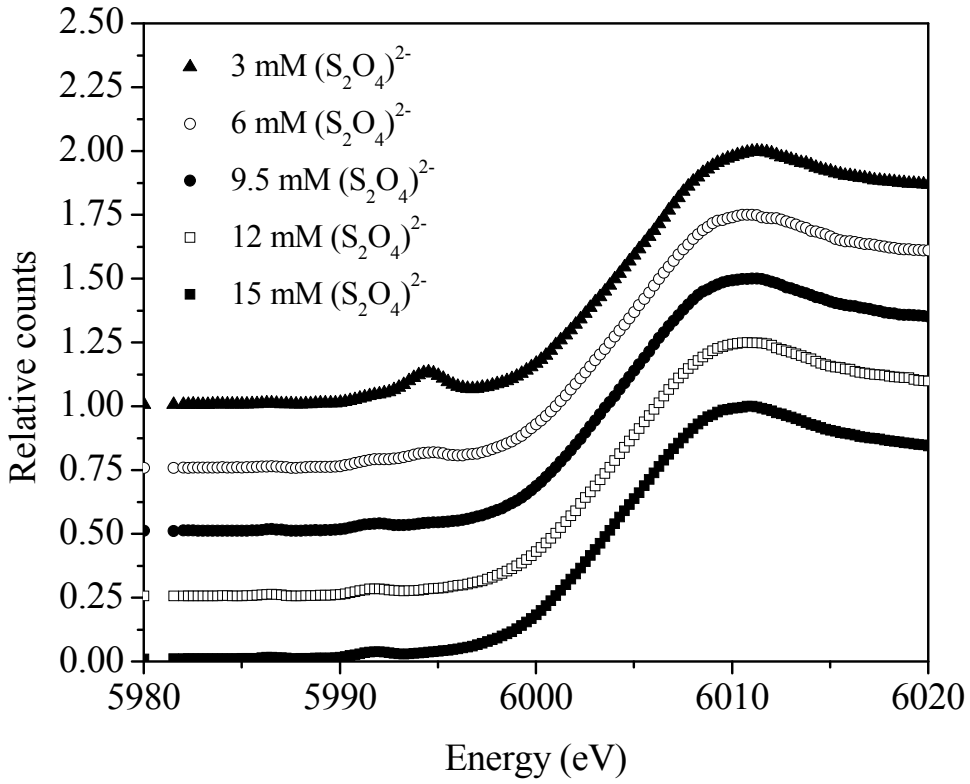

Figure 2.3 X-ray absorption near edge structure (XANES) spectroscopy spectra showing chromium (Cr) peaks for the precipitate formed during the reduction study.

Fitting the scans in Athena confirmed that a greater proportion of the precipitate was composed of Cr(VI) for the 3 and 6 mM $Na_2S_2O_4$ treatments (Figure 2.4). In other words, when more Cr(VI) was present in the aqueous form, more Cr(VI) was also present in the solid form.

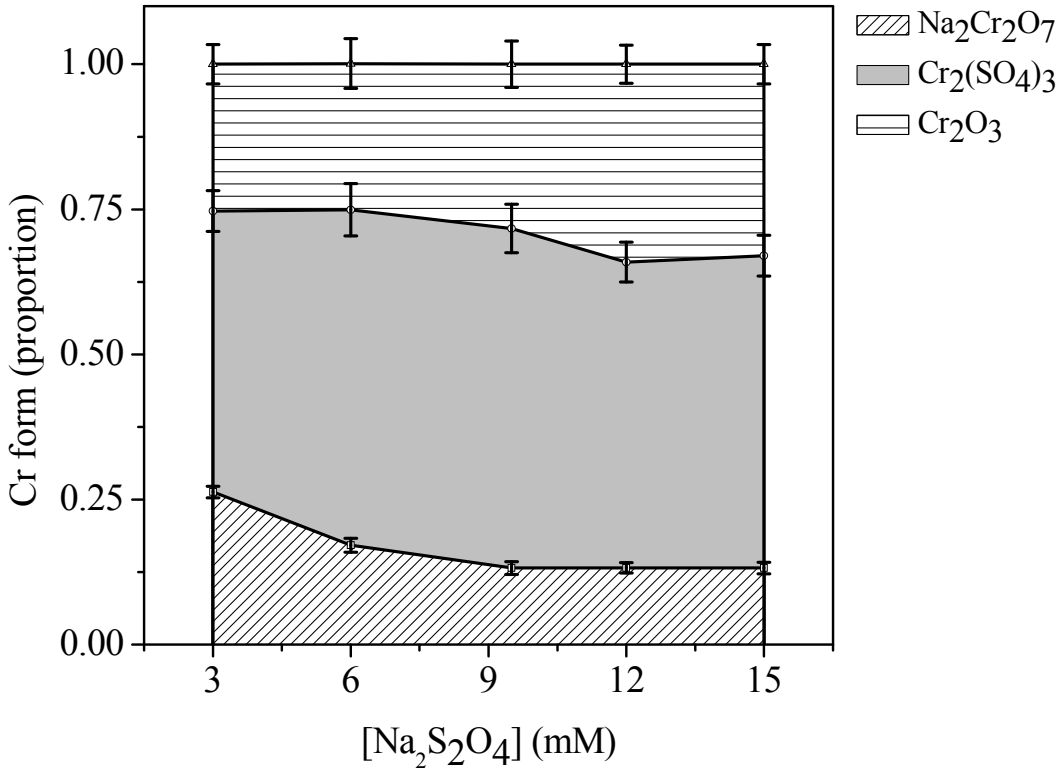

Figure 2.4 Chromium (Cr) form in the precipitate produced during the reduction reaction. Vertical bars represent standard deviation.

Regardless of $Na_2S_2O_4$ concentration, Cr(III) was the dominant Cr species in the precipitate. Analysis of XANES data indicated that the Cr(III) precipitate was a mixture of Cr_2O_3 and $Cr_2(SO_4)_3$.

The use of $Na_2S_2O_4$ as a reducing agent for Cr(VI) has been studied under field conditions by Khan and Puls (2003). They found that aqueous Cr(VI) concentrations in

the aquifer zone reduced by $Na_2S_2O_4$ decreased below detectable levels within 24 h of treatment (Khan and Puls 2003). Therefore, they also determined that $Na_2S_2O_4$ was effective in reducing Cr(VI) to Cr(III) for the purpose of groundwater remediation (Khan and Puls 2003).

2.4.3 Reduction-Sorption

Analysis of variance results showed that $Na_2S_2O_4$, Fe oxide, and their interaction were all significant for the percent of total Cr removed from solution (Table 2.1).

Table 2.1 Iron (Fe) oxide and sodium dithionite ($Na_2S_2O_4$) effects on aqueous total chromium (Cr) removal.

Effect	**Aqueous Total Cr Removed** (%)
Fe oxide	
Ferric-derived	93.0
Ferrous-derived	75.3
$Na_2S_2O_4$ concentration (mM)	
0	45.6
3	74.7
6	88.7
9.5	97.4
12	99.0
15	99.4
	P
Fe oxide	<0.0001
$Na_2S_2O_4$ concentration	<0.0001
Fe Oxide × $Na_2S_2O_4$ concentration	<0.0001

Averaged across all $Na_2S_2O_4$ levels, the ferric-derived oxide treatment removed approximately 18% more total Cr from solution than the ferrous-derived oxide treatment. This is consistent with observations by Murata (2010) who also found greater aqueous Cr removal with the ferric-derived oxide treatment. Both oxide treatments exhibited a trend where aqueous total Cr removal increased as a quadratic function of $Na_2S_2O_4$ concentration (Figure 2.5).

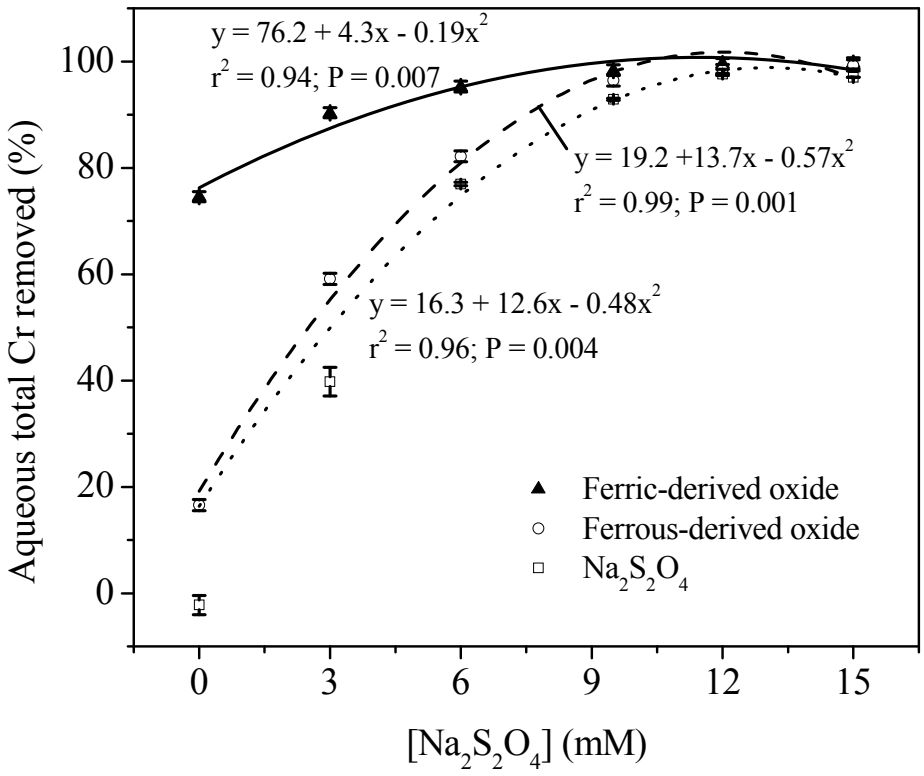

Figure 2.5 Decrease in aqueous total chromium (Cr) concentrations following reduction-sorption treatment of hexavalent chromium- [Cr(VI)] contaminated water. Vertical bars represent standard error of the mean.

The quadratic fit for Cr removal due to treatment with $Na_2S_2O_4$ and the ferrous-derived oxide was similar to the fit for Cr removal due to treatment with $Na_2S_2O_4$ alone. At

$Na_2S_2O_4$ levels ≤6 mM, the ferric-derived oxide treatment removed significantly more total Cr from solution than the ferrous-derived oxide treatment. This was likely due to differences in the oxides' abilities to sorb Cr(VI). Within the 0-9.5 mM $Na_2S_2O_4$ range, the difference in aqueous total Cr removal between the two oxide treatments decreased with increasing $Na_2S_2O_4$ concentration. At high $Na_2S_2O_4$ levels, the ferric- and ferrous-derived oxide treatments were no longer statistically different and aqueous total Cr removal was unaffected by $Na_2S_2O_4$ concentration. All $Na_2S_2O_4$ treatments ≥9.5 mM resulted in the near complete removal of Cr from solution. This was due to the removal of Cr as a Cr(III)-dominated precipitate.

The effect of $Na_2S_2O_4$ concentration on aqueous total Cr removal found in this study differed from the effect seen by Murata (2010). In that study, optimal aqueous Cr removal occurred at a $Na_2S_2O_4$ concentration of 9 mM (Murata 2010). This was because no Cr(III) precipitation occurred and at 9 mM $Na_2S_2O_4$ both Cr(VI) and Cr(III) were present in solution and could make use of positive and negative sorption sites on the oxides (Murata 2010).

This reduction-sorption treatment was more successful in removing Cr from solution than the treatment used by Taylor et al. (2000). In that study, $Na_2S_2O_4$ was used as a reducing agent and the clay minerals smectite, illite, vermiculite, and kaolinite were used as sorbents (Taylor et al. 2000). Although the $Na_2S_2O_4$ reduced nearly all the Cr(VI) to Cr(III), most of the Cr(III) remained in the aqueous form rather than being precipitated or sorbed on the clays (Taylor et al. 2000).

No measurable Cr(III) sorption was evident in this experiment because of precipitation of the Cr(III) following $Na_2S_2O_4$ treatment. Sorption curves for the Cr(VI) data showed that sorption followed the L-curve isotherm for both oxide treatments. This is a very common sorption isotherm found in soil chemistry studies (Sposito 1989). The L-curve is characterized by a relatively steep slope at low equilibrium sorbate concentrations in solution which decreases as equilibrium sorbate concentration increases (Sposito 1989). The L-curve isotherm indicates that the aqueous Cr(VI) had a high affinity for the Fe oxides at low surface coverage and that the number of sorption sites decreased as Cr surface excess increased (Sposito 1989). Murata (2010) also reported that the L-curve sorption isotherm adequately described Cr(VI) sorption. Comparison of nonlinear regression fits of the Langmuir and Freundlich sorption models to Cr(VI) sorption data indicated that the Freundlich model provided valid fits for both oxide treatments (Figure 2.6).

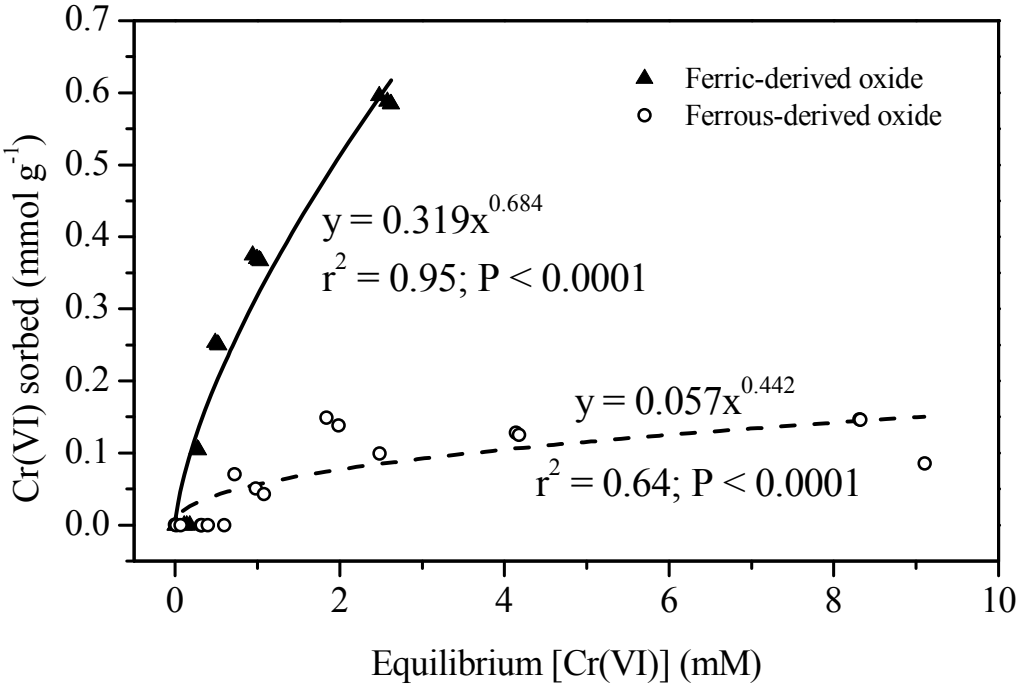

Figure 2.6 Freundlich isotherms for sorption of hexavalent chromium [Cr(VI)] by ferric- and ferrous-derived oxides.

The Freundlich K parameter was significantly greater for the ferric-derived oxide than the ferrous-derived oxide, while the n parameter did not differ significantly between the two oxides (Table 2.2).

Table 2.2 Sorption parameter estimates according to the Freundlich model. Error values represent standard error of the mean.

Oxide	**K ((mmol g^{-1})(mmol L^{-1})$^{1/n}$)**	**1/n**
Ferric-Derived	0.319 ± 0.013a[†]	0.685 ± 0.047
Ferrous-Derived	0.057 ± 0.011b	0.442 ± 0.113

[†]Means in the same column followed by the same letter are not significantly different ($P < 0.05$) according to the Tukey test.

The Freundlich K is related to the sorption capacity of the oxide for Cr(VI) (Summers et al. 2010). The higher K value for the ferric-derived oxide treatment indicates that the

ferric-derived oxide could sorb and retain more Cr(VI) per mass of oxide than the ferrous-derived oxide. This difference in Cr(VI) sorption capacity explains the difference in aqueous total Cr removal between the two oxide treatments. These results agree with Murata (2010) who also found that the ferric-derived oxide had a greater ability to sorb Cr(VI) than the ferrous-derived oxide. In some groundwater contamination scenarios, Cr(VI) sorption by the ferric-derived oxide may provide sufficient remediation without the addition of $Na_2S_2O_4$. The Freundlich parameter 1/n represents relative sorption strength with smaller values indicating greater strength (Snoeyink and Summers 1999). Similar Cr(VI) sorption strength was observed for two Fe oxides.

The Langmuir model provided a valid fit for the Cr(VI) sorption data for ferrous-derived oxide treatment (Figure 2.7) but not the ferric-derived oxide treatment. When fits of the Freundlich and Langmuir models were compared for the ferrous-derived oxide treatment, the Langmuir model was found to have a 99.9% probability of being the more correct model.

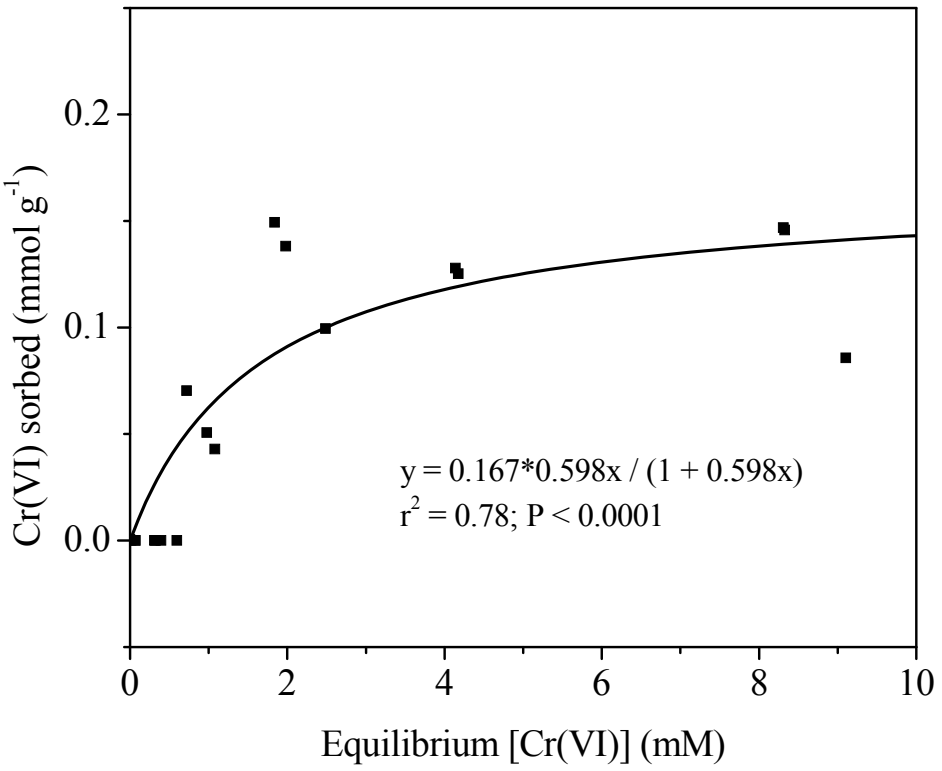

Figure 2.7 Langmuir isotherm for the sorption of hexavalent chromium [Cr(VI)] by ferrous-derived oxide.

The Langmuir parameter estimates were S_{max}=0.167 ± 0.028 mmol g^{-1} and K=0.598 ± 0.281 L $mmol^{-1}$. The S_{max} parameter is indicative of the maximum sorption possible. In other words, the ferrous-derived oxide can sorb a maximum of approximately 0.167 mmol of Cr(VI) per gram of oxide. The sorption constant K is indicative of relative binding strength between the sorbate and the sorbent. In this case, the value of K is moot because the Langmuir model was not a good fit for the ferric-derived oxide data so K values cannot be compared. Therefore, it was not possible to make any conclusion about the strength or permanence of Cr(VI) sorption in this study. However, Ajouyed et al.

(2010) reported that the iron oxides hematite and goethite could be used as Cr(VI) sorbents for remediation purposes, particularly under low-pH conditions. Superior Cr(VI) sorption at low pH was thought to have occurred due to the neutralization of negatively charged sorption sites by H^+ ions and the resulting increase in attraction between Cr(VI) anions and the Fe minerals (Ajouyed et al. 2010).

X-ray absorption near edge spectroscopy for the recovered oxides and precipitates showed that the proportion of Cr(VI) in the sorbed state increased with decreasing $Na_2S_2O_4$ concentration. This pattern was observed in both the ferric- and ferrous-derived oxide treatments. As with the Cr precipitate scans, a larger Cr(VI) pre-edge peak can be observed in scans corresponding to lower $Na_2S_2O_4$-addition treatments (Figures 2.8 and 2.9).

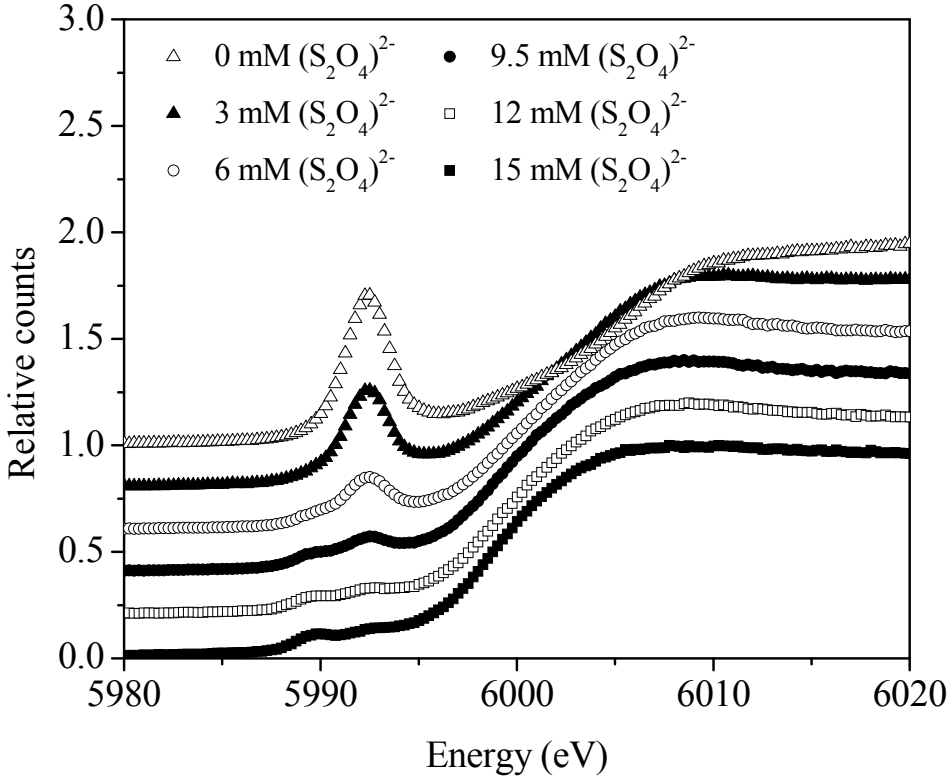

Figure 2.8 X-ray absorption near edge structure (XANES) spectroscopy spectra showing chromium (Cr) peaks for the sorbed Cr produced during the ferric-derived oxide treatment.

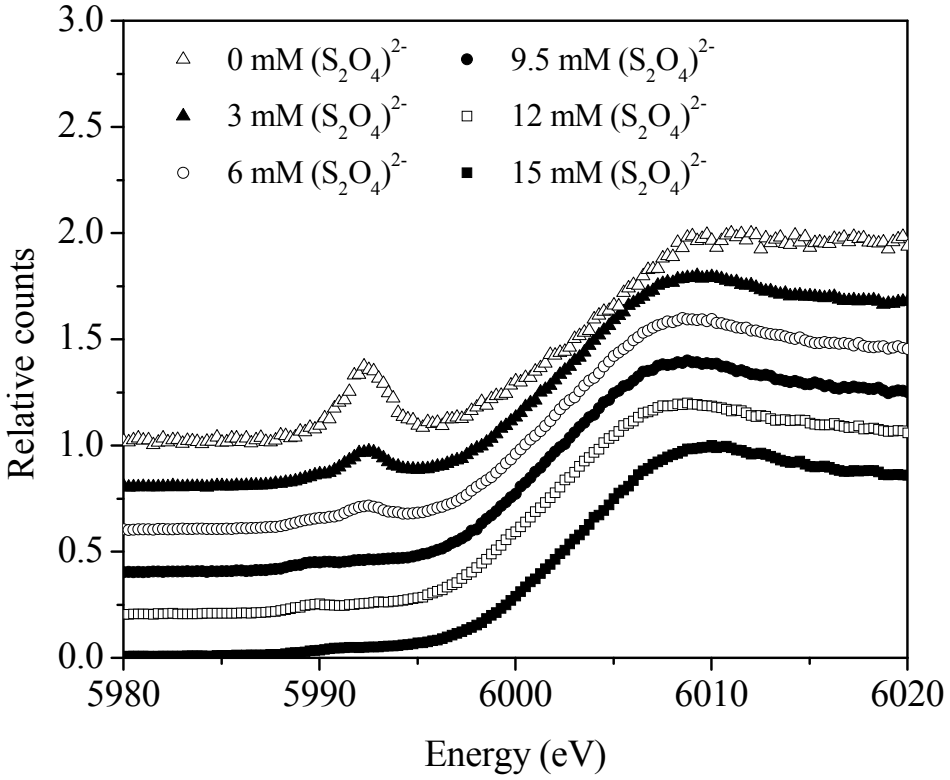

Figure 2.9 X-ray absorption near edge structure (XANES) spectroscopy spectra showing chromium (Cr) peaks for the sorbed Cr produced during the ferrous-derived oxide treatment.

Analysis of XANES data indicated that the trend of increasing sorbed Cr(VI) content with decreasing $Na_2S_2O_4$ addition was more pronounced in the ferric-derived oxide treatment (Figure 2.10).

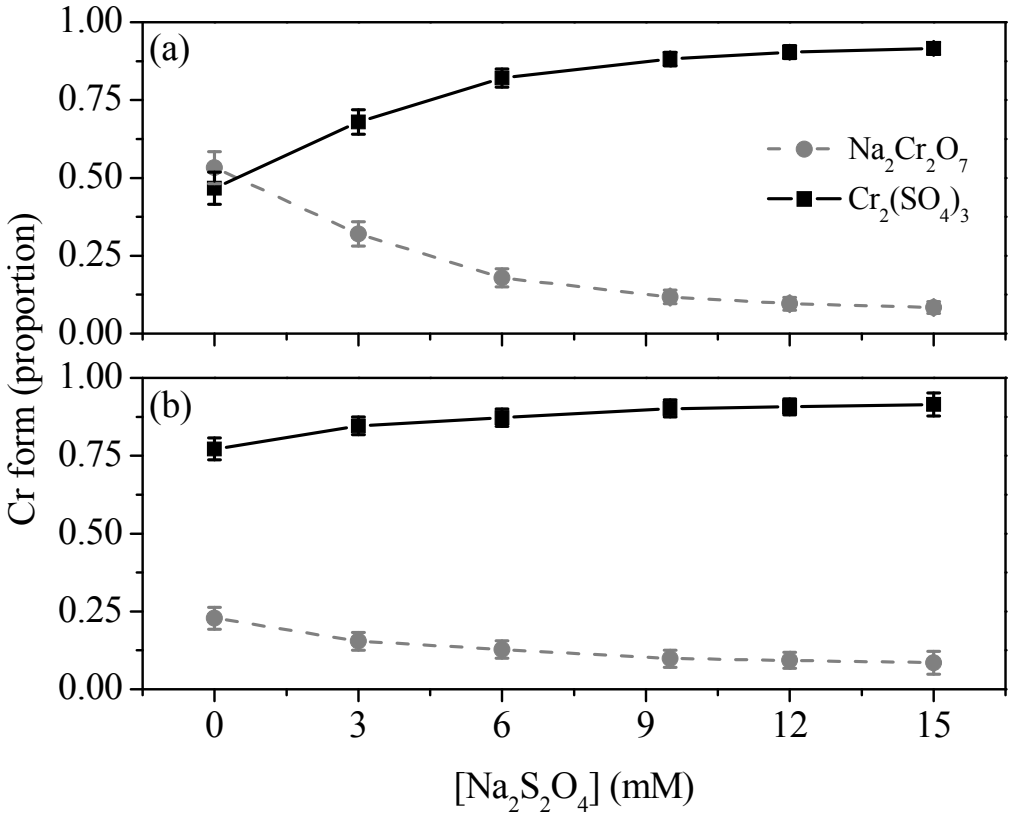

Figure 2.10 Form of chromium (Cr) in the sorbed state for the ferric-derived oxide (a) and ferrous-derived oxide (b) treatments as affected by sodium dithionite ($Na_2S_2O_4$) concentration. Vertical bars represent standard deviation.

When no $Na_2S_2O_4$ was added, and the maximum sorbed Cr(VI) was observed, approximately 53% of the total Cr sorbed by ferric-derived oxide was Cr(VI) while only about 23% of the total Cr sorbed by ferrous-derived oxide was Cr(VI). This finding further supports the observation that the ferric-derived oxide was superior to the ferrous-derived oxide in terms of Cr(VI) sorption.

2.5 Conclusions

The two Fe oxides prepared for this study differed with respect to physicochemical properties. The oxides required different amounts of NaOH in their preparation by titration and had different colours, moisture contents, and XANES spectra. Identification of the oxide types and more extensive examination of their differences were beyond the scope of this study and would require mineralogical analysis.

Sodium dithionite was effective in reducing Cr(VI) to Cr(III). The acid-consuming nature of the reaction raised the solution pH enough to allow for the precipitation of Cr(III). Since Cr(III) precipitates are fairly insoluble and very unlikely to be converted back to Cr(VI), this is not considered a drawback for this remediation method. When a greater proportion of the aqueous Cr was in the Cr(VI) oxidation state, a greater proportion of the Cr precipitate was also in the Cr(VI) form. Trivalent chromium dominated the precipitate regardless of the amount of $Na_2S_2O_4$ added.

Results from the reduction-sorption study showed that the ferric-derived oxide was more effective in removing Cr from solution than the ferrous-derived oxide. This observation was especially apparent at low levels of $Na_2S_2O_4$ addition, when more Cr(VI) was available in solution. It was proposed that the reason behind Cr removal differences between the two oxides was Cr(VI) sorption capacity. Sorption modelling according to the Freundlich model confirmed that the ferric-derived oxide had a greater capacity to sorb Cr(VI) than did the ferrous-derived oxide. However, the Langmuir sorption model provided a better fit to the ferrous-derived oxide data. This indicates that the two oxides may differ in their sorption mechanisms, ostensibly due to their structural differences.

The higher moisture content of the ferric-derived oxide may be indicative of its greater surface area to which the Cr could sorb.

Overall, these results suggest that chemical reduction of Cr(VI) using $Na_2S_2O_4$ followed by Cr immobilization on iron oxides can be an effective groundwater remediation strategy. In some Cr(VI)-contamination scenarios, the addition of the ferric-derived oxide alone may remove sufficient quantities of Cr from solution to meet guideline concentrations. The Fe oxide could be added to the contaminated groundwater system either through an injection well or as a permeable reactive barrier (PRB) installed downgradient of the plume. When higher Cr removal rates are required, the addition of 3-6 mM $Na_2S_2O_4$ could be used to increase remediation effectiveness. Adding high concentrations (12-15 mM) of $Na_2S_2O_4$ without any Fe oxide could also be used to achieve high Cr removal rates. However, this option would be much less cost effective.

2.6 References

Ajouyed, O., Hurel, C., Ammari, M., Ben Allal, L., and Marmier, N. 2010. Sorption of Cr(VI) onto natural iron and aluminium (oxy)hydroxides: Effects of pH, ionic strength and initial concentration. J. Hazard. Mater. 174:616-622.

Bartlett, R.J. and James, B.R. 1996. Chromium. In: Sparks, D.L., editor, Methods of soil analysis. 1st ed. SSSA and ASA, Madison, WI, U.S.A. p. 683-702.

Bartlett, R.J. and James, B.R. 1979. Behaviour of chromium in soils: III. Oxidation. J. Environ. Qual. 8:31-34.

Bartlett, R.J. and Kimble, J.M. 1976*a*. Behaviour of chromium in soils: I. Trivalent forms. J. Environ. Qual. 5:379-382.

Bartlett, R.J. and Kimble, J.M. 1976*b*. Behaviour of chromium in soils: II. Hexavalent forms. J. Environ. Qual. 5:383-386.

Blowes, D.W., Ptacek, C.J., Benner, S.G., McRae, C.W.T., Bennett, T.A., and Puls, R.W. 2000. Treatment of inorganic contaminants using permeable reactive barriers. J. Contam. Hydrol. 45:123-137.

Blowes, D.W., Ptacek, C.J., and Jambor, J.L. 1997. In-situ remediation of Cr(VI)-contaminated groundwater using permeable reactive walls: Laboratory studies. Environ. Sci. Technol. 31:3348-3357.

Canadian Council of Ministers of the Environment (CCME). 1999*a*. Canadian water quality guidelines for the protection of agricultural water uses: Hexavalent chromium and trivalent chromium. In: Canadian environmental quality guidelines. Publ. 1299. Canadian Council of Ministers of the Environment, Winnipeg, MB.

Canadian Council of Ministers of the Environment (CCME). 1999*b*. Canadian water quality guidelines for the protection of aquatic life: Hexavalent chromium and trivalent chromium. In : Canadian environmental quality guidelines. Publ. 1299. Canadian Council of Ministers of the Environment, Winnipeg, MB.

Canadian Environmental Protection Act (CEPA). 1994. Chromium and its compounds. Priority substances list assessment report En 40-215/39E. Environment Canada and Health Canada, Ottawa, ON.

Cheslock-Fitzgerald, M. 1990. Trivalent and hexavalent chromium, their reactions with minerals and potential phytotoxicity in soils. M.Sc. Thesis. University of Manitoba, Winnipeg, MB.

Goh, T.B., Huang, P.M., Dudas, M.J., and Pawluck, S. 1987. Effect of iron on the nature of precipitation products of aluminum. Can. J. Soil Sci. 67:135-145.

Graham, M.C., Farmer, J.G., Anderson, P., Paterson, E., Hillier, S., Lumsdon, D.G., and Bewley, R.J.F. 2005. Calcium polysulfide remediation of hexavalent chromium contamination from chromite ore processing residue. Sci. Total Environ. 364:32-44.

Graham, M.C., Farmer, J.G., Anderson, P., Paterson, E., Hillier, S., Lumsdon, D.G., Bewley, R.J.F. 2006. Calcium polysulfide remediation of hexavalent chromium contamination from chromite ore processing residue. Sci. Total Environ. 364:32-44.

Han, I., Schlautman, M.A., and Batchelor, B. 2000. Removal of hexavalent chromium from groundwater by granular activated carbon. Water Environ. Res. 72:29-39.

James, B.R. 2001. Remediation-by-reduction strategies for chromate-contaminated soils. Environ. Geochem. Hlth. 23:175-179.

Kabata-Pendias, A. 2011. Trace elements in soils and plants. 4th ed. CRC Press, Boca Raton, FL, U.S.A.

Keely, J.F. 1989. Performance evaluations of pump-and-treat remediations. EPA/540/4-89/005. U.S. Environmental Protection Agency, Ada, OK, U.S.A.

Khan, F.A. and Puls, R.W. 2003. In situ abiotic detoxification and immobilization of hexavalent chromium. Ground Water Monit. R. 23:77-84.

McBride, M.B. 1994. Ion sorption. In: Environmental chemistry of soils. Oxford University Press, New York, NY, U.S.A. p. 122-143.

Motulsky, H and Christopolous, A. 2003. Comparing models using Akaike's Information Criteria (AIC). In: Fitting Models to Biological Data using Linear and Nonlinear Regression: A Practical Guide to Curve Fitting. GraphPad Software Inc., San Diego, CA, U.S.A. p. 143-148.

Murata, A.P. 2010. Characterizing the fate of aqueous phase hexavalent chromium following addition of sodium dithionite and iron oxides. Undergraduate Thesis. University of Manitoba, Winnipeg, MB.

Niu, S.-F., Liu, Y., Xu, X.-H., and Lou, X.-H. 2005. Removal of hexavalent chromium from aqueous solution by iron nanoparticles. J. Zhejiang Univ. Sci. 6B:1022-1027.

Ontario Ministry of the Environment. 1998. Thirty seven municipal water pollution plants monitoring study. Volume 2: Appendix A - Individual plant reports. Water Resources Branch, Ontario Ministry of the Environment, Toronto, ON.

Oze , C., Bird, D.K., and Fendorf, S. 2007. Genesis of hexavalent chromium from natural sources in soil and groundwater. Proc. Natl. Acad. Sci. USA. 104:6544-6549.

Palmer, C.D. and Wittbrodt, P.R. 1991. Processes affecting the remediation of chromium-contaminated sites. Environ. Health Persp. 92:25-40.

Pawlisz, A.V., Kent, R.A., Schneider, U.A., and Jefferson, C. 1997. Canadian water quality guidelines for chromium. Environ. Toxic. Water Qual. 12:185-193.

Penner-Hahn, J.E. 2004. X-ray absorption spectroscopy. In: J.A. and Meyer, T.J., editors, Comprehensive coordination chemistry II: From biology to nanotechnology. Vol. 2. Elsevier, San Diego, CA, U.S.A. p. 159-186.

Powell, R.M., Puls, R.W., Hightower, S.K., and Sabatini, D.A. 1995. Coupled iron corrosion and chromate reduction: Mechanisms for subsurface remediation. Environ. Sci. Technol. 29:1913-1922.

Schulze, D.G. and Bertsch, P.M. 1995. Synchrotron X-ray techniques and their applications. In: Sparks, D.L.,editor, Synchrotron X-ray techniques in soil, plant, and environmental research. Advances in Agronomy 55. Academic Press, San Diego, CA, U.S.A. p. 15-54.

Snoeyink, V.L. and Summers, R.S. 1999. Adsorption of organic compounds. In: Letterman, R.D., editor, Water quality and treatment: A handbook of community water supplies. 5th ed. American Water Works Association and McGraw-Hill, New York, NY, U.S.A. p. 13.1-13.83.

Sposito, G. 1989. Soil adsorption phenomena. In: The chemistry of soils. Oxford University Press, New York, NY, U.S.A. p. 149-168.

Statistics Canada. 1991. Imports by commodity - December 1991. Ministry of Industry, Science and Technology, Ottawa, ON.

Summers, R.S., Knappe, D.R.U., and Snoeyink, V.L. 2010. Adsorption of organic compounds by activated carbon. In: Edzwald, J.K., editor, Water quality and treatment: A handbook on drinking water. 6th ed. American Water Works Association, American Society of Civil Engineers, and McGraw-Hill, New York, NY, U.S.A. p. 14.1-14.27.

Taylor, R.W., Shen, S., Bleam, W.F., and Tu, S. 2000. Chromate removal by dithionite-reduced clays: Evidence from direct X-ray adsorption near edge spectroscopy (XANES) of chromate reduction at clay surfaces. Clay. Clay Miner. 48:648-654.

U.S. Department of the Interior (USDI) and U.S. Geological Survey (USGS). 2012. Chromium. In: Mineral Commodity Summaries 2012. U.S. Geological Survey, Reston, VA, U.S.A. p. 42-43.

USEPA. 1999. Integrated Risk Information System (IRIS) on Chromium VI. U.S. Environmental Protection Agency, Washington, DC, U.S.A.

USEPA. 2000. In situ treatment of soil and groundwater contaminated with chromium. Technical resource guide EPA/625/R-00/004. United States Environmental Protection Agency, Washington, DC, U.S.A.

Wilkin, R.T., Su, C., Ford, R.G., and Paul, C.J. 2005. Chromium-removal processes during groundwater remediation by a zerovalent iron permeable reactive barrier. Environ. Sci. Technol. 39:4599-4605.

3. PHYTOEXTRACTION REMEDIATION OF SOILS CONTAMINATED WITH MULTIPLE TRACE ELEMENTS

3.1 Abstract

Soils which have been contaminated due to industrial activities often contain complex mixtures of trace elements. Affected sites are frequently large in extent and therefore ill-suited to conventional remediation techniques such as soil excavation and disposal. Phytoremediation is a promising remediation strategy since it is inexpensive, can be applied in situ, and has been found to be effective for numerous individual trace element contaminants. The purpose of this study was to examine the use of agronomic (*Brassicae*) and native (*Deschampsia caespitosa*) plant species for the phytoremediation of low- and high- organic matter (OM) soils contaminated with multiple trace elements. Growth room experiments were conducted to quantify germination and early growth in the contaminated soils and to measure above-ground biomass production, shoot trace element concentrations, trace element uptake, and change in soil trace element concentrations over multiple harvests. Results showed that *D. caespitosa* had better early growth in the contaminated soils than any of the *Brassicae*. *Deschampsia caespitosa* plants grown in the contaminated high-OM soil accumulated significantly higher concentrations of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn) in their above-ground tissue 25 wk after plant establishment compared to plants grown in the contaminated low-OM and non-contaminated soils. However, the

trace element concentrations were low from an environmental perspective and decreased by the next harvest (44 wk after plant establishment). Calculated bioconcentration factors were all < 1, the threshold value for plants to be considered suitable for phytoextraction purposes. Plants grown in the contaminated soils produced less biomass than those grown in a fertilized non-contaminated low-OM soil. Maximum trace element uptake was ≤ 30 μg pot^{-1} (67 μg kg^{-1} of dry soil) for all trace elements except Zn which had uptake of 940 μg pot^{-1} (2.1 mg kg^{-1} of dry soil). Only As and Pb concentrations in the high-OM contaminated soil and Cr concentration in the low-OM contaminated soil decreased after the phytoextraction treatment. These results indicate that the treatment period (equivalent to ≤ 3 growing seasons) employed in this study was not long enough to effectively reduce trace element concentrations in the highly contaminated soils tested. This is consistent with numerous previous studies, which showed that most contaminated sites will require more than 5 yr to remediate using phytoextraction.

3.2 Introduction

3.2.1 Phytoextraction

Industrial sites are often contaminated with multiple trace elements (Quartacci et al. 2006). Remediation of these sites is complex due to variation in the physical, chemical, and biological behaviours of different contaminants. For industrial activities such as smelting, where the release of contamination is atmospheric, remediation efforts can be further complicated due to the vast extent of contamination. Phytoremediation, the use of plants for contamination clean-up, is a promising remediation strategy for such sites since

it is inexpensive and can be performed in situ (McGrath and Zhao 2003; Marques et al. 2009; Kabata-Pendias 2011). When compared to conventional soil remediation techniques such as excavation and reburial, phytoremediation can be up to 1,000 times less expensive (Memon and Schroder 2008). Phytoremediation techniques for trace elements can be divided into two main categories. Phytostabilization is the use of plants to stabilize impacted soil or immobilize the contaminants held in said soil so as to reduce or prevent contaminant transport (McGrath and Zhao 2003; Marques et al. 2009). Phytoextraction is the use of plants to take up contaminants from impacted material and transport them into harvestable plant tissue (Van Nevel et al. 2007; Marques et al. 2009; Kabata-Pendias 2011). This process concentrates the contaminants and decreases the volume of impacted material (Salt et al. 1995*a*; Raskin et al. 1997). The plant tissue is then stored in an area where it can no longer pose a threat to environmental or human health (Kumar et al. 1995; Nowack et al. 2006). Incineration or composting may be used to further reduce the volume of contaminated material (Kumar et al. 1995; Raskin et al. 1997; Nowack et al. 2006; Memon and Schroder 2008).

As with all phytoremediation techniques, the main advantages of phytoextraction are its low cost and in situ applicability (McGrath and Zhao 2003, Kabata-Pendias 2011). After planting, only minor costs and maintenance are required for field management and harvesting (Memon and Schroder 2008). According to Salt et al. (1995*a*), remediation of one acre of sandy loam soil to a depth of 50 cm would cost at least $400,000 using conventional methods including excavation and storage. Raskin et al. (1997) estimate the cost of excavation and reburial at a hazardous waste site to be $1,000,000 per acre of soil area. In contrast, remediation estimates are only $60,000-100,000 per acre using

phytoextraction (Salt et al. 1995*a*). This is particularly important in the case of large or remote contaminated sites whose extent or distance from treatment or disposal centres renders conventional dig-and-dump or ex situ remediation methods so expensive that they become economically infeasible. Another advantage of phytoextraction is its relatively low release of carbon dioxide. In fact, plants can be considered living pumps which remove contaminants from the soil by using solar power (Salt et al. 1995*a*). Even if the harvested plant biomass is incinerated, no more carbon dioxide will be released than the amount the plants originally took from the atmosphere (Memon and Schroder 2008). Disadvantages of phytoextraction relate to limitations in its application. Phytoextraction is a relatively slow treatment method and is only effective within the root zone (Van Nevel et al. 2007). It is not uncommon for remediation time frames to be in the range of decades (Memon and Schroder 2008). Another disadvantage of phytoextraction is the risk of contaminant redistribution in the environment via litter fall and herbivore consumption (Van Nevel et al. 2007). Litter fall is mainly a concern for tree species which are not being considered in this study. The introduction of contaminants into the food chain by herbivore consumption could possibly be discouraged by the use of animal deterrents in and around the area being remediated.

A simple model for phytoextraction efficiency can be written as

$$\text{Efficiency} = f(\text{biomass, concentration}) \qquad \textbf{[1]}$$

In this model, biomass refers to shoot tissue since only the above-ground portion of the plants can be easily harvested (McGrath and Zhao 2003). Bioconcentration is an indicator of how well a plant species can accumulate contaminants from the soil. It is usually represented by bioconcentration factor (BCF), the ratio of a contaminant's

concentration in the shoot tissue to its concentration in the soil (McGrath and Zhao 2003). According to McGrath and Zhao (2003), species with BCF <1 for a given contaminant are not suitable phytoextractors for that contaminant because of the long time frame that would be required to attain significant reduction in soil contamination regardless of biomass yield. Optimal plant species for phytoextraction can be described as being able to tolerate contamination, accumulate contaminants in harvestable tissue, and quickly produce large quantities of harvestable biomass (Salt et al. 1995*a*). Hyperaccumulators are plant species which are exceptionally capable of taking up trace elements above background soil concentrations (Marques et al. 2009; Kabata-Pendias 2011); they generally have BCF >1, a shoot to root contaminant concentration ratio > 1, and hypertolerance to contamination (McGrath and Zhao 2003). Threshold shoot trace element concentrations required for hyperaccumulator status depend on the contaminant in question. For example, hyperaccumulators can concentrate Cu or Pb above 1000 mg kg^{-1} and Zn above 10,000 mg kg^{-1} in shoot tissue (Kabata-Pendias 2011). Hyperaccumulators are rare with less than 0.2% of angiosperms being defined as such (McGrath and Zhao 2003).

Brassica juncea (L.) Czern. (Indian mustard) is among the hyperaccumulator species most commonly studied in phytoextraction projects and is known to accumulate large amounts of lead (Pb), zinc (Zn), nickel (Ni), copper (Cu), cadmium (Cd), chromium (Cr), uranium (U), and manganese (Mn) in its tissues (Environment Canada 2003, Kababa-Pendias 2011). Most phytoextraction studies, however, focus on one or two contaminants which have been intentionally added to a growth medium. For example, Salt et al. (1995*b*) grew *B. juncea* plants in a hydroponic solution containing 0.1 µg mL^{-1}

Cd. Concentrations of Cd in dried shoot and root tissue were found to be 1100 and 6700 times the concentration in solution, respectively (Salt et al. 1995*b*). Some studies have addressed the slow speed of phytoextraction by looking at the use of chelating agents to increase the bioavailability and uptake of trace element contaminants. Vassil et al. (1998) conducted a study where *B. juncea* plants were grown in hydroponic solutions containing various 0.5 mM Pb and various concentrations (0.1-2.5 mM) of the chelating agent ethylenediaminetetraacetic acid (EDTA). The study showed that Pb was transported as a complex with EDTA and that the addition of EDTA to the system greatly increased Pb uptake, with concentrations up to 75 times the concentration in solution being measured in the shoot tissue (Vassil et al. 1998). In a study by Epstein et al. (1999), *B. juncea* was grown in pots of sandy clay loam soil amended with different concentrations of Pb and supplied with different concentrations of EDTA upon flowering. The results supported those for the hydroponic system found by Vassil et al. (Epstein et al. 1999). While these studies have successfully demonstrated the ability of *B. juncea* plants to take up trace elements from growth media, contaminant concentrations in shoot tissue may be much higher than those that could be expected from applied phytoextraction treatments. This is because the bioavailability of trace elements in hydroponic solutions or spiked soils is higher than in soils which have previously been contaminated and have had time to reach stable equilibria (Quartacci et al. 2006). These studies also ignore the effect of multiple contaminants on trace element uptake.

Very little research has been done on the phytoextraction of soils contaminated by multiple trace elements even though most polluted soil contains several substances at toxic levels (Quartacci et al. 2006). One of the few papers which studied a complex

mixture of trace element contaminants was a greenhouse experiment conducted by Kumar et al. (1995). *B. juncea* was grown in a mixture of sand and perlite treated with a 10 mL solution containing 2 mg L^{-1} Cd, 50 mg L^{-1} trivalent Cr, 3.5 mg L^{-1} hexavalent Cr, 10 mg L^{-1} Cu, 100 mg L^{-1} Ni, 500 mg L^{-1} Pb, and 100 mg L^{-1} Zn (Kumar et al. 1995). Results showed that *B. juncea* was capable of accumulating Cd, hexavalent Cr, Cu, Ni, and Zn at concentrations 52, 58, 7, 31, and 17 times their respective growth medium concentrations (Kumar et al. 1995). *B. juncea* was also able to accumulate Pb, a highly immobile contaminant, to levels of 108 mg g^{-1} dry root tissue and 35 mg g^{-1} dry shoot tissue (Kumar et al. 1995). A study by Quartacci et al. (2006) examined the effect of nitrilotriacetate (NTA) and citric acid chelating agent addition on the *B. juncea* uptake of trace elements from a growth medium contaminated with 40 mg kg^{-1} Cd, 190 mg kg^{-1} Cu, 1330 mg kg^{-1} Pb, and 3330 mg kg^{-1} Zn. They reported that NTA addition increased the availability of all elements except Cr but decreased biomass production; therefore, only total uptake of Cu and Zn was significantly improved (Quartacci et al. 2006). Citric acid was not found to affect shoot trace element concentrations (Quartacci et al. 2006).

Overall, much of the existing literature focuses on simplified contaminated growth media which have been treated with a known concentration of only one or two trace elements (Salt et al. 1995*b*, Vassil et al. 1998, Epstein et al. 1999). Therefore, a gap in knowledge exists regarding the effectiveness of phytoextraction on commonly occurring soils contaminated with a complex mixture of several trace elements. Furthermore, there is a dearth of published research on the phytoextraction of organic soils contaminated with multiple trace elements.

3.2.2 Contaminated Soil Source

The contaminated soil for this study was collected from the town of Flin Flon, MB (55°N, 102°W). Flin Flon is a mining community located about 750 km north-northwest of Winnipeg on the MB-SK border. The city is situated on the Paleozoic-Precambrian Shield boundary in a low hilly Boreal forest area composed mostly of jack pine (*Pinus banksiana* Lamb.), black spruce (*Picea mariana* (Mill.) B.S.P.), white spruce (*Picea glauca* (Moench) Voss), trembling aspen (*Populus tremuloides* Michx.), and balsam poplar (*Populus basamifera* L.) (Franzin et al. 1979; Hogan and Wotton 1984; Henderson et al. 1998; McMartin et al. 1999). Mineral operations in the Flin Flon area began with the establishment of Cu-Zn mines by Hudson Bay Mining and Smelting Co., Ltd. in 1927 and smelting in 1930 (Franzin et al. 1979). The smelter was closed in mid-June 2010. During its operation, the Flin Flon complex produced Cu, Zn, and Cd metals on site and sent Pb, gold (Au), silver (Ag), selenium (Se), and tellurium (Te) materials off-site for further processing (Franzin et al. 1979). Historically, smelter emissions were released from low stacks (30 m) until 1974, when a 251-m stack was constructed (Franzin et al. 1979; Hogan and Wotton 1984; McMartin et al. 1999). Emission quantities and composition has varied over time due to changing ore material and processing, recovery, and smelting technologies (McMartin et al. 1999; Intrinsic Environmental Sciences Inc. 2010). Particulate emissions have been estimated at about 7150 t yr^{-1} prior to 1974, about 6834 t yr^{-1} beginning 1974, and about 632 t yr^{-1} following technology changes in 1995 (McMartin et al. 1999). Historically, predominant emission components have included sulfur dioxide (SO_2), Zn, iron (Fe), and Pb (Intrinsic Environmental Sciences Inc. 2010). Other trace elements emitted include arsenic (As), Cu, Cd, mercury (Hg), Ag, aluminium

(Al), magnesium (Mg), Mn, Se, antimony (Sb), Ni, Cr, and cobalt (Co) (Intrinsic Environmental Sciences Inc. 2010).

Franzin et al. (1979) conducted a study of the atmospheric deposition of trace elements in the Flin Flon area and determined that the smelter was a significant source of Zn, Cd, Pb, As, and Cu to an area up to 250,000 km^2 in size. Hogan and Wotton (1984) studied the distribution and effects of metal contaminants near the smelter in Flin Flon after approximately 50 yr of operation. Within 5 km of the smelter, tree mortality, reduction in species diversity, stunted growth, and soil erosion were reported (Hogan and Wotton 1984). They measured elevated levels of Cu, Zn, and Pb in soil samples up to 35-40 km from the smelter and noted that metal concentrations were highest at the surface of the soil profile due to the atmospheric nature of contamination (Hogan and Wotton 1984). Soil pH was not correlated with distance from the smelter, indicating that stack emissions had not caused soil acidification (Hogan and Wotton 1984). McMartin et al. (1999) reported that surface organic horizon concentrations of As, Cd, Cu, Hg, Pb, and Zn were elevated nearby the smelter and decreased with distance in all directions. The trace element distribution was slightly skewed to the southeast in accordance with prevailing wind conditions (McMartin et al. 1999). Distance to background concentrations were determined to be 70 km for Cd, 76 km for Pb, 84 km for Zn, 85 km for Hg, 90 km for Cu and 104 km for As (McMartin et al. 1999). These results supported those of Zoltai (1988) who reported trace element contamination of peat to distances of 65 km for Pb, 77 km for Zn, and 110 km for Cu and As. Henderson et al. (1998) reported that the majority of Zn in the surface organic horizon at sites within 50 km of the smelter was in labile forms with an average of 54% in the soluble organic phase. This supported the findings

by Hogan and Wotton (1984) that 50-60% of Zn and 18-36% of the Cu in the surface organic horizon at sites within 70 km of the smelter was bioavailable.

Given the large extent of contamination found at Flin Flon, a cost efficient remediation method such as phytoextraction is desirable. The distribution of contamination in the soil profile is suited to phytoextraction because it is concentrated near the surface, well within the root zone. The slow speed of phytoextraction is not a major concern for the Flin Flon site because the contamination is not considered to be an immediate threat to human health (Intrinsic Environmental Sciences Inc. 2010).

3.2.3 Deschampsia caespitosa

Deschampsia caespitosa (L.) Beauv. (tufted hairgrass) is a perennial grass which forms dense tussocks with numerous shoots (Darris et al. 1995; FAO 2004). It occurs naturally in temperate humid regions across the northern Hemisphere (Darris et al. 1995; FAO 2004). Tufted hairgrass is an incredibly robust species which can tolerate drought conditions due to its deep roots, grow in soils with pH 4.3-8.1, survive at altitudes as great as 2750 m, and tolerate waterlogging and frost (FAO 2004). Gunn et al. (1995) documented the changes in the area surrounding several Sudbury, ON smelters following a nearly 90% reduction in SO_2 which began around 1960. The emission controls which caused the SO_2 reduction did not greatly reduce the acidity or metal concentrations in soils surrounding the smelters (Gunn et al. 1995). The soils were reported to contain elevated levels of Ni, Cu, Co, Ag, Fe, and Pb (Hutchinson and Whitby 1974). Gunn et al. (1995) found that *D. caespitosa* was among the first vascular species to recolonize the more severely impacted barrens surrounding the smelters approximately 10 yr after the

SO_2 emission reductions began. *D. caespitosa* from the Sudbury area was found to be tolerant to Cu and Ni (Cox and Hutchinson 1979; Rauser and Winterhalder 1985). The same studies also reported Zn-tolerance which was suggested as having developed coincidentally alongside the Cu- and Ni-tolerances since elevated levels of Zn were not observed in the Sudbury soil (Cox and Hutchinson 1979; Rauser and Winterhalder 1985). Tolerance to trace element contamination can occur either when plants exclude or have metabolic tolerance for the contaminants (Memon and Schroder 2008; Marques et al. 2009). Since *D. caespitosa* can naturally survive in trace element-contaminated soils, it is reasonable to propose that it may have the potential to take up contaminants as well. The perennial nature of *D. caespitosa* would make phytoextraction particularly cost-efficient since no replanting would be required in between harvests.

3.2.4 Objectives

The objectives of this study were to:

1. Quantify the germination and early growth of three *Brassica* species and *D. caespitosa* in low- and high-organic matter (OM) soils contaminated with multiple trace elements;
2. Compare above-ground biomass yields and trace element uptake by plants grown in fertilized vs. non-fertilized contaminated and non-contaminated soils; and
3. Quantify changes in soil trace element concentrations following multiple harvests of plants growing in contaminated soils.

3.3 Materials and Methods

3.3.1 Soils

Bulk soil samples (0-15 cm layer) were collected from Flin Flon, MB at sites located within 250 m of each other and approximately 5 km southwest of the Hudson Bay Mining and Smelting Co. smelting complex. Five of the samples were relatively low in OM while the other five were high in OM. Each group of five samples was mixed to give two composite soils: a low-OM contaminated (CLOM) soil with 34 mg kg^{-1} OM and a high-OM contaminated (CHOM) soil with 236 mg kg^{-1} OM. The contaminated soils were analyzed for trace element concentrations by inductively-coupled plasma mass spectrometry (ICP-MS) according to a procedure adapted from USEPA method 200.2 at a commercial laboratory in Winnipeg, MB.

Two non-contaminated soils were included in the study for comparison. The first, referred to as the non-contaminated low-OM (NLOM) soil, was a 2:1:1 mixture of soil, peat, and sand commonly used as a growth medium for potted plants. The second, called the non-contaminated high-OM (NHOM) soil, was a peat also used as a growth medium for potted plants. These non-contaminated soils were analyzed for trace element concentration by ICP-MS following aqua regia digestion at a commercial laboratory in Vancouver, BC. Both the non-contaminated and contaminated soils were analyzed for nitrate-nitrogen (NO_3-N), Olsen phosphorus (Olsen P), potassium (K), pH, OM, and cation exchange capacity (CEC) at a commercial laboratory in Northwood, ND, U.S.A. Field capacity moisture contents of all growth media were also determined by measuring the moisture content of covered sample cores which had been allowed to drain freely for 3 d.

3.3.2 Seed Germination

A growth room study was carried out in order to examine the germinability and early growth of three *Brassica* species (*B. carinata* A. Braun, *B. juncea*, and *B. napus* L.) in the contaminated soils. *Brassica juncea* was chosen due to its frequent use in phytoremediation research and its known hyperaccumulator status (Environment Canada 2003), while *B. carinata* was included because of its robustness and potential for high biomass production. Inclusion of *B. napus* was based on its potential for biofuel production. One seed of each *Brassica* species was planted in each of 24 germination cells containing one of the four soils. The growth room was maintained at a day temperature of 22°C for 16 h and a night temperature of 15°C for 8 h, with relative humidity set at 60%. The germination trays were watered regularly. No nutrient amendments were applied. Maximum germination counts and plant height 4 wk after seeding were recorded. The study was done in triplicate with a randomized complete block design.

A second growth room germination study was conducted using *D. caespitosa* due to its robustness and potential for contaminant extraction. Two *D. caespitosa* seeds were planted in each of 24 germination cells for each of the four soil types. The growth room was maintained at a day temperature of 22°C for 16 h and a night temperature of 15°C for 8 h, with relative humidity set at 60%. The germination trays were watered regularly and no fertilizer was added. The germination trays were covered with transparent plastic sheets until the grass grew tall enough to touch them. This was done in order to prevent the soils from drying out too quickly while still allowing airflow. Maximum germination

counts and plant height 4 wk after seeding were recorded. The study was done in triplicate with a randomized complete block design.

Analysis of variance (ANOVA) of the germination and early growth data was performed using PROC MIXED in SAS version 9.2 (SAS Institute, 2008). For the *Brassica* germination study, the effects of species and soil type on germination percentage and plant height after 4 wk were determined. For the *D. caespitosa* germination study, the effect of soil type on germination percentage and plant height after 4 wk was determined.

3.3.3 Phytoextraction

The multiple harvest phytoextraction experiment was conducted in a growth room in order to determine the phytoextraction capability of *D. caespitosa*. Two pre-germinated *D. caespitosa* seedlings were transplanted into each pot. The individual seedlings were transplanted into pots containing the same soil type in which they had been pre-germinated. There were 12 pots for each soil type. The mass of dry soil in each pot was 1.31 kg for the low-OM soils, 450 g for the CHOM soil, and 246 g for the NHOM soil. Half of the pots of each soil type were fertilized with urea and monoammonium phosphate at rates of 40 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} N. These rates were based on nutrient recommendations from MAF (2001) and FAO (2004). The growth room was maintained at a day temperature of 22°C for 16 h and a night temperature of 15°C for 8 h, with relative humidity set at 60%. The pots were watered regularly to approximately 70% of field moisture capacity.

The first *D. caespitosa* harvest was carried out 14 wk after transplanting. The second harvest occurred 11 wk later and the third harvest occurred 19 wk after that. At each

harvest, above-ground biomass samples were collected by cutting the grasses at a height of approximately 3 cm from the soil surface. Above-ground tissue samples were not collected during the first harvest for plants grown in the CLOM soil because the biomass was insufficient for trace element analysis. Soil samples were collected during each harvest using a small (~2 cm diameter) soil probe. Two soil cores were taken from each pot; one sample was collected close to the *D. caespitosa* plants and the other was collected approximately 8 cm from the plants. After each harvest, pots assigned to the fertilizer treatment received urea and monoammonium phosphate at rates of 40 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} N.

Shoot and soil samples collected at each harvest were air-dried. The dry mass of each plant tissue sample was measured and recorded. Shoot samples were then finely ground using a ceramic mixer mill (8000M, SPEX Sample Prep, Metuchen, NJ, U.S.A.). Soil samples were ground using an agate mortar and pestle and passed through a 100-µm sieve. The above-ground plant tissue samples were digested in a microwave oven (MARS 5, CEM Corp., Matthews, NC, U.S.A.). Fifty-milligram (dry wt.) subsamples were digested in 1 mL of 15.8 M HNO_3. The samples were brought to 200°C in the microwave over a 10-min period. The samples were then held at 200°C for 15 min. For each run of 12 plant samples, one blank, two certified reference materials, and three duplicates were included. Once the samples cooled, the extract was diluted 20 times and analyzed for trace element concentrations using ICP-MS (Elan DRC II, Perkin Elmer, Waltham, MA, U.S.A). Soil samples from the final harvest were sent to a commercial laboratory in Vancouver, BC for aqua regia digestion and trace element analysis by ICP-MS.

Since the first harvest of plant tissue from the CLOM soil did not occur until the second harvest for the other plants, biomass values from the first two harvests were combined for the NLOM, NHOM, and CHOM pots. This may have affected the biomass collected from the clean mineral, clean organic, and contaminated organic pots. A study by Stohlgren et al. (1989) found that additional harvests in a *D. caespitosa-Carex rostrata* Stokes. plant community resulted in a 15-20% decrease in biomass production. However, the effect of repeated harvests specifically on *D. caespitsoa* was not determined (Stohlgren et al. 1989). In any case, the data transformation allowed for the statistical analysis of the biomass and trace element data. The period of plant growth from seeding until the first harvest for contaminated mineral pots and the second harvest for all other pots was classified as Time 1. The period of growth leading up to the second harvest for contaminated mineral pots and the third harvest for all other pots was classified as Time 2. Mean shoot trace element concentrations for the two time periods were determined for the NLOM, NHOM, and CHOM pots by the total trace element uptake from each pot for that period and dividing it by the corresponding total biomass yield for the same period. Trace element uptake was calculated for each phytoextraction period by multiplying biomass yield by trace element concentration. Change in soil trace element concentration was calculated as the difference between the initial and post-phytoextraction soil trace element concentrations. Analysis of variance was performed using PROC MIXED in SAS to determine the effects of soil type, fertilizer application, and time on biomass, shoot trace element concentration, trace element uptake, and change in soil trace element concentration. When necessary, data were transformed to fulfil the normality of residuals assumption of the ANOVA procedure. For shoot

concentrations of As, Cr, and Cu, the -0.25 power transformation was used. The 0.25 power transformation was used for the Cd, Pb, and Zn shoot concentration data. Shoot Se concentration data were omitted from the analysis because nearly all concentrations were below the analytical detection limit. For the other trace elements, values below the detection limit were replaced by concentrations equal to half of the detection limit. Statistical analysis of the trace element uptake data also required transformations. As, Cd, Cu, Pb, and Zn data were transformed to the power of 0.25. The -0.25 power transformation was used for Cr uptake data. Statistical analysis of the change in trace element content of the contaminated soils following phytoextraction treatment required data transformations for Cr and Pb. For both elements, the data were made positive by adding a constant prior to applying a 0.25 power transformation.

3.4 Results and Discussion

3.4.1 Soils

The four soils used in this study were found to vary in their basic chemical properties (Table 3.1). Both contaminated soils had very low NO_3-N concentrations while the NLOM soil had high NO_3-N concentrations (Marx et al. 1996). Nitrate-nitrogen measurements for the NHOM soil were omitted because the results were likely invalid due to an overestimate of bulk density. It is probable that NO_3-N was low in the NHOM soil. Olsen-P analyses showed that the NHOM and CLOM soils had low P concentrations (Marx et al. 1996). The NLOM and CHOM soils had moderate P levels (Marx et al. 1996).

Table 3.1 Chemical properties of the contaminated and non-contaminated soils. Error values represent standard deviation.

Property	Soil			
	NLOM	**NHOM**	**CLOM**	**CHOM**
Nitrate-N (mg kg^{-1})	43.7 ± 6.5	-	0.5 ± 0	1.5 ± 1.0
Olsen-P (mg kg^{-1})	12 ± 2	3 ± 1	6 ± 1	12 ± 1
K (mg kg^{-1})	189 ± 28	19 ± 2	231 ± 4	126 ± 10
pH	7.6 ± 0.1	4.4 ± 0.1	6.7 ± 0.2	6.3 ± 0.1
OM (%)	3.9 ± 0.4	96.3 ± 1.0	3.4 ± 0.1	23.6 ± 2.3
CEC (meq)	26.5 ± 2.5	7.6 ± 0.8	15.6 ± 1.7	12.3 ± 0.1

Both low-OM soils had moderate K levels while the CHOM soil had low K levels and the NHOM soil had very low K levels (Marx et al. 1996). Soil pH was nearly neutral for the NLOM soil and both contaminated soils. The NHOM soil was at the very acidic end of acceptable growing conditions for *D. caespitosa* (FAO 2004). The low-OM soils had similar OM concentrations while the NHOM soil had a much greater OM concentration than the CHOM soil. Cation exchange capacity increased in the following order: NHOM < CHOM < CLOM < NLOM.

Due to the differences in soil properties the non-contaminated soils cannot be considered experimental controls for the contaminated soils. Instead, the non-contaminated soils were included to allow for comparison in germination, growth, and trace element uptake between plants grown in the contaminated soils and plants grown in commonly used non-contaminated soils. Based on its chemical properties, the NLOM soil was determined to offer the best growing conditions of all four soils.

ICP-MS analyses confirmed that the composite soils from Flin Flon contained multiple trace elements, most of which exceeded Canadian Council of Ministers of the Environment (CCME) soil quality guidelines (Table 3.2).

Table 3.2 Concentrations of selected trace elements in the contaminated soils compared to the Canadian soil quality guidelines for agricultural land (CCME 2007).

Trace Element	**Concentration** (mg kg^{-1})		
	CLOM Soil	**CHOM Soil**	**Canadian Soil Quality Guideline**
As	22	159	12
Cd	7.4	28.3	1.4
Cr	111	45	64
Cu	440	1590	63
Pb	67	4500	70
Se	3	10	1
Zn	2000	7460	200

In general, the CHOM soil was more heavily impacted and contained higher concentrations of As, Cd, Cu, Pb, Se, and Zn than the CLOM soil. The opposite was true for Cr. The CHOM soil was expected to contain higher trace element concentrations due to cycling within the soil. Trace elements become enriched in the upper portion of soil OM through long-term upwards movement by plant roots and atmospheric interception by vegetative canopy followed by accumulation and decay of plant litter (Stevenson 1994). Therefore, the CHOM soil would contain trace elements of atmospheric origin as well as those translocated from deeper in the profile.

3.4.2 Seed Germination

Analysis of variance for *Brassica* species germination percentage data indicated a significant species effect but non-significant soil type and interaction effects (Table 3.3).

Table 3.3 Species and soil effects on germination percentage and plant height 4 wk after seeding for *Brassica* plants.

Effect	**Germination** (%)	**Height** (cm)
Species		
B. carinata	49.7b[†]	7.18
B. juncea	96.5a	8.91
B. napus	90.6a	9.34
Soil		
NLOM	79.6	11.80
NHOM	82.4	12.35
CLOM	75.0	6.94
CHOM	78.7	2.82
	P	
Species	<0.0001	<0.0001
Soil	0.40	<0.0001
Species × soil	0.99	<0.0001

[†]Means in the same column followed by the same letter are not significantly different (P > 0.05) according to the Tukey test.

Brassica carinata had a significantly lower germination percentage than either *B. juncea* or *B. napus*. The non-significant soil effect agrees with research by Meng et al. (2008) which showed that germination counts for *B. napus* seeded on filter papers in various Cd solutions was not negatively impacted until very high aqueous Cd concentrations of > 200μM (22 mg L^{-1}) were applied.

Plant height after 4 wk was significantly affected by *Brassica* species, soil type, and the interaction between the two (Table 3.3). In general, *Brassicae* grown in the non-contaminated soils had greater early growth than *Brassicae* grown in the contaminated soils (Figure 3.1).

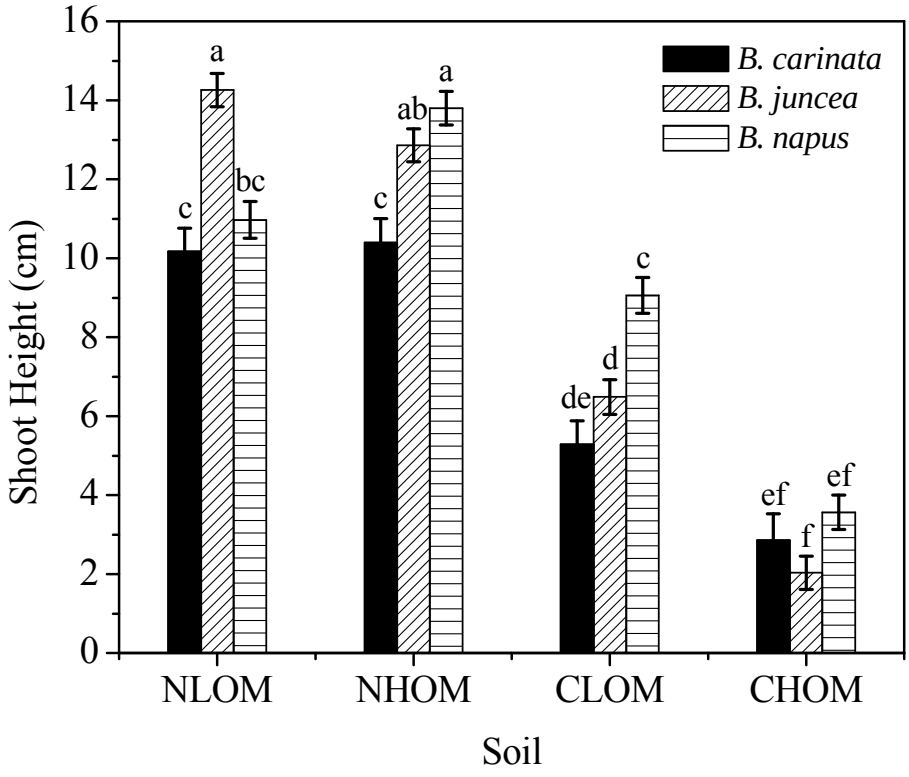

Figure 3.1 Four-week growth of *Brassica* species in various soils. Vertical bars represent standard error of the mean. Columns labelled with the same letter are not significantly different ($P > 0.05$) according to the Tukey test.

However, the height of *B. napus* plants grown in the CLOM soil was not significantly different from the height of *B. napus* plants grown in the NLOM soil or *B. carinata* plants grown in either non-contaminated soil. The significant interaction arose because within each soil type, the ranking of the *Brassicae* was not the same. All *Brassica* species grown in the CHOM soil were extremely stunted and exhibited a purple colouration. The

Brassicae did not die during the study but growth appeared to have stopped completely by 4 wk after seeding. Purpling is typically indicative of elevated levels of anthocyanin, a pigment which accumulates due to various plant stresses (McCauley et al. 2009). In frequent cases, purpling is associated with P deficiency (McCauley et al. 2009). Since the purpling was observed in plants germinated in the CHOM soil which actually had higher Olsen-P concentrations than either the CLOM soil or the NHOM soil, P deficiency is likely not the cause of the discolouration in this study. Instead, it is possible that plant stress due to high trace element concentrations resulted in the purpling. The contaminants could have interfered with biochemical reactions or the uptake of essential nutrients (Kabata-Pendias 2011). Quartacci et al. (2006) also reported stunted growth of *B. juncea* seedlings sown in soil contaminated by multiple trace elements. Instead of a purple discolouration, however, the plants exhibited chlorosis and nectrotic areas (Quartacci et al. 2006).

Soil type did not have a significant effect on *D. caespitosa* germination percentage but did have a significant effect on *D. caespitosa* height after 4 wk (Table 3.4). Plants grown in the NLOM soil had the greatest early growth. Plants grown in the NHOM and CLOM soils had statistically similar heights 4 wk after seeding.

Table 3.4 Soil effect on germination percentage and plant height 4 wk after seeding for *Deschampsia caespitosa*.

Effect	**Germination** (%)	**Height** (cm)
Soil		
NLOM	32.6	7.26a[†]
NHOM	54.9	5.39b
CLOM	50.7	4.55b
CHOM	86.8	3.63c
	P	
Soil	0.062	<0.0001

[†]Means in the same column followed by the same letter are not significantly different (P > 0.05) according to the Tukey test.

As with the height of *Brassicae* 4 wk after seeding, plants grown in the CHOM soil were stunted. However, the *D. caespitosa* did not exhibit any discolouration and growth did not appear to have stopped. This may have been due to the plants' tolerance to contamination. As a result, *D. caespitosa* was chosen for further study in the phytoextraction experiment.

3.4.3 Phytoextraction

When transplanted from the germination cells, the *D. caespitosa* had multiple thin shoots. As the plants grew, it became apparent that some were not *D. caespitosa*. This occurred in the case of two of the 24 contaminated mineral plants and 18 of the 24 contaminated organic plants. The unknown grasses were larger than the *D. caespitosa* and had broader shoots. Since no unknown plants were present in the clean soils, it is believed they germinated from seeds stored in the contaminated soils. These plants were replaced with

D. caespitosa still growing in the germination trays two weeks after the original transplant.

During the next 12 wk of plant growth, *D. caespitosa* plants grown in the NLOM soil appeared to be the healthiest. They appeared very green and seemed to have the highest above-ground biomass yield. The plants from the CHOM soil also appeared healthy and plants from seven of the pots had begun to flower by the end of 12 wk. Plants grown in the NHOM soil appeared to be highly dependent on fertilizer application. The pots which were not fertilized had very small plants. The pots which were fertilized had much larger plants but the shoots were chlorotic. *Deschampsia caespitosa* plants grown in the CLOM soil produced very little biomass. At the time of the first harvest, above ground plant tissue was only harvested for *D. caespitosa* grown in the NLOM, NHOM, and CHOM soils. The plants from the CLOM soil had not produced enough mass to allow for trace element analysis.

After the first harvest, all plants continued to grow. After approximately 8 wk, the plants in the CHOM soil developed a pinkish brown colouration. Plants from the fertilized NHOM soil still appeared yellowish and plants from all other treatments had a few yellow and brown shoots. At the time of the second harvest, 11 wk after the first, *D. caespitosa* plants from all soil types had produced sufficient biomass for trace element analysis so above ground biomass samples were collected from all pots.

After the second harvest, the plants from all soil types appeared to be growing at a much slower rate. After about 12 wk, the plants from the CHOM soil were green again. After 19 wk, a third harvest was conducted and above-ground tissue was collected from all

pots. It is possible that the slow growth during Time 2 occurred as a stress symptom due to the lack of rest or winter period in the study.

Analysis of variance for above-ground biomass indicated significant effects of soil type, fertilizer, and time as well as significant interactions between soil and fertilizer, soil and time, and soil, fertilizer, and time (Table 3.5).

Table 3.5 Soil, fertilizer, and time effects on above-ground biomass yield for *Deschampsia caespitosa* plants.

Effect	**Biomass** (g, dry wt.)
Soil	
NLOM	7.31
NHOM	2.51
CLOM	1.24
CHOM	2.50
Fertilizer	
Yes	4.54
No	2.24
Time	
1	4.81
2	1.98
	P
Soil	<0.0001
Fertilizer	<0.0001
Time	<0.0001
Soil × fertilizer	<0.0001
Soil × time	<0.0001
Fertilizer × time	0.13
Soil × fertilizer × time	0.005

During Time 1, *D. caespitosa* plants growing in fertilized NLOM soil produced the greatest biomass followed by *D. caespitosa* plants growing in unfertilized NLOM soil (Figure 3.2).

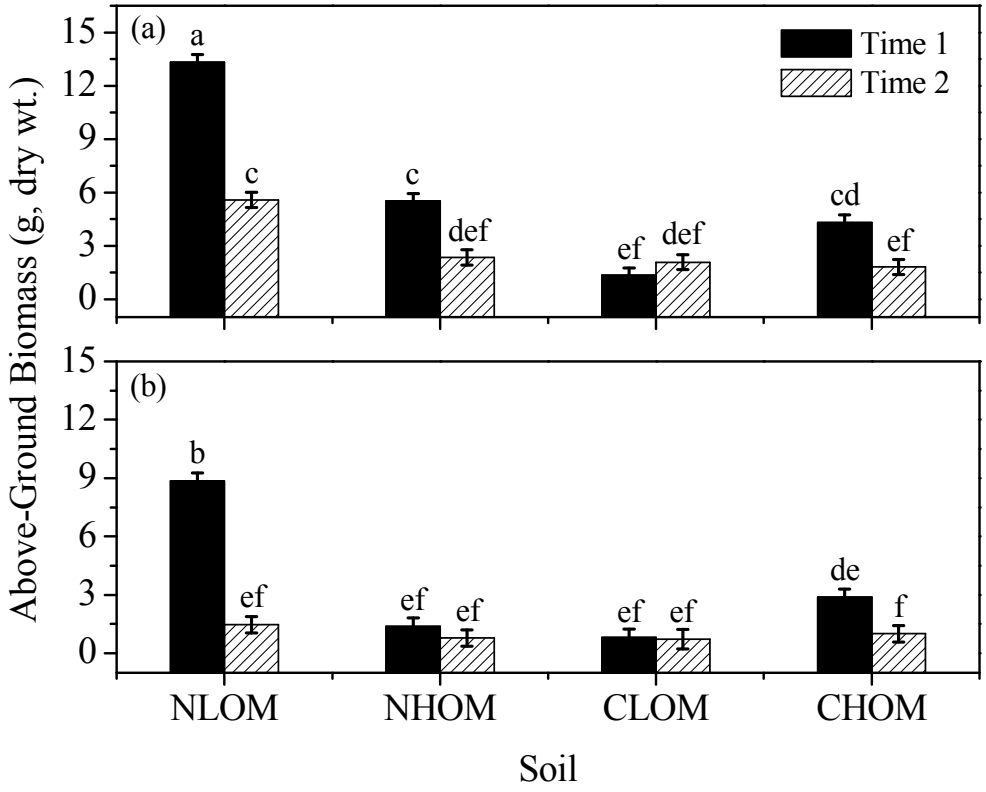

Figure 3.2 Above ground biomass of *Deschampsia caespitosa* grown in various soils with fertilizer (a) and without fertilizer (b). Vertical bars represent standard error of the mean. Columns in the same panel labelled with the same letter are not significantly different ($P > 0.05$) according to the Tukey test.

The treatments which produced the least above ground biomass were the fertilized and unfertilized CLOM soils and the unfertilized NHOM soil. Biomass yield from Time 2 was highest for plants growing in the fertilized NLOM soil. All the other Time 2 soil-fertilizer treatments had statistically similar biomass yields. The high biomass production of plants grown in the NLOM soil was expected due to that soil's superior chemical

properties such as NO_3-N concentration, Olsen-P concentration, and CEC. All pots except the fertilized CLOM treatment had greater yields during Time 1 than Time 2. The difference for the fertilized CLOM soil treatment was likely due to the low biomass of plants grown in the CLOM soil during the first part of Time 1. Also, the shoot harvest of plants grown in the other three soils partway through Time 1 may have encouraged further biomass production. Within each time period, fertilized pots produced more above-ground biomass than unfertilized pots of the same soil type. This was expected because none of the soils had sufficient nutrient concentrations for optimum growth.

Trace element analysis results showed that shoot concentrations of Cd, Cr, Pb, and Zn were significantly affected by soil, time, and the soil by time interaction (Table 3.6). Soil and the soil by time interaction were significant for the concentration of As in the above-ground biomass. Soil and time were significant for the concentration of Cu in above-ground plant tissue.

Table 3.6 Soil, fertilizer, and time effects on shoot trace element concentrations for *Deschampsia caespitosa.*

Effect	Shoot Concentration ($\mu g\ g^{-1}$)					
	As	**Cd**	**Cr**	**Cu**	**Pb**	**Zn**
Soil						
NLOM	0.52	0.14	2.28	5.17	0.22	26
NHOM	0.42	0.11	2.35	6.36	0.42	105
CLOM	0.52	3.58	1.88	10.95	0.52	371
CHOM	6.58	9.21	5.14	57.40	19.30	2598
Fertilizer						
Yes	0.79	1.30	2.57	10.40	1.43	343
No	0.82	1.38	2.71	11.25	1.60	310
Time						
1	0.91	2.20	3.77	18.01a[†]	3.31	542
2	0.71	0.76	1.90	6.88b	0.57	182
	P					
Soil	<0.0001	<0.0001	0.003	<0.0001	<0.0001	<0.0001
Fertilizer	0.87	0.74	0.81	0.75	0.69	0.55
Time	0.07	<0.0001	0.0073	0.0012	<0.0001	0.0005
Soil × fertilizer	0.85	0.61	0.99	0.95	0.49	0.71
Soil × time	<0.0001	0.0002	0.003	0.49	<0.0001	<0.0001
Fertilizer × time	0.94	0.82	0.81	0.58	0.34	0.94
Soil × fertilizer × time	0.78	0.53	0.99	0.65	0.71	0.90

[†]Means in the same column followed by the same letter are not significantly different ($P > 0.05$) according to the Tukey test.

It can be seen in Figure 3.3 that As, Cd, Cr, Cu, Pb, and Zn after Time 1 were all by far the most concentrated in shoots from CHOM soil.

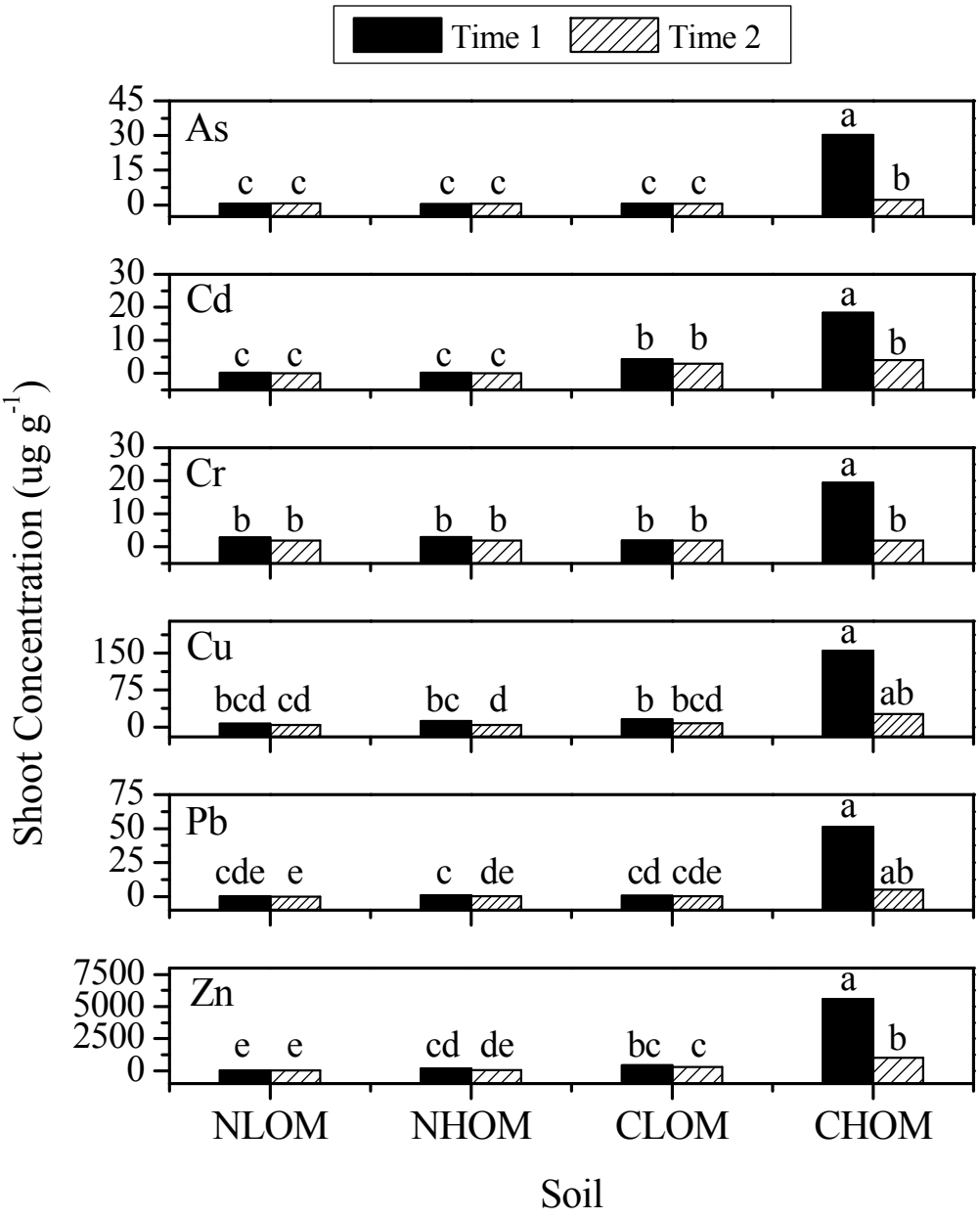

Figure 3.3 Concentrations of trace elements in above-ground plant tissue. Columns in the same panel labelled with the same letter are not significantly different ($P > 0.05$) according to the Tukey test.

At the end of Time 1, shoots from the CHOM soil contained 30 μg g^{-1} As. At the end of Time 2, the plants grown in CHOM soil still contained significantly more As than any of

the other treatments but the concentration was much lower at 2.2 μg g^{-1}. All the other treatments resulted in shoot As concentrations below 0.7 μg g^{-1}. Shoot Cd concentration in plants from the CHOM soil after Time 1 was 18 μg g^{-1}. The next highest shoot Cd concentrations ranging from 2.9-4.3 μg g^{-1} were found in shoots from the CHOM soil after Time 2 and shoots from the CLOM soil after Times 1 and 2. Cadmium concentrations in the above-ground biomass of plants grown in the non-contaminated soils were all below 0.3 μg g^{-1}. For Cr, shoot concentration was 19 μg g^{-1} for plants from the CHOM soil after Time 1. All other treatments resulted in shoot Cr concentrations less than 3.0 μg g^{-1}. Shoot Cu concentrations were 154 μg g^{-1} and 26 μg g^{-1} for plants from the CHOM soil after Times 1 and 2, respectively. All other shoot Cu concentrations were below 16.0 μg g^{-1}. Lead was present at 51 μg g^{-1} in shoots from the CHOM soil after Time 1. The next highest shoot concentration was 5.3 μg g^{-1} in shoots from the CHOM soil after Time 2. All other treatments resulted in shoot Pb concentrations below 1.0 μg g^{-1}. Shoot Zn concentration was 5580 μg g^{-1} for shoots from the CHOM soil after Time 1. After Time 2, plants from the CHOM soil had a shoot Zn concentration of 1010 μg g^{-1}. All other shoot Zn concentrations were below 450 μg g^{-1}.

It is clear that the above-ground biomass from plants grown in CHOM soil after Time 1 had much higher trace element levels than any of the other shoots. By the end of Time 2, the concentrations had dropped significantly. This may have occurred due to the most available forms of the trace element contaminants being taken up during Time 1. By Time 2, the contaminants remaining would be in forms that could not easily be removed from the soil. Only a fraction of the total trace element content of a soil is in the aqueous, easily dissolved, or easily desorbed forms which are available to plants (Salt et al. 1995*a*;

Marques et al. 2009). Trace elements strongly bound to organic matter or included in silicate mineral structures, precipitates, and insoluble compounds such as oxides and hydroxides are not considered to be phytoavailable (Salt et al. 1995*a*). In a repeated harvest phytoextraction study using *Pteris vittata* L. to remove As from contaminated soils, a similar decrease in shoot concentration was reported for the second harvest (Gonzaga et al. 2008). However, this reduction was attributed to stress caused by a drastic first harvest method which also resulted in reduced growth during the period leading up to the second harvest (Gonzaga et al. 2008). For plants growing in most of the studied soils, shoot As concentrations from the third harvest had returned to the level of those from the first harvest since *P. Vittata* is highly effective in As solubilisation (Gonzaga et al. 2008). A decrease in available forms of Cd and Zn following three phytoextraction harvests was reported by Keller and Hammer (2004) who studied the treatment of trace element-contaminated soils with *Thlaspi caerulescens* J. and C. Presl. The decrease was attributed to the inability of the soil system to replenish bioavailable pools of trace elements as quickly as they were being phytoextracted (Keller and Hammer 2004). Reduced shoot trace element concentrations for plants grown in the CHOM soil during Time 2 could have also resulted from reduced uptake due to toxicity effects or plant stress caused by the lack of winter period.

Trace element concentrations in shoots from the CLOM soil were relatively low and statistically similar to concentrations in shoots from the non-contaminated soils except in the case of Cd. It was expected that shoots from the CLOM soil would have lower trace element concentrations than shoots from the CHOM soil because soil trace element concentrations were lower in the CLOM soil.

Table 3.7 shows the BCF calculated for each trace element based on its concentrations in soil and shoot tissue.

Table 3.7 Trace element concentrations in above-ground *Deschampsia caespitosa* tissue and soil and the resulting bioconcentration factors (BCFs).

Element	Maximum Shoot Concentration (mg kg^{-1})		Soil Concentration (mg kg^{-1})		BCF	
	CLOM	CHOM	CLOM	CHOM	CLOM	CHOM
As	0.58	30	22	159	0.03	0.19
Cd	4.3	18	7.4	28.3	0.58	0.64
Cr	1.9	19	111	45	0.02	0.42
Cu	16	154	440	1590	0.04	0.10
Pb	0.78	51	67	4500	0.01	0.01
Zn	442	5580	2000	7460	0.22	0.75

For both contaminated soils, the BCFs for every trace element studied were well below one. Therefore, phytoextraction using *D. caespitosa* would require more than 100 harvests and the treatment is considered unfeasible unless trace element uptake can be improved (McGrath and Zhao 2003). Also, although *D. caespitosa* appears to tolerate the contaminants, it cannot be considered a hyperaccumulator for As, Cd, Cr, Cu, Pb, or Zn.

In general, the BCFs for plants grown in the CHOM soil are higher than the BCFs for plants grown in the CLOM soil. This may be due to greater quantities of phytoavailable trace elements in the CHOM soil which was found to contain greater total trace element concentrations. It is important to note, however, that the BCFs for plants grown in the CHOM soil decrease over time, possibly due to the preferential removal of the most phytoavailable forms of the trace elements or reduced uptake due to either contaminant toxicity or stress associated with the lack of winter period. The length of this study was

insufficient to determine whether the long-term average BCFs for plants grown in the CHOM soil would be higher than those for plants grown in the CLOM soil.

The addition of a chelating agent to the contaminated soils may improve trace element uptake. Chelating agents bind with the trace elements to form soluble complexes and decrease precipitation and sorption in the soil, thus making the trace elements more bioavailable (Marques et al. 2009). However, the increased trace element availability may also result in a greater risk of contaminant transport to groundwater by leaching, particularly on coarse-textured soils (Marques et al. 2009). Soil acidification by the addition of ammonium-containing fertilizers or soil acidifiers could also increase the phytoavailablity of the trace element contaminants (Salt et a. 1995*a*).

The Cu and Pb BCFs for plants grown in the CHOM soil in this study were comparable to those (0.13 and 0.02, respectively) found for *B. juncea* plants grown in a sandy soil contaminated with multiple trace elements (Quartacci et al. 2006). The Cd and Zn BCFs for plants grown in the CHOM soil were higher than those (0.50 and 0.45, respectively) reported by Quartacci et al. (2006). However, the addition of the chelating agent NTA resulted in the Cd, Cu, and Zn BCFs (0.88, 0.40, and 0.90, respectively) being higher for the *B. juncea* plants than for the *D. caespitosa* plants grown in the CHOM soil (Quartacci et al. 2006). Kumar et al. (1995) reported much higher Cd, Cu, Pb, and Zn BCFs for *B. juncea* than those found by Quartacci et al. (2006). They determined BCFs of 52, 7, 1.7, and 17 for Cd, Cu, Pb, and Zn, respectively. These BCFs may be elevated due to differences in the growth media used in the two studies. The growth medium used by Kumar et al. (1995) was a sand-Perlite mixture spiked with various contaminants rather than a naturally-existing soil impacted by long-term contamination used by Quartacci et

al. (2006). Therefore, the trace elements were likely much more bioavailable in the Kumar et al. (1995) study. Kumar et al. (1995) also used much lower contaminant concentrations than Quartacci et al. (2006). This may have resulted in greater BCFs due to reduced stress to the plants.

Soil, time, and the soil by time interaction effects were significant for As, Cd, Cu, Pb, and Zn uptake (Table 3.8). Fertilizer also had a significant effect on Cr uptake.

Table 3.8 Soil, fertilizer, and time effects on the uptake of trace elements by *Deschampsia caespitosa*.

Effect	Uptake (μg pot^{-1})					
	As	**Cd**	**Cr**	**Cu**	**Pb**	**Zn**
Soil						
NLOM	0.20	0.05	0.58	2.85	0.08	8.60
NHOM	0.05	0.01	0.21	1.21	0.05	12.28
CLOM	0.04	0.23	0.12	0.92	0.03	25.18
CHOM	1.33	1.06	0.40	8.75	2.39	308.98
Fertilizer						
Yes	0.26	0.20	0.37a[†]	3.16	0.25	54.70
No	0.14	0.12	0.19b	2.00	0.15	27.45
Time						
1	0.42	0.34	0.53	6.75	0.58	87.67
2	0.07	0.06	0.14	0.69	0.05	14.42
	P					
Soil	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
Fertilizer	0.14	0.15	0.04	0.28	0.27	0.08
Time	0.0003	0.001	0.002	0.0006	<0.0001	0.001
Soil × fertilizer	0.50	0.85	0.29	0.38	0.87	0.79
Soil × time	<0.0001	<0.0001	0.0001	0.003	<0.0001	<0.0001
Fertilizer × time	0.46	0.78	0.11	0.59	0.57	0.97
Soil × fertilizer × time	0.38	0.51	0.75	0.32	0.90	0.67

[†]Means in the same column followed by the same letter are not significantly different ($P > 0.05$) according to the Tukey test.

Figure 3.4 shows that the greatest trace element uptake for As, Cd, Cr, Cu, Pb, and Zn occurred in plants growing in the CHOM soil during Time 1.

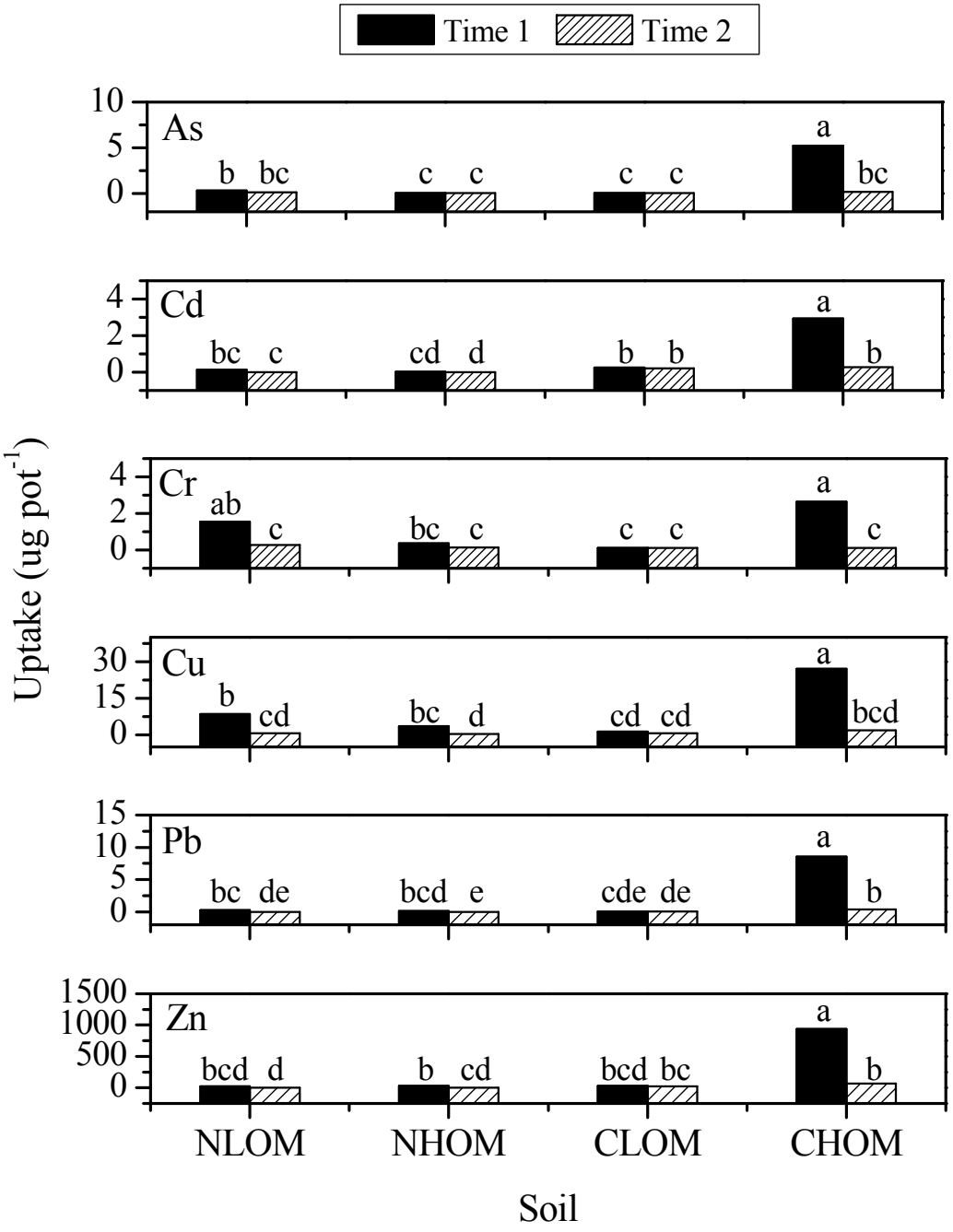

Figure 3.4 Trace element uptake by *Deschampsia caespitosa*. Columns in the same panel labelled with the same letter are not significantly different ($P > 0.05$) according to the Tukey test.

This was because that treatment resulted in much higher shoot trace element concentrations than all the other treatments. Even though above-ground biomass after Time 1 for plants grown in the CHOM soil was only moderate, the high trace element concentrations translated into the highest trace element uptake. In the case of Cr, shoot concentration in plants from the NLOM soil after Time 1 was still lower than shoot concentration in plants from the CHOM soil after Time 2, but not to such an extreme extent. Therefore, when multiplied by Time 1 biomass, which was very high for plants grown in the NLOM soil, Cr uptake was similar between the NLOM and CHOM soil treatments. Cu uptake from the NLOM soil after Time 1 was elevated for the same reason. Fertilizer application was not a significant factor in trace element uptake for any contaminant except for Cr. Cr uptake was significantly higher for fertilized treatments (0.4 μg pot^{-1}) than for unfertilized treatments (0.2 μg pot^{-1}).

Although statistically significant amounts of trace element contaminants were taken up by plants grown in the CHOM soil during Time 1, it is important to note the actual uptake values from an environmental perspective. All Time 1 trace element uptake values other than Zn were below 30 μg pot^{-1} (67 μg kg^{-1} of dry soil) for plants grown in the CHOM soil. Zn uptake was higher at about 940 μg pot^{-1}(2.1 mg kg^{-1} of dry soil), but still very low compared to soil Zn content. Therefore, from an environmental standpoint, even the Time 1 contaminant uptake by plants grown in the CHOM soil did not result in significant contaminant uptake. This means that phytoextraction by *D. caespitosa* is not a suitable short-term remediation treatment for soils contaminated with multiple trace elements. However, it does not prohibit the possibility of using phytoextraction by *D. caespitosa* for long-term clean-up efforts.

Trace element uptake by *D. caespitosa* in this study was lower than that reported for *B. juncea* by Quartacci et al. (2006). Uptake of Cd, Cu, Pb, and Zn was greater for *B. juncea* by factors of 10, 2, 5, and 2, respectively, when no chelating agent was added (Quartacci et al. 2006). The addition of NTA resulted in Cu and Zn uptake being greater for *B. juncea* by a factor of 3 (Quartacci et al. 2006). When no chelating agent was added, the greater trace element uptake by *B. juncea* can be explained by its larger above-ground biomass production compared to *D. caespitosa*. When NTA was added, increased trace element phytoavailability also played a role in the superior contaminant uptake.

Results for the change in trace element content of the contaminated soils following phytoextraction showed that soil type was the only significant factor affecting the change in soil concentrations of As, Cd, Cr, and Pb (Table 3.9). The interaction between soil type and fertilizer was significant for the change in soil Zn content. Neither soil type, fertilizer, nor the interaction was significant for the change in Cu or Se concentrations.

Table 3.9 Soil and fertilizer effects on the change in soil trace element concentrations following phytoextraction treatment.

Effect	Change in Soil Concentration (%)						
	As	**Cd**	**Cr**	**Cu**	**Pb**	**Se**	**Zn**
Soil							
CLOM	7.4a[†]	17.3a	-17.1b	1.1	39.6a	6.8	-5.8
CHOM	-27.8b	0.15b	1.6a	6.2	-37.3b	-0.49	11.6
Fertilizer							
Yes	-9.5	5.6	-11.5	4.8	-12.5	6.3	1.7
No	-10.9	11.9	-6.9	2.5	-3.7	-0.02	4.1
				P			
Soil	<0.0001	0.0003	0.046	0.60	0.012	0.49	0.11
Fertilizer	0.81	0.12	0.43	0.79	0.62	0.55	0.80
Soil × fertilizer	0.06	0.11	0.17	0.09	0.95	0.37	0.049

[†]Means in the same column followed by the same letter are not significantly different ($P > 0.05$) according to the Tukey test.

The phytoextraction treatment resulted in a much greater soil As and Pb reduction in the CHOM soil than in the CLOM soil. Since much more As and Pb were taken up by *D. caespitosa* plants grown in the CHOM soil than in the CLOM soil, it follows logically that the concentrations of As and Pb in the CHOM soil would decrease significantly more than the concentrations of As and Pb in the CLOM soil. In contrast, soil Cr decreased more in the CLOM soil than in the CHOM soil. This does not seem to agree with above-ground tissue Cr concentration data and Cr uptake data. *D. caespitosa* plants grown in the CHOM soil contained higher concentrations of Cr and had higher Cr uptake values. It is possible that *D. caespitosa* plants grown in the CLOM soil took up Cr from the soil and stored it in root tissue. However, analysis of root tissue was not within the scope of this study so this suggestion requires further research. Although the change in soil Cd

following phytoextraction was statistically different for the two contaminated soils, soil Cd concentration did not appear to decrease in either soil. The apparent increase in soil Cd in the CLOM soil is likely within the margin of error for the measurement. Soil Zn appeared to decrease by the greatest percentage in the fertilized CLOM pots and by the least in the fertilized CHOM pots. However, the differences in the change in soil Zn for the various treatments were not significant. The change in soil Cu and Se concentrations were not significantly affected by soil type and appeared to be unchanged by phytoextraction. Overall, the *D. caespitosa* phytoextraction treatment can only be considered effective in reducing soil levels of As and Pb in the CHOM soil and Cr in the CLOM soil. The soil trace element results support the shoot trace element concentration and uptake findings saying that the *D. caespitosa* phytoextraction treatment was unsuccessful in providing adequate contaminant reduction over a short time period.

3.5 Conclusion

Both contaminated soils, with low to moderate nutrient levels, provided less suitable growth conditions than the NLOM soil. Analysis of trace element concentrations in the soils indicated that the CHOM soil was more highly contaminated than the CLOM soil.

Brassica carinata, *B. juncea*, and *B. napus* all exhibited extremely stunted growth in the CHOM soil. *Deschampsia caespitosa* showed less stunted growth and was therefore considered to have greater potential for biomass production and trace element uptake in the contaminated soils.

The fertilized NLOM soil resulted in the greatest above-ground biomass production during each phytoextraction period. After Time 1, biomass from plants grown in the CHOM soil was greater than biomass from plants grown in the CLOM soil. After Time 2, the contaminated soils had similar biomass yields. The finding that *D. caespitosa* plants grown in the contaminated soils could not give the optimal biomass produced by plants grown in the fertilized NLOM soil ultimately translates into decreased phytoextraction efficiency.

Results from analyses of shoot trace element concentrations showed that As, Cd, Cr, Cu, Pb, and Zn were all much more highly concentrated in shoots grown in the CHOM soil after Time 1 than any other treatment. The large decrease in shoot trace element concentrations in plants grown in the CHOM soil from Time 1 to Time 2 can be attributed to the preferential uptake of more bioavailable contaminant forms during the earlier phytoextraction period. For all trace elements other than Cd, concentrations in shoots grown in the CLOM soil were statistically similar to concentrations found in shoots from the non-contaminated soils. This may have occurred because the CLOM soil was low in contaminants in bioavailable forms. Bioconcentration factors calculated for each trace element indicate that *D. caespitosa* is not a hyperaccumulator and is not suitable for phytoextraction use unless trace element uptake can be improved. When the relatively low biomass production of plants grown in the CHOM soil was taken into consideration, trace element uptake was still found to be greatest for plants grown in the CHOM soil after Time 1. However, the study period was much too short for adequate contaminant removal and therefore the amounts of trace elements taken up were low from an environmental standpoint. Although shoots from plants grown in the CHOM soil

did contain statistically elevated levels of As, Cd, Cr, Cu, Pb, and Zn, low biomass production meant that actual uptake values were below 30 μg pot^{-1} (67 μg kg^{-1} of dry soil) for every contaminant other than Zn (2.1 mg kg^{-1} of dry soil). Analysis of the change in soil trace element concentration following phytoextraction showed that more As and Pb were removed from the CHOM soil than from the CLOM soil. Soil Cr decreased more in the CLOM soil than in the CHOM soil. For all other trace elements, no significant decrease in soil concentration was apparent.

Overall, *D. caespitosa* plants did not take up large enough amounts of trace elements to provide adequate remediation of the contaminated soils. Therefore, the *D. caespitosa* phytoextraction treatment cannot be considered effective for the short-term (≤ 3 seasons) phytoremediation of soils contaminated by multiple trace elements. Nevertheless, by tolerating the trace elements and therefore providing a vegetative cover, *D. caespitosa* can effectively minimize or prevent the off-site migration of contaminants.

3.6 References

Canadian Council of Ministers of the Environment (CCME). 2007. Canadian soil quality guidelines for the protection of environmental and human health: Summary tables. Canadian Council of Ministers of the Environment, Winnipeg, MB.

Cox, R.M. and Hutchinson, T.C. 1979. Metal co-tolerances in the grass *Deschampsia caespitosa*. Nature 279: 231-233.

Darris, D., Lambert, S., and Young III, W. 1995. Seed production of tufted hairgrass. Plant materials technical note 16. U.S. Department of Agriculture, Natural Resources Conservation Service, Portland, OR, U.S.A.

Environment Canada. 2003. Phytorem: Potential Green Solutions for Metal Contaminated Sites. Environment Canada, Ottawa, ON.

Epstein, A.L., Gussman, C.D., Blaylock, M.J., Yermiyahu, U., Huang, J.W., Kapulnik, Y., and Orser, C.S. 1999. EDTA and Pb-EDTA accumulation in *Brassica juncea* grown in Pb-amended soil. Plant Soil 208:87-94.

Franzin, W.G., McFarlane, G.A., and Lutz, A. 1979. Atmospheric fallout in the vicinity of a base metal smelter at Flin Flon, Manitoba, Canada. Environ. Sci. Technol. 13:1513-1522.

Food and Agriculture Organization of the United Nations (FAO). 2004. *Deschampsia cespitosa*. In: Site-specific grasses and herbs. Food and Agriculture Organization of the United Nations, Rome, Italy.

Gonzaga, M.I.S., Santos, J.A.G., and Ma, L.Q. 2008. Phytoextraction by arsenic hyperaccumulator *Ptersi vittata* L. from six arsenic-contaminated soils: Repeated harvests and arsenic redistribution. Environ. Pollut. 154:212-218.

Henderson, P.J., McMartin, I., Hall, G.E., Percival, J.B., and Walker, D.A. 1998. The chemical and physical characteristics of heavy metals in humus and till in the vicinity of the base metal smelter at Flin Flon, Manitoba, Canada. Environ. Geol. 34:39-58.

Hogan, G.D. and Wotton, D.L. 1984. Pollutant distribution and effects in forests adjacent to smelters. J. Environ. Qual. 13: 377-382.

Hutchinson, T.C. and Whitby, L.M. 1974. Heavy-metal pollution in the Sudbury mining and smelting region of Canada: I. Soil and vegetation contamination by nickel, copper, and other metals. Environ. Conserv. 1:123-132.

Intrinsic Environmental Sciences Inc. 2010. The Flin Flon soils study: An assessment of exposure and human health risks in the Flin Flon area: Summary document and integration of results. Hudson Bay Mining and Smelting Co., Flin Flon, MB.

Kabata-Pendias, A. 2011. Trace elements in soils and plants. 4th ed. CRC Press, Boca Raton, FL, U.S.A.

Keller, C. and Hammer, D. 2004. Metal availability and soil toxicity after repeated croppings of *Thlaspi caerulescens* in metal contaminated soils. Environ. Pollut. 131:243-254.

Kumar, P.B.A.N., Dushenkov, V., Motto, H., and Raskin, I. 1995. Phytoextraction: The use of plants to remove heavy metals from soils. Environ. Sci. Technol. 29:1232-1238.

Manitoba Agriculture and Food (MAF). 2001. Forage crops. Field crop production guide. Revised ed. Manitoba Agriculture and Food, Winnipeg, MB.

Marques, A.P.G.C., Rangel, A.O.S.S., and Castro, P.M.L. 2009. Remediation of heavy metal contaminated soils: Phytoremediation as a potentially promising clean-up technology. Crit. Rev. Env. Sci. Tec. 39:622-654.

Marx, E.S., Hart, J., and Stevens, R.G. 1996. Soil test interpretation guide. Oregon State University, Corvallis, OR, U.S.A.

McCauley, A., Jones, C., and Jacobsen, J. 2009. Plant nutrient functions and deficiency and toxicity symptoms. Montana State University. Bozeman, MT, U.S.A.

McGrath, S.P. and Zhao, F.-J. 2003. Phytoextraction of metals and metalloids from contaminated soils. Curr. Opin. Biotech. 14: 277-282.

McMartin, I., Henderson, P.J., and Nielsen, E. 1999. Impact of a base metal smelter on the geochemistry of soils of the Flin Flon region, Manitoba and Saskatchewan. Can. J. Earth Sci. 36:141-160.

Memon, A.R. and Schroder, P. 2008. Implications of metal accumulation mechanisms to phytoremediation. Environ. Sci. Pollut. R. 6:162-175.

Meng, H., Hua, S., Shamsi, I.H., Jilani, G., Li, Y., and Jiang, L. 2008. Cadmium-induced stress on the seed germination and seedling growth of *Brassica napus* L., and its alleviation through exogenous plant growth regulators. Plant Growth Regul. 58:47-59.

Quartacci, M.F., Argilla, A., Baker, A.J.M, and Navari-Izzo, F. 2006. Phytoextraction of metals from a multiply contaminated soil by Indian mustard. Chemosphere 63:918-925.

Raskin, I., Smith, R.D., and Salt, D.E. 1997. Phytoremediation of metals: Using plants to remove pollutants from the environment. Curr. Opin. Biotech. 8:221-226.

Rauser, W.E. and Winterhalder, E.K. 1985. Evaluation of copper, nickel, and zinc tolerances in four grass species. Can. J. Bot. 63:58-63.

Salt, D.E., Blaylock, M., Kumar, N.P.B.A., Dushenkov, V., Ensley, B.D., Chet, I., and Raskin, I. 1995*a*. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Nat. Biotechnol. 13:468-474.

Salt, D.E., Prince, R.C., Pickering, I.J., and Raskin, I. 1995*b*. Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol.109: 1427-1433.

Soil Classification Working Group (SCWG). 1998. Organic order. In: The Canadian system of soil classification. 3rd ed. NRC Research Press, Ottawa, ON.

Stevenson, F.J. 1994. Organic matter reactions involving metal ions in soil. In: Humus chemistry: Genesis, composition, reactions. 2nd ed. John Wiley and Sons, New York, NY, U.S.A. p. 378-404.

Stohlgren, T.J., DeBenedetti, S.H., and Parsons, D.J. 1989. Effects of herbage removal on productivity of selected high-Sierra meadow community types. Environ. Manage. 13:485-491.

Van Nevel, L., Mertens, J., Oorts, K., and Verheyen, K. 2007. Phytoextraction of metals from soils: How far from practice? Environ. Pollut. 150:34-40.

Vassil, A.D., Kapulnik, Y., Raskin, I., and Salt, D.E. 1998. The role of EDTA in lead transport and accumulation by Indian mustard. Plant Physiol. 117:447-453.

Zoltai, S.C. 1988. Distribution of base metals in peat near a smelter at Flin Flon, Manitoba. Water Air Soil Poll. 37:217-228.

4. OVERALL SYNTHESIS

4.1 Summary of Research

Trace element contamination of the environment is a common occurrence due to industrial activities such as fossil fuel combustion, mining, manufacturing, and waste disposal (Knox et al. 1999; Hooda 2010; Kabata-Pendias 2011). At elevated concentrations, trace elements can pose serious risks to environmental and human health. These risks can be minimized through the use of various remediation methods which detoxify, immobilize, or remove contaminants from the environment (Sims and Sims 1991; Khan et al. 2004; Singh et al. 2009; Zvomuya and Murata 2012). The purpose of this thesis was to examine remediation strategies for two different trace element contamination scenarios.

Chapter 2 of this thesis focussed on the remediation of groundwater contaminated with hexavalent chromium [Cr(VI)]. Several laboratory experiments were conducted to examine a multi-stage treatment which first reduced various concentrations of aqueous Cr(VI) to Cr(III) through the addition of the reducing agent sodium dithionite ($Na_2S_2O_4$) and then immobilized the Cr by sorption on different types of synthetic iron (Fe) oxides. Addition of $Na_2S_2O_4$ to water artificially contaminated with Cr(VI) solutions resulted in a decrease in total aqueous Cr concentrations due to the formation of a Cr(III)-dominated precipitate. Subsequent addition of Fe oxides derived from ferric chloride ($FeCl_3$) or ferrous chloride ($FeCl_2$) resulted in further removal of Cr from the aqueous phase. The

ferric-derived oxide was more effective in decreasing aqueous Cr concentrations due to its greater ability to sorb Cr(VI). Treatment of Cr(VI)-contaminated water with the ferric-derived oxide alone may provide sufficient aqueous Cr removal in some situations. In scenarios where greater Cr removal is required or desired, 3-6 mM of $Na_2S_2O_4$ could be added to the groundwater as well.

Chapter 3 of this thesis focussed on the remediation of high- and low-organic matter (OM) soils contaminated with multiple trace elements, including arsenic (As), cadmium (Cd), Cr, copper (Cu), lead (Pb), selenium (Se), and zinc (Zn). Growth room experiments were conducted to examine the ability of *Deschampsia caespitosa* (L.) Beauv. to grow in and phytoextract contaminants from the soils over multiple harvests. Early growth of *D. caespitosa* was found to be less inhibited by the contaminated soils than was early growth of *Brassica juncea* (L.) Czern., a commonly studied hyperaccumulator, as well as *Brassica carinata* A. Braun, and *Brassica napus* L. Trace element analysis of above-ground biomass indicated that *D. caespitosa* plants grown in the contaminated high-OM soil contained elevated concentrations of As, Cd, Cr, Cu, Pb, and Zn. However, the trace element concentrations were low from a phytoextraction perspective. It was also determined that *D. caespitosa* plants grown in the contaminated soils could not produce the optimal above-ground biomass observed for plants grown in fertilized non-contaminated low-OM soil. Low biomass yield combined with low trace element concentrations resulted in environmentally insignificant levels of trace element uptake by plants grown in contaminated high-OM soil and statistically insignificant uptake by plants grown in contaminated low-OM soil. Only As and Pb in the contaminated high-OM soil and Cr in the contaminated low-OM soil were found to decrease due to the

phytoextraction treatment. Overall, it was concluded that *D. caespitosa* was not effective in the short-term phytoremediation of soils contaminated with multiple trace elements.

Results from both chapters highlight the importance of contaminant and environmental characteristics in the development and implementation of trace element remediation strategies. Consideration should be given to both the nature and number of contaminants. For trace element contaminants such as Cr, whose behaviour is linked to speciation, highly effective remediation methods which include redox reactions can be developed. For situations involving multiple trace element contaminants, remediation techniques proven effective for single-contaminant sites may not be viable. Environmental conditions must also be considered when developing remediation strategies. For example, Cr precipitation in the Cr(VI) remediation study was dependent upon the pH of the system. Also, alteration of soil conditions, including the addition of chelating agents or soil acidifiers, may help improve the phytoextraction ability of *D. caespitosa*.

4.2 Practical Implications

Results of this thesis research can be utilized in the development of trace element remediation techniques for soil systems. The reduction-sorption treatment of Cr(VI)-contaminated groundwater studied in Chapter 2 was deemed effective and could be applied as an in situ clean-up method following successful field-scale testing. This remediation strategy would involve the construction of injection wells or a permeable reactive barrier to allow for the addition of Fe oxide to the contaminated system (Blowes et al. 1997; Blowes et al. 2000; Khan and Puls 2003). In some cases, Cr(VI) sorption by the Fe oxide would provide sufficient Cr remediation. In scenarios which call for higher

Cr removal rates, $Na_2S_2O_4$ could also be added to the contaminated plume through the injection well. Pumping wells could be included to increase the flow of groundwater through the treatment zone (Blowes et al. 1997).

The *D. caespitosa* phytoextraction treatment studied in Chapter 3 was not successful on a short time scale but still has valuable information to contribute regarding the development of phytoremediation strategies for soils contaminated with complex mixtures of trace elements. Results showed that *B. juncea*, a known hyperaccumulator of several individual contaminants, grew extremely poorly in the heavily contaminated high-OM soil. Therefore, although many previous studies which only examined one or two contaminants report excellent phytoextraction by *B. juncea*, the species may not be suitable for soils containing several trace elements (Salt et al. 1995, Vassil et al. 1998, Epstein et al. 1999). Since such soils are very common, alternative phytoremediation species must be considered. This study showed that *D. caespitosa* grew much better than *B. juncea* in the contaminated high-OM soil. However, during the timeframe tested in this research, *D. caespitosa* performed poorly in terms of trace element accumulation. There is opportunity to improve the phytoextraction capability of *D. caespitosa*, however, whether it be by the addition of chelating agents to increase trace element bioavailability or by the selection or development of varieties which are better adapted for contaminant uptake (Salt et a. 1995*a*; Marques et al. 2009). Regardless of trace element uptake, *D. caespitosa* could be seeded on highly contaminated land to effect phytostabilization. The ability of the plants to grow in the soils used in this study indicate that it could be useful in providing a vegetative cover to prevent wind and water erosion of soils containing multiple trace elements (McGrath and Zhao 2003; Marques et al. 2009).

4.3 Recommendations for Further Study

The research described in this thesis raises further scientific questions and provides openings for additional research regarding the remediation of trace element contamination. In Chapter 2, differences in the sorptive ability of the two synthetic Fe oxides were reported but the cause of said differences remains unknown. Mineralogical analysis of the Fe oxides would provide information on structural and compositional differences. This information would be useful for explaining the observed sorptive differences but also for developing methods of preparing even more effective Fe oxides.

In this study, Cr was removed from solution either as insoluble Cr(III) precipitates or as sorbed Cr(VI). In a similar study, Cr was also removed as sorbed Cr(III) (Murata 2010). Trivalent chromium is known to be more easily sorbed than Cr(VI) but it would be useful to conduct a sequential extraction study to quantitatively determine the ease with which immobilized Cr can return to the aqueous phase (Bartlett and James 1996; Kabata-Pendias 2011).

The phytoextraction study conducted as part of this thesis focussed mainly on the trace element of above-ground biomass. A study on the trace element content of the plant roots is currently underway. Results from this study will provide useful information on the trace element distribution within *D. caespitosa* plants. It could also explain why a decrease in soil Cr concentration was observed for the low-OM contaminated soil even though no significant uptake into shoot tissue was found.

It would also be useful to conduct a sequential extraction study to determine the bioavailability of the trace elements in the contaminated soils over time during phytoextraction treatment. Results could explain the difference in trace element uptake from two contaminated soils and the significant decrease in trace element concentrations over time seen in the above-ground tissue of plants grown in the high-OM contaminated soil.

Since bioconcentration of trace elements by *D. caespitosa* was very low, research could be conducted to study the effect of different chelating agents and soil acidifiers on trace element bioavailability and uptake. Such a study should also include research on the effect of the amendments on trace element leaching. If phytoextraction agents are found to be capable of increasing trace element transport into the shoot tissue of *D. caespitosa* plants without causing excessive leaching risks, then phytoextraction using *D. caespitosa* could become a viable remediation technique.

4.4 References

Bartlett, R.J. and James, B.R. 1996. Chromium. In: Sparks, D.L., editor, Methods of soil analysis. 1st ed. SSSA and ASA, Madison, WI, U.S.A. p. 683-702.

Bartlett, R.J. and Kimble, J.M. 1976. Behaviour of chromium in soils: I. Trivalent forms. J. Environ. Qual. 5:379-382.

Blowes, D.W., Ptacek, C.J., and Jambor, J.L. 1997. In-situ remediation of Cr(VI)-contaminated groundwater using permeable reactive walls: Laboratory studies. Environ. Sci. Technol. 31:3348-3357.

Epstein, A.L., Gussman, C.D., Blaylock, M.J., Yermiyahu, U., Huang, J.W., Kapulnik, Y., and Orser, C.S. 1999. EDTA and Pb-EDTA accumulation in *Brassica juncea* grown in Pb-amended soil. Plant Soil 208:87-94.

Hooda, P.S., editor. 2010. Trace elements in soils. John Wiley and Sons, Chichester, U.K.

Kabata-Pendias, A. 2011. Trace elements in soils and plants. 4th ed. CRC Press, Boca Raton, U.S.A.

Khan, F.A. and Puls, R.W. 2003. In situ abiotic detoxification and immobilization of hexavalent chromium. Ground Water Monit. R. 23:77-84.

Khan, F.I., Husain, T., and Hejazi, R. 2004. An overview and analysis of site remediation technologies. J. Env. Mgmt. 71:95-122.

Knox, A.S., Gamerdinger, A.P., Adriano, D.C., Kolka, R.K., and Kaplan, D.I. 1999. Sources and practices contributing to soil contamination. In: Adriano, D.C., Bollag, J.M., Frankenberger, Jr., W.T., and Sims, R.C., editors, Bioremediation of contaminated soils. Agron. monogr. 37. ASA, Madison, WI, U.S.A. p. 53-87.

McGrath, S.P. and Zhao, F.-J. 2003. Phytoextraction of metals and metalloids from contaminated soils. Curr. Opin. Biotech. 14: 277-282.

Marques, A.P.G.C., Rangel, A.O.S.S., and Castro, P.M.L. 2009. Remediation of heavy metal contaminated soils: Phytoremediation as a potentially promising clean-up technology. Crit. Rev. Env. Sci. Tec. 39:622-654.

Salt, D.E., Blaylock, M., Kumar, N.P.B.A., Dushenkov, V., Ensley, B.D., Chet, I., and Raskin, I. 1995*a*. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Nat. Biotechnol. 13:468-474.

Salt, D.E., Prince, R.C., Pickering, I.J., and Raskin, I. 1995*b*. Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol.109: 1427-1433.

Sims, R.C. and Sims J.L. 1991. Remediation techniques for contaminated soils. In: Site characterization for subsurface remediation. Seminar publication EPA/625/4-91/026. U.S. Environmental Protection Agency, Washington, DC, U.S.A. p. 215-241.

Singh, A., Kuhad, R.C., and Ward, O.P. 2009. Biological remediation of soil: An overview of global market and available technologies. In: Singh, A., Kuhad, R.C., and Ward, O.P., editors, Advances in applied bioremediation. Soil biology 17. Springer, Dordrecht, Netherlands. p. 1-20.

Vassil, A.D., Kapulnik, Y., Raskin, I., and Salt, D.E. 1998. The role of EDTA in lead transport and accumulation by Indian mustard. Plant Physiol. 117:447-453.

Zvomuya, F. and Murata, A.P. 2012. Soil contamination and remediation. In: El-Shaarawi, A.H. and Piegorsch, W.W., editors, Encyclopedia of environmetrics. 2nd ed. John Wiley and Sons, Chichester, U.K. (in press).

APPENDICES

I. Qualitative Description of Iron Oxide Synthesis

The ferric chloride ($FeCl_3$) began as an orange powder and remained orange when made into a 0.05 M solution. When titrated with 0.5 M sodium hydroxide (NaOH), a clear liquid, the solution turned slightly darker where the NaOH droplets hit. As more NaOH was added, the sample gradually became more red coloured and eventually turned dark reddish brown. The ferrous chloride ($FeCl_2$) began as a yellowish orange powder with a tinge of green. Once mixed into a 0.05 M solution, it was orange in colour. When titrated with NaOH, the solution turned greenish brown where the drops of NaOH landed. As more NaOH was added, the sample progressed from orange through to reddish orange and brown before becoming a very dark greenish black colour.

II. Qualitative Description of Hexavalent Chromium Reduction

The potassium chromate (K_2CrO_4) began as a bright yellow solution. The addition of hydrochloric acid (HCl) caused the solution to become slightly darker. As the amount of sodium dithionite ($Na_2S_2O_4$) added increased, the solution turned increasingly green. After the 24-hour reaction period, a precipitate was observed in all the treatments where $Na_2S_2O_4$ was added. The colour of the precipitate differed with the amount of $Na_2S_2O_4$

added. At lower concentrations of $Na_2S_2O_4$, the precipitate appeared yellowish brown. At higher concentrations of $Na_2S_2O_4$, the precipitate appeared green.