REDUCTION AND BIOAVAILABILITY OF CHROMIUM IN SOILS

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LIST OF PUBLICATIONS

Journal papers

1. Girish Choppala, Bolan NS, Megharaj M, Chen Z and Naidu R. (2011). The influence of biochar and black carbon on reduction and bioavailability of chromate in soils. Journal of Environmental Quality (Published; doi:10.2134/jeq2011.0145).
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5. Jin Hee Park, Dane Lamb, Periyasamy Panneerselvam, Girish Choppala, Nanthi Bolan (2010). Role of organic amendments on enhanced bioremediation of heavy metal (loid) contaminated soils. Journal of Hazardous Materials. 185: 549-574.
6. Dane Lamb, Kartik Venkatraman, Nanthi Bolan, Girish Choppala, Nanjappa Ashwath, Ravi Naidu. (2011). Phytocapping: an alternative technology for the sustainable management of landfill sites. Critical Reviews in Environmental Science and Technology (Review proposal accepted, manuscript submitted).
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Prospective papers

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Conference proceedings

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ABSTRACT

Improper disposal of industrial wastes including effluents often leads to contamination of both water and soil, and therefore posing a danger to flora and fauna. Of all heavy metals, chromium (Cr) is being used by many industries including petroleum refining, chrome tanning, timber treatment and cooling towers at different stages of their manufacturing. Chromium is a redox active heavy metal that exists in several oxidation states, of which hexavalent [Cr(VI)] and trivalent [Cr(III)] species are reported to be prevalent in the environment. Chromium(VI) is highly mobile, toxic, mutagenic and teratogenic, whereas Cr(III) is relatively immobile and is an essential dietary micronutrient for humans. Therefore, mitigation of toxicity of Cr(VI) in contaminated soil is of prime importance so that environmental pollution due to Cr is reduced.

Diverse mechanisms such as immobilization through adsorption and reduction by abiotic and biotic pathways have been proposed to decrease toxicity of Cr in soils. A remediation strategy through reduction of soluble, mobile and toxic form of Cr(VI) to insoluble and less toxic Cr(III), appears to be the most practical method in soils.

This study attempted to mitigate Cr toxicity in soils by reducing Cr(VI) to Cr(III) and its subsequent immobilization in contaminated soils. The specific objectives were to: (i) quantify simultaneously both Cr(III) and Cr(VI) species using different complexing agents on lC-ICP-MS; (ii) examine sorption and mobility of Cr species in a range of soils; (iii) elucidate the influence of amendments on the relative retention of Cr(VI) and Cr(III) in soils; (iv) examine the effect of organic carbon sources on reducing Cr(VI) and concurrent immobilization of Cr(III); and (v) estimate the impact of various organic carbon sources on the phytoavailability of Cr(VI) in contaminated soil.

In the initial phase, a method was developed for simultaneous quantification of Cr(III) and Cr(VI) by IC-ICP-MS using different ligands, where cationic Cr(III) was converted to anionic complex. Of the ligands tested, Nitrilo triacetic acid trisodium salt (NTA 3 Na^{+}) and 2-hydroxyethyl ethylene diamine triacetic acid (HEDTA) achieved high UV response and high selectivity for Cr(III). Based on these results, HEDTA was used to complex Cr(III) and analyse Cr species in soil solution and wastewater sources.

The sorption and mobility of Cr species as affected by soil properties were examined using batch experiments. The adsorption data were fitted to Freundlich and Langmuir

isotherm equations. Results indicated that adsorption of Cr(III) was higher than Cr(VI) in all soils tested. The adsorption of Cr(III) increased when pH and CEC levels also increased. These parameters had no significant effect on Cr(VI) adsorption, whereas the amount of Fe (crystalline) and positive charge increased Cr(VI) adsorption. The mobility of Cr(VI) was substantially higher than Cr(III) in soils.

The influence of lime, iron (III) oxide, elemental sulfur and cow manure amendments on the adsorption of Cr(III) and Cr(VI) was examined. These soil amendments affected the retention of Cr(III) and Cr(VI) in soils. Adding lime increased the adsorption of Cr(III), while elemental sulfur and cow manure increased the adsorption of Cr(VI). This increase in adsorption was attributed to change in pH and surface charge of soils. Cow manure also reduced Cr(VI) to Cr(III) and this process enhanced the subsequent retention of Cr(III).

The chemodynamics of Cr(VI) reduction in the presence of carbon sources such as black carbon, biochar and cow manure was examined. The results indicated that reduction of Cr(VI) was higher in acidic soils than alkaline soils. Adding black carbon and biochar to alkaline soils increased the reduction of Cr(VI). Black carbon emerged as being more effective in reducing Cr(VI) than biochar and cow manure, which was attributed to the difference in pH and functional groups in these amendments.

Finally, the phytoavailability of Cr(VI) in the presence of black carbon and biochar was examined using a glasshouse experiment. The results demonstrated that Cr(VI) decreased plant biomass production. Most of the Cr(VI) accumulated at roots and again, applying biochar and black carbon reduced Cr(VI) to Cr(III). Consequently the level of phytotoxicity in soils decreased.

In conclusion, the mobility and bioavailability of Cr(VI) was higher than Cr(III) in most of the soils tested. However, applying organic amendments such as black carbon and biochar proved to be effective in the mitigation of mobility and phytotoxicity of Cr(VI) in contaminated soils.

DECLARATION

I declare that:

This thesis presents work carried out by myself and does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university. To the best of my knowledge it does not contain any materials previously published or written by another person except where due reference is made in the text; and all substantive contributions by others to the work presented, including jointly authored publications, are clearly acknowledged.

Girish Choppala

Signed _____ Date _____

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Chapter 1

GENERAL INTRODUCTION

1.1 Legacy of chromium toxicity

Since the Industrial Revolution, large volumes of contaminants have been released into the environment. Many industries use heavy metals in their processes. Contamination of water and soil by heavy metals is inevitable. Such metals persist for a long time and has a detrimental effect on the environment. Chromium (Cr) is one such toxic heavy metal, released by both Pedogenic and anthropogenic sources. Industries such as electroplating, steel, leather tanning, timber treatment, cooling towers, dye production, and anodising of aluminium utilize Cr. The Toxic Release Inventory of USEPA [United States Environmental Protection Agency] revealed that nearly 52,600 Mg of Cr is released annually into the environment (TRI01, 2003). Around 157,290 hectares of land is under winery cultivation (ABS, 2009) and approximately 3400 Mg Cr(VI) is present in Australian vineyard soils.

Chromium exists in various oxidation states, from -2 to +6. However, only Cr(III) and Cr(VI) are prevalent in the environment. Chromium(VI) is a strong oxidiser, present as a tetrahedral oxoanion in aqueous solutions and is highly soluble, mobile in groundwater, and meta-stable in soils. Chromium(III) bonds to six oxygens in an octahedral complex, is relatively insoluble, immobile and less toxic (Brown et al., 1999). Chromium(VI) is carcinogenic, mutagenic, and teratogenic (De Flora, 2000). In contrast, Cr(III) is an essential dietary nutrient for humans in minute quantities (50-200 µg/day). Regarding Cr(VI), the maximum permissible limit is only 0.05 mg/L (WHO, 1996) while the threshold level for Cr(III) in drinking water and waste water is 5 and 170 mg/L, respectively (Acar and Malkoc, 2004; USEPA,1976). Hence, it is of great importance to reduce the impact of Cr toxicity on the environment.

1.2 Measurement of species of chromium

The toxicity of Cr is species-specific, so the quantification of total Cr concentration in environmental samples may not be sufficient to estimate the distribution of various Cr species. Detailed information about oxidation states is essential to understand the toxicity of Cr in the environment. Therefore the quantification of various species of Cr [Cr(VI) and

Cr(III)] separately, rather than as total Cr is important. Demands have increased for analytical techniques that accurately estimate metal speciation and many techniques including ion chromatography connected to inductively coupled plasma-mass spectroscopy (ICP-MS) are currently available. Total Cr can be estimated by ICP-OES or ICP-MS, Cr(VI) can be measured using 1, 5-diphenyl carbazide on spectrophotometry; concentration of Cr(III) is calculated indirectly by subtracting Cr(VI) from total Cr. However, the simultaneous determination of Cr(VI) and Cr(III) using a single chromatography column is difficult because Cr(III) is in a cationic form, whereas Cr(VI) exists as an anion.

The best possible approach involves a derivatization process, whereby Cr(III) is complexed with a ligand to form an anionic complex, which can be simultaneously separated from Cr(VI) by anion-exchange chromatography (Tomlinson et al., 1994; Byrdy et al., 1995; Gurleyuk and Wallschlager, 2001). Aminopolycarboxylic acids such as 1, 2-cyclohexanediamine tetracetic acid (CDTA), ethylene diamine tetracetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA) were used to convert Cr(III) into anionic complexes (Semenova et al., 1996; Jung et al., 1997; Himeno et al., 1998).

1.3 Adsorption of chromium species in soil

Bioavailability of heavy metals in soils is controlled mostly by adsorption-desorption reactions at the mineral-water interface and adsorption results in the removal of metals from soil solution (McBride, 1994). The study of adsorption of Cr(VI) and Cr(III) is important to understand the toxicity and bioavailability of these two species in soil. Adsorption of Cr species depends on soil properties, which in turn are influenced by soil constituents such as organic matter (OM), Fe and Al oxides, clay minerals (Vega et al., 2006). Organic matter, clays and sesquioxides that are components of soil are known to adsorb Cr (Davis and Leckie, 1980; Zachara et al., 1989). While Cr(III) is readily retained by soils, Cr(VI) retention occurs only in soils that are rich in positive charge. Chromium(III) precipitates as $Cr(OH)_3$ or chelated with organic compounds and readily adsorbs to mineral surfaces in most soils (Wittbrodt and Palmer, 1996).

Mobility of Cr(VI) in natural environments is mostly controlled by sorption onto metal oxide surfaces; involving surface complexation reactions in which the oxygen moiety of Cr(VI) displaces hydroxyl groups on the metal oxide. Chromium(VI) oxyanion species have high mobility in soils due to predominantly negatively charged soil particles, so electrostatic repulsion eliminates Cr(VI) from adsorption (Hsu et al., 2009). Hence, the detoxification of

Cr(VI) through reduction to Cr(III) and its subsequent adsorption is considered to be an effective method for remediating Cr contaminated soils.

Chromium(VI) forms a weak outer sphere surface complex on iron(III) [Fe(III)] oxides and subsurface soils (Qafoku et al., 2009). Chromium(VI) mobility is controlled mainly by both electrostatic and specific adsorption mechanisms. The release of hydroxyl ions, confirmed by an increase in pH is attributed to the exchange of Cr(VI) with hydroxyl ions and forms a weak bond with surface of soil. The Cr(VI) adsorption increases negative surface charge and decreases surface potential of soils (Jiang et al., 2008). Iron(III) oxide, which is high in positive charge is likely to control Cr(VI) adsorption in soils and their other soil properties such as pH, CEC, clay, organic matter exert less influence.

In contrast Cr(III) adsorbs readily to soil colloids by forming electrostatic bonds and subsequently precipitates with hydroxyl ions or gradually dehydrates and crystallizes as Cr_2O_3 (Brose and James, 2010). The pH of soil, cation exchange capacity (CEC), and clay greatly influence Cr(III) adsorption. The abundance of soil negative charge retains cationic Cr(III), and increases the adsorption.

1.4 Reduction of Cr(VI) in soils

The removal of Cr(VI) by adding biomass derived from plants, fungi, bacteria, yeast as bio-sorbents for Cr(VI) have increased due to cost-effective remediation technique (Nourbakhsh, 1994; Ucun et al., 2002; Ozdemir et al., 2003). However, the mobility, toxicity and bioavailability of Cr depend on its oxidation state, so the best remediation strategy to mitigate Cr contamination is to reduce soluble, mobile and toxic Cr(VI) to insoluble and less toxic Cr(III) (James, 2001). Soil pH (i.e. supply of protons), Cr(VI) adsorption (i.e. free Cr(VI) species) and organic matter (i.e. supply of electrons) are the primary factors influencing the Cr redox reactions (Bartlett, 1991; Banks et al., 2006). Chromium reduction occurs readily in the presence of organic matter including manures composts, humic acids and fulvic acids (Losi et al., 1994; Wittbrodt and Palmer, 1995; Wittbrodt and Palmer, 1996; Bolan et al., 2003). Other properties that induce Cr(VI) reduction soil and water include Fe(II) bearing minerals and sulfide compounds (Fendorf and Li, 1996; Patterson et al., 1997). To reduce Cr(VI) in soils, the availability of protons and electrons are important (Eqn. 1.1).

$$CrO_4^{2-} + 7H^+ + 3e^- \rightarrow Cr^{3+} + 4H_20 \qquad (1.1)$$

Although the impact of soil pH and organic amendments on reducing Cr(VI) has been examined (Bolan et al., 2003; Graham and Bouwer, 2010), no comprehensive study has examined the effect of three important rate limiting factors (i.e. supply of free Cr(VI) ions, protons and electrons) on Cr(VI). Therefore, the effect of adsorption of Cr(VI), protons (pH) and electrons (organic amendments) on Cr(VI) reduction in both acidic and alkaline soils are examined in this study.

1.5 Objectives of the research

The overall objective of this research is to investigate the potential value of organic amendments on the reduction of Cr(VI) to Cr(III) and its subsequent immobilization in contaminated soils. The specific objectives of this research include:

- Examine the efficiency of complexation of Cr species with different ligands and their separation using IC-ICP-MS.
- Analyse the physico-chemical properties of wide range of soils across Australia and to evaluate the effect of soil properties on the sorption of Cr(III) and Cr(VI).
- Study the influence of inorganic amendments on the immobilization of Cr species in soil.
- Understand the effect of organic amendments on the reduction of Cr(VI) in soil.
- Investigate the toxicity and bioavailability of Cr(III) and Cr(VI) as influenced by amendments.

1.6 Thesis structure

Chapter 1 *Introduction* gives an overall outline of contamination, speciation, toxicity, sorption of Cr(III) and Cr(VI), and reduction of Cr(VI) in soil. This chapter lists the research objectives and how thesis is structured.

Chapter 2 *Literature review* elucidates the relevant studies on sources and contamination of Cr, adsorption-desorption and redox reactions in soil and water, and factors that affect mobility and bioavailability. This chapter also discusses various treatment methodologies used to mitigate Cr toxicity in contaminated soils.

Chapter 3 *Simultaneous determination of chromium species by ion chromatography coupled with inductively coupled plasma mass spectrometry* analyses different methods used to quantify Cr species in soil and describes the method developed for simultaneous determination of Cr species in soil solutions using different complexing agents. Evaluation

of the developed method in the simultaneous measurement of Cr species in contaminated soil pore water and some waste water effluents is also discussed.

Chapter 4 *Relative sorption and mobility of chromium species in a range of soil types* evaluates the influence of several soil properties on the sorption of Cr species in a wide range of Australian soils. In addition, the desorption, mobility and leaching of Cr(VI) in soils of different pH are also reported.

Chapter 5 *Influence of amendments on the relative sorption of chromium species in soils* evaluates the impact of inorganic amendments such as lime, elemental sulphur, red mud [Fe(III) oxide] and cow manure on the sorption of Cr(III) and Cr(VI) in contaminated soils. These amendments have the potential to manipulate the pH, surface charge and electron dynamics, thereby influencing the sorption and mobility of Cr in soils.

Chapter 6 *Chemo-dynamics of Cr(VI) reduction in soils: implications to remediation* discuss the effect of supply of Cr(VI) species (i.e. Cr(VI) adsorption), protons (i.e. pH) and electrons (i.e. organic amendments) on Cr(VI) reduction. The influence of pH as affected by lime and elemental sulphur [S(O)] application to reduce Cr(VI) is discussed. Moreover, the potential of black carbon, biochar and cow manure to decrease Cr(VI) is reported in this chapter.

Chapter 7 *Impact of diverse organic carbon sources on phytoavailability of chromium(VI) in soils* investigates the effect of toxicity of Cr(VI) on plant biomass production, distribution of Cr(VI) in shoots, roots and relative water content. This chapter reports the decreased toxicity of Cr(VI) when organic amendments are applied.

Chapter 8 *Summary and conclusions* provides overall research conclusions and a summary of the major research findings including a conceptual diagram. This chapter suggests possible future directions in research with advanced techniques such as synchrotron-based speciation studies.

Chapter 2

LITERATURE REVIEW

2.1 Introduction

Chromium (Cr), which was first discovered by the French chemist Louis Nicolas Vauquelin in 1797, is the 21st most abundant element (about 122 mg/kg) and sixth most abundant transition metal in the earth's crust. Chromium belongs to Group VI-B in the periodic table with the atomic number 24, molecular weight of 52 and has a high melting point (1857^{0}C) (Adriano, 1986).

Chromium is a redox active metal and exhibits a wide range of oxidation states, from -2 to +6. However, Cr(III) and Cr(VI) are predominant in the environment. Chromium(III) exists as mononuclear [$CrOH^{2+}$, $Cr(OH)_2^{+}$, $Cr(OH)_4^{-}$], neutral [$Cr(OH)_3$] and poly-nuclear [$Cr_2(OH)_2$, $Cr_3(OH)_4^{5+}$] species. Chromium(VI) exists as chromate (CrO_4^{2-}), bichromate ($HCrO_4^{-}$) and dichromate ($Cr_2O_7^{2-}$). Chromium(VI) species (CrO_4^{2-}) at high concentrations (>520 mg/L) undergoes dimerization and forms dichromate ($Cr_2O_7^{2-}$) (Losi et al., 1994).

The main source of Cr is chromite ore [Fe, Mg)O(Cr, Al, Fe)$_2$O$_3$] (Adriano, 1986). Most of the chromium (80%) produced is used in metallurgical applications (Barnhart, 1997). Since the Industrial Revolution, the utilization of Cr has increased and is used in many industries (Kuo and Bembenek, 2008). Improper disposal of the effluents from these industries leads to the contamination of terrestrial environments and often mixes with surrounding water bodies and is detrimental to flora and fauna (Avudainayagam et al., 2003; López-Luna et al., 2009).

Chromium(III) is considered to be a hard Lewis acid and tends to form strong octahedral coordination complexes with oxygen and donor ligands (Mohan and Pittman, 2006). Chromium(III) is less mobile and insoluble, an essential dietary nutrient (5-200 µg/day) and regulates glucose metabolism in humans (Lukaski, 1999). In contrast, Cr(VI) is readily soluble, highly mobile and is classified as carcinogenic, mutagenic and teratogenic (Fendorf, 1995). The threshold level for Cr(III) in waste water is 5 mg/L (Acar and Malkoc, 2004), total Cr in drinking and ground water is 0.1 mg/L but for Cr(VI), the maximum permissible limit is only 0.05 mg/L (WHO, 1996).

In this chapter, the sources and reactions of Cr in the environment, including adsorption-desorption, redox reactions in soils are discussed. The remediation strategies such as abiotic and biotic reduction, immobilization and phytoremediation of Cr are also reported.

2.2 Sources of Chromium

Naturally and anthropogenically derived Cr can present in various environmental media including surface and groundwater, seawater, soil and sediments, rocks and air.

2.2.1 Natural Sources

The natural source of Cr is derived mainly from soils and sediments and the concentration of Cr depends on the nature of parent material. It has been reported that the average Cr concentration in the continental crust is 125 mg/kg, commonly ranging between 80-200 mg/kg (NAS, 1974; Oze et al., 2007). Chromium levels average from 20-35 mg/kg in granitic igneous rock, limestone, and sandstones to 220 mg/kg in basaltic igneous rock and 205-15,300 mg/kg in ultramafic rocks (Sumner and Naidu, 1997). Surface soils of gabbroic origin in Scotland contain nearly 100 mg/kg Cr, whereas serpentine soils contain approximately 49,000 mg/kg in surface soils (Naidu et al., 2000). Chromium levels vary within the atmosphere. Most Chromium in the atmosphere derives from windblown dust, forest fires, meteoric dust, sea salt spray or particles and volcanic eruptions and ranges from 0.015 to 0.03 $\mu g/m^3$ (Nriagu et al., 1988).

2.2.2 Anthropogenic sources

Soil and groundwater can become contaminated with Cr and other metals from the waste sludge disposal of industries (Figure 2.1). The total annual input of Cr into soils has been estimated to be between 4.35×10^5 and 1.18×10^6 metric tons, and by 2000 Cr production was estimated around 105.4 million tons (Nriagu and Pacyna, 1988; Han et al., 2004). Chromium in food is mainly in the Cr(III) form and can be found in such products as brewer's yeast, calf liver, cheese, and wheat germ. Phosphate fertilizers are also a significant source of Cr(III) input to soils (McGrath and Smith, 1990). Chromic Acid (H_2CrO_4) is used as a fungicide and insecticide. It is used for wood and lumber protection treatments and sodium dichromate ($Na_2Cr_2O_7$) is registered as an insecticide used to prevent termite damage to wood products.

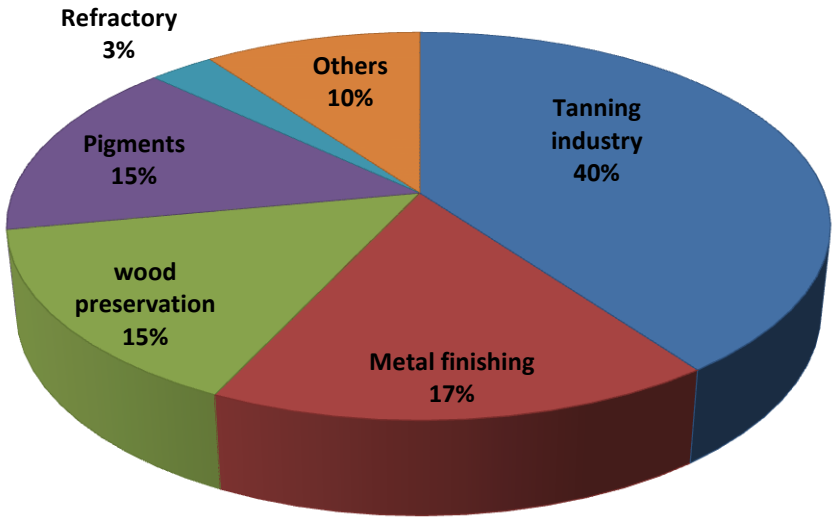


Figure 2.1 Consumption of Chromium in different industries (Kamaludeen et al., 2003).

Chromium has become one of the major soil contaminants, derived mainly from the disposal of tannery and treatment wastes, and Cr ore processing residue (COPR), metal plating, anodizing, ink manufacture, dyes, pigments, glass, ceramics, glues, tanning, wood preservatives, textiles, and corrosion inhibitors in cooling water (McGrath and Smith, 1990; Meegoda et al., 1999; Tarcan et al., 2010). Both Cr(III) and Cr(VI) may occur in wastewaters derived from these sources. Past practice, notably in plating operations (where Cr(VI) is predominant) was to dispose of Cr-wastewater into dry well.

The largest amount of Cr is applied directly to land surface by the disposal of trapped and bottom fly ash derived from coal fired power stations and also iron and steel industry is the largest anthropogenic source of Cr emissions worldwide (Nriagu and Pacyna, 1988).The disposal of large quantities of this ash on soil can result in elevated Cr concentrations relative to natural background levels, notably around coal-fired power plants. However, little Cr is taken up by vegetation due to the precipitation as $Cr(OH)_3$ (Furr et al., 1976; Wu et al., 2008).

Chromium(VI) also emitted from oil combustion, sewer sludge incineration, cement production, municipal waste incinerations and refractories. Chromium electroplating operations are conventionally conducted in baths containing chromic acid. During the plating process, bubbles of gas are emitted through the surface of the bath. These bubbles carry entrained Cr(VI) into the air (Kuo and Wang, 2002). Firebrick lining of glass furnaces is another source of Cr(VI) emissions. Lead chromate ($PbCrO_4$), a pigment used in yellow paint, for instance traffic lane demarcations, is a minor source of Cr (VI) (Jacobs

and Testa, 2005). In sea water, Cr is estimated at a concentration of 0.6 µg/L. Uncontaminated surface water Cr concentrations are estimated as 1 µg/L, and varies with the mineralogy of the soil. Immobilization of Cr occurs by precipitation into solid phases as a result of changes in the pH or oxidation state.

2.3 Properties of Chromium

Chromium is a silvery, lustrous malleable metal and has a high resistance to oxidizing acids, so it is used in anti-corrosive alloys. Chromium has four stable isotopes - ^{50}Cr, ^{52}Cr, ^{53}Cr, and ^{54}Cr – but only ^{52}Cr has high abundance, up to 83.76% (Adriano, 1986). The pure metallic form rarely occurs naturally. Chromium does not evaporate, but it can be present in air as particles.

2.3.1 Oxidation states

Chromium exists in different oxidation states, ranging from 2^- to 6^+. However, only 3^+ and 6^+ are prevalent in the natural environment. Cr(III) is thermodynamically more stable than Cr(VI) (Kota and Stasicka, 2000). The oxidation states of Cr when below three are reducing and above three are oxidizing (Adriano, 1986).

2.3.2 Speciation of chromium in soils

Hexavalent chromium is a strong oxidizing agent and exists only in oxygenated species that are very stable and pH dependent (Nieboer and Jusys, 1988):

$$H_2CrO_4 \Leftrightarrow H^+ + HCrO_4^- \ (K_{a1}=10^{0.6}) \quad (2.1)$$

$$HCrO_4^- \Leftrightarrow H^+ + CrO_4^{2-} \ (K_{a2}=0^{-5.9}) \quad (2.2)$$

where K is the dissociation constant. From the above reactions we observe that at very low pH values (near 0), H_2CrO_4 is the dominant species, while between the pH values of 0 and 5.9, $HCrO_4^-$dominates and, at or above pH 6.0, CrO_4^{2-}prevails (Eqns. 2.1, 2. 2; Figure 2.2). Since the pH in environmental matrices would generally not be expected to fall near zero, only $HCrO_4^-$and Cro_4^{2-}should be present in natural systems.

Trivalent chromium [Cr(III)] is a more stable form (NAS, 1974; Cary, 1982), and its chemistry is more complex than that of hexavalent Cr [Cr(VI)]. Because it has relatively less affinity for 0_2, Cr(III) is known to form numerous complexes, with both organic and inorganic ligands. However, among the ligands OH^-, SO_4^{2-}, NO_3^-, and CO_3^{2-}(evaluated at

concentrations commonly encountered in the natural environment), only OH^- was found to significantly complex with Cr(III) (Rai et al., 1986). Within environmental systems, Cr(III) exists as a hydroxyl species including $Cr(OH)_2^+$, $Cr(OH)_3$ and $Cr(OH)_4^-$ (Rai and Zachara, 1988). Speciation of Cr(VI) and Cr(III) will generally depend on a variety of environmental parameters, including pH, concentration, and available ligands. The behaviour of both Cr(VI) and Cr(III) species, and the inter-conversion between these two forms must be understood when considering the environmental properties of Cr.

2.4 Reactions of Chromium in soil and ground water

Chromium in the natural environment is greatly influenced by redox reactions, which alter its physical and chemical properties. Oxidation-reduction reactions involve the transfer of electrons. In redox reactions some components lose electrons and some components gain electrons from this transfer. The redox transformation of Cr(III) to Cr(VI), the oxidation of Cr(III) or the reduction of Cr(VI) to Cr(III) requires another redox couple (of oxidizing / reducing agent) which accepts or donates the necessary electrons (Figure. 2.2). In natural waters, sediments and soils, the significant redox couples (reducing agents/oxidation agents) are H_2O/O_2 (aq); Mn(II)/Mn(VI); NO_2/NO_3; Fe(II)/Fe(III); S^{2-}/SO_4^{2-}; CH_4/CO_2 (Richard and Bourg, 1991; Buerge and Hug, 1997).

The pH of soils plays a crucial role in the reactions of Cr in soils. The adjustment of pH is often easier but a change in oxidation state is more permanent, especially in the subsurface environment, due to the extremely large activation energies associated with many redox reactions. Changes in pH accompanied by fixation in an immobile phase often affect the mobility but not the toxicity of a metal. Changes in oxidation state usually affect both the mobility and the toxicity. Chromium generally exists as immobile Cr(III) under reducing conditions and the highly mobile and toxic Cr(VI) under oxidizing conditions. Consequently, redox manipulation is the preferred method for immobilizing Cr. Chromium(VI) can be reduced to Cr(III) by several methods and Cr(III) will not easily reoxidize to Cr(VI). Chromium immobilization includes complex formation, surface sorption and precipitation.

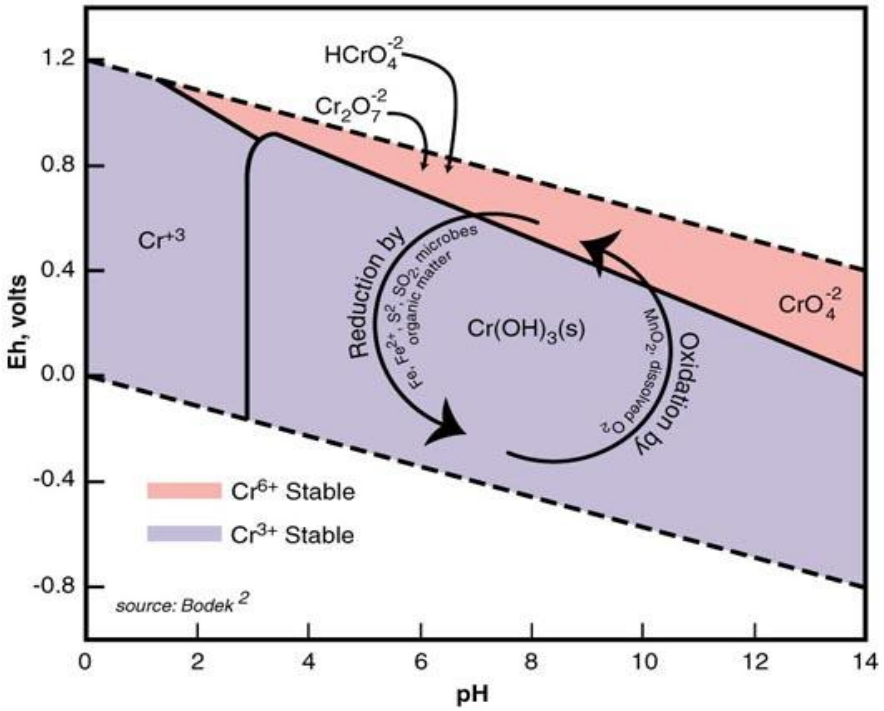


Figure 2.2 Stability diagram showing aqueous speciation of Chromium at various Eh and pH values (Rai et al., 1989)

Cr(VI) can be transported great distances in ground water owing in part to its high solubility, but it may be transformed by reduction to and precipitated as Cr(III) (Jardine et al., 1999; Tokunaga et al., 2001b). Chromium(VI) can be reduced readily to Cr(III) in the presence of organic matter, especially at lower pH (Bartlett and Kimble, 1976; Bloomfield and Pruden, 1980). Chromium(III) is less soluble and not easily transported into groundwater, however it can be oxidized by manganese oxides in soils (Schroeder and Lee, 1975; Bartlett and James, 1979).

2.4.1 Oxidation of Chromium

Many oxidizing agents are known to oxidize Cr(III) to Cr(VI) but only a few of them are found in the environment in sufficient concentrations to initiate this reaction. Water, MnO_2, O_3, H_2O_2 and PbO_2 are good sources of oxygen for oxidation of Cr(III) (Kimbrough et al., 1999). Mn oxides are effective in oxidizing Cr(III) (Bartlett and James, 1979; Schroeder and Lee, 1987). Dissolved oxygen can also oxidize Cr(III) to Cr(VI) but the rate of oxidation using dissolved oxygen is relatively slow (Rai et al., 1986).

Manganese (Mn) can act as a transporter of electrons between dissolved oxygen and Cr(III), and therefore Mn indirectly oxidizes Cr(III) to Cr(VI). In oxidizing or moderately anoxic environments, Cr exists as Cr(VI). Aerobic thermal oxidation of Cr(III) also

produces Cr(VI), however the kinetics of the reaction (Eqn. 2.3) are limited at room temperature.

$$2Cr_2O_3 + 3\ O_2 \rightarrow H_2CrO_4 \qquad (2.3)$$

In addition, incineration of tannery sludge also increases Cr(VI) concentration in leachates, which may be attributed to the oxidation of Cr(III) that is present in the sludge (Apte et al., 2005).

2.4.2 Reduction of Cr(VI) to Cr(III)

In the presence of electron donors, Cr(VI) can be reduced to Cr(III), which is less toxic. Iron(II), reduced sulfur and organic matter are chief sources of electrons to reduce Cr(VI) (Kotas and Stasicka, 2000). Both, chemical (abiotic) and biological (biotic) processes can reduce Cr(VI) to Cr(III). Although abiotic reduction is 100 times faster than biotic reduction rate, the biotic reduction process is more common in soils (Wielinga et al., 2001).

2.4.2.1 Reduction by Fe(II)

Ferrous ion [Fe(II)] plays a dominant role in the reduction of Cr(VI) to Cr(III) in water and soil (Figure 2.3). Discharges of industrial wastes generate dissolved Fe(II) ions into environmental waters and weathering of Fe(II)-containing minerals will release Fe(II). Oxides (e.g. goethite, hematite), sulfides (pyrite) and silicates (Fayalite, olivine) are some examples of Fe(II) containing minerals (Palmer and Puls, 1994). At neutral and alkaline conditions, Fe(II) controls the reduction of Cr(VI) in natural anaerobic systems at redox levels less than +100 mV (Anderson et al., 1994; Pettine et al., 1998). The rate of reduction of Cr(VI) depends on the dissolution and oxygenation of Fe(II) in soils (Fendorf and Li, 1996).

2.4.2.2 Reduction by sulfur

Reduced sulfur such as S^{2-}, H_2S, $S_2O_3^{2-}$ can be a major factor in the reduction of Cr(VI) to Cr(III) in soils. Most sulfides (S^{2-}) are not soluble but some dissolved S(II) ions may reduce Cr(VI), which come from discharge of industrial wastes and decomposition of organic matter. The rate of sulfide-induced Cr(VI) reduction is initially rapid, slows down within a few minutes but reduction will be completed after one day (Schroeder and Lee, 1975).

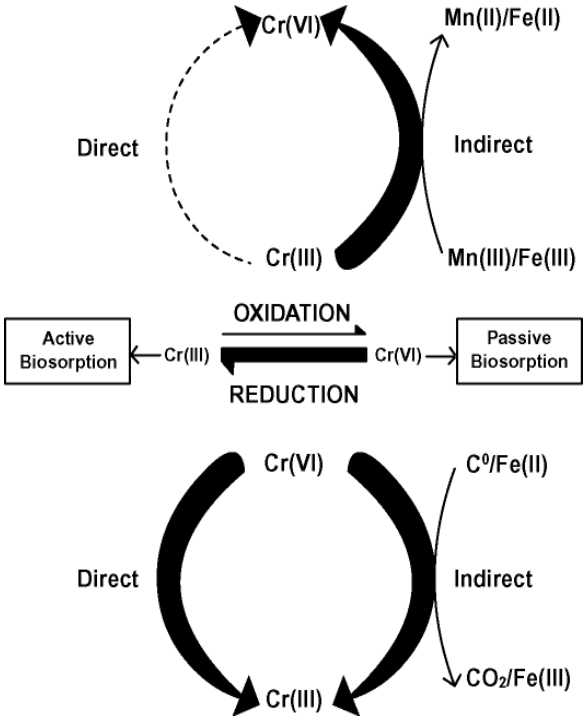


Figure 2.3 Flow chart of effect of reducing and oxidizing agents on Cr (Bolan et al., 2003).

2.4.2.3 Reduction by Organic Matter

Organic matter is a good source of electrons to reduce Cr(VI) in soils. Humic and fulvic acids are the main source of organic carbon and it is also present in small quantities in amino acids (Wittbrodt and Palmer., 1995; Bolan et al., 2011). Eckert et al. (1990) observed that the rate of reduction decreased with an increase in pH; half-life for Cr(VI) was 3 days at very low pH when reduced with humic acids, and might take several days at pH range from 4 to 7.

Incorporation of organic matter into the soil increases dissolved organic carbon, which stimulates both microbes as a source of energy and also serves as a pool of electrons with acidic and basic functional groups (Bolan et al., 2010). Chromium(VI) can be reduced to Cr(III) in the natural environment, where a ready source of electrons is available. Suitable conditions for this reduction to occur are often met where organic matter is present and can act as an electron donor. The reduction rate is increased in acid rather than alkaline soils (Bartlett and Kimble, 1976; Cary et al., 1977; Park et al., 2010).

2.4.3 Precipitation/Dissolution reactions of Chromium

Solubility of Cr and kinetics of dissolution governs the precipitation/dissolution reactions (Kimbrough et al., 1999). Chromium(III) species do not occur naturally and are less stable

in the environment. Precipitation of Cr(III) as the mixed Fe-Cr hydroxide $(Cr,Fe)(OH)_3$ enhances the precipitation of Cr(III) in waters at neutral pH levels (Sass and Rai, 1987). CrO_4^{2-} and $Cr_2O_7^{2-}$ anions of Cr(VI) are water soluble at all pHs but the rates of precipitation and dissolution reactions depend on pH.

Removal of Cr(III) from industrial effluent can be achieved using alkaline materials such as lime and magnesium oxide to precipitate Cr(III) as chromic hydroxide. Precipitation is reported to be most effective at pH 8.5 - 9.5, due to the low solubility of chromic hydroxide in that range (Patterson, 1985). Reduction of Cr(VI) to Cr(III), and subsequent hydroxide precipitation of Cr(III) ion, is the most common method for treating Cr(VI) contaminated industrial effluent (Loyaux-Lawniczak et al., 2001; James, 2001).

2.4.4 Sorption and desorption reactions of Chromium

The phenomenon of sorption is important in the transport and fate of a solute. Redox, precipitation reactions and sorption reactions are influenced by the complex environment which is inherent in soils. pH, surface area, density of active sites often influence equilibrium of sorption (Sposito, 1983; Kimbrough et al., 1999; Bolan et al., 1999).

It is crucial to increase the retention of Cr in soils in order to reduce groundwater contamination and uptake by plants. The retention of Cr(III) can be increased by elevating the pH level in the soil. The retention of Cr (VI) can be increased either by increasing the positive charge on the soil particles or by reducing Cr(VI) to Cr(III). Iron, manganese oxides, and clay minerals strongly sorb Cr(III) and this sorption is rapid; about 90% of Cr(III) is sorbed by clay minerals and Fe oxides within 24 hours (Bartlett and Kimble, 1976). Moreover, the sorption of Cr(III) increases with when pH rises because the clay surface becomes more negatively charged, and increases the amount of organic matter content in the soil (Paya Perez et al., 1988).

However, Cr(III) adsorption decreases when other inorganic cations or dissolved organic ligands are present (Richard and Bourg, 1991; Avudainayagam et al., 2001). Chromium(III) is strongly retained on soil particles whereas Cr(VI) is very weakly sorbed onto soil particles and is readily available for plant uptake and leaching to groundwater (Bolan et al., 2003; Banks et al., 2006). Leaching studies involving Cu, Cr and As added through CCA solution have indicated that Cr(VI) is readily leached compared to As and Cu (Carey et al., 1996b; Ko et al., 2007).

Anions of Cr, $HCrO_4^-$ and CrO_4^{2-} species are sorbed by Mn, Al and Fe oxides and hydroxides (positively charged surfaces) and clay minerals (Rai et al., 1986; James and

Bartlett, 1983). Amorphous iron (Fe) is the adsorbate found at the highest concentration in most aquifer materials and Fe hydroxides strongly sorb Cr(VI) (James and Bartlett,1983). Chromium(VI) sorption is favoured on sorbents, which are positively charged at low to neutral pH. Addition of organic amendments such as plant bark has been shown to increase the sorption of Cr(VI) (Bolan and Thiagarajan, 2001). Cellulose in the bark absorbs Cr(VI) strongly and forms Cr(VI)-cellulose complexes (Pizzi, 1981).

Low cost adsorbents such as agricultural by-products (sugar beet pulp, coir pith, rice straw, and saw dust), activated carbon of chars and bio-sorbents (algae, fungi, bacteria, chitosan in fungal cell wall) can be used for sorption of Cr(VI) and Cr(III) (Mohan and Pittman, 2006). The bark of the tree *Pinus radiata* bark contains a large number of cation exchange sites and therefore has a large retentive capacity of Cr(III) (Mahimairaja et al., 2000). Sorption of Cr(VI) by clays, soils and natural aquifer materials is low to moderate in pH ranges common to groundwater. Competing anions have a detrimental effect on Cr(VI) sorption, depending on dissolved concentrations of the competing anion and CrO_4^{2-}, their relative affinities for the solid surface and surface site concentration (Rai et al., 1986).

2.5 Factors Affecting Mobility and Availability of Chromium

Some factors play a vital role in mobility, transformation, speciation and availability of Cr in the environment. These include pH, oxidation state of Cr, presence of oxidizing and reducing material, redox potential and oxides of Iron and Manganese.

2.5.1 pH

The concentration of protons affects the solubility of Cr species and their sorption, controls the dynamics of Cr redox reactions in soils and its bioavailability to plants (James and Bartlett, 1988; Losi et al., 1994). Chromium(III) is more stable in acidic conditions and Cr(VI) is stable in alkaline conditions. Reduction is more rapid in acid than in alkaline soils. Organic constituents of the soil become more effective in reducing Cr(VI) with decreasing pH. In raising the soil pH, the organic matter may solubilize and thus may increase the reduction of Cr(VI) during its determination with the acid-diphenyl carbazide reagent. In clay mineral equilibration, Griffin et al. (1977) noticed that the water-soluble Cr(VI) decreased as the pH of kaolinite and montmorillonite suspensions decreased. This can be attributed to $HCrO_4^-$ adsorption by pH-dependent exchange sites on clay colloids. However, it is more likely that the anaerobic system maintained in that equilibrium

favoured Cr(VI) reduction to Cr(III) and facilitated the Cr(III) sorption by clay exchange sites. The solubility of Cr(III) decreases as the solution pH is raised above four.

2.5.2 Oxidation state of Chromium

The oxidation state of Cr is very important in relation to its mobility and role in plant and human nutrition. Chromium(VI) is more toxic in plant and human nutrition as explained earlier and also known to be more mobile in surface and subsurface environments than Cr(III). Hexavalent chromium eluted prior to trivalent chromium in municipal solid waste at anaerobic conditions (Artiole and Fuller, 1979). Again, this difference is due to the anionic nature of the Cr(VI) species, while Cr(III) exists as a cation and its hydrolytic forms are prone to precipitate starting at about pH 4.5.

Conversion of Cr(III) to Cr(VI) can be very slow or negligible in soils, and may be due to the absence of electron acceptors such as high-valence Mn and Fe. From the Eh-pH stability field (Figure. 2.2), at low redox potential and low pH, Cr(III) species are dominant. At neutral pH, Cr(VI) could be a predominant form of Cr in some natural systems. At pH 7, the concentration of CrO_4^{2-} could be equal to the concentration of $Cr(OH)_2^{+}$ in well-aerated soils.

Organic amendments such as biological waste materials (poultry and livestock manures), biosolids, domestic refuse, peat and topsoil increase the amount of dissolved organic carbon in soils either by acting as a source of DOC or by increasing the solubility of the soil organic matter (Schindler et al., 1992). Other organic compounds such as citric acid, gallic acid, acetic acid and other low molecular weight organics may serve a dual role as chelators for Cr(III) or electron donors for reducing Cr(VI) (James and Bartlett, 1983). These compounds may exist in soils as degradation products of organic waste or as metabolites excreted by plant roots. Organic amendments have a high cation exchange capacity and form strong complexes with metals; furthermore they decrease the bioavailability of toxic metals to plants in contaminated soils (Hetrick et al., 1994; Park et al., 2010). These amendments provide a source of energy to the microorganisms and thereby reduce Cr(VI) to Cr(III) (Paul and Beauchamp, 1989; Jardine et al., 1999).

The presence of manganese oxides plays a key role in the oxidation of Cr since they act as electron acceptors in the reaction. Adsorption of Cr(III) by Mn oxides is a first step in its oxidation by Mn (Bartlett and James, 1979). In aerobic soils, Mn oxides typically occur as surface coatings of clays and Fe oxides. Manganese oxides have high adsorptive capacities for heavy metals because of their large surface areas and high negative

charges. Soils having high free Fe and Mn oxides would significantly retard the migration of Cr(VI) (Korte et al., 1976) (Figure. 2.4).

Long (1983) found that Mn oxide and to a lesser extent, Fe(III) oxide play critical role in the adsorption of Cr(VI) by sediments. Under field conditions, the Mn oxides exist as reactive forms and serve as the electron link between Cr(III) and atmospheric O_2 and the amount of Cr(III) oxidized is proportional to the amount of Mn reduced (Bartlett and James, 1979). The oxidation of Cr(III) by Mn oxides is more rapid than by O_2. Because of slow kinetics, Cr(III) prefers to participate in other reactions (sorption/precipitation), which are faster than reacting with dissolved oxygen, hence the oxidation of Cr(III) by oxygen is unlikely (Palmer and Wittbrodt, 1990).

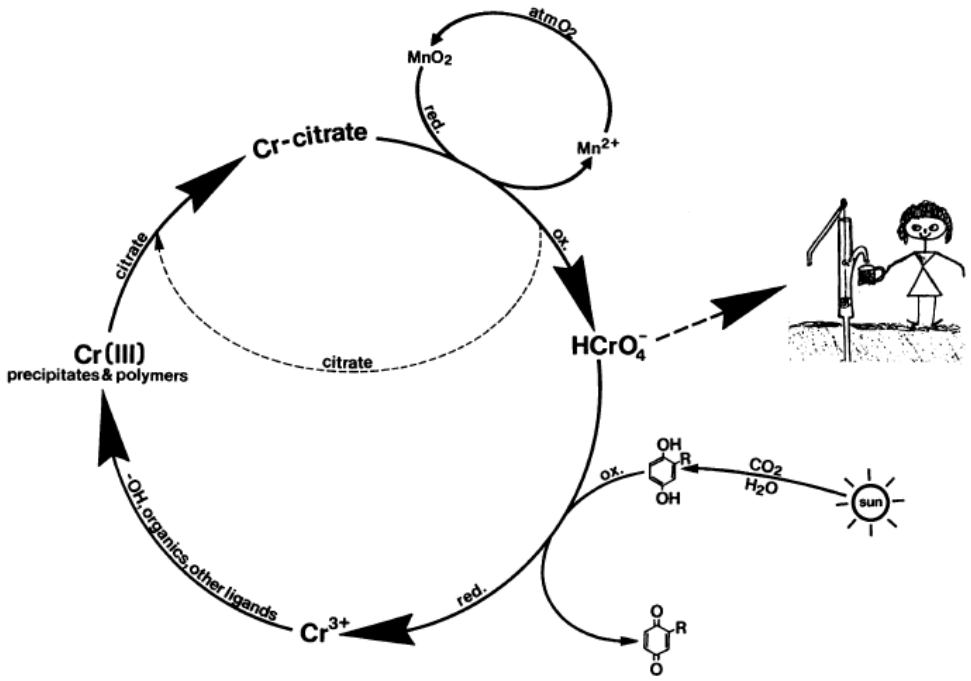


Figure 2.4 Chromium Cycle in the environment (Bartlett, 1991).

The presence of Fe oxides, especially in low-pH materials, facilitates the adsorption of Cr(VI) (Rai et al., 1989; Zachara et al., 1989). However, the presence of competing ions such as SO_4^{2-} and dissolved organic C, which compete for adsorption sites, can supress Cr(VI) adsorption. Electron donors are necessary for reduction to proceed, and organic matter (OM) and Fe(II) can serve as electron doors in soils and waste disposal system (Buerge and Hug, 1999). Reduction might be insignificant in soils low in OM and soluble Fe (Bartlett and Kimble, 1976b; Korte et al., 1976).

2.6 Treatment technologies to remediate toxicity of Cr(VI)

Mitigation of Cr(VI) toxicity requires knowledge about the conditions of site, flexibility in remediation design and creativity in optimisation strategies. Once the conditions of the soil are understood, behaviour of Cr in ground water and treatment methods can be predicted and remedial alternatives for Cr(VI) can be formalised.

2.6.1 Reduction of toxicity

The main remediation method to reduce toxicity of Cr(VI) is converting the oxidation state of Cr from +6 (toxic) to +3 (relatively less toxic) through reduction.

2.6.1.1 Abiotic reduction

In abiotic reduction (chemical reduction), reducing agents such as S^{2-}, Fe(0) or Fe(II) (Eqn. 2.3 and 2.4) donate electrons to Cr(VI). This chemical reduction targets both mitigation of toxicity and removal of Cr.

$$Fe + CrO_4^{2-} + 0.5\ H_2O \rightarrow Fe(OH)_3 + 0.5\ Cr_2O_3 \quad (2.3)$$

$$6\ Fe^{2+} + 2\ CrO_4^{2-} + 13\ H_2O \rightarrow 6\ Fe(OH)_3 + Cr_2O_3 + 8\ H^+ \quad (2.4)$$

Abiotic reduction is highly dependent on the existing site specific physical and chemical conditions such as pH, permeability, lithology, and water depth, concentrations of metals in water and soil, and alkalinity. *In-situ* reduction involves injection of electron donors such as H_2S, sodium dithionite ($Na_2S_2O_4$), sodium metabisulphite ($NaHSO_3$), Calcium metabisulphite ($CaHSO_3$), calcium polysulfide (CaS_5), Fe(II), Fe(0) and tin(II) chloride ($SnCl_2$) (Hawley et al., 2005). The pH of these donors needs to be adjusted to optimize electrostatic surface interactions between Cr(VI) anionic species and electron donor. Oxidized or impure Fe was the most effective, with no reduction occurring when pure Fe(0) was used (Powell et al., 1995). This was explained by the hypothesis that Fe(0) needs a reaction-initiating catalyst to participate in the redox reaction, for oxidation of Fe(0) to Fe(II). Surface area of Fe is another factor, which will affect redox reactions. Ponder et al. (2000) reported that reduction was 21 times greater when iron nanoparticles (10nm to 30 nm in diameter) were used compared to commercial iron.

2.6.1.2 Biotic reduction

Microorganisms that reduce Cr(VI) to Cr(III) include bacteria like *Pseudomonas*, *micrococcus, Escherichia*, *Enterobacter*, *Bacillus*, *Aeromonas*, *Achromobacter* and *Desulfomamaculum*, algae, yeasts and fungi (McLean and Beveridge, 1999; Cervantes et al, 2001). Microorganisms use organic amendments (plant residues, peat, and manures) as a source of energy. This mechanism involves the catalytic removal of high energy electrons from organic carbons to release oxidized CO_2. Sulfur is released in varying states of oxidation, organic phosphate is mineralized to release inorganic phosphate compounds and nitrogen is reduced to ammonium form. These ammonium and sulfur compounds are ultimately used as electron donors to reduce Cr(VI), and finally release fully oxidized sulfur as SO_4^{2-}, nitrogen as nitrate and Cr(VI) as Cr(III).

Biological reduction reactions need the presence of external electron donors like Fe, Mn or oxidized organic matter. This process is the same as chemical reduction but is biologically mediated and kinetically advantageous under aerobic conditions. Sulfur reducing bacteria produces H_2S, which acts as a reducing agent. Fein et al. (2002) reported that bacterial surfaces can also catalyse Cr(VI) reduction.

Biological factors play a vital role in the reduction of Cr(VI) and subsequent immobilization of Cr(III) in soils. Heterotrophic microorganisms are involved in the reduction of Cr(VI) to Cr(III) either by aerobic or anaerobic means, depending on organism, in both soil and water environments (Lovely and Phillips, 1994). Chromium(VI) reducing microorganisms are ubiquitous in soils and can enhance the reduction of Cr(VI) under ideal conditions (Turick et al., 1996).

Organic carbon concentrations, pH and dissolved oxygen affect the microbial reduction rates. Reduction reactions are sensitive to oxygen concentrations, so under anaerobic conditions, both biotic and abiotic reduction mechanisms are competitive (Hawley et al., 2005). In enhanced bioremediation (biostimulation), oxygen and/or nutrients are injected to stimulate indigenous microorganisms and this process involves recirculating water amended by a carbon source in the contaminated soil to stimulate bacterial activity, thereby reducing Cr(VI) faster (Hawley et al., 2005). Organic amendments like leaf litter, biosolids, poultry and livestock manures improve the physical nature of soil by increasing water retention and providing nutrients to plants in a slow release form (Tordoff et al., 2000; Wong, 2003). Organic amendments have a high cation exchange capacity, increase retention of metals in the soil and form stable organic heavy metal complexes, thereby decreasing the phytotoxicity by lowering the availability of heavy metals to plants (Park et

al., 2010). Organic amendments are a ready source of electrons and donate them to strong oxidizing metals like Cr(VI), which then reduces to less toxic Cr(III) (Hetrick et al., 1994).

Organic amendments are efficient in reducing highly mobile Cr(VI) to immobile and stable Cr(III) by subsequent precipitation of $Cr(OH)_3$ (Bolan and Duraisamy, 2003). Black carbon prepared from the dry biomass of rice straw is highly effective in reducing Cr(VI) toxicity (Hsu et al., 2009). Biomass of seaweed is proven as an effective reducing and immobilizing agent of Cr(VI) (Park et al., 2006). Soybean meal and rice bran have also been shown to decrease Cr(VI) concentration in soils, attributed to increase in DOC content (Chiu et al., 2009). Soy broth serves as an organic carbon source and reduces Cr(VI) in soils; and re-oxidation of Cr(III) is dependent on the availability of organic carbon and Mn redox status in the soils (Tokunaga et al., 2007).

2.6.1.3 Phytoremediation

Plants tend to reduce Cr toxicity by converting highly toxic and readily mobile Cr(VI) to less toxic Cr(III). Phytoaccumulation, one of the most common forms of Cr(VI) phytoremediation, comprises the uptake of the Cr from the soil to the plant roots and the shoots of plants. The plant *Leptospermum scoparium* was found to contain soluble Cr in the leaf tissue as trioxalatochromate [Cr(III)] ion (Lytle et al., 1998). Phytostabilization is most useful for low concentration contamination (Bolan et al., 2011). Chromium(VI) is toxic to plants because it generates free OH radicals and it further breaks down to Cr(III). The energetic O^- radicals can mutate DNA and lead to other toxic effects. Cr(VI) compromises plant growth and causes root damage (Salunkhe et al., 1998).

Indian mustard seedlings grown in aerated water absorb Cr(VI) by a factor of 100 to 250 times the concentration in water (Salt et al., 1997). *Brassica juncea* and *B. nigra* have the highest metal accumulating ability and Cr had the highest extraction coefficient, followed by Cd, Ni, Sn and Cu (Kumar et al., 1995). The wetland plants, duckweed are also hyper-accumulators of various metals including Cr (Zayed et al., 1998).

Water hyacinth (*Eichhornia crassipes*) can accumulate high concentrations of Cr(VI) (up to 6 mg/g dry mass) and plant roots can grow in 10 mg/L Cr(VI) and *Herniaria hirusta* was also found to be a Cr accumulator (Cervantes et al., 2001). Sulfur-loving plants such as cauliflower, kale and cabbage showed high concentrations of Cr accumulation (135 to 160 mg/kg in roots, 1.6 to 2.0 mg/kg in shoots) (Cervantes et al., 2001) with *Brassica spp*.

accumulating the most. Sulfur's transport system may be responsible for the high Cr uptake ability of these species.

Considerable work remains to be done on the development of phytoremediation technologies, and the mechanisms for uptake are not yet clear. Neither are the effects of organic matter and other soil amendments on uptake efficiency, possibly owing to the uncertainty associated with the mechanisms of uptake and/or precipitation.

2.7 Conclusion

- Industrialization has increased the utilization and improper disposal of Cr into the environment. Industries such as electroplating, steel industries, cooling towers and refractory, timber treatment discharge high concentrations of Cr.

- Chromium is a redox active heavy metal and the toxicity of Cr depends on its oxidation state. Chromium (III) is less mobile and an essential nutrient, whereas Cr(VI) is highly toxic.

- The toxicity of Cr can be mitigated by immobilization through adsorption using low cost adsorbents such as saw dust, activated carbon and algae.

- The remediation of Cr(VI) contaminated soil by reducing it to non-toxic Cr(III) can be achieved by materials that have the capacity to increase DOC in soils and also donate electrons.

- Addition of organic amendments increases microbial community and respiration, promotes reduction of Cr(VI) and subsequently decreases phytotoxicity to plants.

Chapter 3

SIMULTANEOUS DETERMINATION OF CHROMIUM SPECIES USING ION CHROMATOGRAPHY WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

3.1 Introduction

Increasingly, heavy metals are being disposed in the natural environment and this poses a great threat to people, flora and fauna. The two prominent species of Cr, trivalent [Cr(III)] and hexavalent [Cr(VI)], behave differently in the environment and they have very different characteristics. Chromium(III) at lower levels is an essential micronutrient, whereas Cr(VI) is highly toxic even at lower concentrations (Bolan et al., 2003; Lee Jardine et al., 2011). Since the toxicity of Cr is species-specific, detailed information about the oxidation state is essential if we are to understand the toxicity of Cr.

Quantification of total chromium concentration in environmental samples may not be sufficient to estimate the distribution of various Cr species because of Cr(III) and Cr(VI) have contrasting properties. Detailed information about the oxidation states is essential to understand the toxicity of Cr. Several methods are available to quantify Cr species in environmental samples (Sperling et al., 1992; James et al., 1995; Adria-Cerezo et al., 2000).

Demands for analytical techniques have increased so that Cr speciation can be determined. Currently, many techniques including ion chromatography coupled with inductively coupled plasma-mass spectroscopy (ICP-MS) are available for metal speciation. However, the simultaneous determination of Cr(VI) and Cr(III) using a single chromatography column is difficult due to the differences in their ionic nature (while Cr(VI) is anionic and Cr(III) is in the cationic form). Hence, conversion of cationic Cr(III) to anionic form and subsequent separation of these two anionic species with a single anion-exchange chromatography hyphened with ICP-MS is possible.

Ion chromatography (IC) coupled with inductively coupled plasma (ICP-MS) has been developed for Cr speciation because ICP-MS offers high sensitivity and multi-isotope detection, as Cr exists in four different stable isotopes with various abundance - ^{50}Cr, ^{52}Cr, ^{53}Cr and ^{54}Cr (Camara et al., 2000; Adriano, 2001; Michalk et al., 2002). However, this problem can be resolved using two approaches. The first uses an anion-exchange column and a cation-exchange guard column in series or a mixed mode column to retain both species and analyze them on ICP-MS (Pantsar-Kallio et al., 1996; Barnowski et al., 1997). The second approach involves a derivatization process, whereby Cr(III) is complexed with a ligand to form an anionic complex, which can be simultaneously separated from Cr(VI) employing anion-exchange chromatography (Tomlinson et al., 1994; Byrdy et al., 1995; Gurleyuk and Wallschlager, 2001).

Amino-polycarboxylic acids like 1, 2-cyclohexanediamine tetracetic acid (CDTA), ethylene diamine tetracetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA) have been used to convert Cr(III) into anionic complexes (Semenova et al., 1996; Jung et al., 1997; Himeno et al., 1998). Achieving high ultra-violet (UV) response to detect low quantities and selectivity to estimate Cr species on IC-ICP-MS, ligands that form complexes with metal species should satisfy the following requirements, including: (i) complexes should have high UV absorbance; (ii) complexes should be stable during analysis; and (iii) the ligand should form only one single distinct complex with Cr(III) (Liu et al., 1999).

Although several researchers examined the effect of ligands on the complexing capability of Cr(III) (Jen et al., 1993; Dabek-Zlotorzynska et al., 1998; Chen et al., 2001), their application in the simultaneous measurement of Cr(III) and Cr(VI) species in environmental samples has not been reported.

3.2 Objectives

The overall objective of this study is to estimate both Cr(III) and Cr(VI) simultaneously using ion chromatography coupled with inductively coupled plasma mass spectrometry . The specific objectives include:

- To evaluate the effect of various ligands on Cr(III) complexation.
- To optimise pre-column derivatization conditions including pH and concentration of buffering solution.
- To quantify and separate Cr(III) and Cr(VI) by ligands in soil pore water and wastewater sources.

3.3 Hypothesis

Simultaneous separation and estimation of Cr(III) and Cr(VI) is difficult since both these species have contrasting characteristics. Chromium(III) is a cation, whereas Cr(VI) exists as an oxy-anion. Hence the hypothesis tested was that converting cationic Cr(III) to anionic Cr(III) can be achieved by reacting with complexing ligands that form stable complexes with Cr(III).

3.4 Experiments

Major experiments/analyses conducted for simultaneous estimation of Cr(III) and Cr(VI) using ligands are listed in Table 3.1.

Table 3.1 The major experiments conducted for quantification of Cr species.

Title	Chemicals and parameters	Measurements
Effect of various ligands on quantification of Cr species	DTPA, PDA, HEDTA and NTA $3Na^+$	Retention time, peak abundance and intensity
Influence of concentration of eluent on separation of peaks	10 and 20mM of mobile phase (NH_4NO_3)	Retention time, peak abundance and intensity
Impact of pH of eluent on intensities of peaks	4, 6, 7	Retention time, peak abundance and intensity
Simultaneous measurement of Cr species	Soil pore water and wastewater sources (piggery, winery, dairy and sewage)	Cr(III) and Cr(VI) concentrations

3.5 Materials and methods

3.5.1 Reagents and solutions

Millipore water (18.2 MΩ/cm, Milli-Q plus system, Millipore, Bedford, MA, USA) was used in the experiments. Chromium(VI) standard solution (0.1 mM) was prepared from

potassium dichromate ($K_2Cr_2O_7$) (AR grade, Sigma-Aldrich, Sydney). Chromium(III) stock standard solution (0.1 mM) was prepared from chromium nitrate (Cr $(NO_3)_3$) (BDH, UK). Intermediate and working solutions were prepared daily.

Different ligands such as HEDTA (N-(2-hydroxyethyl ethylene diamine triacetic acid); NTA-3Na^+ (Nitrilo triacetic acid, tri sodium salt); DTPA (Diethylene triamine pentaacetic acid); PDA (2, 6 pyridine dicarboxylic acid)] were dissolved in appropriate concentrations to examine their ability to complex with Cr(III).

3.5.2 Sample preparation

Each ligand (3ml of 0.1 mM) was separately put into a beaker and mixed vigorously with Cr(III) (6 ml of 0.1 mM), and the samples were heated on a hot water bath at 80^0C for an hour to decrease the activation energy. This promoted the reaction of Cr(III) with ligands because Cr(III) is kinetically inert at room temperature (Jung et al., 1997). After cooling to room temperature, 10 µL Cr(III)-ligand and 10 µL Cr(VI) (0.1 mM) were put into a 1ml glass vial and mixed with 980 µL Milli-Q water and used for both Cr(III) and Cr(VI) analysis. Solution was injected using an 1100 auto sampler into liquid chromatography (flow rate was 2.0ml/min at 30^0C column temperature) with an injection volume of 20 µl.

3.5.3 Instruments and apparatus

Inductive coupled plasma-mass spectrometer (ICP-MS) (7500c, Tokyo, Japan) was used as an element specific mass detector coupled to a liquid chromatographic module (LC) (Agilent, Tokyo, Japan) equipped with a separation column (G3154A/101). Porous polymethacrylate resin was packed into the separation column that has 50 µmol/g exchange capacity. The outlet of the LC was connected directly to the ICP-MS by polyetheretherketone (PEEK) tubing (70 cm, 0.13 mm internal diameter). Chromium was detected at mass-to-charge ratio (m/z) 52. The conditions of IC-ICP MS are listed in Table 3.2. Data process and analysis was controlled using Agilent Chemstation software.

3.5.4 Effect of concentration of mobile phases on the separation of Cr(III) and Cr(VI)

Several factors need to be optimized to achieve high sensitivity, selectivity and reproducibility of peak intensities of Cr species. One such factor is to optimize the concentration of mobile phase solution, and separating Cr(III) and Cr(VI) can be achieved

by using appropriate solutions. Hence, mobile phase was prepared by dissolving the required amounts of NH_4NO_3 in Milli-Q water to prepare 10, 20, 30 mM solutions.

Table 3.2 Optimised settings of IC-ICP-MS used for analysis

Instrument	Parameters	Settings
ICP-MS 7500C	Nebuliser	Polycarb 400
	Nebuliser flow(L/min)	0.24
	Plasma RF power (kW)	1.45
	Monitored ion	Cr^{52}
	Plasma gas flow-Argon ((L/min)	15
	Auxiliary gas flow- Argon ((L/min)	1.0
	Carrier gas flow- Argon ((L/min)	1.15
Sample introduction	Pump rate (rpm)	25
	Spray chamber temperature (^{0}C)	3
Ion chromatography (IC)	Column model	G3154A/102
Agilent 1100	Mobile phase	20 mM NH_4NO_3 at pH 4.0
	Flow rate (L/min)	1.0
	Injection volume(µL)	50
	Column temperature (^{0}C)	30

3.5.5 Effect of pH of mobile phase on complexation of Cr(III)

The optimal conditions for complexation of Cr(III) with different ligands were investigated. For optimising chromatographic separation, pH of the mobile phase was investigated by adjusting to 4, 6 and 7 using diluted nitric acid (HNO_3) or ammonium hydroxide (NH_4OH).

3.5.6 Estimation of Cr species in soil pore water

The pore water samples collected from contaminated soils using Rhizon, were used to estimate C(III) and Cr(VI) simultaneously by using HEDTA and NTA-$3Na^+$ as Cr(III) complexing agents. The method for collection of pore water from soil samples by using Rhizon samplers is described in Section 7.5.4 in Chapter 7. Initially each ligand and water sample were mixed at 1:2 ratio, heated at 80^OC for an hour and filtered through 0.45 µM filter tips. In a 1ml glass vial, 20 µl of the sample was taken and mixed with 980 µl Milli-Q water. The sample solution was injected into liquid chromatography (flow rate was 2.0

ml/min at 30^0C column temperature) with an injection volume of 20 µl using an 1100 auto sampler and analysed on ICP-MS.

3.5.7 Analysis of Cr species in soil and effluent samples

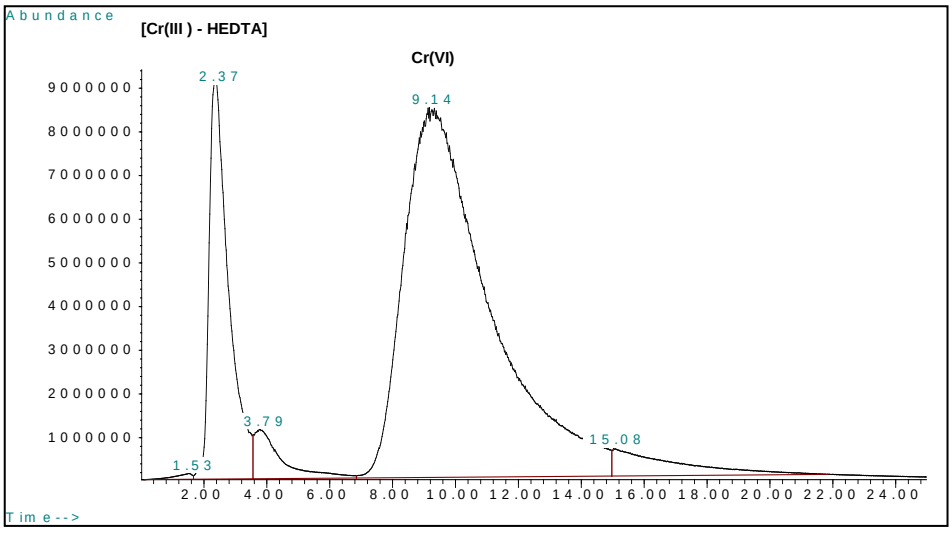
The proposed method was tested to analyse Cr(III) and Cr(VI) in different soils (acidic, neutral and alkaline) and effluents (waste water, piggery, winery, dairy and sewage) from South Australia that have been spiked with both Cr(III) and Cr(VI). The soils were spiked with 100 mg/kg Cr(III) and Cr(VI) at 1:10 ratio and mixed for an hour. The effluents were spiked with 50 mg/L Cr(III) and Cr(VI) and mixed for an hour. For independent analysis, total Cr was estimated on ICP-OES and Cr(VI) was estimated on spectrophotometer using 1, 5-Diphenyl carbazide as colour developing reagent. Chromium(III) was measured by subtracting Cr(VI) from total Cr. The Cr species in both soil and effluents were analysed simultaneously by the proposed method as described in Section 3.5.6.

3.6 Results and discussion

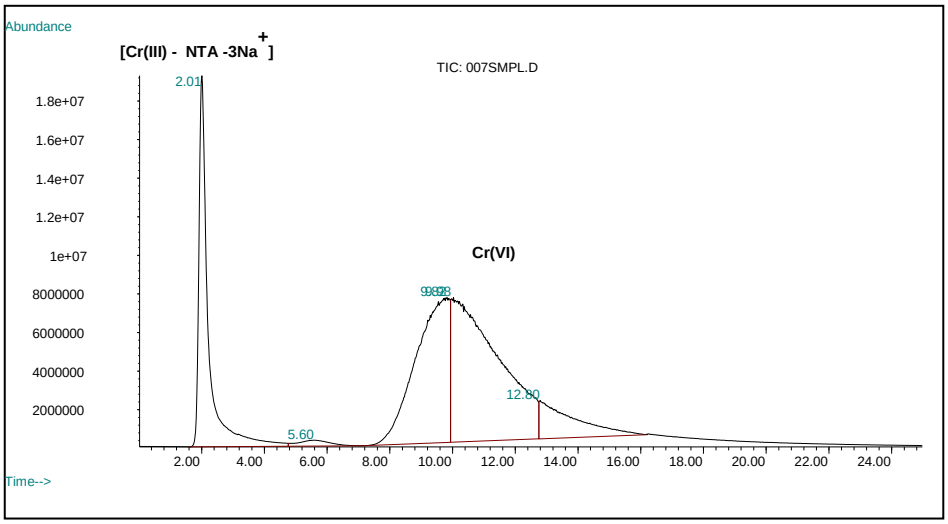
3.6.1 Effect of various ligands on complexing Cr(III)

The spectra of ICP-MS indicated that except for NTA, all the chromatograms overlapped, which was attributed to the unfavourable conditions for the separation of both [Cr(III)-ligand] complex and Cr(VI) in the IC column. To increase further resolution, solutions of both [Cr(III)-ligand] and Cr(VI) were diluted to 0.01 mM. 10 µL of 0.01 mM [Cr(III)+ ligands], 10 µL of 0.01 mM Cr(VI) and 980 µl deionized water were mixed in a glass bottle, the injected quantity was increased from 20 to 30 µL and the eluent (NH_4NO_3) strength was made up to 20 mM.

The results from IC-ICP-MS chromatograms indicated that separation for Cr(III) and Cr(VI) was achieved using NTA-$3Na^+$ and HEDTA as complexing agents, which could be attributed to the formation of stable Cr-NTA $3Na^+$ and Cr-HEDTA complexes. This results in the separation of retention time because the different negative charge of $[Cr\text{-}NTA\text{-}3Na^+]^{-1}$, $[Cr\text{-}HEDTA]^{-1}$ and Cr(VI); resulted in the resolution of Cr(III) and Cr(VI) (Sarzanini and Bruzzoniti, 2001). This clearly shows that NTA- 3 Na^+ and HEDTA can be used as ligands for the formation of Cr(III) complex and subsequent separation from Cr(VI) (Figure 3.1). To further resolve peaks well, the factor- resolution was tested in the following section.



3.1 (a) [Cr(III)-HEDTA] and Cr(VI)



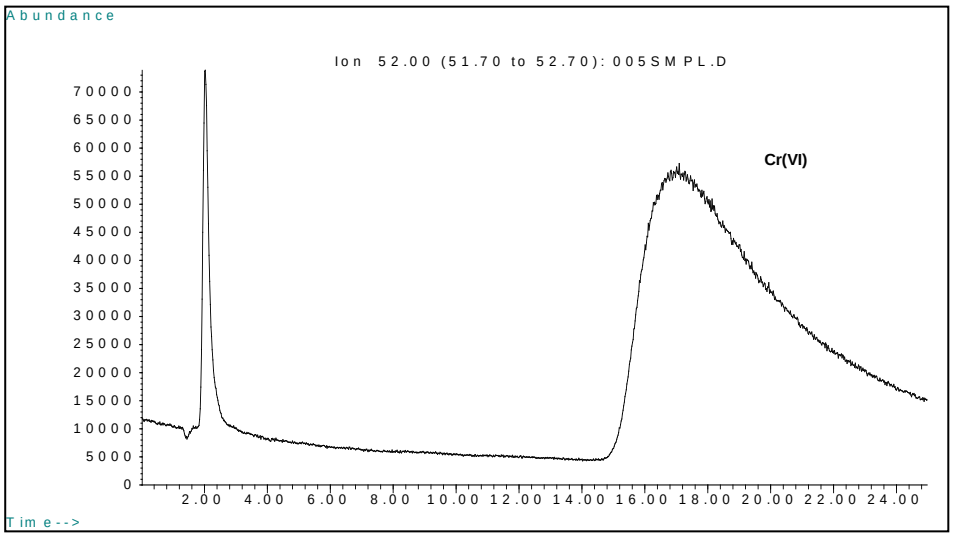
3.1 (b) [Cr(III) –NTA-3Na^{+}] and Cr(VI)

Figure 3.1 Effect of different ligands on complexation of: (a) Cr(III) **–** [Cr(III)-HEDTA] and Cr(VI); (b) Cr(III) –NTA-3Na^{+}] and Cr(VI).

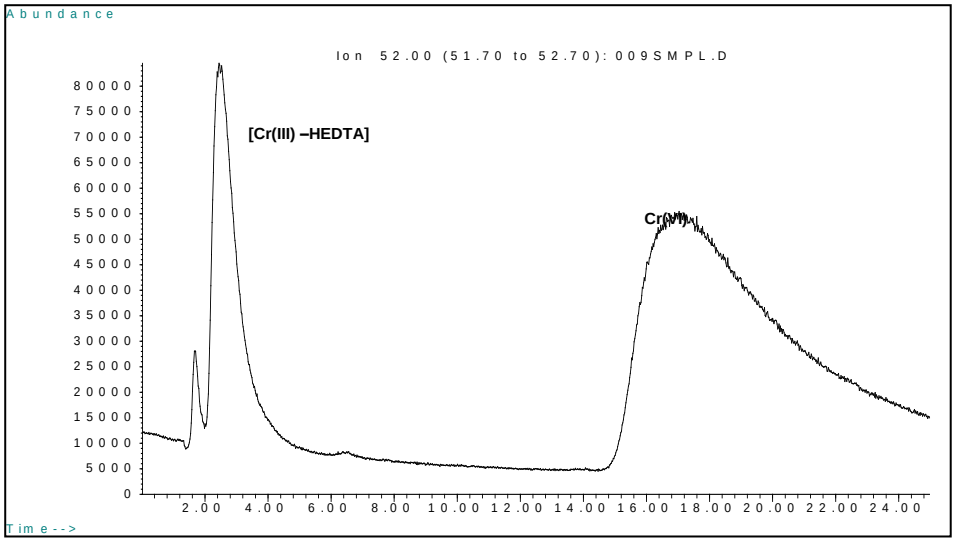
3.6.2 Effect of concentration of mobile phase on complexation of Cr(III)

Different concentrations of NH_4NO_3 (10, 20, 30 and 40 mM) eluent were examined and the results demonstrated that using 20 mM NH_4NO_3 resulted in clear separation (for [Cr(III)-ligand] and Cr(VI)) possible. Figure 3.2 presents the chromatograms obtained at different concentrations (10, 20 mM) of mobile phase. The retention time decreased with

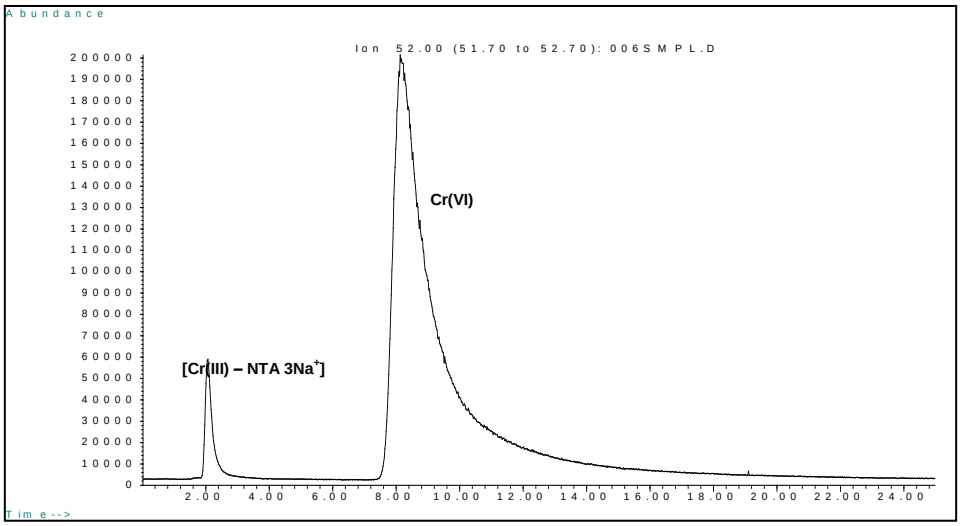
increasing NH_4NO_3 concentration and, consequently, the peak intensities were increased. Good peak shape and resolution were obtained using a mobile phase containing 20 mM NH_4NO_3. This indicates that the higher the concentration of competing ions in the eluent the more effectively d target ions are displaced from the stationary phase and they are more rapidly eluted from the column (Weiss, 1995). The parameters used for the IC-ICP-MS system employed in the quantification of Cr species are listed in Table 3.1.



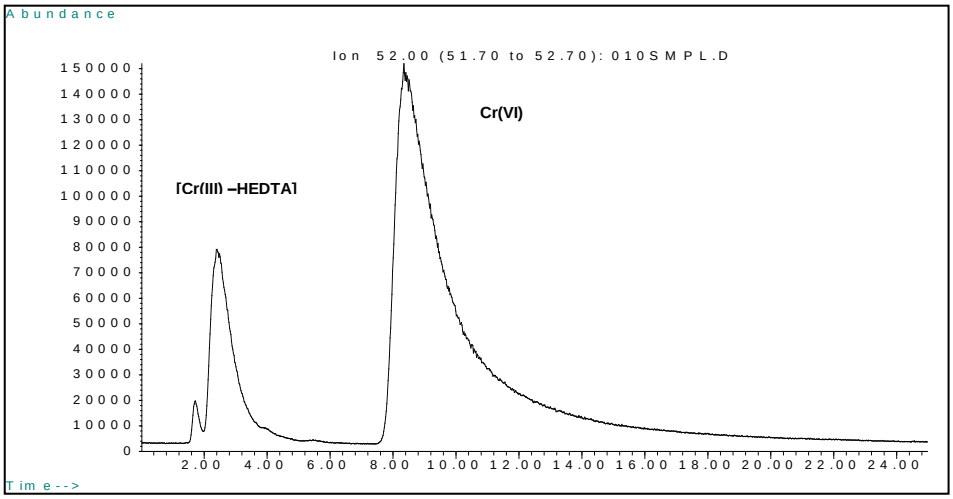
3.2 (a) 10 mM NH_4NO_3 [Cr(III) –NTA-3Na^+] and Cr(VI)



3.2 (b) 10 mM NH_4NO_3 [Cr(IIII)-HEDTA] and Cr(VI)



3.2 (c) 20 mM NH_4NO_3 [Cr(III) –NTA-$3Na^+$] and Cr(VI)



3.2 (d) 20 mM NH_4NO_3 [Cr(IIII)-HEDTA] and Cr(VI)

Figure 3.2 Influence of concentration of buffering solution on the intensity and resolution of peaks that correspond to Cr(III)-ligand and Cr(VI).

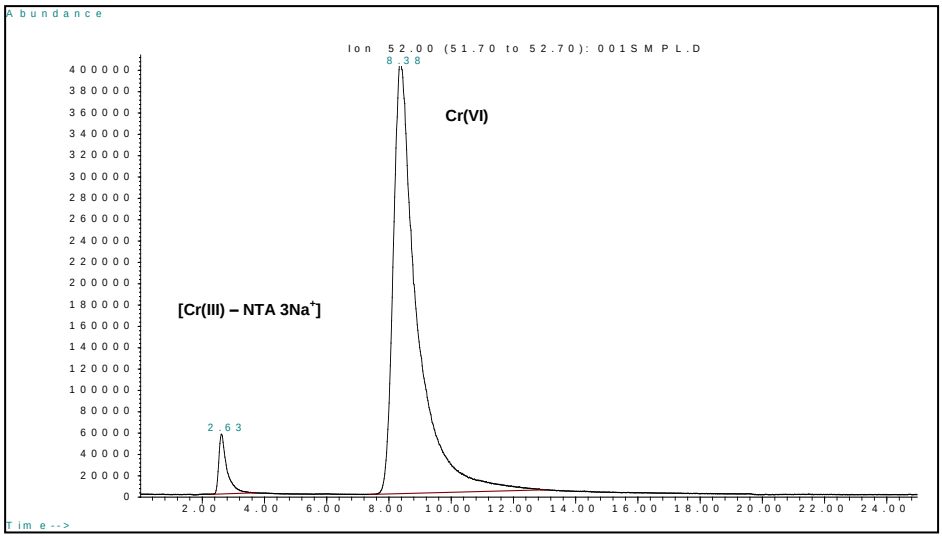
3.6.3 Effect of pH on complexation of Cr(III)

The mobility of ions in the column is governed by charge-to-size ratio of target metal ions as well as pH of the mobile phase. Of the different pHs tested on the mobility of Cr species, the pH of mobile phase at 4.1 enhances the separation of both [Cr(III)-ligand] and Cr(VI) species, which was evident from the high intensity of the peaks.

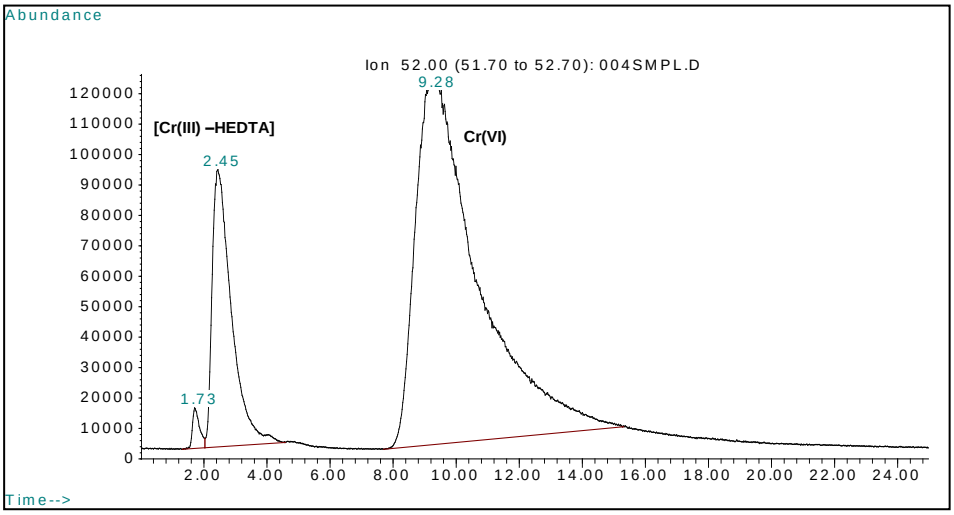
In contrast, at other pHs tested (i.e. pH 5, 6 and 7), the peaks are broadened (Figure 3.3). This effect of pH may be due to change in the net charge of chromate in solution (Jung et al., 1997). The pH of mobile phase solution also controls the oxidation state of Cr(VI) in the solution. Chromium (VI) can be present in solution as bichromate ($HCrO_4^-$), chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$). At pH below 2, Cr(VI) exists as H_2CrO_4, whereas $HCrO_4^-$ and $Cr_2O_7^{2-}$occur in the pH range between 2 and 6; at pH < 9, CrO_4^{2-} prevails in the environment (Bartlett, 1991).

3.6.4 Stability of Cr(III)-ligand complexes

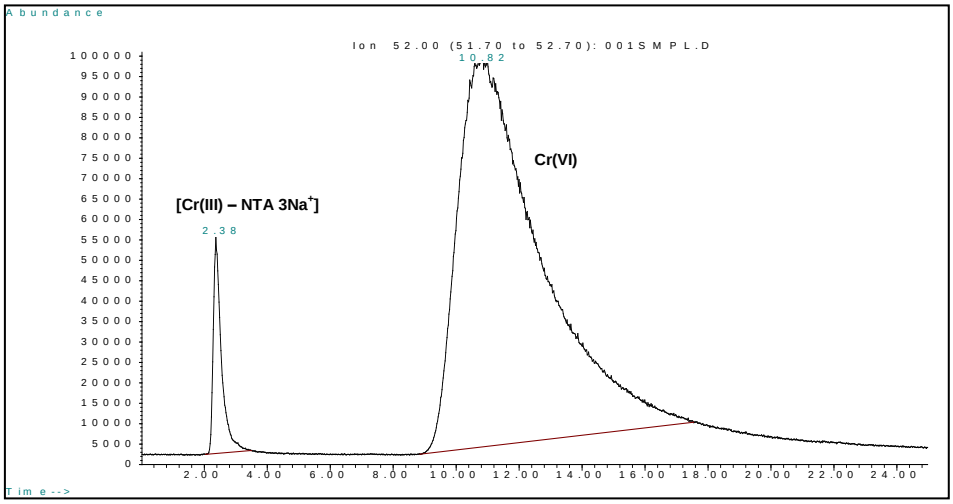
For a successful quantification of Cr species, anionic complexes should be stable and should not undergo ligand exchange during analysis (Inoue et al., 2005). Among four ligands tested, only two ligands, HEDTA and NTA-3Na$^+$, produced stable complexes with Cr(III), as indicated by high intensity and high resolution peaks with low retention time. Ligands such as DTPA and PDA performed poorly in terms of their ability to complex with Cr(III) as indicated by weak peaks corresponding to Cr(III) in chromatograms (Figure. 3.3). However, chromatograms for $[Cr(HEDTA)]^-$ and $[Cr(NTA\text{-}3Na)]^-$ indicated that during analysis, Cr complexes did not undergo ligand-exchange with NO_3^- (competing ion) in buffering solution.



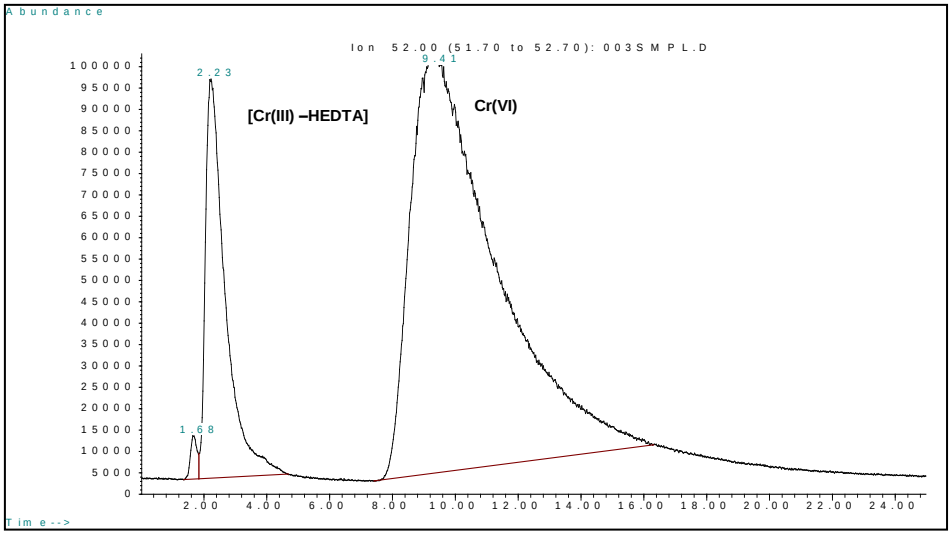
3.3 (a) pH 4: [Cr(III) –NTA-3Na$^+$] and Cr(VI)



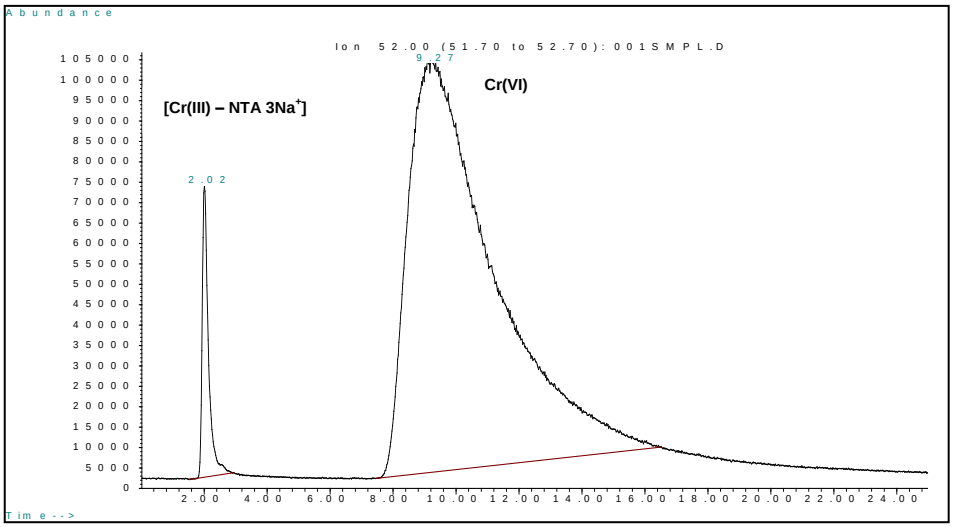
3.3 (b) pH 4: [Cr(IIII)-HEDTA] and Cr(VI)



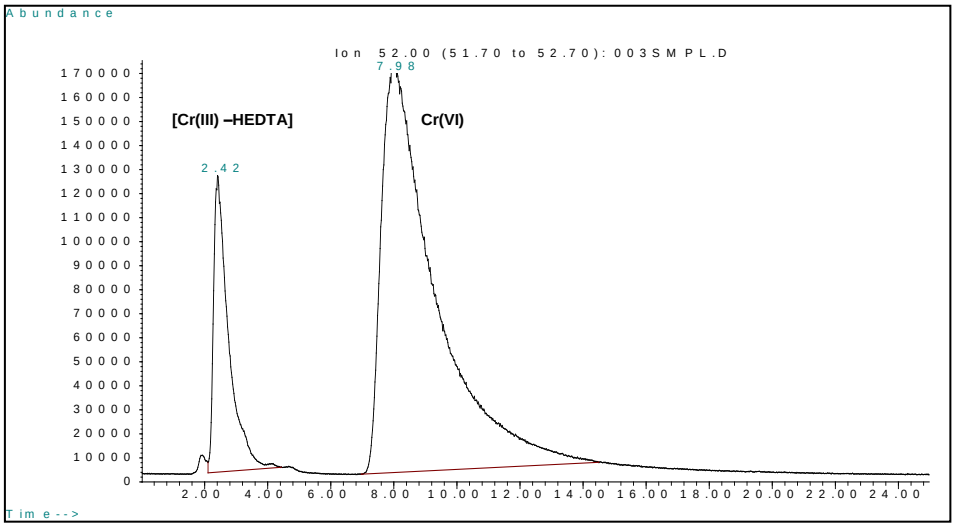
3.3 (c) pH 6: [Cr(III) – NTA-3Na^{+}] and Cr(VI)



3.3 (d) pH 6: [Cr(IIII) – HEDTA] and Cr(VI)



3.3 (e) pH 7 [Cr(III) –NTA-3Na+] and Cr(VI)



3.3 (f) pH 7: [Cr(IIII)-HEDTA] and Cr(VI)

Figure 3.3 Influence of concentration of buffering solution on the intensity and resolution of peaks that correspond to Cr(III)-ligand and Cr(VI).

3.6.5 Estimation of Cr species in water soil pore water

To analyse Cr species, calibration curves were plotted between peak area and concentration of the [Cr(ligand)]. All the plotted calibration curves were linear (Table 3.3), and correlation coefficients (r^2) ranged from 0.991 to 0.999 within the concentration range of 0.5 to 2.5 mM.

Table 3.3 Correlation coefficients of Cr species determined using ligands

Ligand	Species	Regression line	Coefficient
HEDTA	Cr(III)	Y=2.01 E + 09x – 2.02 E + 04	0.998
	Cr(VI)	Y=5.13 E + 06x – 4.05 E + 07	0.991
NTA-3Na^{+}	Cr(III)	Y=3.44 E + 07x – 1.27 E + 05	0.999
	Cr(VI)	Y=4.73 E + 05x – 1.58 E + 02	0.999

Chromatograms of soil pore water have high noise, which may be due to the interference of other ions in soil matrix. Initially the peak intensities for [Cr(ligand)] were weak, and after increasing the concentration of ligands from 0.01 mM to 0.02 mM, the intensities of peaks were increased, which corresponds to [Cr(ligand)].

3.6.6 Quantification of Cr species in soil and waste water samples

The results showed that the proposed method increased the recovery of Cr(III) and Cr(VI) species in both soils and effluents. The new method improved Cr(III) recovery by 5.91, 11.07 and 1.75% in acidic, neutral and alkaline soils, respectively over the conventional method. Moreover Cr(VI) recovery was improved by 6.17, 6.51 and 6.75% in acidic, neutral and alkaline soils, respectively (Table 3.4). For piggery, winery, dairy and sewage effluents, respectively, recovery in Cr(III) was higher (i.e. 3.65, 3.57, 3.78 and 3.79%) For Cr (VI) recovery the percentages for recovery in piggery, winery, dairy and sewage effluents were 2.32, 4.77, 1.98 and 1.76%.

Chromium(III) was recovered in higher amounts than Cr(VI). This may be attributed to the poor methodology that was used to estimate Cr(III) conventionally. The recovery of Cr(III) was higher in soils than effluents. This may be due to the fact that complexing ligands reduce the adsorption and increase mobility in soils by converting cationic Cr(III) to the less strongly sorbed Cr(III)-ligand. The relative difference between conventional and this improved method in measuring Cr species in pore water effluents is less, which may be due to the less sorption of the Cr species.

Table 3.4 The relative comparison of conventional method and proposed method for estimating Cr species in different soils and effluents.

Samples	Amount of Cr species spiked (soils: mg/kg; effluents: mg/L)		Cr concentration using independent analysis (soils: mg/kg; effluents: mg/L)		Cr concentration using simultaneous analysis (proposed method) (soils: mg/kg; effluents: mg/L)	
	Cr(III)	Cr(VI)	Cr(III)*	Cr(VI)**	Cr(III)	Cr(VI)
Acidic soil	100	100	85.42±0.16	84.47±0.05	91.32±0.14	90.47±0.25
Neutral soil	100	100	83.64±0.09	85.52±0.17	94.71±0.21	92.03±0.14
Alkaline soil	100	100	78.66±0.15	88.93±0.05	80.41±0.16	95.68±0.35
Soil Porewater	100	100	87.51±0.04	91.64±0.13	92.28±0.08	94.15±0.17
Wastewater	50	50	44.51± 0.11	46.24±0.22	46.35±0.14	48.92±0.27
Piggery effluent	50	50	42.14±0.08	43.83±0.16	45.79±0.12	46.15±0.14
Winery effluent	50	50	42.65±1.06	43.17±0.13	46.21±0.16	47.94±0.06
Dairy shed effluent	50	50	41.06±0.45	44.73±0.57	44.84±0.09	46.71±0.18
Sewage effluent	50	50	37.84±0.28	43.09±0.14	41.63±0.14	44.85±0.13

*Cr(III) = [(total by ICP-OES) – (Cr(VI) by calorimetric)]; **Cr(VI) by calorimetric method (USEPA, 1996)

3.7 Conclusion

- In this chapter is was shown that simultaneous determination of Cr(III) and Cr(VI) can be achieved by using complexing ligands to convert Cr(III) to anionic species and subsequent analysis of these species using IC-ICP-MS.

- It also emerged that buffering solutions' parameters such as pH and concentration play an important role in separating both [Cr(III)-ligand] complex and Cr(VI) species, resulting in high peak intensities.

- The optimum buffering solution pH was 4.0 while concentration was 20 mM for simultaneous quantification of Cr(III) and Cr(VI), which was evident when both Cr(III)-ligand and Cr(VI) peaks were separated with high intensities.

- The recovery of Cr(III) species was higher than Cr(VI) when the proposed method was used in soils and effluents, which may be due to the indirect estimation of Cr(III) that was not reliable.

- Finally the amount of Cr(III) estimated using proposed method was higher in soils, and this was attributable to the conversion of cationic Cr(III) to anionic [Cr(III)-ligand] ⁻that has less sorption capacity in soils. The proposed method to estimate Cr species in soil solutions needs to be modified in order to supress interferences of other ions with complexing capacity of ligands.

Chapter 4

RELATIVE SORPTION AND MOBILITY OF CHROMIUM SPECIES IN A RANGE OF SOIL TYPES

4.1 Introduction

A whole range of anthropogenic activities such as stainless steel industries, petroleum refining, metalliferous electroplating tannery and timber treatment, mining and smelting, and pesticides discharge huge amounts of heavy metals into the natural environment (Kim et al., 2003; Bolan et al., 2003). Migration and bioavailability of heavy metals in the terrestrial environment make it important for us to understand the impact of metal-containing wastes on soils throughout the world. Effluent from tannery, timber, and electroplating industries are enriched with heavy metals including chromium (Cr) (Sarin and Pant, 2005). The oxidation state of Cr in contaminated sites is an important indicator of toxicity. Chromium is used as Cr(III) in tannery industries and as Cr(VI) in timber treatment and electroplating (Barnhart, 1997). Geochemical factors and processes that control the environmental chemistry and fate of Cr include pH, dispersion, advection, adsorption, redox transformations, complexation and precipitation (Adriano, 2001).

Copper-chromate-arsenate (CCA) treated timber is commonly used for fence posts in vineyards and this activity can result in the release of Cr species to soil environments (Robinson et al., 2006). The main sources of Cr(VI) contamination in Australia are the timber treatment industries and tannery effluents (Naidu et al., 2000). Around 157,290 hectares of land is under winery cultivation (ABS, 2009) and approximately 3400 Mg Cr(VI) is present in Australian vineyard soils (Robinson et al., 2004). When considering vineyards alone in Australia, around 6 million CCA treated posts (broken) are disposed of annually and constitute the major contamination source of Cr(VI) in soils and ground water. Previous studies (Chirenje et al., 2003; Hingston, 2001; Robinson et al., 2006) have confirmed that CCA leaches from treated timber into the surrounding soil and water.

While Cr(III) is thermodynamically stable and strongly retained in soil particles, Cr(VI) species such as chromate (CrO_4^{2-}), bichromate ($HCrO_4^-$) and dichromate ($Cr_2O_7^{2-}$) are weakly sorbed to soils under alkaline to slightly acidic conditions, leading to their

movement to sub-surface environments (James and Bartlett, 1983; Wittbrodt and Palmer, 1995; Carey et al., 1996). Chromium(VI) is a strong oxidiser, exists as tetrahedral oxygenated species in aqueous solutions, is highly soluble in aqueous and furthermore, is mobile in groundwater and surface waters (Nieboer and Jusys, 1988). In contrast, Cr(III) binds with six oxygens in an octahedral complex, is relatively insoluble, immobile and less toxic (Brown et al., 1999).

Chromium(VI) is a more stable form, in equilibrium with atmospheric oxygen, but in the presence of soil organic matter it reduces to Cr(III) (Cary et al.,1977; Grove and Ellis, 1980; Bolan et al., 2003; Banks et al., 2006; Jiang et al., 2008). Ferrous iron and H_2S can also reduce Cr(VI) to Cr(III), which is subsequently adsorbed onto soil particles (Eary and Rai, 1989). Although in most soils Cr(VI) species are reduced to Cr(III), in some soils that are rich in oxidising compounds such as oxidised manganese, Cr(III) is oxidised to Cr(VI) species when pH is greater than 5 (Bartlett and James, 1979; Fendorf and Zasoski, 1992). The redox reactions of Cr(VI) are largely controlled by the adsorption in soils, the supply of oxidising/reducing (redox) compounds and microbial activity. Clays and sesquioxides that are components of soil are also known to adsorb Cr (Davis and Leckie, 1980; Zachara et al., 1989). The detoxification of Cr(VI) through reduction to Cr(III) and its subsequent adsorption is considered to be an effective method for remediating Cr contaminated soil.

Bioavailability of heavy metals is controlled mostly by adsorption-desorption reactions at the mineral-water interface, and adsorption is a common mechanism to remove metals and takes place at solute-mineral interface (McBride, 1994). Adsorption of metals depends on soil properties, which in turn is influenced by soil constituents such as organic matter (OM), Fe and Al oxides, and clay minerals (Vega et al., 2006). The study of adsorption of Cr(VI) and Cr(III) is important in soils to understand toxicity and bioavailability of these two species. Mobility of Cr(VI) in natural environments is mostly controlled by sorption onto metal oxide surfaces; the oxygen moiety of Cr(VI) replaces by hydroxyl groups on the metal oxide surface and forms an inner-sphere complex. Saw dust and used tyres have been used to remove Cr(VI) from aqueous solution via sorption (Hamadi et al., 2001).

Since Cr(VI) and Cr(III) have opposite characteristics, they behave differently in soils. While Cr(III) retains strongly in soil particles, Cr(VI) shares a smaller charge, compared to other anions such as arsenic species, forms a weaker bond on adsorption (McBride, 1994) and it also exhibits reduced adsorption at near-neutral pH values (Grossl et al., 1997). Hence it is necessary to understand the dynamics of these two species in soils.

Among the properties that were reported (Table 4.1) on sorption of Cr species, there was no relative comparison of adsorption, desorption and mobility of these two Cr ions by various soil types in Australia.

4.2 Objectives

The overall objective of this research is to investigate the effect of different physio-chemical soil properties on the relative sorption and mobility of Cr(III) and Cr(VI) in a wide range of soils. The specific objectives of experiments described in this chapter include,

- To determine physio-chemical properties of a wide range of soils in Australia
- To evaluate the relationship between soil properties and the adsorption of Cr(III) and Cr(VI) in soils
- To measure the amount of Cr(III) and Cr(VI) desorbed from two different soils that vary in pH (acidic, ADH; alkaline, KPB)
- To estimate the leaching of Cr(III) and Cr(VI) in two different soils
- To predict the relative mobility of Cr(III) and Cr(VI) as affected by soil properties and their adsorption characteristics in soils.

4.3 Hypothesis

The sorption of Cr(III) and Cr(VI) is influenced by soil properties such as pH, CEC, surface charge, organic matter, clay, iron (Fe) and aluminium (Al) oxides, water extractable sulphate and phosphate. The mobility of Cr(VI) is higher in alkaline than acidic soils. In contrast, Cr(III) mobility is higher in acidic and near neutral soils than alkaline soils.

4.4 Experiments

The various experiments conducted for the adsorption of Cr(III) and Cr(VI) in contaminated soils are listed in Table 4.1.

Table 4.1 The major experiments and the treatments used in this Chapter

Experiments	Treatments	Measurements
Adsorption	12 soils* – BIT, DKT, UCT, UCB, ADH, RHB, RHT, RBB, PWT, CLV, WAL, KPB. Cr(III) (0-1000 mg/kg); Cr(VI) (0-500 mg/kg)	pH and adsorption parameters
Desorption	Acidic (ADH) and alkaline (KPB) soils Cr(III) (0, 600-1000 mg/kg); Cr(VI) (0-500 mg/kg)	Cr(III) and Cr(VI) concentrations (cumulative)
Mobility	12 soils	Bulk density, volumetric water content
Leaching	Acidic (ADH) and alkaline (KPB) soils Cr(III) (0 and 1000 mg/kg); Cr(VI) (0 and 500 mg/kg)	Cr(III) and Cr(VI) concentrations in leachates (cumulative)

4.5 Materials and methods

4.5.1 Soil sampling

All soil samples were collected from uncontaminated land. Twelve different surface soils ranging in depth from 0-10 cm for representative soil profiles in South Australia, Queensland, and New South Wales were used in this study. The collected soils vary widely in their origin, mineralogy and soil properties (Table 4.2). The air-dried soil samples were disaggregated with agate mortar and sieved using a steel screen with an opening of 2 mm. This ensured uniformity and they were stored in polythene bags for further analysis.

4.5.2 Characterization of soils

The soil samples were analysed for pH, clay content, cation exchange capacity (CEC), surface charge, organic matter, different fractions of iron and aluminum, water extractable sulphate (SO_4^{2-}) and phosphate (PO_4^{3-}).

Soil pH was measured in water and 0.01 M $CaCl_2$ at a soil: solution ratio of 1:2.5 (w/v ratio) using a pH meter. Soil sample (5 g) was weighed into a 50 ml polypropylene centrifuge tube, mixed with 12.5 ml of 0.01 M $CaCl_2$, shaken in an end-over-end shaker for

30 minutes and pH was measured with a pH electrode (TPS Smartchem-lab, Australia) after calibration. The cation exchange capacity (CEC) of soils was determined by a method described by Gillman and Sumpter (1986). This technique involves the displacement of cations on exchange sites with 0.2 M NH_4Cl. The soil (2 g) soil was taken in 50 ml polypropylene centrifuge tube, mixed with 20 ml 0.2 M NH_4Cl, and shaken for 2 hours. The samples were centrifuged at 1789 *g* for 20 minutes, filtered through 0.45 µm membrane filter tips and analysed for displaced cations (Ca, Mg, K and Na) by ICP-MS (Agilent, 7500ce, USA). Total organic carbon was measured by Walkley and Black (1934) dichromate oxidation method. A factor of 1.72 was used to convert organic carbon to organic matter.

Clay percentage was estimated by pipette method (Miller and Miller, 1987) after dispersion in sodium hexametaphosphate and sodium hydroxide. Water extractable SO_4^{2-} and PO_4^{3-} were measured using ion chromatography. Two grams of soil was put into a polypropylene centrifuge tube and equilibrated with 20 ml of Milli-Q water and shaken (end-over-end) for 16 hours. The suspension solution was then centrifuged at 1789 *g* for 20 min, filtered through 0.45 µm membrane filter tips, and analysed for S and P using ion chromatography (Dionex, ICS 2000-RFIC, Japan). Different fractions of iron (Fe) and aluminium (Al) were extracted; with dithionate-citrate-bicarbonate for crystalline Fe and Al (Mehra and Jackson, 1960); ammonium oxalate for amorphous Fe and Al (McKeague and Day, 1966); sodium pyrophosphate for organic bound Fe and Al (McKeague, 1967) and were analysed on ICP-MS.

4.5.3 Surface charge

To examine the influence of soil surface charge on the sorption of Cr(III) and Cr(VI), positive and negative surface charges were estimated by measuring Cl^- and Na^+ retention following a ion-retention method of Schofield (1949). Two grams of (<0.5 mm) air dried soil samples was mixed with 40 ml 1M NaCl solution in a weighed centrifuge tube for 30 minutes, centrifuged (20 min, 1789 *g*), and soil was retained for four further washings with 0.1M NaCl. The soil was then shaken with 0.02 M NaCl solution for 5 min to reduce the concentration of entrained solution. The tubes were weighed and the adsorbed Na^+ and Cl^- ions were then displaced and extracted by shaking for 20 mins with 20 ml quantities of 1M NH_4NO_3 solution. Concentration of Cl^- and Na^+ was measured on ion chromatography and ICP-MS, respectively.

4.5.4 Batch Sorption

Distilled and double deionised water (Millipore, Billerica, MA) was used to prepare all the solutions. Chromium(III) solution was prepared by dissolving chromium nitrate $Cr(NO_3)_3$, Cr(VI) from potassium dichromate ($K_2Cr_2O_7$) and both chemicals were purchased from Sigma-Aldrich, Australia.

Adsorption isotherm represents the amount of solute adsorbed per unit weight of adsorbent as a function of equilibrium concentration in the solution at constant temperature. A batch technique was used to perform all adsorption studies. 2.0g of each of soil sample was taken (triplicates) in 50 ml polypropylene centrifuge tubes, mixed with 20 ml of Cr solutions [$Cr(NO_3)_3$ for Cr(III) and $K_2Cr_2O_7$ for Cr(VI)]. The solution concentrations used were 0-1000 mg/L for Cr(III) and 0-500 mg/L for Cr(VI) with 0.1M KNO_3 as the background electrolyte. The treated samples were shaken on an end-over-end shaker at room temperature (22 ± 2^0 C). After 16 hours of shaking, suspensions were centrifuged at 1789 *g*, filtered through 0.45-µm Millipore filter tips and analysed for Cr remaining in the solution. This entailed the use of an Inductively Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) (Perkin-Elmer Optima-5300, Japan). The amount of Cr species sorbed by soils was calculated as follows:

$$S_{ads} = \frac{(C_o - C_e) * V}{W} \qquad (4.1)$$

where S_{ads} is the amount sorbed (mg/kg), C_o is the initial concentration (mg/L), C_e is the equilibrium concentration (mg/L), V is the solution volume (L), and W is the weight of the air-dried soil sample (kg) (Eqn. 4.1). However, there was no difference between total Cr(VI) and KH_2PO_4 extractable Cr(VI), confirms the negligible reduction of Cr(VI) in soils.

4.5.5 Adsorption isotherm models

Sorption isotherm interprets information about adsorption capacity and retention strength by which the sorbate is held onto the soil. The sorption of solutes in soils is often described using Langmuir and Freundlich isotherms. Langmuir adsorption isotherm describes the build-up of molecules of adsorbate on the surface of adsorbent and assumes that the number of surface sites is limited (Langmuir, 1918). The Langmuir isotherm parameters were calculated using the Eqn. 4.2

$$S_{ads} = \frac{b\, X_m\, C_e}{(1 + b\, C_e)} \quad (4.2)$$

where S_{ads} is the quantity of solute adsorbed (mg/kg), C_e is solute concentration at equilibrium, b is binding constant that reflects the relative rates of adsorption at equilibrium, and X_m is maximum adsorption capacity. The Freundlich model (Freundlich, 1928) (Eq. 4.3) is an empirical equation based on the distribution of solute between the solid phase and aqueous phase at equilibrium:

$$S_{ads} = K_f\, C_e^{n} \quad (4.3)$$

where S_{ads} is the amount of Cr sorbed (mg/kg), C*e* is concentration of Cr at equilibrium, K_f is sorption coefficient (L/kg), and n is sorption intensity (dimensionless). The lower the n denotes the degree of deviation from isotherm linearity.

4.5.6 Desorption of Cr in soils

To estimate the desorption of Cr(III) and Cr(VI) in acidic (ADH) and alkaline (KPB) soils, batch experiments were conducted. Soil samples (2 g) were equilibrated with Cr(III) (0, 600-1000 mg/kg) and Cr(VI) (0-500 mg/kg) for 16 hours at 1:10 (soil: solution) ratio using an end-over-end shaker at 15 rpm at a constant temperature of 22 ± 2^{O}C. The samples were centrifuged at 1789 *g* for 20 minutes. For desorption of Cr(III) and Cr(VI), 1M KNO_3 and $NaNO_3$, respectively were used as electrolytes (Jiang et al., 2008). The weights of the tubes with entrained solutions were recorded. Following adsorption measurements, the soils were equilibrated for two hours with electrolytes at 1:10, soil and solution ratio and repeated 5 times until there was no further Cr in desorbing solutions and weights of soil and entrained solutions were recorded after each step. The concentrations of Cr(III) and Cr(VI) in desorbing solutions (cumulative) were analysed using ICP-OES and spectrophotometer, respectively.

4.5.7 Leaching Cr in soils

For the leaching experiment, acidic (ADH) and alkaline (KPB) soils were incubated with 1000, 500 mg Cr(III) and Cr(VI)/kg, respectively at field capacity. Following three days' incubation, soils were transferred and packed in 60 ml syringe columns (3 cm in diameter X 7.5 cm in heights) and leached with 0.001M $CaCl_2$. The columns were leached for 6 pore volumes and the leachate samples were collected for every pore volume of the

respective soils (10.1 ml for ADH and 11.4 ml for KPB). The leachate Cr(VI) concentration was measured immediately after collection by spectrophotometer using 1, 5-Diphenyl Carbazide as colour developing reagent. Chromium(III) was measured by subtracting Cr(VI) from the total Cr (analysed on ICP-OES).

4.5.8 Mobility of Cr species in different soil types

The mobility of Cr species was also estimated in terms of retardation factor (R_f) according to the following equation (Kookana et al., 1990):

$$R_f = 1 + [(D_b / \theta_s)^n K_f C^{n-1}] \quad (4.4)$$

where R_f is retardation factor (which is an index of mobility of Cr species), D_b is bulk density (g/cm^3), θ_S is volumetric water content (m^3/m^3). K_f and n are Freundlich sorption constants, and C is equilibrium solution concentration (Eqn. 4.4).

4.5.9 Statistical analysis

The effect of soil properties on the sorption of Cr(III) and Cr(VI) was examined using correlation analysis. The software Statistical Product and Service Solutions (SPSS) 17.0 l was used to conduct all the statistical analyses to find the relationship between the soil properties and chromium sorption capacity. Sorption isotherms data were fitted to the Freundlich and Langmuir adsorption models using Grapher™ software (Golden Software, Version 7, USA). The mean standard deviation (STD) values were obtained from the triplicates of each sample using Microsoft Excel.

4.6 Results and discussion

4.6.1 Properties of soils

The selected properties of 12 soils are listed in Table 4.2. The pH of these soils ranged from 3.07 to 8.10 and CEC ranged from 2.1 to 7.7 cmol/kg. Organic matter content ranged from 0.38 to 6.7% and clay content from 3.1 to 62.3%. Water extractable SO_4^{2-} values were within 0.97 to 9.56 mg/kg and water extractable PO_4^{3-} concentrations were from 0.14 to 2.52 mg/kg.

4.6.2 Sorption isotherms as a function of Cr concentration

The sorption capacity of Cr(III) and Cr(VI) in soils, as indicated by the values of K_f and X_m were calculated using the Freundlich and Langmuir equations, respectively. Although it is difficult to compare two adsorption isotherm equations, the regression coefficients (R^2) for the Freundlich equation were higher than the Langmuir equation (Table 4.2), indicating that the Freundlich isotherm explained Cr sorption better than the Langmuir isotherm. Similarly, Agarwal et al. (2006) reported that the Freundlich adsorption isotherm exhibited better fit than the Langmuir adsorption isotherm for removing aqueous Cr(VI).

Freundlich sorption coefficients (K_f) ranged from 120 to 2240 for Cr(III) and from 0.03 to 173 for Cr(VI) suggesting that sorption of Cr(III) is far higher than Cr(VI). The higher sorption of Cr(III) may be attributed to its stronger affinity to the negatively charged surface of soils. In contrast, low sorption of Cr(VI) may have resulted from the repulsion between Cr(VI) ions and negatively charged colloids of soils. Bradl (2004) concluded with reference to the contrasting adsorption properties of Cr species, that Cr(III) sorption was higher than Cr(VI), in a range of 30-300 times by clay minerals.

Table 4.2 The major experiments and the treatments used in this Chapter.

Soil	Source	Classification	pH	CEC (cmol kg^{-1})	clay %	OM %	SO_4^{2-} (mg/kg)	PO_4^{3-} (mg/kg)
BIT	QLD	Podosol	3.07±0.03	2.1±0.23	3.1±0.32	2.07±0.23	BDL	2.067±0.46
DKT	NSW	Tenosol	4.00±0.01	2.9±0.28	13.2±0.38	1.25±0.23	BDL	0.938±0.28
UCT	QLD	Kurosol	4.06±0.03	4.1±0.12	9.2±0.21	1.29±0.19	BDL	BDL
UCB	QLD	Kurosol	4.10±0.07	3.4±0.17	18.5±0.16	0.49±0.27	6.48±0.06	1.538±0.068
ADH	SA	Kurosol	4.60±0.11	3.9±0.15	23.7±0.06	1.06±0.21	2.3±0.2	0.375±0.194
RHB	NSW	Ferrosol	4.62±0.06	4.3±0.25	53.8±0.15	2.05±0.08	9.36±0.11	1.378±0.146
RHT	NSW	Ferrosol	4.83±0.03	7.6±0.49	42.6±0.03	4.63±0.03	4.97±0.13	1.413±0.347
RBB	QLD	Ferrosol	5.58±0.04	5.9±0.32	62.3±0.12	0.38±0.16	8.63±0.28	1.212±0.076
PWT	QLD	Vertosol	6.53±0.04	6.1±0.11	47.3±0.15	1.58±0.31	8.85±0.15	2.525±0.174
CLV	SA	Sodosol	7.15±0.09	6.9±0.14	38.6±0.25	2.58±0.47	6.56±0.38	0.582±0.345
WAL	SA	Calcarasol	7.58±0.05	7.6±0.16	10.7±0.14	0.97±0.05	9.56±0.27	BDL
KPB	SA	Chromosol	8.10±0.06	7.7±0.34	45.4±0.07	6.74±0.58	BDL	0.14±0.067

BDL, below detectable level (detection limit-0.05 mg/L)

Bribie Island, QLD-**BIT**; Drake, NSW-**DKT**; Kulpara, SA-**KPB**; Redland Bay, QLD-**RBB**; Richmond Hill NSW, **RHT**; Richmond Hill NSW-**RHB**; Pittsworth QLD-**PWT**; Upper Coomera; QLD-**UCT**; Upper Coomera-B horizon; QLD-**UCB**; Wallaroo, SA-**WAL**; Adelaide Hills, SA-**ADH**; Clare Valley,SA-**CLV.**

Table 4.3 Estimated sorption coefficients of Cr(III) and Cr(VI) in different soils measured by Freundlich and Langmuir adsorption isotherms. Data are the means ± standard deviation of three replicates.

Soil type	Freundlich isotherm						Langmuir isotherm					
	Cr(IIII)			Cr(VI)			Cr(III)			Cr(VI)		
	K_f	n	R^2	K_f	n	R^2	X_m	b	R^2	X_m	b	R^2
BIT	141.3±0.806	0.90±0.024	0.984	3.65±0.151	0.35±0.064	0.975	1341	0.011	0.726	1433	0.0023	0.753
DKT	121.3±0.749	0.90±0.47	0.978	0.4±0.08	0.35±0.037	0.953	1351	0.010	0.617	741	0.0023	0.644
UCT	230.8±0.176	0.88±0.25	0.983	5.05±0.066	0.30±0.044	0.976	1526	0.0168	0.774	1878	0.0020	0.835
UCB	139.6±0.574	0.76±0.073	0.971	1.43±0.034	0.37±0.051	0.979	1486	0.011	0.566	1043	0.0012	0.65
ADH	702.2±0.434	0.73±0.185	0.924	19.1±0.208	0.14±0.134	0.984	1626	0.08	0.795	2391	0.0026	0.889
RHB	441.8±0.616	0.62±0.154	0.987	34.2±0.241	0.25±0.069	0.981	1981	0.030	0.587	2137	0.0041	0.877
RHT	1042.0±0.948	0.89±0.348	0.986	6.24±0.047	0.17±0.036	0.988	2752	0.101	0.545	5004	0.0008	0.872
RBB	620.1±0.475	0.40±0.216	0.953	172.1±0.478	0.19±0.027	0.983	1898	0.047	0.894	2165	0.0119	0.668
PWT	731.2±0.537	0.92±0.174	0.944	0.05±0.041	0.57±0.032	0.987	5837	0.102	0.715	403	0.0017	0.811
CLV	1259.8±0.494	0.84±0.451	0.985	0.08±0.003	0.28±0.076	0.946	179	1.158	0.787	279	0.0022	0.762
WAL	2025±0.546	0.91±0.167	0.991	0.04±0.001	0.19±0.095	0.984	87	2.125	0.626	133	0.0023	0.805
KPB	2238.7±0.57	0.88±0.132	0.986	0.03±0.018	0.20±0.051	0.984	44	1.673	0.763	146	0.0021	0.853

4.6.3 Sorption of Cr(III) and Cr(VI) as a function of soil properties

4.6.3.1 Effect of pH on sorption of Cr(III)

Soil pH plays a pivotal role in the adsorption of heavy metal cations because it affects the solubility of hydroxides, organic matter solubility, and surface charge of iron and aluminium. Metal cations in the solution retain to the soil surface by adsorption, complexation and precipitation (McBride, 1994; Sparks, 1995). Sorption of Cr(III) in the soils followed the expected trend, and rose when the pH level in the soil increased (Fig. 4.1). Of these soils, alkaline soil (KPB) has the highest sorption capacity (k_f =2238 L/kg).

Soil pH affects the solubility, adsorption and speciation of metal(loid) ions in several ways, foremost of which include: (a) modification of surface charge in variable charge soils; (b) altering the speciation of metal(loid)s; and (c) influencing the redox reactions of the metal(loid)s (Adriano, 2001). Adsorption of metals almost invariably increases with increasing soil pH (Tiller, 1989; Yang et al., 2006). Three possible reasons have been advanced for this phenomenon (Naidu et al., 1994). Firstly, in variable-charge soils, an increase in pH causes an increase in surface negative charge resulting in higher cation adsorption. Secondly, an increase in soil pH is likely to increase hydroxy species of metal cations (MOH_n^+) (Eqn. 4.5), which are adsorbed preferentially over mere metal cation. The dissolved Cr(III) species at higher pH are surrounded by an inner hydration sheath of six water molecules with octahedral symmetry and forms hydroxocomplexes (Ziemniak et al., 1998). Thirdly, precipitation of Cr^{3+} as $Cr(OH)_3$ is likely to result in greater retention at pH values above 10.

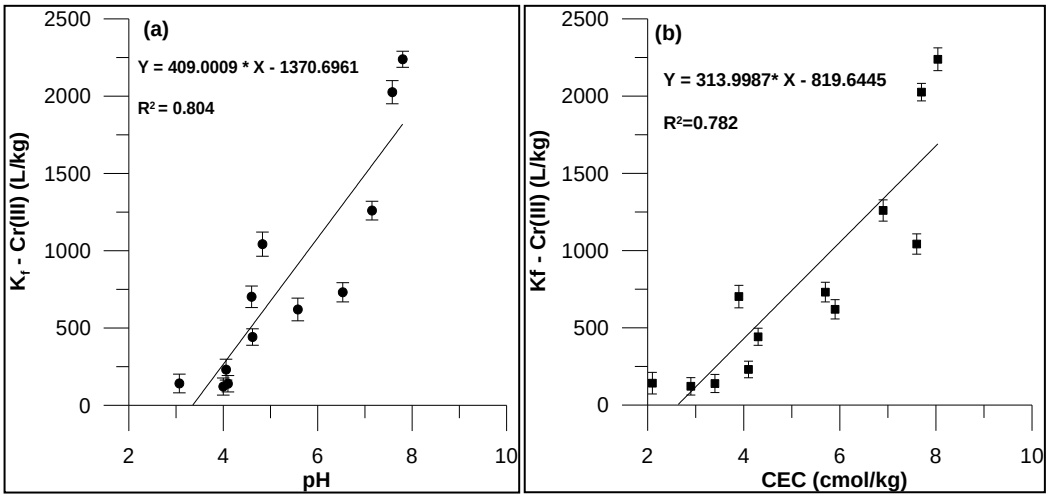


Figure 4.1 Relationship between pH (a) and CEC (b) on the adsorption of Cr(III) ($P \leq 0.05$).

Chromium(III) adsorption is highly influenced by pH and cation exchange capacity (CEC). The increased adsorption of Cr(III) with an increase in pH (Fig. 4.1a) may be attributed to the adsorption of Cr(III)-hydroxy complexes and cation exchange reactions (Fig. 4.1b) (Grove and Ellis, 1980). Cao et al. (2011) found that high adsorption of Cr(III) was attributed to the high CEC in soils. At soil pH below 5.5, Cr(III) adsorbs on the negative surface charge of soils and above pH 5.5, Cr(III) precipitates as $Cr(OH)_3$ and accumulates on the soil surface (Stewart et al., 2003) (Eqn 4.5).

$$Cr^{3+}\ (aq) + n\ H_2O \rightarrow Cr(OH)_n^{3-n} + nH^+ \qquad (4.5)$$

The increase in the sorption of Cr(III) with an increase in pH could be explained by the fact that competition between protons and Cr(III) decreased; as the pH increases protons in the soil deplete. Soil negative charge increased with the pH (Fig. 4.2a), so the sorption of Cr(III) would indicate adsorption on negative charges on soil clay surface or surface complexation. At the same time, decrease in positive surface charge results in lower electrostatic repulsion between surface and Cr(III).

4.6.4 Influence of soil properties on the sorption of Cr(VI)

In all soils, Cr(VI) adsorption was much lower than that of Cr(III). While Cr(III) adsorption increased as pH increased, the effect of pH on the Cr(VI) adsorption was not consistent (Fig. 4.3a). The change in adsorption of Cr(VI) was not significant when compared to other metal ions with a change in pH of soil. The low sorption of Cr(VI) was attributed to the ionic nature, as Cr(VI) exists as an oxyanion and the adsorption mechanism differs from cation adsorption (Gao et al., 1997). Chromium(VI) species have weaker sorption in soils and the capability of soil for Cr(VI) sorption is much lower than other heavy metals (Tzou et al., 1998).

Adsorption of Cr(VI) is controlled by the availability of surface sites and rate of complexation of the dissolved species with surface sites (Sposito, 1994). The amount of clay that contains hydroxides and oxyhydroxides of Fe and Al, which in turn carry both positive and negative charge on their surface, can influence the sorption of anions. While CEC increased adsorption of Cr(III) in soil, there was no significant effect of CEC on the adsorption of Cr(VI) (Fig. 4.3b). However, adsorption was higher in soils containing high Fe, which may be attributed to high positive charge (Griffin et al., 1977; Rai et al., 1989; Zachara et al., 1989). High surface area of clay content in soil increases Cr(VI) sorption by providing positive adsorption sites. However, the clay content (Fig. 4.3c) in the soils used

in this study has less effect than positive charge (Fig. 4.3d) and crystalline Fe (Fig. 4.2b) on sorption of Cr(VI) in soil.

Fractions of iron (Fe) and aluminium (Al) oxides are major adsorbents of anions (Yu, 1997). McKeague and Day (1966) concluded that oxalate extraction was efficient in dissolving amorphous iron oxide, whereas dithionite citrate (Fe_{DC}) extraction could dissolve a large proportion of the crystalline iron. Sodium pyrophosphate extracts organic bound (OB) Fe and Al from the soils. Among all different fractions of Fe and Al (crystalline, amorphous and organic bound), only Fe_{DC} (crystalline Fe) was positively correlated with the sorption of Cr(VI) (Fig. 4.2b).

Water extractable SO_4^{2-} and PO_4^{3-} had no effect on or relationship to the sorption of Cr(VI). However, external addition of SO_4^{2-} and PO_4^{3-} has been shown to reduce the adsorption of CrO_4^{2-} due to competition for the positive sites on soil surface (Pradhan et al., 1999).

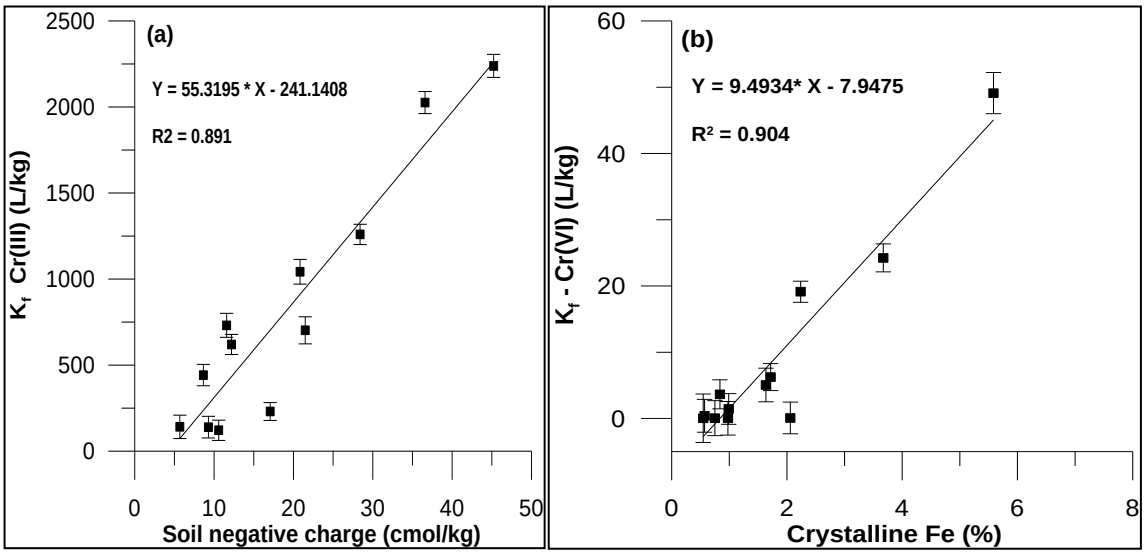


Figure 4.2 Sorption of Cr(III) influenced by (a) soil surface charge, and (b) sorption of Cr(VI) by Fe_{DC} ($P \leq 0.05$).

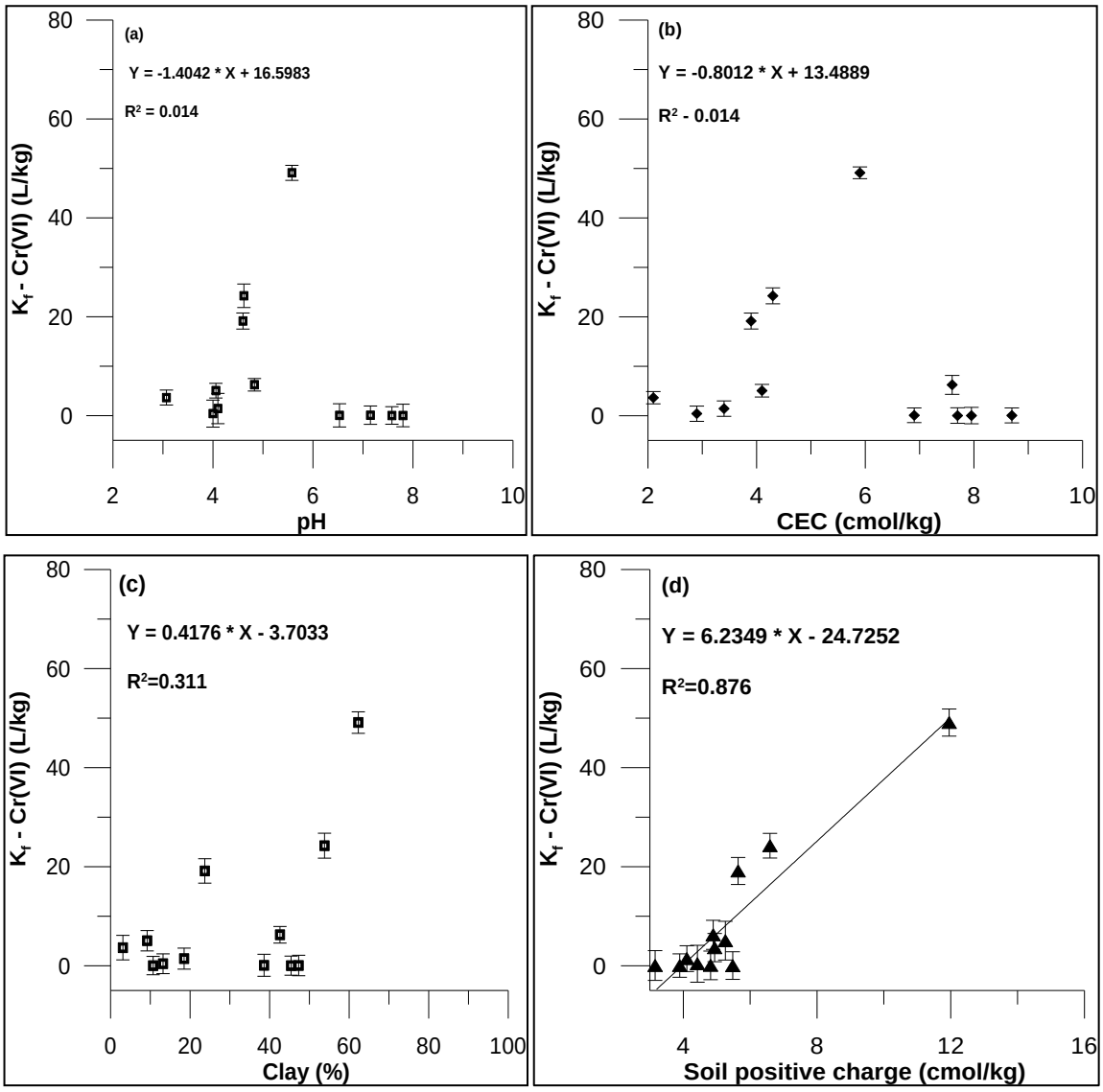


Figure 4.3 Effect of pH (a), CEC (b), clay (c) and positive charge (d) on the adsorption of Cr(VI) in soils.

4.6.5 Desorption of Cr in soils

The results showed that desorption of Cr(VI) was higher than Cr(III) in soils. After equilibration of ADH and KPB soils (Fig. 4.4) with 100 mg/kg Cr(VI), 92.69 and 98.60 mg/kg cumulative Cr(VI) desorbed, respectively. On the other hand 500.82 and 52.45 mg/kg cumulative Cr(III) desorbed from ADH and KPB soils, respectively which had been equilibrated with 600 mg/kg Cr(III). Following equilibration, the cumulative Cr(VI) desorbed was higher in alkaline soil than in acidic soil. The decrease in Cr(VI) desorption from ADH soil was attributed to its low pH, where net charge is positive and increases Cr(VI) retention. In KPB soil, due to its high alkaline nature (pH 8.10), Cr(VI) is readily mobile, hence the desorption was high. The Cr(VI) anions, chromate (CrO_4^{2}), bichromate ($HCrO_4^{-}$)

and dichromate ($Cr_2O_7^{2-}$) have weak sorption capacity in alkaline to slightly acidic soils (Palmer and Wittbrodt, 1994). Hence, Cr(VI) desorbs at a high rate in most of the soils.

The decreased desorption of Cr(III) in both ADH and KPB soils may be due to the formation of Cr(III)-hydroxy complexes. Moreover, Cr(III) has a strong tendency to bind to several ligands that render it insoluble and immobile in soils (Bartlett and James, 1984). The splitting energy (Δ_o) of Cr(III) in crystal field splitting theory is higher than for Cr(VI), forms stable octahedral complexes and forms strong bonds with electron donor groups and extremely immobile in soil (Naidu et al., 2000).

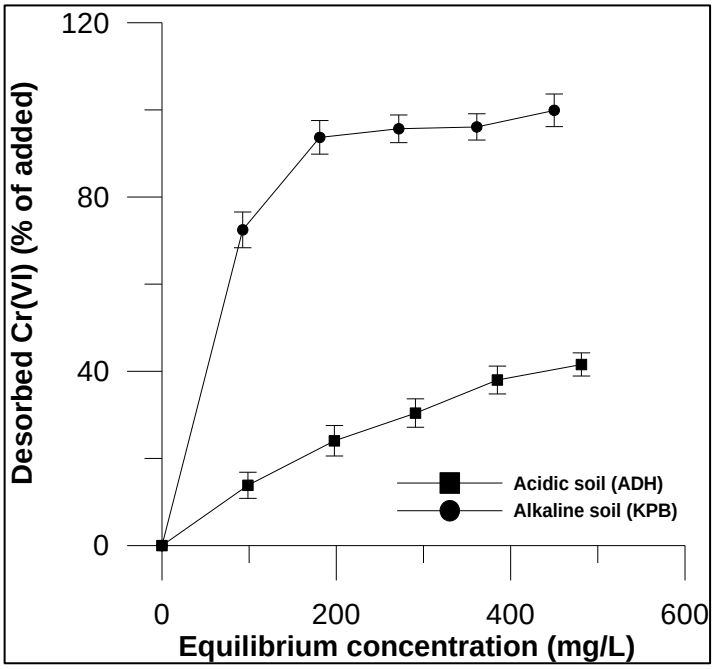


Figure 4.4 Desorption of Cr(VI) in acidic (ADH) and alkaline (KPB) soils.

4.6.6 Leaching of Cr in soils

The concentration of Cr(III) in the leachate in both ADH and KPB soils was lower than the Cr(VI) concentration. The total leached Cr(III) and Cr(VI) concentrations in ADH and KPB soils were 25.8 and 8.5% of total added Cr, respectively in ADH soil, and 14.9 and 34.6%, respectively in KPB soil (Fig. 4.5).

Chromium(III) leached at a higher rate in acidic soil than alkaline soil. In comparison, the mobility of cations is lower than anions in most of the soils. Being a cation, Cr(III) retains to negatively charged soil particles and colloids, and consequently any leaching was limited. In addition, precipitation of Cr(III) as $Cr(OH)_3$ that retards leaching, is another

possible mechanism for Cr(III) having less leaching capacity (Banks et al., 2006). Leaching of Cr(VI) is higher in both acidic and alkaline soils. In alkaline soil (KPB), After 3 days of incubation, there was no change in Cr(VI) concentration, most of the Cr(VI) was leached, whereas in acidic soil (ADH), half of the amount of Cr(VI) added was in labile form (Bloomfield and Pruden, 1980). The amount of Cr(VI) leached from Cr contaminated soils was found to be unchanged between pH 4.5 to 12 (Weng et al., 1994). Moreover, Cr(VI) from Cr contaminated alkaline soils is highly mobile and readily leaches into ground water (Mahimairaja et al., 2000; Naidu et al., 2000).

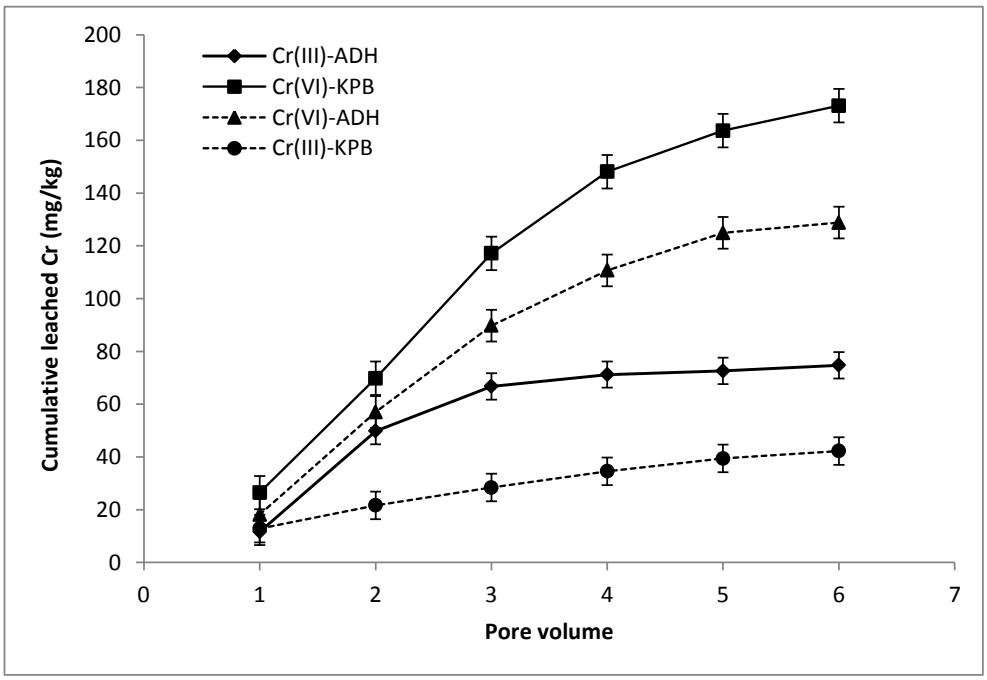


Figure 4.5 Cumulative concentration of Cr(VI) and Cr(III) in acidic and alkaline soils.

4.6.7 Mobility of Cr

Retardation factor (R_f) provides clues about the interactions between the soil matrix and solute (Wierenga and Van Genuchten, 1989). The retardation factors for Cr(III) and Cr(VI) obtained for the 12 soils are shown in Table 4.4. The values of R_f are higher for Cr(III), indicating that Cr(III) is retained strongly in soils and is less mobile (Fig. 4.6). In contrast, Cr(VI) retardation values are very low, suggesting that Cr(VI) is highly mobile and bioavailable.

As the pH of the soil increased, the R_f values of Cr(III) also increased. However these decreased for Cr(VI), indicating that Cr(III) has high adsorption with low mobility, whereas Cr(VI) has low adsorption with high mobility. The low mobility of Cr(III) with an increase in

pH can be attributed to both specific and non-specific adsorption and precipitation of Cr(III) in soils. An increase in soil pH reduces the availability of most toxic heavy metals in soils.

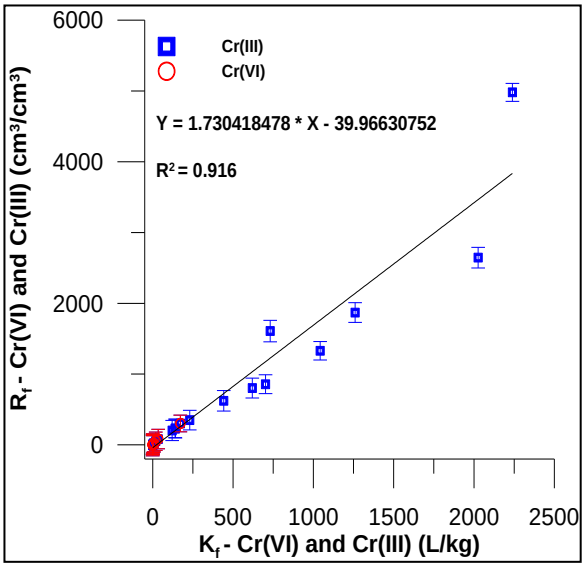


Figure 4.6 Relationship between adsorption, as measured by k_f and mobility, as measured by R_f.

Table 4.4 Physical parameters of some selected soils in Australia. Data are the means ± standard deviation of three samples.

Soil	D_b	V_m	Retardation factor	
	(g/cm^3)	(m^3/m^3)	(cm^3/cm^3)	
			Cr(III)	Cr(VI)
BIT	1.443±0.025	0.219±2.054	274.525±0.051	20.913±0.142
DKT	1.268±0.034	0.307±0.211	200.217±0.087	3.253±1.624
UCT	1.517±2.148	0.258±5.043	393.613±8.347	25.027±.0.416
UCB	1.532±0.131	0.311±0.247	252.846±2.503	8.632±0.148
ADH	1.379±0.257	0.286±2.568	876.254±0.445	58.531±2.408
RHB	1.265±0.442	0.271±0.571	650.458±5.038	89.998±0.147
RHT	1.251±4.039	0.289±2.342	1338.32±0.134	23.992±2.089
RBB	1.493±0.025	0.270±0.479	858.846±0.019	342.176±0.514
PWT	1.505±1.354	0.202±3.636	2298.24±7.471	2.251±0.548
CLV	1.116±0.547	0.386±0.196	1696.91±0.258	1.412±1.175
WAL	1.015±0.023	0.415±3.347	2402.12±0.485	1.007±0.698
KPB	1.023±0.091	0.409±0.482	2690.31±4.198	1.010±3.047

The leaching percentage of Cr(VI) and Cr(III) from both acidic and alkaline soils, and the retardation factor (R_f) that was calculated using bulk density, volumetric water content and K_f from sorption experiments revealed that there was a negative correlation between leaching and R_f in these two soils (Fig. 4.7).

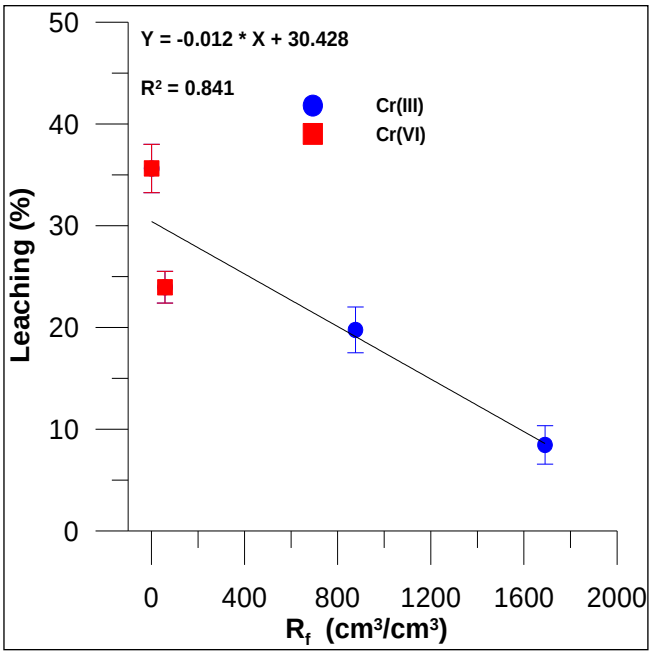


Figure 4.7 Mobility of Cr(VI) and Cr(III) as calculated from leaching and retardation factor.

4.7 Conclusion

- In all soils, Cr(III) adsorption was higher than that of Cr(VI). While Cr(III) adsorption increased with an increase in pH and CEC, there was no significant effect of these two parameters on the sorption of Cr(VI) in soils.

- Organic matter and clay content increased the sorption of Cr(III) but did not influence the sorption of Cr(VI). Desorption of Cr(VI) is higher than that of Cr(III) in soils and this feature was attributed to the pH of soils.

- Furthermore, Cr(VI) desorbed more from alkaline and acidic soils, whereas Cr(III) had the opposite trait. Chromium(VI) and Cr(III) leaching was high in alkaline and acidic soils, respectively, and this was consistent with their adsorption behaviour.

- Mobility of Cr(III) and Cr(VI) as measured by retardation factor suggested that Cr(VI) is more mobile than Cr(III) in soils.

Chapter 5

INFLUENCE OF AMENDMENTS ON THE RELATIVE SORPTION OF CHROMIUM SPECIES IN SOILS

5.1 Introduction

Industrial intensification has increased the utilization and release of heavy metals into the environment. Some of the industries such as tannery, electroplating, paints, cooling towers and timber treatment discharge effluents that contain chromium (Cr). The concentrations of Cr levels in the effluents should be reduced in order to maintain ecosystem integrity.

Adsorption is an effective method to decrease the mobility and availability, thereby arresting further contamination. There are several methods to mitigate Cr toxicity in soil and aquatic systems, that include, chemo and biosorption, and reduction of toxic Cr(VI) to less toxic Cr(III) species using various organic and inorganic amendments, leaf powders, seaweed, black carbon ((Mohan et al., 2008; Hsu et al., 2009).

The behaviour of Cr in soil depends on the physiochemical properties including, pH, organic matter, CEC and oxidation state of Cr (Banks et al., 2006). The retention of Cr in soils is important to decrease ground water and soil contamination, and in turn, retention is mainly controlled by the sorption, precipitation, redox reactions and nucleation reactions (Bradl, 2004). An increase in soil pH increases the retention of Cr(III) in soils. Whereas positive charge of iron oxides in the soil and soil colloids increases Cr(VI) retention (Stewart et al., 2003).

Several researchers have reported the utilization of amendments such as agricultural by-products, activated carbon, and minerals for the retention of Cr in soil (Singh et al., 1992; Leyva-Ramos et al., 1995; Samantaroy et al., 1997). However, there was no comprehensive research on the comparative retention of Cr(III) and Cr(VI) using amendments such as iron oxide, cow manure, sulphur (elemental) and lime in soils. Iron oxide carries both positive and negative charge in soil based on pH and point of zero net

charge (PZNC) and therefore can be used to retain both anions and cations in soils (Van Ranst et al., 1998).

Furthermore, results in Chapter 4 have indicated that there was a strong relationship between dithionate extractable Fe (Crystalline) and Cr(VI) adsorption in soils (Section 4.6.4, Chapter 4). The presence of positive charge also influences Cr(VI) adsorption in soils. Whereas, for Cr(III) adsorption, pH, CEC, clay and native organic matter have significant positive effect. Cow manure adds significant amount of organic matter into the soil and encourages microbial population. In addition, organic matter in cow manure comprises several functional groups, capable of donating electrons to reduce and retain Cr in soils. Addition of lime increases the pH by releasing hydroxyl ions and also increases surface negative charge in soil; and incorporation of elemental sulfur decreases soil pH by releasing H^+ (Haynes and Naidu,1998; Cui et al., 2004; Bolan et al., 2004).

In this experiment, we examined the effect of two groups of soil amendments for the retention of Cr species in soil. The lime and elemental sulfur were used to change the pH; iron oxide and cow manure were used to change the surface charge in soil.

5.2 Objectives

The overall objective of this experiment was to evaluate the influence of some common soil amendments on the relative sorption of Cr species in soil.
The specific objectives include

- To demonstrate the effect of lime and elemental S on the soil pH.
- To investigate the effect of iron oxide and organic amendments on the surface charge of soils.
- To evaluate the influence of pH on the retention of Cr species and
- To evaluate the effect of organic amendments on the retention and reduction of Cr species in soils.

5.3 Hypothesis

The retention of Cr(III) and Cr(VI) is influenced by soil pH, surface charge and availability of electrons. In the presence of organic matter, Cr(VI) is reduced to Cr(III) and subsequently immobilized on negatively charged soil surface. The hypothesis tested was that the addition of lime and elemental sulfur increases the retention of Cr(III) and Cr(VI),

respectively due to change in pH; addition of iron oxide and organic matter increases the positive charge thereby increasing the retention of Cr(VI) in soil.

5.4 Experiments

The major experiments conducted to examine retention of Cr using different amendments are listed in Table 5.1.

Table 5.1 The major experiments conducted and used in chapter 5

Experiment	Treatments	Measurements
Effect of pH on retention of Cr	Neutral soil: pH altered using sulfur (elemental) and lime - based on soil buffering capacity. Cr concentration range: 0-500 mg/kg	Buffering capacity, pH, adsorption of Cr(VI) and Cr(III) species.
Effect of surface charge on Cr retention in soils	Neutral soil: mixed with iron oxide (5%) Cr concentration range: 0-500 mg/kg	CEC, adsorption of Cr(VI) and Cr(III) species.
Effect of organic amendments on Cr retention	Neutral soil: mixed with cow manure as organic amendment (5%) Cr concentration range: 0-500 mg/kg	DOC, pH, adsorption of Cr(VI) and Cr(III) species pH, CEC, OM.

5.5 Materials and methods

5.5.1 Soils and amendments

The neutral soil (CLV) was collected from Clare valley, South Australia. The soil was mixed thoroughly, air dried under shade, sieved through 2mm steel screen and stored in plastic containers until further analysis. Iron(III) oxide was purchased from Chem-supply, South Australia. Lime, elemental sulfur and cow manure were purchased from a local agricultural store. Cow manure was ground and sieved through 2mm steel screen.

To change the pH of the soils, lime and sulphur (elemental) were mixed at various levels (1, 2 and 3 units) based on the buffering capacity (10.13 mmol/kg) and incubated at field capacity for one month. The soil was mixed with iron oxide and cow manure at 5% addition (w/w basis) and incubated for a month at field capacity.

5.5.2 Characterization of soil

The soil was analysed for pH, CEC (cmol/kg) and organic matter (%), buffering capacity and surface charge. The pH of soil was estimated using 0.01M $CaCl_2$, CEC using NH_4Cl to displace cations in soil solution; and total organic carbon was measured by Walkley and Black (1934) dichromate oxidation method. A factor of 1.72 was used to convert organic carbon to organic matter).

The detailed methodologies of determination of pH, CEC and organic matter were mentioned in Section 4.5.2. in Chapter 4. The buffering capacity of soil was estimated by titrating soil with increasing volumes of 0.2 M hydrochloric acid (HCl) and sodium hydroxide (NaOH) (Aitken and Moody, 1994). The solutions were added to 20 g soil at different volumes from 2 to 6 ml, added appropriate amount of water to make total volume of solution to 8 ml and soil suspension sample was shaken in an end-over-end shaker for 25 mins at $24^{O}C$. The soils were further mixed with 42 ml water to equilibrate for further 5 minutes and recorded the pH. Titration curves were plotted using pH of soil suspension and acid/base input; buffering capacity was deduced from drawing a tangent to the curve of best fit drawn in the graph and results were expressed in mmol OH^- or H^+/kg soil/pH.

The surface charge of the soils was estimated using 0.5 M $CaCl_2$ as background electrolyte at soil and solution ratio of 1:20. The soil and solution mixture was shaken end-over-end for 1 hour, centrifuged at 1758 *g* and decanted the supernatant solution. This step was repeated twice. The equilibrated soil samples were centrifuged at 1789 *g* for 20 min and filtered through 0.45 µm filter tips. The supernatant solution was discarded and weights of the soil solution along with test tubes were noted down in every step. The soils then were washed two times with MilliQ water and again the soils were equilibrated with 0.01 M $CaCl_2$ for an hour. After discarding the supernatant solution after centrifugation, 20 ml 0.5 M KNO_3 was mixed to the soil and equilibrated again in an end-over-end shaker for an hour. The supernatant solutions after centrifugation were collected and this step was repeated three times. The surface negative and positive charges were calculated from the concentrations of Ca^{2+} and Cl^- that were measured on ICP-MS and Ion chromatography, respectively (Schofield, 1967; Naidu et al., 1994).

5.5.3 Adsorption experiments

Batch sorption experiment was conducted to measure the extent of sorption at various concentrations of Cr(III) and Cr(VI) by the amended soil samples. Experiments were conducted at 24^0C and carried out in triplicates with a background electrolyte ionic strength of 0.1 M KNO_3. Chromium(III) and Cr(VI) solutions were prepared in 0.1M KNO_3 using chromium sulfate and potassium dichromate, respectively. Adsorption of Cr was measured at various concentrations in the range between 0-500 mg/L. Soil samples were mixed with Cr solutions at a soil: solution ratio of 1:10 by shaking on an end-over-end shaker for 16 hours. The resulting samples were centrifuged at 1789 *g* for 20 min and filtered through 0.45 µm filter tips.

The concentration of total Cr was measured by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and Cr(VI) species was measured by spectrophotometry at 540nm wavelength using 1, 5-diphenyl carbazide as a colour developing reagent. Chromium(III) was calculated by subtracting Cr(VI) from total Cr. The amount of adsorbed Cr was calculated from the difference between the added amount and that remaining in solution after equilibration of 16 hours. The amount of Cr(III) and Cr(VI) sorbed by soils was calculated from Eqn. 4.1 in Section 4.5.4 in Chapter 4.

5.5.4 Adsorption isotherm

The most commonly used isotherms; Langmuir (Eqn. 4.2) and Freundlich (Eqn. 4.3) were fitted to Cr isotherm data for the soil used in this chapter. The parameters of Langmuir and Freundlich isotherms were mentioned in Section 4.5.5 in Chapter 4.

5.6 Statistical analysis

The impact of soil amendments on the retention of Cr(VI) and Cr(III) was examined by correlation analysis. Statistical analysis was done using Statistical Product and Service Solutions (SPSS) 18.0 to derive the relationship between the Cr retention capacity and soil properties as affected by soil amendments and Grapher 7.0 was used to draw the sorption isotherms.

5.7 Results and discussion

5.7.1 Soil properties

Soil physical and chemical properties are listed in Table 5.2. The soil pH was 7.15, CEC was 6.93 cmol/kg and clay content was 38.6 %. The pH buffering capacity of soil was 10.13 mmol OH^- or H^+/kg soil/pH. The pH of neutral soil (7.15) was altered to 8.23, 9.12 and 10.36 using lime; to 6.24, 5.13 and 4.38 using elemental sulphur (S(0)). When elemental sulphur is added to soils, it is oxidised to sulphuric acid and further dissociates into H^+ ions and SO_4^{2-} ions (Eqn 5.1). Addition of lime neutralises H^+ ions, Al^{3+} ions precipitates into non-toxic forms, Ca^{2+} and Mg^{2+} from the lime increases % base saturation, thereby pH raises (Eqn 5.2).

$$2S^o + 2H_2O + 3O_2 \rightarrow 2SO_4^{2-} + 4H^+ \quad (5.1)$$

$$CaCO_3 + H_2O \rightarrow Ca^{2+} + HCO_3^- + OH^- \quad (5.2)$$

5.7.2 Adsorption of chromium

The results revealed that the Freundlich equation flitted better than the Langmuir equation as indicated by higher correlation coefficient values, which is applicable for non-specific adsorption on heterogeneous soil surface (Table 5.3). The better fit of Freundlich isotherm for adsorption of Cr(VI) and Cr(III) species was also supported by other studies (Lazaridis and Asoudihou, 2003; Bishnoy et al., 2004; Dubey and Gopal, 2007; Li et al., 2007)

Adsorption of Cr as measured by Freundlich sorption coefficients (K_f) for Cr(III) and Cr(VI) was 1259.8 and 0.079 L/kg, respectively, suggesting that Cr(III) sorption is far higher than Cr(VI). Adsorption of Cr was highly influenced by the addition of amendments (Bolan and Duraisamy, 2003; Chiu et al., 2009). The adsorption of Cr(III) was higher in soils that were incubated with lime and iron oxide. However, the addition of iron oxide increased the retention of Cr(VI) in soils; cow manure increased the sorption of Cr(VI) and also promoted the reduction of Cr(VI), followed by immobilization of Cr(III).

In the presence of lime and iron oxide, there was no change in the oxidation state of Cr added. In contrast, when Cr(VI) was added to soil that was mixed with cow manure, after equilibration, soil solution comprises both Cr(VI) and Cr(III), suggesting that Cr(VI) was reduced to Cr(III) and subsequently adsorbed.

5.7.2.1 Effect of lime on retention of Cr

Addition of lime greatly increased the adsorption of Cr(III) in the soils. As the pH of soil using lime increased to 8.23, 9.12 and 10.36; sorption coefficients (K_f) for Cr(III) in these soils were 4017.9, 5390.1 and 6491.9 L/kg, respectively (Figure 5.1a). In contrast, the K_f values of Cr(VI) slightly decreased with addition of lime with a K_f value of 0.11, 0.09 and 0.06 L/kg in the soils that have pH 8.23, 9.12 and 10.36, respectively (Fig. 5.1b). Several reasons contribute to the pH-induced immobilization of metals in soils. Lime often increases the pH-induced surface negative charge and leads to the retention of cations (Naidu et al., 1994; Bolan et al., 2003). In addition, an increase in soil pH results in the formation of Cr(III) hydroxyl species ($Cr(OH)_2^+$), which have greater affinity for retention on soil colloids and finally Cr(III) mostly precipitates as $Cr(OH)_3$ in alkaline soils (Bartlett, 1992).

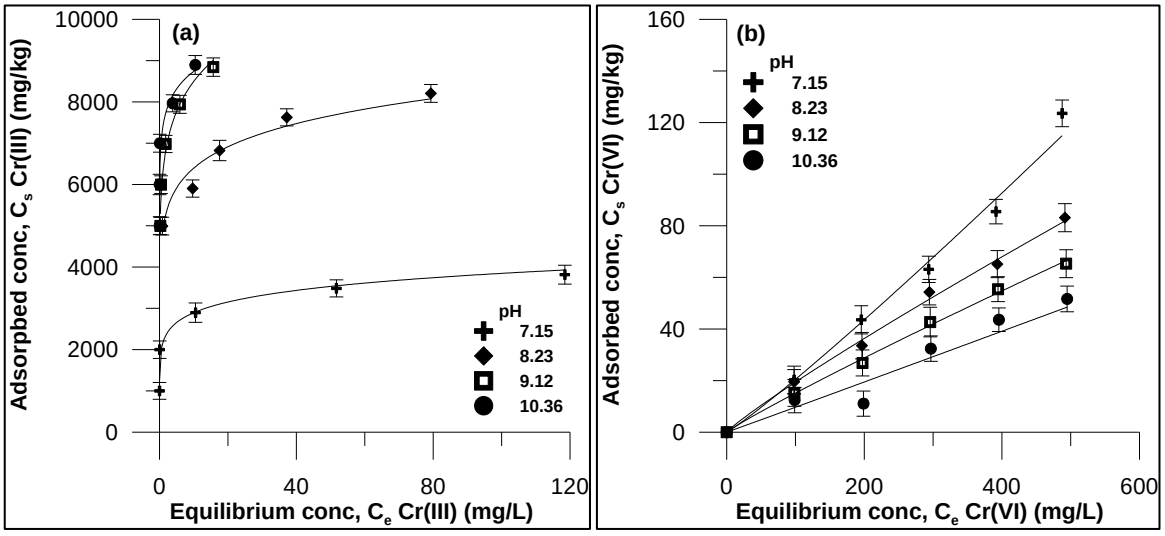


Figure 5.1 Effect of lime on adsorption of Cr(III) (a), and Cr(VI) in neutral soil (b).

Retention of Cr species is unlikely to change the toxicity, however reduces the risk in the environment (Fendorf, 1995). Soil solution pH is one of the major factors determining the surface properties of soils (Sposito, 1983; Barrow, 1985; Bolan et al., 1997). An increase in pH in variable charge soils leads to the formation of negative charge on hydroxyl-Fe and Al surfaces that arise from the deprotonating potential determining $M\text{-}OH_2^{0.5}$ and $M\text{-}OH^{0.5-}$ groups. Hence the activity of hydroxyl ions increases and favours the specific adsorption of Cr(III) (Bradl., 2004). Once the sorption sites are saturated by retaining Cr(III) ions, polymerization of $Cr(OH)_3$ occurs and finally precipitates on the surface of soil colloids (McBride, 2000).

The adsorption of Cr(VI) species (k_f = 0.079) by the soil used in this experiment is low compared to Cr(III) adsorption (k_f = 1259.8). The difference in the adsorption of Cr species may be attributed to the difference in amount of negative (28.4 cmol/kg) and positive (4.81 cmol/kg) charges in the soil. In contrast to Cr(III) adsorption, the addition of lime decreased the Cr(VI) adsorption in soils. An increase in soil pH decreases the sorption of Cr(VI) due to decrease in positive charge on soil surfaces (Bolan and Thiagarajan, 2001) (Table 5.3). The increased pH resulting from liming also diminishes the reduction of Cr(VI) to Cr(III) as the availability and activity of H^+ decreases in alkaline soils, since Cr(VI) reduction is dependent on the activity of H^+ ions (Eary and Rai, 1991; Chrysochoou et al., 2010). Moreover, it has been shown that abiotic reduction can be accelerated in the presence of organic amendments at low pH (Deiana et al., 2007).

Table 5.2 The effect of amendments on soil properties

Amendment	Level (%) (w/w)	pH	CEC (cmol/kg)	OM (%)
Control		7.15	6.92	2.58
Lime	0.037	8.23	7.46	2.74
	0.074	9.12	7.91	2.83
	0.111	10.36	8.35	2.95
Elemental Sulfur	0.16	6.23	6.33	2.51
	0.32	5.12	5.98	2.34
	0.48	4.08	5.41	2.02
Fe oxide	5.0	7.68	5.87	2.54
Manure	5.0	5.36	7.53	5.84

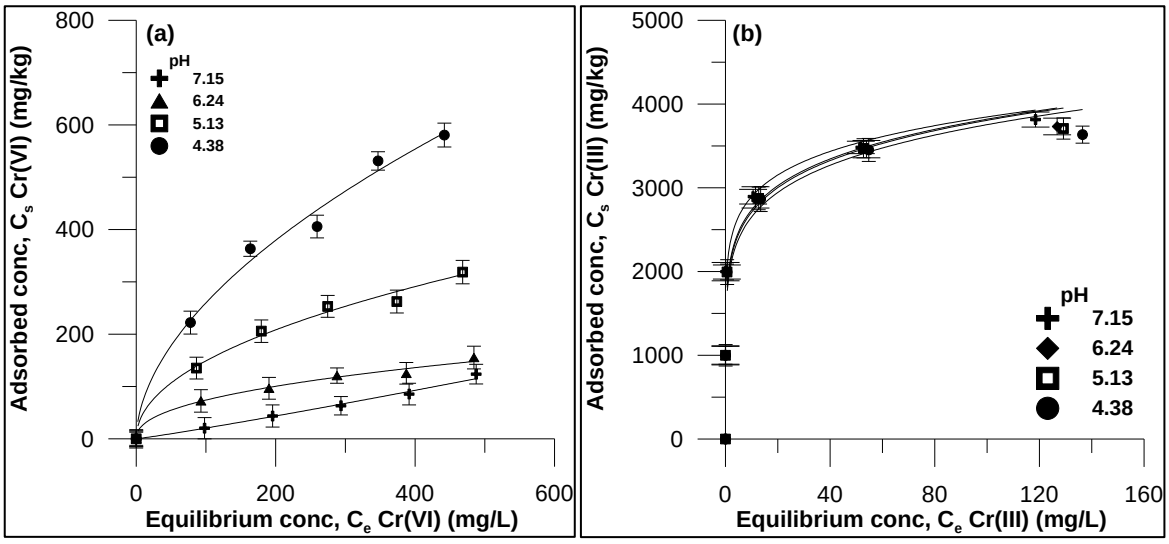


Figure 5.2 Influence of addition of elemental sulfur on adsorption of Cr(VI) (a), and Cr(III) in soil (b).

Chromium(VI) adsorption is favoured if the soil surface contains positive charge, by surface complexation (Bradl, 2004). Addition of lime increases net negative charge in soils (Bolan et al., 1999).

5.7.2.2 Effect of elemental sulfur on the retention of Cr in soils

The addition of S(0) decreased the pH of soils, thereby slightly decreasing the adsorption of Cr(III). However it resulted in a significant increase in Cr(VI) adsorption. The incubation of soils with S(0) decreased the pH of soil from 7.15 to 6.23, 5.12 and 4.08. The adsorption coefficients of Cr(III) were 1259.8, 1043.5, 1012.1 and 956.1 L/kg in soils with values of 7.15, 6.23, 5.12 and 4.08, respectively (Figure 5.3a).

Dissolved Cr concentration in soil solution is dominated by the ionic forms in acidic conditions such as hydroxyl species; $Cr(OH)_2^+$, $Cr_2(OH)_4^+$ and $Cr_3(OH)_6^+$. but in alkaline condition, Cr(III) species undergo either ion-exchange reactions with soil surface or precipitates as $Cr(OH)_3$. The solubility of Cr(III) hydroxides is low and the sorption of Cr(III) is stronger than Cr(VI) sorption on clay minerals. However in acidic soils, H^+ ions replace the cations from the surface of soils, hence the sorption of Cr(III) reduced with a decrease in soil pH.

Elemental sulphur addition increased the adsorption of Cr(VI) in soils. Freundlich coefficients of soils were 9.84, 16.13 and 21.05 L/kg in soils that have pH; 6.24, 5.13 and 4.38, respectively (Figure. 5.3).

It is estimated that each Cr(VI) ion can react and occupy 3-4 hydroxyl ion surface sites (Benjamin and Bloom, 1981). There are different mechanisms that could explain the adsorption of Cr(VI) in low pH soils. The positive charge in low pH soils can retain Cr(VI). In acidic soils, mineral dissolution increases and Cr(VI) will be reduced or retained by Fe(II) bearing minerals (Qafoku et al., 2010).

5.7.2.3 Effect of Iron (III) oxide on the retention of Cr in soils

Addition of Fe(III) oxide influenced the adsorption of both Cr(III) and Cr(VI). The adsorption coefficient (K_f) for Cr(VI) was increased from 0.079 (control soil) to 91.15 L/kg after incubation of soil with 5% iron oxide for three days. In contrast, sorption of Cr(III) decreased from 1259.85 (control soil) to 1185.42 L/kg in soil that was incubated with 5% Fe(III) oxide.

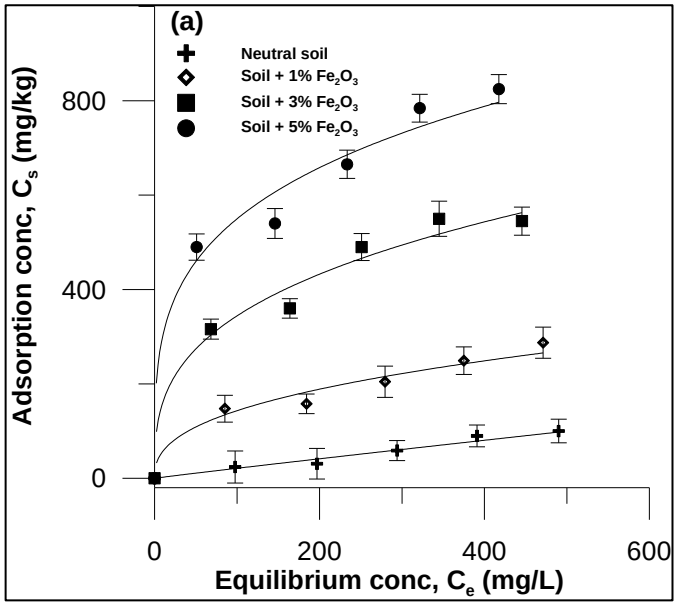


Figure 5.3 Effect of addition of Fe(III) on adsorption of Cr(VI) in neutral soil

Chromium(VI) participates in surface complexation reaction with hydroxy species of iron oxides (Fendorf, 1995). Chromium(VI) adsorption has often been shown to increase with increasing Fe content, which may be attributed to high positive charge (Zachara et al., 1989). Iron(III) oxide addition provides charge to the soil, thereby influencing Cr retention. Since Iron oxide carries high point of zero net charge (PZNC), it can effectively adsorbs oxyanions such as Cr(VI) in the pH in range of 2-7 (Zachara et al., 1987). Chromium(VI) forms inner sphere surface complexation with positively charged Fe(III) oxide in soils. Similarly, Khaodhiar et al. (2000) observed an increase in Cr(VI) and arsenate [As(V)] adsorption by soil in the presence of iron(III) oxide.

In general, cations participate in isomorphic substitution with Fe on clay or retained by oxyhydroxides surfaces. However, the increase in positive charge of soils with the addition of Fe(III) oxide may be attributed to the decrease in Cr(III) sorption due to the repulsion between similar charge of Fe(III) oxide and cationic Cr(III). Removal of Fe(III) oxide using dithionate method increased the adsorption of cationic heavy metals such as cadmium, copper, zinc and lead (Elliot and Huang, 1985).

5.7.2.4 Effect of cow manure on the sorption of Cr in soils

In the presence of cow manure, adsorption of Cr(III) and Cr(VI) increased from 1259.8 to 1284.1 L/kg and from 0.079 to 4.46, respectively (Table 5.3).

Increased adsorption of Cr(III) in cow manure amended soil is attributed to the increase in cation exchange capacity. However, the adsorption of Cr(VI) as measured from the decrease in Cr(VI) in equilibrium solution results from two main mechanisms; adsorption and reduction. In the presence of cow manure, the reduction process dominates, resulting in the reduction of Cr(VI) to Cr(III) (Shafey, 2004). Organic amendments stimulate microbial population after incorporating in the soils and biotic reduction of Cr(VI) is higher in soils than chemically mediated reduction (Banks et al., 2006). The supply of carbon and protons are considered to be the major factors in enhancing the reduction of Cr(VI) (Losi et al., 1994). In soils, DOC is identified as one of the potential drivers of Cr(VI) reduction (Jardine et al., 1999).

The increased reduction of Cr(VI) in the presence of cow manure may also due to the increased microbial activity (Ros et al., 2003). Organic amendments can also increase the retention of Cr(VI) in soils. The protonated active functional groups in biosorbents will retain Cr(VI) in low pH. At lower pH levels, Cr(VI) reduces to Cr(III) (Liu et al., 2006).

Table 5.3 Adsorption coefficients of Cr(III) and Cr(VI) in soils with different amendments

	Freundlich isotherm						Langmuir isotherm					
	Cr(IIII)			Cr(VI)			Cr(III)			Cr(VI)		
	K_f	n	R^2	K_f	n	R^2	X_m	b	R^2	X_m	b	R^2
CLV (control)	1259.8±4.417	0.13±0.026	0.980	0.079±0.024	0094 ±0.015	0.855	3815	0.0005	0.745	123	0.0161	0.788
CLV + LIME												
pH: 8.23	4017.9±7.635	0.11±0.005	0.954	0.11±0.005	1.07±0.045	0.897	8206	0.0003	0.814	83	0.0241	0.761
pH: 9.12	5390.1±6.013	0.13±0.011	0.961	0.09±0.003	0.90±0.024	0.913	8842	0.0002	0.708	65	0.0306	0.816
pH: 10.36	6491.9±4.818	0.08±0.002	0.872	0.06±0.001	0.92±0.018	0.732	8895	0.0002	0.711	51	0.0387	0.648
CLV+ S(0)												
pH: 6.23	1012.1±6.814	0.14±0.034	0.988	9.84±0.042	0.44±0.031	0.976	3732	0.0053	0.842	155	0.0128	0.814
pH: 5.12	956.1±4.485	0.15±0.006	0.976	16.13±0.178	0.48±0.057	0.937	3708	0.0054	0.863	318	0.0062	0.855
pH: 4.08	1460.9±3.941	0.15±0.013	0.903	21.06±0.124	0.54±0.043	0.958	3635	0.0053	0.747	580	0.0034	0.782
CLV+ Fe oxide												
5% **(w/w)**	1185.4±5.841	0.13±0.01	0.924	91.15±0.275	0.32±0.008	0.816	4506	0.0004	0.722	824	0.0024	0.674
CLV+ Cow manure (5%) (w/w)	1284.1±2.274	0.20±0.04	0.913	4.46±0.048	1.01±0.042	0.934	3725	0.0004	0.835	305	0.0057	0.713

5.8 Conclusion

- The adsorption of Cr(VI) and Cr(III) can be increased by the addition of soil amendments. The two important soil parameters; pH and surface charge are vital in determining the adsorption of Cr species.

- While addition of lime increased the Cr(III) adsorption, there was a slight decrease in Cr(VI) adsorption. This was attributed to the increased pH of soils, where Cr(III) precipitates or adsorbs to hydroxyl ions. Increase in soil pH decrease positive charge, hence the adsorption of Cr(VI) was decreased.

- Elemental sulfur decreased the soil pH, hence showed opposite effect of lime on Cr(VI) and Cr(III) species. The increased adsorption of Cr(VI) was due to the increase in protonated surface sites, where Cr(VI) participates in surface complexation or specific adsorption. In contrast, Cr(III) adsorption decreased.

- Iron(III) oxide addition increased the positive surface charge in soils. Adsorption of Cr(VI) increased with the addition of Fe_2O_3 in soils; however Cr(III) sorption decreased which was related to the repulsion between iron oxides and cationic Cr(III).

- Addition of cow manure increased both Cr(VI) and Cr(III) adsorption. Furthermore Cr(VI) was reduced to Cr(III), may be due to the functional groups in DOC of cow manure. Cow manure increased cation exchange capacity in soils, thereby increased Cr(III) adsorption.

Chapter 6

CHEMO-DYNAMICS OF CHROMIUM REDUCTION IN SOILS: IMPLICATIONS FOR REMEDIATION

6.1 Introduction

Both adsorption-desorption and reduction-oxidation (redox) reactions are significant aspects for the speciation, transport, toxicity and ultimately the fate of Cr in the environment (James, 2002). While Cr(VI) is mobile and toxic, Cr(III) behaves as a hard Lewis acid, forms complexes with several ligands and is strongly retained, less mobile and non-toxic (Richard and Bourg, 1991). The toxicity of Cr(VI) can be mitigated by adsorption and reduction. Mineral surfaces that have high positive charge such as iron and aluminium oxides adsorb Cr oxyanions between pH 2-7. The presence of competing inorganic ligands interferes with Cr(VI) sorption and their capacity to compete with Cr(VI) sorption follows: $HPO_4^{2-}/H_2PO^{4-} > HCO^{3-} > SO_4^{2-} > NO^{3-}$ (Azizian and Nelson, 1993).

Remediation of Cr(VI) in the environment can be accomplished by the addition of immobilizing materials, thereby reducing the mobility and bioavailability of Cr(VI) (Bolan Thiagarajan, 2001; Janos et al., 2009). Recent trends in the removal of Cr(VI) by adding biomass derived from plants, fungi, bacteria, yeast as bio-sorbents for Cr(VI) has been increased due to cost-effective remediation techniques (Nourbakhsh, 1994; Ucun et al., 2002; Ozdemir et al., 2003). However, the mobility, toxicity and bioavailability of Cr depends mainly on its oxidation state, so the best remediation strategy to mitigate Cr contamination is first to reduce soluble, mobile and toxic Cr(VI) to less toxic Cr(III) followed by its immobilization (James, 2001). Soil pH (i.e. supply of protons), Cr(VI) adsorption (i.e. supply of free Cr(VI) ions in soil solution) and organic matter (i.e. supply of electrons) are the primary factors that influence Cr redox reactions (Bartlett, 1991; Banks et al., 2006).

Chromium reduction occurs readily when organic matter is present, such as manures, composts, humic acids and fulvic acids, phenols, powdered leaves, organic acids, and brown sea weeds (Losi et al., 1994; Wittbrodt and Palmer, 1995; Wittbrodt and Palmer, 1996; Bolan et al., 2003). The added organic amendments release dissolved organic carbon

(DOC), which are rich in functional groups, donate electrons to reduce Cr(VI) (Tokunaga et al., 2003; Hsu et al., 2009). Other processes that induce Cr(VI) reduction in soil and water include Fe(II) bearing minerals and sulfide compounds (Fendorf and Li, 1996; Patterson et al., 1997). The toxicity of Cr(VI) can be mitigated through the reduction mechanism. However, for reaction to proceed the availability of free Cr(VI) ions is important for it to undergo either microbial or chemical reduction. In Chapter 5, the results disclosed that the presence of iron(III) oxide decreases the availability of Cr(VI) in the soil.

Although the effect of soil pH and organic amendments on the reduction of Cr(VI) has been examined (Bolan et al., 2003; Graham and Bouwer, 2010), no comprehensive study has yet analysed the simultaneous effect of three important rate limiting factors (i.e. supply of free Cr(VI) ions, protons and electrons) on Cr(VI). This study investigated the effects of Cr(VI) adsorption (i.e. availability of free Cr(VI) species in soil solution), soil pH (i.e. supply of protons) and various electron donor carbon sources (black carbon (BC), chicken waste biochar (CWB) and cow manure (CM) on the reduction of Cr(VI) in acidic and alkaline soils.

6.2 Objectives

The overall objective is to examine the effect of Cr(VI) adsorption, soil pH and various electron donor carbon sources on the reduction of Cr(VI) in soils. The specific objectives include:

- To examine the effect of iron(III) oxide addition (i.e. availability of free Cr(VI) species in soil solution) on the reduction of Cr(VI).
- To compare the impact of various pH soils (i.e. supply of protons) on Cr(VI) reduction and
- To estimate the efficiency of black carbon (BC), chicken waste biochar (CWB) and cow manure (CM) on the reduction of Cr(VI) in soils.

6.3 Hypothesis

The sorption of Cr(VI) decreases the bioavailability of free Cr(VI) ions for the reduction to occur in soils. The pH of soil plays an important role in the reduction by donating protons, organic carbon sources, which will serve as a source of electrons for Cr(VI) reduction.

6.4 Experiment

In this chapter, the major experiments conducted to observe the chemodynamics of Cr(VI) reduction in soils are listed in Table 6.1.

Table 6.1 The major experiments conducted and used in Chapter 6

No.	Title of experiment	Treatments	Measurements
1	Effect of adsorption on Cr(VI) reduction	Five natural soils; ADH, RBB, PWT, CLV, KPB* Neutral soil mixed with 1, 3, 5% Iron(III) oxide	Total Cr, CEC, OM, sorption parameters and Cr(VI).
2	Effect of pH on Cr(VI) reduction	Five natural soils: 5 (different pH levels). 5% Iron(III) oxide	K (rate constant), $t_{1/2}$ (half-life), pH, Cr(VI)
3	Effect of organic carbon sources on Cr(VI) reduction	1 natural soil: (Alkaline soil, pH:8.10) Organic carbon sources: 3 (Black carbon, Chicken manure biochar and cow manure)	Total Cr, CEC, Cr(VI), functional groups (FTIR)

*Details of the soils are documented in Table 4.2, Chapter 4.

6.5 Materials and methods

In this study, the effect of Cr(VI) adsorption on its reduction was examined by: (i) amending a low Cr(VI) adsorbing soil with increasing levels (0- 5%) of iron(III) oxide (May and Baker Ltd, Dagenham, England); (ii) using a range of soils with different Cr(VI) adsorption capacity. The effect of pH on Cr(VI) reduction was examined by: firstly, using five soils with different pH values; and secondly, amending the neutral soil (CLV) to different pH values (4.16 to 11.43) using 0.1M $Ca(OH)_2$ or 0.1M HCl. The effect of electron donor sources (i.e. organic carbon) on Cr(VI) reduction was examined by employing three carbon sources, BC, CMB and CM.

6.5.1 Soils collection and physicochemical characterization

Five different soils were collected from uncontaminated sites in the Adelaide Hills (ADH), Clare Valley (CLV) and Kulpara Bay (KPB) in South Australia, and Pittsworth (PWT) and Redland bay (RBB) in Queensland. These soils' classifications are documented in Table 4.2 in Chapter 4.

To examine the effect of pH on Cr(VI) reduction, a neutral soil (CLV) sample with a pH buffering capacity of 10.13 mmol OH^- or H^+/kg/pH was treated with four levels of 0.1M $Ca(OH)_2$ or 0.1M HCl to achieve a pH range of 5.2 to 7.9. These samples were then incubated in a glasshouse for 4 weeks and subsequently used for Cr(VI) reduction measurements.

The soil samples were analysed for pH, CEC and organic matter. The procedures for estimating pH, CEC and organic matter in the soil samples were described in Section 4.5.2, Chapter 4.

6.5.2 Organic carbon sources and their characterization

Black carbon (BC) was prepared from a South Australian common weed plant, *Solanum elaeagnifolium*. The weed plants were collected and washed with water, dried in air under shade for one week and oven dried at 80^0C for 2 days. The plant residue (100g) was placed in a ceramic cup and pyrolysed aerobically in a muffle furnace for 3 hours at 300^0C (Chun et al., 2004). Nearly 25-30% of the weight of dried weed plant material was converted to BC after pyrolysis. The charred residues were ground to less than 250µm size and treated three times with 1 M HCl for 4 hours and then four times with 1:1 mixture of 1M HCl -1M HF to remove silica and other inorganic materials without damaging the surface property of BC (Chun et al., 2004; Qiu et al., 2008). The protonated BC was washed several times with distilled water until no further change in pH occurred (Qiu et al., 2008). Black carbon was dried in an oven at 60^OC for three days and stored in a glass container. Cow manure was purchased from a local agricultural shop and chicken waste biochar (CWB) was obtained from Pacific Pyrolysis (NSW, Australia).

The BC and CWB samples before and after treatment with Cr(VI) were analysed for Fourier transform infrared spectroscopy (FTIR) to monitor and confirm the role of surface functional groups in Cr(VI) reduction. The infrared spectra of untreated and Cr(VI)-loaded BC and CWB (100mg Cr(VI)/kg) was recorded using Perkin-Elmer spectrum 400 FTIR with DRIFT at

Flinders University, South Australia. The amendment powders were blended with spectroscopic grade KBr at the ratio of 1:10 in an agate mortar and pressed in order to prepare translucent pellets. Spectra of the samples were scanned between 500 - 4000 cm^{-1} at a resolution of 4.0 cm^{-1}.

6.5.3 Chromium (VI) adsorption

Adsorption of Cr(VI) in alkaline soil (KPB) was measured using a batch experiment at known Cr concentrations (0-500 mg/kg) using $K_2Cr_2O_7$ [Cr(VI)] with 0.1 M KNO_3 as the background electrolyte. Three g of each of soil sample (triplicates) was placed in 50 ml centrifuge tubes, mixed with 30 ml of Cr(VI) solution and shaken on an end-over-end shaker at room temperature (22 ± 2^0 C). After 16 hours of shaking, suspensions were centrifuged at 1789 *g*, filtered through a 0.45-µm Millipore filter, and measured Cr(VI) by spectrophotometry using 1, 5 diphenyl carbazide (USEPA, 1996). The amount of Cr(VI) sorbed by soils was calculated from Eqn. 4.1 and the adsorption data were fitted to Freundlich Eqn. 4.3.

6.5.4 Effect of adsorption on Cr(VI) reduction

The effect of adsorption of Cr(VI) on reduction was examined by increasing the positive charge in the soil and measured Cr(VI) reduction. The soil samples ADH, RBB, PWT, CLV and KPB were mixed with 5% iron(III) oxide since the addition of iron oxide has shown to increase Cr(VI) through an increase in positive charge (In Section 5.7.2.3, Chapter 5). The adsorption was measured following section 6.5.3 and reduction was measured as mentioned in 6.5.5.

6.5.5 Chromium (VI) reduction

Cr(VI) reduction as influenced by Cr(VI) sorption (in the presence of Iron(III) oxide), pH (soils amended to various pH values) and organic amendments (BC, CWB and CM) was examined using a batch technique.

Soil samples were mixed with two levels of Cr(VI) (0 and 500 mg Cr/kg soil) using $K_2Cr_2O_7$ and incubated at field capacity. Subsamples were taken at various intervals and extracted with 1 M KH_2PO_4 at a soil: solution ratio of 1:10 for 16 h and the solution was filtered through 0.45 µm filter tips. The concentration of Cr(VI) in the soil extracts was estimated colorimetrically using 1, 5-diphenyl carbazide as colour developing reagent.

To examine the effect of organic amendments (BC, CWB and CM) on the reduction of Cr(VI) the alkaline soil (KPB) was selected since it has the lowest amount of protons and carbon available for Cr(VI) reduction. Alkaline soil samples were mixed with two different levels of organic amendments (0 and 50 mg/kg) and subsequently mixed with two levels of Cr(VI) (0 and 500 mg Cr/kg soil) to monitor Cr(VI) reduction.

The amount of Cr(VI) reduction was estimated from the decrease in the concentration of Cr(VI) in soil solution using Eqn. 6.1

$$C_r = \left[C_i - \left(\frac{C_f * V}{W} \right) \right] \quad (6.1)$$

where C_r is the amount reduced (mg/kg), C_i is the initial concentration (mg/kg) and C_f is the concentration of KH_2PO_4 extractable Cr(VI) (mg/L), V is the solution volume (L) and W is the weight of the air-dried soil sample (kg). The decline in Cr(VI) concentrations may be due to both adsorption and reduction mechanisms.

The first-order decay rate equation as described by Lan et al. (2007) was used to calculate the Cr(VI) reduction rate. In Eqn. 6.2, the net rate of change in residual Cr(VI) will equal the reduction rate (V_{dec}) which is expressed by the following first-order differential equation:

$$V_{dec} = -\left(dC / dt \right) = kC \quad (6.2)$$

where *k* is the first-order reduction rate constant (per time), and C is the concentration of residual Cr(VI) in the soil (mg/L). The equation has the following solution:

$$C = C_0 e^{-kt} \quad (6.3)$$

where C_0 is the initial amount of Cr(VI) recovered at day 0 of the experiment in the soil (mg/L) (Eqn. 6.3). The rate constant *k* was estimated using the nonlinear least square method. The Cr(VI) half-life ($t_{1/2}$ = time taken to reduce Cr(VI) concentration to half of the initial value; i.e. $C = C_0/2$) was calculated using Eqn. 6.4.

$$t_{1/2} = \frac{0.693}{K} \quad (6.4)$$

6.6 Statistical analyses

All All measurements including pH, Cr(VI) concentration were calculated from 3 replicates of each treatment. All calculations and standard deviations of the replicates were determined using Microsoft Excel (2010). The relationships between (i) Freundlich coefficient (iron(III) oxide addition) and half–life of Cr(VI) reduction (natural and amended soils); (ii) pH of soils and half–life of Cr(VI) reduction; (iii) measured and theoretical amount of H^+ ions change during reduction; and (iv) DOC and half–life of Cr(VI) reduction were derived using regression analysis in Grapher ^{TM} software (Golden Software, version 7, USA).

6.7 Results and Discussion

6.7.1 Soil physical and chemical characteristics

The five soils used for Cr(VI) adsorption and reduction experiments varied considerably in terms of physical and chemical properties (Table 6.2). The pH varied from acidic (4.60) in ADH soil to alkaline (8.10) in KPB soil. Organic matter is in the range of 0.4 to 6.7 % and CEC ranged from 3.8 to 7.7 cmol/kg.

Table 6.2 Selected characteristics of soils used in this study

Soil	Source	pH	CEC (cmol/kg)	OM (%)	Fe (total) (%)	Al (total) (%)
ADH	SA	4.60	3.9	1.06	1.26	0.21
RBB	QLD	5.58	5.6	0.38	13.46	1.42
PWT	QLD	6.53	6.1	1.58	2.68	0.37
CLV	SA	7.26	6.3	2.58	2.17	0.14
KPB	SA	8.10	7.7	6.74	1.91	0.33

Of the five soils, the soil from Redland Bay (RBB), Queensland had the highest amount of Fe and Al, suggesting the presence of ferro and aluminosilicates. The iron and aluminium oxides present in RBB soil was the result of lava from a volcano that erupted millions of years ago.

The pH of BC and CWB varied from 3.5 to 8.8. Surface areas (BET) of BC and CWB are 3.35 and 7.27 m^2/g, respectively. Scanning Electron Microscope image demonstrated that BC and CWB had fine pore structure, which reflected the large surface area (Figure 6. 1). The SEM images were not much different between BC and CWB in that they showed fibrous channels.

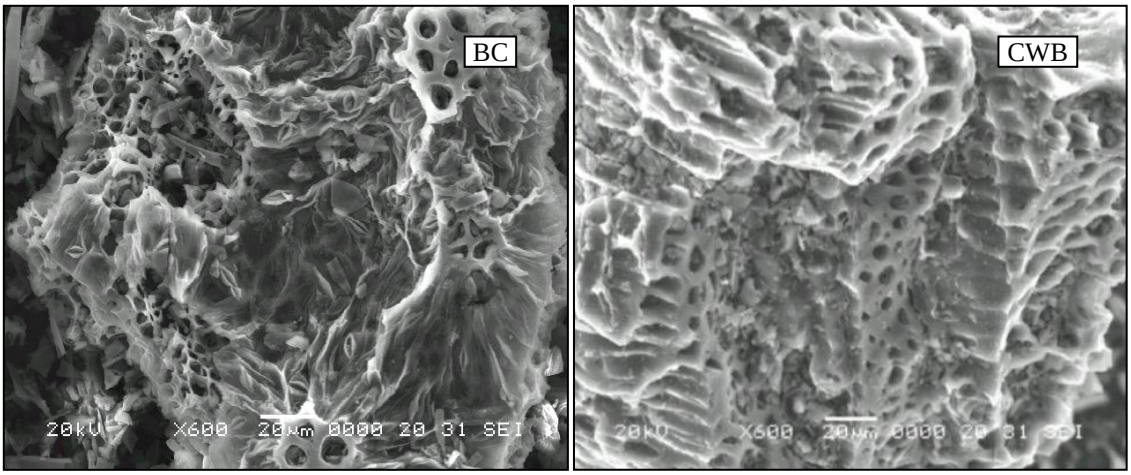


Figure 6.1 Scanning Electron Microscope (SEM) image of black carbon (BC), chicken waste biochar (CWB).

6.7.2 Adsorption of Cr(VI)

The adsorption of Cr(VI) in alkaline soil (KPB) as measured by Freundlich sorption coefficient (K_f) was low, only 0.039. The low sorption in alkaline soil may be due to the occupation of a positive charge on the iron oxide by the hydroxyl ions present in neutral soil. Adsorption of Cr(VI) was less at pH values greater than 6.0 and this may be due to the dual competition of both $CrO_4^{2-}$and $OH^-$to occupy the surface of adsorbent where OH^- prevails (Garg et al., 2007).

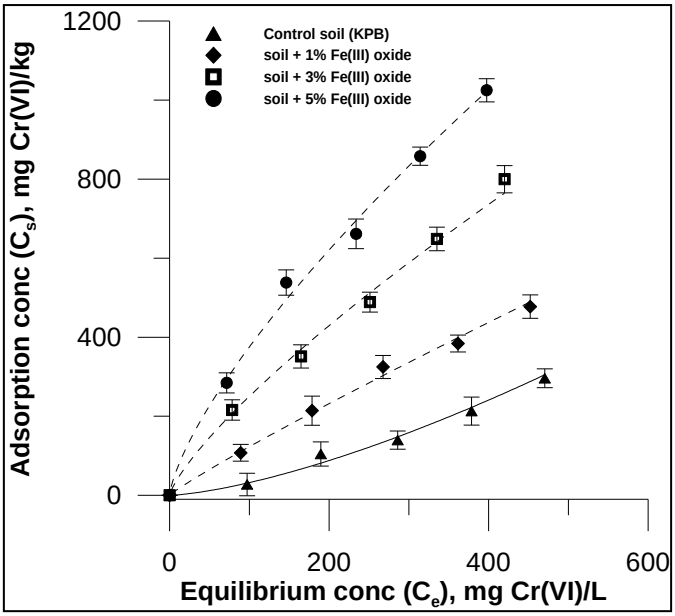


Figure 6.2 Adsorption of Cr(VI) in alkaline soil (KPB) with increasing levels of Fe(III) oxide

The K_f values increased with increasing levels of Fe oxide addition (Figure. 6.2). The addition of 1, 3 and 5% Fe(III) oxide significantly increased K_f from 0.039 (control soil) to 1.817, 6.969, and 13.251, respectively.

Adsorption of Cr(VI) is controlled by the availability and accessibility of surface sites, relative charges of the surface, and rate of complexation of the dissolved species with surface sites (Sposito, 1994). The amount of clay that contains hydroxides and oxy-hydroxides of Fe and Al can influence the sorption of anions. Mineral phases such as iron(III) oxide have a proton-specific surface area with high zero point of charge and the capacity to adsorb Cr(VI) is higher in pH range between 2-7 (Zachara et al., 1987). Chromium(VI) participates in surface complexation reaction with hydroxy species of iron oxides (Fendorf, 1995).

Adsorption of Cr(VI) was higher in soils containing high Fe and Al, which may be attributed to high positive charge (Rai et al., 1989; Zachara et al., 1989). Altundogan (2005) has observed that the impregnation sugar beet pulp with iron(III) oxide decreased Cr(VI) concentration in aqueous media which may be attributed to the iron(III) oxide–induced Cr(VI) adsorption. Addition of iron(III) oxide increases the positive surface area and Cr(VI) removal from aqueous solution is due to the sorption .Iron(III) oxide addition is unlikely to result in the reduction of Cr(VI) because of lack of supply of electrons for Cr(VI) reduction in soils.

6.7.3 Effect of adsorption of Cr(VI) on its reduction

Cr(VI) adsorption as indicated by K_f value (Eq 6.1) ranged from 0.1 10.76 L kg^{-1} in soils and it increased when more iron(III) oxide was added to the alkaline soils (Section 6.7.2). Half-life ($t_{1/2}$) values calculated using first order rate equation (Eqn. 6.5) ranged from 1.37 to 4.19 days (Figure. 6.3) for natural soils.

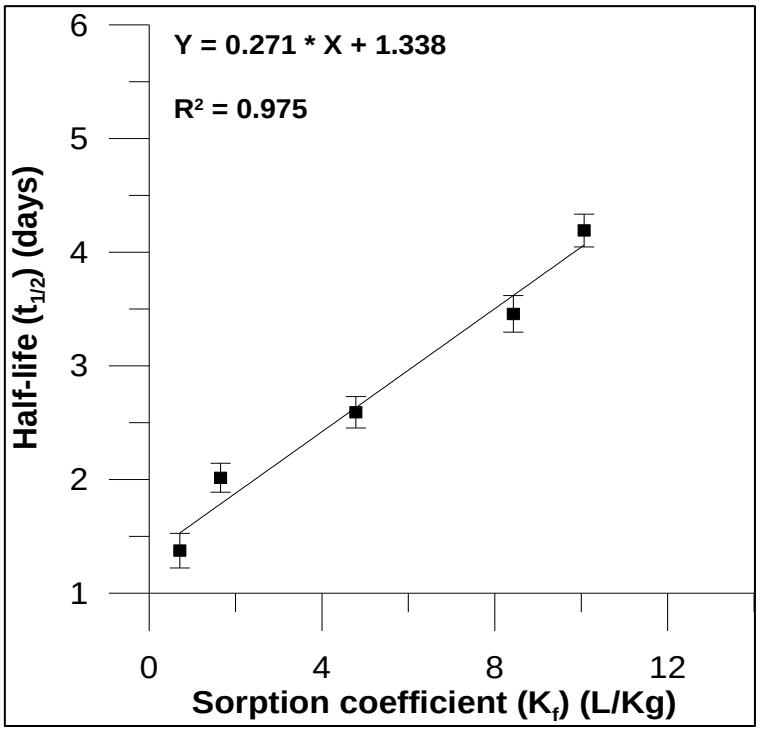


Figure 6.3 Relationship between Cr(VI) adsorption as measured by K_f values and Cr(VI) reduction as measured by half-life for soils, which have been amended with 5% Fe(III) oxide

Chromium(VI) reduction decreased with an increase in Cr(VI) adsorption, as shown by a positive relationship between K_f and $t_{1/2}$ values. Adsorption of heavy metals decreases the mobility and bioavailability in soils (Traina and Laperche, 1999). Adsorption of Cr(VI) reduces the availability of Cr(VI) species to microbial organisms for reduction. In acidic soils, the presence of iron and aluminium oxides increases the retention of Cr(VI), thus Cr(VI) was more protected from reduction (James and Bartlett., 1983). Moreover, the retained Cr(VI) ions on oxides of Fe and Al or organic matter may not be reduced by organic acids such as gallic acid, which are present in the organic matter if the pH of the soil is more than 3 (Fendorf, 1995).

Adsorbed Cr(VI) may have been embedded within deep inside the soil–organic matter complex and iron oxide, where bacteria may be unable to attack or bacteria may be unable to take up the adsorbed Cr(VI) phase (Ogram et al., 1985). The adsorbed Cr(VI) reduces when desorption was higher, so that dissolved Cr(VI) will be available for microbes to reduce. It has been shown that the rate of reduction of Cr(VI) decreases with soil depth as the microbial population decreases (Campbell et al., 1991), which emphasizes the importance of microbial activity. It is also possible that Cr(VI) adsorption also increases with depth and this may explain the decrease in reduction. As the soil depth increases, the organic matter content and microbial population decreases (Bolan and Baskaran, 1997). In soils, Cr(VI) undergoes inner–sphere surface complexation (Jiang et al., 2008). Therefore once Cr(VI) is adsorbed, microbial degradation is less likely and persists in the environment for a long time.

6.7.4 Effect of pH on the reduction of chromate

The rate of Cr(VI) reduction as measured by half-life ($t_{1/2}$) values were 1.37, 2.01, 2.59, 3.45 and 4.19 days for ADH, RBB, PWT, CLV and KPB soils, respectively (Figure. 6.4). In the pH amended neutral soil (CLV), the rate of reduction decreased with increasing pH. Furthermore there was a positive relationship between pH and rate of Cr(VI) reduction as measured by half-life values (Table 6.3).

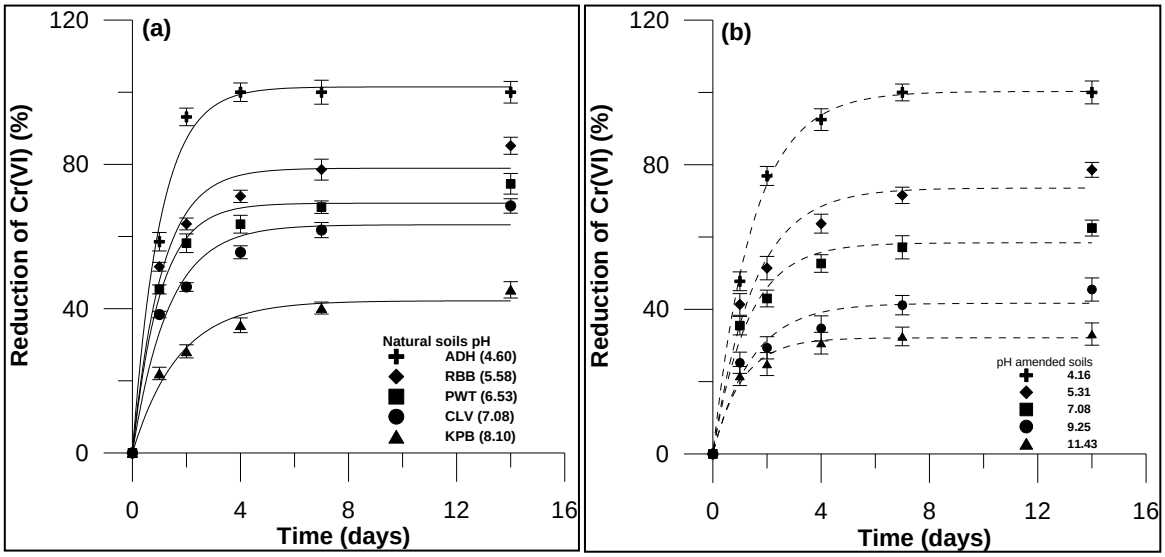


Figure 6.4 Reduction of Cr(VI) in different soils; (a) in natural soils and, (b) in pH amended CLV soil.

The reduction of Cr(VI) to Cr(III), which is a proton-consuming (or hydroxyl release) reaction (Eqn. 6.7), was found to increase when soil pH decreased (Eary and Rai, 1991). The decrease in Cr(VI) reduction with an increase in pH may be attributed to the depletion of protons (Figure. 6.5)

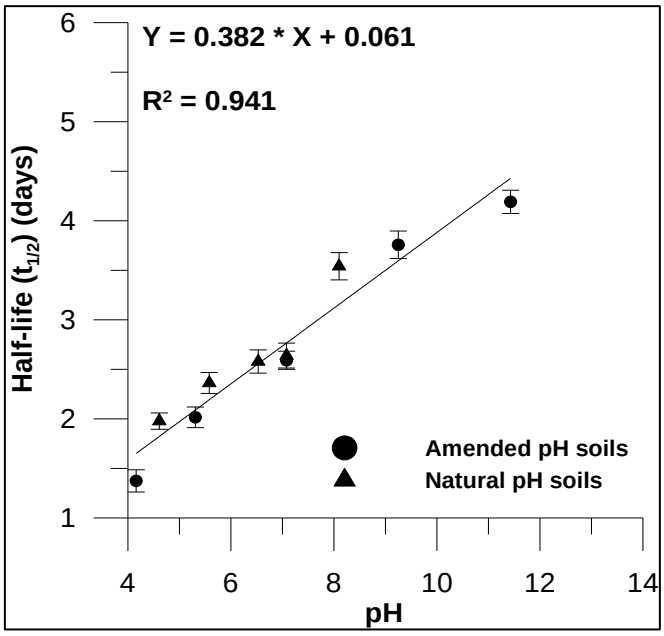


Figure 6.5 Relationship between pH and half-life for unamended soils and pH-amended soil (CLV).

Table 6.3 The rate constants and half-life values for Cr(VI) reduction in soil amended to various pH values

pH of soil	Rate constant (K) (mg/kg/day)	Half-life ($t_{1/2}$) (days)
4.16	0.52	1.47
5.31	0.34	2.16
7.08	0.27	2.57
9.25	0.19	3.23
11.43	0.17	3.94

When the Cr(VI) level reduced, there was a simultaneous decrease in the number of protons, resulting in an increase in soil pH. The increase in pH during reduction can be

explained by the fact that as the reduction proceeded, protons and electrons were consumed, thereby increasing the pH (Bolan et al., 2003).

The theoretical amounts of protons required for Cr(VI) reduction in the incubation experiment were calculated using Eqn. 6.7. The amount of protons consumed during Cr(VI) reduction was also measured from the differences in the pH values between the Cr(VI) treated and the untreated soils, and the soil's pH buffering capacity. There was a good relationship between the theoretical and measured amounts of protons required for Cr(VI) reduction, but the measured amounts were slightly less than the calculated values (Figure. 6.7).

$Cr(III) + Soil \rightarrow Cr^{+3} + H_2O + H^+$ (Adsorption-proton release reaction) (6.5)

$Cr(III) + H_2O \rightarrow Cr(OH)_3 + H^+$ (precipitation - proton release reaction) (6.6)

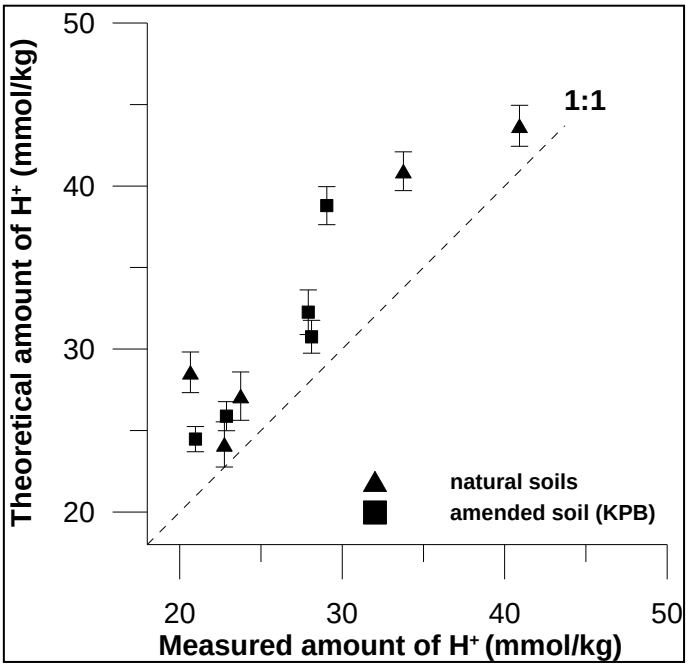


Figure 6.6 Relationship between measured and theoretical amounts of protons consumed during reduction (OC amended: soils mixed with black carbon, cow manure and biochar; natural pH soils: Acidic (4.61), neutral (7.26) and alkaline (8.10); pH amended neutral soil (CLV) (4.16, 5.31, 7.08, 9.25 and 11.43).

It is possible that both Cr(VI) reduction (i.e. proton-consuming reaction; Eqn. 6.7 and 6.9) and Cr(III) immobilization (i.e. proton release reaction; Eqns. 6.5 and 6.6) reactions can occur simultaneously in soils, which may explain the discrepancy between the theoretical and measured protons consumption (Harris and Arnold, 1995). The net effect of Cr(VI)

reduction followed by Cr(III) immobilization is a decrease in H^+ consumption, resulting in a less Cr(VI) reduction-induced increase in pH than predicted. This may be attributed to the higher theoretical than measured H^+ consumption during the Cr(VI) reduction process.

$$CrO_4^{2-} + 8H^+ + 3e^- \rightarrow Cr(OH)_3 + 4H_2O \quad (6.7)$$

6.7.5 Effect of organic amendments on reduction

The amounts of Cr(VI) reduced as estimated from the decrease in the concentration of Cr(VI) in soil solution, increased with an increase in time. After the addition of Cr(VI), the maximum Cr(VI) reduction occurred within 1 week following Cr(VI) addition (Figure 6.7). Reduction of Cr(VI) to Cr(III) in soils has often been found to be rapid, reaching its maximum within a relatively short time, i.e. in days (Ross et al., 1981).

The rate of Cr(VI) reduction increased with the addition of organic amendments and there was a significant difference in the rate of reduction among the amendments. The rate of reduction followed: Black carbon > cow manure > biochar > control soil (Figure 6.7). The rate of reduction values indicate that the addition of some of the organic amendments, such as biochar caused an almost two-fold increase in the rate of reduction of Cr(VI) in the soil. This is consistent with the findings of Losi et al. (1994) and Bolan et al. (2003), where a substantial increase in the rate of reduction of Cr(VI) in the presence of organic amendments such as manure and compost occurred.

The reduction rates of the organic amendments (Fig. 6.7) were described by the Eqn. 6.8.

$$Y = Y_m(1 - \exp^{-rx}) \quad (6.8)$$

where Y is the amount of Cr(VI) reduced (mg/kg), Y_m is the maximum amount of reduced Cr(VI) (mg/kg), *r* is rate constant and *x* indicates the days of incubation.

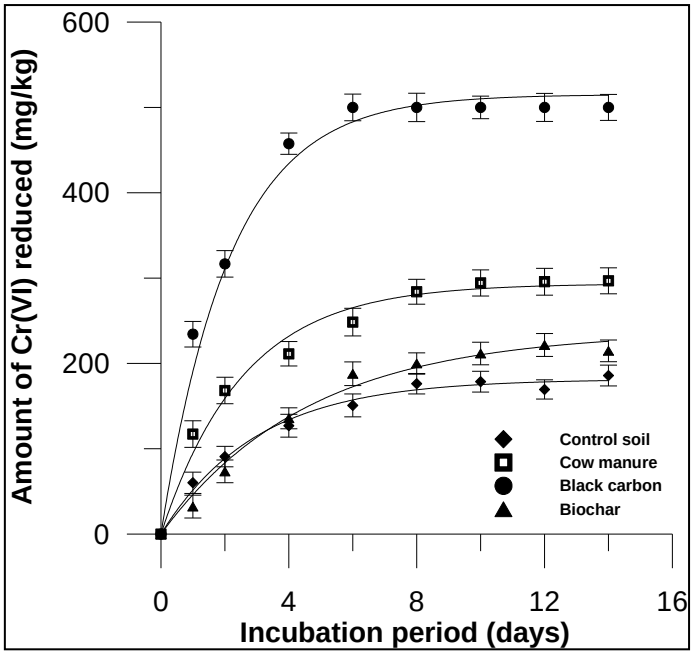


Figure 6.7 Effect of organic amendments on the reduction of Cr(VI) in soil (KPB)

Reduction rate constants (K) were increased with the addition of amendments from 0.018 (control soil) to 0.021, 0.045 and 0.084 mg kg^{-1}/day upon the addition of CMB, and black carbon, respectively. Consequently, half–life (t1/2) values declined from 38.5 (control soil) to 33.0, 15.4, 8.25 days when CMB, CM and BC were added, respectively. As the time passed the rate of reduction became constant. This could be due to the depletion of electrons from the functional groups of organic matter and perhaps oxidation of reactive organic matter in the added amendments.

The results indicated that of the three amendments examined, BC is highly effective. The high efficiency of BC may be attributed to the presence of several oxygen-containing acidic (carbonyl, lactonic, carboxylic, hydroxyl and phenol) and basic (chromene, ketone and pyrone) functional groups (Goldberg, 1985; Boehm, 1994). Black carbon is composed of polycyclic aromatic sheets with several fused rings. The sheets are highly porous with high surface areas. The edges of these sheets are reactive and they contain several functional groups (Palotas et al., 1996; Harris and Tsang, 1997). These sheets have the capacity to act as π-electron donors to reduce Cr(VI) (Wang et al., 2010). The pronated BC has low pH, which may be for a reason for the increase in the rate of reduction. The resultant Cr(III) participates in either adsorption or surface complexation and binds to BC (Hsu et al., 2009).

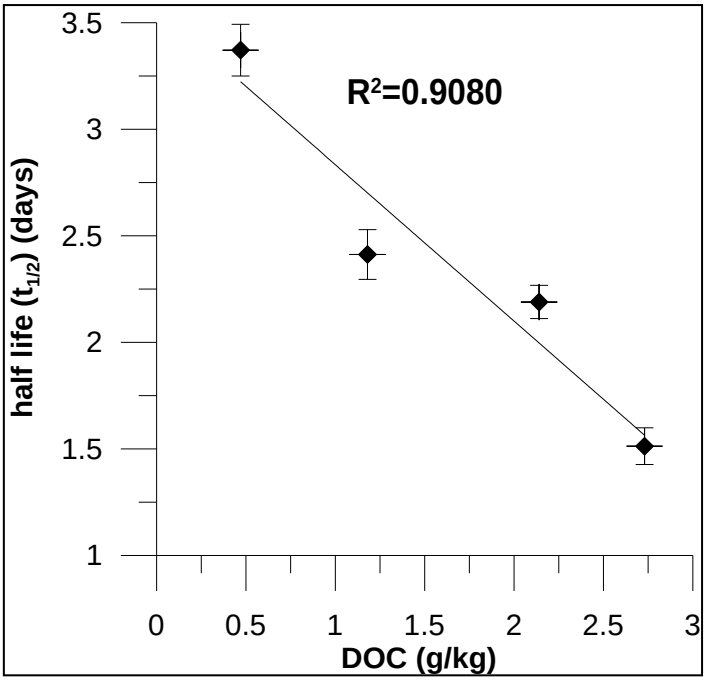


Figure 6.8 Relationship between DOC and half-life.

In the presence of cow manure, Cr(VI) is reduced due to the presence of high dissolved organic carbon (DOC). Moreover, the activity of microorganisms plays a pivotal role in reducing Cr(VI) since cow manure is rich in nutrients (Bader et al., 1999).

There was a negative relationship between the half-life of Cr(VI) reduction and concentration of DOC in added organic amendments (Figure 6.8). This relationship suggests that the rate of reduction increased as DOC concentration increased. Bolan et al. (2003) concluded that easily oxidisable carbon and DOC have a strong linear relationship with the reduction of Cr(VI), which further suggests that only certain components of DOC (Eqn. 6.9) act as electron donors for the reduction.

$$Cr_2O_7^{2-} + \text{organic carbon} + 14\ H^+ \rightarrow 2Cr^{+3} + CO_2 + H_2O \qquad (6.9)$$

Biochar was less effective in the reduction process of Cr(VI) despite the presence of several functional groups. The acidic functional groups will dissociate its protons and act as Brønsted acids in soil pH ranging from 5-7. The high pH (9.2) of the biochar might prevent the dissociation and oxidation of phenolic and hydroxyl groups, thereby limiting the supply of protons for Cr(VI) reduction.

After 4 days of incubation, the reduction mediated by biochar was increased. This could be attributed to the biotic and microbial oxidation of biochar (Zimmerman, 2010), thereby increasing the supply of protons for Cr(VI) reduction. Biochar contains 28.9 g/kg of nitrogen (N), oxidation of this ammoniacal N releases protons, thereby contributing to the reduction of Cr(VI). Elovitz and Fish (1995) identified hydroquinones in natural organic matter (NOM) as potential electron donors for the reduction of Cr(VI) in soils (Wang et al., 2010).

Organic amendments induce Cr(VI) reduction via both biotic (microorganisms) and abiotic (electrons donation) pathways (Losi et al., 1994). In addition, Pérez-Piqueres et al. (2006) have shown that the addition of manure increases the microbial activity as measured by respiration in soils. These microorganisms may produce enzyme *chromate reductase* which could be the primary responsible factor for the reduction of Cr(VI). Sulfate-reducing bacteria are also responsible for the reduction of Cr(VI) in soils (Smith and Gadd, 2000). As the time passed the rate of reduction became constant. This could be due to the depletion of electrons from the functional groups of organic matter and perhaps oxidation of reactive organic matter in the added amendments. Organic amendments supply readily oxidisable organic carbon, and this supports the reduction of Cr(VI) in soils (Losi et al., 1994).

6.7.6 Confirmation of the activity of functional groups

The presence of surface functional groups and the modifications mediated by BC after Cr(VI) reduction was confirmed by FTIR spectra (Figure 6.9). There were several modifications in the spectra of BC after reacting with Cr(VI) (Table 6.4). The absorption peak at 3203 cm^{-1} indicates the presence of free and intermolecular hydroxyl (-OH) groups, confirming the release of hydroxyl ions by Cr(VI) reduction (El-Shafey, 2005). The broad peak observed at 2929.5 cm^{-1} was assigned to the stretching vibrations of C-H groups and amine (NH_2) groups. The peak at 1704.4 cm^{-1} in the untreated BC represents native free–COOH stretching, which was not present after treatment with Cr(VI), indicating its participation in reduction and confirming the process of carboxylation of BC surface (Murphy et al., 2008). The strong intensity of the band at 1442.9 cm^{-1} is attributed to the formation of carbonyl/ carboxylic groups on the surface of BC. These carboxylic groups may serve as binding sites for Cr(III) species resulting from the reduction of Cr(VI) (Hsu et al., 2009).

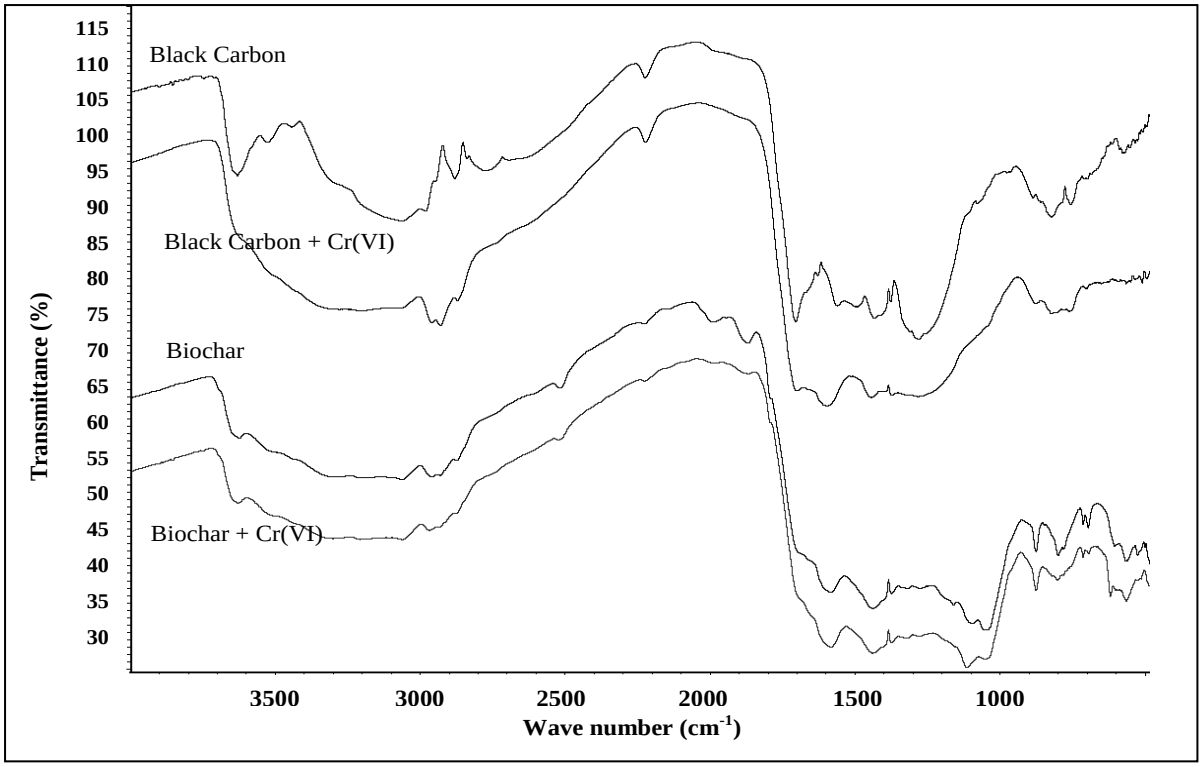


Figure 6.9 Stretching frequencies observed for black carbon and biochar, before and after treatment with Cr(VI).

The intensity of the peaks at 1442.9 and 1283.0 cm^{-1} in Cr(VI) treated BC was reduced, which was attributed to the C-O-CH_3 (methodxyl) deformation and bending of C-OH (hydroxyl) in phenolic structures respectively (Samal et al., 1995; Pandey, 1999).

The decrease in peak intensities indicated the oxidation of these functional groups in BC upon following Cr(VI) reduction. Since Cr(VI) is a strong oxidizing agent, primary and secondary alcohols reduced Cr(VI) and these alcohols were converted into carbonyl compounds (Elangovan et al., 2008). Phenolic groups in the BC play an important role in supplying electrons for the reduction of Cr(VI) (Hsu et al., 2009).

Table 6.4 Identification of functional groups in BC by FTIR and the changes in these groups resulting from Cr(VI) reduction.

Absorption peak (cm^{-1})	Wavelength range (cm^{-1})	Assignment	Changes in functional groups
3400-3300	3500-3200	Hydroxyl (-OH) groups	Broad peak
2929-2881	3000-2850	C-H stretching	Peak height decreased
2224-2220	2250-2100	Di-substituted alkynes	Transmission decreased
1705-1700	1710-1690	C=O stretch in carboxylic acids	-COOH oxidized
1600-1590	1640-1550	Imines and oxines	Carbonyl group formed
1450-1430	1450-1375	C-H bending in alkanes	Deformation of C-O-CH_3
380-1370	1400-1310	Anti-symmetrical stretch of –COO in carboxylic acids	-COOH intensity decreased
1290-1280	1300-1000	C-O stretch in ethers	Bending of C-OH in phenolic groups
825-822	990-690	C-H bending	Transmission decreased

The increased intensity at 1596.2 cm^{-1} in BC after reacting with Cr(VI) indicates the consequent formation of carbonyl and carboxylic groups. All the above-mentioned changes in the functional groups confirmed the participation of functional groups in reducing Cr(VI) and subsequent adsorption of Cr(III) species.

The FTIR spectrum of untreated CWB showed peaks at 3061.8, 1584.2, 1439.5, 1115.1, 875.6 and 802.5 cm^{-1}. The peak at 3061.8 is attributed to the presence of hydroxyl groups and the peak at 1584.2 was due to the stretching vibrations of conjugated C=C bonds and carboxyl groups (Hsu et al., 2009). The vibrations of bonds in ring skeletal C-O and bond stretching in C-C contribute to bands at 1115.1 cm^{-1} (Shen et al., 2010). The band at 875.6 resulted from the out-of-plane bending of aromatic C-H bonds (Yang et al., 2007). Aromatic C-H in plane vibrations resulted in the peak at 802.5 cm^{-1} (Moreno-Castilla et al., 2000). The above mentioned changes in adsorption peak intensities in FTIR spectra confirmed the active role of functional groups for the reduction of Cr(VI) in soils.

6.8 Conclusion

- This chapter has demonstrated that the addition of iron(III) oxides greatly decreased the reduction of Cr(VI) in soils. The increased adsorption of Cr(VI) by iron oxides decreased the bioavailable fraction of Cr(VI) to the microorganisms for this reduction. Hence free Cr(VI) ions in soils is essential for the reduction to proceed.

- Chromium(VI) reduction decreased when pH rose and there was an increase in pH with Cr(VI) reduction. While Cr(VI) reduction is a proton (H^+)-consuming process, Cr(III) immobilization (precipitation/adsorption) is a H^+-releasing process, so the predicted values of H^+ consumption for Cr(VI) reduction were slightly higher than the measured values.

- It emerged in this chapter that black carbon containing high surface area with easily oxidisable functional groups is efficient in the reduction of Cr(VI). This was confirmed by the changes in the different functional groups on the surface of BC resulting from Cr(VI) reduction.

- Dissolved organic carbon (DOC) greatly influences Cr(VI) reduction, increasing the reduction of toxic Cr(VI) to less toxic and relatively immobile Cr(III).

- Finally, the reduction of Cr(VI) to Cr(III) by black carbon and the concurrent immobilization of Cr(III) due to a rise in the level of pH is an effective and economical method to mitigate Cr(VI) contaminated soils.

Chapter 7

IMPACT OF DIVERSE ORGANIC CARBON SOURCES ON PHYTOAVAILABILITY OF CHROMIUM(VI) IN SOIL

7.1 Introduction

The amount of Cr(VI) release into the environment is ever increasing due to industrial activity. Chromium(VI) is toxic to most of the organisms and plants. There are several methodologies to prevent toxicity of Cr(VI) including adsorption and reduction. Chitosan, sea weed, bacteria, algae are some of the biosorbents for removal of Cr(VI) (Boddu et al., 2003; Lin et al., 2009). Sorbents such as sludge biomass, agricultural waste and saw dust reduce the mobility of Cr(VI) in water and soils and are ideal for large scale application since they are less expensive. However, these adsorbing materials may contribute additional load to the environment or cause hazards to ecosystems once they are used and disposed with residual Cr(VI) (Tzou et al., 2003).

Previous results have shown that Cr(VI) toxicity can be mitigated through both adsorption and reduction mechanisms. Adsorption of Cr(VI) decreases the bioavailable fractions of Cr(VI) but won't eliminate the Cr(VI) from soils. Both toxicity and bioavailability depend on speciation of Cr, hence the best possible remediation strategies is to convert toxic, mobile and soluble Cr(VI) to less harmful and insoluble Cr(III) by means of reduction mechanisms (James, 2001).

The reduction of Cr(VI) to Cr(III) and its subsequent immobilization depends on the biogeochemical reactions that are controlled by microorganisms. Organic matter promotes the bio-reduction of Cr(VI), serves as source of energy for microorganisms. Biogeochemical reactions also control the degradation of organic matter by several microorganisms and ultimately serve as terminal electron acceptors (Kyziol et al., 2006). Humic and fulvic acids, carbohydrates and protein, several manures, black carbon and seaweed enhance the reduction process (Wittbrodt and Palmer, 1996; Bolan and Thiagarajan, 2001; Bolan et al., 2003; Park et al., 2004; Zhou et al., 2008). Reduced chemical species such as

sulphite, , dithionite, Fe(II) and sulfides supply electrons to reduce Cr(VI) to Cr(III) (Pal and Yost, 1995; Fendorf and Li, 1996; Zhou et al., 2001; Su and Ludwig, 2005). However, excessive addition of these chemicals may increase the redox potential of soils, thereby increasing the solubility of other heavy metals (Skipper and Turco, 1995)

The results in Chapter 6 have demonstrated that organic amendments such as black carbon, cow manure and biochar have decreased the Cr(VI) concentration in alkaline soils by enhancing the reduction process. The high efficiency of black carbon was attributed to the role of functional groups, which donate electrons for Cr(VI) to get reduced. Cow manure increased dissolved organic carbon in soils and DOC is responsible for Cr(VI) reduction. Biochar enhanced Cr(VI) reduction by undergoing microbial oxidation thereby providing electrons (Section 6.7.5 in Chapter 6).

The plant growth in contaminated soils is inhibited due to the toxicity of excessive levels of heavy metals. Chromium species are toxic, non-essential to plants and are detrimental to plant growth (Shanker et al., 2005). Chromium renders growth retardation, accumulates, clots in vascular bundles of roots and shoots. In soils, the solubility of Cr(III) species are considered to be less than Cr(VI) (Srivastava et al., 1999). Plants grown in Cr(VI) contaminated soils are subjected to higher phytotoxicity than those grown in Cr(III) contaminated soils (Han et al., 2004). Phyto-availability of Cr(VI) is higher than Cr(III) and toxic to many plants, translocates easily into the plant tissues along with sulfates and damages plant tissues (Adriano, 1986; Han et al., 2004).

Application of organic amendments to the soils increase dissolved organic carbon (DOC) either by acting as source of DOC or solubilisation of soil organic matter (Schindler et al., 1992). Numerous researchers have confirmed that application of organic amendments also reduces Cr toxicity in soils. However, only limited work has been accounted on the beneficial effect of organic amendments on the remediation of phytotoxicity in soils (Table 7.1). Moreover, there was no comprehensive study on the role of pH of organic amendments in decreasing Cr(VI) phytotoxicity in contaminated soils. Sunflower (*Helianthus annuus* L) plant has been proven as hyper-accumulator of chromium (Shahandeh and Hossner, 2000). In this study, we examined the effect of various organic carbon sources that vary in pH and carbon fractions (Black carbon, chicken waste biochar and cow manure) on the mitigation of Cr(VI) phytotoxicity in soils.

Table 7.1 Selected references on the remediation of Cr(VI) phytotoxicity by organic amendments

Amendment	Plant used	Observation	Reference
Cow manure	*Festuca arundiace*	Reduced Cr in roots and no change in Cr concentration in shoots	Banks et al., 2006
Biosolids compost	*Brassica juncea*	Reduced Cr concentration in plant tissue	Bolan et al., 2003
Cattle compost and straw	*Lactuca sativa*	Dry matter of shoot and roots increased	Rendina et al., 2005
Hog dung and cattle dung compost	*Triticum vulgare*	Growth of seedlings increased	Lee et al., 2005
Bark of *Pinus radiata*	*Helianthus annuus*	Reduced availability of Cr for plant uptake	Bolan et al., 2001
Dairy sludge	*Jatropa curcas*	Reduced Cr uptake by plant	Yadav et al., 2009
Biosolids compost	*Sesbania punicea* and *Sesbania virgata*	Decreased Cr in plant extract	Branzini and Zubillaga, 2010
Farm yard manure	*Spinacea oleracea*	Increased root and shoot growth	Singh et al., 2007
Dry cattle manure	*Medicago sativa*	Increased plant biomass yield	Losi et al., 1994

7.2 Objectives

The overall objective of this study is to examine the impact of diverse organic carbon sources (Black carbon, chicken waste biochar and cow manure) on the phytoavailability of Cr(VI) in soils. The specific objectives include:

- To investigate the toxicity of Cr(VI) to plants using a hyper-accumulator, *Helianthus annuus* L.
- To evaluate the uptake of Cr(VI) in plant tissue.
- To examine the distribution of Cr(VI) in shoots and roots.
- To Illustrate the relationship between soil solution and Cr(VI) uptake in plants.

7.3 Hypothesis

Addition of organic amendments increases DOC in soils, functional groups in the DOC donate electrons and reduces Cr(VI) to Cr(III), thereby decreases the toxicity and phytoavailability.

7.4 Experiments

The major experiments conducted to examine the phytoavailability of Cr(VI) as effected by organic carbon sources are listed in table 7.2.

Table 7.2 The major experiments and the treatments used for phytoavailability experiment

Title	Treatments	Measurements
Plant growth	**Soils:** Acidic (ADH), Alkaline (KPB) **Cr(VI) levels:** 0, 50, 100, 250 mg/kg **Organic amendments:** Black carbon (BC), Chicken waste biochar (CWB) and cow manure (CM)	Dry plant biomass (root and shoot), Total Cr
Pore water analysis	**Soils:** Acidic (ADH), Alkaline (KPB) **Cr(VI) levels:** 0, 50, 100, 250 mg/kg **Organic amendments:** Black carbon (BC), Chicken waste biochar (CWB) and cow manure (CM) **Days of pore water collection**: 0, 7, 14, 21, 28	pH and water extractable Cr

7.5 Materials and methods

7.5.1 Soils collection and characterization

Bulk samples of two different soils, were collected from uncontaminated surface soils from South Australia. The collected soils vary widely in their origin, mineralogy, and soil properties. The acidic Adelaide Hills soil (ADH) is classified as Kurosol and the alkaline

Kulpara Bay soil (KPB) as Chromosol. The air-dried soil samples were disaggregated with agate mortar and sieved with steel screen with an opening of 2 mm to ensure the uniformity and were stored in polythene bags until further analysis.

7.5.2 Preparation of soil treatments

Both acidic and alkaline soils were selected to examine the effect of soil pH on phytoavailability of Cr(VI) and impact of amendments. Soil samples were treated with different levels of Cr(VI) (0, 50, 100 and 250 mg/kg) using $K_2Cr_2O_7$ as Cr(VI) source and incubated at field capacity. These soil samples were subsequently mixed with 5% organic amendments and transferred to pots.

7.5.3 Plant growth experiment

The method developed by Stanford and Dement (1957) was used for the plant growth experiment in this chapter. This method permits the measurement of metal absorption by plants during relatively short periods of root-soil contact. In the usual pot-type plant assay, the seed is germinated in the soil material to be tested and permitted to grow for a specified period of time. Such a technique imposes limitations such as: (i) possible secondary effects of soil treatment on seed germination and seedling growth, prior to the time which the metal may affect the plant growth; (ii) undue length of time for chemical or microbial activities to occur in the soil resulting in masking of the effects of the original treatment; and (iii) the usual problems of obtaining uniform seedling stands for suitable controls. The advantages of Stanford-Dement bioassay are: (i) short-term effects on higher plants where dry matter production is not the parameter of concern; (ii) simple and economical to use and allows for adequate replication; and (iii) only small quantities of soil are needed.

Transparent round plastic pots with 700 ml capacity and bottoms removed were nested in similar containers which had intact bottoms and filled with 500 g of sand. Ten seeds of sunflower (*Helianthus annuus*) were sown directly into the sand at 5 mm depth and the pots were covered loosely with lids for 3 days to maintain the moisture content and thereby achieving germination. The pots were watered twice with deionized water every day for 10-12 days during the germination period. After this period, Hoagland nutrient solution (Hoagland and Arnon, 1950) was added to each pot twice a day to the required moisture content. The plants were thinned to 6 per pot after two weeks (Plate 7.1).

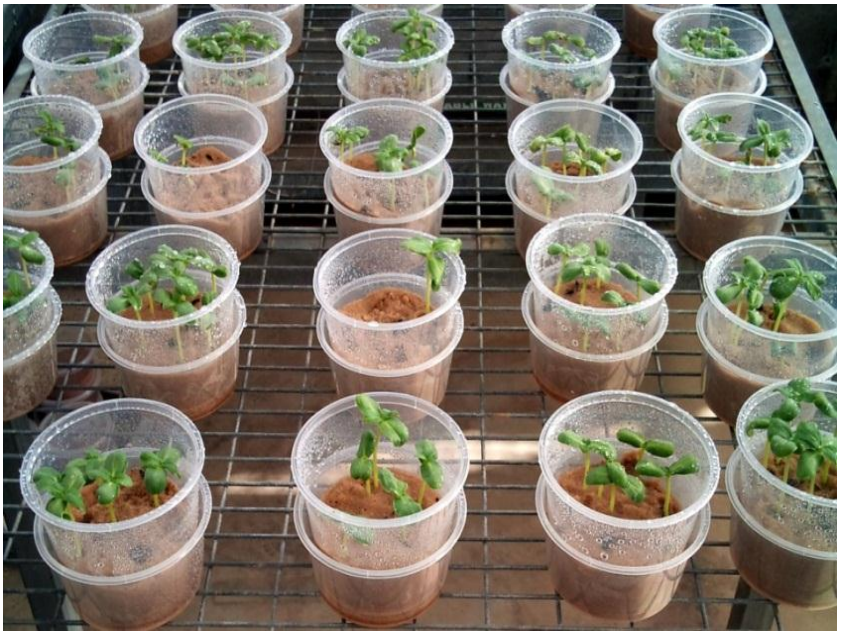


Plate 7.1 Plants grown using Stanford and Dement method

After 5 weeks of plant growth, the plastic containers containing plants were nested in the second plastic container holding 200 g of soil, thus placing the dense mat of roots in intimate contact with the soil. The soils were wetted to 70% water holding capacity (WHC) before the transfer of plant containing pots. Rhizon soil moisture samples were inserted into selected soil samples to allow sampling of soil pore water during the experiment. Moisture losses were replenished by periodic water/nutrient solution additions. Plants were harvested at 9 weeks after sowing.

7.5.4 Rhizon sample analysis

Rhizon samplers (one per pot, Rhizosphere Research Products, Wageningen, Netherlands), which are porous only to liquids were placed horizontally at 1 cm from the bottom of pot. Each treatment was carried out in triplicate. Vacuum extraction method was used by using 10 ml disposable syringes to collect pore water. Vacuum was applied over 12 hours period, during night and the liquid samples were collected at 1, 2, 3 and 4 weeks after the establishment of the pot experiment, and analysed for Cr using ICP-OES on the same days of collection. Cr(VI) was analysed by the method described in Section 4.5.7 in Chapter 4. The amount of Cr(III) was calculated by subtracting Cr(VI) from total Cr in pore water.

7.5.5 Plant biomass analysis

The plant materials were analysed for metal content using nitric acid digestion (Zarcinas et al. 1987). The ground plant material (0.1-0.5 g) was weighed directly into a 75 mL digestion tube with 5 mL of concentrated nitric acid and left to cold digest in a fume cupboard overnight. After keeping the samples overnight, the tubes were heated using a temperature controlled digestion block (AI Scientific Block Digestion System AIM 500) programmed to slowly increase the temperature to 140^0C until 1 mL of digest remained in the tube. The tubes were brought to room temperature prior to the dilution with deionised water. The concentration of Cr(III) in the digested plant solution by Inductively Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) (Perkin-Elmer Optima-5300, Japan). Relative water content of the shoots was estimated from the Eqn. 7.1

$$\mathrm{RWC}(\%) = \frac{(\text{fresh wt.} - \text{dry wt.})}{\text{fresh wt}} \times 100 \qquad (7.1)$$

7.6 Statistical analysis

All measurements including pH, Cr concentration and plant growth were calculated from triplicates of each treatment. Statistical analysis for correlation among weight of dry biomass, Cr concentration in biomass and Cr in soil pore water was performed by using Excel (Microsoft, 2010) and Grapher ^{TM} software (Golden Software 7, USA).

7.7 Results and discussion

7.7.1 Uptake and accumulation of Cr(VI)

The results showed that dry biomass production was slightly higher in alkaline soils when compared to acidic soils. Plants grown in acidic and alkaline soils (control) produced dry biomass of 3.437 and 3.791 g/pot, respectively. The soil pH affects the nutrient availability and root growth of plants. The excessive amount of H^+ ions in soil solution influences the root cell membrane potential. In the soils, the solubility of Al increases in soils below pH 4.5, and displaces cations such as calcium, potassium and magnesium in soil solution, thereby decreases the plant growth (Bates et al., 2002).

Plants from the both acidic and alkaline contaminated soils showed a distinctive decrease in biomass (fresh and dry), and relative water content (RWC). There was a positive correlation (R^2=0.77) between fresh biomass and RWC (Figure 7.1). As the Cr(VI) concentration in the soils increases, the water content in the plant decreases, indicating the potential stress of Cr(VI) on plants (Su et al., 2005).

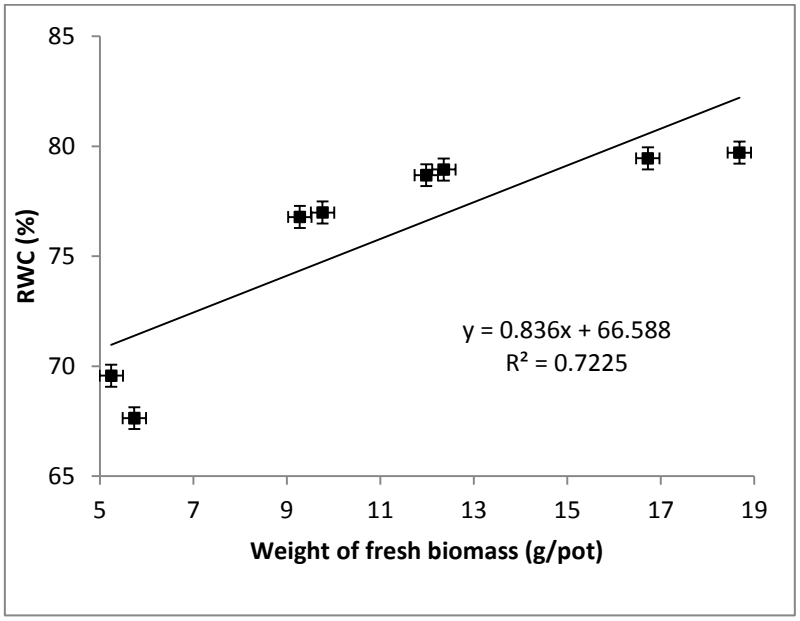


Figure 7.1 Relationship between weight of fresh biomass and relative water content

The plant growth decreased with increasing levels of Cr in soils and biomass. Biomass of sunflower plant grown in 250 mg Cr(VI)/kg acidic and alkaline soils greatly depressed, 1.595 and 1.5962 g/pot, respectively (Table 7.3) suggesting the toxicity of Cr(VI) at higher concentrations. Biomass was higher in acidic contaminated soils than alkaline soils. This difference may be due to the effect of pH on Cr reduction. As the pH of the soils decreases, the reduction of Cr(VI) increases (James, 1985).



Plate 7.2 Effect of Cr(VI) on the sunflower plant - visible lesions of interveinal chlorosis.

As the Cr(VI) in the soils increased, the concentration of Cr in the roots and shoots increased . The accumulation of Cr is more in roots than shoots. The high accumulation of Cr in roots may be due the immobilization of Cr in root vacuoles, inhibiting the transfer to shoots, which is natural response to toxicity and also Cr inhibits the root cell division (Barcelo et al., 1986; Shanker et al., 2004). Chromium(VI) replaces magnesium (Mg) in several enzymes and decreases chlorophyll content in shoots and also depletes carotenoids in plant tissue (Vajpayee et al., 2000) (Plate 7.2). In addition, Cr inhibits Hill's reaction and decreases biomass of the plant (Panda and Choudhury, 2005). However, total uptake of Cr(VI) increased in both root and shoot when the concentration of Cr(VI) increased in contaminated soils (Table 7.3) (Plate 7.3).



Plate 7.3 Phytotoxic symptoms of Cr(VI) in shoot, (a) stems of plants grown in soil without Cr(VI) and (b) stems of plants grown in Cr(VI) contaminated soils

Table 7.3 Biomass production, accumulation of Cr in shoots and roots in sunflower plant in Cr(VI) contaminated soils.

Soil type	Addition Cr(VI) (mg/kg)	Biomass		RWC* (%)	Shoot (mg/kg)	Root (mg/kg)	Total Cr in biomass (mg/kg soil)	Shoot/ root
		Dry (g/pot)	Fresh (g/pot)					
Control (ADH)	0	3.437	16.724	79.448	BDL*	BDL	BDL	BDL
Control (KPB)	0	3.791	18.683	79.708	BDL	BDL	BDL	BDL
Contaminated (ADH)	50	2.602	12.356	78.941	1.157	4.346	2.752	0.266
	100	2.247	9.763	76.984	1.813	7.638	4.726	0.237
	250	1.595	5.735	69.566	2.196	12.47	7.336	0.186
Contaminated (KPB)	50	2.554	11.982	78.684	1.157	3.426	2.292	0.337
	100	2.154	9.277	76.781	1.349	6.097	3.723	0.212
	250	1.562	5.241	67.637	2.014	11.45	6.732	0.175

*ADH-Adelaide Hills soil (acidic); KPB-Kulpara Bay soil (alkaline); BDL-Below detectable level (<0.05 mg Cr(VI)/L); RWC-Relative Water Content

Chromium(VI) has the capacity to damage the cell membrane due to its high oxidation power, so higher Cr(VI) may have passively transferred along with sulfates and phosphates (Vazquez et al., 1987). There was a negative relationship between Cr concentration in shoots and RWC indicating that Cr affects cell turgor (Figure 7.2) (Su et al., 2005). The accumulation of Cr in roots may result from Cr binding to organic molecules such as carbohydrates and proteins or due to the formation of simple Cr salts (Han et al., 2004).

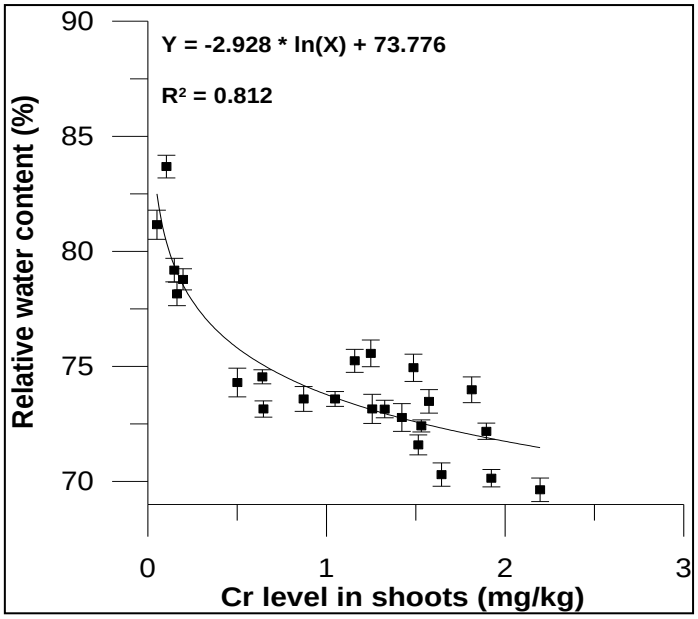


Figure 7.2 Relationship between concentration of Cr in shoots and RWC.

7.7.2 Effect of organic amendments on the plant uptake and translocation of Cr(VI)

Application of amendments decreased Cr(VI) toxicity in plants in both acidic and alkaline contaminated soils, thereby increasing dry biomass. The biomass production was more in acidic soil than in alkaline soils. Application of black carbon to contaminated soils has enhanced dry biomass production, followed by biochar and cow manure.

In acidic soils, addition of 5% black carbon increased the plant dry matter yield by 23, 26 and 31 % in soils treated with 50, 100 and 250 mg Cr(VI)/kg, respectively (Figure 7.3). Biochar application increased dry matter yield by 16, 20 and 22 % in acidic soils treated with 50, 100 and 250 mg Cr(VI)/kg, respectively (Figure 7. 3). Cow manure (5%, w/w basis) inclusion into acidic contaminated soil increased dry biomass by 14, 18 and 21% in acidic soils, which have 50, 100 and 250 mg Cr(VI)/kg, respectively. Addition of BC, biochar and cow manure decreased Cr accumulation in biomass by 67, 55 and 34%, respectively in acidic soil treated with 50 mg Cr(VI)/kg. At higher Cr(VI) concentrations (250 mg/kg), the decrease in Cr accumulation was more in roots (54%). Whereas at shoots, the decrease in Cr accumulation was only 43% compared to plant grown in Cr contaminated soils without BC application.

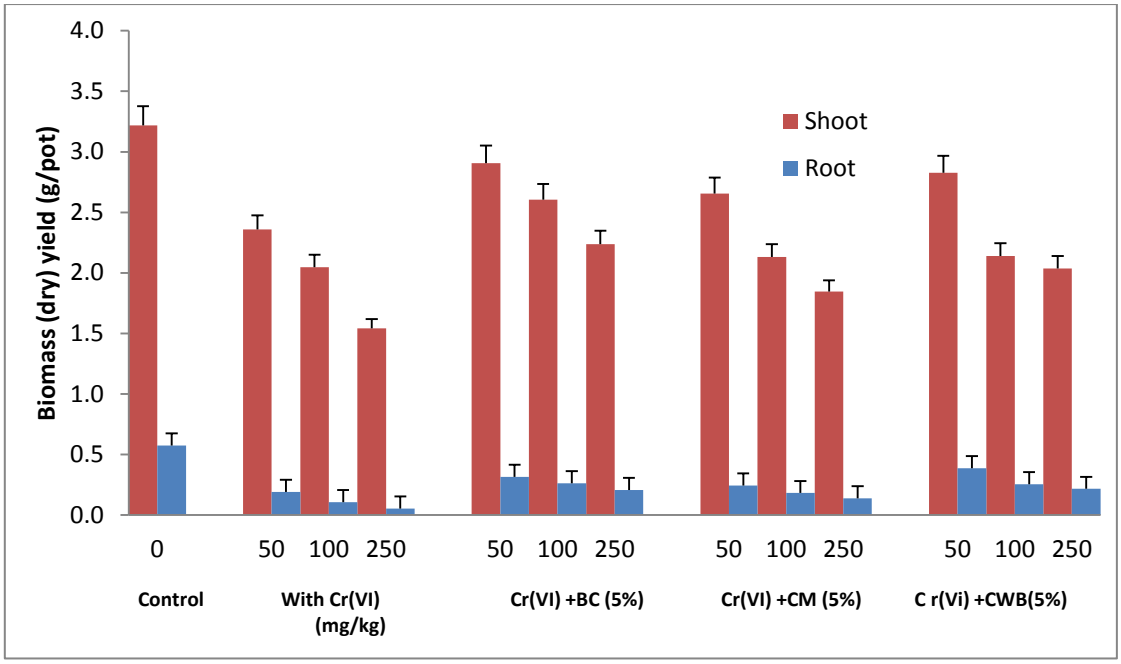


Figure 7.3 Relationship between average plant dry biomass (g/pot) and Cr(VI) concentration in acidic soils.

In alkaline soils, amended with BC (5%, w/w basis) increased dry matter yield by 21, 22 and 25% in soils treated with 50, 100 and 250 mg Cr(VI)/kg, respectively. Biochar addition (5%, w/w basis) increased dry matter yield by 15, 22 and 27% in 50, 100 and 250 mg Cr(VI)/kg, respectively in contaminated alkaline soil. Cow manure (5%, w/w basis) addition increased dry biomass by 12, 14 and 19% in 50, 100 and 250 mg Cr(VI)/kg, respectively. Moreover, black carbon, biochar and cow manure decreased Cr in plant dry matter by 71, 34 and 13%, respectively in 50 mg Cr(VI)/kg contaminated soil.

The increased biomass after the application of amendments is due to favourable conditions for Cr(VI) reduction. For Cr(VI) to get reduced, both protons and electrons are essential (Eqn. 7.2).

$$CrO_4^{2-} + 8H^+ + 3e^- \rightarrow Cr(OH)_3 + 4H_2O \qquad (7.2)$$

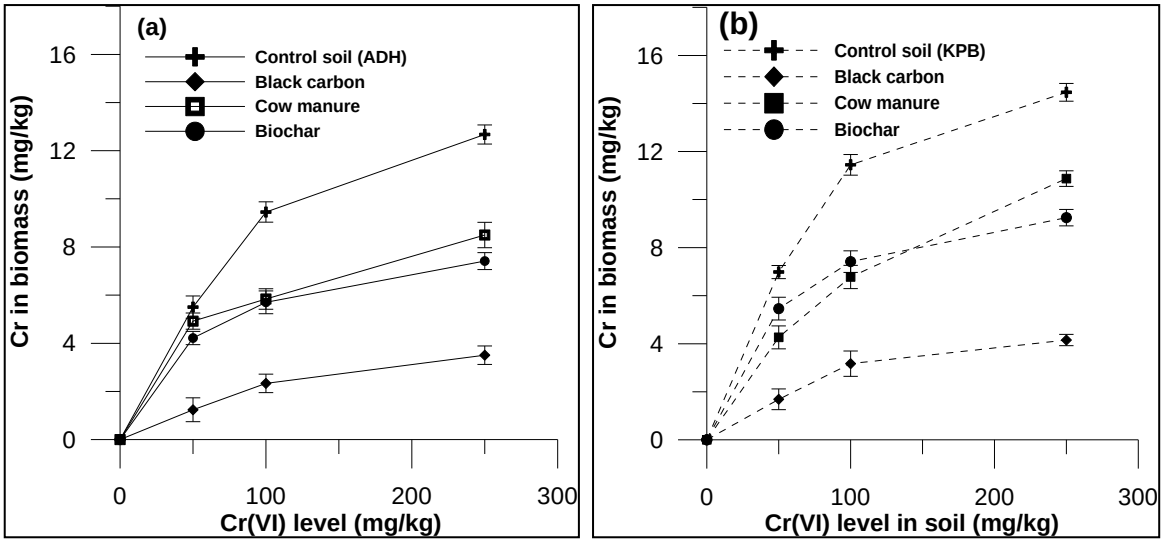


Figure 7.4 Effect of different organic amendments on Cr in biomass (a) in acidic soil (ADH) and, (b) in alkaline soil (KPB).

Addition of organic amendments increases the availability of free electrons in the soils (Park et al., 2010). The acidic soils have more number of protons for Cr(VI) reduction, hence the reduction in acidic soil is generally higher than alkaline soil.



100 mg Cr(VI)/kg + 5% Black carbon **100 mg Cr(VI)/kg**

Plate 7.4 The effect of biochar on plants grown in Cr(VI) contaminated soils

However, organic amendments have a profound effect on the reduction of Cr(VI) in alkaline contaminated soil. Application of BC to Cr(VI) contaminated acidic soil substantially decreased the Cr concentration in plant tissue (Fig. 7.4a, b) (Plate 7.4).

7.7.3 Relationship between Cr concentration and plant growth

There was a negative relationship between concentration of Cr and production of biomass in sunflower plant (Fig. 7.5). Chromium accumulation was more in biomass that grown in alkaline soil than in acidic soil. Acidic soil contains more number of H^+, which diminish the transport of Cr(VI) into plant tissue by reducing into Cr(III). As the reduction precedes, the pH of the soils increases, thereby Cr(III) also immobilizes.

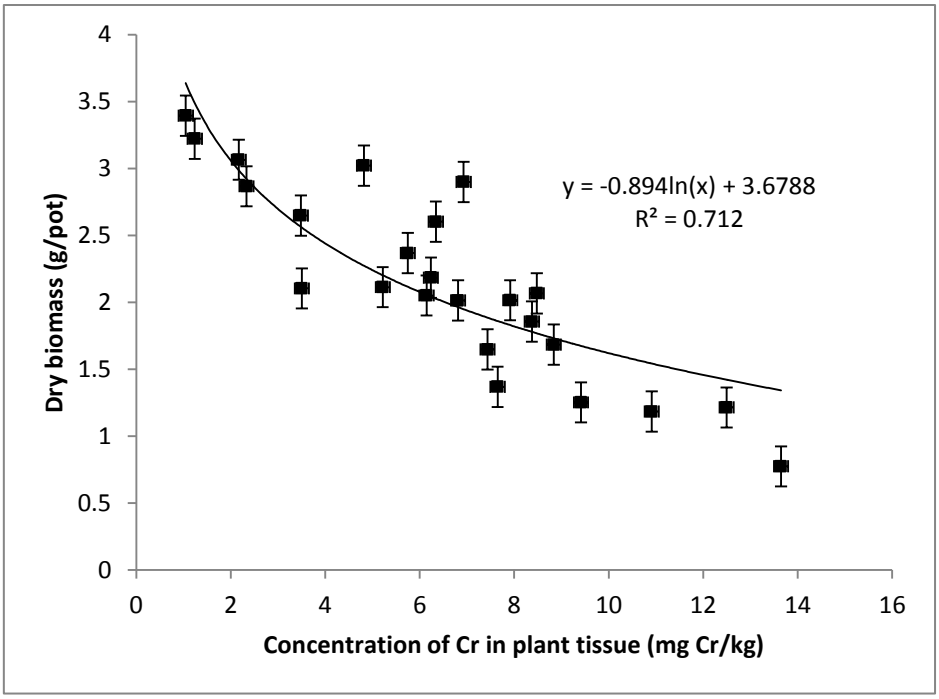


Figure 7.5 Relationship between and Cr(VI) level and plant biomass.

7.7.4 Soil pore water analysis

The results from the soil pore water collected by Rhizon sampler showed that Cr(VI) concentration increased with increasing levels of Cr(VI) application to soils. However, the concentration of Cr decreased with increasing time, which is attributed to the reduction of Cr(VI) to Cr(III). Earlier incubation experiments have shown that the concentration of Cr(VI) decreased with time, which was due to reduction of Cr(VI) to Cr(III) (Section 6.7.4 in Chapter 6).

7.7.4.1 Chromium (VI) in pore water from acidic soil

There was a significant decrease in the concentration of Cr(VI) in pore water upon the addition of BC to the contaminated acidic soils. The decrease in Cr(VI) concentration is more in acidic soils that was incubated with 50 mg Cr(VI)/kg soil than alkaline soils. Addition of 5% BC decreased Cr(VI) from 0.84 mg/L in 1 day after addition of BC to 0.11 mg within 14 days (Table 7.4). At 100mg Cr(VI)/kg level, the pore water concentration was decreased from 2.681 to 0.714 mg Cr(VI)/L in 28 days in the absence of amendment (Figure7.6a). Whereas, addition of BC decreased 100% available Cr(VI) in porewater in 21 days.

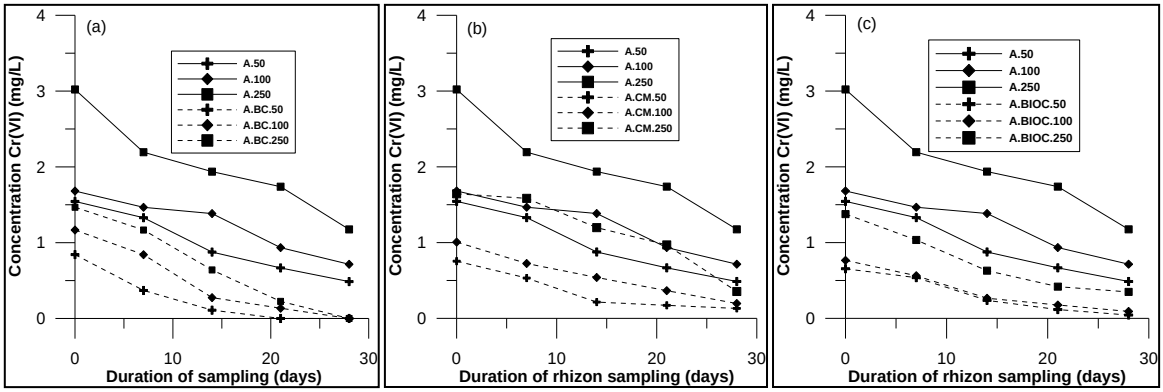


Figure 7.6 The effect of black carbon (a), cow manure (b) and biochar (c) on pore water concentration of Cr(VI) in acidic soils.

The addition of cow manure and biochar also decreased Cr(VI) in acidic soil pore water (Fig 7.6b) and the effect was more pronounced with biochar than cow manure. Cow manure decreased Cr(VI) from 57 to 68% in 28 days in contaminated soil (250mg Cr(VI)/kg) whereas biochar decreased from 63 to 79 mg/L during 28 days (Fig 7.6c).

The effect of BC addition on the Cr(VI) concentration was due to the reduction of Cr(VI) to non-toxic Cr(III). Black carbon contains high surface area, which is highly reactive with many acidic functional groups, thereby supplying electrons for Cr(VI) reduction (Wang et al., 2010). As the concentration of Cr(VI) increased in soils, the effect of BC on reduction of Cr(VI) in pore water was decreased. This may be because of the electrons from the BC are not sufficient for the reduction. Black carbon reduces Cr(VI) in two step mechanism. Functional groups on BC adsorbs Cr(VI); and subsequently reduces to Cr(III) and the resultant Cr(III) is immobilized through surface complexation with surface on BC (Hsu et al., 2009).

Table 7.4 Effect of organic amendments on Cr(VI) concentration in pore water in acidic soil.

Days	Cr(VI) in pore water (mg/L) In acidic soil			Black carbon addition (5%, w/w basis)			Cow manure addition (5%, w/w basis)			Biochar addition (5%, w/w basis)		
	50	100	250	50	100	250	50	100	250	50	100	250
	mg/kg											
	Percentage decrease in Cr concentration in porewater*											
	1.544	2.681	3.021	45	56	58	33	55	57	51	59	63
7	1.329	1.967	2.194	72	57	47	37	53	47	55	66	68
14	0.876	1.385	1.938	88	80	67	41	61	42	68	72	73
21	0.666	0.935	1.738	100	86	87	62	61	66	70	72	76
28	0.487	0.714	1.175	100	100	100	63	64	68	77	87	79

*Decreased Cr conc. in pore water (%) = [(Cr conc. without amendment – Cr conc. with amendment) / Cr conc. without amendment] * 100

Biochar is highly porous and has high adsorptive capacity. Once the biochar mixes with water, the surface area increases because of swelling and creates more biochar/water contact (Mohan et al., 2011). Hence the phytoavailability of Cr(VI) decreases in the presence of biochar.

7.7.4.2 Chromium (VI) in pore water from alkaline soil

The pore water collected from alkaline soil had high concentrations of Cr(VI) within a day after incubation. Application of BC, CM and CWB decreased Cr(VI) in pore water. In the alkaline soil, addition of 5% BC decreased Cr(VI) pore water concentration by 91% within 21 days in 250 mg Cr(VI)/kg. Whereas CWB decreased Cr(VI) concentration by 88% in 21 days and both BC and CWB decreased Cr(VI) concentration by 100% in 28 days in treated soil (Fig 7.7a; 7.7c). Cow manure addition has limited effect on Cr(VI) in pore water concentration, reducing Cr(VI) concentration only by 56% in 28 days in 250 mg Cr(VI)/kg contaminated soil (Fig 7.7b).

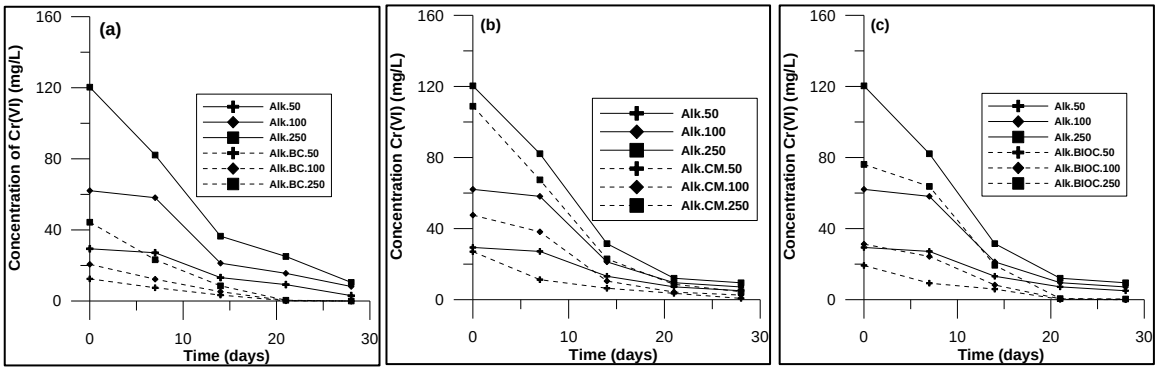


Figure 7.7 The effect of amendments; (a) amended with black carbon, (b) cow manure and (c) biochar on Cr(VI) level in alkaline soil pore water.

Soil parameters such as pH, Eh and moisture content affect the reduction of Cr(VI), and Cr(VI) mobility is high in alkaline soils (James, 1994; Wittbrodt and Palmer, 1995). However, addition of BC, CM and CWB decreased Cr(VI) concentration in both acidic and alkaline soils through reduction, thereby inhibited the phytotoxicity of Cr(VI) to plants, which was confirmed by decreased Cr(VI) concentrations in soil pore water. Organic amendments were more effective in alkaline soils where the reduction is low in the absence of organic amendments than in acidic soils.

7.7.4.3 Effect of pore water Cr(VI) concentration on chromium uptake by plants and its biomass

The results showed that the uptake of Cr(VI) into plant biomass increased with an increase in free Cr(VI) species in both acidic and alkaline soils (Fig. 7.8a). The positive correlation between pore water Cr(VI) concentration and plant tissue Cr(VI) concentration was attributed to the passive translocation of Cr(VI) into plant tissue along with sulfate ions because of its chemical similarity (Zayed and Terry, 2003). The speciation of Cr in plant materials were not determined due to the limitations of the acid digestion method, however other studies suggested the reduction of Cr(VI) in several plants (Howe et al., 2003; Xu and Xaffe, 2006).

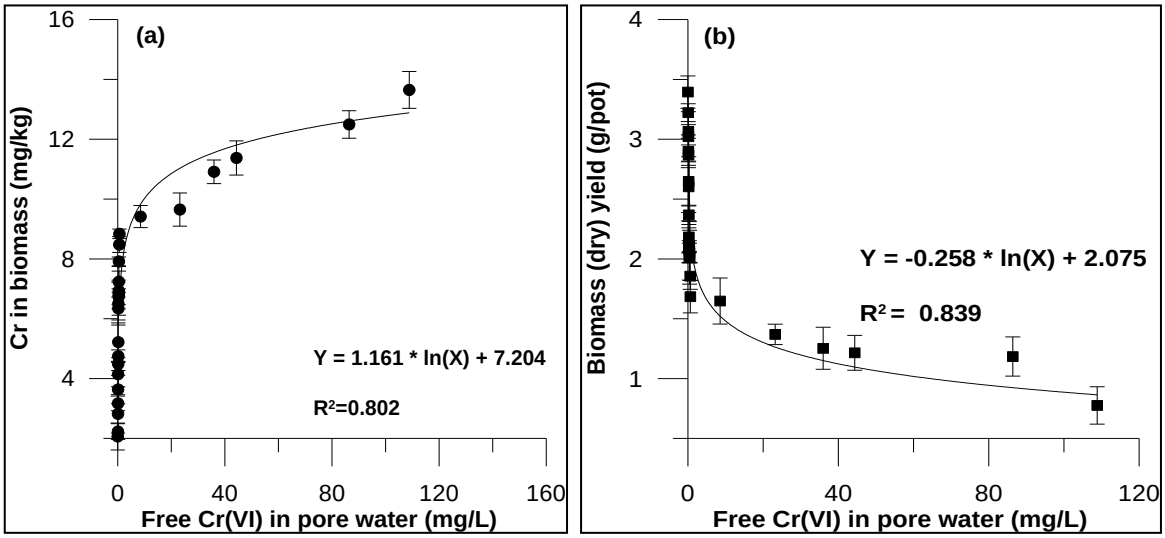


Figure 7.8 (a) The effect of pore water Cr(VI) on Cr tissue concentration, and (b) on plant biomass production in contaminated soils.

In contrast, there was negative relationship between free Cr(VI) species in pore water and plant biomass production (Fig. 7.8b). This relationship shows the toxicity of free Cr(VI) species in the soils, thereby decreasing plant growth. Hence the mitigation of available Cr(VI) species is of prime importance for sustainable environment.

7.8 Conclusion

- Chromium (VI) is toxic to plant, thereby reducing dry-biomass of both root and shoots. The higher concentrations of Cr in rootssuggested that Cr accumulates mostly in roots, and at higher Cr concentrations of Cr(VI) in soils Cr translocates into shoot.

- As the chromium concentration in plant tissue increased, the production of biomass decreased, suggesting the phytotoxic effect of Cr(VI) in soils.

- With an increase in free Cr(VI) species in pore water, Cr accumulation in the plant tissue concentration increased and the plant dry biomass decreased.

- Application of organic amendments were found to be effective in decreasing phytoavailability of Cr(VI) in soils.

- Black carbon was shown to play significant role in reducing Cr(VI) phytotoxicity, followed by biochar and cow manure.

- In acidic and alkaline soils, addition of organic amendments decreased phytotoxic symptoms, as shown by increased plant growth. However, the effect of organic amendments was more pronounced in alkaline soil.

- The availability of free Cr(VI) in alkaline contaminated soils is higher than acidic soil and the application of organic amendments decreased Cr(VI) concentrations in pore water.

Chapter 8

SUMMARY AND CONCLUSIONS

This chapter summarizes the key outcomes arising from this research and issues that need further consideration. In this study, the simultaneous quantification of chromium (Cr) species using complexing ligands was tested. The soil properties that determine the fate and availability of Cr species in soils were evaluated and the impact of soil amendments on the immobilization of Cr species was addressed. The effect of diverse organic carbon sources on the reduction and bioavailability of Cr(VI) in contaminated soils was evaluated. Furthermore the results have demonstrated the potential value of utilizing new organic carbon sources to mitigate Cr(VI) toxicity in soils.

8.1 Specific contributions

8.1.1 Simultaneous estimation of Cr species

The two predominant Cr species in the environment - Cr(III) and Cr(VI) - have contrasting toxic properties. While Cr(III) is an essential nutrient at low concentrations (>200 µg/day), Cr(VI) causes cancer in people even in minute quantities (<0.05 µg/L). It is therefore vitally important to accurately quantify these two species. The method we developed was efficient in the simultaneous measurement of Cr(VI) and Cr(III) in soil solutions and leachates.

The results showed that complexing ligands have the ability to convert cationic Cr(III) to anionic species with an ion chromatography connected to an inductively coupled plasma-mass spectrophotometer (IC-ICP-MS). The parameters of the mobile phase in the IC column play an important role in the separation of both [Cr(III)-ligand] complex and Cr(VI) by mass/charge ratio, as indicated by peak intensities. This method, however, needs to be improved to eliminate interference by other ions during the complexation of Cr(III) with ligands.

8.1.2 Chromium adsorption

The mobility and bioavailability of Cr is controlled by adsorption and reduction reactions in soil (Figure 8.1), which are critical in understanding the fate and mobility of solutes in the environment. Chromium ions, Cr(III) and Cr(VI), have different adsorption characteristics, resulting in different behaviour in soils. Results indicated that Cr(III) adsorption was higher than Cr(VI) in the soils used in this study. The soil parameters, pH and CEC, control the adsorption of Cr(III), and increase as pH and CEC also increase. However, these parameters had no significant effect on the adsorption of Cr(VI) in soils.

Organic matter and clay content also contributed to the adsorption of Cr(III), producing a positive relationship with Cr(III) adsorption. Chromium(VI) species participates in both outer-sphere and inner-sphere complexation and its desorption was higher than that of Cr(III). The leaching of Cr(III) was less than Cr(VI) in soils. The mobility of Cr species as estimated by retardation factor suggested that Cr(VI) mobility was higher than Cr(III) in soils.

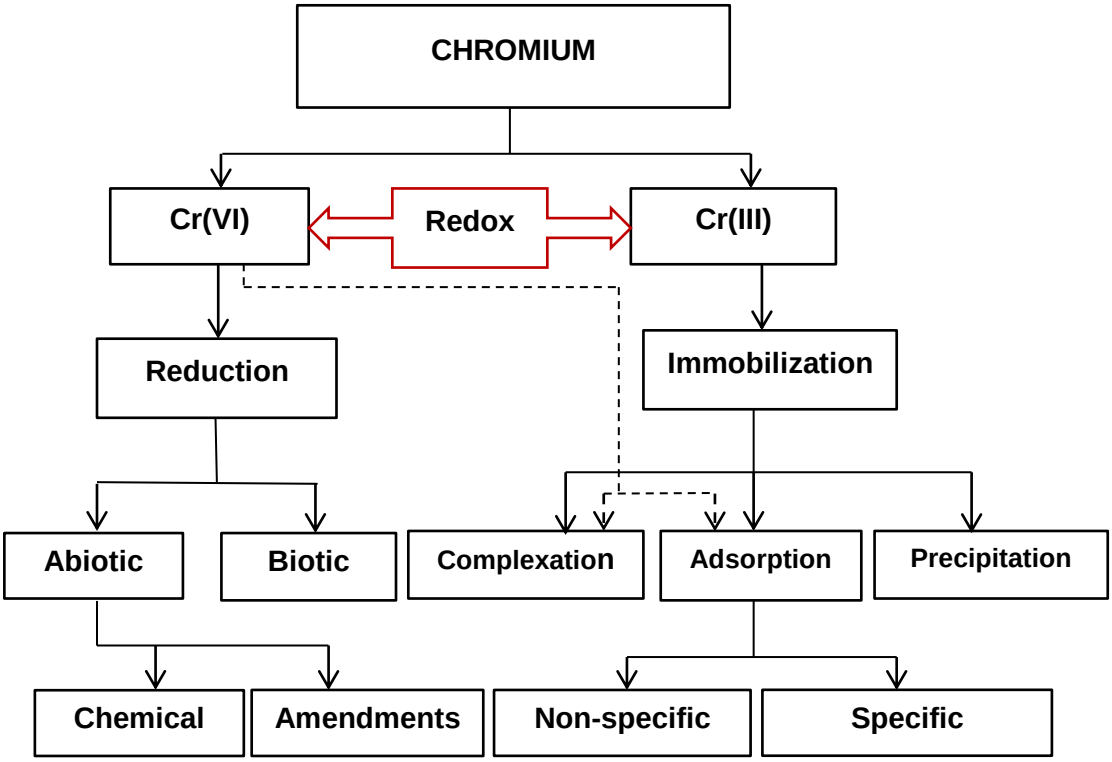


Figure 8.1 Schematic diagrams of chromium dynamics and mitigation of Cr toxicity in soils.

8.1.3 Effect of soil amendments on sorption of Cr in soils

Soil properties such as pH, surface charge and redox reactions are critical in the retention of Cr(III) and Cr(VI) in soils. An increase in retention of these two species is important to reduce their damaging impact on the environment. The effect of lime and elemental sulphur [S(0)] (i.e. proton dynamics), iron(III) oxide (i.e. surface charge) and cow manure (i.e. functional groups) on Cr(III) and Cr(VI) retention was examined. The results showed that adding lime increased soil pH, thereby increasing the retention of Cr(III) in soils. In contrast the addition of S(0) decreased soil pH, subsequently increasing the retention of Cr(VI).

Surface charge of the soils was influenced by the addition of Fe(III) oxide, hence Cr(VI) adsorption was increased. Cow manure enhanced the reduction of Cr(VI) to Cr(III) and the resultant increase in pH increased the immobilization of Cr(III) in contaminated soils through adsorption and precipitation reactions.

8.1.4 Reduction of Cr(VI) in contaminated soils

The mitigation of Cr(VI) toxicity can be achieved either by immobilization or reduction to Cr(III) in soils. Immobilization may only inhibit the mobility, but cannot eliminate the toxicity from the contaminated sites. Hence, the best possible method to eliminate the toxicity is to reduce Cr(VI) to relatively non-toxic Cr(III) in soils. The application of protonated black carbon (BC) prepared from local plant weed (*Solanum elaeagnifolium*), biochar (CWB) and cow manure (CM) on the reduction of Cr(VI) in the contaminated soils was examined. Furthermore, the effect of adsorption Cr(VI) on its subsequent reduction to Cr(III) was also examined. An increase in positive charge resulting from the Fe(III) oxide decreased Cr(VI) reduction in soils, since increased adsorption reduced the bioavailable fraction of Cr(VI) to microorganisms. Hence free Cr(VI) ions are crucial for reduction to proceed.

Addition of BC, CWB and CM to Cr(VI) contaminated soils increased Cr(VI) reduction and the efficiency was in the order of BC>CWB>CM. Furthermore, these amendments increased DOC in soils, which may be the key property contributing to enhanced Cr(VI) reduction. Since Cr(VI) reduction is a proton consumption reaction, as the reduction proceeds, pH of the soil solution increased simultaneously. The increased pH resulted in the subsequent immobilization Cr(III) in soils through adsorption and precipitation reactions. The active role of functional groups in Cr(VI) reduction in presence of black carbon and biochar was further confirmed by the FTIR spectrophotometry.

8.1.5 Phytoavailability of Cr(VI) in soils

Chromium(VI) is highly mobile in soil solution and toxic to soil microorganisms and plants. The effect of black carbon and biochar on the microbial respiration and phytoavailability in Cr(VI) contaminated soils was examined. The microbial respiration as measured by CO_2 release, decreased in Cr(VI) contaminated soils. However, the addition of black carbon mitigated the inhibitory effect of Cr(VI) on microbial activity.

The plant biomass decreased in Cr(VI) contaminated soils, where the effect was more dominant in alkaline soils compared to acidic soils. Again, the addition of black carbon and biochar increased the biomass. The Cr concentration in plant tissues decreased with the application of these amendments. There was a positive relationship between Cr(VI) level in pore-water and the amount of Cr in plant biomass. This may be due to free Cr(VI) ions being present in pore water, which translocates passively by plant vessels into the biomass (Cervantes et al., 2001). The Cr(VI) concentration in pore-water decreased in the presence of amendments, which was attributed to Cr(VI) reduction.

8.2 Implications of the research findings

Excessive concentrations of heavy metals including Cr released from industrial activities adversely affect biological functions in soil. Several industries such as dye production, leather tanning, anodising of aluminium, cooling towers, electroplating, and steel production all use this heavy metal at different stages of their manufacturing cycle (Banks et al., 2006). The toxicity, mobility and bioavailability of Cr mainly depend on its speciation.

The most common species, Cr(III) and Cr(VI), prevail in the environment with different physiochemical properties and biochemical reactivities. Chromium(VI) chemically meta-stable in soil and exists as chromate (CrO_4^{2-}, $Cr_2O_7^{2-}$) and bichromate ($HCrO_4^-$, H_2CrO_4, and $HCr_2O_7^-$) complexes in strongly oxidizing environments (Faybishenko et al., 2008). In contrast, Cr(III) is an essential dietary nutrient (>200 µg/day), mediates normal glucose metabolism, cholesterol and fat in humans and forms stable inorganic and trivalent hexaqua ions [$(Cr(H_2O)_6)^{3+}$] and hexacoordinate stable complexes.

Chromium(VI) in soils can be effectively reduced to Cr(III) by using organic carbon (OC) sources such as biosolids compost, farmyard and poultry manures through different mechanisms. Organic carbon stimulates microbial communities and increases dissolved

organic carbon (DOC), which act as an electron source for the reduction of Cr(VI) (Bolan et al., 2003). The potential of OC in reducing Cr(VI) depends on the contaminant concentration, microbial activity and reactivity of OC. The other mechanism suggested in the reduction of Cr(VI) is where organic acids and humic acids participate as potential reductants.

Adding organic amendments immobilizes and decreases the availability of toxic heavy metals (Park et al., 2011). However, when organic matter decomposes, adsorbed metals may be released into the soils (Namgay et al., 2010). Black carbon derived from plant biomass significantly increases retention of cations in soils and enhances soil fertility, whilst also decreasing offsite pollution in agricultural landscapes (Van Zwieten et al., 2010). Incorporation of biochar into the soil increases water holding capacity and nutrient availability to plants, and reduces acidity in soils, thereby decreasing aluminium toxicity to plants and soil microorganisms.

It has been reported that several organic carbon (OC) sources including brown seaweed, phenols, powdered leaves, biosolids composts, farm yard manure, chicken manure, and organic acids are able to reduce Cr(VI) to Cr(III) with subsequent immobilization of Cr(III) (Bolan et al., 2003; Hsu et al., 2009). Black carbon and biochar appear to be effective in the mitigation of Cr contamination as they are highly reactive with many functional groups, and able to donate electrons to reduce Cr(VI) in soil. The addition of organic amendments increases DOC and microbial respiration, which in turn enhances biotransformation of metal(loid)s including Cr reduction (Park et al., 2010).

The soil pH, surface charge and functional groups of organic carbon influence the retention of Cr(III) and Cr(VI) in soils. The retention of Cr(VI) was higher in acidic soils and soils that have high positive charge. Cow manure increased the reduction of Cr(VI) in soils. The protonated black carbon have the highest Cr(VI) reducing capacity , followed by biochar. Addition of black carbon to alkaline contaminated soil enhanced Cr(VI) reduction, microbial respiration, and also decreased phytotoxicity of Cr(VI) in soil.

Since the Industrial Revolution, heavy metals, including Cr have been used in ever-increasing quantities. Accurately estimating two predominant species, Cr(III) and Cr(VI) Cr species, is essential. The improved method developed in this study can be used to achieve simultaneous quantification of both Cr(III) and Cr(VI) at very low concentrations on IC-ICPMS. The mobility of Cr species depends on the soil properties. Chromium(III) is relatively immobile whereas the mobility and bioavailability of Cr(VI) is high. Landfill

leachates with high heavy metal concentration often enter into fresh water wells and this problem can be arrested by adding organic and inorganic amendments that change pH and soil surface charge, thereby increasing the adsorption of heavy metals.

Chromium(VI) contaminated soils are detrimental to plants, microbes and sustainable agriculture. Application of black carbon, biochar and cow manure decreased Cr(VI) concentration by means of reduction. Among organic amendments black carbon is highly effective. Addition of black carbon and biochar increases soil microbial activity and decreases phytotoxicity. Once the contaminated soils are remediated, the microbial activity and plant growth can be enhanced and subsequently promote sustainable rehabilitation/revegetation.

8.3 Areas of future research

This research has highlighted several areas where productive research could be carried out in the future.

- In this study five different ligands were examined for the complexation and conversion of cationic Cr(III) to anionic Cr(III) in soil solution. The stability constants of the complexed ligands need to be examined.

- The effect of soil properties such as pH, CEC, organic matter, clay content and water extractable sulphate and phosphates on Cr adsorption was investigated. Chromium(VI) adsorption is also influenced by the presence of carbonates, bicarbonates and nitrates, which needs to be examined.

- The retention and mobility of Cr(III) and Cr(VI) was carried out only at the laboratory scale. The influence of myriad of soil properties (physical, chemical and biological) and environmental parameters (rainfall, temperature) on the retention and mobility of Cr(VI) in different soils needs to be examined under field conditions.

- A better understanding of the mechanism of adsorption of Cr(VI) should be further illustrated to confirm the relative extent of inner-sphere and outer-sphere complexation. This will greatly help to increase the retention of Cr(VI) in contaminated soil.

- In tannery effluent contaminated soils, Cr(III) concentration is often higher than Cr(VI) species since $Cr_2(SO_4)_3$ used as collagen (hide protein) stabilizing agent. However, the Cr(VI) concentration in these sites has been shown to increase with time. Hence, an in-depth study should be done on the mechanisms that oxidise Cr(III) to Cr(VI).

- The addition of black carbon and biochar has increased the reduction of Cr(VI) in alkaline soils. The role of functional groups was also confirmed by using FTIR. The most interesting area that future research could focus on discovering the active role of electrons in Cr(VI) reduction using synchrotron-based applications.

- Addition of organic amendments increased DOC in the soils. The DOC is composed of several functional groups. The characterization of carbon, such as aliphatic and aromatic carbon in DOC needs to be quantified.

- The transfer of electrons plays an important role in Cr(VI) reduction. Therefore redox measurements during Cr(VI) reduction need to be undertaken.

- Future research should aim to examine the effect of black carbon and biochar over the long-term in order to discover if re-oxidation of Cr(VI) in contaminated soil is possible. The potential value of other low cost amendments such as chitosan-based bio-waste, sea weed and the char-liquid from biochar plants on the reduction of Cr(VI) in water and soil must be further examined.

REFERENCES

ABS. (2009). Australian bureau of statistics. 1301.0 - Year Book, Australia.

Acar, F. and Malkoc, E. (2004). The removal of chromium (VI) from aqueous solutions by *Fagus orientalis* L. *Bioresource technology* **94,** 13-15.

Adriá-Cerezo, D. M., Llobat-Estellés, M. and Maurí-Aucejo, A. R. (2000). Preconcentration and speciation of chromium in waters using solid-phase extraction and atomic absorption spectrometry. *Talanta* **51,** 531-536.

Adriano, D. C. (1986). Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals. 2nd edition. Springer - New York.

Adriano, D. C., Wenzel, W. W., Vangronsveld, J. and Bolan, N. S. (2004). Role of assisted natural remediation in environmental cleanup. *Geoderma* **122,** 121-142.

Agarwal, G., Bhuptawat, H. K. and Chaudhari, S. (2006). Biosorption of aqueous chromium (VI) by Tamarindus indica seeds. *Bioresource Technology* **97,** 949-956.

Aitken, R. and Moody, P. (1994). The effect of valence and ionic-strength on the measurement of pH buffer capacity. *Australian Journal of Soil Research* **32.** 975-984.

Alloway, B. J. (1990). 2 Soil processes and the behaviour of metals. *Heavy metals in soils.*

Alloway, B.J. (1995). Heavy metals in soils. Springer.

Altundogan, H. S. (2005). Cr (VI) removal from aqueous solution by iron (III) hydroxide-loaded sugar beet pulp. *Process Biochemistry* **40,** 1443-1452.

Anderson, C., Deram, A., Petit, D., Brooks, R., Stewart, B. and Simcock, R. (2001). Induced hyperaccumulation: metal movement and problems. *Trace Elements in Soil: Bioavailability, Flux, and Transfer* 63 -76.

Anderson, R. (1989). Essentiality of chromium in humans. *Science of Total Environment* **86,** 75-81.

Artiole, J. and Fuller, W. H. (1979). Effect of Crushed Limestone Barriers on Chromium Attenuation in Soils. *Journal of environmental quality* **8,** 503-510.

Avudainayagam, S., Megharaj, M., Owens, G., Kookana, R., Chittleborough, D. and Naidu, R. (2003). Chemistry of chromium in soils with emphasis on tannery waste sites. *Reviews of Environmental Contamination and Toxicology* **178,** 53-91.

Avudainayagam, S., Naidu, R., Kookana, R., Alston, A. M., McClure, S. and Smith, L. (2001). Effects of electrolyte composition on chromium desorption in soils contaminated by tannery waste. *Australian Journal of Soil Research* **39,** 1077-1090.

Azizian M. F., Nelson. O. P., (1993). Hexavalent chromium adsorption kinetics and equilibrium in a natural soil. *Iranian Journal of Science and Technology* **17,** 81-89.

Bader, J. L., Gonzalez, G., Goodell, P. C., Ali, A. M. S. and Pillai, S. D. (1999). Aerobic reduction of hexavalent chromium in soil by indigenous microorganisms. *Bioremediation Journal* **3,** 201-211.

Baes, C. and Mesmer, R. (1986). The Hydrolysis of Cations. *RE Krieger, Malabar.*

Banks, M., Schwab, A. and Henderson, C. (2006). Leaching and reduction of chromium in soil as affected by soil organic content and plants. *Chemosphere* **62,** 255-264.

Baral, S., Das, N., Roy Chaudhury, G. and Das, S. (2009). A preliminary study on the adsorptive removal of Cr (VI) using seaweed, *Hydrilla verticillata*. *Journal of Hazardous Materials* **171,** 358-369.

Barcelo, J., Poschenrieder, C. and Gunse, B. (1986). Water relations of chromium VI treated bush bean plants (*Phaseolus vulgaris* L. cv. Contender) under both normal and water stress conditions. *Journal of Experimental Botany* **37,** 178-187.

Barnhart, J. (1997). Chromium chemistry and implications for environmental fate and toxicity. *Soil and Sediment Contamination: An International Journal* **6,** 561-568.

Barnowski, C., Jakubowski, N., Stuewer, D. and Broekaert, J. A. C. (1997). Speciation of chromium by direct coupling of ion exchange chromatography with inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry* **12,** 1155-1161.

Barrow, N. (1986). Reaction of anions and cations with variable-charge soils. *Advances in Agronomy* **38**. 183-230.

Bartlett, R. and James, B. (1979). Behaviour of chromium in soils: III. Oxidation. *Journal of Environmental Quality* **8,** 31-35.

Bartlett, R. and James, B. (1988). Mobility and bioavailability of chromium in soils. *Advances in Environmental Science and Technology* **20,** 267-304.

Bartlett, R. and Kimble, J. (1976). Behaviour of chromium in soils: II. Hexavalent forms. *Journal of Environmental Quality* **5,** 383-386.

Bartlett, R. J. (1991). Chromium cycling in soils and water: links, gaps, and methods. *Environmental Health Perspectives* **92,** 17-24.

Bartlett, R.J and James, B.R. (1988). Mobility and Bioavailability of chromium in soils; *In*" Chromium in Natural and human environments" (Nriagu J.O and Nieboer.E. Eds.). 267-304. Wiley-Interscience, New York.

Baruthio, F. (1992). Toxic effects of chromium and its compounds. *Biological Trace Element Research* **32,** 145-153.

Bates, T. R., Dunst, R. M., Taft, T. and Vercant, M. (2002). The vegetative response of' Concord' grape vines to soil pH. *HortScience* **37,** 890-893.

Benjamin M. M. and N. S. Bloom. (1981). Effects of strong binding of anionic adsorbates on adsorption of trace metals on amorphous iron oxyhydroxide. Tewari P.H. Plenum Press, New York.

Besselievre. EB and M. Schwartz. (1976). Treatment of industrial wastes. 2nd edition. McGraw-Hill - New York.

Bishnoi, N. R., Bajaj, M., Sharma, N. and Gupta, A. (2004). Adsorption of Cr (VI) on activated rice husk carbon and activated alumina. *Bioresource Technology* **91,** 305-307.

Bisogni Jr, J. J. and Lawrence, A. W. (1975). Kinetics of mercury methylation in aerobic and anaerobic aquatic environments. *Journal (Water Pollution Control Federation).* **47,** 135-152.

Bloem, J., D. W. Hopkins and A. Benedetti. (2006). Microbiological methods for assessing soil quality. CABI.

Bloomfield, C. and Pruden, G. (1980). The behaviour of Cr (VI) in soil under aerobic and anaerobic conditions. *Environmental Pollution Series A, Ecological and Biological* **23,** 103-114.

Boddu, V. M., Abburi, K., Talbott, J. L. and Smith, E. D. (2003). Removal of hexavalent chromium from wastewater using a new composite chitosan biosorbent. *Environmental Science and Technology* **37,** 4449-4456.

Boehm, H. (1994). Some aspects of the surface chemistry of carbon blacks and other carbons. *Carbon* **32,** 759-769.

Bolan, N. and Baskaran, S. Biodegradation of 2, 4-D herbicide as affected by its adsorption-desorption behaviour and microbial activity of soils. *Australian Journal of Soil Research* **34,** 1041-1053.

Bolan, N. S. and Duraisamy, V. P. (2003). Role of inorganic and organic soil amendments on immobilization and phytoavailability of heavy metals: a review involving specific case studies. *Australian Journal of Soil Research* **41,** 533-556.

Bolan, N. S. and Thiagarajan, S. (2001). Retention and plant availability of chromium in soils as affected by lime and organic matter amendments. *Australian Journal of Soil Research* **39,** 1091-1104.

Bolan, N. S., Adriano, D. C. and Curtin, D. (2003c). Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. *Advances in Agronomy* **78,** 215-272.

Bolan, N. S., Adriano, D. C., Natesan, R. and Koo, B. J. (2003b).Effects of organic amendments on the reduction and phytoavailability of chromate in mineral soil. *Journal of Environmental Quality* **32,** 120-128.

Bolan, N. S., Naidu, R., Syers, J. K. and Tillman, R. W. (1999). Surface charge and solute interactions in soils. *Advances in Agronomy* **67,** 87-140.

Bolan, N., Naidu, R., Choppala, G., Park, J., Mora, M. L., Budianta, D. and Panneerselvam, P. (2010). Solute interactions in soils in relation to the bioavailability and environmental remediation of heavy metals and metalloids. *Pedologist* **53,** 1-18.

Bradl, H. B. (2004). Adsorption of heavy metal ions on soils and soils constituents. *Journal of Colloid and Interface Science* **277,** 1-18.

Brose, D. A. and James, B. R. (2010). Oxidation− Reduction Transformations of Chromium in Aerobic Soils and the Role of Electron-Shuttling Quinones. *Environmental Science and Technology* **44,** 9438–9444.

Brown, G. E., Foster, A. L. and Ostergren, J. D. (1999). Mineral surfaces and bioavailability of heavy metals: A molecular-scale perspective. *Proceedings of the National Academy of Sciences* **96,** 3388-3395.

Brown, S. L., Chaney, R. L., Angle, J. S. and Ryan, J. A. (1998). The phytoavailability of cadmium to lettuce in long-term biosolids-amended soils. *Journal of Environmental Quality* **27,** 1071-1078.

Buerge, I. J. and Hug, S. J. (1997). Kinetics and pH dependence of chromium (VI) reduction by iron (II). *Environmental Science and Technology* **31,** 1426-1432.

Burke, T., Fagliano, J., Goldoft, M., Hazen, R. E., Iglewicz, R. and McKee, T. (1991). Chromite ore processing residue in Hudson County, New Jersey. *Environmental Health Perspectives* **92,** 131-137.

Byrdy, F. A., Olson, L. K., Vela, N. P. and Caruso, J. A. (1995). Chromium speciation by anion-exchange high-performance liquid chromatography with both inductively coupled plasma atomic emission spectroscopic and inductively coupled plasma mass spectrometric detection. *Journal of Chromatography A* **712,** 311-320.

Cámara, C., Cornelis, R. and Quevauviller, P. (2000). Assessment of methods currently used for the determination of Cr and Se species in solutions.*Trends in Analytical Chemistry* **19,** 189-194.

Cao, X., Ma, L., Gao, B. and Harris, W. (2009). Dairy-manure derived biochar effectively sorbs lead and atrazine. *Environmental Science and Technology* **43,** 3285-3291.

Carey, P., Mclaren, R. and Adams JA. (1996). Sorption of cupric, dichromate and arsenate ions in some New Zealand soils. *Water, Air and Soil Pollution* **87,** 189-203.

Carey, P., McLaren, R., Cameron, K. and Sedcole, J. (1996b). Leaching of copper, chromium, and arsenic through some free-draining New Zealand soils. *Australian Journal of Soil Research* **34,** 583-597.

Cary, E. (1982). In: Biological and Environmental Aspects of Chromium (S. Langard ed.).

Cary, E. E., Allaway, W. H. and Olson, O. E. (1977). Control of chromium concentrations in food plants. 2. Chemistry of chromium in soils and its availability to plants. *Journal of Agricultural and Food Chemistry* **25,** 305-309.

Cervantes, C., Campos-Garcia, J., Devars, S., Gutierrez-Corona, F., Loza-Tavera, H., Torres-Guzman, J. C. and Moreno-Sanchez, R. (2001). Interactions of chromium with microorganisms and plants. *FEMS microbiology reviews* **25,** 335-347.

Chen, J. M. and Hao, O. J. (1998). Microbial chromium (VI) reduction. *Critical Reviews in Environmental Science and Technology* **28,** 219-251.

Chen, S. S., Hsu, B. C. and Hung, L. W. (2008). Chromate reduction by waste iron from electroplating wastewater using plug flow reactor. *Journal of hazardous materials* **152,** 1092-1097.

Chen, Z. L., Megharaj, M. and Naidu, R. (2007). Speciation of chromium in waste water using ion chromatography inductively coupled plasma mass spectrometry. *Talanta* **72,** 394-400.

Chen, Z., Naidu, R. and Subramanian, A. (2001). Separation of chromium (III) and chromium (VI) by capillary electrophoresis using 2, 6-pyridinedicarboxylic acid as a pre-column complexation agent. *Journal of Chromatography A* **927**. 219-227.

Cheng, C. H., Lehmann, J., Thies, J. E., Burton, S. D. and Engelhard, M. H. (2006). Oxidation of black carbon by biotic and abiotic processes.*Organic Geochemistry* **37,** 1477-1488.

Chiu, C. C., Cheng, C. J., Lin, T. H., Juang, K. W. and Lee, D. Y. (2009). The effectiveness of four organic matter amendments for decreasing resin-extractable Cr (VI) in Cr (VI)-contaminated soils. *Journal of Hazardous Materials* **161,** 1239-1244.

Chlopecka, A. and Adriano, D. (1997). Influence of zeolite, apatite and Fe-oxide on Cd and Pb uptake by crops. *Science of the Total Environment* **207,** 195-206.

Chrysochoou, M., Ferreira, D. R. and Johnston, C. P. (2010). Calcium polysulfide treatment of Cr (VI)-contaminated soil. *Journal of Hazardous Materials* **179**. 650-657.

Chun, Y., Sheng, G., Chiou, C. T. and Xing, B. (2004). Compositions and sorptive properties of crop residue-derived chars. *Environmental Science and Technology* **38,** 4649-4655.

Cui, Y., Wang, Q., Dong, Y., Li, H. and Christie, P. (2004). Enhanced uptake of soil Pb and Zn by Indian mustard and winter wheat following combined soil application of elemental sulphur and EDTA. *Plant and Soil* **261,** 181-188.

Dabek-Zlotorzynska, E. and PC, L. (1998). Capillary electrophoresis: the state-of-the-art in metal speciation studies. *Analytica Chimica Acta* **359,**1-26.

Das, S. K. and Guha, A. K. (2007). Biosorption of chromium by Termitomyces clypeatus. *Colloids and Surfaces B: Biointerfaces* **60,** 46-54.

Davis, J. A. and Leckie, J. O. (1980). Surface ionization and complexation at the oxide/water interface. 3. Adsorption of anions. *Journal of Colloid and Interface Science* **74,** 32-43.

De Flora, S. (2000). Threshold mechanisms and site specificity in chromium (VI) carcinogenesis. *Carcinogenesis* **21,** 533-541.

Deiana, S., Premoli, A. and Senette, C. (2007). Reduction of Cr (VI) by caffeic acid. *Chemosphere*. 67. 1919-1926.

Deng, B. and Stone, A. T. (1996). Surface-catalysed chromium (VI) reduction: Reactivity comparisons of different organic reductants and different oxide surfaces. *Environmental Science and Technology* **30,** 2484-2494.

Dubey, S. P. and Gopal, K. (2007). Adsorption of chromium (VI) on low cost adsorbents derived from agricultural waste material: A comparative study. *Journal of Hazardous Materials* **145,** 465-470.

Eary, L. and Rai, D. (1988). Chromate removal from aqueous wastes by reduction with ferrous ion. *Environmental Science and Technology* **22,** 972-977.

Eary, LE and Rai, Dhanpat. (1991). Chromate reduction by subsurface soils under acidic conditions. *Soil Science Society of America Journal* **55,** 676-683.

Eckert, J., Stewart, J., Waite, T., Szymczak, R. and Williams, K. (1990). Reduction of chromium (VI) at sub-μg l− 1 levels by fulvic acid. *Analytical Chimica Acta* **236,** 357-362.

Elangovan, R., Philip, L. and Chandraraj, K. (2008). Biosorption of chromium species by aquatic weeds: Kinetics and mechanism studies. *Journal of Hazardous Materials* **152,** 100-112.

Elliott, H., Liberati, M. and Huang, C. (1986). Effect of iron oxide removal on heavy metal sorption by acid subsoils. *Water, Air and Soil Pollution* **27**. 379-389.

Elovitz, M. S. and Fish, W. (1994). Redox interactions of Cr (VI) and substituted phenols: kinetic investigation. *Environmental Science and Technology* **28,** 2161-2169.

Elovitz, M. S. and Fish, W. (1995). Redox interactions of Cr (VI) and substituted phenols: products and mechanism. *Environmental Science and Technology* **29,** 1933-1943.

El-Shafey, E. (2005). Behaviour of reduction–sorption of chromium (VI) from an aqueous solution on a modified sorbent from rice husk. *Water, Air and Soil Pollution* **163,** 81-102.

Fanning, P. E. and Vannice, M. A. (1993). A DRIFTS study of the formation of surface groups on carbon by oxidation. *Carbon* **31,** 721-730.

Faybishenko, B., Hazen, T. C., Long, P. E., Brodie, E. L., Conrad, M. E., Hubbard, S. S., Christensen, J. N., Joyner, D., Borglin, S. E. and Chakraborty, R. (2008). In situ long-term reductive bioimmobilization of Cr (VI) in groundwater using hydrogen release compound. *Environmental Science and Technology* **42,** 8478-8485.

Fein, J. B., Fowle, D. A., Cahill, J., Kemner, K., Boyanov, M. and Bunker, B. (2002). Nonmetabolic Reduction of Cr (VI) by Bacterial Surfaces Under Nutrient-Absent Conditions. *Geomicrobiology Journal* **19,** 369-382.

Fendorf, S. E. (1995). Surface reactions of chromium in soils and waters. *Geoderma* **67,** 55-71.

Fendorf, S. E. and Li, G. (1996). Kinetics of chromate reduction by ferrous iron. *Environmental Science and Technology* **30,** 1614-1617.

Fendorf, S. E. and Zasoski, R. J. (1992). Chromium (III) oxidation by. delta.-manganese oxide (MnO2). 1. Characterization. *Environmental Science and Technology* **26,** 79-85.

Fendorf, S., Eick, M. J., Grossl, P. and Sparks, D. L. (1997). Arsenate and chromate retention mechanisms on goethite. 1. Surface structure. *Environmental Science and Technology* **31,** 315-320.

Freundlich. H.(1926). Colloid & capillary chemistry. Methuen & co. ltd. USA.

Furr, A. K., Kelley, W. C., Bache, C. A., Gutenmann, W. H. and Lisk, D. J. (1976). Multi element uptake by vegetables and millet grown in pots on fly ash amended soil. *Journal of Agricultural and Food Chemistry* **24,** 885-888.

Gao, S., Walker, W. J., Dahlgren, R. A. and Bold, J. (1997). Simultaneous sorption of Cd, Cu, Ni, Zn, Pb, and Cr on soils treated with sewage sludge supernatant. *Water, Air and Soil Pollution* **93,** 331-345.

Garg, U. K., Kaur, M., Garg, V. and Sud, D. (2007). Removal of hexavalent chromium from aqueous solution by agricultural waste biomass. *Journal of Hazardous Materials* **140,** 60-68.

Gillman, G. P. and Sumpter, E. A. (1986). Modification to the compulsive exchange method for measuring exchange characteristics of soils. *Australian Journal of Soil Research* **24,** 61-66.

Ginder-Vogel, M., Borch, T., Mayes, M. A., Jardine, P. M. and Fendorf, S. (2005). Chromate reduction and retention processes within arid subsurface environments. *Environmental Science and Technology* **39,** 7833-7839.

Goldberg. E. D. (1985). Black carbon in the environment: properties and distribution. John Wiley & Sons.

Graham, A. M. and Bouwer, E. J. (2009). Rates of hexavalent chromium reduction in anoxic estuarine sediments: pH effects and the role of acid volatile sulfides. *Environmental Science and Technology* **44,** 136-142.

Grate, J. W. and Taylor, R. H. (1996). Sequential injection method with on-line soil extraction for determination of Cr (VI). *Field Analytical Chemistry and Technology* **1,** 39-48.

Griffin, R. A., Au, A. K. and Frost, R. R. (1977). Effect of pH on adsorption of chromium from landfill-leachate by clay minerals. *Journal of Environmental Science and Health, Part A* **12,** 431-449.

Grossl, P. R., Eick, M., Sparks, D. L., Goldberg, S. and Ainsworth, C. C. (1997). Arsenate and chromate retention mechanisms on goethite. 2. Kinetic evaluation using a pressure-jump relaxation technique. *Environmental Science and Technology* **31,** 321-326.

Grove, J. H. and Ellis, B. G. (1980). Extractable chromium as related to soil pH and applied chromium. *Soil Science Society of America Journal* **44,** 238-242.

Gurleyuk, H. and Wallschlager, D. (2001). Determination of chromium (III) and chromium (VI) using suppressed ion chromatography inductively coupled plasma mass spectrometry presented at the 2001 European Winter Conference on Plasma Spectrochemistry, Lillehammer, Norway, February 4-8, 2001. *Journal of Analytical Atomic Spectrometry* **16**. 926-930.

Hamadi, N. K., Chen, X. D., Farid, M. M. and Lu, M. G. Q. (2001). Adsorption kinetics for the removal of chromium(VI) from aqueous solution by adsorbents derived from used tyres and sawdust. *Chemical Engineering Journal* **84,** 95-105.

Han, F. X., Sridhar, B., Monts, D. L. and Su, Y. (2004). Phytoavailability and toxicity of trivalent and hexavalent chromium to Brassica juncea. *New Phytologist* **162.** 489-499.

Harris, P., Burian, A. and Duber, S. (2000). High-resolution electron microscopy of a microporous carbon. *Philosophical Magazine Letters* **80,** 381-386.

Hawley, E. L., Deeb, R. A., Kavanaugh, M. C. and Jacobs, J. A. (2005). Treatment technologies for chromium (VI). *Chromium (VI) handbook.* 275.

Haynes, R. and Naidu, R. (1998). Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. *Nutrient Cycling in Agroecosystems* **51**. 123-137.

Hetrick, B., Wilson, G. and Figge, D. (1994).*Environmental Pollution* **86,** 171-179.

Himeno, S., Nakashima, Y. and Sano, K. (1998). Simultaneous determination of chromium (VI) and chromium (III) by capillary electrophoresis. *Analytical Sciences* **14,** 369-373.

Hingston, J., Collins, C., Murphy, R. and Lester, J. (2001). Leaching of chromated copper arsenate wood preservatives: a review. *Environmental Pollution* **111**. 53-66.

Hoagland, D. R. and Arnon, D. I. (1950). The water-culture method for growing plants without soil. Circular. California Agricultural Experiment Station. 347.

Howe, J. A., Loeppert, R. H., DeRose, V. J., Hunter, D. B. and Bertsch, P. M. (2003). Localization and speciation of chromium in subterranean clover using XRF, XANES, and EPR spectroscopy. *Environmental Science and Technology* **37,** 4091-4097.

Hsu, N. H., Wang, S. L., Liao, Y. H., Huang, S. T., Tzou, Y. M. and Huang, Y. M. (2009). Removal of hexavalent chromium from acidic aqueous solutions using rice straw-derived carbon. *Journal of Hazardous Materials* **171,** 1066-1070.

Hsu, N. H., Wang, S. L., Lin, Y. C., Sheng, G. D. and Lee, J. F. (2009). Reduction of Cr (VI) by Crop-Residue-Derived Black Carbon. *Environmental Science and Technology* **43,** 8801-8806.

Jacobs, J. and Testa, S. M. (2005). Overview of chromium (VI) in the environment: Background and history. *Chromium (VI) Handbook. CRC Press, Boca Raton, Fla.* 1-21.

James, B. (1996). The challenge of remediating chromium-contaminated soil. *Environmental Science and Technology* **30,** 248A-251A.

James, B. (2002). Chemical transformations of chromium in soils: relevance to mobility, bioavailability and remediation. *The chromium file* **8.**

James, B. R. (1994). Hexavalent chromium solubility and reduction in alkaline soils enriched with chromite ore processing residue. *Journal of Environmental Quality* **23,** 227-233.

James, B. R. (2001). Remediation-by-reduction strategies for chromate-contaminated soils. *Environmental Geochemistry and Health* **23,** 175-179.

James, B. R. and Bartlett, R. J. (1983a). Behaviour of Chromium in Soils. VI. Interactions Between Oxidation-Reduction and Organic Complexation. *Journal of Environmental Quality* **12,** 173-176.

James, B. R. and Bartlett, R. J. (1983b). Behaviour of chromium in soils: VII. Adsorption and reduction of hexavalent forms. *Journal of Environmental Quality* **12,** 177-181.

James, B. R., Petura, J. C., Vitale, R. J. and George, R. (1997). Oxidation-Reduction Chemistry of Chromium: Relevance to the Regulation and Remediation of Chromate-Contaminated Soils. *Chromium in Soil: Perspectives in Chemistry, Health, and Environmental Regulation.* **6,** 569-580.

James, B. R., Petura, J. C., Vitale, R. J. and Mussoline, G. R. (1995). Hexavalent Chromium Extraction from Soils: A Comparison of Five Methods. *Environmental Science and Technology* **29,** 2377-2381.

Janos, P., Hula, V., Bradnová, P., Pilarová, V. and Sedlbauer, J. (2009). Reduction and immobilization of hexavalent chromium with coal-and humate-based sorbents. *Chemosphere* **75,** 732-738.

Jardine, P., Fendorf, S., Mayes, M., Larsen, I., Brooks, S. and Bailey, W. (1999). Fate and transport of hexavalent chromium in undisturbed heterogeneous soil. *Environmental Science and Technology* **33,** 2939-2944.

Jen, J. F., Ou-Yang, G. L., Chen, C. S. and Yang, S. M. (1993). Simultaneous determination of chromium (III) and chromium (VI) with reversed-phase ion-pair high-performance liquid chromatography. *Analyst* **118,** 1281-1284.

Jiang, J., Xu, R., Wang, Y. and Zhao, A. (2008). The mechanism of chromate sorption by three variable charge soils. *Chemosphere.* **71,** 1469-1475.

Jiang, X., Luo, Y., Zhao, Q., Baker, A., Christie, P. and Wong, M. (2003). Soil Cd availability to Indian mustard and environmental risk following EDTA addition to Cd-contaminated soil. *Chemosphere* **50,** 813-818.

Jung, G. Y., KIM, Y. S. and Lim, H. B. (1997). Simultaneous Determination of Chromium (III) and Chromium (VI) in Aqueous Solution by Capillary Electrophoresis with On-Column UV-VIS Detection. *Analytical Sciences* **13,** 463-467.

Kamaludeen, S. P. B., Megharaj, M., Juhasz, A. L., Sethunathan, N. and Naidu, R. (2003). Chromium-microorganism interactions in soils: remediation implications. *Reviews of environmental contamination and toxicology* **178,** 93-164.

Kayser, A., Wenger, K., Keller, A., Attinger, W., Felix, H., Gupta, S. and Schulin, R. (2000). Enhancement of phytoextraction of Zn, Cd, and Cu from calcareous soil: the use of NTA and sulfur amendments. *Environmental Science and Technology* **34,** 1778-1783.

Khaodhiar, S., Azizian, M. F., Osathaphan, K. and Nelson, P. O. (2000). Copper, chromium, and arsenic adsorption and equilibrium modelling in an iron-oxide-coated sand, background electrolyte system. *Water, Air and SOIL Pollution* **119**. 105-120.

Kim, I. S., Kang, K. H., Johnson-Green, P. and Lee, E. J. (2003). Investigation of heavy metal accumulation in *Polygonum thunbergii* for phytoextraction. *Environmental pollution* 126. 235-243.

Ko, B. G., Vogeler, I., Bolan, N. S., Clothier, B., Green, S. and Kennedy, J. (2007). Mobility of copper, chromium and arsenic from treated timber into grapevines. *Science of the Total Environment* **388,** 35-42.

Kookana, R. S., Gerritse, R. G. and Aylmore, L. A. G. (1990). Effect of organic cosolvent on adsorption and desorption of linuron and simazine in soil. *Australian Journal of Soil Research* **28,** 717–725.

Korte, N., Skopp, J., Fuller, W., Niebla, E. and Alesii, B. (1976). Trace element movement in soils: influence of soil physical and chemical properties. *Soil Science* **122,** 350-357.

Kota, J. and Stasicka, Z. (2000). Chromium occurrence in the environment and methods of its speciation. *Environmental Pollution* **107,** 263-283.

Kumar, P. B. A. N., Dushenkov, V., Motto, H. and Raskin, I. (1995). Phytoextraction: The Use of Plants To Remove Heavy Metals from Soils. *Environmental Science and Technology* **29,** 1232-1238.

Kuo, S. and Bembenek, R. (2008). Sorption and desorption of chromate by wood shavings impregnated with iron or aluminum oxide. *Bioresource Technology* **99,** 5617-5625.

Kuo, Y. M. and Wang, C. S. (2002). Effect of rise distance on droplets generated from bubble bursting on the surface of chromic acid solutions. *AIHA Journal* **63**. 5-10.

Kyziol, J., Twardowska, I. and Schmitt-Kopplin, P. (2006). The role of humic substances in chromium sorption onto natural organic matter (peat). *Chemosphere* **63,** 1974-1982.

Lan, Y., Deng, B., Kim, C. and Thornton, E. C. (2007). Influence of soil minerals on chromium(VI) reduction by sulfide under anoxic conditions. *Geochemical transactions* **8,** 4.

Langmuir, I. (1918). The adsorption of gases on plane surfaces of glass, mica and platinum. *Journal of the American Chemical Society* **40,** 1361-1403.

Lazaridis, N. and Asouhidou, D. (2003). Kinetics of sorptive removal of chromium (VI) from aqueous solutions by calcined Mg-Al-CO3 hydrotalcite. *Water research* **37,** 2875-2882.

Leyva-Ramos, R., Fuentes-Rubio, L., Guerrero-Coronado, R. M. and Mendoza-Barron, J. (1995). Adsorption of trivalent chromium from aqueous solutions onto activated carbon. *Journal of Chemical Technology and Biotechnology* **62** 64-67.

Li, H., Wang, Q., Cui, Y., Dong, Y. and Christie, P. (2005). Slow release chelate enhancement of lead phytoextraction by corn (*Zea mays* L.) from contaminated soil—a preliminary study. *Science of the Total Environment* **339,** 179-187.

Li, Q., Zhai, J., Zhang, W., Wang, M. and Zhou, J. (2007). Kinetic studies of adsorption of Pb (II), Cr (III) and Cu (II) from aqueous solution by sawdust and modified peanut husk. *Journal of hazardous materials* **141,** 163-167. .

Lin, Y., Wang, S., Shen, W., Huang, P., Chiang, P., Liu, J., Chen, C. and Tzou, Y. (2009). Photo-enhancement of Cr (VI) reduction by fungal biomass of *Neurospora crassa*. *Applied Catalysis B: Environmental* **92,** 294-300.

Liu, B. F., Liu, L. B. and Cheng, J. K. (1999). Analysis of inorganic cations as their complexes by capillary electrophoresis. *Journal of Chromatography A*. **834**. 277-308.

Liu, C. C., Wang, M. K., Chyow-San Chiou, ., Li, Y. S., Lin, Y. A. and Huang, S. S. (2006). Chromium removal and sorption mechanism from aqueous solutions by wine processing waste sludge. *Industrial and Engineering Chemistry Research* **45,** 8891-8899.

López-Luna, J., González-Chávez, M., Esparza-García, F. and Rodríguez-Vázquez, R. (2009). Toxicity assessment of soil amended with tannery sludge, trivalent chromium and hexavalent chromium, using wheat, oat and sorghum plants. *Journal Of Hazardous Materials* **163**. 829-834.

Losi, M. and Frankenberger, W. (1994). Chromium-resistant microorganisms isolated from evaporation ponds of a metal processing plant. *Water, Air and Soil Pollution* **74,** 405-413.

Losi, M. E., Amrhein, C. and Frankenberger, W. T., Jr. (1994). Environmental biochemistry of chromium. *Reviews of Environmental Contamination and Toxicology* **136.** 91-121.

Losi, M., Amrhein, C. and Frankenberger Jr, W. (1994). Bioremediation of Chromate-Contaminated Groundwater by Reduction and Precipitation in Surface Soils. *Journal of Environmental Quality* **23,** 1141-1150.

Losi, M., Amrhein, C. and Frankenberger Jr, W. (1994). Factors affecting chemical and biological reduction of hexavalent chromium in soil. *Environmental Toxicology and Chemistry* **13,** 1727-1735.

Lovley, D. R. and Phillips, E. J. P. (1994). Reduction of Chromate by Desulfovibrio vulgaris and Its c3 Cytochrome. *Applied and Environmental Microbiology* **60,** 726-728.

Loyaux-Lawniczak, S., Lecomte, P. and Ehrhardt, J. J. (2001). Behaviour of hexavalent chromium in a polluted groundwater: redox processes and immobilization in soils. *Environmental Science and Technology* **35,** 1350-1357.

Lukaski, H. C. (1999). Chromium as a supplement 1. *Annual Review of Nutrition* **19,** 279-302.

Lytle, C. M., Lytle, F. W., Yang, N., Qian, J. H., Hansen, D., Zayed, A. and Terry, N. (1998). Reduction of Cr (VI) to Cr (III) by wetland plants: Potential for in situ heavy metal detoxification. *Environmental Science and Technology* **32,** 3087-3093.

Mahimairajah, et al. (2000). Extent and severity of contamination around tanning industries in Vellore district. In "ACIAR Proceedings" Anonymous pp. 75-82.

McBride. M.B. (1994). Environmental chemistry of soils. Oxford University Press, New York.

McGrath, S. and Smith, S. (1990). 7 Chromium and nickel. *Heavy Metals in Soils.*

McKeague, J. and Day, J. (1966). Dithionite-and oxalate-extractable Fe and Al as aids in differentiating various classes of soils. *Canadian Journal of Soil Science* **46,** 13-22.

McKinley, W. S., Pratt, R. C. and McPhillips, L. C. (1992). Cleaning up chromium. *Civil Engineering-ASCE* **62,** 69-71.

McLean, J., Lee, J. and Beveridge, T. (2002). Interactions of bacteria and environmental metals, fine-grained mineral development and bioremediation strategies. *Interactions between Soil Particles and Microorganisms: Impact on the Terrestrial Ecosystem* **8,** 227–261.

Meegoda, J. N., Kamolpornwijit, W., Vaccari, D. A., Ezeldin, A., Noval, B., Mueller, R. and Santora, S. (1999). Remediation of chromium-contaminated soils: bench-scale investigation. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management* **3,** 124-131.

Megharaj, M., Avudainayagam, S. and Naidu, R. (2003). Toxicity of Hexavalent Chromium and Its Reduction by Bacteria Isolated from Soil Contaminated with Tannery Waste. *Current Microbiology* **47,** 51-54.

Mehra, O. and Jackson, M. (1960). Iron oxide removal from soils and clays by a dithionite-citrate buffered with sodium bicarbonate. *Clays and Clay Minerals* **7,** 317-327.

Michalke, B. (2002). The coupling of LC to ICP-MS in element speciation–Part II: Recent trends in application .*Trends in Analytical Chemistry* **21,** 154-165.

Miller, W. and Miller, D. (1987). A micro-pipette method for soil mechanical analysis. *Communications in Soil Science and Plant Analysis* **18**. 1-15.

Mohan, D. and Pittman Jr, C. U. (2006). Activated carbons and low cost adsorbents for remediation of tri-and hexavalent chromium from water. *Journal of Hazardous Materials* **137,** 762-811.

Mohan, D., Pittman Jr, C. U., Bricka, M., Smith, F., Yancey, B., Mohammad, J., Steele, P. H., Alexandre-Franco, M. F., Gómez-Serrano, V. and Gong, H. (2007). Sorption of arsenic, cadmium, and lead by chars produced from fast pyrolysis of wood and bark during bio-oil production. *Journal of Colloid and Interface Science* **310,** 57-73.

Mohan, D., Rajput, S., Singh, V. K., Steele, P. H. and Pittman Jr, C. U. (2011). Modelling and evaluation of chromium remediation from water using low cost bio-char, a green adsorbent. *Journal of Hazardous Materials* **188**, 319-333.

Moreno-Castilla, C., Lopez-Ramon, M. and Carrasco-Marin, F. (2000). Changes in surface chemistry of activated carbons by wet oxidation. *Carbon* **38,** 1995-2001.

Mouni, L., Merabet, D., Robert, D. and Bouzaza, A. (2009). Batch studies for the investigation of the sorption of the heavy metals Pb2 and Zn2 onto Amizour soil (Algeria). *Geoderma* **154**. 30-35.

Mukherjee, A. B. (1998). Chromium in the environment of Finland. *The Science of the Total Environment* **217,** 9-19.

Murphy, V., Hughes, H. and McLaughlin, P. (2008). Comparative study of chromium biosorption by red, green and brown seaweed biomass. *Chemosphere* **70,** 1128-1134.

Naidu, et al. (2000). Fate of Chromium at tannery waste-contaminated sites at Mount Barker, South Australia. In 'Towards better management of soils contaminated with tannery waste. In "ACIAR proceedings, No 88" (R Naidu, IR Willet, S Mahimairaja, R Kookana, K Ramasamy), pp. 57-70.

Naidu, R., Bolan, N. S., Kookana, R. S. and Tiller, K. (1994). Ionic-strength and pH effects on the sorption of cadmium and the surface charge of soils. *European Journal of Soil Science* **45**, 419-429.

Namgay, T., Singh, B. and Singh, B. P. (2010). Influence of biochar application to soil on the availability of As, Cd, Cu, Pb, and Zn to maize (Zea mays L.). *Australian Journal of Soil Research* **48,** 638-647.

NAS (1974) Chromium. National academy of Sciences, Washington, DC.

Nieboer, E. and Jusys, A. (1988). Biologic chemistry of chromium. *Advances in Environmental Science and Technology* **20,** 21-79.

Nourbakhsh, M. (1994). A comparative study of various biosorbents for removal of chromium (VI) ions from industrial waste waters. *Process Biochemistry* **29,** 1-5.

Nriagu, J. O. and Pacyna, J. M. (1988). Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature.* **333,** 134-139.

Ogram, A., Jessup, R., Ou, L. T. and Rao, P. (1985). Effects of sorption on biological degradation rates of (2, 4-dichlorophenoxy) acetic acid in soils. *Applied and Environmental Microbiology* **49**. 582.

Oze, C., Bird, D. K. and Fendorf, S. (2007). Genesis of hexavalent chromium from natural sources in soil and groundwater. *Proceedings of the National Academy of Sciences* **104,** 6544-6549.

Pal, D. and Yost, K. W. (1995). Fixation and stabilization of chromium in contaminated materials. Fixation and stabilization of chromium in contaminated materials. US patent no. 5397478. USA

Palmer, C. D. and Puls, R. W. (1996). Natural attenuation of hexavalent chromium in ground water and soils. *Epa Environmental Assessment Sourcebook.* 12.

Palmer, C. D. and Wittbrodt, P. R. (1991). Processes affecting the remediation of chromium-contaminated sites. *Environmental Health Perspectives* **92,** 25-40.

Palotás, Á. B., Rainey, L. C., Feldermann, C. J., Sarofim, A. F. and Vander Sande, J. B. (1996). Soot morphology: An application of image analysis in high-resolution transmission electron microscopy. *Microscopy research and technique* **33,** 266-278.

Panda, S. and Choudhury, S. (2005). Chromium stress in plants. Brazilian Journal of Plant Physiology **17**. 95-102.

Pantsar-Kallio, M. and Manninen, P. K. G. (1996). Speciation of chromium in waste waters by coupled column ion chromatography-inductively coupled plasma mass spectrometry. *Journal of Chromatography A.* **750,** 89-95.

Park JH, Choppala G, Bolan NS, Chung JW and Chuasavathi T. (2011) Biochar reduces the bio-availability and phytotoxicity of heavy metals. *Plant and Soil* **348,** 439-451.

Park, D., Yun, Y. S. and Park, J. M. (2004). Reduction of hexavalent chromium with the brown seaweed Ecklonia biomass. *Environmental Science and Technology.* **38,** 4860-4864.

Park, J. H., Lamb, D., Paneerselvam, P., Choppala, G., Bolan, N. and Chung, J. W. (2010). Role of organic amendments on enhanced bioremediation of heavy metal (loid) contaminated soils. *Journal of Hazardous Materials* **185,** 549-574.

Patterson, R. R., Fendorf, S. and Fendorf, M. (1997). Reduction of Hexavalent Chromium by Amorphous Iron Sulfide. *Environmental Science and Technology* **31,** 2039-2044.

Patterson. J. W. (1985). Industrial wastewater treatment technology. Butterworth Publishers, Stoneham, MA.

Paya Perez, A., Gotz, L., Kephalopoulos, S. and Bignoli, G. (1988). Sorption of chromium species on soil. Heavy Metals in the Hydrocycle. 59–66.

Pérez-Piqueres, A., Edel-Hermann, V., Alabouvette, C. and Steinberg, C. (2006). Response of soil microbial communities to compost amendments. *Soil Biology and Biochemistry* **38,** 460-470.

Pizzi, A. (1981). The Chemistry and Kinetic Behaviour of Cu-Cr-As/B Wood Preservatives. I. Fixation of Chromium on. *Journal of Polymer Science: Polymer Chemistry Edition.* **19,** 3093-3121.

Ponder, S.M., Darab, J.G., and Mallouk, T.E. (2000). Remediation of Cr (VI) and Pb (II) Aqueous Solutions Using Supported, Nanoscale Zero-valent Iron. *Environmental Science and Technology* **34,** 2564-2569.

Powell, R. M., Puls, R. W., Hightower, S. K. and Sabatini, D. A. (1995). Coupled Iron Corrosion and Chromate Reduction: Mechanisms for Subsurface Remediation. *Environmental Science and Technology* **29,** 1913-1922.

Qafoku, N. P., Evan Dresel, P., Ilton, E., McKinley, J. P. and Resch, C. T. (2010). Chromium transport in an acidic waste contaminated subsurface medium: The role of reduction. *Chemosphere* **81,** 1492-1500.

Qiu, Y., Cheng, H., Xu, C. and Sheng, G. D. (2008). Surface characteristics of crop-residue-derived black carbon and lead (II) adsorption. *Water Research* **42,** 567-574.

Rai, D. and Zachara, J. (1986).*Geochemical behaviour of chromium species.* EA-4544.

Rai, D., Eary, L. E. and Zachara, J. M. (1989). Environmental chemistry of chromium. *The Science of the Total Environment* **86,** 15-23.

Rai, D., Sass, B. M. and Moore, D. A. (1987). Chromium (III) hydrolysis constants and solubility of chromium (III) hydroxide. *Inorganic Chemistry* **26,** 345-349.

Reddy, K. R., Parupudi, U. S., Devulapalli, S. N. and Xu, C. Y. (1997). Effects of soil composition on the removal of chromium by electrokinetics. *Journal of Hazardous Materials* **55,** 135-158.

Richard, F. C. and Bourg, A. (1991). Aqueous geochemistry of chromium: a review. *Water Research* **25,** 807-816.

Robinson, B., Greven, M., Green, S., Sivakumaran, S., Davidson, P. and Clothier, B. (2006). Leaching of copper, chromium and arsenic from treated vineyard posts in Marlborough, New Zealand. *Science of the Total Environment* **364,** 113-123.

Robinson, B., Greven, M., Sivakumaran, S., Green, S. and Clothier, B. (2004). Results of an initial survey of the leaching from treated posts in vineyards in the Marlborough region. *A Report to the Marlborough District Council, HortResearch Client Report.* **12173.**

Rondon, M. A., Lehmann, J., Ramírez, J. and Hurtado, M. (2007). Biological nitrogen fixation by common beans (Phaseolus vulgaris L.) increases with bio-char additions. *Biology and Fertility of Soils* **43,** 699-708.

Ros, M., Hernandez, M. T. and García, C. (2003). Soil microbial activity after restoration of a semiarid soil by organic amendments. *Soil Biology and Biochemistry* **35,** 463-469.

Ross, D., Sjogren, R. and Bartlett, R. (1981). Behaviour of chromium in soils: IV. *Toxicity of microorganisms. Journal of Environmental Quality* **10,** 145-148.

Salt, D. E., Pickering, I., Prince, R. C., Gleba, D., Dushenkov, S., Smith, R. D. and Raskin, I. (1997). Metal Accumulation by Aquacultured Seedlings of Indian mustard. *Environmental Science and Technology* **31,** 1636-1644.

Salunkhe, P., Dhakephalkar, P. and Paknikar, K. (1998). Bioremediation of hexavalent chromium in soil microcosms. *Biotechnology Letters* **20,** 749-751.

Samal, R., Panda, B., Rout, S. and Mohanty, M. (1995). Effect of chemical modification on FTIR spectra. I. Physical and chemical behaviour of coir. *Journal of Applied Polymer Science* **58,** 745-752.

Sarin, V. and Pant, K. (2006). Removal of chromium from industrial waste by using eucalyptus bark. *Bioresource Technology* **97,** 15-20.

Sass, B. M. and Rai, D. (1987). Solubility of amorphous chromium (III)-iron (III) hydroxide solid solutions. *Inorganic Chemistry* **26,** 2228-2232.

Schindler, D., Bayley, S., Curtis, P., Parker, B., Stainton, M. and Kelly, C. (1992). Natural and man-caused factors affecting the abundance and cycling of dissolved organic substances in precambrian shield lakes. *Hydrobiologia* **229**. 1-21.

Schofield, R. (1950). Effect of pH on electric charges carried by clay particles. *European Journal of Soil Science* **1**. 1-8.

Schroeder, D. C. and Lee, G. F. (1975). Potential transformations of chromium in natural waters. *Water, Air, and Soil Pollution* **4,** 355-365.

Semenova, O. P., Timerbaev, A. R., Gagstadter, R. and Bonn, G. K. (1996). Speciation of chromium ions by capillary zone electrophoresis. *Journal of High Resolution Chromatography* **19,** 177-179.

Shahandeh, H. and Hossner, L. (2000). Plant screening for chromium phytoremediation. *International Journal of Phytoremediation* **2,** 31-51.

Shanker, A. K., Cervantes, C., Loza-Tavera, H. and Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment International* **31,** 739-753.

Shen, Y. S., Wang, S. L., Huang, S. T., Tzou, Y. M. and Huang, J. H. (2010). Biosorption of Cr (VI) by coconut coir: Spectroscopic investigation on the reaction mechanism of Cr (VI) with lignocellulosic material. *Journal of Hazardous Materials* **179,** 160-165.

Skipper, H. and Turco, R. (1995). Bioremediation Science and Applications. *Soil Science Society of America* **104,** 55-86.

Smith, W. and Gadd, G. (2000). Reduction and precipitation of chromate by mixed culture sulphate-reducing bacterial biofilms. *Journal of Applied Microbiology* **88,** 983-991.

Sparks. D.L. (2003). Environmental soil chemistry. Academic Pr. USA

Sperling, M., Xu, S. and Welz, B. (1992). Determination of chromium (III) and chromium (VI) in water using flow injection on-line preconcentration with selective adsorption on activated alumina and flame atomic absorption spectrometric detection. *Analytical Chemistry* **64,** 3101-3108.

Sposito. G.(1984). The surface chemistry of soils. Oxford University Press. USA

Sposito. G.(1994). Chemical equilibria and kinetics in soils. Oxford University Press, USA.

Srivastava, S., Prakash, S. and Srivastava, M. (1999). Chromium mobilization and plant availability—the impact of organic complexing ligands. *Plant and Soil* **212,** 201-206.

Stanford, G.D.M., DeMent, J.D. (1957). A Method for Measuring Short-Term Nutrient Absorption by Plants: I. Phosphorus. *Soil Science Society of America Journal* **21,** 612-617.

Stewart, M., Jardine, P., Barnett, M., Mehlhorn, T., Hyder, L. and McKay, L. (2003). Influence of soil geochemical and physical properties on the sorption and bioaccessibility of chromium (III). *Journal of Environmental Quality* **32,** 129-137.

Stumm, W., J. J. Morgan and J. I. Drever. (1996). Aquatic chemistry. Wiley New York.

Su, C. and Ludwig, R. D. (2005). Treatment of hexavalent chromium in chromite ore processing solid waste using a mixed reductant solution of ferrous sulfate and sodium dithionite. *Environmental Science and Technology* **39,** 6208-6216.

Su, Y., Han, F. X., Sridhar, B. and Monts, D. L. (2005). Phytotoxicity and phytoaccumulation of trivalent and hexavalent chromium in brake fern. *Environmental Toxicology and Chemistry* **24,** 2019-2026.

Sumner, M.E., and Naidu, R. (1997). Potential for management of nickel and chromium in ultramafic and contaminated soils. In "The ecology of ultramafic and metalliferous areas" (Jaffré, T., Reeves R.D. and Becquer, T), pp. 249-259.

Suseela, K., Sivaparvathi, M. and Nandy, S. (1987). Removal of chromium from tannery effluent using powdered leaves. *Leather Science* **34,** 149-156.

Taghizadeh-Toosi, A., Condron, T. J., Sherlock, L. M., Anderson, R. R., Craigie, C. R. and Robin, A. (2011). Biochar Incorporation into Pasture Soil Suppresses in situ Nitrous Oxide Emissions from Ruminant Urine Patches. *Journal of Environmental Quality* **40,** 468-476.

Tarcan, G., Akıncı, G. and Danışman, M. A. (2010). Assessment of the Pollution from Tannery Effluents upon Waters and Soils in and Around Kula Vicinity, Turkey. *Water, Air, & Soil Pollution* **213,** 1999-210.

Tiller, K. Urban soil contamination in Australia. *Australian Journal of Soil Research* **30,** 937-957.

Tokunaga, T. K., Wan, J., Firestone, M. K., Hazen, T. C., Olson, K. R., Herman, D. J., Sutton, S. R. and Lanzirotti, A. (2003). In Situ Reduction of Chromium (VI) in Heavily Contaminated Soils through Organic Carbon Amendment. *Journal of Environmental Quality* **32,** 1641-1649.

Tokunaga, T. K., Wan, J., Firestone, M. K., Hazen, T. C., Schwartz, E., Sutton, S. R. and Newville, M. (2001). Chromium diffusion and reduction in soil aggregates. *Environmental Science and Technology* **35,** 3169-3174.

Tokunaga, T. K., Wan, J., Lanzirotti, A., Sutton, S. R., Newville, M. and Rao, W. (2007). Long-term stability of organic carbon-stimulated chromate reduction in contaminated soils and its relation to manganese redox status. *Environmental Science and Technology* **41,** 4326-4331.

Tomlinson, M. J., Wang, J. and Caruso, J. A. (1994). Speciation of toxicologically important transition metals using ion chromatography with inductively coupled plasma mass spectrometric detection. *Journal of Analytical Atomic Spectrometry* **9,** 957-964.

Tordoff, G., Baker, A. J. M. and Willis, A. (2000). Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere* **41,** 219-228.

Traina, S. J. and Laperche, V. (1999). Contaminant bioavailability in soils, sediments, and aquatic environments. *Proceedings of the National Academy of Sciences of the United States of America* **96,** 3365-3371.

TRI01. (2003). Toxic Chemical Release Inventory 2001. Data contained in the Toxic Chemical Release Inventory (TRI). U. S. Environmental Protection Agency Office of Environmental Information. http://www.epa.gov/triexplorer/

Turick, C., Apel, W. and Carmiol, N. (1996). Isolation of hexavalent chromium-reducing anaerobes from hexavalent-chromium-contaminated and noncontaminated environments. *Applied Microbiology and Biotechnology* **44,** 683-688.

Tzou, Y., Chen, Y. and Wang, M. (1998). Chromate sorption by acidic and alkaline soils. *Journal of environmental science and health. Part A, Environmental Science and Engineering* **33,** 1607-1630.

Ucun, H., Bayhan, Y. K., Kaya, Y., Cakici, A. and Faruk Algur, O. (2002). Biosorption of chromium (VI) from aqueous solution by cone biomass of Pinus sylvestris. *Bioresource Technology* **85,** 155-158.

US EPA 7196A. (1995). Chromium, Hexavalent (colorimetric). Test Methods for Evaluating Solid Waste, Physical/Chemical Methods.SW-846. 3rd Edition.

US EPA. (2000). *In-situ* treatment of soil and ground water contaminated with chromium-technical resource guide. *United States Environmental Protection Agency.*

Vajpayee, P., Tripathi, R., Rai, U., Ali, M. and Singh, S. (2000). Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphean alba* L. *Chemosphere* **41,** 1075-1082.

Van Zwieten, L., Kimber, S., Morris, S., Chan, K., Downie, A., Rust, J., Joseph, S. and Cowie, A. (2010). Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant and Soil* **327,** 235-246.

Vazquez, M., Poschenrieder, C. and Barcelo, J. (1987). Chromium(VI) induced structural and ultra-structural changes in bush bean plants (Phaseolus vulgaris L.). *Annals of Botany* **59.** 427-438.

Vega, F. A., Covelo, E. F. and Andrade, M. L. (2006). Competitive sorption and desorption of heavy metals in mine soils: Influence of mine soil characteristics. *Journal of Colloid and Interface Science* **298,** 582-592.

Venitt, S. and Levy, L. (1974). Mutagenicity of chromates in bacteria and its relevance to chromate carcinogenesis. *Nature* **250,** 493-495.

Walkley, A. and Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* **37,** 29-38.

Wang, X. S., Chen, L. F., Li, F. Y., Chen, K. L., Wan, W. Y. and Tang, Y. J. (2010). Removal of Cr (VI) with wheat-residue derived black carbon: Reaction mechanism and adsorption performance. *Journal of Hazardous Materials* **175,** 816-822.

Weng, C., Huang, C., Allen, H. E., Cheng, A. H. D. and Sanders, P. F. (1994). Chromium leaching behaviour in soil derived from chromite ore processing waste. *Science of the Total Environment* **154,** 71-86.

Whitten K. W. and Gailey KD. (1984). General chemistry. Saunders College Pub. (Philadelphia).

Wielinga, B., Mizuba, M. M., Hansel, C. M. and Fendorf, S. (2001). Iron Promoted Reduction of Chromate by Dissimilatory Iron-Reducing Bacteria. *Environmental Science and Technology* **35,** 522-527.

Wittbrodt, P. R. and Palmer, C. D. (1995). Reduction of Cr (VI) in the Presence of Excess Soil Fulvic Acid. *Environmental Science and Technology* **29,** 255-263.

Wittbrodt, P. R. and Palmer, C. D. (1996). Effect of temperature, ionic strength, background electrolytes, and Fe(III) on the reduction of hexavalent chromium by soil humic substances. *Environmental Science and Technology* **30,** 2470–2477.

Wong, M. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere* **50,** 775-780.

World Health Organisation. (1996). Health criteria and other supporting information.

Wu, Y., Zhang, S., Guo, X. and Huang, H. (2008). Adsorption of chromium (III) on lignin. *Bioresource Technology* **99**. 7709-7715.

Xu, S. and Jaffe, P. R. (2006). Effects of Plants on the Removal of Hexavalent Chromium in Wetland Sediments. *Journal of Environmental Quality* **35**, 334-341.

Yang, H., Yan, R., Chen, H., Lee, D. H. and Zheng, C. (2007). Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel* **86,** 1781-1788.

Zachara, J. M., Ainsworth, C. C., Cowan, C. E. and Resch, C. T. (1989). Adsorption of chromate by subsurface soil horizons. *Soil Science Society of America Journal* **53,** 418-428.

Zachara, J. M., Girvin, D. C., Schmidt, R. L. and Resch, C. T. (1987). Chromate adsorption on amorphous iron oxyhydroxide in the presence of major groundwater ions. *Environmental Science and Technology* **21,** 589-594.

Zayed, A. M. and Terry, N. (2003). Chromium in the environment: factors affecting biological remediation. *Plant and Soil* **249,** 139-156.

Zayed, A., Gowthaman, S. and Terry, N. (1998). Phytoaccumulation of Trace Elements by Wetland Plants: I. Duckweed. *Journal of Environmental Quality* **27,** 715-721.

Zhou, M., Liu, Y., Zeng, G., Li, X., Xu, W. and Fan, T. (2007). Kinetic and Equilibrium Studies of Cr (VI) Biosorption by Dead *Bacillus licheniformis* Biomass. *World Journal of Microbiology and Biotechnology* **23,** 43-48.

Ziemniak, S., Jones, M. and Combs, K. (1998). Solubility and phase behaviour of Cr(III) oxides in alkaline media at elevated temperatures. *Journal of Solution Chemistry* **27,** 33-66.

Zimmerman, A. R. (2010). Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environmental Science and Technology* **44,** 1295-1301.