CHROMIUM DYNAMICS IN SOIL

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ABSTRACT

Due to increasing awareness of potential Cr toxicity, there is a pressing need to establish sensitive and robust Cr fractionation and speciation methodologies that will be enable separation of the two redox Cr species (Cr^{III} and Cr^{VI}) from different environmental phases and their quantification. The intention of this work was to assess the behaviour of Cr species, especially Cr^{VI}, in soils and the factors controlling Cr solubility, fractionation, redox transformation rates and uptake by plants. The analysis methods relied on alkaline extraction in TMAH, liquid chromatography (LC) to separate the chromium species and inductively couple plasma mass spectrometry (ICP-MS) for quantification of chromium. The interference of $^{40}Ar^{12}C^{+}$ background peak at mass 52 was reduced by using the CCT-KED facility of the ICP-MS. A solution of 50 mM TRIS buffer, 40 mM NH_4NO_3, 10^{-5} M ammonium-EDTA at pH 7.0 was used as the chromatographic eluent. The method developed is suitable for determining Cr^{VI} in soil, following alkaline extraction in TMAH, but not for Cr^{III} due to poor recovery, redox transformation and strong binding of Cr^{III} with humic acid despite attempts to preserve the trivalent species using EDTA and heating.

The extraction method was applied to assessing Cr speciation and fractionation in a wide range of soil ecosystems collected from urban sites in Wolverhampton, Nottingham, London and a historical sewage sludge disposal farm in Nottinghamshire. To predict soil Cr^{VI} content the use of TMAH-extractable Cr (Cr_{TMAH}) was better (R=0.911) compared to total soil Cr content (Cr_{total}; R=0.554). The same analytical approaches were also applied to the development of a method to determine isotopically exchangeable Cr^{VI} in soils. This employed isotopically enriched $^{50}Cr^{VI}$ as a 'spike' isotope added to soils suspended in varying concentrations of TMAH in an attempt to resolve a consistent fraction of isotopically exchangeable, or 'labile', $Cr^{VI}O_4^{2-}$ in soil. It was apparent that, because of the slow exchange kinetics of Cr^{VI} in soils, it was difficult to determine a consistent isotopically exchangeable fraction. Nevertheless, the investigation did suggest a refinement of the simple TMAH extraction protocol could enable direct determination of labile soil Cr^{VI}. The kinetics of Cr^{VI} interaction with a geocolloid (humic acid) was assessed and humic acid was found capable of both reducing Cr^{VI} and binding with the resulting Cr^{III} species. Finally, Cr uptake by maize grown on a historical sewage sludge disposal farm was assessed with several approaches to finding a correlation between Cr in soil and Cr uptake by plants. The concentration of Cr^{VI} in soil, and its solubility, could be reasonably well predicted from Cr_{total} or Cr_{TMAH} and soil

properties. However, restricted uptake of Cr^{VI} by the maize plants, and probably reduction of Cr^{VI} to Cr^{III} in the root system, made it impossible to predict Cr transfer to shoots or the speciation of the Cr in maize shoots. Overall, due mainly to the apparent ability of the maize plants to control uptake and speciation of Cr^{VI}, the produce was considered safe to be consumed by ruminants as regards Cr^{VI} content.

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GLOSSARY

$Ca(NO_3)_2$	calcium nitrate
CCT-KED	Collision Cell Technology-Kinetic Energy Discrimination
Cps	count per second
Cr^{VI}	chromium (VI)
Cr^{III}	chromium (III)
Cr_{TMAH}	TMAH-extracted Cr
Cr^{VI}_{TMAH}	TMAH-extracted Cr^{VI}
Cr^{VI}_{CaNO3}	$Ca(NO_3)_2$-suspended Cr^{VI}
Cr_{total}	total Cr
$Cr_{total-S}$	total Cr in soil (mg kg^{-1})
$Cr_{total-P}$	total Cr in plant (mg kg^{-1})
Cr_E	labile Cr
Cr^{VI}_E	labile chromium (VI)
EDTA	Ethylene diamine tetraacetic acid
EU	European Union
Fe_{total}	total Fe
HA	humic acid
HNO_3	nitric acid
HPLC	High Performance Liquid Chromatography
ICP-MS	Inductively couple plasma-mass spectrometry
ICPS	integrated count per second
LN	London
LOI	Loss of ignition
Mn_{total}	total Mn
N_2	Nitrogen

Na_2CO_3	sodium carbonate
NaOH	sodium hydroxide
NG	Nottingham
NH_4-EDTA	ethylenediamine tetra acetic acid tetraammonium salt
RF	radio frequency
RSD	Residual standard deviation
SB	Sutton Bonington
S.E	Standard error
SEC	Size Exclusion Chromatography
SR	Stoke Rochford
StB	Stoke Bardolph
TMAH	tetra-methyl ammonium hydroxide
TRIS	tris(hydroxymethyl)aminomethane
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Agency (USFDA)
WV	Wolverhampton

1 INTRODUCTION

Chromium is a Group 6 transition metal. It is commonly present in trivalent and hexavalent forms in the environment (Cr^{III} and Cr^{VI}). These two redox species exist as cationic (Cr^{3+}, $CrOH^{2+}$) and anionic (CrO_4^{2-}) forms in soils and so demonstrate contrasting geochemical and biochemical behaviour (Katz and Salem, 1994; Fendorf, 1992; Shanker et al., 2005). Cr^{III} is an important micronutrient for humans and animals as it has an essential role in the maintenance of normal glucose and lipid metabolism (Anderson, 1989, 1997). A recommended daily intake (RDI) for adults of 50 – 200 µg of Cr^{III} per day has been suggested by the US National Research Council (NRC, 1989). In contrast, Cr^{VI} is both carcinogenic and toxic; an oral Reference Dose (RfD) has been established at just 0.003 mg kg^{-1} day^{-1} Cr^{VI} by the USEPA (EPA, 1998).

1.1 USES OF CHROMIUM

A recent review of literature by Dhal et al., (2013) found that 90% of total chrome ore production was used by the metallurgical industry in the production of stainless steel, alloyed and non-ferrous alloy. The remaining 10% of total chrome ore produced was mainly used in the refractory industry (glass, ceramics etc), in cement production and in various chemical industries such as the production of chromic acid used in wood preservation and anti-corrosion compounds for (eg) reactor cooling water (Dhal et al., 2013; Alloway, 2012; Dresel et al., 2008).

1.2 ENVIRONMENTAL CHEMISTRY OF CHROMIUM

Chromium can be found in all phases of the environment, including air, water and soil. In the atmosphere, Cr (particulate) concentration varies from 5.0 x 10^{-6}-1.2 x 10^{-3} µg m^{-3} in air (Nriagu, 1988). Chromium concentration in fresh water ranges from 0.1 to 117 µg L^{-1} whereas in seawater the concentration range is 0.2 to 50 µg L^{-1} (Shanker et al., 2005). In European soils, the background concentrations of Cr are from 5 to 68 mg kg^{-1} with the lowest in sandy soils and more in soils originating from volcanic rocks (Utermann, 2006). More Cr can be found in ultramafic rocks compared to granitic rock with chromite as the most common mineral (Nriagu, 1988; Reimann and Caritat, 1998)

Chromium can exist in several oxidation states between -2 and +6 but only the +3 and +6 states are environmentally relevant. Trivalent Cr^{III} is the stable form in most natural systems where hydrolytic products; $Cr(OH)^{2+}$,$Cr(OH)_2^{+}$,$Cr(OH)_3^{-}$, are the dominant *inorganic* aqueous species at pH values from 4 to 6 controlling sorption to geocolloids and solubility (Figure 1.0). However, in natural waters Cr^{III} is most likely to be bound to humic and fulvic acid or other organic ligands (Kotas and Stasicka, 2000). Cr^{VI} is stable in natural systems only at high pH (Figure 1.0) and is readily reduced to Cr^{III} by functional groups on humic and fulvic acids and subsequently bound to organic hydroxyl or carboxyl groups (Unceta et al., 2010). The presence of small concentrations of Cr^{VI} in the environment is normal - a result of oxidation of geogenic Cr^{III}. However, high concentrations of Cr^{VI} are likely to be due to direct contamination with Cr^{VI} from anthropogenic activities (Bartlett, 1991). In neutral to basic conditions Cr^{VI} is in the form of CrO_4^{2-} (James and Bartlett, 1993; Kotas and Stasicka, 2000). Reduction of Cr^{VI} (CrO_4^{2-}, $HCrO_4^{-}$) is promoted under acidic conditions (Deltombe et al., 1966) in the presence of electron donors:

$$HCrO_4^- + 7H^+ + 3e^- \rightleftharpoons Cr^{3+} + 4H_2O \quad \text{and}$$

$$CrO_4^{2-} + 5H^+ + 3e^- \rightleftharpoons Cr(OH)_3 + H_2O$$

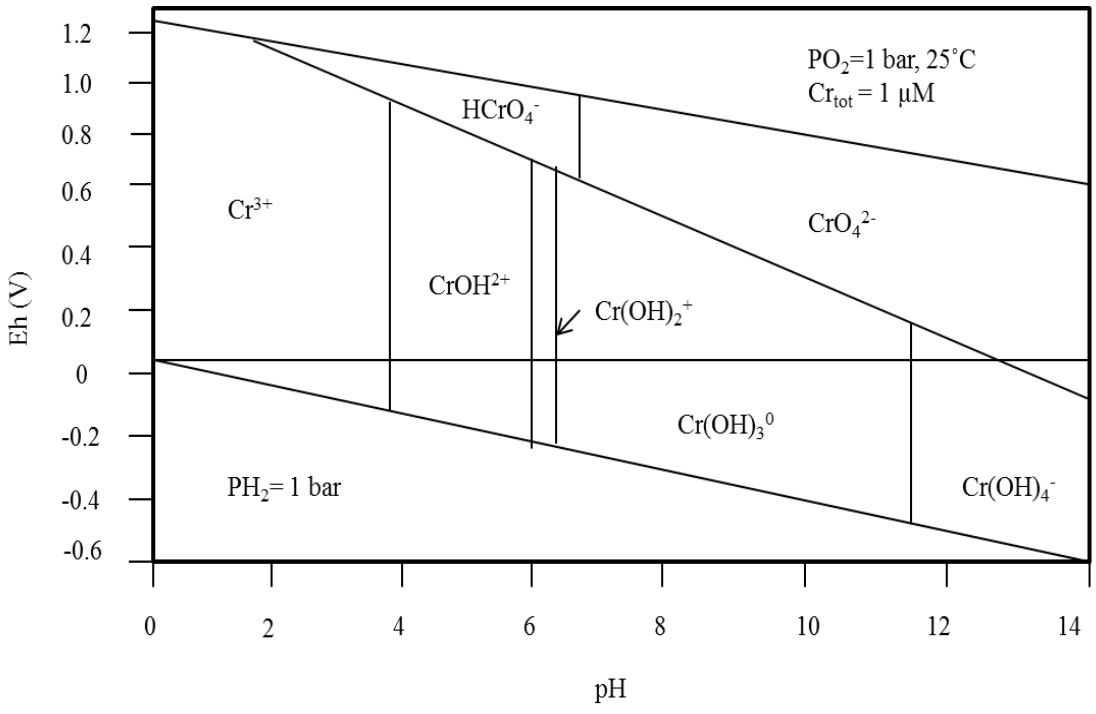


Figure 1.1 A simplified redox potential (Eh-pH diagram) for aqueous Cr at 25˚C (adapted from Palmer and Wittbrodt, 1991)

1.2.1 Chromium in soil

(i). Origins of Cr in soil

Natural Sources

In common with most trace elements, the present of chromium in soil is the result of weathering of Cr from minerals within parent material and so its concentration varies depending in the mineralogy of the parent material (Economou-Eliopoulus et al., 2011). The highest concentration of Cr is found in mafic (170-200 mg kg^{-1}) and ultramafic (1600-3400 mg kg^{-1}) rocks whereas the lowest concentrations are in acid igneous and sedimentary rock ranging from 5 to 120 mg kg^{-1} (Kabata Pendias, 2001; Alloway, 2013).

Anthropogenic sources

The use of phosphate fertilizer may contribute to the presence of Cr in soil; the National Research Council of Canada report that Cr concentrations in rock phosphate deposits vary from 30 to 300 mg kg^{-1} (NRCC, 1976). A later study by Becquer et al. (2003) observed an increase in Cr^{VI} concentration in the soil pore solution after large phosphate fertilizer inputs. The application of sewage sludge also contributes to elevated Cr concentrations in soil. Localised industrial activity that utilizes Cr will also produce soil hotspots: Zayed and Terry (2003) found 25.9 g kg^{-1} of total Cr in soil in the vicinity of a chrome production facility in the USA.

(ii). Fractionation and speciation of Cr in soil.

It is generally agreed in the literature that Cr^{III} in the form of $Cr(OH)_3$ is only slightly mobile in very acidic media and almost completely precipitated above pH 5.5 (Bartlett and Kimble, 1976). Above pH 5.5, Cr^{III} is present as hydroxyl species such as $Cr(OH)_2^+$ and strongly bound to inorganic geocolloids such as alumina-silicate clays and iron oxides and to humic substances via carboxy groups (Kyziol et al., 2006).

By contrast, Cr^{VI} is soluble in both acidic and alkaline soils and may be lost from soil by leaching, or retained in soil by absorption onto Fe (hydro)oxides such as goethite, Al oxides and other positively charged colloids (James, 1988), especially at low pH values. The mobility of Cr^{VI} in soil (CrO_4^{2-} and $HCrO_4^-$ ions) is due to electrostatic repulsion of soil particles at high pH (Alloway, 2013; Fendorf, 1995).

(iii). Behaviour of Cr species in soil

The behaviour of Cr in soil depends on pH, redox potential, presence of potential electron donors and Cr adsorption on colloids; both Cr^{III} and Cr^{VI} may form various hydrolysis and deprotonated products for example $CrOH^{2+}$, $Cr(OH)^{4-}$ is the hydroxyl species of Cr^{III} (Rai et al., 1987).

Oxidation of Cr^{III}

Oxidition of Cr^{III} to the more toxic Cr^{VI} species can occur in soil due to the presence of electron acceptors such as manganese oxides and, potentially, dissolved oxygen (Eary and Rai, 1987; Bartlett and James, 1979; Fendorf and Zasoski, 1992; Jardine et al, 1999; Kozuh et al., 2000; Guha et al., 2001; Pantsar-Kallio et al., 2001; Kim and Dixon, 2002; Oze et al., 2007). However, in a study by Schroeder and Lee (1975) there was little interaction between dissolved oxygen and Cr^{III} compared to the effect of Mn oxide. The oxidation of Cr^{III} by Mn oxide first involves adsorption of Cr^{III} on to the surface of Mn oxide, which is partly determined by the availability of dissolved Cr^{III} species (Manceau and Charlett, 1992; Fendorf and Zasoski, 1992; Eary and Rai, 1987). The presence of Cr^{III} subspecies in soil, such as $Cr(H_2O)_6^{3+}$ and freshly precipitated $Cr(OH)_3$ may affected the oxidation process (James et al., 1995,1996; Vitale et al., 1994,1995), whilst Cr_2O_3 and aged $Cr(OH)_3$ are resistant to oxidation as they tend to be strongly bound to soil humus (Bartlett and James, 1988; Huo et al., 2000).

Reduction of Cr^{VI}

This reaction is vitally important in reducing the toxicity of Cr^{VI} in soil and preventing its transfer to surface water and groundwater.

Organic matter

The presence of organic carbon stimulates microbial activity and can cause an increase in the concentration of dissolved organic carbon which acts as an electron donor for the reduction of Cr^{VI} (Bolan et al., 2003). Some organic carbon sources, such as biosolid compost, farmyard and poultry manures can also effectively reduce Cr^{VI} through an abiotic mechanism (Park et al., 2008; Tokunaga et al., 2003). Nakayasu et al. (1999) found that low molecular weight organic acids, such as tannic acid and gallic acid, were more reactive in reducing Cr^{VI} compared to humic acid which may be due to the redox potential of these organic materials.

Their findings were in agreement with Nakayama et al. (1981b) and James and Bartlett (1983b) who also found soluble low molecular weight organic matter (LMWOM), such as ascorbic and gallic acid, was an effective reductant of Cr^{VI}. Various types of humic acid have been investigated and shown to reduce Cr^{VI} (Lu et al., 1997; Wittbrodt and Palmer, 1995; Scaglia et al., 2013), even though the conversion of Cr^{VI} to HA-Cr^{III} and the reaction mechanism has not been extensively studied or quantified. Some contrasting evidence from Leita et al., (2013) found and suggested a mechanism which involved the interaction of Cr^{VI} with humic acid to form Cr^{VI}-HA micelles rather than initial reduction to Cr^{III} followed by bonding to humic oxyacid groups.

Ferrous iron

Iron (II) species not only reduce Cr^{VI}, but will also enhance the removal of (the resulting) aqueous Cr^{III} due to the formation of $(Fe,Cr)(OH)_3$ precipitates (Sass and Rai, 1987; Chon et al., 2006). The formation of this solid Cr^{III} phase then also avoids the potential for oxidation to Cr^{VI} (Fendorf et al., 1996). Eary and Rai, (1988) observed that Cr^{VI} reduction by aqueous Fe^{II} was completed within 5 minutes at pH values greater than 4. In a forest wetland soil, as well as in sand and gravel of a suboxic aquifer, Fe^{II} was recognized as the dominant reductant of Cr^{VI} at redox potentials less than +100mV (Masscheleyn et al., 1992; Anderson et al., 1994). At some stage in the acidification of soil, the reducing capability of Fe^{II} became equal to that of organic material in reducing Cr^{VI} (Eary and Rai, 1991). Zhilin et al., (2004) studied the influence of Fe^{II} in reducing Cr^{VI} by using capillary zone electrophoresis and found that by increasing the concentration of Fe^{II}, Cr^{VI} reduction was faster. Lee et al., (2003) found that 'zero-valent' Fe^0 metal from waste was more efficient in reducing Cr^{VI} in soil than a commercially available for of Fe^0. Chon et al., (2006) found that, among the ferrous iron-bearing mineral, pyrite can reduce Cr^{VI} two times faster than biotite in acidic conditions.

Sulfide

The reduction reaction of Cr^{VI} with sulphide depends on the pH with a faster reaction under acidic conditions and a slower response in alkaline conditions (Beukes et al., 1999). The presence of clay minerals, such as montmorillonite and kaolinite, can also increase the reduction rate of Cr^{VI} by sulfide due to adsorption of Cr^{III} (Buerge and Hug, 1999). In contrast, Lan et al., (2007) found that kaolinite, montmorillonite, amorphous SiO_2 and TiO_2 actually inhibited Cr^{VI} reduction by sulfide due to adsorption of elemental S produced from

the reaction with these minerals. However, reduction of Cr^{VI} by Fe^{II} is much faster than the reduction of Cr^{VI} by sulfide at pH 7 to 9 (Lan et al., 2007).

1.2.2 Chromium in plants.

Which species is taken up most easily by plants – Cr^{III} or Cr^{VI} ?

Various authors have explored the preference of plants for uptake of Cr species (Cr^{VI} or Cr^{III}), with most studies simply comparing which added species showed the greatest uptake. Huffman and Allaway (1973) found that the uptake and translocation of ^{51}Cr differed only slightly for Cr^{III} and Cr^{VI}, with 28 % of Cr^{VI} and 21 % of Cr^{III} remaining in the nutrient solution planted with beans after 9 days. For wheat, 6 % of Cr^{VI} and 5 % of Cr^{III} remained in the nutrient solution.However, the chemical form of Cr in the nutrient solutions after addition of the Cr treatment was not known and so it is possible that transformation occurred, especially in the presence of soluble organic rhizosphere compounds. In a study on the uptake of Cr species by wheat planted in a greenhouse pot experiment, Cary et al., (1977) found that Cr^{VI} was the species most easily taken up by plants. They found that adding 2.0 mg kg^{-1} of Cr^{VI} in soil, in solution, increased the total concentration of Cr in grain from 17 to 43 mg kg^{-1} and straw from 183 to 1353 mg kg^{-1}, respectively.

The accumulation of Cr^{III} and Cr^{VI} in barley, maize, cauliflower, radish, carrot, beetroot, lettuce, tomato and mung beans supplied with a Cr-enriched nutrient solution was studied by Lahouti and Peterson (1979). More Cr was found in the roots of the crops when Cr^{III} was supplied in the nutrient solution as compared to Cr^{VI}. They also determined that Cr^{VI} entered the plant via the sulphate pathway whereas Cr^{III} uptake was passive. Shewry and Peterson (1974) found that uptake of $^{51}CrO_4^{2-}$ in barley seedlings, *Hordeum vulgare L.* was inhibited in the presence of SO_4^{2-} and other group VI anions like SeO_4^{2-}, MoO_4^{2-} and WO_4^{2-} with uptake of $^{51}CrO_4^{2-}$ in the order $WO_4^{2-} > SeO_4^{2-} > MoO_4^{2-} > SO_4^{2-}$.

Zayed et al., (1998) measured Cr uptake by vegetable crops cultured hydroponically in a greenhouse at 25-30°C and supplied with either Cr^{VI} or Cr^{III}; they found that more Cr^{VI} was accumulated in the roots and shoots of cauliflower, kale and cabbage compared to Cr^{III}. The same finding was observed by Shahandeh and Hossner, (2000) as the accumulation of Cr in Brassica species was ten times greater in roots and shoots if the plants were supplied with

Cr^{VI} rather than Cr^{III}. In contrast, Zhang *et al*., (2008) found the preference for Cr uptake by willow roots from hydroponic solution was Cr^{III} > Cr^{III}-EDTA(1:0.5) > Cr^{III}-EDTA (1:1) > Cr^{VI} > Cr^{VI}-EDTA(1:0.5) = Cr^{VI}-EDTA(1:1).

Srivastava et al., (1999) studied the uptake of Cr^{VI} and Cr^{III} (supplied at 5 µg mL^{-1}) by maize in the presence of carboxylic and amino acids and found that concentration of Cr^{VI} in roots was 3 to 6 times higher compared to the concentration of Cr^{III} in shoots. Uptake of Cr^{III} was increased by increasing the ratio of Cr^{III}:carboxylic and amino acid. The source-to-plant transfer coefficient was six times higher with a 1:100 ratio of Cr^{III}:carboxylic and amino acid compared to a control treatment without the addition of carboxylic and amino acid.

What happens to Cr following uptake by plants?

Various studies have been carried out on Cr following its uptake by plants, even though there was no clear mechanism indicating a preference for Cr species. Skeffington et al. (1976) found that when plants were supplied with either Cr^{III} or Cr^{VI} ions, CrO_4^{2-} was the only species detected in plant tissue by using solvent extraction and high-voltage paper electrophoresis. In contrast, Zayed et al., (1998) showed, using XAS (X-ray absorption spectroscopy) techniques that only Cr^{III} species were detected in all plant tissue tested, regardless of whether these plants were treated with $Cr^{VI}O_4^{2-}$ or Cr^{III}. Their data strongly indicated a conversion of CrO_4^{2-} to Cr^{III} in root tissue since there was no Cr^{VI} species in the roots of plants that were previously supplied with CrO_4^{2-}.

Huffman and Allaway (1973) found that Cr concentrations in bean plants followed the sequence: roots > leaves> stems > chaff (pods) > seeds. Whilst in wheat, the order was: roots > stems > leaves > chaff (pods) > seeds. The apparent exclusion of Cr from seeds may result from lack of a direct connection of vascular tissue to developing seeds (Esau, 1965). Mishra et al., (1997) found that the accumulation of Cr was greater in roots than in shoots and grains in paddy rice grown in sand and soil culture.

Plant type - do different plants have different preferences for Cr ?

Crops vary in their ability to take up and accumulate Cr in their tissues. In a range of studies, the highest Cr concentrations found in non-food species include species of fern with 5720 mg kg^{-1} in roots and 215 mg kg^{-1} in shoots and the least in spinach with 3.14 mg kg^{-1} in root and

2.18 mg kg^{-1} in shoot. (Singh, 2001; Hong et al., 1999; Peralta et al., 2001; Mishra et al., 1997; Kocik and Ilavsky, 1994; Shahandeh and Hossner, 2000a; Su et al., 2005; Zayed et al., 1998; Huffman and Allaway, 1973a; Turner and Rust, 1971; López-Luna et al., 2009).

Does aerial deposition contribute to Cr loading in leaves ?

Aerial deposition can contribute to Cr loading in leaves but there are contradicting arguments from previous work regarding the efficacy of a water-washing step in reducing extraneous Cr concentration on leaves. Early investigations by Larsen et al., (1992) and Voutsa et al., (1996) found that atmospheric deposition of Cr on leaves could not be removed by washing. However, other workers have found that washing with water can reduce Cr concentrations in leaves (e.g. Mignorance and Oliva, 2006; Nabulo et al., 2010).

Damage to plants from Chromium?

Symptom of Cr toxicity appear as wilting of tops, root injury and chlorosis in young leaves with chlorotic bands on cereals and brownish-red leaves typical features (Baszynski et al., 1981). Cr^{III} does not appear to affect shoot growth, in terms of shoot length, dry weight, and total leaf area, whereas root length and root dry weight were significantly affected by Cr^{III} in a study by Shahandeh and Hossner, (2000). However, Cr^{VI} is toxic to plants and may cause membrane damage due to its high oxidation power. Increased concentration of Cr in nutrient solutions (up to 10^4 µM) are reported to disorganize the fine structure of chloroplasts and chloroplast membrane of *Lemna minor* (duckweed) (Baszynski et al., 1981). Chatterjee and Chatterjee (2000) reported that an excess of Cr in plants caused poor protein formation resulting from the disruption of the N metabolism. Increased levels of Cr^{VI} in nutrient solution decreased CO_2 assimilation and other parameters associated with the photosystem of *Lolium perenne* leaves (Vernay et al, 2007). Schiavon et al., (2008) notice a significant decrease of the S uptake rates by plants under Cr-stress.

It has been judged that Cr^{VI} is harmful to plants at concentrations greater than 25 mg kg^{-1} and may cause plant death at concentrations in soil greater than 100 mg kg^{-1} (Shahandeh and Hossner, 2000a).

Is there are risk of Cr^{VI} transfer into the wider food chain?

The risk of transfer of toxic metal into the human food chain is increased in the case of vegetables such as raw salad crops and other green leaf vegetables such as spinach and cabbage, due to increasing demand on agricultural production and the need to utilize contaminated soils and soil in the peri-urban environment (Economou-Eliopoulos et al., 2011; Patra et al., 2001; Srikath and Reddy, 1991; Nabulo et al., 2010). Moreover, in urban agriculture, wastewater and solid organic wastes are often the principal sources of irrigation and fertilizer used to enhance the yields of staple crops and vegetables. The use of such soil amendments can contribute significantly to metal loadings in urban soils and agricultural produce (Nabulo et al., 2010). In a comparison made between washed and unwashed fifteen green leaves vegetables grown on contaminated urban soils in and around Kampala City, Uganda. Nabulo et al., (2010) found Cr was the highest element with the ratio of unwashed:wash plant (<2.5) compared to Pb, Cu, Zn, Ni and Cd due to the aerial deposition of Cr on the leaves.

1.3 ADVERSE EFFECTS OF CHROMIUM CONTAMINATION

1.3.1 Health Effects

Katz and Salem (1994) reported that Cr^{VI} is 10 to 100 times more toxic than Cr^{III}. From a study on rats, 50 to 100 mg kg^{-1} Cr^{VI} and 1900 to 3000 mg kg^{-1} Cr^{III} were the respective doses that can cause death (Flora et al., 1990). The National Institute for Occupational Safety and Health (NIOSH) has categorised Cr^{VI} as carcinogenic and acknowledge that it can lead to cancer of the lung, nasal passage and sinuses in workers in industries that utilize Cr compounds, such as the production of cement, steel, paints and pigments, and may suffer direct and prolonged exposure to Cr^{VI} airborne particulates (NIOSH 1988b, 2002, 2005a, 2013). Gibb et al., (2000b) investigated cumulative Cr^{VI} exposure to 2,357 chromate production workers in Baltimore, Maryland from 1947 to 1974 and confirmed a statistical connection with lung cancer.

1.3.2 Environment

Barnhart, (1997) reported that the leather industry causes a high input of Cr to the biosphere, and is responsible for about 40% of total industrial use. In India, about 2000-3200 tons of

elemental Cr was released to the environment from tanning industries (Chandra et al., 1997). Bini et al., (2008) reported that Cr concentration in soils near a leather tannery in Italy was up to 10,000 mg kg^{-1} (mean 210 mg kg^{-1}). In addition, it has been estimated that about 2% of Cr from solid waste of leather dust in Pakistan can reach the environment (Khan, 2001).

In the UK, chromium emissions to the environment are predominantly derived from public power generation and other forms of fuel combustion, waste incineration and other industrial processes. A total of 60 tonnes of chromium was emitted to the environment in 1995 (Salway et al, 1997). Pawlet et al., (2001) reported that the maximum concentration of total Cr (65,100 mg kg^{-1}) and Cr^{VI} (15,600 mg kg^{-1}) from 15 sites in South East Glasgow was due to chromite ore processing residue that being used as infill materials on land.

In a recent survey by the Environmental Working Group (EWG), a non-profit American environmental organisation, it was found that tap water from 31 of 35 American cities contained measurable Cr^{VI} up to 13 µg L^{-1}. The five cities with the highest Cr^{VI} concentrations followed the sequence: Norman, Oklahoma > Honolulu, Hawai > Riverside, California > Madison, Wisconsin > San Jose, California (Figure 1.1) (EWG, 2010). Discharge of Cr^{VI} from steel and pulp mills, metal plating and leather tanning industry resulted in the presence of Cr^{VI} in these water supplies (EWG, 2010).

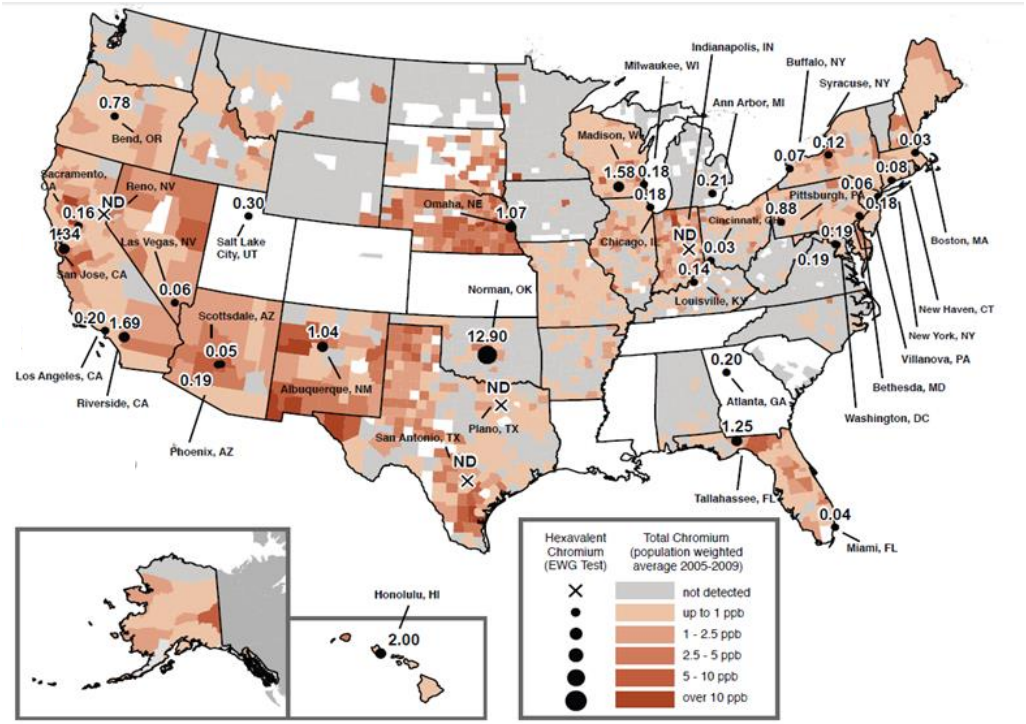


Figure 1.2 The concentration of Cr^{VI} at test sites (different sizes of black dots reflect the level found) from an EWG survey. The brown shaded areas represent population-adjusted average concentrations of total Cr by sites (EWG, 2010).

1.4 STANDARDS AND ASSESSMENT

Due to increasing awareness of the need to protect human populations and the environment from Cr toxicity, authorities have set the limits for (total) Cr and Cr^{VI} in various media.

1.4.1 Drinking water standards

The limit for Cr in water varies between countries. As a guideline, the USEPA has set the maximum concentration of total Cr in drinking water to 100 µg L^{-1} (EPA, 1991). The UK Drinking Water Inspectorate has adopted a tighter standard for total Cr of 50 µg L^{-1}, but no standard for Cr^{VI} is currently available (DEFRA, 2009). California authorities have proposed a safe limit for Cr^{VI} in drinking water of 0.06 µg L^{-1} (OEHHA, 2009). This is sufficiently low that it may challenge the sensitivity of instruments used for water analysis and so may be regarded as a standard based on 'detectable presence'.

1.4.2 Soil standard

In 2013, DEFRA introduced a new guideline on health criteria value with suggested changes to exposure parameters of Cr^{VI} in 'marginally' contaminated land designated as residential with or without consumption of home grown produce (6.1 mg kg^{-1}), allotment (120 mg kg^{-1}), commercial (33 mg kg^{-1}), public open space for residential (7.7 mg kg^{-1}) or 'park' (220 mg kg^{-1}) of Cr^{VI} (DEFRA, 2013). However, there is no concrete study on the toxicity of Cr^{VI} in soil that directly links to adverse health effect, as most of the studies focussed on air borne Cr^{VI} that was released during the industrial activities. Therefore a study was conducted in Chapter 7 that will further give some overview on the uptake of Cr^{VI} by plant (maize) and a possibility of Cr^{VI} being transferred to the human population.These may support as to why DEFRA introduced the same exposure parameters of Cr^{VI} (6.1 mg kg^{-1}) with or without consuming the home grown produce.

1.4.3 Air standard

Due to its toxicity, USEPA has listed Cr^{VI} as one of the 188 hazardous air pollutants in 1990 (EPA, 2004). NIOSH has recommended a maximum concentration limit of 0.2 µg m^{-3} of Cr^{VI} airborne exposure for daily workers (8 hr per day) during a 40 hr working week to limit the risk of lung cancer (NIOSH, 2013).

1.5 REMEDIATION OF CHROMIUM CONTAMINATED MEDIA

The main purpose of remediation treatments of Cr-contaminated soils, water or sediment is the conversion of the easily mobile and highly toxic Cr^{VI} species to the immobile and relatively benign species, Cr^{III}. A number of studies have been undertaken to understand the environmental problems and the effectiveness of remedial treatments of Cr-contaminated land (Powlett et al., 2001; Bini et al., 2008; Geelhoed et al., 2003; Hiller et al., 2003; Pawlett et al., 2001). In a laboratory scale trial to remediate soils with high Cr concentrations, Pawlett et al., (2001) found that treatment with organic matter such as horse dung manures and molasses, sulphite reducing bacteria and ferrous sulphate only reduced Cr^{VI} up to 9% Cr^{VI} from total Cr after 8 months. This may have been due to various parameters such as time scale, pH or temperature restricting microbial activity. Biosorption of Cr by bacteria such as *Pseudomonas spp*. from tannery effluent is effective in converting elevated contents of Cr^{VI} to Cr^{III} in soil and contaminated media compared to chemical and electrochemical methods (Srivastave et al., 2008; Ozdemir et al., 2004).

The use of plants such as *Brassica juncea* (Indian mustard) to phyto-remediate Cr contaminated soil has been attempted but has met with limited success. In trials, more Cr was found in roots compared to shoots and plants died when Cr concentrations of approximately 100 mg kg^{-1} in the shoots was attained (Shahandeh and Hossner, 2000; Bluskov et al., 2005). Non-food plants, such as species of ferns, have also been tested as candidate plant species for phytoremediation; for example *Pteris vittata* accumulated Cr concentrations in shoots up to 215 mg kg^{-1} (Su et al., 2005).

1.6 PROJECT AIMS

The principal aims of this work are to develop techniques of isotopic labelling and chemical speciation which enable increased understanding of (i) chromium dynamics (solubility and speciation) in soils, (ii) consequential bioavailability to plants and (iii) likely transfer to human populations. In order to achieve these aims, four experimental objectives have been set which include:

1. developing a robust analytical method (HPLC-ICP-MS) for speciating Cr in natural solutions and extracts of soil;
2. developing combined speciation-isotopic techniques to further understand the underlying mechanisms observed in various aspects of method development (1) and in applications (3);
3. applying the tools developed in (1) and (2) to real world investigations, focussing on the dynamics of Cr speciation in soils, natural solutions and, especially, in plants;
4. developing a kinetic model of Cr^{VI} interactions with an important geocolloid with Cr^{VI} reduction capability and Cr^{III} binding capacity - humic acid.

The thesis is organized in the following sequence. In Chapter 2 the general methods used in soil characterisation such as pH, loss on ignition, total metal content and also tetra-methyl ammonium hydroxide (TMAH) extraction of soil and Cr speciation analysis are described. Initial work in preparing and validating the concentration of ^{50}Cr stable isotope species is also presented.

Chapter 3 presents the development of methods for speciating Cr in acidic and calcareous soil extracts. This includes the optimisation of ICP-MS conditions and choice of internal standard,

followed by development of a chromatographic method for Cr speciation. The chromatographic work includes establishing an appropriate eluent, optimising the concentration of a Cr^{III}-complexing agent (EDTA) to facilitate transit through the chromatographic column and assessing the need for heating (duration and temperature) to stabilize Cr^{III} complexes with EDTA. The stability of Cr^{III}, Cr^{VI} and mixed Cr species in an established extractant (0.28 M Na_2CO_3 with 0.50 M NaOH) and in 1% TMAH or water was also assessed. The efficiency of recovery of Cr^{III} and Cr^{VI} in heated TMAH extractions of acidic and calcareous soils, incubated with EDTA concentrations of 0.001 M to 0.05 M was tested and the redox behaviour of ^{52}Cr in the extracts was investigated using ^{50}Cr-labelled species.

Chapter 4 involves screening the presence of Cr^{VI} in a range of soils from urban areas in Wolverhampton, Nottingham and London and in soils from a dedicated disposal farm historically amended with sewage sludge using the methods developed in Chapter 3. The measured Cr fractions resolved using acid digestion and alkaline extraction (TMAH) and their relationship with soil properties was investigated. From the combined dataset, a predictive algorithm was developed to estimate Cr fractionation (Cr^{VI} and Cr^{III}) from soil properties.

Chapter 5 presents the development of an isotopic dilution method to assess reactive (isotopically exchangeable) Cr^{VI} in soil. In the initial work, the need for speciation of Cr in solution and to determine whether $^{50}Cr^{VI}$ redox equilibrium is achieved within the timescale of the E-value measurement was assessed. A detailed comparison was made of a range of alkaline (0.001 to 10 % TMAH) suspending solutions, and the neutral salt 0.01 M $Ca(NO_3)_2$. There was an attempt to optimise ^{50}Cr spike level and equilibration time in the determination of isotopically exchangeable Cr^{VI} 'E-values'.

Chapter 6 is a study of the kinetics of Cr^{VI} reduction by humic acid extracted from a peat soil. An aliquot of $^{50}Cr^{VI}$ was added to a humic acid solution and its speciation and conversion to HA-Cr^{III} was monitored through time using LC-ICP-MS; the conversion to organically-bound Cr^{III} was confirmed by size exclusion chromatography (SEC-ICP-MS). The roles of various relevant variables were tested including contact time between Cr^{VI} and humic acid, initial Cr^{VI} concentration and pH value. A kinetic model of the interaction of Cr^{VI} with humic acid was developed based on the results obtained.

Chapter 7 investigates the uptake of total Cr and Cr^{VI} in fodder maize growing on soil historically amended with sewage sludge at the Severn Trent Water sewage disposal site at Stoke Bardolph, Nottinghamshire. Correlations between Cr fractions in the soils and maize plants were undertaken to investigate the transfer of Cr from soil-to-plant and the extent to which the plant is able to exercise control over transfer and, crucially, speciation. A site specific risk assessment of transfer of Cr to human and animal foodchains was attempted.

Finally, Chapter 8 covers general conclusions and recommendations for further work.

2 GENERAL MATERIALS AND METHODS

This chapter describes general materials and methods used throughout this work including pH, loss of ignition, dissolved organic carbon, humic acid preparation and determination as well as trace element concentration. It also describes the preparation of $^{50}Cr^{III}$ and $^{50}Cr^{VI}$ spike solutions, tetra-methyl ammonium hydroxide (TMAH) extraction of soil and plant materials and Cr speciation analysis.

2.1 Soil sampling and pre-treatment

Soil samples were collected using a stainless steel spade, auger or trowel and sealed in plastic bags for transport. They were then air dried in aluminium trays, gently disaggregated using a pestle and mortar (where necessary) and sieved to obtain a <2 mm fraction, removing stones and plant debris. Processed samples were stored in clean polyethylene containers. A sub-sample of each soil was ground to a fine powder in an agate ball mill (Retsh, Model PM400) and stored in the same way.

2.2 Standard methods

2.2.1 Soil pH

Soil pH was determine in soil:water suspensions (1:2.5) after shaking on an end over end shaker for 30 minutes. The pH of the soil suspension was measured using a Hanna Instrument pH meter 209 with a combined glass electrode (Ag/AgCl) PHE 1004 calibrated with buffer solutions of pH 4.01 (1.0 M of $KHC_8H_4O_4$) and pH 7 (mixed solution of 1.0 M KH_2PO_4 and 1.0 M Na_2HPO_4), allowing 5 minutes for the reading to stabilize.

2.2.2 Loss on ignition

Approximately 5 g of oven-dried <2 mm sieved soil samples were ignited at 550˚C in ceramic crucibles in a muffle furnace for 16 h. Crucibles were re-weighed after cooling and the % loss was calculated.

2.2.3 Humic acid preparation

About 400 g of Irish moss peat soil was added to a large beaker and filled to ¾ capacity with 0.1 M NaOH. The beaker was then sealed with parafilm and a low flow rate of N_2 was introduced to create a nitrogen blanket. The solution was left overnight and then centrifuged at 3000 rpm for 20 min. The supernatant was acidified to pH 2 with 10% HCl (which was prepared by diluting 13.5 ml of 37% HCl in 50 ml milliQ water) before centrifuged at 1500

rpm for 15 min to separate the fulvic and humic fraction. The supernatant (fulvic acid fraction) was disposed off and the flocculated humic acid was packed into pre-softened Visking dialysis tubing before dialysis against a mixture of 1% HCl/HNO_3 (which was prepared by diluting 2.70 ml of 37% HCl and 1.50 ml of 70% HNO_3 in 95.8 ml of milliQ water) for a week to remove metal contaminant and silica. The dialysis continued for another week against Milli-Q water (replaced daily).The purification step ceased after the conductivity in the Milli-Q water was below than 20 µS cm^{-1}.The purified humic acid was then freeze dried for 4 days and then stored in a universal tube in a desiccator before use.

2.2.4 Spectrophotometric determination of humic acid

The humic acid (prepared according to Section 2.3.4) concentration was determined by measuring absorbance at 400 nm in a 1 cm cell using a UV-Vis Spectrophotometer (CECIL, CE1011) (Gan et al., 2007). A humic acid stock solution (0.08 g mL^{-1}) was prepared in 0.1 M NaOH with pH adjusted to 7.5 using HNO_3. Samples were diluted to obtain absorbance values in the range of 0.0 to 1.0. The concentration of humic acid was calculated according to Equation 2.1;

$$HA = \frac{(A - 0.025)}{0.0044} \qquad 2.1$$

Where HA = humic acid concentration (mg L^{-1}), A-0.025 = the true value of measured absorbance describe the equation where 0.025 is the background value of 0.1 M NaOH

2.2.5 Extraction of Fe and Mn Oxides

The extraction of amorphous Fe, Al and Mn oxides was undertaken by adapting method described by Anschutz et al., (1998). Triplicate samples of 0.3 g of finely ground soil were suspended in 25 mL solution containing 0.22 M trisodium citrate, 0.11 M sodium hydrogen carbonate and 0.1 M sodium dithionate in polycarbonate centrifuge tubes and shaken with lids left slightly open to let gas escape but retain water vapour by condensation for 16 hours in a water bath at 40˚C. Samples were centrifuge and filtered (<0.22 µm) before dilution (1:10) with Milli-Q water prior to analysis. Total Fe and Mn concentrations were assayed by ICP-MS as described in Section 2.3 and concentrations of Fe_2O_3 and MnO_2 were then calculated.

2.2.6 Acid digestion of soil and plant samples

Samples (approximately 200mg) of finely ground soil were digested in PFA vials within a 48-place Teflon-coated graphite block digester (Model A3, Analysco Ltd, Chipping Norton, UK) with 2 ml HNO_3 (70%, TAG) and 1 ml $HClO_4$ (70%, AR) at 80˚C for 8 hr then at 100˚C for 2 hr. A volume of 2.5 ml HF (40%, AR) was then added to the digestion vessels and the samples heated to 120˚C for 8 hr. After that, a further 2.5 ml HNO_3 (70%, AR) and 2.5 ml Milli-Q water were added to the dry residue and heated for 30 min at 50˚C. Acids were Analytical Reagent (AR) or Trace Analysis Grade (TAG) from Fischer Scientific, UK. The digested soil samples were diluted to 50 ml with Milli-Q water and stored at room temperature in universal sample bottles pending elemental analysis. The samples were then diluted to 1 in 10 with Milli-Q water using a compudil-D auto diluter (Hook and Tucker Instruments) immediately prior to multi element analysis by ICP-MS (see section 2.3.1)

For plant samples, 200 mg of finely ground material was digested using 9 ml HNO_3 (70%, TAG) and 1ml H_2O_2 (30%, AR) in microwave for 45 min at 150˚C. The digested samples were make up to 15 ml and further diluted to 1 in 10 with Milli-Q water before multi element analysis by ICP-MS (see section 2.3.1)

2.2.7 Chromium extraction and equilibration

Chromium in TMAH (Cr_{TMAH})

A modified version of the Watts and Mitchell (2009) method was used to extract Cr from soil sample. Five grams of <2 mm soil samples were suspended in 25 ml of 10% TMAH and heated for 3 hours. The soil suspension was then centrifuged at 3000 rpm for 20 min and filtered using 0.2 µm Millex Milipore filters. The filtrate was diluted 1:10 and total Cr and Cr^{VI} were determined as described in Section 2.4.2. Detailed method development work to determine the optimum conditions for maintaining the stability of Cr species in the extractant and soil extracts was undertaken (see Chapter 3).

A modified version of the Watts and Mitchell (2009) method was used to extract Cr from plant sample. Five millilitres of 10% TMAH was added to 0.5 g of dried and ground sample. The solution was heated for 3 hr at 70˚C with shaking after 1.5 hr. Five millilitres of Milli-Q water was then added to each sample before shaking and centrifugation at 3000 rpm for 15 min. The solution was then filtered using 0.2 µm Millex Milipore filters and diluted to 1% TMAH before the analysis.

Equilibration in $Ca(NO_3)_2$

Two grams of <2 mm sieved soil was equilibrated with 0.01 M $Ca(NO_3)_2$ for 3 days. After equilibration, the soil suspension was centrifuged at 3000 rpm for 20 min and filtered using 0.2 µm Millex Milipore filters. The filtrate was analysed for Cr and C^{VI} using ICP-MS and HPLC-ICP-MS, respectively.

2.3. ICP-MS analysis

2.3.1 Multi - element analysis

Multi-element analysis of digested plant and soil samples was carried out using ICP-MS according to the settings described in Table 2.1. An internal standard; Sc (50 µg L^{-1}), Ge (10 µg L^{-1}), Rh (10 µg L^{-1}) in 2% HNO_3 was employed. Two sets of external multi-element calibration standards were used for major elements (concentration range from 0-30 mg L^{-1}) and trace elements (concentration ranged from 0-100 µg L^{-1}). Sample processing was undertaken using Plasmalab software with internal cross calibration between pulse counting and analogue detector modes where required. All elemental concentrations were converted to mg kg^{-1} dry weight of soil and plant (Equation 2.2).

$$C_{soil} = \frac{(C_{sol} - C_{blank}) \times V}{W_{soil}} \qquad 2.2$$

where C_{soil} is the elemental concentration (mg kg^{-1}) in the soil; C_{sol} and C_{blank} are the concentrations (µg L^{-1}) in the soil and blank digests, corrected for dilution, V is the digest volume (50 mL) and W_{soil} is the mass of soil digested (c. 200 mg).

2.3.2 Chromium analysis and speciation in extracts

Total Chromium in TMAH (Cr_{TMAH})

Stock standards for Cr analysis in TMAH extracts were prepared from 1000 mg L^{-1} of Cr^{VI} in water, Lot No. 16-109Cr(VI), SPEXertificate. Working standards of Cr^{VI} (0 to 100 µg L^{-1}) were freshly diluted in 1% TMAH before each analytical run. An internal standard of ^{45}Sc, ^{74}Ge, ^{103}Rh at approximately 10 µg L^{-1} in 1% TMAH were added to the sample via a T-piece before the nebulizer. The washout solution was also 1% TMAH in order to make sure full wash-out of chromium between samples. The ICP-MS settings are tabulated in Table 2.1.

Chromium speciation

Speciation was undertaken using a Dionex ICS-3000 LC coupled to ICP-MS. The LC was controlled with a computer using Chromeleon Software (Dionex, version 6.80SR12) and data processing was carried out with Plasmalab software. Samples were introduced through a concentric glass venturi nebulizer from the chromatography column output with solution flow rate maintained at 1.2 mL min^{-1}.The LC-ICP-MS operating conditions are summarized in Table 2.1. During speciation, no internal standards were used as drift correction was applied by using repeated 20 µg L^{-1} Cr^{VI} standard was run after every ten samples for allow manual drift correction. Chromium species peak integration to derive integrated-peak-per-second (ICPS) values was carried out manually with Plasmalab software and final concentration was calculated, incorporating drift correction.

In the initial stage (Chapter 3), pH of the eluent was established at 9.4. However, at later stage (Chapter 4, 5,7), the pH of the eluent has been changed to pH 7 as Tanoshima et al., (2012) found out better and faster separation between Cr^{VI} and Cr^{III} can be achieved at this pH. The limit of detection (LOD) of the method was calculated based on 3 times standard deviation of 10 blank (1% TMAH) solution and was established at 0.04 µg L^{-1} Cr^{VI} and 0.06 µg L^{-1} Cr^{III} respectively. Limit of quantification (LOQ) is calculated based on ten times standard deviation of 10 blank (1% TMAH) solution; 0.13 µg L^{-1} Cr^{VI} and 0.2 µg L^{-1} Cr^{III}.

Determination of complexes of Cr with dissolved organic matter was also carried out by using a Dionex ICS-3000 LC coupled to ICP-MS with different column used. In this attempt, Size Exclusion Column (SEC; GE Healthcare, Superose 12 10/300 GL) comprises of a stationary phase of cross-linked agarose matrix with a separation range from 1 to 300 KDa was used. A guard column (Hamilton, PEEK PRP X-100) was used upstream of the SEC column to prevent blockage. Samples were injected with an autosampler controlled by a Chromeleon software and introduced directly from the SEC column to the nebulizer for Cr analysis by ICP-MS in CCT-KED mode. The operating condition was summarized in Table 2.1

Table 2.1 Typical operating conditioned for Inductively Couple Plasma - Mass Spectrometry (ICP-MS) and liquid chromatography (LC)

ICP-MS	
Instrument	ICP-MS, Thermo-Fisher Scientific X-Series^{II}, Germany operated in CCT-KED mode
Instrument power	1400 W
Nebulizer glass flow rate	0.79 – 0.83 L min^{-1}
Argon auxiliary glass flow rate	1.0 L min^{-1}
Quadrupole parameters	Minimum 1500 µS, maximum 15000 µS Minimum and maximum settle times 1000 µS for isotopic measurement
Reaction cell gas	4.00 mL min^{-1}, 7% H_2 in He
Resolution	0.26 amu at 10% peak maximum
Quadrupole dwell time (ms)	20 ms for internal standards (^{45}Sc, ^{72}Ge, ^{103}Rh) For isotopic measurement; ^{50}Cr (5 ms), ^{51}V (5 ms), ^{52}Cr (5 ms), ^{53}Cr (5 ms). A ^{49}Ti isotope with 2 ms dwell time was introduced to allow the quadrupole to stabilize before measuring ^{50}Cr)
Detection mode	Pulse counting (analogue if CPS > 1.5 M)
Auto sampler	Cetac ASX-520 with 4 x 60-place sample racks
LC	
Instrument	Dionex ICS-3000, HPLC
Column	Anion exchange :Hamilton-PRP X100, (5 µm, 4.1 x 50 mm) SEC: Superose 12 10/300 GL column (GE Healthcare)
Mobile phase	Anion exchange : 50 mM TRIS, 40 mM NH_4NO_3, 0.005 M of NH_4-EDTA, pH adjusted to 9.4 in the intial stage but later on adjusted to 7.0 using HNO_3 SEC : 50mM TRIS, 40mM NH_4NO_3, 0.005 M of NH_4-EDTA, pH adjusted to 8.4 using HNO_3
Flow rate	1.2 mL min^{-1}
Sample loop	100 µL
Elution mode	Isocratic

2.3.3 Cr isotope analysis

Preparation of $^{50}Cr^{III}$ and $^{50}Cr^{VI}$ stock solutions.

Forty milligrams of ^{50}Cr metal (99.70% enrichment) was purchased from ISOFLEX USA, certificate no: 24-01-50-3986 on 25 March 2011. The preparation of $^{50}Cr^{III}$ and $^{50}Cr^{VI}$ stock solutions was according to EPA Method 6800, with some modification. The Cr metal was dissolved in 4 mL of 6 M HCl with heating to 80 $^{\circ}$C on a hotplate; the resulting solution was greenish blue, suggesting Cr^{III} was the main species. Heating was continued to evaporate excess HCl and Cl_2 gas and to form a $Cr^{III}Cl_3$ precipitate. The precipitate was then dissolved in 10 mL of 1 % HNO_3 and half of the solution was retained as a Cr^{III} stock solution following dilution to 100 mL with 1 % HNO_3. The remaining solution (5 mL) was again heated on a hotplate with 0.25 mL of 30 % H_2O_2 and 4 mL of conc. NH_4OH added to oxidize the $^{50}Cr^{III}$ to $^{50}Cr^{VI}$. After completion of the oxidation process (no further H_2O_2 fumes), the sample was diluted to 100 mL with water; the solution was bright yellow, indicating the presence of Cr^{VI}. The $^{50}Cr^{III}$ and $^{50}Cr^{VI}$ stock solutions were then analyzed for total Cr and $^{50}Cr^{III}$ and $^{50}Cr^{VI}$ species using ICP-MS and LC-ICP-MS, respectively.

For calibration and assay of the ^{50}Cr stock solutions, four standard solutions of Cr (normal isotopic abundances) were prepared (200, 400, 600 and 1000 µg L^{-1}) from commercial Cr^{III} and Cr^{VI} stock solutions (1000 mg L^{-1} Cr^{III} in 2% HNO_3, Lot number 16-102CR(III), SPEXertificate and 1000 mg L^{-1} of Cr^{VI} in water , Lot number 16-109Cr(VI), SPEXertificate). These were diluted with Milli-Q water, adjusted to 0.05 M ammonium-EDTA, and incubated in a water bath at 70°C to simulate sample preservation in typical alkaline soil and plant extractants (see Section 3.6.4). The average concentration of Cr in the primary $^{50}Cr^{III}$ and $^{50}Cr^{VI}$ stock solutions are 204 mg L^{-1} and 162 mg L^{-1} respectively. The $^{50}Cr^{VI}$ results were slightly lower than $^{50}Cr^{III}$, possibly due to losses that may have occurred due to frothing after adding peroxide to oxidize $^{50}Cr^{III}$ to $^{50}Cr^{VI}$.

Speciation analysis was undertaken to verify the concentrations of $^{50}Cr^{III}$ and $^{50}Cr^{VI}$ species and to confirm complete oxidation of $^{50}Cr^{III}$ to $^{50}Cr^{VI}$. The chromatograms shown in Figures 2.1 shows $^{50}Cr^{III}$ and $^{50}Cr^{VI}$ peaks at 9.7 and 9.0 minutes respectively, suggesting that the preparation of ^{50}Cr species stock solutions was successful. The measured concentrations of $^{50}Cr^{III}$ and $^{50}Cr^{VI}$ species in the two ^{50}Cr

isotopic stock solutions were slightly higher than the total ^{50}Cr assay: 209 mg L^{-1} and 167 mg L^{-1} for $^{50}Cr^{III}$ and $^{50}Cr^{VI}$, respectively. The concentrations measured by direct ICP-MS analysis have been used throughout this thesis.

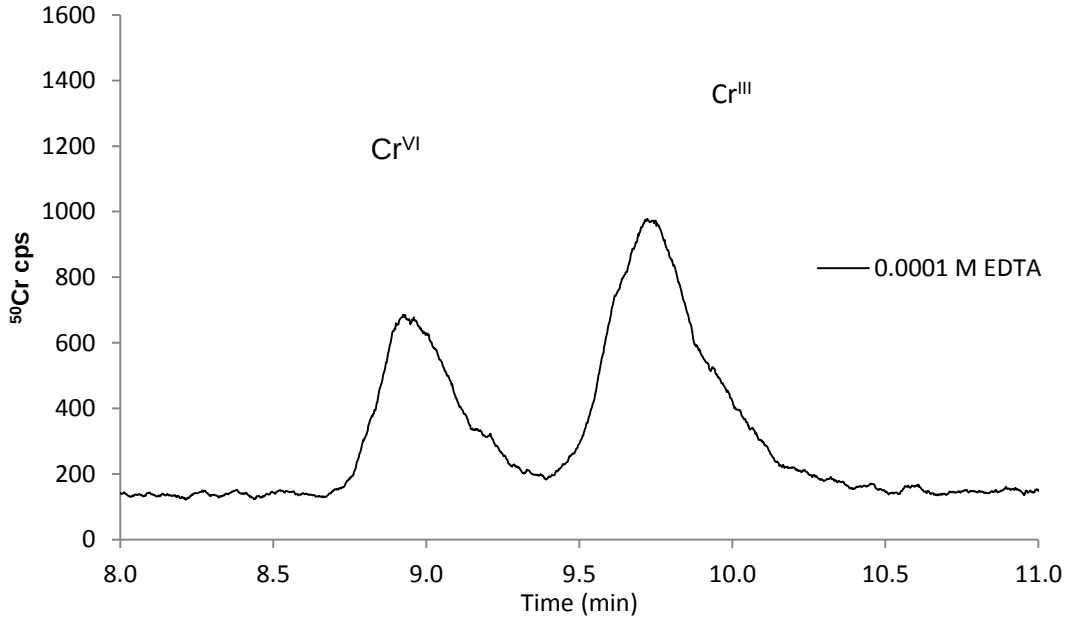


Figure 2.1 Chromatogram showing dilution of a mixture of $^{50}Cr^{VI}$ and $^{50}Cr^{III}$ isotopic stock solution (20 µg L^{-1} $^{50}Cr^{VI}$ and 20 µg L^{-1} $^{50}Cr^{VI}$ in EDTA (0.001 M) with heating at 70 °C for 2 h). A PRP-X100 Anion exchange column (50 x 4.1 mm) with mobile phase 50 mM TRIS, 40 mM NH_4NO_3, 10^{-5} M EDTA and pH adjusted to 9.4 using HNO_3 was used. Data were plotted using a moving average interval of 30 time periods of 132 ms.

Mass discrimination factor (K-factor) for ^{52}Cr and ^{50}Cr Isotopes

Mass discrimination occurs when the ICP-MS instrument produces a non-uniform or inefficient response across the measured mass range (Yang et al., 2003; Vogl et al., 2010). A problem attributed to mass discrimination can be resolved using correction factor (K-factor) which is the true to measured isotope ratios. The sensitivity of each inorganic form of Cr isotope analyzed by LC-ICP-MS is shown in Table 2.2. The sensitivities of each Cr isotope were calculated by dividing total analyte count per

second by the concentration of Cr isotope (µg L^{-1}).The average measured mass discrimination factor (K-factor) for ^{50}Cr:^{52}Cr was 0.8567.

Table 2.2 Mass discrimination factor for ^{52}Cr and ^{50}Cr isotopes.

Isotope	Inorganic form	Conc. (µg L^{-1})	Total CPS	Sensitivity (CPS/µg L^{-1})	^{50}Cr:^{52}Cr
^{50}Cr	$^{50}Cr^{III}$	8.35	10100	1208	0.8567
		16.7	20400	1224	
		25.0	30800	1229	
		41.7	51700	1238	
	$^{50}Cr^{VI}$	8.35	9150	1096	
		16.7	19900	1192	
		25.0	30600	1223	
		41.7	50100	1200	
			Avg	1201	
^{52}Cr	$^{52}Cr^{III}$	167	235000	1404	
		335	475000	1420	
		502	718000	1430	
		837	1210000	1441	
	$^{52}Cr^{VI}$	167	216000	1291	
		335	466000	1394	
		502	720000	1433	
		837	1180000	1405	
			Avg	1402	

3 METHOD DEVELOPMENT

3.1 OVERVIEW AND OBJECTIVES

In understanding Cr dynamics in soil, an accurate and precise detection of Cr species is essential. From a toxicological standpoint there is most interest in Cr^{VI} in soils and plants which usually extracted in an alkaline matrix (Rahman et al., 2005, Bank et al., 2006, Grabarczyk et al., 2006) prior to assay by (e.g.) AAS or ICP-MS. Currently, EPA 3060A (James et al., 1995) is the only accredited method for extracting Cr^{VI} from soluble, adsorbed and precipitated forms of Cr compounds in soils, sludges, sediments and similar waste materials. The extracting reagent is a combination of 0.28 M Na_2CO_3 and 0.5 M NaOH. When applied to soils this will clearly mobilize adsorbed anionic Cr^{VI} ions but may also either precipitate Cr^{III} ions or mobilise these as dissolved humic and fulvic complexes (James et al., 1995). The alkaline conditions can help to prevent reduction of Cr^{VI} however, creation of Cr^{VI} from oxidation of Cr^{III} may occur in soils with high concentrations of MnO_2 and high Cr^{III}/Cr^{VI} ratios (Unceta et al., 2010). James et al., (1997) found that a hot alkaline solution (0.28 M Na_2CO_3/0.50 NaOH) was very effective for extracting Cr^{VI} from soil compared to using phosphate buffer and 0.1 M NaOH. Pettine et al., (2005) found that the present of humic complexes in alkaline extracts using EPA 3060A interfered with the analysis of Cr^{VI} by spectrophotometric diphenylcabazide method as the humic complexes absorbed at 540 nm, the wavelength used for measuring the Cr complex with DPC leading to overestimation of Cr^{VI} concentration. An alternative alkaline extractant, dilute tetra-methyl ammonium hydroxide (TMAH) solution, has been used recently by researchers to solubilize iodine (Yamada et al., 1999) and water has been used to quantify 'soluble' forms of Cr^{VI} in numerous soils under diverse redox conditions (James et al., 1995; Panichev et al., 2003; Mandiwana et al., 2007). Therefore, the use 0.28 M Na_2CO_3 and 0.50 NaOH, TMAH and water will also being considered to extract Cr^{VI} in this chapter.

The method development work described in this chapter assesses the stability of chromium in soil extracts. Initially, attention was focused on the optimization of the ICP-MS in standard and CCT-KED operating modes and choice of internal standard. The next phase was the development of a chromatographic method which included (i)

establishing an appropriate eluent, (ii) optimising the concentration of a Cr^{III}-complexing agent (EDTA) to facilitate transit through the chromatographic column and (iii) assessing the need for heating (duration and temperature) to encourage complex-formation of EDTA with Cr^{III} (Gurleyuk et al., 2001). The samples analysed by hyphenated High Performance Liquid Chromatography-Inductively Couple Plasma Mass Spectrometry (HPLC-ICP-MS) included single species standards of Cr^{III} and Cr^{VI} and mixed Cr species. The overall objective was to try and obtain good separation between the two inorganic Cr species and a stable chromatographic chemistry that prevented changes in speciation and loss of analyte by adsorption within the system. The stability of Cr species in different soil extraction matrices was also assessed; compositions included water, 1 % TMAH and 0.28 M Na_2CO_3 with 0.50 M NaOH as established alkaline extractants (James et al., 1995). At the end of the method development work, the stability of individual Cr^{III}, Cr^{VI} and mixed Cr species spiked into extracts from an acidic and calcareous soil was assessed. The behaviour of ^{52}Cr species in soil also was monitored by using ^{50}Cr isotope as an audit trail.

3.2 Chromium analysis by ICP-MS mode

3.2.1 Standard vs CCT-KED operational modes.

Chromium is one of several elements that have poor detection limits by ICP-MS due to spectral interferences generated by ions derived from the plasma gas, matrix components, or the solvent/acid used (Thomas, 2004). Most of the interference in determination of $^{52}Cr^{+}$ arises from $^{40}Ar^{12}C$ formation in the plasma; this interference cannot be completely eliminated by traditional quadrupole mass analyzer technology (Thomas, 2004). Clearly this is more likely to be an issue in bi-carbonate based extractants, as typically used for Cr^{VI} extraction from soils (Thomas, 2004). With the introduction of collision/reaction cells immediately prior to the analyzer quadrupole, polyatomic species can be effectively dissociated by collision, usually with helium gas. In the XSeries^{II} ICP-MS, a collision/reaction gas (typically 7% hydrogen in helium) is bled into a hexapole cell which operates in RF-only mode. The RF field has the effect of focussing the ions, which then collide and react with molecules of the collision/reaction gas. By a number of different ion-molecule collision and reaction mechanisms, polyatomic interfering ions such as $^{40}Ar^{40}Ar^{+}$, $^{40}Ar^{16}O^{+}$ and $^{40}Ar^{12}CH^{+}$ will either be converted to harmless non-interfering species or the analyte will be

converted to ions with a mass which is not subject to interference. For example, hydrogen gas can be used to eliminate the interference of $^{40}Ar^{12}CH^{+}$ in the determination of $^{53}Cr^{+}$ (Thomas, 2004):

$$^{40}Ar^{12}CH^{+} + H_2 = H_3^{+} + {}^{40}Ar^{12}C$$

However, complex secondary reactions in the cell may generate undesirable interfering species. These species can then be eliminated using discrimination based on kinetic energy or mass filtering. Kinetic Energy Discrimination (KED) is achieved by setting the collision cell potential to a slightly more negative value than the mass filter (quadrupole) potential. This causes the collision product ions generated in the cell to have a lower kinetic energy and so these are not transmitted through to the analytical quadrupole (Thomas, 2004).

3.3 Chromium isotopes and internal standard

The isotopic masses, abundances and ionization potentials of Cr, the plasma gas (Ar) and the internal standards (Sc, Ge, Rh and Ir) used in this study are tabulated in Table 3.1.

Table 3.1 Properties of Cr and internal standards used to test extraction matrices.

Element	m/z ratio	Isotope abundance (%)	[a]First ionization energy (eV)
Cr	50	4.3	6.77
	52	83.8	6.77
	53	9.5	6.77
	54	2.4	6.77
Ar	40	99.6	15.8
Sc	45	100	6.56
Ge	74	35.9	7.89
Rh	103	100	7.46
Ir	193	62.7	8.97

[a] http://www.science.co.il/PTelements.asp?s=ionization

An internal standard is a non-analyte that is added to the blank solution, standards and sample before the analysis, or introduced to the sample stream immediately before the nebuliser through a T-piece (Thomas, 2004). Internal standardization is used to correct for changes in analyte sensitivity caused by variations in the concentration and type of the matrix components found in the sample. Throughout this chapter, internal standard

is only applied during the determination of total Cr using ICP-MS and was not applied during speciation work.

3.4 Objectives

The objectives within this chapter were therefore:

- to develop effective extraction systems (reagents and procedures) for soils and plants in order to speciate and fractionate Cr in both those media;
- to develop robust Cr speciation methodologies (using HPLC-ICP-MS)

3.5 MATERIALS AND METHODS

3.5.1 Operational mode and solution matrix.

Two operational modes were tested: 'standard' mode and 'Collision Cell Technology with Kinetic Energy Discrimination' (CCT-KED) mode. Test solutions of single Cr species, including 50 µg L^{-1} Cr^{III} and 50 µg L^{-1} Cr^{VI} were prepared from Cr^{III} and Cr^{VI} stock solutions (1000 mg L^{-1} Cr^{III} in 2% HNO_3, Lot No. 16-102CR(III), SPEXertificate and 1000 mg L^{-1} of Cr^{VI} in water, Lot No. 16-109Cr(VI), SPEXertificate). These were diluted in water, 1 % TMAH and 0.28 M Na_2CO_3/0.50 M NaOH in the presence of 0.001 M EDTA to simulate suspension in typical alkaline soil and plant extractants. The resulting standards were then used to measure the sensitivity of each isotope in the alkaline extractants and assess their vulnerability to interferences. Four internal standards were used, including ^{45}Sc, ^{74}Ge, ^{103}Rh and ^{193}Ir (10,000 mg L^{-1} Sc in 5% HNO_3, Lot No: AE14-23Sc, SPEXertificate; 10,000 mg L^{-1} Ir in 10% HCl, Lot No 11-147IR; 10, 0000 mg L^{-1} Rh in 10% HCL, Lot No 12-99RH; 10,000 mg L^{-1} Ge in 10% HCl). The four chromium isotopes measured were ^{50}Cr, ^{52}Cr, ^{53}Cr and ^{54}Cr.

3.5.2 Effect of matrix on Cr speciation by HPLC-ICP-MS.

The stability of Cr^{III} and Cr^{VI} in water, 1 % TMAH and 0.28 M Na_2CO_3/0.50 M NaOH were studied using hyphenated HPLC-ICP-MS. Samples with approximately 20 µg L^{-1} Cr^{III} and Cr^{VI} species were prepared in the three contrasting matrices with,

or without, addition of 0.001 M EDTA and heated at 70°C for 1 hour to encourage EDTA-Cr^{III} complex formation. The chromatographic mobile phase used during the study was 50 mM TRIS, 40 mM NH_4NO_3 with a PRP-X100 anion exchange column, dimensions 250 x 4.6 mm column (Hamilton). The pH of the mobile phase was adjusted to 9.4 using 12 mL of 25% TMAH.

3.5.3 Effect of EDTA and heating on the stability of Cr species

Samples with 20 µg L^{-1} Cr^{III}, 20 µg $L^{-1}Cr^{VI}$ and mixed species (10 µg L^{-1} Cr^{III} and 10 µg L^{-1} Cr^{VI}) with four different concentration of EDTA (0.01, 0.001, 0.0001 and 0.00001 M EDTA) in water and 1% TMAH were prepared. All samples were heated at 70°C to encourage Cr^{III}-EDTA complex formation. The same mobile phase was used see Section 3.5.2

3.5.4 Speciation and fractionation of Cr in soil extracts

Speciation of Cr in soil extracts

TMAH extracts of two contrasting topsoils, acidic and calcareous, were used to investigate the stability of Cr^{III} and Cr^{VI} in soil extracts. Two concentrations of EDTA, 0.01 M and 0.001 M, and incubation times from 1 to 3 hours were also tested. The acidic soil (pH 4.12 in water) was obtained from a deciduous woodland close to the University Farm at Sutton Bonington, Leicestershire (SB) ($52^o49'49"$ N, $1^o14'19"$ W; Elev. 52 m) and the calcareous soil (pH 7.43 in water) was from an arable field at Stoke Rochford, Lincolnshire (SR) ($52^o51'25"$ N, $0^o38'58"$ W, Elev. 122 m). Both were air dried and sieved to <2 mm. About 5 g of soil was shaken overnight in 25 mL of 10% TMAH, in 50 mL centrifuge tubes, at 200 rpm. The suspensions were then centrifuged for 20 min at 3000 rpm and 1 mL of the supernatant was combined with either 1 mL of 0.1 M EDTA or 5 mL of 2 mM EDTA and made up to 10 mL with MilliQ water to produce EDTA concentrations of either 0.01 M or 0.001 M. Samples were incubated at 70^oC in a water bath for 1, 2 or 3 hours. Solutions were analyzed for Cr^{VI} and Cr^{III} by HPLC-ICP-MS (Section 2.4.2).

The same procedure was used to determine the stability of spiked Cr samples in which 1 mL of 100 µg L^{-1} Cr^{VI} or 100 µg L^{-1} Cr^{III} solutions were added to the supernatant solution of the TMAH extraction of each soil immediately after adding the EDTA

(above) and made up to 10 mL with MilliQ water. The final concentration of Cr^{III} and Cr^{VI} in the soil solution was 10 µg L^{-1} for each species.

Further work was carried out by adding 200 µg L^{-1} of the single species Cr^{VI}, 200 µg L^{-1} of Cr^{III} or 100 µg L^{-1} of mixed Cr species (Cr^{III} and Cr^{VI}) to a higher concentration of 0.05 M EDTA in the supernatant (above). The solution was also analyzed for multielement concentrations using CCT-KED ICP-MS. The fulvic acid concentration of the soil solution was determined by measuring absorbance at 400 nm in a 1 cm cell using a UV-Vis Spectrophotometer (CECIL, Ce1011) (Gan et al., 2007). A fulvic acid stock solution (0.08 g mL^{-1}) (Gan et al., 2007; Marshall, 1992) was prepared in NaOH with pH adjusted to 7 using HNO_3.

Speciation and fractionation of ^{50}Cr and ^{52}Cr species in Soil

TMAH extracts of two contrasting topsoils, acidic (SB) and calcareous (SR) were used to investigate the kinetics of $^{50}Cr^{III}$, $^{52}Cr^{VI}$, $^{50}Cr^{VI}$ and $^{52}Cr^{III}$ in soil extracts over 3 days. Chromium-50 stock solution was prepared according to section 3.5.4. A mix of different Cr isotope species; either 50 µg L^{-1} $^{50}Cr^{III}$ and 50 µg L^{-1} $^{52}Cr^{VI}$, or 50 µg L^{-1} $^{50}Cr^{VI}$ and 50µg L^{-1} $^{52}Cr^{III}$, were spiked into the soil extracts. Pre and post-complexation of either $^{50}Cr^{III}$ or $^{52}Cr^{III}$ with three different concentrations of $(NH_4)_2$-EDTA; 0.005 M, 0.02 M and 0.05 M of EDTA, were also investigated.

For the pre-complexation experiment with 0.05 M EDTA, 5 mL of 0.1 M EDTA and 0.5 mL of 1000 µg L^{-1} of $^{50}Cr^{III}$ or $^{52}Cr^{III}$ were pipetted into an ICP tube, incubated for 2 hr at 70°C, and then left to cool. After that, 1 ml of 1% TMAH or TMAH soil extract, 0.5 mL of either 1000 µg L^{-1} of $^{52}Cr^{VI}$ or 1000 µg L^{-1} $^{50}Cr^{VI}$ were pipette into the tube and made to 10 mL with Milli-Q water. Samples were then left to equilibrate at room temperature for three days. The same preparation was also carried out for 0.02 M and 0.005 M EDTA samples with 0.04 M or 0.1 M stock EDTA solutions, respectively.

For post-complexation with 0.05 M EDTA, 1 mL of either 1% TMAH (for control) or TMAH soil extracts, 5 mL of 0.1 M EDTA, 0.5 mL of 1000 µg L^{-1} of $^{50}Cr^{III}$ or $^{52}Cr^{III}$, 0.5 mL of 1000 µg L^{-1} of $^{52}Cr^{VI}$ or $^{50}Cr^{VI}$ were pipetted into an ICP tube. The solutions were made up to 10 mL with Milli-Q water and incubated for 2 hr at 70°C before leaving to equilibrate at room temperature for three days. The same preparation was

also carried out for 0.02 M and 0.005 M EDTA with 0.04 M or 0.1 M EDTA as stock EDTA solution, respectively. All of the solutions were analyzed for ^{50}Cr and ^{52}Cr species by HPLC-ICP-MS.

TMAH extracts of two contrasting topsoils, acidic (SB) and calcareous (SR) were again used to investigate solubility and speciation of $^{50}Cr^{III}$, $^{52}Cr^{VI}$, $^{50}Cr^{VI}$ and $^{52}Cr^{III}$ in soil extracts. A mix of different Cr isotopes species, either (i) 5 µg L^{-1} $^{50}Cr^{III}$ and 5 µg L^{-1} $^{52}Cr^{VI}$ or (ii) 5 µg L^{-1} $^{50}Cr^{VI}$ and 5 µg L^{-1} $^{52}Cr^{III}$, were spiked into the soil extracts. Post-complexation with six different concentrations of $(NH_4)_2$-EDTA; 0.0001, 0.0005, 0.001, 0.005, 0.01 and 0.05 M and incubation at 70°C for 2 hr were investigated. Samples were analyzed for ^{50}Cr and ^{52}Cr species by HPLC-ICP-MS (Section 2.4.2 & 2.4.3). In this experiment, the pH of the eluent was adjusted to 7 by titration with HNO_3; this had the effect of reducing the runtime to ~7 min per sample (Tanoshima et al., 2012).

3.6 RESULTS AND DISCUSSION

3.6.1 ICP-MS operation: comparing standard and CCT-KED modes

Operating the ICP-MS in standard mode.

The variation in signal (uncorrected cps) for the four internal standards (Sc, Ge, Rh and Ir), representing a mass range from 45 to 193, in the three contrasting matrices are shown in Figure 3.1. For all four elements the response varied between the matrices. Matrix suppression was clearly present in the Na_2CO_3/NaOH matrix, probably due to Na ionization; the effect was greatest for Ir. There was a slight signal enhancement, relative to water, in TMAH; the effect was greatest for Sc. The origin of this effect is not clear, but it is known that C^+ ions can enhance ionization of some elements, such as Se, causing an increase in analyte sensitivity.

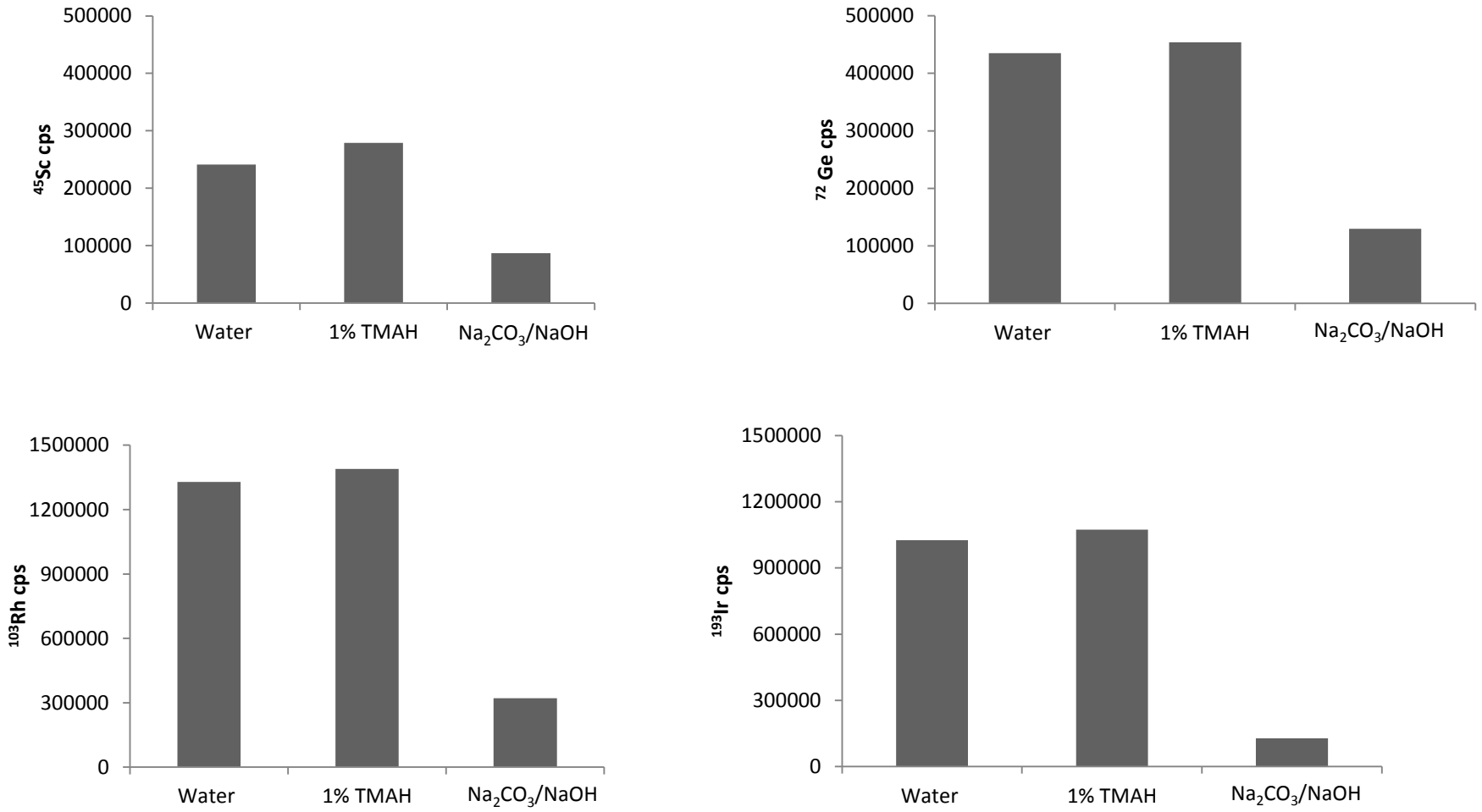


Figure 3.1 ICP-MS signal (uncorrected CPS) of four internal standards (Sc, Ge, Rh and Ir) in three contrasting matrices, water, 1 % TMAH and 0.28 M Na_2CO_3 / 0.50 M NaOH with the instrument in standard mode. All solutions contained 0.001 M EDTA and were heated at 70 $^{\circ}$C for 1 hr to enhance EDTA-Cr^{III} complex formation. Results are based on single unreplicated measurements.

The instrument response in standard mode (uncorrected cps) to ^{52}Cr, present initially as Cr^{III} or Cr^{VI} and dissolved in the three matrices (water, TMAH and Na_2CO_3/NaOH) is shown in Figure 3.2. As seen for the four internal standards, the sensitivity in 1 % TMAH was apparently greater than in water. However, the effect was considerably greater for ^{52}Cr and it is highly likely that this was due to a polyatomic interference ($^{40}Ar^{12}C$) rather than enhanced sensitivity alone. Again, as seen for the internal standards, the sensitvity in the Na_2CO_3/NaOH was considerably less than in water, probably due to matrix suppression caused by Na ionization suppressing ionization of Cr in the plasma. However, the suppression of signal in the Na_2CO_3/NaOH was considerably less than was seen for the internal standards (Fig. 3.1); this may have been due to $^{40}Ar^{12}C$ formation with some of the carbonate. Thus the net signal for ^{52}Cr in the Na_2CO_3/NaOH matrix appears to be both suppressed due to Na ionization effects and enhanced by polyatomic interferences.

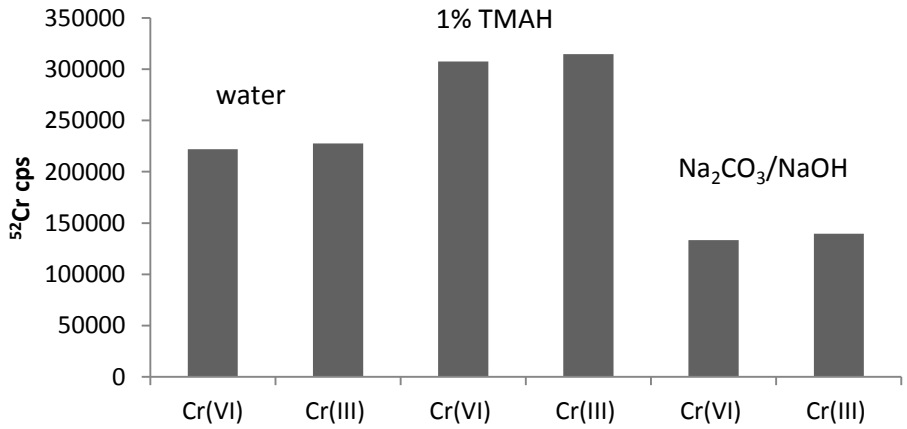


Figure 3.2 ICP-MS response (uncorrected cps) to 20 µg L^{-1} of ^{52}Cr present as Cr^{III} and Cr^{VI} dissolved in 3 contrasting matrices (water, 1 % TMAH and 0.28 M Na_2CO_3/0.50 M NaOH) with the instrument in standard mode. All solutions contained 0.001 M EDTA and were heated at 70 ^{o}C for 1 hr to enhance EDTA-Cr^{III} complex formation. Results are based on single unreplicated measurements.

The effect of blank-subtraction and implementation of the internal standard to correct the ^{52}Cr signal in the three matrices is shown in Figure 3.3. Blank subtraction virtually eliminated the effect of polyatomic $^{40}Ar^{12}C$ formation in TMAH which produced a signal very similar to the water matrix. The blank signals for water,

TMAH and the Na_2CO_3/NaOH matrices are shown in Table 3.2 using all four internal standards individually for correction. The blank signal for 1% TMAH was higher than in water which may be due to polyatomic interference. The highest blank signal was in the Na_2CO_3/NaOH matrix. The greater degree of suppression of the internal standards by Na ionization (Fig. 3.1) produced a greatly 'over-corrected' signal for Cr in the Na_2CO_3/NaOH matrix. This was particularly severe when using Rh and Ir as internal standards. From this study, Sc and Ge was found to be the most robust internal standards for ^{52}Cr analysis using standard mode ICP-MS in all three extractants with blank subtraction. However, the dual effects of Na^+ ion suppression and $^{40}Ar^{12}C$ formation in the Na_2CO_3/NaOH matrix would present problems, even after blank subtraction, if there was any variation in carbon concentration between the samples, due to humic acid mobilization, or dilution of Na prior to assay.

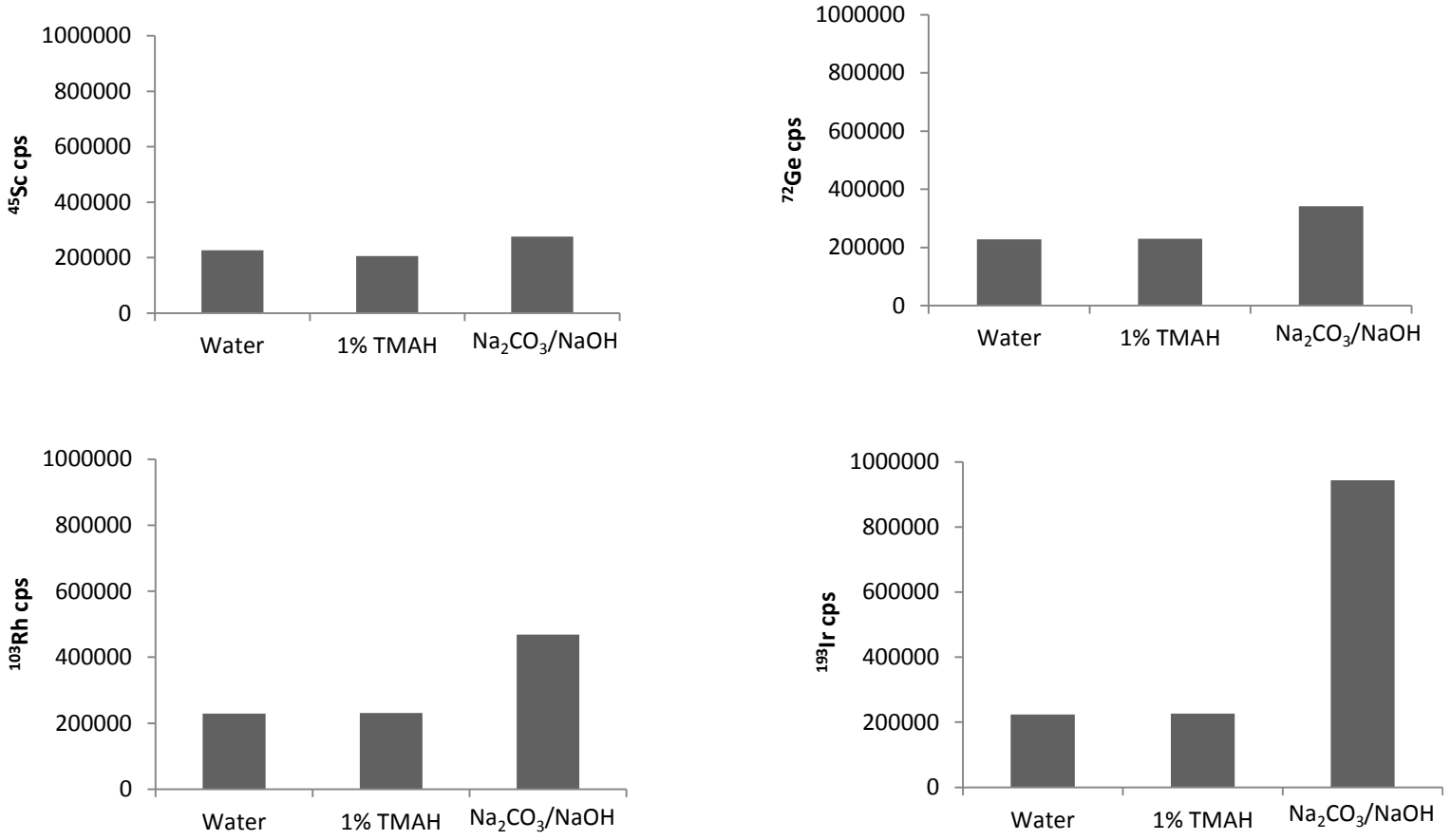


Figure 3.3 ICP-MS response (corrected cps) for ^{52}Cr corrected by blank subtraction and using each of the four alternative internal standards (Sc, Ge, Rh and Ir) dissolved in 3 contrasting matrices (water, 1 % TMAH and 0.28 M Na_2CO_3/0.50 M NaOH). Chromium concentration was 20 µg L^{-1} Cr^{III} and the instrument was in standard mode. All solutions contained 0.001 M EDTA and were heated at 70 °C for 1 hr to ensure EDTA-Cr^{III} complex formation. Results are based on single unreplicated measurement.

Table 3.2 ICP-MS signal (cps) at ^{52}Cr using one of four internal standards (Sc, Ge, Rh and Ir) in blank solutions of water, 1 % TMAH and 0.28 M Na_2CO_3/0.50 M NaOH, with the instrument in standard mode. All solutions contained 0.001 M EDTA and were heated at 70 °C for 1 hr. Results are based on single unreplicated measurement.

	ICP-MS signal for ^{52}Cr (cps) when using four individual internal standards			
Extractants	Sc	Ge	Rh	Ir
Water	2196	2196	2193	2177
1 % TMAH	67694	75535	75145	71910
0.28 M Na_2CO_3/0.50M NaOH	113895	132129	117400	168980

The response of the Cr isotopes, ^{50}Cr, ^{53}Cr and ^{54}Cr, in the different matrices with 20 µg L^{-1} Cr, analyzed by ICP-MS in standard mode is shown in Figure 3.4. The responses of ^{50}Cr and ^{53}Cr were as seen for ^{52}Cr but there was some form of matrix enhancement observed in ^{54}Cr in the Na_2CO_3/NaOH matrix

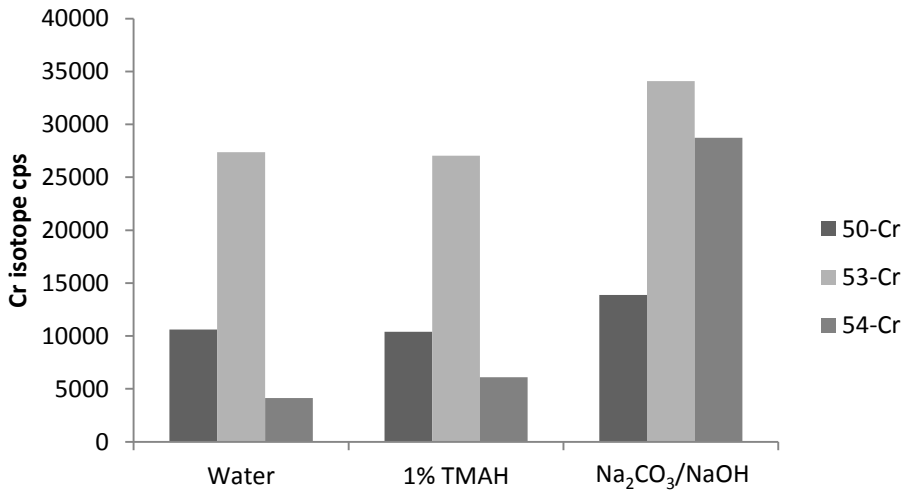


Figure 3.4 ICP-MS response (corrected cps) for the minor Cr isotopes ^{50}Cr, ^{53}Cr and ^{54}Cr dissolved in 3 contrasting matrices (water, 1 % TMAH and 0.28 M Na_2CO_3/0.50 M NaOH) using Ge as an internal standard. Total Cr concentration was 20 µg L^{-1} Cr^{VI} and Cr^{III} and the instrument was in standard mode. All solutions contained 0.001 M EDTA and were heated at 70^{o}C for 1 hr to enhance EDTA-Cr^{III} complex formation. Results are based on single unreplicated measurement.

It was found that by using standard mode, water and 1% TMAH were the suitable extractants. The recommended internal standards to be used were Sc and Ge as the signals were consistent throughout the analysis using ICP-MS. However, blank subtraction is needed in order to remove the polyatomic interference. Therefore, it is useful to have a look at how the collision cells work.

Operating the ICP-MS in CCT-KED mode.

The variation in signal (uncorrected cps) of CCT-KED ICP-MS for the four internal standards (Sc, Ge, Rh and Ir) in three contrasting matrices is shown in Figure 3.5. There was clearly an overall reduction in sensitivity by employing CCT-KED, as the ratio of responses (standard: CCT-KED) for the internal standards ranged from 2 to 20. Scandium gave the highest ratio of standard: CCT-KED (20 times higher in standard mode), followed by Ge at 6.5, whilst for Rh and Ir, sensitivity in CCT-KED mode was halved. Severe matrix suppression caused by the ionization of Na^+ in the plasma still occurred for all the internal standards.

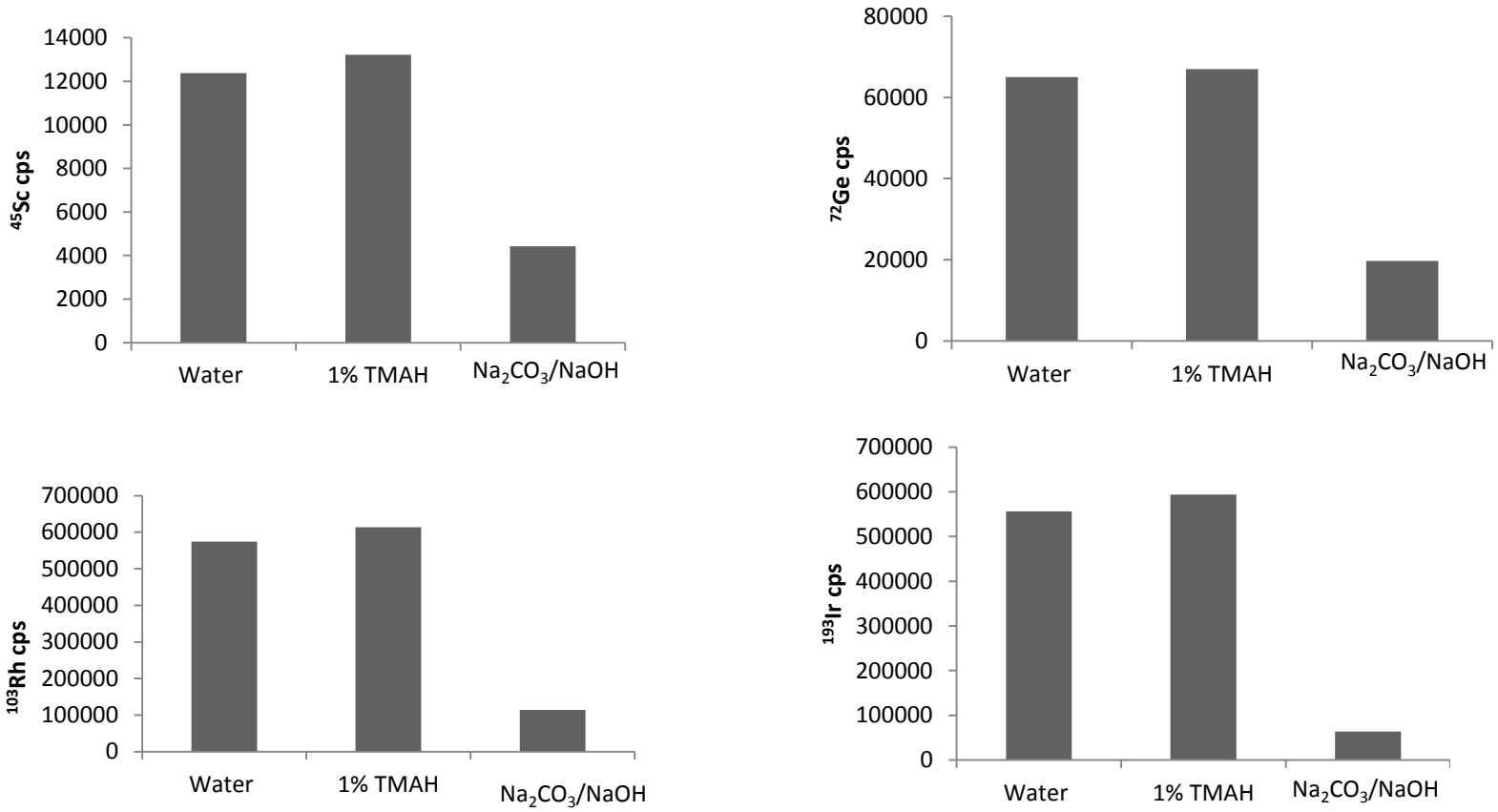


Figure 3.5 ICP-MS signal (uncorrected CPS) for four internal standards (Sc, Ge, Rh and Ir) in three contrasting matrices, water, 1 % TMAH and 0.28 M Na_2CO_3/0.50 M NaOH with the instrument in CCT-KED mode. All solutions contained 0.001 M EDTA and were heated at 70^{o}C for 1 hr to enhance EDTA-Cr^{III} complex formation. Results are based on single unreplicated measurement.

The ICP-MS response to ^{52}Cr present as Cr^{III} and Cr^{VI} dissolved in the 3 matrices (water, 1 % TMAH and 0.50 M Na_2CO_3/0.28 M NaOH) in CCT-KED mode is shown in Figure 3.6. The sensitivity was about 10 times lower compared to standard ICP-MS mode. However, the advantage of CCT-KED mode is that it can reduce polyatomic interferences. This appears to have been successful as approximately the same signal was observed for water and 1% TMAH extracts suggesting that interference from $^{40}Ar^{12}C$ had been eliminated. However, the ionization suppression in the plasma caused by Na^+ in the 0.28 M Na_2CO_3/0.50 M NaOH matrix was still evident. From this study, CCT-KED mode was chosen for further work with ^{52}Cr as the chosen isotope due to its high natural abundance (Table 3.1). It was also clear that TMAH may be preferred as the alkaline extractant, over the traditional sodium bicarbonate solution. Although Figure 3.3 suggests that standard mode (with blank correction), utilizing Ge or Rh as internal standards, may provide a viable analysis method, a strategy of polyatomic elimination, rather than 'equalization', is preferable to allow for changes in carbon concentration between samples and standards.

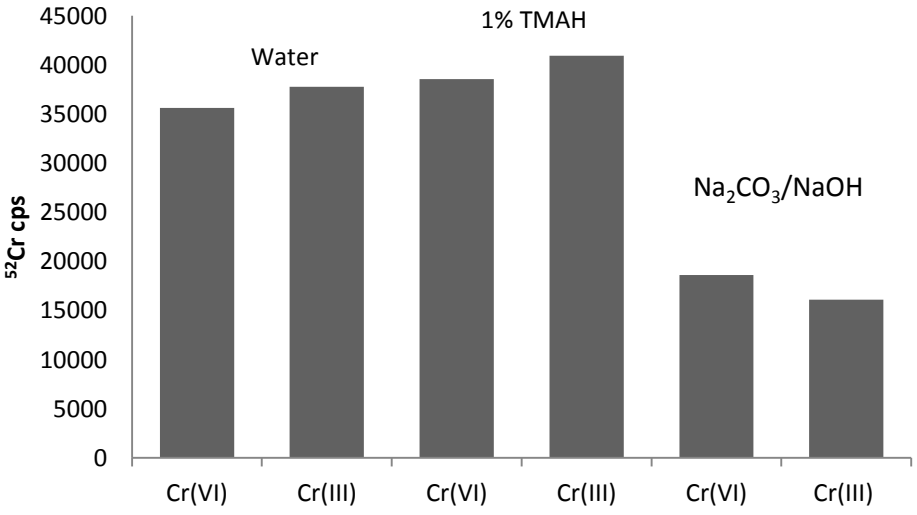


Figure 3.6 ICP-MS response (uncorrected cps) to 20 µg L^{-1} of ^{52}Cr present as Cr^{III} or Cr^{VI} and dissolved in 3 contrasting matrices (water, 1 % TMAH and 0.28 M Na_2CO_3/0.50 M NaOH) with the instrument in CCT-KED mode. All solutions contained 0.001 M EDTA and were heated at 70^oC for 1 hr to ensure EDTA-Cr^{III} complex formation. Results are based on single unreplicated measurement.

The effect of blank-subtraction and implementation of the internal standards to correct ^{52}Cr cps in the three matrices is shown in Figure 3.7. There was negligible effect of blank subtraction in CCT-KED mode. As can be seen in Figure 3.8, clearly there was no polyatomic interference in all the internal standards without any blank correction. The blank signals for water, 1 % TMAH and the Na_2CO_3/NaOH matrices are shown in Table 3.3 using all four internal standards individually for correction. The main effect shown in Figure 3.7 is that water and TMAH produce very consistent responses whereas in the Na_2CO_3/NaOH matrix the response at mass 52 is very dependent on differences between the effect of Na^+ ionization on the internal standard and on the analyte (^{52}Cr). So with Sc as internal standard the main effect is Na^+ suppression of ^{52}Cr while with Ir as internal standard the main effect is suppression of Ir causing a large compensation effect on the reported ^{52}Cr signal.

Table 3.3 ICP-MS signal (cps) at ^{52}Cr using four internal standards (Sc, Ge, Rh and Ir) in blank matrices: water, 1 % TMAH and 0.28 M Na_2CO_3/0.50 M NaOH with instrument in CCT-KED mode. All solutions contained 0.001 M EDTA and were heated at 70 °C for 1 hr. Results are based on single unreplicated measurement.

	ICP-MS signal for ^{52}Cr (cps) when using four different internal standards			
Solution matrix	Sc	Ge	Rh	Ir
Water	27	26	26	26
1 % TMAH	1076	954	926	943
0.28 M Na_2CO_3/0.50 M NaOH	3218	954	14023	20218

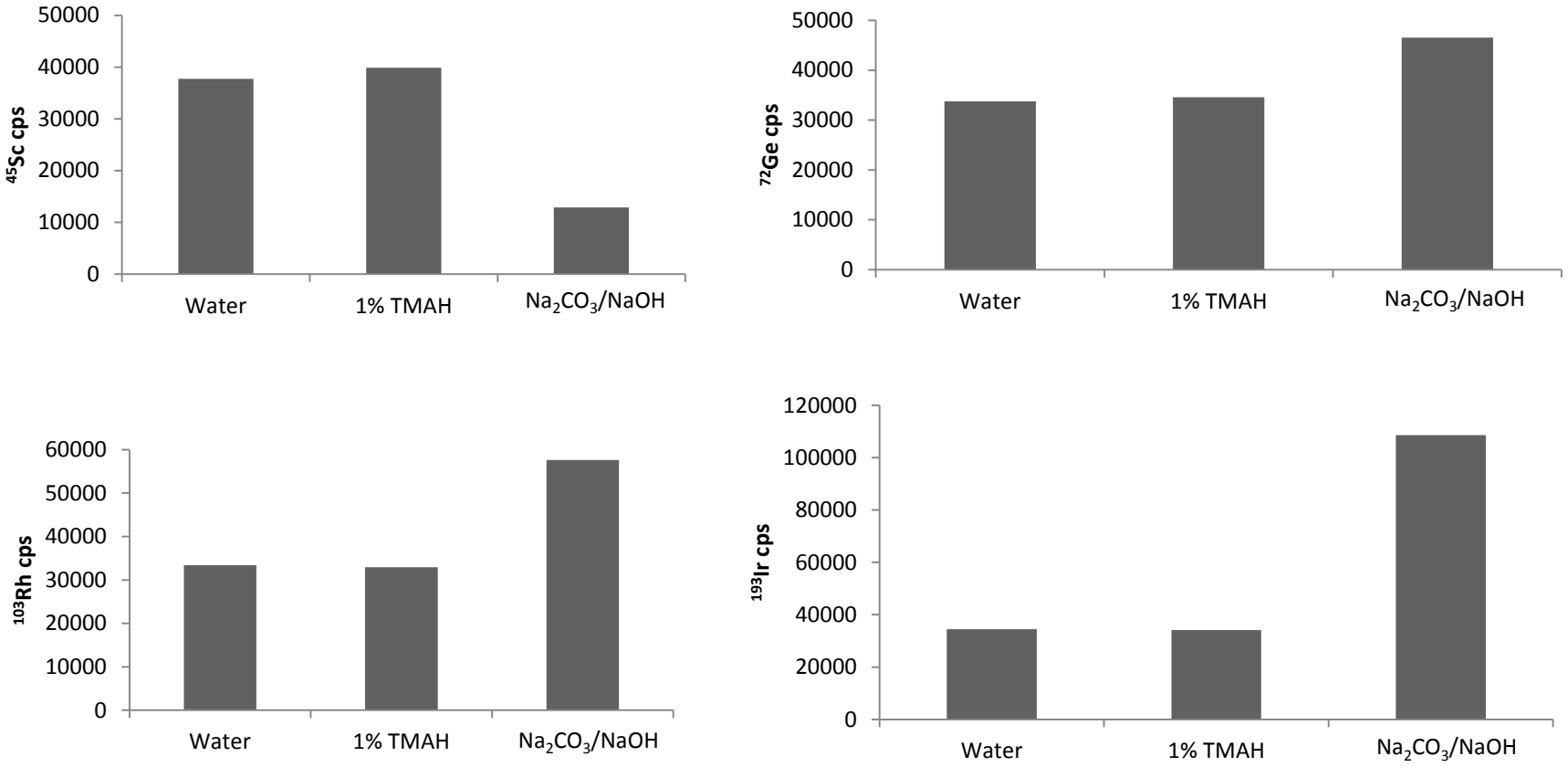


Figure 3.7 ICP-MS response (corrected cps) for ^{52}Cr corrected by blank subtraction for four alternative internal standards (Sc, Ge, Rh and Ir) dissolved in 3 contrasting matrices (water, 1 % TMAH and 0.28 M Na_2CO_3/0.50 M NaOH). Chromium concentration was 20 µg L^{-1} Cr^{III} and the instrument was in CCT-KED mode. All solutions contained 0.001 M EDTA and were heated at 70^{o}C for 1 hr to enhance EDTA-Cr^{III} complex formation. Results are based on single unreplicated measurement.

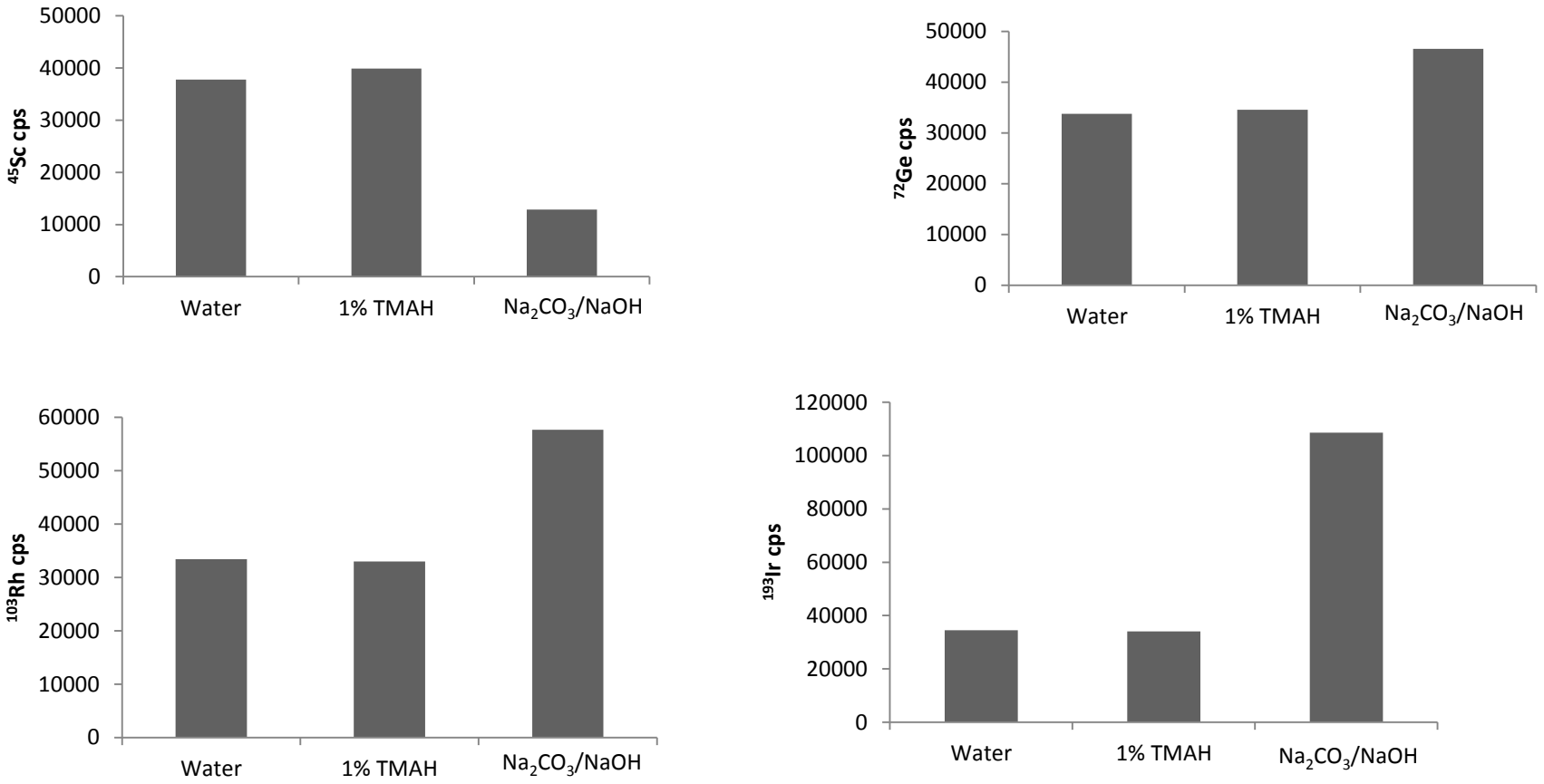


Figure 3.8 ICP-MS response (uncorrected cps) for ^{52}Cr corrected by blank subtraction for four alternative internal standards (Sc, Ge, Rh and Ir) dissolved in 3 contrasting matrices (water, 1 % TMAH and 0.28 M Na_2CO_3/0.50 M NaOH). Chromium concentration was 20 µg L^{-1} Cr^{III} and the instrument was in CCT-KED mode. All solutions contained 0.001 M EDTA and were heated at 70^{o}C for 1 hr to enhance EDTA-Cr^{III} complex formation. Results are based on single unreplicated measurement.

The response of Cr isotopes, ^{50}Cr, ^{53}Cr and ^{54}Cr in different matrices (20 µg L^{-1}) Cr analyzed by ICP-MS in CCT-KED mode is shown in Figure 3.9. The responses of ^{50}Cr and ^{53}Cr were as seen for ^{52}Cr but there was some form of matrix enhancement observed for ^{54}Cr in the Na_2CO_3/NaOH matrix, similar to that observed in standard mode.

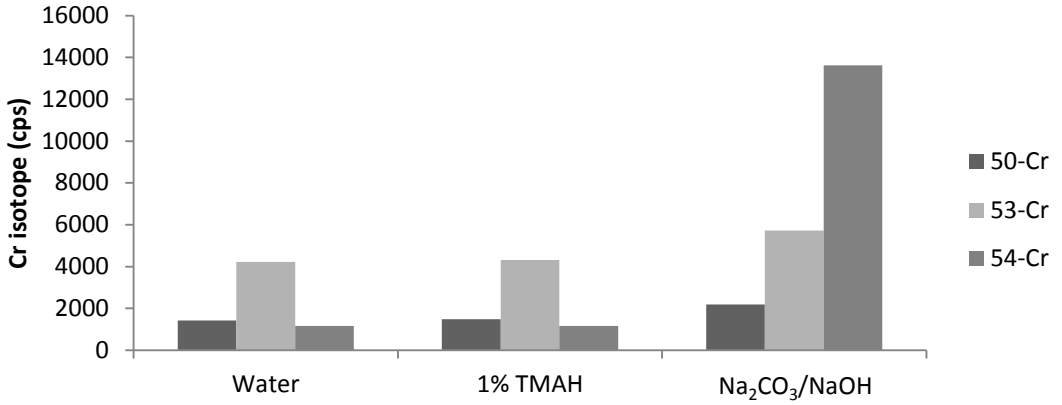


Figure 3.9 ICP-MS response (corrected cps) for the minor Cr isotopes ^{50}Cr, ^{53}Cr and ^{54}Cr dissolved in 3 contrasting matrices (water, 1 % TMAH and 0.28 M Na_2CO_3/0.50 M NaOH) using Ge as an internal standard. Total Cr concentration was 20 µg L^{-1} Cr^{III} and the instrument was in CCT-KED mode. All solutions contained 0.001 M EDTA and were heated at 70^{o}C for 1 hr to ensure EDTA-Cr^{III} complex formation. Results are based on single unreplicated measurement.

Water and 1% TMAH were also the suitable extractants for Cr when analysed by using ICP-MS in CCT-KED mode. The polyatomic interference can be removed using this mode, without the need to do blank subtraction.

3.6.2 Effect of solution matrix on Cr speciation

Mobile phase for Cr speciation

In an initial study, the chromatographic mobile phase used was that proposed by Gurleyuk et al., (2001) using 50 mM NH_4NO_3, 1x10^{-5} M ammonium-EDTA and 2% methanol; the pH was adjusted to 8 using TMAH. However, Cr species could not be separated in all the matrices as both Cr species appeared at the same retention time. An alternative approach was tried using 50 mM TRIS buffer, 40 mM NH_4NO_3, 1x10^{-5} M ammonium-EDTA with pH adjustment to 9.4 using TMAH. All chromatographic runs were undertaken in CCT-KED mode without the use of an internal standard. The latter is based on the assumption that the small sample volume (20 µL) and its

separation within the column produces an identical matrix for both standards and samples (i.e. the eluent) during the period of peak evolution. Longer term drift is corrected simply by using repeated standards.

Stability of Cr species in water

As can be seen in the chromatograms shown in Figure 3.10, there was no effect of heating or the presence of EDTA (0.001 M) on Cr^{VI}. The Cr^{VI} peak appeared at about 9 minutes and all samples produced virtually identical peaks for all treatments. Figure 3.11 shows similar chromatograms for Cr^{III} which produced a peak at about 9.8 minutes but with clear differences between treatments. The largest peak, comparable to the equivalent signal for Cr^{VI} (Fig. 3.10) was for heated solutions containing EDTA. There was about 7% conversion from Cr^{III} to Cr^{VI} without heating in the presence of EDTA but most of the Cr^{III} was simply lost in the high pH matrix, possibly through sorption or precipitation within the guard column. Without EDTA, with or without heating, virtually all Cr^{III} was lost with some evidence of a small proportion of the Cr^{III} undergoing oxidation to Cr^{VI} as shown by a minor peak at 9.0 minutes

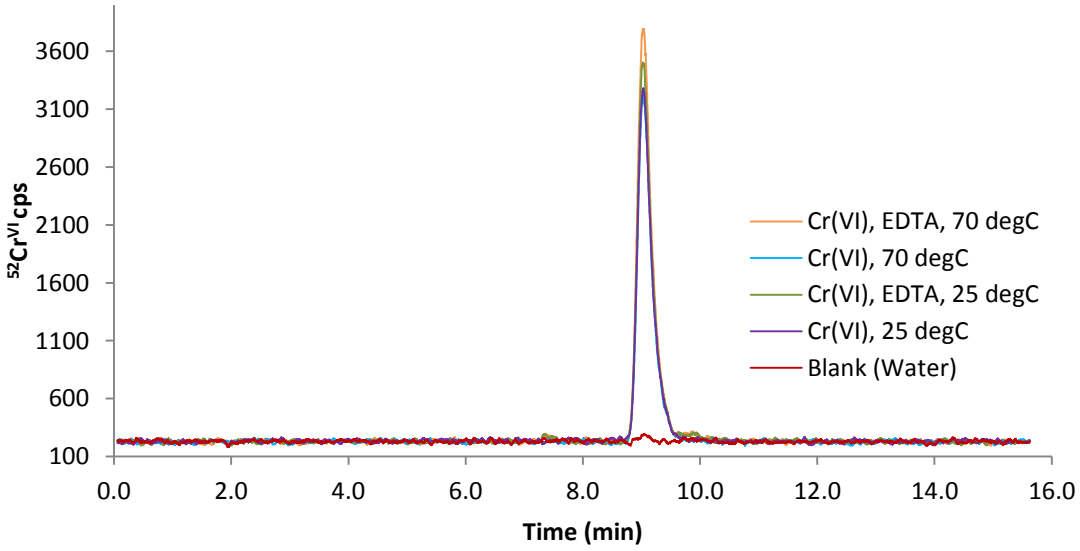


Figure 3.10 Chromatograpms (cps vs time) of 20 µg L^{-1} Cr^{VI} in water with, and without, added EDTA (0.001 M) and heating at 70 °C for 1 hr. A PRP-X100 Anion exchange column (50 x 4.1 mm) with mobile phase 50 mM TRIS, 40 mM NH_4NO_3, 10^{-5} M EDTA and pH adjusted to 9.4 using TMAH was used. Data were plotted using a moving average interval of 30 time periods of 121 ms.

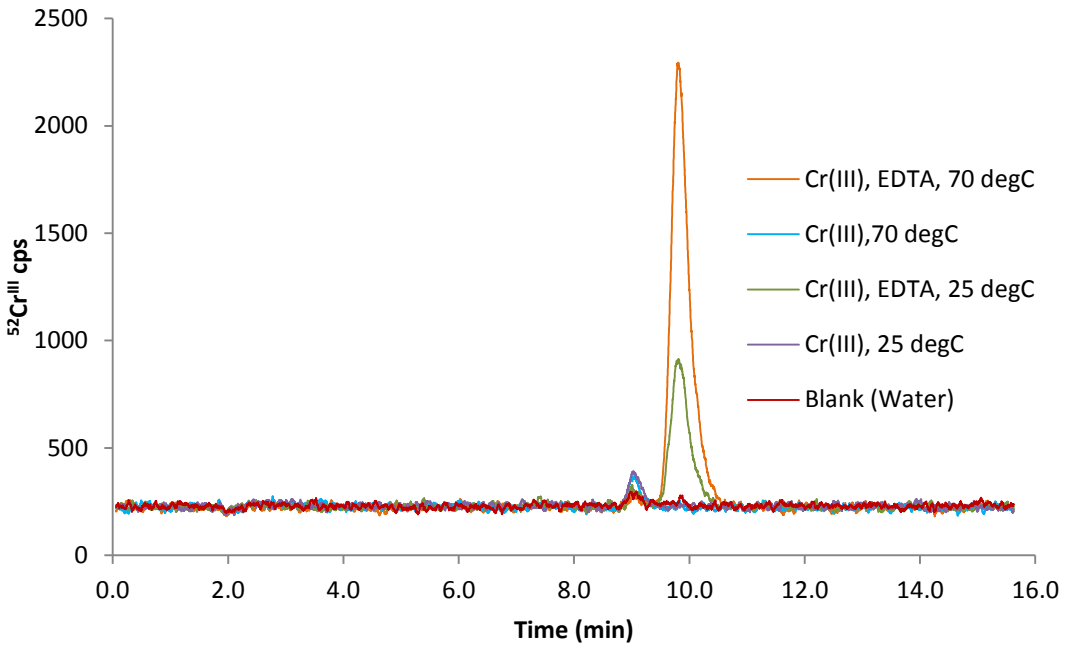


Figure 3.11 Chromatograms (cps vs time) of 20 µg L^{-1} Cr^{III} in water with and without added EDTA (0.001 M) and heating at 70 °C for 1hr. A PRP-X100 Anion exchange column (50 x 4.1 mm) with mobile phase 50 mM TRIS, 40 mM NH_4NO_3, 10^{-5} M EDTA and pH adjusted to 9.4 using TMAH was used. Data were plotted using a moving average interval of 30 time periods of 121 ms.

Stability of Cr species in 1 % TMAH

In 1 % TMAH, a Cr^{VI} peak appeared at about 9 minutes (Fig. 3.12). At room temperature (nominally 25°C), the recovery of Cr^{VI} was similar with or without the presence of EDTA. The recovery of Cr^{VI} with heating and EDTA gave a similar result although heating without EDTA (blue line) gave a lower recovery. Overall, Cr^{VI} appears to be a reasonably robust species as it exhibited similar behaviour in water (Fig. 3.10) and 1 % TMAH (Fig. 3.12). The peak for trivalent Cr^{III} appeared at about 10 minutes (Fig. 3.13). In TMAH, only the presence of EDTA and heating preserved Cr^{III} but even in this case about 20 % of Cr^{III} was converted to Cr^{VI}. Without heating (but with EDTA) the Cr^{III} peak was considerable lower due mainly to oxidation to Cr^{VI} as shown by an enhanced Cr^{VI} peak at 9 minutes. Without EDTA, almost all Cr^{III} was lost by precipitation or adsorption with the remainder being converted to Cr^{VI} (Fig. 3.13). It is clear from these results that both EDTA and heating at 70°C are crucial to preserve Cr^{III} in solution under alkaline conditions. The same

trend was observed with water as the solution matrix (Fig. 3.11) but the alkaline conditions increased both the loss of Cr^{III} and its conversion to Cr^{VI}. Even with heating and EDTA (orange line, Figure 3.13) there was evidence of conversion to Cr^{VI} which suggests a higher concentration of EDTA may be needed in order to preserve all Cr^{III} in 1 % TMAH.

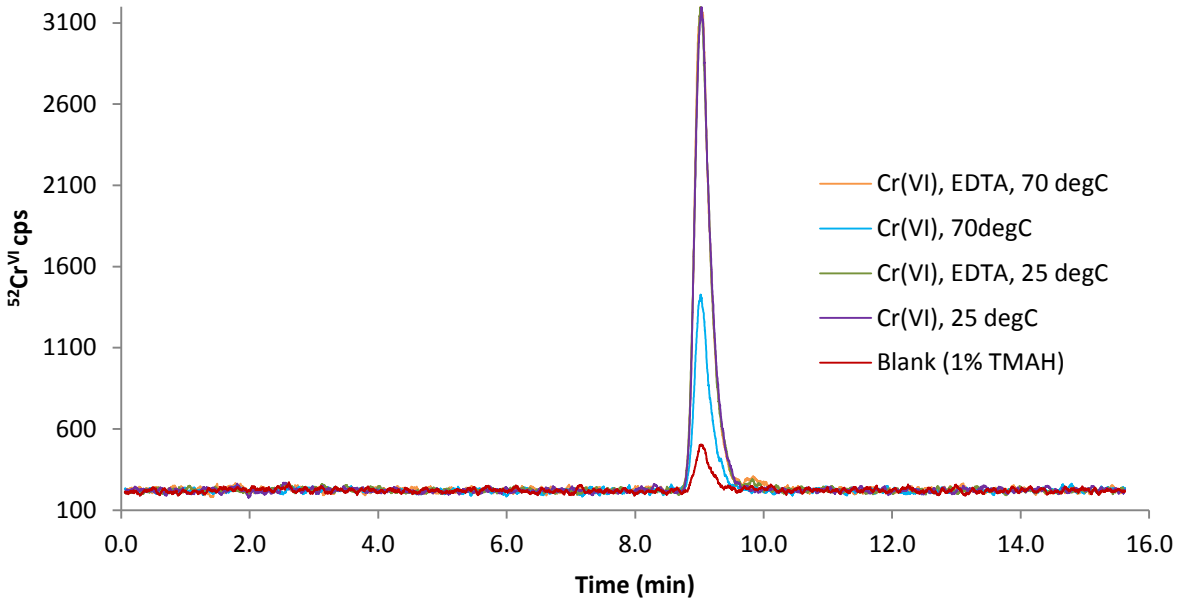


Figure 3.12 Chromatograms (cps vs time) of 20 µg L^{-1} Cr^{VI} in 1 % TMAH with and without added EDTA (0.001 M) and heating at 70 °C for 1hr. A PRP-X100 Anion exchange column (50 x 4.1 mm) with mobile phase 50 mM TRIS, 40 mM NH_4NO_3, 10^{-5} M EDTA and pH adjusted to 9.4 using TMAH was used. Data were plotted using a moving average interval of 30 time periods of 121 ms.

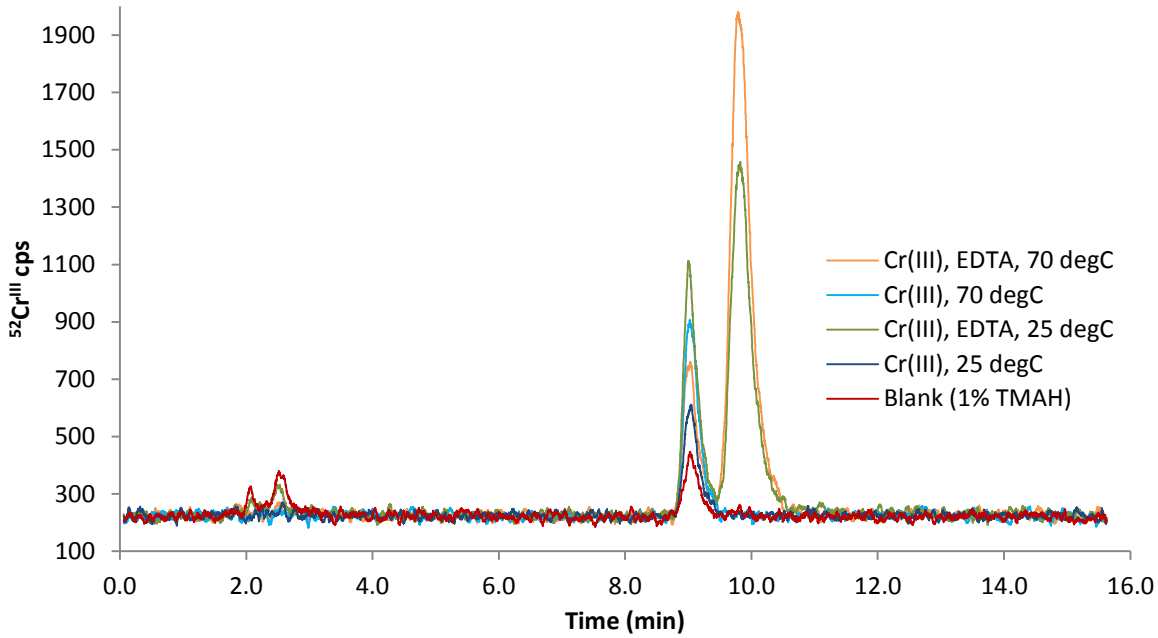


Figure 3.13 Chromatograms (cps vs time) of 20 µg L^{-1} Cr^{III} in 1 % TMAH with and without added EDTA (0.001 M) and heating at 70 °C for 1hr. A PRP-X100 Anion exchange column (50 x 4.1 mm) with mobile phase 50 mM TRIS, 40 mM NH_4NO_3, 10^{-5} M EDTA and pH adjusted to 9.4 using TMAH was used. Data were plotted using a moving average interval of 30 time periods of 121 ms.

Stability of Cr species in 0.28 M Na_2CO_3/0.5 M NaOH.

In the sodium bicarbonate matrix, there were two early peaks at about 2 and 2.6 minutes which were probably due to the emergence of carbonate/bicarbonate ions to form $^{40}Ar^{12}C$ species in the plasma (Fig 3.14) despite the use of CCT-KED mode to suppress polyatomic interference (Section 3.3). Cr^{VI} was again quite robust as there was no effect from the presence of EDTA (green and purple lines) nor from heating (orange and blue lines) (Fig 3.14.). For Cr^{III} in the Na_2CO_3/NaOH matrix (Fig. 3.15), it was clear that all Cr had either been lost or oxidised to Cr^{VI}. The only identifiable Cr peak (Cr^{VI}) was considerably smaller than in Figure 3.9, suggesting substantial loss. According to Khamis et al., (2009), aqueous Cr^{III} in the solution can react with carbonate and form a precipitate:

$$2[Cr(H_2O)_6]_{(aq)}^{3+} + 3CO_{3(aq)}^{2(} \rightarrow 2[Cr(H_2O)_3(OH)_3]_{(s)} + 3CO_{2(g)} + 3H_2O_{(l)}$$

It is possible that loss occurred within the guard column or during transition through the anion exchange column. The same peaks at 2.0 and 2.8 min seen in Figure 3.14 appeared in Figure 3.15; again it is thought that this might be $^{40}Ar^{12}C$ interference due to carbonate and bicarbonate transition but since a substantial proportion of Cr^{III} had been converted to Cr^{VI} the possibility of, for example, a neutral Cr^{VI} complex species cannot be ruled out entirely.

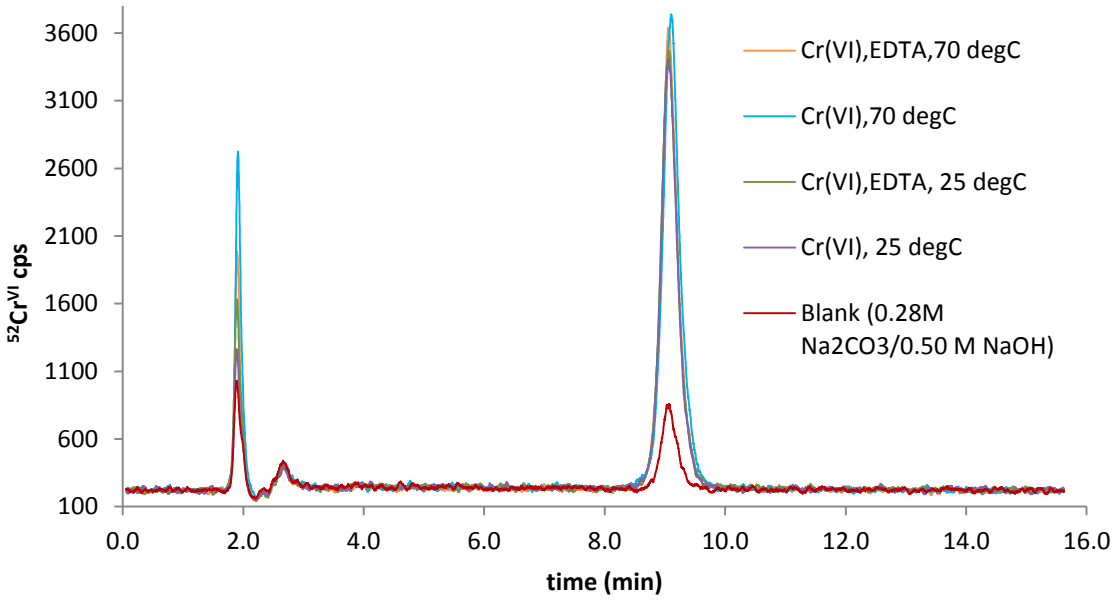


Figure 3.14 Chromatograms (cps vs time) of 20 µg L^{-1} Cr^{VI} in 0.28 M Na_2CO_3/0.50 M NaOH with and without added EDTA (0.001 M) and heating at 70 °C for 1hr. A PRP-X100 Anion exchange column (50 x 4.1 mm) with mobile phase 50 mM TRIS, 40 mM NH_4NO_3, 10^{-5} M EDTA and pH adjusted to 9.4 using TMAH was used. Data were plotted using a moving average interval of 30 time periods of 121 ms.

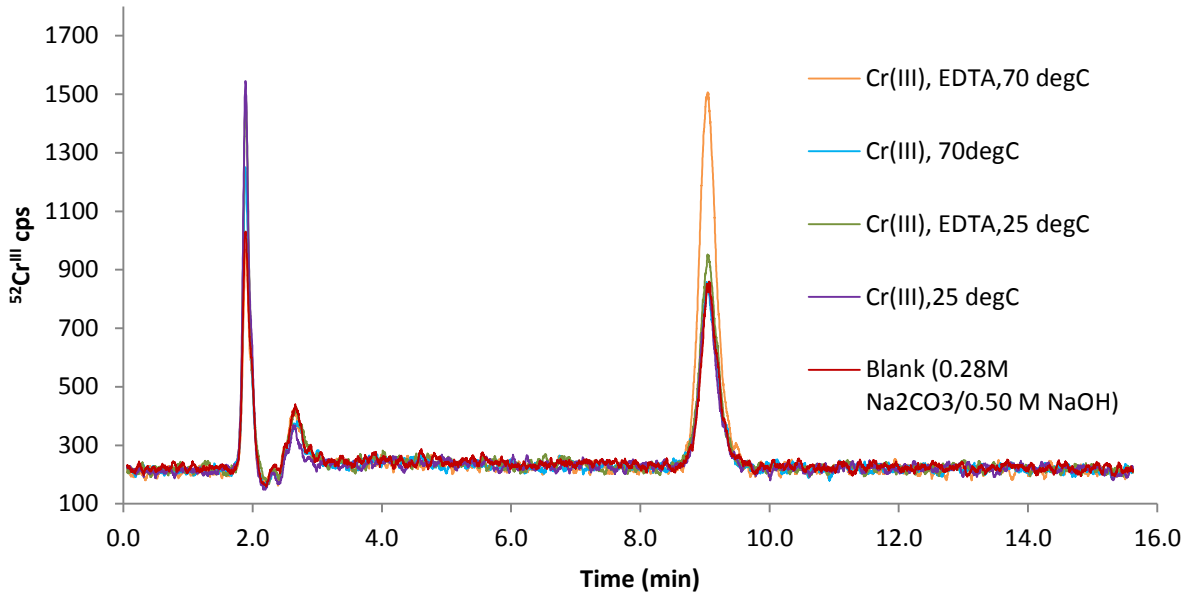


Figure 3.15. Chromatograms (cps vs time) of 20 µg L^{-1} Cr^{III} in 0.28 M Na_2CO_3/0.50 M NaOH with and without added EDTA (0.001 M) and heating at 70 °C for 1hr. A PRP-X100 Anion exchange column (50 x 4.1 mm) with mobile phase 50 mM TRIS, 40 mM NH_4NO_3, 10^{-5} M EDTA and pH adjusted to 9.4 using TMAH was used. Data were plotted using a moving average interval of 30 time periods of 121 ms.

3.6.3 The effect of EDTA concentration, heating time and temperature on the stability of Cr species

As TMAH and water gave promising results as potential extractants, they were chosen for further investigation of the effect of EDTA concentration and heating towards Cr species. The concentration of EDTA was varied from 10^{-2} to 10^{-5} M using 20 µg L^{-1} Cr^{III}, 20 µg L^{-1} Cr^{VI} or mixed Cr species (10 µg L^{-1} Cr^{VI} and 10 µg L^{-1} Cr^{III}) in a matrix of 1 % TMAH and with heating at 70°C to encourage Cr^{III}-EDTA complex formation.

Cr^{III} in water

Chromatograms of Cr^{III} in a water matrix with various concentrations of EDTA are shown in Figure 3.16; peak integrations are shown in Figure 3.17. Concentrations of EDTA in the range 10^{-2} – 10^{-4} M gave very similar recoveries of Cr^{III} (analysis of variance, ANOVA, p=0.630) but there was substantial loss of Cr^{III} (18%) at 10^{-5} M EDTA (Fig. 3.17) with no sign of conversion to Cr^{VI}.

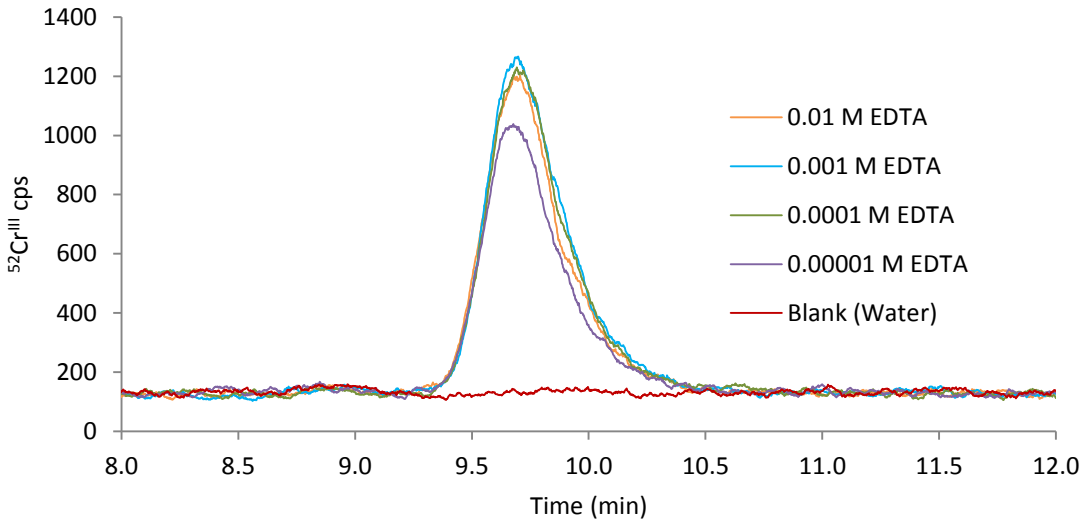


Figure 3.16 Chromatograms (cps vs time) of 20 µg L^{-1} Cr^{III} in water with EDTA concentrations ranging from 10^{-5} to 10^{-2} M with heating at 70 °C for 1hr. A PRP-X100 Anion exchange column (50 x 4.1 mm) with mobile phase 50 mM TRIS, 40 mM NH_4NO_3, 10^{-5} M EDTA and pH adjusted to 9.4 using TMAH was used. Data were plotted using a moving average interval of 30 time periods of 121 ms.

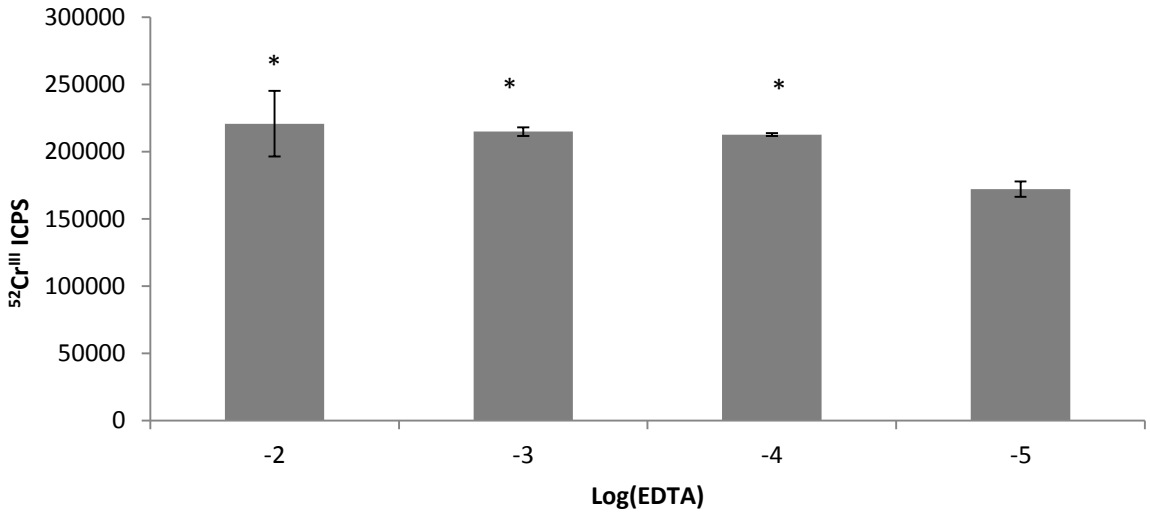


Figure 3.17 Integrated chromatogram peaks for Cr^{III} (ICPS) in water extracts stabilized with different concentrations of EDTA ranging from 10^{-5} to 10^{-2} M. Total Cr concentration was 20 µg L^{-1} Cr^{III} and the instrument was in CCT-KED mode. All solutions were heated at 70°C for 1 hr to ensure EDTA-Cr^{III} complex formation; chromatograms are shown in Fig. 3.16. Error bars show standard error of triplicate measurements of each Cr^{III} solution with different EDTA concentration. Stars above each bar indicated no significant different ($p>0.05$) on the results obtained.

Increasing the incubation temperature from 70˚C to 90°C for one hour, at an EDTA concentration of 10^{-3} M, produced no significant change in Cr^{III} recovery (t-test, $p>0.05$) (Fig. 3.18). However, surprisingly, there was an apparent loss of Cr^{III} (about 36%) by prolonging the incubation time to 2 hours at 70°C (Figure 3.18); this also produced more variable results (larger standard error).

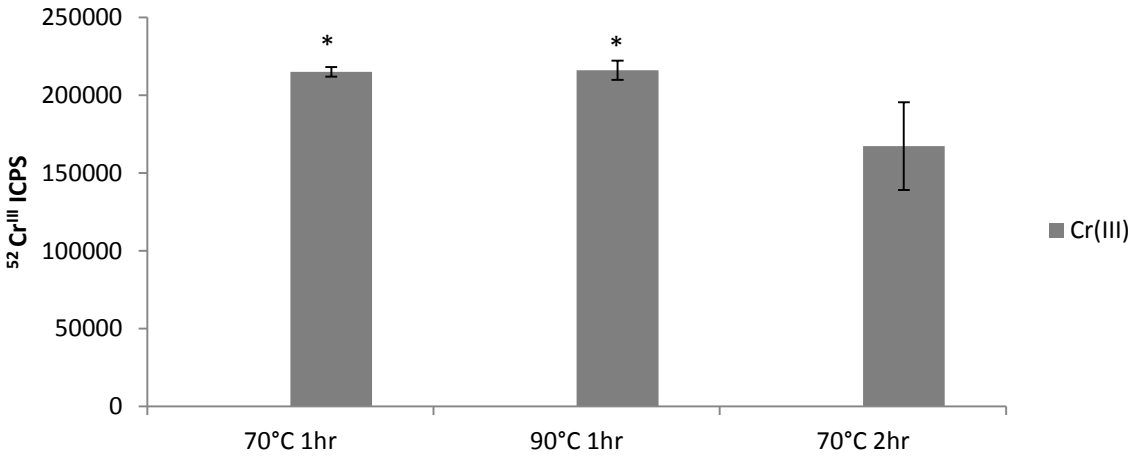


Figure 3.18 Integrated chromatogram peaks for Cr^{III} (ICPS) in water extracts and heated at 70°C and 90°C for 1 and 2 hr. Total Cr concentration was 20 µg L^{-1} Cr^{III} and the instrument was in CCT-KED mode. All solutions contained 0.001 M EDTA. Error bars show standard error of triplicate measurements of each Cr^{III} standard solution. Stars above each bar indicated no significant different ($p>0.05$) on the results obtained.

Cr^{III} in TMAH

In TMAH, the Cr^{III} peak appeared at about 9.75 minutes (Fig. 3.19) but there was clear evidence of conversion to Cr^{VI} in the alkaline matrix with a peak at about 9.0 minutes for some of the samples. This was as seen before in Figure 3.13 and is potentially a serious limitation if the extractant used to measure Cr^{VI} actually creates Cr^{VI} from soil Cr^{III}. The extent of conversion was dependent on the EDTA concentration used to stabilise the Cr^{III}. At 0.01 M EDTA, there was virtually no conversion of Cr^{III} to Cr^{VI} (c. 1 %). At EDTA concentrations of 10^{-3}, 10^{-4} and 10^{-5} M, about 6 %, 35 % and 87 % respectively of the recoverable Cr was in the form of Cr^{VI}. As well as conversion to Cr^{VI}, it was also clear that there was (again) loss of Cr^{III} from the system at the lower EDTA concentrations – possibly through precipitation or

adsorption within the guard column. The chromatograms shown in Figure 3.19 were integrated to quantify the peaks attributable to Cr^{III} and Cr^{VI} (Fig. 3.20). The highest signal (cps) for 20 µg L^{-1} Cr^{III} in 1 % TMAH was at 0.01 M EDTA so this concentration may be the optimum concentration for Cr^{III}-EDTA complex. At lower concentrations, 10^{-3} M – 10^{-5} M, there was clearly progressive loss of Cr^{III} and greater conversion of Cr^{III} to Cr^{VI}. The overall loss of Cr^{III} was 38 % and 93 % at 10^{-4} and 10^{-5} M EDTA, respectively (Fig. 3.20). As seen previously (Fig. 3.20) greater loss of Cr^{III} (and conversion to Cr^{VI}) was observed in 1 % TMAH as compared to water (Fig. 3.17), especially at lower concentrations of EDTA, 10^{-4} and 10^{-5} M. On overall, EDTA does affect the formation of both Cr^{VI} (analysis of variance, ANOVA, $p<0.05$) and Cr^{III} (ANOVA, $p<0.05$) (Fig 3.20).

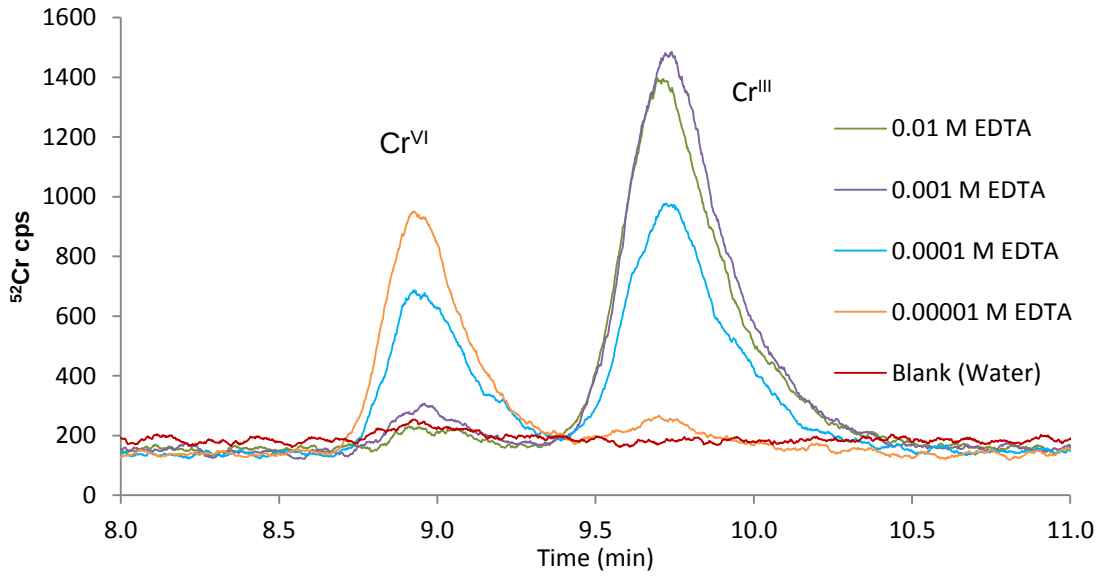


Figure 3.19. Chromatograms (cps vs time) of 20 µg L^{-1} Cr^{III} in water with concentration of EDTA ranging from 10^{-5} to 10^{-2} M and heating at 70 °C for 1 hr. A PRP-X100 Anion exchange column (50 x 4.1 mm) with mobile phase 50 mM TRIS, 40 mM NH_4NO_3, 10^{-5} M EDTA and pH adjusted to 9.4 using TMAH was used. Data were plotted using a moving average interval of 30 time periods of 121 ms.

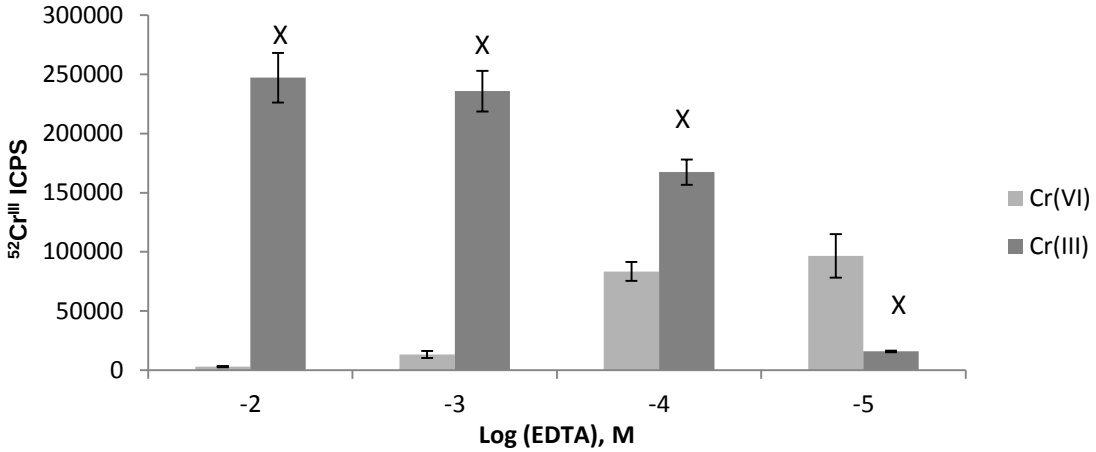


Figure 3.20 Integrated chromatogram peaks for Cr^{III} (ICPS) in 1 % TMAH extracts of different concentrations of EDTA ranging from 10^{-5} to 10^{-2} M. Total Cr concentration was 20 µg L^{-1} Cr^{III} and the instrument was in CCT-KED mode. All solutions were heated at 70 °C for 1 hr to ensure EDTA-Cr^{III} complex formation; chromatographs are shown in Fig. 3.19.. Error bars show standard error of triplicate measurements of each Cr^{III} solution. X above each bar indicated a significant different ($p<0.05$) on Cr^{III} results.

Prolonging the incubation time to two hours appeared to increase the loss of Cr^{III} in the presence of 10^{-3} M EDTA but with less conversion to Cr^{VI}. Increasing the incubation temperature to 90°C slightly increased ($p>0.05$) the recovery of Cr^{III} and with less conversion to Cr^{VI}. (Fig. 3.21)

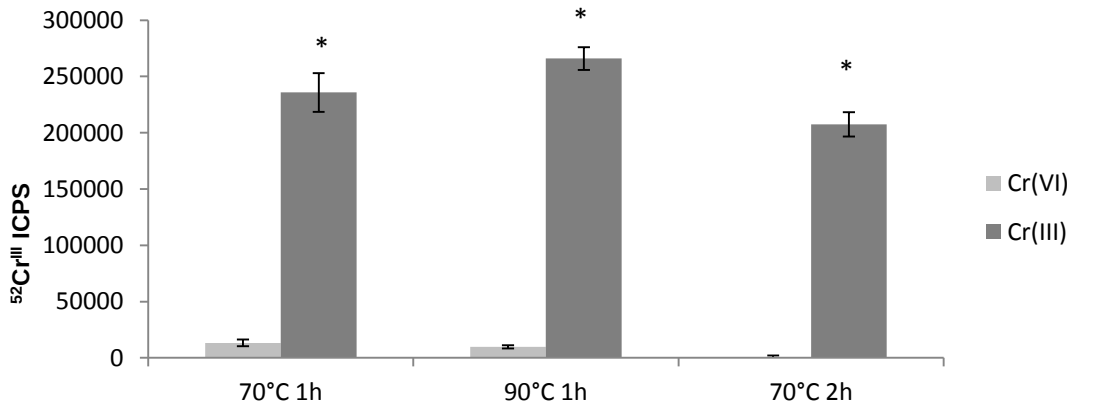


Figure 3.21 Integrated chromatogram peaks for Cr^{III} and Cr^{VI} (ICPS) in 1 % TMAH extracts and heated at 70 °C and 90 °C for 1 and 2 hr. Total Cr concentration was 20 µg L^{-1} Cr^{III} and the ICP-MS was in CCT-KED mode. All solutions contained 0.001 M EDTA. Error bars show standard error of triplicate measurements of each Cr^{III} and Cr^{VI}. Stars above each bar indicated no significant different ($p>0.05$) on Cr^{III} results at the same EDTA concentration.

Cr^{VI} in water

The change in integrated signal for Cr^{VI} peaks, in a water matrix, with EDTA concentration in the range of 10^{-2} – 10^{-5} M is shown in Figure 3.22. There was only a slight, and inconsistent, effect of EDTA on Cr^{VI} peak area suggesting that Cr^{VI} is a robust species in a wide range of EDTA concentrations. No significant different (analysis of variance, ANOVA, p=0.630) was observed in the response of Cr^{VI} at different concentration of EDTA.

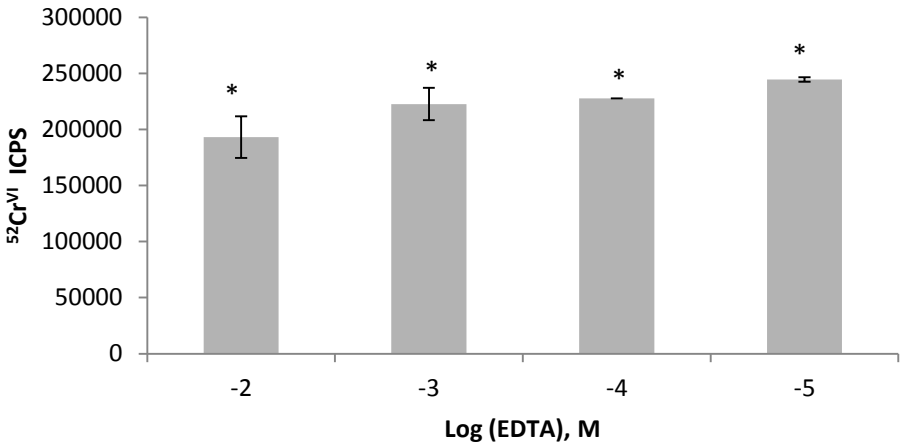


Figure 3.22 Integrated chromatogram peaks for Cr^{VI} (ICPS) in water with concentration of EDTA ranging from 10^{-5} to 10^{-2} M. Total Cr concentration was 20 µg L^{-1} Cr^{VI} and the ICP-MS was in CCT-KED mode. All solutions were heated at 70°C for 1 hr. Error bars show standard error of triplicate measurements of each Cr^{VI} solution. Stars above each bar indicated no significant different (p>0.05) on the results obtained.

Prolonging the heated incubation time to two hours appeared to slightly increase the loss of Cr^{VI} in the presence of 10^{-3} M EDTA. Increasing the incubation temperature to 90°C appeared to have an effect similar to that produced by prolonging the incubation (Fig. 3.23). There was no significant different between the concentration of Cr^{VI} either by increasing the temperature of the extract from 70˚C to 90˚C or prolong the incubation time from 1 to 2 hours (analysis of variance, ANOVA, p>0.05)

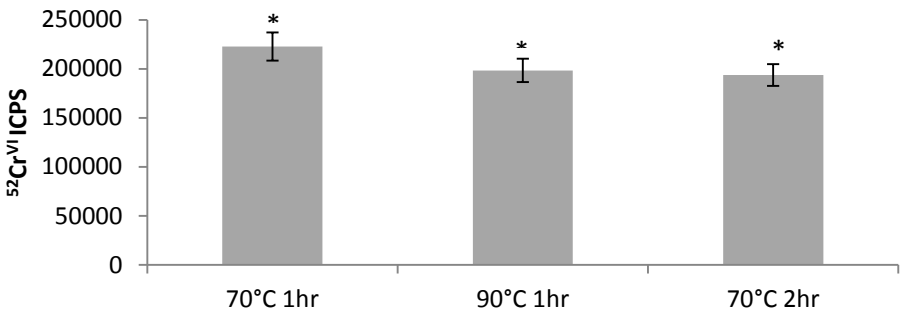


Figure 3.23 Integrated chromatogram peaks for Cr^{VI} (ICPS) in water with 0.001 M EDTA and heated at 70 °C or 90 °C for 1 or 2 hr. Total Cr concentration was 20 µg L^{-1} Cr^{III} and the ICP-MS was in CCT-KED mode. Error bars show standard error of triplicate measurements of each Cr^{VI} solution. Stars above each bar indicated no significant different ($p > 0.05$) on the results obtained.

Mixed Cr^{III} and Cr^{VI} species in water

The integrated chromatogram peaks for Cr^{III} and Cr^{VI} in water are shown in Figure 3.24. Good recovery was obtained for both Cr species in water with EDTA concentrations ranging from 10^{-5} to 10^{-2} M and incubated for 1 hour. No significant different in the results of Cr^{III} and Cr^{VI} at different concentration of EDTA ($p>0.05$).

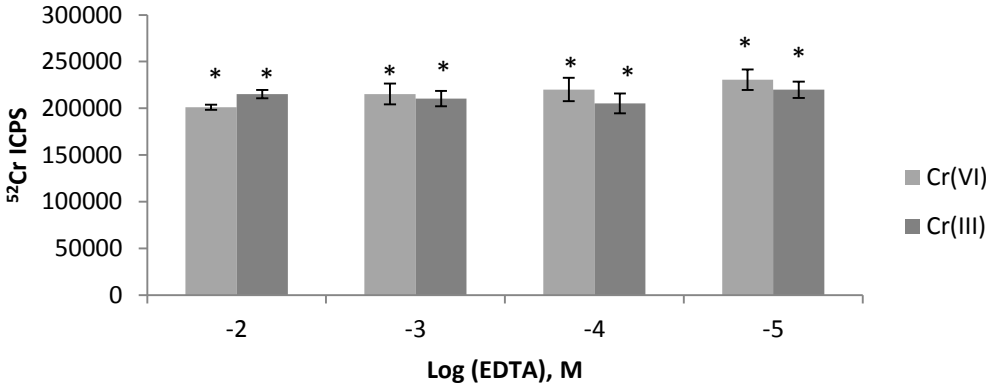


Figure 3.24 Integrated chromatogram peaks for Cr^{III} and Cr^{VI} (ICPS) in water with a range of concentrations of EDTA ranging from 10^{-5} to 10^{-2} M. Total Cr concentration was a mix of the two inorganic Cr species (10 µg L^{-1} Cr^{VI} and 10 µg L^{-1} Cr^{III}) and the instrument was in CCT-KED mode. All solutions were heated at 70°C for 1 hr with 0.001 M EDTA. Error bars show standard error of triplicate measurements of each Cr^{III} and Cr^{VI} solution. Stars above each bar indicated no significant different ($p>0.05$) either for Cr^{VI} or Cr^{III} results obtained.

Prolonging the incubation time to two hours appeared to slightly increase the loss of Cr^{III} (ANOVA, $p>0.05$) and Cr^{VI} (ANOVA, $p>0.05$) in the presence of 10^{-3} M EDTA. A lower Cr^{VI} signal was observed when increasing the incubation temperature to 90°C for one hour, as compared to incubation at 70°C, indicating a slight loss of Cr^{VI} (ANOVA, $p>0.05$). (Fig. 3.25)

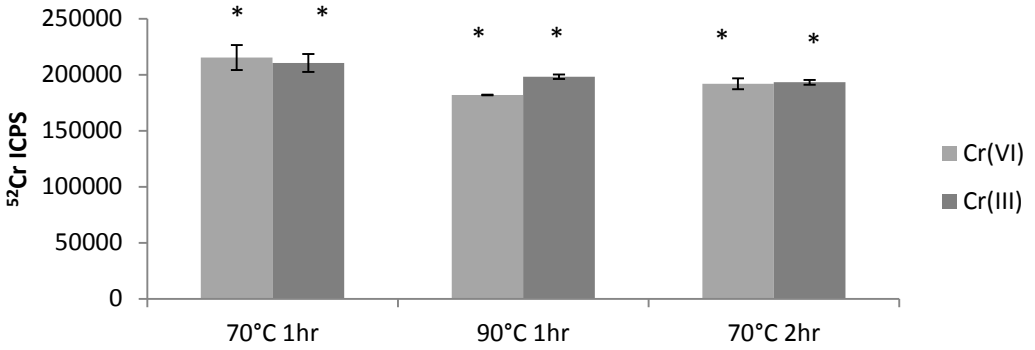


Figure 3.25 Integrated chromatogram peaks for Cr^{III} and Cr^{VI} (ICPS) in water with EDTA concentrations ranging from 10^{-5} to 10^{-2} M. Total Cr concentration was a mix of Cr species (10 µg L^{-1} Cr^{VI} and 10 µg L^{-1} Cr^{III}) and the ICP-MS was in CCT-KED mode. All solutions were heated at 70°C with 0.001 M EDTA. Error bars show standard error of triplicate measurements of each Cr^{III} and Cr^{VI} solution.Stars above each bar indicate no significant difference ($p>0.05$) between mean peak areas.

Cr^{VI} in 1 % TMAH

The change in integrated signal for Cr^{VI} peaks with EDTA concentration is shown in Figure 3.26. There was only a slight, and inconsistent, effect of EDTA and longer incubation time (3 hr) on peak area. The highest response (cps) of Cr^{VI} to EDTA concentration was at 10^{-2} M (176,000 cps) but the variation of the signal was only about 15% from the lowest to the highest response (ANOVA, $p>0.05$). It appears that Cr^{VI} produces a very consistent response at any concentration of EDTA.

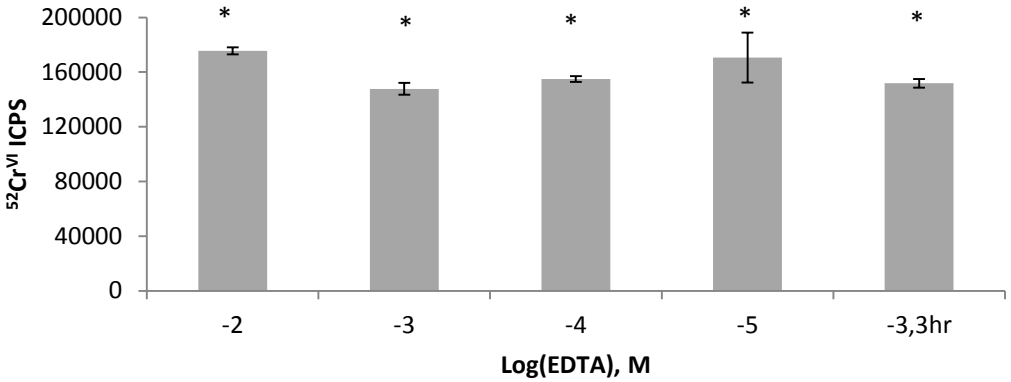


Figure 3.26 Integrated chromatogram peaks for Cr^{VI} (ICPS) in 1 % TMAH extracts with EDTA concentrations ranging from 10^{-5} to 10^{-2} M. Total Cr concentration was 20 µg L^{-1} as Cr^{VI} and the instrument was in CCT-KED mode. Most solutions were heated at 70°C for 1 hr; one sample at an EDTA concentration of 0.001 M was heated for 3 hr, and is labelled -3, 3 hr. Error bars show standard error of triplicate measurements of each Cr^{VI} solution with different EDTA concentrations. Stars above each bar indicate no significant difference ($p>0.05$) between mean peak areas.

Mixed Cr^{III} and Cr^{VI} species in 1 % TMAH

The chromatograms of mixed Cr species, 10 µg L^{-1} of Cr^{III} and 10 µg L^{-1} of Cr^{VI} are shown in Figure 3.27. The retention time of Cr^{VI} was 10.8 minutes followed by a Cr^{III} peak at 11.6 minutes. The chromatograms shown in Figure 3.27 were integrated to quantify the peaks attributable to Cr^{III} and Cr^{VI} (Fig. 3.28). As seen in the single species chromatograms, reducing the concentration of EDTA from 0.01 M resulted in a progressive loss of Cr^{III}. Furthermore, at 10^{-5} M EDTA only, there was evidence of an enhanced Cr^{VI} signal due to oxidation of Cr^{III} (Fig. 3.28). No consistent or substantial effect on Cr^{VI} at EDTA concentration from 10^{-4} M to 10^{-2} M was seen (ANOVA, $p=0.745$), apart from an increased concentration below 10^{-4} M EDTA (Figure 2.8). However, there are significant different ($p<0.05$) on the response of Cr^{III} at different concentration of EDTA from 10^{-5} M to 10^{-2} M.

Prolonging the incubation time to 3 hours, the response of Cr^{VI} and Cr^{III} remained stable at 71 000 cps in 10^{-3} M EDTA (Fig. 3.28) although there appeared to be a small loss of about 15 % for Cr^{VI} and 6% for Cr^{III}, compared to the one hour incubation at 10^{-3} M EDTA. Three hours is the suggested time for the extraction of iodine in TMAH (Yamada et al., 1999).

It is clear that, within the protocols described, loss of Cr^{III} occurs if the EDTA concentration is below 10^{-3} or 10^{-2} M, also conversion to Cr^{VI} occurs at lower EDTA concentrations. Extending heating time beyond 1 hr has only a limited and inconsistent effect on Cr^{III} and less effect on Cr^{VI}.

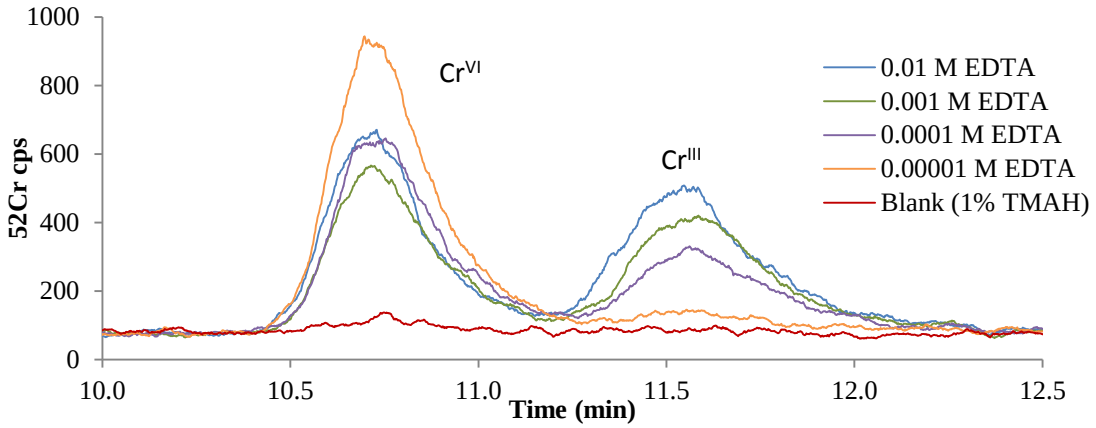


Figure 3.27 Chromatograms (cps vs time) of mixed Cr species (10 µg L^{-1} Cr^{VI} and 10 µg L^{-1} Cr^{III}) in 1 % TMAH with EDTA concentration ranging from 10^{-2} to 10^{-5} M and heated at 70 °C for 1 hr. A PRP-X100 Anion exchange model (50 x 4.1 mm) with mobile phase 50 mM TRIS, 40 mM NH_4NO_3, 10^{-5} M EDTA and pH adjusted to 9.4 using TMAH was used. Data were plotted using a moving average interval of 30 time periods of 121 ms.

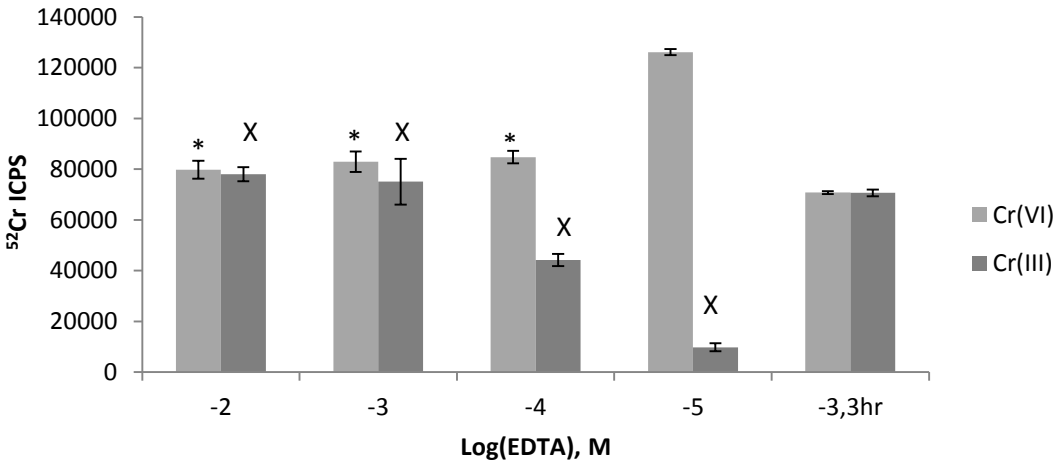


Figure 3.28 Integrated chromatogram peaks for Cr^{III} and Cr^{VI} (ICPS) in 1 % TMAH with concentrations of EDTA ranging from 10^{-5} to 10^{-2} M. Total Cr concentration was a mix of Cr species (10 µg L^{-1} Cr^{VI} and 10 µg L^{-1} Cr^{III}) and the instrument was in CCT-KED mode. All solutions were heated at 70°C for 1 hr except for one sample incubated for 3 hr with 0.001 M EDTA (labelled -3,3hr). Error bars show standard error of triplicate measurements of each Cr^{III} and Cr^{VI} solution. Stars above each bar indicated no significant different ($p>0.05$) between mean peak areas for Cr^{VI}. X above each bar refers to significant different ($p<0.05$) between mean peak areas for Cr^{III}.

3.6.4 Speciation and Fractionation of Cr in Soil

Speciation of Cr in soil extracts

Speciation of Cr was undertaken, as described previously, in filtered 1 % TMAH extractions of two contrasting soils at 0.001 and 0.01 M EDTA. The nature and stability of Cr species in both unspiked and spiked extracts of acidic and calcareous soil incubated for 1 to 3 hours at 70 °C was studied. Sample preparation was according to Section 3.5.5. Integrated chromatogram peaks for Cr^{VI} and Cr^{III} in unspiked soil solutions is shown in Figure 3.29. The presence of Cr^{VI} in the acidic woodland (SB) and calcareous arable (SR) soils was clearly indicated with a higher concentration in SB than SR and a small increase in signal with heated incubation time. There was no significant different ($p>0.05$) in the the concentration of Cr^{VI} either in SB or SR soils extracted in 1% TMAH for 2 or 3 hours. No signal for Cr^{III} was seen at 0.001 M EDTA in either the unspiked SB and or SR soil TMAH extracts (Fig. 3.29).

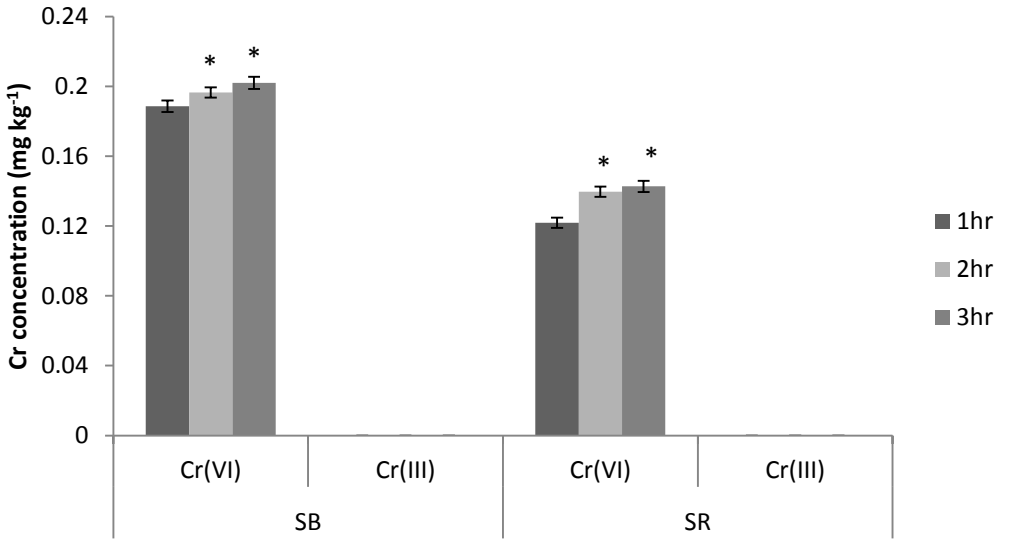


Figure 3.29 Concentration (mg kg^{-1}) of Cr^{III} and Cr^{VI} in 1 % TMAH extracts of unspiked acidic woodland (SB) and unspiked calcareous arable (SR) soils, incubated at 70°C for 1 to 3 hr with 0.001 M EDTA. Error bars show standard error of triplicate measurements of each soil extract. Stars above each bar indicated no significant different ($p>0.05$) between the mean concentrations of $Cr^{VI.}$.

At a higher EDTA concentration (0.01 M) Cr^{III} was clearly present in both (unspiked) soil extracts (Fig. 3.30). It was demonstrated previously (see Section 3.6.3, Fig 3.26) that Cr^{VI} was unaffected by the concentration of EDTA. However, the concentration for Cr^{VI} in 0.01 M EDTA for the SB soil (Fig. 3.30) was slightly lower than in 0.001 M EDTA (Fig. 3.29); it is possible that this indicates some degree of reduction of Cr^{VI} to Cr^{III} in the acidic SB soil in 0.01 M EDTA. A paired t-test (Minitab software) suggested that there was no significant different in the result of longer incubation time from 2 to 3 hours for Cr^{VI} in SB ($p>0.05$) (Fig 3.29).

In the SR soil, there was also a slightly lower concentration of Cr^{VI} in 0.01 M as compared to 0.001 M EDTA (Figs 3.30 and 3.29) possibly indicating that the presence of Cr^{III} in the SR soil solution included a contribution from reduction of Cr^{VI}. The concentration of Cr^{III} was higher in SR compared to the SB soil for all EDTA-heated incubation times (1 - 3 hr) (Fig. 3.30). This may reflect the fractionation within the soil or the strength of humic-Cr^{III} bonding which the EDTA has to overcome to produce the EDTA-Cr^{III} complex measured. An ANOVA (Minitab software) also suggested that there was no significant different in the result of longer incubation time from 1 to 3 hours for Cr^{III} in SB ($p>0.05$) and SR samples ($p>0.05$) (Fig 3.30).

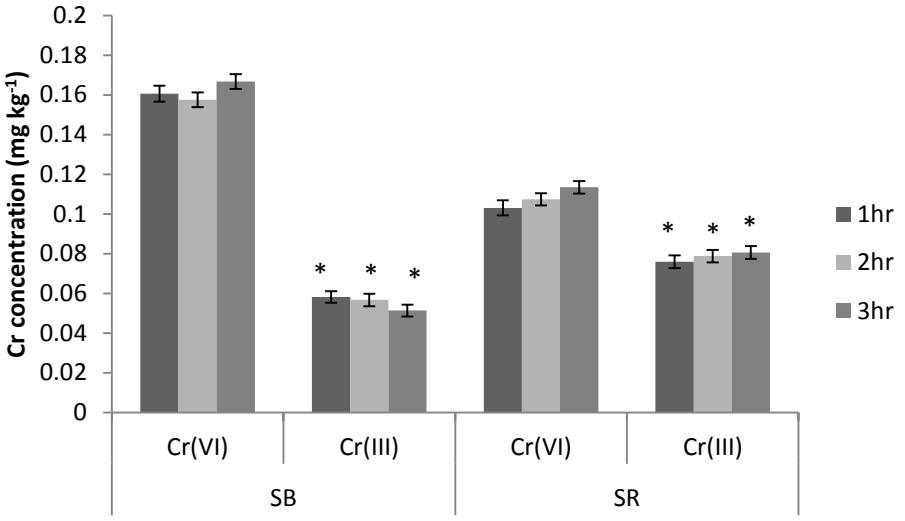


Figure 3.30 Concentration (mg kg^{-1}) of Cr^{III} and Cr^{VI} in 1 % TMAH extracts, from the unspiked acidic woodland (SB) and unspiked calcareous arable (SR) soils, incubated at 70 ^{o}C for 1 to 3 hr with 0.01 M EDTA. Error bars show standard error of triplicate measurements of each soil extract. Stars above each bar indicated no significant different ($p>0.05$) between the mean concentrations of Cr^{III}.

Soil extracts spiked with 0.01 mg kg^{-1} of both Cr^{III} and Cr^{VI} were speciated in a similar way. The concentration of spiked SB and SR soil extracts in 0.01 M and 0.001 M EDTA are shown in Figures 3.37 and 3.38. No Cr^{III} was detected in the mixed Cr spike in both soil extracts incubated for 1 to 3 hr in 0.001 M EDTA (Fig. 3.31). It appears that at this concentration, the complexing power of EDTA is not sufficient to retain Cr^{III} in the alkaline soil extracts (Fig. 3.31) or that possibly oxidation from Cr^{III} to Cr^{VI} occurred as Cr^{VI} was the only species present when both soil solutions were spiked with 0.01 mg kg^{-1} of both Cr^{III} and Cr^{VI} in 0.001 M EDTA.

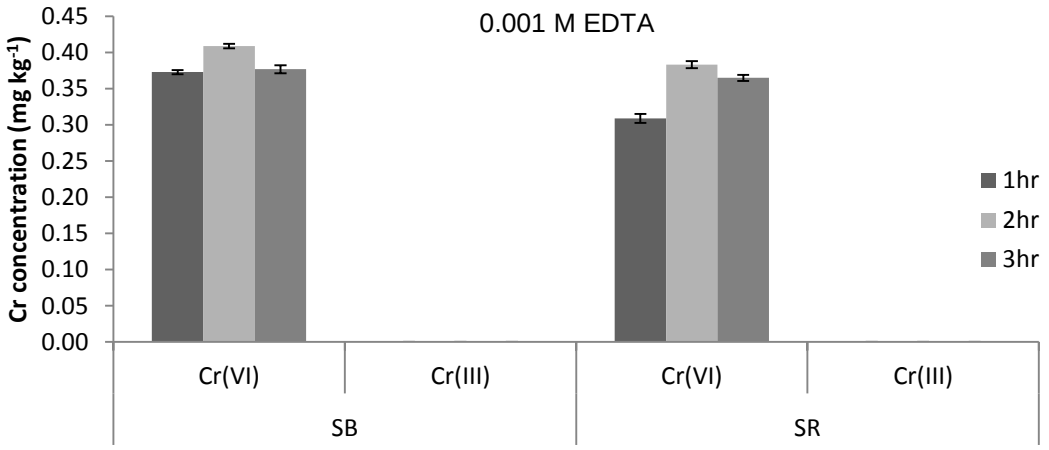


Figure 3.31 Concentration (mg kg^{-1}) of Cr^{III} and Cr^{VI} in 1 % TMAH extracts of an acidic woodland (SB) and calcareous arable (SR) soils, both were spiked with a mix of 0.01 mg kg^{-1} Cr^{VI} and 0.01 mg kg^{-1} Cr^{III}, incubated at 70 $^{\circ}$C for 1 to 3 hr with 0.001 M EDTA. Error bars show standard error of triplicate measurements of each soil extract.

With a higher concentration of EDTA, 0.01 M, there was clear evidence of the presence of Cr^{III} in both soils (Fig. 3.32). The concentration of the Cr^{III} spike was greater in SR soil than in SB for all heated incubation times (1 - 3 hr). The concentration of Cr^{VI} in a mixture of 0.01 mg kg^{-1} Cr^{VI} and 0.01 mg kg^{-1} Cr^{III} spike in SB and SR was double the Cr^{VI} concentration in the unspiked soils (Fig. 3.30), but the concentration of Cr^{III} was only slightly increased (Fig. 3.32) as compared to Cr^{III} signal in the unspiked SB and SR soils (Fig 3.30).This may be due to loss of Cr^{III} or oxidation of Cr^{III} to Cr^{VI} in SB and SR. There was no significant different in the concentration of Cr^{III} (ANOVA, p=0.706) and Cr^{VI} (ANOVA, p=0.598) in SR by prolong the incubation time from 1 to 3 hours (Fig 3.32)

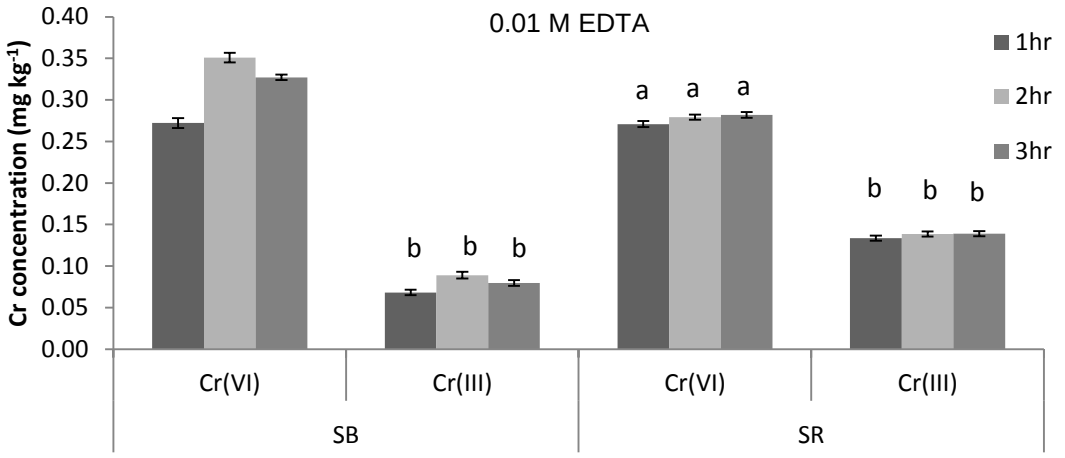


Figure 3.32 The concentration (mg kg^{-1}) of Cr^{III} and Cr^{VI} in 1 % TMAH extracts of an acidic woodland (SB) and calcareous arable (SR) soils, both spiked with a mix of 0.01 mg kg^{-1} Cr^{VI} and 0.01 mg kg^{-1} Cr^{III}, incubated at 70 °C for 1 to 3 hr with 0.01 M EDTA. Error bars show standard error of triplicate measurements of each soil extract. Identical letters above each bar indicate no significant differences ($p>0.05$) between the mean concentrations; different letters indicate significant differences ($p<0.05$) between mean concentrations.

The preservation of added Cr species in the mixed spiked systems incubated with 0.001 M (Fig. 3.33) and 0.01 M EDTA (Fig. 3.34) for 1 to 3hr was assessed by subtracting the concentration of Cr^{III} and Cr^{VI} in unspiked for soil extracts SB and SR from their mix spiked concentrations incubated with either 0.001 M or 0.01 M EDTA.

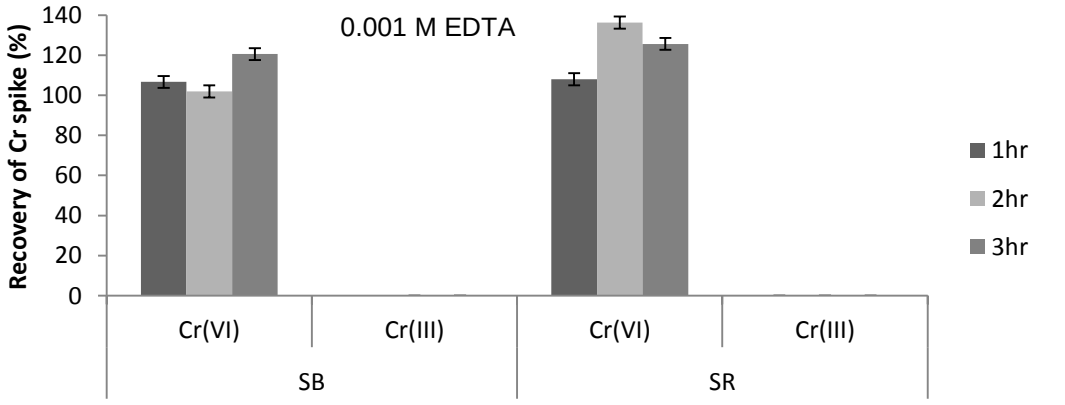


Figure 3.33 Percentage recovery of Cr^{III} and Cr^{VI} in 1 % TMAH extracts after soil solutions were incubated with 0.001 M EDTA for 1 to 3 hr with a mixed 0.01 mg kg^{-1} Cr^{VI} and 0.01 mg kg^{-1} Cr^{III} spike in acidic (SB) and calcareous (SR) soil solutions. Error bars show standard error of triplicate measurements of each soil extract.

Generally, preservation of added Cr^{VI} was close to 100% in both soils and at both EDTA concentrations. There was evidence that some Cr^{III} can be preserved in 0.01 M EDTA regardless of incubation time (1 to 3 hr) (Fig. 3.34). The preservation of Cr^{III} was greater in the calcareous soil (SR) extract compared to the acidic soil (SB) and more consistent with heating time. Nevertheless, Figure 3.34 also shows that the recovery for Cr^{III} was only about 40% of the spike added in the case of SR and less in the SB extracts. The recovery of Cr^{VI} in 0.001 M EDTA (Fig. 3.33) was slightly higher than the recovery of Cr^{VI} in 0.01 M EDTA (Fig. 3.34) which may be due to oxidation of Cr^{III} to Cr^{VI}. Again, this suggests that a higher concentration of EDTA is needed in order to preserve Cr^{III} in soil extracts. In overall, two hours is sufficient to extract the Cr species in both alkaline and acidic soils (Figure 3.30). As can be seen in Fig 3.34, there was no significant different ($p>0.05$) on the recovery of either Cr^{VI} or Cr^{III} in SR after extracted from 1 to 3 hours at 70 ˚C in 1% TMAH.

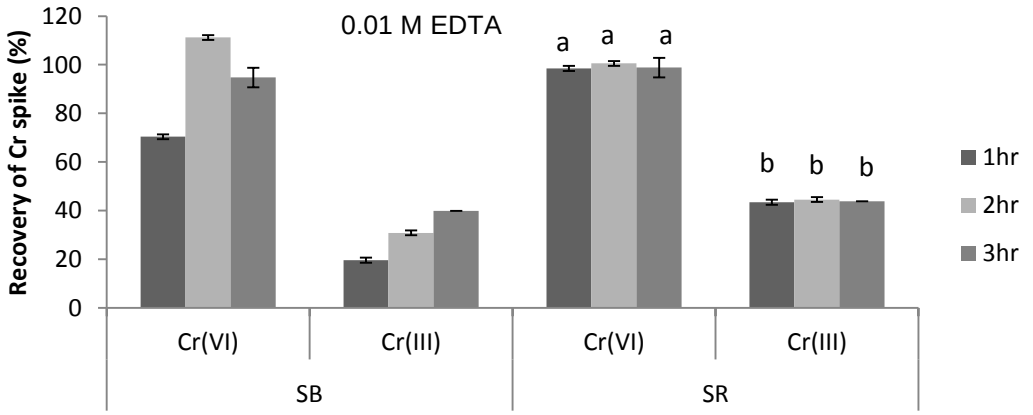


Figure 3.34 Percentage recovery of Cr^{III} and Cr^{VI} spikes in 1 % TMAH extracts after soil solutions were incubated at 0.01 M EDTA for 1 to 3 hr with a mixed of 0.01 mg kg^{-1} Cr^{VI} and 0.01 mg kg^{-1} Cr^{III} spike in acidic (SB) and calcareous (SR) soil solutions. Error bars show standard error of triplicate measurements of each soil extract. Identical letters above each bar indicate no significant differences ($p>0.05$) between mean concentrations; different letters indicate significant differences ($p<0.05$) between mean concentrations.

The preservation of Cr^{III} and Cr^{VI} in TMAH extractable acidic (SB) and calcareous (SR) soil was further studied using 0.05 M EDTA to preserve Cr^{III} at an incubation time of 2 hr. In this experiment, the soil solutions were spiked with higher concentration of Cr species; 0.10 mg kg^{-1} single Cr^{VI}, 0.10 mg kg^{-1} single Cr^{III} species and 0.10 mg kg^{-1} of each Cr^{VI} and Cr^{III} species as a mixed spike. The concentrations

of Cr^{VI} and Cr^{III} for unspiked soil solution are shown in Figure 3.35. At 0.05 M EDTA, there was clear evidence of the presence of Cr^{III} in both soil solutions incubated at 70 °C for 2 hr. However, at this concentration of EDTA (0.05 M), the higher concentration of Cr^{III} compared to Cr^{VI} seen may be due to reduction of Cr^{VI} to Cr^{III}. This conclusion is supported by comparing the concentration of Cr species for unspiked SB and unspiked SR incubated with 0.01 M EDTA for two hours in which lower concentrations of Cr^{III} than Cr^{VI} were observed (Fig. 3.30). The other possibility is simply that the higher concentration of EDTA overcame the strength of humic-Cr^{III} bonding which resulted in the higher Cr^{III} concentration observed in Figure 3.35. It was found that the concentration of humic acid in SB was 6533 mg kg^{-1} double the concentration of humic acid in SR, (3354 mg kg^{-1}).

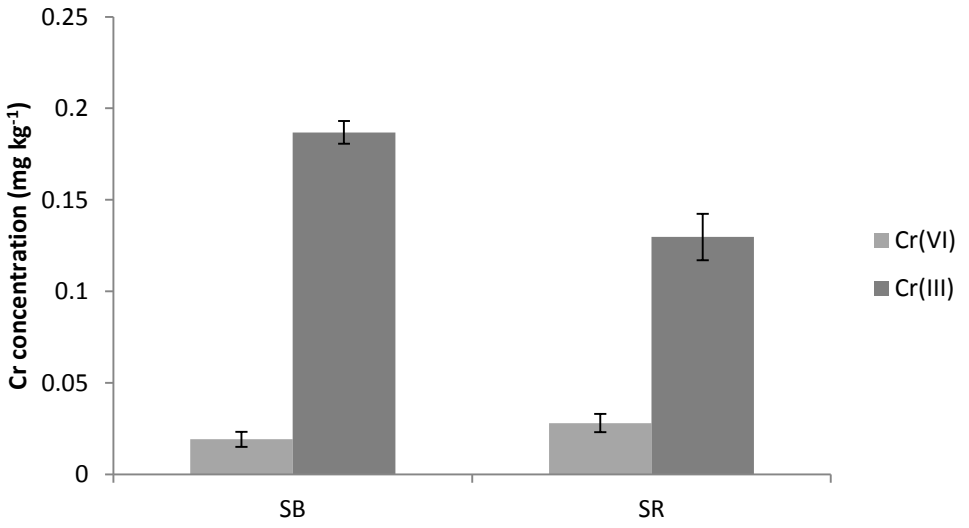


Figure 3.35 Concentration of Cr^{III} and Cr^{VI} (mg kg^{-1}) in 1 % TMAH extracts, from unspiked acidic woodland (SB) and calcareous arable (SR) soils, incubated at 70 ^{o}C for 2 hr with 0.05 M EDTA. Error bars show standard error of triplicate measurements of each soil extract.

The behaviour of the single and mixed Cr spiked SB and SR solutions with 0.05 M EDTA is shown in Figure 3.36. There was evidence of *oxidation* of Cr^{III} to Cr^{VI} when SR soil solution was spiked with 0.10 mg kg^{-1} Cr^{III}. However, more *reduction* of Cr^{VI} to Cr^{III} was observed when 0.10 mg kg^{-1} Cr^{VI} was spiked in SB as compared to SR (Fig. 3.36). In the mixed 0.10 mg kg^{-1} Cr spike, again a reduction of Cr^{VI} to Cr^{III} was observed in SB extracts.

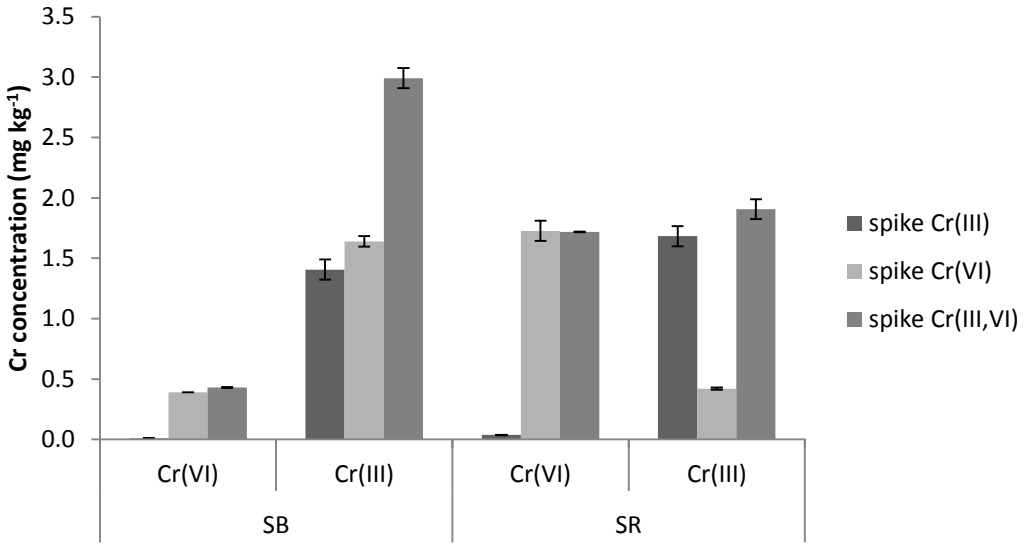


Figure 3.36 Concentration of Cr^{III} and Cr^{VI} (mg kg^{-1}) in 1 % TMAH extracts, from single 0.10 mg kg^{-1} Cr^{VI}, single 0.10 mg kg^{-1} Cr^{III} and mixed Cr (0.10 mg kg^{-1} and 0.10 mg kg^{-1} Cr^{III}) spiked acidic woodland (SB) and calcareous arable (SR) soils, incubated at 70 °C for 2 hr with 0.05 M EDTA. Error bars show standard error of triplicate measurements of each soil extract.

The net recovery of Cr^{III} and Cr^{VI} spikes in acidic and calcareous soil extracts in 0.05 M EDTA (Fig. 3.37) was confirmed by subtracting the unspiked concentrations of Cr^{III} and Cr^{VI} (Fig. 3.35) from the mixed spike Cr concentrations (Fig. 3.36). In the following discussion, data in Figure 3.37 is described from left to right along the X-axis. The SB(Cr^{VI}) data showed very poor recovery of Cr^{VI} and no evidence of any oxidation of Cr^{III} to Cr^{VI}. By contrast, the SB(Cr^{III}) chromatographs showed reasonable, but only partial, recovery of Cr^{III} where this species was added, and evidence of substantial reduction of Cr^{VI} to Cr^{III} where the oxidised form (Cr^{VI}) was added and where the mixed spike was used. In the latter case the 'recovery' was around 140% as a combination of added Cr^{III} and reduction of the Cr^{VI} to additional Cr^{III}. For the extraction of the alkaline soil (SR), again there was no evidence of oxidation of Cr^{III} to Cr^{VI} but good, and almost identical, recovery of added Cr^{VI} in the single and mixed spike. There was evidence of some reduction of Cr^{VI} to Cr^{III} since the recovery of added Cr^{VI} was around 80%. Reduction to Cr^{III} was supported by the recovery of some Cr^{III} (< 20%) where Cr^{VI} was added and the slightly enhanced Cr^{III} concentration in the mixed spike relative to the Cr^{III} single spike.

It is clear from this study that the redox behaviour of Cr added to alkaline soil extracts depends on the type of soil and its constituents. This is perhaps surprising because the solution composition of the extracting reagent (1 % TMAH and 0.05 M EDTA) might be expected to determine the fate of added Cr. Therefore, it is worth studying the interconversion of Cr species further by using isotopic dilution technique where 'blank subtraction' is not required and the distinction between native and added Cr is unequivocal. Apart from that, studying different Cr isotope, such as ^{50}Cr may understand the behaviour of ^{52}Cr.The ^{50}Cr can act as an audit trail of ^{52}Cr as the presence of ^{50}Cr is almost negligible in soil.

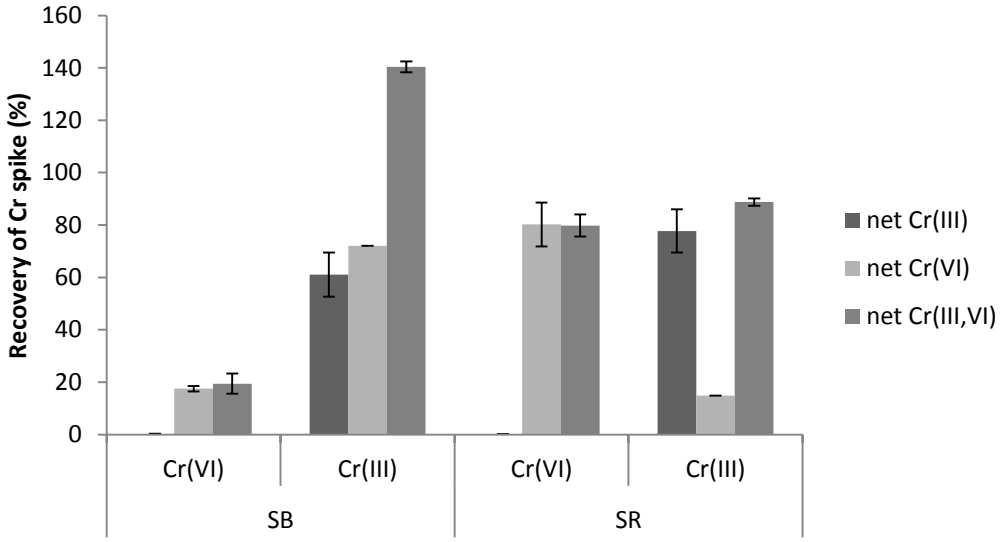


Figure 3.37 Percentage recovery for Cr^{III} and Cr^{VI} added to 1 % TMAH extracts of acid woodland (SB) and alkaline arable (SR) soils. Soil solutions were incubated in 0.05 M EDTA for 2 hr at 70^{o}C and spiked with single 0.20 mg kg^{-1} Cr^{III}, single 0.20 mg kg^{-1} Cr^{VI} or mixed 0.10 mg kg^{-1} Cr^{VI} and Cr^{III} standards. Error bars show standard error of triplicate measurements of each soil extract.

Speciation and fractionation of ^{50}Cr and ^{52}Cr species in Soil

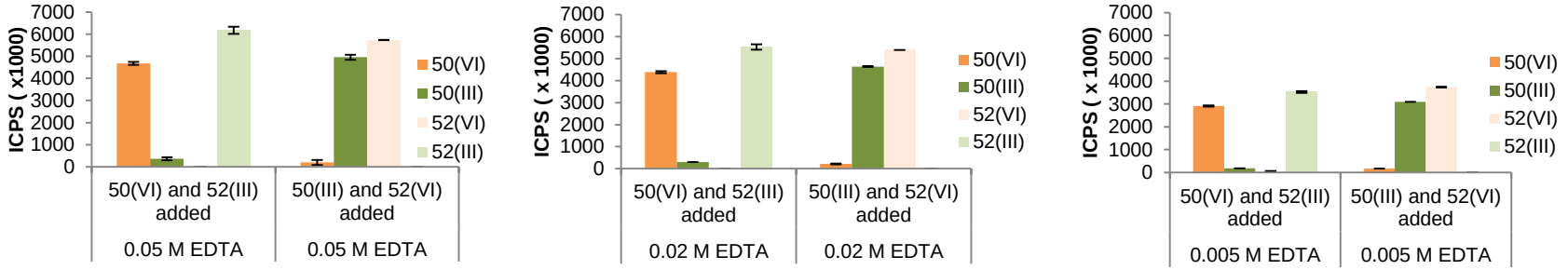
Throughout this work, a ^{50}Cr isotope (both Cr species) was used to assess the stability of Cr^{III} and redox reaction of ^{52}Cr species in the TMAH soil extract. Two sets of Cr^{III} complexation, pre and post complexation at different range of EDTA was used to mobilize Cr^{III} in the soil extract. Pre-complexation refers to complexing Cr^{III} with EDTA by heating (to accelerate the reaction) prior to using it as a tracer whilst post-complexation is where the Cr^{III} spike was added and then heating with the soil extract.

The sample preparation was according to section 3.5.5. Chromium-50 isotope was found stable (90-100% recovery) in 1% TMAH up to 5 days. The pre-complexation results are shown in Figure 3.38.The first three graphs from left to right refer to TMAH control solutions of a mixture of either 50 µg L^{-1} $^{50}Cr^{VI}$ and $^{52}Cr^{III}$, or 50 µg L^{-1} $^{50}Cr^{III}$ and $^{52}Cr^{VI}$ at different EDTA concentration (0.05 M, 0.02 M and 0.005 M). The results showed that Cr^{III} (for both isotopes) was well preserved by pre-complexation with ammonium EDTA. There was no effect of different concentration of EDTA to Cr^{VI}.

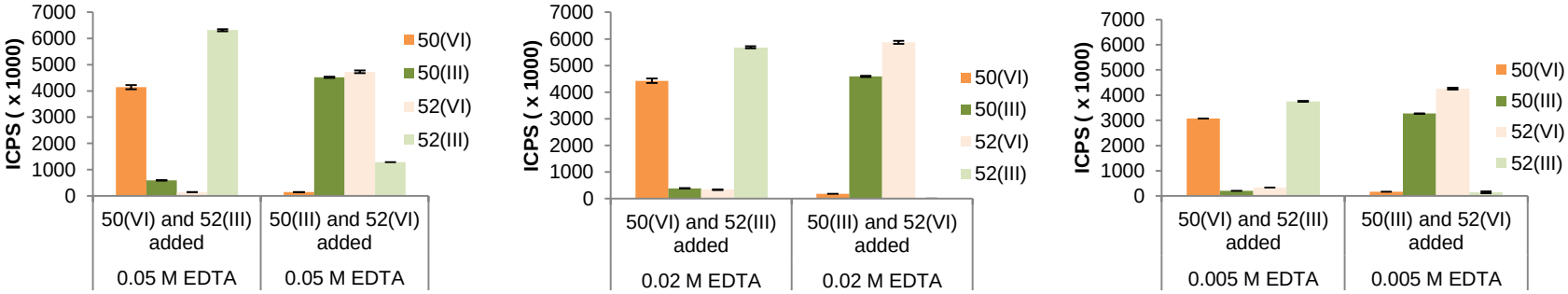
The second row of Figure 3.38 shows the pre-complexed experiment for a mixture of either $^{50}Cr^{VI}$ and $^{52}Cr^{III}$, or $^{50}Cr^{III}$ and $^{52}Cr^{VI}$ at different EDTA concentrations (0.05 M, 0.02 M and 0.005 M) in acidic (SB) soil extracts. Again, Cr^{III} (for both isotopes) was well preserved. A slight loss of Cr^{VI} (for both isotopes) was observed that may be due to reduction in 0.05 M EDTA. At 0.02 M EDTA, there was a slight oxidation of $^{52}Cr^{III}$ to $^{52}Cr^{VI}$.

In calcareous soil (SR) extracts (third row of Figure 3.38), both $^{52}Cr^{III}$ and $^{50}Cr^{III}$ remained stable and was well preserved when pre-complexed with EDTA. However, there is evidence of reduction of $^{52}Cr^{VI}$ to $^{52}Cr^{III}$ at all EDTA concentrations.

TMAH Control solutions_PRE a) b) c)



TMAH SB Soil Extracts_PRE a) b) c)



TMAH SR soil extract_PRE a) b) c)

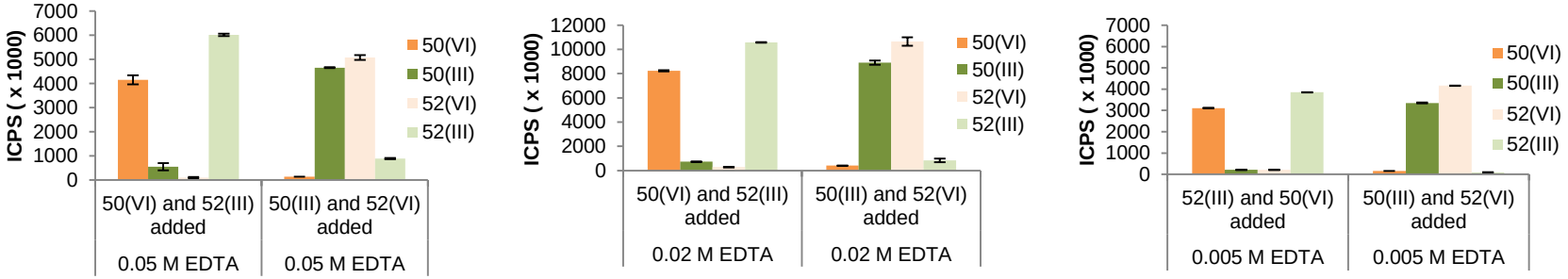


Figure 3.38 The HPLC-ICP-MS signal (ICPS) of a mixture of 50 µg L^{-1} of $^{52}Cr^{III}$ or $^{50}Cr^{III}$ precomplexation with different EDTA concentration; a)0.05 M, b) 0.02 M and c) 0.005 M with either 50 µg L^{-1} $^{50}Cr^{VI}$ or 50 µg L^{-1} $^{52}Cr^{VI}$ in control solutions, SB and SR soil extracts. Error bars show standard error of triplicate measurements for each control and soil solution

Results of systems where post-complexation was used are shown in Figure 3.39. The first row of graphs are for TMAH control solutions with a mixture of either 50 µg L^{-1} $^{50}Cr^{VI}$, and $^{52}Cr^{III}$ or 50 µg L^{-1} $^{50}Cr^{III}$ and $^{52}Cr^{VI}$ at different EDTA concentrations (0.05 M, 0.02 M and 0.005 M). The results showed some slight evidence of reduction of $^{50}Cr^{VI}$ and oxidation of $^{50}Cr^{III}$ but this was not apparent for $^{52}Cr^{VI}$ which may indicated minor change in the ^{50}Cr standard solution.

Different behaviour of Cr was observed in acidic SB soil extracts where post-complexation rather than pre-complexation was used. Slight evidence of Cr^{VI} reduction was seen in the intermediate EDTA concentration (0.02 M). Substantial oxidation and loss (adsorption) of Cr^{III} (both isotopes) was observed as the EDTA concentration was reduced from 0.05 M to 0.005 M.

In calcareous soil extracts, there was clear evidence of reduction at the highest EDTA concentration for both isotopes and as the EDTA concentration is reduced, there was substantial loss and oxidation of Cr^{III} (Fig 3.39)

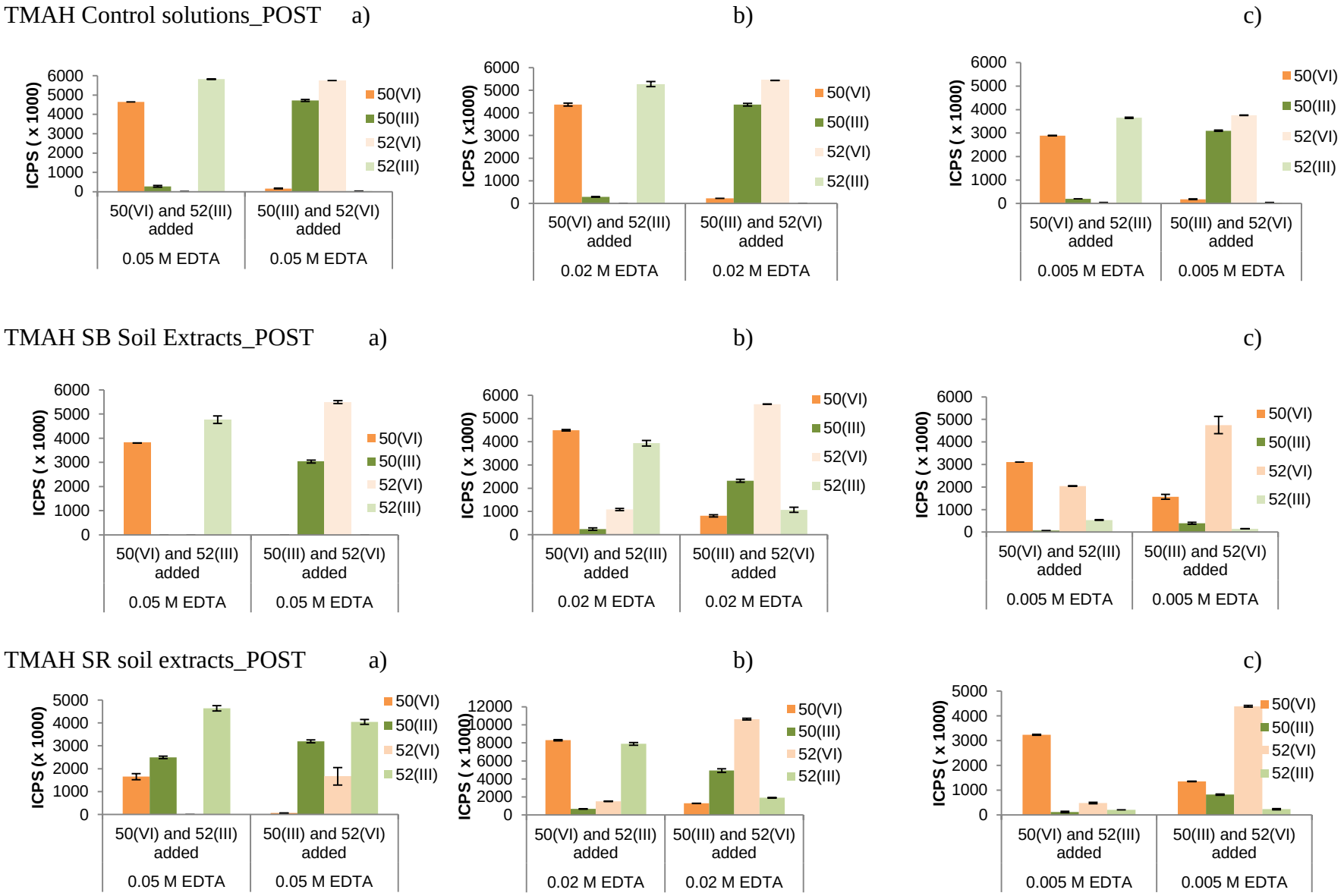


Figure 3.39 The HPLC-ICP-MS signal (ICPS) of a mixture of either 50 µg L^{-1} $^{50}Cr^{VI}$ and $^{52}Cr^{III}$, or 50 µg L^{-1} $^{50}Cr^{III}$ and $^{52}Cr^{VI}$ at different EDTA concentrations; a) 0.05 M, b) 0.02 M and c) 0.005 M in control solutions, SB and SR soil extracts. Error bars show standard error of triplicate measurements for each control and soil extract.

The results in previous section showed that the concentration of EDTA appeared to affect the extent of Cr^{VI} reduction to Cr^{III}, therefore a further experiment was undertaken to explore this aspect in greater detail. The concentrations of Cr presented throughout this section refer to a 1-in-10 dilution of the TMAH soil extract.

TMAH extracts of two contrasting topsoils, acidic (SB) and calcareous (SR) were again used to investigate solubility and speciation of $^{50}Cr^{III}$, $^{52}Cr^{VI}$, $^{50}Cr^{VI}$ and $^{52}Cr^{III}$ in soil extracts. A mix of different Cr isotopes species, either (i) 5 µg L^{-1} $^{50}Cr^{III}$ and 5 µg L^{-1} $^{52}Cr^{VI}$ or (ii) 5 µg L^{-1} $^{50}Cr^{VI}$ and 5 µg L^{-1} $^{52}Cr^{III}$, were spiked into the soil extracts. Post-complexation with six different concentrations of $(NH_4)_2$-EDTA; 0.0001, 0.0005, 0.001, 0.005, 0.01 and 0.05 M and incubation at 70°C for 2 hr were investigated. Samples were analyzed for ^{50}Cr and ^{52}Cr species by HPLC-ICP-MS (Section 2.3.2 & 2.3.3). In this experiment, the pH of the eluent was adjusted to 7 by titration with HNO_3; this had the effect of reducing the runtime to ~7 min per sample (Tanoshima et al., 2012).

Recovery of ^{52}Cr species in SB and SR soil extracts

The total Cr concentration in the TMAH extracts of both soils was determined by ICP-MS in CCT-KED mode (Section 2.4.2). The ^{52}Cr concentrations in the SB and SR soil extracts were 22.4 ± 0.30 µg L^{-1} and 21.6 ± 0.24 µg L^{-1} respectively.

Concentrations of Cr^{VI} and Cr^{III} species (for both Cr isotopes) in unspiked SB and SR soil solutions incubated with six different concentrations of EDTA are shown in Figure 3.40a and 3.40b. The concentrations of the identifiable inorganic Cr species were considerably less than the total Cr concentrations, especially in the case of the SR soil extract. This implies that the majority of Cr dissolved by the TMAH extraction procedure is probably humic-bound Cr^{III}; the remaining Cr is likely to be either inorganic Cr^{VI} or EDTA-complexed Cr^{III} extracted from dissolved humic acid. The presence of $^{52}Cr^{VI}$ in both soils was clearly indicated with a higher concentration in the acidic woodland soil (SB) than the calcareous soil (SR).

Cr^{VI} (for both isotopes) was the only inorganic species present in both soil solutions at lower concentrations of EDTA (0.0001 and 0.0005 M). At lower concentration of EDTA (0.0001M to 0.001 M) there was no significant different (ANOVA, p=0.528)

in the concentration of $^{52}Cr^{VI}$ in unspiked SB (Figure 3.40 a) which indicated that Cr^{VI} was a stable Cr species. The presence of $^{52}Cr^{III}$ was seen at EDTA concentrations >0.001 M and it increased with EDTA concentration. This would be expected if EDTA had extracted Cr^{III} from the relatively large pool of humic-bound Cr^{III} present in both soil extracts. However, at EDTA concentrations greater than 0.001 M the increase in Cr^{III} was concurrent with a decrease in Cr^{VI}, suggesting reduction of inorganic Cr^{VI} to $^{52}Cr^{III}$ may also have contributed to the increase in the EDTA-Cr^{III} complex measured.

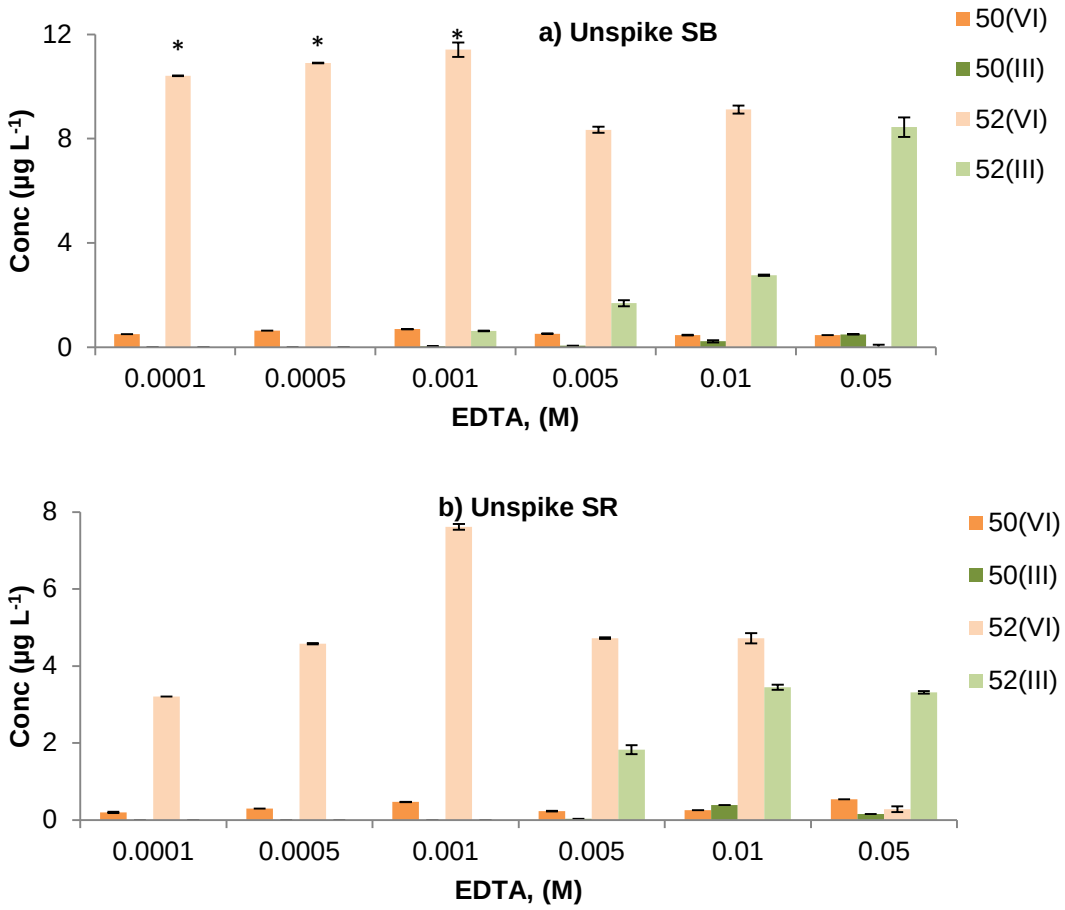


Figure 3.40 Concentrations (µg L^{-1}) of Cr^{VI} and Cr^{III} (for both Cr isotopes) in a) unspiked SB and b) unspiked SR soil solutions incubated with six different concentrations of EDTA (0.0001, 0.0005, 0.001, 0.005, 0.01 and 0.05 M) and heated at 70°C for 2 hr. Error bars show standard error of triplicate measurements of each soil extract. Stars above each bar indicated no significant different ($p<0.05$) on the results obtained.

The preservation of added ^{52}Cr species (5 µg L^{-1}) in the ^{52}Cr-spiked solutions, incubated with 0.0001 – 0.05 M EDTA for 2 hr, was assessed by subtracting the concentration of $^{52}Cr^{VI}$ and $^{52}Cr^{III}$ in unspiked SB or SR soil extracts from their mixed spiked concentration across the range of EDTA concentrations (Fig. 3.41). Good recovery (86-100%) of the $^{52}Cr^{VI}$ spike (Figs 3.41a and 3.41c) was observed for EDTA concentrations of 0.0001 - 0.01 but poor recovery of $^{52}Cr^{VI}$ was observed at 0.05 M EDTA due probably to reduction of $^{52}Cr^{VI}$ to $^{52}Cr^{III}$. This observation was inline with the previous finding on a reduction of $^{52}Cr^{VI}$ to $^{52}Cr^{III}$ in SB and SR soil at 0.05 M EDTA (Figs 3.35-3.38)

At lower concentrations of EDTA (0.0001 to 0.001 M), loss of the $^{52}Cr^{III}$ spike (Figs 3.41b and 3.41d) was observed due to oxidation of $^{52}Cr^{III}$ to $^{52}Cr^{VI}$. An increase in the preservation of $^{52}Cr^{III}$ was seen with increasing concentration of EDTA from 0.005 to 0.05 M. Approximately 50% of the 5 µg L^{-1} $^{52}Cr^{III}$ spike was recovered in the SR soil extract at EDTA concentrations of 0.01 and 0.05 M, but lower recovery (26%) was observed in the SB extract. However, there is some uncertainty regarding the origin of $^{52}Cr^{III}$ at higher EDTA concentrations in $^{52}Cr^{VI}$-spiked solutions; the EDTA-$^{52}Cr^{III}$ may arise from reduction of $^{52}Cr^{VI}$ or from extraction from humic-bound $^{52}Cr^{III}$. This uncertainty can only be confirmed unequivocally by looking at $^{50}Cr^{VI}$-spiked solutions.

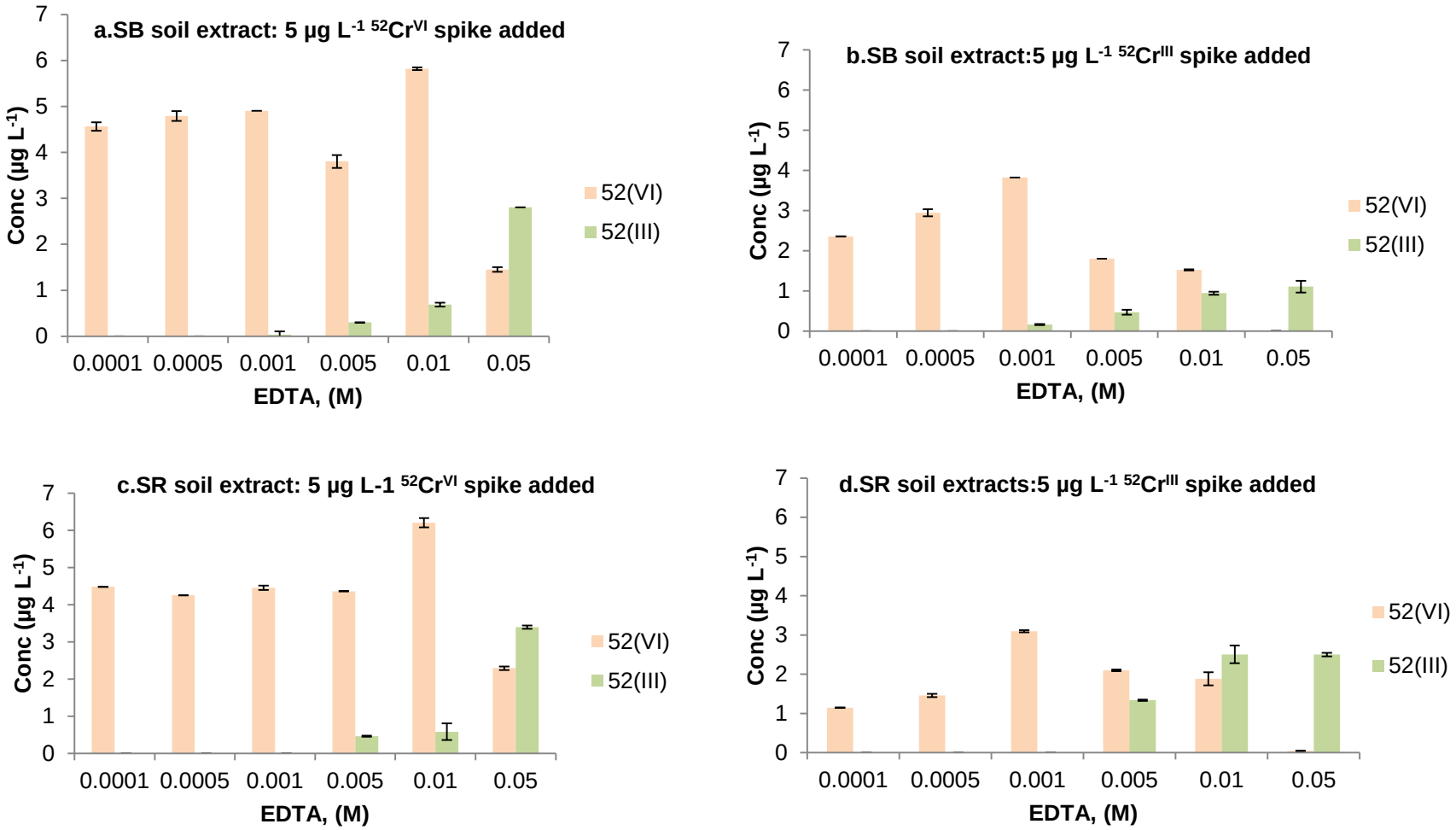


Figure 3.41 Concentration (µg L^{-1}) of $^{52}Cr^{VI}$ (orange) and $^{52}Cr^{III}$ (green) remaining in the solution after subtracting the concentration of $^{52}Cr^{VI}$ and $^{52}Cr^{III}$ in unspiked for SB and SR soil extracts from their mixed spiked concentration with 0.0001, 0.0005, 0.001, 0.005, 0.01 or 0.05 M EDTA. All of the samples were incubated at 70°C for 2 hr. Error bars show standard error of triplicate measurements of each soil extract.

Recovery of ^{50}Cr species in SB and SR soil extracts

The apparent redox transformation of ^{50}Cr in the alkaline soil extract at high EDTA concentrations was further investigated using extracts spiked with $^{50}Cr^{VI}$ to distinguish between Cr^{III} released from solubilised humic acid (almost exclusively ^{52}Cr) and created by reduction of inorganic $^{50}Cr^{VI}$. The preservation of ^{50}Cr species in the spiked ^{50}Cr species incubated with 0.0001 – 0.05 M EDTA for 2 hr was assessed by subtracting the concentration of $^{50}Cr^{VI}$ and $^{50}Cr^{III}$ in unspiked SB or SR soil extracts from their mixed spiked concentration across the range of EDTA concentrations (Fig. 3.42).

The two top graphs in Fig. 3.42 (3.42a and 3.42b) show the concentrations of $^{50}Cr^{VI}$ and $^{50}Cr^{III}$ remaining in the SB soil extract after spiking with either $^{50}Cr^{VI}$ (3.42a) or $^{50}Cr^{III}$ (3.42b); the lower graphs (3.42c and 3.42d) are data for the SR soil extract. Again, a good recovery (100-108 %) of the 5 µg L^{-1} spike of $^{50}Cr^{VI}$ in SB and SR soil extracts was observed for EDTA concentrations of 0.0001 – 0.01 M. Reduction of $^{50}Cr^{VI}$ to $^{50}Cr^{III}$ was clearly observed at 0.01 and 0.05 M EDTA in SB (Fig. 3.42a) and 0.05 M EDTA in SR (Fig. 3.42c) extracts, respectively. This confirms that the appearance of $^{52}Cr^{III}$ at high EDTA concentrations in the unspiked soil extracts (Figures 3.40a and 3.40b) was due to reduction of inorganic $^{52}Cr^{VI}$ rather than release humic bound Cr^{III} as an EDTA complex.

Loss of $^{50}Cr^{III}$ spike at low EDTA concentrations (0.0001 - 0.001 M) was observed in SB and SR soil extracts (Figs 3.42b and 3.42d). This was partly due to oxidation of $^{50}Cr^{III}$ to $^{50}Cr^{VI}$ under the alkaline conditions of the TMAH extract, as shown by the concurrent appearance of $^{50}Cr^{VI}$. However, transformation of $^{50}Cr^{III}$ to $^{50}Cr^{VI}$ was not quantitative and there was an overall loss of the $^{50}Cr^{III}$ – possibly to humic bound forms at low EDTA concentrations. There was possibly some evidence that increasing EDTA concentrations (in the range 0.0001 – 0.005 M) increased the amount of $^{50}Cr^{III}$ transformed to $^{50}Cr^{VI}$ rather than lost to humic-bound forms. At still higher EDTA concentrations (0.005 – 0.05 M) the preservation of $^{50}Cr^{III}$ was better, presumably due partly to more effective complexation but also because redox conditions apparently favoured Cr^{III} (Figs 3.42b and 3.42d). Approximately 68 % of $^{50}Cr^{III}$ was recovered in the SR soil solution from 5 µg L^{-1} $^{50}Cr^{III}$ spiked at 0.05 M EDTA. Compared to $^{52}Cr^{VI}$ (Figure 3.41), reduction of $^{50}Cr^{VI}$ to $^{50}Cr^{III}$ was observed at 0.01 M EDTA whereas for

$^{52}Cr^{VI}$, reduction occurred at concentrations 0.005 M EDTA. Some minor (apparent) differences between the isotopes might have been caused by the presence of a large concentration of humic bound $^{52}Cr^{III}$ (but not $^{50}Cr^{III}$).

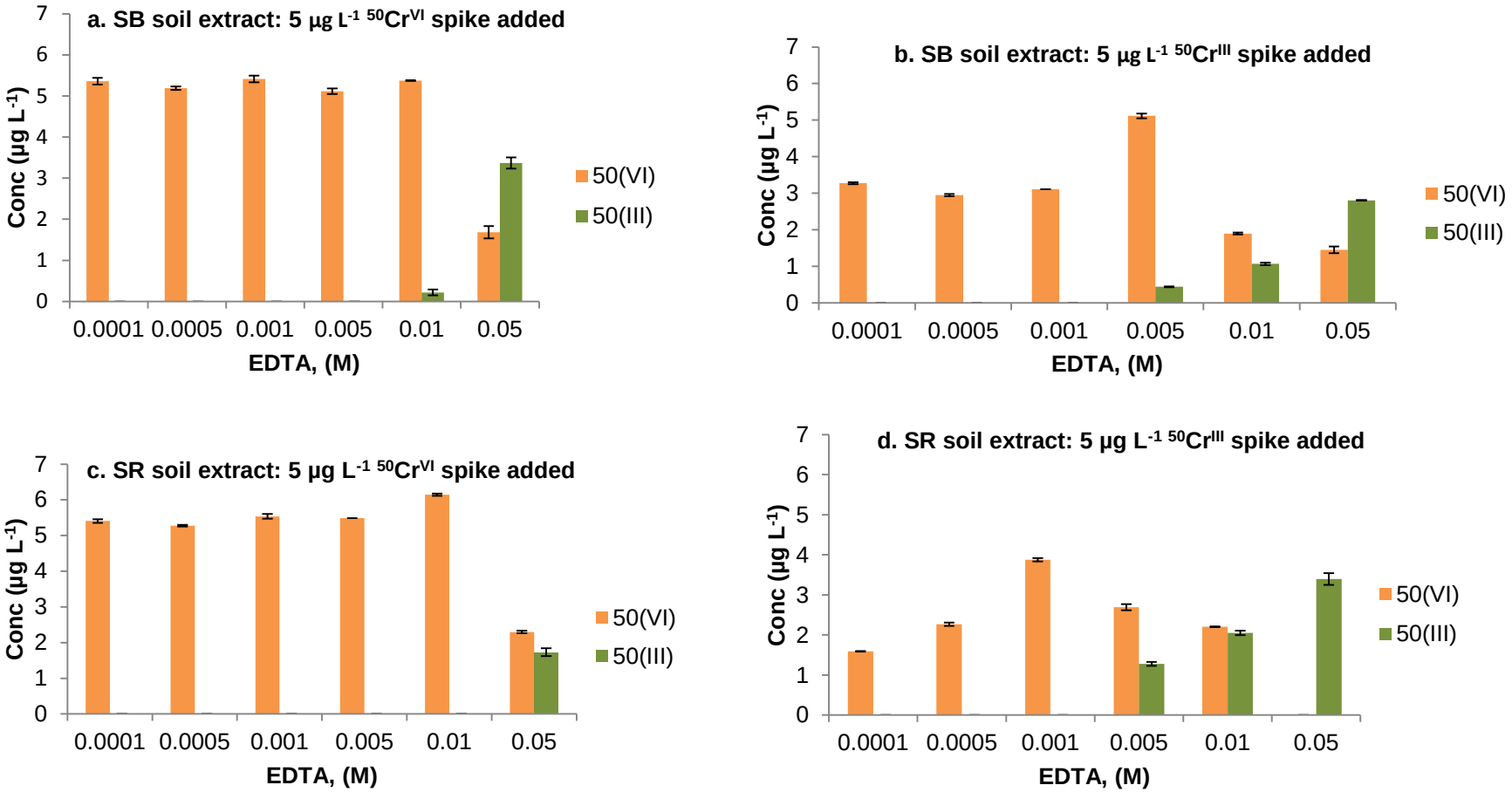


Figure 3.42 Concentration (µg L^{-1}) of $^{50}Cr^{VI}$ (orange) and $^{50}Cr^{III}$ (green) remaining in the solution after subtracting the concentration of $^{50}Cr^{VI}$ and $^{50}Cr^{III}$ in unspiked for SB and SR soil extracts from their 5 µg L^{-1} spiked concentrations with 0.0001, 0.0005, 0.001, 0.005, 0.01 or 0.05 M EDTA. All of the samples were heated for 2 hr at 70 °C.Error bars show standard error of triplicate measurements of each soil extract.

3.7 CONCLUSIONS

In this study, a reliable method for Cr speciation was developed using hyphenated HPLC-ICP-MS (in CCTKED mode). The use of CCT-KED is the preferred mode because it reduces polyatomic interferences on ^{52}Cr. However, although polyatomic interferences were successfully reduced by employing this approach, the sensitivity of ^{52}Cr was 10 times lower than in standard mode. Germanium and Rhodium were found to be the best internal standards in CCT-KED mode as their response was consistent and they were not affected by variation in solution matrix (water, 1% TMAH and 0.28 M Na_2CO_3/0.50 M NaOH). For ICP-MS, tetra-methyl ammonium hydroxide (TMAH) was found to be a preferable matrix compared to the EPA 3060A method (a combination of 0.28 M Na_2CO_3/0.50 M NaOH) due to Na^+ suppression and $^{40}Ar^{12}C$ formation that caused interferences with ^{52}Cr measurement in a carbonate matrix. A loss of Cr^{III} due to oxidation to Cr^{VI} in the carbonate matrix was observed when speciation work was undertaken. Therefore the carbonate extraction was found to be unsuitable in this study even though it works well for colorimetric methods although with much poorer sensitivity than that provided by ICP-MS. A good separation of Cr^{III} and Cr^{VI} in TMAH was initially obtained with the need for a complexing agent, EDTA and heating at 70˚C to mobilize Cr^{III}-EDTA in solution. However, there was a clear evidence of oxidation of Cr^{III} to Cr^{VI} in TMAH which was dependent on EDTA concentration. A progressive loss of Cr^{III} was observed at 10^{-2} to 10^{-4} M EDTA with greatest loss at the lowest EDTA concentrations (10^{-5} M). This observation is important because if alkaline extractions of soil promote oxidation of Cr^{III} to Cr^{VI} then this could result in overestimation of Cr^{VI} concentrations and exaggeration of risk.

Cr^{VI} was found to be a stable species in alkaline (TMAH) solution with negligible effects from EDTA concentration and heating. The mobile phase used for chromium speciation was a mixture of 50 mM TRIS, 40 mM NH_4NO_3 and 1 x 10^{-5} M ammonium EDTA at pH 7 with PRP-X100 anion exchange column (250 x 4.6 mm) as a stationary phase.

The efficiency of extraction of Cr^{III} and Cr^{VI} in TMAH extraction of acidic and calcareous soils incubated with 0.001 to 0.05 M EDTA at 70˚C was tested. The extraction time (1 to 3 hr) had little influence on the concentration of Cr species extracted. Changing the concentration of EDTA caused a loss of Cr^{III} at lower EDTA

concentrations (0.001 M) in both soil extracts with more loss in the acidic soil compared to the calcareous soil. Considerable concentrations of both Cr^{VI} and Cr^{III} were measured in the TMAH extraction, stabilised with 0.01 M EDTA, for both soils. Reduction of Cr^{VI} to Cr^{III} was observed in both the acidic and calcareous soils at higher EDTA concentrations (0.05 M). More reduction was observed in the acidic than in the calcareous soil which led to the assumption that the fate of Cr speciation in soil extracts depends on the soil and hence, presumably, its constituents. This assumption (whether the concentration of Cr species was affected by soil properties) will be further investigated in Chapter 4.

Further investigation on the effect of EDTA concentration (0.0001 to 0.05 M) on Cr^{VI} reduction to Cr^{III} in TMAH extracts of acidic and calcareous soil was carried out by using the enriched stable isotopes $^{50}Cr^{III}$ and $^{50}Cr^{VI}$ as tracers. This approach provided clearer evidence of the redox reaction of Cr in both soil extracts as the presence of native ^{50}Cr species in the soils was negligible. A loss of Cr^{III} in alkaline soil solution was found to be due to oxidation to Cr^{VI} and a marked preference for complexation with humic acid compared to EDTA. This reaction was observed at low EDTA concentrations (< 0.001 M) added to soil solution extracts and was validated by using the $^{50}Cr^{VI}$ species as a tracer. Higher EDTA concentrations (> 0.01 M) prevented oxidation of Cr^{III} to Cr^{VI} but could also result in reduction of Cr^{VI} to Cr^{III} in the TMAH soil extract. Therefore, the coexistence of humic-bound Cr^{III} or, to a lesser extent, EDTA-bound Cr^{III} extracted from dissolved HA should be considered as it causes an underestimation of Cr^{VI} concentration. It was also clear that the speciation analysis needed to be carried out as soon as possible after extraction for soil extracts with high organic matter to avoid the interaction of organic matter with Cr^{VI} that leads to reduction to Cr^{III}. The interaction of Cr^{VI} with soil organic ligands (humic acid) will be further investigated and discussed in Chapter 6.

4 SPECIATION AND FRACTIONATION OF CHROMIUM IN URBAN SOILS AND A SEWAGE SLUDGE DISPOSAL FARM

4.1 INTRODUCTION

As Cr^{VI} is the main concern due to its toxicity, the ability to predict Cr^{VI} from different Cr fractions in soil such as Cr extracted from soil using acid (Cr_{total}), alkaline (Cr_{TMAH}) or other soil properties would be useful as it would avoid the need for more complex speciation analysis. Xiao et al., (2013) found a narrow range of Cr^{VI} concentration from 0.21 to 0.53 mg kg^{-1} in different soil types in seven cities in China with the lowest in Ustic Cambosols and highest in Molisol. Han et al., (2004) found that the distribution of Cr^{VI} in contaminated soil was strongly affected by Cr loading level and time, with more Cr^{VI} being found in newly Cr contaminated soil due to a weaker binding with solid phase compared to the presence of Cr in native soil. Wittbrodt and Palmer (1996) found that the majority of organic fractions in most soil is humic substance that represent a significant reservoir of electron donor for reduction of Cr^{VI}.

In another attempt by Gray and McLaren (2006), they found that the concentration of Cr is affected by pH, total carbon content and oxalate-extractable Al. However, Grybos et al., (2007) found an unclear Cr mechanism, due to species transformation and/or solution speciation in solubilisation interrelated to metal/metalloid-adsorbing soil constituents. They found that an increase in the concentration of Cr was due to the reduction and dissolution of oxide surfaces. A study by Bhattacharya et al., (2005) on Cr fractionations in municipal solid waste compost found that Cr was associated with different soil fractions in the order: organic matter bound > Fe, Mn oxide bound > residual > exchangeable > carbonate > water soluble.

4.1.1 Objectives

The objectives were therefore;

- to quantify the concentrations of Cr^{VI} in a diverse range of soil ecosystems, including urban areas and a site of sewage sludge disposal to assess the controlling influence of soil properties
- to develop a model to predict Cr^{VI}_{TMAH} based upon soil properties.

The relationships between different fractions of Cr ($Cr_{total,}$ Cr_{TMAH} and Cr^{VI}_{TMAH}) and soil properties such as pH, LOI, Mn_{total} and Fe_{total} were investigated. In order to predict the concentration of Cr^{VI}_{TMAH}, two multiple regression models were developed based on Cr_{total} (which has been used by geochemical modelling) or Cr_{TMAH} with or without soil properties using all four datasets.

4.2 MATERIALS AND METHODS

4.2.1 Sampling site description

Soils (n=124) were obtained from four different sites in the UK; Nottingham (NG), Wolverhampton (WV), London (LN) and Stoke Bardolph sewage sludge disposal farm near Nottingham (StB). The fifty surface soils from Wolverhampton and Nottingham have quite different industrial legacies. Site selection was based on the distribution pattern of total soil metal concentrations relating to past and present industrial and domestic land uses. Brownfield sites, rail transport sidings, urban roadsides, waste disposal facilities, recreation areas, garden, urban nature reserves and woodland and parkland areas are all represented. Data relating to these soils had been previously published in an investigation of metal speciation and bioavailability in risk assessment (Thornton et al., 2008). The soils were sub-sample from the urban geochemistry division of the British Geological Survey (BGS) sample archive (Thornton et al., 2008) and some of the samples were very limited to proceed with aluminium determination.

Thirty six London topsoils (5 to 20 cm) were sub-sampled from the Geochemical Baseline Survey of the Environment (GBASE) “London Earth” sample archive of the British Geological Survey (BGS) (Johnson et al., 2005). These soils cover 13 different land uses and a range of soil properties including pH, organic matter content and total Pb content.

Thirty eight soils were sampled at the sewage reprocessing facility at Stoke Bardolph (52˚57’0’’N -1˚2’0’’W). The farm area is approximately 700 ha (60 fields) and has been managed within the guideline governing ‘dedicated sites’ set out by the 1989 Sludge Regulations. Sewage sludge has been applied at different rates in different

areas for approximately 100 years. Currently about 80% of this land area is used for growing pasture and fodder crops, mainly forage maize and winter wheat.

4.2.2 Soil characterization

200 g of soil samples were air dried and sieved to <2mm. Soil pH values were measured in 0.01 M $CaCl_2$ soil suspension (1:10 soil:solution ratio). Soil properties were measured in triplicate including; loss on ignition (LOI), total Fe (Fe_{total}) and total Mn (Mn_{total}) were determined by extraction with a mixture of sodium dithionate, sodium citrate and sodium bicarbonate as according to Section 2.2.5 and total Cr (Cr_{total}) was undertaken with acid digestion (according to Section 2.2.6). The extraction of Cr and Cr^{VI} from soil with TMAH, (Cr_{TMAH} and Cr^{VI}_{TMAH}) was undertaken as described in Section 2.2.7 and the analysis was undertaken as described in Section 2.3.1 and 2.3.2.

4.2.3 Statistical analysis

Statistical analysis was carried out using Minitab version 16.2.2. Some parameters e.g. Cr_{TMAH}, Cr_{total}, Mn_{total}, and Fe_{total} were log-transformed to normalize their distribution. Correlation matrices were prepared to consider statistical significance of soil parameters at $p<0.05$, $p<0.01$ and $p<0.001$.

4.2.4 Modelling of Cr^{VI} using total Cr and Cr_{TMAH} with soil properties

A multiple regression model was used to predict Cr^{VI}_{TMAH} from Cr_{total}, $Cr_{TMAH,}$ pH, LOI, Fe_{total} and Mn_{total} as shown in Equation 4.1.

$$Ln\ (Cr^{VI}_{TMAH}) = Ln\ [\ k_0 + k_1\ (pH) + k_2\ (LOI) + k_3\ (Cr_{total}) + k_4\ (Fe_{total}) + k_5\ (Mn_{total})\ ]$$

Eq 4.1

Where the regression coefficients k_0, k_1, etc can be positive or negative. The overall fit for all measured and modelled Cr^{VI} was determined based on correlation coefficient, R and the residual standard deviation (RSD). The intention in developing this model is to see if it is possible to predict the concentration of Cr^{VI} in the form of Cr^{VI}_{TMAH} in soil without a requirement to undertake speciation analysis. Aluminium was not

considered as one of the soil variables due to insufficient sample to proceed with the analysis.

4.3 RESULTS AND DISCUSSION

4.3.1 Soil characterisation

Wolverhampton (WV)

Soil properties for the WV soils are summarized in Figure 4.1 and Table 4.1. Soil pH in $CaCl_2$ covered the range 3.61 to 7.36 and organic matter (% LOI) was between 3.23 % and 51.3 %. Cr_{total} ranged from 21.6 to 552 mg kg^{-1} with the majority of soils < 85 mg kg^{-1} with an exception of two soils with 108 mg kg^{-1} and 552 mg kg^{-1} Cr_{total}. Cr_{TMAH} ranged from 0.128 and 28.3 mg kg^{-1} with a mean value of 3.53 mg kg^{-1}. Cr^{VI}_{TMAH} was typically < 40% of Cr_{TMAH} and ranged from 0.095 to 2.05 mg kg^{-1} with two soils higher than this range (8.41, 18.6 mg kg^{-1}). Fe_{total} and Mn_{total} ranged from 9.70 to 66.8 g kg^{-1} (mean 24.8 g kg^{-1}) and 162 to 3030 mg kg^{-1} (mean 617 mg kg^{-1}), respectively.

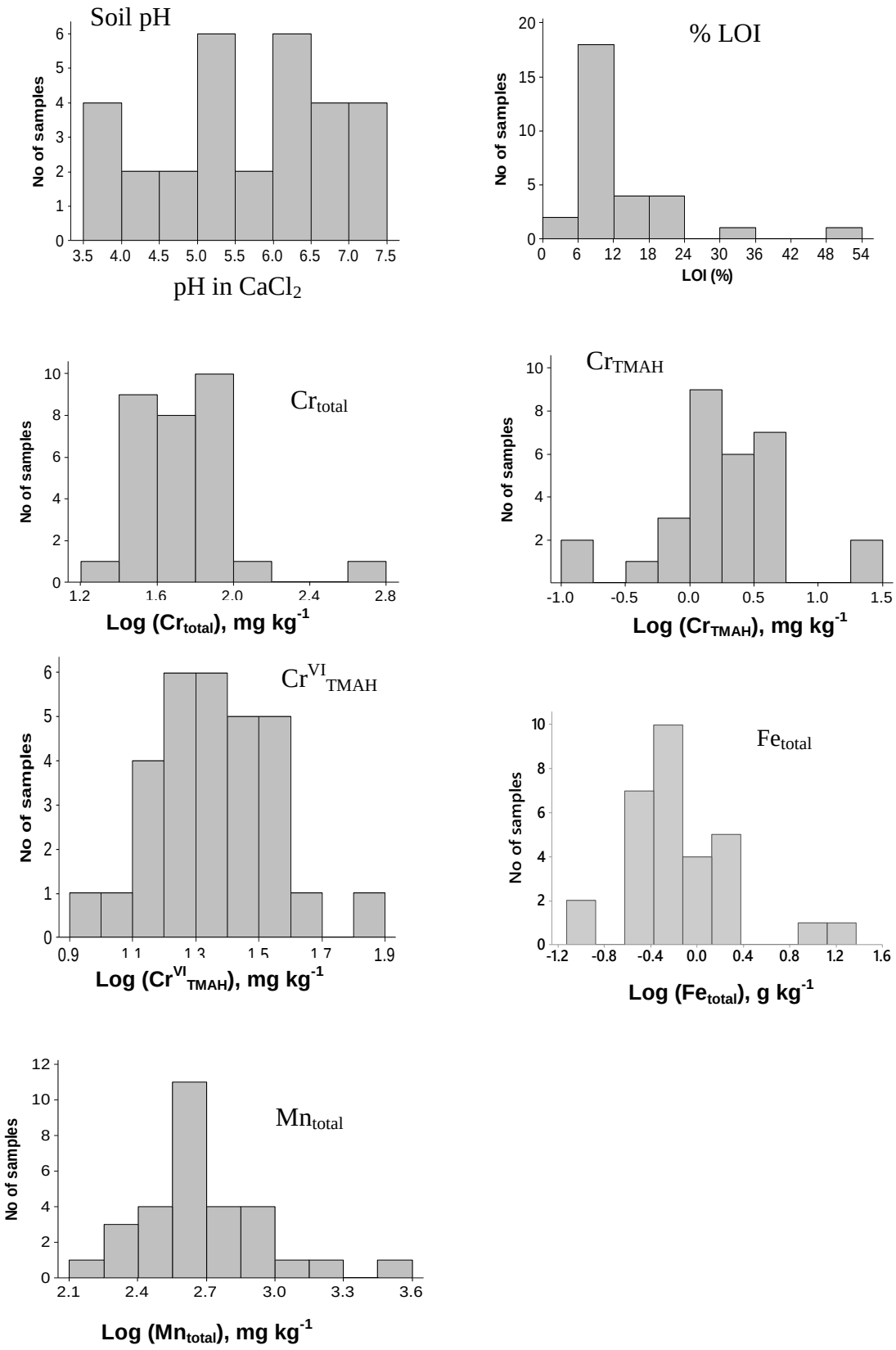


Figure 4.1 Soil characteristics of WV soils shown as histograms for soil pH, % LOI Log(Cr_{total}) (mg kg^{-1}), Log(Cr_{TMAH}) (mg kg^{-1}), Log(Cr^{VI}_{TMAH}) (mg kg^{-1}), Log(Fe_{total}) (g kg^{-1}) and Log(Mn_{total}) (mg kg^{-1}).

Table 4.1 Summary of measured soil properties and land use, for the Wolverhampton (WV) soils (n=30). Values in brackets show the number of replicates for determination of each value.

Samples	pH (n=1)	LOI % (n=1)	Cr_{total} mg kg^{-1} (n=3)	Cr_{TMAH} mg kg^{-1} (n=3)	Cr^{VI}_{TMAH} mg kg^{-1} (n=3)	Fe_{total} g kg^{-1} (n=3)	Mn_{total} mg kg^{-1} (n=3)	Landuse
WV1	4.43	7.69	26.0	1.30	0.48	12.8	222	Grassland (Golfcourse)
WV2	3.61	6.02	21.6	3.06	1.14	13.3	359	Grassland (Golfcourse)
WV3	5.83	22.5	70.8	4.51	0.44	28.5	1060	Park
WV4	5.55	7.14	27.3	2.98	0.65	18.4	397	Grassland (open land)
WV5	6.11	17.4	108	3.67	1.53	35.7	775	Old industrial (gas work)
WV6	6.48	17.5	552	28.3	18.6	66.8	1920	Wasteland (Old landfill)
WV7	6.99	10.3	76.0	3.85	0.86	43.1	945	Grassland
WV8	5.45	17.0	57.7	1.72	0.63	29.3	775	Woodland
WV9	5.26	8.25	31.4	1.65	0.53	22.2	512	Grassland
WV10	6.74	10.9	69.4	4.17	2.02	34.0	926	Old industrial (tyre factory)
WV11	4.22	8.78	57.6	3.44	0.83	17.0	321	Domestic garden
WV12	5.42	6.21	26.4	1.25	0.37	13.0	366	Grassland
WV13	5.23	9.40	39.4	2.47	0.71	16.7	484	Park
WV14	4.76	51.3	49.4	2.49	0.84	29.6	574	Nature reserve (railway)
WV15	6.30	20.0	51.2	2.04	0.50	31.7	3030	Nature reserve (railway)
WV16	3.83	30.3	66.9	3.78	2.05	9.70	162	Vegetated colliery spoil heap
WV17	5.38	8.08	33.4	1.64	0.47	15.9	421	Grassland
WV18	4.55	11.6	53.6	18.1	8.41	22.4	451	Grassland
WV19	5.31	12.6	78.0	0.91	0.39	18.8	309	Grassland
WV20	3.95	10.2	25.8	3.51	1.95	10.8	206	Grassland
WV21	6.34	7.43	82.4	0.15	0.12	23.8	397	Brownfield
WV22	7.36	6.24	72.3	0.13	0.10	21.5	437	Brownfield
WV23	7.02	3.23	65.3	1.39	0.73	20.8	422	Brownfield
WV24	6.95	7.67	52.8	1.25	0.70	21.0	495	Brownfield
WV25	3.96	18.7	81.1	3.16	1.70	28.4	562	Park
WV26	6.39	6.23	31.1	0.91	0.25	15.1	295	Grassland
WV27	7.04	5.73	55.6	0.94	0.27	38.8	684	Brownfield
WV28	7.12	7.84	48.9	0.53	0.25	28.3	475	Brownfield
WV29	6.70	21.5	69.2	1.37	0.38	37.6	180	Deciduous woodland
WV30	6.40	7.40	30.4	1.13	0.32	17.6	332	Park

Correlations between soil properties are shown in Table 4.2. A weak correlation was observed between Fe_{total} and LOI (R=0.503, p<0.01) as well as Mn_{total} and LOI (R=0.508, p<0.01) and Fe_{total} and Mn_{total} were correlated with each other (R=0.935, p<0.001). A significant correlation exists between Cr^{VI}_{TMAH} and Cr_{TMAH} (R=0.922, p<0.001). As shown in Chapter 3, TMAH extraction of soil mobilizes Cr^{VI} and Cr^{III}-HA and this explains this relationship. Significant correlations were also observed between Cr_{total} and Cr_{TMAH} (R=0.859, p<0.001) and Cr_{total} and Cr^{VI}_{TMAH} (R=0.875, p<0.001) which suggested that TMAH extaction can be used as a surrogate for acid digestion.

Table 4.2 Correlation matrix for measured soil properties in WV soils; p-values are shown in brackets with significant correlations in bold type.

Soil variable	**pH**	**LOI**	**Cr_{TMAH}**	**Cr^{VI}_{TMAH}**	**Cr_{total}**	**Fe_{total}**
LOI	-0.010					
	0.957					
Cr_{TMAH}	-0.011	0.271				
	0.954	0.147				
Cr^{VI}_{TMAH}	0.027	0.313	**0.922**			
	0.886	0.092	*(<0.001)*			
Cr_{total}	-0.047	**0.379**	**0.859**	**0.875**		
	0.806	*(<0.05)*	*(<0.001)*	*(<0.001)*		
Fe_{total}	**0.388**	**0.503**	-0.177	-0.108	0.101	
	(<0.05)	*(<0.01)*	0.350	0.569	0.596	
Mn_{total}	0.344	**0.508**	-0.175	-0.080	0.142	**0.935**
	0.063	*(<0.01)*	0.354	0.675	0.453	*(<0.001)*

Nottingham (NG)

Soil properties are summarized in Figure 4.2 and Table 4.3 for the NG soils. Soil pH in $CaCl_2$ ranged from 3.98 to 7.18 with 75 % of the soil having a pH of more than 6. About 20% of NG samples were collected from brownfield sites with a narrow pH range from 6.1 to 7.2 but a wide range of % LOI (5 - 26.9%). The % LOI for the whole dataset ranged from 3.12 % to 26.9 %. Cr_{TMAH} ranged from 0.11 to 10 mg kg^{-1} with two soils higher than this range (403, 497 mg kg^{-1}) with a mean value of 47.8 mg kg^{-1}. Cr^{VI}_{TMAH} was typically <40% of Cr_{TMAH} and ranged between 0.21 and 148 mg kg^{-1}. Cr_{total} ranged from 23.7 to 83.7 with four soils higher than this range (172, 252, 870, 1370 mg kg^{-1}. Fe_{total} and Mn_{total} ranged from 16.1 to 79.9 g kg^{-1} and 294 to 2300 mg kg^{-1}, respectively. The highest concentration of Fe_{total} and Mn_{total} were found in NG11 and NG12 which were samples from soil near a disused valve factory and both of these elements were used as raw materials during the production of valve.

Samples NG14 and NG15 had the highest concentrations of Cr_{total}, Cr_{TMAH} and Cr^{VI}_{TMAH} and were collected from Stoke Bardolph sewage farm and a more detailed study on this site will be considered later in this chapter.

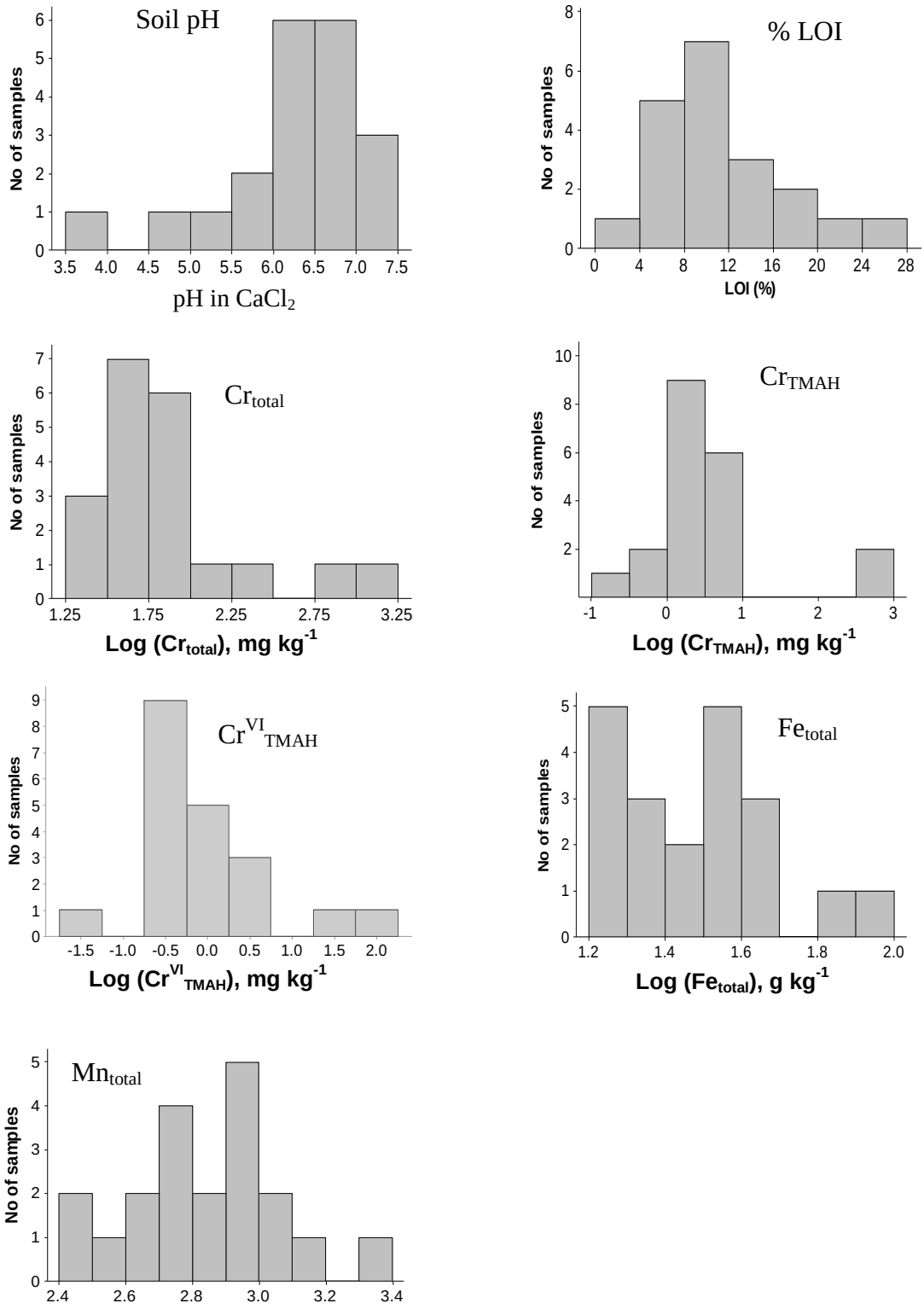


Figure 4.2 Soil characteristics of NG soils shown as histograms for soil pH, % LOI, Log(Cr_{total}) (mg kg^{-1}), Log(Cr_{TMAH}) (mg kg^{-1}), Log(Cr^{VI}_{TMAH}) (mg kg^{-1}) Log(Fe_{total}) (g kg^{-1}) and Log(Mn_{total}) (mg kg^{-1}).

Table 4.3 Summary of measured soil propoerties and land use for Nottingham (NG) soils (n=20). Values in brackets show number of replicates for determination of each value.

Samples	pH (n=1)	LOI % (n=1)	Cr_{total} mg kg^{-1} (n=3)	Cr_{TMAH} mg kg^{-1} (n=3)	$Cr^{VI}{}_{TMAH}$ mg kg^{-1} (n=3)	Fe_{total} g kg^{-1} (n=3)	Mn_{total} mg kg^{-1} (n=3)	Land use
NG1	4.51	11.8	69. 8	1.75	0.43	38	1080	Grassland (football field)
NG2	5.23	9.04	23.7	1.51	0.55	16.1	588	Churchyard
NG3	6.48	10.5	70.8	5.46	1.64	35.8	905	School
NG4	6.04	8.39	53.1	2.63	0.39	21.8	441	Domestic garden
NG5	6.83	5.85	38.1	1.37	0.38	19.0	460	Domestic garden
NG6	6.75	13.9	55.5	3.32	1.66	46.6	785	Railway
NG7	6.71	8.00	46.6	2.33	0.29	27.5	739	Grassland (imported)
NG8	3.98	16.2	83.7	2.33	0.97	42.5	954	Grassland (racecourse)
NG9	5.98	8.20	30.4	1.90	0.72	17.3	332	Colliery spoil heap
NG10	6.39	10.3	34.2	8.83	1.96	21.3	616	Colliery spoil heap
NG11	7.02	13.2	172	9.57	5.55	63.2	1440	Disused valve factory
NG12	7.04	19.1	252	5.34	1.83	79.9	2300	Disused valve factory
NG13	6.87	3.12	25.6	0.11	0.03	17.5	294	Grassland (amenity)
NG14	6.18	20.0	1370	497	148	20.9	585	Sewage farm
NG15	5.70	13.6	780	403	46.7	19.6	310	Sewage farm
NG16	7.18	7.53	55.4	2.32	0.21	32.3	846	Brownfield
NG17	6.07	26.9	60.6	1.37	0.33	45.1	954	Brownfield
NG18	6.66	7.29	57.1	3.56	1.54	30.8	909	Brownfield
NG19	6.62	7.68	59.2	0.78	0.24	34.2	1050	Brownfield
NG20	6.33	5.33	49.4	0.64	0.29	34.6	625	Brownfield

Correlations between soil properties are shown in Table 4.4. A significant correlation (with R >0.9, p<0.001) was observed between Cr^{VI}_{TMAH}, Cr_{TMAH} and Cr_{total}. LOI correlated significantly with all Cr fractions as well as Fe_{total} (p<0.05). However, there was no correlation between all Cr fractions and pH with Fe_{total} and Mn_{total}. Some earlier findings observed that Fe^{II} could enhanced the removal of aqueous Cr^{III} by the formation of $(Fe,Cr)(OH)_3$ precipitate (Sass and Rai, 1987; Chon et al., 2006).

Table 4.4 Correlation matrix for measured soil properties in NG soils; p-values are given in brackets with significant correlations in bold.

Soil variable	pH	LOI	Cr_{TMAH}	Cr^{VI}_{TMAH}	Cr_{total}	Fe_{total}
LOI	-0.228					
	0.333					
Cr_{TMAH}	-0.072	**0.460**				
	0.763	*(<0.05)*				
Cr^{VI}_{TMAH}	-0.093	**0.493**	**0.965**			
	0.698	*(<0.05)*	*(<0.001)*			
Cr_{total}	-0.033	**0.559**	**0.869**	**0.859**		
	0.891	*(<0.05)*	*(<0.001)*	*(<0.001)*		
Fe_{total}	0.125	**0.488**	-0.048	0.041	0.215	
	0.600	*(<0.05)*	0.841	0.863	0.362	
Mn_{total}	0.084	0.436	-0.034	0.046	0.157	**0.894**
	0.725	0.054	0.888	0.847	0.507	*(<0.001)*

London (LN)

London (LN) soils also covered a wide range of soil properties and land use, summarized in Figure 4.3 and Table 4.5. Soil pH ranged from 3.29 to 7.68, with 95% soil pH >6. Compared to WV and NG soils, a lower range of % LOI was observed (4.25 to 17.5%). Cr_{total} ranged from 50.4 to 100 mg kg^{-1} with 5 soils higher than this range (157, 159, 168, 203, 277 mg kg^{-1}) with a mean value of 89.1 mg kg^{-1}. Fe_{total} and were in the range of 10.1 to 67.3 g kg^{-1} with a mean value of 31.8 g kg^{-1}. Mn_{total} ranged from 325 to 1310 mg kg^{-1} with an exception of one soil lower than this range (85 mg kg^{-1}). Compared to WV and NG soils, the London soils cover a smaller range

of Cr_{TMAH} (10.75 – 13.3 mg kg^{-1}) and Cr^{VI}_{TMAH} (0.41 – 11 mg kg^{-1}). Many of the soils (44%) were sampled from urban domestic gardens (Table 4.5) which would not be expected to be contaminated with anthropogenic Cr. A possible explanation for these urban soil is because it has low level of Cr^{VI}_{TMAH} loading with 74% having Cr^{VI}_{TMAH} concentration less than 2.0 mg kg^{-1}. Thus, it is likely that a large proportion of Cr in these soils is geogenic, bound with primary minerals and relatively inert.

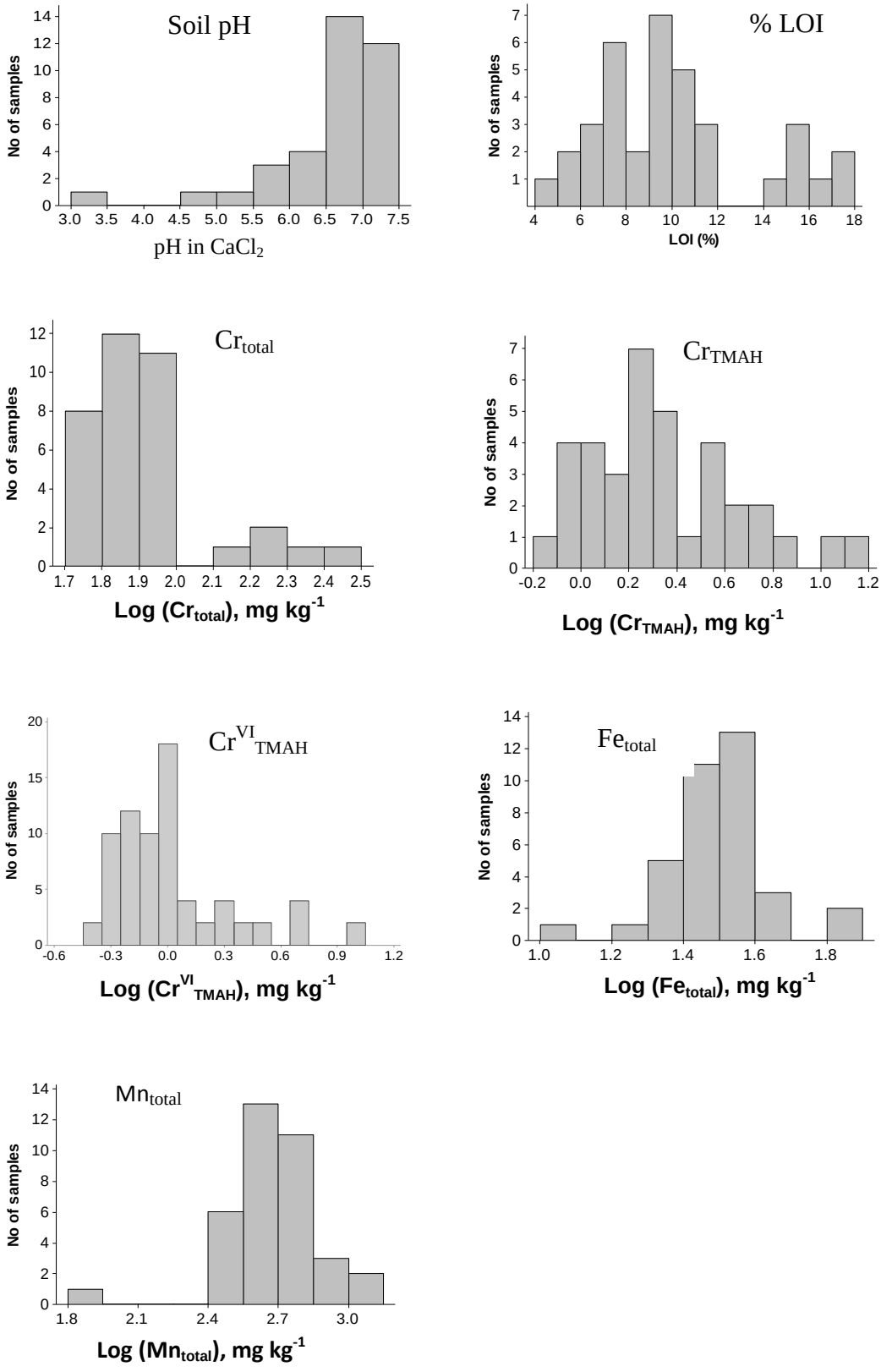


Figure 4.3 Soil characteristics of LN soils shown as histograms for soil pH, % LOI, Cr_{total} (mg kg^{-1}), Cr_{TMAH} (mg kg^{-1}), $Cr^{VI}{}_{TMAH}$ (mg kg^{-1}), Mn_{total} (mg kg^{-1}) and Fe_{total} (g kg^{-1}).

Table 4.5 Summary of measured soil properties and land use, for London (LN) soils (n=36). Values in brackets show the number of replicates for determination of each value.

Sample	pH (n=1)	LOI % (n=1)	Cr_{total} mg kg^{-1} (n=3)	Cr_{TMAH} mg kg^{-1} (n=3)	Cr^{VI}_{TMAH} mg kg^{-1} (n=3)	Fe_{total} g kg^{-1} (n=3)	Mn_{total} mg kg^{-1} (n=3)	Landuse
641188	7.30	9.33	277	10.9	5.04	36.9	627	Urban open space
641198	7.14	6.66	58.4	1.13	0.54	26.0	349	Commercial and residential
641201	6.93	10.0	65.9	1.80	0.75	29.6	449	Commercial and residential
641221	6.98	9.73	52.4	2.72	0.97	36.0	527	Recreational area
650221	7.07	7.08	73.4	3.20	0.95	33.0	581	Commercial and residential
650324	6.98	15.4	83.4	0.88	0.50	35.0	488	Commercial and residential
650773	7.24	9.50	64.9	0.83	0.41	32.0	403	Grass moor
650803	6.74	7.52	76.2	2.21	0.92	29.3	604	Urban domestic garden
650903	5.98	11.5	90.6	1.07	0.65	32.4	434	School
650930	6.90	7.34	99.8	0.75	0.47	43.4	418	Urban Domestic Garden
650932	5.57	10.4	54.8	1.63	0.8	22.1	341	Major road/verge
651006	6.69	6.71	66.7	1.43	0.75	26.1	643	Major road/verge
651013	6.92	5.95	65.3	3.34	0.95	32.3	519	Deciduous woodland
651133	5.80	9.22	73.7	1.76	0.69	26.7	349	Park
651344	7.21	8.27	85.7	2.36	0.96	33.3	449	Domestic garden
651438	5.00	6.78	68.4	1.33	0.85	25.9	713	Grass moor
651654	3.29	9.17	60.1	4.06	1.95	10.1	85.0	Deciduous woodland
651954	6.75	7.89	168	7.37	2.62	38.9	658	Urban domestic garden
652570	6.74	16.6	159	2.01	1.08	44.8	744	Urban domestic garden
652631	7.23	17.5	91.2	6.03	5.11	39.9	635	Urban domestic garden
653407	6.29	14.0	62.8	3.18	1.66	21.7	573	Urban domestic garden
653588	4.95	11.3	50.4	0.84	0.5	16.6	356	Urban domestic garden
654281	6.62	15.5	66.0	1.65	0.87	27.6	503	Urban domestic garden
654464	7.20	4.25	84.4	2.14	1.06	31.1	403	Major road/verge
654542	7.12	17.3	83.8	1.04	0.64	33.1	457	Urban domestic garden
654699	7.25	7.32	157	4.24	1.23	67.3	1310	Urban domestic garden
654950	6.75	15.5	59.4	1.55	1.05	21.6	341	Urban domestic garden
654976	7.05	7.65	203	5.69	2.02	65.1	1130	Commercial and residential
655522	6.78	9.67	84.0	2.07	1.08	26.4	472	Urban domestic garden
655783	7.07	9.96	55.9	1.01	0.47	20.8	767	Urban domestic garden
655952	6.00	10.1	90.7	3.80	3.10	26.4	325.3	Urban domestic garden
656000	6.44	10.3	85.0	13.3	11.1	32.3	457	Urban domestic garden
656271	7.31	10.9	74.2	1.80	1.20	27.2	426	Hospital Ground
656359	6.61	5.74	64.6	1.70	0.59	24.3	426	Urban open space
656463	6.03	8.21	69.9	1.97	0.62	34.8	643	Park
656571	6.90	11.5	81.9	0.97	0.56	33.8	341	Urban domestic garden

Correlations between soil properties are given in Table 4.6. Significant correlations were observed between Cr^{VI}_{TMAH} and Cr_{TMAH} (R=0.910, p<0.001) and between Cr_{total} and Cr_{TMAH} (R=0.588, p<0.001), Fe_{total} with pH (R=0.521, p<0.001) and Fe_{total} with Cr_{total} (R=0.653, p<0.001). Mn_{total} was also correlated with Fe_{total} (0.782, p<0.001) in this soil. However, there was no correlation between Fe_{total} and pH in WV and NG.

Table 4.6 Correlation matrix for measured soil properties in LN soils; p-values are given in brackets with significant correlations in bold type.

Soil variables	**pH**	**LOI**	**Cr $_{TMAH}$**	**Cr^{VI}_{TMAH}**	**Cr_{total}**	**Fe_{total}**
LOI	0.036					
	0.833					
Cr $_{TMAH}$	0.068	-0.052				
	0.692	0.762				
Cr^{VI}_{TMAH}	0.016	0.111	**0.910**			
	0.928	0.518	*(<0.001)*			
Cr_{total}	0.288	-0.034	**0.588**	0.325		
	0.088	0.844	*(<0.001)*	0.053		
Fe_{total}	**0.521**	-0.063	0.278	0.116	**0.653**	
	(<0.001)	0.714	0.101	0.499	*(<0.001)*	
Mn_{total}	**0.379**	-0.109	0.229	0.046	**0.539**	**0.782**
	(<0.05)	0.527	0.178	0.789	*(<0.01)*	*(<0.001)*

Stoke Bardolph

Soils from the Stoke Bardolph sewage disposal farm sites covered a wide range of soil properties (see Figure 4.4 and Table 4.7). Compared with WV, and NG soils, they have a narrower range of pH from 6.43 to 7.68, similar to the pH range for the majority of LN soils. A lower % LOI was also observed compared to WV and NG, ranging from 3.52 to 23.4 %. Cr_{total} ranged from 45.7 to 1670 mg kg^{-1} as a consequence of contamination from sewage sludge application; with the soil sampled from Field 13 contained the highest Cr_{total} for any of the soils studied. This site also covered a wider range of Cr^{VI}_{TMAH} (0.03 – 21.1 mg kg^{-1}) and Cr_{TMAH} (0.15 – 81.5 mg kg^{-1}). Lower Cr_{total}, Cr_{TMAH} and Cr^{VI}_{TMAH} was observed in Fields 48-54 which may be a consequence of lower sludge inputs to these fields.

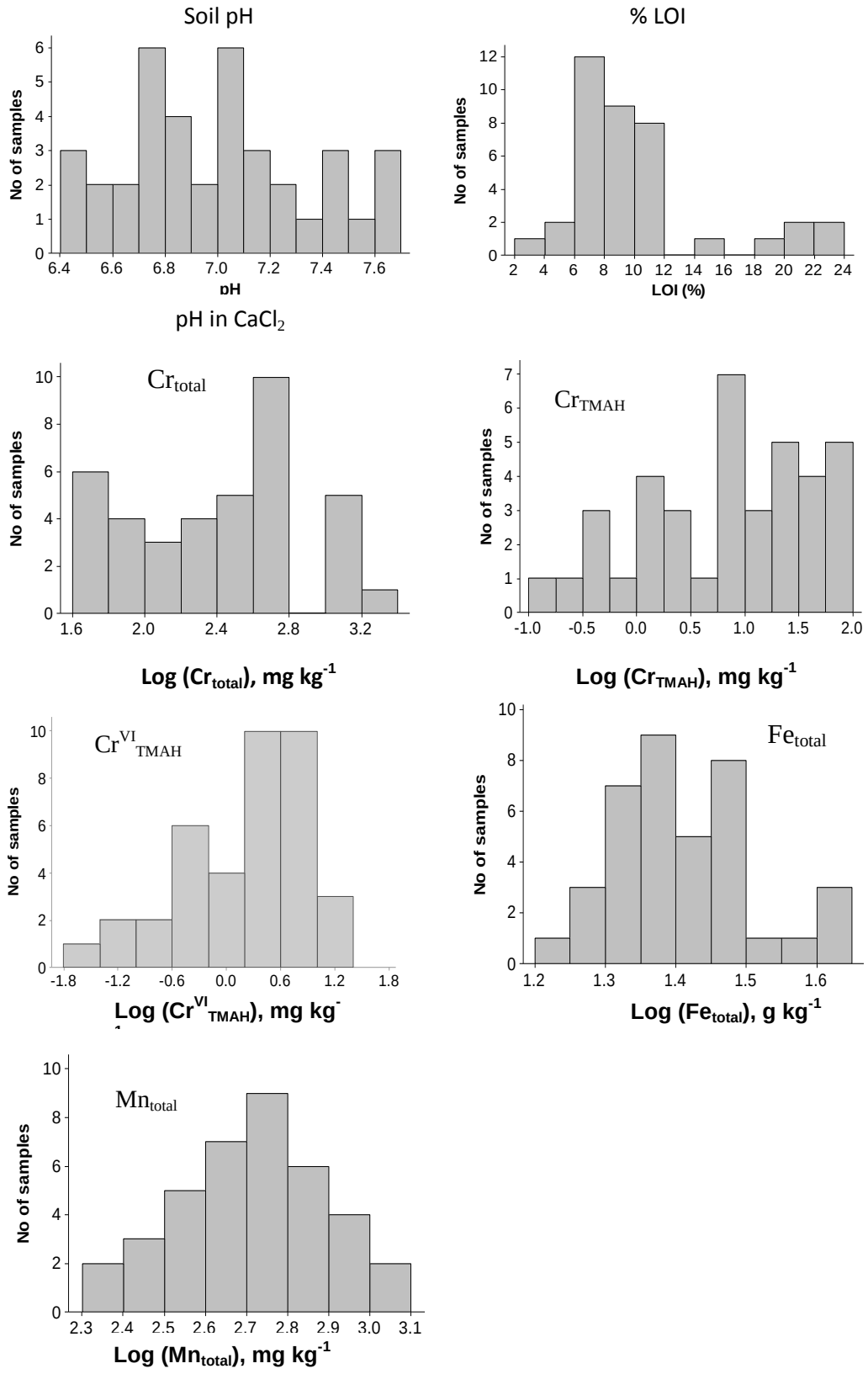


Figure 4.4 Soil characteristics of StB soils shown as histograms for soil pH, % LOI, Log(Total Cr), (mg kg^{-1}), Log(Cr_{TMAH}), (mg kg^{-1}), Log(Cr^{VI}_{TMAH}), (mg kg^{-1}), Log(Total Fe_{total}), (g kg^{-1}) and Log(Total Mn_{total}), (mg kg^{-1}).

Table 4.7 Summary of measured soil properties in Stoke Bardolph (StB) (n=38). Values in brackets show the number of replicates for determination of each value.

Field	pH (n=1)	LOI % (n=1)	Cr_{total} mg kg^{-1} (n=3)	Cr_{TMAH} mg kg^{-1} (n=3)	$Cr^{VI}{}_{TMAH}$ mg kg^{-1} (n=3)	Fe_{total} g kg^{-1} (n=3)	Mn_{total} mg kg^{-1} (n=3)
3	7.08	6.07	140	4.18	0.57	26.7	817
4	6.79	9.63	319	20.4	2.45	32.5	720
5	7.63	3.52	66.6	1.68	0.37	17.8	431
6A	6.63	10.2	479	14.0	2.76	25.0	307
6B	6.53	8.77	538	30.2	3.99	19.9	288
8A	6.43	20.7	1310	74.9	7.56	22.2	566
8B	6.49	21.7	1470	57.4	7.33	24.0	685
9A	6.58	15.3	1110	65.2	21.1	31.2	688
9B	6.48	23.4	1670	81.5	4.23	22.1	585
11	7.07	9.44	487	14.2	3.24	25.4	992
12	6.76	22.6	1360	78.8	11.0	21.0	458
13	7.12	18.9	1060	41.4	3.60	25.0	546
17	7.29	7.86	54.0	0.68	0.44	29.9	1170
19	7.36	6.38	59.6	1.52	0.36	22.2	583
20	6.72	10.3	476	28.8	6.03	24.2	328
21	7.08	6.89	45.7	1.42	1.06	25.2	911
22	6.82	10.1	615	34.7	8.13	22.2	417
23A	6.77	10.3	410	28.0	6.24	23.8	465
23B	6.72	9.55	428	18.7	2.82	20.6	242
24A	6.89	11.1	615	43.9	8.97	18.2	320
24B	6.71	10.6	555	50.9	10.8	17.6	205
29	7.47	7.53	190	9.18	0.98	20.1	547
31	6.66	7.00	70.7	2.57	1.10	23.9	969
33	6.95	6.39	99.3	3.07	0.63	23.1	622
34	7.06	10.4	483	9.65	4.41	29.0	453
36	7.45	6.77	102	1.17	0.35	36.3	1150
37	7.00	11.5	391	8.66	1.71	29.7	750
40A	7.07	8.96	334	5.64	4.96	27.8	737
40B	6.94	8.15	344	12.1	2.92	26.6	384
40C	7.19	7.82	160	8.49	3.09	24.1	394
44	7.28	6.57	162	5.87	1.80	23.5	514
46	7.62	9.74	204	1.86	0.99	43.3	302
47	6.83	9.66	287	9.66	8.84	28.9	355
48	7.46	9.03	104	0.45	0.23	42.0	610
49	7.68	7.66	94.2	0.36	0.05	44.6	758
52	7.12	6.76	43.2	0.32	0.09	31.2	437
53	6.81	5.90	48.4	0.22	0.17	28.4	493
54	7.53	4.51	59.7	0.15	0.03	31.3	570

Again, significant correlations were observed between Cr_{total}, Cr_{TMAH} and Cr^{VI}_{TMAH} (Table 4.8). There was a significant correlation between Cr^{VI}_{TMAH} and Cr_{TMAH} as well as Cr^{VI}_{TMAH} with pH (R = 0.778, p<0.001 and R = -0.573, p<0.001, respectively). In these soils pH was poorly correlated to Fe_{total} and Mn_{total} (R=0.375, p<0.001 and R=0.314, p<0.05 respectively). Cr_{total}, Cr_{TMAH} and Cr^{VI}_{TMAH} were closely correlated with % LOI and pH and these two soil properties were likely masking the effect of Fe_{total} and Mn_{total}. This finding was in agreement with a study on Cr fractionation in municipal solid waste compost by Bhattacharya et al., (2005) who also found that most of Cr fraction was associated with organic matter.

Table 4.8 Correlation matrix for measured soil properties in StB soils;p-values are given in brackets with significant correlations in bold type.

Soil variable	**pH**	**LOI**	**Cr_{TMAH}**	**Cr^{VI}_{TMAH}**	**Cr_{total}**	**Fe_{total}**
LOI	**-0.575**					
	(<0.001)					
Cr_{TMAH}	**-0.679**	**0.894**				
	(<0.001)	*(<0.001)*				
Cr^{VI}_{TMAH}	**-0.573**	**0.576**	**0.778**			
	(<0.001)	*(<0.001)*	*(<0.001)*			
Cr_{total}	**-0.645**	**0.964**	**0.947**	**0.674**		
	(<0.001)	*(<0.001)*	*(<0.001)*	*(<0.001)*		
Fe_{total}	**0.494**	-0.184	**-0.406**	-0.288	-0.316	
	(<0.01)	0.269	*(<0.05)*	0.080	0.053	
Mn_{total}	0.228	-0.096	-0.241	-0.234	-0.166	**0.324**
	0.169	0.567	0.146	0.158	0.320	*(<0.05)*

Comparison of results from the four sites

Data for all four sites (WV, NG, LN and StB) is compiled in Figure 4.5 and a correlation matrix (Table 4.9). When the data sets are considered together Cr_{total} is significantly correlated with % LOI (R=0.352, p<0.001), Cr_{TMAH} (R=0.579, p<0.001) and Cr^{VI}_{TMAH} (R=0.486, p<0.001). Good correlations are also observed between Cr^{VI}_{TMAH} and Cr_{TMAH} (R=0.94, p<0.001) and between Mn_{total} and Fe_{total} (R=0.631, p<0.001). If these results are compared with the correlations between soil properties for the three urban sites (WV, NG and LN) only (Table 4.10), the correlations between % LOI and Cr fractions disappear.

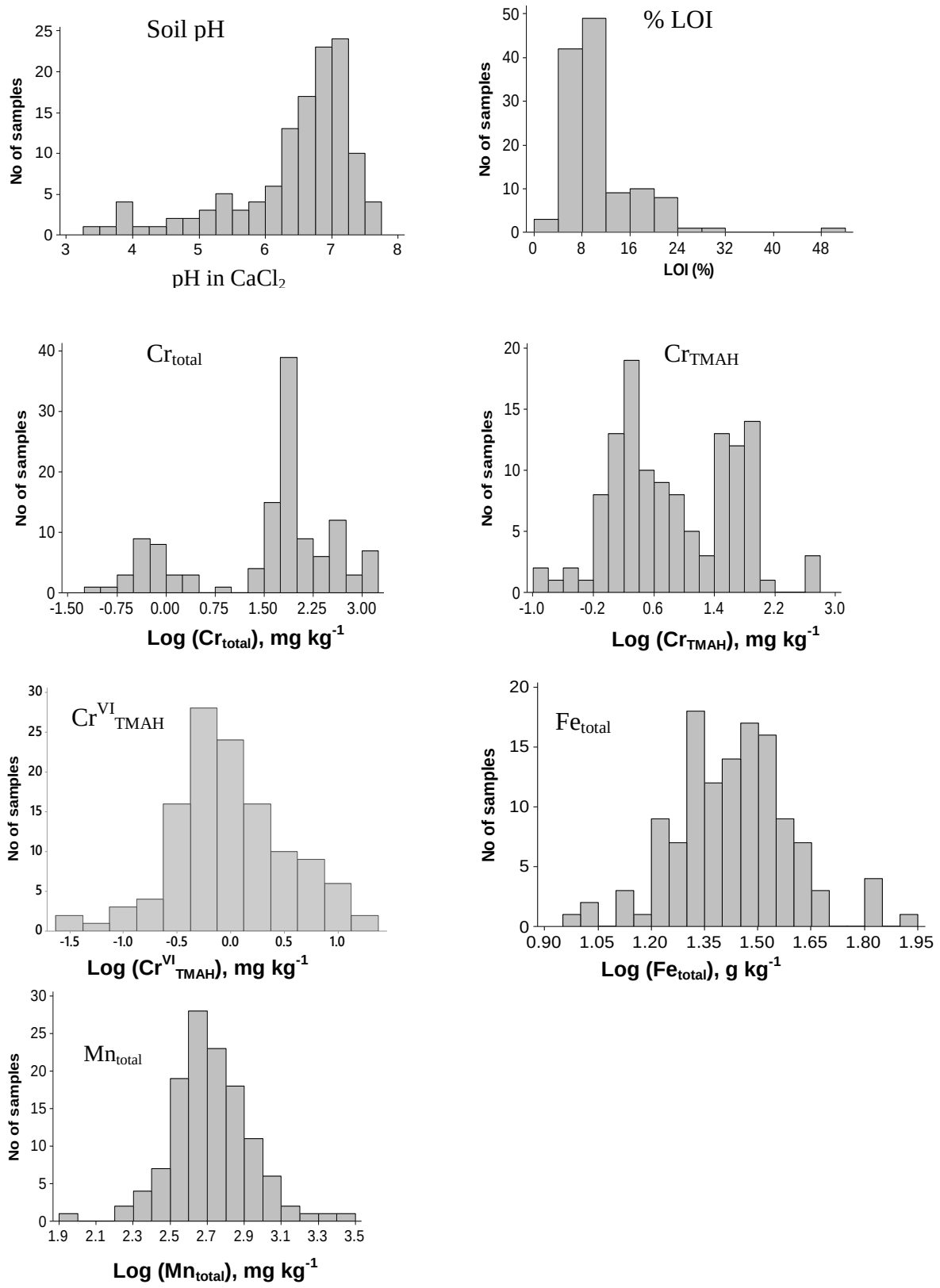


Figure 4.5 Soil characteristics of the four soil sets shown as histograms for soil pH, % LOI, Log (Cr_{total}) (mg kg^{-1}), Log (Cr_{TMAH}) (mg kg^{-1}), Log (Cr^{VI}_{TMAH}) (mg kg^{-1}), Log (Fe_{total}), (g kg^{-1}) and Log (Mn_{total}), (mg kg^{-1}).

Table 4.9 Correlation matrix for different soil properties for all four soil sets; p-values are shown in brackets with significant correlations in bold type.

Soil variables	pH	LOI	Cr_{TMAH}	Cr^{VI}_{TMAH}	Cr_{total}	Fe_{total}
LOI	**-0.279**					
	(<0.01)					
Cr_{TMAH}	-0.041	**0.207**				
	0.651	*(<0.05)*				
Cr^{VI}_{TMAH}	-0.034	**0.185**	**0.914**			
	0.709	*(<0.05)*	*(<0.001)*			
Cr_{total}	0.088	**0.352**	**0.579**	**0.486**		
	0.331	*(<0.001)*	*(<0.001)*	*(<0.001)*		
Fe_{total}	**0.362**	0.133	-0.116	-0.053	-0.046	
	(<0.001)	0.140	0.199	0.558	0.613	
Mn_{total}	0.163	**0.182**	-0.057	-0.005	0.011	**0.631**
	0.070	*(<0.05)*	0.533	0.956	0.903	*(<0.001)*

Table 4.10 Correlation matrix for different soil properties in the urban soils only; p-values are given in brackets with significant correlations in bold type.

Soil variables	pH	LOI	Cr_{TMAH}	Cr^{VI}_{TMAH}	Cr_{total}	Fe_{total}
LOI	**-0.244**					
	(<0.05)					
Cr_{TMAH}	-0.035	0.140				
	0.748	0.199				
Cr^{VI}_{TMAH}	-0.016	0.163	**0.923**			
	0.884	0.134	*(<0.001)*			
Cr_{total}	0.001	0.178	**0.853**	**0.866**		
	0.992	0.102	*(<0.001)*	*(<0.001)*		
Fe_{total}	**0.451**	0.176	-0.088	-0.041	0.190	
	(<0.001)	0.106	0.420	0.706	0.080	
Mn_{total}	0.205	**0.227**	-0.038	0.009	0.184	**0.667**
	0.059	*(<0.05)*	0.730	0.936	0.089	*(<0.001)*

4.3.2 The effect of soil properties and contaminant sources on Cr^{VI}

In most of the soils, Cr^{VI}_{TMAH} is observed to be up to 70% of Cr_{TMAH} and up to 5.7 % Cr_{total} with exception of 3 soils with higher percentage of Cr^{VI} to Cr_{total} (10.8, 13.1 and 15.7%). The concentration of Cr^{VI}_{TMAH} observed in the four soil sets is compared in Figure 4.6. Median values of Cr^{VI} in WV and NG are 0.626 and 0.491 mg kg^{-1}, respectively. Higher median values were observed in LN (0.92 mg kg^{-1}) and StB (2.45 mg kg^{-1}). It can be clearly seen in Figure 4.6 that the variability of Cr^{VI} was according to the following inter quartile range (IQR): StB > NG > LN > WV which can be calculated by difference between the 25^{th} percentile and 75^{th} percentile of the data. The higher variability of Cr^{VI}_{TMAH} in StB may be due to a higher mobility of Cr^{VI} in the sewage sludge disposal site at high pH ranging from 6.43 to 7.68 and also resulting in highly variable organic matter content (Table 4.7). This high variability of Cr^{VI} is in agreement with Fonseca et al., (2009) who showed that as the pH increased, the adsorption of Cr^{VI} decreased resulting in a higher mobility of Cr^{VI} in a loamy sand soil. A lower variation in Cr^{VI}_{TMAH} at the three urban sites may be due to the land use as most of the samples were collected from domestic garden, and grassland. Therefore, soil properties have been demonstrated to control the mobility of Cr^{VI} in these soils.

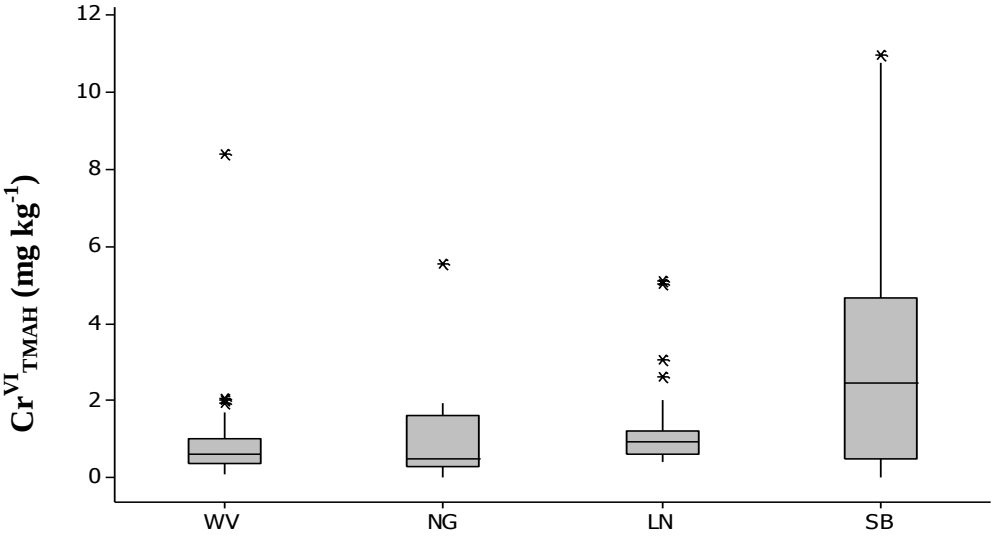


Figure 4.6 Box and whisker plot showing the range of Cr^{VI}_{TMAH} concentrations in the four soil sets; (WV n=35; NG n=18, LN n=34, and StB n=35. The upper and lower quartiles represent the highest and lowest 25% of the data respectively; whiskers indicate variability outside these lower quartiles. Outliers are marked with an asterisk; four extreme outliers (NG14:148 mg kg^{-1}; NG15:46.7 mg kg^{-1}; WV6: 18.6 mg kg^{-1} and 9A : 21.1 mg kg^{-1}) have been removed.

4.3.3 Prediction of Cr^{VI}_{TMAH} using multiple regression

Several attempts were made to predict Cr^{VI}_{TMAH} using $Cr_{total,}$ a ratio of $Cr^{VI}_{TMAH}/Cr_{total}$ followed by using Cr_{TMAH} with and without a function of soil properties (pH, LOI, Fe_{total} and Mn_{total}). Gray and McLaren (2006) demonstrated that a multiple linear regression model dependent upon solution pH, total metal content, total carbon and soil metal oxide content could be used to predict water soluble Cr in contaminated soil. They found a significant correlation ($p<0.001$) between the logarithm of Cr solubility and the predicted logarithm of pH, total Cr and amorphous Al with R=0.66, however, no Cr speciation was carried out in their work. Modelling using these four diverse datasets should increase our understanding of the soil factors and land uses that influence Cr^{VI}.

Prior to the make the prediction, a correlation between the ratio of Cr^{VI}_{TMAH} and Cr_{total} against Cr^{VI}_{TMAH} has been tested in Figure 4.7. This will ensure that the correlations are not artifacts produced by outliers or clustering of data in groups. There was no correlation was found

between Cr^{VI}_{TMAH}:Cr_{total} and Cr^{VI}_{TMAH} with the data are well distributed across the scatter plot graph (Figure 4.7)

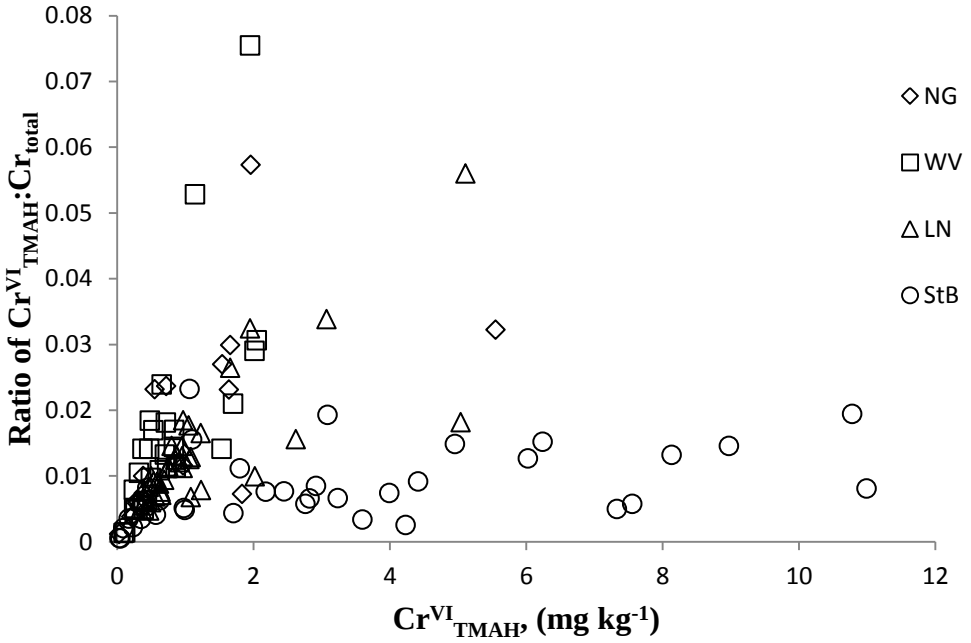


Figure 4.7 A correlation between ratio of Cr^{VI}_{TMAH} and Cr_{total} against Cr^{VI}_{TMAH} in four soil datasets: Wolverhampton (WV), Nottingham (NG), London (LN) and Stoke Bardolph (StB), n=122.

The results of the multiple regression model (see Equation 4.1, Section 4.2.4) are compared to measure Cr^{VI}_{TMAH} in Figure 4.8 and Table 4.11. Cr^{VI}_{TMAH} outliers (NG14, NG15, WV 6 and 9A) were excluded from the model. Fields with lower Cr_{total} from StB (such as Field 49 and 54) were underpredicted. The LN soils form a tight group with most around the 1:1 line or within 1 RSD. WV soils again formed a tight group but deviated further from the 1:1 line than the LN soils. Loss on ignition (% LOI), Cr_{total} and Mn_{total} were significant variables ($p<0.05$) for predicting Cr^{VI}_{TMAH} but pH and Fe_{total} were not significant variables ($p>0.05$) (Table 4.11). Although Fe and Mn oxides are known to be important sorbents for a range of metals (Trivedi and Axe, 2000), the inclusion of amorphous Fe and Mn oxide contents instead of Fe_{total} and Mn_{total} in the regression (Equation 4.1) provided no apparent improvement in model RSD values.

Table 4.11 Best fit coefficients for predicting Cr^{VI}_{TMAH}. Data is from four sites (n=123) with pH, %LOI, Cr_{total}, Fe_{total} and Mn_{total} as determining variables. Model residual standard deviation values (RSD) and correlation coefficients (R, predicted vs observed) are included. Significant values are shown in bold where p is <0.05. The non significant values, pH was removed from the multiple regression.

Coefficient	All sites (n=124)
k_o	0.00
k1 (LOI), %	**0.0296 (p<0.05)**
k_2 (Cr_{total}), mg kg^{-1}	**0.0060 (p<0.05)**
k_3 (Mn_{total}), mg kg^{-1}	**0.0387 (p<0.05)**
RSD	0.074
R	0.554

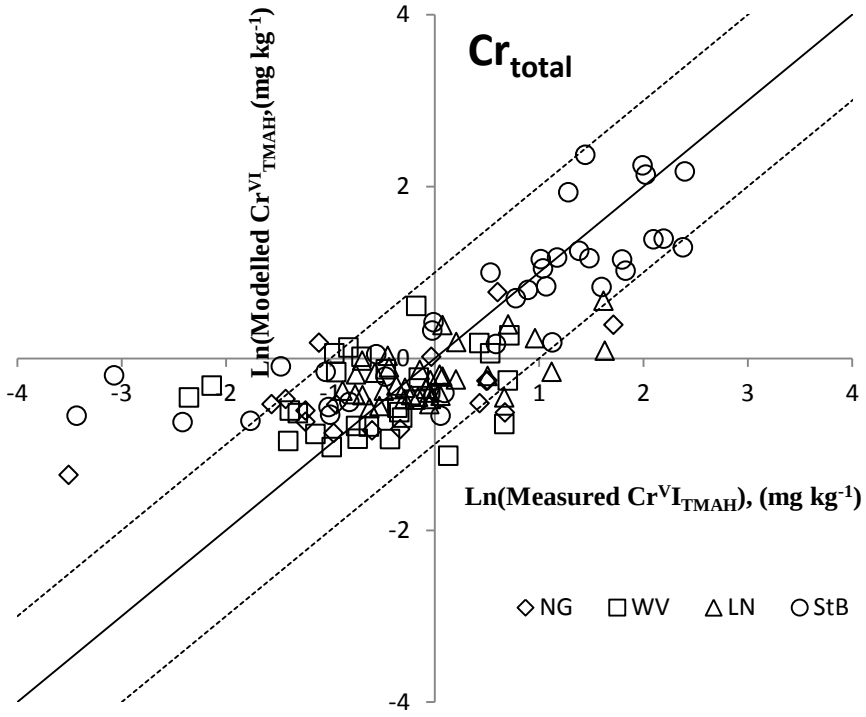


Figure 4.8 Predicted vs measured Cr^{VI}_{TMAH} (mg kg^{-1}, Ln scale) in soils from four soil datasets: Wolverhampton (WV), Nottingham (NG), London (LN) and Stoke Bardolph (StB), (n=122) using C_{total} and other soil parameters. The solid line represents the 1:1 line and the dashed line represent ±1 RSD for the model.

Another attempt is to predict Cr^{VI} concentration using on a ratio of Cr^{VI} to Cr_{total} and soil parameters (Figure 4.9). However, as seen in Table 4.12, a weaker correlation (R=0.4312) was observed compared to prediction using absolute concentration of Cr_{total} (0.5540) (Table 4.11). Therefore, it is suggested to use absolute concentration rather than using ratio to proceed with the prediction of Cr^{VI}.

Table 4.12 Best fit coefficients for predicting Cr^{VI}_{TMAH}. Data is from four sites (n=123) with pH, %LOI, Cr_{total}, Fe_{total} and Mn_{total} as determining variables. Model residual standard deviation values (RSD) and correlation coefficients (R, predicted vs observed) are included. Significant values are shown in bold where p is <0.05. pH, LOI, Fe_{total} and Mn_{total} were found as non significant (p>0.05) variables and removed from the multiple regression.

Coefficient	All sites (n=124)
k_o	0.0000
k_1 (Cr^{VI}/Cr_{total}), mg kg^{-1}	**0.0060 (p<0.05)**
RSD	0.0689
R	0.4312

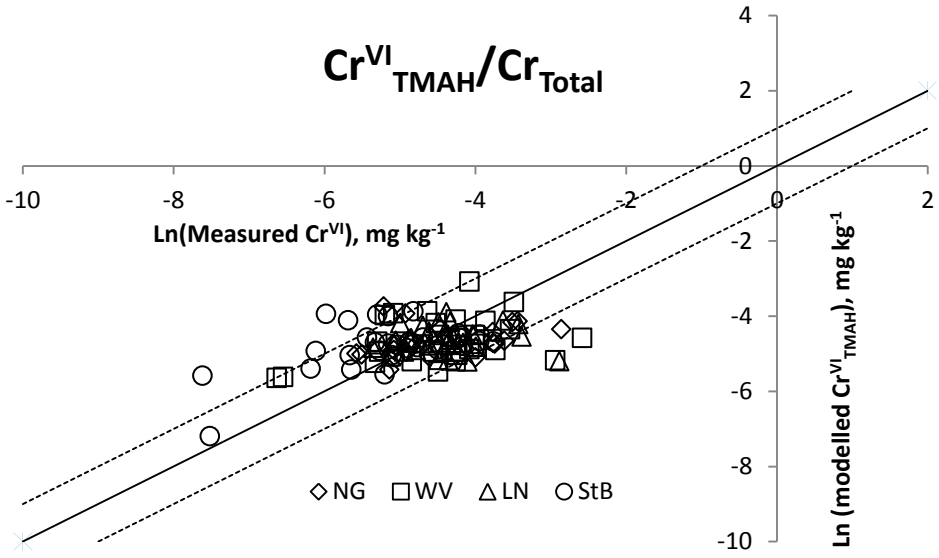


Figure 4.9 Predicted vs measured Cr^{VI}_{TMAH} (mg kg^{-1}, Ln scale) in soils from four soil datasets: Wolverhampton (WV), Nottingham (NG), London (LN) and Stoke Bardolph (StB), (n=122) using a ratio of Cr^{VI}_{TMAH} and $Cr_{total,}$ and other soil parameters. The solid line represents the 1:1 line and the dashed line represent ±1 RSD for the model.

Again the correlation data check was carried out between Cr^{VI}_{TMAH}:Cr_{TMAH} and Cr^{VI}_{TMAH}. There was no correlation between the ratio of Cr^{VI}_{TMAH}:Cr_{TMAH} and Cr^{VI}_{TMAH} and the data are well distributed along the graph (Figure 4.10). Then, another prediction on the concentration of Cr^{VI}_{TMAH} was made using Cr_{TMAH} and LOI, pH, Fe_{total} and Mn_{total} in the same way i.e, replacing the variable of Cr_{total} with Cr_{TMAH} (Table 4.13, Figure 4.11). Better prediction of Cr^{VI} concentration was obtained using Cr_{TMAH}, pH, Fe_{total} and Mn_{total} with a good correlation (R=0.874) and lower residual standard deviation (RSD) (Table 4.13) compared to Cr_{total} and other soil properties. In general, values are less clustered and are closer to the 1:1 line. An improved fit for LN soils was also observed using this model with all values falling within ± 1 RSD. However, among the 5 variables tested, only Cr_{TMAH} (kCr_{TMAH}= 0.2622) and LOI (k_{LOI}=0.0115) were found to be significant ($p<0.05$) (Table 4.13).

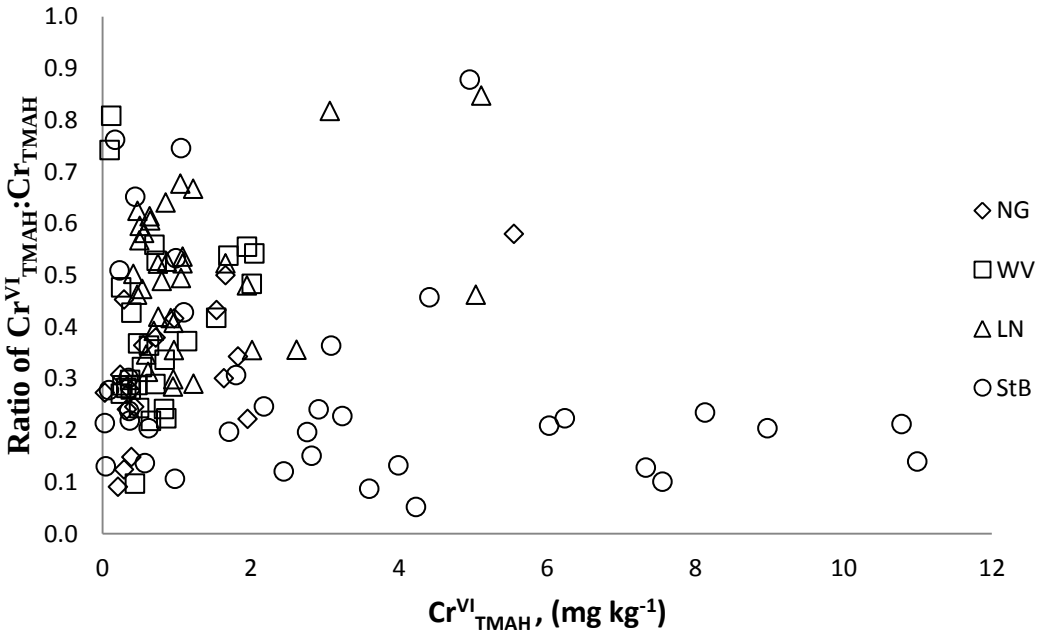


Figure 4.10 A correlation between ratio of Cr^{VI}_{TMAH} and Cr_{TMAH} against Cr^{VI}_{TMAH} in four soil datasets: Wolverhampton (WV), Nottingham (NG), London (LN) and Stoke Bardolph (StB), n=122.

Table 4.13 Best fit coefficients for predicting Cr^{VI}_{TMAH}. Data is from four sites (n=123) with pH, %LOI, Cr_{total}, Fe_{total} and Mn_{total} as determining variables. Model residual standard deviation values (RSD) and correlation coefficients (R, predicted vs observed) are included. Significant values are shown in bold where p is <0.05. pH, Fe_{total} and Mn_{total} were found as non significant (p>0.05) variables and removed from the multiple regression.

Coefficient	All sites (n=122)
k_o	0.00
k_1 (LOI), %	**0.0116 (p<0.05)**
k_2 (Cr_{TMAH}), mg kg^{-1}	**0.2622 (p<0.05)**
RSD	0.0489
R	0.8076

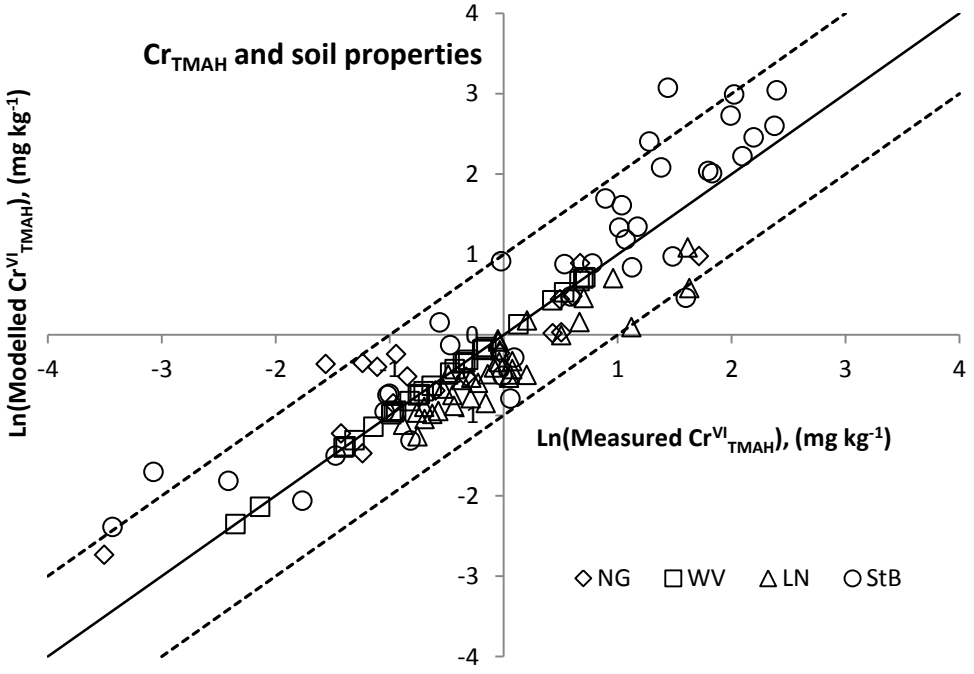


Figure 4.11 Predicted vs measured Cr^{VI}_{TMAH} (mg kg^{-1}, Ln scale) in soils from four soil datasets: Wolverhampton (WV), Nottingham (NG), London (LN) and Stoke Bardolph (StB), (n=122) using Cr_{TMAH} and other soil parameters. The solid line represents the 1:1 line and the dashed lines represent ±1 RSD for the model.

Another attempt is to examine the reliability of Cr_{TMAH} without soil properties in predicting the concentration of Cr^{VI}_{TMAH} using a multiple liner regression model. A better prediction of Cr^{VI} was observed with R=0.9106 (Table 4.14). Although some overprediction and underprediction was observed, however, most data fell within ± 1 RSD except some

overprediction of soils from StB. This may be due to the presence of high organic matter that can reduce the concentration of Cr^{VI} soil by time (Wittbrodt and Palmer, 1996)

Table 4.14 Best fit coefficient for predicting Cr^{VI} on soils from all four sites (n=122) using Cr_{TMAH} as determining variable. Model residual standard deviation values (RSD) and correlation coefficient (R, predicted vs observed) for all sites are included. Significant values are shown in bold where p is <0.05.

Coefficient	All sites (n=122)
ko	0.0792
k_1 (Cr_{TMAH}), mg kg^{-1}	**0.2798 (p<0.05)**
RSD	0.0483
R	0.9106

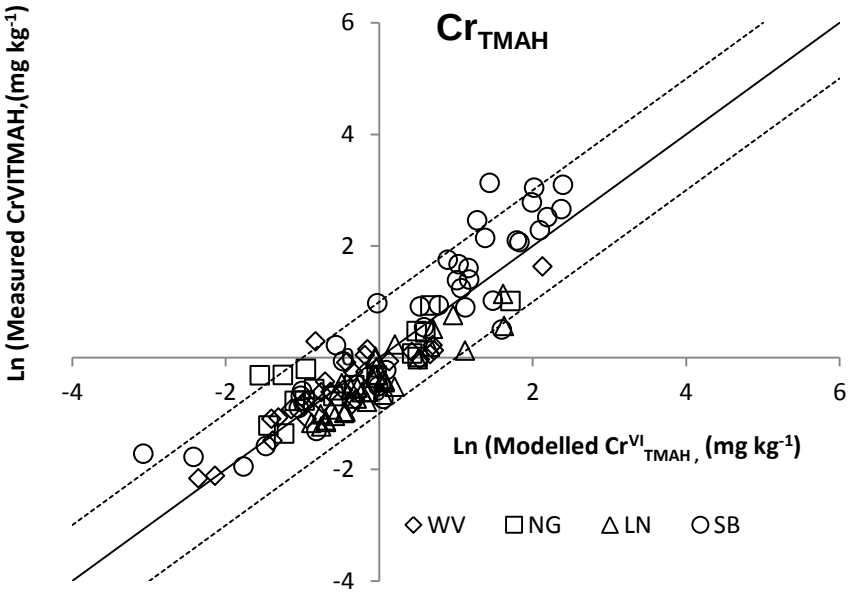


Figure 4.12 Predicted vs measured of Cr^{VI}_{TMAH} (mg kg^{-1}, Ln scale) in soils from four soil datasets: Wolverhampton (WV), Nottingham (NG), London (LN) and Stoke Bardolph (StB). (n=124). The prediction was made using Cr_{TMAH} as determining variable. The solid line represent 1:1 line and the dashed line represents ±1 RSD.

4.5 CONCLUSIONS

In general, the Cr^{VI} concentrations measured for these urban and sewage amended soils were lower than the Cr^{VI} screening soil value imposed by DEFRA (DEFRA, 2013). Results showed that TMAH can be used as an extractant for predicting the concentration of Cr^{VI} (i,e, Cr^{VI}_{TMAH}) in soil. Both soil properties and source of contamination affected the concentration of Cr^{VI}_{TMAH}, with higher concentrations found in sewage farm soils and lowest concentrations in urban areas. London (LN) soils had the lowest concentration of Cr^{VI}_{TMAH}.

Several attempts were carried out to predict the concentration of Cr^{VI} using multiple regression model and the speciation work can be neglected. However, an overprediction of Cr^{VI} was observed in soil with the highest concentration of Cr_{total} and underpredicted some soil with lower Cr_{total} when using Cr_{total} and soil parameters as the determining variables. Acid digestion of soil resulted in reduction of Cr^{VI} to Cr^{III}, however, Cr^{VI} was stable in alkaline condition. The ratio of Cr^{VI} to Cr_{total} does not help in the prediction of Cr^{VI}. An improved model fit was observed when Cr_{TMAH} was used as an input variable instead of Cr_{total}. Alkaline extraction of Cr (Cr_{TMAH}) mobilizes Cr^{VI} alongside a fraction of soluble Cr^{III} bound with HA (see Chapter 3). The reliability of Cr_{TMAH} as the determining variables in predicting the concentration of Cr^{VI} has been demonstrated with or without other soil parameters. However, the concentration of Cr^{VI} in some of the soils in StB cannot be predicted which may be due to nature of samples and the presence of high organic matter input. The presence of Cr^{VI} might be decreased by prolong the reaction with organic (Chapter 6). Therefore, the speciation work which involved alkaline extraction of Cr in soil and the use of HPLC-ICP-MS is still crucial in order monitor the concentration of Cr^{VI} in contaminated site. As Cr^{VI} toxicity is of concern, further investigation was therefore undertaken to determine the lability of Cr^{VI} in soil (Chapter 5).

5 USE OF ISOTOPIC DILUTION AND SOLUTION SPECIATION IN MEASURING LABILE CHROMIUM AND Cr^{VI} IN SOIL

5.1 INTRODUCTION

It is important to consider the isotopically exchangeable fraction (E-value) in assessing the mobility and availability of an element in soil (Gäbler et al., 1999). Measuring E-values involves determining the distribution of an added isotope between solid and solution phases of a soil suspension. This measurement is normally carried out using a *stable* isotopic dilution approach due to the increasing availability of ICP-MS instrumentation (Gäbler et al., 1999; Young et al., 2000; Nolan et al., 2004; Marzouk et al., 2013). The methodology has developed substantially over the past 30 – 40 years but the technique has only been widely applied to the simple divalent metal cations, such as Cd, Pb, Zn, Cu and Ni. Thus, for example, Marzouk et al. (2013), in a large catchment scale study, simultaneously measured E-values of Zn, Cd and Pb in Pb/Zn contaminated soil suspended with 0.01 M $Ca(NO_3)_2$, equilibrated for 3 days and spiked with ^{70}Zn, ^{108}Cd and ^{204}Pb. They found that the range of E-values as a proportion (%) of total metal content was as follows: Zn (2-54%), Cd (3-77 %) and Pb (7-99%).

The use of isotope techniques in order to investigate anions such as phosphorus dynamics in soil-plant systems are widely carried out using radioactive ^{32}P and ^{33}P isotopes. Cerenkov counting technique has been employed to calculate β- radiation emitted by both P isotopes. The limitation by employing ^{32}P is due to its short half life (14.3) and may effect and limited the duration of an experiment (Barrow, 1983; Lookman et al., 1995). A study by Lookman et al., (1997) using solid state ^{31}P Nuclear Magnetic Resonance (NMR) found several pool of P in soils which is categorized as rapidly desorb, slow exchange and precipitated in condensed Ca phosphate and strongly sorbed onto Al oxides.

However, studies which apply isotopic dilution to study Cr fractionation in soils are extremely limited and difficulties have been reported which may relate partly to uncertainties over the lability of Cr^{III} species in solution and transfers between different oxidation states; the latter issue is discussed later. Thus, Gäbler et al, (1999) found differences in the proportion of isotopically exchangeable Cr in soil, depending on the suspending matrix used: EDTA (1.31 %); NH_4NO_3 (0.0545 %) and water (0.0618 %). This suggests that different equilibrating solutions render different pools of soil Cr exchangeable with the spike isotope.

Gäbler et al., (2007) measured Cr E-values in 115 soil samples suspended in 0.01 M $Ca(NO_3)_2$ by stable isotopic dilution. They spiked soil suspensions with $^{53}Cr^{III}$ and found Cr E-values in the range 0.024 to 9.9 mg kg^{-1} (0.8 – 5.8 % of the soil Cr content). However, speciation analysis was not carried out to determine whether the measured values of Cr_E truly represented labile Cr^{III} or if redox changes to the spike isotope had occurred.

It is widely accepted that chromium exists in two oxidations states in soil: Cr^{III} and Cr^{VI} (Kotaś et al., 2000; Zhilin et al., 2004; Unceta et al., 2010). Potentially, both species may be present as either 'non-labile', and able to exchange with the solution phase only through a kinetically moderated process, or 'labile' and so capable of rapid exchange between the solid and solution phases. Thus labile Cr^{VI} and Cr^{III} in soil can be rapidly exchanged with Cr^{III} and Cr^{VI} species in the soil solution, respectively (Figure 5.1). However, total Cr^{III} measured in solution may be a combination of both labile Cr^{III}-DOC and non-labile Cr^{III}-DOC complexes, possibly also Cr^{III} occluded within nano-particulate Fe^{III}-oxide particles etc. Such mixed fractionation can lead to an over-estimation of 'labile' Cr^{III} concentration. Preliminary work (Chapter 3) using a $^{50}Cr^{III}$ spike in TMAH soil extracts with 0.0001 M and 0.0005 M EDTA showed poor recovery (30-40 %) of total Cr in solution as a chromatographically identifiable EDTA-Cr^{III} complex. This suggests strongly preferential complexation with DOC instead of EDTA (Figures 3.48b & 3.48d) and points to the existence of non-labile DOC-Cr^{III} complexes in solution. Farmer et al., (2002) measured Cr^{VI} and Cr^{III} concentrations in soil pore-water and groundwater samples collected from disposal sites for cromite ore processing residue (COPR) in SE Glasgow and South Lanarkshire (1988-2000) by using Speciated Isotopic Dilution Mass Spectrometry (SIDMS). They found that the concentration of Cr^{III} in the water samples analysed by SIDMS was lower (<0.01 to 5.8 mg L^{-1}) than Cr^{III} simply estimated as the difference in concentration between total Cr and diphenylcarbazide-assayed Cr^{VI} (<0.01 to 95.2 mg L^{-1}). This result may have been due to the presence of non-labile Cr^{III}-organic complexes being absorbed by the analytical column of the HPLC. By contrast, the concentration of Cr^{VI} in the samples was similar when measured by both methods.

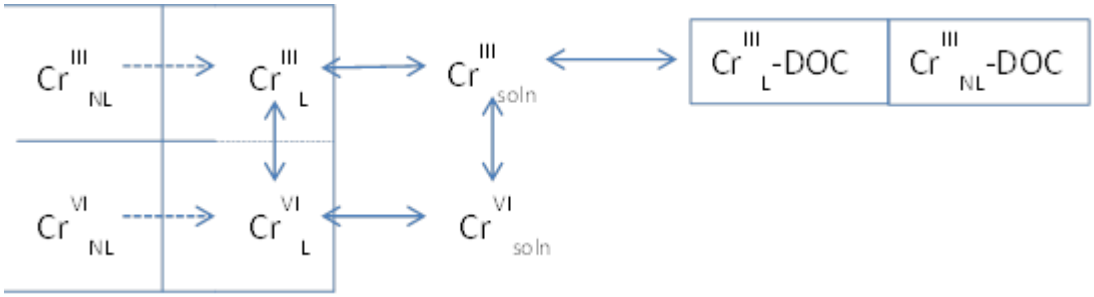


Figure 5.1. Schematic representation of chromium dynamics in soil suspensions. Subscripts 'L' and 'NL' refer to labile and non-labile forms. Chromium in solution is either present as free ions (soln) or as organic (humic) complexes (-DOC); the latter may be labile or non-labile.

Due to the complexity of measuring Cr^{III} as an independent species, and the greater importance of the Cr^{VI} form, it was decided to focus on trying to determine isotopically exchangeable Cr^{VI} in soils. Therefore, in this study, $^{50}Cr^{VI}$ was used as the spike isotope to determine exchangeable or labile Cr^{VI} in soil suspensions. Some preliminary work using $^{50}Cr^{VI}$ showed that it can be used as a tracer in monitoring changes in the oxidation state of ^{52}Cr in separated TMAH soil extracts (Chapter 3; Figures 3.47 & 3.48). Cr^{VI} is also a stable species in TMAH, it is largely conserved in transit through anion exchange chromatography columns and is unlikely to form complexes with DOC as they are both anionic species.

The need for speciation in measurements of E-value.

Hamon et al., (2004) demonstrated the need for speciation in isotopic dilution experiments by showing that the solid⇌solution partition coefficient (K_d) differs between As species. The measured solid⇌solution distribution of a spike isotope, added as one particular species, is therefore not compatible with a measurement of native elemental analyte in solution which comprises a mix of species – and so the 'normal' calculation of E-value gives an erroneous result. However, it is also true that speciation can be ignored if the soil suspension is in a state of both isotopic *and* redox equilibrium so that the added spike isotope has proportionately distributed itself between all labile forms, regardless of redox state. The E-value calculated is then valid but it is also a compound term, including all labile redox forms present. This point was later made by Hamon et al., (2008). The kinetics of Cr redox changes in soil are not clear and so it cannot be guaranteed that an added spike of $^{50}Cr^{VI}$ will

reach equilibrium with labile trivalent $^{52}Cr^{III}$ forms. Therefore, there is a need for isotopic Cr speciation because (i) redox equilibrium cannot be assumed, (ii) there may be non-labile DOC-Cr^{III} species in solution and (iii) there is greater interest in determining the concentration of labile Cr^{VI} species as opposed to a hypothetical compound 'labile Cr' fraction which includes Cr in both oxidation states.

There have been very few studies which have attempted to measure labile Cr^{VI} in soil by isotopic dilution. Therefore, several aspects of the experimental protocols need to be considered in order to optimise the procedure and also to provide background information that may help interpret the results which will be discussed within this chapter.

a) Chromium speciation.

In Chapter 3, TMAH was shown to be a suitable extractant for Cr^{VI} in soil, therefore it was chosen in this work as a suspending solution for (partially) mobilizing Cr^{VI} into the soil solution. However, TMAH will also solubilize humic-bound Cr^{III} and so it cannot be assumed that all Cr in solution is present as Cr^{VI}. This also applies even for neutral electrolytes such as 0.01 M $Ca(NO_3)_2$. The two inorganic Cr species will clearly have different K_d values and so spiking with $^{50}Cr^{VI}$ and then relying on the measurement of an isotope ratio based on total ^{50}Cr and ^{52}Cr in solution is likely to give erroneous results. For example, if the added $^{50}Cr^{VI}$ spike all remains in the Cr^{VI} form but the TMAH extractent mobilizes humic-bound Cr^{III}, as well as $^{52}Cr^{VI}$, then the isotopic ratio in solution is an incompatible mix of species (Eq 5.1):

$$\frac{^{50}Cr}{^{52}Cr} = \left(\frac{^{50}\mathrm{Cr}^{\mathrm{VI}}}{Humic - {}^{52}Cr^{III} + {}^{52}\mathrm{Cr}^{\mathrm{VI}}} \right) \qquad (5.1)$$

Alternatively, if the added $^{50}Cr^{VI}$ spike actually achieves both isotopic and redox equilibrium then the measurement of isotopic ratio, regardless of species, does provide a meaningful measurement of labile Cr (both Cr^{III} and Cr^{VI} combined), because there will be proportional distribution of the added ^{50}Cr isotope between all species involving both Cr^{III} and Cr^{VI} (Eq 5.2).

$$\frac{^{50}Cr}{^{52}Cr} = \left(\frac{Humic - {}^{50}Cr^{III} + {}^{50\mathrm{V}}\mathrm{Cr}^{\mathrm{VI}}}{Humic - {}^{52}Cr^{III} + {}^{52}\mathrm{Cr}^{\mathrm{VI}}} \right) \qquad (5.2)$$

Even if redox equilibrium is established and Equation 5.2 is valid, however, it must be remembered that the resulting E-value provides an estimate of the overall Cr labile pool (both

Cr^{III} and Cr^{VI} combined), whereas the key interest probably lies with Cr^{VI} for toxicological reasons. The significance of a 'compound' E-value is doubtful because it would comprise different mixes of labile Cr^{III} and Cr^{VI} in different soils.

b) Suspending solution matrix

In the earlier work of this chapter, several TMAH concentrations were tested as a means of partially solubilizing Cr^{VI} in order to establish a true solid-solution equilibrium which permits measurement of the $^{50}Cr^{VI}$ spike distribution between the solid and solution phases of the soil suspension - rather than having the spike entirely adsorbed or wholly in solution. This method was also compared with the more conventional neutral electrolyte, 0.01 M $Ca(NO_3)_2$, as the suspending solution. The experimental objective was to try to optimise a suspending solution matrix which does not mobilize non-labile Cr, or cause speciation changes, but solubilizes enough labile Cr^{VI} to provide robust measureable values of the isotopic ratio of $^{50}Cr^{VI}$ / $^{52}Cr^{VI}$ in solution.

c) Spike isotope concentration

As for (a) above, the objective in choosing a spike concentration is to produce a suspension which (i) produces analytically robust measurements of $^{50}Cr^{VI}$ in solution and (ii) in which Rss, the measured ratio of $^{50}Cr^{VI}/^{52}Cr^{VI}$, is sufficiently different from the background ratio that it is possible to calculate a reliable E-value. Nolan et al., (2004) also showed that there is need to consider the absolute value of individual isotope concentrations in solution in order to get an accurate E-value measurement.

d) Spike isotope equilibration time

If the spike isotope is in contact with the soil for a long period of time, then it will mix with non-labile metal. In addition, there is a greater risk of speciation changes with extended contact times and a greater likelihood of anaerobic conditions in the soil suspension. However, depending on the kinetics of labile isotope mixing, it is also important to allow sufficient time for the spike isotope to mix with the labile Cr^{VI} pool in soil. The time required for isotopic equilibration may also depend on the suspending solution matrix. It is therefore important to examine the effect of isotope equilibration time on i) the spike isotope ($^{50}Cr^{VI}$) equilibration between the solid and solution phases, ii) the spike isotope speciation in solution, iii) the Rss values and iv) the final calculated E-values.

The overall aim of the work in this chapter was to establish an isotopic dilution method for Cr^{VI} in soil, or at least assess the validity of the isotopic dilution approach in assessing the lability of Cr^{VI} in soils.

The experimental objectives of the work were to:

- compare alkaline and neutral suspending solutions as suitable liquid phases in the determination of Cr^{VI} E-values;
- optimise the isotope ($^{50}Cr^{VI}$) spike concentration and the equilibration time used;
- assess the need for speciation of Cr in solution and determine whether $^{50}Cr^{VI}$ redox equilibrium is achieved within the timescale of the E-value measurement.

5.2 MATERIALS AND METHODS

A soil sample collected from a sewage sludge disposal farm was used for a preliminary study of the isotopic dilution technique. The sewage farm is run by Severn Trent at Stoke Bardolph near Nottingham, England (GR: SK 643 406 Latitude 52˚ 57' 0'' N, Longitude 1˚ 2' 0'' W). Soil samples were air dried, homogenized and finely ground (see Section 2.2).

5.2.1 Preparation of $^{50}Cr^{VI}$ enriched solution

The preparation of $^{50}Cr^{VI}$ stock solution was according to Section 2.3.3.

5.2.2 Effect of equilibration time, concentration of $^{50}Cr^{VI}$ spike and TMAH concentration on labile Cr and Cr^{VI}

Two grams of soil were suspended in 30 ml TMAH at a range of concentrations (0.01, 0.1, 1.0 and 10 %) and shaken end-over-end in Oak Ridge polypropylene centrifuge tubes for 24 hr. In each suspension, 0.4 mL of ^{50}Cr solutions containing either 603 µg $^{50}Cr\ L^{-1}$, 1206 µg $^{50}Cr\ L^{-1}$ or 1809 µg $^{50}Cr\ L^{-1}$ was added which was equivalent to a 50, 100 or 150 % change in the natural abundance of $^{50}Cr^{VI}$ in the labile Cr pool for the soil. The concentration of labile $^{50}Cr^{VI}$ was initially estimated from the concentration of $^{52}Cr^{VI}$ extracted in 1% TMAH adjusted for the gravimetric isotopic abundance of the ^{50}Cr isotope (0.0417). Four spike isotope contact times with the soil suspensions were tested: 1, 2, 3 and 4 days. After equilibration, the soil suspension was centrifuged at 3000 rpm for 20 min and filtered using 0.2 µm Millex Milipore filters. The filtrate was divided into two; the first 10 mL was directly assayed for Cr_{TMAH} and Cr_E value by CCT-KED ICP-MS (Section 2.3.2) and the second (1

mL) was used to measure the species Cr^{VI}_{TMAH} and Cr^{VI}_{E} value by HPLC-ICP-MS (Section 2.3.2). In both cases, ^{50}Cr and ^{52}Cr isotopes were determined. All measurements were undertaken as soon as possible after sampling.

5.2.3 Effect of TMAH concentration on Cr_E and Cr^{VI}_E

The same soil (using 2 g in 30 ml TMAH) was further studied using a range of lower TMAH concentrations: 0.01, 0.03, 0.05, 0.07, 0.09, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 % for the second experiment, based on a suitable range of TMAH concentrations proposed from the first experiment. In each suspension, 0.4 ml of 1807 µg L^{-1} $^{50}Cr^{VI}$ was added which was equivalent to a 150 % change in the natural abundance of $^{50}Cr^{VI}$ in the labile Cr pool for the soil. The solution was then equilibrated for 3 days. After equilibration, the soil suspension was centrifuged at 3000 rpm for 20 min and filtered using 0.2 µm Millex Milipore filters prior to assay of Cr species, as described above.

5.2.4 Effect of TMAH concentration and the use of 0.01 M $Ca(NO_3)_2$ on Cr_E and Cr^{VI}_E

A third experiment was carried out using 0.001, 0.01, 0.02 and 0.04 % TMAH and a standard electrolyte (0.01 M $Ca(NO_3)_2$) commonly used to suspend soils for determination of cationic trace metal E-values. Different spike concentrations of $^{50}Cr^{VI}$ (30, 60, 90 and 120 %) were added to the soil suspension and equilibrated for 3 days. After equilibration, the soil suspension was then centrifuged at 3000 rpm for 20 min and filtered using 0.2 µm Millex Milipore filters prior to determination of Cr species.

5.2.5 Optimising equilibration time and spike level of $^{50}Cr^{VI}$ in 0.01 M $Ca(NO_3)_2$ for determination of Cr_E and Cr^{VI}_E

In the fourth experiment, the contact time between the equilibrated soil solution and the spike was varied from 1 to 7 days. Again, 2 g of soil sample were suspended in 0.01 M $Ca(NO_3)_2$ and shaken end over end for 24 hr prior to spiking. To each suspension, 0.4 ml of solutions containing 362, 723, 1085 and 1447 µg L^{-1} $^{50}Cr^{VI}$ was added which was equivalent to 30, 60, 90 and 120 % change in the natural abundance of $^{50}Cr^{VI}$ in the soil. The same treatment and analysis of the samples was carried out as described for the previous experiments.

5.2.6 Calculating E- values

Throughout this Chapter, the isotopically exchangeable Cr E-values were determined from the isotope abundance ratio of the spike isotope (^{50}Cr) to Cr background isotopes (^{52}Cr) (Eq

5.3). The same equation was also used for measuring E-values specifically for Cr^{VI} (Cr^{VI}_E), but the isotopic abundances used were then specifically for $^{50}Cr^{VI}$ or $^{52}Cr^{VI}$ species.

$$Cr\ E\text{-}value\ (mg\,kg^{-1}) = \left(\frac{^{52Cr}M}{W}\right)\left(\frac{^{50Cr}C_{spike}\ ^{50Cr}V_{spike}}{^{50Cr}M_{spike}}\right)\left(\frac{^{50Cr}IA_{spike} - {}^{52Cr}IA_{spike}Rss}{^{52Cr}IA_{soil}Rss - {}^{50Cr}IA_{soil}}\right) \qquad (5.3)$$

W is the weight of soil used (kg), V_{spike} is the volume of spike added (L), IA is isotopic abundance (mole basis), M is the average atomic mass of ^{52}Cr in the soil or ^{50}Cr in the spike, C_{spike} is the gravimetric concentration of ^{50}Cr in the spike solution and Rss is the ratio of $^{50}Cr/^{52}Cr$ in the spike soil solution.

Prior to measurement of the E-value, a minor isobaric interference of ^{50}Cr from ^{50}V was corrected according to Equation 5.4.

$$Net\ ^{50}Cr(cps) = \left(^{50}Cr_s\right) - \left(\frac{^{50}V_{IA}\ ^{51}V_s}{^{51}V_{IA}}\right) \qquad (5.4)$$

where S is the ICP-MS signal (count per second) and IA is isotope abundance (mole basis)

5.3 RESULTS AND DISCUSSION

5.3.1 Effect of equilibration time, concentration of $^{50}Cr^{VI}$ spike and TMAH concentration on labile Cr

Cr_{TMAH} and Cr_E

In this preliminary experiment, the concentration of TMAH in soil suspension was varied from 0.01, 0.1, 1.0 to 10.0 % with three concentrations of ^{50}Cr spike representing 50,100 or 150 % of the anticipated concentration of labile $^{50}Cr^{VI}$ in the soil. A solution of 10 % TMAH was chosen alongside the lower concentration as it is used to extract Cr^{VI} from soil (Chapter 2, Section 2.2.7); solutions were diluted (1:10) prior to analysis. The objective was to determine the optimum TMAH range that will solubilize labile Cr and provide robust measurements of the isotope ratio of $^{50}Cr/^{52}Cr$ in solution.

The concentrations of the basic (compound) E-value (Cr_E) and TMAH extractable Cr (Cr_{TMAH}) determined in 0.01% TMAH are shown in Figure 5.2a. At all spike concentrations and equilibration times, Cr_{TMAH} was fairly constant. By contrast, Cr_E was greater than

Cr_{TMAH} and increased with isotope equilibration time. It therefore seems likely that the TMAH (0.01 %) did not solubilize all the labile Cr ($Cr_E > Cr_{TMAH}$) but it is also clear that the spike isotope continued to access (mix with) an increasing proportion of Cr in the solid phase over 4 days, possibly suggesting that it may be difficult to easily distinguish an immediately exchangeable fraction using isotopic dilution approaches.

At 0.1% TMAH (Fig. 5.2b), the ^{50}Cr spike appeared to reach equilibrium with the native Cr quite rapidly; there was little change in Cr_E with equilibration time. However, at all levels of ^{50}Cr spike the Cr_E value was about the same concentration as Cr_{TMAH} except in soil samples suspended in 0.1 % TMAH for 1 or 2 days. This would suggest that the added ^{50}Cr spike has simply equilibrated with Cr in the solution phase because 0.1 % TMAH has successfully solubilised all the labile Cr in the soil – so none of the spike isotope has been adsorbed. The values of Cr_E were also broadly comparable to the values measured after 4-days equilibration in 0.01 % TMAH, or even slightly less, which is difficult to explain.

For soils suspended in 1.0 and 10.0 % TMAH, the concentration of Cr_{TMAH} was about 3-5 times greater than Cr_E (Figure 5.2c and 5.2d). This must be because the higher TMAH concentrations (1 and 10%) mobilized non-labile form of Cr, such as Cr^{III}-DOC complexes, as well as Cr^{VI} in solution. The concentrations of Cr_E were also considerably greater than measured in 0.01 and 0.1 % TMAH, suggesting perhaps that the higher TMAH concentrations had solubilized (and rendered labile) previously non-labile forms of Cr. Concentrations of Cr_E value also appeared to increase with increase in spike level of ^{50}Cr from 50 to 150 % in 1.0 and 10 % TMAH.

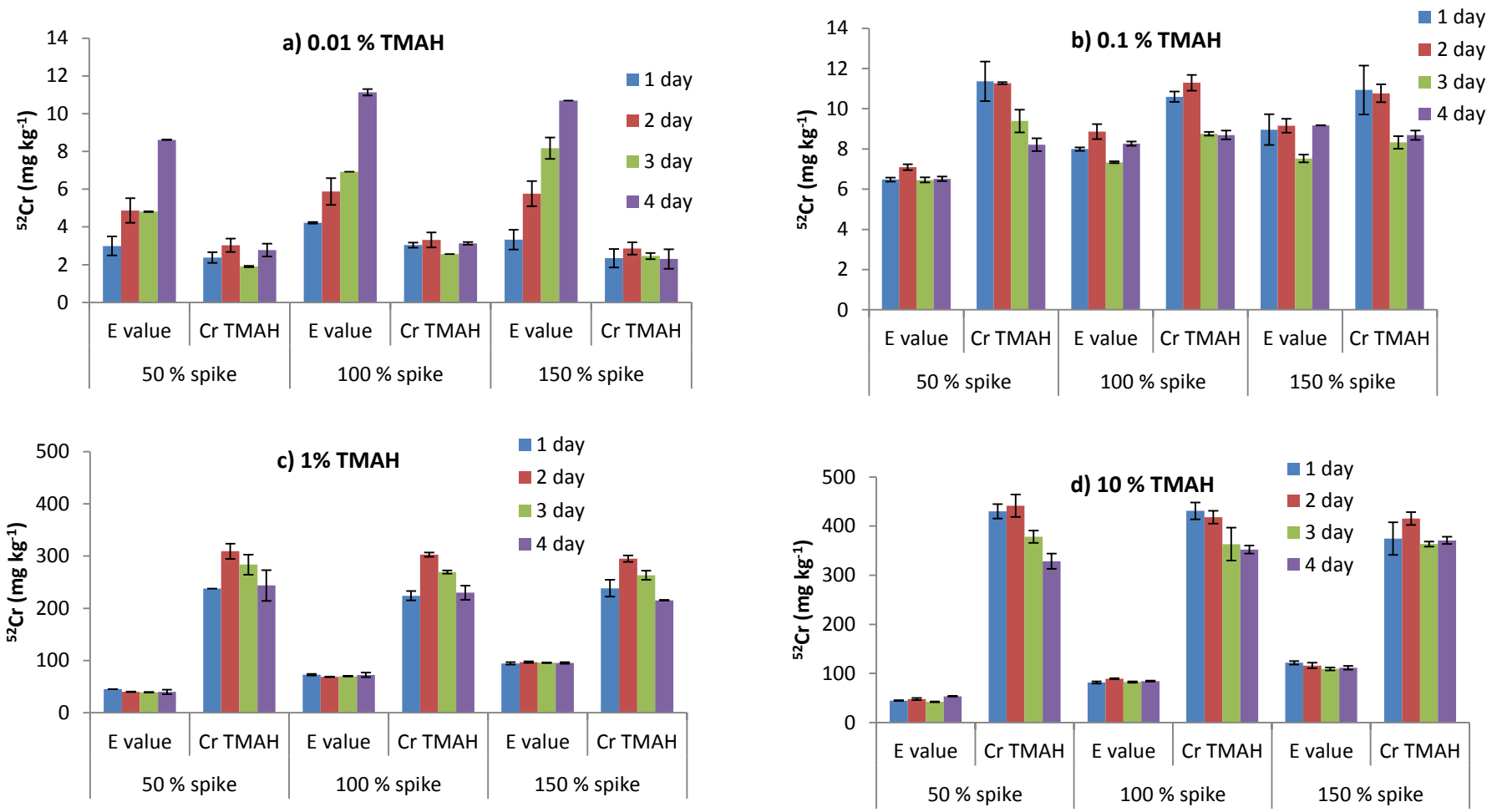


Figure 5.2 The concentrations of the simple form of the E-value (Cr_E) and Cr_{TMAH} measured on soil suspended in a range of TMAH concentration; 0.01, 0.1, 1.0 and 10 % and spiked with 50, 100 and 150 % of $^{50}Cr^{VI}$ following equilibration for 1 to 4 days. The error bars show standard errorr of three replicates.

Isotope ratio, Rss

The ratio of isotopic abundances Rss in the equilibrated solution is calculated from the ratio of the isotopic abundance of the spike isotope (^{50}Cr) to that of the native isotope (^{52}Cr) in the soil with the inclusion of a mass discrimination factor to convert measured intensity (cps) ratios to true isotopic ratios. The Rss values of ^{50}Cr/^{52}Cr at different ^{50}Cr spike levels and equilibration times are shown in Figure 5.3. At 0.01 and 0.1 % TMAH, a higher Rss value can be observed for all ^{50}Cr spike levels compared to the background Rss (shown as a zero spike level) value showing the enrichment of the spike isotope in solution. In the case of 0.01 % TMAH (Fig. 5.3a) there was a clear decline in Rss value with isotope equilibration time as the $^{50}Cr^{VI}$ spike slowly equilibrated with Cr pools in the soil solid phase. By contrast, the suspensions in 0.1 % TMAH were much more stable; this contrast is reflected in the trend in E-values already discussed. In order to measure a reliable E-value, the isotope ratio (Rss) should be sufficiently different from background. Therefore, these TMAH ranges (0.01 to 0.1 %) could be used for further investigations. As the suspending TMAH concentration was increased to 1 and 10 %, the Rss value became virtually identical to the native Rss value (Fig. 5.3c and 5.3d). At these concentrations of TMAH, so much native (non-labile) soil Cr has been solubilized that Rss values are indistinguishable from background and the E-value is completely unreliable.

A further complication lies in the lack of discrimination between Cr^{III} and Cr^{VI} so that the measurement of Rss in spiked solutions is actually a compound of several species – for example chromate (Cr^{VI}) ions and humic-bound Cr^{III} mobilized by the TMAH (Eq 5.5).

$$Rss = \frac{^{50}Cr}{^{52}Cr} = \left(\frac{Humic\text{–}^{50}Cr^{III} + ^{50}Cr^{VI}}{Humic\text{–}^{52}Cr^{III} + ^{52}Cr^{VI}} \right) \qquad (5.5)$$

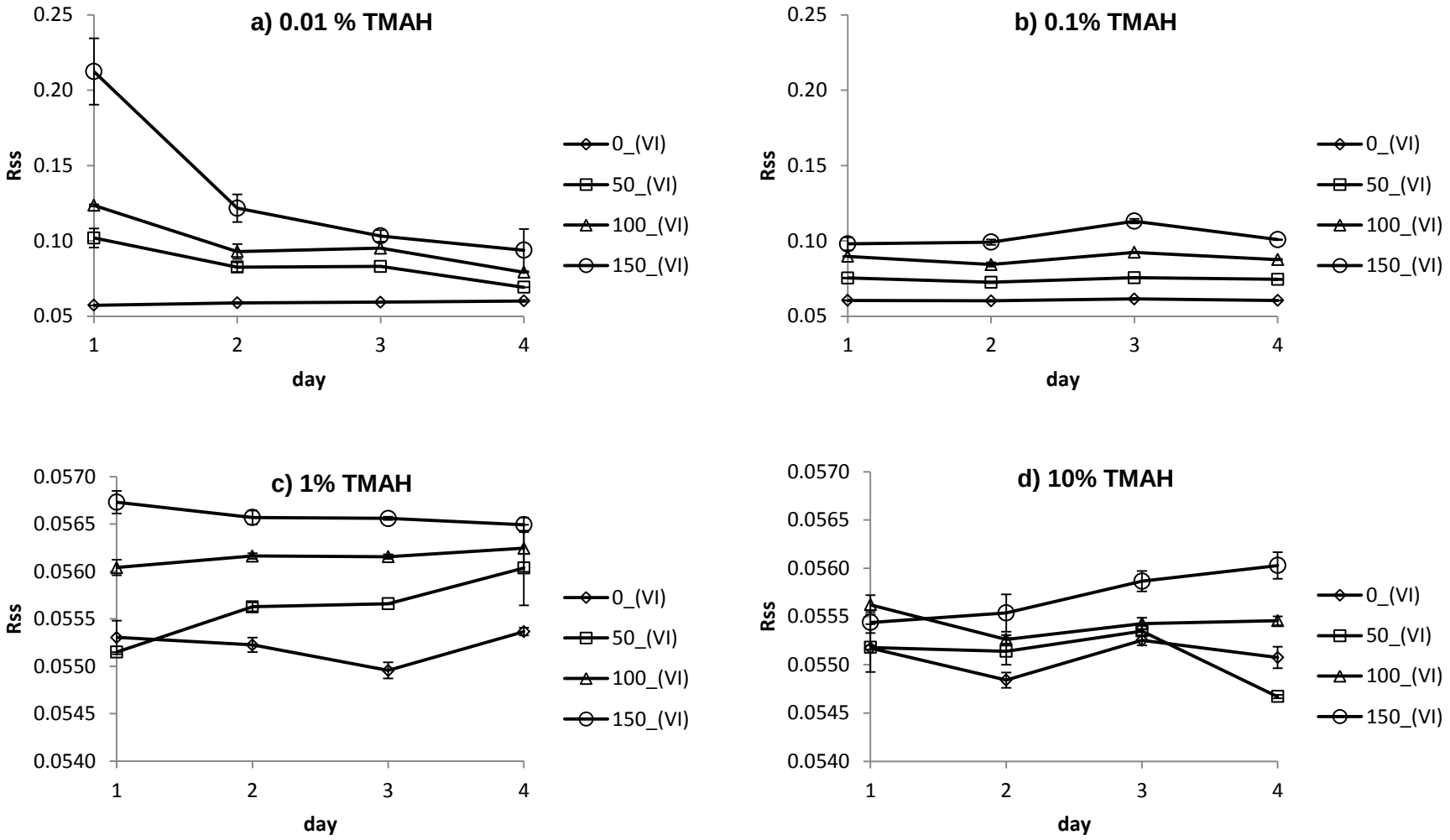


Figure 5.3 The isotope ratio (Rss) of $^{50}Cr/^{52}Cr$ at different ^{50}Cr spike; 0, 50, 100 and 150% of ^{50}Cr at various TMAH concentrations; 0.01, 0.1, 1.0 and 10.0 % and equilibrate for 1 to 4 days. The error bars show standard errors of three replicates.

Cr^{VI}_{TMAH} and Cr^{VI}_{E}

Speciation (LC-ICP-MS) was also undertaken on the soil solutions in order to measure TMAH-extracted Cr^{VI} (Cr^{VI}_{TMAH}) and labile Cr^{VI} (Cr^{VI}_{E}) (Fig. 5.4). In 0.01% and 0.1% TMAH, the E-value Cr^{VI}_{E} was almost two orders of magnitude smaller than Cr_E measured without speciation (Figs 5.2a and 5.2b). Otherwise the pattern shown was similar in that (i) Cr^{VI}_{E} was larger than Cr_{TMAH} in 0.01% TMAH (Fig. 5.3a), suggesting that the spike isotope had been partially adsorbed in the solid phase and (ii) values of Cr^{VI}_{E} in 0.01% TMAH increased with equilibration time towards the values for Cr^{VI}_{E} measured in 0.1% TMAH (Fig. 5.4b). Values of Cr^{VI}_{E} measured in 0.1% TMAH were much more stable with equilibration time and spike level and were very similar to Cr_{TMAH}, suggesting that all labile Cr^{VI} had been solubilised and the spike isotope was virtually all in solution. All these factors combined suggest that simple extraction with 0.1% TMAH provides a good estimate of a relatively stable E-value. The slow isotopic equilibration shown in 0.01% TMAH suggests that the kinetic restrictions on establishing a solid ⇌ solution equilibrium for labile Cr^{VI} may negate the development of an effective E-value protocol.

In 1% and 10% TMAH the apparent values of Cr^{VI}_{E} were much higher. The concentration of Cr^{VI}_{TMAH} was about the same as Cr^{VI}_{E} in 1.0 % TMAH at 50 and 100 % spike, but a higher concentration of Cr^{VI}_{E} was found in the 150 % spike. However, the concentration of Cr^{VI}_{E} showed an apparent decrease with time at 10 % TMAH while Cr^{VI}_{TMAH} extracted was higher than in 1% TMAH. It is very likely that the larger concentrations of TMAH (1% and 10%) mobilized non labile Cr^{VI} into solution and may also have caused redox changes which compromised the analysis. A further complication lies with the reliability of measured Rss values across the range of TMAH concentrations and, specifically, their discrimination against the unspiked soil suspensions.

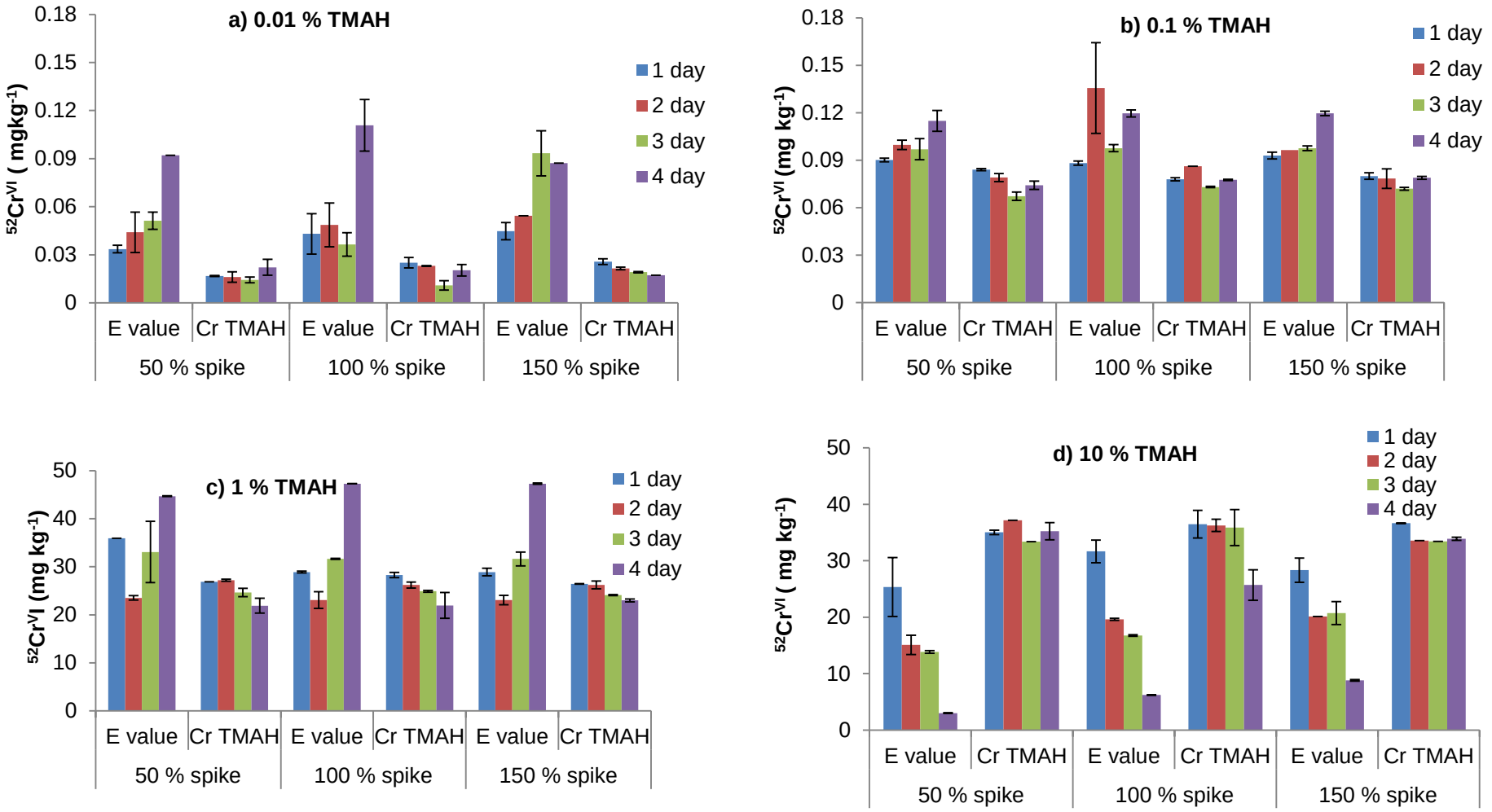


Figure 5.4 The concentrations of the speciated form of E-value (Cr^{VI}_{E}) and Cr^{VI}_{TMAH} measured on soil suspended in a range of TMAH concentration; 0.01, 0.1, 1.0 and 10 % and spiked with 50, 100 and 150 % of $^{50}Cr^{VI}$ following equilibration for 1 to 4 days. The error bars show standard errors of three replicates.

Isotope ratio, Rss of $^{50}Cr^{VI}/^{52}Cr^{VI}$

The isotopic abundance ratios (Rss) of $^{50}Cr^{VI}/^{52}Cr^{VI}$ at different levels of ^{50}Cr spike, equilibration times and TMAH concentrations are shown in Figure 5.5. The isotopic abundance ratios of Cr^{VI} showed larger differences across the range of spike concentrations compared to Rss values calculated without speciation (Fig 5.3). In 0.1% TMAH, the decline in Rss value with time as the spike gradually moves towards an equilibrium state is clear. The stability of Rss in 0.1% TMAH, by contrast, reflects the fact that virtually all labile Cr^{VI} is in solution. For both 0.01% and 0.1% TMAH the difference in Rss across the spike concentrations is clear and provides a relatively robust analysis. However, for 1% and 10% TMAH the mobilization of (presumably) non-labile Cr into solution has resulted in very little discrimination against background Rss values and, although there is a qualitative trend with spike concentration, it is likely that the measured Rss values at different $^{50}Cr^{VI}$ spike concentrations are not analytically robust.

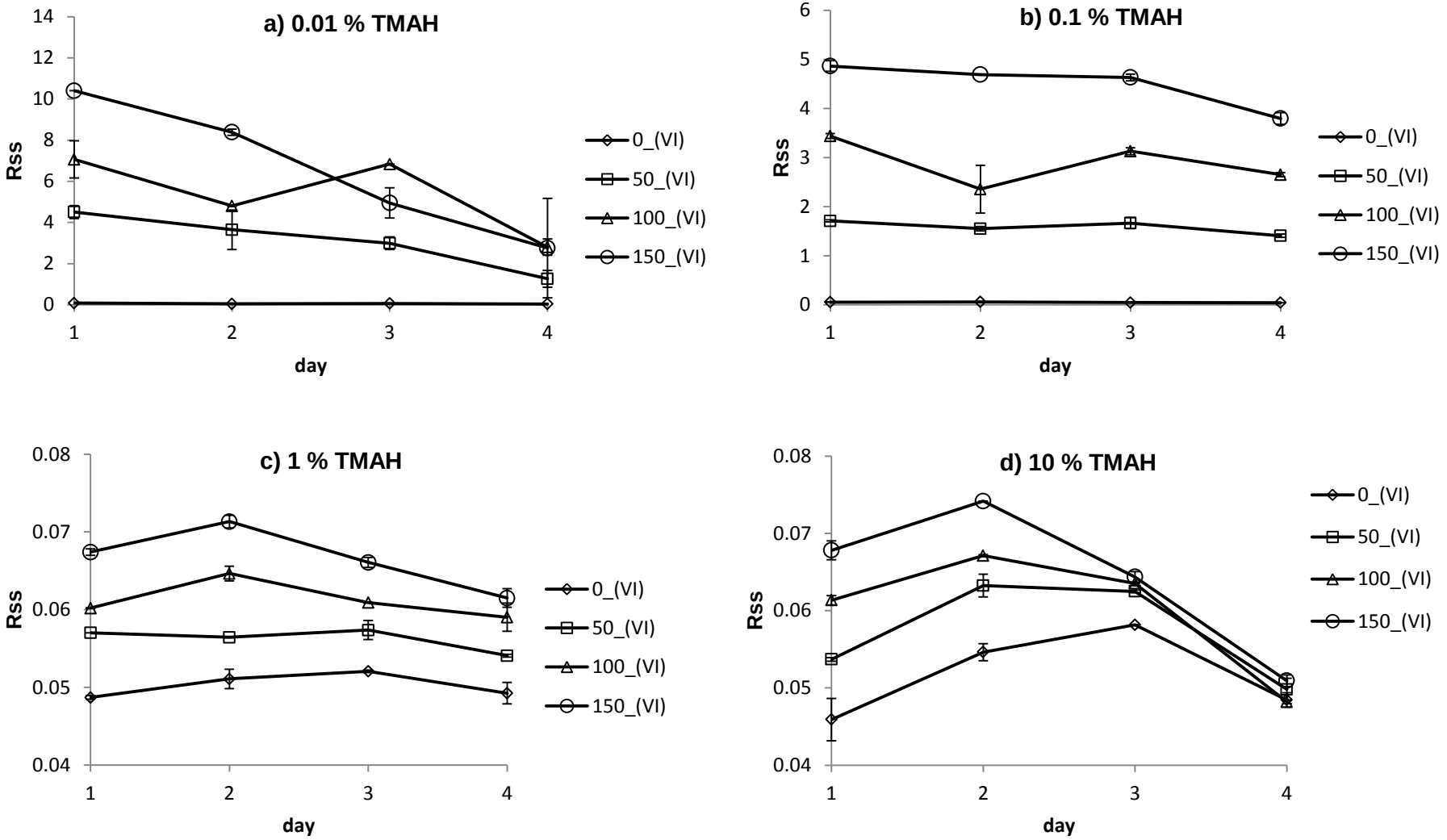


Figure 5.5 The isotope ratio (Rss) of $^{50}Cr^{VI}/^{52}Cr^{VI}$ at different concentration of spiked; 0, 50, 100 and 150% of $^{50}Cr^{VI}$ in soil, TMAH concentrations; 0.01, 0.1, 1.0 and 10.0 % and equilibration from 1 to 4 days. The error bars show standard errors of three replicates.

5.3.2 Effect of different ranges of TMAH on Cr_E and Cr^{VI}_E

As 0.01% and 0.1 % TMAH gave promising results in determining Cr_E and Cr^{VI}_E, this range was chosen for further investigation. In this experiment, the soil samples were equilibrated for 3 days in a range of TMAH concentrations from 0.01 to 1.0 % at the 150 % $^{50}Cr^{VI}$ spike level. The aim of this experiment was to establish a suitable range of TMAH in partially solubilizing Cr^{VI} with minimal mobilization of non-labile Cr or redox changes. The objective was to establish a true solid ⇌ solution equilibrium which permits measurement of the $^{50}Cr^{VI}$ spike distribution between the solid and solution phases of the soil suspension rather than having the spike entirely absorbed or wholly in solution.

The solution concentration of $^{50}Cr^{VI}$ in unspiked and spiked soil suspended at different TMAH concentrations is shown in Figure 5.6. Adsorption of the spike isotope can be clearly seen at low concentrations (0.01 and 0.05 %) of TMAH. The proportion of $^{50}Cr^{VI}$ spike in the solution phase increased with TMAH concentration until at 0.05 to 0.09 % TMAH, all the $^{50}Cr^{VI}$ spike added was in solution. At still higher TMAH concentrations there was then clear evidence of native soil $^{50}Cr^{VI}$ mobilization (0.1 to 1.0 % TMAH). The spiked and un-spiked soil solution trends ran in parallel with $^{50}Cr^{VI}$ in solution in the spiked system rising above the spike level just as the unspiked system showed a marked increase in native $^{50}Cr^{VI}$ mobilisation.

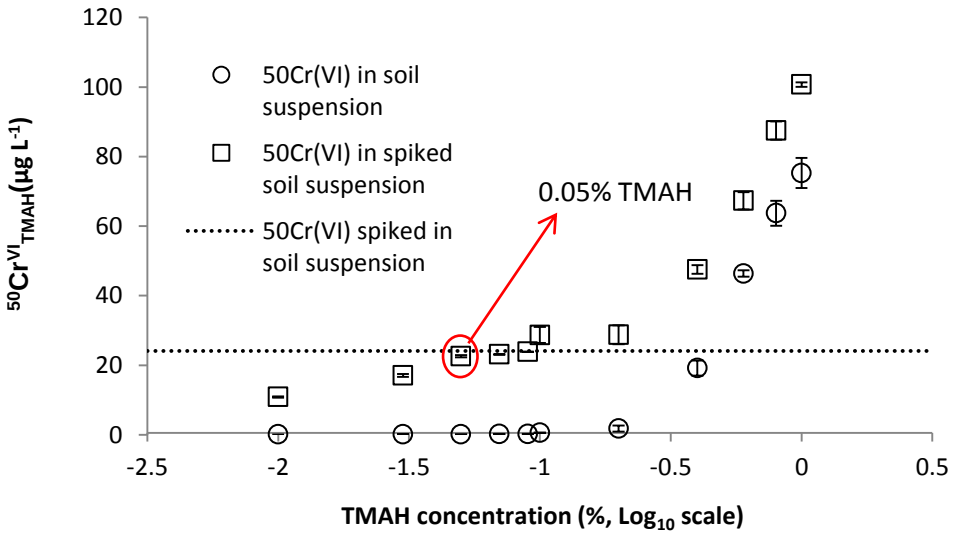


Figure 5.6 The solution concentration of $^{50}Cr^{VI}$ (µg L^{-1}) in spiked and un-spiked soil suspensions at different TMAH concentrations (0.01 to 1.0 %). The horizontal dotted line shows the spike level, equivalent to all the added $^{50}Cr^{VI}$ spike being in solution. The error bars show standard errors of three replicates.

The stability of the $^{50}Cr^{VI}$ spike in the TMAH soil solutions was assessed by subtracting the concentration of $^{50}Cr^{VI}$ in the un-spiked soil solutions from the concentration of $^{50}Cr^{VI}$ in the spiked soil solutions (Figure 5.7). At 0.01 to 0.07 % TMAH, about (50-95%) of $^{50}Cr^{VI}$ was recovered in the soil solution, so the remaining $^{50}Cr^{VI}$ can be assumed to be adsorbed on the soil surface (Figure 5.6). At higher TMAH concentrations (0.1 - 1.0 %) the $^{50}Cr^{VI}$ spike was conserved in solution and subtraction of the native $^{50}Cr^{VI}$ mobilised provided a reasonably convincing mass balance on the spike level.

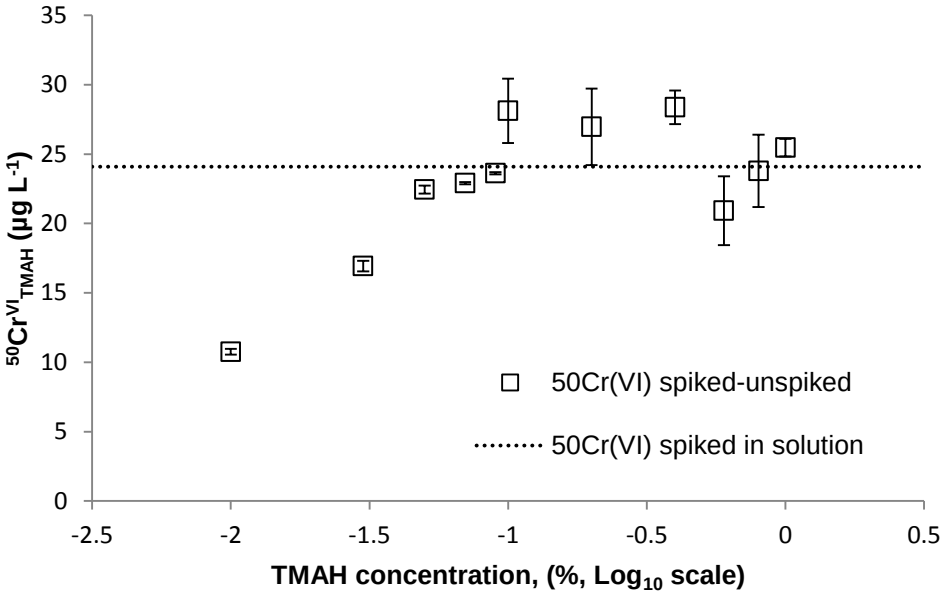


Figure 5.7 The concentration of $^{50}Cr^{VI}$ (µg L^{-1}) spike remaining in the soil suspension after spiking with 24.1 µg L^{-1} of $^{50}Cr^{VI}$ (dotted line) at different TMAH concentrations (0.01 to 1.0 %). The error bars show standard errors of three replicates.

The distribution of $^{50}Cr^{VI}$ spike in the soil suspension from low to high TMAH concentration can be clearly explained by dividing the overall trend in isotopic abundance ratio (Rss) into three sections (Figure 5.8). For this purpose the Rss value was corrected for native $^{50}Cr^{VI}$ mobilization by (i) subtracting the background Rss value (0.05186) and subtracting the measured Rss value in an unspiked soil suspension (spiked-unspiked); both approaches gave very similar results (Fig. 5.8).

Part A - Rss increased with TMAH concentration due to *greater relative solubility* of the $^{50}Cr^{VI}$ spike over that of native $^{50}Cr^{VI}$ in soil. The evidence for this can be seen in Figure 5.6, as more spike $^{50}Cr^{VI}$ was in solution from 0.01 to 0.05 % TMAH compared to the trend in

native soil $^{50}Cr^{VI}$. It should be noted that the degree to which a fixed pool of labile Cr^{VI} is solubilised should, theoretically, have no effect on the Rss value. This is because the spike isotope should mix completely with the entire labile pool and therefore produce the same level of isotopic abundance in the solution phase (and labile adsorbed phase), regardless of the proportion of the labile pool which is in solution. The fact that Rss increases with solubility suggests that the ability of adsorbed labile Cr^{VI} to isotopically exchange is actually kinetically constrained. Although there is rapid adsorption of the added $^{50}Cr^{VI}$ spike, there is much slower desorption of labile $^{52}Cr^{VI}$ from the soil as part of the approach to isotopic equilibrium.

Part B – When all the spike is in solution, inevitably the TMAH mobilizes more native $^{52}Cr^{VI}$ and possibly oxidises humic-$^{52}Cr^{III}$ causing a decrease in Rss.

Part C – A stable Rss value which approaches background levels is established as more non labile $^{52}Cr^{VI}$ is aggressively mobilized by TMAH and the $^{50}Cr^{VI}$ spike level is simply swamped by native $^{52}Cr^{VI}$ in the soil suspension.

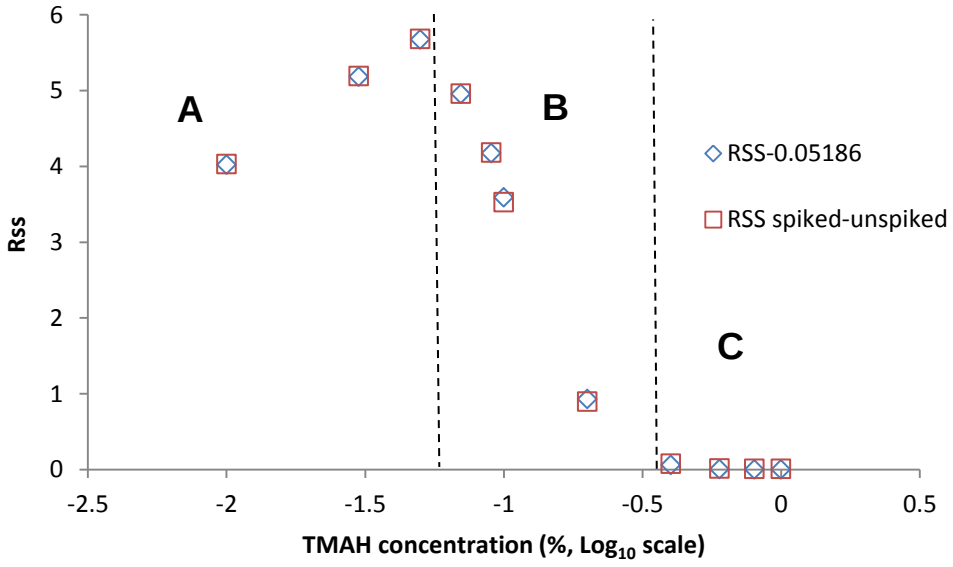


Figure 5.8 The isotope ratio of $^{50}Cr^{VI}/^{52}Cr^{VI}$ (Rss) at different TMAH concentrations (log_{10} scale). A value of 0.05186 refers to the background Rss of $^{50}Cr^{VI}$:$^{52}Cr^{VI}$ in standard 20 µg L^{-1} $^{50}Cr^{VI}$ solution; the unspiked value refers to the Rss of an unspiked soil TMAH solution (blank).

The total concentration of ^{50}Cr in the spiked and unspiked soil TMAH suspensions (0.01 to 1.0 % TMAH) are shown in Figure 5.9. There was only a small difference in the concentration of ^{50}Cr in the spiked soil suspension compared to soil suspension at < 0.2 % TMAH. Thus at >0.2 % TMAH non labile Cr in the soil started to be mobilized.

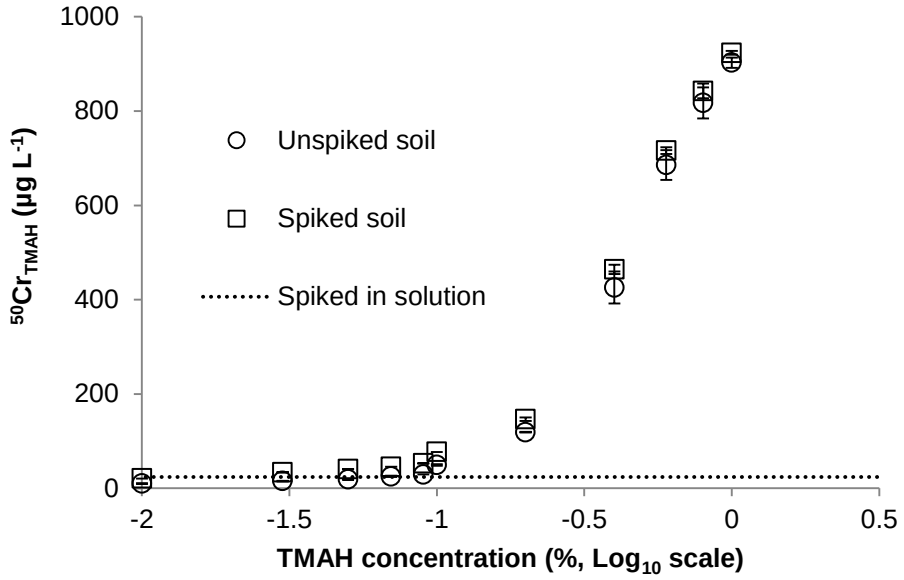


Figure 5.9 The concentration of ^{50}Cr (µg L^{-1}) in spiked and unspiked soil suspensions in different TMAH concentrations (0.01 to 1.0 %). The error bars show the standard errors of three replicates.

The concentrations of Cr_{TMAH} and Cr_E are shown in Figure 5.10 (a) and (b) and the speciated values are shown in Figure 5.10(c) and (d). The pattern shown is consistent with the previous experiment. Values of Cr_E (unspeciated) greatly exceeded those of Cr^{VI}_E due to the inclusion of trivalent Cr in the calculation. At the lower TMAH range (<0.2%), the concentration of Cr^{VI}_{TMAH} and Cr^{VI}_E were about 100 times lower than Cr_{TMAH} and Cr_E. At higher TMAH concentrations (>0.5%) non labile Cr was mobilized into the soil solution causing an increase in the apparent values of Cr_E and Cr^{VI}_E. From the results, soil suspension in 0.01 to 0.05 % TMAH appears to provide a reasonably robust suspending matrix for the determination of E-values provided speciation is undertaken. Figure 5.10 (c) shows that there is a restricted range of TMAH concentration (0.05 – 0.09%) over which $Cr^{VI}_E \approx Cr_{TMAH}$ indicating that all the spike stays in solution but very little non-labile Cr has been mobilized.

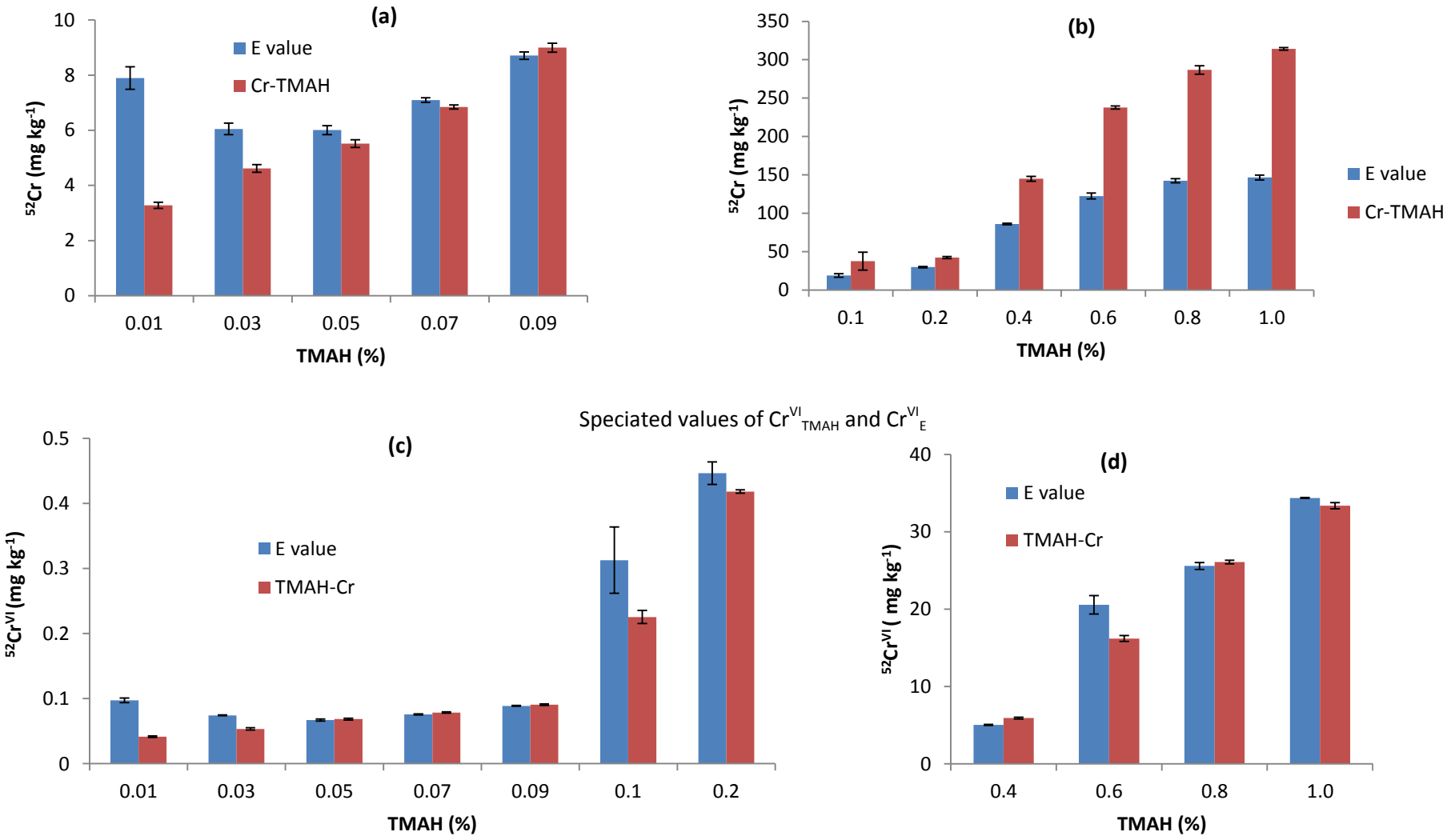


Figure 5.10 The concentrations of Cr_E and Cr_{TMAH} (a & b) and $Cr^{VI}{}_E$ and $Cr^{VI}{}_{TMAH}$ (c &d),(mg kg^{-1}) at a range of TMAH concentration (0.01 to 1.0%), spiked at a level of 150 % of $^{50}Cr^{VI}$, and equilibrated for 3 days. The error bars show standard errors of three replicates.

5.3.3 Comparison of 0.01 M $Ca(NO_3)_2$ and TMAH concentrations (0.001 to 0.04 %) on Cr^{VI}_E

This experiment was carried out to compare the E-values determined using an established neutral electrolyte, 0.01 M $Ca(NO_3)_2$, as the soil suspending matrix, with the range of TMAH concentrations which appeared to give reasonably stable E-value results (0.001, 0.01, 0.02 and 0.04 %). A range of ^{50}Cr spikes (30, 60, 90 or 120 % change in the natural abundance of the (estimated) labile Cr^{VI} pool in the soil) was tested and spiked suspensions were equilibrated for 3 days. Some studies have suggested using 0.1 M $CaCl_2$ as the suspension electrolyte, but chloride may produce Cl^-O^+ polyatomic isobaric interferences with the ^{52}Cr analyte (Kan et al., 2004, 2006; Vanhaecke et al., 2006; Goessler et al., 2008; Unceta et al., 2010) and so a nitrate electrolyte was chosen.

The concentrations of $^{50}Cr^{VI}$ in the solution phase of unspiked and spiked soil suspensions, at a range of TMAH concentrations and 0.01 M $Ca(NO_3)_2$, are shown in Figure 5.11. As expected, there was a progressive increase in $^{50}Cr^{VI}$ concentration in solution with increasing TMAH concentration (and with spike level). In 0.01 M $Ca(NO_3)_2$ a much lower range of $^{50}Cr^{VI}$ concentrations was observed compared to the solution concentrations of $^{50}Cr^{VI}$ soil suspended in TMAH. This reflects, in part, the stronger absorption of $^{50}Cr^{VI}$ in soil at lower pH (6.03 after 3 days equilibration) compared to the higher pH values measured in the TMAH soil suspension (Table 5.1).

Table 5.1 Effect of equilibration time and solution matrix on the soil suspension pH

Solution matrix	pH value		
	24 hr	3 days	Difference
0.001 % TMAH	6.98	6.78	0.20
0.01 % TMAH	7.36	6.79	0.57
0.02 % TMAH	7.80	6.96	0.84
0.04 % TMAH	8.56	7.79	0.77
0.01 M $Ca(NO_3)_2$	6.39	6.03	0.36

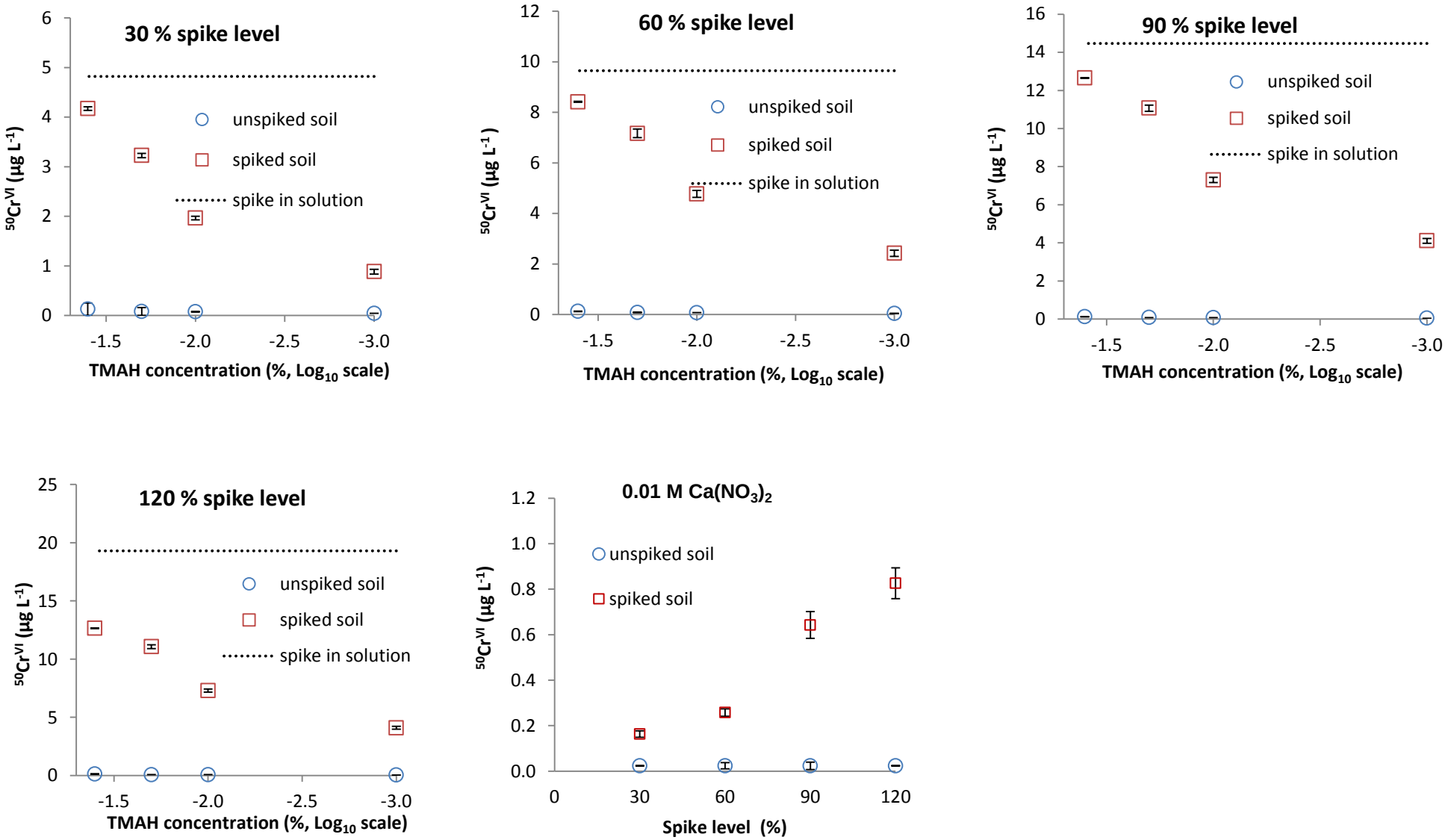


Figure 5.11 Concentration of $^{50}Cr^{VI}$ (µg L^{-1}) in spiked and unspiked soil suspensions for a range of TMAH concentrations (0.001, 0.01, 0.02, 0.04 %) and 0.01 M $Ca(NO_3)_2$ at different $^{50}Cr^{VI}$ spike levels (30, 60, 90 and 120 % of native $^{50}Cr^{VI}$ in soil) equilibrated for 3 days. The horizontal dotted line indicates the spike level; the error bars show the standard errors of three replicates.

Values of Rss (ratio of $^{50}Cr^{VI}$ to $^{52}Cr^{VI}$ isotopic abundances in spiked suspensions) at different concentrations of TMAH and 0.01M $Ca(NO_3)_2$, and at various $^{50}Cr^{VI}$ spike levels, are shown in Figure 5.12. The values of Rss increased with increasing spike level, as expected, but also with TMAH concentration due to preferential solubilisation of the $^{50}Cr^{VI}$ spike over native soil $^{52}Cr^{VI}$, as seen previously (Figure 5.8). Again this supports the idea that the labile adsorbed Cr^{VI} is kinetically constrained from rapid isotopic mixing, as discussed previously. Thus, even lower values of Rss were observed at all levels of $^{50}Cr^{VI}$ spike in 0.01 M $Ca(NO_3)_2$ compared with the TMAH suspensions because of strong adsorption of the spike (Fig. 5.12). It is obviously important to allow sufficient time for the spike isotope to fully mix with the adsorbed labile Cr^{VI} pool in soil and the time required appears to depend on the suspending solution matrix. As seen in the previous experiments, complete and rapid mixing may only occur when the suspending solutions has effectively solubilised all the labile pool. Hamon et al., (2008) suggested a post-equilibration time of 1 to 3 days is sufficient for isotopic equilibrium to be achieved and this sort of time period is normally adopted for most applications. In 0.01 M $Ca(NO_3)_2$ it appears that full isotopic mixing has not occurred and the difference between spike and background is possibly too small which may affect the reliability of the E-value measured. As seen earlier, the concentration of $^{50}Cr^{VI}$ is too low in 0.01 M $Ca(NO_3)_2$ (Figure 5.11) and there is a need to prolong the equilibration of the $^{50}Cr^{VI}$spike in the soil-$Ca(NO_3)_2$ electrolyte in order to allow more 'labile' $^{50}Cr^{VI}$ to solubilize (without mobilizing non-labile Cr^{VI}) into solution to provide robust measureable values of the true labile isotopic ratio of $^{50}Cr^{VI}/^{52}Cr^{VI}$ in solution.

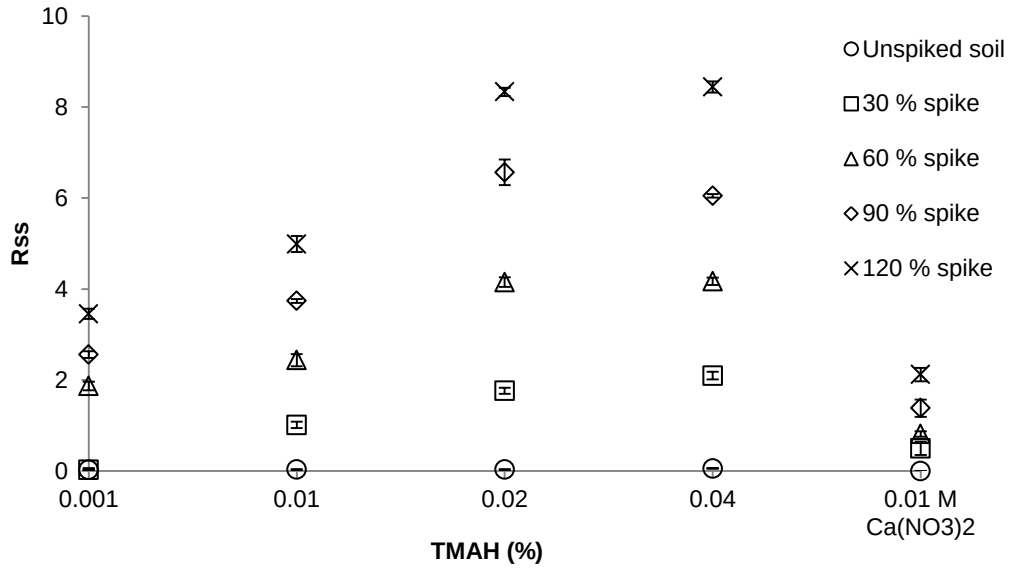


Figure 5.12 The isotopic ratio (Rss) $^{50}Cr^{VI}/^{52}Cr^{VI}$ at different concentrations of TMAH (0.001, 0.01, 0.02 and 0.04 %) and 0.01 M $Ca(NO_3)_2$ with various $^{50}Cr^{VI}$ spiked levels (30, 60, 90 and 120 %) equilibrated for 3 days. The error bars show the standard errors of three replicates.

The effect of $^{50}Cr^{VI}$ spike levels on Cr^{VI}_{E} and Cr^{VI}_{TMAH} at different TMAH concentrations and neutral electrolyte is shown in Figure 5.13. A continuous spectrum of adsorption strengths can be seen from 0.001 to 0.04 % TMAH as the concentration of Cr^{VI}_{E} decreased with increasing TMAH concentration due to greater solubility of native $^{52}Cr^{VI}$ – which would then be interpreted as the spike isotope ($^{50}Cr^{VI}$) mixing with a smaller labile pool (increase in Rss), as discussed previously. In general, the concentration of Cr^{VI}_{E} was higher in soil-$Ca(NO_3)_2$ suspensions compared to TMAH (Figure 5.13) for the same reasons: strong adsorption of the spike isotope is not matched by desorption of $^{52}Cr^{VI}$ from the native soil labile pool due to kinetic constrainst on desorption. Increasing the $^{50}Cr^{VI}$ spike level caused slightly lower values of Cr^{VI}_{E} in soil-$Ca(NO_3)_2$. This may have been due to more weakly held $^{50}Cr^{VI}$ at higher spike levels (Rss increases if more spike stays in solution) but there is also greater uncertainty in the measurements as Rss was close to background at low spike levels. Clearly, longer equilibration times would be needed to reach isotopic equilibrium and achieve reliable Cr^{VI}_{E} measurements in 0.01 M $Ca(NO_3)_2$ and this would increase the risk of anaerobic conditions and isotopic mixing with the otherwise non-labile fraction of Cr^{VI}.

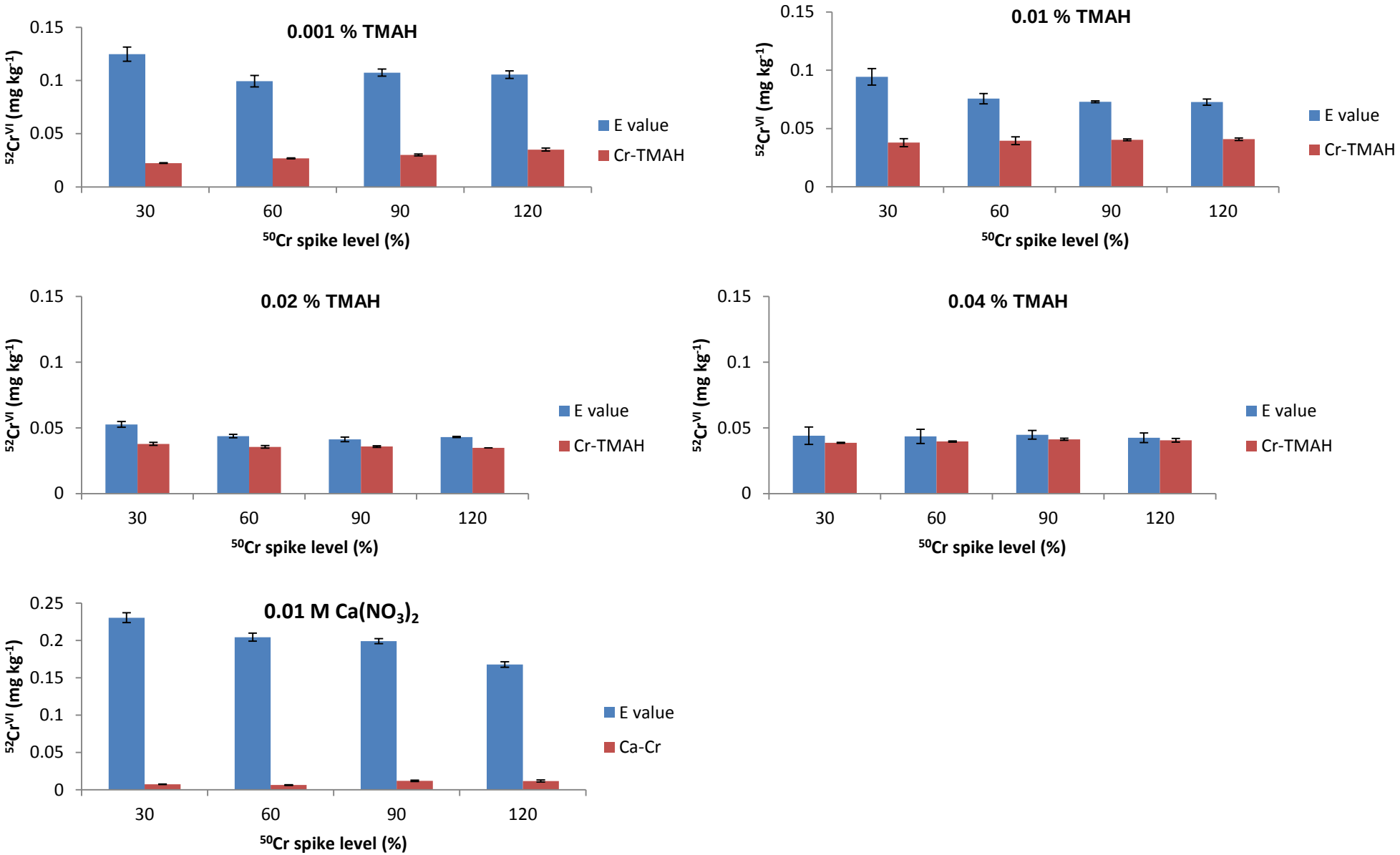


Figure 5.13 The concentration of $^{52}Cr^{VI}$ E value and Cr_{TMAH} at a range of TMAH concentrations (0.001, 0.01, 0.02, 0.04) and 0.01 M $Ca(NO_3)_2$ with different level of $^{50}Cr^{VI}$ spike levels (30, 60, 90 and 120 %) and equilibrated for 3 days. The error bars show standard errors of three replicates.

5.3.4 Optimising equilibration time and $^{50}Cr^{VI}$ spike concentration in 0.01 M $Ca(NO_3)_2$ for the measurement of Cr^{VI}_E

This experiment was carried out in an attempt to optimise the equilibration time of a ^{50}Cr spike a in 0.01 M $Ca(NO_3)_2$ matrix. The time period chosen was from 1 to 7 days with different levels of $^{50}Cr^{VI}$ spike from 30 to 120% of the natural abundance $^{50}Cr^{VI}$ in the soil. The concentration of $^{50}Cr^{VI}$ in the soil suspension at different levels of $^{50}Cr^{VI}$ spike and equilibration time was measured in order to assess the behaviour of the spike added (Figure 5.14a). The concentration of $^{50}Cr^{VI}$ was increased by increasing the $^{50}Cr^{VI}$ spike level and there was a progressive loss of $^{50}Cr^{VI}$ from solution with increased equilibration time up to about 5 days. A closer look at the change in concentration of $^{50}Cr^{VI}$ from 4 to 7 days equilibration time can be seen at Figure 5.14 (b). Unfortunately, the concentration of $^{50}Cr^{VI}$ was too low to be reliably measured from 4 to 7 days equilibration because of limitations in the sensitivity of the ICP-MS instrument. The limit of detection for the HPLC-ICP-MS system based on signal (S)/noise ratio was 0.04 µg $^{50}Cr^{VI}$ L^{-1}.

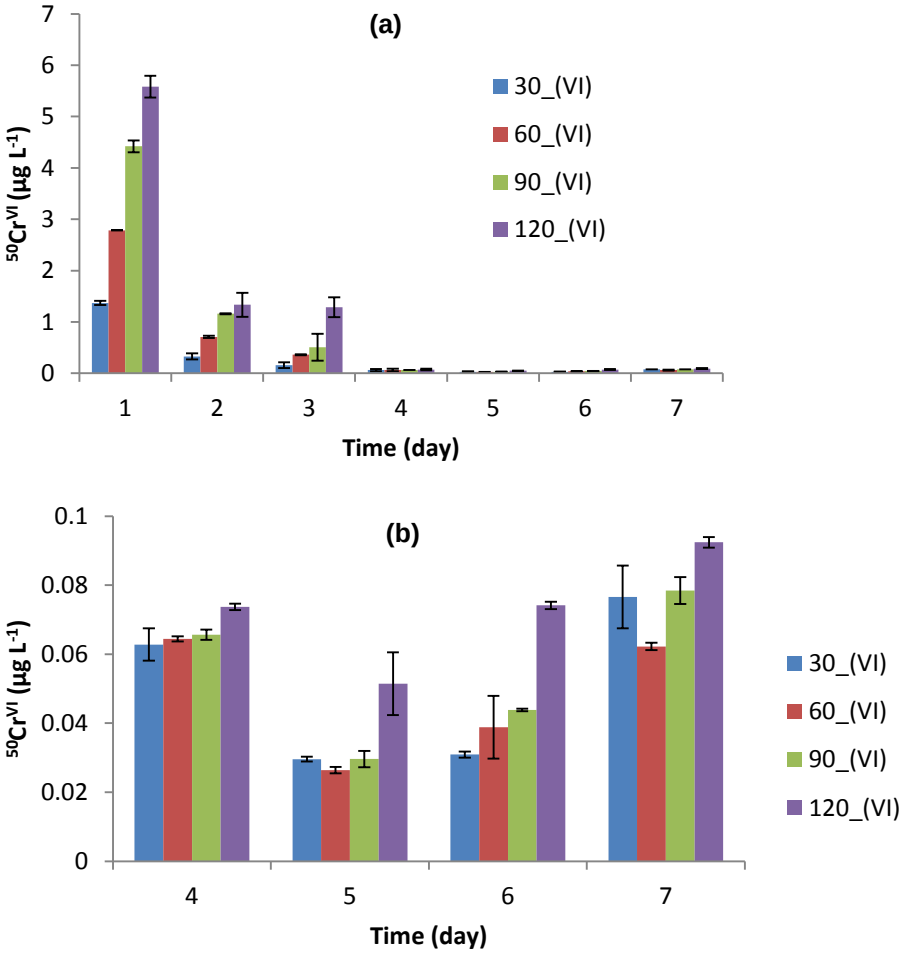


Figure 5.14 The concentration of Cr^{VI}_{CaNO3} (mg kg^{-1}) in the solution phase of SB soil suspensions equilibrated in 0.01 M $Ca(NO_3)_2$ for a)1 to 7 days and b) 4 to 7 days (a smaller scale) and spiked with 30 to 120 % levels of $^{50}Cr^{VI}$. The error bars show the standard errors of three replicates.

The natural abundance of ^{52}Cr is 83.4% (about 20 times higher than $^{50}Cr^{VI}$) and thus should be reliably detected by HPLC-ICP-MS (Figure 5.15). Surprisingly, the solution concentration of $^{52}Cr^{VI}$ in the 0.01 M $Ca(NO_3)_2$ soil suspension decreased with time. This may have been due to reduction of Cr^{VI} to humic-Cr^{III} or possibly precipitation as $CaCrO_4$ in the $Ca(NO_3)_2$ matrix (Figure 5.15). This adds a further complication to the problem of slow desorption of $^{52}Cr^{VI}$ discussed previously. It appears that although the rapid $^{50}Cr^{VI}$ spike adsorption is not matched, through isotopic mixing, by $^{52}Cr^{VI}$ desorption the limited solubilisation of $^{52}Cr^{VI}$ that does occur actually becomes re-adsorbed with extended equilibration time in a $Ca(NO_3)_2$ matrix.

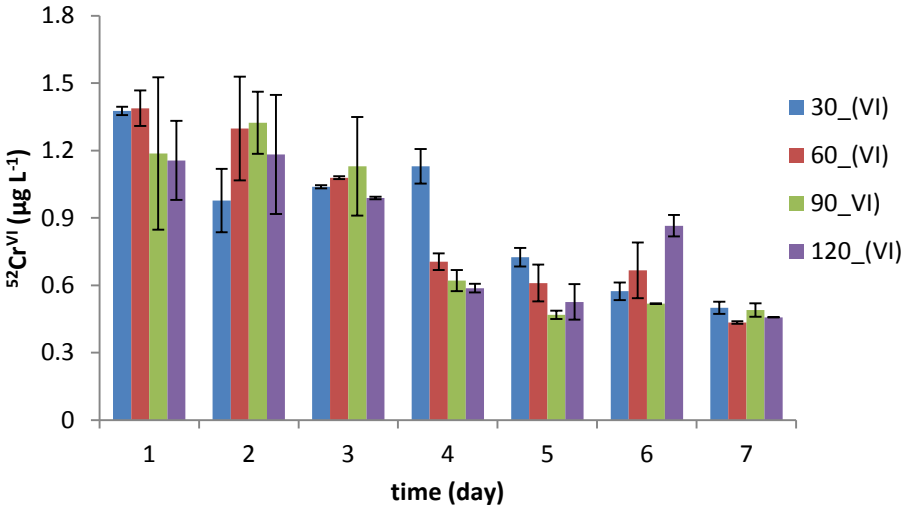


Figure 5.15 The concentration of $^{52}Cr^{VI}$ (µg L^{-1}) in the solution phase of SB soil suspensions equilibrated in 0.01 M $Ca(NO_3)_2$ for 1 to 7 days and spiked with 30 to 120 % of $^{50}Cr^{VI}$. The error bars show standard errors of three replicates.

The concentration of Cr^{VI}_E increased with equilibration time from 1 to 7 days (Figure 5.16). This is as expected and reflects the slow process of isotopic mixing between the native Cr^{VI} and spike $^{50}Cr^{VI}$, although the data for days 5 – 7 may be suspect because of uncertainty in the measurement of $^{50}Cr^{VI}$ at such small concentrations, as discussed previously. The behaviour of the spike $^{50}Cr^{VI}$ can be observed in Figure 5.17. Despite the progressive loss of native $^{52}Cr^{VI}$ from solution (Figure 5.15), longer equilibration times between the spike and soil-$Ca(NO_3)_2$ suspension resulted in an even greater rate of decrease in $^{50}Cr^{VI}$ spike concentration and so a progressive decrease in Rss value (Figure 5.17).

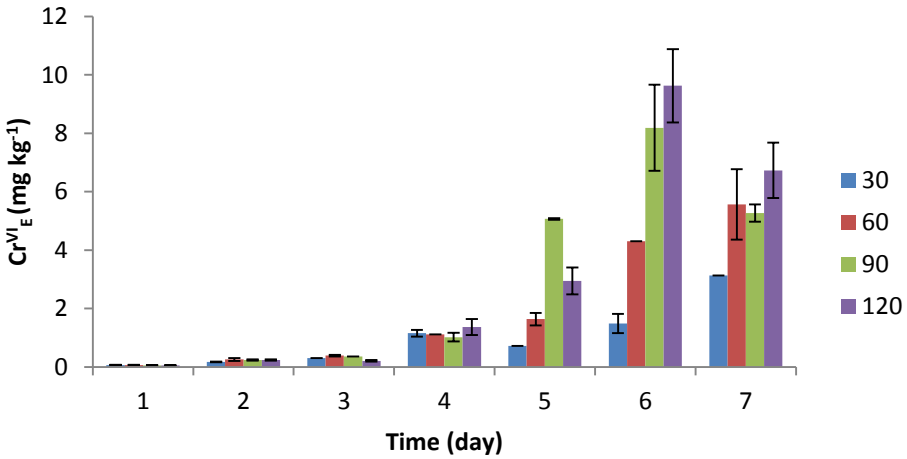


Figure 5.16 The concentration of Cr^{VI}_E (mg kg^{-1}) in SB soil samples equilibrated in 0.01 M $Ca(NO_3)_2$ for 1 to 7 days and spiked with 30 to 120 % of $^{50}Cr^{VI}$. The error bars show standard errors of three replicates.

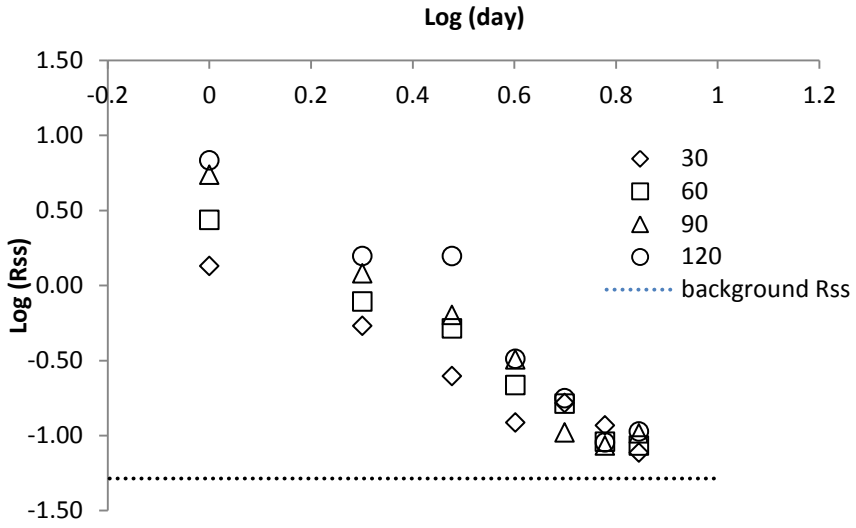


Figure 5.17 Change in Rss values with time for SB soil samples equilibrated in 0.01 M $Ca(NO_3)_2$ for 1 to 7 days and spiked with 30 to 120 % of $^{50}Cr^{VI}$. The horizontal dotted line indicates the background Rss.

5.4 CONCLUSIONS

Results from the experimental work presented in Chapter 5 suggest the need for Cr speciation in assessing the lability of Cr^{VI} in contaminated soils. The distribution of $^{50}Cr^{VI}$ spike between solid and solution phase of soil suspension was monitored by varying the concentration and type of the suspending solution matrix, concentration of $^{50}Cr^{VI}$ spike and spike isotope equilibration time. When the $^{50}Cr^{VI}$ spike was added to soil suspended at 0.01 to 10 % TMAH, it failed to reach equilibrium at low TMAH concentrations (0.01 - 0.1 %). At higher TMAH concentrations (0.1 – 10 %), non-labile Cr^{VI} and humic-bound Cr^{III} was mobilized into the solution producing higher concentrations of Cr_{TMAH} than Cr_E and, eventually, almost identical values of Rss in the spiked and unspiked systems. It was therefore necessary to follow the trend in isotopic abundance of $^{50}Cr^{VI}$ with equilibration time, and with TMAH concentration (0.001 – 0.04 %), to understand the kinetic process of isotopic exchange and its relationship with the labile $^{52}Cr^{VI}$ pool. The kinetics of Cr^{VI} exchange were simply too slow to use the $^{50}Cr^{VI}$ isotope in a conventional E-value protocol. Essentially, the only convincing E-values for Cr^{VI} were measured in systems (TMAH concentrations) where virtually all the labile Cr^{VI} was in solution but non-labile Cr had not been mobilized. Use of the isotope at least helped to highlight a restricted range of TMAH concentrations where these conditions applied.

Speciation of Cr^{VI} was vital in order to give a true ratio of $^{50}Cr^{VI}$:$^{52}Cr^{VI}$ in solution and thus provide a reliable calculation of Cr^{VI}_E (subject to the caveats discussed above). Measuring an 'unspeciated' E-value (Cr_E) did not give meaningful results, partly because of the mobilization of non-labile humic-bound Cr^{III} into solution. For example, the concentration of Cr_E was up to 80 times higher compared to the measured value of Cr^{VI}_E even when isotopic spike remained entirely in solution, so that $Cr_{TMAH} = Cr_E$. A much smaller labile Cr^{VI} pool (speciated E-value) was measured in such cases and the majority of Cr remaining in solution was labile and non-labile Cr^{III}-DOC species.

A limitation that was found in this study was to get a consistent fraction of labile Cr^{VI} in soil. The trend of Cr^{VI}_E depends on the zone of TMAH concentration such as below or above the point at which labile Cr^{VI} is in solution. At the lowest TMAH concentration (0.01%) where not all spike was in solution, higher Cr^{VI}_E was observed due to slow desorption kinetics of native $^{52}Cr^{VI}$. As TMAH increased (0.03-0.09%), labile $^{52}Cr^{VI}$ is dissolved, which the isotope mixes with, and E values decline. Then as TMAH increases further (0.1 – 1.0%) non labile Cr^{VI} is dissolved and the E value increases substantially. An attempt to prolong the contact time between the spike and native Cr^{VI} in 0.01 M $Ca(NO_3)_2$ also resulted in an inconsistent Cr^{VI}_E because of the slow exchange kinetics between the spike and native Cr^{VI} in $Ca(NO_3)_2$ suspensions and in TMAH concentrations that failed to mobilize all labile Cr^{VI} into solution.

Due to the slow isotopic exchange kinetics, the behaviour of labile Cr^{VI} depended on the suspending solution, and level of $^{50}Cr^{VI}$ spike used. For instance, at the same equilibration time, the concentration of Cr^{VI}_E was lower in TMAH (0.001 to 0.04%) compared to 0.01 M $Ca(NO_3)_2$ at different level of spike. The exchange between the spike and native Cr^{VI} was slower in $Ca(NO_3)_2$ simply because of the larger K_d value for the $Ca(NO_3)_2$ matrix compared to any of the TMAH concentrations used. Greater adsorption of $^{50}Cr^{VI}$ spike and a continuous reduction of native Cr^{VI} was observed by prolonging the equilibration time in $Ca(NO_3)_2$. The risk of Cr^{VI} spike reduction and binding to mobilized humic acid in the TMAH matrices potentially undermines the principles of the E-value measurement and so further work was undertaken on Cr^{VI} interaction with DOC over longer timescales in the following chapter (Chapter 6).

6 INTERACTION OF Cr^{VI} WITH HUMIC ACID

6.1 INTRODUCTION

The majority of soil organic matter consists of relatively stable humified material. Humic substances are complex macromolecules and include aromatic components that are heavily substituted with oxygen-containing functional group (Stevenson, 1985). Humic acid (HA), fulvic acid (FA) and humin are the three fractions of humic substance that are operationally defined depending on their solubilities (Aiken et al., 1985). The fraction that is soluble in basic solution is humic acid and has higher carbon content and molecular weight than fulvic acids. Both humic and fulvic acids have a significant capacity to promote redox reactions and may be the major soil reservoir of electron donors for the reduction of Cr^{VI} and subsequent bonding of Cr^{III} (Wittbrodt and Palmer, 1996).

Any reaction that can reduce Cr^{VI} to Cr^{III}, and bind the latter species, is important as it can reduce the risk from Cr^{VI} to human and environmental health (Choppala et al., 2013). Earlier work investigating the reaction of Cr^{VI} with humic acid found Cr^{VI} reduction to an organic Cr^{III}-complex was dependent on pH, temperature and the concentration of humic acid with studies conducted at a range of pH from 2 to 10 (Wittbrodt and Palmer, 1996 ; Lu et al., 1997). The ability of humic acid to bind with Cr^{III} has been demonstrated by Fukushima et al., (1995) who found that the binding capacities of peat HAs and commercially available HA with Cr^{III} were correlated with the carboxylic content of HA and the bonding between Cr^{III} and carboxylate was confirmed by Fourier Transform Infrared Spectroscopy (FTIR). However, the stability and solubility of organic-Cr^{III} complexes depends on the characteristics of the chelating agent and soil pH value (James and Barlett, 1983a; Fendorf, 1994; Fukushima et al., 1995; Krajnc et al., 1995). Marx et al., (1999) studied the stability of Cr^{III}-HA complexes involving four samples of soluble humic substances, isolated from water sources of different origins, using an isotopic labelling approach. A spike of $^{53}Cr^{III}$ isotope was added to HA solutions after equilibrating the solution for 2 days with natural $^{52}Cr^{III}$. They found different Cr isotopic ratios in unseparated and spiked humic samples and in their ultrafiltrate just 1 hr after spiking; this indicated that only a small fraction of isotope exchange between free and humic-complexed Cr ions had occurred. The $^{52}Cr/^{53}Cr$ ratio was stable from 1 to 77 days which suggested that the high molecular weight Cr^{III}-HA complex possessed long term kinetic stability and was likely, therefore, to be mobile in the natural

water samples. The factors controlling the kinetics of the reaction between the natural humic substances and Cr were not explored further.

A more complex reaction pathway for changes in Cr speciation was proposed by Lu et al., (1997) with Cr^{V} as an intermediate product during the reduction of Cr^{VI} to Cr^{III} in the presence of HA. They found that complete reduction of Cr^{VI} to Cr^{V} by HA at pH 3 was achieved by heating the mixtures at 100 ˚C for 6 hours. Lu et al., (1997) found that different sources of humic acid gave the same results in reducing Cr^{VI} and suggested that this was because of the similarity of their functional groups. Similarly, in a study on Cr^{VI} reduction in humic and fulvic acid at pH 3, Wittbrodt and Palmer (1996) found greater differences between reduction rate coefficients for Cr^{VI} in HA and FA from the same soil compared to HA extracted from different soils. In contrast, Scaglia et al., (2013) found that HA extracted from waste such as from garden-lignocellulose and kitchen food waste (60:40) reduced 15% more Cr^{VI} compared to coal-derived humic acid, at pH 2.5, due to the presence of more reactive functional group such as phenols and thiols. Humic acid extracted from peat was found to be more efficient in reducing Cr^{VI} compared to 'Leonardite' HA due to a higher concentration of polysaccharide in the former which represents 23% of total carbon content (Zhilin et al., 2004). Other work by Leita et al., (2009) investigated the interaction of Cr^{VI} in the presence of HA by UV-vis spectrometry, differential pulse stripping voltammetry, and X-ray absorption spectroscopy (XAS). They found that Cr^{VI} interaction with HA was via the formation of Cr^{VI}-HA micelles rather than reduction to Cr^{III} and subsequent bonding. The Cr^{VI} K edge Extended X-ray Absorption Fines Structure (EXAFS) results showed that the interaction with HAs did not induce any structural modifications of the CrO_4^{2-} complex as there was the same coordination number (4.2) and distance (1.61 Å) observed with or without HA at pH 6.5.

A variety of other techniques have been used to study the interaction of Cr with humic acid included capillary zone electrophoresis, differential pulse stripping voltammetry, colorimetric method and size exclusion chromatography (SEC) (Wittbrodt and Palmer, 1997; Scaglia et al., 2013; Sadi et al., 2002 and Leita et al., 2007). Size exclusion chromatography (SEC) is one separation technique used for characterization of the molecular size distribution of humic substance and separation of inorganic and organic species (Zhou et al., 2000; Sadi et al., 2002; Leita et al., 2007). Sadi et al., (2002) used SEC to investigate elemental Cr binding with humic acid extracted from compost. They found that most (76.7%) of the Cr was distributed within the molecular weight fraction 2 – 15 kDa of humic substances with 18.2%

in the >15 kDa and 5.2% in the <2 kDa fractions. Leita et al., (2007) investigated Cr mobility and distribution of organic Cr complexes over a range of pH values: 4.7, 7.3 and 10.3 in HA and FA extracted from compost. The mobility of Cr increased as pH increased with greater organic-Cr concentrations found in the <40 kDa molecular weight fraction compared to higher molecular weights.

The work described in this chapter assesses the interaction of Cr^{VI} with humic acid. ^{50}Cr as Cr^{VI}, was added to humic acid solutions and incubated to measure the conversion of $Cr^{VI}O_4^{2-}$ to humic-bound Cr^{III} with time. The distribution of Cr^{VI} in the presence of humic acid and conversion to HA-Cr^{III} was confirmed by SEC-ICP-MS. The concentrations of Cr^{VI} and HA-Cr^{III} were accurately measured by anion exchange chromatography and SEC-ICP-MS respectively. Various relevant variables were tested including contact time between Cr^{VI} and humic acid, remaining Cr^{VI} concentration and pH value. From the resulting data, a kinetic model of the interaction of Cr^{VI} with humic acid was developed.

6.1.1 The experimental objectives within this chapter were to:

- confirm the transfer of free Cr^{VI} ions (CrO_4^{2-}) to humic-bound Cr^{III};
- quantify the rate of reduction of Cr^{VI} in contact with humic acid (HA);
- determine the effect of pH on reduction rate and examine the underlying mechanisms for this;
- develop a kinetic model predicting the interaction of Cr^{VI} with humic acid.

6.2 MATERIALS AND METHODS

6.2.1 Preparation of HA stock solution

The preparation of humic acid was according to Section 2.2.3. A 1000 mg L^{-1} of HA stock solution was prepared by dissolving 0.2 g of the freeze dried humic acid in 200 ml of 0.1 M NaOH and the pH was adjusted to 7.2 by using HNO_3. The solution was then filtered to < 1.2 µm and stored in polyethylene bottle at 6 °C before use.

6.2.2 Interaction of $^{50}Cr^{VI}$ with HA: effect of Cr concentration.

A $^{50}Cr^{VI}$ stock solution was prepared according to Section 2.3.3. The humic acid, 200 mg L^{-1} was spiked with $^{50}Cr^{VI}$ to give final concentrations of 20, 40 and 100 mg L^{-1} of $^{50}Cr^{VI}$. The mixtures were stored in polyethylene ICP sample tubes at 20°C. In the initial experiment,

spiking was carried out every day from 1 to 10 days. At the end of the incubation period, the separation of organic (humic-Cr^{III}) and inorganic Cr^{VI} species was achieved using SEC-ICP-MS according to Section 2.3.2 (Table 2.1a for ICP-MS and Table 2.1 b for SEC).

A second experiment was undertaken to test longer incubation times for Cr^{VI} reaction with HA. The sample preparation was split into 3 parts: 1 to 10 days, 6 to 14 days and 12 to 21 days (Table 6.1) with the same approach used as in the previous experiment; the sample preparation was scheduled so that the sample with longest incubation time was prepared first.

Table 6.1 Sample preparation, analysis date and incubation time for Cr^{VI} in HA solutions.

Preparation of samples	Analysis date	Incubation time (days)
21/05/13 to 30/05/13	30/05/13 to 31/05/13	1 to 10
26/05/13 to 04/06/13	09/06/13 to 10/06/13	6 to 14
01/0613 to 10/06/13	21/06/13 to 22/06/13	12 to 21

The use of SEC-ICP-MS was important to confirm that the progressive loss of Cr^{VI} was due to humic binding of Cr^{III} and to examine the molecular size distribution of the HA-Cr^{III} complexes. However, analytically, anion exchange chromatography (LC-ICP-MS) provided a much faster and more accurate determination of remaining Cr^{VI}. Therefore, a third investigation was undertaken using an anion exchange column (LC-ICP-MS), partly to measure the concentration of free Cr^{VI} ions more accurately than possible with SEC. In this experiment, three different concentrations of $^{50}Cr^{VI}$ were spiked into 200 mg L^{-1} humic acid solution to give final concentrations of 20, 40 and 100 µg $^{50}Cr^{VI}$ L^{-1}. The solutions were prepared from 1 to 31 days and stored in a temperature controlled room at 20°C and then analysed for Cr^{VI} using LC-ICP-MS as described in Section 2.3.2.

A fourth experiment was undertaken to determine the effect of pH on Cr^{VI} reduction kinetics. Aliquots (100 mL) of a stock solution containing 1000 mg L^{-1} HA were equilibrated for 24 hr at pH 6.0, 7.0 and 8.0 before being spiked with $^{50}Cr^{VI}$; pH adjustment was undertaken using 50% TAG HNO_3. Two different concentrations of $^{50}Cr^{VI}$ were spiked into 200 mg L^{-1} humic acid solution to give final concentrations of 20 and 100 µg L^{-1}. The solutions stored in a temperature controlled room for 30 days at 20˚C. The final (fifth) set of experiments tested four pH values (6.2, 7.2, 8.4 and 9.1) over a longer incubation time, up to 40 days. This final

trial was undertaken to expand the investigation but it was also in response to the observation that pH drifted in the previous trial. Therefore, the incubated Cr^{VI} - HA solutions were stored under a nitrogen gas (N_2) blanket throughout the trial. The solution was then analysed for Cr^{VI} using LC-ICP-MS

A typical chromatogram of Cr distribution by SEC is shown in Figure 6.1. The retention time of HA-Cr^{III}, including high (excluded) and low molecular weight fractions, ranged from 6 to 15 min and free Cr^{VI} ions from 15 to 17 min. The integration of Cr^{VI} and HA-Cr^{III} peaks was carried out manually using the ICP-MS 'Plasmalab™' software and a final concentration calculated, incorporating drift correction in a separate spreadsheet (MS, Excel) to obtain final results. However, it was difficult to select a cut-off point between the range of HA-Cr^{III} fractions and the discrete Cr^{VI} peak as can be seen in an example shown in Figure 6.1. Therefore two approaches were used to integrate the $^{50}Cr^{VI}$ peak; 45˚ and 90˚ baselines between HA-Cr^{III} and Cr^{VI} (Figure 6.1a and b). The average (mean) signal (integrated counts per second (ICPS) from both approaches was used to estimate the concentration of HA-Cr^{III} and Cr^{VI} throughout this study.

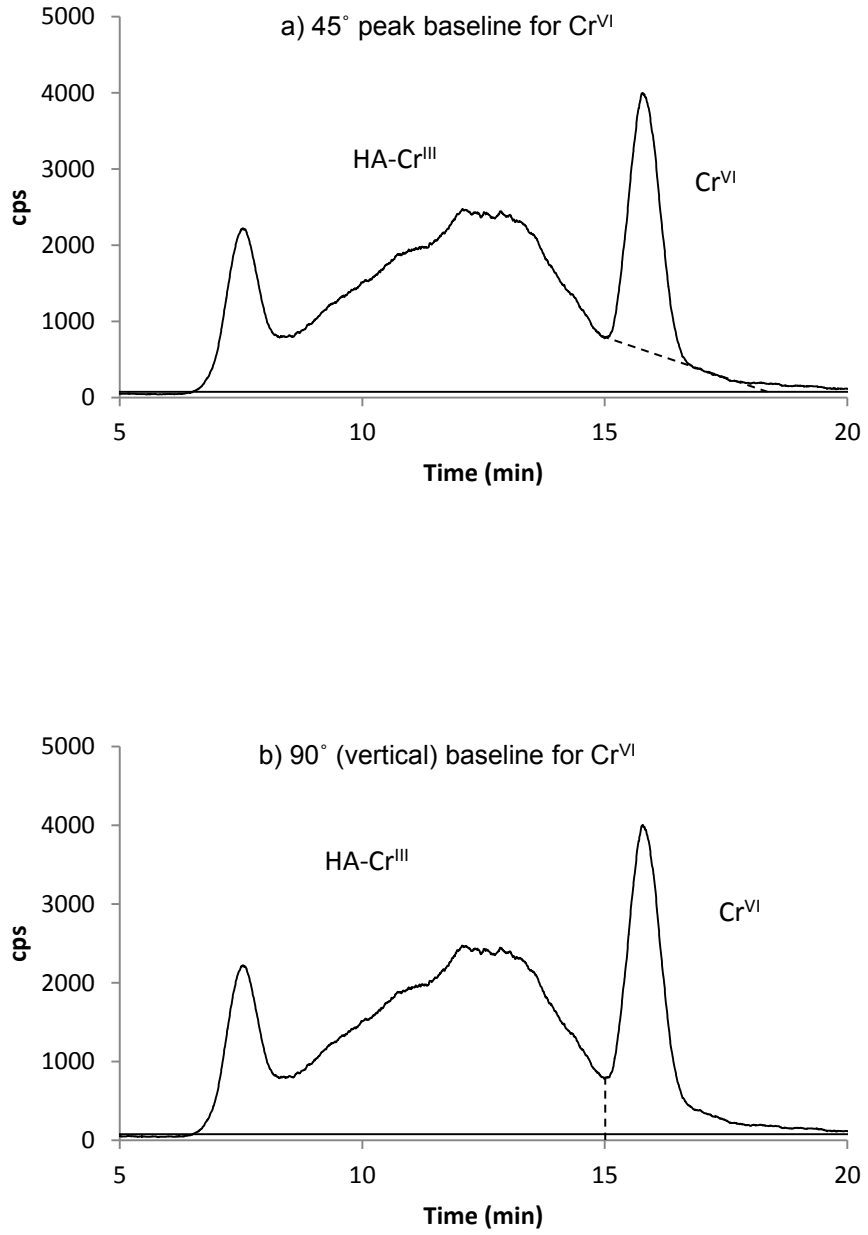


Figure 6.1 Size exclusion chromatograms showing the distribution of HA-Cr^{III} and Cr^{VI} fractions. HA solutions (200 mg L^{-1} at pH 7.2) were spiked with 100 µg L^{-1} of $^{50}Cr^{VI}$ and incubated at 20˚C for 10 days. The integration of HA-Cr^{III} and Cr^{VI} was carried out using a) 45˚ and b) 90˚ 'baselines' for Cr^{VI} shown by the dotted line. A Superose 12 10/300 column (GE Healthcare) with mobile phase 50 mM TRIS, 40 mM NH_4NO_3, 0.005 M of NH_4-EDTA, pH adjusted to 8.4 using 5% HNO_3. Data were plotted using a moving average interval of 100 time periods of 355 ms.

6.2.3 Modelling Cr^{VI} reaction kinetics with humic acid

The reaction of Cr^{VI} with humic acid was modelled using an irreversible first-order rate equation:

$$Cr_t = Cr_0 e^{-kt} \qquad \text{Eq 6.1}$$

Where,

Cr_t =concentration of $^{50}Cr^{VI}$ in solution at time, t (µg L^{-1}), k = the reaction rate constant (day^{-1}), t = time (day), Cr_0 = initial concentration of $^{50}Cr^{VI}$ at t =0

The reaction rate constant (k) was estimated using a non-linear least square method by minimizing the residual standard deviation (RSD) between the measured and modelled values of Cr_t using the *Solver* function in Microsoft Excel. The Cr^{VI} half life ($t_{1/2}$) is the time taken to reduce Cr^{VI} concentration to half of the initial value, i.e $Cr_{t½} = 0.5Cr_0$; $t_{1/2}$ was calculated as:

$$t_{1/2} = \frac{-\ln(0.5)}{k} = \frac{0.693}{k} \qquad \text{Eq 6.2}$$

6.3 RESULTS AND DISCUSSION

6.3.1 SEC-ICP-MS analysis

In the preliminary experiment, three initial $^{50}Cr^{VI}$ concentrations (Cr_o) (20, 40 or 100 µg L^{-1}) were equilibrated in solutions containing an excess of humic acid (pH = 7.2; 200 mg L^{-1}). The HA-Cr mixtures were then kept in a temperature controlled room at 20°C for 1 to 10 days. The results are shown in Figure 6.2. From 1 to 3 days, $^{50}Cr^{VI}$ was apparently stable with no evidence of reaction with humic acid. Fukushima et al., (1995) observed relatively slow formation of HA-Cr^{III} complexes when HA solution was initially spiked with Cr^{III}, compared to a faster reaction of HA with Cu^{2+} and Ni^{2+}, but did not see any lag in the reaction as observed here. A gradual decrease in $^{50}Cr^{VI}$ concentration was observed starting on day 4 followed by a further slow reduction of Cr^{VI} to HA-Cr^{III} up to 10 days at which point the experiment was terminated. Lu et al., (1997) also found incomplete reduction of Cr^{VI} with humic substance at pH 7 after 10 days. The same trend was observed for all $^{50}Cr^{VI}$ spike levels. A different scenario was observed in TMAH soil extracts spiked with $^{50}Cr^{VI}$ (Chapter 3, Figure 3.38), whereby the reduction of $^{50}Cr^{VI}$ to HA-Cr^{III} occurred within two hours

probably as a consequence of heating the TMAH solutions at 70˚C resulting in faster $^{50}Cr^{VI}$ reduction.

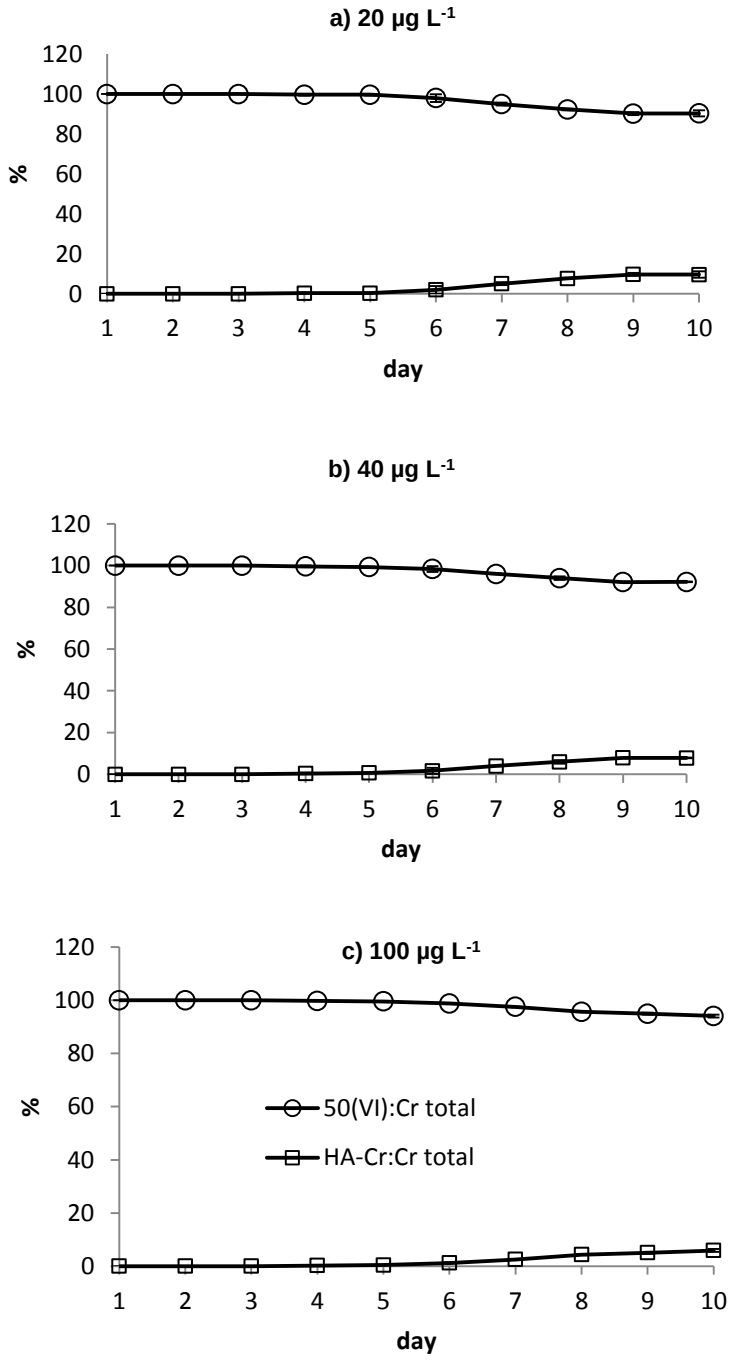


Figure 6.2 Change in $^{50}Cr^{VI}$ and HA-Cr concentrations as a proportion of total Cr at different initial $^{50}Cr^{VI}$ spike : a) 20 µg L^{-1} b) 40 µg L^{-1} and c) 100 µg L^{-1} added to 200 mg L^{-1} HA at pH 7.2 and at 20˚C as a function of time up to 10 days. Error bars represent the standard errors of duplicate measurements.

Among the three concentrations of Cr studied (20, 40, 100 µg L^{-1}) the fastest reaction was observed at 20 µg L^{-1} (Figure 6.3). This may suggest that the capacity of the humic acid to reduce Cr^{VI} is a rate-limiting factor and so at the highest ratio of humic acid:Cr^{VI} in the solution, the rate of Cr^{VI} reduction is greatest. It should also be noted that, although there was a systematic difference between the three Cr concentrations, the overall effect of HA:Cr ratio on reduction rate was about 4%.

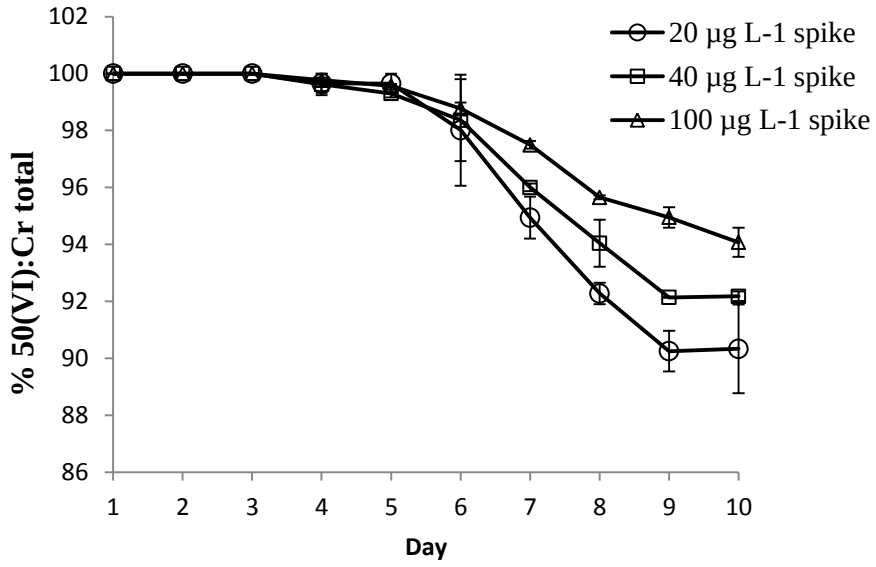


Figure 6.3 Change in proportion of $^{50}Cr^{VI}$ remaining at different initial $^{50}Cr^{VI}$ spike concentrations (20 µg L^{-1}, 40 µg L^{-1} and 100 µg L^{-1}), added to 200 mg L^{-1} HA at pH 7.2 and at 20˚C, as a function of time up to 10 days. Error bars represent the standard errors of duplicate measurements.

6.3.2 Extending the contact time between Cr^{VI} and HA

The relatively slow reaction between HA and Cr^{VI} suggested the need for longer incubation times to resolve the kinetics of Cr^{VI} interactions with HA. Therefore, a second experiment was carried out using incubation times from 1 to 21 days. For logistical reasons, sample preparation was split into 3 parts: 1 to 10 days, 6 to 14 days and 12 to 21 days.

The progressive reaction of Cr^{VI} with HA and the relative molecular weight distribution of HA-Cr complexes can be seen by comparing the size exclusion chromatograms from 1 to 21 days (Figure 6.4). Comparing a control chromatogram (Figure 6.4a) with Figure 6.4(b), there was no measureable interaction of Cr^{VI} with HA after one day incubation. This is consistent

with the previous experiment which suggested that reduction of Cr^{VI} was only apparent after 3 days (Figure 6.2). However, by prolonging the incubation from 8 to 21 days, a gradual build-up of a large peak can be seen at about 8 to 15 minutes in the chromatogram (Figure 6.4 c-e) with a concurrent decreased of the Cr^{VI} signal at 15-17 min (Figure 6.4 c-e). This is strong evidence for an underlying mechanism that involves reduction of Cr^{VI} to HA–Cr^{III}. In solution, Cr^{VI} appears as soluble low molecular weight anionic species such as CrO_4^{2-}, $Cr_2O_7^{2-}$ or $HCrO_4^-$ and does not bind to humic substance.

A small peak was observed from 6 to 8 min (Figure 6.4 a) in a control (unspiked) 200 mg L^{-1} HA solution which represents a high molecular weight (chromatographically excluded) HA fraction. This suggests that the 'native' Cr, present as a residue following the HA preparation process, was entirely associated with a humic fraction which had a very high molecular size range. The height of the peak increased with longer contact times between Cr^{VI} and HA (Figure 6.4 b-d) but the majority of the newly HA-bound Cr was spread across a much wider molecular size range and not wholly excluded. This may suggest that there is a gradual re-distribution of Cr held initially within low molecular weight HA-Cr^{III} fractions to higher molecular weight HA-Cr^{III} fractions with time. In most previous studies, native Cr^{III}-HA was mainly found to be bound to relatively low molecular weight fractions (Marx and Heuman, 1999; Leita et al., 2007). However, Sadi et al., (2002) found that Cr (no Cr species stated) was distributed with high molecular weight fractions of humic substances. In the current study, after 21 days, there was complete reduction and re-distribution of $^{50}Cr^{VI}$ to Cr^{III}-HA (Figure 6.4 e).

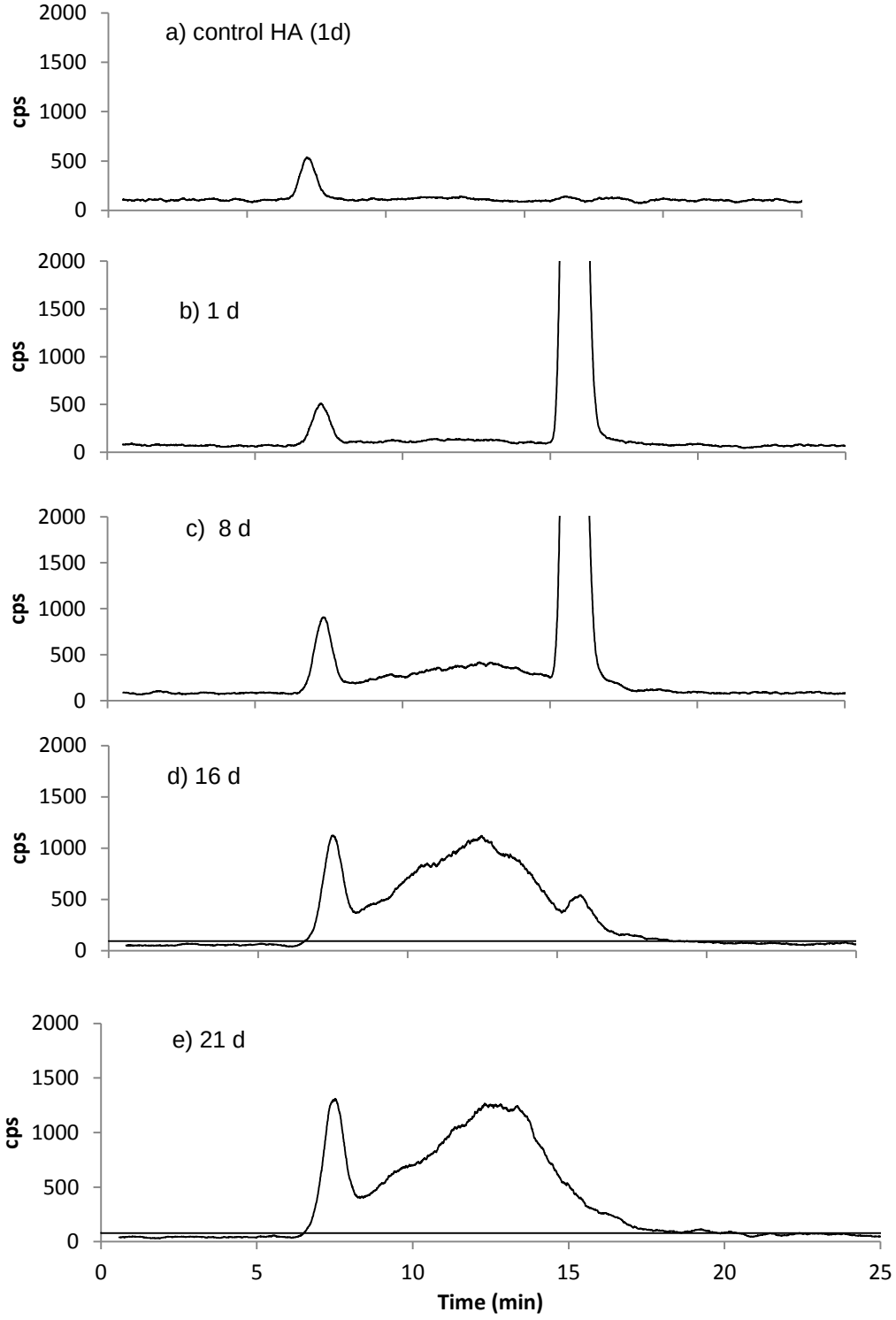


Figure 6.4 Size exclusion chromatograms showing the gradual re-distribution of Cr^{VI} to HA-Cr^{III} when spiking HA solutions (200 mg L^{-1}at pH 7.2 and at 20˚C) with 20 µg $^{50}Cr^{VI}$, incubated at 20˚C for 1 to 21 days (b)-(e); a control (unspiked) sample (a) is included. A Superose 12 10/300 column (GE Healthcare) with mobile phase 50 mM TRIS, 40 mM NH_4NO_3, 0.005 M of NH_4-EDTA, pH adjusted to 8.4 using 5% HNO_3. Data were plotted using a moving average of 100 time periods of 355 ms.

A mass balance for Cr was attempted. The apparent loss of $^{50}Cr^{VI}$ in the solution was calculated by subtracting the remaining $^{50}Cr^{VI}$ concentration in solution from the concentration of the original spike and compared with the measured concentration of HA-bound Cr (Figure 6.5). The loss of Cr^{VI} in solution was presumed to be due to the conversion of Cr^{VI} to HA-Cr^{III}; even though the recovery was not 100%, the correspondence was reasonable (Figure 6.5).

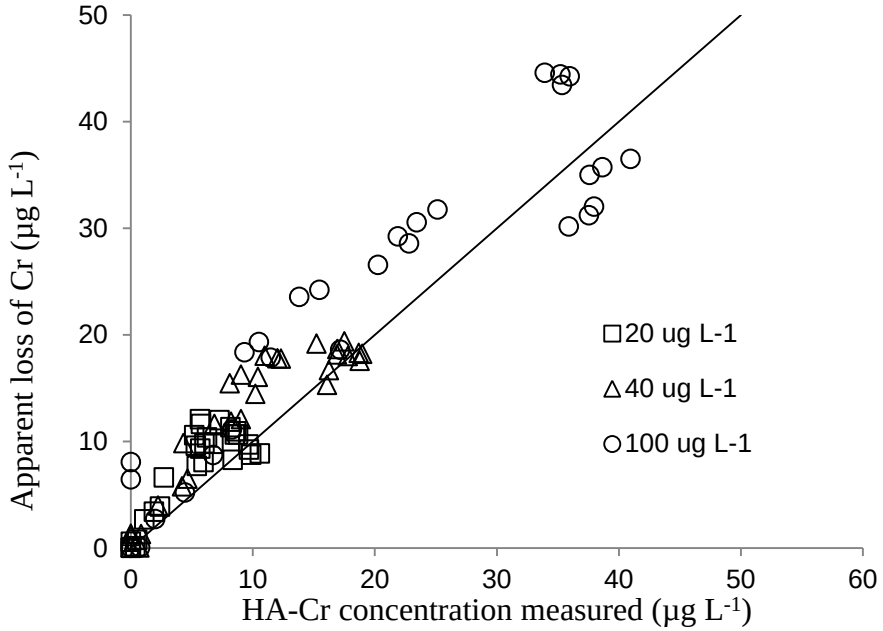


Figure 6.5 The progressive loss of Cr^{VI} and production of HA-Cr (µg L^{-1}) at different concentrations of $^{50}Cr^{VI}$ spike (20, 40 and 100 µg L^{-1}) incubated with HA solutions (200 mg L^{-1} at pH 7.2 and at 20°C). Data points represent average of duplicate measurement.

The concentration of $^{50}Cr^{VI}$ remaining in the solution after being incubated with 200 mg L^{-1} HA at 20°C from 1 to 21 days at different concentrations of $^{50}Cr^{VI}$ spike is shown in Figure 6.6. Although the preparation of the $^{50}Cr^{VI}$ and HA incubation samples was split into three parts, the reduction of $^{50}Cr^{VI}$ from all three sub-experiments was expected to form a single continuous trend with time. However, as can be seen from Figure 6.6 the three sets of data formed discrete trends. This rather odd result may be explained by time-dependent changes in the pH of the HA stock solutions.

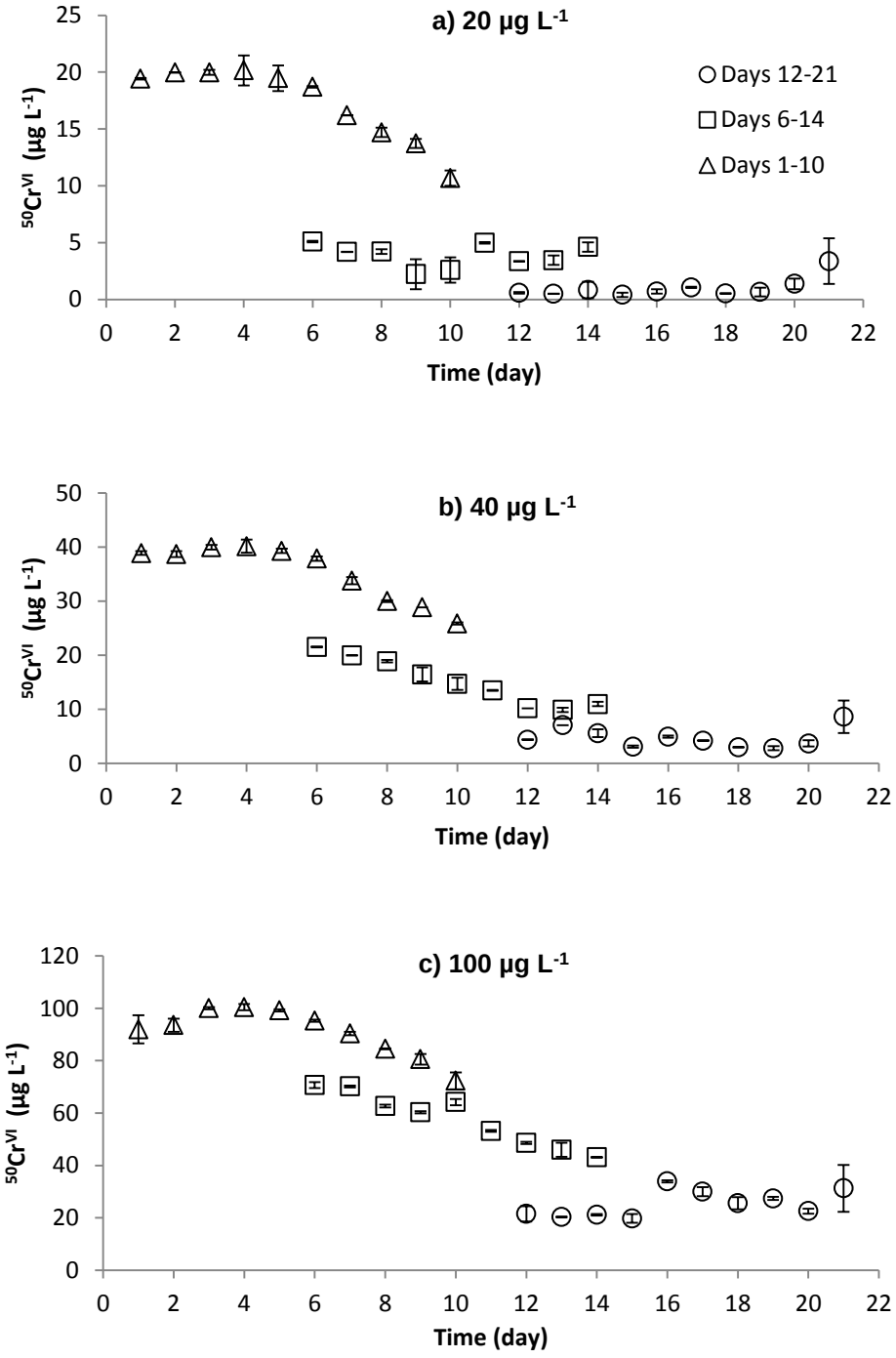


Figure 6.6 Changes in $^{50}Cr^{VI}$ concentrations with time following spiking with $^{50}Cr^{VI}$ at a range of concentrations: a) 20 µg Cr L^{-1} added, b) 40 µg Cr L^{-1} added c)100 µg L^{-1} added to 200 mg L^{-1} HA at pH 7.2 and at 20˚C as a function of time. Sample preparation was split into 3 parts: 1 to 10 days (triangles), 6 to 14 days (squares) and 12 to 21 days (circles). Error bars represent standard errors from duplicate measurements.

6.3.3 Application of a first order kinetic model

Effect of contact time on Cr^{VI} reduction kinetics by HA

As conversion of Cr^{VI} to HA-Cr^{III} was confirmed by the SEC-ICP-MS experiments described above, further investigation (Third experiment) used an anion exchange column (LC-ICP-MS) to more accurately measure free Cr^{VI} with a much shorter analysis time. In the SEC experiments, the runtime was about 25 minutes per sample and the delineation of the free $^{50}Cr^{VI}$ peak presented difficulties (Figure 6.4) whereas the LC-ICP-MS assay took less than 5 minutes and provided clear isolated $^{50}Cr^{VI}$ peaks. Leita et al., (2007) found that the anion exchange chromatographic method was suitable for determining Cr^{VI} but not for Cr^{III}-complexes due to a low recovery (below 40%) suggesting that some Cr^{III}-complexes may retained in the column. In this experiment, three different concentrations of $^{50}Cr^{VI}$ (20, 40 and 100 µg L^{-1}) were spiked into 200 mg L^{-1} humic acid solution at pH 7.5 and each mixture was kept from 1 to 31 days at 20°C. As the mixture was prepared at the same time (i.e once at the beginning of experiment), the effect of changes in pH of stock solutions that has been observed in previous experiment was eliminated. The objective of this experiment was to test the validity of an irreversible first-order (IFO) model describing the reduction of Cr^{VI} and its subsequent complexation as HA-bound Cr^{III}.

A straight line was observed when plotted the $\log_{10}$ concentration of $^{50}Cr^{VI}$ versus time (Figure 6.7) confirms the reduction was according to the first order kinetics. The concentration of $^{50}Cr^{VI}$ was progressively reduced over 31 days at all $^{50}Cr^{VI}$ spike concentrations (Figure 6.7).The reduction of $^{50}Cr^{VI}$ concentration in solution as a function of time was modelled assuming that the reaction proceeds to a zero concentration of $^{50}Cr^{VI}$. In view of the apparent reaction rate dependence on HA:Cr ratio seen previously (Figure 6.2), the IFO model was independently fitted to each spike concentration (Figure 6.7) to further assess this effect. The model for $^{50}Cr^{VI}$ reduction at each spike concentration was well correlated with the measured value; the residual standard deviations (RSD) varied between 1-8 µg L^{-1} across the spike levels. The half life of the Cr^{VI} spike for 20 and 40 µg $^{50}Cr^{VI}$ L^{-1} was about 8 days. Whereas, the half-life was longer at 100 µg $^{50}Cr^{VI}$ L^{-1} spike solution due to the lower ratio of HA: $^{50}Cr^{VI}$ (Table 6.2). For the HA solutions at 100 µg L^{-1} and possibly at 40 µg L^{-1} there was some evidence of the initial delay (lag) in the onset of reduction seen previously. However, this had a minimal effect on the overall fit of the IFO model across the 30 day period.

Table 6.2 The rate constant (k, d^{-1}) and half life at different $^{50}Cr^{VI}$ spike concentrations.

Spike concentration ($\mu g\ L^{-1}$)	Rate constant, k (d^{-1})	Half life $t_{½}$ (d)	RSD ($\mu g\ L^{-1}$)
20	0.0820	8.45	1.15
40	0.0890	7.80	2.77
100	0.0717	9.67	7.90

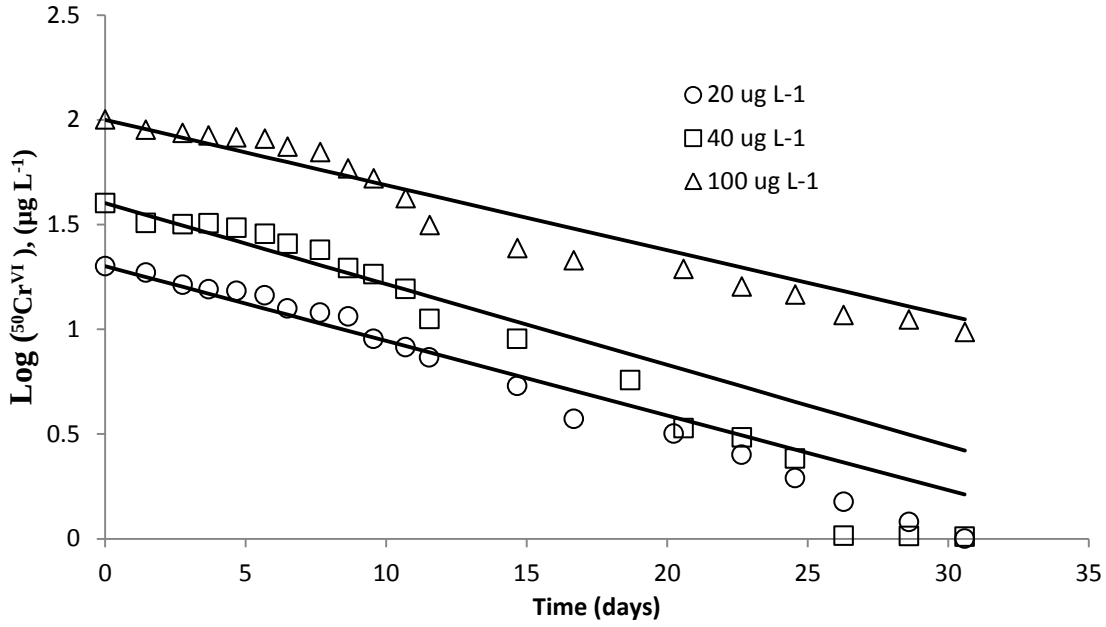


Figure 6.7 The measured (average from duplicate) and Irreversible First Order (IFO) model (line) for $^{50}Cr^{VI}$ reduction (log_{10} scale) in humic acid. Different concentrations of $^{50}Cr^{VI}$ (20, 40 and 100 $\mu g\ L^{-1}$) were incubated with 200 mg L^{-1} humic acid prepared at pH 7.5 and incubated for 1 to 31 days at 20°C.

Effect of pH value on Cr^{VI} reduction kinetics by HA

A fourth experiment was carried out to specifically investigate the effect of pH on Cr^{VI} reduction reaction rate. A range of three pH values (nominally 6.5, 7.5 and 8.5) was used with incubation for up to 30 days with two different concentrations of $^{50}Cr^{VI}$ spike added to each pH treatment (20 and 100 $\mu g\ L^{-1}$). The pH values of the three HA stock solutions were measured immediately after pH adjustment and again after 35 days of incubation. In all three

cases there was a fall in the pH value of the humic acid stock solutions after 35 days (Table 6.3) which may have been due to different levels of CO_2 ingress to the samples.

The pH values of the individual HA-Cr samples were not measured because of limited volume; the solutions were prepared in ICP auto-sampler tubes (5 mL). The pH of the mixture was assumed to be the same as HA stock solution as the amount of $^{50}Cr^{VI}$ added was too small to make any change to the pH, considering the strong buffer capacity of the HA. In addition, the stock and intermediate $^{50}Cr^{VI}$ standard solutions were prepared and diluted in Milli-Q water to avoid addition of acid to the HA solutions.

A reduction in Cr^{VI} concentration was observed at all pH values ranging from 6.5 to 8.5 with faster reduction of the 20 µg $^{50}Cr^{VI}$ L^{-1} spike compared to the 100 µg $^{50}Cr^{VI}$ L^{-1} spike (Figure 6.8). However, a comparatively large drop in pH in the more alkaline stock HA solution (from 8.55 to 6.65) produced a similar reduction reaction rate to that of the samples at pH 6.53 (Figure 6.8 & Table 6.3).

Table 6.3 The rate constant, k and half-life at different $^{50}Cr^{VI}$ spike concentrations and pH values

pH		Rate constant, k (1/d)		Half life, $t_{1/2}$ (d)	
Initial	(after 35 days)	20 µg L^{-1} $^{50}Cr^{VI}$ spike	100 µg L^{-1} $^{50}Cr^{VI}$ spike	20 µg L^{-1} $^{50}Cr^{VI}$ spike	100 µg L^{-1} $^{50}Cr^{VI}$ spike
8.55	6.65	0.0270	0.0180	25.7	38.5
7.50	7.20	0.0961	0.0673	7.22	10.3
6.53	6.71	0.0367	0.0241	18.9	28.8

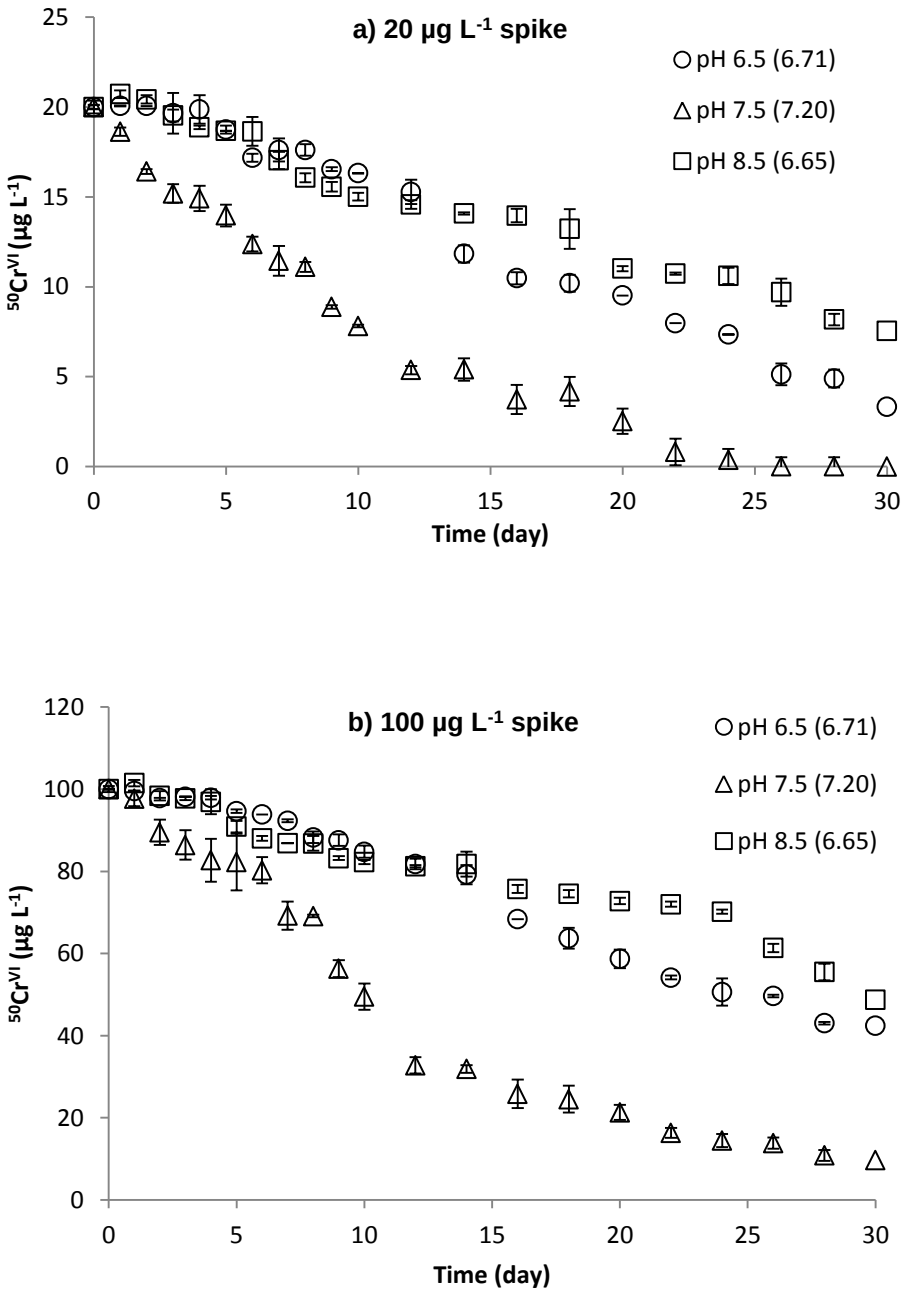


Figure 6.8 Change in $^{50}Cr^{VI}$ concentration with time following spiking with $^{50}Cr^{VI}$ at a) 20 µg L^{-1} and b) 100 µg L^{-1} incubated at 20°C with HA solutions (200 mg L^{-1}) at different pH values: 6.5, 7.5 and 8.5 (actual pH after 30 days shown in parenthesis). Error bars represent standard errors from duplicate measurements.

Again there appeared to be some evidence of an initial 'lag' in reduction rate (Figure 6.8), as seen previously. Given the change (drop) in pH values seen, and the greater reaction rate at lower pH values (Table 6.3) it seems likely that the apparent lag is simply the result of a slower reaction rate at initially high pH values being followed by a faster reaction rate as pH value falls during the incubation.

Effect of controlling pH values on Cr^{VI} reduction kinetics by HA

This final (fifth) experiment was undertaken to address the failure to maintain constant pH values seen in the previous experiment, and the clear effect of pH on Cr^{VI} reduction kinetics. A further investigation was carried out on Cr^{VI} reduction at four different pH values: 6.0, 7.0, 8.0 and 9.0. Two Cr^{VI} final concentrations (20 and 100 µg L^{-1}) were spiked into humic acid solutions (200 mg L^{-1}) and the samples were stored under nitrogen at 20˚C in order to control the pH value during the incubation.

The pH of each HA stock solution was measured at 4-day intervals, from 4 to 40 days (Table 6.4). There was a clear drift in pH value of the HA stock solutions, despite storage under N_2 gas. As the HPLC auto-sampler rack can accommodate 99 samples, the time taken to analyse one batch of samples was about 7 hours. Therefore, the pH of the solution could change during the analysis period due to microbial growth as the analysis was performed at room temperature. However, it would be reasonable to take the average pH changes of the stock solution in order to represent the pH of the HA-Cr solution at the point of measurement by LC-ICP-MS (Table 6.4).

Table 6.4 The pH and average pH from duplicate measurements (Avg) of each HA stock solution measured at 4-day intervals, (except 8-day in the initial measurement) from 0 to 40 days

	Days									
	0	8	12	16	20	24	28	32	36	Avg
pH	6.24	6.53	6.76	7.05	6.80	6.85	6.74	6.80	7.03	6.76
	7.16	6.93	7.22	7.22	7.37	7.09	7.15	7.21	7.20	7.17
	8.44	7.56	7.36	7.71	7.68	7.50	7.75	7.61	7.74	7.71
	9.12	7.64	7.76	7.72	7.82	7.60	7.84	7.82	7.90	7.91

The rate constant, k was estimated using a non-linear least square method by minimizing the RSD between measured and modelled values of Cr_t using solver function in Microsoft Excel. The relationship between reaction rate and equilibrium (average) pH (Table 6.4) was reasonably fitted which shows a clear effect of pH on the rate constant (Figure 6.9). Apart from a clear effect of pH on rate constant, as expected, but there is also an effect of Cr concentrations which indicates a faster rate of reduction of Cr^{VI} to HA-Cr^{III} at lower concentrations of Cr. This suggests that the reaction is limited to some extent by the availability of reduction sites on the humic acid.

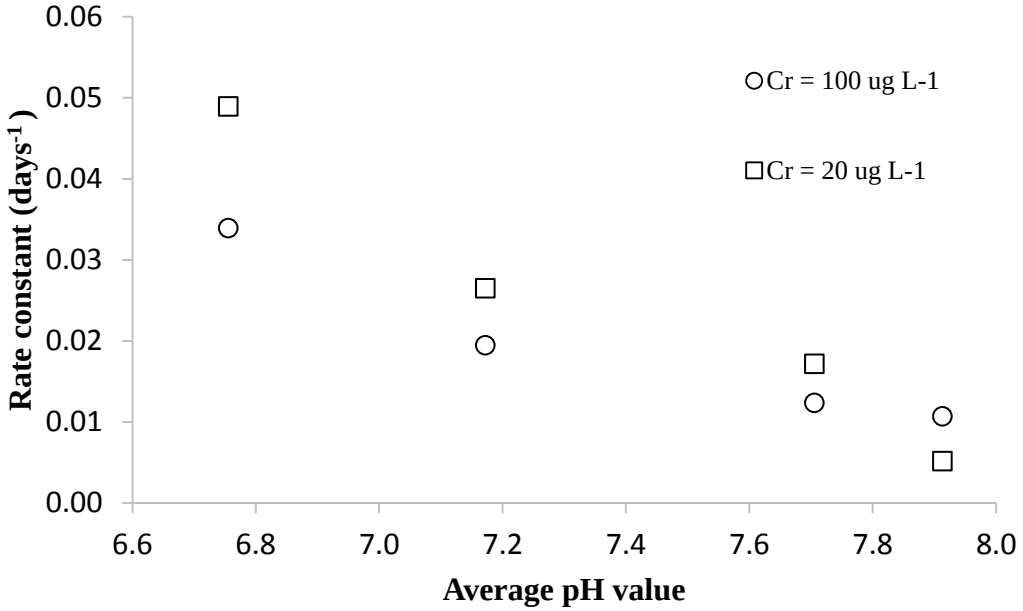


Figure 6.9 Relationship between rate constant and average pH from duplicate measurements for $^{50}Cr^{VI}$ (20 and 100 µg L^{-1}) incubated in 200 mg L^{-1} humic acid solutions at 20°C and stored under N_2 gas.

The incremental change in Cr^{VI} was then modelled using a fix reaction rate at each equilibrium pH and the incubation time period. For example, for a $^{50}Cr^{VI}$ spike of 20 µg L^{-1} in HA at equilibrium pH at 6.76 at t=4 days, Cr_0 (Eq. 6.1) is 20 µg L^{-1}, k is the estimated value and t is the real contact time. This process was then followed incrementally over the whole incubation time period with a fix reaction rate. The same approach was then used to model the changes in Cr_t (Eq 6.1) at each equilibrium (average) pH using an estimated values of k as describe above and a fix reaction rate was used to model the incremental change in Cr^{VI} throughout the incubation time period.

The measured and modelled values of two $^{50}Cr^{VI}$ concentrations (20 µg L^{-1} and 100 µg L^{-1}) at different average pH levels through time are shown in Figures 6.10 a, b. At 20 µg L-^{1} $^{50}Cr^{VI}$, the model overpredicted the concentration of $^{50}Cr^{VI}$ in the initial incubation time from 8 to 17 days and underpredicted the $^{50}Cr^{VI}$ concentrations towards the end incubation time at pH 6. There was a good agreement between measured and modelled $^{50}Cr^{VI}$ at pH 7, 8 and 9. Eventhough at pH 7 and 8 the model was underpredicted in the initial stage of incubation period and some overprediction can be seen at pH 9, but the relative standard deviation between measured and modelled $^{50}Cr^{VI}$ concentration is less than 2 µg L^{-1}, which can be considered as good.

In general, at 100 µg L^{-1} $^{50}Cr^{VI}$ (Figure 6.10 b) most of the developed model underpredicted the concentration of $^{50}Cr^{VI}$ except for pH 6. It should be noted that microbial growth, CO_2 production have not been investigated in this study.

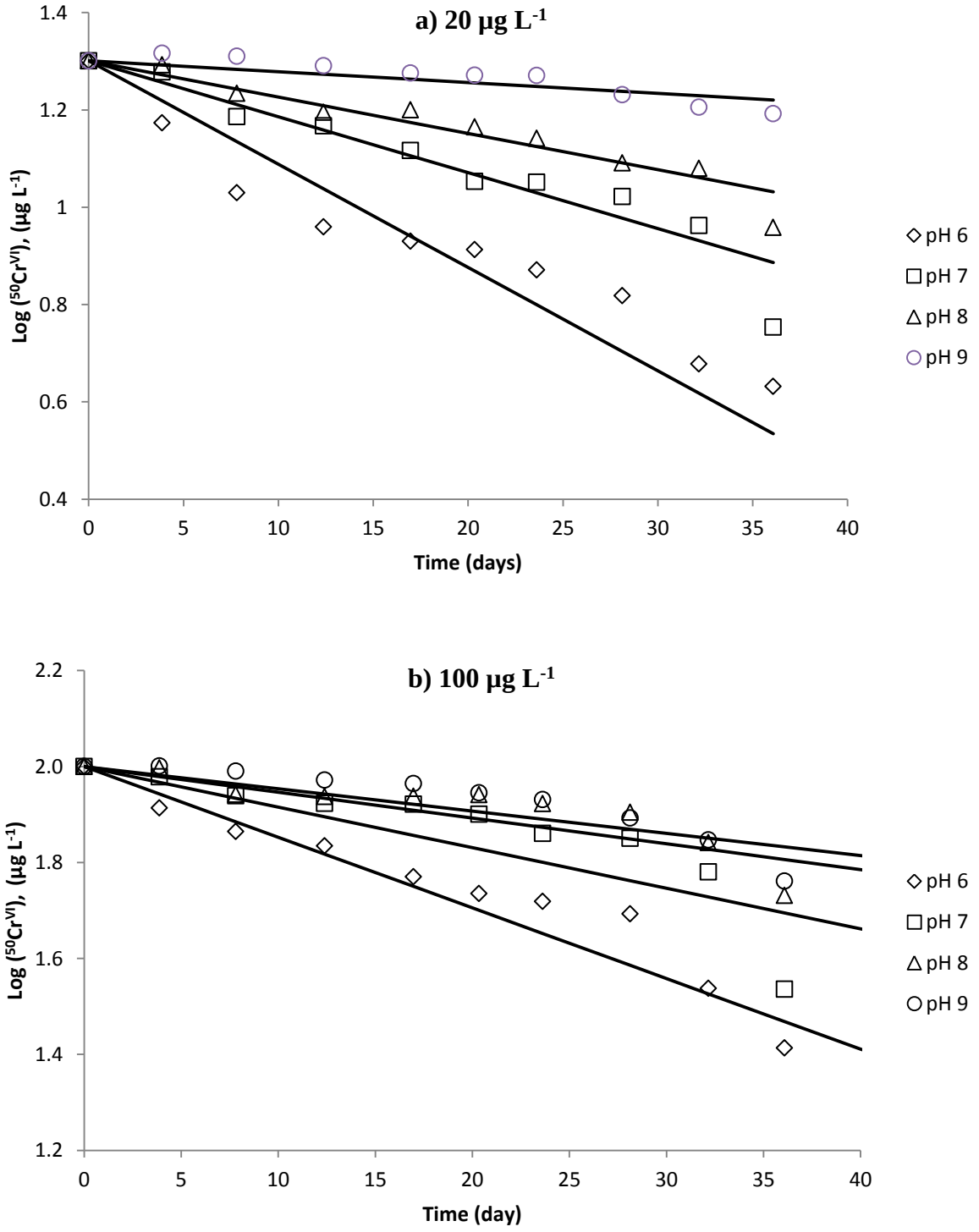


Figure 6.10 Reduction of $^{50}Cr^{VI}$ (log_{10} scale) to humic-bound Cr^{III} as a function of time at two concentrations of $^{50}Cr^{VI}$ (a. 20 µg L^{-1} and b. 100 µg L^{-1}) incubated at 20°C with 200 mg L^{-1} humic acid for 36 days at four initial pH values (pH 6.0, 7.0, 8.0, 9.0). Measured data using average from duplicate (symbols) and a pH-dependent 'Irreversible First Order' (IFO) model fit (line) are shown.

Park et al., (2008) proposed two mechanisms of Cr^{VI} reduction (direct and indirect) by biomaterial. A process of 'direct' reduction involved Cr^{VI} contact with electron donor groups in the biomaterial and subsequent reduction to Cr^{III} or form Cr^{III} complexes. A second 'indirect' mechanism involved binding of Cr^{VI} with positively charged groups, followed by reduction to Cr^{III} by adjacent electron donor group and release of the reduced Cr^{III}.

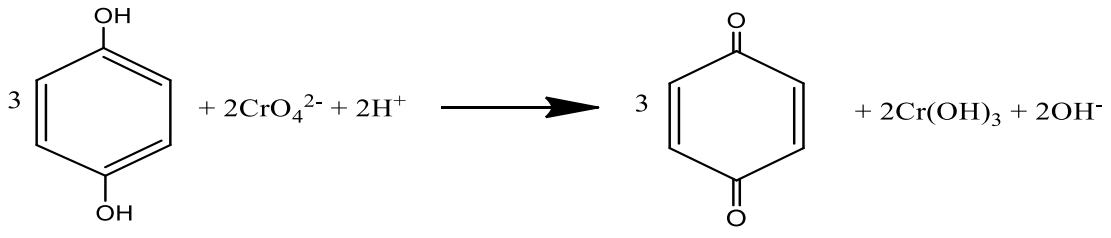
When considering the reaction of Cr^{VI} with humic acid to form HA-Cr^{III} complexes, there are several reasons to expect pH-dependence arising from a range of underlying mechanisms.

i) HA surface charge

When considering the reaction of (anionic) $Cr^{VI}O_4^{2-}$ with negatively charged dissolved humic acid there must be a barrier to chromate ions accessing electron donor groups on the surface of the humic molecules because of electrostatic repulsion. The humic molecule undergoes progressive dissociation of carboxyl groups up to around pH 8 with increasing negative surface charge. Therefore it would be expected that $Cr^{VI}O_4^{2-}$ ions would have to overcome an increasing energy barrier to interact with the humic surface as pH rises and this would be manifested as a slower rate of reduction.

ii) Reduction reaction and proton-electron stoichiometry

Oxidation of Cr^{III} to Cr^{VI} in soil can be catalysed by some Mn^{VI} oxides (Bartlett and James, 1979; Oze et al., 2007) but these are not expected to be present in purified HA suspensions. Reduction of Cr^{VI} may be due to the presence of organic functional groups in HA such as hydroquinone (HQ) that act as electron donors (Elovitz et al., 1994,1995; Brose et al., 2010). Several investigations of the interaction of Cr^{VI} with biomaterials such as lignin, hops and seaweed also found a reduction of Cr^{VI} to Cr^{III} that was subsequently bound to carboxyl and phenolic groups (Cardea-Torresday et al., 2000; Parson et al., 2002; Dupont et al., 2002; Park et al., 2008). The reaction of Cr^{VI} with hydroquinone can be represented as follows;



hydroquinone p-benzoquinone

There is limited information available in the literature on the underlying mechanisms of reduction of Cr^{VI} with hydroquinone (HQ) to benzoquinone (BQ). Therefore, the following speculative reaction sequence is postulated:

$$3HA\text{-}HQ \rightleftharpoons 3HA\text{-}BQ + 6e^- + 6H^+ \quad (1)$$

$$2Cr^{VI}O_4^{2-} + 6e^- + 16H^+ \rightleftharpoons 2Cr^{III} + 8H_2O \quad (2)$$

$$Cr^{III} + HA\text{-}(COOH)_3 \rightleftharpoons HA\text{-}COO_3Cr^{III} + 3H^+ \quad (3)$$

Combining (1) and (2),

$$2Cr^{VI}O_4^{2-} + 3HA\text{-}HQ + 10H^+ \rightleftharpoons 3HA\text{-}BQ + 2Cr^{III} + 8H_2O \quad (4)$$

Expressing (4) in terms of a single Cr:

$$Cr^{VI}O_4^{2-} + 1.5HA\text{-}HQ + 5H^+ \rightleftharpoons 1.5HA\text{-}BQ + Cr^{III} + 4H_2O \quad (5)$$

Combining (5) and (3)

$$Cr^{VI}O_4^{2-} + 1.5HA\text{-}HQ + HA\text{-}COOH_3 + 5H^+ \rightleftharpoons 1.5HA\text{-}BQ + HA\text{-}COO_3Cr^{III} + 3H^+ + 4H_2O \quad (6)$$

$$Cr^{VI}O_4^{2-} + 1.5HA\text{-}HQ + HA\text{-}COOH_3 + 2H^+ \rightleftharpoons 1.5HA\text{-}BQ + HA\text{-}COO3Cr^{III} + 4H_2O \quad (7)$$

The final equation indicates that lowering the pH should increase the rate of reduction (H^+ activity appears on the left hand side of the reduction equation), and this is in keeping with observed trends discussed above.

iii) Complex formation between Cr^{III} and Humic acid.

The final factor to consider is the strength of complex formation between Cr^{III} and HA which will increase as pH rises due to carboxyl ionization, until complex formation with OH^- or HCO_3^- ions and possibly precipitation, acts against this. It might therefore be expected that reduction rate would be more restricted at low pH values due to limited Cr^{III} complex formation. This would act against the previous two factors discussed. However, it seems intuitively likely that the rate of Cr^{III} complex formation is unlikely to be the rate-determining step, considering the rapid reaction normally seen between polyvalent cations and humic acid and the range of pH values over which Cr^{III} complex formation with HA is likely to occur.

6.4 CONCLUSIONS

In this study, Cr^{VI} interactions with humic acid (extracted from a peat soil) solution were measured over 30 days and modelled using an irreversible first order rate equation. Anion exchange chromatography can be used to accurately measure the concentration of Cr^{VI} faster than SEC. However, the use of SEC has allowed information to be gained about the transformation of free Cr^{VI} to Cr^{III} bound to low molecular weight HA fraction and spread across to a higher molecular size range with time. The loss of $^{50}Cr^{VI}$ observed over time was shown to be due to conversion of Cr^{VI} to HA-Cr^{III}. This finding answers some uncertainty raised from Chapter 5.

The rate of Cr^{VI} reduction was affected by pH, the ratio of HA:Cr and contact time between Cr^{VI} and HA with a clear effect has been demonstrated in this chapter. An attempts to control the pH of HA stock solution was made by storing the HA solution under a N_2 gas blanket and the pH was measured at 4 days interval. However, this attempt still failed to maintain the HA solution at the desired pH. The average pH of HA stock solution was used to mimick the pH of HA:Cr solution as the solution prepared was too little in order to measure the pH.

A kinetic model of Cr^{VI} reduction was developed using an irreversible first-order rate reaction using a fixed rate constant measured by minimising the RSD of modelled and measured Cr^{VI} concentration at each average pH value. A better kinetic model was predicted based on the reduction of 20 µg L^{-1} Cr^{VI} to humic-bound Cr^{III} compared to a higher concentration of Cr^{VI}. In general, at higher concentration of Cr^{VI} (100 µg L^{-1}), the model underpredicted the

reduction of Cr^{VI} to humic-bound Cr^{III} due to several reason; the surface charge of HA, reduction reaction and proton electron stoichiometry and complex formation between Cr^{III} and HA. A mechanism of reduction of Cr^{VI} with hydroquinone to benzoquinone has been postulated which suggests that the rate of reduction increases at lower pH.

7 UPTAKE OF CHROMIUM, FROM SOIL HISTORICALLY AMENDED WITH SEWAGE SLUDGE, BY MAIZE (*Zea mays* L.): A CASE STUDY

7.1 INTRODUCTION

Studies on the dynamics of Cr in the soil-plant system under field conditions are relatively scarce. Most published studies have been carried out under controlled conditions in plant growth facilities and have included the addition of Cr to the growth media. Some have involved hydroponics, in order to easily monitor Cr speciation in solution. A few of the published studies were intended to identify the potential for plants to act as a Cr hyperaccumulator and involved non-food species such as ferns (Table 7.1). It can be concluded that, although Cr uptake depends on the type of plant and the Cr species added, Cr was generally accumulated to a greater degree in roots compared to shoots. This indicates a barrier to xylem transfer to shoots and, in the case of Cr^{VI} uptake, may involve reduction to Cr^{III} and subsequent sorption to ligand sites within the root.

Lopez-Luna et al., (2009) investigated the accumulation of Cr in wheat, oat and sorghum seedlings grown in a growth chamber in a Cr free garden soil amended with sewage sludge with a total Cr concentration at 65,000 mg kg^{-1} collected from a leather industry facility in Mexico. They amended the soil with sludge to achieve a total Cr concentration in soil in the range of 500 to 8000 mg kg^{-1} for growing wheat and a lower range (0 to 4000 mg kg^{-1}) for oats and sorghum. They found that increasing sludge application to the soil increased the total concentration of Cr in roots in all plants studied: up to 52.5 mg kg^{-1}, and 20.6 mg kg^{-1} in the roots of wheat, oats and sorghum respectively. However, less than 8 mg kg^{-1} was detected in sorghum shoots and no Cr was detected in wheat and oat seedlings. A recent study by Allué et al., (2013) determined Cr fractionation in a loamy-clay soil amended with variable sludge loadings (0 to 20,000 mg kg^{-1}) to reach a range of 100 to 150 mg kg^{-1} total Cr in soil and the uptake of Cr by fenugreek. Again it was found that Cr concentrations were greater in roots compared to shoots. They also found that by increasing sludge dose, more Cr was found in the moderately reducible fraction extracted by acid oxalate compared to the residual fraction determined by $HF/HClO_4$ digestion. However, no Cr speciation work was undertaken, either in the sludge-amended soil or in the plant tissue. Gupta and Sinha (2006) also undertook fractionation of soil amended with sludge and found that most Cr was present in carbonate and residual fractions which probably limited the Cr availability to plants under the circumneutral pH conditions in arable soils.

Several physiological and geochemical processes may affect the transfer of Cr^{VI} to plants from soil. For example, the presence of MnO_2 is important in facilitating oxidation of Cr^{III} to Cr^{VI} in soil and thereby increasing the supply of the Cr^{VI} form. Similarly, as pH increases Cr^{VI} becomes more soluble and therefore potentially more available to plants (Unceta et al., 2010) while Cr^{III} is probably less available at high soil pH. As Cr is not an essential element for plants, there is no specific uptake mechanism for Cr in plants and it is generally agreed that Cr^{VI} is taken up via the sulphate pathway so that chromate enters root cells using the same transport system as sulphate (Smith et al., 1989; Shanker et al., 2005).

Based on some limited work under field conditions, Cr uptake is also dependent on plant type. Bhattacharya et al., (2005) studied chromium uptake by rice grown in soil amended with municipal solid waste compost (MSWC). They found that Cr uptake in straw (16.5 mg kg^{-1}) was higher compared to grain (0.77 mg kg^{-1}). Zarcinas et al., (2004) assessed the concentration of Cr in 13 crops in Thailand. They found that the highest uptake of Cr was found in rice in the range 0.10 to 2.7 mg kg^{-1} in plants from 0.15 to 159 mg kg^{-1} in the soil. In a recent study, Liao et al., (2011) examined the transfer of Cr from soil to vegetables including Chinese cabbage, celery and lettuce grown in areas near ferro-alloy manufacturing industries in Hunan, China. They found that the uptake of Cr was dependent on plant type according to the following order: Chinese cabbage>lettuce>celery. Liao et al., (2011) also observed that no Cr^{VI} was detected in the plants, using a spectrophotometric method, suggesting that reduction of Cr^{VI} to Cr^{III} occurs within plant tissue.

Aerial deposition, rather than systemic uptake via roots, can be the main reason for elevated Cr concentrations in (or on) leaves (Larsen et al., 1991; Voutsa et al., 1996; Mignorance and Oliva, 2006; Nabulo et al., 2010). Nabulo et al. (2010) showed that aerial deposition of Cr was the main source in green leaf vegetables grown in Kampala, Uganda. The relationship between trace element content of cabbage, carrot, leek, lettuce and endive in agricultural soil in an industrial area of Thessaloniki, Greece was studied by Voutsa et al., (1996). They found that concentration of Cr in endive was about three times higher than the same plant growing on uncontaminated sites and attributed this to atmospheric deposition within the industrial location.

This chapter further investigated the solubility of different Cr fractions in soil including soluble Cr in 0.01 M $Ca(NO_3)_2$ (Cr_{CaNO3}) and soluble Cr^{VI} in 0.01 M $Ca(NO_3)_2$ (Cr^{VI}_{CaNO3}) with soil properties and the uptake of total Cr ($Cr_{total\text{-}P}$ and $Cr_{TMAH\text{-}P}$) and Cr^{VI} ($Cr^{VI}_{TMAH\text{-}P}$) in

maize (*Z. mays*) grown on soil historically amended with sewage sludge at Stoke Bardolph (StB), Nottinghamshire which is also one of the site being studied in Chapter 4. Therefore, some correlation between Cr fractions in these soils (total Cr ($Cr_{total\text{-}s}$), TMAH extractable Cr ($Cr_{TMAH\text{-}s}$), TMAH extractable Cr^{VI} ($Cr^{VI}_{TMAH\text{-}s}$)) with soil properties were already discussed in Chapter 4 (Section 4.3.2). The sites specific risk assessment also was carried out in this chapter in order to investigate whether the maize plant in this area are safe to be consume by ruminant or any potential risk on Cr and transfer to the human population.

Table 7.1 Published studies on the relationship between chromium concentration in growth media and its uptake in crops.

Cr concentration in medium (mg kg^{-1})	Cr species	Concentration (mg kg^{-1}) and accumulation pattern			Crop/Plant	Reference
		Root	Shoot	Root : Shoot		
0 to 5	Not available	Not available	22.4*	Not available	Soybean	Turner and Rust, (1971)
0 to 44	$Cr^{III,}$ Cr^{VI}	92% of total ^{51}Cr in plant	Not available	Not available	Bean	Huffman and Allaway, (1973a)
		95% of total ^{51}Cr in plant	Not available	Not available	Wheat	Huffman and Allaway, (1973a)
0 to 200	Not available	More Cr in roots than shoots		Not available	Sunflower, maize and bean	Kocik and Ilavsky (1994)
0.5 to 25 ^{51}Cr	Not available	More Cr in roots than shoots		Not available	Paddy/rice	Mishra et al., (1997)
1	Cr^{III} , Cr^{VI}	350	2.00	175	Cauliflower, kale and cabbage	Zayed et al., (1998)
1	Not available	2980	44.0	66.8	Smart weed	Hong et al., (1999)
0 to 500	Not available	116	2.40	48.3	Sorghum	Shahandeh and Hossner, (2000a)
		212	5.80	36.6	Sunflower	Shahandeh and Hossner, (2000a)
0 to 40	Cr^{VI}	More Cr in roots than shoots		Not available	Lucerne	Peralta et al., (2001)
0 to 135	Cr^{III} , Cr^{VI}	3.14	2.18	1.44	Spinach	Singh (2001)
0 to 1000	Cr^{VI}	5720	215	26.6	Brake fern	Su et al., (2005)
50 to 500	Cr^{III}	553	215	2.57	Brake fern	Su et al., (2005)
0 to 2000	Cr^{III}	13.0*	7.10*	1.83*	Wheat	López-Luna et al., (2009)
	Cr^{III}	14.0*	8.39*	1.67*	sorghum	López-Luna et al., (2009)
0 to 4000	Cr^{III}	28.0*	8.19*	3.42*	Oat	López-Luna et al., (2009)
0 to 500	Cr^{VI}	13.9*	10.8*	1.29*	Wheat	López-Luna et al., (2009)
	Cr^{VI}	35.4*	6.27*	5.65*	Sorghum	López-Luna et al., (2009)
	Cr^{VI}	27.0*	8.87*	3.04*	Oat	López-Luna et al., (2009)

*average values

7.2 MATERIALS AND METHODS

7.2.1 Sampling site description

Thirty six soil samples from Stoke Bardolph sewage farm were sampled according to Section 2.1; the site description is given in Section 4.2.1 and a satellite view on the location is shown in Figure 7.1. The sample coding systems which represent the field sites is shown in Figure 7.2.

7.2.2 Soil characterization

To measure Cr solubility, soil (2 g) samples were equilibrated with 30 mL 0.01 M $Ca(NO_3)_2$ for three days to measure soluble forms of Cr. The soil suspension was then centrifuged at 3000 rpm for 20 min and filtered using 0.2 µm Millex Milipore filters prior to analysis of Cr_{CaNO3} and Cr^{VI}_{CaNO3} by ICP-MS and HPLC-ICP-MS, according to Section 2.4.2 but with the following amendment. For determination of Cr_{CaNO3}, working standards of Cr^{VI} (0 to 100 µg L^{-1}) and internal standards of ^{45}Sc, ^{74}Ge, ^{103}Rh at approximately 10 µg L^{-1} in 0.01 M $Ca(NO_3)_2$ were used. In order to determine Cr^{VI}_{CaNO3} (HPLC-ICP-MS), a repeated 20 µg L^{-1} Cr^{VI} standard prepared in 0.01 M $Ca(NO_3)_2$ was run after ten samples for drift correction.

7.2.3 Plant sampling and sample preparation

Plant samples (maize) were cut using clean secateurs and placed in a white nylon sack for transport (Plate 7.1). Upon return to the laboratory, each sample was cut into small pieces and washed to remove soil dust in tap water and deionised water before being oven dried at 60˚C for 4 days in paper bags. Following drying, samples were cut into smaller pieces and ground using a cutting mill. Ground samples were stored in clean polyethylene bottles.

7.2.4 Measuring Cr_{TMAH} and Cr^{VI}_{TMAH} in maize plant

A modification of the TMAH extraction method of Watts and Mitchell (2009) was used to extract Cr from plant samples. Five millilitres of 10% TMAH was added to 0.5 g of dried and ground plant sample. The solution was heated for 3 hr at 70˚C and shaken after 1.5 hr. Five millilitres of Milli-Q water was added to each sample before shaking and centrifuging at 2400 g for 15 min. The solution was then filtered using 0.2 µm Millex Milipore syringe filters and diluted to 1% TMAH before analysis of Cr_{TMAH-P} and Cr^{VI}_{TMAH-P} by ICP-MS and HPLC-ICP-MS, respectively (Section 2.4.2).

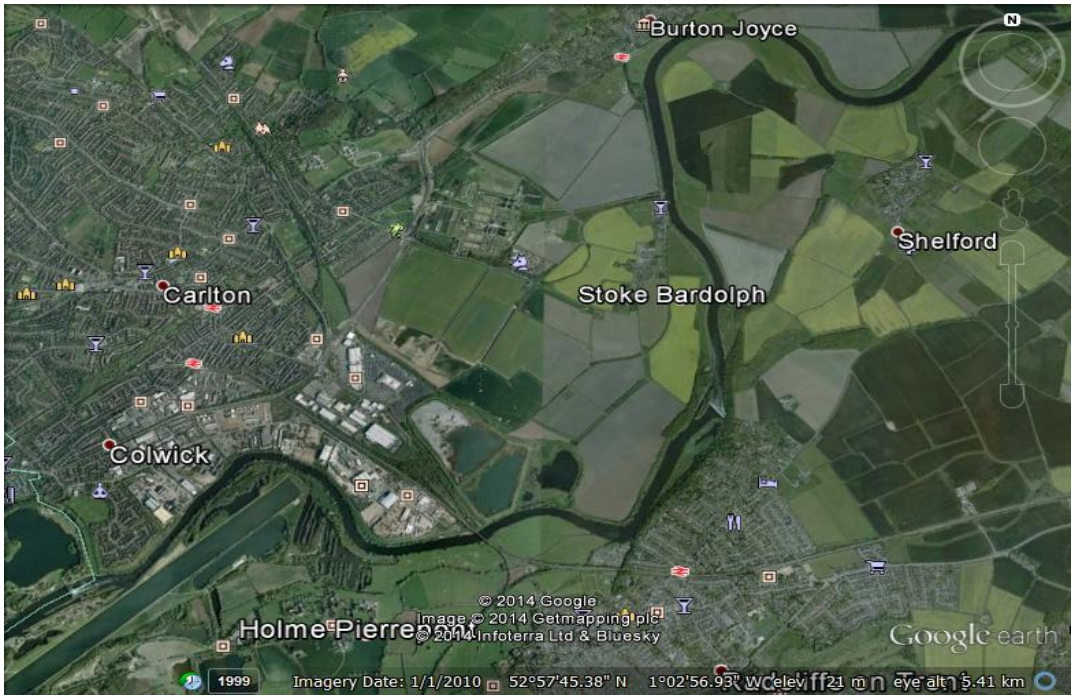


Figure 7.1 A satellite view of the Stoke Bardolph site. Source ‘’Stoke Bardolph’ 52˚ 57’ 0’’ N, and 1˚ 2’ 0’’ W’’. Google Earth. Accessed on 8th May 2014.

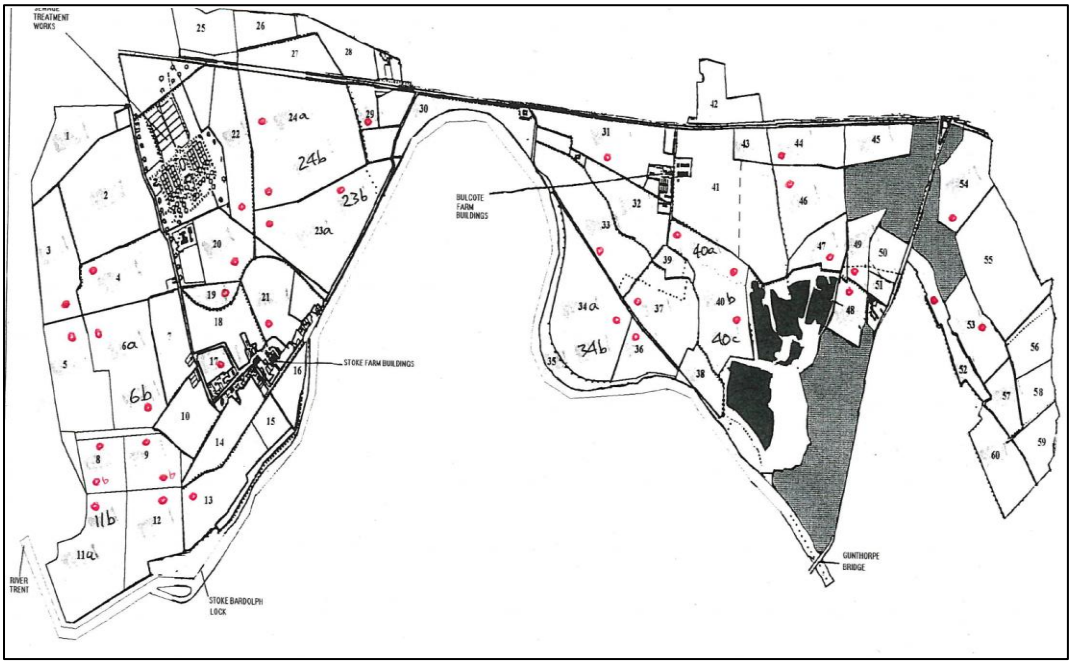


Figure 7.2 Map of the sewage sludge disposal field site in Stoke Bardolph. The red dots referred to the individual sampling sites; the sample coding system was according to the field numbers used by Severn Trent Water Ltd.

Plate 7.1 Maize sampling in one of the fields at Stoke Bardolph on 7th August 2013.

7.3 RESULTS AND DISCUSSION

7.3.1 Cr solubility in soil

The concentration of Cr_{CaNO3} ranged from 0.260 to 13.5 µg L^{-1} (median 4.15 µg L^{-1}) whilst Cr^{VI}_{CaNO3} ranged from 0.325 to 10.0 µg L^{-1} (median 1.63 µg L^{-1}) (Table 7.2).Correlations between soil properties and Cr_{CaNO3} and Cr^{VI}_{CaNO3} in the StB soils are shown in Table 7.3.

Table 7.2 Summary of different Cr fractions ($Cr_{total\text{-}S}$, $Cr_{TMAH\text{-}S}$, $Cr^{VI}_{TMAH\text{-}S}$, Cr_{CaNO3}, Cr^{VI}_{CaNO3}) from Stoke Bardolph soils (n=38). Data is arranged in ascending order of $Cr_{total\text{-}S}$ and *data from Chapter 4. Results for Cr_{CaNO3} and Cr^{VI}_{CaNO3} were taken from the average of triplicate measurements.

Fields	pH*	LOI* %	$Cr_{total\text{-}S}$* mg kg^{-1}	$Cr_{TMAH\text{-}S}$* mg kg^{-1}	$Cr^{VI}_{TMAH\text{-}S}$* mg kg^{-1}	Cr_{CaNO3} µg L^{-1}	Cr^{VI}_{CaNO3} µg L^{-1}
52	7.12	6.76	43.2	0.32	0.09	0.26	0.35
21	7.08	6.89	45.7	1.42	1.06	0.39	0.36
53	6.81	5.90	48.4	0.22	0.17	0.34	0.38
17	7.29	7.86	54.0	0.68	0.44	0.64	0.80
19	7.36	6.38	59.6	1.52	0.36	1.09	1.35
54	7.53	4.51	59.7	0.15	0.03	0.33	0.55
5	7.63	3.52	66.6	1.68	0.37	0.82	1.08
31	6.66	7.00	70.7	2.57	1.10	1.21	3.16
49	7.68	7.66	94.2	0.36	0.05	0.48	0.76
33	6.95	6.39	99.3	3.07	0.63	2.24	1.04
36	7.45	6.77	102	1.17	0.35	2.69	10.0
48	7.46	9.03	104	0.45	0.23	1.02	1.63
3	7.08	6.07	140	4.18	0.57	2.41	3.25
40C	7.19	7.82	160	8.49	3.09	3.90	2.46
44	7.28	6.57	162	5.87	1.80	2.12	1.85
29	7.47	7.53	190	9.18	0.98	2.79	0.94
46	7.62	9.74	204	1.86	0.99	2.60	2.00
47	6.83	9.66	287	8.84	2.18	4.71	2.18
4	6.79	9.63	319	20.4	2.45	2.40	1.52
40A	7.07	8.96	334	5.64	4.96	4.39	4.42
40B	6.94	8.15	344	12.1	2.92	5.94	4.53
37	7.00	11.5	391	8.66	1.71	5.48	4.92
23A	6.77	10.3	410	28.0	6.24	3.66	1.37
23B	6.72	9.55	428	18.7	2.82	5.96	1.89
20	6.72	10.3	476	28.8	6.03	4.47	1.21
6A	6.63	10.2	479	14.0	2.76	5.48	1.78
34	7.06	10.4	483	9.65	4.41	8.11	2.89
11	7.07	9.44	487	14.2	3.24	6.18	7.26
6B	6.53	8.77	538	30.2	3.99	7.53	2.29
24B	6.71	10.6	555	50.9	10.8	5.59	NA
24A	6.89	11.1	615	43.9	8.97	4.86	2.60
22	6.82	10.1	615	34.7	8.13	6.52	2.33
13	7.12	18.9	1060	41.4	3.60	7.56	2.59
9A	6.58	15.3	1110	65.2	21.1	8.69	0.54
8A	6.43	20.7	1310	74.9	7.56	11.06	0.33
12	6.76	22.6	1350	78.8	11.0	9.49	1.07
8B	6.49	21.7	1470	57.4	7.33	9.03	0.50
9B	6.48	23.4	1670	81.5	4.23	13.5	0.34

NA (not available)

A good correlation also was observed between Cr_{CaNO3} and $Cr_{total\text{-}S}$ (r=0.922) which suggests that Cr in the soil solution or pore water is at least partly controlled by $Cr_{total\text{-}s}$. However, there was no correlation between Cr^{VI}_{CaNO3} with other soil properties and Cr fraction in soil which may be due to reduction of Cr^{VI} at pH6.03 in soil equilibrated with 0.01 M $Ca(NO_3)_2$ after 3 days (Table 5.1, Chapter 5).

Table 7.3 Correlation matrix for measured soil properties, pH, LOI, $Cr_{total\text{-}S}$, $Cr_{TMAH\text{-}S}$, $Cr^{VI}_{TMAH\text{-}S}$, Cr_{CaNO3}, Cr^{VI}_{CaNO3} (r-values and p-values in brackets) in SB soils. Significant correlations are in bold and italic type.

	pH	LOI	$Cr_{total\text{-}s}$	$Cr_{TMAH\text{-}s}$	$Cr^{VI}_{TMAH\text{-}s}$	Cr_{CaNO3}
Cr_{CaNO3}	**-0.673**	**0.856**	**0.922**	**0.856**	**0.636**	
	(<0.001)	*(<0.001)*	*(<0.001)*	*(<0.001)*	*(<0.001)*	
Cr^{VI}_{CaNO3}	0.161	-0.186	-0.171	-0.246	-0.134	0.027
	(0.341)	(0.271)	(0.311)	(0.142)	(0.428)	(0.875)

Ge et al., (2000) found that plant root uptake depended on the fraction of contaminant that can be transfered from soil to soil solution. Chromium fractions and species in soil ($Cr_{TMAH\text{-}S}$ and $Cr^{VI}_{TMAH\text{-}S}$) could be predicted from $Cr_{total\text{-}S}$ and showed a good linear relationship in Figure 7.3 a) and b). Chromium solubility was also found to be a function of $Cr_{total\text{-}S}$ (Fig. 7.3c) although only a very small proportion of Cr was solubilised from soil in the 0.01 M $Ca(NO_3)_2$ suspension. As Fig. 7.3c showed an asymptotic trend, it was also clear that the solubility of Cr declined as $Cr_{total\text{-}S}$ increased, suggesting that while sludge addition increased soil Cr content, it also increased the capacity of the soil to adsorb or fix Cr, possibly through the co-addition of organic matter or phosphate.

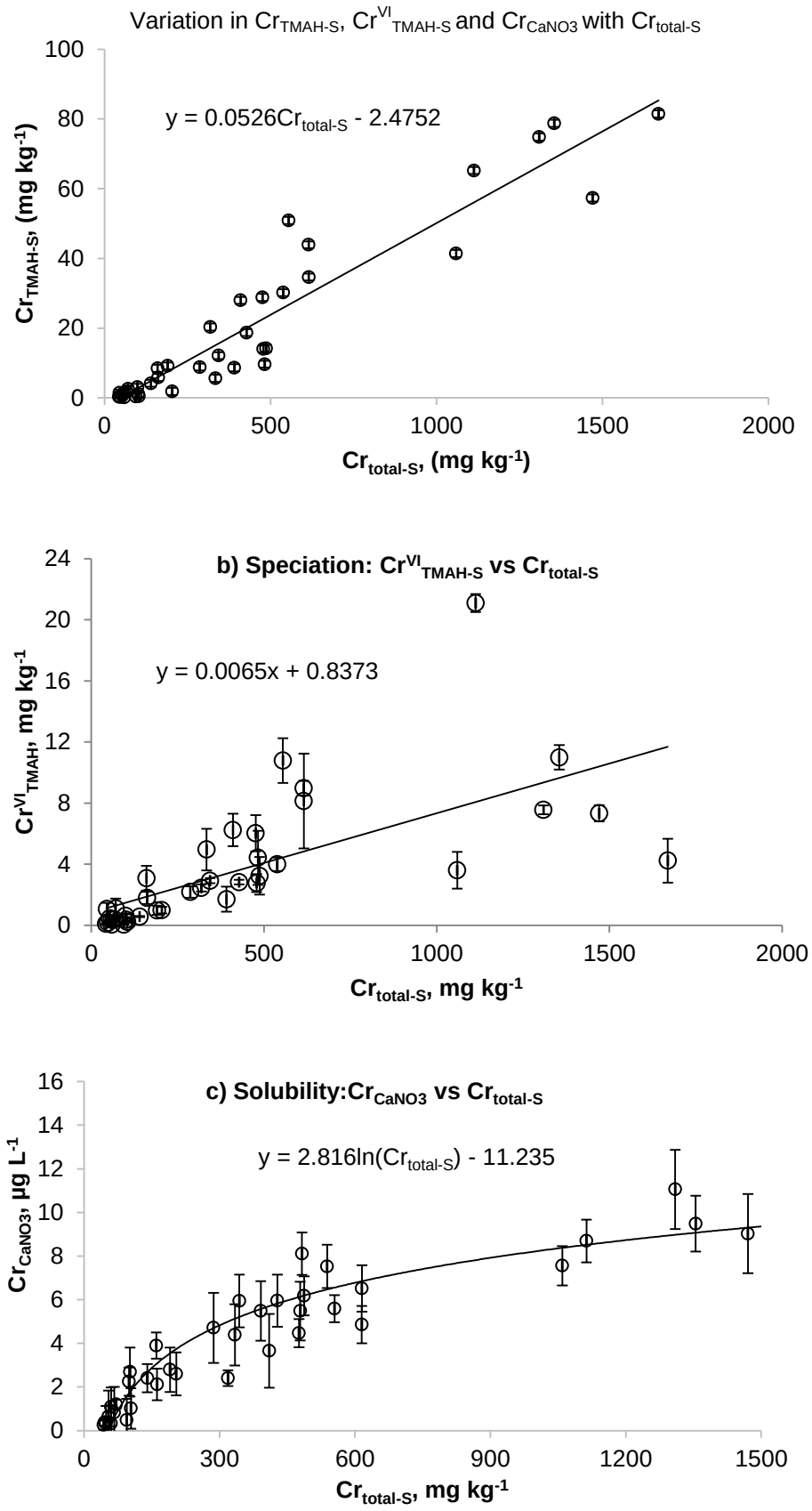


Figure 7.3 Variation in the Cr fractionation, speciation and solubility (a. Cr_{TMAH-S}; b. Cr^{VI}_{TMAH-S}; and c. Cr_{CaNO3}) with $Cr_{total-S}$ concentration in Stoke Bardolph soils. Error bars represent the standard error of triplicate measurements.

7.3.2 Chromium uptake and speciation in maize plants

In this study, the whole plant above ground was processed (dried, ground and extracted) as a single product to simulate the normal post-harvest processing of fodder maize. Total Cr concentration in the maize plants ($Cr_{total\text{-}P}$) ranged from 0.105 to 2.76 mg kg^{-1} (median 0.279 mg kg^{-1}) (Table 7.3). The Cr uptake could be partly from atmospheric deposition to leaves despite washing as there are some contradicting arguments from previous work regarding the efficacy of a water-washing step in reducing extraneous Cr concentration on leaves (Larsen et al., 1991; Voutsa et al., 1996; Nabulo et al., 2010). The Stoke Bardolph site is unlikely to be subject to substantial aerial pollution, and the variation in $Cr_{total\text{-}P}$ between fields was substantial, suggesting a soil (rather than atmospheric) source of plant Cr. Nevertheless there exists the possibility that local soil re-suspension may contribute to aerial deposition to leaves. Marwa et al. (2012) found the average concentration of total Cr in maize shoot and grain from a field survey of 40 farms from various underlying rock geology and anthropogenic activities in Tanzania ranged from 0.18 to 1.72 mg kg^{-1} and 0.01 to 0.38 mg kg^{-1}, respectively, which is lower than in the current study. The concentration of $Cr_{TMAH\text{-}P}$ ranged from 0.0052 to 0.146 mg kg^{-1} (median 0.0492 mg kg^{-1}). This fraction probably consists of a mixture of Cr^{VI} and Cr^{III}-organic forms in the plant but $Cr^{VI}_{TMAH\text{-}P}$ in the maize plants ranged between 0.003 – 0.0185 mg kg^{-1} (median 0.0116 mg kg^{-1}) and so the majority of the TMAH-extractable Cr was Cr^{III} in some combined form.

Table 7.4 Concentration of different Cr fraction ($Cr_{total-P}$, Cr_{TMAH-P}, Cr^{VI}_{TMAH-P}) (mg kg^{-1}) in maize samples (n=38) from Stoke Bardolph summarized using mean and standard error (*SE*) of triplicate measurements. Data are arranged in ascending order of $Cr_{total-P.}$

Field	$Cr_{total-P}$ (mg kg^{-1})		Cr_{TMAH-P} (mg kg^{-1})		Cr^{VI}_{TMAH-P} (mg kg^{-1})	
	mean	*SE*	mean	*SE*	mean	*SE*
44	0.105	0.0006	0.0615	0.0006	0.015	0.0017
34	0.123	0.0009	0.0800	0.0036	0.012	0.0004
53	0.136	0.0011	0.0404	0.0028	0.003	0.0022
36	0.160	0.0013	0.0386	0.0014	0.011	0.0014
5	0.160	0.0028	0.0398	0.0041	0.011	0.0010
47	0.161	0.1190	0.0541	0.0049	0.017	0.0028
6b	0.168	0.0101	0.0180	0.0027	0.012	0.0018
40b	0.171	0.0165	0.0423	0.0038	0.013	0.0010
54	0.188	0.0420	0.0052	0.0091	0.003	0.0012
6a	0.194	0.0033	0.0188	0.0043	0.011	0.0009
40c	0.195	0.0178	0.0502	0.0106	0.011	0.0023
46	0.199	0.0028	0.0907	0.0021	0.015	0.0009
40	0.207	0.0005	0.0481	0.0086	0.015	0.0012
3	0.215	0.0004	0.0192	0.0064	0.016	0.0009
33	0.232	0.0066	0.0709	0.0441	0.013	0.0007
4	0.242	0.0003	0.0199	0.0041	0.012	0.0002
12	0.247	0.0030	0.0607	0.0005	0.012	0.0011
8a	0.270	0.0009	0.0364	0.0004	0.010	0.0017
52	0.278	0.0021	0.1460	0.0004	0.006	0.0022
48	0.280	0.0222	0.0640	0.0036	0.018	0.0019
9b	0.281	0.0010	0.0562	0.0043	0.013	0.0026
11	0.285	0.0026	0.0526	0.0037	0.010	0.0020
49	0.356	0.0001	0.0471	0.0013	0.015	0.0022
37	0.383	0.0006	0.0759	0.0051	0.013	0.0007
23a	0.404	0.0029	0.0273	0.0147	0.012	0.0010
19	0.412	0.0002	0.0250	0.0052	0.010	0.0021
22	0.453	0.0007	0.0462	0.0130	0.012	0.0014
8b	0.455	0.0019	0.0297	0.0032	0.012	0.0014
13	0.494	0.0011	0.0637	0.0022	0.011	0.0012
31	0.502	0.0009	0.0363	0.0106	0.012	0.0016
29	0.518	0.0006	0.0518	0.0071	0.011	0.0017
24b	0.519	0.0017	0.0528	0.0056	0.011	0.0020
17	0.539	0.0019	0.0434	0.0024	0.009	0.0016
20	0.598	0.0008	0.1116	0.0049	0.012	0.0029
24a	0.704	0.0003	0.0628	0.0041	0.010	0.0030
23b	0.751	0.0003	0.0490	0.0270	0.013	0.0002
9a	0.760	0.0008	0.0289	0.0042	0.010	0.0008
21	2.761	0.0006	0.0296	0.0016	0.011	0.0008

The relationship between $Cr_{total\text{-}P}$ and $Cr^{VI}_{TMAH\text{-}P}$ in the maize plants is shown in Figure 7.4, there was no correlation between $Cr_{total\text{-}P}$ and $Cr^{VI}_{TMAH\text{-}P}$ or between $Cr_{TMAH\text{-}P}$ and $Cr^{VI}_{TMAH\text{-}P}$ ($r=0.08$, $p>0.05$) (Table 7.5). An extreme value of $Cr_{total\text{-}P}$ from Field 21 (2.76 mg kg^{-1}) was treated as an outlier and excluded; nevertheless, it was clear that the variation of $Cr_{total\text{-}P}$ (RSD=0.433 mg kg^{-1}) was considerably greater than that of $Cr^{VI}_{TMAH\text{-}P}$.

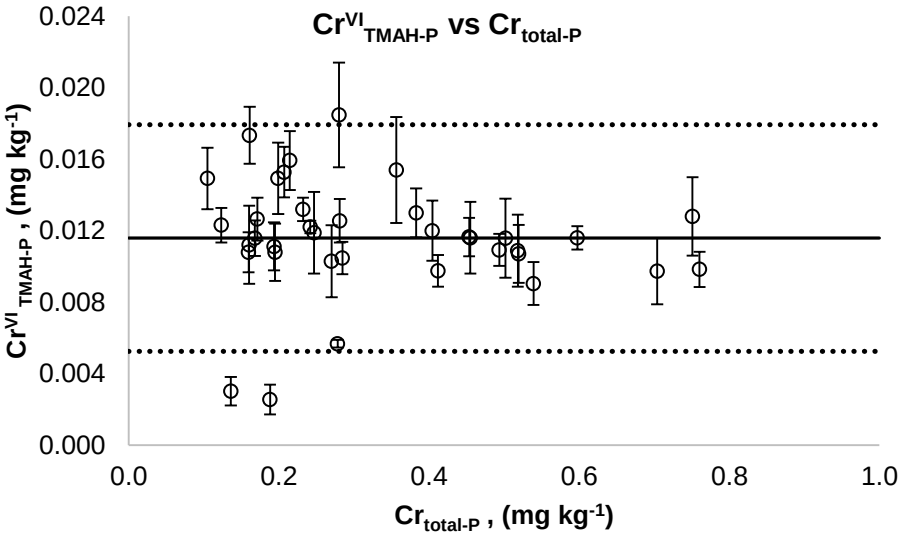


Figure 7.4 TMAH extractable Cr^{VI} ($Cr^{VI}_{TMAH\text{-}P}$) as a function of total Cr concentration ($Cr_{total\text{-}P}$) in whole maize plants. The solid line refers to the mean concentration of $Cr^{VI}_{TMAH\text{-}P}$ and the broken lines delineate ± 2 SD values. Error bars represent the standard error between three replicates.

Table 7.5 Correlation matrix for Cr fractionation in maize plants: $Cr_{total\text{-}P}$, $Cr_{TMAH\text{-}P}$, $Cr^{VI}_{TMAH\text{-}P}$ (r-values and p-values in brackets) in SB soils.

	$Cr_{total\text{-}P}$	$Cr_{TMAH\text{-}P}$
$Cr_{TMAH\text{-}P}$	-0.087 (>0.05)	
$Cr^{VI}_{TMAH\text{-}P}$	-0.064 (>0.05)	0.080 (>0.05)

7.3.3 Transfer of Cr from soil to plant

Several fractions of soil Cr ($Cr_{total\text{-}S}$, $Cr_{TMAH\text{-}S}$ and Cr_{CaNO3}) were initially investigated as potential controlling factors for transfer of Cr from soil to plant (Figure 7.5a to 7.5c). The variation in $Cr_{total\text{-}P}$ with $Cr_{total\text{-}S}$ is shown in Figure 7.5 a: there was no correlation between $Cr_{total\text{-}P}$ and $Cr_{total\text{-}S}$. Transfer from soil to plant was extremely limited, with less than 0.04% of $Cr_{total\text{-}S}$ being taken up by plant in the form of $Cr_{total\text{-}S}$. There was also no correlation between $Cr_{TMAH\text{-}P}$ and $Cr_{total\text{-}S}$ (Fig 7.5b). Only about 10% of $Cr_{TMAH\text{-}P}$ was in the form of Cr^{VI} ($Cr^{VI}_{TMAH\text{-}P}$) and the rest is likely to be Cr^{III} bound to organic ligands. However, there was also no correlation between $Cr_{total\text{-}S}$ and $Cr^{VI}_{TMAH\text{-}P}$ with the latter remaining virtually constant at about 0.10 to 0.18 mg kg^{-1} across a range of $Cr_{total\text{-}S}$ concentrations from 100 to 1800 mg kg^{-1} (Fig 7.5 c). Only below a $Cr_{total\text{-}S}$ level of 100 mg kg^{-1} there was some evidence of lower $Cr^{VI}_{TMAH\text{-}P}$ concentrations; this trend was absent from Figures 7.5a and 7.5b.

Variation in $Cr_{total-P}$, Cr_{TMAH-P} and Cr^{VI}_{TMAH-P} with $Cr_{total-S}$

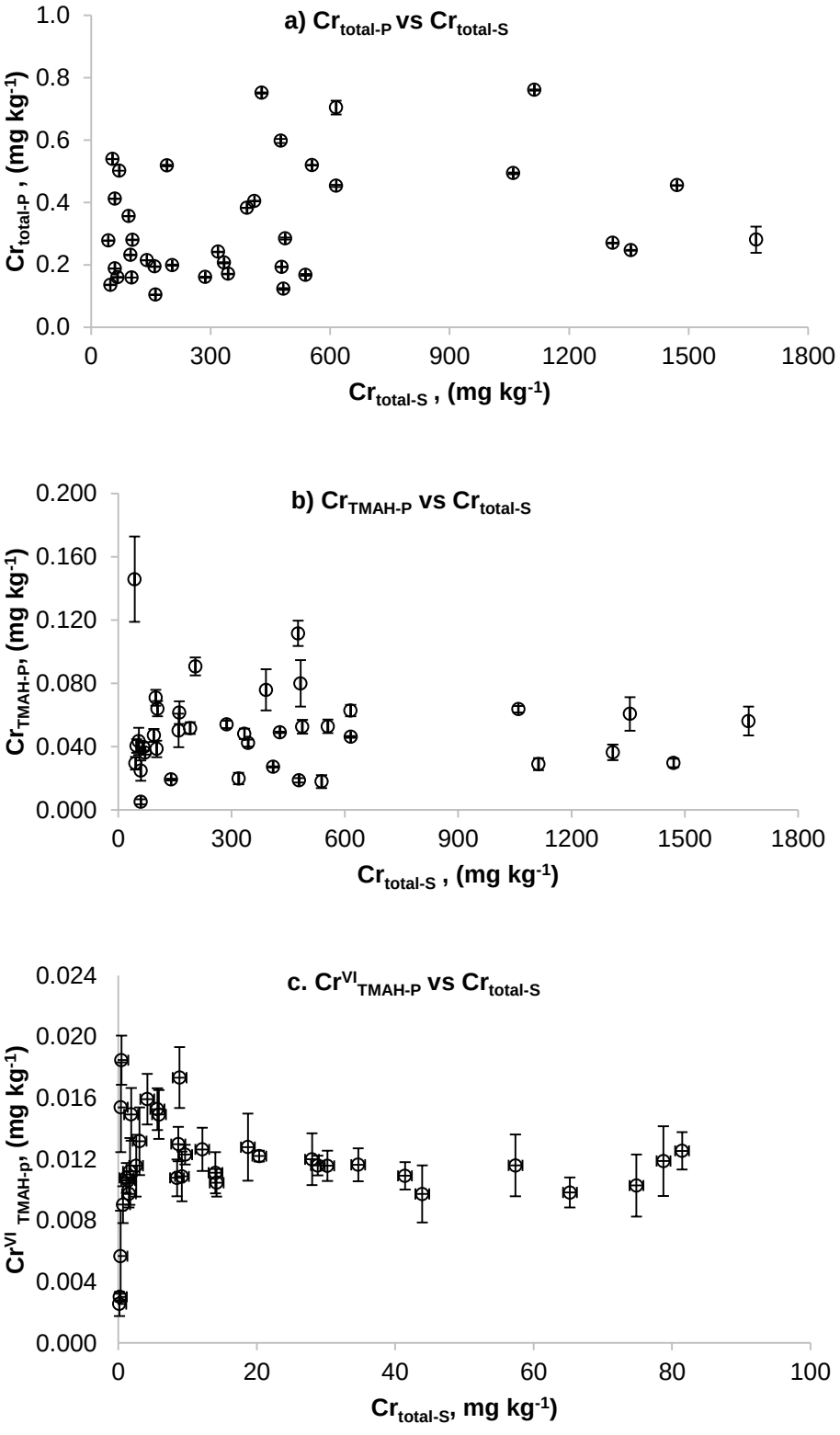


Figure 7.5 Variation in the concentration of Cr in maize plants (a. $Cr_{total-P}$; b. Cr_{TMAH-P}; and c. Cr^{VI}_{TMAH-P}) with $Cr_{total-S}$ concentration in Stoke Bardolph soils ($Cr_{total-S}$). Error bars represent standard error of triplicate measurements.

A similar lack of correlation was found between $Cr_{total\text{-}P}$, $Cr_{TMAH\text{-}P}$ and $Cr^{VI}_{TMAH\text{-}P}$ and TMAH-extractable soil Cr ($Cr_{TMAH\text{-}S}$) (Figure 7.6). Only 20% of $Cr_{total\text{-}P}$ was also extractable in TMAH ($Cr_{TMAH\text{-}P}$) whilst $Cr_{TMAH\text{-}S}$ was about 5% than $Cr_{total\text{-}S}$. An invariant value for $Cr^{VI}_{TMAH\text{-}P}$, which is restricted within the range 0.010 to 0.014 mg kg^{-1} at more than 20 mg kg^{-1} $Cr_{TMAH\text{-}S}$, suggests that the plant exercises control over the uptake, or redox status, of Cr^{VI} (Figure 7.6 c).

Variation in $Cr_{total-P}$, Cr_{TMAH-P} and Cr^{VI}_{TMAH-P} with Cr_{TMAH-S}

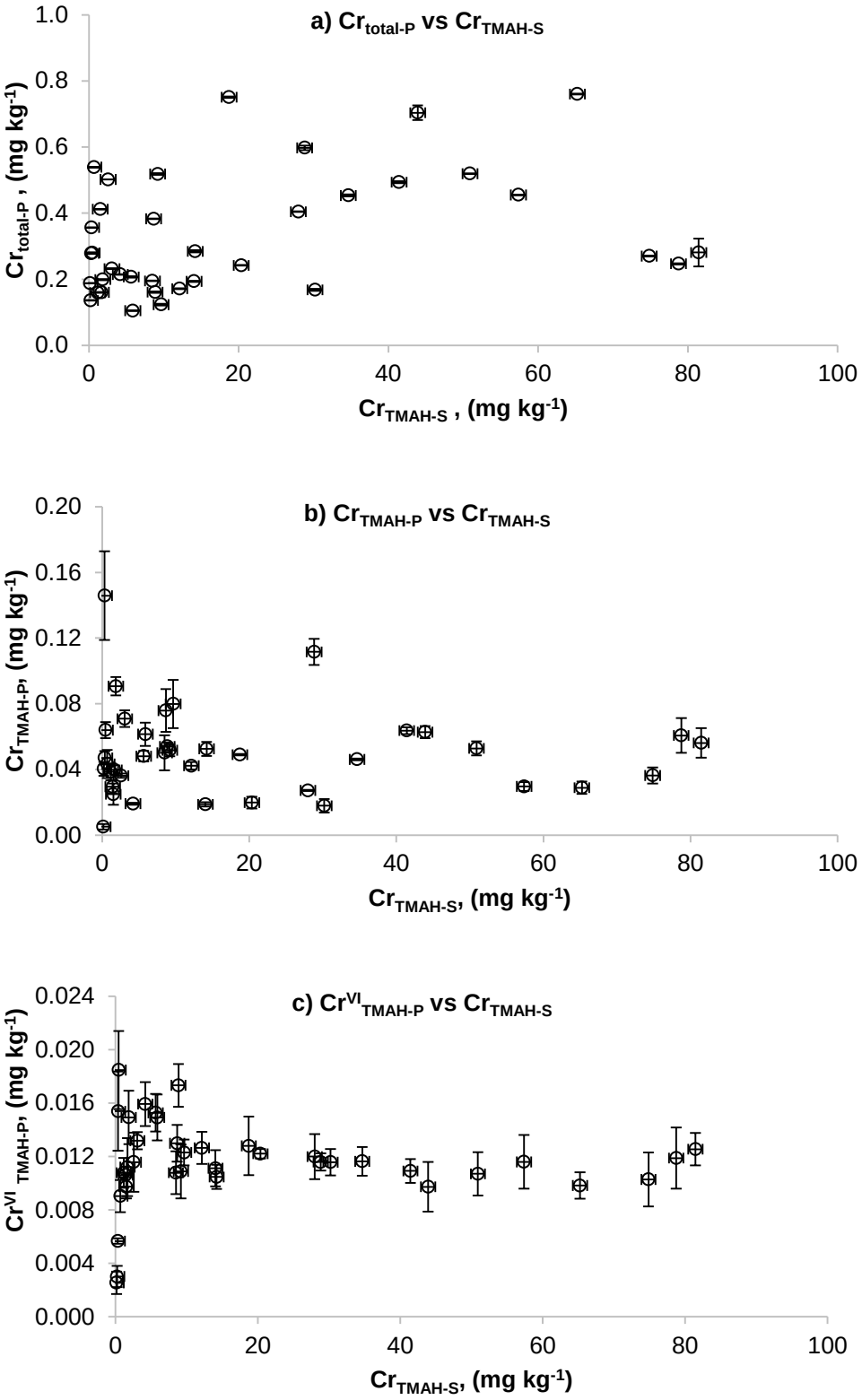


Figure 7.6 Variation in the concentration of Cr in maize plants (a. $Cr_{total-P}$; b. Cr_{TMAH-P}; and c. Cr^{VI}_{TMAH-P}) with Cr extractable by TMAH from Stoke Bardolph soils (Cr_{TMAH-S}). Error bars represent the standard error of triplicate measurements.

The concentration of Cr_{CaNO3} was in the range of 0.260 to 13.5 µg L^{-1} (Figure 7.7). There was no correlation between $Cr_{total\text{-}P}$ and the Cr_{CaNO3} in soil solution (Figure 7.6 a). Again, there was no correlation between $Cr_{TMAH\text{-}P}$ and Cr_{CaNO3} in soil solution (7.7 b). This finding is also in agreement with Khan (2001) as they also found no correlation between extractable Cr in soil and Cr in plants grown on a soil contaminated by tannery effluent. As can be seen in Fig 7.7 c), there was restricted uptake of $Cr^{VI}_{TMAH\text{-}P}$ by plants from the soluble Cr^{VI} fraction in soil (Cr^{VI}_{CaNO3}) which again appears to suggest that plant roots tightly control either Cr^{VI} uptake or Cr redox status in the plant. Lytle et al., (1998) found that a wetland plant, water hyacinth, was able to reduce Cr^{VI} to Cr^{III} in fine lateral roots when the nutrient culture was initially supplied with 10 mg L^{-1} Cr^{VI}.

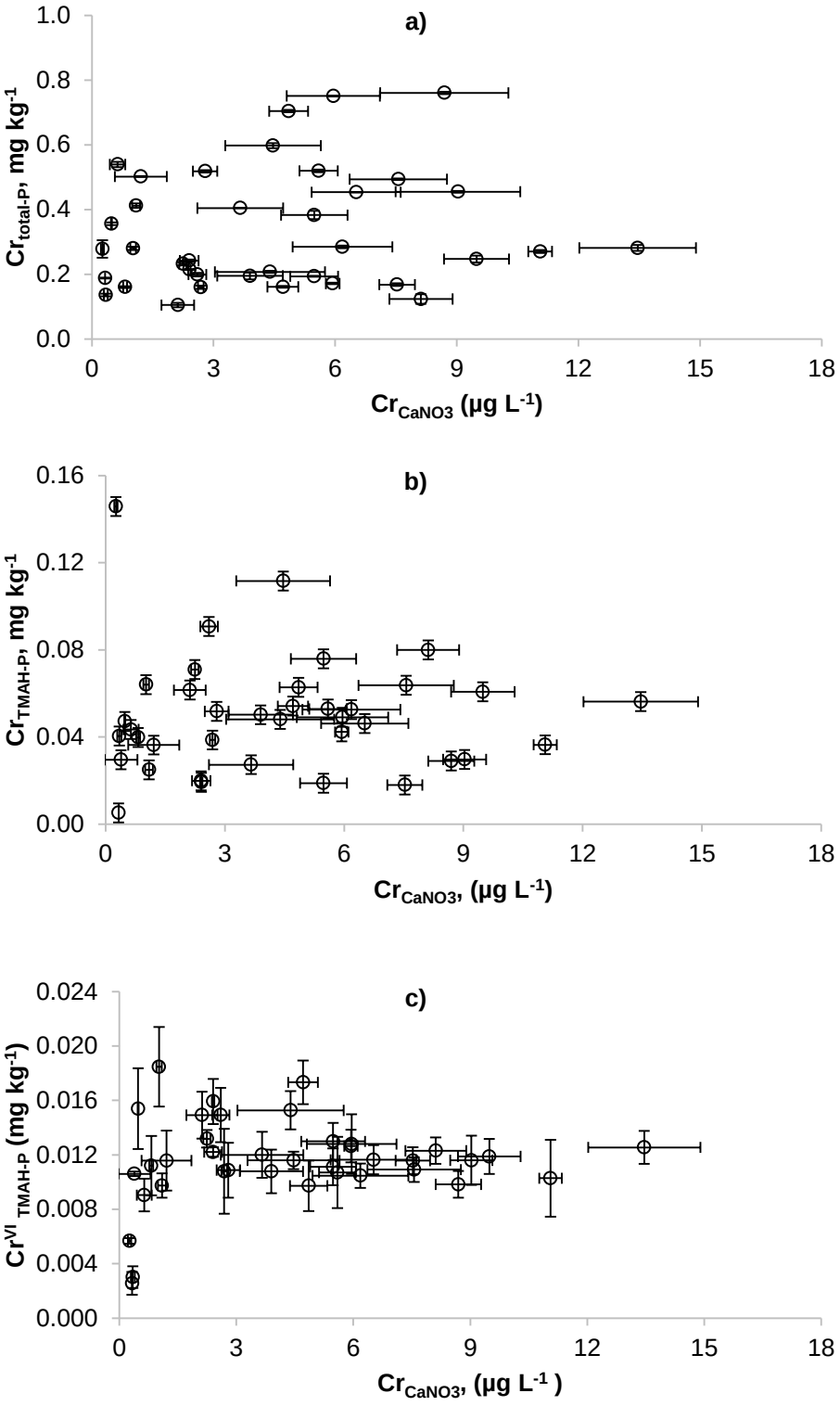


Figure 7.7 Variation in the concentration of Cr in maize plants (a. $Cr_{total\text{-}P}$; b. $Cr_{TMAH\text{-}P}$; and c. $Cr^{VI}_{TMAH\text{-}P}$) with total Cr concentration in a 0.01 M $Ca(NO_3)_2$ extraction of Stoke Bardolph soils (Cr_{CaNO3}). Error bars represent the standard error of triplicate measurements.

The correlations between Cr fractions in plant and Cr^{VI} in soil (Cr^{VI}_{CaNO3} and Cr^{VI}_{TMAH}) are shown in Figures 7.8 and 7.9, respectively. There was no correlation between $Cr_{total\text{-}P}$, $Cr_{TMAH\text{-}P}$ or $Cr^{VI}_{TMAH\text{-}P}$ with Cr^{VI}_{CaNO3} (Figure 7.8). The same finding was also observed between the Cr fractions in plants ($Cr_{total\text{-}P}$, $Cr_{TMAH\text{-}P}$ or $Cr^{VI}_{TMAH\text{-}P}$) and $Cr^{VI}_{TMAH\text{-}S}$ (Figure 7.9). Again, this may suggest that Cr^{VI} is not the principal reservoir of plant-available Cr in these soils or it might indicate that either uptake of Cr^{VI} is highly restricted or that Cr^{VI} is reduced within the plant following uptake. Several published studies have demonstrated that Cr^{VI} is reduced within plants following uptake of Cr^{VI} (Liao et al., 2011; Zayed et al., 1998).

Variation in $Cr_{total-P}$, Cr_{TMAH-P} and Cr^{VI}_{TMAH-P} with Cr^{VI}_{CaNO3}

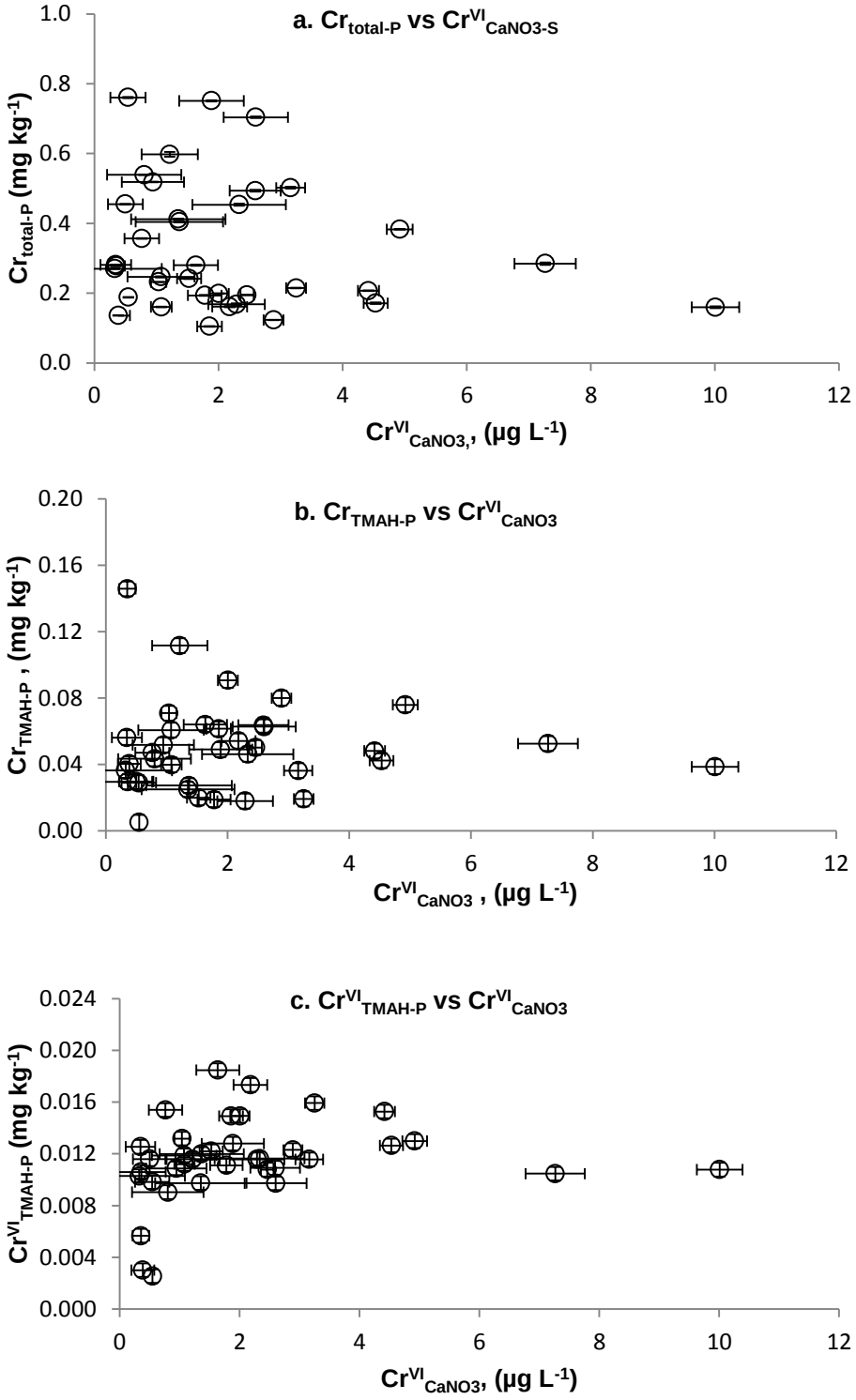


Figure 7.8 Variation in the concentration of Cr in maize plants (a. $Cr_{total-P}$; b. Cr_{TMAH-P}; and c. Cr^{VI}_{TMAH-P}) with Cr^{VI} concentration in a 0.01 M $Ca(NO_3)_2$ extraction of Stoke Bardolph soils (Cr^{VI}_{CaNO3}). Error bars represent standard error of triplicate measurements.

Variation in $Cr_{total-P}$, Cr_{TMAH-P} and Cr^{VI}_{TMAH-P} with Cr^{VI}_{TMAH-P}

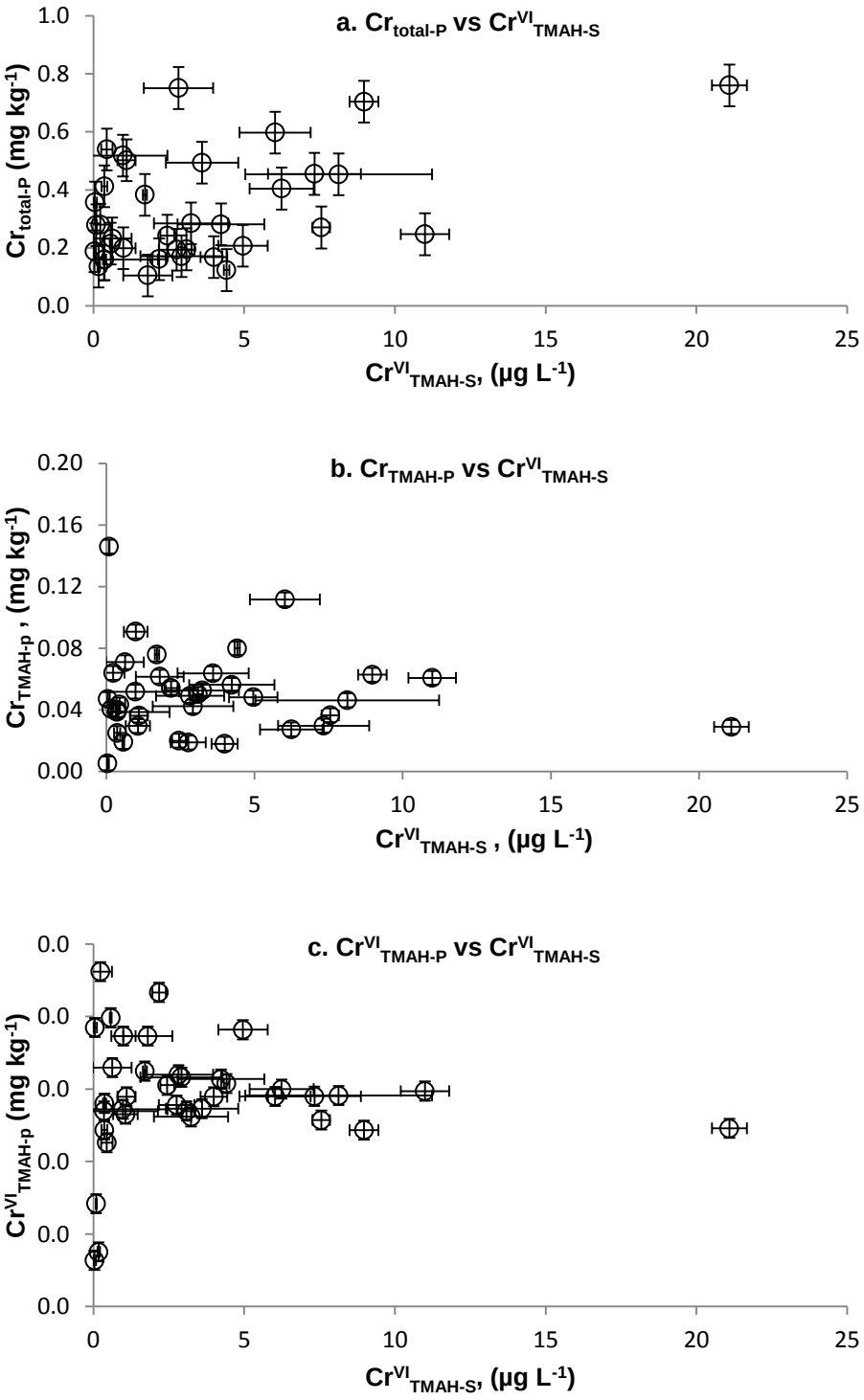


Figure 7.9 Variation in the concentration of Cr in maize plants (a. $Cr_{total-P}$; b) Cr_{TMAH-p}; and c. Cr^{VI}_{TMAH-P}) with Cr^{VI} in TMAH extraction of Stoke Bardolph soils (Cr^{VI}_{TMAH-S}). Error bars represent standard error of triplicate measurements.

7.3.4 Site-specific risk assessment

The potential toxicity of Cr to animals and human population is dependent on its chemical form in food plants such as vegetable crops and cereals – it is widely accepted that Cr^{VI} is considerably more toxic. Most Cr in the maize plants (86 – 100%) was present as Cr^{III} - determined as the difference between $Cr^{VI}_{TMAH\text{-}P}$ and $Cr_{total\text{-}P}$.

Cr^{III}

Tri-valent Cr is an essential micronutrient in animals. An estimate of the safe and adequate daily dietary intake for adults of 50 – 200 µg of Cr^{III} per day has been recommended by the US National Research Council in 1989 (NRC, 1989), followed by an 'adequate intake' value (AI) introduced in 2001 due to insufficient research to establish a reliable Recommended Daily Allowance (RDA) (Institute of Medicine, Food and Nutrition Board, Washington, 2001). The Cr AI value varies with age and gender. For example, Cr intake for adult women (19 to 50 years) is 25 µg per day which is lower than that recommended for adult men in the same age group (35 µg per day). However, although Cr^{III} is an essential micronutrient for humans, it can be toxic at high concentrations. Thus, an oral reference dose of Cr^{III} has been established within the United States Environmental Protection Agency (USEPA) Integrated Risk Information System (IRIS) (USEPA, 1998) at 1.5 mg kg^{-1} per day - this is equivalent to an intake of 105 mg per day for a 70 kg adult. Thus, even with the highest measured Cr concentration in the maize plants (2.76 mg kg^{-1}) there seems very little risk if food crops were produced from those soils which yielded an equivalent Cr concentration. Similarly, a fully grown Holstein dairy cow, weighing around 700 kg and eating dry matter at a rate of around 3% of body mass (21 kg) per day would consume the equivalent of 58 mg Cr^{III} which amounts to only 0.083 mg kg^{-1} day^{-1} against a (human) RfD of 1.5 mg kg^{-1} day^{-1}.

Cr^{VI}

For the previous example, the consumption of Cr^{VI}, for the worst case situation (highest plant Cr content and greatest proportion of Cr^{VI}) would amount to a daily dose to the cow of 0.0116 mg kg^{-1} day^{-1} of Cr^{VI} – considerably greater than the USEPA reference dose for Cr^{VI} intake by humans of 0.003 mg kg^{-1} day^{-1} and suggesting a hazard quotient of 3.9. Clearly there are very considerable uncertainties in such an analysis and human RfD values are generally quite conservative. To date, there is no concentration limit for Cr under the EU

Feed Regulation Directive 2002/32/EC and amendment 2003/100/EC (EC2002, 2003). For the metals and metalloids listed, the maximum concentration limits in feed are: As (2.3 mg kg^{-1}), Cd (1.1 mg kg^{-1}), Pb (45 mg kg^{-1}) and F (170 mg kg^{-1}). Furthermore, no Cr^{III} or Cr^{VI} limits have been established by the United States Food and Drug Agency (USFDA) for animal feed.

Overall, the evidence of virtually invariant Cr^{VI}_{TMAH-P}, despite considerable Cr enrichment in the soil, suggests that plant controls over Cr^{VI} content mean that the fodder maize grown on these heavily sludged soils can be safely used for animal feed. It is more likely that the limitations to arable production on such soils will lie with potential transfer of other heavy metals into crops.

7.4. CONCLUSIONS

The uptake of Cr from soil contaminated with sewage sludge to plant (maize) was investigated. There was good agreement between different fractions of Cr in soil, such as between $Cr_{total-S}$, Cr_{TMAH-S} and Cr^{VI}_{TMAH-S}, as well as $Cr_{total-S}$ and $Cr_{CaNO3,}$ which suggested that $C_{total-S}$ controlled the solubility of Cr in soil. However, there was no correlation between the total Cr concentration in soil and Cr uptake by maize plants. There was also no correlation between different fractions of Cr in soil and different fractions of Cr in the plants. It can be concluded that the maize plants are able to exercise control over Cr^{VI} uptake and transfer to shoots, possibly by reduction of Cr^{VI} to Cr^{III} and subsequent binding to ligand sites in the roots. This was proven by a very restricted range of Cr^{VI}_{TMAH-P} values in plants over a wide range of Cr fractions in the soil ($Cr_{total-S}$, Cr_{TMAH}, Cr_{CaNO3}, Cr^{VI}_{TMAH}, Cr^{VI}_{CaNO3}).

As Cr^{III} can be toxic at large concentration, eventhough with Cr^{III} fraction more than 85% in this plant, there was only a little risk of transfer to human and animal. The lack of variation in Cr^{VI}_{TMAH}, even at elevated soil Cr levels, suggests that fodder maize grown on sites with a history of sewage sludge amendment is potentially safe for consumption by ruminants provided the other potentially toxic elements are in compliance with EU Feed Regulation Directives.

8 GENERAL CONCLUSIONS

The work presented in this thesis is an investigation of Cr speciation and stable isotopic dilution methods as tools for assessing the transformations and lability of Cr^{VI} and Cr^{III} species in soils and their subsequent uptake by plants.

The thesis comprises five themes:

I. developing Cr speciation methodology in alkaline soil extracts (Chapters 2 and 3)
II. assessing Cr speciation and fractionation in a wide range of soil ecosystems collected from urban sites in Wolverhampton, Nottingham, London and a historical sewage sludge disposal farm (Chapter 4);
III. developing isotopic dilution methodology for Cr and Cr^{VI} (Chapter 5)
IV. assessing the kinetics of Cr^{VI} interaction with a geocolloid (humic acid) capable of both reducing Cr^{VI} and binding the resulting Cr^{III} species (Chapter 6)
V. examining the factors affecting Cr uptake by maize plants grown on a historically contaminated sewage sludge disposal farm (Chapter 7).

8.1 Main findings

8.1.1 Chromium in soil

In an earlier attempt to optimize ICP-MS for Cr analyis, CCT-KED mode using Sc and Ge as internal standards provided less polyatomic interference but with 10 times lower sensitivity compared to ICP-MS in standard mode. To quantify Cr speciation, LC was used to separate Cr^{III} and Cr^{VI} by employing a Hamilton-PRP X100, (5 µm, 4.1 x 50 mm) anion exchange column with 50 mM TRIS, 40 mM NH_4NO_3, 0.005 M of NH_4-EDTA as mobile phase and pH adjusted to 7.0. This resulted in a faster analysis time (7 minutes) compared to previous trials at pH 9.4 (15 minutes). After separation, Cr^{III} and Cr^{VI} were detected by ICP-MS in CCT-KED mode with better separation obtained in a TMAH matrix compared to the established alkaline soil extractant, EPA 3060 (0.25 M Na_2CO_3 with 0.50 M NaOH). As Cr^{III} was not stable in solution under alkaline conditions, EDTA (0.001M and 0.01 M) was tested, with heating, as a means of retaining Cr^{III} in solution in TMAH. However, the use of EDTA

in soil TMAH extracts largely failed to retain all Cr^{III} in the form of a soluble Cr^{III}-EDTA complex due partly to preferential binding of Cr^{III} to dissolved humic acid. Poor recovery of Cr^{III} was also partly due to oxidation to Cr^{VI} and adsorption of humic-bound Cr^{III} in the analytical column. By contrast, there was good recovery of Cr^{VI} in soil TMAH extracts through the chromatography column. Therefore the extraction and analysis methodology developed is recommended for determination of Cr^{VI} in soil although a consensus agreement among soil testing laboratories following a proficiency testing program is required. This should provide a more sensitive and reliable method for assay of Cr^{VI} in soil against a background of increasing awareness of the health and safety issues associated with Cr^{VI} in the environment.

Soil characteristics of urban areas, from Wolverhampton, Nottingham, London, and from a historical sewage sludge disposal farm in Nottinghamshire, were used to test for relationships between Cr^{VI} concentration and soil characteristics. A multiple regression approach was adopted, using total soil Cr content (Cr_{total}), organic matter content, total soil Fe (Fe_{total}), total soil Mn (Mn_{total}) and pH as inputs. The model generally underestimated Cr^{VI} concentration, particularly in soils with the highest concentration of Cr_{total}. However, prediction based on TMAH-extractable Cr (Cr_{TMAH}) greatly improved agreement between measured and modelled Cr^{VI}. This approach may be useful in the prediction of Cr^{VI} in soil subject to routine geochemical survey, without the need for chromatographic speciation analysis.

Isotopic dilution employing $^{50}Cr^{VI}$ can be used to determine isotopically exchangeable Cr^{VI} in soils provided: i) coupling with speciation analysis in used to determine Cr^{VI} and ii) the solution matrix is engineered to ensure that all labile Cr^{VI} is in solution, rather than adsorbed. The use of more ‘aggressive’ suspending solutions (eg higher TMAH concentrations) risk mobilizing non labile Cr^{VI}. Unfortunately, weaker solutions that fail to mobilize all labile Cr^{VI} provide variable results due to the slow kinetics of the $^{50}Cr^{VI}$ isotopic equilibration. Refinements such as optimizing the spike level of $^{50}Cr^{VI}$ used and testing different isotopic equilibration times in a range of suspending solutions were tested. Results from this study suggest that a range of 0.001 to 0.04% TMAH meet the required criteria. Thus, although isotopic dilution is not recommended as a routine technique, mainly due to the slow exchange kinetics of Cr^{VI} in soils, nevertheless the use of the technique appears to indicate that a low concentration of TMAH (c. 0.01 % TMAH) may provide the basis for a new extraction

method which aims to approximate a truly labile Cr^{VI} pool in soils. Further refinement of this approach is required.

From the experimental results in Chapters 3 and 6, it is clear that dissolved organic matter plays an important role in determining the fate and redox behaviour of Cr^{VI} in soil. Following several trials to investigate the redox behaviour of Cr in soil, and by optimizing EDTA concentration to stabilize Cr^{III} species, the use of the ^{50}Cr isotope and speciation by LC-ICP-MS ($^{50}Cr^{III}$ and $^{50}Cr^{VI}$) provides clear evidence that conversion of Cr^{VI} to dissolved humic-bound Cr causes underestimation of Cr^{VI} in soil. A follow up investigation on the interaction of $^{50}Cr^{VI}$ in the presence of HA confirmed the reduction of Cr^{VI} to Cr^{III} and subsequent binding to HA. However, the transformation of Cr^{VI} to Cr^{III} in the presence of HA was substantially affected by pH of the HA solution, contact time and the ratio of Cr^{VI}:HA.

8.1.2 Cr uptake by plants and risk assessment

The solubility of Cr in soil historically subject to a wide range of sewage application levels can be predicted from extractable Cr (Cr_{TMAH}) and soil properties. However, there was no correlation between Cr concentration in soil and uptake by maize and, from the available data, it appears that prediction of Cr uptake, and Cr speciation in plants, from soil properties and Cr fractionation, is not possible.

It was apparent that maize plants exercise strict control over the uptake of Cr^{VI} or Cr^{III} as a very restricted range of Cr^{VI} concentrations was found in plants growing in a soil with a comparatively large range of Cr concentrations. This may have been due to reduction of Cr^{VI} to Cr^{III} within the root system. Although there is limited guidance on acceptable Cr (and Cr^{VI}) levels in animal feed, a worst case hazard quotient of 3.9 was estimated for the fodder maize analysed in this study. However, considering the highly restricted concentration of Cr^{VI} found in the maize shoot tissue it appears that transfer of Cr^{VI} to agricultural animals is not hazardous and there is perhaps even less risk to human populations (from Cr^{VI}) if such soils were used for food production.

8.2 Future work

From the main findings highlighted above, the issues that require further investigation to improve knowledge of Cr dynamics in soils and uptake by plants are listed below.

- Further investigation of possible redox coupling (eg humic and fulvic acids, Fe^{II}, Mn^{VI}) governing rates of change in Cr speciation and fractionation.
- An expanded investigation of the measurement of Cr^{VI} E-values using a wider range of soil Cr content and sources, and soil properties, using a more carefully focussed range of TMAH concentrations in the general range of 0.001 to 0.04%.
- Further use of the $^{50}Cr^{VI}$ isotope dilution method to quantify the kinetics of Cr speciation and fractionation dynamics in a wide range of whole soils with variable sources of Cr contamination.
- An investigation of the potential use of diffusive gradient thin film techniques (DGT) in measuring labile Cr^{VI} species in soils.
- Testing the use of the $^{50}Cr^{VI}$ isotope in a more detailed investigation of plant uptake by looking at speciation in different plant compartments and the kinetics of transfer within the plant.
- Comparing the behaviour of Cr^{VI} in soil with other divalent anion such as phosphate.

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