Abiotic reduction of Cr(VI) by humic acids : kinetics and removal mechanism

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The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

Dedication

To my beloved parents for their love, endless support and encouragement in all aspects of my life.

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Abstract

The contamination of groundwater by hexavalent chromium is a worldwide problem that has resulted mainly from the poorly managed disposal of hexavalent chromium wastes. Numerous different sources of wastes can produce Cr(VI)-contaminated groundwater with a wide range of pH values. The reduction of Cr(VI) to Cr(III) is of great importance because the hazard of Cr(VI) decreases when it is reduced to the less toxic form, Cr(III). Humic acids obtained from different sources are capable of Cr(VI) reduction. This study compared Cr(VI) reduction over a wide range of pH values (3-11) using humic acids extracted from lignite, peat, soil, and sludge phyto conditioning residue. The reduction of Cr(VI) by all humic acids was pH dependent.

All humic acids reduced Cr(VI) to Cr(III) in a first-order reaction with respect to Cr(VI) concentration but exhibited a partial order of approximately 0.5 with respect to [H+]. Among the humic acids, peat humic acid showed the highest rates of reactions, where Cr(VI) reduction was observed at all pH values investigated (3-11). ^{13}C NMR and pyrolysis GC-MS spectroscopy indicated that the reduction of Cr(VI) into Cr(III) by the humic acids resulted in a loss of aromatic compounds and hydroxyl groups. X-ray absorption spectroscopy indicated that at all pH values the resulting Cr(III) was associated with the partially degraded humic acid in an inner sphere adsorption complex. The reaction mechanism of Cr(VI) by the humic acid’s functional groups is likely to be similar to that by simple organic moieties, which controlled by chromate ester formation followed by the electron transfer decomposition step. The initial step is rapid in acidic systems but far less favourable in alkaline conditions. The finding of this study highlights the potential use of organic matter rich with humic acids for the in-situ reduction of Cr(VI) from aqueous systems over a broad range of environmental conditions.

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Abbreviations

COPR	Chromite Ore Processing Residue
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
HAs	Humic acids
AHA	Aldrich humic acid
rAHA	Refined Aldrich humic acid
PHA	Peat humic acid
SHA	Soil humic acid
SPCR-HA	Sludge phyto conditioning residue-humic acid
XRF	X-ray fluorescence spectroscopy
XAS	X-ray absorption spectroscopy
XANES	X-ray absorption near edge structure
EXAFS	Extended x-ray adsorption fine structure
^{13}C-NMR)	Carbon-13 nuclear magnetic resonance spectroscopy
Pyro-GCMS	Pyrolysis gas chromatography-mass spectroscopy
PRBs	Permeable reactive barriers

Introduction, Aim, and Objectives

1.1 Introduction

Chromium is an important metal from economic and environmental points of view. It has been used in various chemical and manufacturing processes such as in alloys, electroplating, leather tanning, and wood preservation (Barnhart, 1997, Darrie, 2001, Guertin et al., 2005, International Chromium Development Association, 2016). Unfortunately, poor storage, leakage, and improper disposal practices frequently cause the release of chromium to the environment (Burke et al., 1991, Puls et al., 1999, Geelhoed et al., 2002, Whittleston et al., 2011, Ding et al., 2016, Izbicki and Groover, 2016, Matern et al., 2016). The varied sources of contamination have released Cr(VI) into soils and groundwater over a wide range of pH values. For example, electroplating processes can release very acidic solutions. On the other hand, disposal practices of Chromite Ore Processing Residue (COPR) from poorly managed sites have released a high level of Cr(VI) into soils and groundwater at pH > 12. Highly alkaline chromate contaminated plumes are considered more challenging compared with other contaminated sites because the high alkalinity impedes the natural attenuation processes such as the adsorption by Fe and Al oxides and hydrous oxides, clay minerals and colloids, where the adsorption is favourable under acidic to slightly alkaline conditions (James and Bartlett, 1983, Zachara et al., 1987, Rai et al., 1989, Fendorf, 1995). Hence, it has been still under investigation and needs a thorough understanding.

Recently, there has been an increasing interest in remediation strategies that focus on Cr(VI) reduction into less toxic and less mobile form of Cr(III). In-situ remediation techniques have been developed and directed toward the removal of highly hazardous substances including Cr(VI). Several reactive materials have been investigated to remove Cr(VI), such as zero valent iron (ZVI) and Fe(II) containing substances. However, both reactive materials, zero-valent iron (ZVI) and Fe(II) containing substances have a limitation for use that results from the passivation under alkaline conditions (He et al., 2004, Fuller et al., 2013). Recent developments in the field of in-situ remediation have led to a renewed interest in using environmentally friendly materials in the remediation process. It has been reported that soil organic matter is an efficient reactive material for the remediation of Cr(VI) (Eary and Rai, 1991, Fendorf, 1995). Humic substances represent the majority of soil organic matter that plays a significant role in the geochemical behaviour and migration of metals. It can be fractionated into three main groups based on their solubility in water; humin, humic acids, and fulvic acids. Humic acids, the principal fraction of humic substances, are composed of a complex and heterogeneous mixture of polydispersed substances that are rich in aromatic compounds, aliphatic chains and functional groups (Boguta and Sokolowska, 2016). They have various quantities of different functional groups based on the origin, age, climate and extraction conditions (Kipton et al., 1992, Stevenson, 1994). These functional groups are mainly composed of carboxylic, phenolic, alcoholic hydroxyl, amino and imino groups, quinine and ketonic carbonyl and sulfhydryl groups (Sposito and Weber, 1986, Stevenson, 1994). However, it is believed that the solubility and reactivity of humic substances is mainly related to phenolic and carboxylic groups (de Melo et al., 2016). The presence of phenolic and carboxylic acids is responsible for the ability of humic acids to form complexes (chelates) with metal ions (Pehlivan and Arslan, 2006, Erdogan et al., 2007). In the past decades, the interaction of humic acids with Cr(VI) has attracted much attention due to the high toxicity of Cr(VI) and the potential feasibility in using materials rich with humic acids in remediation technique (Zhilin et al., 2004, Scaglia et al., 2013). Most studies of Cr(VI) reduction by humic acids have focused on the reduction capacity of humic acid and the related kinetics (Wittbrodt and Palmer, 1997, Hsu et al., 2009). Despite the large volume of literature that confirms the high capacity of the humic acids for the removal of Cr(VI), a little information is available about the speciation of Cr(VI) and the removal mechanism by these materials. The removal mechanisms and products have not been fully understood especially under highly alkaline conditions. As well, few researchers have addressed how organic-rich secondary materials enhance the reduction of Cr(VI), especially under highly alkaline conditions. The literature information about speciation of Cr(VI) and the removal mechanisms by humic acids is ambiguous and lacking an in-depth understanding of the phenomenon.

This research study aims at investigating the chemical reactions between humic acids (HAs) and chromate over a wide range of pH values focussing on the reaction kinetics and mechanisms. The output of this research is expected to provide more rigorous and in-depth understanding of the reactivity of these materials under highly alkaline conditions and the suitability of organic-rich secondary materials for an in-situ treatment of Cr(VI) contaminated groundwater.

1.2 Organic Rich Secondary Material Permeable Reactive Barriers: Aim and Objectives

Aim

Investigate the chemical reaction between humic acids (HAs) and chromate over a wide range of pHs and the suitability of organic-rich secondary materials for the treatment of Cr(VI) contaminated groundwater.

To achieve the above aim, the following hypothesis and objectives were investigated.

Hypothesis: The removal kinetics of Cr(VI) by humic acids is highly affected by its chemical characteristics.

Objective: Characterize the chemistry of the selected humic acids using various analytical and spectroscopic techniques.

Hypothesis: The reaction between humic acids and Cr(VI) is pH dependent and the reaction rate increases with decreasing in pH.

Objective: Determine the pH dependence of Cr(VI) removal in batch reaction of Cr(VI) solution and HAs.

Hypothesis: Cr(VI) removed from aqueous solutions by humic acids is reduced into Cr(III) over the pH range (3-11).

Objective: Determine Cr speciation after Cr(VI) has reacted with the humic acids at pH range (3-11) using x-ray adsorption spectroscopy.

Hypothesis: Cr(VI) reacts with the oxygen-containing functional groups on the humic acids, such as phenols, changing the chemical structure of these groups.

Objective: Determine the changes in the humic acids chemistry that result from the exposure to chromate solution.

1.3 Thesis Structure

The overall structure of this thesis takes the form of seven chapters. The second chapter is a literature review concerning recent research in the field. The third chapter is concerned with the methodology used for this research study. The fourth chapter represents the findings of the research, focusing on the characteristics of the selected humic acids. Chapter five is concerned with the kinetics of the reaction between Cr(VI) and humic acids in the aquatic system as a function of pH. Chapter six focuses on the removal mechanism of Cr(VI) by the humic acids. The final chapter draws together the entire thesis, tying up the conclusions and includes a discussion of the implication of the findings to future research into this field.

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Chapter 2 Literature Review

2.1 Chromium: Background

Chromium is a vital transition metal. It was discovered in 1770 in the red lead mineral (crocoite, $PbCrO_4$) by Pallas a Russian geologist (Weeks, 1945). In 1797, the French chemist Vauquelin extracted chromium oxide from crocoite; then and after only one year, he succeeded in separating chromium metal from its oxide by a reduction process (Vauquelin, 1798, Weeks, 1945). Vauquelin named the discovered metal chroma, which is a Greek word that means colour (Liddell et al., 1968) because chromium compounds have distinctive colours.

Chromium is the 21st most abundant element in the Earth's crust with an average concentration of 100 µg/g (Nriagu, 1988). The naturally-occurring chromium compounds in the secondary geochemical environments are produced by weathering, erosion, and transport (Morrison et al., 2009). The average concentrations of chromium in these systems, soils, groundwater, and surface water, are 200 mg/kg (Motzer, 2005), < 1.0 µg/l, and (0.5 – 2) µg/l respectively (WHO, 2003). Chromium is found in nine oxidation states from -2 to +6, but only Cr(III) and Cr(VI) are environmentally stable. It is rarely found as an element or metal in the Earth's crust, but almost it is found in compounds or in the form of ions (Testa, 2004). Cr(IV) and Cr(V) can be found as unstable intermediates during the reactions of Cr(III) and Cr(VI) (Kotaś and Stasicka, 2000). In addition, there are 26 chromium isotopes, only four of them are stable. These isotopes include ^{50}Cr, ^{52}Cr, ^{53}Cr, and ^{54}Cr; their natural abundance is 4.345%, 83.789%, 9.501% and 2.365%, respectively (Winter, 2018). Chromium is found in 82 minerals, but spinel minerals $(Mg, Fe)(Al, Cr, Fe)_2O_4$ are the main commercial source of chromium (Darrie, 2001). In spinel minerals, the percentage of chromite ($FeCr_2O_4$) ranges from 15% to 65% (Darrie, 2001). Indeed, chromium has been mined commercially only in the form of chromite ore (Papp, 1994).

Chromium can be considered as a strategic metal, which has a high industrial value in the world owing to its various and widespread uses in industry, such as the alloy industry, chromium plating, leather tanning, wood preservation, wax, chromate pigments, refractories, ceramics, catalysts and organic acids

(Barnhart, 1997, USGS, 2013, International Chromium Development Association, 2016). However, the majority of chromite ore has been exploited by the metallurgical industry (USGS, 2012).

As a result of the many and varied uses of chromium, the world mine production is growing from year to year, for instance, it raised from 26,400 in 2014 to 27,000 thousand metric tons in 2015 (USGS, 2016). Most of the chromite ore has been produced from South Africa followed by Kazakhstan and Turkey. In 2016, these countries, taken together, produced about 76% of the total world mine production of chromite (USGS, 2017).

The manufacturing and various uses of chromium in industry have led, on occasion, to a severe anthropogenic contamination in water and soils (Burke et al., 1991, Puls et al., 1999, Whittleston et al., 2011a, Ding et al., 2016, Izbicki and Groover, 2016, Matern et al., 2016). Wastes emitted from these industries have drastically raised the concentration of chromium in both soils and water. For example, the total chromium concentration in groundwater is typically less than 10 µg/L, whereas it is reported that in some chromium contaminated areas Cr(VI) concentrations are between 300 – 500 µg/L (Hawley et al., 2004). In comparison, the United States Environmental Protection Agency has determined the maximum concentration level goal (MCLG) for total chromium in drinking water to be 100 µg/L (USEPA, 2018), at that time the maximum permissible limit of Cr(VI) is only 50 µg/L (WHO, 2003). As a result, a wide interest in chromium contamination has emerged in the scientific community and public sectors because chronic exposure to chromium has an adverse effect on human health and environment.

2.2 Health and Environmental Effects of Chromium

The two stable oxidation states of chromium, Cr(III) and Cr(VI) have completely different properties and reactivity. Cr(III) is immobile from slightly acidic to alkaline conditions (Palmer and Puls, 1994). It is considered as an important and essential trace element for living organisms, which has an influence on the biological function of the humans and animals (Lukaski, 1999). A number of studies have found that Cr(III) enhances the insulin action, which regulates the metabolism of glucose and lipids (Lukaski, 1999, Kotaś and Stasicka, 2000). It is recommended that the daily intake of Cr for adults should range between 50 to 200 µg (Williamson and Wyandt, 2000). The main sources of Cr(III) for human are food, such as fish, vegetables and fruits (WHO, 2003).

On the other hand, Cr(VI) has been considered one of the top 20 hazardous substances on the substance priority list (ATSDR, 2017). Researchers have thoroughly studied the effect of Cr(VI) on the human body and other biological systems. They found that acute exposure to Cr(VI) by several pathways can cause different physiological problems. Inhalation of Cr-bearing suspended solids is the most serious exposure pathway. It has been known that acute exposure to Cr-bearing dust causes human lung cancer (Leonard and Lauwerys, 1980, Kondo et al., 2003). One of the oldest studies about the lung cancer associated with a chronic occupational exposure to Cr(VI) compounds was in the united states in 1947 (Holmes et al., 2008). The mortality data of the chromate company showed that 21.8% of the workers' deaths were due to lung cancer compared to 1.4% in their reference population (Holmes et al., 2008). A more recent study carried out on animals as a model has been suggested that the oral exposure to Cr(VI) by drinking water contaminated with Cr(VI) can be considered a potential source of cancer (Costa and Klein, 2006). Although the majority of studies have been confirmed the carcinogenic effect of Cr(VI), the interaction mechanism between Cr(VI) and the human cells are still uncertain. It has been suggested that the toxicity of Cr(VI) arises from several reasons. First, it has an ability to diffuse readily across cell through the sulphate channels (Alexander and Aaseth, 1995) and acts as an oxidizing agent (Kotaś and Stasicka, 2000). Second, Inside the cells, free radicals are formed during the reduction of Cr(VI) to Cr(III) and have a toxicological effect. Finally, the produced Cr(III) has an ability to coordinate with organic compounds and consequently inhibit some metallo-enzyme systems (Kotaś and Stasicka, 2000). A few recent studies have proposed mechanisms for Cr(VI)-related cancer based on three paradigms; multistage carcinogenesis, epigenetic modifications and genomic instability (Cheng et al., 1999, Qian et al., 2001, Holmes et al., 2008). In addition to the carcinogenic effect of Cr(VI), it has been found that the inhalation of Cr-bearing suspended solids causes many other diseases such as asthma, perforation in the nasal septum, bronchitis, liver, and larynx inflammation (Burke et al., 1991, Kotaś and

Stasicka, 2000, Guertin, 2005). Moreover, skin contact with Cr(VI) compounds stimulates dermal edema, erythema, dermal necrosis, dermal corrosion, eschar formation, ,diarrhea, and hypoactivity (Gad et al., 1986).

The effects of chromium compounds on the plants also depend on its oxidation states. CrO_4^{2-} and $HCrO_4^-$ ions are the most mobile species of chromium in soils that can be easily taken by plants and leached into deep layers of soils (Dhal et al., 2013). According to Cervantes et al. (2001) and Fendorf (1995), Cr(VI) can be extremely damaging to living cells and low concentrations of Cr(VI) as 0.5 ppm in solutions or 5 ppm in soils can poison plants.

Studies show little agreement on what is the effect of Cr(III) on plants; however, Fendorf (1995) argues that Cr(III) is safe to plant. Whereas Cervantes et al. (2001) claims that Cr(III) has a relatively low toxicity to plants due to its very low solubility, which impedes its transfer to plants. In contrast, Panda and Choudhury (2005) argue that Cr(III) is an extremely toxic to plants even at low concentration. It damages the plant cells and influences on the plant growth, pigment content, water balance and finally the plant is damaged. Recent research has shown that the plant roots transform Cr(III) to Cr(VI) which readily penetrates to other parts of the plant (Chattopadhyay et al., 2011).

2.3 Sources of Chromium in Contaminated Soils and Natural Water

Soils and natural water have been considered the principal sinks of chromium that has been originated from natural and/or anthropogenic resources. Indeed, the high concentration of chromium in these systems mainly emerges from its manufacturing and use in industry under occasionally improper wastes disposal in poorly managed sites (Burke et al., 1991, Puls et al., 1999, Geelhoed et al., 2002, Whittleston et al., 2011b, Ding et al., 2016, Izbicki and Groover, 2016, Matern et al., 2016).

Chromium manufacturing process has been considered the major anthropogenic source of chromium that has been led to a severe contamination in soils and natural water over several decades around the world. The manufacturing process that uses chromite ore a commercially raw material to produce chromium chemicals. It is used directly to produce sodium dichromate that is an intermediate chemical used to manufacture different kinds of chromium chemicals (Kogel, 2006). Historically, the manufacturing process called “high lime”. It involves mixing the ore with lime (CaO) and soda ash (Na_2CO_3) followed by roasting to a temperature of (1100 – 1500 ºC) to convert the insoluble Cr(III) compounds into sodium chromate (Na_2CrO_4), which is highly soluble salt (Burke et al., 1991, Sreeram and Ramasami, 2001). The lime is added as a mechanical filler allowing oxygen to oxidize Cr(III) (Breeze, 1973). Then the mixture is leached out with water to extract the soluble Na_2CrO_4 (Palmer and Wittbrodt, 1991). Sodium chromate is reacted with sulphuric acid to produce sodium dichromate (Breeze, 1973). The remaining mud may be leached again to get additional Cr(VI) (Palmer and Wittbrodt, 1991). At the end of the manufacturing process, a waste residue is produced. This residue is known as chromite ore processing residue (COPR) which was excessively used as a filling material and in the construction works (Burke et al., 1991). As well, large amounts of COPR was dumped in landfill (Gancy and Wamser, 1976). The uncontrolled COPR disposal has caused with time a complicated environmental problem which impedes many remediation techniques. It contains a high concentration of Cr(III) and Cr(VI), which is ranging from (4% - 6%) (w/w) total chromium also it has a high pH value (11.5 - 12) (Geelhoed et al., 2002). As well as, the continuous leaching of its content of chromate salts may continue for decades due to the fact that COPR contains chromium salts that are very slowly soluble (Burke et al., 1991). It is found that the pore water in contact with COPR has a pH of (11-12) (Geelhoed et al., 2002). Moreover, the concentration of Cr(VI) in groundwater at a site where the COPR was dumped reached up to 1.6 mM (Geelhoed et al., 2002).

In addition to the COPR problem, soils and water have been also contaminated with chromium by several pathways, such as seepage from industrial lagoons, leaks from industrial metal processing or facilities, and infiltration of leachate from landfills (Testa, 2004). High concentrations of chromium have been reported in soils and groundwater in both the developed and developing countries (Okrent and Xing, 1993, Srinath et al., 2002) by chromium containing industries such as tanning industries, chrome-plating, and wood preservation processes. For example, in India, tannery effluents are classified as the major aquatic pollutant among all industrial wastes (Agrawal et al., 2006, Rangasamy et al., 2015). Tanneries release about 2000-3000 ton/year of chromium into the environment, with chromium aqueous concentration ranging between 2000 and 5000 mg/l (Rangasamy et al., 2015) causing a heavily polluted groundwater near by these industries (Brindha and Elango, 2012). Moreover, in the USA about 1300 sites may have groundwater contaminated with chromium (Fruchter, 2002).

In spite the fact that the manufacturing and uses of chromium represent the principal sources of contamination, occasionally natural processes may lead to elevated chromium concentrations in soils and groundwater. It has been reported that chromium is mainly in the Cr(III) oxidation state in the natural systems (Steinpress et al., 2005). Its concentrations may differ widely based on the nature of its parent materials (Naidu and Kookana, 1998). Some parent materials such as granitic, standstone, and limestone rocks have relatively low concentrations of chromium with an average from 20 to 35 mg/kg (Bowen, 1979). On the other hand, ultramafic rocks have a high concentration of chromium that may reach up to 1800 mg/kg (Bowen, 1979). Therefore, soils originate from ultramafic rocks can have high Cr contents (Stueber and Goles, 1967, Schwertmann and Latham, 1986, Becquer et al., 2003). Furthermore, geochemical processes such as weathering may enhance the potential oxidation of Cr(III) resulting from the parent materials to Cr(VI), particularly by manganese (IV) oxides (Bartlett and James, 1979, Eary and Rai, 1987, Fendorf and Zasoski, 1992). These natural processes are thought to be responsible for chromium concentrations in groundwater above the World Health Organisation maximum for drinking water (50 µg/l; (WHO, 2003)) at a number of locations around the world, particularly in the south-west United States (Robertson, 1991, Ball and Izbicki, 2004, Steinpress, 2005) and Italy (Fantoni et al., 2002).

2.4 Aqueous Geochemistry of Chromium

Chromium can be found in the natural systems only as Cr(III) and Cr(VI). The chromium oxidation state is not only associated with its toxicity but also it is strongly responsible for its aqueous concentration and mobility in the various geologic environments (Rai et al., 1989). In the natural environment, the two stable oxidation state Cr(VI) and Cr(III) have widely different properties. Cr(VI) species are highly soluble, bioavailable and mobile in most soils and water systems (Fendorf et al., 1992, Kotaś and Stasicka, 2000). While Cr(III) species are immobile due to their limited solubility, complexation with insoluble organic matter, and adsorption by negatively charge surfaces that have been common in soils and sediments. Understanding the geochemistry of chromium including its speciation, reactivity, and the natural processes that control its bioavalibility are essential factors to evaluate and predict the potential risk of chromium species. However, this risk sometimes is not related only to the presence of Cr(VI) species but also to the potential re-oxidation of Cr(III) to Cr(VI) and to what extent these species can be retained on soil or sediment minerals (Fendorf, 1995).

In fact, the occurrence of chromium species in the geologic environments depends mainly on the oxidation-reduction conditions, which is expressed as (Eh), and on the pH values (Figure 2.1) (Palmer and Wittbrodt, 1991). Under oxidizing conditions, Cr(VI) is dominant. While under reducing conditions, Cr(III) is the prevalent form. In the natural environment Cr(VI) presents in various forms which depend primarily on the pH values and on the Cr(VI) concentration in that environment. It may exist as H_2CrO_4 (chromic acid), $HCrO_4^-$ (hydrogen chromate), CrO_4^{2-} (chromate) or $Cr_2O_7^{2-}$ (dichromate). H_2CrO_4 is formed only under highly acidic condition (pH 1) (Palmer and PuIs, 1994). $HCrO_4^-$ is prevalent when the pH value is less than 6.5 and also the Cr(VI) concentration is less than 30 mM (Palmer and Puls, 1994). Moreover, above pH 6.5 CrO_4^{2-} becomes the predominant form (Palmer and Wittbrodt, 1991). As well, $Cr_2O_7^{2-}$ is dominant when the concentration of Cr(VI) is more than 30 mM and the pH is between 4 and 6 (Palmer and PuIs, 1994, Szecsody et al., 2005). Indeed, according to the most common conditions in soils and groundwater, $HCrO_4^-$ and CrO_4^{2-} are the most expected forms of Cr(VI) under oxidation conditions.

In reducing aqueous systems chromium is found in the 3+ oxidation state as the aqueous cation Cr^{3+} when the pH is below 3.5 (Palmer and PuIs, 1994). Moreover, as the pH increases Cr(III) composes different forms of hydroxo complexes (Rai et al., 1989). It is found that $CrOH^{2+}$ is the predominant hydroxo complex at pH (3.8 – 6.3) (Rai et al., 1989). Whereas at pH values between 6.3 and 11.5 $Cr(OH)_3$ is formed, which is highly insoluble (Rai et al., 1989) and at highly alkaline environment (pH > 11.5) $Cr(OH)_4^-$ is the dominant form (Rai et al., 1989b). It has been reported that polymers of Cr(III) can occur in the environment (Stuenzi and Marty, 1983). However, the experimental results of Rai et al. (1987) show that no evidence for the presence of Cr(III) polymers even at high temperature, where the polynuclear complexes are expected to form rapidly.

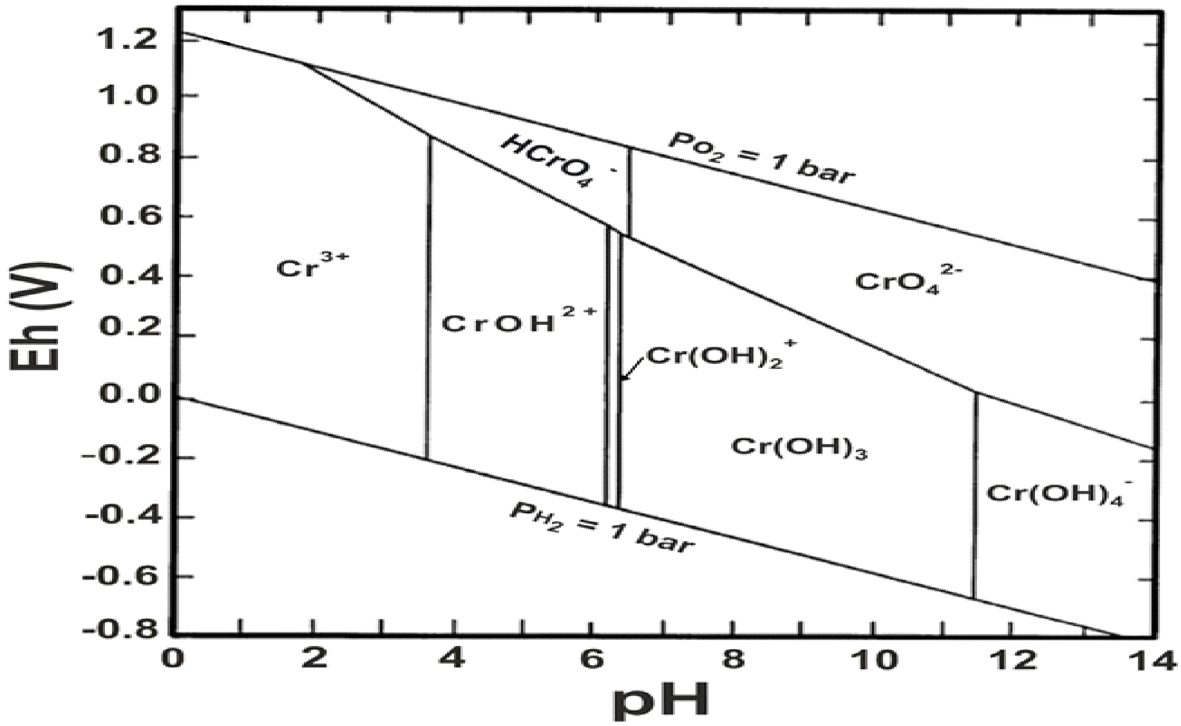

Figure 2. 1: Eh-pH diagram illustrates the chromium speciation in an aqueous system (Figure taken from Palmer and Wittbrodt, 1991)**.**

2.4.1 Reactions of Chromium in Soils and Groundwater

In geological environments the aqueous concentration of Cr(VI) is controlled by adsorption/desorption, precipitation/dissolution, and redox reactions, while the aqueous concentration of Cr(III) is controlled mainly by precipitation/dissolution processes, redox reactions, hydrolysis and complexation (Rai et al., 1989, Richard and Bourg, 1991) (see Figure 2.2). Understanding the chemistry of chromium requires a deep knowledge of the chemistry and mineralogy in these environments in order to be able to determine the potential geochemical reactions that enhance the storing or releasing of Cr(VI) in these natural systems.

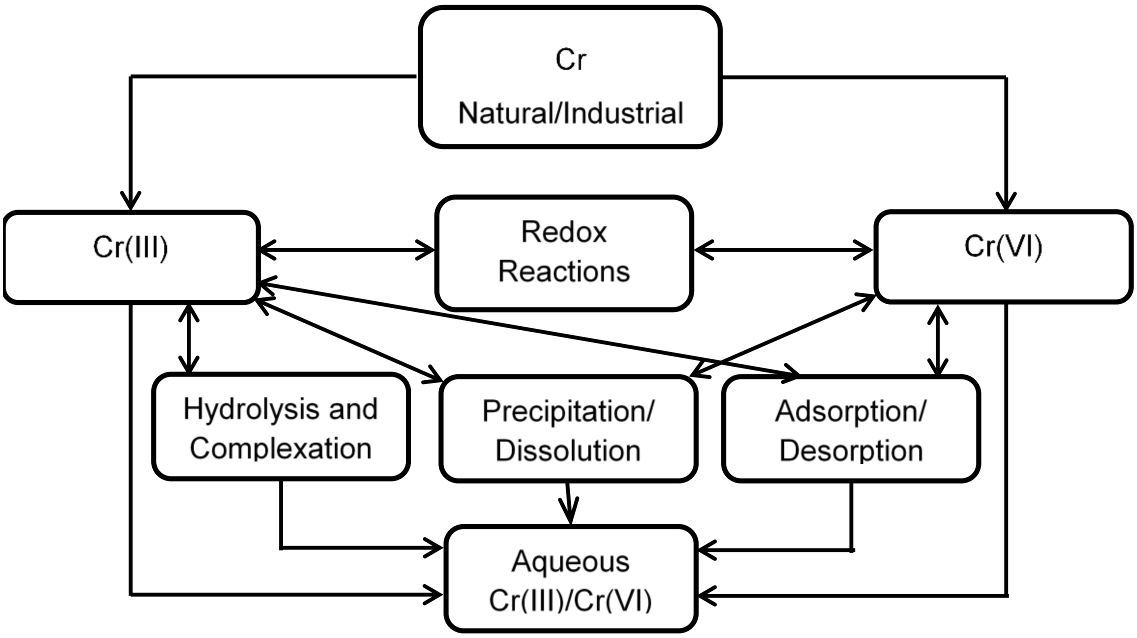

Figure 2. 2: Schematic diagram of chromium in the geological environments, (after Rai et al., 1989, Richard and Bourg, 1991).

2.4.1.1 Reactions of Cr(III) in soils and groundwater

The knowledge of Cr(III) behavior in the natural systems is an important issue originating from the potential hazard of re-oxidation under specific environmental conditions. Indeed, Cr(III) has been considered the stable form of chromium in many natural systems and that depends fundamentally on several physical and chemical processes. In the absence of complexing agents, except H_2O or OH^-, Cr(III) can form $Cr(OH)_3$ as an insoluble product that significantly limits its mobility and bioavailability in soils and water systems over a wide range of pH values 6.5 to 11.5 (Rai et al., 1987). It can be also removed from the aqueous systems that contain Fe(III) by forming $(Cr_x, Fe_{1-x})(OH)_3$ under slightly acidic to alkaline conditions (Sass and Rai, 1987, Eary and Rai, 1989, Rai et al., 1989, Palmer and Wittbrodt, 1991).

The other soluble products of Cr(III) can be retained appreciably in minerals or soil organic matter by adsorption and/or complexation processes. Cr(III) species can be sorbed by negatively charged surfaces that are common in soil and sediments, such as aluminosilicate clay minerals (Fendorf and Zasoski, 1992, Fendorf et al., 1994). It has been reported that Cr(III) is adsorbed rapidly on the clay minerals and iron oxides surfaces (Richard and Bourg, 1991). As other cationic metals, the adsorption of Cr(III) on these surfaces increases with pH (Rai et al., 1984). In the presence of organic matter, Cr(III) can form complexes under neutral to alkaline conditions (Palmer and Puls, 1994). The formed complexes between Cr(III) and organic ligands are more stabilized than their complexes with H_2O and OH^- (Kotaś and Stasicka, 2000).

In spite the fact that Cr(III) species are immobile in most soils and water systems and are not toxic, the potential for re-oxidation into Cr(VI) can be considered another environmental threat. So far it has only been shown that Cr(III) can be oxidized in soils, water, and waste materials by dissolved oxygen and manganese dioxides (Schroeder and Lee, 1975, Eary and Rai, 1987, Fendorf and Zasoski, 1992). The oxidation of Cr(III) by the dissolved oxygen has been reported by several researchers. Laboratory studies reveal that the reaction proceeds with a slow rate under conditions simulating those in natural water (Schroeder and Lee, 1975). It has been found that less than 2% of Cr(III) was oxidized to Cr(VI) in 2 weeks at a pH range 5.5 to 9.9 (Schroeder and Lee, 1975). However, these results differ by the experiments of Eary and Rai (1987) who did not detect any aqueous Cr(VI) of a 19 µM Cr(III) solution in a 24 days period at a pH 4, 12.0, and 12.5. Despite the disagreement between the above studies, the potential oxidation of aqueous Cr(III) species by dissolved oxygen does not usually pose a significant environmental risk.

Moreover, in order to determine whether dissolved oxygen might catalyse the oxidation of Cr(III) by other oxidants such as manganese oxides, the rate of Cr(III) oxidation by β-MnO_2 was studied at pH 4 under aerated and de-aerated conditions (Eary and Rai (1987). Their results suggest that the oxidation rates in aerated samples were slightly higher than that in the de-aerated samples. The slightly higher rate does not have a significant effect and it has been expected that the oxidation of Cr(III) occurs by direct reaction with β-MnO_2 surface (Eary and Rai, 1987).

On the other hand, manganese oxides have been proved to be the principal oxidants for Cr(III) under environmental surface conditions (Schroeder and Lee, 1975, Bartlett and James, 1979, Richard and Bourg, 1991). The proposed overall reaction has been described by the following equation (Fendorf and Zasoski, 1992):

$$Cr^{3+} + 1.5\ \delta\text{-}MnO_{2(s)} + H_2O \leftrightarrow HCrO_4^- + 1.5\ Mn^{2+} + H^+ \qquad 2.1$$

The overall oxidation process has been suggested to occur as a result of three-step process (Bartlett and James, 1979, Eary and Rai, 1987). First, Cr(III) is adsorbed on manganese oxides surface. Second, Cr(III) is oxidized to Cr(VI) on MnO_2 surface. Finally, the reaction products, Cr(VI) and Mn(II), are desorbed.

Because manganese oxides are the most effective oxidants of Cr(III) in soil, sediment and natural water, several studies have focused on the effective factors on the oxidation process. It has been found that numerous solution and surface factors either enhance or retard the oxidation process by manganese oxides, which involve: Cr(III) and manganese oxides contents, pH values, ionic strength, reaction products, manganese oxides characteristics, and organic matter content (Eary and Rai, 1987, Johnson and Xyla, 1991, Fendorf and Zasoski, 1992, Fendorf, 1995, Feng et al., 2006, Reijonen and Hartikainen, 2016).

The effect of pH on the oxidation of Cr(III) by different types of manganese oxides has been examined. Experimental data have shown that the oxidation of Cr(III) by δ-MnO_2 is inhibited at pH values greater than 3.5 or 4 (Fendorf and Zasoski, 1992, Fendorf et al., 1992). It has been proposed that the inhibition is due to the formation of chromium hydroxide precipitate on manganese oxide surface (Charlet and Manceau, 1992, Fendorf and Zasoski, 1992, Fendorf et al., 1992). This precipitate works both as a barrier to electron transfer between aqueous Cr(III) species and the oxidized forms of Manganese (Mn(IV) / Mn(III)) and as a redox stable sink for Cr(III) (Fendorf et al., 1992). A recent study by Feng et al. (2006) has been conducted using different types of manganese oxides. Their results show that Cr(III) can be strongly oxidized by manganese oxides only at low pH values, where Cr(III) species are aqueous. When the pH increases from 3.0 to 3.5, the amount of oxidized Cr(III) by manganese oxides decreases considerably. Above pH 5.0-5.5, $Cr(OH)_3$ precipitates and inhibits the oxidation process.

Based on these studies, it is deduced that under common pH values of most soils, which typically range between a slightly acidic to a moderately alkaline, the oxidation of Cr(III) species is very limited due to the formation of $Cr(OH)_3$ precipitate on manganese oxides surfaces.

The oxidation kinetics of Cr(III) on the surface of manganite have been also investigated as a function of ionic strength (Johnson and Xyla, 1991). It has been expected that the ionic strength affects the activity coefficients in solutions, as well as, on the surface charge of solids. However, the experimental results showed that the oxidation reaction is independent of ionic strength (Johnson and Xyla, 1991).

The effect of the initial concentration of Cr(III) on the oxidation process has been investigated. Johnson and Xyla (1991) examined the oxidation of Cr(III) by manganite as a function of initial Cr(III) concentrations. Their experimental results show that the reaction is first order with respect to manganite adsorption density and Cr(III) concentration as long as the adsorbed Cr(III) on the surface is below a critical adsorption density (0.2 $\mu mol/m^2$). Above this value, a high rate of reaction was not observed. Probably, when the concentration of Cr(III) is high, a limited amount of Cr(III) would be oxidized due to the formation of $Cr(OH)_3$ precipitate, which alters the surface properties of manganese oxides and consequently the surface is inhibited. (Fendorf and Zasoski, 1992).

The amount of manganese oxides and their surface characteristics are important factors that have been examined. The experimental investigations revealed that the rates of aqueous Cr(III) oxidation are directly proportional to the amount of manganese oxides and their specific areas (Schroeder and Lee, 1975, Eary and Rai, 1987). Moreover, the various forms of manganese oxides may have a different capacity for oxidizing Cr(III). For example, the oxidation rate of Cr(III) on the surface of manganite, γ-MnOOH, is (10-10,000) times faster than other forms of manganese oxides (Johnson and Xyla, 1991). It has been believed that the point of zero charge and the surface energy of Mn-oxides are important reasons for the variation in their oxidation capability. Manganese oxides with lower zero points of charge and higher surface energy such as $\delta\text{-}MnO_2(s)$ (pzc = 2.3) may oxidise Cr(III) more rapidly than those having higher zero points of charge and lower surface energy such as $\beta\text{-}MnO_{2(s)}$ (pzc = 7.3) (Eary and Rai, 1987).

A few studies have been investigated the influence of the reaction products on the oxidation processes. It has been postulated that Mn(II) may compete with Cr(III) for adsorption sites and consequently limits the oxidation of Cr(III) (Amacher and Baker, 1982, Eary and Rai, 1987). However, microscopic and spectroscopic data revealed that the oxidation process is inhibited due to the formation of $Cr(OH)_3$ precipitate and the oxidation products do not limit the oxidation process (Charlet and Manceau, 1992, Fendorf et al., 1992).

In addition to the previous factors, organic matter content in the natural systems may have a significant impact on the oxidation of Cr(III) by Mn-oxides. The oxidation of Cr(III) by Mn-oxides could be substantially inhibited due to the presence of organic matter that binds strongly with Cr(III) by complexation (Douglas et al., 1986, Johnson and Xyla, 1991). This finding clearly reveals that natural systems rich with organic matter content affect the geochemical distribution of Cr and it is expected that lower risk of Cr(III) oxidation comparing to other systems that contain lower organic content.

The most important conclusion can be drawn from all above studies is that the hazard arising from Cr remobilization may vary considerably from one field site to another. In general, field characterisation is essential to understand the contribution of all possible and influenced factors, so that each site should be evaluated individually before choosing a suitable remediation method. The presence of Mn-oxides does not necessarily mean a high environmental risk due to the influence of other natural processes that may significantly inhibit the oxidation of Cr(III) or control the oxidized chromium.

2.4.1.2 Reactions of Cr(VI) in soils and groundwater

The sorption behavior of Cr(VI) species will determine its mobility in soils and groundwater systems. In such systems it exhibits typical anionic sorption behavior, where its adsorption decreases with pH (Richard and Bourg, 1991, Hawley et al., 2005), and it is not significantly retained by the negatively charged soil colloids (Fendorf, 1995). However, Cr(VI) can be sorbed by several inorganic constituents in soils such as Fe and Al oxides and hydrous oxides, clay minerals and colloids under acidic to slightly alkaline conditions (James and Bartlett, 1983, Zachara et al., 1987, Rai et al., 1989, Fendorf, 1995). It has been reported that the hydrous oxides of iron and aluminium have often a net positive charge and consequently a chemical affinity for Cr(VI) species (Fendorf, 1995). A direct atomic-level evidence using EXAFS analysis shows that Cr(VI) forms an inner-sphere complex on goethite (FeO(OH)), with both monodentate and bidentate complexes (Fendorf, 1995). Similarly, it would be expected that Cr(VI) forms an inner-sphere complex on the surface of aluminium hydrous oxides (Fendorf, 1995). The formation of inner-sphere complexes between Cr(VI) and these surfaces highlights the importance of iron and aluminum hydrous oxides as adsorbents that would be significantly diminished the desorption of Cr(VI). Based on these results, it has been expected that soils with high content of Fe and Al hydrous oxides may decrease the potential risk of Cr(VI) mobility. However, some soils and sediments have a low content of Fe and Al hydrous oxides and consequently aqueous Cr(VI) will be a serious environmental problem. Moreover, in systems where the concentration of competing anions is high, Cr(VI) adsorption becomes less important (Richard and Bourg, 1991, Fendorf, 1995, Hawley et al., 2005).

In some environments, Cr(VI) can be immobilised by a precipitation process. Cr(VI) can be controlled in the soils by barium chromate ($BaCrO_4$) (Kim and Dixon, 2002). It has been proposed that a solid solution of $Ba(S,Cr)O_4$ may form in environments that contain $BaSO_4$ and $BaCrO_4$ (Rai et al., 1988, Rai et al., 1989). However, few soils contain significant quantities of barium sulphate minerals.

Reduction reactions are another essential process that control the mobility and bioavailability of Cr(VI). In biological reduction (biotic reduction), microorganisms such as bacteria (e.g. *Streptomyces sp., Micrococcus sp., Pseudomonas sp., Pseudomonas fluorescens, Bacillus sp., and Ochrobactrum sp.*), and fungi (e.g. *Aspergillus flavus, Aspergillus sp. and Aspergillus niger*) can reduce Cr(VI) to Cr(III) by aerobic and anaerobic pathways (Wang and Xiao, 1995, Park et al., 2005, Elangovan et al., 2006, Bennett et al., 2013, Thatoi et al., 2014). As microbial reduction of Cr(VI) can occur under both aerobic and anaerobic conditions, it can be important under aerobic conditions (Fendorf et al., 2001) where the rate of abiotic reduction of Cr(VI) becomes much slower (Singh et al., 2005, Chrysochoou and Ting, 2011). Numerous studies have been attempted to describe the reduction mechanisms of Cr(VI) by microorganisms. It has been found that the mechanisms by which these microorganisms reduce Cr(VI) are variable and depend on the species of microorganisms (Joutey et al., 2015). For example, Alkaliphilic Bacteria (*Tissierella, Clostridium, and Alkaliphilusspp*) can reduce Cr(VI) by extracellular electron transport-mediated Fe(III) reduction process (Fuller et al., 2014). The electron shuttles (flavins) transfer the electrons from the bacteria cells into Fe(III), which in turn is reduced into Fe(II). Then Cr(VI) is chemically reduced into Cr(III) by Fe(II) (Fuller et al., 2014). Enzymatic extracellular reduction has been also reported with *Escherichia coli* ATCC 33456, where Cr(VI) is reduced in the external medium by reductase that mediates the electron transport to Cr(VI) (Shen and Wang, 1993). Intracellular Cr(VI) reduction has been also proposed, which involves the diffusion of chromate through a facilitated transport system followed by intercellular reduction of Cr(VI) to Cr(III) (Arslan et al., 1987). Although the majority of chromium inside the cells is Cr(III), traces of Cr(VI) is determined by spectrophotometric analysis (Arslan et al., 1987). However, it has been found that intracellular reduction of Cr(VI) to Cr(III) by *Bacillus subtilis* bacteria promotes mutagenesis and cells death by the oxidative stress-induced DNA damage (Santos-Escobar et al., 2014).

In chemical reduction, Fe(II), reduced sulphur, and organic matter can reduce Cr(VI) in soils and water (Schroeder and Lee, 1975, Eary and Rai, 1987, Rai et al., 1989, Eary and Rai, 1991, Palmer and Wittbrodt, 1991). Ferrous iron in both solutions or in mineral phases is able to reduce Cr(VI) to Cr(III) (Palmer and Wittbrodt, 1991). The reaction products Cr(III) and Fe(III) hydrolyse, bind and then precipitate as $(Cr_x Fe_{1-x})(OH)_3$ (Sass and Rai, 1987, Eary and Rai, 1989, Rai et al., 1989, Palmer and Wittbrodt, 1991). The precipitate stabilizes the reduced chromium and keeps it in an immobilized state as well as diminishes the possibility of re-oxidation to Cr(VI) over the pH range of about 4-12 (Eary and Rai, 1988, Eary and Rai, 1989, Fendorf et al., 1992, Fendorf, 1995). The reduced precipitate was described by the following reaction (Fendorf, 1995):

$$xCr(III) + (1-x)Fe(III) + 3H_2O \longrightarrow (Cr_xFe_{1-x})(OH)_{3(s)} + 3H^+ \qquad (2.2)$$

Where x varies from 0 to 1.

Moreover, the reduction rate of Cr(VI) by ferrous iron has been well demonstrated. Results obtained from literature have shown that aqueous Fe(II) can reduce Cr(VI) in much more rapid rates than ferrous iron-containing minerals such as hematite and biotite (Eary and Rai, 1988, Eary and Rai, 1989). It has been reported that the reduction of Cr(VI) by ferrous iron-containing minerals depends on the dissolution rates of ferrous iron of these minerals (Eary and Rai, 1989). Besides the effect of ferrous iron phase on the reduction of Cr(VI), pH and dissolved oxygen have been also investigated. According to the experimental data of Eary and Rai (1988), it has been found that aqueous Fe(II) is an efficient reductant for Cr(VI) at pH < 10 even under aerated condition. This reaction was very rapid and completed within (1-2) minutes

(Eary and Rai, 1988). The stoichiometric relation between the oxidised Fe(II) and the reduced Cr(VI) was 3:1 under a pH range (2-10), while under hyper alkaline conditions, the molar ratios became significantly higher (Eary and Rai, 1988). This finding is supported by Fendorf and Li (1996) study, which reveals that dissolved oxygen would limit the reduction of Cr(VI) at pH values > 8 even at macromolar concentrations. The research study by J. Chen et al. (2015) also found that at pH 3.0 – 9.0 the reduction of Cr(VI) by Fe(II) was close to 100%, while at pH higher than 12.0 the reduction decreased to about 60%. The decline in Cr(VI) reduction at high pH values could be attributed to the oxidation of Fe(II) by dissolved oxygen being larger than that by Cr(VI) (J. J. Chen et al., 2015, Eary and Rai, 1988). All of these findings imply that under natural system conditions, Fe(II) bearing minerals are significantly retard the transformation of Cr(VI) to subsurface environment. However, most of the time, within soils and sediments the vadose zone does not contain sufficient amount of Fe(II) to reduce Cr(VI) (Jardine et al., 1999).

Reduced sulphur such as S, S^{2-}, $S_2O_3^{2-}$, H_2S, iron sulphide and dithionite is also another important reductant that reduces Cr(VI) to Cr(III) (Hawley et al., 2004). The reduction of Cr(VI) by hydrogen sulphide and iron sulphide was investigated and it was found that the effectiveness of these materials decreases as the pH increases (Kim et al., 2001). Similarly, the results obtained by J. Chen et al. (2015) suggest that the reduction of Cr(VI) by sulphides is largely pH dependent and at pH > 7 the removal of Cr(VI) is less than 10%. Furthermore, iron sulphide was tested and it was found that it can reduce Cr(VI) but the rate of reaction is slow so that this material is not efficient to treat a large volume of water (Powell et al., 1998). The dominant aqueous sulphide species is H_2S below pH 7 and HS^{-1} above, whereas the dominant aqueous Cr(VI) species is $HCrO_4^-$ and CrO_4^{2-} below and above pH 6.45 (Pourbaix, 1966), and thus electrostatic repulsion may limit Cr(VI) reduction by sulphides at alkaline pH.

In addition to the inorganic soil constituents, soil organic matter exerts a major role in the retention of organic and inorganic pollutants. In recent decades, there has been a growing body of literature that recognizes the importance of soil and other natural organic matter as a reducing agent for many contaminants including Cr(VI). Soil organic matter is particularly important in systems where the concentrations of aqueous ferrous iron or Fe(II) bearing solids are limited.

It has been reported that soil organic matter in soluble or insoluble phase is capable of reducing Cr(VI) to Cr(III) (Fendorf, 1995). Cr(III) reduced by organic matter may precipitate as $Cr(OH)_3$ or bind to the soil organic matter (Palmer and PuIs, 1994). The reduction of Cr(VI) by organic matter most probably involves a reaction with humic substances, which represent the main fraction of natural organic matter (Trevisan et al., 2010). Humic substances are able to interact with different materials including metal ions, organic compounds, oxides, hydroxides and minerals and form water-soluble and water-insoluble complexes (Trevisan et al., 2010). The diversity of functional groups found in humic materials provides a range of possible metal binding sites (Livens, 1991). In addition, humic acids (an important component of soil organic matter) have a high content of acidic functional groups so that it has a high buffer capacity in a wide pH range (Pertusatti and Prado, 2007). The buffer capacity and chemical reduction, give a special importance of the soil organic matter in highly alkaline contaminated sites such as chromium ore processing residue contaminated sites, where the high alkalinity forms the greatest challenge that limits the using of other natural reactive materials such as Fe(II) and reduced sulphur.

2.5 Humic Substances

2.5.1 Formation of Humic Substances

A brief review of the formation of humic substances is a key requirement to understand their properties and their behavior with metal ions. Indeed, Humic substances, which represent the majority of the natural soil organic matter (International Humic Substances Society, 2007), are the dark-coloured and heterogeneous organic matter produced by the decay and microbiological transformation of plant and animal remains by bacteria and fungi (Stevenson, 1994, Swift, 1999, Sutton and Sposito, 2005, Brookes et al., 2008). During the last decades, a considerable information has been published about the chemical and physical characteristics of humic substances, but the formation of humic substances is still the least understood topic of humus chemistry (Stevenson, 1982). Several mechanisms have been proposed to explain how humic substances are formed, which include modified lignin theory, sugar-amine condensation theory, polyphenol theory, and micelle model.

According to the modified lignin theory, the remaining fraction of lignin (that is not attacked by microorganisms) is modified and becomes a humus material (Waksman, 1932). It has been proposed that the modification process includes loss of methoxyl (OCH_3) groups, generation of o-hydroxyphenols, and oxidation of aliphatic side chains to produce COOH groups (Flaig et al., 1975, Flaig, 1988). Then humic acids are produced from the condensation of the modified lignin with the microbially produced protein (Senesi et al., 2009). Despite the evidence that has been cited by Waksman (1932) to support this theory, it has later faced criticism from other scientists. The major objection to this theory is that the role of lignin seems to be overestimated (Saiz-Jimenez and De Leeuw, 1987). The modified lignin may represent a major contributor in humus formation only in excessively wet environments where fungi that decompose lignin are not found (Stevenson, 1994). Moreover, in aerobic soils lignin is decomposed by microorganisms before the synthesis of humus (Stevenson, 1994).

Sugar-amine condensation theory is one of the oldest theories that describe the formation of humic substances. According to this theory, the produced sugars and amino acids from microbial metabolism are subjected to nonenzymatic condensation (Senesi et al., 2009). The reaction between reducing sugars (ketoses and aldoses) and amino compounds takes place and that leads to the formation of N-substituted glycosylmine (Mirsal, 2008). The dehydration and fragmentation of this product produce the brown nitrogenous polymers, which in turn are thought to form the bulk of humic acids (Mirsal, 2008). This theory has been criticized by Burdon (2001), because the concentrations of a free monosaccharide, proteins, peptides, and amino acids in soils are not sufficient to account for the amount of humic substances found in soils.

The polyphenol theory states that polyphenols are humic acid precursors that can be produced by two different pathways (Peña-Méndez et al., 2005). They originate from the biodegradation of lignin by microorganisms and are then oxidized by enzymes into quinones. The resulting quinones are polymerized into humic substances (Stevenson, 1994). Polyphenols are also synthesised by microorganisms from non-lignin substances like cellulose then it follows the same mechanism as explained for polyphenols from lignin origin (Stevenson, 1994).

However, more recent evidence does not support the traditional models (modified lignin, sugar-amine condensation, and polyphenol), and it is now thought that humic substances are better regarded as supramolecular associations (the “micelle model”) of relatively small and chemically diverse organic molecules (Wershaw, 1994, Kögel-Knabner, 2000, Sutton and Sposito, 2005). These ordered aggregates are held together by relatively weak hydrogen bonds (H-bonds) and hydrophobic interactions, rather than the stronger covalent bonds that would be associated with a polymer model of humic substances (Piccolo, 2001, Simpson et al., 2002). The main constituent of the supramolecular humic associations are amphiphilic molecules produced by enzymatic depolymerization of aromatic plant components (e.g. lignin and tannin) (Wershaw, 1999, Kögel-Knabner, 2000). These have both hydrophobic parts, composed of relatively unaltered segments of plant polymers, and hydrophilic parts, consisting primarily of carboxylic acid groups, and cluster together into micelle-like particles (Piccolo et al., 1996, von Wandruszka, 1998). Other biomolecules from plant degradation including lipids, lignin, nonlignin aromatic species, carbohydrates, and proteins, are intimately associated with either hydrophobic or hydrophilic domains within the clusters (Zang et al., 2000, Fan et al., 2004).

2.5.2 Fractionation of Humic Substances

Regardless of the detailed structure of the humic substances, what is not disputed is that humic substances are extremely heterogeneous on a micro-scale, but exhibit remarkable uniformity on a macro-scale (MacCarthy, 2001). The difficulties in characterising this complex material have led to its being subdivided it into three operationally defined fractions depending on solubility under acidic or alkaline conditions (Stevenson, 1994, Wershaw, 1994, Sutton and Sposito, 2005). Humin is defined as the insoluble fraction under all pH conditions, humic acids (HAs) are soluble under alkaline conditions but not acidic conditions, and fulvic acids (FAs) which are soluble under all pH conditions (Stevenson, 1994).

Among the above fractions, humin has the largest molecular weight, which ranges from around 100,000 to 10,000,000 Daltons (Pettit, 2004). In addition, The elemental analysis and spectroscopic data (diffuse reflectance infrared fourier transform spectroscopy, and solid-state spectroscopy) reveals that humin has the lowest aromatic content comparing to the other fractions in soil humic substances (Kang et al., 2003). It has also lower total acidity, carboxyl, and phenolic groups than the corresponding fulvic and humic acids (Pettit, 2004). As the ability of humic substances to form stable complexes with metal ions is based on their content of oxygen-containing functional groups (Eshwar et al., 2017), it has been expected that humin has the least affinity for metal ions. For example, experimental data has shown that the reduction capability of Cr(VI) by humin, that is derived from soil, is relatively lower than that by the corresponding humic acid (Zhang et al., 2018). According to this study, humin has lower phenols and hydroxyl contents than humic acids, where these functional groups are the main electron donors for Cr(VI) reduction.

Both fulvic acids and humic acids can be considered major fractions in humic substances (Tang et al., 2014). The percentages of fulvic and humic acids in various natural and waste-derived organic matter differ considerably from one source to another. This variation is probably due to the numerous conditions under which humic substances are formed. For example, The humic substances obtained from forest soils are characterized by their lower humic acid/fulvic acid ratio than peat and grass land soils (Kononova, 1966). Table (2.1) shows the humic acid/fulvic acid ratios (HA/FA) of terrestrial, aquatic and waste-derived organic matter (van Zomeren and Comans, 2007, Schellekens et al., 2017). From this table, it is observed that both the terrestrial and waste-derived organic matter have a higher proportion of humic acids than fulvic acids. Moreover, the humic acid/fulvic acid ratio increases in the order: soil < landfill waste < compost waste < peat.

Another interesting point is that the humic acid/fulvic acid ratio generally decreases with increasing soils depth. According to the data obtained by (Canellas and Façanha, 2004), it has been found that the humic acid/fulvic acid ratio decreased from 0.6 into 0.1 when the soil samples were obtained at depth intervals extended from (0-5 cm) into (20-40 cm) from the soil surface.

Comparison of the molecular composition of fulvic acids and their corresponding humic acids reveals similarities on a macroscale and significant differences on a microscale. It has been reported that no significant differences between the two fractions for reactive functional groups, which include carbohydrates, phenols, benzenes, and lignin phenols (Schellekens et al., 2017). However, it has been found that fulvic and humic acids differ in their average molecular weight, %aromaticity, elemental proportions, and acidic functional group's contents. The average molecular weight of fulvic acids ranges between 500 and 5000 Daltons, while the average molecular weight of humic acids is much higher and ranges between 50,000 and 100,000 Daltons (Stevenson, 1994). The aromatic content in fulvic acid is typically lower than that in humic acid from the same source (Pettit, 2004). The elemental proportions also differ significantly between fulvic and humic acids. In general, the carbon content of fulvic acids varies from 40.7 to 50.6 percent; oxygen content varies from 39.7 to 49.8 percent (Stevenson, 1994). Humic acids have higher carbon content that varies from 53.8 to 58.7 percent and lower oxygen content that varies from 32.8 to 38.3 percent (Stevenson, 1994). The total acidity of fulvic acids is considerably higher than that in humic acids. According to Hayes and Swift (1990) the total acidity of fulvic and humic acids is (6.4 – 14.2 mmole/g) and (5.6 – 8.9 mmole/g), respectively. The higher acidity of fulvic acids is an attributed to their higher carboxyl groups content comparing to that in humic acids (Hayes and Swift, 1990).

As the structure of both humic and fulvic acids contains a considerable amount of reactive functional groups, particularly –OH and –COOH groups, they can be considered an important complexing agents with metal ions in natural systems (Buffle et al., 1977). Experimental data has shown that the binding capacity of fulvic acids with metal ions (e.g., Pb, Cd, and Zn) is greater than that with the corresponding humic acids (Boruvka and Drábek, 2004). Although the greater ability of fulvic acids to interact with metal ions comparing to humic acids, humic acids have a greater importance in controlling the mobility of metals due to their lower solubility than fulvic acids in the pH range of most natural systems.

Table 2.1: Humic acid/fulvic acid ratios (HA/FA) of some natural and waste-derived organic matter (van Zomeren and Comans, 2007, Schellekens et al., 2017)**.**

Source	(HA/FA)
Soil (U.S.)	1.73
Peat ((Devoke, UK)	40.7
Dissolved organic matter-rich pond	0.23
Compost from municipal waste	25.7
Landfill waste	6.22

2.5.3 Properties and Functionality of Humic Acids

Humic acids can originate from many different materials, such as coal, peat, soil, and sewage sludge, so a significant variation in their material properties and functionality should be expected.

2.5.3.1 Elemental Composition

Humic acids consist primarily of the elements C, H, O, N, and S (in typical order of mole %). Humic acids also usually contain small amounts of amorphous silica and aluminosilicate impurities carried over from the extraction process, which results in an ash after ignition enriched in Si, Al, K, Fe and Ca. The principal element compositions are usually expressed as weight percentages on a moisture and ash free basis. The elemental composition is considered by some to be indicative of the source of humic acid and its degree of humification (Stevenson, 1994).

Table 2.2 shows the principal element composition of humic acids from different sources (Lu et al., 2000, Novak et al., 2001, Kimuro et al., 2017). Carbon and oxygen contents are typically in the range (50% - 60%) and (30% - 35%), respectively. Hydrogen, nitrogen, and sulphur are typically in the range (4% - 6%), (2% - 4%) and (0.0% - 2%), respectively (Schnitzer and Khan, 1972).

In addition to the elemental composition, atomic ratios have been also used as indicators of the degree of aromaticity, which in turn, is thought by some to be an indicator of the degree of humification (Giovanela et al., 2010). It has been found that samples exhibit a low H/C ratio also exhibit a high aromatic content and vice versa (Rice and MacCarthy, 1991). The evaluation of the data in Table 2.2 suggests that the degree of aromaticity (humification) increases in the order: groundwater humic acid < peat humic acid < swamp water-HA < soil humic acid < coal humic acid. In addition to the degree of humification, H/C and O/C ratios have been also used to illustrate the variations in humic substances as a function of origin (Rice and MacCarthy, 1991).

Table 2.2: Elemental composition and atomic ratios of humic acids from different resources**.**

Test	Soil-HA*	Peat-HA*	Coal-derived HA*	Groundwater-HA**	Swamp water-HA***
C (%)	54.28	55.09	61.37	61.82	48.59
H (%)	2.98	4.54	3.29	6.46	3.36
N (%)	2.20	1.42	1.27	3.11	0.90
S (%)	0.81	0.54	0.52	-	-
O (%)	37.90	35.71	33.19	27.60	47.20
O/C	0.52	0.49	0.41	0.34	0.73
H/C	0.66	0.99	0.64	1.25	0.83

*(Novak et al., 2001)

**(Kimuro et al., 2017)

***(Lu et al., 2000).

2.5.3.2 Oxygen-Containing Functional Groups

The general structures of humic acids obtained from different origins show the presence of various types of functional groups. It has been found that irrespective of the sources or environments of humic acids, they contain primarily the following functional groups in different proportions: oxygen, nitrogen, and sulphur containing functional groups (Vairavamurthy et al., 1997). However, among these groups, it has been found that oxygen-containing functional groups are predominant with various functionalities, which include carboxyl, carbonyl, alcoholic and phenolic hydroxyl, and methoxyl groups (Kuwatsuka et al., 1978, Vairavamurthy et al., 1997). Furthermore, it has been also reported that among oxygen-containing functional groups, carboxyl and phenolic groups represent the most dominant and reactive groups (Andjelkovic et al., 2006).

Oxygen-containing functional groups exhibit an important influence on soils and natural water through its effect on buffering, exchange capacity, and controlling the mobility and bioavailability of inorganic components (Barančíková and Makovníková, 2003, Pertusatti and Prado, 2007). It has been documented that humic substances interact with more than 50 elements including nutrients, toxic metals, halogens and radionuclides (Rahman et al., 2010). Due to the abilities of these groups to bind with metal ions, much of the current literature concerning on soil and water contamination has been paid attention to this significant role.

It has been reported that the binding ability with metal cations is related to the pH (Tipping, 2002) that is mainly attributed to the ionization of carboxylic and phenolic groups (Stevenson, 1994). The total content of protons that can be dissociated in humic acids is between 4 and 6 meq/g of HA (Tipping, 2002). However, humic acids are polyprotic weak acids, and the carboxylic and phenolic groups have a different tendency for ionization. The majority of groups dissociate at $\mathrm{pH} < 7$ are carboxylic (Tipping, 2002). On the other hand, phenolic compounds are weaker acids than most carboxylic acids with higher average pKa values comparing to those for carboxylic groups (Stevenson, 1994).

The amount of oxygen-containing functional groups may differ considerably among the different types of humic acids. It has been reported that the differences in the functional groups for the various types of humic acids are attributed to their different biogeochemical origins and the degree of humification (Kuwatsuka et al., 1978, Mengchang et al., 2008). The results obtained by Kuwatsuka et al. (1978) experiments show that the contents of carboxyl and carbonyl groups of various types of soil humic acids increase with increasing humification, while the contents of phenolic and alcoholic hydroxyl groups and methoxyl groups decrease. Table 2.3 shows the carboxyl and phenolic contents in humic acids extracted from different origins (Saito and Hayano, 1980, Campitelli et al., 2006, Janoš et al., 2008).

Table 2.3: Carboxyl and phenolic contents in different kinds of humic acids (meq per g of humic acid).

	Total acidity	Carboxyl	Phenolic
Brown coal-derived humic acid [a]	7.57	3.02	4.55
Peat humic acid [a]	8.15	2.78	5.37
Soil humic acids [b]	11.61	5.67	5.94
Marine humic acid [c]	5.80	3.27	2.53

[a] (Janoš et al., 2008)

[b] (Campitelli et al., 2006)

[c] (Saito and Hayano, 1980)

2.5.3.3 Molecular Weight

Normally, humic acids' molecular weights are determined as average molecular weights, due to the difficulty to separate it into distinct molecular fractions (Chin et al., 1994). It has been found that the unfractionated humic acids often have an average molecular weight that varies from thousands to several hundred thousand Daltons (Tipping, 2002). Data obtained by several studies for humic acids from different origins are summarised in Table (2.4). These data reveal that significant differences of the average molecular weights between the different humic acids and as well as for the same type of humic acid and that probably due to the variation in the surrounding environment, method of extraction and the used method of analyses. This variation may also reflect the intrinsic difficulty in measuring the molecular weight of micellular supramolecules.

Numerous studies have been investigated the influence of humic acids molecular weight fractions on its mobility, chemical composition, and reactivity with metal ions (Hirata, 1983, Chin et al., 1994, Chin et al., 1997). It has been found that a substantial variation in the chemical composition between the molecular weight fractions (Swift et al., 1992). Rashid and King (1971) and Fukushima et al. (1996a) results revealed a direct relationship between the humic acid’s molecular weight fraction and the acidic properties. This is supported by Richard et al. (2007) study, which revealed that carboxylic groups are mainly concentrated in low molecular weight fractions, while aliphatic moieties like polysaccharide and peptides are concentrated in high molecular weight fractions. The above findings are consistent with a recent study that conducted by Chen et al. (2011). They confirmed that low molecular weight humic acid is enriched with polar C in aliphatic region and aromatic content. As a result of this, they found that low molecular weight fraction of humic acid exhibited greater capacity of Cr(VI) reduction. In general, the metal holding capacity increases with decreasing the molecular weight fraction of the same humic acid (Rashid, 1971).

Table 2.4: Weight average molecular weight of various types of humic acids.

Humic acid	Reported weight average molecular weights (Daltons)	Reference
Aquatic humic acid	2,500[a]	[a](Rodríguez and Núñez, 2011)
Aldrich HA (Coal derived HA)	4,500[a]	[b](Chin et al., 1994)
	4,100[b]	[c](Beckett et al., 1987)
	14,500[c]	[d](Janoš and Zatřepálková, 2007)
	24,400[d]	
Peat HA	37,800[d]	[e](Reid et al., 1990)
	(˜8,000-˜17,000)[e]	[f](Kulikova et al., 2010)
	18,600[f]	[g](Tipping, 2002)
Soil HA	(25,000-200,000+)[g]	

2.5.4 Structure of Humic Acids

Although humic acids characteristics have been studied for decades, the knowledge of their structural formula remains limited due to their structural complexity and diversity of their sources. Until now, there is no single structural formula can be used to describe humic acids from various sources (Diallo et al., 2003). However, over the decades, a number of structural models have been proposed for the average structures of various types of humic acids (Kononova, 1966, Schulten and Schnitzer, 1993, Ghabbour et al., 2001, Diallo et al., 2003, Albers et al., 2008). Indeed, the derivation of these models is mainly based on the information provided by the detailed physical and chemical analyses such as elemental composition, functional groups, spectroscopic data, and degradation products. The structural models obtained from these analyses suggest that humic acids share the main structural features. The structural network of humic acids consists of aromatic rings and aliphatic substances that link together by covalent bonds and attach to various oxygen and nitrogen functional groups such as COOH, phenolic and alcoholic hydroxyls, ketones, ester, ethers, and nitrogen in heterocyclic structures and as nitriles (Schulten and Schnitzer, 1993, Schnitzer, 1994, Schulten, 1994). However, these models differ mainly between them in the aromatic to aliphatic ratios and in the kind and arrangement of reactive functional groups (Stevenson, 1994). For example, the structural model proposed by Schulten and Schnitzer (1993) of soil humic acids (Figure 2.3) shows a high molecular weight macromolecule (5540 Da) that is enriched with carboxylic, phenolic and alcoholic hydroxyl groups. The COOH groups that are clearly placed on both aromatic rings and aliphatic chains of the macromolecule. Models have been used to suggest structures for aquatic and sludge humic acids that show more aliphatic features than those derived from agricultural soils (Stevenson, 1994).

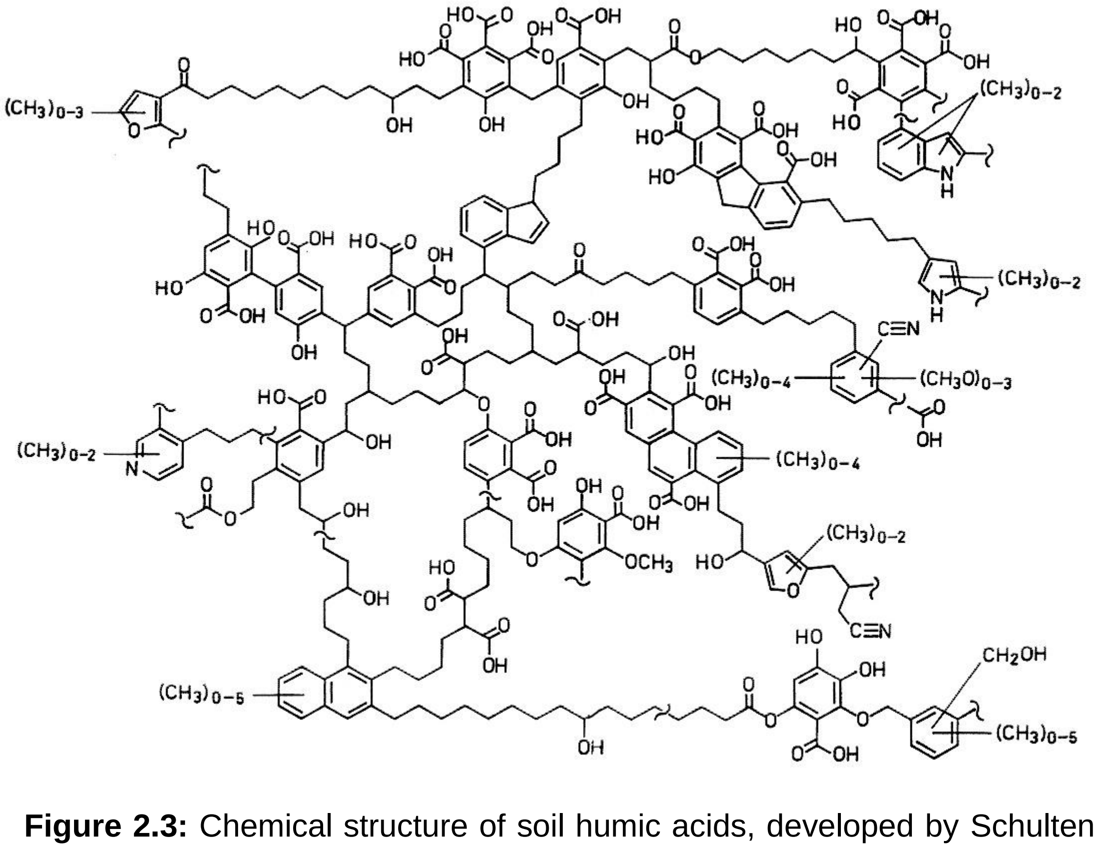

Figure 2.3: Chemical structure of soil humic acids, developed by Schulten and Schnitzer (1993) based on pyrolysis and spectroscopic analysis, oxidative/reductive degradation, colloid chemical and electron microscope results.

2.5.5 Humic Acids – Metal Ions Interactions

Humic acids and their interaction with metal ions in aquatic environments have a special environmental interest. In addition to the role of humic acids as electron donors, they can be considered important complexing agents of metal ions. The ability of humic acids to form complexes with metal ions is mainly due to their high content of oxygen-containing functional groups (Stevenson, 1994). These groups can considerably control the mobility and bioavailability of toxic metal ions, such as Pb(II), Cd(II), Hg(II), Zn(II), Al(III), and Mn(II), Ni(II) (Pehlivan and Arslan, 2006, Dinu, 2015, Jiang et al., 2015), which are mainly introduced into the natural systems from anthropogenic sources.

Due to their heterogeneity, and their large number of reactive sites, humic acids have binding affinities for metal cations that range from weak interactions to a highly stable coordinate linkage (Stevenson, 1994). These interactions may involve either formation of inner-sphere complexes or outer sphere complex with metal cations. A stable inner sphere complex is formed when the donor atom in the ligand displaces strongly coordinated H_2O molecules and binds directly to the central metal ion/atom by coordinate covalent bonds (Kerndorff and Schnitzer, 1980, Logan et al., 1997). This kind of binding may occur through either a monodentate ligand (ligand with one donor atom) or polydentate ligand (ligand with more than one donor atoms) (Tipping, 2002). The complex produced by a monodentate ligand forms a coordinate linkage, while the complex produced by a polydentate ligand forms a chelate (ring) with higher stability compared to a coordinate complex (Tipping, 2002). In contrast to the inner sphere complex, outer sphere complex is formed when the donor atom binds to the metal ion indirectly via water molecule by weak electrostatic interactions (Kerndorff and Schnitzer, 1980). Indeed, experimental data suggest that inner sphere complexes are the predominant form of complexation with transition metals such as Pb, Fe, and Cd (Lakatos et al., 1977, Logan et al., 1997). However, the outer sphere complexes are also formed between humic acids and some transition metal ions, but in conjunction with the inner sphere complex (Senesi et al., 1986, Ohta et al., 2011). Also, outer sphere complexation at the weaker sites becomes relatively important as the stronger sites become saturated (Stevenson, 1994).

Several factors have been reported to effect metal ions-humic acids interactions, which include the chemical characteristics of the humic acids, the nature of metal ions, and the acidity of the surrounded environment (Dinu, 2015). The characteristics of humic acids, including the kind and content of functional groups and molecular weight distribution, are essential factors that govern the humic acids-metal ions interaction (Fukushima et al., 1996b, Lishtvan et al., 2006). The major effective ligands in humic acids include oxygen/nitrogen and sulphur-functional groups, which represent the coordination sites of humic acids (Pehlivan and Arslan, 2006, Jiang et al., 2015). It has been reported that some metal ions, which classified as hard acids such as Fe(III), Al(III), Cr(III) have high affinity to hard bases in humic acids, which include O/N-containing functional groups. On the other hand, soft acids such as Pb(II), Cu(II), Zn(II), and Hg(II) have high affinity to soft bases in the humic acids that include S/P-containing functional groups.

The ability of humic acids to form HA-metal complexes, and their metal ion binding capacity, have also been found to depend on the humic acid size fraction being tested. According to the experimental data of Fukushima et al. (1996b), the lowest molecular weight fractions of humic acid have the largest Cu(II) binding abilities. The higher binding capacities of the lowest molecular weight fractions for Cu(II) is related to their proportionately higher content of carboxylic groups (Fukushima et al., 1996b). Similarly, Christl et al. (2001) studied the binding of Cu to humic acid size fractions, (30-100 kD) and HA > 300 kD. Their results show that at low concentrations of Cu, the amount of Cu binds to the two size fractions of humic acid nearly identical, which reflects that an enough binding sites are available to bind Cu ions in the two fractions. However, at high concentrations of Cu, the binding of Cu to the smaller size fraction was significantly greater than that to the larger fraction. This has been explained as a result of a larger content of carboxyl and phenolic groups in the smaller fraction than that in the larger size fraction.

The complexation affinity of humic acids for metal ions has been also investigated with respect to the pH of the surrounding environment. Metal ions complexing is considered to result from the surface charge on the humic acids. At low pH values, humic acid molecules are aggregated and form colloids with very little or no charge (Ritchie and Posner, 1982). With increasing pH, acidic functional groups such as carboxylic acids and phenols deprotonate and the surface charge becomes increasingly negative (Ghosh and Banerjee, 1997, Evangelou et al., 2002).

2.5.6 Interaction between Humic Acids and Chromium Ions: Kinetics and Mechanisms

The kinetics of Cr(VI) reduction by humic acids have been investigated as a function of pH and reactants concentrations. It has been found that the rate of Cr(VI) reduction is strongly pH dependent, increasing with decreasing pH (Fukushima et al., 1997, Wittbrodt and Palmer, 1997, Nakayasu et al., 1999, Zhilin et al., 2004). When the pH values ≥ 6 the rate of reaction decreases to negligible rates (Wittbrodt and Palmer, 1997). It has been believed that the sharp decline in the reduction rate of Cr(VI) at pH ≥ 6 is due to the formation of CrO_4^{2-} (see Figure 2.1. Eh-pH diagram), which has a little contribution in the reaction with organic matter (Elovitz and Fish, 1995, Wittbrodt and Palmer, 1995). However, it has been observed that at sites contaminated with hyperalkaline chromium ore processing residue, Cr accumulation in high organic soils at pH ~10.5 (Higgins et al., 1998, Whittleston et al., 2011a, Ding et al., 2016). Moreover, the kinetics of Cr(VI) reduction by phenols, as model precursors of humic acids, show three different pathways for the reduction of Cr(VI) at different ranges of pH (Elovitz and Fish, 1995). The reduction of Cr(VI) by phenols is most rapid at pH 1 where the dominant pathway is second order with respect to $[H^+]$. The reduction rate decreases at $2 < pH < 5$ where the dominant pathway is first order with respect to $[H^+]$. At pH ≥ 5, the reduction rate is the lowest where the dominant pathway is independent of $[H^+]$.

The reduction rate has been also investigated as a function of reactants' concentrations. The kinetic data has shown that the reduction rate increases considerably with increasing the initial concentration of soil humic acid (Wittbrodt and Palmer, 1997). On the other hand, the reaction rate constants decrease with increasing the initial concentrations of Cr(VI) (Wittbrodt and Palmer, 1997, Li et al., 2008).

According to the kinetic data has been obtained by Wittbrodt and Palmer (1997), an empirical rate equation (equation 2.3) has been developed for the reduction of Cr(VI) by soil humic acid over specific environmental conditions. The equation display a non- linear reduction of Cr(VI) by soil humic acid (SHA) with time and it is valid under the following conditions; pH (2-7), $Cr(VI)_0$ (0.02-0.2 mM), SHA (25-250) mg/l (Wittbrodt and Palmer, 1997).

$$R = -\left(k_0 + k[H^+]^{1/2}\right)[HCrO_4^-]^{1/2}X_e^{-1} \qquad 2.3$$

Where:

k_0: (8.3 ± 1.2) * 10^{-12}, S^{-1}

k: (2.04 ± 0.05) * 10^{-9}, $l^{1/2}$ $mol^{-1/2}$ S^{-1}

Xe: equivalent fraction of soil humic acid oxidized

Recently, the mechanisms of oxidation of humic acids functional groups by Cr(VI) have received more attention. Due to the complexity of humic acids, which have various reactive moieties, model precursors of humic acids have been used to understand the reaction mechanisms. It has been found that the oxidation of organic moieties; alcohols, aldehydes, and phenols, by Cr(VI) closely follow similar multi-steps mechanism. It has been confirmed that in acidic solutions the oxidation of these moieties starts with the rapid and reversible formation of chromate ester intermediate (Lee and Stewart, 1967, Wiberg and Schafer, 1967, Elovitz and Fish, 1995). This step involves the attachment of Cr(VI) via an oxygen linkage to a carbon atom (Lee and Stewart, 1967, Wiberg and Schafer, 1967, Elovitz and Fish, 1995, Z. F. Chen et al., 2015). Then this intermediate decomposes in the rate-limiting step that involves carbon-hydrogen bond cleavage (Lee and Stewart, 1967). Infact, the decomposition of the chromate ester is accompanied by a transfer of one or two electrons to the Cr(VI) center (Lee and Stewart, 1967, Wiberg and Schafer, 1967). Subsequently, the decomposition products depend on how chromate ester decomposes (see Figure 2.4 as an example) (Solomons and Fryhle, 2002). For example, when there are electron donating groups, such as methyl substituents attached to the phenolic ring, one electron is transferred and the products involve the formation of dimers and larger organic polymers and Cr(V) species. On the other hand, two electrons are transferred when the donating groups contain methoxy substituents, and the products involve the formation of monomeric quinones and Cr(IV) species.

Since chromate ester decomposition produces one or two electrons, as mentioned above, the intermediate Cr(IV) and Cr(V) species proceed the reaction to produce Cr(III) as the final Cr species in all experimental systems (Lee and Stewart, 1967, Wiberg and Schafer, 1967, Elovitz and Fish, 1995, Z.

F. Chen et al., 2015). Cr(IV) disproportionates to Cr(V) and Cr(III) (Haight et al., 1971), while Cr(V) disproportionate to Cr(IV) and Cr(VI) (Buxton and Djouider, 1996). Cr(IV) can oxidize alcohol and phenol moieties in single electron transfer reactions, while Cr(V) can oxidize these moieties in two electrons transfer reactions (analogous to the Cr(VI)-pathway), and thus can produce Cr(III) (Bruckner, 2002).

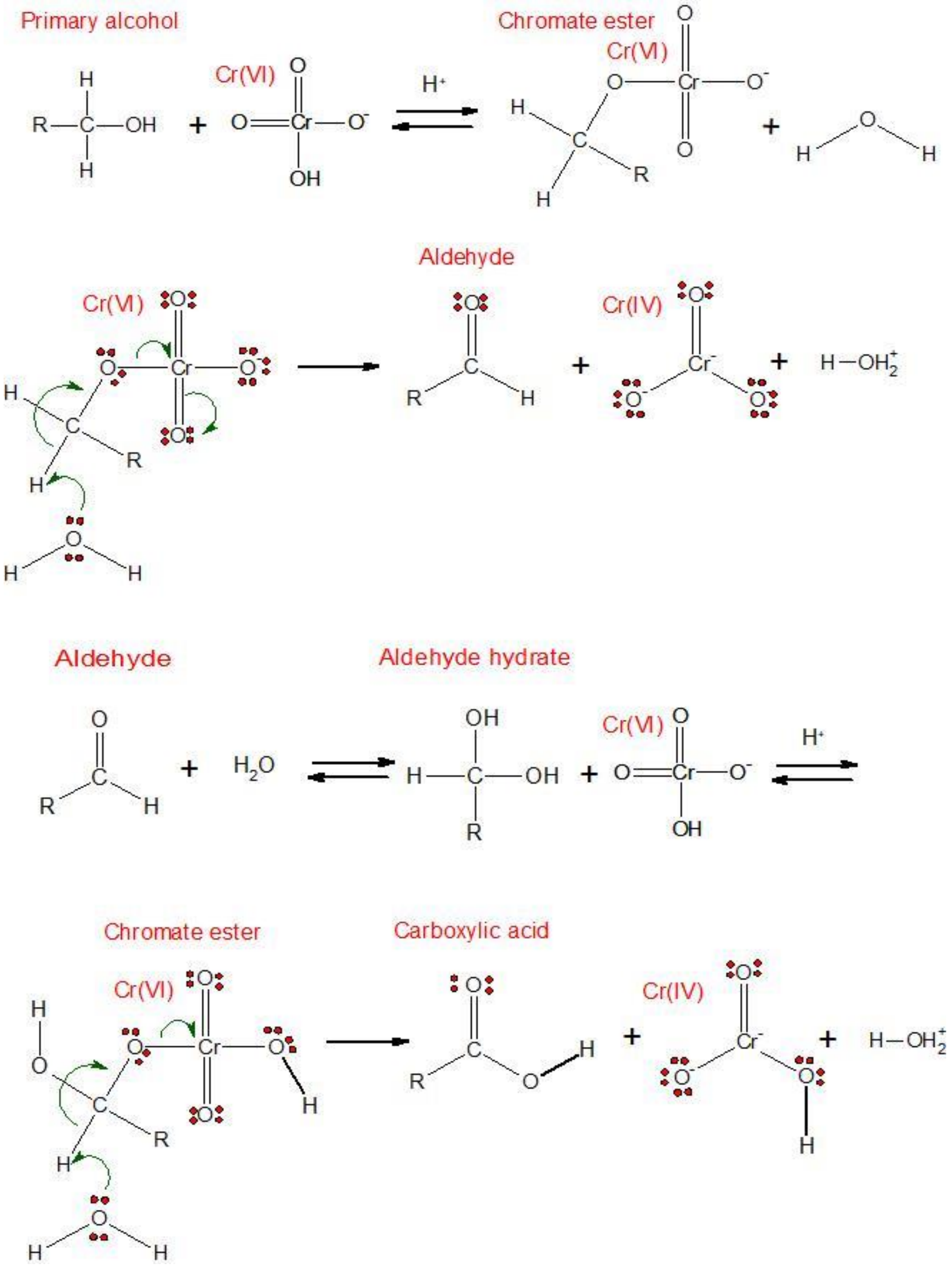

Figure 2.4: Reaction products and mechanism for the reduction of Cr(VI) by A) primary alcohol and B) aldehyde; adapted from (Solomons and Fryhle, 2002).

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Chapter 3: Materials, Methods, and Experimental Design

3.1 Materials

3.1.1 Cr(VI) Solutions

A 0.033 mol/L potassium chromate (K_2CrO_4) solution was obtained from Fluka Company, Germany (analytical brand). All solutions used in this study were prepared by diluting this stock solution in deionized water (0.64 µs/cm).

3.1.2 Humic Acids

Four different humic acids were used in this study: lignite derived humic acid, peat humic acid, sludge phyto-conditioning residue humic acid, and soil humic acid.

Aldrich humic acid (AHA) - A commercially produced humic acid was obtained as a sodium salt from Sigma-Aldrich, UK. This product is extracted from lignite (Poynton, 2016). AHA to be used in the batch experiments was dissolved in deionised water (DIW) and its pH was adjusted to the desired value using 1 N HCl or NaOH. AHA to be used in some characterization tests was further refined (refined Aldrich humic acid: rAHA). It was dissolved in DIW and the pH was increased to 12 using 0.5 N NaOH. The mixture was shaken for an hour and acidified to pH < 2 using 4N HCl. The precipitate was collected by centrifugation (~3,000g, 45 min). It was washed twice with a small amount of DIW and centrifuged again. The final precipitate was collected at pH 2, dried at 40 ºC, and disaggregated using a mortar and pestle.

Peat humic acid (PHA) - Irish moss peat was obtained from Westland Horticulture Ltd, UK. Humic acids were extracted using the procedure described below (section 3.1.3).

Sludge phyto-conditioning residue humic acid (SPCR-HA) - Sludge phyto-conditioning residue (SPCR), a high organic matter secondary material produced by Yorkshire Water. This material is made by mixing dewatered sewage sludge with locally recovered green waste (shredded trees, grass, etc.) in a 1:1 ratio, composting this mixture in windrows for around 8 weeks, and then using this material to grow ryegrass in a 0.80 m deep beds (White et al., 2008). SPCR was collected from Esholt Sludge Treatment Works in April 2015. Humic acids were extracted using the procedure described below (section 3.1.3).

Soil humic acid (SHA) - Soil with moderate clay-loam contents was collected by D.I. Stewart in December 2016 from the ‘Big Substation’ field at the University of Leeds Farm (Headley Hall Farm; 53°52'7.65" N and 1°19'37.73" W). This field is in arable rotation. The sample was taken using a spade (sample depth 0-20 cm). Humic acids were extracted using the procedure described below (section 3.1.3).

3.1.3 Humic Acid Extraction Process

Extraction of humic acids follows the main steps recommended by Stevenson (1994). Air-dried and sieved samples (< 2 mm) of peat (40 g), SPCR (40 g) and soil (80 g) were placed into 500 ml round screw cap borosilicate glass bottles and 0.1 N HCl was added at a solid to liquid ratio of 1:5. The mixtures were shaken at 200 rpm for 4 hrs using a reciprocating shaker (Infors HT, Multitron Pro). The acid washed solids were collected after centrifugation (~3,000g, 30 min). Then the solids were suspended in 0.5 N NaOH at a solid to liquid ratio of 1:10 for peat and SPCR (organic rich samples) and 1:5 for soil and shaken for 24 hours. After centrifugation (~3,000g, 45 min) the supernatant was collected and stored at room temperature. This procedure was repeated twice using 0.5 N NaOH and deionised water (DIW) for an hour (each). The collected supernatant was acidified to pH < 2 by addition of 4 N HCl. The humic acid precipitate was recovered by centrifugation (~3000 g, 45 min). Then, the humic acids were dissolved with NaOH and centrifuged (~3,000g, 45 min) to remove fine particles that entered during the decanting of the supernatant liquor. The process of acidification, collection of the precipitate, and re-dissolution in NaOH was repeated once more to maximize the removal of inorganic impurities. After these steps, the peat humic acid was acidified and centrifuged as above. Peat humic acid precipitate was washed several times with a small amount of deionised water, centrifuged, and the final product was collected at pH 2. After that, the product was dried at 40 ºC and disaggregated using a mortar and pestle and stored in sealed light-resistant bottles under laboratory atmosphere.

Those steps in the above procedure that Stevenson (1994) identified as air-sensitive were carried out under a nitrogen atmosphere. To maximize humic acids output, the extraction procedure using fresh samples were repeated several times. The procedure of humic acids extraction and purification processes is illustrated in Figure (3.1).

3.1.4 Refining of SHA and SPCR-HA

Both soil humic acid (SHA) and sludge phyto conditioning residue-humic acid (SPCR-HA) required further purification due to their high ash content (37%, 19%), respectively. The humic acid solutions were centrifuged at a high speed (~11,000 g, 30 min). Then the supernatants were collected and filtered using 0.2 µm PES syringe filter (Sartorius Minisart syringe filters). Finally, the humic acids were acidified, washed, dried, de-aggregated and stored as mentioned above.

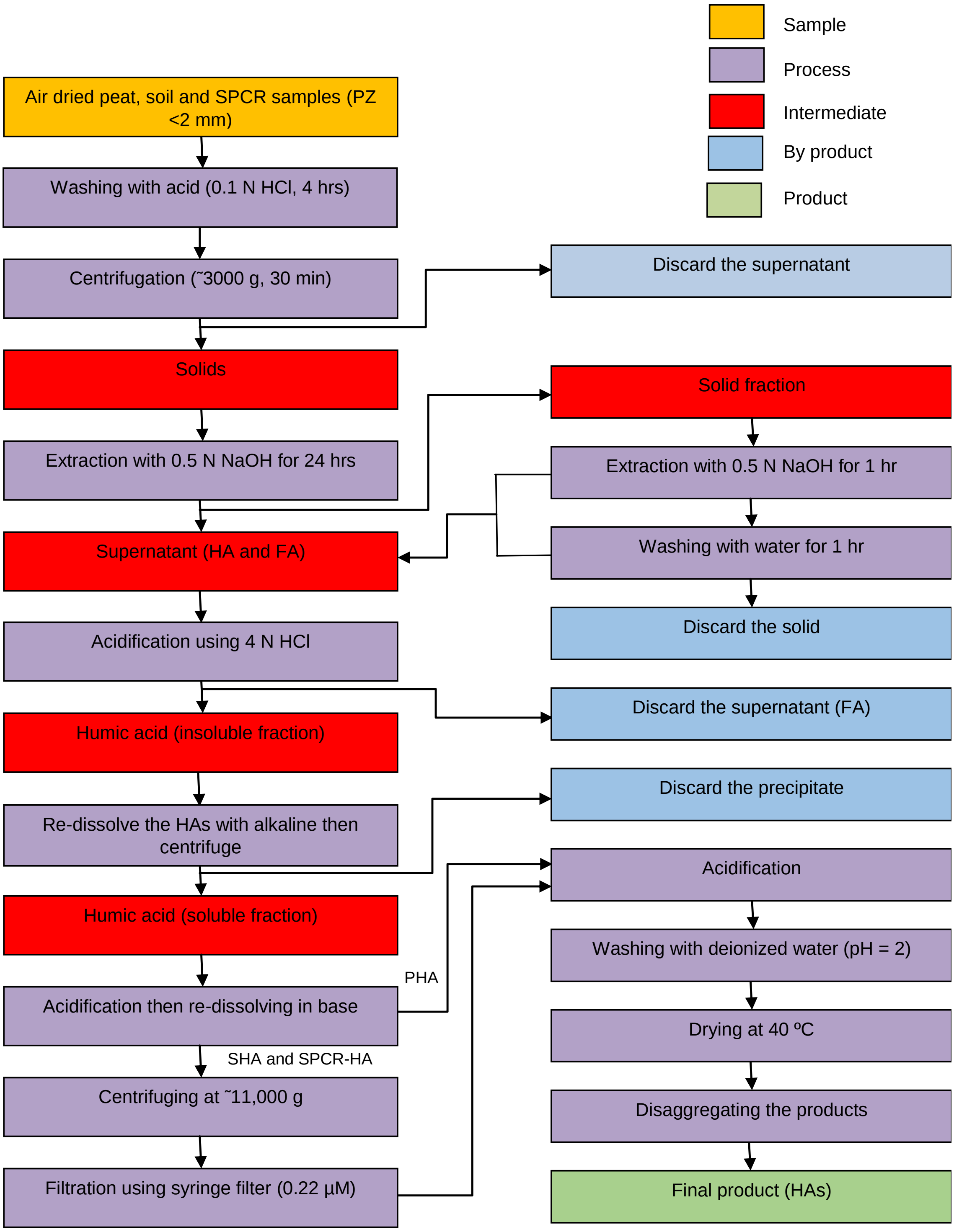

Figure 3.1: Scheme for the extraction and purification processes of humic acids.

3.2 Methods

3.2.1 Moisture Content

3.2.1.1 Moisture content of the humic acids' parent materials

The moisture content of the parent materials from which humic acids were extracted (peat, soil, and SPCR) were determined following the ASTM standard (D2974-87), method B (ASTM, 1993). According to this method representative samples (6 replicates) of the parent materials were dried at room temperature for a day and then placed in oven-dried and pre-weighted porcelain crucibles. Both the samples and the crucibles were weighted to the nearest 0.01 g and the masses of the parent materials were determined by the difference. The samples were then oven dried at 105 ºC for at least 16 hours. After a complete drying, the masses were recorded and the moisture content was calculated for the samples as a percentage of the air-dried samples using the following equation:

$$Moisture\ content, \% = \frac{W_{i\_}W_f}{W_i} \times 100 \qquad (3.1)$$

Where:

W_i = mass of the parent materials, air-dried at room temperature, g.

W_f = mass of the oven-dried parent materials at 105 ºC, g.

3.2.1.2 Moisture content of the humic acids

The moisture content was determined for the humic acids following the ASTM standard (D2974-87), method A (ASTM, 1993). The procedure of this method is similar to that explained above in Section 3.2.1.1 except that the moisture content was expressed as a percent of the as-received mass, which was at 40 °C using the following equation:

$$Moisture\ content, \% = \frac{W_{i-}W_f}{W_i} \times 100 \qquad (3.2)$$

Where:

W_i = mass of the humic acid, dried at 40 ºC, g.

W_f = mass of the oven-dried humic acid at 105 ºC, g.

3.2.2 Ash Content

The ash content was determined by ignition of moisture-free samples at 750 ºC following the ASTM standard (D2974-07a) (ASTM, 2010). 6 replicates of oven-dried samples (at 105 °C) were placed in pre-weighted porcelain crucibles that had previously been heated to 750 ºC prior to current usage. Both the sample and the crucible were weighted to the nearest 0.01 g and the masses of the humic acids were determined by the difference. The samples were placed in a Muffle furnace at 750 ºC for 6 hours. After a complete ignition, the samples were placed to cool to about 150 ºC and then placed in a desiccator for cooling to the room temperature. Then the samples were re-weighed and the ash content was calculated as a percentage of remaining residues after ignition at 750 °C relative to the oven-dry masses using the following equation:

$$Ash\ content, \% = \frac{C}{B} \times 100 \qquad (3.3)$$

Where:

C = mass of the residue ignited at 750 °C, g.

B = oven-dried sample, g.

3.2.3 Organic Matter Content

The organic matter content of the materials from which humic acids were extracted (peat, soil, and SPCR) were determined using the loss of ignition method at 550 ºC. 6 replicates of oven-dried (at 105 °C) and sieved samples (< 2 mm) were placed in pre-weighted porcelain crucibles that had previously been heated to 550 ºC prior to current usage. The moisture free samples were then ignited in a Muffle furnace at 550 ºC for 6 hrs. After a complete ignition, the samples were placed to cool in a desiccator for cooling to the room temperature. Then the samples were weighted and the organic contents were calculated and expressed as a percentage of the oven-dried mass using the following equation:

$$Organic\ matter, \% = \frac{A - B}{A} \times 100 \qquad (3.4)$$

Where:

A = oven-dried mass, g.

B = Ignited mass, g.

3.2.4 E4/E6

The absorbance ratio E4/E6 of the humic acid solutions, which refers to the absorbance ratio at 465 and 665 nm, was determined according to Chen et al. (1977) procedure. 4 mg of humic acid was dissolved in 10 ml of 0.05 N $NaHCO_3$ solution. The samples were agitated for 4 hours at 200 rpm using a mechanical shaker (Infors HT Multitron Pro) until the humic acids were completely dissolved. Then, the absorbance was measured at 465 nm and 665 nm by comparison with a reference blank of 0.05 N $NaHCO_3$ solution using Thermo Scientific Biomate 3 spectrophotometer.

3.2.5 Redox Potential

The redox potential was measured using Ultrameter II (Myron L Company). A saturated paste of humic acid was prepared by mixing 1 g of humic acid with 1 ml of degassed DIW. The sample was homogenized and then the redox potential was measured immediately at 24 ºC.

3.2.6 Elemental Analyses CHNSO

The weight percentages of the major elements in the HAs; carbon, hydrogen, nitrogen, sulphur, and oxygen were determined using Thermo Scientific FLASH

2000 CHNS/O Analyser. Solid samples were prepared for the analysis by grinding representative masses to fine particle size (212-355 µm). These samples were homogenised and dried at 40 °C for the analyses. Triplicate samples (2-4 mg) were weighted in tin and silver micro-capsules using micro-analytical balance (± 0.01 mg) for CHNS and O analyses, respectively. The samples masses were registered and the capsules were sealed.

For CHNS analyses, the combustion method was used. The samples weighed in tin capsules were placed in the auto-sampler and introduced into a combustion reactor (furnace) with a high purity oxygen gas stream (BOC Grade N5.0). These capsules were burned in the furnace at 900°C and the combustion products were carried by a helium gas stream (BOC CP Grade) into an oxidation/reduction reactor in order to ensure full conversion of elemental C, H, N and S into CO_2, H_2O, N_2, and SO_2 respectively. These gases were separated by gas chromatography column and finally identified by a thermal conductivity detector (TCD).

For oxygen determination, pyrolysis method was used. The silver capsules containing the samples were placed in the auto-sampler and delivered to the pyrolysis reactor with helium gas stream (BOC CP Grade). Inside the reactor, oxygen reacts with carbon that presents in the reactor at 1,050 °C forming carbon monoxide. The produced carbon monoxide was then separated by gas chromatography column and finally detected by a thermal conductivity detector (TCD).

For both CHNS and O analyses, standard materials were used for calibration (Elemental Microanalysis Ltd.). Before running the unknown samples, the instrument was calibrated by running a blank followed by high purity standards that covered the range of element content in the HAs samples. Each standard was measured in duplicate. The generated calibration curve was then used to calculate the weight percentages of the elements in the HAs samples taken into consideration the initial mass of each sample that was used for the analyses. In addition, the final results of the elemental composition were normalized to 100% of the organic composition and expressed as free moisture and ash contents. The test was performed in the School of Chemical and Process Engineering lab (University of Leeds) by A. Cunliffe for samples prepared by the Author.

3.2.7 Functional Groups Content

Total acidity and carboxyl groups were determined quantitively using barium hydroxide and calcium acetate methods, respectively. The phenolic acidity was calculated as the difference between the total acidity and carboxyl groups. Details of the functional groups related work are furnished below.

A) Total acidity

The total acidity (carboxyl, phenolic and/or enolic-OH) was determined using the barium hydroxide method used by Schnitzer and Khan (1972). Triplicate suspensions of 100 mg humic acids and 20 ml of 0.2 N $Ba(OH)_2$ were prepared in 125 ml round screw cap glass bottles. Triplicate blanks composed of the reagents were prepared and treated in the same manner as the samples. The bottles were flushed out with N_2 gas for 4 minutes and closed immediately and tightly with screw caps. Then the bottles were shaken at room temperature for 24 hours using a mechanical shaker (Infors HT, Multitron Pro) at 120 rpm. After that, the samples were filtered at 2.7 µm pore size, ashless, chemical resistant filter paper (Whatman cat no. 1542090). The residue in the bottle and that collected on the filter paper were washed with CO_2 free DIW. The washing was added to the filtrate and the unreacted $Ba(OH)_2$ was titrated down to pH 8.4 using 0.5 N HCl. The total acidity was calculated and the values were expressed as (milliequivalents/g humic acid) using the following Equation (3.5):

$$Total\ acidity = \frac{1000(V_b - V_s)N_a}{Mass\ of\ sample\ (mg)} \qquad (3.5)$$

Where:

V_b: Volume of the acid consumed by the blank, ml.

V_s: Volume of the acid consumed by the sample, ml.

N_a: Normality of the acid ($eq.L^{-1}$).

B) Carboxyl groups

The carboxyl groups were determined using the calcium acetate method used by Schnitzer and Khan (1972). Triplicate suspensions of 100 mg humic acids, 10 ml of 1 N $Ca(CH_3COO)_2$ solution, and 40 ml of CO_2 free DIW were prepared in 125 ml round screw cap glass bottles. Triplicate blanks composed of the reagents were prepared and treated in the same manner as the samples. The bottles were flushed out with N_2 gas for 4 minutes and closed immediately and tightly with screw caps. Then they were shaken at room temperature for 24 hours using a mechanical shaker (Infors HT, Multitron Pro) at 120 rpm. After that, the samples were filtered at 2.7 µm pore size, ashless, chemical resistant filter paper (Whatman cat no. 1542090). The residue in the bottle and that collected on the filter paper were washed with CO_2 free DIW. The washing was added to the filtrate and then the liberated acetic acid was titrated up to pH 9.8 using 0.1 N NaOH. The carboxyl content was calculated and the values were expressed as (milliequivalents/g humic acid) using equation (3.6).

$$COOH = \frac{1000(V_s - V_b)N_b}{Massofsample\ (mg)} \qquad (3.6)$$

Where:

V_s: Volume of base consumed by the sample, ml.

V_b: Volume of base consumed by the blank, ml.

N_b: Normality of the standard base (eq.L^{-1}).

C) Phenolic acidity

Phenolic acidity was calculated directly by the difference between the total acidity and carboxyl groups contents that were calculated as explained above.

3.2.8 Acid-Base Titration

Acid-base properties of the humic acids were investigated using direct discontinuous base titrations following Janoš et al. (2008). Humic acid (0.25 g) was suspended in 1N NaCl (25 mL) in 100 ml polypropylene screw-cap bottles, and an increasing volume of 0.5 N NaOH was added to successive bottles. The final volume was adjusted to 50 mL using DIW to give a final ionic strength equivalent to I = 0.5 NaCl. The samples were purged with nitrogen for 4 minutes, and the bottles were sealed. The samples were equilibrated for 72 hours with discontinuous shaking, and the final pH values were measured.

The analyses were carried out in triplicate.

3.2.9 Metal Content by XRF

The concentrations of the major and trace inorganic constituents in the humic acids dried at 40 ºC were determined by x-ray fluorescence (XRF) spectroscopy (X-5000 Mobile XRF System - Olympus IMS). The sample was prepared by grinding the humic acid into a fine powder (˜< 50 µm) using an agate mortar and pestle. The sample was then homogenized, and a representative mass was packed in a plastic sample holder. The sample holder was sealed with a thin film and placed in the instrument.

In this technique, x-rays bombard the sample, and if the energy of this radiation is higher than the ionization energy of an atom; one or more electrons are excited from the inner shell of the atom. As a result, the electron structure of the atom becomes unstable and immediately an electron from a higher energy orbital, such as L or M shell jumps into the empty position and fills the vacancy and the excited atom relaxes back to a ground state. As a result, X-rays of a specific energy were emitted which are characteristic of the energy difference between the initial and final orbital involved. The emitted X-Rays are collected as a function of energy by a High-Resolution Silicon Drift Detector. The energy of emitted X-rays are characteristic of specific elements in the sample. In addition, each element can be quantified based on its peak intensity that is directly proportional to its concentration in the sample. Minor and trace elements (<2000 ppm) were quantified using a standardless method (i.e. Compton Normalization); and all other elements where quantified by calibration using certified reference materials with a matched matrix composition. The test was performed in the School of Earth and Environment lab (University of Leeds) by A. Bray for samples prepared by the Author.

3.2.10 UV-VIS Spectroscopy Method

UV/VIS spectroscopy is a widely used method for the quantitative analyses of many inorganic and organic aqueous species. A light source creates a range of wavelengths that enters through the first slit of the monochrometer as a straight beam of light. When the beam strikes the prism or grating, it splits into several wavelengths that dispersed in the space and only single wavelength of light passes through the wavelength selector (the second slit in the monochrometer). As the beam of light of a single wavelength passes through a colored sample, light is absorbed by the analyte. As a result, an excitation of electrons from the lower energy level to higher energy level takes place. As the beam exits the sample cell, it strikes a detector (photocell) that measures the intensity of light passes through the sample cell by creating an electric current. Similarly, the intensity of light leaving a reference cell (contains all reagents except the analyte) is measured to remove the absorption of light due to the cuvette or the reagent. Then the ratio of the intensity of light leaving the sample cell to the intensity of the incident beam is determined, which is known as a transmittance (T). As the sample cell contains more atoms or molecules that absorb the light, the intensity of light passes through the sample has decreased, and consequently, the transmittance has also decreased. The relationship between the percent transmittance and the concentration of the sample is expressed by an algorithmic function:

$$\%T = 100 \times \left(\frac{P}{P_o}\right) = e^{-\varepsilon bc} \qquad (3.7)$$

Where:

T: transmittance

P: the transmitted intensity

P_o: the intensity of the incident beam

ε: molar absorptivity, L mol^{-1} cm^{-1}

b: path length of the cuvette containing the sample, cm

c: concentration of the analyte in the sample, mol L^{-1}

A simple linear function has been created by converting the percent transmittance into absorbance by taking the negative logarithm of the transmittance and this is what has been known as Beer – Lambert law:

$$Absorbance = -\log_{10} T = \varepsilon bc \qquad (3.8)$$

According to this Law, the absorbance is directly proportional to the concentration. This relationship remains true as long as the solution is relatively diluted. To estimate the concentrations of unknown samples, a calibration curve is generated by measuring the absorbance values of known concentrations (standards). A plot of the absorbance values versus the concentrations is prepared. When the absorbance of the unknown solution is measured at the same wavelength, the corresponding concentration is calculated using the calibration curve.

3.2.10.1 Aqueous Cr(VI)

Aqueous Cr(VI) concentrations were determined spectrophotometrically using the adapted version of USEPA method 7196a (USEPA, 1992). 0.2 mL centrifuged fresh supernatant (passed through a 3 kDa filter) was placed in 3.5 mL glass cuvette (light path = 10 mm) and then the sample was acidified with 1.8 mL of 0.01M H_2SO_4. 0.2 mL of 1,5 diphenylcarbazide solution (250 mg 1, 5 Diphenylcarbazide in 50 mL Acetone) was then added to the mixture. The sample was gently mixed by a pipette to homogenize the mixture. Similarly, a blank using deionised water was treated as the sample and used as a reference. After waiting for 10 minutes to allow the red-violet colour of the sample solution to develop, the blank solution was placed into the cell holder in the UV-VIS spectrophotometer and the background reading was measured at 540 nm then the instrument was zeroed. After that, the absorbance reading of the sample was recorded at the same wavelength. A calibration curve was constructed at the time of analyses using at least 6 standard solutions including a blank (Figure 3.2). The standard solutions were prepared from K_2CrO_4 stock solution in (10-400) µM range and the developed equation of the calibration curve was used to transform the absorbance obtained from the tested samples into concentration. Samples had concentrations exceeded the highest value of the calibration curve were re-measured after a suitable dilution with DIW. The detection limit was 4.2 µM, therefore any reading below this concentration was referred to as undetectable.

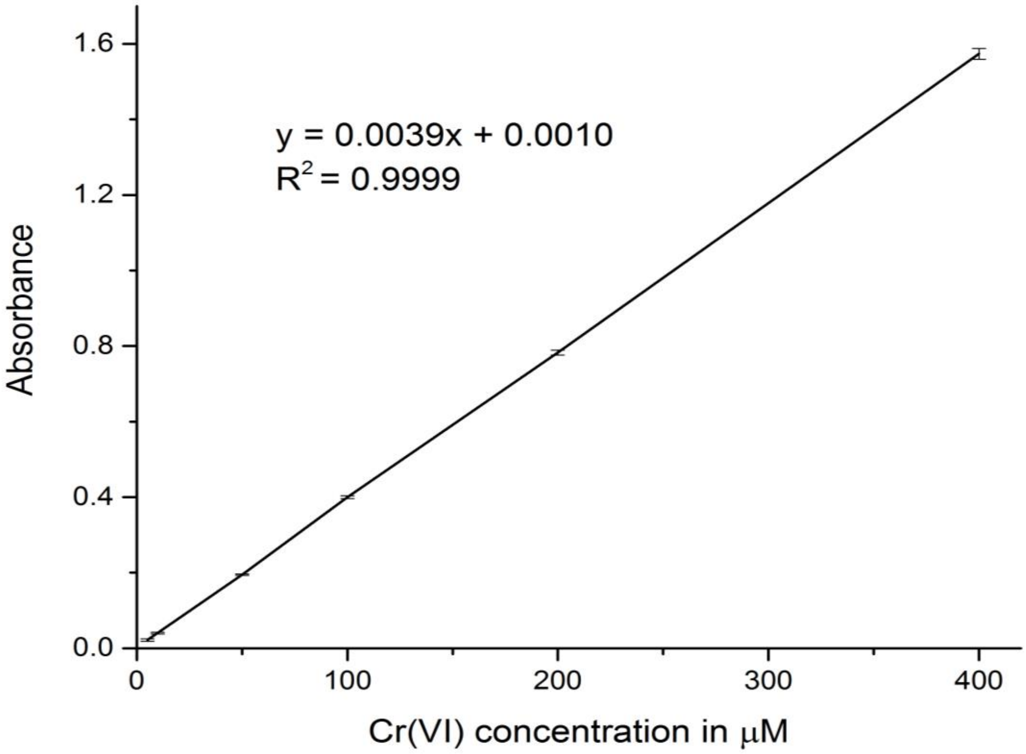

Figure 3.2: Example of the calibration curve of Cr(VI) by UV-VIS spectrophotometer at λ = 540 nm.

3.2.11 Total Cr Determination Using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Method

ICP-OES is a technique that is used for the identification and quantification of elements in aqueous samples. The instrument is mainly composed of two units; inductively coupled plasma (ICP) and optical emission spectrometry (OES). A sample is introduced into a nebulizer via a peristaltic pump. The nebulizer turns the sample into droplets and portion of these droplets goes down into a drain and the remains enter a torch. The torch contains a very hot plasma flame (7000 – 10000 °C), which is typically generated from argon gas. The hot plasma ionizes the elements (atoms) into a higher state and when the electrons fall back down into their ground states, light with different wavelengths is emitted that are characteristic of these atoms. The light emitted enter into the OES unit and the wavelengths are separated by a prism. In the detector, the elements are identified based on the light wavelengths and the intensity of each light can be measured. By comparing the light intensity with a standard curve of the metal that is being interested in, the metal concentration is determined. To measure the metal content in a solid sample, it should be digested and the extract is analysed after preparation as aqueous samples.

3.2.11.1 Total Aqueous Cr

Total Cr in the aqueous solutions was measured after the reaction of Cr(VI) and the HAs had reached an equilibrium state using Inductively Coupled Plasma Optical Emission Spectrometry (Thermo Scientific iCAP 7400 radial ICP-OES). The samples were collected as described below in section (3.3.1) and filtered using 0.2 µm PES syringe filter (Sartorius Minisart syringe filters). Before analysis, the samples were diluted with 18.2 MΩ H_2O (1:5). Matrix-matched calibration curves were prepared and the instrument was calibrated between 0.1-10 mg L^{-1} Cr, as shown in Figure 3.3. Yttrium was used as an internal standard and added to each sample dilution and calibration standard to 2 mg L^{-1}. The dilution regime for each sample was 1.0 ml sample, 3.9 ml DIW (18.2 MΩ), and 0.1 ml yttrium internal standard (at 100 mg L^{-1}). The instrument setup and parameters are summarized below in Table 3.1. The test was performed in the School of Earth and Environment lab (University of Leeds) by S. Reid for samples prepared by the Author.

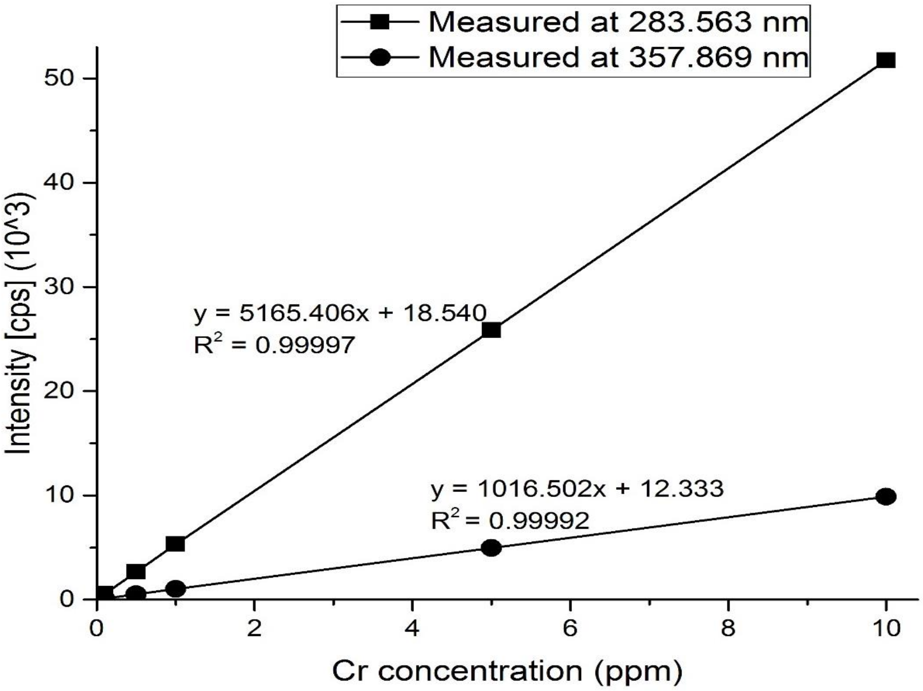

Figure 3.3: Calibration curves of Cr by ICP-OES at λ = A) 283.563 nm, and B) 357 nm.

Table 3.1: iCAP 7400 radial ICP-OES set up and parameters

Parameter	Setting
Nebuliser type	Glass concentric ring
Nebuliser gas flow	0.5 ml min^{-1}
Spray chamber	Glass, cyclonic
Torch Height	8 mm
Plasma power	1500 W
Analytical wavelengths used for Cr	283.563 nm, 357.869 nm
Analytical wavelengths used for Yttrium (Internal standard)	371.030 nm
Detection limit (ppm)	0.147
%uncertainty	1.59

3.2.11.2 Total Cr Associated with the Solid Phase

Total Cr associated with the solid phase of humic acid was determined after acid digestion by following USEPA method 3050B (USEPA, 1996) (samples collection was illustrated in Section 3.3.1). The humic acid (0.15 - 0.25 g) was added to a digestion vessel with 5 ml of a 1:1 dilution of 70% HNO_3 in DIW. The sample was covered with a watch glass and heated in a block heater (Grant QBD4) to 95 ± 5 ºC for (10-15) minutes without boiling. After that 10 ml of 70%, HNO_3 was added gradually until no brown fume was generated. This step was repeated for some samples by adding a further amount of 70% HNO_3 to get a complete reaction with HNO_3 (no brown fume was generated). After this step, 1 ml of DIW and 4 ml of (30%) H_2O_2 was added gradually. When the peroxide reaction had ceased and the general appearance of the sample was unchanged, 3 ml of concentrated HCl was added to the sample and heated for 15 minutes. After the sample was cooled down, the digestate solution was diluted to 25 ml with DIW. Then the digestate was filtered by 0.2 µm PES syringe filter (Sartorius Minisart syringe filters), and the filtrate was analyzed for total Cr by ICP-OES similarly as mentioned above in section (3.2.11.1).

3.2.12 X-ray Absorption Spectroscopy (XAS)

X-ray absorption spectroscopy (XAS) is an advanced and non-destructive analytical technique that is widely used for structural characterisation of matter. To characterise an element, x-ray with a specific radiation energy passes through a sample. When the energy of x-ray matches the binding energy of an electron in an atom, the electron in the core level (e.g., 1s electrons) absorbs the x-ray photon or part of its kinetic energy and then ejects from its core electronic state into the excited electronic state or into the continuum. The ejection of the electron from its core-shell creates a core hole and that making the atom unstable. The atom can restore their stability in mainly two ways; x-ray fluorescence or Auger electron emission. In x-ray fluorescence, an electron from a higher energy shell fills the core hole and that accompanied by an emission of a fluorescent photon. The released photon energy equals the difference between the two shells energy, which is typically in the x-ray region and with an energy characteristic of that atom (Weller et al., 2014). Occasionally, this photon energy causes an ejection of an auger electron if it is higher than the auger electron binding energy in the shell. Due to the various potential final states of the photoelectrons, the interpretation of the data is based on the sum of all possible final states of photoelectrons. The energy emitted by the atom is collected and then processed in the analyser to generate a spectrum. The spectrum involves two main regions (see Figure 3.4). The first region is called X-ray Absorption Near-Edge Structure (XANES) and the second region is called Extended X-ray Absorption Fine Structure (EXAFS). In XANES region, the produced spectrum shows a sharp absorption edge that corresponds to the excited core electron of the atom. For example, if 1s electron is excited, the edge is called k edge, while if 2s or 2p electron is excited, the edge is called L edge. The absorption edge is used as a feature to identify the elements, where each element has distinct absorption edge. The shape of the XANES region of the spectrum can indicate the oxidation state of an atom and its local coordination environment. In EXAFS region, a region of x-ray spectrum starts from 30 eV to up 1000 eV above the edge (Gupta, 2012), the wave-like nature of this region is produced when the ejected photo-electron is scattered by neighbouring atoms. The interferences between the outgoing electron and the back-scattered ones lead to the oscillations in the absorption (Gupta, 2012). After modulation of this region, the data can give information about the type and number of neighbouring atoms, and the distance to neighbouring atoms.

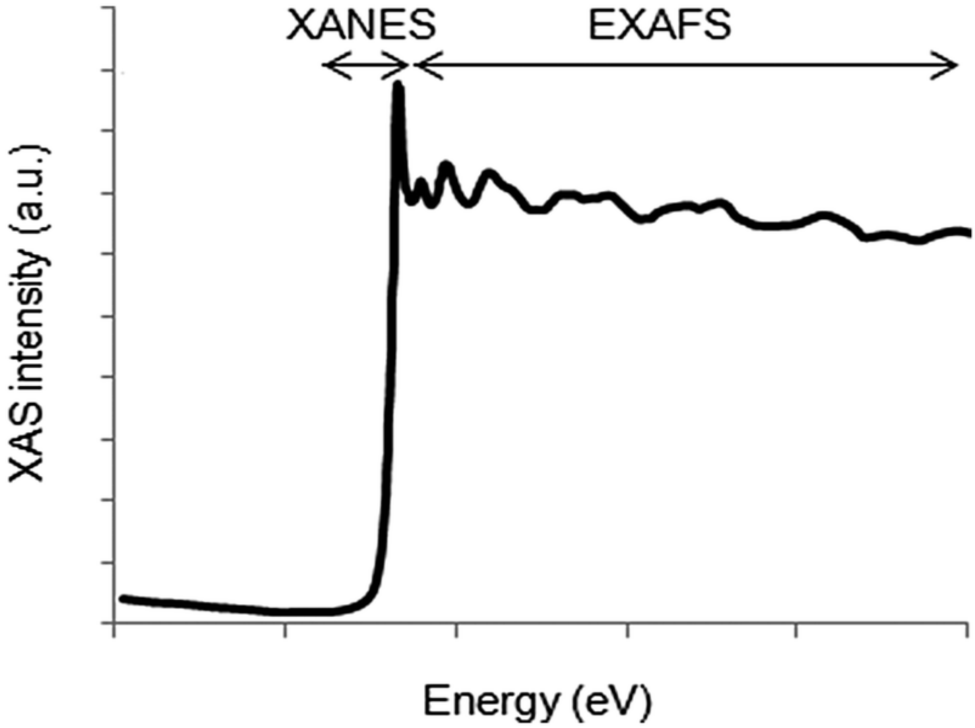

Figure 3.4: An example of x-ray absorption spectrum that shows the XANES region and EXAFS region. Adapted from (van Oversteeg et al., 2017).

3.2.12.1 X-ray Absorption Spectroscopy (XAS), Data Collection, and Analysis

Selected humic acids samples were recovered from Cr(VI) (1100 µM) batch experiments (samples preparation was explained in section 3.3.1).

Samples and standards were pressed into pellets (without diluent), sealed in Kapton tape and then analysed in fluorescence mode (0.1 - 2 % Cr) or transmission mode (> 2 % Cr) as appropriate. The Cr K-edge (5989 eV) XAS data were gathered from two separate sessions, the first one on Beamline B18 (PHA samples) and the second one on Beamline I18 (AHA samples) at the Diamond Light Source.

At Beamline B18 the incident beam had a typical operating voltage and current of 3GeV and 300mA, respectively. The x-rays at B18 are generated in a bending magnet source. The beam is vertically collimated by a Si mirror and then passes through a double crystal Si monochromator. After that It was focused onto the sample to give a spot size of 200 x 250 µm. Sr K edge (16105 keV) XAS spectra were gathered from fluorescence using a 9 element Ge solid state detector at room temperature x-rays. Multiple short scans (approx. 5 min.) were collected at this beamline from each sample and each subsequent scan was assessed over time to ensure no X-ray induced changes in Cr speciation occurred.

At Beamline I18 the data was collected while operating at a typical operating voltage and current of 3 GeV and 250 mA, respectively, using a nitrogen cooled Si(111) double crystal monochromator and focussing optics. Two plane mirrors was used to reduce the harmonic content of the beam and also Kirkpatrick-Baez mirrors were used to produce a relatively unfocused beam (approximately 50 µm diameter at the sample). Data was gathered in fluorescence mode using a 9 element solid state Ge detector at room temperature. The sample stage was systematically moved between around ~20 min scans to exposure a fresh part of the sample for each scan to ensure no X-ray induced changes in Cr speciation occurred. Data acquisition was undertaken and processed by I.T. Burke (supervisor) on beamline B18 and G. Cibin (Diamond Light Source staff) on beamline I18 from samples prepared by the Author.

3.2.12.2 EXAFS Data Analysis and Fitting

Multiple XAS scans from each sample were summed and averaged using Athena software version 0.8.056 (Ravel and Newville, 2005) to maximise the signal/noise ratio and XANES data was plotted. Cr K-edge EXAFS data was also collected and fitted to molecular coordination models using Artemis software version 0.8.056. Shell-by-shell fitting was performed by estimating initial parameters for single scattering pathways of backscattering atoms and then interactively these parameters were refined. The refinement was carried out, specifically, for the number of atoms (± 25%), interatomic distances, Fermi energy and Debye-Waller factor (σ^2). This procedure was performed initially with single scattering and multiple scattering pathways representing the CrO_6 octahedral and subsequent Cr and/or C single scattering pathways were added. Additional pathways were then accepted only where they improved the overall fit quality by greater than 10%, which was determined by the reduction of the reduced Chi-squared measure of fit (Burke, 2006).

3.2.13 Carbon-13 Nuclear Magnetic Resonance Spectroscopy (^{13}C-NMR)

^{13}C-nuclear magnetic resonance spectroscopy (^{13}C-NMR) is a powerful non-destructive tool that is used to determine the type of carbon atoms that present in the sample (e.g., aromatic, carbonyl, alkyl). The fundamental principle of ^{13}C-NMR is based on the presence of carbon 13 nuclei that have an odd number of neutrons. In the absence of an external magnetic field, these nuclei spin randomly. Inside the ^{13}C-NMR machine, a strong electromagnetic radiation is applied to a sample (radio frequency) which aligns the ^{13}C nuclei in a particular way. ^{13}C nuclei, which have a low energy, spin with the magnetic field, while those that have a high energy spin in the opposite direction of the magnetic field. When the radio frequency equals to the precessional frequency, the nucleus that spins with the magnetic field is excited and spin in the opposite direction. Then the same nucleus jumps down to its base state and that gives up some energy. The produced energy matches the energy gap between the excited state and base state. The NMR machine detects this energy, which depends on the type of atoms that are next to the ^{13}C atom. NMR data is presented as a series of chemical shifts that indicate the carbon environment. The arrangement of ^{13}C peaks depends on the shielding effect of electrons. Carbon nuclei that are shielded by more electrons have a lower chemical shift while those are shielded by less electrons have a higher chemical shift.

3.2.13.1 Carbon-13 Nuclear Magnetic Resonance Spectroscopy (^{13}C-NMR), Data Collection and Analysis

The HAs samples reacted with 8000 µmol Cr(VI) at pH3 were prepared as explained in section (3.3.2). Control samples were prepared by adjusting the pH to 3 using 1 M NaOH. When pH became stable, the solid humic acids were centrifuged (~3,000 g, 45 min). The humic acids precipitates were separated and the supernatants were collected and centrifuged again at a higher speed (~11,000 g, 30 min) through a 3 kDa filters (AmiconUltra 0.5 centrifugal filter). The humic acids precipitates on the filters were collected and added to the solids that were collected previously. All samples were dried anaerobically and divided to be used for ^{13}C-NMR and pyrolyses GCMS analyses.

Humic acids samples that had been reacted with 8,000 µmol Cr(VI) at pH 3, and control HA samples, were packed into a 4 mm diameter zirconium rotor tubes. Cross-polarisation magic-angle-spinning ^{13}C-NMR spectra were obtained on a Bruker 400 MhzAvance II spectrometer, with a double-bearing magic-angle-spinning probe head (BL4 type) and sample spinning set using a Bruker MAS II control unit. The samples were transferred to a rotors tube (4 mm in diameter) and the spectra were collected after overnight running to get a good signal to noise ratio. Measurement parameters were ~100.6 MHz frequency, 90-degree proton pulse length, 2.5 microseconds, 2 milliseconds contact time, and 5 second delay time, while the spinning speed was 10 kHz. 5,012 and 10,000, 15,000 scans were collected and averaged for the samples. Chemical shifts calibration were performed using an alpha-glycine spectrum (calibrated on the glycine peak at 43.5ppm) (Baker, 2017).

NMR spectra were divided into chemical shift regions; 0-45 ppm (alkyl C), 45-110 ppm (O-alkyl C), 110-160 ppm (aromatic C) and 160-220 ppm (carbonyl C) (Kögel-Knabner, 2000, Golchin et al., 1997). These regions are very similar to those used by Knicker et al. (2005), who sub divided the aromatic region into sub regions; aromatic-C bonded to C/H (110-140 ppm) and to O (140-160 ppm). The test was performed in the School of Physics and Astronomy lab (University of Leeds) by D. Baker and M. Ries for samples prepared by the Author.

3.2.14 Pyrolyses Gas Chromatography-Mass Spectroscopy (Pyro-GCMS)

Pyrolysis-Gas chromatography/Mass spectrometry (Py-GC/MS) was conducted on HAs samples that had been reacted with 8,000 µmol Cr(VI) at pH 3, and control HA samples that had been conditioned to the same pH value (samples preparation is described in Section 3.2.13.1) After the samples preparation, a fine, homogenized sample (2-5 mg) was placed in between quartz wool plugs in a small pyrolysis tube that was then placed in the cold platinum filaments of the pyrolysis unit (CDS Pyroprobe® Model 5000). Samples were heated at a rate of 20 ˚C per millisecond to 500 ˚C (held for 20 seconds). The fragments (pyrolysates) were then carried by He gas into the gas chromatogram (GCMS-QP2010SE, Shimadzu). Pyrolysates were separated on an Rtx-1701 capillary column before transferred to the mass spectrometer, where they were detected by their mass to charge ratio using quadrupole mass detector. The absolute and relative intensity of each peak on the pyrogram was determined by performing the area integration using Shimadzu GC solution software (version 4.11 SU2). The test was performed in the School of Chemical and Process Engineering lab (University of Leeds) by A. Ross and the Author for samples prepared by the Author, while the data analyses was performed by F. Gill and the Author.

3.3 Experimental Design

3.3.1 Cr(VI) Reduction by Humic Acids

Cr(VI)-humic acid suspensions were prepared by adding 1 g of humic acid and 90 ml of deionised water to 120 ml glass serum bottles. The suspensions were prepared in triplicate and equilibrated at pH 3, 5, 7, 8.5, 9 and 11 using either 1 M HCl or NaOH. The suspensions were intermittently shaken using a mechanical shaker, and the pH was adjusted until the pH reading was stable for at least one day. The bottles were then sealed and autoclaved at 121℃ for 15 minutes. After cooling, the suspensions volume were made up to 96.7 ml using autoclaved deionised water, and the pH was readjusted, if necessary, to maintain pH within ± 0.03 units of the target value. The suspensions were then purged with nitrogen for five minutes. Finally, 3.3 ml of potassium chromate solution (1/30 M K_2CrO_4; Fluka, Germany) was added to each bottle for a 100 ml final volume (initial [Cr(VI)] =1,100 µmol L^{-1}). The bottles were sealed with butyl rubber stopper (Sigma-Aldrich Company Ltd. UK) and capped with aluminium crimps (Sigma-Aldrich Company Ltd. UK). Control samples were prepared in triplicate using the potassium chromate solution and autoclaved, N_2 purged, deionised water. The bottles were then incubated in the dark at 20±1°C for ~50 days. During this period, the bottles were periodically sampled aseptically for geochemical analysis. These samples were obtained by shaking the bottles to homogenize their contents and 2 ml of suspension was extracted using N_2 gas-filled syringes. The collected samples were centrifuged (~11,000 g, 30 min) through a 3 KDa filter (Amicon ultra 0.5 centrifugal filter), and the filtrates were divided for Cr(VI) and pH determination. After completing the experiment (~50 days), each sample was separated into an aqueous solution and solid-phase humic acid. Aqueous subsamples were taken for Cr(VI) and pH measurement as described above. In order to separate the solid humic acids, an equal volume of aluminium sulphate solution (5g $Al_2(SO_4)_3.16H_2O$ per liter) was added to the remaining sample (two volumes of aluminium sulphate were added to the pH 11 sample). The mixtures were shaken manually for a few seconds then centrifuged at 3,226 g for one minute. The supernatant was immediately separated and analysed for total Cr using Inductively Coupled

Plasma Optical Emission Spectrometry (Thermo Scientific iCAP 7400 radial ICP-OES). The separated solid phase was then washed three times with deionised water. Each time the solid was suspended in distilled water and shaken manually for a few seconds, and then centrifuged at 3,226 g for a minute and the supernatant was discarded. Half the solid samples was air-dried in an anaerobic cabinet and then used for x-ray adsorption spectroscopy (XAS) analysis. The other half was oven-dried at 100 ºC and was analysed after digestion for total Cr.

The above procedure was repeated to prepare Cr(VI)-AHA and Cr(VI)-PHA suspensions at initial Cr(VI) concentrations equal 550 and 2,200 µmol L^{-1} per g HA, respectively.

3.3.2 Surface Loading Experiment

The loading capacity of the humic acids for Cr(VI) was investigated at pH 3 (loading capacity refers to the amount of Cr added per mass of humic acid). The humic acids samples were prepared with the various amount of Cr(VI). The suspension preparation was similar to that described above in Section 3.3.1, except potassium chromate solution (0.033 mol L^{-1} K_2CrO_4) (Fluka Company, Germany) was added to give the following initial Cr(VI) loading per g humic acid: 110, 1,000, 2,000, 4,000, and 8,000 µmol. Bottles were incubated in the dark at 20±1°C and sampled aseptically for Cr(VI) analysis to determine when the reactions reached to the equilibrium state. After equilibrium (˜30 days), the bottles were sampled for Cr(VI), total Cr, and pH determination. The aqueous subsamples for Cr(VI) and total Cr analyses were passed through a 3 kDa filter (Amicon ultra 0.5 centrifugal filter). The amount of Cr associated with the solid phase was calculated based on the difference between the initial and final Cr in the aqueous phase. The solid phase HAs was separated from the remaining suspension (sample contained initial Cr(VI) loading of 8,000 µmol/g) by filtration. Then the solid sample was air-dried in an anaerobic cabinet and retained for ^{13}C NMR and pyrolysis GC-MS analysis.

Samples were carried out in duplicate.

3.3.3 Stability of Cr(III)-Humic Acids Experiment

The stability of Cr(III)-humic acids complexation was investigated at pH 3 in the presence of $MgCl_2$ as a competing ion. Triplicate suspensions of Cr(VI)-humic acids (1100 µM Cr(VI)/g humic acid) were prepared at pH 3 and incubated in the dark at 20±1°C (a detailed description of suspension preparation, sampling and chemical analyses in section 3.3.1, 3.2.10.1, and 3.2.11.1). When the reactions reached the equilibrium state, the aqueous concentrations of Cr(VI) and total Cr were determined after centrifugation (~11,000 g, 30 min) through a 3 kDa filter (Amiconultra 0.5 centrifugal filter) using UV-VIS spectrophotometer and ICP-OES, respectively. After that 104.73 mg of $MgCl_2$ was added to the suspensions (the initial concentration of Mg^{2+} was 11,000 µM). The actual initial concentration of Mg^{+2} was slightly (˜5 %) higher than the calculated value due to the sampling before adding Mg^{2+}. The suspensions were flushed with N_2 and sealed immediately with butyl rubber stoppers (Sigma-Aldrich Company Ltd. UK) and aluminium crimps (Sigma-Aldrich Company Ltd. UK). The Bottles were sampled after 48 hrs for aqueous Cr(VI) and total Cr analysis and then Cr(III) concentration was measured by the difference between total Cr and Cr(VI).

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Chapter 4: Chemical and Spectroscopic Characteristics of Humic Acids

4.1 Method

The methods for the characterization of the raw materials (peat, soil, and SPCR), extraction and purification of the humic acids, and chemical and spectroscopic characterization of the humic acids were reported in details in Chapter 3.

4.2 Results

4.2.1 Characterization of Humic Acids’ Parent Materials

The raw materials that were used for the extraction of humic acids; peat, soil, and SPCR were characterized with respect to some important properties as reported in Table 4.1. The organic matter content in the raw materials was considerably different. Peat contained the largest organic content of nearly (93.4%), whereas the soil sample contained the lowest value (5.3%). The yield of the humic acids that was obtained from these materials was also considerably varied and ranged between 1.5 to about 214 g HA per kg of air-dried raw material. PHA produced the largest yield of humic acid, while soil produced the lowest one. In addition, the percentage of the humic acid relative to the organic matter content in each material was estimated (moisture-free basis) and it was found that the humic acids formed about 24.5% and 27.2% of organic matter in peat and SPCR, respectively. In contrast, the humic acid formed only 2.9% of organic matter in the soil.

For comparison, high-quality lignite may compose of 80% to 90% humic matter, (Tan, 2014). However, the commercial-grade lignite composes of 30% to 60% humic matter Stevenson and Cole (1999).

Table 4.1: General characteristics of peat, soil, and SPCR

Test	Peat	Soil	SPCR
% Moisture content (air-dried basis)	12.7 ± 0.1[b]	1.7 ± 0.4	12.2 ± 0.2
% Organic matter (oven-dried basis)	93.4 ± 0.3	5.3 ± 0.2	43.0 ± 0.3
Humic acid yield (g HA/kg air-dried raw material)	213.8 ± 16.6	1.5 ± 0.0	102.6 ± 7.1
% of humic acid relative to the organic matter content[a]	24.5	2.9	27.2

[a] The values were expressed on a moisture-free basis.

[b] Standard deviation.

4.2.2 Chemical and Spectroscopic Characterization of the Humic Acids

4.2.2.1 Moisture and Ash Content

The moisture and ash content of the humic acids are summarized in Table 4.2. The range of the moisture content of air-dried humic acids was found to be between 3.1% to 14.9%. The lowest value was for SHA and the highest value was for AHA. Ash content varied considerably and ranged from nearly 2% for PHA to 27% for AHA.

Table 4.2: Moisture and ash content in humic acids.

Test	AHA	rAHA[a]	PHA	SHA	SPCR-HA
% Moisture content (air dried basis)	14.9 ± 0.6[b]	4.0 ± 0.7	7.5 ± 0.2	3.1 ± 0.7	6.1 ± 1.3
% Ash content (oven dried basis)	26.9 ± 1.6	18.0 ± 0.2	1.8 ± 0.4	7.2 ± 0.2	10.4 ± 0.6

[a] Refined Aldrich humic acid.

[b] Standard deviation.

4.2.2.2 Elemental Composition

The elemental compositions and atomic ratios of the humic acids are presented in Table 4.3. The percentages of C and O in the humic acids ranged from 50.43% to 60.12% and from 31.53% to 37.32%, respectively. The percentages of C increased in the order SHA < SPCR-HA < rAHA < PHA, while the percentages of O increased in the reverse order (PHA < rAHA < SPCR-HA < SHA). The percentages of N and H ranged from 1.75% to 6.5% and from 5.15% to 5.57%, respectively. The percentages of N increased in the order rAHA < PHA < SPCR-HA < SHA, while the percentages of H increased in the order SPCR-HA < rAHA < SHA < PHA. Sulphur had percentages that ranged from 0.26% to 0.60%. The above percentages of C, H, N, and O fall within the ranges that have been reported in literature of humic acids derived from coal, peat, soil, and sewage sludge (Rice and MacCarthy, 1991, Novak et al., 2001, Amir et al., 2004, Zaccone et al., 2007, Klavins and Purmalis, 2013). However, the high standard deviation of sulphur data makes these values unreliable. The ranges of atomic ratios, H/C, O/C, and N/C, were (1.05-1.3), (0.39-0.56), and (0.03-0.11), respectively. All atomic ratios were higher in SHA and SPCR-HA than in rAHA and PHA.

4.2.2.3 Redox Potential

Table 4.3 lists the redox potential values at 24 °C for the humic acids samples. All humic acids samples exhibited a positive redox potential that ranged between 275 and 386 mV. The results also showed that rAHA and SPCR-HA had a greater redox potential than PHA and SHA.

4.2.2.4 E4/E6

The optical properties of the humic acids, which represent the absorbance value at 465 nm to the absorbance at 665 nm, are summarized in Table 4.3. The results showed that E4/E6 ratios for rAHA, PHA, and SPCR-HA ranged between 4.25 and 5.44 and the values increased in the order PHA < rAHA < SPCR-HA. These values are in good agreement with previous findings in the literature for lignite, peat, and sludge derived humic acids (Deiana et al., 1990, Gao et al., 1999). In contrast, it has been found that SHA exhibited a much higher ratio than those reported in the literature for soil humic acids. This ratio has been typically found to be approximately less than 6.0 (Tu et al., 1993, Campitelli et al., 2006, Kachari et al., 2015).

Table 4.3: Elemental composition, atomic ratios, redox potential, and E_4/E_6 of the humic acids.

Test	**rAHA**	**PHA**	**SHA**	**SPCR-HA**
C (%)[a]	59.92±2.79[d]	60.12±1.40	50.43±0.39	52.24±0.32
N (%)	1.75±0.40	2.51±0.07	6.50±0.07	5.04±0.04
H (%)	5.26±1.56	5.57±0.29	5.45±0.06	5.15±0.05
S (%)	0.41±0.24 (0.00-0.74)[e]	0.26±0.16 (0.00 -0.46)	0.30±0.27 (0.10 -0.67)	0.60±0.30 (0.20-0.81)
O (%)	32.66±0.35	31.53±1.92	37.32±0.54	36.97±0.60
H/C[b]	1.05	1.11	1.30	1.18
O/C	0.41	0.39	0.56	0.53
N/C	0.03	0.04	0.11	0.08
Redox potential (mV) at (24 ºC)	386	301	275	382
E4/E6[c]	4.88±0.03	4.25±0.16	10.25±0.49	5.44±0.12

[a] The elemental composition were calculated based on the moisture and ash free basis.

[b] H/C: atomic ratio of hydrogen to carbon. O/C: atomic ratio of oxygen to carbon. N/C: atomic ratio of nitrogen to carbon.

[c] Ratio of absorbance at 465 and 665 nm was determined following the method of Chen et al. (1977).

[d] Standard deviation

[e] Range of sulphur values

4.2.2.5 Inorganic Elements

The principal inorganic elements in the humic acids samples are summarized in Table 4.4. These elements include Al, Si, P, S, K, Ca, Ti, and Fe (Na was not detected by XRF). The total metal percentages of rAHA, PHA, SHA, and SPCR-HA were found to be 15%, 1.3%, 2.8%, and 6.6%, respectively. It was found that SPCR-HA contained the greatest Fe content comparing to the other humic acids.

Table 4.4: Major inorganic impurities of the humic acids determined by XRF.

Element (W %)[a]	rAHA	PHA	SHA	SPCR-HA
Al	3.55 ± 0.06[b]	0.18 ± 0.02	0.18 ± 0.01	0.23 ± 0.01
Si	7.22 ± 0.03	0.24 ± 0.01	0.13 ± 0.00	0.17 ± 0.00
P	0.02 ± 0.00	0.03 ± 0.00	0.31 ± 0.00	0.38 ± 0.01
S	0.76 ± 0.00	0.70 ± 0.00	0.66 ± 0.00	0.58 ± 0.00
K	0.51 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Ca	0.01 ± 0.00	ND[c]	0.05 ± 0.00	0.03 ± 0.00
Ti	0.52 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.05 ± 0.00
Fe	2.41 ± 0.01	0.05 ± 0.00	1.41 ± 0.02	5.14 ± 0.03

[a] Dry weight basis

[b] Standard deviation

[c] ND: non detectable

4.2.2.6 Acidic Functional Groups

The concentrations of the acidic functional groups analysed by barium hydroxide and Ca-acetate methods are shown in Table 4.5. It can be seen from the data that the total acidity content of all humic acids lay in a narrow range of values (6.4-6.7) meq/g. The highest content of the total acidity was found in

PHA and the lowest value was found in rAHA. The carboxylic groups content was noticeably higher in rAHA than that in the other humic acids. The highest content of phenolic groups was found in PHA, while the lowest value was found in rAHA. In general, it was observed that the phenolic groups content was higher than the carboxyl groups content in all humic acids samples.

The acidic functional groups were also obtained from the direct discontinuous base titration method (see Figure 4.1 and Table 4.5). The total acidity computed by this method was substantially lower than that obtained by barium hydroxide method. Furthermore, compared to the barium hydroxide and Ca-acetate methods, all humic acids had lower contents of phenolic groups and higher contents of carboxyl groups.

Table 4.5: Carboxylic, phenolic and total acidity of the humic acids (meq/g)

Functional groups	rAHA	PHA	SHA	SPCR-HA
Total acidity - $Ba(OH)_2$ method ①	6.4 ± 0.4[a]	6.7 ± 0.1	6.6 ±0.3	6.5±0.2
Carboxyl acidity - Ca-acetate method ②	3.1 ± 0.1	2.6 ± 0.0	2.6 ±0.1	2.9 ±0.2
Phenolic acidity (difference between① and ② above)	3.3	4.1	4.0	3.6
Total acidity - titration method (sum of ③ and ④b)	5.7	5.8	4.5	5.4
Carboxyl acidity - titration method ③	3.7	3.3	3.1	3.8
Phenolic acidity - titration method ④	2.0	2.5	1.4	1.6

Note: Carboxylic and phenolic acidity were calculated from the base titrations following Ritchie and Purdue (Ritchie and Perdue, 2003).

[a] Standard deviation.

Carboxylic acidity = Base consumption up to pH 8

Phenolic acidity= 2*(Base consumption between pH 8 and 10)

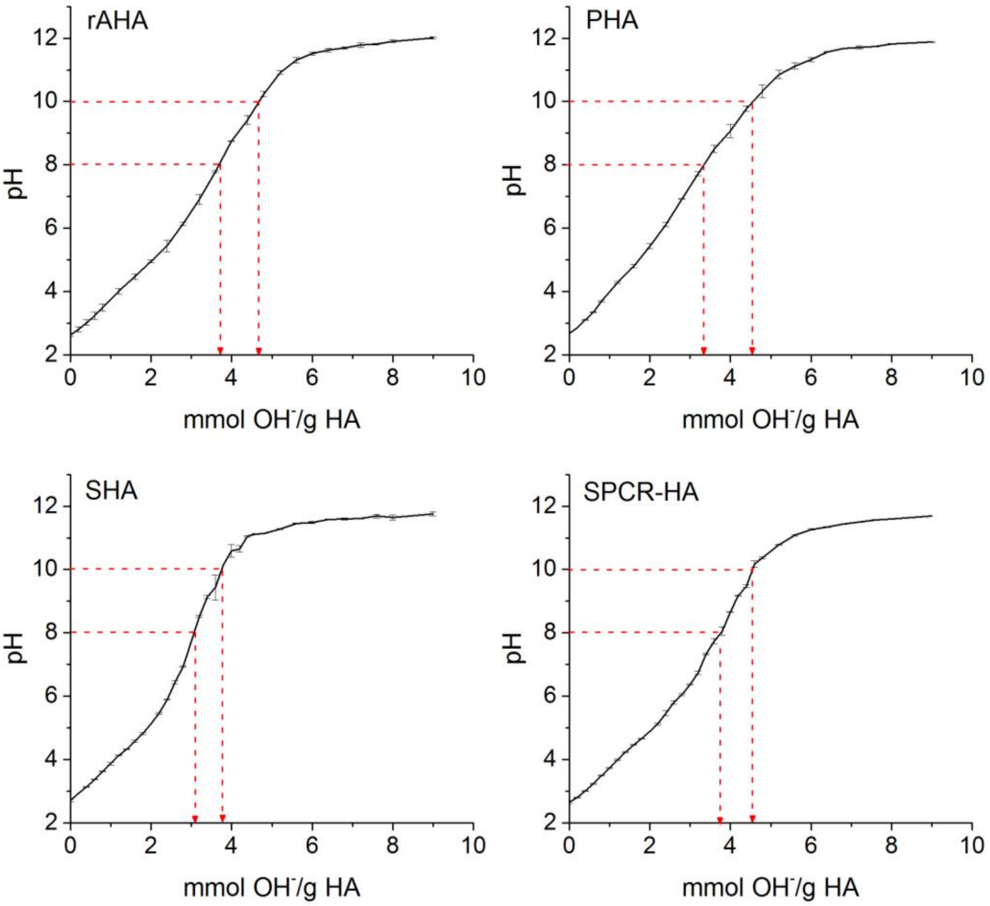

Figure 4.1: Base titration curves for rAHA, PHA, SHA, and SPCR-HA in 0.5N NaCl (error bars show the standard deviation of triplicate measurements and, where not visible, are less than the size of the symbols used).

4.2.2.7 Solid State ^{13}C-NMR

Figure 4.2 illustrates the ^{13}C-NMR spectra of the humic acids. The peak assignments for the carbon classes were determined and divided into four main groups at the following chemical shifts, 0-45 ppm (alkyl C), 45-110 ppm (O-alkyl C), 110-160 ppm (Aromatic C), and 160-220 ppm carbonyl C (Golchin et al., 1997, Kögel-Knabner, 2000). Moreover, the aromatic region were sub divided into subregions, which include aromatic C bonded to C/H (110-140 ppm) and to O (140-160 ppm) (Knicker et al., 2005). The contents of C-containing functional groups were identified semi-quantitatively from the relative peak areas of different carbon type. The total aromaticity was determined by dividing the peak areas of the total aromatic C (110-160 ppm) over the peak areas of the aliphatic and aromatic C (0-160 ppm) (Schnitzer et al., 1991).

The results that are summarized in Table 4.6 indicate that PHA, SHA, and SPCR-HA had a much larger content of O-alkyl C than rAHA. The relative proportion of aromatic C was between 25.0% and 38.7% and increased in the order SHA < SPCR-HA < PHA < rAHA. The sub-area associated with C-O (140-160 ppm) were also higher in AHA and PHA (10.1% and 9.6%, respectively) than that in SHA and SPCR-HA (7.4% and 9.1, respectively). Carbonyl carbon content ranged between 12.1-15.1%. It was more abundant in SHA than in other humic acids, while rAHA was characterized by the lowest content of carbonyl C. The degree of aromaticity was between 29.4% and 44.0% and increased in the order SHA < SPCR-HA < PHA < rAHA. However, all humic acids contained a larger content of aliphatic-C comparing to the aromatic-C.

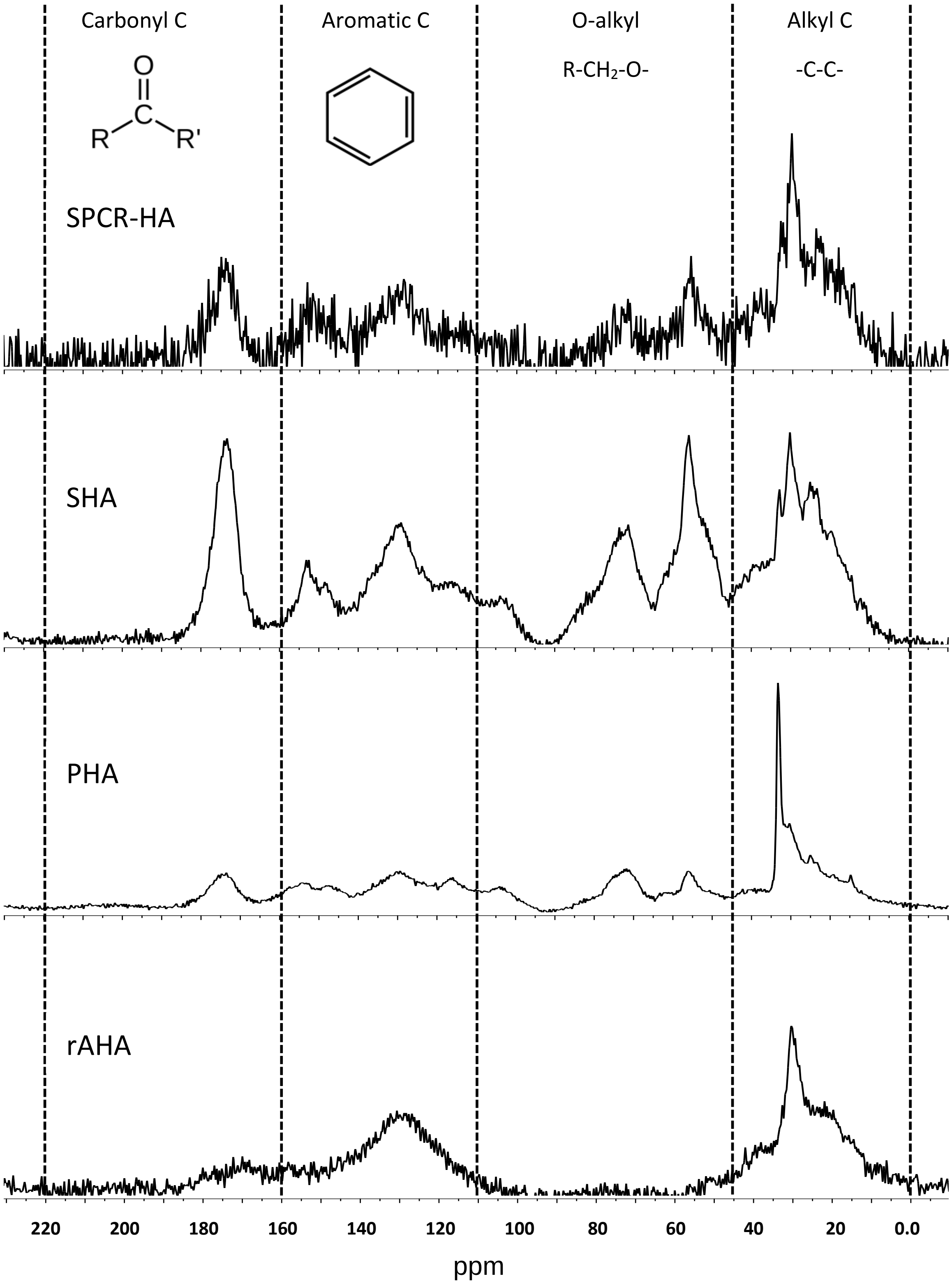

Figure 4.2: ^{13}C-NMR spectra of the humic acids at pH 3. Curves are normalised to an equal area under the curves. The spectra are operationally divided into chemical shift regions characteristic of different C bonding environments (Golchin et al., 1997, Kögel-Knabner, 2000).

Table 4.6: Proportion of different types of carbon in the humic acids determined by CP MAS ^{13}C-NMR. The spectra were operationally divided into chemical shift regions characteristic of different C bonding environments following Golchin et al. (1997) and Kögel-Knabner (2000).

Type of organic carbon (% of total area)	**rAHA**	**PHA**	**SHA**	**SPCR-HA**
Alkyl C (0-45 ppm)	47.1	33.9	28.7	39.3
O-alkyl C (45-110 ppm)	2.1	24.8	31.2	20.9
Aromatic C (110-160 ppm)	38.7	27.8	25.0	26.3
Carbonyl C (160-220 ppm)	12.1	13.5	15.1	13.5
Aromaticity (%)	44.0	32.1	29.4	30.4

4.3 Discussion

4.3.1 Humic acids' Parent Materials Characterization

The findings of the parent materials analysis, peat, soil, and SPCR, suggest that the content of organic matter varied widely with the source of the parent materials. Among these materials, peat can be considered the most valuable source of organic matter due to its high humic acid yield. In addition, the fractions of the humic acids relative to the organic contents also considerably varied. In peat and SPCR the humic acid fractions were within the expected range. It has been reported that about 20% of the solid organic material in peat is humic substances (Tipping, 2002). Moreover, it has been found that about 17% of the total organic carbon in sewage sludge is represented by humic carbon (Riffaldi et al., 1982). The amount of extracted humic acid from the soil sample can be considered very low compared to the average values were reported in the literature (Posner, 1966). It has been reported that the yield of humic acid that is extracted by NaOH from soil ranges between 5.7 to 6.9 g/kg soil (Posner, 1966). However, a low yield of humic acids has been reported in the literature for some types of soils such as the fine sandy loamy soil (1.7 and 0.8 g/kg at a depth of 10 and 20 cm, respectively). The low humic acid content in the soil sample, which was used in this study (moderate clay-loam soil), relative to the organic content might be related to the association of the humic acid with clay or humin fractions. As a result, the humic acid could not be completely extracted with a relatively low concentration of NaOH (0.5 N). There is, however, another possible explanation that might be related to the soil profile. The soil sample was collected at (0-20 cm) from the upper surface and probably the majority of the organic material was partially decomposed. Indeed, the low humic acids' yield from soils comparing to peat and SPCR may limit its use for broader implications as a reactive material.

4.3.2 Humic Acids Characterization

The chemical and spectroscopic characterization of the humic acids revealed significant differences between them. The ash content was much higher in AHA than other types of humic acids, which suggests that a high mineral content associated with AHA. Data of the inorganic impurities content also shows that AHA had a considerably higher percentage of the inorganic impurities than other humic acids.

The elemental composition and atomic ratios of the humic acids suggest a variation in their values from one source to another. In general, the high C and low O, H, and N contents in the humic acids have always been considered as an index for a high degree of humification (Stevenson, 1994). The results of this study found that rAHA and PHA contained more C and lower N and O than SHA and SPCR-HA (Table 4.3), which indicates a higher degree of humification of rAHA and PHA than SHA and SPCR-HA. The atomic ratios have been also used as an indicator of the degree of aromaticity and the maturity of humic matter (González-Vila et al., 1992, Abakumov et al., 2018). The lower values of H/C, O/C, and N/C for rAHA and PHA than SHA and SPCR suggest that the parent materials of rAHA and PHA are more stable and more humified than the younger age humic acids, SHA and SPCR-HA. Based on the atomic ratios of the humic acids, the average chemical formulas of rAHA, PHA, SHA, and SPCR-HA would be $C_{40}H_{42}O_{16}N$, $C_{40}H_{44.3}O_{15.7}N_{1.4}$, $C_{40}H_{51.9}O_{22.4}N_{4.4}$, and $C_{40}H_{47.4}O_{21.2}N_{3.3}$, respectively.

The E4/E6 ratio has been also used as an indicator of the humification, particle size, and molecular weight of humic substances (Chin et al., 1994). A negative correlation between E4/E6 and the degree of humification has been reported (Chen et al., 1977). In this study, comparing the E4/E6 values of the humic acids shows that rAHA and PHA were more humified and consequently had a higher molecular weight than SHA and SPCR-HA. This finding shows a good agreement between the E4/E6 ratios and the atomic ratios, as indicators of the degree of humification for humic substances.

The total acidity estimated by barium hydroxide and direct base titration methods indicates that all humic acids (except SHA estimated by titration method) have approximately similar total acidity (per unit mass of the total material). This finding is contrary to the expectations for humic acids that were extracted from different origins. The insignificant differences in the total acidity between the humic acids are probably due to the ash content. The ash content is often a result of amorphous silica and aluminosilicate impurities attached with the humic acid (Tan, 1977). These minerals have a pH buffering effect, which may cause an increase in the total acidity. As a result, it has been expected that the actual total acidity of rAHA, SHA, and SPCR-HA (ash content: 18.0%, 7.2%, and 10.4%, respectively) are likely to be lower than their estimated values. The carboxylic groups that were estimated by both methods, barium hydroxide and direct base titration methods indicate that rAHA and SPCR-HA have a higher content of carboxylic groups than PHA and SHA. The phenolic groups that were also estimated by the two methods reflect an increase of the phenolic groups' contents in two different order. However, in both methods, PHA has the highest phenolic content. In fact, the differences between the humic acids in their carboxylic and phenolic acidity suggest that the available binding sites in each humic acid may differ appreciably with the system's pH values.

The integrated areas of ^{13}C-NMR spectrum indicate significant differences between the humic acids in their content of C groups. The older material AHA and PHA, which were extracted from Miocene age lignite (6-26 Ma; Germany) and Holocene age peat (<12 ka, Ireland) respectively, exhibited a higher aromaticity than the younger materials, SHA and SPCR-HA. This finding shows an agreement with the atomic ratios. The correlation between the aromaticity and atomic ratios; H/C, O/C, and N/C (Figure 4.3) shows a good negative linear correlation; Pearson’s r equals -0.79, -0.62, and -0.75 respectively, suggesting the effect of the humic acids’ age on the degree of humification. Moreover, the ^{13}C-NMR spectra also showed that the sub-area associated with C-O (140-160 ppm), which includes phenols, was higher in AHA and PHA than that in SHA and SPCR-HA. As it has been confirmed that phenols have an ability to reduce Cr(VI) into Cr(III) (Elovitz and Fish, 1994, Elovitz and Fish, 1995), it would be expected that AHA and PHA may exhibit a higher ability to reduce Cr(VI) than SHA and SPCR-HA.

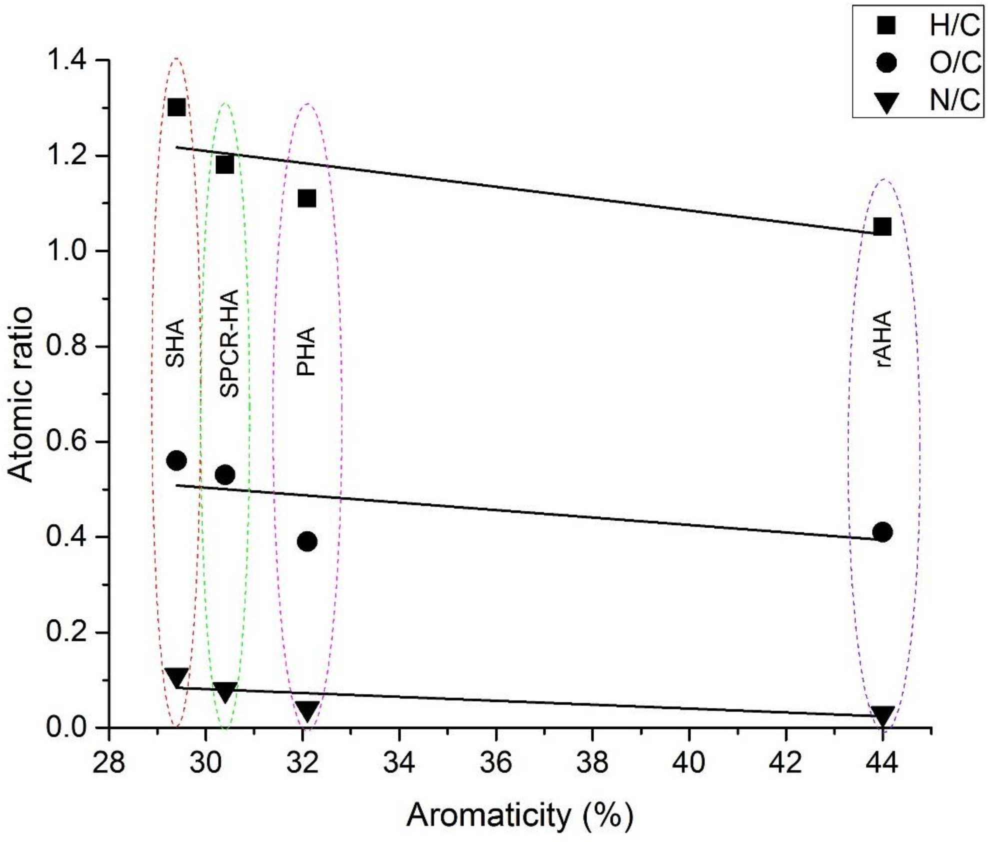

Figure 4.3: The relationship between the aromaticity and the atomic ratios of the humic acids.

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Chapter 5: Abiotic Reduction of Cr(VI) by Organic Compounds Representative of Soil Organic Matter in Aquatic System: Reaction Kinetics as a Function of pH

This aim of the experiments reported in this chapter is to investigate and compare the reaction of aqueous Cr(VI) with humic acids derived from different sources over a wide range of pH values. The study involves the determination of the rate of reaction as a function of solution pH, Cr speciation after the reaction with the humic acids, the maximum capacity of the humic acids under specific environmental conditions, and the stability of the formed complexes after the reduction of Cr(VI).

5.1 Results

5.1.1 The Rate Aqueous Cr(VI) is Removed from Solution with Humic Acids

5.1.1.1 The Effect of Initial pH on Cr(VI) Removal Rates by AHA

The initial pH values of AHA systems increased slightly during the first day but tended quickly towards stable values (Figure 5.1, A). The maximum increase of the pH values was observed at pH 3 and 5 and then at pH 7. Under alkaline conditions, the change in the initial pH values was minimal. The initial pH values were used to name the systems in this chapter.

The removal of Cr(VI) by AHA at a wide range of pH values is shown in Figure (5.2, A). At pH 3, Cr(VI) was removed completely from the solutions over a period of 16 days. About 50% of Cr(VI) was removed on the first day, while it took 15 days to remove the second half of aqueous Cr(VI). At pH 5, the majority of aqueous Cr(VI) was removed from the solutions (93% removal) over a period of 51 days. More than 50% of Cr(VI) was removed in the first 5 days, whereas it took 46 days to remove around 40% of aqueous Cr(VI). At pH 7, only 38% of Cr(VI) was removed from solutions over the entire period of experiments and the majority of removal occurred in the first 5 days. At pH 8.5, the removal of Cr(VI) was only 10% over the entire period of the experiment. At pH 9 and 11, Cr(VI) removal from solutions was not detected.

5.1.1.2 The Effect of Initial pH on Cr(VI) Removal Rates by PHA

Figure (5.1, B) shows the pH of the PHA systems. The initial pH values increased slightly during the first day only in the systems at pH ≤ 7. The removal of Cr(VI) by PHA is illustrated in Figure (5.2, B). Cr(VI) was completely removed from solutions at pH 3 and 5 in 1 and 2 days, respectively. In these systems, the majority of Cr(VI) was removed in the first 4 hours. At pH 7, Cr(VI) was also completely removed from solutions, but at a slower rate. Under alkaline conditions, pH 8.5 and 9, the majority of Cr(VI) was removed from solutions (95% and 85% removal, respectively) during 51 days. Similar to the previous systems, the reaction was rapid in the early period of the experiment and then it became slower with time. Under hyper alkaline conditions, pH 11, although six replicates were used, there was no clear pattern for the behavior of Cr and the percentage of the removed aqueous Cr(VI) varied between 12% and 100%, and the average was 57%.

5.1.1.3 The Effect of Initial pH on Cr(VI) Removal Rates by SHA

The pH values of SHA systems increased slightly during the first day in the systems at pH ≤ 7 (Figure 5.1, C). The removal of Cr(VI) is shown in Figure (5.2, C). It is apparent from this Figure that Cr(VI) was completely removed at pH 3 and pH 5 in 5 and 15 days, respectively. At pH 7, approximately 80% of Cr(VI) was removed during the entire period of the experiment. Under alkaline condition (pH 8.5 and 9), 45% and 35% of Cr(VI) was removed from solutions, respectively. Under hyper alkaline conditions (pH 11), no removal of Cr(VI) from solutions was detected. In all systems where Cr(VI) was removed, the reaction was rapid at the beginning of the experiment (in the first 2 days) and then noticeable decreases of Cr(VI) removal was observed with time.

5.1.1.4 The Effect of Initial pH on Cr(VI) Removal Rates by SPCR-HA

Figure (5.1, D) shows the changes in pH during (SPCR-HA)-Cr(VI) experiment. Based on this figure, the initial pH values increased in all systems at pH ≤ 7 on the first day of the experiment and then the values tended to be stable. The removal of Cr(VI) by SPCR-HA is illustrated in Figure 5.2, D. Cr(VI) was completely removed from solutions at pH 3 and 5 in 2 days. At pH 7, Cr(VI) was also completely removed from the solutions but it took about 16 days. At pH 8.5 and 9, the removal of Cr(VI) was approximately 85% and 60%, respectively. At pH 11, the removal of Cr(VI) was not detected. In all systems where Cr(VI) was removed from the solutions, the removal of Cr(VI) took place rapidly in the early period of reaction and then decreased with time.

5.1.1.5 Comparison of the Reactivity of the Humic Acids as a Function of pH

From the data in Figure 5.1, it is apparent that the initial pH values in all systems increased slightly in solutions at pH ≤ 7 during the first day of the experiments and then became stable. A comparison of the reactivity of the humic acids over a pH range of 3-11 (Figure 5.2) reveals that Cr(VI) was removed from solutions by all humic acids in a similar manner over time, the removal of Cr(VI) increased with decreasing the pH values. Under acidic conditions, Cr(VI) was completely removed (i.e.< 6% remaining in AHA system at pH 5) by all humic acids but at different rates. It was found that Cr(VI) removal from solutions by PHA and SPCR-HA was much faster than by AHA and SHA. At pH 7, Cr(VI) was completely removed from solutions only by PHA and SPCR-HA (taking ~15 days). At the same pH value, it was found that Cr(VI) was partially removed by SHA and AHA (80% and 38% removed, respectively). Under alkaline conditions, the rates of reaction were significantly decreased. At pH 8.5, Cr(VI) was extensively removed only by PHA and SPCR-HA (95%, 85% removed after 51 days, respectively), while the removal was much lower by AHA and SHA (10%, 45%, respectively) over the entire period of experiments.

At pH 9, there was partial Cr(VI) removal by PHA, SPCR-HA, and SHA (85%, 60%, and 35%, removed, respectively), while Cr(VI) removal by AHA could not be detected at this pH value.

5.1.2 Effect of Initial Cr(VI) Concentration on the Cr(VI) Removal Rate

5.1.2.1 The Effect of Initial Cr(VI) Concentration on the Cr(VI) Removal Rate by AHA

In AHA-550 µM Cr(VI) system, the initial pH values of the solutions at pH ≤ 7 slightly increased during the first 24 hrs and then tended to remain nearly stable (Figure 5.3, A). The pH values of these solutions increased by only about 0.5 of a pH unit. Figure (5.4, A) shows the removal of 550 µM Cr(VI) by AHA over the pH range 3-11. It was found that Cr(VI) was completely removed at pH 3 and 5 over 5 and 18 days, respectively. At pH 7, 60% of Cr(VI) was removed after 51 days. At pH 8.5, only a small amount of Cr(VI) was removed (~10%). Above pH 8.5, the removal of Cr(VI) by AHA was not detected. As Figure (5.4, A) shows, at the pH values 3, 5, and 7, there was rapid removal of Cr(VI) at the beginning of the reaction and then the reaction proceeded at a slower rate.

5.1.2.2 The Effect of Initial Cr(VI) Concentration on the Cr(VI) Removal Rate by PHA

In PHA-2200 µM Cr(VI) system, the initial pH values of the solutions at pH 3 and 5 increased significantly during the first 2 days and then tended to remain nearly stable (Figure 5.3, D). The pH values of pH 3 solutions increased by 2 pH units, while the pH value of pH 5 increased by 1.2 pH units. The initial pH values of the solutions at pH 7 was also increased by about a pH unit, but that took about 16 days to get a stable pH value. Figure (5.4, D) shows the removal of 2200 µM Cr(VI) by PHA over the pH range 3-11. Cr(VI) was completely removed at pH 3 and 5 in 11 days. At pH 7, 85% of Cr(VI) was removed after 51 days. At pH 8.5 and 9, Cr(VI) was partially removed from the solutions and the percentages were 44% and 32%, respectively. At pH 11, only 11% of Cr(VI) was removed during the experiment.

5.1.2.3 Comparison of the Reactivity of the Humic Acids as a Function of Cr(VI) Concentration

Comparing the results of AHA-550 µM Cr(VI) and AHA-1100 µM Cr(VI) (Figure 5.3, A and B) indicate that in both systems, the initial pH values drifted during the first day at pH ≤ 7 and then tended to remain nearly stable, and the drift was slightly larger at the higher Cr(VI) concentration. For example, in AHA-550 µM Cr(VI) experiments, the pH value increased from 3 to 3.4 while it increased from 3 to 4.1 in 1100 µM Cr(VI) experiment.

The results of the Cr(VI) removal (Figure 5.4, A and B) show a complete removal of Cr(VI) by both systems only at pH 3, but it was much faster at a lower initial concentration of Cr(VI). At pH 5, Cr(VI) was completely removed in the AHA-550 µM Cr(VI) system in 18 days, while it took 51 days to remove 93% of Cr(VI) in the AHA-1100 µM Cr(VI) system. At pH 7, Cr(VI) was partially removed in both systems, but the percentage of removal was higher in AHA-550 µM Cr(VI) system than that in AHA-1100 µM Cr(VI) system (60% and 38% removed, respectively). At pH 8.5, only 10% of Cr(VI) was removed in both systems. At pH 9 and 11, the removal of Cr(VI) by AHA was neither detected at 550 µM Cr(VI) nor at 1100 µM Cr(VI).

Similarly, comparing the results of PHA-1110 µM Cr(VI) and PHA-2200 µM Cr(VI) (Figure 5.3, C and D) indicates that over the pH range 3-7, the higher initial Cr(VI) concentration resulted in more pH drift in the acidic range than the lower concentration. For instance, the pH value increased from 3 to 3.7 in 1100 µM Cr(VI) system, while it increased from 3 to 5 in the 2200 µM Cr(VI) system. Figure 5.4, C and D shows that the rate of reaction was much greater at the lower concentration of Cr(VI) (1100 µM) than that at the higher concentration of Cr(VI) (2200 µM). When the initial concentration of Cr(VI) was 1100 µM, Cr(VI) was completely removed at pH 3 and 5 in 1 and 2 days, respectively while it took 11 days in 2200 µM Cr(VI) system. At pH 7, Cr(VI) was completely removed after 16 days in 1100 Cr(VI) system, while it took 51 days to remove 85% of Cr(VI) in the 2200 µM Cr(VI) system. At pH 8.5 and 9, Cr(VI) was partially removed from both systems after 51 days and the percentages were 95% and 85% in 1100 µM Cr(VI) system and 44% and 32% in 2200 Cr(VI) system. At pH 11, there was no clear pattern for the removal of Cr(VI) in 1100 µM Cr(VI) system as mentioned above in section 5.1.2, while only 11% of Cr(VI) was removed in 2200 µM Cr(V) system.

5.1.3 Cr Association after Reaction with HAs

After the reaction between Cr(VI) and humic acids for 51 days, the partitioning of Cr between the aqueous and solid phases was determined as a function of pH. Moreover, speciation analysis of aqueous Cr was carried out.

5.1.3.1 Cr Association after Reaction Between 1100 µM Cr(VI) and AHA

Figure (5.5, A) shows the distribution of 1100 µM Cr after the reaction with AHA. At pH 3, the majority of Cr(VI) initially in the solution became associated with the solid phase, and no Cr(VI) was detected in the aqueous phase. A small amount of Cr was present in the solution at this pH value, and this was probably aqueous Cr(III) (aqueous Cr(III) equals the difference between total aqueous Cr and aqueous Cr(VI)). A similar pattern was observed at pH 5, although a small amount of Cr(VI) was also detected in the solution. At pH 7, there was a roughly equal distribution of Cr between the solid phase and free aqueous Cr(VI). At pH ≥ 8.5, there was very little Cr associated with the solid phase, and most Cr remained as Cr(VI) in the solution.

5.1.3.2 Cr Association after Reaction Between 1100 µM Cr(VI) and PHA

The data in Figure (5.5, B) indicates that at pH < 7, The majority of the Cr(VI) initially in the solutions was associated with the solid phase and no aqueous Cr(VI) was detected in these systems (although a small amount of Cr was present in the solution at pH 3 probably as aqueous Cr(III)). At pH 8.5 and pH 9, about 90% and 85% of Cr, respectively was associated with the solid phase, and the remaining Cr in the aqueous phase was present as Cr(VI). In the tests at pH 11, although six replicates were used, there was no clear pattern for the behaviour of Cr and the amount of aqueous Cr(VI) varied between 1% and 88%, and the average was 43%.

5.1.3.3 Cr Association after Reaction Between 1100 µM Cr(VI) and SHA

It is apparent from Figure (5.5, C) that the majority of the Cr(VI) initially in solution was associated with the solid phase, in the systems with pH < 7, and no free aqueous Cr(VI) was detected in these systems. The Cr that remained in the aqueous phase at pH 3 (23%) is inferred to be Cr(III), as is the small amount of Cr that remained in solutions at pH 5 (7%). In the system at pH 7, a large proportion of Cr was associated with the solid phase, and about 20% of aqueous Cr(VI) remained in solution. In the system at pH 8.5, there was an equal distribution of Cr between the solid phase and free aqueous Cr(VI). At pH 9 there was more than 60% of Cr remained as aqueous Cr(VI) in solution. In the system at pH 10.5 most Cr remained as aqueous Cr(VI) in solution.

5.1.3.4 Cr Association after Reaction Between 1100 µM Cr(VI) and SPCR-HA

In this system, the majority of the Cr(VI) initially in solution is transferred to the humic acid in all tests with pH < 8.5, and no free aqueous Cr(VI) was detected in the solutions (Figure 5.5, D). The Cr that remained in solution at pH 3 (23%) is inferred to be Cr(III), as is the small amount of Cr that remained in solutions at pH 5 and 7. In the systems at pH 8.5, most of the Cr was associated with the solid phase, but a small amount of aqueous Cr(VI) remained in solution (~16%). At pH 9 there was approximately an equal distribution of Cr between the solid phase and free aqueous Cr(VI). However, in the systems at pH 10.5, there was very little Cr associated with the solid phase, and most Cr remained as Cr(VI) in solution.

5.1.3.5 Cr Association after Reaction Between 550 µM Cr(VI) and AHA

Figure (5.6, A) shows that at pH 3 and 5, the majority of Cr(VI) initially in the solutions were associated with the solid phase, and no Cr(VI) was detected in the aqueous phase. About 20% of Cr was detected in the solutions at pH3, which was probably as Cr(III). At pH 7, the majority of Cr(VI) initially in the solution was associated with the solid phase. Above pH 8.5, there was very little Cr associated with the solid phase, and most Cr remained as Cr(VI) in the solution.

5.1.3.6 Cr Association after Reaction between 2200 μM Cr(VI) and PHA

Figure (5.6, D) shows that at pH ≤ 5 the majority of the Cr(VI) initially in the solutions was associated with the solid, and no aqueous Cr(VI) was detected in these systems. A small amount of Cr was present in the solution at pH 3 (5%) was attributed to aqueous Cr(III). At pH 7, Cr was mainly associated with the solid phase and a small amount of Cr remained in the solution as Cr(VI) (14%). At pH 8.5 and pH 9, the percentage of Cr associated with the solid phase were 55% and 42%, respectively and the remaining Cr in the aqueous phase was present as Cr(VI). At pH 11, only 15% of Cr was associated with the solid phase and the majority of Cr remained in the aqueous phase as Cr(VI).

5.1.3.7 Comparison of Cr Distribution after the Reaction with the Humic Acids as a Function with pH

The speciation analysis of Cr shows that the majority of Cr was associated with the humic acids at pH 3 in all systems (Figure 5.5). The remaining Cr in the solutions is inferred to be Cr(III). The amount of Cr(III) in the solutions was higher in SHA and SPCR-HA (~20%) than that in the AHA and PHA (~10%) systems. At pH 5, the majority of Cr was associated with the solid phase in all systems, although a small amount of Cr(VI) existed in AHA solutions. The remaining Cr in these systems existed as a free Cr(III) in the solutions. At pH 7, more than 90% of Cr was associated with PHA, SHA, and SPCR-HA, while only 50% of Cr was associated with AHA and the remaining Cr was exist as aqueous Cr(VI). At pH 8.5, it was found that the highest percentage of Cr associated with the humic acids were with PHA, while the lowest percentage was with AHA. At pH 9 and 11, the order of the humic acids according to their increasing ability to bind Cr was AHA < SHA < SPCR-HA < PHA.

5.1.3.8 Comparison of Cr Distribution after the Reaction with AHA and PHA as a Function with Initial Cr(VI) Concentration

In AHA systems (550 µM Cr(VI) and 1100 µM Cr(VI)), it was found that the percentages of Cr associated with AHA under acidic conditions were similar regardless of the initial concentration of Cr(VI) (Figure 5.6, A and B). However, the proportion of Cr in the aqueous phase in these systems (inferred to be aqueous Cr(III)), was higher in the 550 µM Cr(VI) system than that in the 1100 µM Cr(VI) system, although the inferred aqueous Cr(III) concentrations were similar. At pH 7, it was found that 85% of Cr bound with AHA when the initial concentration of Cr(VI) was 550 µM Cr(VI), while there was a roughly equal distribution of Cr between the solid phase and free aqueous Cr(VI) in 1100 µM Cr(VI) system. Under alkaline conditions, the percentages of Cr associated with AHA were low in both systems, although it was slightly higher in the 550 µM Cr(VI) system than that at 1100 µM Cr(VI) system at pH 8.5 and 9.

In the PHA systems (Figure 5.6, C and D), the majority of the Cr(VI) initially in the solutions became associated with the solid phase in both systems, 1100 µM Cr(VI) and 2200 µM Cr(VI), with pH ≤ 5, and no aqueous Cr(VI) was detected in these systems. A small amount of Cr was present in the solution at pH 3 and is inferred to be aqueous Cr(III) and the proportion of the initial concentration was nearly two times higher in 1100 µM Cr(VI) system than that in 2200 µM Cr(VI) system (i.e. the aqueous Cr(III) concentrations were similar). At pH 7, Cr was completely associated with the solid phase in the 1100 µM Cr(VI) system, while in the 2200 µM Cr(VI) system a small amount of Cr remained in the solution as Cr(VI) (14%). At pH 8.5 and pH 9, the majority of Cr was associated with the solid phase in 1100 µM Cr(VI) system (90% and 85%, respectively), while in 2200 µM Cr(VI) system about 55% and 42%, respectively, of Cr was associated with the solid phase. In both systems, the remaining Cr in the aqueous phase was present as Cr(VI). At pH 11, there was no clear pattern for the behaviour of Cr in the1100 µM Cr(VI) system, as illustrated in section 5.1.1.2, whereas only 15% of Cr was associated with the solid phase in 2200 µM Cr(VI) system.

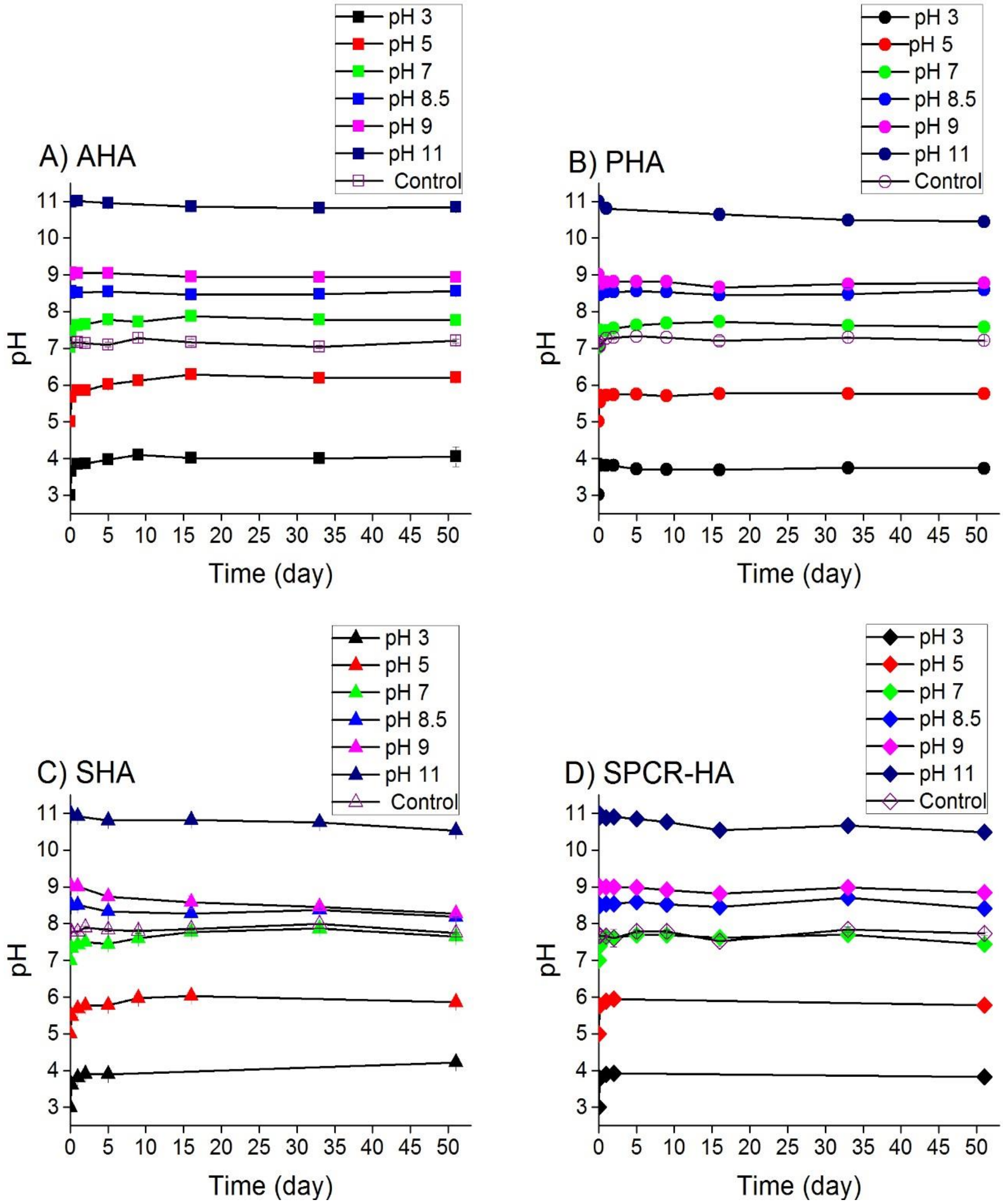


Figure 5.1: A, B, C, and D pH vs. time for AHA-Cr(VI), PHA-Cr(VI), SHA-Cr(VI), and (SPCR-HA)-Cr(VI) suspensions, respectively. [Cr(VI)] added initially: 1,100 µM, [HA]: 1g /100 ml, reaction time: 51 days.

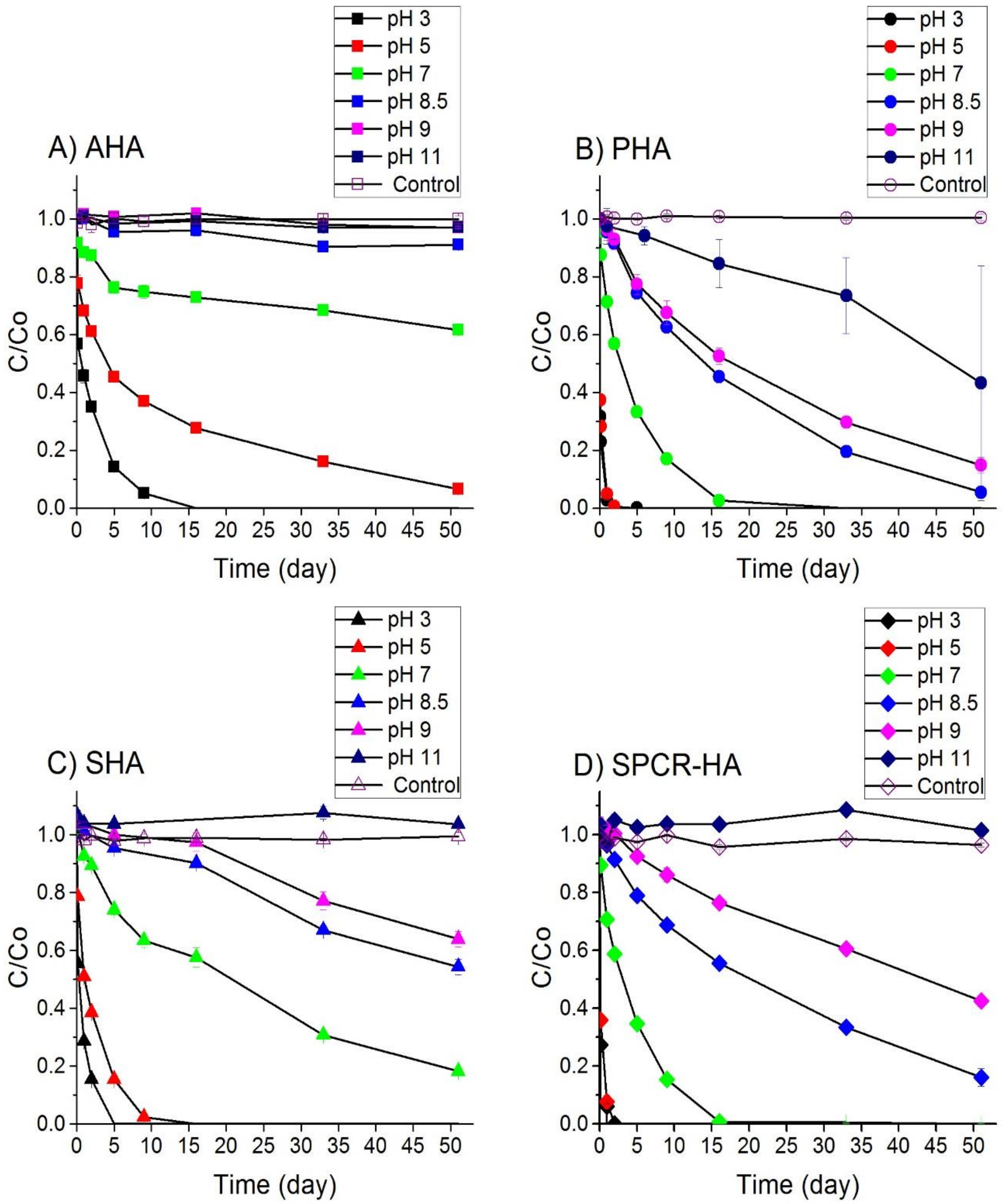


Figure 5.2: A, B, C, and D reduction of Cr(VI) by AHA, PHA, SHA, and SPCR-HA, respectively in aqueous solutions at various pH values. C = $[Cr(VI)]_t$, Co = $[Cr(VI)]o$ = 1,100 µM, [HA] = 1g/100 ml.

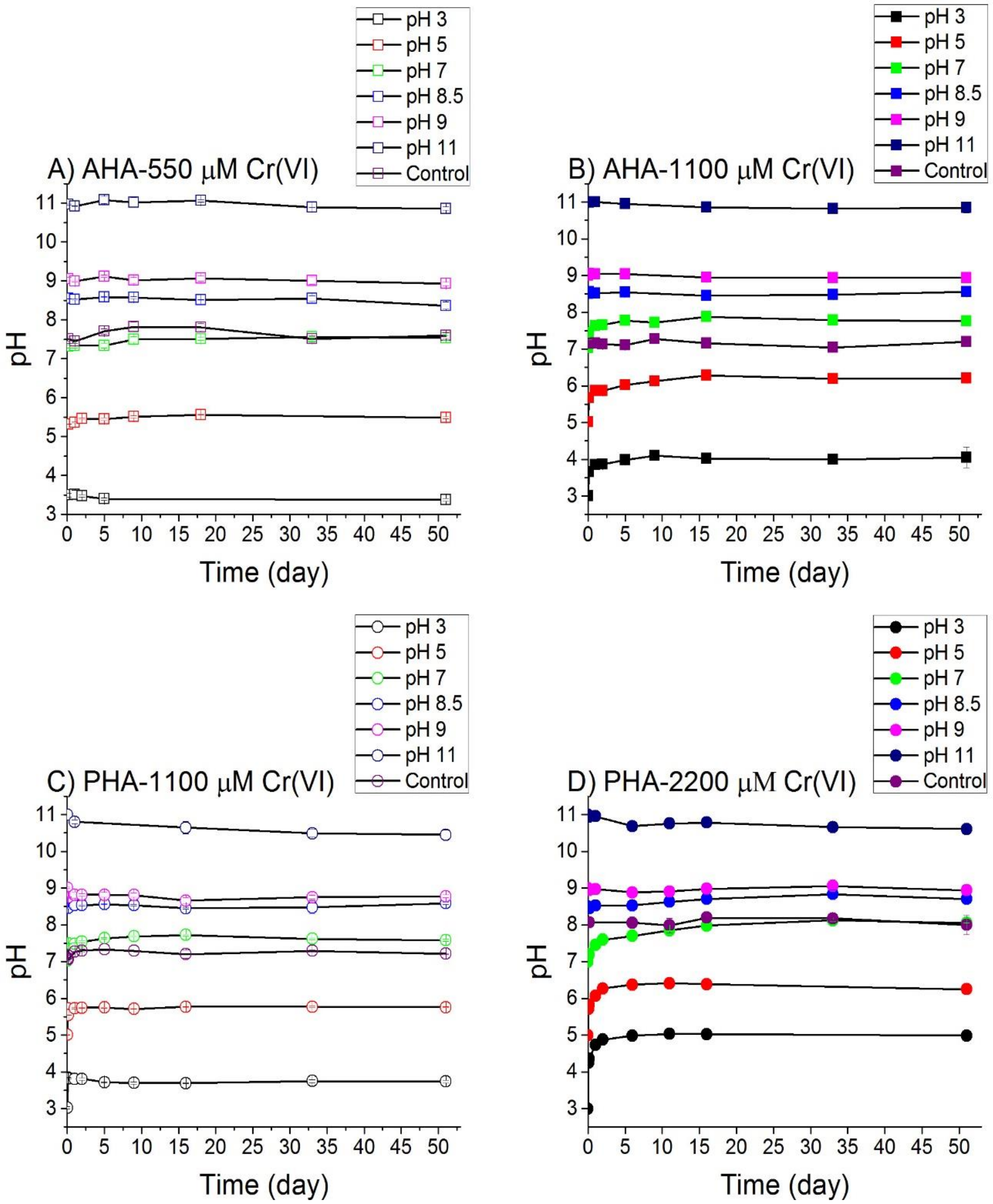


Figure 5.3: pH vs. time for A) AHA-550 µM Cr(VI), B) AHA-1,100 µM Cr(VI), C) PHA-1,100 µM Cr(VI), and D) PHA-2,200 µM Cr(VI) suspensions. [HA]: 1g/100 ml, reaction time: 51 days.

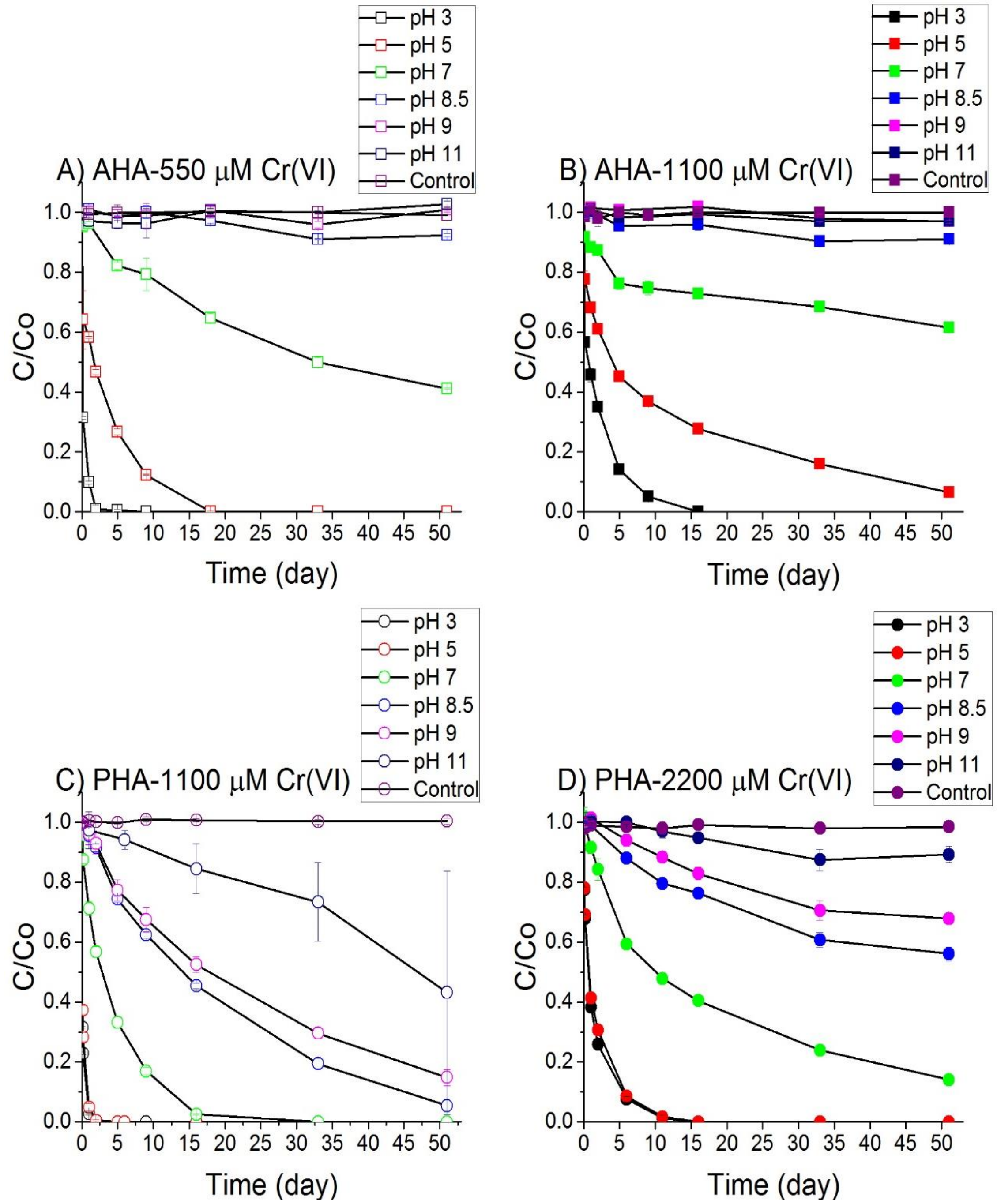


Figure 5.4: Effect of initial Cr(VI) concentration on its removal rate from aqueous solution using AHA and PHA. A) AHA-550 µM Cr(VI), B) AHA-1,100 µM Cr(VI), C) PHA-1,100 µM Cr(VI), D) PHA-2,200 µM Cr(VI), initial pH (3-11), [HA]: 1g/100 ml, reaction time: 51 days.

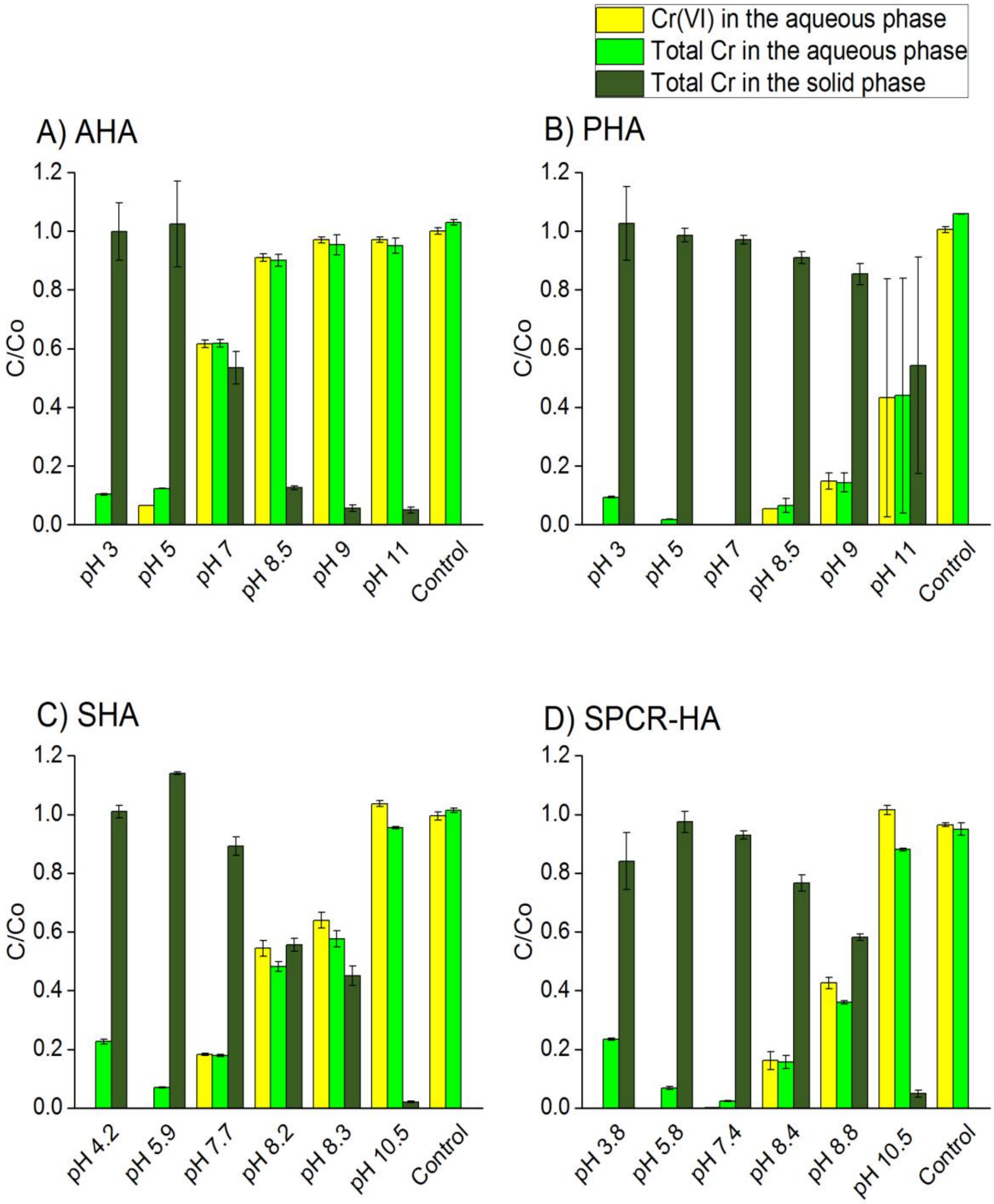


Figure 5.5: Speciation for A) AHA-Cr(VI), B) PHA-Cr(VI), C) SHA-Cr(VI), and D) (SPCR-HA)-Cr(VI) systems at different pH values. C/Co: Concentration of Cr(VI) in the aqueous phase, total Cr in the aqueous phase and total Cr in the solid phase to the concentration of Cr(VI) added initially. [Cr(VI)] added initially: 1,100 µM, [HA]: 1g /100 ml, reaction time: 51 days.

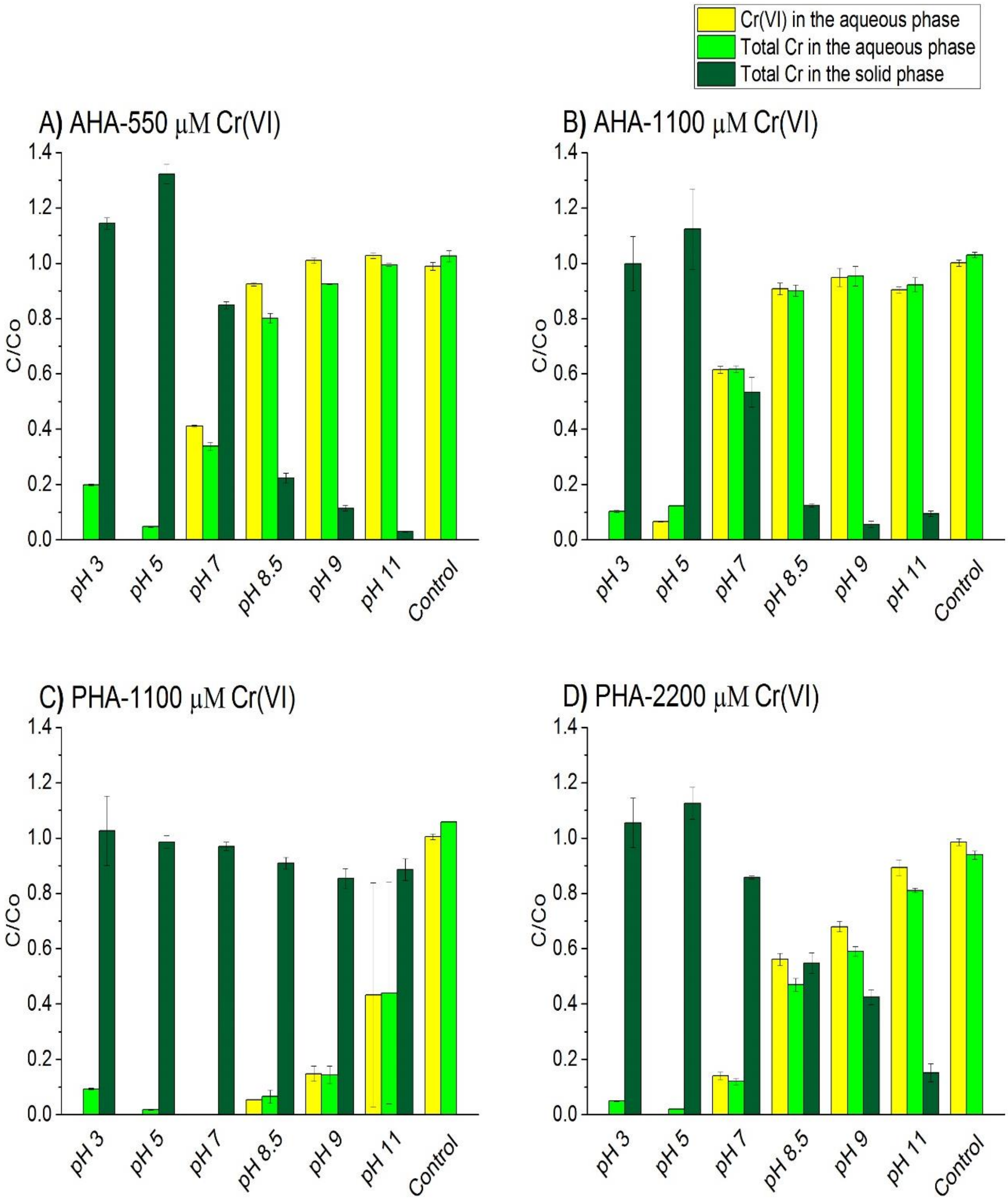


Figure 5.6: Speciation for A) AHA-550 µM Cr(VI), B) AHA-1,100 µM Cr(VI), C) PHA-1,100 µM Cr(VI), and D) PHA-2,200 µM Cr(VI) systems at different pH values. C/Co: Concentration of Cr(VI) in the aqueous phase, total Cr in the aqueous phase and total Cr in the solid phase to the concentration of Cr(VI) added initially. [HA]: 1g /100 ml, reaction time: 51 days.

5.1.4 Surface Loading Experiment

Figure (5.7, A) shows the loading capacities of the humic acids at initial pH 3 and Cr(VI) loading between 110 and 8,000 µmol/g HA. The values were calculated after the reactions of HAs-Cr(VI) reached the equilibrium state (˜30 days) as follow:

$$Removal\ capacity, \mu mol/g = (Co - Ce)\frac{V}{M}$$

Where:

C_o: the initial concentration of Cr(VI), µmol/l.

C_e: the equilibrium concentration of Cr(VI), µmol/l.

V: the solution volume, L.

M: the mass of humic acid, g.

As shown in Figure (5.7 A), at a concentration of 110 µmol/g HA Cr(VI) was removed completely by all humic acids as was illustrated previously in Section (5.1.1). In the range between 1,000 and 4,000 µmol/g HA, the removal capacity of Cr(VI) by PHA was (2-3) times greater than that for the other types of humic acids, while SHA showed the lowest capacity. Moreover, AHA and SPCR-HA showed nearly similar capacity over that range (1,000 – 4,000 µmol/g HA). At 8,000 µmol/g initial concentration, PHA also exhibited the highest removal capacity of Cr(VI), which was 1380 µmol/g HA, while at this initial concentration the removal capacity by other humic acids was only between 500 and 600 µmol/g.

After the reactions reached the equilibrium state, the pH values were measured (Figure 5.7, B) and it was found that in all systems the pH values increased significantly with the increase of initial Cr(VI) content. It raised up to around 7 in the systems containing 8,000 µmol Cr(VI) per g humic acid.

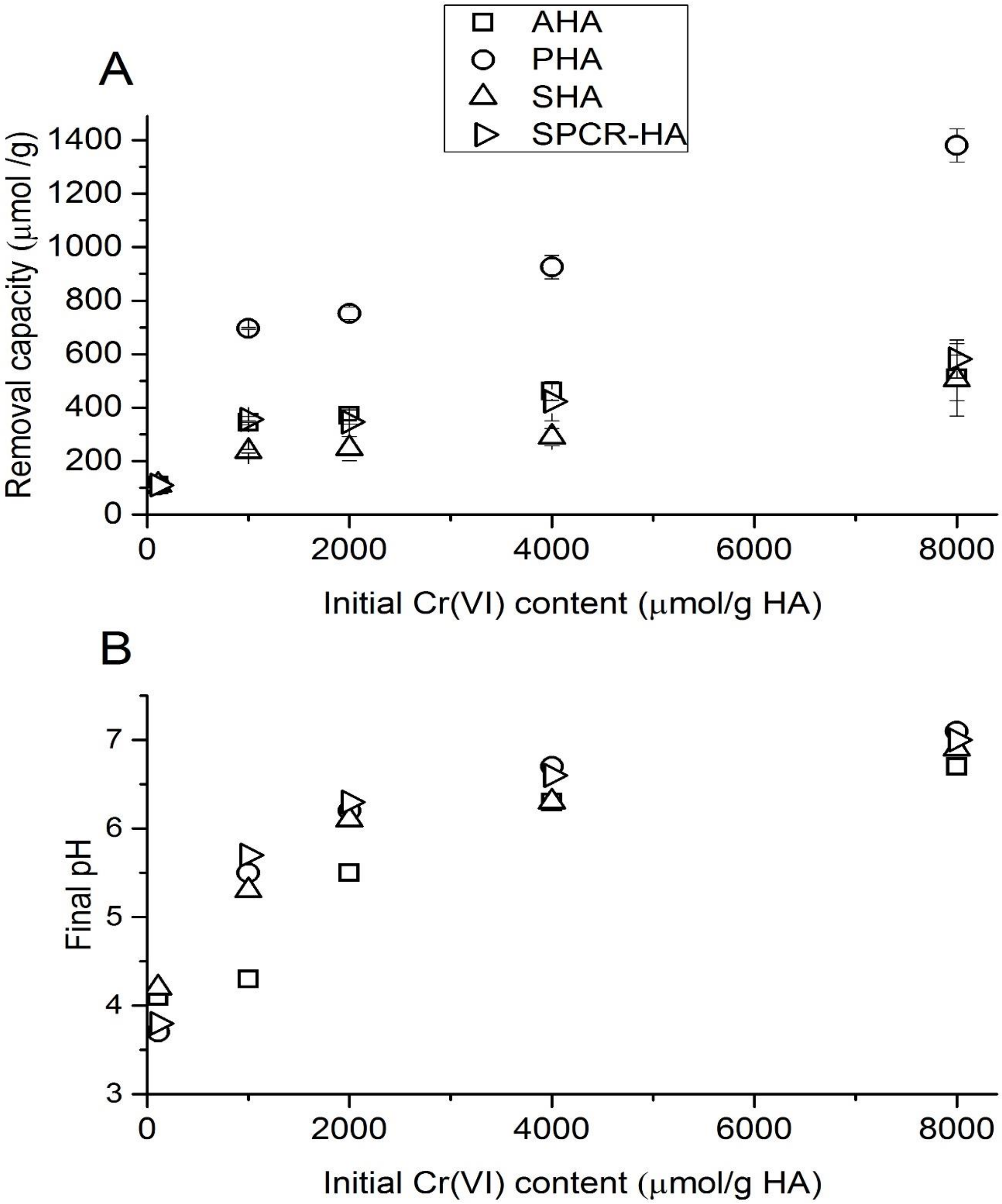


Figure 5.7: A) Removal capacity of the humic acids as a function of the initial Cr(VI) concentration. Initial pH = 3. Experiments duration ˜30 days. B) pH changes after the reactions reached the equilibrium state.

5.1.5 Stability of Cr(III)-Humic Acids Complexation

After the reaction between Cr(VI) and the humic acids at pH 3 reached the equilibrium state, the stability of the formed Cr(III)-HAs complexes were investigated and the results are summarized in Table 5.1. The results show that before adding $MgCl_2$ a small amount of Cr was present in all solutions, which was probably aqueous Cr(III). After adding $MaCl_2$, all humic acids samples exhibited a very little degree of leaching. The increasing of total Cr in the aqueous phase was between 0.2% and 1.4%. The highest value was in PHA sample, while the lowest value was in SHA.

Table 5.1: Chromium distribution before and after the addition of $MgCl_2$ into HAs-Cr(VI) systems. [Cr(VI)] added initially: 1,100 µM, [HA]: 1g /100 ml, [$MgCl_2$]: 11,000 µM, and initial pH = 3.

	AHA	PHA	SHA	SPCR-HA
% Aqueous total Cr before adding $MgCl_2$	9.9±0.3[a]	2.9±0.2	3.1±0.0	3.7±0.0
% Aqueous Cr(VI) before adding $MgCl_2$	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
% Total Cr associated with the solid phase before adding $MgCl_2$[b]	90.1	97.1	96.9	96.3
% Aqueous total Cr after adding $MgCl_2$	10.8±0.3	4.3±0.1	3.3±0.1	4.1±0.0
% Aqueous Cr(VI) after adding $MgCl_2$	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
% Total Cr associated with the solid phase after adding $MgCl_2$[c]	89.2	95.7	96.7	95.9

[a] Standard deviation.

[b, c] Total Cr associated with the solid phase was estimated by the difference between 100% and the percentage of aqueous total Cr.

5.2 Discussion

5.2.1 The Effect of Initial pH and Initial Concentration of Cr(VI) on Cr(VI) Removal by the Humic Acids

The kinetic results indicated that the rate of removal of Cr(VI) by all types of humic acids under investigation was pH dependent. This finding is consistent with findings of past studies by Fukushima et al. (1995), Wittbrodt and Palmer (1997), and Nakayasu et al. (1999) where the reduction of Cr(VI) by humic acids was tested under pH values ≤ 7. It has been proposed that $HCrO_4^-$ is the primary species of Cr(VI) that participate in the redox process, which is dominant at pH < 6.5 (Palmer and Puls, 1994). Although the reduction of Cr(VI) by humic acids decreases with pH, the results of this study confirmed that it can still occur in the pH range where CrO_4^{2-} is the predominant species (pH > 6.5) (Palmer and Wittbrodt, 1991) in proportions vary with the type of humic acids.

Figure 5.2 shows a non-linear removal of Cr(VI) with time. The early rapid decrease of Cr(VI) probably results from the reduction of Cr(VI) by the most easily oxidized functional groups (Wittbrodt and Palmer, 1995, Wittbrodt and Palmer, 1997). As the concentration of these groups decreases with reaction time, the reaction would be taken place with lower reactive functional groups than those at the early stage of the reaction.

The results of surface loading capacity in section (5.1.4), where the initial pH value was 3, indicate that the concentrations of the reactive functional groups in all humic acids were much higher than the concentration of Cr(VI) that was used in these experiments. If it is assumed that the reaction involves a single type of reactive functional group then it is appropriate to describe the data at each pH value using pseudo-first-order kinetics, relative to the Cr(VI) concentration. Accordingly, the rate law proposed in equation 5.1 can be simplified to a pseudo-first-order rate equation:

$$r = k'[Cr(VI)] \qquad (5.1)$$

Where:

k': is a pseudo-first-order rate constant for the specified pH value, which equals k x [initial concentration of HA's reactive functional groups].

Figure 5.8 shows a plot of the logarithmic concentration of Cr(VI) versus time and approximately a linear relationships were obtained for most fitted data. The rate constants and the half-life ($t_{1/2}$) were calculated for the humic acids at the pH values (final pH) where the removal of Cr(VI) was detected (Table 5.2 and Figure 5.9). The majority of the estimated R^2 values were > 0.9, which indicates that pseudo-first-order kinetic is a reasonable fit to the data. Graphs of the pseudo-first-order rate constants k' against pH value indicate that the rate constants decrease as the pH increases (Figure 5.9). If it assumed that the reaction is in some way mediated by protons, and therefore that $k' \propto [H^+]^n$, then the values of the exponent, n, are 0.48, 0.40, 0.59, and 0.48 for AHA, PHA, SHA, and SPCR-HA, respectively (Figure 5.10). This finding is in a good agreement with the results of Wittbrodt and Palmer (1995) and Wittbrodt and Palmer (1997), who found that the rate of Cr(VI) reduction by soil fulvic acid and soil humic acids are proportional to $[H^+]^{0.45}$ and $[H^+]^{0.5}$, respectively at pH values ≤ 7. The dependence of the reaction rates of all humic acids under investigation on the partial orders with respect to the protons suggests that the rate equations were not simple. It would be expected that over the pH range (3-11) the reactions took place by more than one pathway. A proton-dependent pathway is expected under acidic conditions and the rate equation would be a first order with respect to the protons. On the other hand, under alkaline conditions, a proton independent pathway is expected and consequently, the rate equation would be a zero order with respect to the protons. As the reduction of Cr(VI) by the humic acids occurred under acidic and alkaline conditions, it would be expected that the order changed between 1 and 0 and consequently, the overall rate dependence on protons was around 0.5. This finding would be compatible with Elovitz and Fish (1995) model, which suggests that the rate dependence of protons is well accounted by three redox pathways. At pH ≤ 2, two proton pathway is expected. A single proton pathway dominates at 2 < pH < 5. A proton independent pathway dominates at pH ≥ 5.

The reduction rate constants of Cr(VI) at different initial Cr(VI) concentration using AHA and PHA emphasize that the rate constant values decrease with increasing the initial concentration of Cr(VI) over a range of pH values (Figure

5.11, Figure 5.12, and Table 5.3). The rate constants in Table 5.3 shows that when the concentration of Cr(VI) was doubled at initial pH ≤ 5, in AHA and PHA systems, the rate constants were 7 to 8 times lower than that at a low concentration. It is interesting to note that this finding is contrary to thermodynamic expectations, increasing the concentration of Cr(VI) should have no impact on the rate constant. A possible explanation for these results may be the drifting of the initial pH values were higher at the higher concentration of Cr(VI). For example, the initial pH value was drifted from 3 to 3.7 in PHA-1100 µM Cr(VI) system, while it was drifted into pH 5 in the PHA-2200 µM Cr(VI) system. Figure (5.13) shows the rate constants versus the final pH values of AHA and PHA at the two concentrations. This figure illustrates that the high and low concentration tests follow roughly the same trend. The data of AHA fall close to a common trend line. For PHA there is a little more separation between the data, which shows the difficulty of evaluating the rate constant when the system pH is varying.

The speciation analysis of Cr after the reaction of Cr(VI) with the humic acids for 51 days shows that about 5% to 20% of total Cr added initially was present as a free aqueous Cr(III) at pH 3 and 5 (Figure 5.5). This finding indicates that the complexing ability of the humic acids for reduced Cr(III) is pH dependent, increasing with increasing pH. The present finding supports at a study by Fukushima et al. (1997), which found that under acidic conditions (pH 3.2 – 5.1) part of the reduced Cr(VI) by humic acid remained as a free aqueous Cr(III). Moreover, Fukushima et al. (1995) investigated the binding sites of the humic acids by comparing the FTIR spectra of a protonated humic acid and Cr(III)-HA complex. Their results suggest that carboxylate is the binding sites for Cr(III). As a result, it can be expected that at low pH, carboxylic groups are partially deprotonated and then limited negatively charged binding sites are available for Cr(III) species, which present as cations under acidic conditions (see Figure 2.1) and hence, decrease the binding ability of the humic acids.

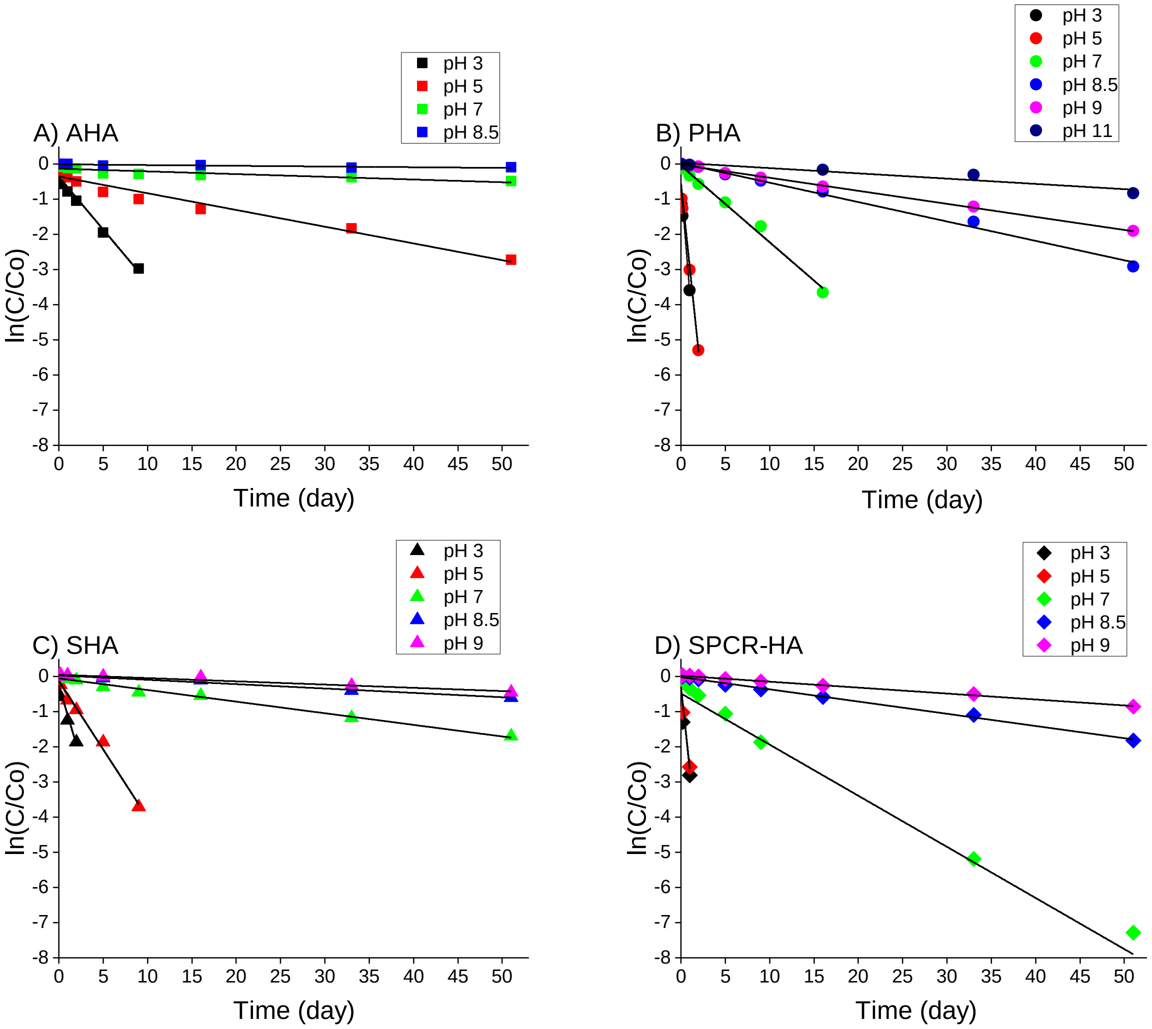


Figure 5.8: A, B, C, and D Pseudo-first-order rate plots for the AHA-Cr(VI), PHA-Cr(VI), SHA-Cr(VI), and (SPCR-HA)-Cr(VI), respectively. C = $[Cr(VI)]_t$, Co = $[Cr(VI)]_o$ = 1100 µM, [HA] = 1g/100 ml.

Table 5.2: Pseudo-first-order rate constants and half-lives for Cr(VI) reduction by AHA, PHA, SHA, and SPCR-HA at various pH values.

	Initial pH	Final pH	K´ (hr^{-1})	R^2	Half-life (hr)
AHA	3.0	4.1	1.25×10^{-2}	0.97	55.4
	5.0	6.2	2.00×10^{-3}	0.96	346.5
	7.0	7.8	3.00×10^{-4}	0.78	2310
	8.5	8.6	8.00×10^{-5}	0.83	8662
PHA	3.0	3.7	1.29×10^{-1}	0.91	5.4
	5.0	5.8	1.00×10^{-1}	0.97	6.9
	7.0	7.6	9.10×10^{-3}	0.99	76.2
	8.5	8.6	2.30×10^{-3}	0.99	301.3
	9.0	8.8	1.50×10^{-3}	0.99	462
	11.0	10.5	6.00×10^{-4}	0.92	1155.2
SHA	3.0	4.2	1.03×10^{-1}	0.90	6.7
	5.0	5.9	1.63×10^{-2}	0.99	42.5
	7.0	7.7	1.38×10^{-3}	0.99	502.3
	8.5	8.2	5.17×10^{-4}	0.98	1340.7
	9.0	8.3	3.85×10^{-4}	0.96	1800.4
SPCR-HA	3.0	3.8	1.04×10^{-1}	0.90	6.72
	5.0	5.8	9.58×10^{-2}	0.94	7.20
	7.0	7.4	5.96×10^{-3}	0.99	116.6
	8.5	8.4	1.45×10^{-3}	0.99	478.1
	9.0	8.8	4.96×10^{-4}	0.98	1398

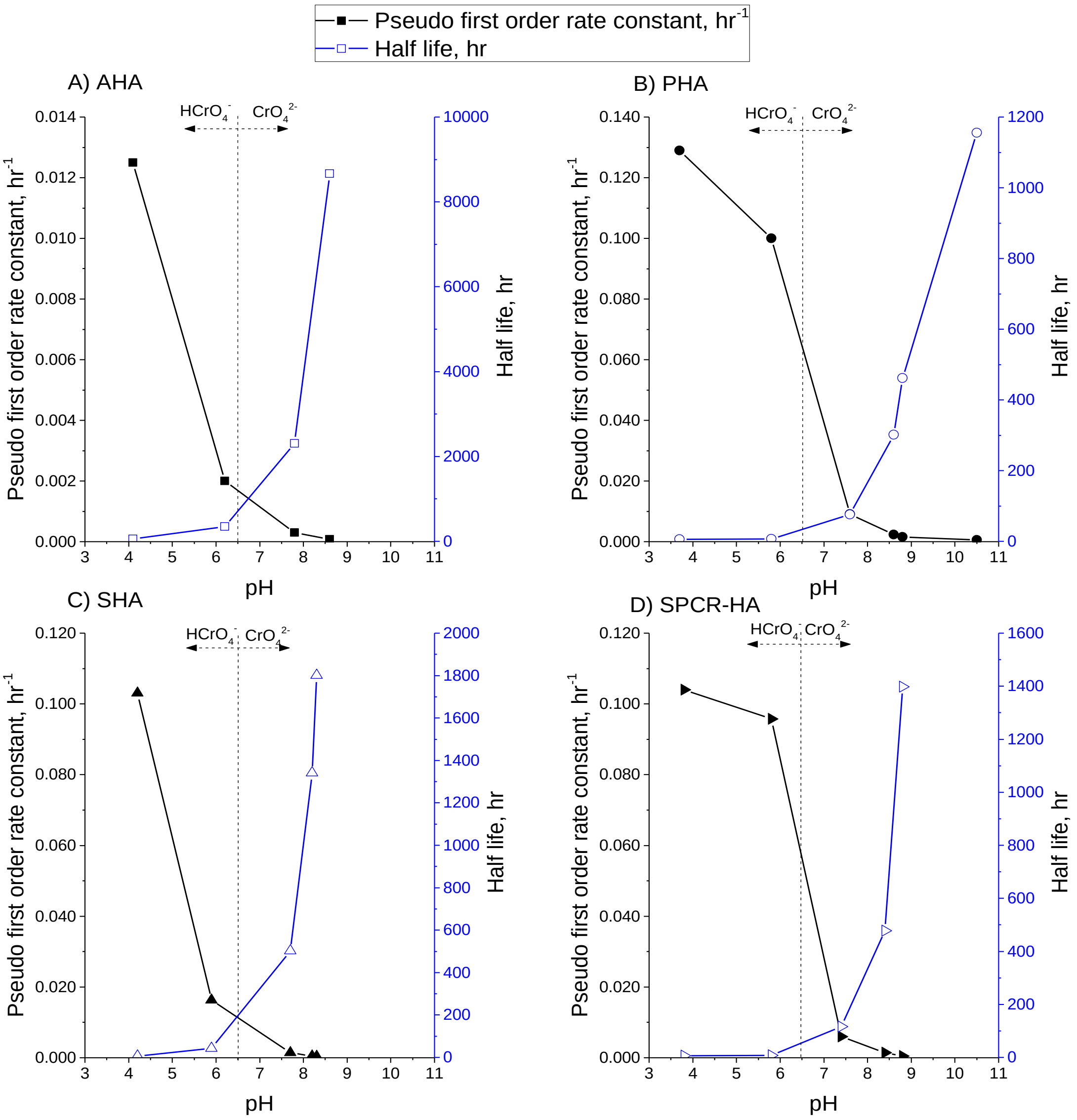


Figure 5.9: Pseudo-first-order rate constants and half-lives for the reduction of Cr(VI) by A) AHA, B) PHA, C) SHA, and D) SPCR-HA. Reactions contained 110 µmol Cr(VI)/g of HA. The dashed line indicates the pH value below and above which $HCrO_4^-$ and CrO_4^{2-} are the dominant aqueous Cr(VI) species, respectively (Pourbaix, 1966).

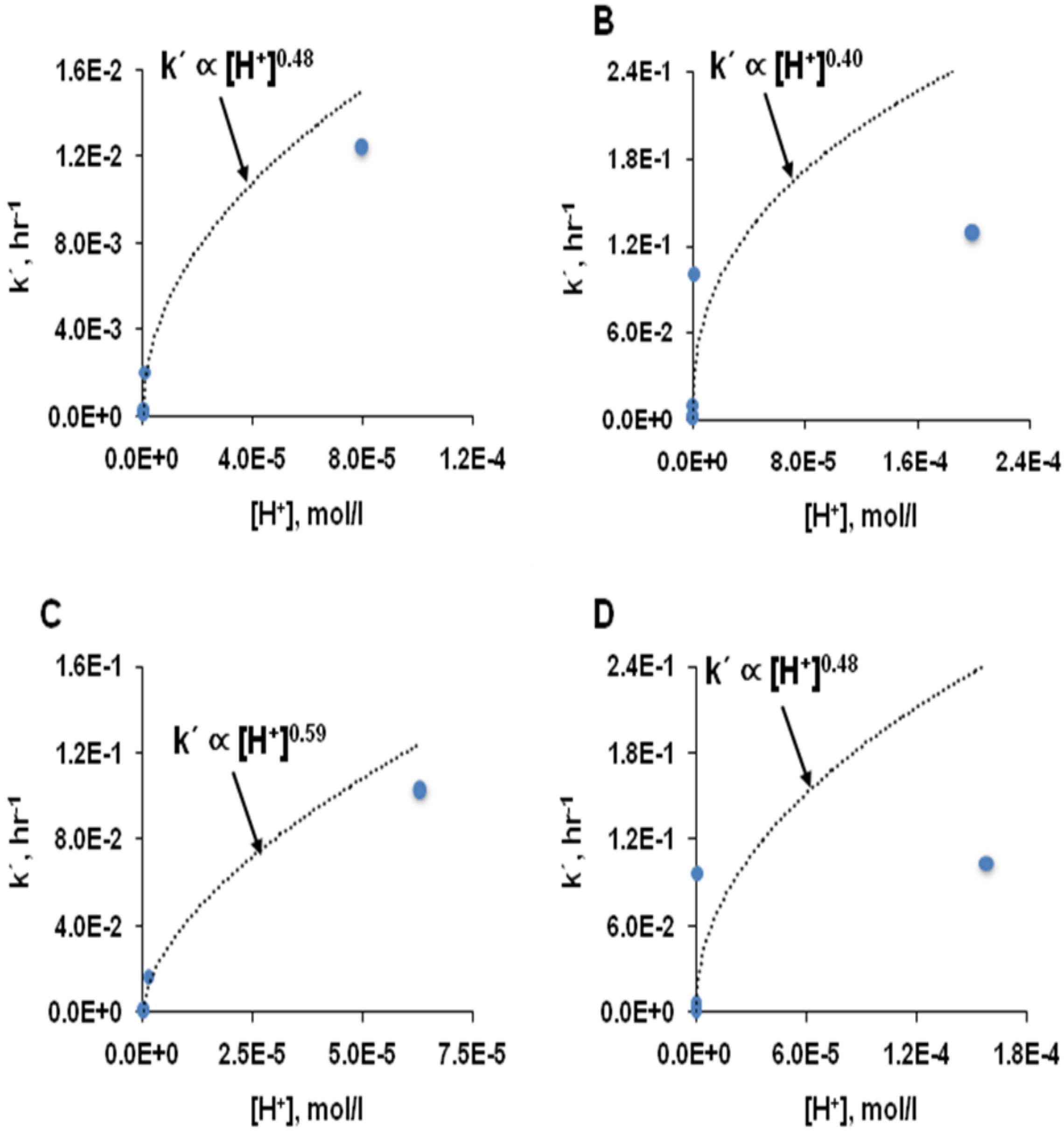


Figure 5.10: Pseudo-first-order rate constants versus [H^+] for A) AHA-Cr(VI), B) PHA-Cr(VI), C) SHA-Cr(VI), and D) (SPCR-HA)-Cr(VI).

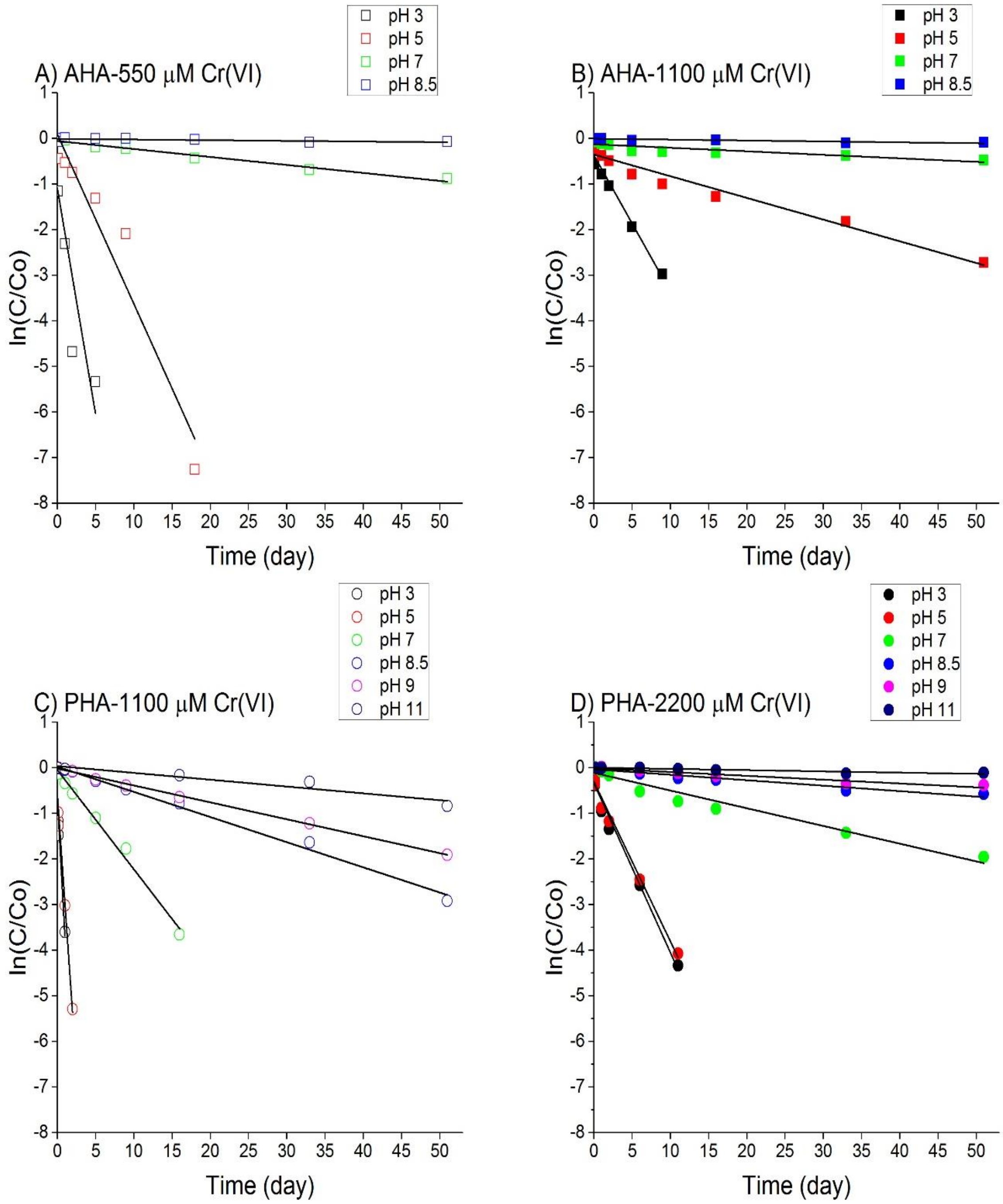


Figure 5.11: A, B, C, and D Pseudo-first-order rate plots for the AHA-550 µM Cr(VI), AHA-1100 µM Cr(VI), PHA- 1100 µM Cr(VI), and PHA- 2200 µM Cr(VI), respectively. C = $[Cr(VI)]_t$, Co = [Cr(VI)]o, and [HA] = 1g/100 ml.

Table 5.3: Rate constants and half-lives for AHA-550 µM Cr(VI), AHA-1100 µM, PHA-1100 µM Cr(VI), and PHA-2200 µM Cr(VI) at various pH values.

	Initial	Final pH	K (hr^{-1})	R^2	Half-life
AHA-550 µM Cr(VI)	3.0	3.4	8.95×10^{-2}	0.97	7.8
	5.0	5.5	1.55×10^{-2}	0.94	46.2
	7.0	7.5	7.29×10^{-4}	0.98	950.8
	8.5	8.4	7.54×10^{-5}	0.84	9192.9
AHA-1100 µM Cr(VI)	3.0	4.1	1.25×10^{-2}	0.97	55.4
	5.0	6.2	2.00×10^{-3}	0.96	346.5
	7.0	7.8	3.00×10^{-4}	0.78	2310
	8.5	8.6	8.00×10^{-5}	0.83	8662
PHA-1100 µM Cr(VI)	3.0	3.7	1.29×10^{-1}	0.91	5.4
	5.0	5.8	1.00×10^{-1}	0.97	6.9
	7.0	7.6	9.10×10^{-3}	0.99	76.2
	8.5	8.6	2.30×10^{-3}	0.99	301.3
	9.0	8.8	1.50×10^{-3}	0.99	462.0
	11.0	10.5	6.0×10^{-4}	0.92	1155.2
PHA-2200 µM Cr(VI)	3.0	5.0	1.55×10^{-2}	0.98	44.8
	5.0	6.2	1.47×10^{-2}	0.99	47.5
	7.0	8.1	1.62×10^{-3}	0.96	428.2
	8.5	8.7	4.96×10^{-4}	0.95	1388.6
	9.0	8.9	3.46×10^{-4}	0.94	1982.8
	11.0	10.6	1.13×10^{-4}	0.86	6134.0

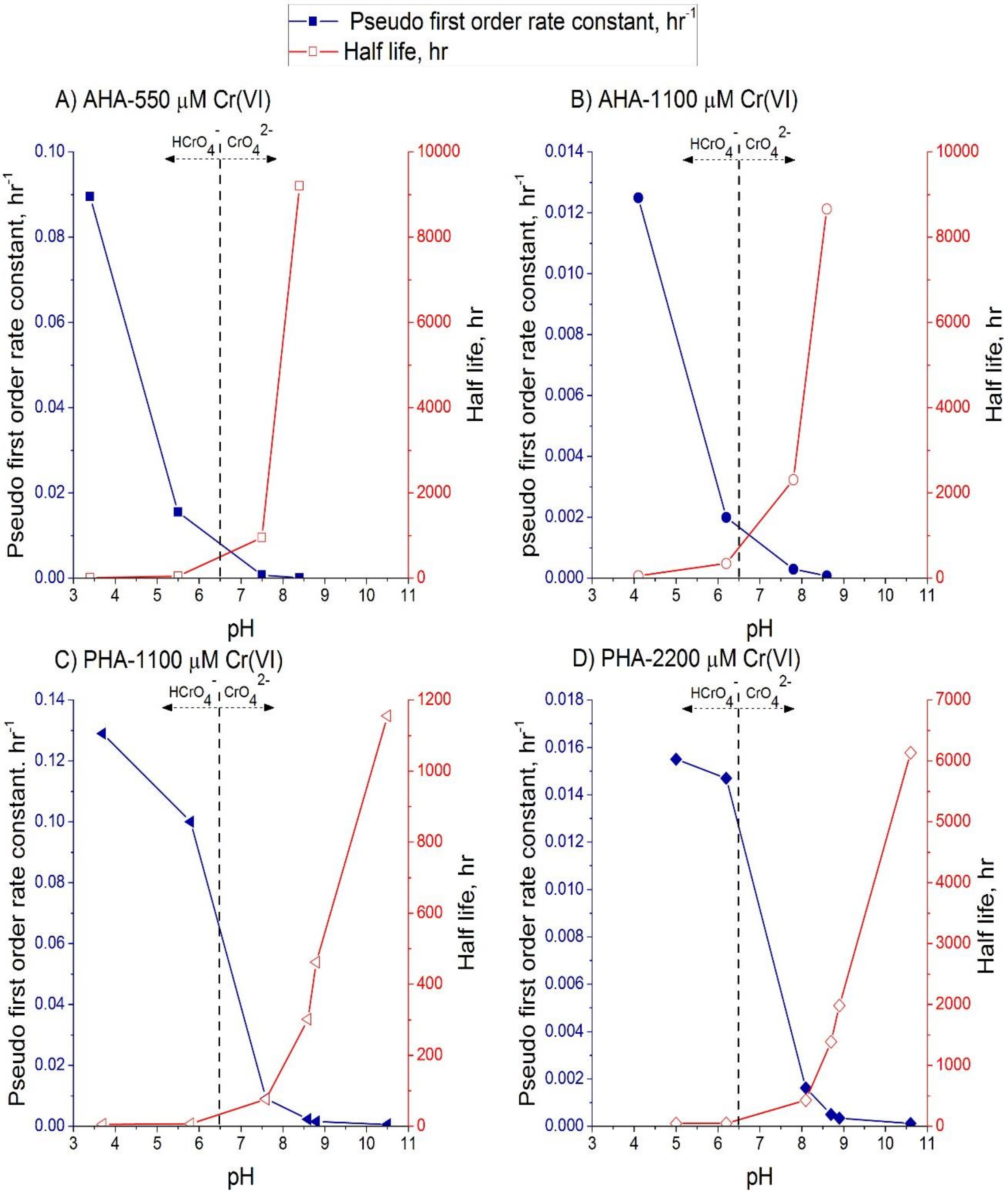
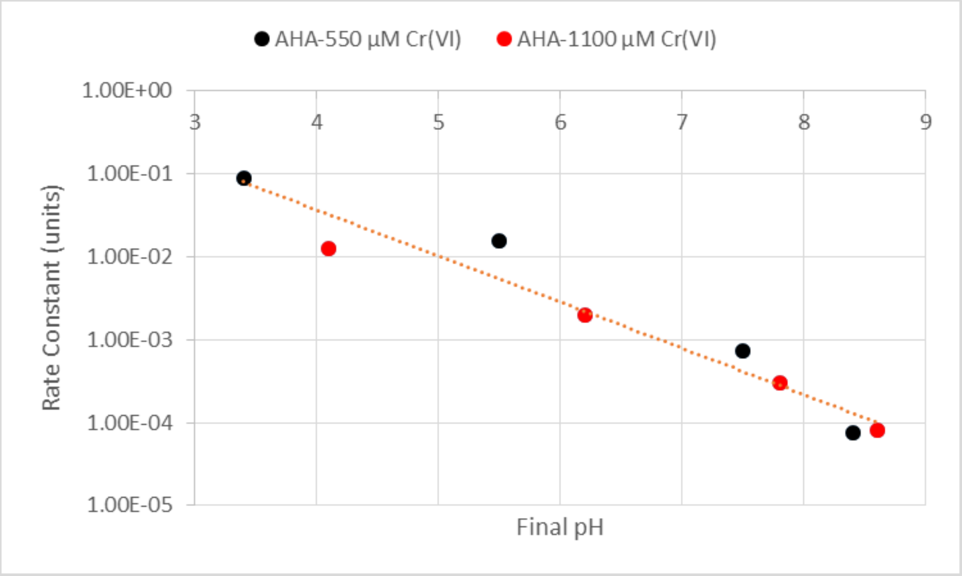


Figure 5.12: Pseudo-first-order rate constants and half-lives for the reduction of Cr(VI) by A) AHA-550 µM Cr(VI), B) AHA-1100 µM Cr(VI), C) PHA-1100 µM Cr(VI, and D) PHA-2200 µM Cr(VI). Reactions contained 110 µmol Cr(VI)/g of HA. The dashed line indicates the pH value below and above which $HCrO_4^-$ and CrO_4^{2-} are the dominant aqueous Cr(VI) species, respectively (Pourbaix, 1966).



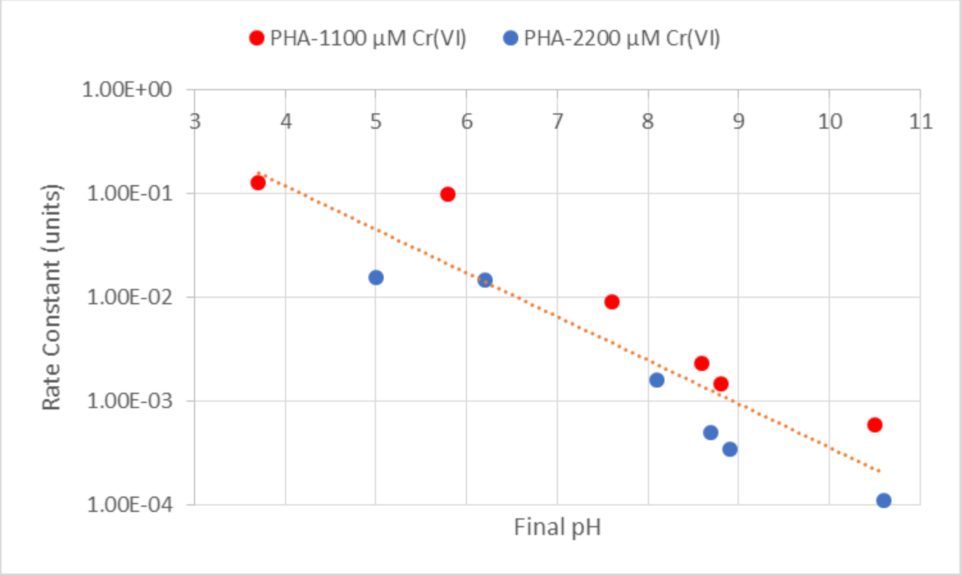


Figure 5.13: Rate constants versus the final pH values for AHA-550 µM Cr(VI), AHA-1100 µM Cr(VI), PHA-1100 µM Cr(VI), and PHA-2200 µM Cr(VI).

5.2.2 Surface Loading Experiment

The data obtained from the surface loading experiment needs to be interpreted with caution due to the drift of the initial pH value (pH 3), which increased considerably with the increase of Cr(VI) concentration. However, the comparison between the humic acids could be more valid when it is carried out at each individual concentration of Cr(VI) (as illustrated in Section 5.1.4) especially the drift of pH values at that concentration was within a narrow range for all humic acids.

The data indicate that the maximum amount of Cr(VI) that can be removed from aqueous systems by a unit mass of the humic acid depends on the type of the humic acid, as illustrated in Figure (5.7). PHA had a much higher capacity to remove Cr(VI) than the other types of humic acids over the range (1,000-8,000 µmol/g HA). The higher capacity of PHA in comparison with the other types of humic acids could be attributed to its reactive functional groups content. It was found that phenolic content (Chapter 4, Table 4.5) was greater in PHA than that in the other types of the humic acids. Phenolic groups have been principally reported as a reducing agent for Cr(VI), therefore increasing the content of phenolic groups increases the capacity of humic acids to reduce Cr(VI) (Scaglia et al., 2013).

5.2.3 Stability of Cr(III)-Humic Acids Complexation

The percentages of Cr(III) that leached from the humic acids-Cr(III) complexes by an exchangeable cation Mg^{2+} were very little (section 5.1.5). This finding suggests that Cr(III)-HAs complexes could be highly stable and are not susceptible for leaching by Mg^{+2}. The other environmentally abundant cations like Ca^{+2}, Na^{+}, and K^{+} may also bind the humic acids and therefore, a potential competition with Cr^{+3} at the binding sites in the humic acids would be expected. However, these mono and divalent cations are held with humic acids by simple cation exchange by the formation of a salt with carboxyl groups (Stevenson, 1994). On the other hand, metals in the first transition series, including Cr, have a potential for forming coordinate linkages with humic acids ligands (Stevenson, 1994). It is therefore likely that such differences in the type of binding contribute to the stability of Cr(III)-HAs complexes against leaching by the most abundant environmentally cations.

5.3 References

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Chapter 6 Reduction Mechanism of Cr(VI) by Humic Acids

The aim of the tests reported in this chapter is to investigate the speciation of Cr attached to humic acids and to discuss the potential removal mechanism. The study involves the determination of the Cr(VI/III) speciation and the local environment around Cr that attached with the humic acids using X-ray absorption near edge structure spectroscopy (XANES) and extended X-ray absorption fine structure spectroscopy (EXAFS), respectively. Furthermore, the study involves a determination of the changes in HAs chemistry that result from the exposure to excess chromate solution using Cross-polarisation magic-angle-spinning ^{13}C NMR spectroscopy and pyrolysis gas chromatography-mass spectroscopy (pyGCMS).

6.1 Results

6.1.1 X-ray Absorption Spectroscopy

6.1.1.1 X-ray Absorption Near Edge Structure (XANES)

XANES spectra collected from Aldrich humic acid (AHA) that had been reacted with Cr(VI) at pH 3, 5, and 7 were almost identical (Figure 6.1). Comparing these spectra with aqueous CrO_4^{2-} standard materials shows the lack of evidence of the characteristic Cr(VI) sharp pre-edge peak at 5994 eV (Peterson et al., 1996). On the other hand, the spectra most closely resemble those collected from the aqueous Cr(III) or poorly crystalline hydrous $Cr(OH)_3$ standards. Furthermore, the XANES spectra of AHA-Cr were clearly different from that of the crystalline Cr_2O_3 standard, which has two distinguished peaks at 6008 eV and 6012 eV.

XANES spectra of Cr(VI) reacted with peat humic acid (PHA) showed a similarity, regardless of the pH value (Figure 6.1). These spectra were qualitatively similar to those collected from AHA-Cr(VI) systems. These findings suggest that Cr(VI) reacted with AHA and PHA was reduced into Cr(III) over the pH values under investigation.

6.1.1.2 Extended X-ray Absorption Fine Structure (EXAFS)

EXAFS fitting revealed that all samples collected from AHA-Cr(VI) and PHA-Cr(VI) tests were the best fit by single scattering and multiple scattering pathways associated with the Cr(III)O_6 octahedra (i.e. 6 O atoms at 1.96 – 1.97 Å), and by inclusion of two additional Cr–C pathways between 2.91 Å and 3.00 Å (Figure 6.2; Table 6.1). Attempts to fit the EXAFS spectra with additional Cr-Cr pathways at 3.0-3.1 Å produced final fits with unrealistically long Cr-Cr pathway lengths (3.3-3.9 Å) and large Debye-Waller factors (0.009-0.010; indicative of overfitting) compared to other pathways, and failed to improve the overall fit quality. Therefore, the data provided no evidence for $Cr(OH)_3$ polymerisation that has been observed previously for some Cr(III)-humic acid associations ((Gustafsson et al., 2014).

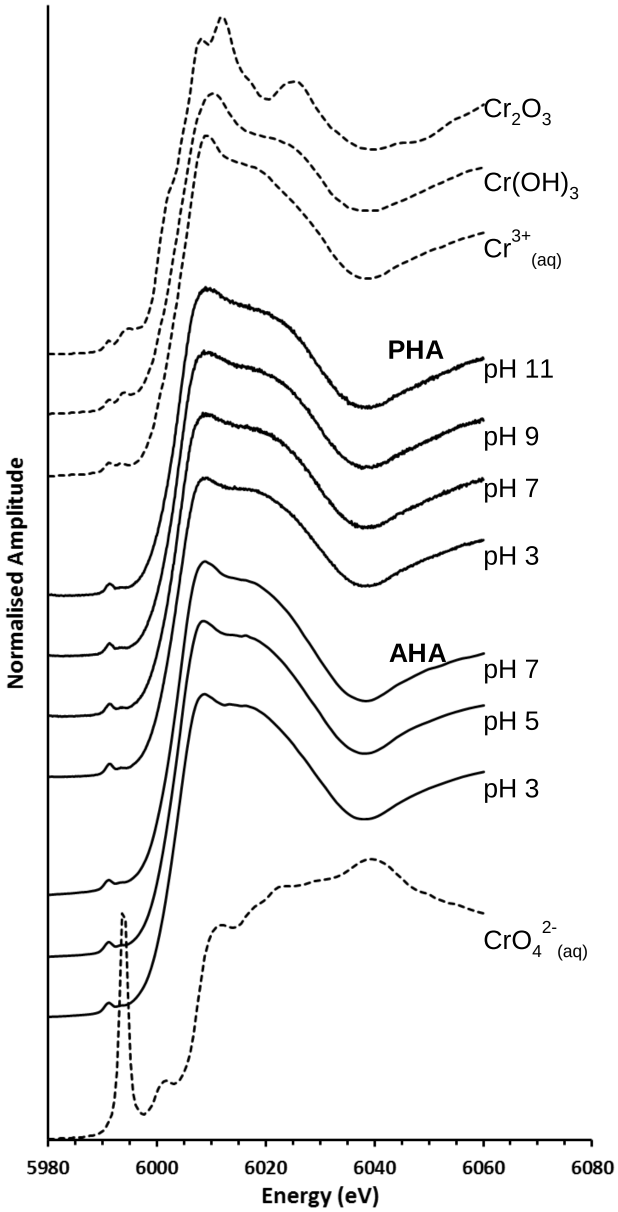

Figure 6. 1: Cr K-edge XANES spectra collected from Aldrich (AHA) and peat (PHA) humic acid samples after reaction with chromate solution for 51 days, and from selected Cr(VI) and Cr(III) containing standards.

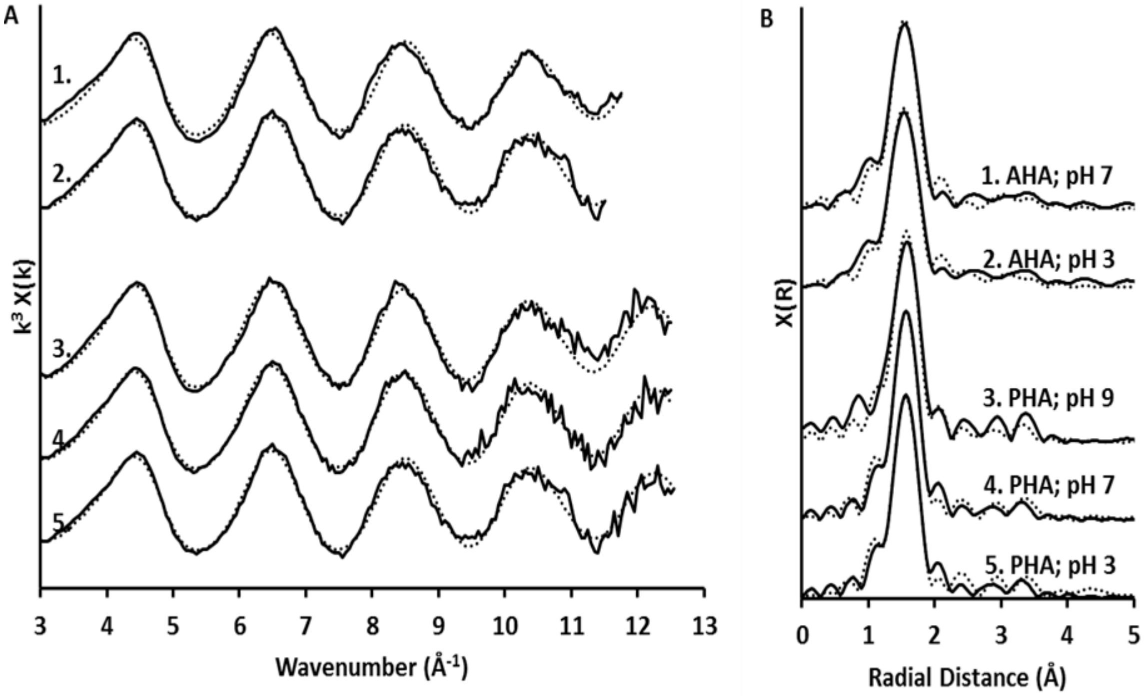

Figure 6.2: A) Cr K-edge EXAFS data collected from Aldrich and peat humic acid samples, and; B) corresponding Fourier transformations. Dotted lines represent bit fit to data calculated in Artemis using pathways and parameters listed in Table 6.2.

Table 6.1: Cr K-edge EXAFS fits, where N is the Occupancy, r is the interatomic distance, σ^2 is the Debye-Waller Factor and reduced χ^2 and *R* are the goodness of fit parameters. Initial set of the goodness of fit parameters relate to fits including just CrO_6 octahedral pathways; second set of parameters relates to best fits including an additional Cr-C pathway (data shown). Uncertainties in the last digit shown in parentheses. MS = multiple scattering pathways as indicated. The normal range of the parameters and factors in the Table are: $\delta e0$ ($^{-}10$ - 10), S^2_0 (0.65-1.10), σ^2 (0.001-0.010), χ^2 (< 600), R (< 0.03),

Experiment Description	**Pathway**	**N**	**r (Å)**	**σ^2 (Å^2)**	**χ^2; *R***
Aldrich Humic acid pH 3	Cr-O	6	1.96(1)	0.003(1)	666; 0.027
$\delta e0$ = -1(1)	Cr-C	2	2.96(3)	0.008(4)	434; 0.016
S^2_0 = 0.77(5)	MS CrO_6	3 x 6	3.93(2)	0.005(2)	
Aldrich Humic acid pH 7	Cr-O	6	1.96(1)	0.003(1)	833; 0.026
$\delta e0$ = 0(1)	Cr-C	2	2.91(5)	0.007(7)	643; 0.018
S^2_0 = 0.81(7)	MS CrO_6	3 x 6	3.91(2)	0.005(2)	
Peat Humic acid pH 3	Cr-O	6	1.97(1)	0.002(1)	137; 0.017
$\delta e0$ = 1(1)	Cr-C	2	2.98(4)	0.007(4)	93; 0.011
S^2_0 = 0.78(5)	MS CrO_6	3 x 6	3.94(2)	0.005(2)	
Peat Humic acid pH 7	Cr-O	6	1.97(1)	0.002(1)	74; 0.014
$\delta e0$ = 1(1)	Cr-C	2	3.00(5)	0.008(4)	55; 0.010
S^2_0 = 0.80(5)	MS CrO_6	3 x 6	3.94(2)	0.005(2)	
Peat Humic acid pH 9	Cr-O	6	1.97(1)	0.002(1)	143; 0.017
$\delta e0$ = 2(1)	Cr-C	2	3.00(5)	0.009(9)	117; 0.014
S^2_0 = 0.83(5)	MS CrO_6	3 x 6	3.95(2)	0.005(2)	

6.1.2 Cross-Polarisation Magic-Angle-Spinning ^{13}C NMR Spectroscopy

6.1.2.1 Cross-Polarisation Magic-angle-Spinning ^{13}C NMR Spectroscopy of AHA

The ^{13}C NMR spectrum of AHA that was equilibrated at pH 3 is shown in Figure 6.3 and the areas under the signal, which was obtained by integration, are reported in Table 6.2. It was found that the alkyl-C accounts for 47.1% of the total C (0-45 ppm; carbon centers singly bonded to either C or H; (Golchin et al., 1997, Kögel-Knabner, 2000). Only 2.1% of the AHA spectrum is in the chemical shift range associated with the alkyl C bonded singly to O (45-110 ppm). It was found that 38.7% of C was in the aromatic and alkene C region of the spectrum (110-160 ppm). However, 10.1% of C was in the sub-range associated with aromatic C-O centers (140-160) ppm (Knicker et al., 2005). The relative content of carbonyl C was 12.1% (160-220 ppm) and the majority of this percentage of C (9.2%) was in the sub-range characteristic of carboxylic and ester moieties (160-185 ppm) (Knicker et al., 2005). The total aromaticity of AHA was 44.0% (total aromaticity equals the peak areas of the total aromatic C (110-160 ppm) over the peak areas of the aliphatic and aromatic C (0-160 ppm); (Schnitzer et al., 1991).

The ^{13}C-NMR spectrum of AHA after reaction with an excess Cr(VI) at initial pH 3, is shown in Figure 6.3 (Table 6.2 reports the area under the signal associated with each part of the spectrum). The portions of the spectra associated with alkyl C and O-alkyl C were 4.9% and 0.4% higher, respectively. On the other hand, the proportion of the spectra usually associated with aromatic C and carbonyl C were 4.8% and 0.6% lower, respectively. The sub-ranges associated with aromatic C-O centers (140-160) ppm and that represents the characteristic of carboxylic and ester moieties (160-185 ppm) were 0.8% and 0.7% lower, respectively. The aromaticity of AHA was 5.6% lower due to the reaction with Cr(VI).

6.1.2.2 Cross-Polarisation Magic-Angle-Spinning ^{13}C NMR Spectroscopy of PHA

The relative contents of the C classes in PHA sample that was equilibrated at pH 3 are reported in Table 6.2. Data presented in the table show that the relative content of the alkyl C was 33.9%. The area of the spectra corresponding to the O-alkyl C was 24.8%. The total aromatic area (110-160 ppm) was 27.8%, and 9.6% of the spectrum occupied the sub-area associated with the aromatic C-O (140-160 ppm). The area corresponding to the carbonyl C was 13.5%, while the sub-area associated with the carboxylic and ester moieties (160-185 ppm) was 9.8%. The aromaticity was found to be about 1/3 of the total area.

After the reaction of PHA with Cr(VI) at initial pH 3, the ^{13}C-NMR spectrum of PHA is shown in Figure 6.3. The relative alkyl C content was 19.6% higher. Both O-alkyl carbon and aromatic contents were 10.1% lower. On the other hand, carbonyl C was 0.5% higher. The sub-areas associated with C-O (140-160 ppm) and the carboxylic and ester moieties (160-185 ppm) were approximately 2% lower. In addition, the results showed that the aromaticity was (11.5%) lower.

6.1.2.3 Cross-Polarisation Magic-Angle-Spinning ^{13}C NMR Spectroscopy of SHA

The ^{13}C-NMR spectrum of SHA (equilibrated at pH 3) is given in Figure 6.3. The percentage of the C accounted for alkyl C was 28.7%. The relative proportion of O-alkyl C was 31.2%. Aromatic C occupied 1/4 of the total area, while the aromatic C-O account for 7.4% of the total area. The fraction of carbonyl C represented 15.1% of the total area, while the sub-area of carboxylic and ester moieties occupied 14.1% of the total area. The aromaticity of the SHA sample was 29.4%.

After the reaction of SHA with Cr(VI) at initial pH 3, the ^{13}C-NMR spectra of SHA is shown in Figure 6.3. The spectrum shows that Alkyl C fraction was 0.3% higher (Figure 6.3 and Table 6.2). Furthermore, the relative area of the O-alkyl C was 5.8% higher. Both, the aromatic C and carbonyl C were 4.4% and 1.8% lower, respectively. The sub-areas, aromatic C-O and carboxylic and ester moieties were 0.7% and 1.1% lower, respectively. The aromaticity was 5.6% lower.

6.1.2.4 Cross-Polarisation Magic-Angle-Spinning ^{13}C NMR Spectroscopy of SPCR-HA

The ^{13}C-NMR spectrum of SPCR-HA (equilibrated at pH 3) is shown in Figure 6.3. The percentage of the C accounted for alkyl C was 39.3%. The relative proportion of O-alkyl C was 20.9%. The alkenes and aromatic C occupied 26.3% of the total area, while the sub-area of aromatic C-O was 9.1%. The carbonyl C fraction represented 13.5% of the total area. The carboxylic and ester moieties occupied 11.6% of the total area. The aromaticity of SPCR-HA was found to be 30.4% of the total area.

After the reaction with Cr(VI) at initial pH 3, the spectrum of SPCR-HA is shown in Figure 6.3. The proportion of Alkyl C was 5.3% higher (Table 6.2). All other fractions, O-alkyl C, aromatic C, and carbonyl C were 1.4%, 0.5%, and 3.5% lower, respectively. The sub-fractions; aromatic C-O and carboxyl C were 0.5% and 1.6% lower, respectively. The aromaticity of the sample was 1.7% lower.

6.1.2.5 Comparison of the Cross-Polarisation Magic-Angle-Spinning ^{13}C NMR Spectroscopy of the Humic Acids

Comparison of the ^{13}C-NMR spectra of AHA, PHA, SHA, and SPCR-HA indicates differences between the four materials (Figure 6.3 and Table 6.2). It was found that AHA had higher Alkyl C and aromatic C content and much lower O-alkyl C content than other types of humic acids. The O-alkyl C was richer in SHA sample. The Carbonyl C did not differ greatly between the humic acids and it was between 12.1% and 15.1%. The aromaticity of the humic acids increased in the order SHA < SPCR-HA < PHA < AHA.

After the reaction between the humic acids and an excess Cr(VI), (8000 µM initial Cr(VI) concentration), the maximum reduction capacity of AHA, PHA, SHA, and SPCR-HA was 511, 1380, 504, and 582 µmol/g HAs, respectively (see Figure 5.7, A). The ^{13}C NMR spectra showed that Alkyl C was increased in all samples and the highest increase was in PHA-Cr sample (Table 6.2). Moreover, the O-alkyl C substantially decreased in PHA sample, while it somewhat decreased in the SPCR-HA sample. On the other hand, AHA and SHA samples showed a small increase of O-alkyl C groups (0.4%, 5.8% increased, respectively). There was very little change in the carbonyl C in any sample (the largest change was a 3.5% decrease in SPCR-HA. The apparent aromaticity of all samples was decrease upon exposure to Cr(VI). The change was highest for PHA (-11.5%) and lowest for SPCR-HA where it was barely detected (-1.7%).

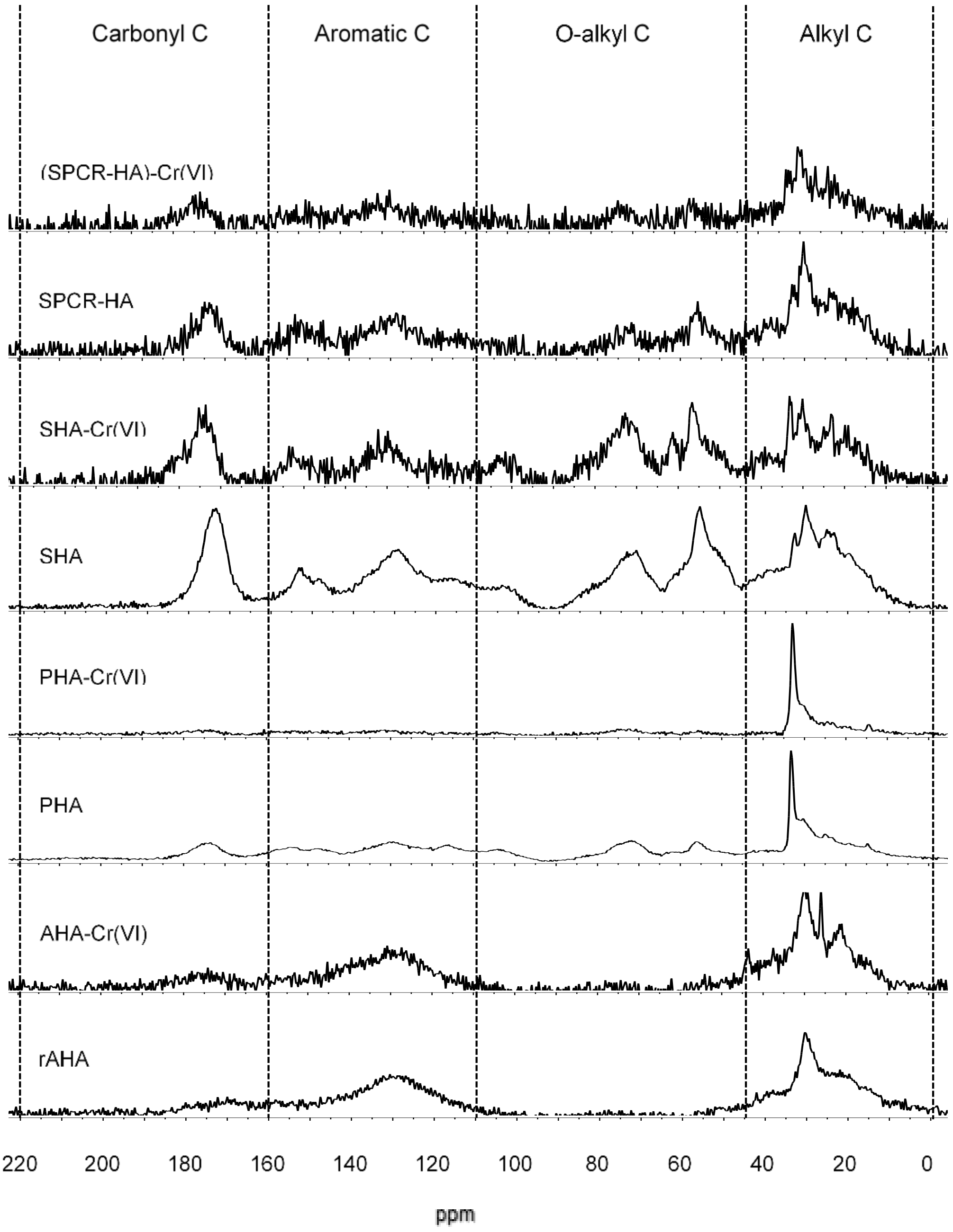

Figure 6.3: ^{13}C-NMR spectra of AHA, PHA, SHA, and SPCR-HA before and after the reaction with an excess Cr(VI) at pH 3. Curves are normalised to equal area under the curves. The spectra are operationally divided into chemical shift regions characteristic of different C bonding environments (Golchin et al., 1997, Kögel-Knabner, 2000)**.**

Table 6.2: Proportionof humic acid carbon in the different bonding environments before and after reaction with excess Cr(VI) determined by CP MAS ^{13}C-NMR (spectra were operationally divided into characteristic chemical shift regions (Kögel-Knabner, 2000, Golchin et al., 1997).

	AHA			PHA			SHA			SPCR-HA		
Type of organic carbon (% of total area)	Before reaction	After reaction	Difference	Before reaction	After reaction	Difference	Before reaction	After reaction	Difference	Before reaction	After reaction	Difference
Alkyl C (0-45 ppm)	47.1	52.0	+4.9	33.9	53.5	+19.6	28.7	29.0	+0.3	39.3	44.6	+5.3
O-alkyl C (45-110 ppm)	2.1	2.5	+0.4	24.8	14.7	-10.1	31.2	37.0	+5.8	20.9	19.5	-1.4
Aromatic C (110-160 ppm)	38.7	33.9	-4.8	27.8	17.7	-10.1	25.0	20.6	-4.4	26.3	25.8	-0.5
Carbonyl C (160-220 ppm)	12.1	11.5	-0.6	13.5	14.0	+0.5	15.1	13.3	-1.8	13.5	10.0	-3.5
Aromaticity* %	44.0	38.4	-5.6	32.1	20.6	-11.5	29.4	23.8	-5.6	30.4	28.7	-1.7

* Aromaticity is defined as (Aromatic C)/(Alkyl C + O-alkyl C + Aromatic C).

6.1.3 Pyrolysis Gas Chromatography-Mass Spectroscopy (pyGCMS)

6.1.3.1 Pyrolysis Gas Chromatography-Mass Spectroscopy of AHA

The pyrogram obtained from Py-GCMS of AHA is shown in Figure 6.4 (A and B). The major pyrolysis products identified in the pyrogram, which have a matching factor (SI) ≥ 90%, are listed in Table A7 (Appendices) with their relative peak areas. The products are also classified into aliphatic and aromatic compounds to facilitate the comparison of the pyrograms before and after the reaction with Cr(VI).

The reaction of AHA with excess Cr(VI) resulted in a change in the AHA pyrogram. A large decrease in the relative size of aromatic fragments accompanied by an increase in the relative size of the aliphatic fragments was observed. Among aromatic fragments, phenols, substituted and unsubstituted were not detected after the reaction with excess Cr (VI) (Table A7, peaks 43, 46, 52). Furthermore, new aliphatic compounds were detected after the reaction with Cr (VI).

6.1.3.2 Pyrolysis Gas Chromatography-Mass Spectroscopy of PHA

PHA pyrogram (Figure 6.4, C) shows a high aromatic content. Based on the pyrogram analysis (Table A7, a matching factor (SI) ≥ 90%), it was found that phenols represented about 80% of the aromatic content and they were mainly as substituted phenols. After the reaction with excess Cr(VI), the pyrogram considerably changed (Figure 6.4, D). Indeed, the substituted phenols greatly decreased and at the same time, the aliphatic fraction increased. A large proportion of the substituted phenols that disappeared due to the reaction with Cr(VI), was methoxy phenols.

6.1.3.3 Pyrolysis Gas Chromatography-Mass Spectroscopy of SHA

Examination of SHA pyrograms indicated that a higher proportion of fragments did not match the library spectrum than with the other HAs. However, the major pyrolysis products were identified in the pyrogram using matching factors (SI) ≥ 85%. An overview of the pyrograms (Figure 6.4, E and F) shows significant changes of SHA due to the reaction with Cr(VI). All fragments associated with the substituted phenols were not detected after the reaction with Cr(VI) (e.g. peaks 45, 52, 56, and 60). On the other hand, the relative size of peaks associated with aliphatic fragments increased.

6.1.3.4 Pyrolysis Gas Chromatography-Mass Spectroscopy of SPCR-HA

As Figures (6.4, G and H) show, there is a clear change of SPCR-HA pyrogram due to the reaction with Cr(VI). The analyses of the pyrograms were by determining the major products that have matching factors (SI) ≥ 85% to decrease the percentage of the unassigned compounds. The information presented in Table A7 shows that the reaction of SPCR-HA with Cr(VI) led to the disappearance of substituted phenols and a considerable decrease in the fragments associated with unsubstituted phenols. In contrast, the fragments associated with the aliphatic compounds increased.

6.1.3.5 Comparison of the Pyrolysis Gas Chromatography-Mass Spectroscopy of the Humic Acids

Direct quantitative comparison of humic acids’ functionality is inappropriate due to the differences in the percentages of the unassigned compounds, which ranged between 4% and 33%. Overall, the pyrograms show differences at a molecular level before and after the reaction with excess Cr(VI) (Table 6.3). This finding indicates that the reaction between the humic acids and Cr(VI) caused changes in the humic acids functionality. The reaction of all humic acids with excess Cr(VI) caused a large decrease in the relative abundance of most aromatic compounds (Figure 6.4). Among aromatic compounds, methoxy-phenolic and other substituted phenolic compounds exhibited the largest decrease in their relative abundance (see Figure 6.5 as a representative of the humic acids). On the other hand, an increased in the relative abundance of most peaks that associated with aliphatic compounds was observed in all humic acids.

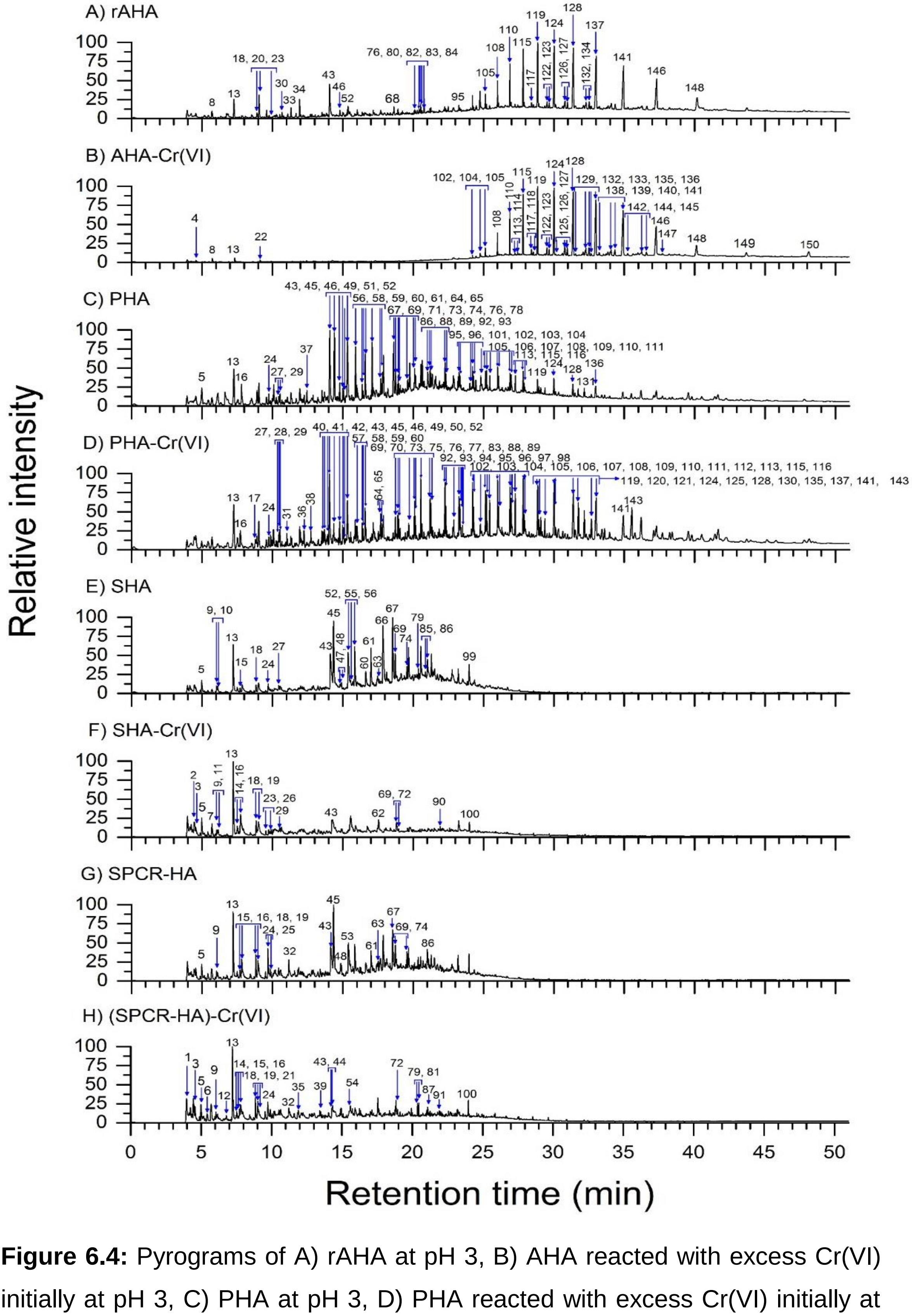

Figure 6.4: Pyrograms of A) rAHA at pH 3, B) AHA reacted with excess Cr(VI) initially at pH 3, C) PHA at pH 3, D) PHA reacted with excess Cr(VI) initially at pH 3, E) SHA at pH 3, F) SHA reacted with excess Cr(VI) initially at pH 3,G) SPCR-HA at pH 3, H) (SPCR-HA)-Cr(VI) reacted with excess Cr(VI) initially at pH 3. Peak identities and intensities are given in Table A7.

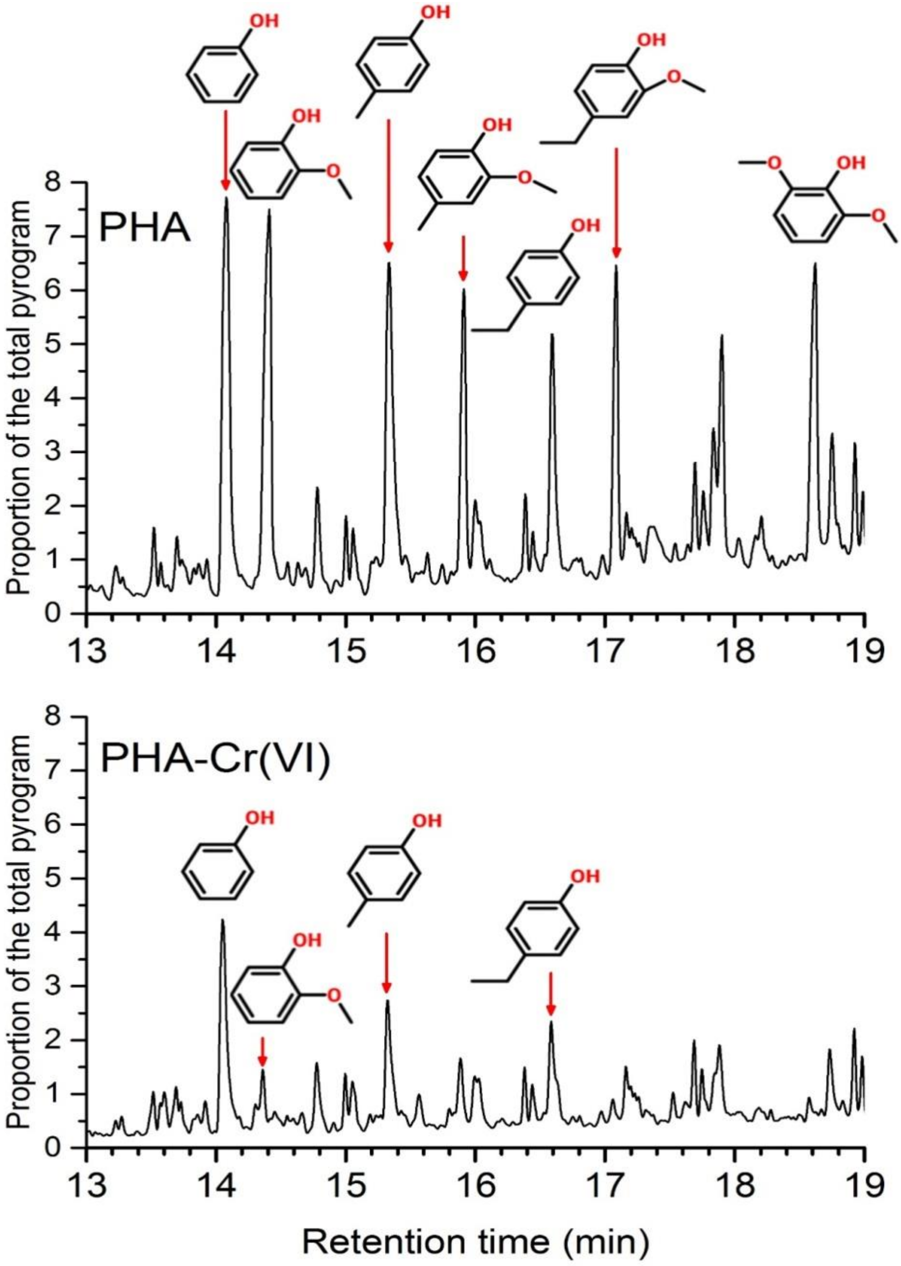

Figure 6.5: Partial pyrograms for PHA before and after reaction with excess Cr(VI) showing a decrease in the relative size of spectral peaks associated with phenolic, methoxyphenolic and other substituted phenolic fragments. Pyrograms have been scaled in proportion to an area that phenol represents of the total pyrogram. Full pyrograms and a table identifying the main thermal degradative products are presented in the Supporting Information

Table 6.3: Principal organic fractions in the humic acids before and after the reaction with 8000 µmol Cr(VI) at pH3, identified by pyrolysis-GC/MS.

	AHA		PHA		SHA		SPCR-HA	
Compounds classification (% of total area)	pH3 *	After the reaction	pH3	After the reaction	pH3	After the reaction	pH3	After the reaction
Total aliphatic compounds	**72.4**	**94.5**	**29.7**	**59.9**	**3.6**	**16.9**	**6.0**	**29.8**
Total aromatic compounds	**18.7**	**1.5**	**49.9**	**20.0**	**71.7**	**59.0**	**60.9**	**37.4**
Phenols	6.3	0.0	39.3	11.3	57.1	8.2	39.2	2.0
Substituted phenols	1.2	0.0	31.6	7.1	51.5	0.0	31.4	0.0
Un-substituted phenols	5.1	0.0	7.7	4.2	5.6	8.2	7.8	2.0
Unassigned compounds	**8.9**	**4.0**	**20.4**	**20.1**	**24.7**	**24.1**	**33.1**	**32.8**

6.2 Discussion

6.2.1 X-ray Absorption Spectroscopy

XANES analysis indicates that the interaction of Cr(VI) with AHA and PHA resulted in a reduction to Cr(III) at all pH values under investigation. EXAFS spectra of these humic acids show a similar interaction pattern with Cr(VI). At all pH values, the reduced Cr was associated with the partially degraded humic acid in an inner sphere adsorption complex. The data obtained from the EXAFS analyses (Table 6.1) suggest that Cr(III) formed 6 bonds with oxygen atoms. The best fit EXAFS model is consistent with octahedral coordinated Cr(III) bound in an inner-sphere complex on the surface of the humic acid involving an average of 2 C containing ligands. However, the poor fitting at wavenumber > $Å^{-1}$ leads to increase uncertainty in parameters for the second shell Cr-C bonds (R, N) and therefore the best fit has relatively high uncertainty in their Debye-Waller factor (σ^2). The finding in this section matches that observed in earlier study by Fukushima et al. (1995). They compared the IR spectra of a protonated humic acid with a solid state Cr(III)-HA complex prepared from a suspension of 10^{-3} M Cr(III) and HA (10 g/l) that reacted for a week in darkness. It was noted that the peaks of carboxylic C=O stretching, and carboxylic C-O-H deformation were lower in the Cr(III)-HA sample than the sample of a protonated humic acid and also a shift of the absorption band of carboxylate COO^- asymmetric stretching to a lower wave length which refers to the Cr(III)-carboxylate binding. Thus, the proposed model that illustrates Cr(III) binding with AHA and PHA can be expressed as $(R\text{-}COO)_2$ –Cr-$(OH_2)_4$.

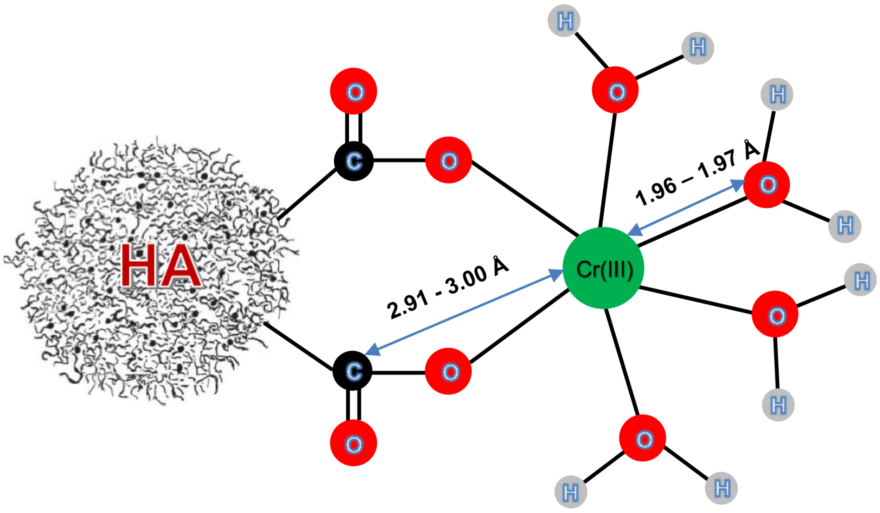

Figure 6.6: Inner sphere complexation between the reduced Cr and humic acids, based on the EXAFS data.

6.2.2 Cross-Polarisation Magic-Angle-Spinning ^{13}C NMR Spectroscopy

Chemical shift regions of the ^{13}C NMR spectra indicate that the humic acids contain different proportions of carbon classes. The proportion of O-alkyl C (45-110 ppm) was very low in AHA in comparison with other types of humic acids. The ^{13}C NMR spectra also indicate that the alkenes and aromatic C (110-160 ppm) increased in the order SHA < SPCR-HA < PHA < AHA. This finding is approximately in agreement with E4/E6 values (Chapter 4, Table 4.3), which suggest that the degree of humification increased in the order SHA < SPCR-HA < AHA < PHA. These results match what it would be expected, where younger materials like soil and SPCR exhibit a lower degree of humification and consequently a lower aromatic content. On the other hand, older material such as AHA that was extracted from Miocene age lignite (6-26 Ma; Germany) and PHA that was extracted from Holocene age peat (< 12 ka, Ireland) exhibit a higher degree of humification and as a result a higher aromatic content than younger materials. Moreover, the sub-area of aromatic C-O (140-160 ppm) was higher in AHA than the other types of humic acids. Indeed, ^{13}C NMR cannot differentiate aromatic C bonded to O in phenolic moieties from those associated with an ether linkage, but the differences in the phenolic acidity estimated by barium hydroxide and Ca-acetate methods (chapter 4, Table 4.5) suggest that a larger proportion of the aromatic C-O centers in AHA are associated with ether linkages than in the other types of humic acids under investigation.

The ^{13}C NMR spectra of the humic acids show changes in all C classes due to the reaction with an excess Cr(VI). All humic acids suffered a loss of aromatic/alkene C but in different proportions. This result may be explained by the fact that the region corresponding to 110-160 ppm contains chemical components that have an ability to reduce Cr(VI). Alkenes can be oxidized by Cr(VI) due to the oxidative cleavage of C-C double bonds. Oxygen is added to the carbon atom and carboxylic acids are produced (Carey and Sundberg, 2007). Reduction of Cr(VI) also resulted in oxidation of aromatic compounds (phenolic and non-phenolic). It has been found that the oxidation of these compounds by Cr(VI) results in cleavage of the intervening C-C bond (Deiana et al., 2007). For example, the reduction of Cr(VI) by caffeic acid (3-(3,4-Dihydroxyphenyl)-2-propenoic acid) involves the release of electrons as a result of the formation of semiquinonic radicals that easily oxidized to quinonic groups and further oxidation leads to the formation of carboxylic groups as shown in Figure (6.7) (Deiana et al., 2007). Similarly, it has been confirmed that the reduction of Fe(III) by caffeic acid can result in ring opening (Deiana et al., 1992, Hynes and O'Coinceanainn, 2004). Such ‘ring opening’ phenomena are consistent with the loss of phenolic and non-phenolic aromatic compounds in the range 110-160 ppm.

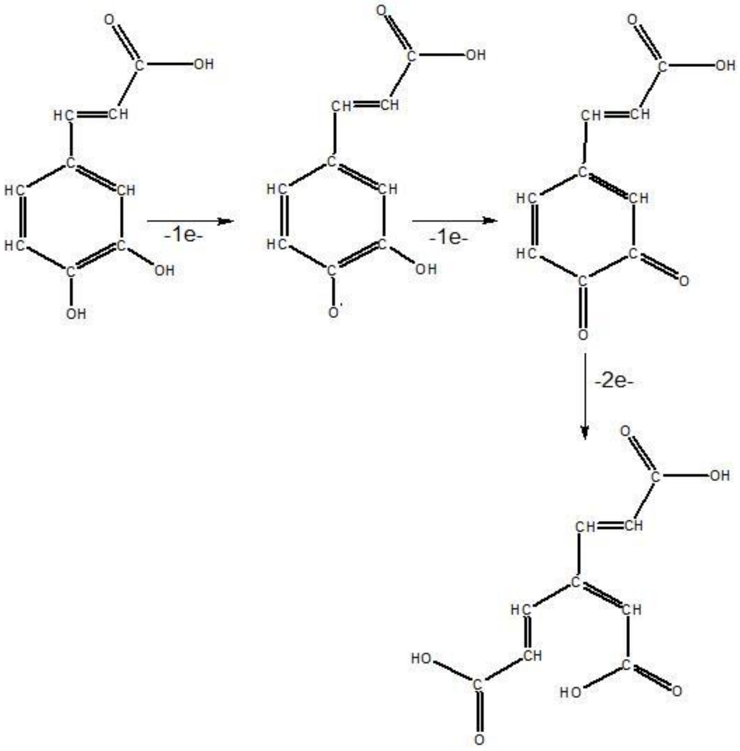

Figure 6.7: Oxidation of caffeic acid by Cr(VI) (Deiana et al., 2007).

Table 6.2 shows a decrease in the relative abundance of O-alkyl compounds in PHA and SPCR-HA (45-110 ppm) due to the reaction with Cr(VI). These compounds probably contain reducing agents, such as primary and secondary alcohols. The oxidation of the primary and secondary alcohols normally lead to the formation of aldehydes and ketones, respectively (Lee and Stewart, 1967, Rahman and Rocek, 1971). When aldehydes are introduced in a further oxidation, carboxylic acids are produced (Rocek and Ng, 1974).

The oxidation of the compounds in the chemical shift range associated with O-alkyl (45-110 ppm) and also those in the chemical shift range associated with alkene and aromatics C (110-160 ppm) must result in an increase in the proportion of the spectrum associated with carbonyl C (160-220 ppm). It is somewhat surprising that ^{13}C NMR did not identify the carbonyl C increase except a slight increase in PHA sample. Zhang et al. (2017) showed that carboxylic groups involve in the complexation with Cr(III) rather than oxidized by Cr(VI). The formation of carboxyl-Cr complex results in shielding effects associated with electron redistribution that causes a decrease in the carbonyl C signal. Therefore, carbonyl groups result from the reduction of Cr(VI) by the humic acids were not detected by ^{13}C NMR due to such shielding. This explanation is compatible with the Cr EXAFS data (section 6.1.1.2), which suggests that the Cr(III) formed an inner sphere adsorption complex with two C atoms.

6.2.3 Pyrolysis Gas Chromatography-Mass Spectroscopy (PyGCMS)

Data obtained by PyGCMS needs to be interpreted with caution. Indeed, the data cannot be considered a representative of the whole sample of humic acid due to the selectivity of pyrolysis technique for the more volatile, while less than half carbon in humic substances is volatile (Stevenson, 1994). In spite of that, significant findings can be obtained that are consistent with the ^{13}C-NMR results, and some more details that can be understood about the changes of the chemical structure that result due to the reaction between the humic acids and Cr(VI). The PyGCMS pyrograms confirm a loss of aromatic fraction during the reaction with Cr(VI) and also an increase in the aliphatic moieties. The loss of the aromatic fraction involves the phenolic and non-phenolic compounds, but it was principally of substituted phenols. Most substituted phenols were determined by the pyrolysis GC/MS (Table A7) associated with methyl and/or methoxy substituents. These substituents have an electron donating character that increases the reducing power of phenols by increasing the electron transfer (Elovitz and Fish, 1994). Overall, the data obtained by pyro-GCMS suggests that the reduction of Cr(VI) to Cr(III) by humic acids involves a reaction with aromatic groups generally, and phenolic moieties in particular.

6.2.4 Mechanism of Cr(VI) Reduction with Humic Acid

The combination of the results obtained by XAS, ^{13}C NMR, and Pyr-GCMS suggest that the reduction of Cr(VI) by the humic acids exhibits a common mechanism regardless of the type of the humic acids. Changes in the humic acids functional groups involved principally the two main fractions; O-alkyl groups and aromatic, while carboxyl groups would be the major site for the complexation of Cr(III). These findings could be generalised as an overall mechanism between Cr(VI) and humic acids regardless of the source of the humic acid.

In spite of the results of this study determined the overall mechanism of Cr(VI) reduction by humic acids' functional groups, the detailed steps of these reactions would be difficult to determine due to the complexity of the humic acids. However the reduction of Cr(VI) with humic acids mainly is achieved by reactive functional groups that involve mainly alcohols, phenols, and aldehydes, it can be therefore hypothesised that the mechanism of humic acid functional groups oxidation is analogue to the mechanism of alcohols, aldehydes, and phenols. The previous knowledge of the reaction mechanism of Cr(VI) and alcohols, phenols, and aldehydes moieties may help to extrapolate the mechanism to Cr(VI)-humic acids systems. Previous studies have been proposed a detailed reaction mechanism of simple alcohols, aldehydes, and phenols (Lee and Stewart, 1967, Wiberg and Schafer, 1967, Wiberg and Schaefer, 1969, Rahman and Rocek, 1971, Rocek and Ng, 1974, Elovitz and Fish, 1995). It has been found that simple alcohols can reduce Cr(VI) in two distinct steps; a rapid and pH-independent step including chromate ester formation followed by the electron transfer decomposition step (Lee and Stewart, 1967, Wiberg and Schafer, 1967, Sengupta et al., 1986). It has been observed spectrophotometrically that under acidic conditions, the interaction of Cr(VI) (principally $HCrO_4^-$) with alcohol moieties starts with a very rapid formation of chromate esters (5-10 msec) (Wiberg and Schafer, 1967). This step involves the addition of the O-H bond of the alcohol moiety to the electrophilic Cr(VI) centre (Lee and Stewart, 1967, Elovitz and Fish, 1995). Subsequently, the second step involves a rate-limiting decomposition of the chromate ester carbonyl compounds that yield an unstable intermediate Cr species, Cr(V) or Cr(IV), and a variety of organic products. The intermediate Cr species are reduced in a quite similar mechanism to the reduction of Cr(VI) (Wiberg and Schaefer, 1969). It has been also reported that the oxidation of the primary alcohols by Cr(VI) yields aldehydes, and if the aldehydes are exhibited for further oxidation by Cr(VI) or the intermediate Cr species, carboxylic acid is produced (Rahman and Rocek, 1971, Bekish, 2012). Secondary alcohols are also exhibited to the oxidation by Cr(VI) and that leads to the formation of ketone (Lee and Stewart, 1967, Wiberg and Schafer, 1967, Wiberg and Schaefer, 1969).

The aliphatic aldehyde moieties can react by a similar mechanism that suggested for the reduction of Cr(VI) by alcohols (Rocek and Ng, 1974)). The final organic product of the reaction is carboxylic groups.

The mechanism for the redox reaction between Cr(VI) and mono-phenolic compounds also occurs via a fast chromate ester formation step (typically < 60 s), followed by a decomposition step that involves an inner sphere electron transfer to the Cr(VI) center, during the decomposition step, either one or two electrons are transferred (Elovitz and Fish, 1995). It has been found that a transfer of one electron is favored when the benzene ring is attached to electron donating groups like methyl substituents and they yield dimers and larger organic polymers, along with reduced Cr(V) species. Two-electron transfer is favored when the benzene ring bind to a strong resonance electron donating substituents such as methoxy phenol and that yields monomeric quinones, along with Cr(IV) species. While the electron-donating substituents increase the reducing power of phenol, it has been found that electron-withdrawing groups can reduce the reducing power of phenol (Elovitz and Fish, 1995). This finding supports the variation of reactivity of the humic acids under investigation (particularly AHA, PHA, and SHA), which showed a relationship between the reduction capacity of the humic acid, phenolic content, and the decrease in the phenolic fraction due to the reaction with Cr(VI). Table 6.4 shows that the pattern in the decrease in the phenolic fraction of AHA, PHA, and SHA was the same as the pattern in the overall capacity to reduce Cr(VI). PHA had the highest capacity to reduce Cr(VI) and at the same time it exhibited the highest decrease of the phenolic fraction. AHA and SHA had approximately similar reduction capacity accompanied with a similar decreasing in the phenolic fraction, although the phenolic content was higher in SHA. This finding suggest that AHA may contain more donating substituents attached with the benzene rings than that in the SHA sample, which results the same reducing capacity, although AHA had a lower phenolic content.

In addition, it has been reported that the reaction mechanism by which the chromate ester decomposes may occur in three different pathways that are differentiated according to the system acidity (Elovitz and Fish, 1995). According to Elovitz and Fish (1995) study, at pH < 2, two proton pathway is expected, while under moderately acidic conditions (2-5) a single proton pathway is dominant. On the other hand, a proton independent pathway is expected at pH > 5. Soil humic substances also can reduce Cr(VI) more rapidly under acidic conditions, and the reaction rate decline sharply above pH 6 (Wittbrodt and Palmer, 1995, Wittbrodt and Palmer, 1997). Indeed it is widely assumed that the bichromate [$HCrO^-$] is the reactive species in the reduction of Cr(VI) by oxidation of organic substrates (Wiberg and Schafer, 1967, Elovitz and Fish, 1994, Elovitz and Fish, 1995, Wittbrodt and Palmer, 1997). As the reaction rate declines sharply to negligible rates when ≥ pH 6, it has been assumed that CrO_4^{2-} (the dominant Cr species at high pH) is unreactive (Elovitz and Fish, 1995). The present study is consistent with the previous findings in term of the rate of reaction between Cr(VI) and all types of humic acids under investigation declined with pH. Moreover, this study contributes additional evidence suggests that under neutral and alkaline conditions CrO_4^{2-} can be reduced by the humic acids but much more slowly. Hence, it could be hypothesised that humic acids as a complex substrate (i.e. where there is a range of reactive moieties) contain more complicated moieties than those studied in the literature. These moieties can enhance the reducing power of humic acids under neutral and alkaline conditions. The results obtained by Elovitz and Fish (1995) support this hypothesis where they found that any difference in the structure of phenols including the type of substituents bind to the benzene ring and also the location of these substituents on the benzene ring considerably effect on the reactivity of these compounds. Consequently, the finding of the present study highlights the significance of humic acids as a reductant of Cr(VI) contaminated sites under the environmental or particular conditions especially those derived from peat and secondary organic materials such as sludge phyto-conditioning residue.

Table 6.4: Reduction capacity of the humic acids, humic acids' phenolic content, and decreasing of phenols after the reaction with Cr(VI).

	AHA	PHA	SHA
Capacity to reduce Cr(VI), µmol/g HA	511	1380	504
Phenol content meq/g HA	3.3	4.1	4.0
Decreasing in phenols, %, (estimated by ^{13}C-NMR)	-0.8	-2.0	-0.7

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Chapter 7 Conclusions, Implication, and Future Work

7.1 Conclusions

7.1.1 Chemical and Spectroscopic Characteristics of Humic Acids

In Chapter 4, the objective was to assess the chemistry of humic acids obtained from different origins and how these characteristics may affect the removal kinetics of Cr(VI) from aqueous systems. Results from this chapter show that the selected humic acids from different origins contained similar classes of chemical substances, but they were considerably different in their content of chemical compounds. The variation in the content of these classes depends on the age of the humic acid. Figure 7.1 shows clearly a consistent pattern between these properties and the geological age of the humic acids, AHA, PHA, and SHA. However, among the humic acids, SPCR-HA was the youngest material that had structural characteristics was not subjected to that pattern. Indeed, SPCR-HA has an indeterminate age, because it contains a mixture of substances which all have strange histories. Figure (7.1) shows that the atomic ratios, O/C, H/C, and N/C increased with decreasing the age of the humic acids. On the other hand, the carboxyl acidity (estimated by Ca-acetate method), aromaticity, and the percentage of phenols (estimated by ^{13}C-NMR) increased with the age of the humic acids and consequently with the degree of humification. Taken together, these results suggest that a different level of reactivity would be expected for the humic acids extracted from origins that have different geological ages. The high content of phenols, which have an ability to reduce Cr(VI) into Cr(III) (Elovitz and Fish, 1994, Elovitz and Fish, 1995) in the older humic acids (AHA and PHA) in comparison with the younger humic acids (SHA and SPCR-HA) suggests that AHA and PHA may exhibit a higher ability to reduce Cr(VI) than SHA and SPCR-HA.

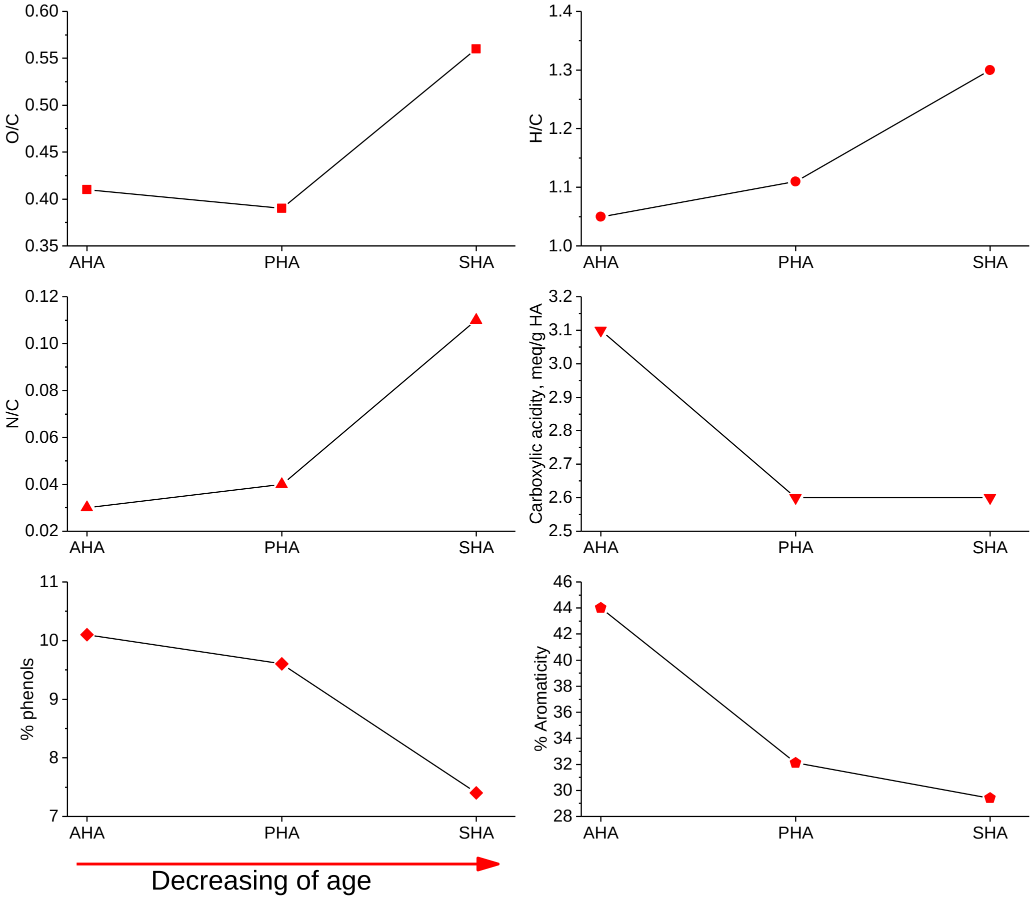

Figure 7.1: Humic acids versus the main characteristics.

7.1.2 Abiotic Reduction of Cr(VI) by Organic Compounds Representative of Soil Organic Matter in Aquatic System: Reaction Kinetics as a Function of pH

Throughout Chapter 5, the effect of pH on the removal of Cr(VI) from aqueous systems by humic acids isolated from different origins was evaluated. Results from this Chapter show that all humic acids exhibited a high capacity to remove Cr(VI) from aqueous systems under acidic conditions with different rates. As the pH increases, the amount of the reduced Cr(VI) and rates of reactions were decreased. Although the rate of reaction decreased with pH the reaction proceeded under alkaline conditions, which suggests that CrO_4^{2-} (the predominant Cr species at pH ≥ 6.5) can be reduced by the humic acids. Comparing to other humic acids under investigation, PHA showed the highest removal capacity accompanied with the highest rates of reactions at all pH values investigated. All humic acids were reduced Cr(VI) to Cr(III) in a first-order reaction with respect to Cr(VI) concentration but exhibited a partial order between 0.40 and 0.59 with respect to [H+]. After the reduction of Cr(VI), the stability of the formed complexes was investigated using magnesium chloride extraction agent and a very small amount of Cr(III) was leached. These results suggest that a very low Cr(III)-exchangeable fraction in all systems existed and the majority of Cr(III) was strongly bound to the humic acids structures. All of the above findings highlight the potential use of humic acids in the remediation of Cr (VI) contaminated sites in a range of environmental conditions.

7.1.3 Reduction Mechanism of Cr(VI) by Humic Acids

In Chapter 6, the objectives were to investigate the speciation of Cr, after Cr(VI) has reacted with the humic acids at pH range (3-11), and to determine the changes in the humic acids chemistry that result from the exposure to Cr(VI) solution. In summary, the speciation of Cr associated with the humic acids using X-ray absorption spectroscopy (XAS) indicates that at all pH values the resulting Cr(III) was associated with the partially degraded humic acid in an inner sphere adsorption complex. The proposed model illustrates that the binding of Cr(III) to the humic acids can be expressed as $(R\text{-}COO)_2$ $–Cr\text{-}(OH_2)_4$. The data obtained from the ^{13}C NMR and pyrolysis GC-MS spectroscopy indicate that the reduction of Cr(VI) into Cr(III) by the humic acids resulted in changes in HAs chemistry. A loss of substituted phenolic moieties and hydroxyl groups from the humic acids exposed to Cr(VI) was observed. The loss of the phenolic and non-phenolic aromatic compounds in all humic acids due to the reaction with Cr(VI) propose ‘ring opening’ phenomena. The reaction mechanism of Cr(VI)-humic acid’s functional groups is likely to be similar to that reported using simple organic moieties (alcohols, aldehydes, and phenols), which involve a rapid chromate ester formation followed by the electron transfer decomposition step (Lee and Stewart, 1967, Wiberg and Schafer, 1967, Wiberg and Schaefer, 1969, Rahman and Rocek, 1971, Rocek and Ng, 1974, Elovitz and Fish, 1995). The number of electrons that can be transferred during the decomposition step depends strongly on the type of substituents attached to the reactive functional groups. Comparing the reactivity of humic acids with simple moieties indicates that humic acids showed a greater reducing power of Cr(VI) especially under neutral and alkaline conditions where simple moieties are un-reactive.

7.2 Implications

In natural environments that are rich with soil organic matter, Cr(VI)-containing leachate will be reduced rapidly to Cr(III) over the range of pH values that lie in the acidic to neutral conditions. The natural reduction of Cr(VI) in such environments prevent the transport of Cr(VI) into the subsurface and consequently into the groundwater. To minimize the transport of Cr(VI) into the groundwater in soils poor in organic matter, Cr(VI)-contaminated plumes could be treated by deploying organic matter that is rich with humic substances underground within an engineered treatment scheme. Permeable reactive barriers (PRBs) could be viable in situ treatment option to treat a contaminated groundwater plume. Recently, this remediation technique has been deployed at many contaminated sites using different reactive materials to treat a range of organic and inorganic contaminants. Full-scale barriers were installed in both confined and unconfined aquifers (Blowes et al., 2000, Di Natale et al., 2008, Robertson et al., 2008), and they have successfully run. Indeed, the success of the PRBs approach is influenced primarily by the dominant direction of the plume. When the direction of the plume is broadly horizontal, the plume can be treated by the PRBs, while it is hard to apply this technique when the plume direction is broadly vertical. In Cr(VI) contaminated sites, small and full-scale applications of PRBs have been also applied using primarily zero-valent iron as a reactive material (Puls et al., 1999a, Puls et al., 1999b, Flury et al., 2009). At some sites, the concentration of Cr(VI) in the released groundwater reduced to less than 0.01 mg/l (Puls et al., 1999a). The previous field applications of PRBs using zero valent iron suggest that the treatment of Cr in the contaminated sites is possible. However, the applying of zero valent iron under alkaline conditions limits the design life of the barrier (Fuller et al., 2013).

The data presented in the result chapters suggests that the use of organic matter rich with humic acids for the in situ remediations of Cr(VI) contaminated groundwater could be successful over a wide range of conditions that are common in the natural environment ($pH \le 7$).To maximise the treatment efficiency and longevity in the barrier, the reactive organic material should contain a humic substance that has a high density of phenolic and hydroxyl sites. Thus, it is important to choose an organic matter that contains more labile humic acids such as peat, sewage sludge, and compost. This will ensure that the rate at which Cr(VI) is reduced, and the total capacity for Cr(VI) reduction will both be high. In addition, the data presented in Chapter 5 clearly shows that the rate of reaction between Cr(VI) and the humic acids was pH dependent, decreases with increasing the pH values, hence, the PRBs have to be constructed with thickness commensurate with the acidity of the contaminated plume. As the pH value increases, the barrier thickness should increase to allow the contaminated plume to flow within the barrier with a residence time that is enough to complete the remediation process. However, as many chromate contaminated sites release leachate at high pH values (above pH 11), particularly chromite ore processing residue leachate, it would be expected that the remediation process of COPR leachate through the barrier will be more complicated in comparison with contaminated plumes at pH values ≤ 7. COPR leachate will increase the solubility of the humic acids due to its high pH value and that will considerably decrease the effective barrier thickness and consequently the lifetime of the barrier. Moreover, the rate of reaction between the humic acids moieties and Cr(VI) will be slow and it is expected to take place on a timescale of weeks. Indeed, the success of the remediation process by the barrier under alkaline conditions requires an installing of a barrier slightly downstream the contaminated site in order to increase the buffering of COPR leachate by the surrounding soil minerals before crossing the barrier. Moreover, the barrier should be designed with enough thickness to achieve two zones concept. The upgradient side of the reactive material works as a buffering zone of the contaminated plume to decrease the pH value of the plume to around pH 8. In addition to the buffering role of that zone, part of Cr(VI) is reduced by the reactive material but at a slow rate. When the plume passes through the downgradient side of the reactive material, Cr(VI) is reduced at a high rate due to the neutralization of the alkaline plume within the buffering zone.

The results presented in Chapter 6 highlight the stability of the reduced Cr within the barrier. Cr(III) strongly binds to carboxylic moieties in an inner sphere complexation, regardless of the pH value. The binding regime between Cr(III) and the humic acid reduces significantly the opportunity for the spread of Cr(III) or re-oxidation into mobile Cr(VI) species by manganese oxides.

7.3 Future Work

Based on the data obtained and discussed in the previous three chapters, it is recommended that further research is undertaken in the following areas:

Competition effects of other metal ions: The published data that described the reduction of Cr(VI)-humic acids were mostly undertaken in the absence of other metal ions. This study illustrated that the reduced Cr was associated with the humic acids by inner-sphere complexation. Hence, the presence of other metal cations may affect the binding of Cr(III) to the humic acids moieties due to the competition between the metals cations on the binding sites, which may increase the opportunity for the spread of Cr(III) and hence re-oxidation into mobile Cr(VI) species in the real applications. Therefore further investigation and experimentation are strongly recommended to be certain whether the metal ions bind to the same sites as Cr, particularly those are accompanied by Cr in the disposal wastes such as Al in the chromium ore processing residue and Cu in wood preservation waste (Geelhoed et al., 2002, Ottosen et al., 2009).

pH effect: While the majority of humic acids-Cr(VI) kinetic data have covered a pH range lower than 7, very little studies have been carried out under alkaline conditions. Based on the fact that the rate of Cr(VI)-humic acids reaction becomes slower with increasing pH, researchers speculated that above pH 7 the reduction of CrO_4^{2-} (the predominant species of Cr(VI)) by humic acids functional groups is not favourable (Elovitz and Fish, 1994, Elovitz and Fish, 1995). However, the findings from this research propose that humic acids extracted principally from peat and sludge phyto conditioning residue can be considered a reasonable reductant of Cr(VI) under such conditions. This finding will serve as a base for future studies to examine the suitability of other types of humic acids (e.g. sewage sludge, compost, etc.) in real applications, especially there are many environmental systems and contaminated sites have pH values greater than 7 and that still represents a challenge for the remediation processes.

Comparison of humic acids from different ages: Although Cr(VI) reduction occurred by all types of humic acids under investigation, the rate at which Cr(VI) is reduced, and also the total capacity for Cr(VI) reduction varied considerably between the humic acids. The reactivity of the humic acids appreciably varies with particularly the density of the phenolic and alcoholic moieties. Hence, further experimental investigations will need to be done to determine the major chemical characteristics of humic acids. While most of the available characterization have been for conventional sources of humic acids (e.g. soil and coal), further experimental investigations would be very interesting for a better understanding of humic acids from secondary materials, such as sludge and compost, and consequently their effectiveness in treating Cr(VI) contaminated sites.

7.4 References

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Appendix A Additional Tables

Table A 1: Data of 1100 µM Cr(VI) removal from aqueous solution by AHA over time as a function of pH, as depicted in Figure 5.1 and 5.2 of chapter 5.

Time (day)	**AHA pH_i^a 3.0**		**AHA pH_i 5.0**		**AHA pH_i 7.0**		**AHA pH_i 8.5**		**AHA pH_i 9.0**		**AHA pH_i 11.0**		**Control**	
	[Cr(VI)] µmol L^{-1}	pH_t^b	[Cr(VI)] µmol L^{-1}	pH_t	[Cr(VI)] µmol L^{-1}	pH_t	[Cr(VI)] µmol L^{-1}	pH_t	[Cr(VI)] µmol L^{-1}	pH_t	[Cr(VI)] µmol L^{-1}	pH_t	[Cr(VI)] µmol L^{-1}	pH_t
0	1100 ± 0	3.0 ± 0.0	1100 ± 0	5.0± 0.0	1100 ± 0	7.0± 0.0	1100 ± 0	8.5± 0.0	1100 ± 0	9.0± 0.0	1100 ± 0	11.0± 0.0	1100 ± 0	7.2± 0.0
0.17	623 ± 6	3.7 ± 0.0	853 ± 32	5.7± 0.0	1008 ± 7	7.5± 0.2	1100 ± 11	8.5± 0.0	1100 ± 24	9.1 ± 0.0	1100 ± 22	11.0± 0.0	1083 ± 19	7.2± 0.0
1	502 ± 25	3.9 ± 0.0	750 ± 17	5.9± 0.0	972 ± 10	7.6± 0.1	1100 ± 22	8.5± 0.0	1122 ± 11	9.0±0.0	1111 ± 0	11.0± 0.0	1111 ± 5	7.2± 0.1
2	385 ± 12	3.9 ± 0.0	671 ± 7	5.9± 0.0	960 ± 7	7.7± 0.0	-	-	-	-	-	-	1079 ± 29	7.1± 0.1
5	156 ± 11	4.0 ± 0.0	498 ± 17	6.0± 0.0	839 ± 22	7.8± 0.0	1045 ± 11	8.5± 0.0	1111 ± 14	9.0± 0.0	1078 ± 11	11.0± 0.0	1101 ± 3	7.1± 0.1
9	56 ± 4	4.1 ± 0.0	405 ± 11	6.1± 0.1	823 ± 25	7.7± 0.0	-	-	-	-	-	-	1024 ± 16	7.3± 0.0
16	ND[c]	4.0 ± 0.0	305 ± 1	6.3± 0.0	801 ± 8	7.9± 0.1	1056 ± 22	8.5± 0.0	1122 ± 6	8.9± 0.0	1089 ± 0	10.9± 0.0	1100 ± 6	7.2± 0.1
33	ND	4.0 ± 0.0	177 ± 1	6.2 ± 0.1	752 ± 13	7.8± 0.0	990 ± 11	8.5± 0.1	1078 ± 14	8.9± 0.0	1067 ± 11	10.8± 0.1	1098 ± 6	7.0± 0.0
51	ND	4.1 ± 0.3	72 ± 1	6.2 ± 0.0	677 ± 14	7.8± 0.0	1001 ± 11	8.6± 0.0	1067 ± 6	8.9±0.0	1067 ± 11	10.8± 0.1	1100 ± 12	7.2± 0.1

[a] Initial pH value.

[b] pH value as a function of time.

[c] Not detected.

Table A 2: Data of 1100 µM Cr(VI) removal from aqueous solution by PHA over time as a function of pH, as depicted in Figure 5.1 and 5.2 of chapter 5.

Time (day)	**PHA pH$_i^a$ 3.0**		**PHA pH$_i$ 5.0**		**PHA pH$_i$ 7.0**		**PHA pH$_i$ 8.5**		**PHA pH$_i$ 9.0**		**PHA pH$_i$ 11.0**		**Control**	
	[Cr(VI)] µmol L^{-1}	pH$_t^b$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$
0	1100 ± 0	3.0± 0.0	1100 ± 0	5.0± 0.0	1100 ± 0	7.0± 0.0	1100 ± 0	8.5± 0.0	1100 ± 0	9.0± 0.0	1100 ± 0	11.0± 0.0	1100 ± 0	7.2± 0.0
0.17	252 ± 4	3.8± 0.0	311 ± 17	5.5± 0.0	963 ± 14	7.5± 0.0	1091 ± 17	8.5± 0.0	1093 ± 13	8.8± 0.0	-	-	1099 ± 1	7.1± 0.0
1	30 ± 4	3.8± 0.0	54 ± 14	5.7± 0.0	783 ± 26	7.5± 0.0	1051± 31	8.5± 0.0	1056 ± 37	8.8± 0.1	1071 ± 68	10.8± 0.1	1108 ± 8	7.3± 0.0
2	ND[c]	3.8± 0.0	6 ± 2	5.7± 0.0	625 ± 20	7.5± 0.0	1007 ± 14	8.5± 0.0	1022 ± 40	8.8± 0.0	-	-	1102 ± 4	7.3± 0.0
5	ND	3.7± 0.0	ND	5.7± 0.0	366 ± 21	7.6± 0.0	818 ± 11	8.6± 0.0	851 ± 39	8.8± 0.1	-	-	1099 ± 3	7.3± 0.0
9	ND	3.7± 0.0	ND	5.7± 0.0	187 ± 13	7.7± 0.0	688 ± 11	8.5± 0.0	743 ± 46	8.8± 0.1	-	-	1111 ± 9	7.3± 0.0
16	ND	3.7± 0.0	ND	5.8± 0.0	29± 2	7.7± 0.0	501 ± 10	8.5± 0.0	578 ± 30	8.7± 0.1	930± 91	10.6± 0.2	1109 ± 6	7.2± 0.1
33	ND	3.7± 0.0	ND	5.8± 0.0	ND	7.6± 0.0	213 ± 20	8.5± 0.2	326 ± 22	8.8± 0.1	807 ± 145	10.5± 0.1	1103 ± 8	7.3± 0.0
51	ND	3.7± 0.0	ND	5.8± 0.0	ND	7.6± 0.0	59 ± 0.0	8.6± 0.0	163 ± 31	8.8± 0.0	475 ± 447	10.5± 0.1	1106 ± 11	7.2± 0.0

[a] Initial pH value.

[b] pH value as a function of time.

[c] Not detected.

Table A 3: Data of Cr(VI) removal from aqueous solution by SHA over time as a function of pH, as depicted in Figure 5.1 and 5.2 of chapter 5.

Time (day)	SHA pH$_i$[a] 3.0		SHA pH$_i$ 5.0		SHA pH$_i$ 7.0		SHA pH$_i$ 8.5		SHA pH$_i$ 9.0		SHA pH$_i$ 11.0		Control	
	[Cr(VI)] µmol L^{-1}	pH$_t$[b]	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$
0	1100 ± 0	3.0± 0.0	1100 ± 0	5.0± 0.0	1100 ± 0	7.0± 0.0	1100 ± 0	8.5± 0.0	1100 ± 0	9.0± 0.0	1100 ± 0	11.0± 0.0	1100 ± 0	7.8± 0.0
0.17	610 ± 17	3.6± 0.0	865 ± 5	5.5± 0.0	1114 ± 9	7.3± 0.1	1143 ± 8	8.5± 0.0	1149 ± 3	9.0± 0.0	1171 ± 20	11.0± 0.0	1110 ± 13	7.8± 0.0
1	315 ± 13	3.8± 0.0	561 ± 5	5.7± 0.0	1019 ± 5	7.4± 0.1	1113 ± 4	8.5± 0.0	1141 ± 9	9.0± 0.0	1144 ± 8	10.9± 0.0	1079 ± 6	7.8± 0.0
2	170 ± 2	3.9± 0.0	424 ± 7	5.8± 0.0	983 ± 4	7.5± 0.1	-	-	-	-	-	-	1096 ± 3	7.9± 0.0
5	ND[c]	3.9± 0.1	170 ± 6	5.8± 0.0	815 ± 19	7.4± 0.1	1050 ± 3	8.3± 0.0	1100 ± 23	8.7± 0.0	1141 ± 7	10.8± 0.0	1076 ± 11	7.8± 0.0
9	ND	-	27± 7	6.0± 0.0	698 ± 27	7.6± 0.1	-	-	-	-	-	-	1086 ± 16	7.8± 0.1
16	ND	-	ND	6.0± 0.0	634 ± 36	7.8± 0.0	992 ± 7	8.3± 0.0	1072 ± 34	8.6± 0.2	-	10.8± 0.0	1088 ± 3	7.9± 0.0
33	ND	-	ND	-	339± 12	7.9± 0.1	737 ± 17	8.4± 0.0	849 ± 33	8.5± 0.1	1184 ± 24	10.8± 0.0	1082 ± 4	8.0± 0.0
51	ND	4.2± 0.0	ND	5.9± 0.0	200 ± 4	7.7± 0.1	598 ± 29	8.2± 0.0	703 ± 30	8.3± 0.0	1141 ± 12	10.5± 0.0	1094 ± 15	7.7± 0.0

[a] Initial pH value.

[b] pH value as a function of time.

[c] Not detected.

Table A 4: Data of Cr(VI) removal from aqueous solution by SPCR-HA over time as a function of pH, as depicted in Figure 5.1 and 5.2 of chapter 5.

Time (day)	SPCR-HA pH$_i^a$ 3.0		SPCR-HA pH$_i$ 5.0		SPCR-HA pH$_i$ 7.0		SPCR-HA pH$_i$ 8.5		SPCR-HA pH$_i$ 9.0		SPCR-HA pH$_i$ 11.0		Control	
	[Cr(VI)] µmol L^{-1}	pH$_t^b$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$
0	1100 ± 0	3.0± 0.0	1100 ± 0	5.0± 0.0	1100 ± 0	7.0± 0.0	1100 ± 0	8.5± 0.0	1100 ± 0	9.0± 0.0	1100 ± 0	11.0± 0.0	1100 ± 0	7.7± 0.1
0.17	301 ± 9	3.8± 0.0	396 ± 17	5.8± 0.0	985 ± 23	7.4± 0.0	1099 ± 52	8.5± 0.0	1139 ± 30	9.0± 0.0	1136 ± 27	10.9± 0.0	1078 ± 2	7.7± 0.1
1	66 ± 10	3.9± 0.0	84 ± 24	5.9± 0.0	779 ± 22	7.6± 0.1	1062± 11	8.5± 0.0	1123 ± 14	9.0± 0.0	1067 ± 10	10.9± 0.0	1070 ± 9	7.7± 0.1
2	ND^c	3.9± 0.0	ND	5.9± 0.0	647 ± 9	7.6± 0.1	1007 ± 11	8.5± 0.0	1105 ± 18	9.0± 0.0	1155 ± 24	10.9± 0.0	1088 ± 5	7.6± 0.2
5	ND	-	ND	-	382 ± 7	7.7± 0.0	869 ± 6	8.6± 0.0	1019 ± 4	9.0± 0.0	1129 ± 5	10.9± 0.0	1072 ± 4	7.8± 0.0
9	ND	-	ND	-	170 ± 11	7.7± 0.0	756 ± 17	8.5± 0.0	948 ± 26	8.9± 0.0	1141 ± 8	10.8± 0.0	1099 ± 3	7.8± 0.0
16	ND	-	ND	-	7± 6	7.6± 0.0	612 ± 10	8.5± 0.0	842 ± 18	8.8± 0.0	1139± 5	10.5± 0.0	1053 ± 9	7.5± 0.1
33	ND	-	ND	-	6± 3	7.7± 0.1	369 ± 15	8.7± 0.0	665 ± 8	9.0± 0.0	1195 ± 20	10.7± 0.0	1084 ± 7	7.8± 0.1
51	ND	3.8± 0.0	ND	5.8± 0.0	1± 1	7.4± 0.0	178 ± 34	8.4± 0.1	468 ± 21	8.8± 0.0	1116 ± 18	10.5± 0.1	1061 ± 8	7.7± 0.0

[a] Initial pH value.

[b] pH value as a function of time.

[c] Not detected.

Table A 5: Data of 550 µM Cr(VI) removal from aqueous solution by AHA over time as a function of pH, as depicted in Figure 5.3 and 5.4 of chapter 5.

Time (day)	**AHA pH$_i^a$ 3.0**		**AHA pH$_i$ 5.0**		**AHA pH$_i$ 7.0**		**AHA pH$_i$ 8.5**		**AHA pH$_i$ 9.0**		**AHA pH$_i$ 11.0**		**Control**	
	[Cr(VI)] µmol L^{-1}	pH$_t^b$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$
0	1100 ± 0	3.0± 0.0	1100 ± 0	5.0± 0.0	1100 ± 0	7.0± 0.0	1100 ± 0	8.5± 0.0	1100 ± 0	9.0± 0.0	1100 ± 0	11.0± 0.0	1100 ± 0	7.5± 0.0
0.17	346 ± 6	3.5 ± 0.1	706 ± 107	5.3 ± 0.0	1047 ± 12	7.3 ± 0.0	1016 ± 24	8.5 ± 0.0	1010 ± 12	9.0 ± 0.0	1008 ± 8	11.0 ± 0.0	1015 ± 3	7.5 ± 0.1
1	109 ± 5	3.5 ± 0.1	641 ± 4	5.4 ± 0.0	1058 ± 7	7.3 ± 0.1	1112 ± 14	8.5 ± 0.0	1100 ± 4	9.0 ± 0.0	1069 ± 21	10.9 ± 0.0	1092 ± 8	7.4 ± 0.0
2	10 ± 14	3.5 ± 0.0	514 ± 9	5.5 ± 0.0	-	-	-	-	-	-	-	-	-	-
5	5 ± 5	3.4 ± 0.0	294 ± 11	5.4 ± 0.0	904 ± 14	7.3 ± 0.1	1083 ± 4	8.6 ± 0.0	1090 ± 5	9.1 ± 0.0	1060 ± 19.8	10.9 ± 0.3	1099 ± 29	7.7 ± 0.1
9	ND^c	-	135 ± 3	5.5 ± 0.0	872 ± 60	7.5 ± 0.1	1100 ± 24	8.6 ± 0.0	1086 ± 47	9.0 ± 0.1	1059 ± 53	11.0 ± 0.1	974 ± 24	7.8 ± 0.1
18	ND	-	1 ± 1	5.6 ± 0.0	712 ± 17	7.5 ± 0.0	1070 ± 9	8.5 ± 0.0	1107 ± 18	9.1 ± 0.1	1106 ± 9	11.1 ± 0.1	1101 ± 11	7.8 ± 0.1
33	ND	-	ND	-	549 ± 19	7.6 ± 0.0	1002 ± 2	8.5 ± 0.1	1055 ± 12	9.0 ± 0.1	1100 ± 5	10.9 ± 0.0	1100 ± 4	7.5 ± 0.1
51	ND	3.4 ± 0.0	ND	5.5 ± 0.0	452 ± 4	7.5 ± 0.1	1017 ± 7	8.4 ± 0.1	1111 ± 11	8.9 ± 0.0	1131 ± 11	10.9 ± 0.0	1088 ± 15	7.6 ± 0.1

[a] Initial pH value.

[b] pH value as a function of time.

[c] Not detected.

Table A 6: Data of 2200 µM Cr(VI) removal from aqueous solution by PHA over time as a function of pH, as depicted in Figure 5.1 and 5.2 of chapter 5.

Time (day)	**PHA pH$_i^a$ 3.0**		**PHA pH$_i$ 5.0**		**PHA pH$_i$ 7.0**		**PHA pH$_i$ 8.5**		**PHA pH$_i$9.0**		**PHA pH$_i$11.0**		**Control**	
	[Cr(VI)] µmol L^{-1}	pH$_t^b$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$	[Cr(VI)] µmol L^{-1}	pH$_t$
0	1100 ± 0	3.0± 0.0	1100 ± 0	5.0± 0.0	1100 ± 0	7.0± 0.0	1100 ± 0	8.5± 0.0	1100 ± 0	9.0± 0.0	1100 ± 0	11.0± 0.0	1100 ± 0	7.2± 0.0
0.17	747 ± 13	4.3 ± 0.0	761 ± 17	5.8 ± 0.0	1089 ± 21	7.2 ± 0.0	1099 ± 3	8.5 ± 0.0	1100 ± 15	8.9 ± 0.0	1100 ± 36	10.9 ± 0.0	1079 ± 11	8.1 ± 0.0
1	422 ± 5	4.7 ± 0.1	456 ± 7	6.1 ± 0.0	1008 ± 23	7.4 ± 0.0	1090 ± 9	8.5 ± 0.1	1115 ± 9	9.0 ± 0.0	1104 ± 7	11.0 ± 0.0	1089 ± 13	-
2	284 ± 14	4.9 ± 0.1	338 ± 10	6.3 ± 0.1	928 ± 40	7.6 ± 0.1	-	-	-	-	-	-	-	-
6	84 ± 11	5.0 ± 0.0	95 ± 3	6.4 ± 0.0	654 ± 17	7.7 ± 0.0	968 ± 13	8.5 ± 0.0	1034 ± 23	8.9 ± 0.0	1100 ± 7	10.7 ± 0.0	1084 ± 17	8.1 ± 0.0
11	14 ± 10	5.0 ± 0.0	18 ± 3	6.4 ± 0.0	526 ± 8	7.8 ± 0.0	876 ± 18	8.6 ± 0.0	972 ± 14	8.9 ± 0.1	1067 ± 26	10.8 ± 0.0	1079 ± 15	8.0 ± 0.2
16	ND^c	5.0 ± 0.0	ND	6.4 ± 0.1	445 ± 3	8.0 ± 0.0	840 ± 18	8.7 ± 0.0	912 ± 23	9.0 ± 0.0	1044 ± 12	10.8 ± 0.0	1091 ± 17	8.2 ± 0.0
33	ND	-	ND	-	264 ± 15	8.1 ± 0.0	668 ± 27	8.8 ± 0.1	777 ± 36	9.1 ± 0.0	962 ± 39	10.7 ± 0.1	1078 ± 10	8.2 ± 0.0
51	ND	5.0 ± 0.1	ND	6.2 ± 0.0	155 ± 16	8.1 ± 0.0	618 ± 25	8.7 ± 0.0	747 ± 21	8.9 ± 0.1	982 ± 30	10.6 ± 0.1	1084 ± 14	8.0 ± 0.3

[a] Initial pH value.

[b] pH value as a function of time.

[c] Not detected.

Table A 7: Principal organic compounds in the humic acids before, and after reaction with 8000 µmol Cr(VI) at pH3, identified by PyGCMS (see Figure 6.4). Principal organic compounds were identified by comparison of the normalized pyrograms with the NIST Standard Reference Database (NIST11s MS library).

	Compound	Aromatic / Aliphatic	Relative Area×100%							
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)	SHA	SHA-Cr(VI)	SPCR-HA	(SPCR-HA)-Cr(VI)
1	1-Butanol	Aliphatic	-	-	-	-	-	-	-	6.04
2	Ethanethiol	Aliphatic	-	-	-	-	-	4.79	-	-
3	Pentanal, 2,3-dimethyl	Aliphatic	-	-	-	-	-	1.83	-	2.51
4	Propylene oxide	Aliphatic	-	0.19	-	-	-	-	-	-
5	Furan, 2-methyl-	Aromatic	-	-	1.68	-	1.44	5.01	2.24	3.52
6	Formaldehyde, dimethylhydrazone	Aliphatic	-	-	-	-	-	-	-	1.86
7	propanenitrile	Aliphatic	-	-	-	-	-	4.49	-	-
8	Benzene	Aromatic	1.16	0.57	-	-	-	-	-	-
9	Butanal, 2-methyl-	Aliphatic	-	-	-	-	0.61	1.20	0.67	1.25
10	Furan, 2,5-dimethyl-	Aromatic	-	-	-	-	0.65	-	-	-
11	Isobutyronitrile	Aliphatic	-	-	-	-	-	1.05	-	-

	Compound	Aromatic / Aliphatic	Relative Area×100%							
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)	SHA	SHA-Cr(VI)	SPCR-HA	(SPCR-HA)-Cr(VI)
12	Hexane, 2,3,4-trimethyl-	Aliphatic	-	-	-	-	-	-	-	1.06
13	Toluene	Aromatic	2.19	0.62	3.52	2.54	5.32	23.13	8.82	16.74
14	1H-Pyrrole, 1-methyl-	Aromatic	-	-	-	-	-	2.46	-	1.5
15	2-Propenoic acid, 1,4-butanediyl ester	Aliphatic	-	-	-	-	0.28	-	0.66	1.71
16	Pyridine	Aromatic	-	-	1.44	0.99	-	8.68	2.39	3.37
17	Pyridine, 2-methyl-	Aromatic	-	-	-	0.55	-	-	-	-
18	Ethylbenzene	Aromatic	0.4	-	-	-	0.51	2.96	2.13	3.82
19	Benzene, 1,4-dimethyl-	Aromatic		-	-	-	-	1.40	0.61	3.66
20	Dimethyl benzene	Aromatic	2.44	-	-	-	-	-	-	-
21	Pyrrole	Aromatic	-	-	-	-	-	-	-	0.95
22	p-Xylene	Aromatic	-	0.28	-	-	-	-	-	-
23	Dimethyl benzene	Aromatic	0.68	-	-	-	-	0.78	-	-
24	1,3,5,7 Cyclooctatetraene	Aliphatic	-	-	0.62	0.46	0.74	-	3.55	4.11

	Compound	Aromatic / Aliphatic	Relative Area×100%							
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)	SHA	SHA-Cr(VI)	SPCR-HA	(SPCR-HA)-Cr(VI)
25	Benzene, (1-methylethyl)-	Aromatic	-	-	-	-	-	-	0.74	-
26	4-Bromoheptane	Aliphatic	-	-	-	-	-	1.0	-	-
27	1H-Pyrrole, 2-methyl-	Aromatic	-	-	0.48	0.46	0.43	-	-	-
28	Anisole	Aromatic	-	-	-	0.62	-	-	-	-
29	1H-Pyrrole, 3-methyl-	Aromatic	-	-	0.98	0.65	-	0.71	-	-
30	Benzene, 1-ethyl-4-methyl-	Aromatic	0.52	-	-	-	-	-	-	-
31	2-Cyclopenten-1-one, 2-methyl-	Aliphatic	-	-	-	0.49	-	-	-	-
32	.alpha.-Methylstyrene	Aromatic		-	-	-	-	-	1.58	1.83
33	Trimethyl benzene	Aromatic	0.77	-	-	-	-	-	-	-
34	Trimethyl benzene	Aromatic	1.56	-	-	-	-	-	-	-
35	Undecane	Aliphatic	-	-	-	-	-	-	-	0.86
36	Benzene, 1-methoxy-2-methyl-	Aromatic	-	-	-	0.5	-	-	-	-

	Compound	Aromatic / Aliphatic	Relative Area×100%							
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)	SHA	SHA-Cr(VI)	SPCR-HA	(SPCR-HA)-Cr(VI)
37	2-Furancarboxaldehyde, 5-methyl-	Aromatic	-	-	0.91	-	-	-	-	-
38	2-Cyclopenten-1-one, 3-methyl-	Aliphatic	-	-	-	0.46	-	-	-	-
39	1-Dodecanol	Aliphatic	-	-	-	-	-	-	-	0.94
40	1-Dodecene	Aliphatic	-	-	-	0.29	-	-	-	-
41	Benzene, 1-ethyl-4-methoxy-	Aromatic	-	-	-	0.33	-	-	-	-
42	Acetophenone	Aromatic	-	-	-	0.38	-	-	-	-
43	Phenol	Aromatic	5.09	-	7.72	4.22	7.65	8.22	7.77	2.03
44	Dodecane, 2-methyl-	Aliphatic	-	-	-	-	-	-	-	1.06
45	Phenol, 2-methoxy-	Aromatic	-	-	6.88	0.64	9.44	-	11.99	-
46	Phenol, 2-methyl-	Aromatic	0.71	-	1.17	1.13	-	-	-	-
47	Tridecane	Aliphatic	-	-	-	-	0.63	-	-	-
48	n-Tridecan-1-ol	Aliphatic	-	-	-	-	0.41	-	0.48	-
49	Tridecane	Aliphatic	-	-	0.57	0.6	-	-	-	-

	Compound	Aromatic / Aliphatic	Relative Area×100%							
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)	SHA	SHA-Cr(VI)	SPCR-HA	(SPCR-HA)-Cr(VI)
50	1-Dodecanol	Aliphatic	-	-		0.77	-	-	-	-
51	1-Tridecene	Aliphatic	-	-	0.56	-	-	-	-	-
52	p-Cresol	Aromatic	0.48	-	5.99	2.06	7.07	-	-	-
53	4-Hydroxytoluene	Aromatic		-	-	-	-	-	5.79	-
54	3-Tetradecene, (E)-	Aliphatic	-	-	-	-	-	-	-	0.87
55	2,5-Pyrrolidinedione, 1-methyl-	Aromatic	-	-	-	-	0.77	-	-	-
56	Creosol(2-methoxy-4methylphenol)	Aromatic	-	-	3.71	-	5.43	-	-	-
57	Phenol, 2,4-dimethyl-	Aromatic	-	-	-	1.22	-	-	-	-
58	Tetradecane	Aliphatic	-	-	0.62	0.53	-	-	-	-
59	1-Tridecene	Aliphatic	-	-	0.36	0.44	-	-	-	-
60	Phenol, 4-ethyl-	Aromatic	-	-	3.54	2.0	1.89	-	-	-
61	Phenol, 4-ethyl-2-methoxy-	Aromatic	-	-	3.77	-	3.92	-	3.42	-
62	Benzenepropanenitrile	Aromatic	-	-	-	-	-	3.21	-	-

	Compound	Aromatic / Aliphatic	Relative Area×100%							
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)	SHA	SHA-Cr(VI)	SPCR-HA	(SPCR-HA)-Cr(VI)
63	1-Pentadecene	Aliphatic	-	-	-	-	0.35		0.67	-
64	Tetradecane	Aliphatic	-	-	0.75	0.66	-	-	-	-
65	1-Pentadecene	Aliphatic	-	-	0.67	0.55	-	-	-	-
66	4-Hydroxy-3-methylacetophenone	Aromatic	-	-	-	-	9.73	-	-	-
67	Phenol, 2,6-dimethoxy-	Aromatic	-	-	4.72	-	8.54	-	5.55	-
68	Naphthalene, 2,6-dimethyl-	Aromatic	0.78	-	-	-	-	-	-	-
69	Indole	Aromatic	-	-	1.02	0.92	3.42	2.48	3.28	-
70	Tetradecane	Aliphatic	-	-	-	0.92	-	-	-	-
71	Octadecane	Aliphatic	-	-	0.98	-	-	-	-	-
72	2-Tridecanone	Aliphatic	-	-	-	-	-	0.69	-	1.13
73	1-Hexadecene	Aliphatic	-	-	0.44	0.55	-	-	-	-
74	Phenol, 2-methoxy-4-(1-propenyl)-, (E)	Aromatic	-	-	0.61	-	1.09	-	1.62	-
75	1H-Indole, 3-methyl-	Aromatic	-	-	-	0.72	-	-	-	-

	Compound	Aromatic / Aliphatic	Relative Area×100%							
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)	SHA	SHA-Cr(VI)	SPCR-HA	(SPCR-HA)-Cr(VI)
76	Octadecane	Aromatic	0.35	-	1.06	1.24	-	-	-	-
77	1-Heptadecene	Aliphatic	-	-		0.61	-	-	-	-
78	1-Pentadecene	Aliphatic	-	-	0.62	-	-	-	-	-
79	1-Dodecanol, 3,7,11-trimethyl-	Aliphatic	-	-	-	-	0.57	-	-	1.37
80	Naphthalene, 2,3,6-trimethyl-	Aromatic	0.38	-	-	-	-	-	-	-
81	1-Dodecanol, 3,7,11-trimethyl-	Aliphatic	-	-	-	-	-	-	-	1.41
82	Naphthalene, 2,3,6-trimethyl-	Aromatic	0.31	-	-	-	-	-	-	-
83	1-Dodecanol, 3,7,11-trimethyl-	Aliphatic	0.68	-	-	2.02	-	-	-	-
84	Naphthalene, 2,3,6-trimethyl-	Aromatic	1.20	-	-	-	-	-	-	-
85	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	Aromatic	-	-	-	-	0.51	-	-	-
86	Ethanone, 1-(4-hydroxy-3-methoxyphenyl)-	Aromatic	-	-	0.82	-	1.81	-	3.0	-
87	1-Octadecene	Aliphatic	-	-	-	-	-	-	-	1.03

	Compound	Aromatic / Aliphatic	Relative Area×100%							
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)	SHA	SHA-Cr(VI)	SPCR-HA	(SPCR-HA)-Cr(VI)
88	Octadecane	Aliphatic	-	-	0.9	1.35	-	-	-	-
89	1-Heptadecene	Aliphatic	-	-	0.6	1.16	-	-	-	-
90	Tetradecanenitrile	Aliphatic	-	-	-	-	-	0.64	-	-
91	2-Tetradecanone	Aliphatic	-	-	-	-	-	-	-	0.88
92	Nonadecane	Aliphatic	-	-	1.32	2.04	-	-	-	-
93	1-Nonadecene	Aliphatic	-	-	0.68	0.95	-	-	-	-
94	2-Pentadecanone, 6,10,14-trimethyl-	Aliphatic	-	-	-	0.69	-	-	-	-
95	Octadecane	Aliphatic	0.33	-	0.89	1.52	-	-	-	-
96	1-Nonadecene	Aliphatic	-	-	0.49	1.05	-	-	-	-
97	1-Nonadecene	Aliphatic	-	-	-	0.46	-	-	-	-
98	2-Heptadecanone	Aliphatic	-	-	-	0.31	-	-	-	-
99	Ethanone,1-(4-hydroxy-3,5-dimethoxyphenyl)-	Aromatic	-	-	-	-	2.09	-	-	-
100	Pentadecanenitrile	Aliphatic	-	-	-	-	-	1.20	-	1.71

	Compound	Aromatic / Aliphatic	Relative Area×100%							
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)	SHA	SHA-Cr(VI)	SPCR-HA	(SPCR-HA)-Cr(VI)
101	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	Aromatic	-	-	0.35	-	-	-	-	-
102	Heneicosane	Aliphatic	-	0.22	1.05	1.92	-	-	-	-
103	1-Nonadecene	Aliphatic	-	-	0.58	0.88	-	-	-	-
104	n-Hexadecanoic acid	Aliphatic	-	0.48	0.91	0.54	-	-	-	-
105	Heneicosane	Aliphatic	0.98	0.65	0.91	1.54	-	-	-	-
106	1-Nonadecene	Aliphatic	-	-	0.93	1.24	-	-	-	-
107	2-Nonadecanone	Aliphatic	-	-	0.64	1.09	-	-	-	-
108	Heneicosane	Aliphatic	1.85	1.7	1.04	1.93	-	-	-	-
109	1-Nonadecene	Aliphatic	-	-	0.56	0.59	-	-	-	-
110	Heneicosane	Aliphatic	3.53	3.76	0.87	1.89	-	-	-	-
111	Octacosanol	Aliphatic	-	-	0.61	1.24	-	-	-	-
112	Behenic alcohol	Aliphatic	-	-	-	0.29	-	-	-	-
113	2-Heptadecanone	Aliphatic	-	0.14	0.73	1.5	-	-	-	-

	Compound	Aromatic / Aliphatic	Relative Area×100%							
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)	SHA	SHA-Cr(VI)	SPCR-HA	(SPCR-HA)-Cr(VI)
114	Octacosane	Aliphatic	-	0.22	-	-	-	-	-	-
115	Heneicosane	Aliphatic	5.61	6.29	1.14	2.47	-	-	-	-
116	Octacosanol	Aliphatic	-	-	0.34	0.66	-	-	-	-
117	Octadecane	Aliphatic	0.34	0.58	-	-	-	-	-	-
118	Octadecane	Aliphatic	-	0.3	-	-	-	-	-	-
119	Heneicosane	Aliphatic	7.66	8.94	0.8	2.56	-	-	-	-
120	Octacosanol	Aliphatic	-	-	-	0.66	-	-	-	-
121	2-Heptadecanone	Aliphatic	-	-	-	0.79	-	-	-	-
122	Octacosane	Aliphatic	0.48	1.03	-	-	-	-	-	-
123	Pentacosane	Aliphatic	0.50	0.72	-	-	-	-	-	-
124	Heneicosane	Aliphatic	8.55	10.18	1.06	3.24	-	-	-	-
125	Octacosanol	Aliphatic	-	0.16	-	0.49	-	-	-	-
126	Tetracontane	Aliphatic	0.49	1.54	-	-	-	-	-	-
127	Octacosane	Aliphatic	0.69	1.15	-	-	-	-	-	-

	Compound	Aromatic / Aliphatic	Relative Area×100%							
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)	SHA	SHA-Cr(VI)	SPCR-HA	(SPCR-HA)-Cr(VI)
128	Heneicosane	Aliphatic	9.90	11.7	0.88	3.19	-	-	-	-
129	Behenic alcohol	Aliphatic	-	0.32	-	-	-	-	-	-
130	Tetracosanoic acid, methyl ester	Aliphatic	-	-	-	2.21	-	-	-	-
131	2-Heptadecanone	Aliphatic	-	-	0.48	-	-	-	-	-
132	Tetracontane	Aliphatic	0.40	0.96	-	-	-	-	-	-
133	Octadecane, 3-methyl-	Aliphatic	-	1.26	-	-	-	-	-	-
134	Octacosane	Aliphatic	0.85	-	-	-	-	-	-	-
135	Squalene	Aliphatic	-	0.31	-	1.09	-	-	-	-
136	Tetracontane	Aliphatic	-	11.76	0.82	-	-	-	-	-
137	Heneicosane	Aliphatic	10.23	-	-	3	-	-	-	-
138	Octacosanol	Aliphatic	-	0.41	-	-	-	-	-	-
139	Octacosanol	Aliphatic	-	0.98	-	-	-	-	-	-
140	Octadecane, 3-methyl-	Aliphatic	-	1.1	-	-	-	-	-	-

	Compound	Aromatic / Aliphatic	Relative Area×100%							
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)	SHA	SHA-Cr(VI)	SPCR-HA	(SPCR-HA)-Cr(VI)
141	Tetracontane	Aliphatic	9.56	11.16	-	1.86	-	-	-	-
142	Octacosanol	Aliphatic	-	0.34	-	-	-	-	-	-
143	Hexacosanoic acid, methyl ester	Aliphatic	-	-	-	2.63	-	-	-	-
144	Tetracontane	Aliphatic	-	0.36	-	-	-	-	-	-
145	Nonacosane	Aliphatic	-	0.71	-	-	-	-	-	-
146	Tetracontane	Aliphatic	7.28	8.09	-	-	-	-	-	-
147	Octacosanol	Aliphatic	-	0.35	-	-	-	-	-	-
148	Tetracontane	Aliphatic	2.13	3.25	-	-	-	-	-	-
149	Tetracontane	Aliphatic	-	1.24	-	-	-	-	-	-
150	Tetracontane	Aliphatic	-	1.95	-	-	-	-	-	-

Appendix B Published paper

Abiotic reduction of Cr(VI) by humic acids derived from peat and lignite: kinetics and removal mechanism. Submitted to Environmental Science and Pollution Research in May 2018 and published December 2018.

Part of the results presented in this thesis has been published in a peer-reviewed journal ‘Environmental Science and Pollution Research’. The following Authors’ Contributions are clarified below.

S.T. Aldmour	Principle author, isolation and characterization of the humic acids, reduction experiments, Pyro-GCMS, data analysis and interpretation, drafting the paper.
I.T. Burke	X-ray absorption spectroscopy (XAS) data processing and analysis, and extensive manuscript review.
A.W. Bray	X-ray fluorescence (XRF) data collection.
D.L. Baker	Cross-polarisation magic-angle-spinning ^{13}C-NMR spectroscopy data collection.
A.B. Ross	Pyrolysis-gas chromatography-mass spectrometry (Pyro-GCMS) data collection.
F. Gill	Pyrolysis-gas chromatography-mass spectrometry (Pyro-GCMS) data analysis.
G. Cibin	X-ray absorption spectroscopy (XAS) data processing and analysis.
M.E. Ries	Cross-polarisation magic-angle-spinning ^{13}C-NMR spectroscopy data collection.
D.I. Stewart	Data analysis and interpretation, and extensive manuscript review.

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RESEARCH ARTICLE

Abiotic reduction of Cr(VI) by humic acids derived from peat and lignite: kinetics and removal mechanism

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Abstract

Hexavalent chromium contamination of groundwater is a worldwide problem caused by anthropogenic and natural processes. We report the rate of Cr(VI) removal by two humic acids (extracted from Miocene age lignite and younger peat soil) in aqueous suspensions across a pH range likely to be encountered in terrestrial environments. Cr(VI) was reduced to Cr(III) in a first-order reaction with respect Cr(VI) concentration, but exhibited a partial order (~ 0.5) with respect to [H+]. This reaction was more rapid with the peat humic acid, where Cr(VI) reduction was observed at all pH values investigated ($3.7 \leq pH \leq 10.5$). ^{13}C NMR and pyrolysis GC-MS spectroscopy indicate that the reaction results in loss of substituted phenolic moieties and hydroxyl groups from the humic acids. X-ray absorption spectroscopy indicated that at all pH values the resulting Cr(III) was associated with the partially degraded humic acid in an inner-sphere adsorption complex. The reaction mechanism is likely to be controlled by ester formation between Cr(VI) and phenolic/hydroxyl moieties, as this initial step is rapid in acidic systems but far less favourable in alkaline conditions. Our findings highlight the potential of humic acid to reduce and remove Cr(VI) from solution in a range of environmental conditions.

Keywords Humic substances · Humic acids · Chromium · Contaminated land · Groundwater

Introduction

Chromium is a strategically important metal that is produced commercially from the chromite ore as sodium dichromate and similar chemicals (Wilbur et al. 2000; Jacobs and Testa 2005; Kogel et al. 2006). It is widely used in alloys, electroplating, leather tanning, timber treatment, wax, chromate pigments, refractories, ceramics, catalysts and organic acids (Barnhart 1997; Darrie 2001; Jacobs and Testa 2005; International Chromium Development Association 2016). Chromium has two environmentally stable oxidation states: Cr(III) and Cr(VI) (Pourbaix 1966; Brito et al. 1997). Cr(III) is an essential trace element for living organisms that has an influence on the biological function of the humans and animals (Lukaski 1999) and has a role in lipid, carbohydrate and glucose metabolism (Vincent 2000; Cefalu and Hu 2004). In contrast, Cr(VI) is toxic to plants (Chandra and Kulshreshtha 2004; Shanker et al. 2005), animals and humans (Costa 1997) and is classified as a mutagenic and carcinogenic material (Leonard and Lauwerys 1980; Kondo et al. 2003; Holmes et al. 2008).

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Occasionally the manufacture and industrial use of chromium can lead to contamination of groundwater and soils (Burke et al. 1991; Puls et al. 1999; Geelhoed et al. 2002; Whittleston et al. 2011; Ding et al. 2016; Izbicki and Groover 2016; Matern et al. 2016). Natural processes have also lead to elevated chromium concentrations in groundwater above the World Health Organisation maximum for drinking water (50 μg/L; (WHO 2003)) at numerous locations around the world (Robertson 1991; Fantoni et al. 2002; Ball and Izbicki 2004; Steinpress 2005). For example, ultramafic rocks can have high Cr contents (Stueber and Goles 1967; Schwertmann and Latham 1986; Becquer et al. 2003), which is mainly in Cr(III) in the parent minerals, but can be oxidised to Cr(VI) during weathering, particularly by manganese (IV) oxides (Bartlett and James 1979; Eary and Rai 1987; Fendorf and Zasoski 1992). While Cr(VI) release can result from many processes, some of the most intractable environmental problems are associated with poor disposal of chromite ore processing residue (COPR) from the high-lime process. While this is an obsolete method for producing chromate chemicals, it is only now being phased-out in newly industrialised countries (e.g. India, China and Bangladesh; Darrie 2001; Gao and Xia 2011; Matern et al. 2016). As a result, there are numerous problematic legacy sites from this technology around the world (Higgins et al. 1998; Geelhoed et al. 2002; Stewart et al. 2007; Whittleston et al. 2011; Matern et al. 2017; Zhou et al. 2018). Water in contact with high-lime COPR has a pH > 12 and can have an aqueous Cr(VI) concentrations in excess of 1 mM (Higgins et al. 1998; Stewart et al. 2010; Matern et al. 2017). When such water inevitably escapes from abandoned waste piles into the geosphere, it produces Cr(VI) plume where the pH varies from hyperalkaline values close to source towards the natural soil value in the far field.

Due to its toxicity and potential mobility (as soluble anionic aqueous species such as CrO_4^{2-}, $HCrO_4^-$ and $Cr_2O_7^{2-}$; Pourbaix 1966; Brito et al. 1997), the accidental release of Cr(VI) into terrestrial ecosystems is a significant cause for concern. In oxidising environments, surface complexation reactions with iron and aluminium oxide minerals can remove Cr(VI) from solution at acidic pH (Rai et al. 1989); however, at neutral and alkaline pH, adsorption to soil minerals is generally weak due to the presence of net negative surface charge at mineral surfaces (Rai et al. 1989; Langmuir 1997). Cr is far less mobile in reducing soil environments because aqueous Fe(II), Fe(II)-containing minerals and reduced sulphur compounds can rapidly reduce Cr(VI) to Cr(III) (Eary and Rai 1988; Rai et al. 1989; Eary and Rai 1991; Palmer and Wittbrodt 1991). Once reduced, Cr(III) will precipitate as $Cr(OH)_3$ in circumneutral conditions (Pourbaix 1966) or, when reduced by Fe(II), as $(Cr_x\ Fe_{1-x})(OH)_3$ (Sass and Rai 1987; Eary and Rai 1988; Rai et al. 1989).

Most soils contain organic matter, which plays an important role in the cycling of many elements in the environment (Gustafsson et al. 2001). Humic substances (the majority of soil organic matter; International Humic Substances Society 2007) are the dark-coloured, heterogeneous organic compounds produced by the decay and transformation of plant and animal residues by bacteria and fungi (Stevenson 1994; Swift 1999; Sutton and Sposito 2005; Brookes et al. 2008). The main humic precursor molecules are formed by depolymerisation and oxidation of plant biopolymers and proteins to produce molecules that contain unaltered polymer segments and phenolic, hydroxyl, carboxyl and amino residues (Wershaw 1986; Stevenson 1994; Swift 1999; Aro et al. 2005). However, there is still debate about how humic substances subsequently form. The traditional ‘polymer model’ assumes that the precursors are microbiologically synthesised into large randomly coiled polymeric macromolecules (Swift 1999), whereas recent evidence suggests that humic substances are supramolecular associations (Wershaw 1994; Kögel-Knabner 2000; Sutton and Sposito 2005). Amphiphilic precursor molecules cluster together into micelle-like particles (Wershaw 1999; Kögel-Knabner 2000), and other biomolecules from plant degradation become associated with either hydrophobic or hydrophilic domains (Piccolo et al. 1996; von Wandruszka 1998; Zang et al. 2000; Piccolo 2001; Simpson et al. 2002; Fan et al. 2004). While most evidence now supports this ‘micelle model’, it does not preclude development of polymer-type bonds, particularly as humic substances can potentially age and degrade over millions of years (Burdon 2001; Knicker et al. 2002; Sutton and Sposito 2005).

Cr(VI) can be reduced to Cr(III) by reaction with organic matter that contains phenolic, hydroxyl and aldehyde moieties (Lee and Stewart 1967; Wiberg and Schafer 1967; Elovitz and Fish 1995; Chen et al. 2015). The reaction with such moieties is thought to involve a chromate ester intermediate that can form with monomeric aqueous H_2CrO_4 and $HCrO_4^-$ species, with the redox step occurring during ester decomposition (Lee and Stewart 1967; Wiberg and Schafer 1967; Elovitz and Fish 1995). Reduction of Cr by this mechanism is rapid in acidic systems, but the rate decreases markedly with increasing pH (Lee and Stewart 1967; Elovitz and Fish 1995; Wittbrodt and Palmer 1997). Usually, it is assumed that Cr(VI) reduction by alcohol, phenolic and aldehyde moieties is negligible when ≥ pH 6 because chromate ester formation is less favourable with the CrO_4^{2-} dianion, which is the dominant Cr(VI) species at high pH (Elovitz and Fish 1995). However, investigations of Cr(VI) mobility at sites contaminated with hyperalkaline (pH > 12) chromium ore processing residue leachate have observed Cr accumulation in organic-rich soils at ~ pH 10.5 (Higgins et al. 1998; Whittleston et al. 2011; Ding et al. 2016), indicating that high pH interactions may occur under field conditions and timescales not observed in short-term laboratory studies.

Although Cr(VI) reduction by humic substances has been well studied in acidic to neutral systems (Wittbrodt and Palmer 1995; Wittbrodt and Palmer 1997; Jardine et al. 1999; Huang et al. 2012), far less is known about potential reactions in the neutral to alkaline pH range relevant to COPR disposal sites. This knowledge gap is important because humic matter are one of the key soil component controlling Cr(VI) mobility in oxic near-surface environments. Further, the introduction of humic matter at a suitable pH point in a Cr(VI) plume could be the basis for groundwater treatment that mitigates environmental damage at otherwise intractable waste disposal sites. This study therefore investigates the reaction between aqueous Cr(VI) and humic acids derived from two sources (peat and lignin) over the range of pH values representative of an environment where an alkaline plume slowly buffers towards the natural pH value of the host soil. The objectives were to (i) investigate the rate at which Cr is removed from aqueous solution by the humic acids using batch exposure tests, (ii) to determine the oxidation state and local bonding environment of resulting solid-associated Cr using X-ray absorption spectroscopy (XAS) and (iii) identify changes humic acid functionality that resulted from the reaction using both ^{13}C nuclear magnetic resonance (NMR) spectroscopy and pyrolysis-gas chromatography-mass spectrometry (PyGCMS). Together, these new data were used to develop a new understanding of the Cr(VI) reduction mechanism occurring with humic substances in the neutral to alkaline pH range.

Materials and methods

Humic acids

Humic acid is the humic fraction that is soluble at pH 12, but progressively precipitated as the pH is buffered to pH 2 (Stevenson 1994; Wershaw 1994; Sutton and Sposito 2005). Aldrich humic acid (AHA), a lignite derived humic acid (Poynton 2016), was acquired as a sodium salt (Sigma-Aldrich, UK). AHA used in some characterisation tests was further refined by dissolution and re-precipitation (rAHA). Peat humic acid (PHA) was obtained from Irish moss peat (Westland Horticulture Ltd., UK) by alkali extraction (Stevenson 1994). (See SI section S1.1 for full details.)

Characterisation of humic acids

Characterisation analyses were carried out in triplicate. Ash contents were determined by ignition of moisture-free samples at 750 °C following ASTM D2974-07a (ASTM 2010). Percentages of C, H, N, S and O were determined using a Thermo Scientific FLASH 2000 CHNS/O elemental analyser (oxygen was determined in pyrolysis mode). The concentrations of inorganic constituents in AHA and PHA were determined by energy dispersive X-ray fluorescence (ED-XRF) spectroscopy (X-5000 Mobile XRF System—Olympus IMS). The total acidity and carboxylic acidity were measured using the barium hydroxide and Ca-acetate methods, respectively, and phenolic acidity was estimated by the difference (Schnitzer and Khan 1972). Carboxylic and phenolic acidity was also estimated through direct discontinuous base titrations that were conducted on 5 g/L humic acid suspensions in 0.5 N NaCl (see SI section 1.1) (Janoš et al. 2008).

Cr(VI)-humic acid batch experiments

Humic acid powder (1 g) was added to DIW (90 mL) in 120-mL glass serum bottles (AHA was used as supplied whereas PHA came from the last step of the extraction protocol). Triplicate suspensions were equilibrated at pH values 3, 5, 7, 8.5, 9 and 11 using either 1 M HCl or NaOH. Samples were intermittently shaken, and the pH was adjusted until the pH value was stable for at least 1 day. The suspensions were then autoclaved at 121 °C for 15 min to ensure that the subsequent long-duration experiments were abiotic. After cooling, autoclaved DIW was added to each bottle to make up the volume to 96.7 mL, and the pH was readjusted, if necessary, to each target value. Air was flushed from the experiments by bubbling nitrogen through the suspensions. Finally, 3.3 mL of potassium chromate solution (1/30 M K_2CrO_4; Fluka, Germany) was added for a 100 mL final volume ($[Cr(VI)] = 1100\ \mu mol\ L^{-1}$). Bottles were sealed with butyl rubber stoppers with aluminium crimps (Sigma-Aldrich Company Ltd. UK). Control samples were prepared using the potassium chromate solution and autoclaved, N_2 purged, DIW. Bottles were incubated in the dark at 20 ± 1 °C and periodically sampled aseptically for geochemical analysis. During sampling, bottles were shaken and 2 mL of suspension was extracted using N_2 gas-filled syringes. Samples were divided for Cr(VI) and pH determination. Subsamples for Cr(VI) analyses were passed through a 3-kDa filter (Amicon ultra 0.5 centrifugal filter).

After testing (~ 50 days), further aqueous subsamples were taken for Cr(VI) and pH analysis, as described above, then an equal volume of aluminium sulphate solution ($Al_2(SO_4)_3.16H_2O$; 5 g/L) was added to the remaining sample to coagulate colloidal humic acid (HA) (two volumes were added to the pH 11 sample). The mixtures were shaken manually for a few seconds then centrifuged at 3226*g* for 1 min, and the supernatant was immediately separated. The solid phase was then washed three times with DIW and centrifuged. Half the solid sample was air-dried in an anaerobic cabinet and retained for XAS analysis. The other half was oven-dried at 100 °C. The supernatant and oven-dried solid samples were analysed for total Cr analysis as described below.

Similar AHA and PHA samples were prepared with an excess of Cr(VI) (8000 μmol Cr(VI)/g HA) to provide solid-phase samples for ^{13}C NMR and PyGCMS analysis. The samples were prepared at pH 3 and allowed to equilibrate for 31 days before the solid phase was separated from the solution by filtration (control samples of AHA and PHA were equilibrated at pH 3).

Aqueous Cr(VI) concentrations were determined colourimetrically (method 7196A; USEPA 1992). Total Cr associated with the humic acid was determined after acid digestion (method 3050B; USEPA 1996). Total Cr in aqueous solutions and acid digestions were determined by inductively coupled plasma optical emission spectrometry (Thermo Scientific iCAP 7400 radial ICP-OES).

X-ray absorption spectroscopy

Cr K-edge XAS spectra were collected from selected humic acid samples recovered from the low concentration Cr(VI) batch experiments (the 'Cr(VI)-humic acid batch experiments' section) on beamlines I18 and B18 at the Diamond Light Source, UK. Reference spectra were also collected for standard laboratory chemicals and precipitated Cr-hydroxide (Saraswat and Vajpe 1984). X-ray absorption near edge (XANES) spectra were summed and normalised using Athena v0.9.24 (Ravel and Newville 2005), and background subtracted extended X-ray absorption fine structure (EXAFS) spectra were fitted to model coordination environments using Artemis v0.9.24 (see SI sections S1.2 and S1.3 for details).

Cross-polarisation magic-angle-spinning ^{13}C-NMR spectroscopy

Humic acid samples were disaggregated and homogenised and packed into 4 mm diameter zirconium rotor tubes. Cross-polarisation magic-angle-spinning (CP/MAS) ^{13}C-NMR spectra were obtained on a Bruker 400 MHz Avance II spectrometer, with a double-bearing magic-angle-spinning probe head (BL4 type) and a Bruker MAS II control unit (see SI for details). Chemical shifts were calibrated using an alpha-glycine spectrum (calibrated on the glycine peak at 43.5 ppm).

Pyrolysis-gas chromatography-mass spectrometry

PyGCMS analysis was performed using a CDS 5000 series pyrolyser (CDS Analytical Inc., Oxford, PA, USA) connected to a Shimadzu QP2010 GC-MS (Shimadzu Corporation, Kyoto, Japan). Samples of approximately 2–3 mg of finely ground and homogenised humic acid were placed between quartz wool plugs in a quartz pyrolysis tube and pyrolysed at a heating rate of 20 °C per millisecond to 500 °C. The pyrolysates were initially trapped on a TENAX adsorbent trap before being desorbed into an Rtx 1701 capillary column (see SI section S1.5).

Results

Characterisation of humic acids

Aldrich humic acid produces 10× more ash upon ignition than PHA (27% and 2%). Refining AHA by alkali extraction/acid precipitation reduces the ash content to 18%. The principle inorganic elements in both humic acids are Al, Si, K, Fe and Ca (SI Table S2: Na is not detectable by ED-XRF), and these form ~ 18% of AHA by elemental mass, whereas these are about 0.5% of PHA by elemental mass. The detailed properties of AHA, rAHA and PHA are reported in full in the Supplementary Information (Table S1 and S2).

The C, H, N, S and O elemental compositions of rAHA and PHA are very similar. AHA contains proportionally more O than rAHA and PHA (assumed to be associated with the fraction removed by refining), but had a similar H/C ratio. The total acidity values of rAHA and PHA determined by the barium hydroxide method were 6.4 and 6.7 meq/g, respectively. The carboxylic acidities determined by the calcium acetate method were 3.1 and 2.6 meq/g, respectively, suggesting the phenolic acidities of rAHA and PHA were 3.3 and 4.1 meq/g, respectively. The carboxylic and phenolic acidities of rAHA and PHA determined from the base titrations were 3.7 and 3.3 meq/g (carboxylic) and 1.9 and 2.1 meq/g (phenolic), respectively (Table 1, SI Fig. S1).

Aqueous Cr speciation and Cr(VI) removal rates determined after contact with humic acid

The rate at which Cr(VI) was removed from free solution by AHA was dependent on the pH of the suspension (Fig. 1). The pH value of these systems changed slightly during the first 24 h, but quickly stabilised at the value used to name the systems. At pH 4.1 Cr(VI) was removed from solution over a period of about 15 days, whereas Cr(VI) removal at pH 6.2 took ~ 50 days. At pH 7.8 and pH 8.6, only partial Cr(VI) removal was observed after ~ 50 days (~ 40% and ~ 10% removal, respectively), with no detectable Cr(VI) removal above pH 9.

The rate at which Cr(VI) was removed from free solution by PHA was also dependent on the pH of the suspension. However, the reaction was significantly faster with PHA than with AHA (e.g. complete Cr(VI) removal at pH 5.8 took ~ 2 days), and the Cr(VI) removal was observed in all tests (85% and 55% of Cr(VI) were removed after ~ 50 days at pH 8.8 and pH 10.4, respectively).

After 51 days, the partitioning of Cr between the free solution and the humic acid and the oxidation state of free aqueous Cr varied with the pH of the systems (Fig. 2). At pH 4.1 in the AHA system > 90% of the Cr(VI) initially in solution was transferred to the humic acid, and no free aqueous Cr(VI) was detected (the small amount of Cr remaining in free

Table 1 Carboxylic, phenolic and total acidity of the humic acids (meq/g)

Functional groups	rAHA	PHA
Total acidity—Ba(OH)$_2$ method (1)	6.4 ± 0.4	6.7 ± 0.1
Carboxyl acidity—Ca-acetate method (2)	3.1 ± 0.1	2.6 ± 0.0
Phenolic acidity (difference between (1) and (2) above)	3.3	4.1
Carboxyl acidity—titration method (3)	3.7	3.3
Phenolic acidity—titration method (4)	1.9	2.1
Total acidity—titration method (sum of (3) and (4) above)	5.6	5.4

Note: Carboxylic and phenolic acidity were calculated from the base titrations following Ritchie and Purdue (2003)

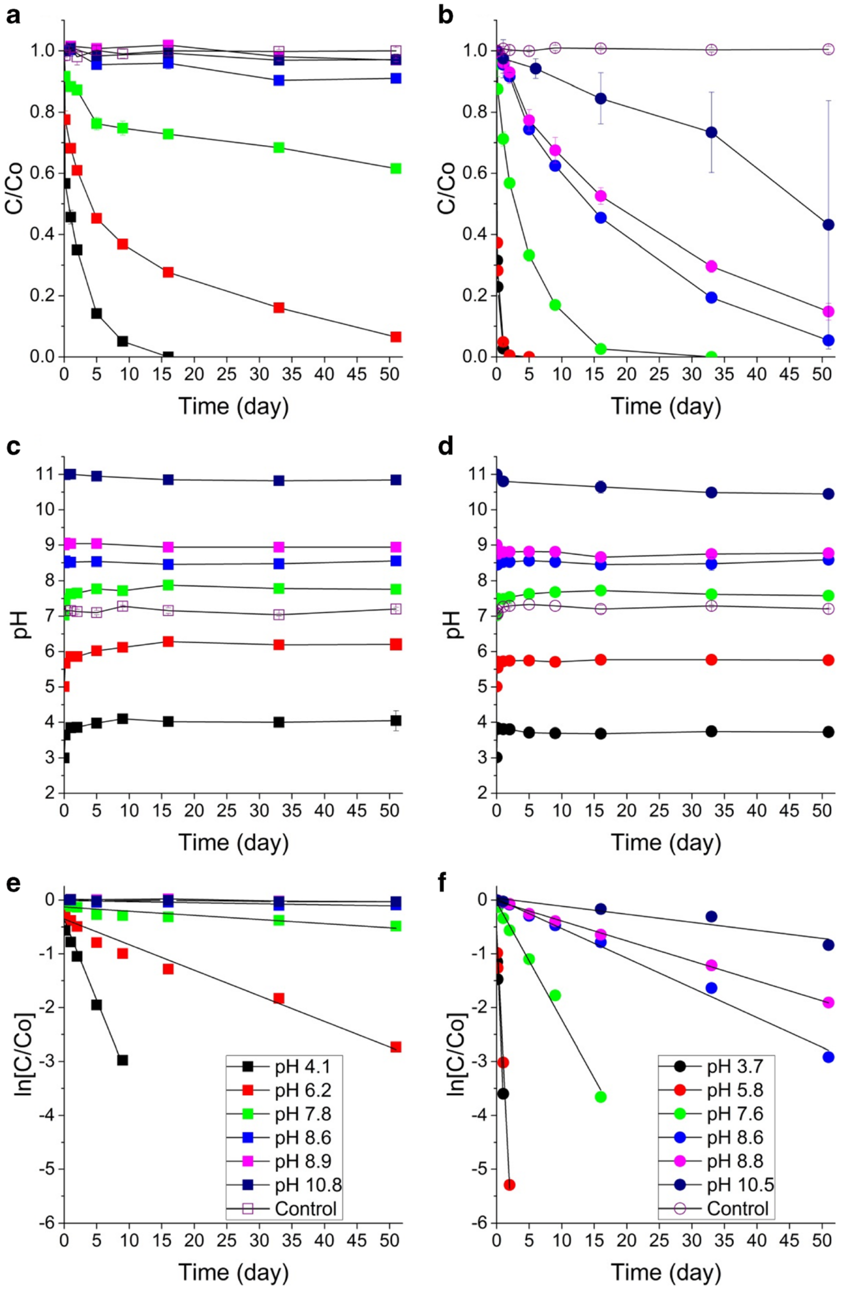

Fig. 1 **a**, **b** Cr(VI) removal from free aqueous solution by AHA and PHA, respectively, at various initial pH values (C/C_0 is the normalised Cr(VI) concentration; $[\mathrm{Cr(VI)}]_0 = 1100\ \mu\mathrm{M}$ and $[\mathrm{HA}] = 1\ \mathrm{g}/100\ \mathrm{mL}$); **c**, **d** solution pH of the AHA and PHA systems, respectively; **e**, **f** pseudo-first-order rate plots for the Cr(VI)-AHA and Cr(VI)-PHA reactions, respectively

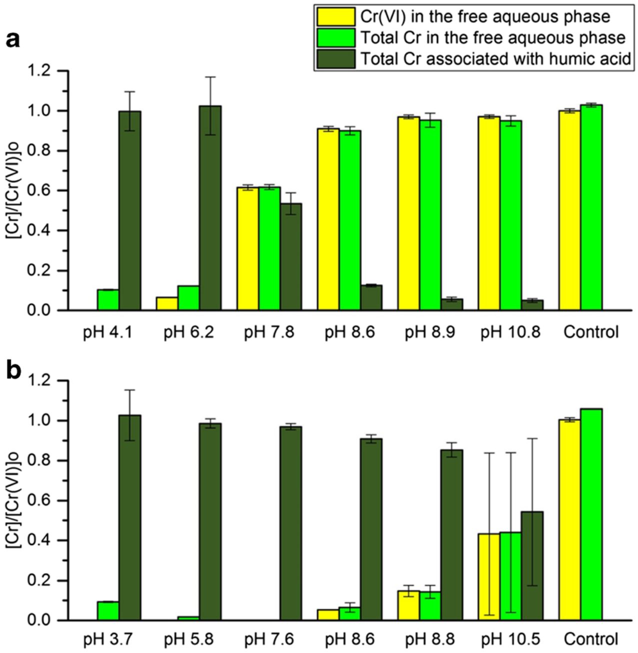

Fig. 2 Speciation of **a** Cr(VI)-AHA and **b** Cr(VI)-PHA systems after 51 days. Solid to liquid ratio: 1 g/100 mL. Initial Cr(VI) concentration in the aqueous phase: $[Cr(VI)]_0 = 1100$ μM

solution was attributed to aqueous Cr(III)). A similar pattern was observed at pH 6.2, although ~ 5% of the Cr remained as aqueous Cr(VI). At pH 7.8, about 60% of the Cr remained as aqueous Cr(VI). Above pH 8.5, there was very little Cr associated with the humic acid, and > 90% remained in the solution as Cr(VI).

In the presence of PHA, most of the Cr(VI) initially in free solution was transferred to the humic acid at pH < 8, and no free aqueous Cr(VI) was detected in these systems (although about 10% of the Cr remained in free solution at pH 3.7 presumably as Cr(III)). In the pH 8.6 and pH 8.8 PHA systems, most of the Cr was associated with the humic acid after 51 days, but ~ 5% and ~ 15% of Cr remained in solution as Cr(VI), respectively. However, at pH 10.5, there was more variation in the behaviour of the PHA system, so six replicates were tested. After 51 days, some Cr was associated with the humic acid in all replicates, but the amount of Cr(VI) remaining in free solution varied between 0 and 90% (average 45%).

AHA and PHA samples that were prepared with excess Cr(VI) for subsequent ^{13}C NMR and PyGCMS analysis buffered the solution from pH 3 to ~ pH 7 in the long-term. AHA removed ~ 500 μmol Cr(VI)/g from solution. PHA removed ~ 1400 μmol Cr(VI)/g from solution.

X-ray absorption spectroscopy

XANES spectra collected from both AHA and PHA samples that had been exposed to Cr(VI) lacked any evidence of the characteristic Cr(VI) pre-edge peak at 5994 eV (Peterson et al. 1996), indicating that only Cr(III) was present in solids regardless of the solution pH during the reaction (S.I. Fig. S2). The XANES spectra from both AHA and PHA sample were qualitatively similar and most closely resemble those collected from the Cr(III) aqueous or poorly crystalline hydrous $Cr(OH)_3$ standards, lacking the detailed structure of the crystalline Cr_2O_3 standard (the absence of structure associated with Cr_2O_3 is probably indicative of Cr(III) binding with HA functional groups, since there is no Cr(III) observed in solution). EXAFS fitting revealed that all samples were best fit by single-scattering and multiple-scattering pathways associated with the $Cr(III)O_6$ octahedra (i.e. 6 O atoms at 1.96–1.97 Å) and by the inclusion of additional Cr-C pathways between 2.91 and 3.00 Å (Fig. 3; SI Table S5). Attempts to fit the EXAFS spectra with additional Cr-Cr pathways at 3.0–3.1 Å produced final fits with unrealistically long Cr-Cr pathway lengths (3.3–3.9 Å) and the large Debye-Waller factors (0.009–0.010; indicative of overfitting) compared to other pathways and failed to improve the overall fit quality. Therefore, the data provided no evidence for $Cr(OH)_3$ polymerisation that has been observed previously for some Cr(III)-humic acid associations (Gustafsson et al. 2014).

Cross-polarisation magic-angle-spinning ^{13}C NMR spectroscopy

Comparison of the ^{13}C-NMR spectra of AHA and PHA indicates differences between the two materials (Table 2 and Fig. 4). Nearly 50% of the AHA spectrum is in the chemical shift range usually associated with alkyl C (0–45 ppm; carbon centres singly bonded to either C or H; Golchin et al. 1997; Kögel-Knabner 2000), with about 1/3 of the PHA spectrum occupying the same range. Conversely, ~ 25% of the PHA spectrum is in the range associated with alkyl C bonded singly to O (45–110 ppm), yet < 5% of the AHA spectrum is in this range. Approximately 40% of the AHA spectrum is in a range associated with alkene and aromatic C (110–160 ppm), whereas ~ 30% of the PHA spectrum is in this range. However, ~ 10% of both spectra are in the sub-range associated with aromatic C–O centres (140–160 ppm (Knicker et al. 2005)). Both humic acids have ~ 10% of their spectra associated with carbonyl C (160–220 ppm), and in both cases, this is mainly in the sub-range characteristic of carboxylic and ester moieties (160–185 ppm) (Knicker et al. 2005).

The ^{13}C-NMR spectra of AHA and PHA both show changes due to the reaction with Cr(VI) in acidic solution (Table 2 and Fig. 4). The proportion of spectra usually associated with aromatic C has decreased by ~ 5% and ~ 10%, respectively. PHA also exhibits a ~ 10% decrease in the proportion of the spectrum associated with alkyl C singly bonded to O (from ~ 25 to ~ 15%), whereas AHA shows little change. In both cases, the proportion of the spectra usually associated with alkyl C (0–45 ppm) has increased by ~ 5% and ~ 20%,

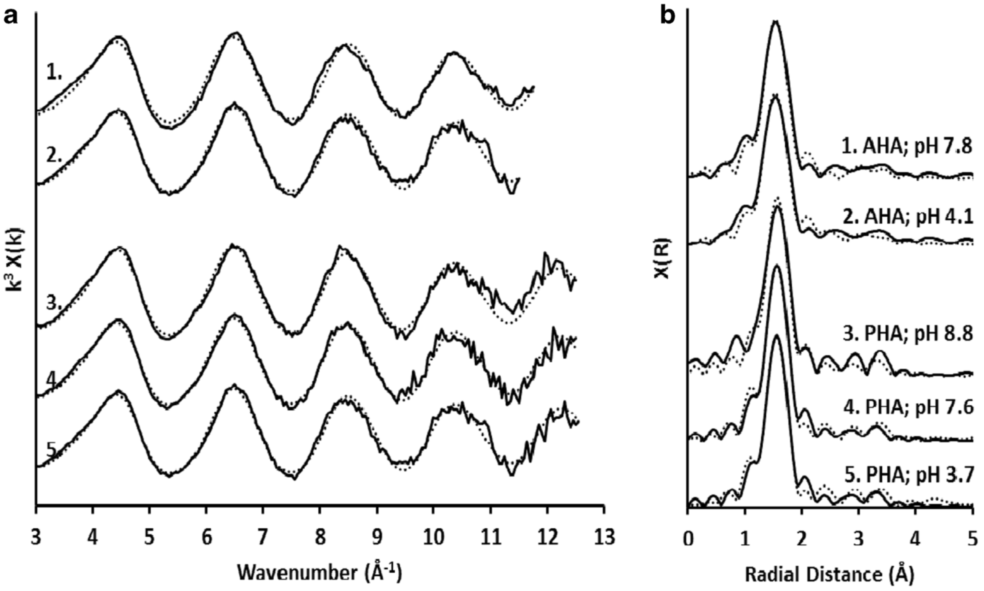

Fig. 3 **a** Cr K-edge EXAFS data collected from Aldrich and peat humic acid samples, and **b** corresponding Fourier transformations. Dotted lines represent bit fit to data calculated in Artemis using pathways and parameters listed in SI Table S5

respectively. Neither material appears to exhibit any increase in the proportion of the spectrum associated with carbonyl C after reaction with Cr (160–220 ppm), but this may be the result of the shielding that occurs due to electron redistribution when carbonyl groups form complexes with Cr(III)(Zhang et al. 2017).

Pyrolysis-gas chromatography-mass spectrometry

Direct quantitative comparison of functionality between the humic acid samples from PyGCMS is inappropriate due to the difference in detector response from different chemical fragments. However, examination of the pyrograms show differences before and after reaction with excess Cr(VI) in acidic solution and thus indicate the changes in humic acid functionality that resulted from the reaction. The reaction of PHA with excess Cr(VI) resulted in a large decrease in the relative size of peaks from products containing phenolic fragments and an increase in the relative size of peaks associated with long-chain aliphatic fragments (Fig. 5 and SI Fig. S3c, d). Peaks for methoxy-phenolic and other substituted phenolic compounds exhibited the largest decrease in relative size. The pyrograms for AHA were less well resolved (possibly a result of the higher ash content), but these also showed a decrease in the relative size of peaks associated with aromatic fragments (SI Fig. S3a, b).

Discussion

Relative differences in reactive sites in lignite and peat derived humic acids

Both the barium hydroxide and direct base titration methods indicate that rAHA and PHA have similar total acidity (per unit mass of the total material). However, the direct base titration and the difference between the barium hydroxide and calcium acetate methods indicate that PHA has 10–25% greater phenolic acidity than rAHA. More importantly, the ash remaining after a loss on ignition (18% in rAHA and 2% in PHA) is often a result of amorphous silica and aluminosilicate impurities present in the humic acid (Tan 1977). Such minerals can buffer pH

Table 2 Proportion of humic acid carbon in the different bonding environments before and after reaction with excess Cr(VI) determined by CP MAS ^{13}C-NMR (spectra were operationally divided into characteristic chemical shift regions (Golchin et al. 1997; Kögel-Knabner 2000))

	AHA			PHA		
Type of organic carbon (% of total area)	Before reaction	After reaction	Difference	Before reaction	After reaction	Difference
Alkyl C (0–45 ppm)	47.1	52.0	+ 4.9	33.9	53.5	+ 19.6
O-alkyl C (45–110 ppm)	2.1	2.5	+ 0.4	24.8	14.7	− 10.0
Aromatic C (110–160 ppm)	38.7	33.9	− 4.8	27.8	17.7	− 10.0
Carbonyl C (160–220 ppm)	12.1	11.5	− 0.5	13.5	14.0	+ 0.5
Aromaticity* (%)	44.0	38.4	− 5.6	32.1	20.6	− 11.5

*Aromaticity is defined as (aromatic C)/(alkyl C + O-alkyl C + Aromatic C)

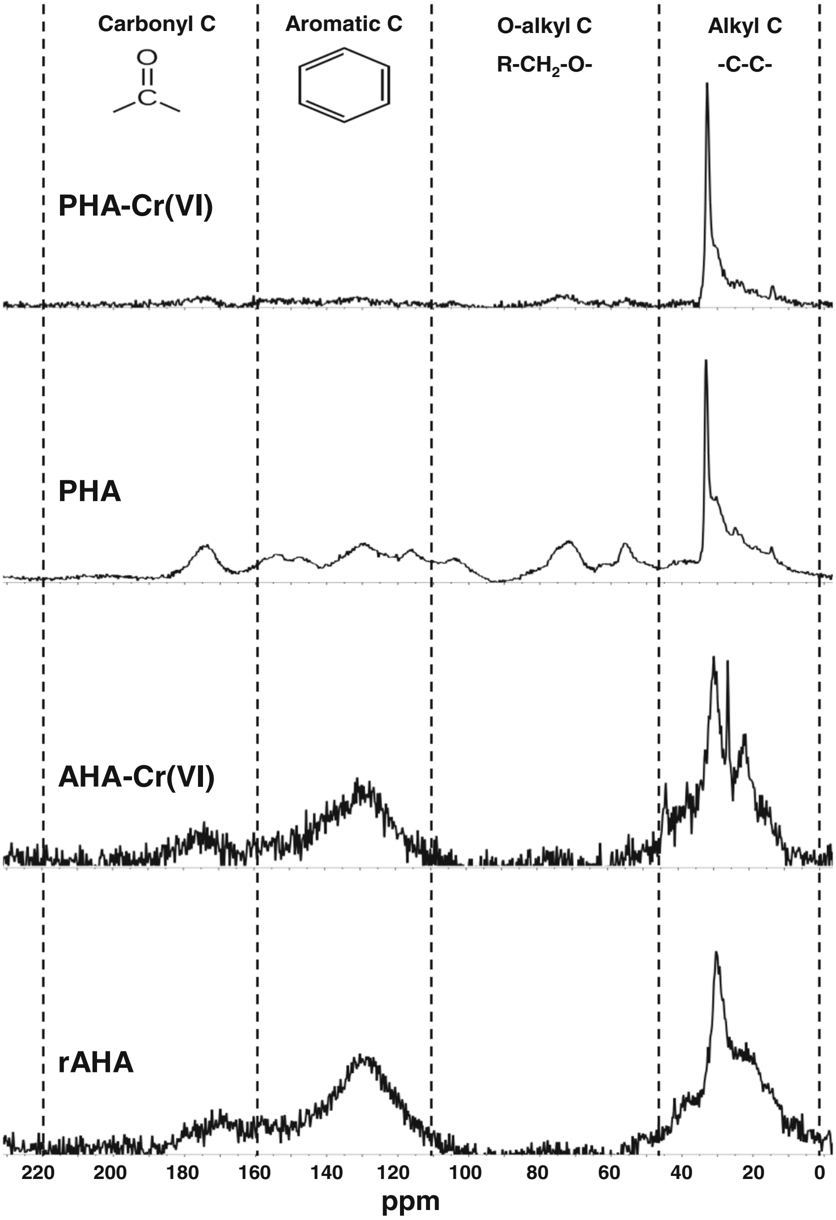

Fig. 4 ^{13}C-NMR spectra of AHA and PHA before and after the reaction with excess Cr(VI) initially at pH 3. Curves are normalised to the equal area under the curves. The spectra are operationally divided into chemical shift regions characteristic of different C bonding environments (Golchin et al. 1997; Kögel-Knabner 2000)

during a titration producing ‘acidity’ in the phenolic range, and when allowance is made for the ash removed by refining (AHA is 27% ash), it is clear that the actual phenolic acidity of PHA is likely to be higher than that of AHA.

Chemical shift regions of the ^{13}C NMR spectra indicate AHA and PHA contain similar proportions of carbonyl C centres associated with either carboxylic acid or ester groups (160–185 ppm). The ^{13}C NMR spectra also indicate that the two HAs contain similar proportions of aromatic/alkene C centres in the range normally associated with aromatic C–O centres (140–160 ppm). ^{13}C NMR cannot differentiate aromatic C bonded to O in phenolic moieties from those associated with an ether linkage (i.e. it cannot differentiate between Ph–O–H from Ph–O–C), but the differences in the phenolic acidity together with lower reactivity with Cr(VI) suggest that a larger proportion of the aromatic C–O centres in AHA may be associated with less reactive ether linkages (characteristic of phenolic polymers) than in PHA. AHA was extracted from Miocene age lignite (6–26 Ma; Germany), whereas PHA was extracted from Holocene age peat (< 12 ka, Ireland). As lignite is essentially compressed and heated peat, the differences in functionality of the two humic acids are probably associated with changes that occur during ageing of the parent materials. This is likely to have involved the formation of linking bonds between phenolic and other aromatic moieties (i.e. reactions similar to the polyphenol pathway assumed in the polymer model of humic substance formation; Stevenson 1994), as oxidative polymerisation of hydroxyphenols and toluenes can be catalysed by enzymes found in plants, fungi and bacteria (Martin and Haider 1980).

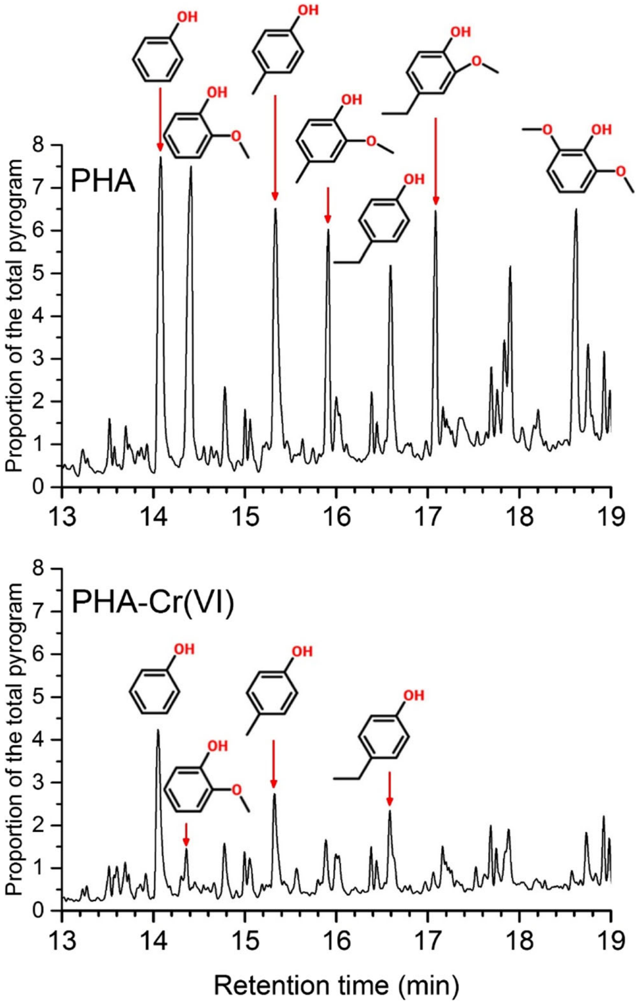

Fig. 5 Partial pyrograms for PHA before and after reaction with excess Cr(VI) initially at pH3 showing a decrease in the relative size of spectral peaks associated with phenolic, methoxy-phenolic and other substituted phenolic fragments. Pyrograms have been scaled in proportion to the area that phenol represents of the total pyrogram. Full pyrograms and a table identifying the main thermal degradative products are presented in the Supporting Information

Controls on the rate of Cr(VI) reduction with humic acid

XANES analysis indicates that the interaction of Cr(VI) with these humic acids resulted in a reduction to Cr(III) at all pH values tested. Also, the similarity of spectral details suggests that Cr(III) produced by reaction with humic acids resides in the same chemical environment regardless of the pH of the system. Changes in the ^{13}C NMR spectra indicate that both humic acids suffered a loss of aromatic/alkene C during the reaction with Cr(VI) in acid solution. PHA also underwent a loss of O-alkyl C bonds (probably hydroxyl groups). Changes in the PyGCMS pyrograms confirm that both HAs suffered a loss of aromatic and an increase in aliphatic moieties during the reaction with Cr(VI) in acid solution. PyGCMS pyrograms suggest that the loss from PHA was principally of substituted phenolic aromatics, whereas with AHA, there was also a loss on non-phenolic aromatics. Collectively, these data suggest that the reduction of Cr(VI) to Cr(III) by humic acids involves a reaction with aromatic groups generally and phenolic moieties in particular. Changes in PHA suggest that aliphatic hydroxyl groups may also have reacted.

Humic acid samples for ^{13}C NMR and pyrolysis GC-MS analysis were reacted with an excess of Cr(VI), and as a result, they buffered the pH from 3 to ~7 (indicating that H$^+$ is consumed by the reaction between Cr(VI) and HA in acidic systems). These samples indicate that AHA can reduce ~500 μmol Cr(VI)/g and PHA ~1400 μmol Cr(VI)/g when the pH ≤ 7. Thus, in the longer term batch tests which were conducted with 110 μmol Cr(VI)/g, HA was available in excess when the pH ≤ 7. In this pH range, the rate of Cr(VI) removal by both HAs is first order with respect to the concentration of Cr(VI) species (Fig. 1e, f). The reaction may still be first order with respect to Cr(VI) concentration at pH > 7. At pH 7.8, AHA reduced only ~40 μmol/g Cr(VI) after 51 days, but the reaction exhibits an approximately linear relationship between log([Cr(VI)]) and time ($r^2 = 0.78$; see SI Table S6). The reaction with PHA exhibited a linear relationship between log([Cr(VI)]) and time at pH 8.6 and 8.8 ($r^2 = 0.99$ and 1.00, respectively).

The rates at which AHA and PHA reduced Cr(VI) is dependent on [H$^+$], which decreases with the increasing pH. The rapidity of Cr(VI) removal from a solution made an accurate rate determination difficult at pH ≤ 4; however, least squares fitting of Eq. (1) to data where pH ≤ 9 yields the values of the exponent, *a*, of 0.48 for AHA and 0.40 for PHA (see SI Fig. S4).

$$k_{\mathrm{obs}} = k_0.[\mathrm{H}^+]^{\mathrm{a}} \tag{1}$$

This is consistent with the trend observed by Wittbrott and Palmer (Wittbrodt and Palmer 1995; Wittbrodt and Palmer 1997), who found that the rate of Cr(VI) reduction by soil fulvic acid and soil humic acid were both proportional to $[\mathrm{H}^+]^{0.45}$ when pH ≤ 7.

Mechanism of Cr(VI) reduction with humic acid

Cr(VI) reduction by humic acid requires that Cr(VI) is first adsorbed in manner that facilitates electron transfer. Experimental studies using simple alcohols and phenolic compounds highlight the formation of a chromate ester as the first step in Cr(VI) reduction (Lee and Stewart 1967; Wiberg and Schafer 1967; Elovitz and Fish 1995). As alcohol and phenolic functional groups are common in humic acid, it is reasonable to expect similar interactions will occur in the experiments reported here, as illustrated in Fig. 6. Humic acids can contain vicinal diols (two hydroxyl groups attached to adjacent carbon atoms), like those found in the humic precursors

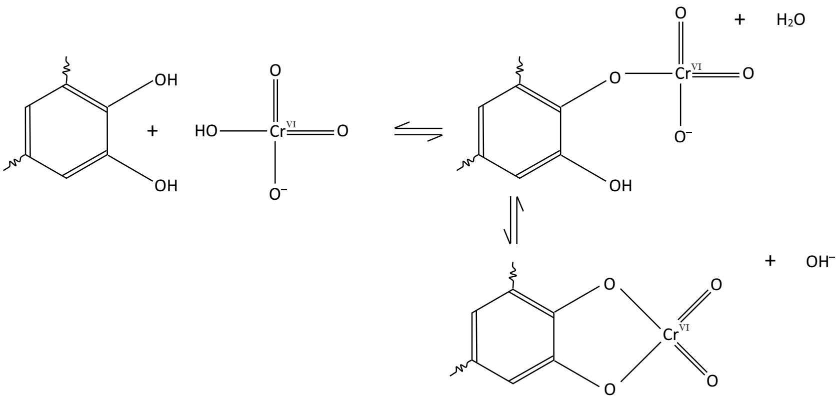

Fig. 6 Formation of a chromate ester with phenolic moieties in humic acid, and a potential cyclic chromate ester with ortho-benzenediol moieties (Wiberg 1965)

caffeic, gallic and tannic acid (Nakayasu et al. 1999; Deiana et al. 2007), and these can also form cyclic chromate ester with Cr(VI) as illustrated in Fig. 6 (Wiberg 1965).

Chromate ester formation is rapid and reversible in acidic conditions (methylphenol reaches equilibrium in < 60 s at pH ≤ 5; Elovitz and Fish 1995). It occurs primarily with monomeric Cr(VI) species and is far more favourable with chromic acid and bichromate species than with the dianionic chromate species (Wiberg 1965; Lee and Stewart 1967; Wiberg and Schafer 1967; Elovitz and Fish 1995), probably because the Cr centre is more electrophylic in the monovalent bichromate than in the divalent chromate anion. Therefore, the rate at which Cr(VI) is reduced by alcohol and phenolic moieties declines sharply when pH ≥ 6 (Elovitz and Fish 1995). However, data presented here shows that Cr(VI) reduction proceeds slowly at alkaline pH, with the same eventual fate for the Cr(III) across the pH range (Fig. 3). This suggests that, while less favourable due to increased electrostatic repulsion, chromate ester formation occurs between deprotonated humic acids and the dianionic chromate species, with the result that Cr(VI) is reduced by humic acids at alkaline pH values on long timescales.

Reduction of Cr(VI) must have resulted in oxidation of the humic acids, but ^{13}C NMR did not identify the functional groups produced. Oxidation of phenolic and alcohol groups by Cr(VI) usually produces ketones and carboxylic acids (Wiberg 1965; Rocek and Riehl 1967; Deiana et al. 2007), and thus, an increase in carbonyl range of the ^{13}C NMR spectra would be anticipated. However, Zhang et al. (2017) showed that Cr(III) sorption to humic acids results in the formation of carbonyl-Cr(III) complexes, and shielding associated with electron redistribution causes a decrease in the ^{13}C NMR signal from carbonyl groups. Thus, it is likely that Cr(VI) reduction by AHA and PHA resulted in the formation of additional carbonyl groups, but these were not detected by ^{13}C NMR due to such shielding. This explanation is compatible with our Cr EXAFS data which suggests that the Cr(III) formed an inner-sphere adsorption complex with two C atoms.

The reduction of Cr(VI) by the humic acids was first order with respect to [Cr(VI)], so the rate-limiting step likely involves a single Cr-containing species. This step is probably chromate ester decomposition (Lee and Stewart 1967; Elovitz and Fish 1995; Wittbrodt and Palmer 1997), which can then result in transfer of one or two electrons to the chromate ion resulting in the production of either Cr(V) or Cr(IV) moieties (Wiberg 1965; Lee and Stewart 1967; Haight et al. 1971; Elovitz and Fish 1995). The reduction is also mixed order with respect to $[H^+]$ which is compatible with Cr(VI)-ester decomposition proceeding concurrently by multiple pathways (with 4-methylphenol ester decomposition can proceed concurrently by proton-activated pathways and a proton-independent pathway; Elovitz and Fish 1995), but it may also indicate that the equilibrium constant for the ester formation may be increasingly less favourable with H_2CrO_4, $HCrO_4^-$ and CrO_4^{2-} species.

Metal reduction by vicinal diols can result in cleavage of the intervening C–C bond (Wiberg 1965; Deiana et al. 1992; Deiana et al. 1995; Deiana et al. 2007). Such 'ring opening' phenomena are consistent with the loss of aromatic and methoxy-phenolic groups observe by NMR and PyGCMS in this study. Cr(IV) moieties produced by electron transfer are unstable and will rapidly disproportionate to form Cr(III) and Cr(V), and Cr(V) can react with alcohol and phenolic groups via the chromate ester in much the same way as Cr(VI) (Wiberg 1965; Haight et al. 1971; Bruckner 2002). Therefore, through several cycles of absorption, ester formation, reduction and disproportionation steps, the Cr(VI) is likely to be eventually converted to the Cr(III) end product. Cr(III) formed from reduction of Cr(VI) remains associated with the partially degraded humic acid at all pH values, although with samples where pH < 4, small amounts of Cr(III) are also present in solution due to the protonation of humic acid surface sites which results in lower sorption of Cr(III) under acidic pH. The lack of Cr-Cr pathways in the Cr-humic acid inner-sphere adsorption complexes points to little or no aqueous Cr(III) accumulation after reduction, such that few Cr(III)-Cr(III) interactions occur during adsorption, and the formation of the Cr(III) dimers observed by Gustafsson et al. (2014) at high pH is not favoured.

Implications

Cr(VI) is reduced to Cr(III) by reaction with humic substances over a wide range of pH values found in the environment. This reaction is rapid in acid and neutral conditions, and therefore, natural soil organic matter will reduce Cr(VI) transport through groundwater when it is present. Also, Cr(VI) contaminated groundwater could be treated by deploying humic substances within an engineered treatment scheme (such as a permeable reactive barrier). Reduction reduces Cr toxicity, and the resulting Cr(III) is strongly held by inner-sphere bonding with humic acids, which significantly reduces the opportunity for the spread of Cr(III) or reoxidation into mobile Cr(VI) species.

The rate at which Cr(VI) is reduced and the total capacity for Cr(VI) reduction are both proportional to the reactivity of the humic acid used and particularly the density of phenolic and hydroxyl sites in the humic substances. Thus, it is important to choose younger sources of organic matter (e.g. sewage sludge, compost), which contain more labile humic substances, to maximise treatment efficiency and longevity in real applications. This choice will be particularly important when pH > 7, where reaction rates are lower.

Treatment of Cr(VI) contaminated groundwater by permeable reactive barrier (PRB) is challenging in alkaline conditions as the reactive materials conventionally deployed within PRBs are not durable in this pH range (e.g. ZVI is passivated at high pH and Fe(II) containing substances, such as green rusts and ferric sulphate solutions, have very short active lifetimes). The reaction of Cr(VI) with humic acids will also not be easy to exploit within a conventional PRB, as it takes place on a timescale of weeks. However, a different remediation strategy may be appropriate, as humic acids become increasingly soluble as the pH rises and a large proportion of humic acids are mobile at high pH. Thus, humic acids will migrate with the groundwater until the pH is sufficiently buffered by reactions with soil minerals for precipitation, creating a diffuse reactive zone downstream of the intervention point.

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Supporting information

Abiotic reduction of Cr(VI) by humic acids derived from peat and lignite: kinetics and removal mechanism

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S1. Materials and methods

S1.1. Humic acid preparation and Standard chemical methods

AHA was refined to produce rAHA. It was dissolved in DIW and the pH was increased to 12 using 0.5N NaOH. The mixture was shaken for an hour, and acidified to pH<2 using 4N HCl. The precipitate was collected by centrifugation (~3000g, 45 min) and was washed twice with small amount of DIW and centrifuged. The final precipitate was dried at 40 ºC, and disaggregated using a mortar and pestle.

Peat (<2mm) was acid washed (0.1 M HCl) and then suspended in 0.5N NaOH at a solid to liquid ratio of 1:10 and shaken for 24 hours. After centrifugation (~3000g, 45 min) the supernatant was recovered and acidified to pH<2 by addition of 4N HCl. The humic acid precipitate was recovered by centrifugation. This procedure was repeated twice more on the humic acid precipitate to maximise the removal of inorganic impurities. The precipitate was washed three times with small amount of DIW and centrifuged. The product was dried at 40 ºC and disaggregated using a mortar and pestle.

The absorbance ratio (E4/E6) of dilute humic acid solutions were determined at 465 and 665 nm using Thermo Scientific Biomate 3 spectrophotometer (Chen et al., 1977). The redox potential of 1:1 humic acid : DIW samples was measured at 24 ºC using Ultrameter II (Myron L Company).

Aqueous Cr(VI) concentrations were determined colorimetrically at 540 nm (Thermo Scientific Biomate 3 spectrophotometer) after reaction with 1,5-diphenylcarbazide under acidic conditions (USEPA, 1992). Total Cr associated with the solid phase was determined after acid digestion by following USEPA method 3050B (USEPA, 1996). The humic acid (0.15 - 0.25 g) was added to 5 ml of a 1:1 dilution of 70% HNO_3 in DIW, and heated in a block heater (Grant QBD4) to 95 ± 5 ºC. A further 10 ml of 70% HNO_3 was added gradually, followed by 1 ml DIW and 4 ml H_2O_2 (30%) was added gradually. When all effervescence had ceased and there was no further change in the general appearance of the sample, 3 ml of concentrated HCl was added. After cooling, the digestate solution was diluted to 25 ml with DIW, filtered using 0.2 µm PES syringe filter (Sartorius Minisart syringe filters), and then analysed for total Cr by ICP-OES. Total aqueous Cr was measured by Inductively Coupled Plasma

Optical Emission Spectrometry (Thermo Scientific iCAP 7400 radial ICP-OES). The pH was measured using a pH meter (Hach HQ40d) calibrated daily at pH4.01, 7, and 10.01. All of the reagents were of analytical grade. All glassware was washed with detergent, soaked overnight in 1 M HCl, and then rinsed with DIW three times.

Direct discontinuous base titrations were conducted where replicate experiments were equilibrated with different amounts of base (Janoš et al., 2008). Humic acid (0.25 g) was suspended aqueous in NaCl with predetermined volume of 0.5N NaOH in screw-cap bottles (total volume 50 mL, $I \approx 0.5$ M). Samples bottles (100 ml polypropylene) were purged with nitrogen for several minutes, and sealed. Samples were equilibrated for 72 hours with intermittent shaking, and the final pH values were measured.Carboxylic and phenolic acidity were calculated from the base titrations following Ritchie and Purdue (Ritchie and Perdue, 2003).

S1.2. X-ray absorption spectroscopy (XAS) data collection and analysis

All samples and standards were analysed as pressed pellets (without diluent) sealed in Kapton tape in fluorescence mode (0.1 - 2 % Cr) or transmission mode (>2 % Cr) as appropriate. The Cr K-edge (5989 eV) XAS data presented in this study were gathered from two separate sessions, oneon Beamline B18 (PHA samples) and one on Beamline I18 (AHA samples) at the Diamond Light Source.

At Beamline B18 the incident beam had a typical operating voltage of 3GeV and a current of 300mA. The x-rays at B18 are generated from a bending magnet source. The beam is vertically collimated by a Si mirror before passing through a double crystal Si monochromator. It was then focused onto the sample to give a spot size of 200 x 250 µm. Sr K edge (16105 keV) XAS spectra were gathered from fluorescence at room temperature x-rays using a 9 element Ge solid state detector.At this beamline multiple short scans (approx. 5 min.) were collected from each sample and each subsequent scan was assessed over time to ensure no X-ray induced changes in Cr speciation occurred.

At Beamline I18 the data was collected while operating at 3 GeV with a typical current of 250 mA, using a nitrogen cooled Si(111) double crystal monochromator and focussing optics. A pair of plane mirrors was used to reduce the harmonic content of the beam and Kirkpatrick-Baez mirrors were used to produce a relatively unfocused beam (approximately 50 µm diameter at the sample). Data was collected in fluorescence mode using a 9 element solid state Ge detector at room temperature.The sample stage was systematically moved between (~20 min.) scans to exposure a fresh part of the sample for each scan to reduce any possibility of X-ray induced changes in Cr speciation.

S1.3 EXAFS data analysis and fitting

Multiple XAS scans from each sample were summed and averaged using Athena v 0.8.056(Ravel and Newville, 2005)to maximise the signal/noise ratio and XANES data was plotted. Cr K-edge EXAFS data was also collected and fit to molecular coordination models using Artemis v 0.8.056.Shell-by-shell fitting was performed by estimating initial parameters for single scattering pathways of backscattering atoms and then interactively refining these parameters. Specifically fits were determined by refining number of atoms (± 25%), interatomic distances, Fermi energy and Debye-Waller factor (σ^2). This procedure was performed initially with single scattering and multiple scattering pathways representing the CrO_6octahedral and subsequent Cr and/or C single scattering pathways were added. Additional pathways were then accepted only where they improved the overall fit quality by greater than 10% (determined by reduction of the reduced Chi-squared measure of fit).

S1.4. CP/MAS ^{13}C-NMR

HA samples that had been reacted with excess Cr(VI) (initially 8000 µmol/g at pH3), and control HA samples that had been conditioned to pH3, were disaggregated and homogenised and packed into a 4 mm diameter zirconium rotor tubes. Cross-polarisation magic-angle-spinning ^{13}C-NMR spectra were obtained on a Bruker 400MHz Avance II spectrometer, with a double-bearing magic-angle-spinning probe head (BL4 type) and a Bruker MAS II control unit. Measurement parameters were ~100.6 MHz frequency, 90 degree proton pulse length, 2.5μs, 2 ms contact time, 5 s delay time, the spinning speed was 10 kHz. 5012 and 10,000 scans were collected and averaged for samples of HA and HA reacted with Cr(VI). Chemical shifts were calibrated using an alpha-glycine spectrum (calibrated on the glycine peak at 43.5ppm).

Free induction decays (FIDs) obtained from ^{13}C CP/MAS ^{13}C-NMR experiments were Fourier transformed to obtain spectra and subsequently zero and first order phase corrections were applied. TheseNMR spectra were operationally divided into chemical shift regions; 0-45 ppm (alkyl C), 45-110 ppm (O-alkyl C),110-160 ppm (aromatic C) and 160-220 ppm (carbonyl C) (Kögel-Knabner, 2000, Golchin et al., 1997). These chemical shift regions are very similar to those used by Knicker et al. (Knicker et al., 2005), who further subdivided the aromatic region into aromatic-C bonded to C/H (110-140 ppm) and to O (140-160 ppm). For each spectrum the proportion of the area under the curve associated each chemical shift region was estimated by numerical integration (reported in Table 2of the main paper).

S1.5. Pyrolysis GC-MS

HA samples were prepared by reaction with excess Cr(VI) (initially 8000 µmol/g at pH 3). Control HA samples were conditioned to pH3. Fine, homogenized HA samples (2-5 mg) were placed between quartz wool plugs in a small pyrolysis tubes. These were placed in the cold platinum filaments of the pyrolysis unit (CDS Pyroprobe® Model 5000). Samples were heated at a rate of 20 ˚C per millisecond to 500 °C (held for 20 seconds). The fragments (pyrolysates) were then carried by He gas into the gas chromatogram (GCMS-QP2010SE, Shimadzu). The pyrolysates were initially trapped on a TENAX adsorbent trap before being desorbed at 300 °C into the split/splitless injector of the GC inlet port via a heated transfer line with a split ratio of 20:1. Pyrolysates were separated on an Rtx-1701 capillary column (60 m long, 0.25 mm id, 0.25 µm film thickness) using a temperature programme of 40 °C (hold time 2 min), ramped to 280 °C at 6 °C/min (hold time 15 min)and a constant column head pressure of 2.07 bar. Pyrolysates were thentransferred to the mass spectrometer, where they were detected by their mass to charge ratio using quadrupole mass detector. The absolute and relative intensity of each peak on the pyrogram was determined by performing the area integration using (Shimadzu GC solution) software.

Table S1. Properties of Aldrich and peat humic acid.

Test	AHA	rAHA	PHA
Ash content %	26.86±1.6	18.0±0.2	1.8±0.4
C (%)[a]	51.1±0.2	59.9±2.8	60.1±1.4
N (%)	1.28±0.04	1.75±0.4	2.51±0.07
H (%)	4.73±1.05	5.26±1.56	5.57±0.29
S (%)[b]	0 - 0.14	0-0.74	0-0.46
O (%)	42.8 ± 1.6	32.7±0.4	31.5±1.9
H/C[c]	1.11	1.05	1.11
O/C	0.63	0.41	0.39
(N+O)/C	0.65	0.43	0.43
E_4/E_6[d]	4.99±0.06	4.88±0.03	4.25±0.16
Redox potential (mV) at 24 ºC [e]	-	386	301

[a] The elemental composition were calculated based on the moisture and ash free basis.

[b] Some replicates were below the detection limit

[c] H/C: atomic ratio of hydrogen to carbon. O/C: atomic ratio of oxygen to carbon. (N+O)/C: atomic ratio of sum of nitrogen and oxygen to carbon.

[d] Ratio of absorbance at 465 and 665 nm determined following the method of Chen et al. (1977).

[e] Redox potential of 1:1 humic acid:DIW samples measured at 24 ºC.

TableS2. Major inorganic impurities of AHA and PHA determined by XRF

Element (W %)	AHA	PHA
Mg	ND*	ND
Al	4.14 ± 0.08	0.18 ± 0.02
Si	6.82 ± 0.04	0.24 ± 0.01
S	0.79 ± 0.00	0.70 ± 0.00
K	2.72 ± 0.01	0.05 ±0.00
Ca	1.99±0.01	ND
Ti	0.43 ± 0.00	0.00 ± 0.00
Fe	2.63 ±0.02	0.05 ± 0.00

*ND: not detected

Table S3. Data of Cr(VI) removal from aqueous solution by AHA over time as a function of pH, as depicted in Fig. 1 of the main text.

Time (day)	AHA pH 4.1		AHA pH 6.2		AHA pH 7.8		AHA pH 8.6		AHA pH 8.9		AHA pH 10.8		Control	
	[Cr(VI)] µmol L^{-1}	pH	[Cr(VI)] µmol L^{-1}	pH	[Cr(VI)] µmol L^{-1}	pH	[Cr(VI)] µmol L^{-1}	pH	[Cr(VI)] µmol L^{-1}	pH	[Cr(VI)] µmol L^{-1}	pH	[Cr(VI)] µmol L^{-1}	pH
0	1100 ± 0	3.0 ± 0.0	1100 ± 0	5.0± 0.0	1100 ± 0	7.0± 0.0	1100 ± 0	8.5± 0.0	1100 ± 0	9.0± 0.0	1100 ± 0	11.0± 0.0	1100 ± 0	7.2± 0.0
0.17	623 ± 6	3.7 ± 0.0	853 ± 32	5.7± 0.0	1008 ± 7	7.5± 0.2	1100 ± 11	8.5± 0.0	1100 ± 24	9.1± 0.0	1100 ± 22	11.0± 0.0	1083 ± 19	7.2± 0.0
1	502 ± 25	3.9 ± 0.0	750 ± 17	5.9± 0.0	972 ± 10	7.6± 0.1	1100 ± 22	8.5± 0.0	1122 ± 11	9.0± 0.0	1111 ± 0	11.0± 0.0	1111 ± 5	7.2± 0.1
2	385 ± 12	3.9 ± 0.0	671 ± 7	5.9± 0.0	960 ± 7	7.7± 0.0	-	-	-	-	-	-	1079 ± 29	7.1± 0.1
5	156 ± 11	4.0 ± 0.0	498 ± 17	6.0± 0.0	839 ± 22	7.8± 0.0	1045 ± 11	8.5± 0.0	1111 ± 14	9.0± 0.0	1078 ± 11	11.0± 0.0	1101 ± 3	7.1± 0.1
9	56 ± 4	4.1 ± 0.0	405 ± 11	6.1± 0.1	823 ± 25	7.7± 0.0	-	-	-	-	-	-	1024 ± 16	7.3± 0.0
16	0	4.0 ± 0.0	305 ± 1	6.3± 0.0	801 ± 8	7.9± 0.1	1056 ± 22	8.5± 0.0	1122 ± 6	8.9± 0.0	1089 ± 0	10.9± 0.0	1100 ± 6	7.2± 0.1
33	0	4.0 ± 0.0	177 ± 1	6.2± 0.1	752 ± 13	7.8± 0.0	990 ± 11	8.5± 0.1	1078 ± 14	8.9± 0.0	1067 ± 11	10.8± 0.1	1098 ± 6	7.0± 0.0
51	0	4.1 ± 0.3	72 ± 1	6.2± 0.0	677 ± 14	7.8± 0.0	1001 ± 11	8.6± 0.0	1067 ± 6	8.9± 0.0	1067 ± 11	10.8± 0.1	1100 ± 12	7.2± 0.1

Table S4. Data of Cr(VI) removal from aqueous solution by PHA over time as a function of pH, as depicted in Fig. 1 of the main text.

Time (day)	PHA pH 3.7		PHA pH 5.8		PHA pH 7.6		PHA pH 8.6		PHA pH 8.8		PHA pH 10.5		Control	
	[Cr(VI)] µmol L^{-1}	pH	[Cr(VI)] µmol L^{-1}	pH	[Cr(VI)] µmol L^{-1}	pH	[Cr(VI)] µmol L^{-1}	pH	[Cr(VI)] µmol L^{-1}	pH	[Cr(VI)] µmol L^{-1}	pH	[Cr(VI)] µmol L^{-1}	pH
0	1100 ± 0	3.0± 0.0	1100 ± 0	5.0± 0.0	1100 ± 0	7.0± 0.0	1100 ± 0	8.6± 0.0	1100 ± 0	9.0± 0.0	1100 ± 0	11.0± 0.0	1100 ± 0	7.2± 0.0
0.17	252 ± 4	3.8± 0.0	311 ± 17	5.5± 0.0	963 ± 14	7.5± 0.0	1091 ± 17	8.5± 0.0	1093 ± 13	8.8± 0.0	-	-	1099 ± 1	7.1± 0.0
1	30 ± 4	3.8± 0.0	54 ± 14	5.7± 0.0	783 ± 26	7.5± 0.0	1051± 31	8.5± 0.0	1056 ± 37	8.8± 0.1	1071 ± 68	10.8± 0.1	1108 ± 8	7.3± 0.0
2	0.0	3.8± 0.0	6 ± 2	5.7± 0.0	625 ± 20	7.5± 0.0	1007 ± 14	8.5± 0.0	1022 ± 40	8.8± 0.0	-	-	1102 ± 4	7.3± 0.0
5	0.0	3.7± 0.0	0.0	5.7± 0.0	366 ± 21	7.6± 0.0	818 ± 11	8.6± 0.0	851 ± 39	8.8± 0.1	-	-	1099 ± 3	7.3± 0.0
9	0.0	3.7± 0.0	0.0	5.7± 0.0	187 ± 13	7.7± 0.0	688 ± 11	8.5± 0.0	743 ± 46	8.8± 0.1	-	-	1111 ± 9	7.3± 0.0
16	0.0	3.7± 0.0	0.0	5.8± 0.0	29± 2	7.7± 0.0	501 ± 10	8.5± 0.0	578 ± 30	8.7± 0.1	930± 91	10.6± 0.2	1109 ± 6	7.2± 0.1
33	0.0	3.7± 0.0	0.0	5.8± 0.0	0.0	7.6± 0.0	213 ± 20	8.5± 0.2	326 ± 22	8.8± 0.1	807 ± 145	10.5± 0.1	1103 ± 8	7.3± 0.0
51	0.0	3.7± 0.0	0.0	5.8± 0.0	0.0	7.6± 0.0	59 ± 0.0	8.6± 0.0	163 ± 31	8.8± 0.0	475 ± 447	10.5± 0.1	1106 ± 11	7.2± 0.0

Table S5. Cr K-edge EXAFS fits, where N is the Occupancy, r is the interatomic distance, σ^2 is the Debye–Waller Factor and reduced χ^2 and *R* are the goodness of fit parameters. Initial set of goodness of fit parameters relate to fits including just CrO_6 octahedral pathways; second set of parameters relates to best fits including an additional Cr-C pathway (data shown). Uncertainties in the last digit shown in parentheses. MS = multiple scattering pathways as indicated.

Experiment Description	Pathway	N	r (Å)	σ^2 (Å^2)	χ^2; *R*
Aldrich Humic acid pH 4.1	Cr-O	6	1.96(1)	0.003(1)	666; 0.027
δe0 = -1(1) S^2_0 = 0.77(5)	Cr-C	2	2.96(3)	0.008(4)	434; 0.016
	MS CrO_6	3 x 6	3.93(2)	0.005(2)	
Aldrich Humic acid pH 7.8	Cr-O	6	1.96(1)	0.003(1)	833; 0.026
δe0 = 0(1) S^2_0 = 0.81(7)	Cr-C	2	2.91(5)	0.007(7)	643; 0.018
	MS CrO_6	3 x 6	3.91(2)	0.005(2)	
Peat Humic acid pH 3.7	Cr-O	6	1.97(1)	0.002(1)	137; 0.017
δe0 = 1(1) S^2_0 = 0.78(5)	Cr-C	2	2.98(4)	0.007(4)	93; 0.011
	MS CrO_6	3 x 6	3.94(2)	0.005(2)	
Peat Humic acid pH 7.6	Cr-O	6	1.97(1)	0.002(1)	74; 0.014
δe0 = 1(1) S^2_0 = 0.80(5)	Cr-C	2	3.00(5)	0.008(4)	55; 0.010
	MS CrO_6	3 x 6	3.94(2)	0.005(2)	
Peat Humic acid pH 8.8	Cr-O	6	1.97(1)	0.002(1)	143; 0.017
δe0 = 2(1) S^2_0 = 0.83(5)	Cr-C	2	3.00(5)	0.009(9)	117; 0.014
	MS CrO_6	3 x 6	3.95(2)	0.005(2)	

Table S6. Rate constants and half-lives for Cr(VI) reduction by AHA and PHA at various pH values.

	pH	K (hr^{-1})	R^2	Half life (hr)
AHA	4.1	1.3×10^{-2}	0.97	55
	6.2	2.0×10^{-3}	0.96	350
	7.8	3.0×10^{-4}	0.78	2300
	8.6	8.0×10^{-5}	0.83	8700
PHA	3.7	1.3×10^{-1}	0.91	5.4
	5.8	1.0×10^{-1}	0.97	6.9
	7.6	9.1×10^{-3}	0.99	76
	8.6	2.3×10^{-3}	0.99	300
	8.8	1.5×10^{-3}	1.00	460
	10.5	6.0×10^{-4}	0.917	1200

Table S7. Principal organic compounds in AHA and PHA before, and after reaction with 8000 µmol Cr(VI) at pH3, identified by PyGCMS (see SI Figure S3). Compounds separated in the pyrograms produced by Py-GC-MS are identified using a NIST Standard Reference database (NIST11s MS library). All compounds listed exhibited a good match (>90%) with the database.

Peak No.	**Compound**	**Aromatic / Aliphatic**	**Relative Area×100%**			
			rAHA	**AHA-Cr(VI)**	**PHA**	**PHA-Cr(VI)**
1	Propylene oxide	Aliphatic	-	0.19	-	-
2	Furan, 2-methyl-	Aromatic	-	-	1.68	-
3	Benzene	Aromatic	1.16	0.57	-	-
4	Toluene	Aromatic	2.19	0.62	3.52	2.55
5	Pyridine	Aromatic	-	-	1.44	1.00
6	Pyridine, 2-methyl-	Aromatic	-	-	-	0.56
7	Ethylbenzene	Aromatic	0.40	-	-	-
8	Dimethyl benzene	Aromatic	2.44	0.28	-	-
9	Dimethyl benzene	Aromatic	0.68	-	-	-
10	1,3,5,7-Cyclooctatetraene	Aliphatic	-	-	0.62	0.46
11	1H-Pyrrole, 2-methyl	Aromatic	-	-	0.48	0.46
12	Anisole	Aromatic	-	-	-	0.62
13	1H-Pyrrole, 2-methyl	Aromatic	-	-	0.98	0.65
14	Benzene, 1-ethyl-4-methyl	Aromatic	0.52	-	-	-
15	2-Cyclopenten-1-one, 2-methyl-	Aliphatic	-	-	-	0.50
16	Trimethyl benzene	Aromatic	0.77	-	-	-
17	Trimethyl benzene	Aromatic	1.56	-	-	-
18	Benzene, 1-methoxy-2-methyl-	Aromatic	-	-	-	0.50
19	2-Furancarboxaldehyde, 5-methyl-	Aromatic	-	-	0.91	-
20	2-Cyclopenten-1-one, 3-methyl-	Aliphatic	-	-	-	0.46
21	1-Dodecene	Aliphatic	-	-	-	0.29
22	Benzene, 1-ethyl-4-methoxy-	Aromatic	-	-	-	0.33
23	Acetophenone	Aromatic	-	-	-	0.38
24	Phenol	Aromatic	5.09	-	7.72	4.24
25	Phenol, 2-methoxy-	Aromatic	-	-	6.88	0.64
26	Phenol, 2-methyl-	Aromatic	0.71	-	1.17	1.13
27	Tridecane	Aliphatic	-	-	0.57	0.60
28	1-Tridecene	Aliphatic	-	-	0.56	0.78
29	p-Cresol (4 methyl phenol)	Aromatic	0.48	-	5.99	2.07
30	Cresol (2-methoxy-4 methylphenol)	Aromatic	-	-	3.71	-
31	Phenol, 2,4-dimethyl	Aromatic	-	-	-	1.23
32	Tetradecane	Aliphatic	--	-	0.62	0.53
33	1-Tridecene	Aliphatic	-	-	0.36	0.45
34	Phenol, 4-ethyl-	Aromatic	-	-	3.54	2.01
35	Phenol, 4-ethyl-2-methoxy-	Aromatic	-	-	3.77	-
36	Tetradecane	Aliphatic	-	-	0.75	0.67
37	1-pentadecene	Aliphatic	-	-	0.67	0.55
38	Phenol, 2,6-dimethoxy-	Aromatic	-	-	4.72	-
39	Naphthalene, 2,6-dimethyl-	Aromatic	0.78	-	-	-

Peak No.	Compound	Aromatic / Aliphatic	Relative Area×100%			
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)
40	Indole	Aromatic	-	-	1.02	0.92
41	Tetradecane	Aliphatic	-	-	0.98	0.92
42	1-Pentadecene	Aliphatic	-	-	0.44	0.56
43	Trans-Isoeugenol	Aromatic	-	-	0.61	-
44	1H-Indole, 3-methyl-	Aromatic	-	-	-	0.72
45	Octadecane	Aliphatic	0.35	-	1.06	1.24
46	1-Pentadecene	Aliphatic	-	-	0.62	0.62
47	Naphthalene, 2,3,6-trimethyl-	Aromatic	0.38	-	-	-
48	Naphthalene, 2,3,6-trimethyl-	Aromatic	0.31	-	-	-
49	1-Dodecanol, 3,7,11-trimethyl-	Aliphatic	0.68	-	-	-
50	1-Dodecanol, 3,7,11-trimethyl-	Aliphatic	-	-	-	2.03
51	Naphthalene, 2,3,6-trimethyl-	Aromatic	1.20	-	-	-
52	Apocynin	Aromatic	-	-	0.82	-
53	Octadecane	Aliphatic	-	-	0.90	1.36
54	1-Octadecene	Aliphatic	-	-	0.6	1.16
55	Nonadecane	Aliphatic	-	-	1.32	2.05
56	1-Nonadecene	Aliphatic	-	-	0.68	0.96
57	2-Pentadecanone, 6,10,14-trimethyl-	Aliphatic	-	-	-	0.70
58	Octadecane	Aliphatic	0.34	-	0.89	1.53
59	1-Nonadecene	Aliphatic	-	-	0.49	1.05
60	1-Nonadecene	Aliphatic	-	-	-	0.46
61	2-Heptadecanone	Aliphatic	-	-	-	0.32
62	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	Aromatic	-	-	0.35	-
63	Heneicosane	Aliphatic	-	0.22	-	-
64	Heneicosane	Aliphatic	-	-	-	1.93
65	1-Nonadecene	Aliphatic	-	-	-	0.88
66	n-Hexadecanoic acid	Aliphatic	-	0.48	-	-
67	Heneicosane	Aliphatic	-	-	1.05	-
68	1-Nonadecene	Aliphatic	-	-	0.58	-
69	n-Hexadecanoic acid	Aliphatic	-	-	0.91	0.54
70	Heneicosane	Aliphatic	0.98	0.65	0.91	1.54
71	1-Nonadecene	Aliphatic	-	-	0.93	1.24
72	2-Nonadecanone	Aliphatic	-	-	0.64	1.09
73	Heneicosane	Aliphatic	1.85	1.70	1.04	1.93
74	1-Nonadecene	Aliphatic	-	-	0.56	0.59
75	Heneicosane	Aliphatic	3.54	3.76	0.87	1.89
76	Octacosanol	Aliphatic	-	-	0.61	1.24
77	Behenic alcohol	Aliphatic	-	-	-	0.29
78	2-Heptadecanone	Aliphatic	-	0.14	0.73	1.50
79	Octacosane	Aliphatic	-	0.22	-	-
80	Heneicosane	Aliphatic	5.61	6.29	1.14	2.47
81	Octacosanol	Aliphatic	-	-	0.34	0.66
82	Octacosane	Aliphatic	0.34	0.58	-	-
83	Octacosane	Aliphatic	-	0.30	-	-
84	Heneicosane	Aliphatic	7.66	8.94	0.8	2.56
85	Octacosanol	Aliphatic	-	-	-	0.66

Peak No.	Compound	Aromatic / Aliphatic	Relative Area×100%			
			rAHA	AHA-Cr(VI)	PHA	PHA-Cr(VI)
86	2-Heptadecanone	Aliphatic	-	-	-	0.79
87	Octacosane	Aliphatic	0.48	1.03	-	-
88	Pentacosane	Aliphatic	0.50	0.72	-	-
89	Heneicosane	Aliphatic	8.56	10.18	1.06	3.24
90	Behenic alcohol	Aliphatic	-	0.16	-	-
91	Octacosanol	Aliphatic	-	-	-	0.49
92	Tetracontane	Aliphatic	0.50	1.54	-	-
93	Octadecane, 3-methyl-	Aliphatic	-	1.15	-	-
94	Octacosane	Aliphatic	0.69	-	-	-
95	Heneicosane	Aliphatic	9.90	11.70	0.88	3.19
96	Behenic alcohol	Aliphatic	-	0.32	-	-
97	Tetracosanoic acid, methyl ester	Aliphatic	-	-	-	2.21
98	2-Heptadecanone	Aliphatic	-	-	0.48	-
99	Tetracontane	Aliphatic	0.40	0.96	-	-
100	Octadecane, 3-methyl-	Aliphatic	-	1.26	-	-
101	Octacosane	Aliphatic	0.85	0.31	-	-
102	Squalene	Aliphatic	-	-	-	1.09
103	Heneicosane	Aliphatic	10.23	11.76	0.82	3
104	Octacosanol	Aliphatic	-	0.41	-	-
105	Octacosane	Aliphatic	-	0.98	-	-
106	Octadecane, 3-methyl-	Aliphatic	-	1.1	-	-
107	Tetracontane	Aliphatic	9.56	11.16	-	1.86
108	Octacosanol	Aliphatic	-	0.34	-	-
109	Hexacosanoic acid, methyl ester	Aliphatic	-	-	-	2.63
110	Tetracontane	Aliphatic	-	0.36	-	-
111	Nonacosane	Aliphatic	-	0.71	-	-
112	Tetracontane	Aliphatic	7.28	8.09	-	-
113	Octacosanol	Aliphatic	-	0.35	-	-
114	Tetracontane	Aliphatic	2.14	3.25	-	-
115	Tetracontane	Aliphatic	-	1.24	-	-
116	Tetracontane	Aliphatic	-	1.95	-	-

The percentages of the unassigned compounds in rAHA, AHA-Cr(VI), PHA, and PHA-Cr(VI) are 8.92%, 4.03%, 24.27%, and 20.23% respectively.

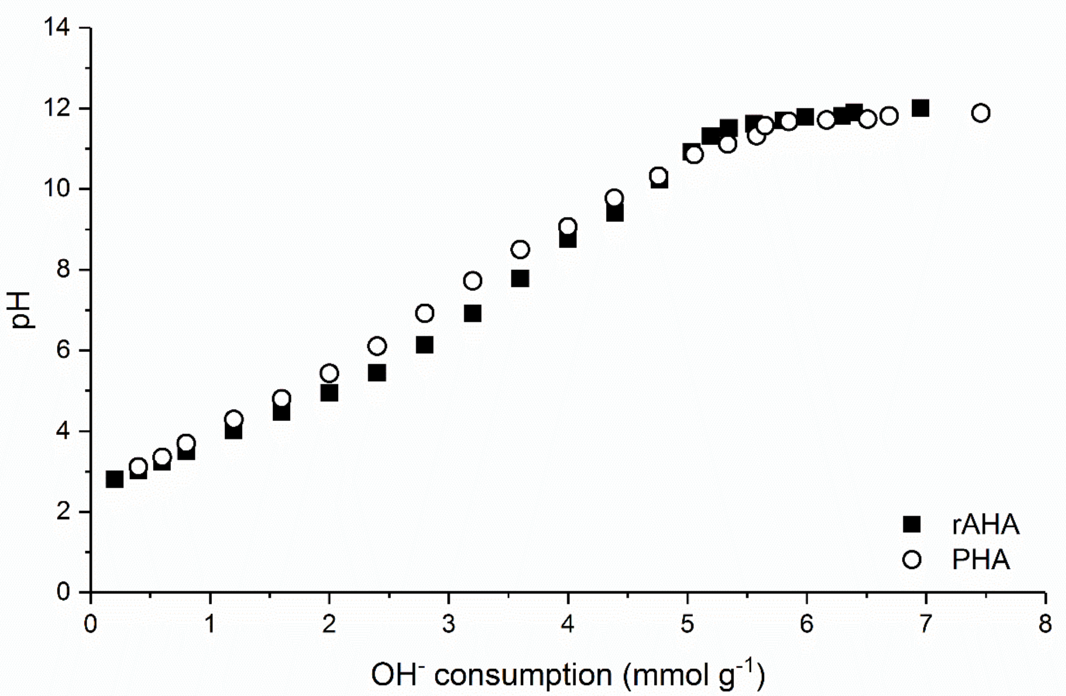

Figure S1. Base titration curves for rAHA and PHA in 0.5N NaCl

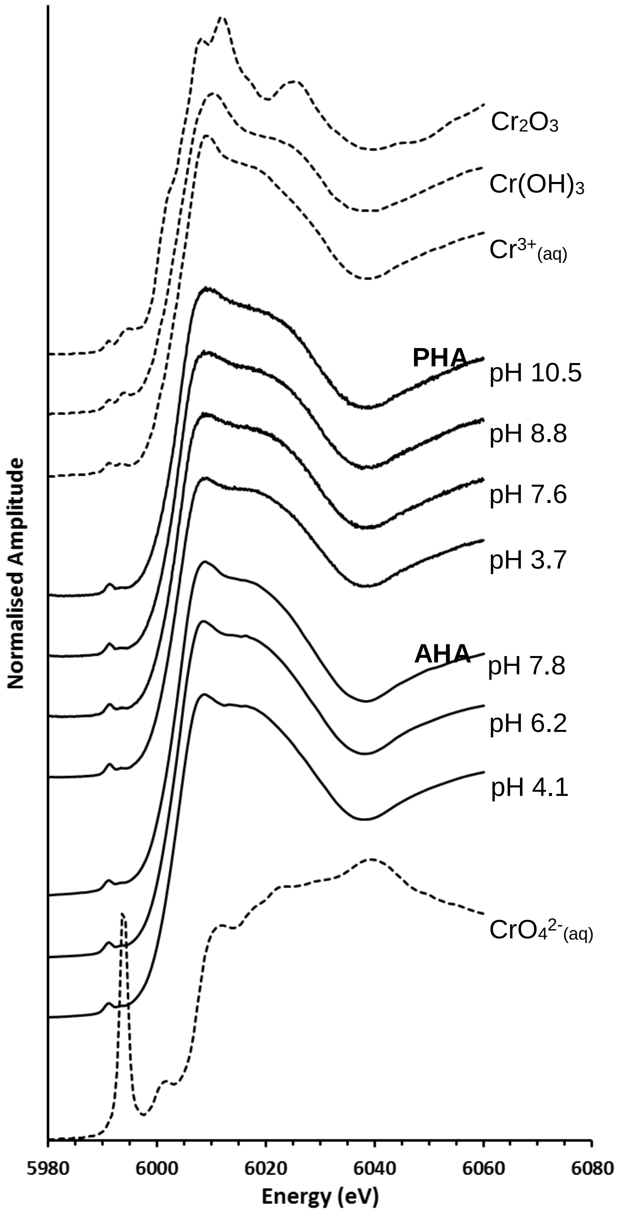

Figure S2. Cr K-edge XANES spectra collected from Aldrich (AHA) and peat (PHA) humic acid samples after reaction with chromate solution for 50 days, and from selected Cr(VI) and Cr(III) containing standards.

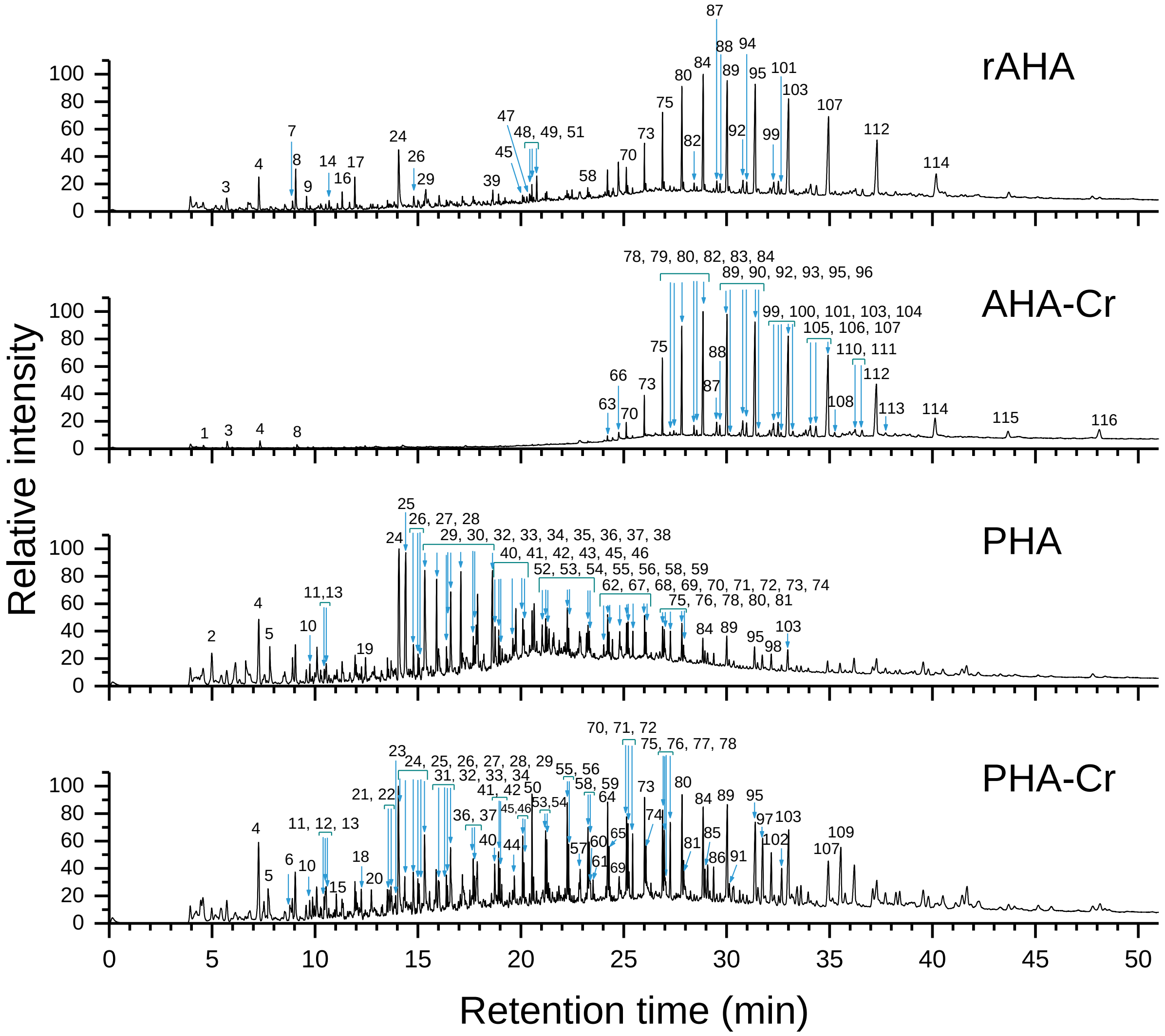

Figure S3. Pyrograms of rAHA at pH 3, AHA reacted with excess Cr(VI) initially at pH 3, PHA at pH 3 and PHA reacted excess Cr(VI) initially at pH 3. Peak identities and intensities are given in Table S7.

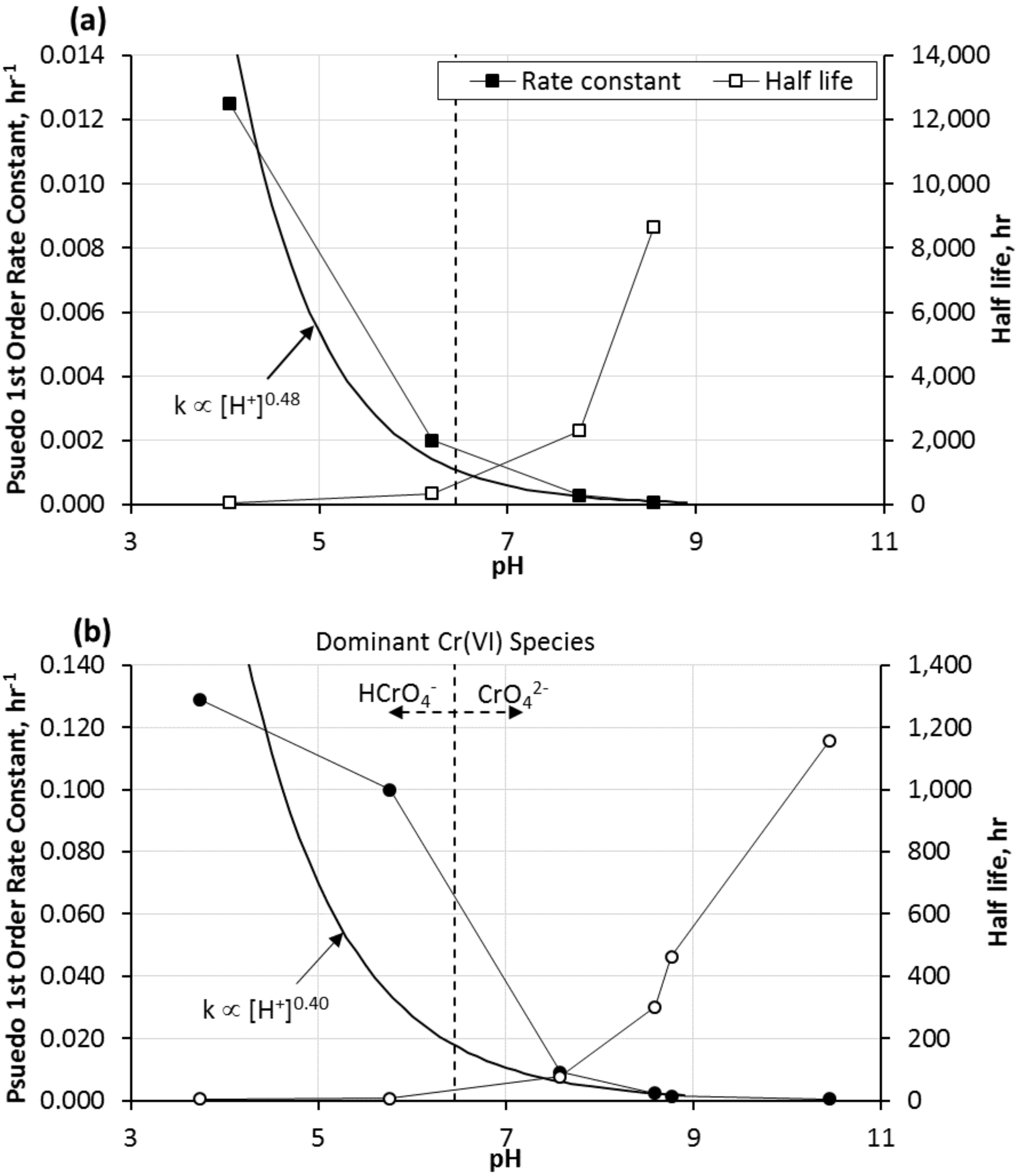

Figure S4. Pseudo first order rate constants and half-lives for the reduction of Cr(VI) by (a) AHA, and (b) PHA. Reactions contained 110 μmol Cr(VI)/g of HA, and an activity coefficient of unity has been assumed to calculate $[H^+]$ from the pH value. Dashed line indicates the pH value below and above which $HCrO_4^-$ and CrO_4^{2-} are the dominant aqueous Cr(VI) species, respectively(Pourbaix, 1966).

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