ABSTRACT

Title of Thesis: OXIDATION-REDUCTION TRANSFORMATIONS OF CHROMIUM IN AEROBIC SOILS AND THE ROLE OF ELECTRON-SHUTTLING QUINONES IN CHEMICAL AND MICROBIOLOGICAL PATHWAYS

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Soils from three toposequences in Maryland with minimal heavy metal contamination were sampled to investigate oxidation-reduction transformations of chromium in whole soil samples. Chromium (VI) reduction to Cr(III) was observed in all 18 samples, and 11 demonstrated enhanced reduction with the electron shuttle anthraquinone-2,6-disulfonate (AQDS). Oxidation of Cr(III) to Cr(VI) was observed in 12 samples, and 7 samples demonstrated diminished oxidation with AQDS. Lactate was added to the Watchung series to enhance biological activity, and high salt concentration was added to inhibit it. Both treatments reduced Cr(VI) to below detection limits by 11 d, suggesting abiotic reduction. The control treatment demonstrated reduction of Cr(VI) without soil. To further investigate, increasing lactate concentrations were added to Cr(VI) and AQDS. Reduction increased with increasing concentration; 60 mM lactate reduced all Cr(VI) within 1 hr. Other organic acids were tested for similar interactions; tartrate and citrate reduced Cr(VI), which was enhanced with AQDS.

OXIDATION-REDUCTION TRANSFORMATIONS OF CHROMIUM IN AEROBIC SOILS AND THE ROLE OF ELECTRON-SHUTTLING QUINONES IN CHEMICAL AND MICROBIOLOGICAL PATHWAYS

By

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Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Biogeochemical and oxidation-reduction (redox) processes in soils are in a constant, dynamic state of non-equilibrium, due to a multitude of interactions among redox-active chemical species, and due to the activity of biological mediators, especially microorganisms (Stumm and Morgan, 1996). A better understanding of these processes can lead to more efficacious remediation and restoration strategies for soils contaminated with metals, and to better land use management practices to protect human health and ecosystem processes. Two of the most pertinent soil characteristics affecting the chemistry of natural soil and water systems are pH and pe, which together can be considered as master variables, because they determine speciation of heavy metals and other elements; and they determine the likelihood for reduction and oxidation reactions to proceed (James, 1996). Investigating the reduction of Cr(VI) to Cr(III) and oxidation of Cr(III) to Cr(VI) by whole soil samples under different experimental conditions will allow for further insight into the nature of complex soil redox processes, and provide a tool for understanding electron transfer reactions under non-equilibrium soil conditions in the field.

Chromium Chemistry in Soil

Chromium (Cr) is a naturally-occurring, redox active transition metal. Though it can exist in a range of oxidation states, Cr(III) and Cr(VI) are the most stable species in soils and natural waters, and in living tissues. Chromium is a contaminant of concern at Superfund sites in the United States, and other sites globally, due to its carcinogenic properties and mobility in soils and natural waters,

principally as the anionic Cr(VI) species, CrO_4^{2-} and $HCrO_4^-$. The U.S. Department of Health and Human Service's Agency for Toxic Substances and Disease Registry publishes the CERCLA Priority List of Hazardous Substances, and ranks Cr(VI) as the number 18 contaminant of concern (Agency for Toxic Substances and Disease Registry Staff, Accessed March 2008).

In contrast, Cr(III) is an essential nutrient with a recommended daily dose of 50-200 μg for adults, ingestible in forms such as the Cr(III)-picolinate chelate, because of its role in the body's metabolism of sugar, protein, and fat (Agency for Toxic Substances and Disease Registry Staff, 2000). Chromium (III) is a strong Lewis acid; it shares three, unpaired d-electrons to acquire a noble gas configuration, and forms both organic and inorganic complexes. In solution, Cr(III) forms the hexaqua ion $[Cr(H_2O)_6]^{3+}$, and is acidic with a pK of 4 for the first hydrolysis product, $Cr(H_2O)_5OH^{2+}$ (Cotton and Wilkinson, 1980). At pH values greater than approximately 5.5, Cr(III) precipitates with OH^- forming $Cr(OH)_{3(s);}$ however, if complexed with organic acids such as fulvic and citric acid, Cr(III) is soluble at pH values up to 6.7 (James and Bartlett, 1983a).

Soluble Cr(III) salts and freshly-precipitated hydroxides oxidize rapidly to Cr(VI) in the presence of Mn(III,IV)(hydr)oxides (Bartlett and James, 1979). The oxidation process is best described as an outer-sphere electron transfer to a decentralized electronic band on the Mn(III,IV)(hydr)oxide (Silvester et al., 1995). The electron transfer was demonstrated to occur most rapidly at pH 3, but was inhibited at higher pH values, possibly due to a change in the electrophoretic mobility of δ-MnO_2 induced by initial Cr(III) concentrations (Fendorf and Zasoski, 1992a).

Higher initial concentrations of Cr(III) resulted in the surface becoming less negatively charged, and less able to adsorb cationic Cr(III) in solution. The electrophoretic mobility of Mn(III,IV)(hydr)oxides in soils is dependent on ion activities, and overall ionic strength of the solution.

In contrast to these findings, Cr(III) oxidation by β-MnO_2 was demonstrated to be most rapid at pH 7 in an aerobic reactor (Apte et al., 2006). A cycle was established in the reactor where the continual generation of Cr(VI), due to re-oxidation of Mn(II) to Mn(III,IV)(hydr)oxides in the presence of oxygen at pH 7, resulted in the interminable dissolution of $Cr(OH)_{3(s)}$ as $Cr(III)_{(aq)}$ was oxidized.

Although Mn(II) is abiotically oxidized by dissolved O_2 and Mn(III,IV)(hydr)oxides, it is also well-recognized that soil microorganisms can act as oxidizers of Mn(II), and the subsequent Mn(III,IV)(hydr)oxides are considered the most reactive forms of Mn in nature (Tebo et al., 2004). Chromium(III) was shown to be indirectly oxidized by biogenic Mn(III,IV)(hydr)oxides produced by *Bacillus* sp. strain SG-1 (Murray and Tebo, 2007). Chromium(III) was also directly oxidized by the bacteria, likely by the same non-specific enzyme employed to oxidize Mn(II). The oxidation of Cr(III) in soils has been demonstrated to be dependent on complex interactions between reactive, abiotic species and biological mediators, which are also important factors in the reduction of Cr(VI) to Cr(III).

Chromium(VI), the thermodynamically most stable form of Cr in systems at equilibrium with the atmosphere, exists as either the $HCrO_4^-$ or CrO_4^{2-} anionic species, making it soluble and potentially mobile in the environment, similar to SO_4^{2-}. In soils, Cr(VI) as CrO_4^{2-} can adsorb via binuclear, bridged complexes, similarly to

SO_4^{2-} or HPO_4^{2-}, in the presence of positively-charged soil colloids. In most soils, however, Cr(VI) is protonated in the form $HCrO_4^-$, which is also tightly held by colloidal surfaces, or in the presence of soil organic matter, can be reduced and precipitated as Cr(III)(hydr)oxides (Bartlett and Kimble, 1976; James and Bartlett, 1983b). The reduction of Cr(VI) by soil fulvic acid was demonstrated to occur more rapidly as pH decreased from 7 to 1, and was best described by an initial rapid reduction period followed by a lower reduction rate as Cr(VI) concentrations diminished (Wittbrodt and Palmer, 1995). Other abiotic reductants in soils include $Fe(II)_{(aq)}$ and H_2S, which are electron-rich, inorganic species effective in reducing Cr(VI) to Cr(III) (Fendorf and Li, 1996; Kim et al., 2001).

In addition to abiotic reduction pathways, microorganisms in soils can play an important role in Cr(VI) reduction. Soil microorganisms have diverse metabolic processes, but all require energy, electron, carbon sources, and terminal electron acceptors for metabolic functions. Microorganisms that derive carbon from organic compounds, such as soil organic matter, are heterotrophic; those that derive it from inorganic carbon, such as CO_2, are autotrophic. Organisms that use organic carbon as an electron source are organotrophic, and those that derive electrons from inorganic forms, such as NH_4^+, are lithotrophic. Table 1 demonstrates the diversity of soil microbial metabolism.

Soil microorganisms are important in the cycling of metals and nutrients in the environment, and for 80 to 90% of carbon mineralization (Hassink et al., 1994). Metabolically, different microorganisms use different electron acceptors, though oxygen is the preferential acceptor in aerobic environments. In the absence of

Energy Source	**Electron Source (reducing equivalents)**	**Carbon Source**	**Terminal Electron Acceptor**
Chemotrophic – external chemical compounds	Lithotrophic – inorganic compounds	Autotrophic – CO_2	Aerobic – O_2
Phototrophic – light	Organotrophic – organic compounds	Heterotrophic – organic compounds	Anaerobic – NO_3, Fe(III), Mn(III,IV), SO_4^{2-}, H_2O

Table 1 Soil microorganisms can be distinguished by their sources for energy, electron, and carbon, as well as their terminal electron acceptor in metabolic processes. These distinctions can be almost freely interchanged. For example, aerobic chemoheterotrophs obtain energy from chemical compounds, electrons and carbon from organic compounds, and use oxygen as a terminal electron acceptor.

oxygen, organisms use an available acceptor that will yield the greatest amount of energy per mole of electron from a given source. The general sequence in reduction of naturally-occurring species in soils and natural waters is NO_3^-, Mn(III,IV)(hydr)oxides, Fe(III)(hydr)oxides, SO_4^{2-}, CO_2, and finally H_2O (Schink, 2006). Thermodynamically, the reduction of Cr(VI) would occur before the reduction of Mn(III,IV)(hydr)oxides, with CH_2O, a general form for soil organic matter, coupled as the electron donor.

Table 2 shows the change in Gibbs free energies associated with the oxidation of soil organic matter, and the reduction of various terminal electron acceptors. These values for the change in Gibbs free energy are based on thermodynamic relationships, and are useful in making predictions about the oxidation state of terminal electron acceptors. However, unlike natural systems, the values presented in this table were generated at standard conditions and equivalent activity for each species. In soils and natural waters at non-equilibrium conditions, there may be a combination of oxidation states among species.

The addition of reduced C compounds and other electron donors to a facultative anaerobic bioreactor resulted in the reduction of contaminated soil with up to 5,100 mg Cr(VI)/kg soil (Krishna and Philip, 2005). Chromium-reducing bacteria reduce Cr(VI) either intracellularly, coupled to metabolic processes, or extracellularly by non-metabolic processes as mediated by cell exudates on the cell wall surface. Chromium (VI) is an oxyanion, and like other anions such as SO_4^{2-} or NO_3^-, it can pass through surface anion transport systems in cellular membranes (Cervantes et al., 2001). Once inside the cell, Cr(VI) is reduced to its lower oxidation states

Electron Source	Terminal Electron Acceptor	ΔG_r (kJ/eq)
CH_2O	O_2	-119
CH_2O	$HCrO_4^-$	-107
CH_2O	MnO_2	-93.0
CH_2O	NO_3^-	-80.4
CH_2O	FeOOH	-73.0
CH_2O	SO_4^{2-}	-28.5

Table 2 A sequence for microbial terminal electron acceptors, where CH_2O is a general formula for a soil organic compound. Microorganisms use electron acceptors based on the highest free energy available, and will progress from O_2 down to SO_4^{2-}. ΔG_r calculated from log K values from given coupled redox reactions (Bartlett and James, 2000).

Cr(V,IV,III) by compounds such as reduced nicotinamide adenine dinucleotide (NADH) and ascorbic acid.

Extracellular reduction is accomplished by soluble, membrane-bound proteins that reduce Cr(VI) on the cell surface, forming insoluble reduction products, so that the metal never enters the cell. For example, the cell wall of Arthrobacter oxydans contains an acid-soluble protein with a positive charge, as shown by electrophoresis, capable of reducing Cr(VI) to an insoluble Cr(III)(hydr)oxide which accumulates on the bacterial surface (Asatiani et al., 2004). Although Mn(III,IV)(hydr)oxides, inorganic and organic reductants, and microorganisms are all agents influencing the redox chemistry of Cr and other elements in soils, the potential for reduction or oxidation depends on proton and electron activities.

Master Variables

Electron activity, or the potential for the electron to do electrical work, is measured as a voltage, Eh, and is often expressed as pe. Although its activity is dimensionless due to the electron having negligible mass, it is analogous to pH as the measure of proton activity. The large charge-to-size ratio of the electron, again similar to the proton, makes it ephemeral in free form, however, it is a strong reducing agent with a potential of -2.7 V relative to hydrogen (Bartlett and James, 2000). In soils and natural waters, the range for pH is 3 to 12, and -10 to 17 for pe (Stumm and Morgan, 1996). These pe values correspond to Eh values of -591 to 1005 mV, where the more positive a value, the lower the electron activity. One use of pe and pH data is to represent which species of an element predominates, and is

thermodynamically favorable, at given electron and proton conditions. Figure 1 is a pe-pH diagram for the Cr system at environmentally relevant conditions.

The controlling effect of pH and pe can be best viewed as a seesaw, where in the presence of reducing agents, such as organic matter or Fe^{2+}, and at low pH values (< 6), reduction of Cr(VI) will be favored, and pe will increase to maintain redox equilibrium (James, 1996). Conversely, in the presence of oxidizing species, such as Mn(III,IV)(hydr)oxides, and at high pH values, oxidation is favored, and pe will decrease. Figure 2 is an illustration of the seesaw analogy for Cr redox in soils. Redox equilibria, however, are metastable, and the seesaw will constantly shift with proton and electron fluctuations through a system. Thermodynamic models predict steady state conditions, but in reality, a dynamic equilibrium is a more accurate representation of natural systems (Stumm and Morgan, 1996). Often, redox reactions in soils are moving towards equilibrium, but the reactions are constantly being perturbed by the influx and efflux of chemical species, thus shifting activities and re-establishing new, partial equilibriums. Because the electron does not exist free in solution, electron flow in soils requires reduction reactions coupled with oxidation reactions. Dioxygen gas is the most ubiquitous electron acceptor in natural systems, and the electron activity of a soil depends, in part, on its presence. Because a saturated soil restricts the diffusion of oxygen, wetter soils allow for more electron flow to other electron acceptors, such as Fe(III)(hydr)oxides and SO_4^{2-} (Bartlett and James, 1993).

When a soil becomes submerged, pe decreases, and the rate of decrease will be a function of easily-oxidized soil organic matter, temperature, time submerged,

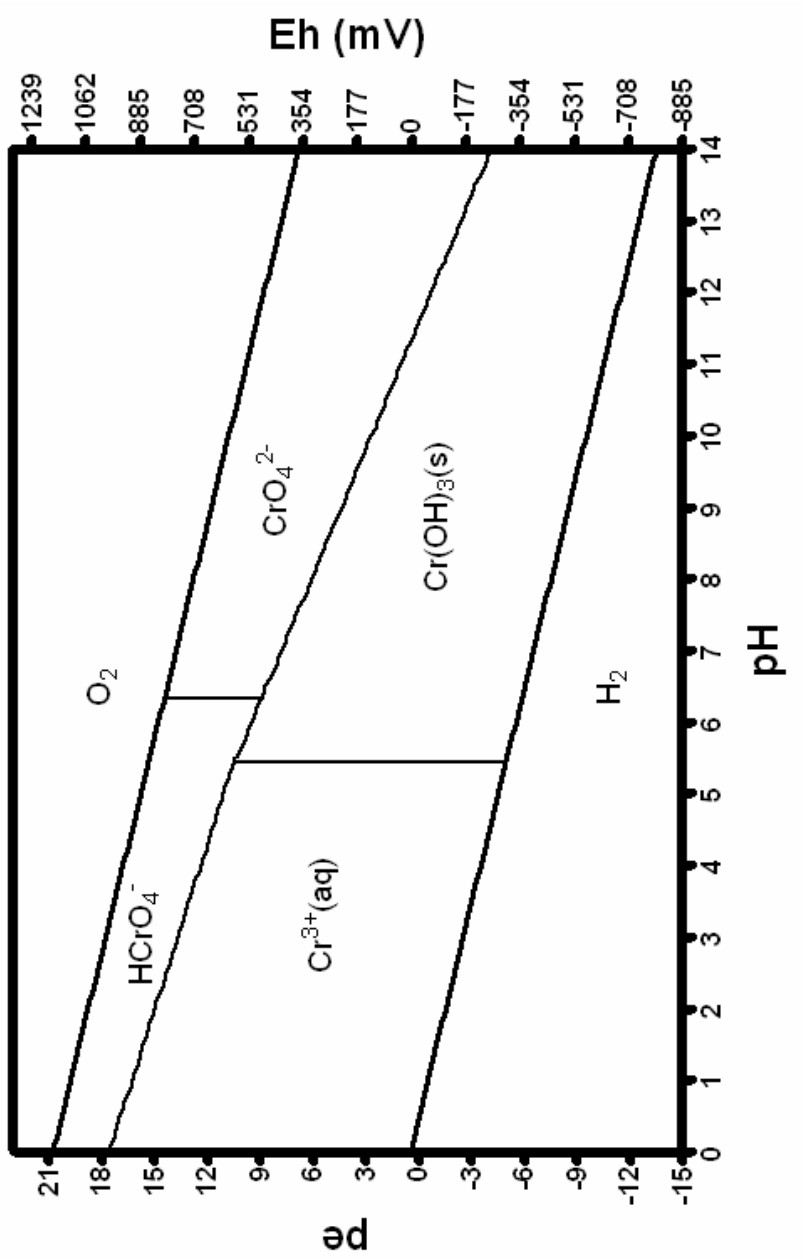


Figure 1 pe-pH diagram for Cr(VI)-Cr(III) redox transitions. Ion activities are 10^{-4} M.

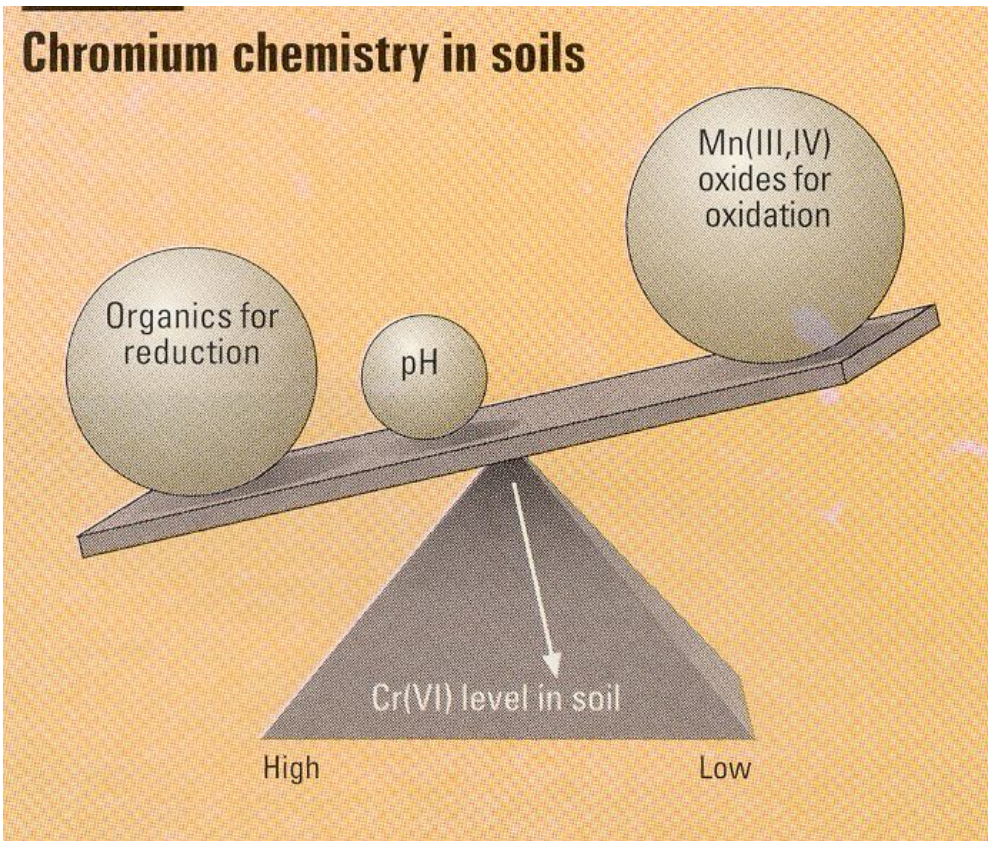


Figure 2 Representation of Cr reduction and oxidation in soils controlled by the seesaw action of pH (James, 1996).

and nature of available electron acceptors (Sparks, 2003), which are also important factors in determining the presence and metabolic requirements of soil microorganisms. Though soils host a variety of microorganisms using a diverse array of electron acceptors for respiration, such as Fe(III)(hydr)oxides in anoxic sediments, soil organic matter is the dominant electron donor, and can be fully oxidized to CO_2 (Lovley, 2000), or partially to organic acids. Understanding the chemistry of meta-stable soil environments is dependant not only on the master variables pH and pe, and their biological mediation, but also on knowledge of pertinent organic compounds, and their redox active functional groups.

Soil Organic Matter

In addition to being a source of electrons, energy, and C for soil microorganisms, soil organic matter serves many functions in a terrestrial environment, which include buffering pH changes, retaining water, stabilizing soil structure, chelating metals, and contributing to pH-dependent cation exchange reactions. The cation exchange capacity of soil organic matter is principally derived from the carboxylic and phenolic groups, though enol and imide groups are also important (McBride, 1994). Though there is still disagreement and speculation about the genesis and characterization of soil organic matter, some generalities concerning chemical properties and molecular structure can be made.

Soil humus is regarded as the amorphous or paracrystalline organic material remaining after dissolved and particulate organic C are accounted for. Soil humus has several distinct qualities, such as aromatic rings with carboxyl, hydroxyl,

carbonyl, and alkyl groups; significant amounts of C-1 to C-20 alkyl chains; aromatic rings and alkyl groups with C-to-C bonds; and simple and polymeric proteinaceous and carbohydrate groups associated along a randomly ordered backbone (Baldock and Nelson, 2000).

The humic substances that constitute soil organic matter, and contribute to the chemical properties of soils are: fulvic acids, operationally defined as soluble in base, acid, and water; humic acids, soluble in base and water; hymatomelanic acid, soluble in ethanol; and humus coal, insoluble in base, acid, and water (Tan, 2003). These operational definitions for humic substance date back to the work of G.J. Mulder in 1862, and expanded on by S. Oden in 1919; however, they are still applicable and in use today. These operational definitions have also been the basis for understanding molecular structures of soil humus, and theories of humification. Understanding the genesis of soil organic matter leads to a fuller understanding of functional groups and molecular structures pertinent to the chemistry of natural systems.

The two most widely-accepted theories of soil organic matter genesis are depolymerization of biological molecules causing direct formation of humic substances; and polymerization of degraded, labile molecules into fulvic acids, then into humic acids upon further humification, and eventually into humus. (Baldock and Nelson, 2000; Tan, 2003). The polymerization, or condensation, theory postulates that degradation of plant derived biopolymers, from abiotic reactions and enzymatic activity, result in labile monomers, such as sugars and amino acids, which abiotically re-polymerize into fulvic acids. Further abiotic and microbial oxidation of these

newly-formed macromolecules results in humic acids consisting of more aromatic structure and phenolic functional groups.

Phenolic groups are important in complexation reactions with metals, and can also be further oxidized by the enzymes phenolase and laccase to produce quinones, which are considered to be the major electron donor and acceptor moieties of humic material (Tan, 2003). In contrast to this oxidation, a one-electron transfer to quinone forms the highly-reactive intermediate semiquinone, and then the second electron transfer forms hydroquinone (Larson, 1997). Hydroquinone is then capable of donating its two electrons in further reduction reactions, making the quinone-hydroquinone species a very dynamic redox constituent in soils and natural waters. Even under aerobic conditions, humic acids express significant reducing capacity, and though Fe(II) bound to humic acids also contributes electrons, relative to humic acids, its contribution can be considered insignificant (Peretyazhko and Sposito, 2006).

In contrast to the polymerization of degradation products resulting in humic macromolecules being randomly coiled (Stevenson, 1994), it is further suggested that humic substances are aggregated supramolecular associations linked by hydrophobic and hydrogen bonding (Sutton and Sposito, 2005). Rather than being well-defined, stoichiometric molecules, they are properly viewed as molecular aggregates of sizes that are between soluble and colloidal forms. These molecules are easily disassociated by the addition of simple organic compounds, such as carboxylic and mineral acids, penetrating the hydrophobically linked interactions between molecules (Piccolo et al., 2001). Organic acids, and α-hydroxy carboxylic acids such as malate, citrate, and lactate in particular, are ubiquitous in the environment due to plant root

exudates and microbiological activity. The potential for these acids to disaggregate soil organic matter could result in the exposure of metal binding phenolic functional groups, and redox-active, quinone structures, otherwise protected within the humic structure. Whether by aggregation or polymerization, the continual abiotic and biological formation of soil organic matter, and disaggregation of humic substances, keeps the soil environment chemically metastable from a redox perspective.

Electron Shuttles

The quinone structure is ubiquitous in the environment; it is found as a component of humic and fulvic acids; vitamin K; and anthraquinones, which occur in plants and fungi. One of the most prolific quinone structures in nature is coenzyme Q10, which is present in all human cells. It is found in high concentrations in heart, lung, and liver tissues and mitochondrial cells, making it one of the most potent, and important inhibitors of free radical reactions (Larson, 1997). Free radicals are species that have an unpaired electron, so they readily donate or accept another electron to pair with it, making them strong reducing and oxidizing agents.

In the soil environment, the superoxide ($\bullet O^{2-}$) and hydroxyl ($OH\bullet$) free radicals are two examples of reactive oxygen species, and possibly are why thermodynamically unfavorable reactions sometimes occur spontaneously (Bartlett and James, 1993). Manganese(II) is an important electron donor, and is one of a few species that can be oxidized by the oxygen free radicals. The oxidized form, Mn(III,IV)(hydr)oxide, is capable of oxidizing hydroquinone into semiquinone, an

organic free radical, and in consequence, is reduced to the free radical supermanganese (Mn^{3+}) (Bartlett and James, 1993).

Peretyazhko and Sposito (2006) demonstrated the natural reducing capacities of International Humic Substance Society (IHSS) humic acids, and also that the microbial reduction of these humic acids increased reducing capacity several-fold. The electron donating kinetics of dissolved organic matter in aquatic systems was shown to occur within the course of 1 day, and capable of shuttling electrons to H_2S and Fe(III) (Bauer et al., 2007). Hydroquinones and quinones not only have been identified as abiotic electron donating and accepting moieties of soil and dissolved organic matter, but are also electron acceptors in microbial respiration (Lovley et al., 1996; Scott et al., 1998).

Soil bacteria were shown to reduce the humic acid analog anthraquinone-2,6-disulfonate (AQDS), which then acted as an electron shuttle in solution to reduce Fe(III) (Kappler et al., 2004). The shuttling activity of AQDS was demonstrated further with Geobacter sulfurreducens continually shuttling electrons to ferrihydrite in solution (Straub and Schink, 2003). Though this process occurs readily in anaerobic sediments, due to the lack of electron scavenging competition from O_2, the aerobic reduction of metals through shuttling processes also occurs. In solution, Shewanella oneidensis aerobically reduced Cr(VI), which was enhanced by the addition of the electron shuttle AQDS (Lowe et al., 2003). The conceptual model for soils is of a similar mechanism occurring with labile humic acids being reduced by soil microorganisms, and in turn, shuttling electrons to abiotically reduce organic and

inorganic constituents. Figure 3 illustrates this conceptual model of shuttling in the environment.

The following equations are stoichiometric, representative redox reactions possible within this model. Equation 1 denotes AQDS reduction, which represents the reduction of labile humic acids in soils by soil microorganisms using it as a terminal electron acceptor as shown in Figure 3. Equation 2 shows the reduction of Cr(VI) by AH_2DS, and equation 3 the oxidation of Cr(III) by a Mn(III,IV)(hydr)oxide.

$$AQDS + 2\,e^- + 2\,H^+ \rightarrow AH_2DS \quad (1)$$

$$HCrO_4^- + 1.5\,AH_2DS + H^+ \rightarrow Cr(OH)_3 + 1.5\,AQDS + H_2O \quad (2)$$

$$Cr(OH)_3 + 3\,\gamma\text{-}MnOOH + 5\,H^+ \rightarrow HCrO_4^- + 3Mn^{2+} + 5H_2O \quad (3)$$

From these equations, the role of pH as a master variable is again illustrated, as protons are consumed in the reduction of Cr(VI) and oxidation of Cr(III). Electron shuttling in soils appears to be a widespread mechanism in abiotic and biological reactions, and to better understand these shuttling processes brings together many important aspects of soil redox chemistry: master variables, soil organic matter and redox active functional groups, soil microorganisms, and the activity of redox active metals.

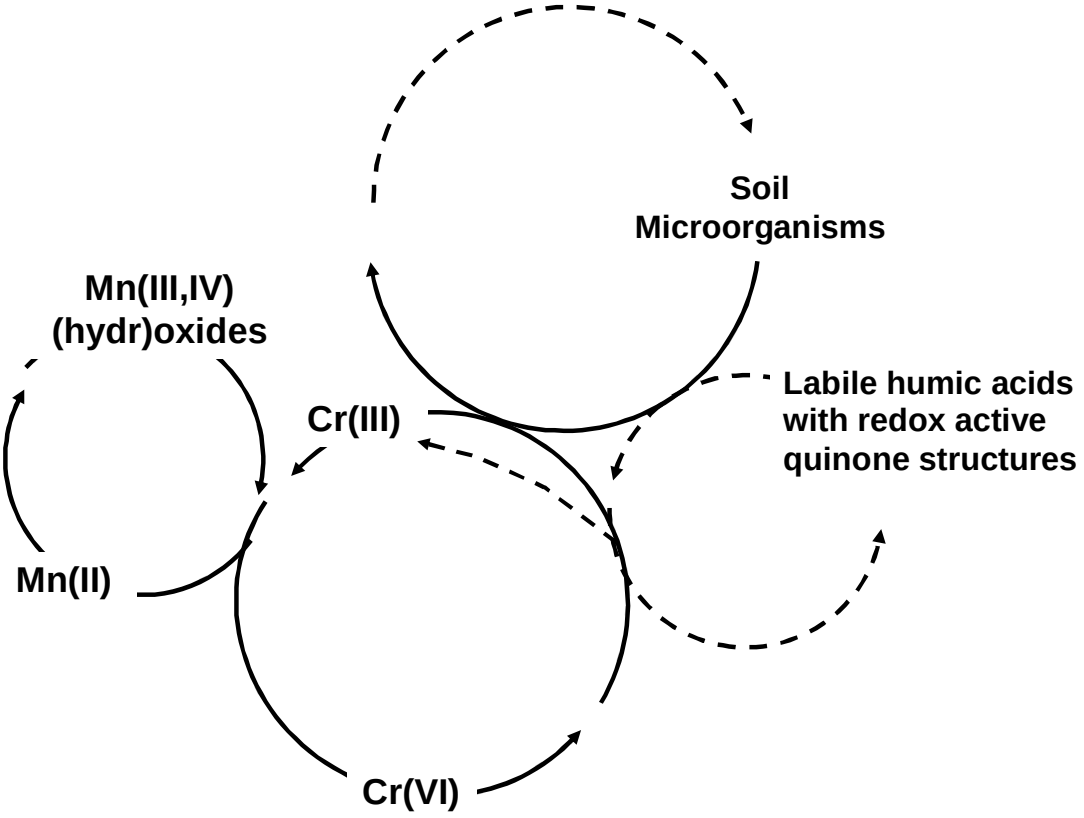


Figure 3 Conceptual model of Cr redox in soils. Solid lines represent demonstrated redox processes, and dashed lines represent proposed shuttling pathways.

Research Objectives

Further insight into the multitude of metastable chemical and biological redox interactions in soils can be gained by investigating these reactions using whole soil samples. Chromium can be reduced and oxidized by both abiotic and biological mechanisms. The intention of this thesis is to investigate redox mechanisms and electron shuttling by characterized, whole soil samples using Cr(VI) and Cr(III) under different treatment regimes.

The remaining thesis will be divided into chapters 2-5. Chapter 2 addresses the following objectives:

- Sample select Maryland soils within three different toposequences.
- Provide a detailed characterization of the samples.

Toposequences were selected for sampling based on presumed differences in soil chemical processes, properties related to drainage class, and other properties affected by landscape position. Chapter 2 details site selection, and soil sampling procedures. At each sampling site, three soils were selected along a landscape gradient, and samples were taken from A and B horizons. The characterization data are presented in tabular form in Appendix B. The characterization data provide the chemical background used to understand observed soil chemical processes and reactions in subsequent chapters.

Chapters 3 and 4 focus on soil reduction and oxidation experiments, and are presented in journal manuscript form. Chapter 3 was developed to address the following objectives:

- Demonstrate the extent of reduction of Cr(VI) and oxidation of Cr(III) added to whole soil samples.
- Investigate the role of the electron shuttle, AQDS, in enhancing reduction and possibly limiting oxidation of Cr.
- Elucidate the kinetics of Cr reduction and oxidation added to whole soil samples, and the role of AQDS in enhancing reduction and possibly limiting oxidation over time.

Chapter 4 builds on the previous trials and attempts to explain redox mechanisms with the following objectives:

- Use chemical means of enhancing and inhibiting biological activity in soils to demonstrate the dominance of either biological or chemical redox pathways for Cr redox reactions.
- Use kinetic reduction data sampled from increasing temperatures to corroborate or reject biological or abiotic redox mechanisms.

The final chapter provides a summary and attempt to expound on trends in the data. It discusses observed redox mechanisms, and presents possible reactions and interactions leading to the results. It presents and discusses remaining, unanswered questions, as well as future research needs.

Chapter 2: Site Selection and Soil Characterization

Introduction

For the purpose of this thesis, three site locations in Maryland with uncontaminated soils were selected for sampling: Beltsville, Wye Island, and Boyds. Each location was chosen based on chemical and pedological differences among the sites. The NRCS Web Soil Survey (WSS) (Soil Survey Staff, Accessed March, 2008a) and Official Soil Series Descriptions (OSD) (Soil Survey Staff, Accessed March, 2008b) were used to select soil locations and series for sampling. Updating the WSS is an ongoing process by the NRCS, and printed soil surveys were also referenced when information on the WSS was unavailable. Though mapping units often have soil inclusions, their descriptions are useful in drawing generalities about soils in a given location. Figures A-1, 2, and 3 in Appendix A show the locations of the series sampled at each site.

Sampling

At each site, samples were taken within a 100-m transect, and along a landscape gradient: summit, back-slope, and foot-slope. This toposequence of soils was selected because different soil characteristics were expected at each position, and variations in observed chemical behavior of soil samples may be inferred from position differences. For example, foot-slope positions would be expected to be higher in soil moisture, and correspondingly, higher in organic matter than summit

locations. Back-slope positions would be considered to have intermediate characteristics.

At each site, soil pits were dug to depths of approximately 25 to 50 cm, and samples were taken from the middle of A and B horizons. The samples were stored in plastic buckets lined with plastic garbage bags to keep in soil moisture. The sampling was done within a 3 d time period in early June, 2006 when soil water potential was at approximately "field capacity" (approximately -5 to -10 kPa). The soils were brought into the lab, passed through a 4 mm polyethylene sieve, mixed thoroughly, and stored back in their respective buckets in the dark at 24º C.

Site Descriptions

The Beltsville soils are located in Prince Georges County, MD, and are Coastal Plain soils from the Christiana, Sunnyside, and Bibb mapping units. These soils are generally regarded as high in Fe oxides, and contain kaolinitic clay. Iron oxides are redox active species that play a role in electron cycling in soils, and kaolinitic clay has low to no shrink-swell capacity. The Christiana series is located at the summit, and is described by the OSD as an Ultisol. The Sunnyside series is on the back-slope, and also is an Ultisol. The Bibb series is a wetter, foot-slope soil, and is an Entisol (Soil Survey Staff, Accessed March, 2008b).

The Wye Island soils are located in Queen Anne's County, MD, and are also Coastal Plain soils, from the Sassafras, Downer, and Elkton mapping units. These soils are low in Fe oxides, clay, and organic matter. From a redox perspective, they are expected to be less likely to reduce or oxidize Cr than the other soil toposequences. The Sassafras series is at the summit of the landscape, and is an

Ultisol. The Downer series is on the back-slope, and is an Ultisol. The Elkton series is a wetter, foot-slope soil, and is an Ultisol (Soil Survey Staff, Accessed March, 2008b).

The Boyds soils are located in Montgomery County, MD, and are Piedmont soils, from the Watchung, Jackland, and Hatboro mapping units. The soils are high in Mn oxides and smectitic clay. Manganese(III,IV)(hydr) oxides are redox active metal oxides especially important in oxidation reactions in soils. Unlike the two previous sequences, which generally progress from well-drained summit soils to poorly-drained foot-slope soils, the soils in this sequence can all be considered somewhat poorly to poorly drained, due to the high shrink-swell activity of smectitic clay. The Watchung series is at the summit, and the Jackland series is on the back-slope. Both are Alfisols. The Hatboro series is on the foot-slope, and is an Inceptisol (Soil Survey Staff, Accessed March, 2008b).

Characterization

During field sampling, horizons from which samples were taken were described for structure, color, and redox concentrations or depletions (redoximorphic features). At the Beltsville location, the Christiana pit was dug at the interface between a hardwood forested area and an agricultural field. The Sunnyside and Bibb pits were located within the forested area. On Wye Island, the Sassafras pit was along the edge of an agricultural field, the Downer pit was at the interface between the field and wooded area, and the Elkton pit was dug in the riparian zone along the water's edge. The Boyds pits were all located in a hardwood forested area within

Little Seneca Park. Tables B-1, 2, and 3 in Appendix B summarize field descriptions for the sampled series.

Before reduction and oxidation trials were begun, a survey of soil properties and characterization data was collected for each horizon of each soil series. The soils were analyzed for water content by drying at 105° C for 24 h (Gardner, 1986), and for pH by both water and salt solution (Thomas, 1996). Also performed was particle size analysis by pipette method to determine textural data for each horizon (Gee and Bauder, 1986), and LECO analysis for % C, N, and H (Nelson and Sommers, 1996). The survey of soil properties is presented in Tables B-4, 5, and 6 of Appendix B. As expected, foot-slope soils for the Beltsville and Wye Island locations were wetter than the respective summit position soils, and had more organic C. All soils can be considered acidic with pH ranging from 3.1 (Christiana Bt2 horizon) to 5.9 (Elkton A horizon).

Ammonium acetate (pH 4.8) extractions were performed to assess exchangeable metals in the soils. For the extraction, 25 mL of a 1.25 M solution was added in triplicate to the field-moist soil equivalent of 5.0 g oven-dried soil and shaken for 1 h. After centrifuging (10 min, 24° C, 10,000 x g), the supernatant liquid was analyzed for Ca, Mg, K, Fe, Mn, Al, and Cr by flame atomic absorption spectrophotometry. The data are presented in Tables B-7, 8, and 9 of Appendix B.

The Beltsville soils were higher in exchangeable Fe, the Boyds soils were higher in exchangeable Mn, and the Wye Island soils were overall lower in exchangeable metals. This survey helps validate the initial assumptions about the chemical nature of these soils. Collectively, the properties and characterization data

provide a basic chemical background to draw from when observing and identifying redox behavior and pathways in these soils.

Chapter 3: Redox Transformations of Chromium and the Role of Electron-Shuttling Quinones in Aerobic Soils

Introduction

Chromium (Cr) is a naturally-occurring, redox-active, transition metal. Though it exists in a range of oxidation states, Cr(III) and Cr(VI) are the most stable species in soils, natural waters, and living tissues. Hexavalent Cr is a concern as an environmental contaminant due to its carcinogenic properties, principally by inhalation, and due to its mobility in soils and natural waters, principally as the anionic Cr(VI) species, CrO_4^{2-} and $HCrO_4^-$. In contrast, Cr(III) in an organically-complexed form is an essential human nutrient, because of its role as a co-enzyme of insulin in the body's metabolism of sugar, protein, and fat (Agency for Toxic Substances and Disease Registry Staff, 2000).

In soils, Cr(VI) mobility can be inhibited by adsorption to positively charged colloidal surfaces in anion exchange, or in the presence of organic matter, by reduction to Cr(III)(hydr)oxides (Bartlett and Kimble, 1976; James and Bartlett, 1983b). The reduction of Cr(VI) by soil fulvic acids was demonstrated to occur more rapidly with decreasing pH, and was best described by an initial rapid reduction period followed by a lower reduction rate as Cr(VI) concentrations diminished (Kozuh et al., 2000; Wittbrodt and Palmer, 1995). Peretyazhko and Sposito (2006) demonstrated the natural reducing capacities of International Humic Substance Society (IHSS) humic acids, and also showed that microbial reduction of humic acids increased reducing capacity several-fold. Additionally, the electron-donating kinetics

of dissolved organic matter in aquatic systems was shown to occur within the course of 1 d, and was capable of shuttling electrons to SO_4^{2-} and Fe(III) (Bauer et al., 2007), converting them to $Fe(II)_{(aq)}$ and H_2S, which are electron-rich, inorganic species also effective in reducing Cr(VI) to Cr(III) (Fendorf and Li, 1996; Kim et al., 2001).

In addition to abiotic reduction pathways, microorganisms in soils play an important role in Cr(VI) reduction. The addition of reduced C compounds to a facultative anaerobic bioreactor resulted in the reduction of Cr(VI) in a contaminated soil with up to 5,100 mg Cr(VI)/kg soil (Krishna and Philip, 2005). Reduced functional groups in soil organic matter can directly reduce Cr(VI), and be a donor and acceptor for electrons in microbial respiration. Quinones, which are considered the main electron donating and accepting moieties of soil and dissolved organic matter, have been shown to also be electron acceptors in microbial respiration (Lovley et al., 1996; Scott et al., 1998). Soil bacteria were shown to reduce the humic acid analog anthraquinone-2,6-disulfonate (AQDS), which acted as a soluble electron shuttle in Fe(III) reduction (Kappler et al., 2004). The shuttling activity of AQDS was demonstrated further with *Geobacter sulfurreducens* continually shuttling electrons to ferrihydrite in solution (Straub and Schink, 2003). Though this process occurs readily in anaerobic sediments, due to the lack of electron-accepting competition from O_2, aerobic reduction of metals through shuttling also occurs. In solution, *Shewanella oneidensis* aerobically reduced Cr(VI), which was enhanced by the addition of AQDS (Lowe et al., 2003). Figure 4 illustrates the transfer of electrons from soil microorganisms to AQDS, and ultimately to Cr(VI).

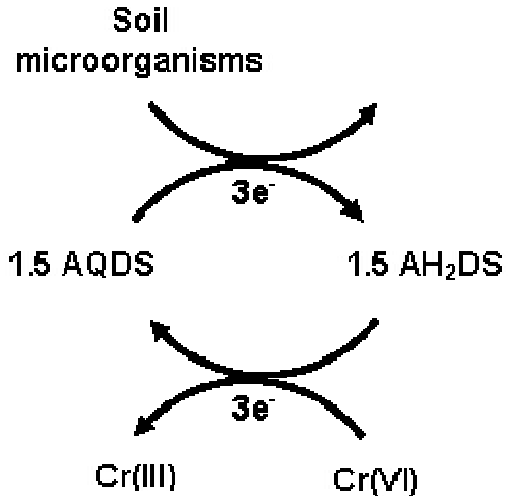


Figure 4 The stoichiometric transfer of electrons from soil microorganisms to AQDS, reducing it to the hydroquinone form AH_2DS, which ultimately reduces Cr(VI) to Cr(III).

The reduction of Cr(VI) to Cr(III) is the primary objective in remediation-by-reduction strategies, but is complicated by the potential for re-oxidation of freshly-reduced and precipitated Cr(III). At pH values greater than approximately 5.5, Cr(III) precipitates with OH- forming $Cr(OH)_{3(s)}$; however, if complexed with organic acids, such as fulvic and citric acid, Cr(III) can stay soluble at pH values up to 6.7 (James and Bartlett, 1983a). Soluble Cr(III) salts and freshly-precipitated hydroxides oxidize rapidly to Cr(VI) in the presence of Mn(III,IV)(hydr)oxides (Bartlett and James, 1979; Fendorf and Zasoski, 1992b). Chromium(III) oxidation by β-MnO_2 was demonstrated to be most rapid at pH 7 in an aerobic reactor, where the continual generation of Cr(VI), due to re-oxidation of Mn(II) to Mn(III,IV)(hydr)oxides in the presence of oxygen, resulted in the oxidation-enhanced dissolution of $Cr(OH)_{3(s)}$ (Apte et al., 2006).

Biogeochemical and redox processes in soils are in a constant, dynamic state of nonequilibrium, due to a multitude of interactions among redox-active chemical species, and due to the activity of biological mediators, especially microorganisms (Stumm and Morgan, 1996). Many studies investigating Cr redox transformations, such as oxidation by Mn(III,IV)(hydr)oxides or reduction by soil microorganisms, have been conducted under experimental conditions outside a soil environment. The objective of this study is to investigate the reduction of Cr(VI) and oxidation of Cr(III), and use of the electron shuttle AQDS, by whole soil samples in order to gain more insight into complex soil redox pathways. A better understanding of these pathways will provide a tool for further understanding electron transfer processes

under nonequilibrium soil conditions in the field, and may provide guidance in the remediation of Cr(VI)-contaminated soils (James, 1996).

Materials and Methods

Soils. Three locations in Maryland with uncontaminated soils at the edge of or within hardwood vegetation were selected for sampling based on pedological and chemical differences of interest. The Beltsville soils selected contain kaolinitic clay, are high in Fe(III)(hydr)oxides, and have up to 25 g C kg soil^{-1}. The Boyds soils contain smectitic clay, are high in Mn(III,IV)(hydr)oxides, and have up to 20 g C kg soil^{-1}. The Wye Island soils were chosen because they are sandier soils, low in metal-(hydr)oxides, and have up to 15 g C kg soil^{-1}. From a redox perspective, they are expected to be less likely to reduce or oxidize Cr than the other soil toposequences.

Three soil pits along a 100-m landscape gradient, or toposequence, were dug at each site to depths of approximately 25 to 50 cm, and samples were taken from the middle of A and B horizons, resulting in a total of 18 samples. The sampling was done within a 3 d time period in early summer when soil water potential was at approximately "field capacity" (approximately -5 to -10 kPa). The soils were brought into the lab, passed through a 4 mm polyethylene sieve, mixed thoroughly, and stored in the dark at 24º C in plastic buckets lined with plastic garbage bags to maintain soil moisture. The A and B horizons of each soil were analyzed for their chemical and physical characteristics, and collectively, provide a useful chemical and pedalogical background for these sites (Appendix B).

Batch Experiments. Field-moist samples of the 18 soils were weighed out, in triplicate in the moist equivalent of 5.0 g oven-dried soil, into 25-mL polycarbonate Oak Ridge type centrifuge tubes. To the first of four sets, 0.2 mM Cr(VI) was added as an aqueous solution of K_2CrO_4, and to another set, 1.0 mM Cr(III) was added as an aqueous solution of $Cr(NO3)_3$. Total solution volume in the centrifuge tubes was brought to 25 mL with distilled water. To the third set, 0.2 mM Cr(VI) and 10 mM anthraquinone-2,6-disulfonate (AQDS) was added. The fourth set received 1 mM Cr(III) and 10 mM AQDS. All treatments also received 0.01 M $NaNO_3$ as a background electrolyte to control for ionic strength, and all sets had controls containing the same chemical treatments with no soil added.

The soil suspensions were shaken on a horizontal shaker at 110 cycles min^{-1} for 24 ± 1 h, at which point 0.25 mL of a 1 M KH_2PO_4/K_2HPO_4 buffer solution was added, and the tubes were shaken for another 1 h to allow the phosphate buffer to displace exchangeable Cr(VI). The addition of P buffer ensures that any loss of Cr(VI) can be attributed to reduction processes, and not sorption to colloidal surfaces. All tubes were centrifuged (10 min, 10,000 x g, 24^o C), and 1 mL aliquots were taken and diluted to 10 mL. A UV-1601PC SPC Shimadzu UV-VIS spectrophotometer adjusted to a wavelength of 540 nm was used for the determination of Cr(VI) by the 1,5-diphenylcarbazide spectrophotometric method (Bartlett and James, 1979).

Two reduction trials, one for 14 d and the other for 72 h, were conducted to evaluate time effects of electron shuttling. For both trials, 2 sets of field-moist samples were weighed out, in triplicate in the moist equivalent of 5.0 g oven-dried soil, into 25-mL polycarbonate Oak Ridge type centrifuge tubes. To the first set, 0.2

mM Cr(VI) was added, to the second, 0.2 mM Cr(VI) plus 10 mM AQDS was added, and total volume in all tubes was brought to 25 mL with distilled water.

For the 14 d trial, all soils were used, and were shaken on an orbital shaker at 100 cycles min^{-1}. Sampling was performed at 1, 2, 7, and 14 d. For the 72 h trial, Christiana, Sunnyside, and Bibb A horizon samples (Beltsville toposequence) were used, and sampling was conducted at 0.25, 2.5, 24, 48, and 72 h. An orbital shaker instead of horizontal shaking was used, because the interaction between the solution and solid phase was thought to better simulate water-solid interactions in a field soil system. The addition of phosphate buffer, centrifugation, and analysis by UV-VIS spectrophotometer were performed in both time trials as previously described for the 24 h batch reduction-oxidation trials.

Results and Discussion

Reduction of Cr(VI). All A and B horizon soil samples reduced Cr(VI), and 11 of the 18 samples demonstrated a significant enhancement of reduction by the addition of AQDS (p-values < 0.05). Figure 5 illustrates percent reduction, and enhancement of reduction by AQDS, for A horizons. Figure 6 illustrates that of B horizons. Graphs are labeled as net reduction, because it is recognized that as Cr(VI) is being reduced, it is likely that oxidation of newly precipitated Cr(III) by Mn(III,IV)(hydr)oxides is occurring simultaneously within the sample.

Of the A horizon soils, Sassafras reduced 0.01 mM Cr(VI) (4.3%) without AQDS, which is the least amount of reduction observed, whereas Hatboro

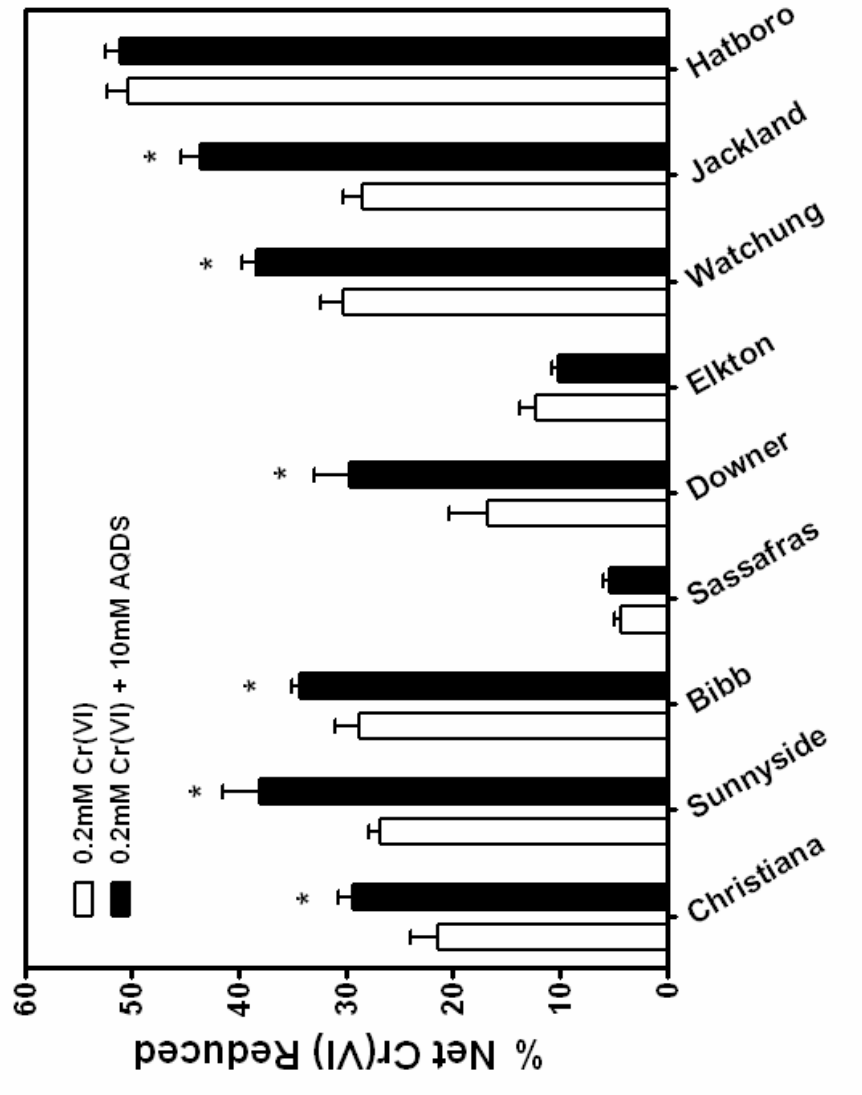


Figure 5 Reduction of 0.2 mM Cr(VI) by A horizons, with and without 10 mM AQDS. Asterisks indicate significant increase in reduction ($p < 0.05$). Bars represent averages of 3 reps ± SEM.

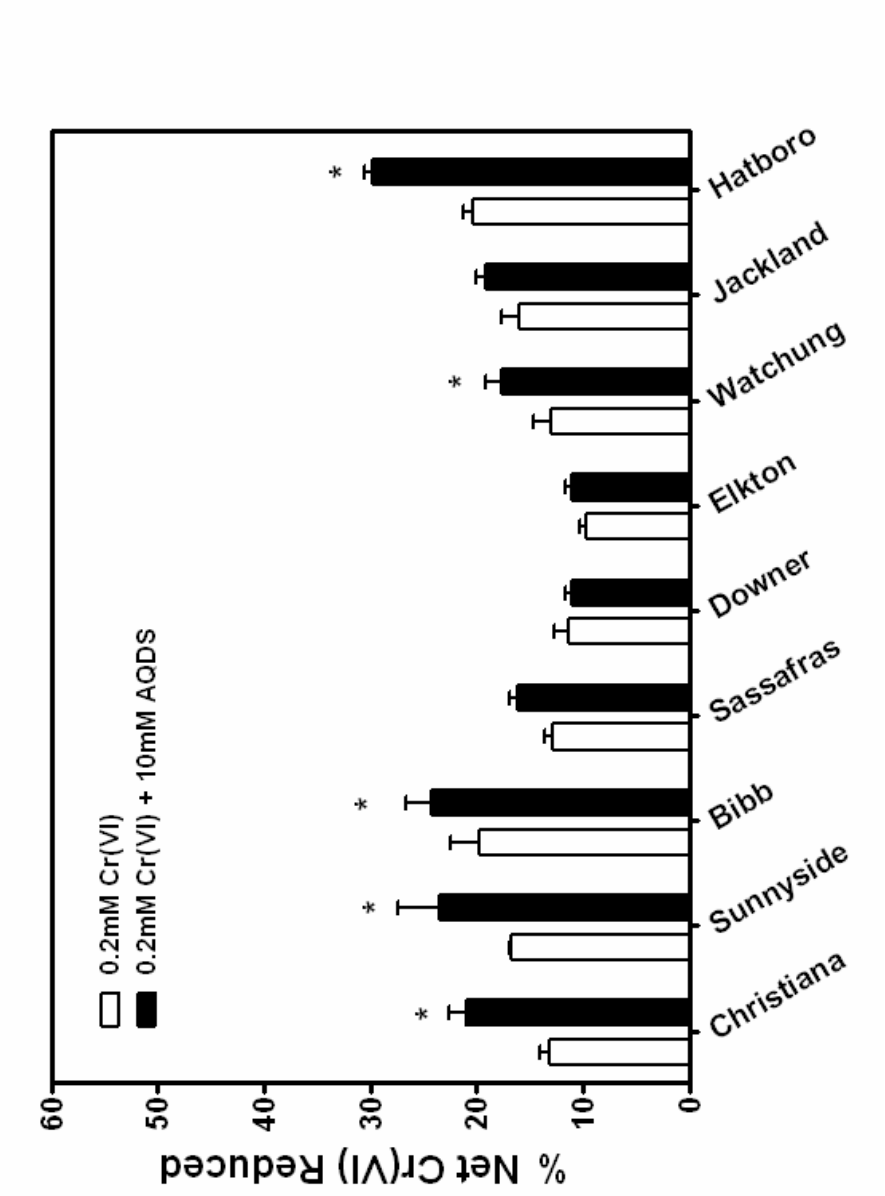


Figure 6 Reduction of 0.2 mM Cr(VI) by B horizons, with and without 10 mM AQDS. Asterisks indicate significant increase in reduction ($p < 0.05$). Bars represent averages of 3 reps ± SEM.

demonstrated the most at 0.1 mM (50%) reduction without AQDS. Jackland demonstrated the largest enhancement of reduction with a 15% increase in reduction with the addition of AQDS. Of the B horizons, Elkton reduced 0.02 mM Cr(VI) (9.3%) without AQDS, which was the least amount observed, whereas Bibb reduced the most at 0.05 mM (24%). Hatboro demonstrated the largest enhancement of reduction with a 10% increase in reduction with the addition of AQDS. Cr(VI) reduction was greatest for A horizons from the Boyds, MD site: Watchung, Jackland, and Hatboro at 0.06 mM (30%), 0.06 mM (28%), and 0.1mM (50%), respectively. Reduction in B horizons for all soils except Sassafras was lower than their respective A horizons. The Sassafras B horizon reduced 0.03 mM (13%) of Cr(VI), whereas the A horizon reduced only 0.01 mM (4.3%).

There is an evident trend in the toposequence of the Beltsville A horizon soils, where reduction increased with progression down the landscape gradient. In this series, Christiana was at the summit, which reduced 0.04 mM (21%) Cr(VI), Sunnyside along the back-slope reduced 0.05 mM (27%), and Bibb at the foot-slope (lowest position) reduced the most at 0.06 mM (29%). Greater reduction in lower position than summit soils ($p < 0.05$) is a pattern also seen in the Boyds and Wye Island toposequences. In the Boyds series, Watchung at the summit of the landscape reduced 0.06 mM (30%), and Hatboro at the lowest position reduced 0.1 mM (50%). At Wye Island, Sassafras is the summit soil, and reduced 0.01 mM (4.3%), and Elkton at the foot-slope reduced 0.02 mM (12%).

These reduction trends may be explained by reducing conditions in soil as a function of soil moisture and organic matter. Soils lower on a landscape generally are

wetter, and have more organic matter than summit soils. This is seen with the Bibb and Elkton foot-slope soils from the Beltsville and Wye Island toposequences. The Bibb soil had 24% soil moisture and 25 g C kg soil^{-1}, whereas Christiana, the summit series, had 12% soil moisture and 12 g C kg soil^{-1}. The Elkton soil from Wye Island had 20% soil moisture and 15 g C kg soil^{-1}, whereas Sassafras, the summit soil, had 7.7% soil moisture and 2.8 g C kg soil^{-1}. Higher soil moistures, sufficient levels of organic C, and the presence of microbiological activity will lower pe, making Cr(VI) reduction favorable and the enhanced reduction by electron shuttles possible.

A correlation analysis between g C kg soil^{-1} and soil moisture for all 9 A horizons indicates a significant, positive relationship ($p < 0.05$, $r^2 = 0.71$). There is also a significant, but not as strong, positive relationship between Cr(VI) reduction and g C kg soil^{-1} ($p < 0.05$, $r^2 = 0.45$) Clearly, there are other biological and chemical factors needed to fully explain Cr(VI) reduction in these soils, however, these data help corroborate the trends observed that wetter soils at lower positions on the landscape are more reducing than summit soils. As mentioned earlier, these results are net reduction, as it is expected that newly precipitated Cr(III)(hydr)oxides are able to be oxidized by naturally occurring Mn(III,IV)(hydr)oxides in soil.

Oxidation of Cr(III). All A horizon samples demonstrated oxidation of Cr(III) to Cr(VI). Figure 7 illustrates oxidation by A horizons, and Figure 8 shows oxidation by B horizons. The Christiana A horizon oxidized 0.08 mM (7.6%), Sunnyside A horizon oxidized 0.09 mM (9.3%), and Bibb A horizon oxidized 0.03 mM (2.6%) Cr(III). The Watchung and Jackland were the strongest oxidizers of all A horizons, oxidizing 0.17 (18%) and 0.12 mM (12%) Cr(III), respectively. Of the B

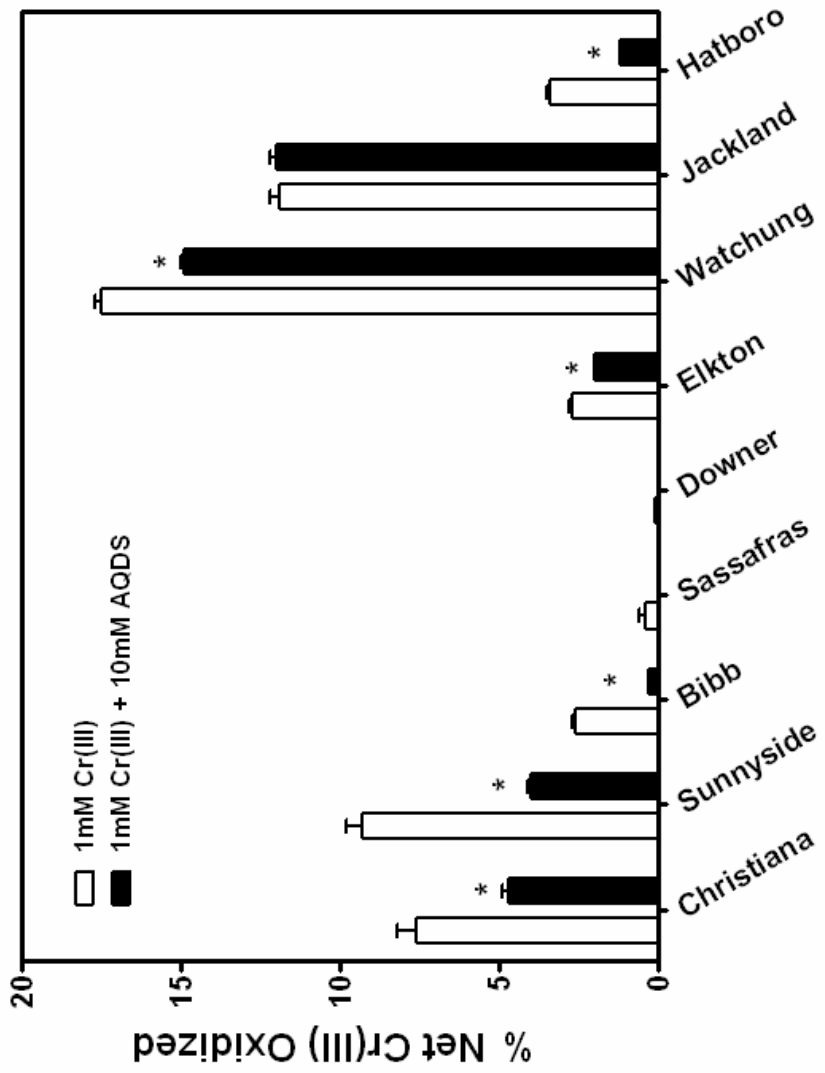


Figure 7 Oxidation of 1.0 mM Cr(III) by A horizons, with and without 10 mM AQDS. Asterisks indicate significant decrease in oxidation ($p < 0.05$). Bars represent averages of 3 reps ± SEM.

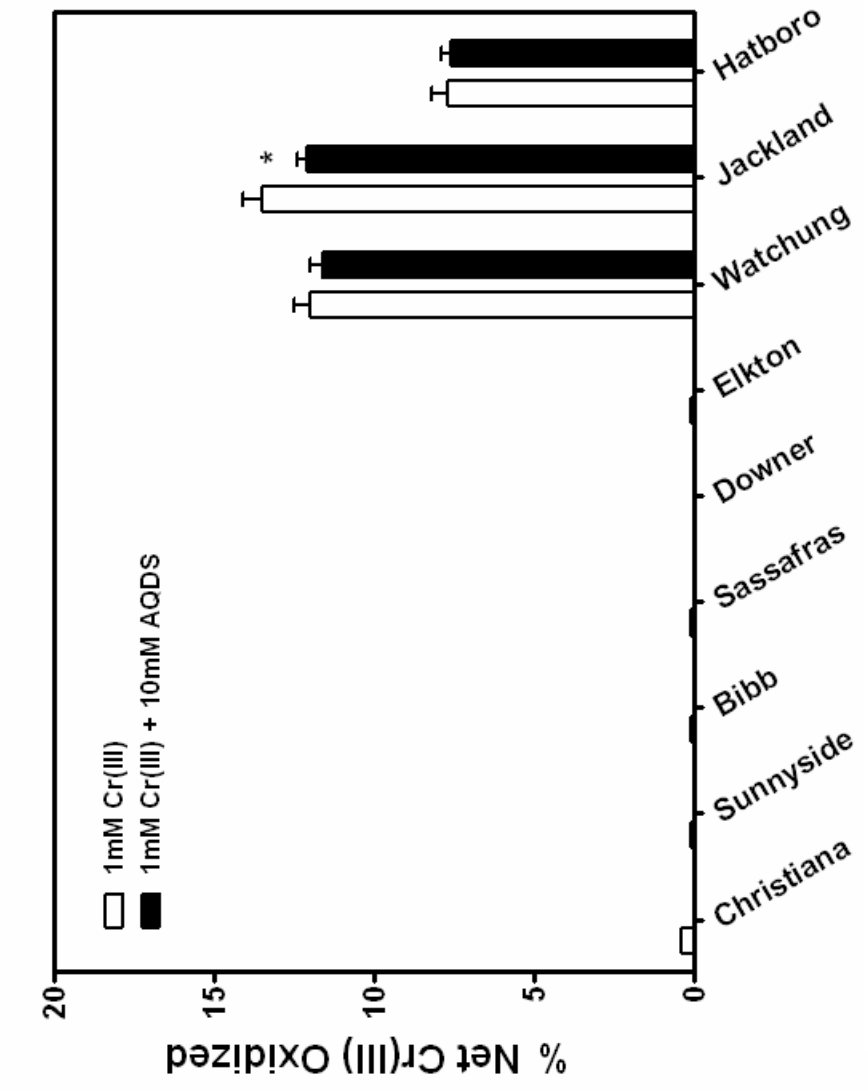


Figure 8 Oxidation of 1.0 mM Cr(III) by B horizons, with and without 10 mM AQDS. Asterisks indicate significant decrease in oxidation ($p < 0.05$). Bars represent averages of 3 reps ± SEM.

horizons, only the Watchung, Jackcland, and Hatboro (Boyds toposequence) oxidized more than 0.01 mM (1%) Cr(III), at 0.12 mM (12%), 0.14 mM (14%), and 0.08 mM (7.7%) Cr(III), respectively. In 7 of the 18 samples, oxidation was significantly reduced by the addition of the electron shuttle AQDS ($p < 0.05$). As with the reduction trial, the soil's position on the landscape seems to play a role in the oxidation of Cr(III). Oxidation by the Christiana and Watchung summit soils was greater than oxidation by their respective foot-slope soils, Bibb and Hatboro.

The soil constituents and reactions involved in oxidation and electron shuttling processes in these soils are elusive. There was no relationship found using correlation analysis between Cr(III) oxidized and exchangeable Mn, soil moisture, or g C kg soil^{-1}. The trial was run under aerobic conditions, and Fe was assumed to be present as insoluble Fe(III,IV)(hydr)oxides and not reactive towards Cr. In comparing Beltsville and Boyds toposequence data for reduction (Figure 5) with those for oxidation (Figure 7), it appears that in soils where reduction is greatest, oxidation occurs the least. This supports the presentation of data as net reduction and oxidation, due to simultaneous redox processes occurring in samples. However, using correlation analysis, there was no significant relationship found between Cr(VI) reduction and Cr(III) oxidation. .

The addition of AQDS enhanced reduction in both reduction and oxidation trials, indicating that shuttling processes favor Cr reduction and not oxidation. However, there was not a significant relationship between % enhanced reduction and soil moisture, g C kg soil^{-1}, or exchangeable Mn. There also does not appear to be a trend with shuttling in landscape positions. Of all 18 soils in the study, 11 were

capable of utilizing the electron shuttle, but no one landscape position consistently utilized shuttling more than another, and it is currently unclear what mechanisms, biological or chemical, are being used in electron transfers from soil to AQDS.

Reduction with time. Reduction by Christiana, Sunnyside, and Bibb A horizons continued for the 14 d period, but for B horizons, it appeared to level off after a 2 d initial reduction period. Figure 9 illustrates reduction by Christiana, Sunnyside, and Bibb A horizons (Beltsville toposequence), and Figure 10 illustrates reduction by B horizons. Data for the other two toposequences show similar trends for A and B horizons, and are presented in Appendix C. At 1 d, Christiana A horizon reduced 0.04 mM (20%) Cr(VI), Sunnyside reduced 0.05 mM (25%), and Bibb reduced 0.05 mM (25%). These values correspond well with A horizon values in the batch reduction study shown in Figure 5. By 14 d, Christiana A horizon reduced 0.14 mM (69%) Cr(VI), Sunnyside reduced 0.15 mM (76%), and Bibb reduced 0.15 mM (74%). There was a significant increase in reduction at 1 d with the addition of AQDS for the Christiana A and B, Sunnyside B, and Bibb B horizons ($p < 0.05$). Of these soils, only the Sunnyside B horizon sustained a significant enhancement in reduction for 14 d, whereas enhancement diminished to no appreciable difference for the other soils.

The reduction curves for these soils seem to follow a first-order reaction for the first 72 h, and then level off for the remainder of the study. This has been previously described and attributed to electron-rich functional groups of soil organic matter, and particularly to fulvic and humic acids, being utilized rapidly, and then

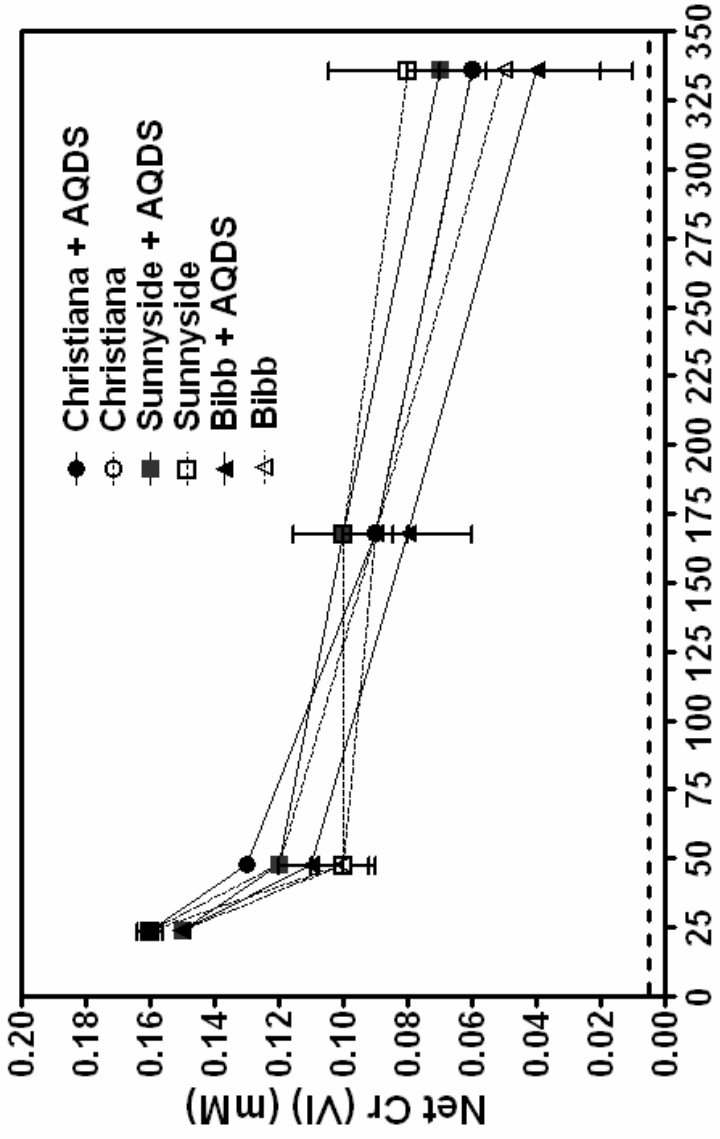


Figure 9 Reduction of 0.2 mM Cr(VI) for 14 days by Beltsville toposequence A horizons, with and without 10 mM AQDS. There was no significant enhancement of reduction after 14 days. Data points represent averages of 3 reps ± SEM.

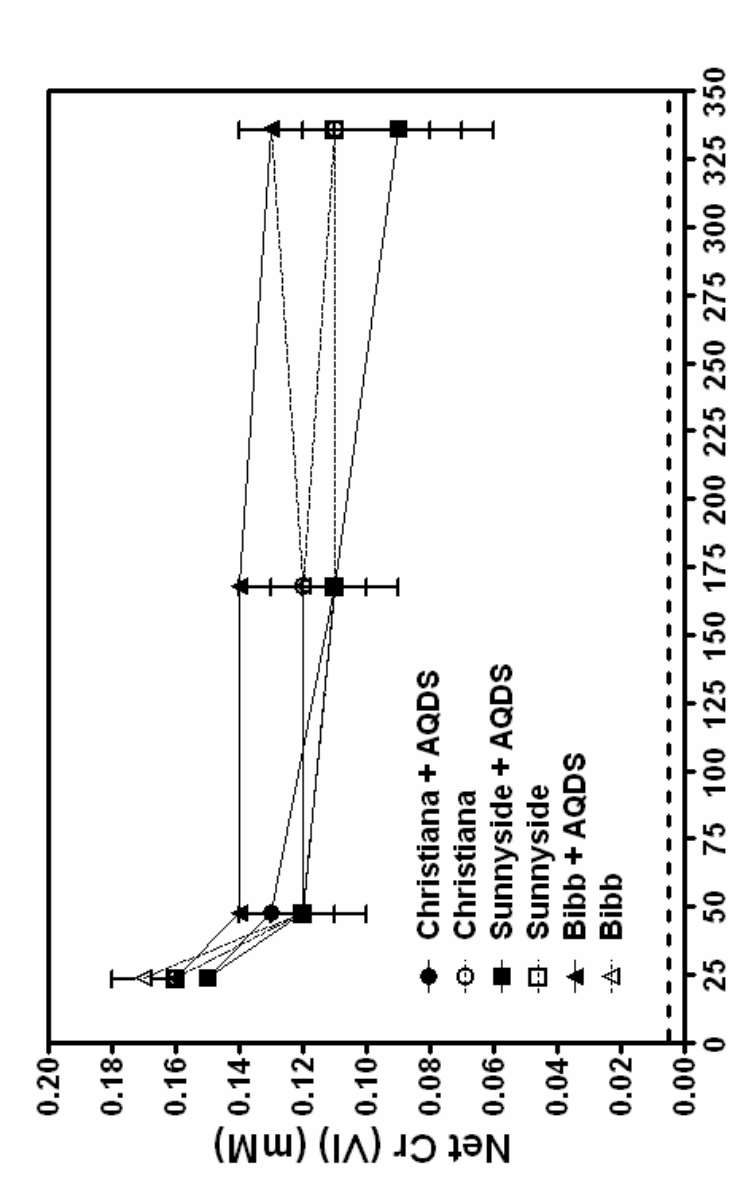


Figure 10 Reduction of 0.2 mM Cr(VI) for 14 days by Beltsville toposequence B horizons, with and without 10 mM AQDS. There was significant Enhancement of reduction ($p < 0.05$) at 14 days for only the Sunnyside soil. Data points represent averages of 3 reps ± SEM.

kinetically slower functional groups donating electrons for the remainder of the time (Kozuh et al., 2000; Wittbrodt and Palmer, 1995). The abiotic reduction of Cr(VI) in this study is attributed to these organic functional groups, because the samples were maintained aerobically for the 14 d period and any Fe or S species would be expected to be in an oxidized form and unable to contribute electrons to Cr.

Because of the kinetically more rapid reduction period in the first few days, the second time trial focused on the 72 h time period. The Christiana, Sunnyside, and Bibb A horizons demonstrated first-order reduction rates, and also for the Christiana and Sunnyside soils, a significant increase in the rate constant with the addition of AQDS. Rate constants were 0.017 h^{-1} for Christiana, 0.019 h^{-1} for Sunnyside, and 0.026 h^{-1} for Bibb. As seen in Table 3, rate constants significantly increased ($p < 0.05$) 71% and 105% with the addition of AQDS for the Christiana and Sunnyside soils, respectively in the initial 72 h of reduction. These results compliment the reduction-oxidation batch trials, demonstrating increased reduction from the Christiana summit, to the Sunnyside, to the Bibb foot-slope soil; it is clear the rate constants for these soils follow this same pattern.

Environmental Significance. The simultaneous reduction and oxidation processes observed in these soils are indicative of the non-equilibrium nature of redox pathways in natural systems. Within the same Bibb A horizon, 0.03 mM of Cr(III) was oxidized to Cr(VI), yet 0.06 mM Cr(VI) was reduced, and 0.07 mM was reduced with the addition of AQDS. Over the course of 14 d, the Bibb A horizon soil reduced 0.15 mM (74%) Cr(VI) in solution. In the pH range of these soils (4-5), Mn(III) is a power oxidant, and is the only natural oxidant of Cr(III) (Bartlett and James, 1993).

Soil	k_1	k_{2AQDS}	Half-life$_1$	Half-life$_{2AQDS}$
Christiana	0.017	0.029*	40.8	23.9*
Sunnyside	0.019	0.039*	36.5	17.8*
Bibb	0.026	0.032	26.7	21.7

Table 3 Rate constants (hr^{-1}) and half-lives (hr), without and with AQDS, for initial 72 hrs in Beltsville A horizon soils. Asterisks indicate significant differences ($p < 0.05$).

These reduction and oxidation trials show that although oxidation and reduction occur simultaneously, with time, reduction pathways prevail. Also, it is clear A horizons are more reactive towards reduction and oxidation transformations of Cr than the respective B horizons. Exchangeable Mn and C are higher in A horizons than B horizons, and as seen with correlation analysis, reduction can be explained in part by the presence of organic C.

The initial use of the electron shuttle AQDS, but diminishing enhancement of reduction with time, is likely due to the chemical reduction of the quinone structure to hydroquinone, or the intermediate radical semiquinone, by electron-rich functional groups in soil organic matter (Bartlett and James, 1993; Scott et al., 1998). Because the soils were used in field moist, aerobic conditions, thermodynamically, it would be expected that Fe would be present as Fe(III)(hydr)oxides), and would not contribute to the reduction of AQDS. The Jackland A horizon demonstrated a 53% enhancement in reduction with the addition of AQDS, which was the greatest of all 18 samples. However, assuming a stoichiometric relationship between AH_2DS and Cr(VI), this enhancement only utilized 0.2% of available reducing equivalents of AQDS.

Though soil microorganisms are capable of reducing AQDS, in microbiological studies, there is often a lag time greater than 1 d for the reduction of electron acceptors or consumption of substrates. The rapid, initial use of AQDS in Cr(VI) reduction in these trials, and the lack of sustained reduction of AQDS, suggests a chemical pathway, and not a biological one, as the dominant reduction mechanism. It is possible the lack of sustained AQDS use could be due to a lack of

nutrients or electron donors for soil microorganisms, or possibly to Cr toxicity, however, Cr(VI)-reducing soil bacteria have exhibited a tolerance up to 5 mM Cr(VI) (Guha et al., 2001). Further work is necessary in order to understand what limits the cycling of AQDS in these soils, and to better understand whether Cr reduction and electron shuttling pathways are chemical or biological.

Chapter 4: Toward a Better Understanding of the Linkages between the Chemical and Microbiological Reduction Pathways of Hexavalent Chromium in Aerobic Soils

Introduction

Chromium (Cr) is a naturally-occurring, redox active transition metal, but as the anionic species, CrO_4^{2-} and $HCrO_4^-$, it is a contaminant of concern because of its carcinogenic properties and mobility in soils and natural waters. Cr(III), however, is an essential nutrient with a recommended daily dose of 50-200 µg for adults, because of its role in the body's metabolism of sugar, protein, and fat (Agency for Toxic Substances and Disease Registry Staff, 2000). Remediation of Cr(VI) by reduction can be accomplished by either biological or chemical methods, though natural reduction processes in soil may be a combination of both pathways.

Chromium exists in soils as $HCrO_4^-$, which can be tightly held by positively-charged colloidal surfaces, or in the presence of soil organic matter, can be reduced and precipitated as Cr(III)(hydr)oxides (Bartlett and Kimble, 1976; James and Bartlett, 1983b). Reduction is accomplished by electron-rich phenolic groups in soil organic matter, which can also be further oxidized by the enzymes phenolase and laccase to produce quinones, considered to be the major electron donor and acceptor moieties of humic material (Tan, 2003).

Conversely to this oxidation, an one electron transfer to a quinone forms the highly reactive intermediate semiquinone, and a second electron transfer forms hydroquinone (Larson, 1997). Hydroquinone is capable of donating its two electrons

in further reduction reactions, making the quinone-hydroquinone couple a very dynamic redox constituent in soils and natural waters. Even under aerobic conditions, humic acids express significant reducing capacity, which is further enhanced when humic acids are first fully reduced by microbial reduction (Peretyazhko and Sposito, 2006).

Soil microbial metabolism also draws from the electron-rich nature of soil organic matter. Reduced C compounds added to a facultative anaerobic bioreactor resulted in the reduction of contaminated soil with up to 5,100 mg Cr(VI)/kg soil (Krishna and Philip, 2005). Chromium reduction by microorganisms is accomplished either intracellularly and possibly coupled to metabolic processes, or extracellularly by non-metabolic processes as mediated by cell exudates on the cell wall surface. Chromium (VI) is an oxyanion, and like other anions, such as SO_4^{2-}, it can pass through surface anion transport systems in cellular membranes (Cervantes et al., 2001). Once inside the cell, Cr(VI) is reduced to its lower oxidation states Cr(V, IV, III) by compounds such as reduced nicotinamide adenine dinucleotide (NADH) and ascorbic acid.

Extracellular reduction is accomplished by soluble, membrane-bound proteins that reduce Cr(VI) on the cell surface, forming insoluble reduction products, so that the metal never enters the cell. The cell wall of *Arthrobacter oxydans* contains an acid-soluble protein with a positive charge, as shown by electrophoresis, capable of reducing Cr(VI) to an insoluble Cr(III)(hydr)oxide which accumulates on the bacterial surface (Asatiani et al., 2004). Soil microorganisms have been shown to

reduce metals directly in soils, and to utilize soluble, humic acids as electron shuttles for metal reduction.

Soil bacteria were shown to reduce the humic acid analog anthraquinone-2,6-disulfonate (AQDS), which then acted as an electron shuttle in solution to reduce Fe(III) (Kappler et al., 2004). The shuttling activity of AQDS was demonstrated further with *Geobacter sulfurreducens* continually shuttling electrons to ferrihydrite in solution (Straub and Schink, 2003). Though this process occurs readily in anaerobic sediments, due to the lack of electron scavenging competition from O_2, the aerobic reduction of metals through shuttling processes also occurs. In solution, *Shewanella oneidensis* aerobically reduced Cr(VI), which was enhanced by the addition of the electron shuttle AQDS (Lowe et al., 2003). Many studies demonstrating Cr(VI) reduction by abiotic and biological agents are often conducted in solution without whole soil samples. The purpose of this study is to demonstrate Cr reduction and oxidation by whole soil samples, and to elucidate the dominance of either chemical or biological redox pathways.

Materials and Methods

Soil. An A horizon sample of the Watchung soil series: fine, smectitic, mesic Typic Albaqualfs (Soil Survey Staff, Accessed March, 2008b) was selected, because its chemical characteristics make the soil a potentially strong reducing and oxidizing soil. The soil contains 20.2 g C kg soil^{-1}, and 18 mg exchangeable Mn kg soil^{-1}, as determined by a 1 h 1.25 M ammonium acetate extraction (Appendix B). In a previous reduction trial, a Watchung soil sample was shaken with 0.2 mM Cr(VI) for

24 h, and reduced 30% of the Cr, with an additional 8.5% reduced with the electron shuttle AQDS. In an oxidation trial, the Watchung soil was shaken with 1 mM Cr(III) for 24 h and oxidized 17.5% of the Cr, but oxidized only 14.9% with AQDS. Sampling of the Watchung soil in the field was conducted in early summer when soil water potential was at approximately "field capacity" (approximately -5 to -10 kPa). The soil was brought into the lab, passed through a 4 mm polyethylene sieve, mixed thoroughly, and stored in the dark at 24º C in plastic buckets lined with plastic garbage bags to keep in soil moisture.

Batch Experiments. Field-moist samples were weighed out equivalent to 5.0 g oven-dried soil into 2 sets of 25-mL polycarbonate Oak Ridge type centrifuge tubes. In the first set, 0.2 mM Cr(VI) was added to the soils as an aqueous solution of K_2CrO_4. Four treatments, designed to enhance or inhibit microbial processes, were then added in triplicate to the Cr(VI) and soil samples: 1) 15 mM lactate to serve as a C and electron source, 2) 15 mM lactate and 10 mM anthraquinone-2,6-disulfonate (AQDS) to serve as an electron shuttle, 3) 15 mM lactate, 10 mM AQDS, and 0.1 M NaCl to create an osmotic potential equivalent to 300 bars, which effectively inhibits microbial metabolism, and 4) no additions. The control (no soil) received 15 mM lactate, 10 mM AQDS, and 0.1 M NaCl. In the second set, these same treatments and control were repeated using 0.2 mM Cr(III), added as an aqueous solution of $Cr(NO_3)_3$.

Total solution volume in all centrifuge tubes was brought to 25 mL with distilled water. Samples were shaken on a horizontal shaker at 110 cycles min^{-1} for 14 d, and sampled at 1, 3, 6, 11, and 14 d. Solution pH and Eh was measured

potentiometrically at each sampling time, 0.25 mL of a 1 M KH_2PO_4/K_2HPO_4 buffer solution was added, and samples were shaken an additional 1 h. The addition of phosphate effectively displaces Cr(VI) from anion exchange sites so that loss in Cr(VI) can be attributed to reduction processes and not sorption. All tubes were centrifuged (10 min, 10,000 x g, 24^o C), and 1 mL aliquots were taken and diluted to 10 mL. A UV-1601PC SPC Shimadzu UV-VIS spectrophotometer adjusted to a wavelength of 540 nm was used for the determination of Cr(VI) by the 1,5-diphenylcarbazide spectrophotometric method (Bartlett and James, 1979). Total Fe and Mn in solution were measured with a Perkin Elmer400 flame atomic absorption spectrophotometer.

A second trial to evaluate reduction kinetics at different temperatures was conducted to further distinguish biological from chemical reduction pathways. Field-moist samples were weighed out equivalent to 5.0 g oven-dried soil into 5 sets of 25-mL polycarbonate Oak Ridge type centrifuge tubes. To each set, 0.2 mM Cr(VI) was added in triplicate, and controls with no soil were included. The 5 sets were shaken on orbital shakers at 100 cyles min-1 in separate incubators at 5, 10, 22, 35, and 40^o C, and were sampled at 1, 3, 5, 10, and 14 d. The addition of phosphate buffer, centrifugation, and analysis by UV-VIS spectrophotometer were performed as previously described in batch experiments.

Results and Discussion

The reduction of Cr(VI) by the Watchung A horizon is illustrated in Figure 11. The sample with soil and no amendments reduced 0.15 mM (74%) Cr(VI) over

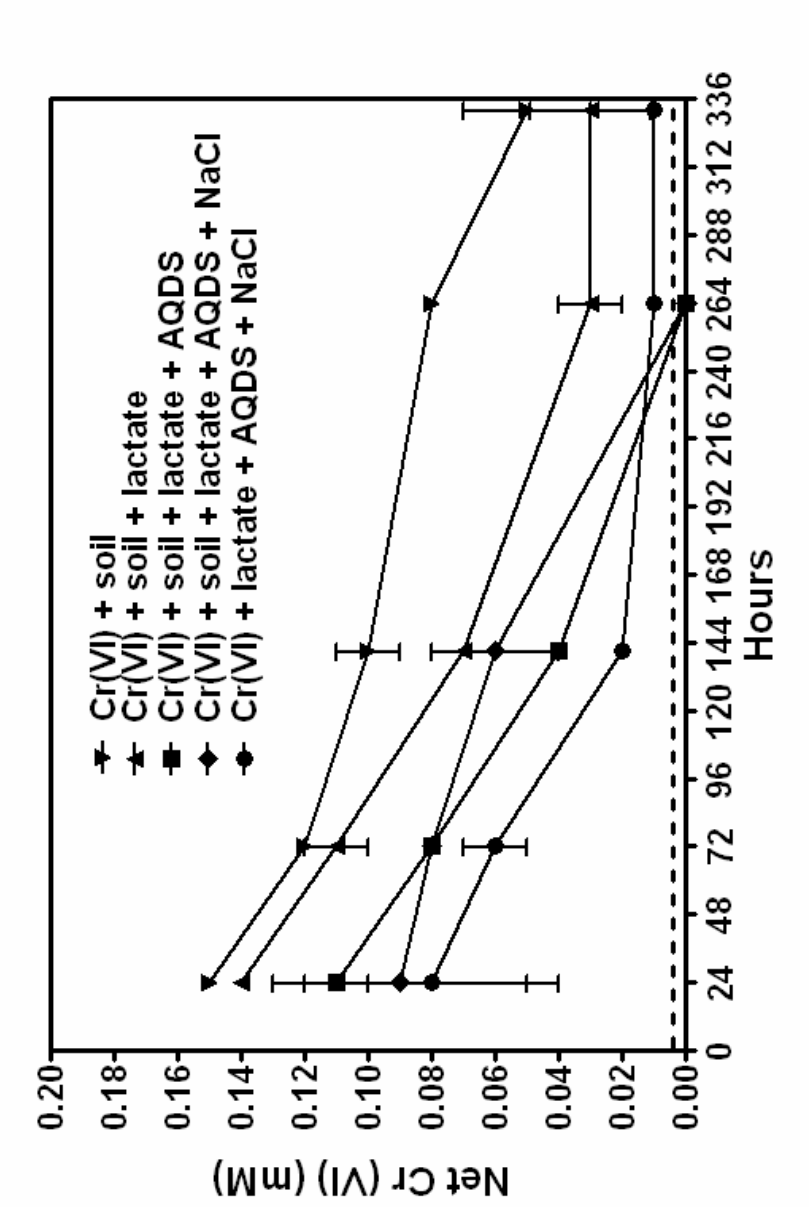


Figure 11 Reduction of 0.2mM Cr(VI) in Watchung A horizon, and enhanced reduction by addition of 15mM lactate and 10mM AQDS, over 14 days. Data points represent averages of 3 reps ± SEM.

14 d. The addition of lactate to the soil, added to stimulate biological activity, increased reduction significantly ($p < 0.05$) to 0.17 mM (85%). The addition of the electron shuttle AQDS to lactate and soil significantly increased reduction to levels below the detection limit by 11 d. This treatment, however, was not significantly different from that which included lactate, AQDS, and 0.1M NaCl, added to inhibit microbial metabolism. This corresponding trend in reduction between these two treatments show that a high salt concentration did not inhibit reduction, suggesting microbial metabolism was not a dominant reduction pathway in this soil, and additionally, electron shuttling by AQDS can occur via chemical pathways.

Bacteria in marine environments are tolerant to salt concentrations, but those that exist in fresh water and upland soils are not. The addition of the high salt concentration in this treatment created a 1% saline environment, which is approximately equal to an osmotic pressure of 300 bars (Brock, 1978). If this environment did not kill bacteria in the soil sample, it would be expected their metabolism would be inhibited. It is further expected that any residual metabolic activity due to bacteria isolated in microenvironments, and not exposed to the high salt conditions, would not be great enough to influence the Cr chemistry observed in these trials. Soil sterilization was explored as an option; however, chemical treatments can leave residues that may interfere with analysis and Mn(III,IV) and Fe(III) (hydr)oxides can be reduced, autoclaving alters soil structure and surface area of clays, and irradiation can create hydroxyl radicals in the soil (Trevors, 1996). A concentrated salt environment ultimately seemed to be the method that would least alter the redox condition of the soil, and effectively inhibit microbiological effects.

The second reduction trial to further explore biological effects demonstrated an increase in reduction rates with increasing temperature. The rates in reduction would be expected to peak, and then decrease at higher temperatures when dominated by biological pathways, whereas the rates would continue to increase linearly with abiotic reduction (Brock, 1978). Figure 12 illustrates a curvilinear relationship between first-order rate coefficients (hr^{-1}) and increasing temperature. This trend of increasing rate coefficients further suggests that abiotic pathways are dominant in the reduction of Cr(VI) in this soil.

In the first reduction trial, the control treatment comprising Cr(VI), lactate, AQDS, and the high salt concentration, but no soil, unexpectedly reduced 0.19 mM (97%) Cr(VI) by 11 d, which was not significantly different than the same treatment with soil. Lactate is often used as a C and electron donor in microbiological studies with metals, because it is not known to reduce or chelate metals. Another trial was run to investigate why reduction occurred in the control treatment. In triplicate, 1.5, 15, 30, and 60 mM lactate was added to 0.2 mM Cr(VI) and 10 mM AQDS. A control treatment with 15 mM lactate and 0.2 mM Cr(VI) was included, and samples were taken at 1, 8, 24, and 48 h. Figure 13 illustrates the reduction of Cr(VI) by lactate via electron shuttling.

There was no reduction at 48 h in the control containing lactate and Cr, but reduction did occur in samples with lactate, Cr, and AQDS. The reduction of Cr(VI) was rapid, and increased with increasing lactate concentrations. The highest concentration of lactate (60 mM) reduced Cr below levels of detection within 1 h, and although 30 mM reduced 0.18 mM (91%) in the initial 1 h, it took until 48 h to

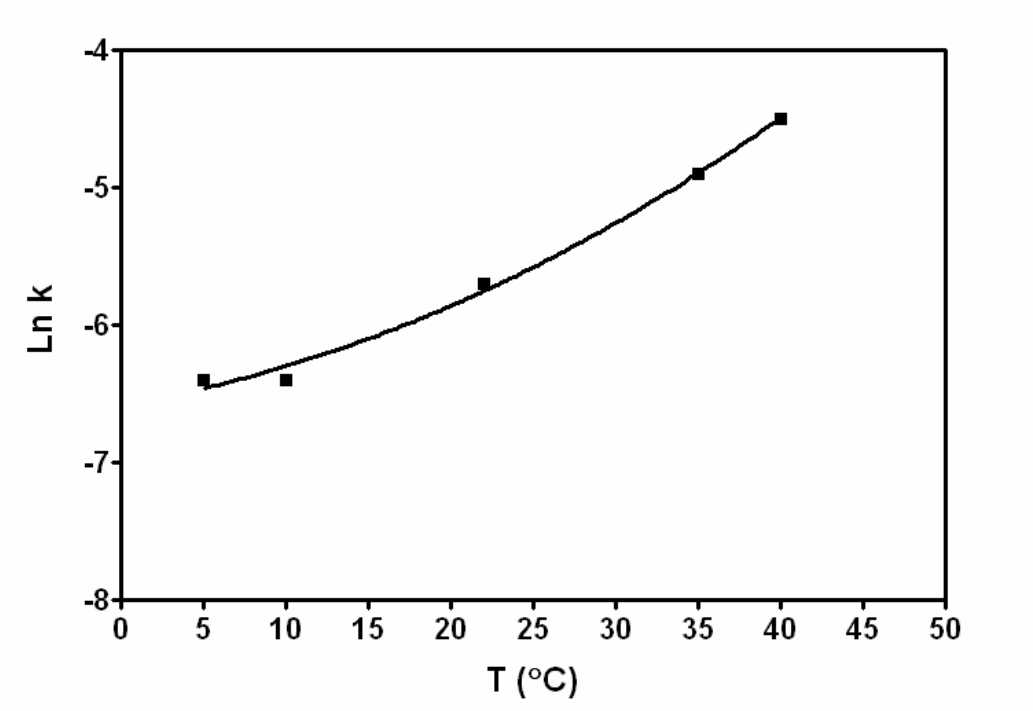


Figure 12 The natural log of rate constants (hr^{-1}) as a function of temperature in the first-order reduction of 0.2mM Cr(VI) over 14 d in Watchung A horizon.

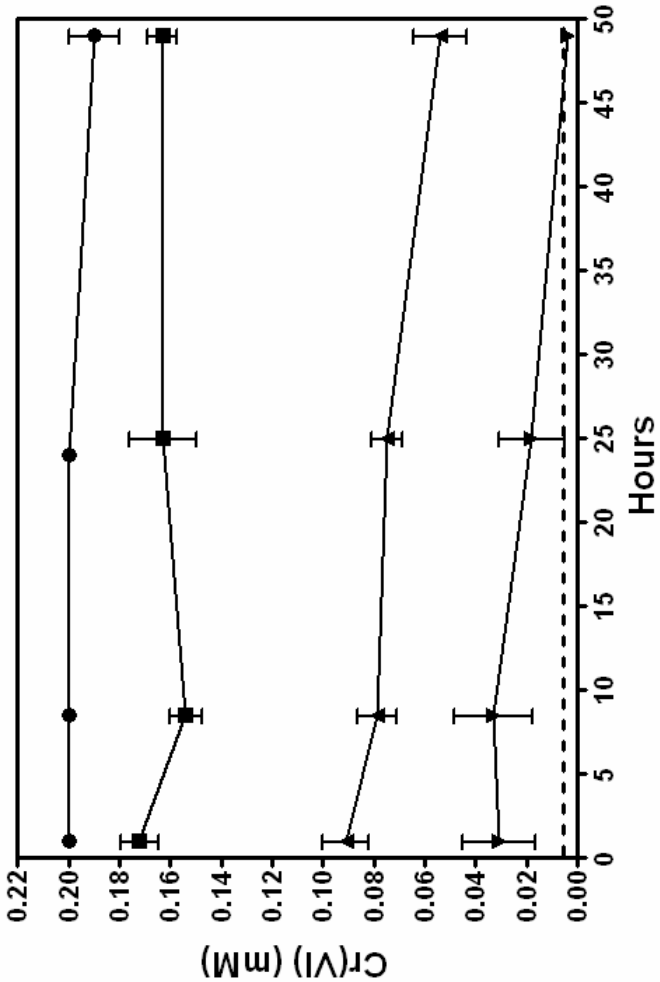


Figure 13 Reduction of 0.2mM Cr(VI) by lactate via 10 mM AQDS over 48 h. ● = control (no AQDS), ■ = 1.5 mM lactate, ▲ = 15mM lactate, and ▼ = 30 mM lactate. The treatment with 60 mM lactate was below level of detection by 1 h. Data points represent averages of 3 reps ± SEM. The dashed line indicates level of detection for Cr.

achieve reduction below the detection limit. The last two samples with 1.5 and 15 mM lactate reduced 0.04 mM (18%) and 0.15 mM (73%) Cr(VI), respectively. It is apparent that AQDS is able to kinetically couple the reduction of Cr by lactate, whereas it otherwise would not occur.

Equation 4 illustrates the stoichiometric transfer of electrons from lactate to AQDS, reducing it to AH_2DS. Equation 5 shows the reduction of Cr(VI) to a Cr(III)(hydr)oxide by AH_2DS, which is re-oxidized back to AQDS, and equation 6 combines equations 4 and 5 into the full reduction of Cr(VI) by lactate via AQDS.

$$AQDS + 2Lactate \rightarrow AH_2DS + 2Pyruvate \quad (4)$$

$$HCrO_4^- + 1.5AH_2DS + H^+ \rightarrow Cr(OH)_3 + 1.5AQDS + H_2O \quad (5)$$

$$HCrO_4^- + 1.5AQDS + 3Lactate + H^+ \rightarrow Cr(OH)_3 + 1.5AQDS + 3Pyruvate + H_2O \quad (6)$$

The addition of lactate to the sample containing soil and no other amendments enhanced reduction 10%. As previously demonstrated, biological pathways do not appear to be contributing to Cr(VI) reduction, and using eq 6 as a model, enhanced reduction can be attributed to the reduction of quinone moieties in the soil, which ultimately reduced Cr(VI). It has been demonstrated that quinone moieties in humic acids contribute up to 79% of the total electron-carrying capacity, as defined by the reduction of Fe(III)-citrate under anaerobic conditions (Ratasuk and Nanny, 2007).

Equations 5 and 6 show the consumption of protons in the reduction of Cr(VI) to a Cr(III)(hydr)oxide. This is corroborated in Figure 14, which illustrates a pH increase for all treatments over 14 d. The addition of lactate and AQDS to the soil

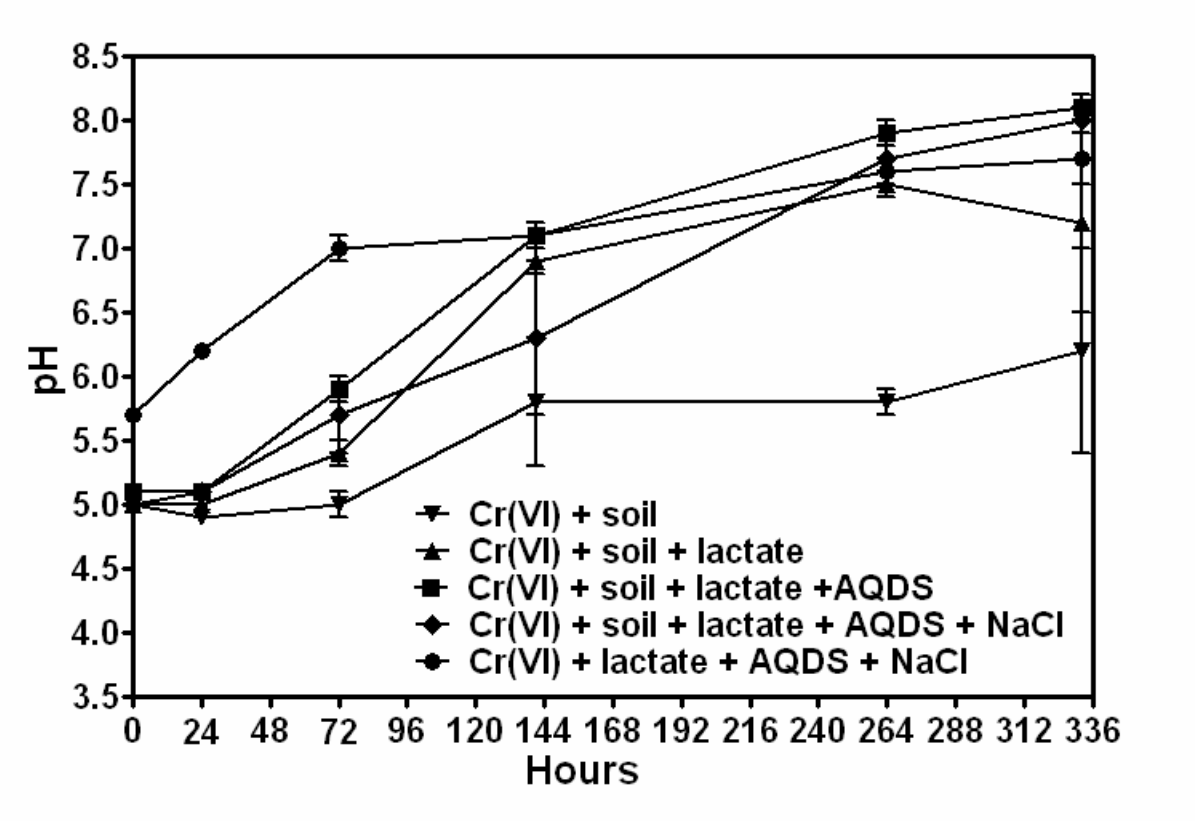


Figure 14 Increase in pH over 14 day reduction of 0.2mM Cr(VI) by Watchung A horizon, and enhanced reduction by addition of 15mM lactate and 10mM AQDS. Data points represent averages of 3 reps ± SEM.

demonstrated the most reduction, and consequently, the greatest increase in pH. At 14 d, this treatment reached a pH of 8.1, whereas the pH in the soil with no amendments increased to 6.2. The initial pH for both samples was 5.0, and because the reduction of AQDS by lactate does not consume protons (eq 4), this greater increase in pH can be partly attributed to the reduction of Cr(VI) to a Cr(III)(hydr)oxide. However, the magnitude of the increase in pH for the lactate and AQDS treatment is considerable, even with complete Cr reduction. Figure 15 demonstrates that additional proton consumption may be attributed to the simultaneous reduction of Mn(III,IV)(hydr)oxides by AH_2DS.

Manganese (II) levels in solution at 14 d for the sample with soil and no amendments were at 4.0×10^{-3} mM, but the addition of lactate and AQDS increased soluble Mn(II) levels to 0.47 mM. Equation 7 illustrates the reduction of an insoluble Mn(III)(hydr)oxide into soluble Mn(II) by AH_2DS, consuming 2 protons.

$$MnOOH + 1/2AH_2DS + 2H^+ \rightarrow Mn^{2+} + 1/2AQDS + 2H_2O \quad (7)$$

The presence of soluble Mn species indicates that a competition exists between Cr(VI) and Mn(III,IV) for electrons carried by AQDS. This competition may hinder the potential for Mn(III,IV)(hydr)oxides in soils to oxidize Cr(III). In the oxidation trial using the same treatments as the reduction trial, the sample with soil and no amendments oxidized 0.01 mM (5%) Cr(III), whereas there was no observable oxidation in other samples. Soluble Mn values in the oxidation trial (data not shown)

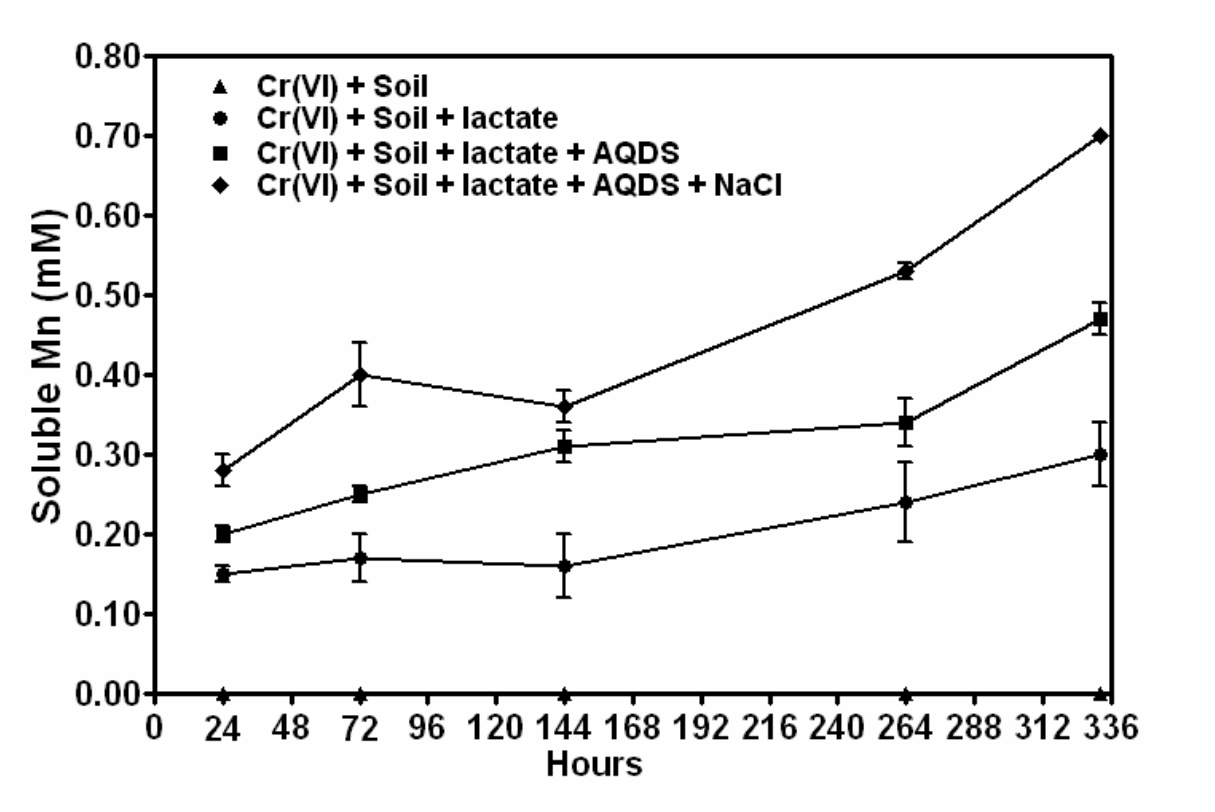


Figure 15 Increase in soluble Mn over 14 d reduction of 0.2 mM Cr(VI) by Watchung A horizon. Treatment with soil and no amendments was just above level of detection, and lies on x-axis. Data points represent averages of 3 reps ± SEM.

closely matched those in the reduction trial illustrated in Figure 15. This suggests that the reduction of Mn(III,IV) may have made it unavailable to oxidize Cr(III), and that any Cr(III) that was oxidized to Cr(VI) was likely rapidly reduced back to Cr(III). These relationships can be further explored with thermodynamic data as presented in Table 4. The reduction of MnOOH by AH_2DS has a ΔG_r of-122.8 kJ/eq, which is more favorable than the reduction of Cr(VI) by AH_2DS at -85.7 kJ/eq. The oxidation of Cr(III) by MnOOH is less favorable than both of these reduction reactions with a ΔG_r of -37.1 kJ/eq. This helps to explain the presence of reduced Mn in solution, and net reduction of Cr(VI) over oxidation of Cr(III).

Although Figures 11 and 15 show that Cr(VI) and Mn(III,IV) reduction occurred over time, indicating conditions were continually becoming more reducing, pe data suggest that a possible equilibrium was being reached. In the first 24 h of the reduction trial, pe decreased from 8.8 to 8.0 for the AQDS and lactate treatment, whereas in the oxidation trial, it increased from 9.9 to 12.2. Figure 16 illustrates the pe fluctuations for both trials. From 24 h to 144 h, pe values increased in the reduction trial, and decreased in the oxidation trial. From 144 h to 332 h, pe values for each sample in the reduction trial, and the corresponding sample in the oxidation trial, appeared to converge. The pe for the lactate and AQDS treatment from the reduction and oxidation trials were converging around 9.0 by 14 d; 9.1 in the reduction trial and 8.6 in the oxidation trial. These results indicate conditions in the oxidation trial became more reducing, and those in the reduction trial became more oxidizing, and that by 14 d, a possible equilibrium in the samples was becoming established.

Species		Equation	E^0 (V)	Log K	ΔG_r^o (kJ/eq)
A	γ-MnOOH	$\gamma\text{-MnOOH} + e^- + 3H^+ \rightarrow Mn^{2+} + 2H_2O$	1.50	25.4	-145.0
B	CrO_4^{2-}	$1/3CrO_4^{2-} + e^- + 5/3H^+ \rightarrow 1/3Cr(OH)_3 + 1/3H_2O$	1.24	21.0	-119.9
C	MnO_2	$1/2MnO_2 + e^- + 2H^+ \rightarrow 1/2Mn^{2+} + H_2O$	1.23	20.8	-118.8
D	$HCrO_4^-$	$1/3HCrO_4^- + e^- + 4/3H^+ \rightarrow 1/3Cr(OH)_3 + 1/3H_2O$	1.11	18.9	-107.9
E	AQDS	$1/2AQDS + e^- + H^+ \rightarrow 1/2AH_2DS$	0.22	3.9	-22.3
F	Lactate	$1/2Pyruvate + e^- + H^+ \rightarrow 1/2Lactate$	0.23	3.9	-22.3
G	CH_2O	$1/4CO_2 + e^- + H^+ \rightarrow 1/4CH_2O + 1.4H_2O$	-0.07	-1.2	6.9
Examples					
γ-MnOOH Reduction by AH_2DS (A and E)				21.5	-122.8
$HCrO_4^-$ Reduction by CH_2O (D and G)				20.1	-114.8
$HCrO_4^-$ Reduction by Lactate (D and F)				15.0	-85.7
$HCrO_4^-$ Reduction by AH_2DS (D andE)				15.0	-85.7
$Cr(OH)_3$ Oxidation by γ-MnOOH (A and D)				6.5	-37.1
AQDS Reduction by CH_2O (E and GI)				5.1	-29.1
AQDS Reduction by Lactate (E and F)				0.0	0.0

Table 4 Reduction half reactions for likely species present in batch trials. These reactions can be combined to form redox reactions, many of which are energetically favorable as indicated by negative ΔG_r values in the given examples. Data from Bartlett and James, 2000; Milazzo et al., 1978; Loach, 1976).

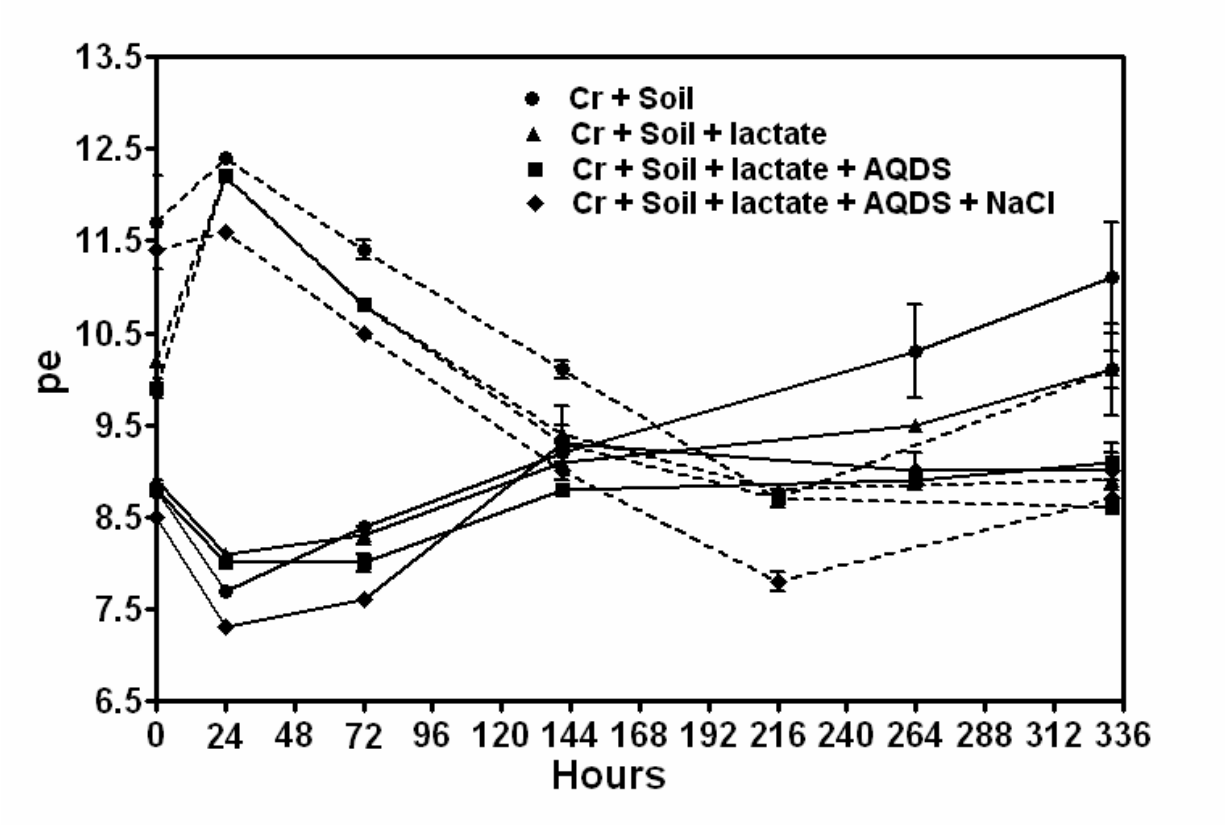
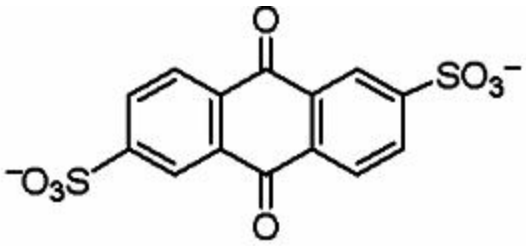


Figure 16 Fluctuations in pe over 14 d reduction and oxidation of 0.2mM Cr(VI) and Cr(III) respectively with Watchung A horizon. Solid trend lines correspond to reduction trial, and dashed trend lines to oxidation. Data points represent averages of 3 reps ± SEM.

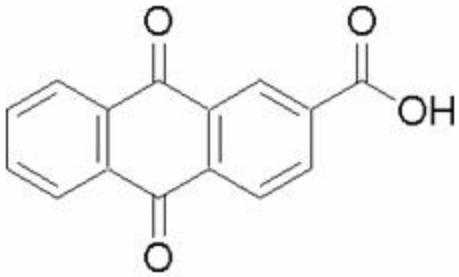
Although the competition between Cr and Mn for electrons carried by AH_2DS was demonstrated, it was unclear how prevalent quinone reduction by organic acids might be in soils and natural waters. To investigate this further, another reduction trial was ensued using 0.2 mM Cr(VI), and 10 mM lactate, citrate, and tartrate. All three organic acids are α-hydroxy carboxylic acids, however, they differ in that lactate has only one carboxylic acid group, tartrate has two, and citrate has three. Additionally, lactate and citrate donate only one electron, whereas tartrate can donate two.

Two AQDS analogs were also included: anthraquinone-2-carboxylic acid (AQCA) and 2-hydroxy-1,4-naphthoquinone (HNQ). The solubility of these compounds was less than AQDS, so to maintain equal concentrations 1 mM of each was included. Figure 17 illustrates the structural similarity of the three quinone compounds. Each treatment received one organic acid and one shuttle compound in order to demonstrate the reducing potential of each organic acid, and how each shuttle may enhance reduction. Additionally, to maintain pH between 6.5 and 7.5, an environmentally and biologically relevant pH range, 40 mM HEPES buffer was included in each treatment. To ensure that only chemical reduction pathways were being utilized, each solution was filter sterilized prior to being added to treatments. Samples were shaken aerobically on an orbital shaker at 24º C, and 1 mL aliquot samples were taken over a period of 24 h.

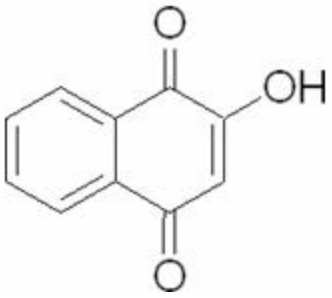
Table 5 presents relevant reduction half reactions, standard electrode potentials, and Gibbs free energies for possible species present in batch trials. Example equations based on the thermodynamic data are also given in table 5 to



anthraquinone-2,6-disulfonate (AQDS)



anthraquinone-2-carboxylic acid (AQCA)



2-hydroxy-1,4-naphthoquinone (HNQ)

Figure 17 Structural illustrations for AQDS, AQCA, and HNQ.

Species		Equation	E^0 (V)	Log K	ΔG_r^o (kJ/eq)
A	CrO_4^{2-}	$1/3CrO_4^{2-} + e^- + 5/3H^+ \rightarrow 1/3Cr(OH)_3 + 1/3H_2O$	1.24	21.0	-119.9
B	$HCrO_4^-$	$1/3HCrO_4^- + e^- + 4/3H^+ \rightarrow 1/3Cr(OH)_3 + 1/3H_2O$	1.11	18.9	-107.9
C	HNQ	$1/2HNQ + e^- + H^+ \rightarrow 1/2HNH_2$	0.47	8.0	-45.7
D	AQDS	$1/2AQDS + e^- + H^+ \rightarrow 1/2AH_2DS$	0.22	3.9	-22.3
E	Lactate	1/2Pyruvate + e^- + H^+ → 1/2Lactate	0.23	3.9	-22.3
F	Citrate	One e^- transfer; oxidized species not given	0.19	3.2	-18.3
G	Tartrate	One e^- transfer; oxidized species not given	0.13	2.2	-12.6
Examples					
$HCrO_4^-$ Reduction by Tartrate (A and G)				16.7	-95.4
$HCrO_4^-$ Reduction by Citrate (A and F)				15.7	-89.6
$HCrO_4^-$ Reduction by AH_2DS (A andD)				15.0	-85.7
$HCrO_4^-$ Reduction by Lactate (A and E)				15.0	-85.7
HNQ Reduction by Tartrate (C and G)				5.8	-33.1
HNQ Reduction by Citrate (C and F)				4.8	-27.4
HNQ Reduction by Lactate (C and E)				4.1	-23.4
AQDS Reduction by Tartrate (D and G)				1.7	-9.7
AQDS Reduction by Citrate (D and F)				0.7	-4.0
AQDS Reduction by Lactate (D and E)				0.0	0.0

Table 5 Reduction half reactions for likely species present in batch trials. These reactions can be combined to form redox reactions, many of which are energetically favorable as indicated by negative ΔG_r values in the given examples. Data from Bartlett and James, 2000; Milazzo et al., 1978; Loach, 1976).

demonstrate the likelihood for a reaction to occur. Figure 18 illustrates a pe-pH diagram for Cr(VI), organic acids, and electron shuttles. Using the table and graph, predictions can be made regarding the reduction by the organic acids and reduced shuttling compounds. For example, tartrate has a standard electrode potential relative to hydrogen (E^o) of 0.130 V, which is below those for citrate and lactate, at 0.190 V and 0.230 V respectively (Milazzo et al., 1978). This places the line for tartrate on Figure 17 below the other two, and so it would be expected to be a stronger reducing agent than citrate and lactate. Correspondingly, the reduction of Cr(VI) by tartrate has a ΔG_r of -95.4 kJ/eq as seen in Table 5, whereas that for citrate and tartare are -89.6 and -85.7 kJ/eq respectively. AQDS has an E^o of 0.230 V, which is lower than that for HNQ at 0.470 V (Loach, 1976), and although HNQ would be expected to be more readily reduced than AQDS, AH_2DS once reduced would be expected to be the stronger reducing agent. The ΔG_r for the reduction of Cr(VI) by AH_2DS is the same as that for lactate at -95.4 kJ/eq.

Figure 19 illustrates reduction of Cr(VI) by the three organic acids with the three shuttling compounds. Tartrate is a strong reducing agent, and reduction is significantly enhanced ($p < 0.05$) with the addition of AQDS. The other shuttling compounds, AQCA and HNQ did not enhance reduction beyond that of tartrate. This trend is also seen in the reduction by citrate and lactate; reduction was enhanced with AQDS, but not by AQCA and HNQ.

The sulfonate groups on AQDS are thought to pull electron density away from the quinone structure, and so are regarded as electron withdrawing groups (EWG), whereas the hydroxyl group on HNQ is thought to push the electron density into the

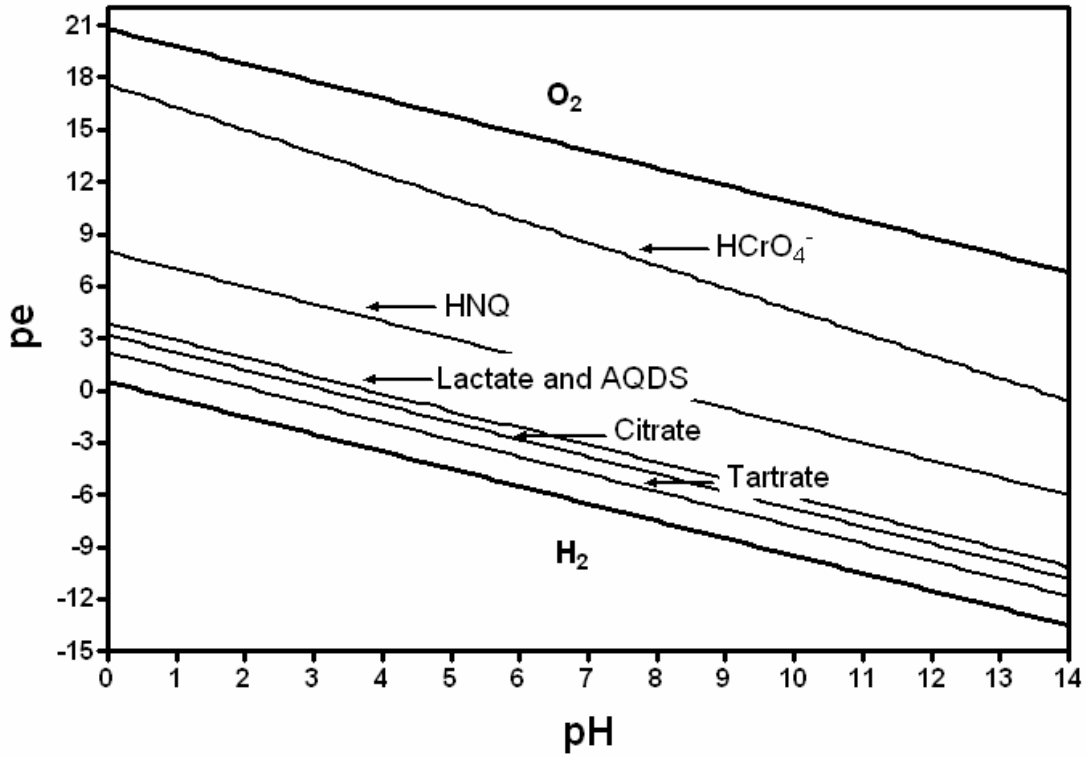


Figure 18 pe-pH diagram illustrating theoretical, experimental conditions. Lines calculated from standard electrode potentials (E^o at pH = 0). Cr data from Bartlett and James, 2000; lactate, citrate, and tartrate data from Milazzo et al., 1978; and AQDS and HNQ data from Loach, 1976. Ion activities are 10^{-4} M.

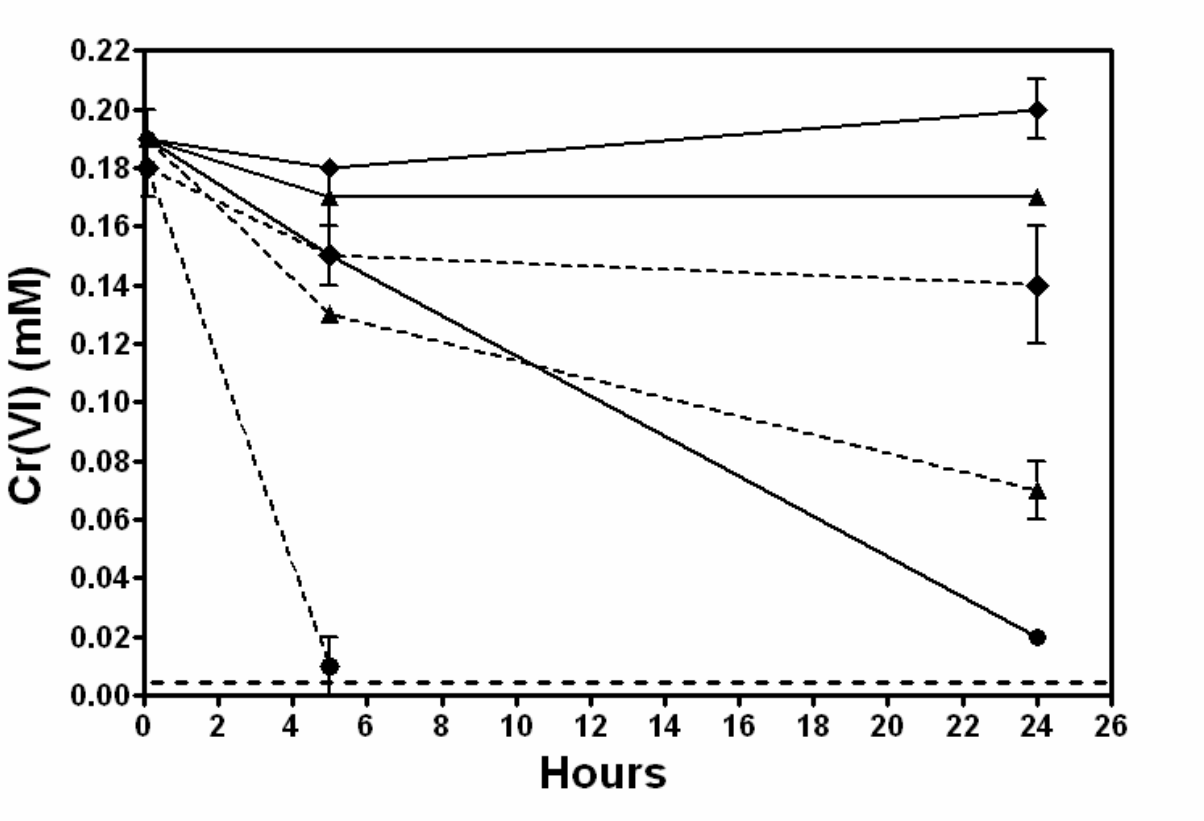


Figure 19 Reduction 0.2 mM Cr(VI) in 24 h by tartrate, citrate, and lactate with and without AQDS treatments. AQDS was the only electron shuttle to significantly enhanced reduction ($p < 0.05$). AQCA and HNQ were not significantly different than reduction by tartrate, citrate, and lactate. ● = tartrate, ▲ = citrate, and ♦ = lactate. Dashed trend lines indicate treatments with AQDS, solid lines without. Data points represent averages of 3 reps ± SEM.

quinone structure of the compound, and is considered an electron donating group (EDG) (Ratasuk and Nanny, 2007). This may explain why AQDS is able to become more of a reducing agent than HNQ, and enhance reduction by tartrate, citrate, and lactate. However, the carboxylic acid group of AQCA is also considered to be an electron withdrawing group, and it would be expected that it would behave similarly to AQDS, but like HNQ, it also did not enhance reduction.

Environmental Significance. The biological reduction of electron shuttles, such as soluble humic acids, is important in soils and natural waters, but this study has shown that abiotic reduction pathways for shuttles and metals also significantly contribute to natural redox cycling. Organic acids produced by microbial activity or plant root exudates can be important reducing agents for quinone moieties in soils, and for soluble humic acids functioning as electron shuttles. A conceptual model for Cr redox cycling in soils is presented in Figure 20.

Even as a simple model of a natural system, it is easily deduced there is much electron flow among different species. Continual electron inputs by soil organic matter and by root exudation of organic acids, as well as fluctuations in oxygen status due to soil moisture, all contribute to a thermodynamically metastable system. In an aerobic soil, such as the one used in this study, the presence of oxygen may have been the sole electron acceptor by soil microorganisms. From a metabolic standpoint, oxygen is a more favorable electron acceptor than Cr(VI), which would explain the dominance of abiotic and not biological reduction pathways in this study. With the presence of Mn(III,IV)(hydr)oxides, oxygen is also important as an oxidant of reduced Mn species.

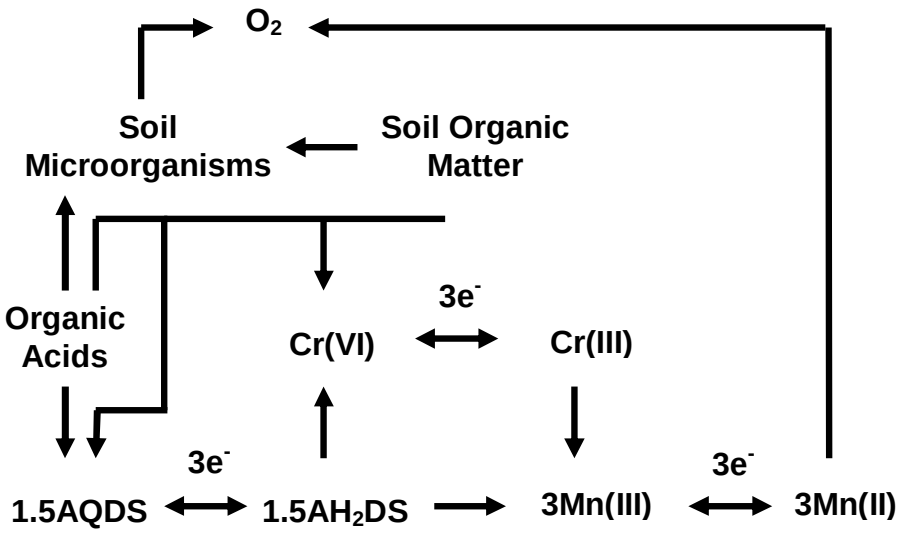


Figure 20 A conceptual model of the Cr redox cycle in an aerobic soil. The redox couples AQDS/AH_2DS, Cr(VI)/Cr(III), and Mn(III)/Mn(II) are shown as 3 electron transfers. AQDS represents naturally derived, soluble quinone-containing humic acids. Arrows between different species indicate direction of electron flow.

Though Mn(III,IV)(hydr)oxides are capable of oxidizing Cr(III) to Cr(VI) in soils, it was demonstrated that the presence of reduced C in soil organic matter, organic acids, and soluble humic acids, as represented by AQDS, overcame any oxidizing potential. It would be expected that in natural systems there would be fluctuations in the oxidizing and reducing potential, but with time, environmental conditions would favor the reduction of Cr(VI), and would keep Cr(III) reduced either as a metal-(hydr)oxide precipitate, or possibly as a soluble organic complex.

The reduction of AQDS by lactate, allowing for an indirect reduction of Cr(VI), where otherwise it would not occur, may further our understanding of soil organic matter and redox cycling in natural systems. Organic acids, such as lactate or tartrate, in soils may be able to reduce quinone moieties in soil organic matter and soluble humic acids, consequently enhancing the reducing capacity of the soil. Further work is needed to understand what other organic acids are capable of reducing these compounds, and to better understand why some compounds, such as AQDS are capable of enhancing reduction, but others, such as HNQ are not. This information would allow for a better understanding of metal cycling in soils, the natural attenuation of a Cr contaminated soil, and possibly of the engineering of a more effective remediation by reduction strategy.

Chapter 5: Summary

Due to a multitude of redox-active chemical species and biological mediators in soils and natural waters, these systems are constantly in a dynamic state of non-equilibrium. This thesis set out to investigate the reduction of chromium(VI) and oxidation of chromium(III) using whole soil samples under different experimental conditions in order to gain further insight into the nature of these redox processes, and to provide a tool for better understanding electron transfer processes under non-equilibrium conditions. It is hoped a better understanding of these processes can lead to more efficacious remediation and restoration strategies for soils contaminated with metals, and to better land use management to protect human health and ecosystem processes.

This thesis demonstrated an extensive capacity for Cr(VI) reduction, and a less prevalent ability for Cr(III) oxidation, in soils sampled from three toposequences in Maryland: Beltsville, Boyds, and Wye Island. For example, the Hatboro A horizon soil demonstrated the ability to reduce 0.10 mM (50%) Cr(VI) within a 24 h period, but was also able to oxidize 0.03 mM Cr(III). Because a soil sample is capable of this dynamic redox transformation, the concept of net reduction and net oxidation should be considered when accessing redox processes in natural systems or in remediation by reduction strategies. Manganese is the only know natural oxidant of Cr(III) in soils, but the reduction of Cr(VI) may be attributed to a number of electron donors, such as microorganisms and humic acids.

There was an apparent trend in reduction along these toposequences, where reduction was greatest in A horizon soils at the lowest point on the landscape. The A horizons of the toe-slope soils: Bibb, Elkton, and Hatboro all reduced more Cr(VI) than their respective summit position soils: Christiana, Sassafras, and Watchung. Soils lower on the landscape are generally wetter, which slows the diffusion of oxygen into soil. This process slows microbiological degradation of organic C in these environments, and as a result, it would be expected that these lower position soils would have higher levels of organic C than summit soils. A correlation analysis showed this relationship to be true ($p < 0.05$, $r2 = 0.71$), however, when testing the relationship between organic C and Cr reduction, a significant, but weaker relationship was found. Clearly there are other variables needed to fully explain Cr reduction, but with a larger sample set, the relationship between organic C and Cr reduction may be better elucidated.

Organic C serves as an electron donor to soil microorganisms, and directly to Cr as humic and fulvic acids. Smaller, soluble humic acids are thought to exist as electron shuttles in natural environments, and have been demonstrated to reduce Fe(III) in sediments and in cultured media. In addition to the tendency for these soils to reduce Cr(VI), most were also able to utilize the addition of the electron shuttle anthraquinone-2,6-disulfonate (AQDS) within the same 24 h period to enhance reduction, and also to diminish oxidation. AQDS consists of a quinone structure on a heterocyclic organic compound, and is thought to resemble the structure of a soluble humic acid molecule. The quinone structure is ubiquitous in biological compounds, such as vitamin K and coenzyme Q, and can be formed by the oxidation of phenol

groups in plant residues and root exudates, as is thought to occur in the process of humification.

These compounds are considered to be reduced as a terminal electron acceptor in the metabolism of soil microorganisms to form either a hydroquinone, or the highly reactive semiquinone species. Although these soils demonstrated reduction over 14 d, the shuttling processes involved appeared to be limited to within the first few days of the trial. This was corroborated with a 72 h trial, which showed significantly reduced half-lives in the Christiana and Sunnyside A horizon soils. There can often be a lag time of up to several days for microbiological growth with the addition of a substrate or terminal electron acceptor, and so this initial 72 h enhancement of reduction and then diminished effect suggests that it was not used for metabolic purposes in this soil.

To better determine whether Cr reduction is attributed to biological or abiotic reduction, two trials were ensued. The first trial fit first-order reaction equations to reduction curves created at 4, 10, 22, 30, and 40º C, and evaluated how rate coefficients changed with increasing temperatures. Biological kinetics reach a peak at an optimum temperature, and then decrease with increasing temperatures, whereas chemical reduction would be expected to increase linearly with higher temperatures. Although this method is more often used in evaluating petroleum hydrocarbons as a substrate for biological degradation, if Cr is serving as a metabolic terminal electron acceptor or is being reduced in a co-metabolic process, the result should be the same.

The log of the rate coefficients plotted as a function of increasing temperature demonstrated a curvilinear, but increasing relationship, suggesting reduction can be

attributed to abiotic pathways in the soil. A log transformation of this data was expected to produce a more linear fit than what was demonstrated, which may be attributed to using a first-order rate equation. As seen with the 72 h trial, Cr reduction is often fit with first-order rate equations when reactions occur within 3 d, however, to allow sufficient time for biological activity the time was extended to 14 d. A second-order rate equation may better fit the data, which would allow for more accurate rate coefficients, but increasing rates with temperature would still be expected.

In the second trial to further investigate biological from chemical reduction pathways, lactate was added to the Watchung A horizon soil to enhance biological activity, and a high salt concentration was added to inhibit activity over a 14 d reduction trial. The addition of the high salt concentration in this treatment created a 1% saline environment, which is approximately equal to an osmotic pressure of 300 bars. If this environment did not kill bacteria in the soil sample, it would be expected their metabolism would be inhibited. It is further expected that any residual metabolic activity due to bacteria isolated in microenvironments, and not exposed to high salt conditions, would not be great enough to influence the Cr chemistry observed in these trials. Soil sterilization was explored as an option, however, chemical treatments can leave residues that may interfere with analysis, autoclaving alters soil structure and surface area of clays, and irradiation can create hydroxyl radicals in the soil. A concentrated salt environment ultimately seemed to be the method that would least alter redox conditions in the soil, and effectively inhibit microbiological activity.

The addition of lactate and AQDS to the soil reduced levels to below detection limits at 11 d, but this treatment was not significantly different than the addition of lactate, AQDS, and the high salt concentration that also reduced Cr to below the detection limit at 11 d. This lack of difference further suggests abiotic pathways prevailed in the reduction of Cr(VI) over the 14 d trial. The control in this trial, which was lactate, AQDS, and a high salt concentration in the absence of soil, demonstrated that in the presence of AQDS, lactate is able to reduce Cr(VI); this reaction suggests a stronger role for quinone structures and organic acids in redox behavior of dissolved and soil organic matter. Further investigation of this control demonstrated that with a constant concentration of AQDS, increasing reduction of Cr occurred with increasing levels of lactate, with 60 mM lactate reducing all Cr within 1 h.

With this observation, it can be proposed that the enhancement in reduction of Cr by the addition of lactate to the Watchung A horizon did not reduce the metal directly, but likely reduced quinone structures in the soil organic matter that were then able to reduce Cr. The addition of lactate and AQDS allowed for both the reduction of quinones in the soil and of AQDS in solution, which combined, were capable of fully reducing all Cr(VI). It was also found that soluble Mn levels in solution increased with the addition of lactate, and further increased with the addition of lactate and AQDS. This suggests the reductive dissolution of Mn(III,IV)(hydr)oxides in soils may “compete” with Cr for electrons from these donating groups in soil organic matter. This also suggests that organic acids and

shuttling compounds may play a stronger role in the abiotic cycling of metals in soils and natural waters.

To investigate the prevalence of these processes, tartrate and citrate were also evaluated for their ability to reduce Cr, and to utilize shuttling compounds to enhanced reduction. Two AQDS analogs were included: anthraquinone-2-carboxylic acid (AQCA) and 2-hydroxy-1,4-naphthoquinone (HNQ). To ensure that only chemical reduction pathways were being utilized, each solution was filter sterilized and sampling was completed within 24 h. Tartrate, a stronger reducing agent than lactate or citrate based on standard electrode potentials, reduced 92% of Cr, whereas citrate reduced 17%, and lactate didn't reduce any Cr(VI). With the addition of AQDS, tartrate reduced Cr to below the detection limit by 5 h, citrate reduced 63% by 24 h, and lactate reduced 30% by 24 h. The other shuttling compounds, AQCA and HNQ did not enhance reduction beyond that of tartrate, citrate, or lactate.

Sulfonate groups on AQDS are thought to be electron withdrawing groups (EWG), and pull electron density away from the quinone structure, whereas the hydroxyl group on HNQ is an electron donating group, pushing electron density into the quinone structure of the compound. This may help explain why AQDS was able to become more of a reducing agent than HNQ, and enhance reduction by tartrate, citrate, and lactate, however, the carboxylic acid group of AQCA is also considered an EWG and would be expected to behave more similarly to AQDS. Clearly, there is more to be learned by further investigating the redox capacities of these shuttling compounds, and for exploring the ability for other naturally produced organic acids to contribute to redox equilibrium in soils and natural waters.

The biological reduction of electron shuttles, such as soluble humic acids, is important in natural systems, but this study has shown that abiotic reduction pathways for shuttles and metals also significantly contribute to natural redox cycling. Organic acids produced by microbial activity or plant root exudates can be important reducing agents for quinone moieties in soils, and for soluble humic acids functioning as electron shuttles. In an aerobic soil, such as the one used in this study, the presence of oxygen may have been the sole electron acceptor by soil microorganisms, because oxygen is a more favorable electron acceptor than Cr(VI) in metabolic processes. With the presence of Mn(III,IV)(hydr)oxides, oxygen is also important as an oxidant of reduced Mn species.

Though Mn(III,IV)(hydr)oxides are capable of oxidizing Cr(III) to Cr(VI) in soils, it was demonstrated that the presence of reduced C in soil organic matter, organic acids, and soluble humic acids, as represented by AQDS, overcame any oxidizing potential. It would be expected that in natural systems there would be fluctuations in the oxidizing and reducing potential, but with time, environmental conditions would favor the reduction of Cr(VI), and would keep Cr(III) reduced either as a metal-(hydr)oxide precipitate, or possibly as a soluble organic complex. Understanding these redox reactions presented in this thesis allows for a better understanding of metal cycling in soils, the natural attenuation of a Cr contaminated soil, and possibly of the engineering of a more effective remediation by reduction strategy.

Appendix A

Sampling Locations

Figure A-1 Boyds, MD soils. Watchung is the summit series, Jackland the back-slope series, and Hatboro the toe-slope series. The Watchung, Jackland, and Hatobor series are all located in a hardwood forested area in Little Seneca Park. Photo taken from the Natural Resources Conservation Service Web Soil Survey (accessed 2008).

Figure A-2 Beltsville, MD soils. Christiana is the summit series, Sunnyside the back-slope series, and Bibb the toe-slope series. The Christiana series is located on the edge of an agriculture field, and the Sunnyside and Bibb series are located in the hardwood and pine forest west of the field. Photo taken from the Natural Resources Conservation Service Web Soil Survey (accessed 2008).

Figure A-3 Wye Island soils. Sassafras is the summit series, Downer the back-slope series, and Elkton the toe-slope series. The Sassafras series is located on the edge of an agriculture field, the Downer series is located at the interface between the wooded area and grass, and the Elkton series is found in the riparian zone along the water. Photo taken from the Natural Resources Conservation Service Web Soil Survey (accessed 2008).

Appendix B

Field Descriptions and

Soil Characterization Data

Series	Taxonomic Class	Horizon	Depth (cm)	Munsell Color	Structure	Redoximorphic Features
Christiana	Fine, kaolinitic, mesic Typic Paleudults	Ap	10	10YR5/6	Granular	None
		Bt2	30	5YR/6	Blocky	None
Sunnyside	Fine-loamy, siliceous, semiactive, mesic Typic Hapludults	Ap	10	7.5YR4/6	Granular	None
		Bt2	38	5YR4/6	Blocky	Few concentrations
Bibb	Coarse-loamy, siliceous, active, acid, thermic Typic Fluvaquents	A	10	10YR3/4	Granular	None
		C1	38	7.5YR5/6	Blocky	Common concentrations 5YR5/8

Table B-1 Field description data for A and B horizons of Beltsville soil series. Series name, taxonomic class, and horizon designation are from NRSC ODS 2008. Depth refers to approximate depth of sampling. Color, structure, and redoximorphic features are from field observations.

Series	Taxonomic Class	Horizon	Depth (cm)	Munsell Color	Structure	Redoximorphic Features
Sassafras	Fine-loamy, siliceous, semiactive, mesic Typic Hapludults	Ap	15	10YR4/6	Granular	None
		Bt2	58	7.5YR4/6	Granular	None
Downer	Coarse-loamy, siliceous, semiactive, mesic Typic Hapludults	Ap	12	10YR5/4	Granular	None
		Bt	46	10YR4/6	Blocky (weak)	None
Elkton	Fine-silty, mixed, active, mesic Typic Endoaquults	A	10	10YR4/6	Granular	None
		Bt	38	10YR4/2	Blocky	Many concentrations 5YR4/4 and few depletions

Table B-2 Field description data for A and B horizons of Wye Island soil series. Series name, taxonomic class, and horizon designation are from NRSC ODS 2008. Depth refers to approximate depth of sampling. Color, structure, and redoximorphic features are from field observations.

Series	Taxonomic Class	Horizon	Depth (cm)	Munsell Color	Structure	Redoximorphic Features
Watchung	Fine, smectitic, mesic Typic Albaqualfs	Ap	18	10YR3/3	Granular	None
		Btg1	30	10YR/4/2	Blocky	Common concentrations and few depletions
Jackland	Fine, smectitic, mesic Aquic Hapludalfs	Ap	15	10YR4/4	Granular	None
		Bt1	30	7.5YR4/4	Blocky	Common concentrations 7.5YR6/8 and few depletions
Hatboro	Fine-loamy, mixed, active, nonacid, mesic Fluvaquentic Endoaquepts	Ap	20	10YR5/4	Granular	None
		Bg1	38	10YR4/2	Blocky	Few concentrations and depletions

Table B-3 Field description data for A and B horizons of Boyds soil series. Series name, taxonomic class, and horizon designation are from NRSC ODS 2008. Depth refers to approximate depth of sampling. Color, structure, and redoximorphic features are from field observations.

Series	Horizon	Water (%)	pH(w)	pH(s)	Organic Matter (%)	Soil Texture (% Sand, Silt, Clay)
Christiana	Ap	12	4.0 ± 0.03	3.4 ± 0.05	2.3	Loam (49, 42, 9)
Christiana	Bt2	17	3.1 ± 0.08	3.4 ± 0.08	0.34	Clay loam (21, 48, 31)
Sunnyside	Ap	12	3.5 ± 0.11	3.5 ± 0.02	2.5	Loam (49, 39, 12)
Sunnyside	Bt2	15	3.5 ± 0.03	2.9 ± 0.03	0.29	Clay loam (28, 48, 28)
Bibb	A	24	3.8 ± 0.07	3.0 ± 0.03	4.9	Loam (50, 38, 12)
Bibb	C1	23	3.6 ± 0.11	3.1 ± 0.03	0.87	Clay loam (24, 36, 30)

Table B-4 Survey of Beltsville soils chemical and physical properties. Organic C determined by LECO %C, H, N, where all C is assumed to be in organic form. pH values are averages of 3 reps ± SEM.

Series	Horizon	Water (%)	pH(w)	pH(s)	Organic Matter (%)	Soil Texture
Sassafras	Ap	7.7	4.4 ± 0.06	3.7 ± 0.06	0.55	Sandy loam (71, 19, 10)
Sassafras	Bt2	11	3.5 ± 0.0	3.1 ± 0.03	0.27	Sandy clay loam (60, 16, 24)
Downer	Ap	8.9	4.5 ± 0.09	3.1 ± 0.05	2.1	Loam (51, 41, 8)
Downer	Bt	7.4	4.3 ± 0.04	3.3 ± 0.02	0.93	Sandy loam (66, 20, 14)
Elkton	A	20	5.9 ± 0.03	5.3 ± 0.08	2.9	Loam (51, 33, 16)
Elkton	Bt	18	5.4 ± 0.04	4.6 ± 0.04	1.4	Sandy loam (74, 19, 7)

Table B-5 Survey of Wye Island soils chemical and physical properties. Organic C determined by LECO %C, H, N, where all C is assumed to be in organic form. pH values are averages of 3 reps ± SEM.

Series	Horizon	Water (%)	pH(w)	pH(s)	Organic Matter (%)	Soil Texture
Watchung	Ap	20	4.6 ± 0.04	4.0 ± 0.03	4.0	Loam (41, 48, 11)
Watchung	Btg1	19	5.0 ± 0.05	4.4 ± 0.02	1.2	Clay loam (34, 34, 32)
Jackland	Ap	15	4.4 ± 0.03	4.0 ± 0.01	3.8	Loam (32, 43, 26)
Jackland	Bt1	24	5.0 ± 0.09	5.0 ± 0.03	1.0	Clay loam (35, 34, 31)
Hatboro	Ap	23	3.5 ± 0.03	3.4 ± 0.01	3.5	Silt loam (23, 57, 20)
Hatboro	Bg1	20	3.6 ± 0.12	3.6 ± 0.02	1.7	Silt loam (31, 57, 12)

Table B-6 Survey of Boyds soils chemical and physical properties. Organic C determined by LECO %C, H, N, where all C is assumed to be in organic form. pH values are averages of 3 reps ± SEM.

Series	Horizon	NH_4OAc Extracted Metals (mg/kg soil)					
		Ca	Mg	K	Fe	Mn	Al
Christiana	Ap	203 ± 2.9	18.7 ± 0.18	40.3 ± .8	44.7 ± 1.9	6.03 ± 0.03	61.2 ± 1.7
Christiana	Bt2	201 ± 3.5	23.7 ± 0.27	115 ± 1.9	26.9 ± 3.0	5.39 ± 0.34	166 ± 4.1
Sunnyside	Ap	75.6 ± 1.4	15.1 ± 0.09	34.9 ± 0.40	27.8 ± 0.90	6.26 ± 0.26	129 ± 4.4
Sunnyside	Bt2	72.9 ± 0.53	50.9 ± 0.33	11.5 ± 0.89	15.1 ± 0.29	3.06 ± 0.11	233 ± 2.9
Bibb	A	158 ± 1.3	27.5 ± 0.18	28.6 ± 0.62	381 ± 5.7	4.09 ± 0.21	129 ± 8.2
Bibb	C1	139 ± 1.3	37.5 ± 0.51	44.1 ± 1.6	129 ± 2.9	14.2 ± 0.10	213 ± 9.5

Table B-7 Survey of exchangeable metals by ammonium acetate extraction in Beltsville toposequence soils. Chromium levels were below level of detection for all samples. Values are replicates of 3 samples in mg kg soil $^{-1}$ ± SEM.

Series	Horizon	NH_4OAc Extracted Metals (mg/kg soil)					
		Ca	Mg	K	Fe	Mn	Al
Sassafras	Ap	138 ± 1.8	17.8 ± 0.06	13.7 ± 0.73	6.08 ± 0.16	3.01 ± 2.6	44.2 ± 1.3
Sassafras	Bt2	54.1 ± 0.41	74.8 ± 0.70	26.6 ± 0.93	10.7 ± 0.22	0.314 ± 0.03	277 ± 7.0
Downer	Ap	29.1 ± 1.7	23.1 ± 0.98	22.8 ± 5.6	19.6 ± 0.18	1.79 ± 0.19	176 ± 4.3
Downer	Bt	23.2 ± 1.5	26.2 ± 0.19	22.1 ± 5.9	10.0 ± 0.25	0.813 ± 0.05	131 ± 6.2
Elkton	A	221 ± 4.4	154 ± 0.31	136 ± 11	3.89 ± 0.20	0.831 ± 0.03	49.9 ± 3.5
Elkton	Bt	87.5 ± 1.0	90.2 ± 0.85	65.0 ± 1.8	27.4 ± 0.45	0.718 ± 0.04	65.8 ± 3.5

Table B-8 Survey of exchangeable metals by ammonium acetate extraction in Wye Island toposequence soils. Chromium levels were below level of detection for all samples. Values are replicates of 3 samples in mg kg soil $^{-1}$ ± SEM.

Series	Horizon	NH_4OAc Extracted Metals (mg/kg soil)					
		Ca	Mg	K	Fe	Mn	Al
Watchung	Ap	879 ± 17	1.10×10^3 ± 15	990 ± 7.5	11.7 ± 0.36	18.0 ± 0.63	27.6 ± 1.0
Watchung	Btg1	1.46×10^3 ± 6.0	137 ± 2.0	116 ± 0.87	30.0 ± 3.3	15.8 ± 0.46	37.4 ± 7.2
Jackland	Ap	994 ± 10	1.29×10^3 ± 22	1.11×10^3 ± 30	62.8 ± 0.19	33.3 ± 0.40	54.8 ± 1.6
Jackland	Bt1	1.68×10^3 ± 11	143 ± 1.3	112 ± 1.6	0.178 ± 0.06	0.283 ± 0.10	28.5 ± 4.8
Hatboro	Ap	18.5 ± 0.36	67.7 ± 2.4	44.2 ± 0.55	1.40 ± 0.59	1.16 ± 0.14	193 ± 1.9
Hatboro	Bg1	97.4 ± 1.8	1.78 ± 0.05	2.33 ± 0.07	0.621 ± 0.06	0.280 ± 0.12	156 ± 1.4

Table B-9 Survey of exchangeable metals by ammonium acetate extraction in Boyds toposequence soils. Chromium levels were below level of detection for all samples. Values are replicates of 3 samples in mg kg soil $^{-1}$ ± SEM.

Appendix C

14 d Reduction Trial for Wye Island and Boyds Toposequences

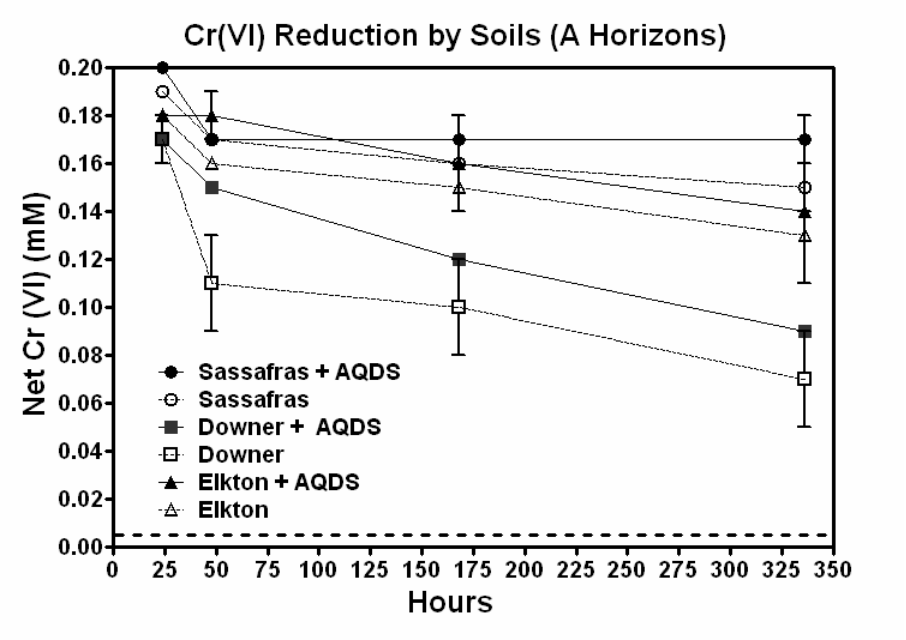


Figure C-1 Reduction of 0.2 mM Cr(VI) for 14 d by A horizons of Wye Island, MD soils, with and without 10 mM AQDS. There was no significant enhancement of reduction after 24 h ($p < 0.05$), but was significant by 14 d for Sassafras and Downer soils.

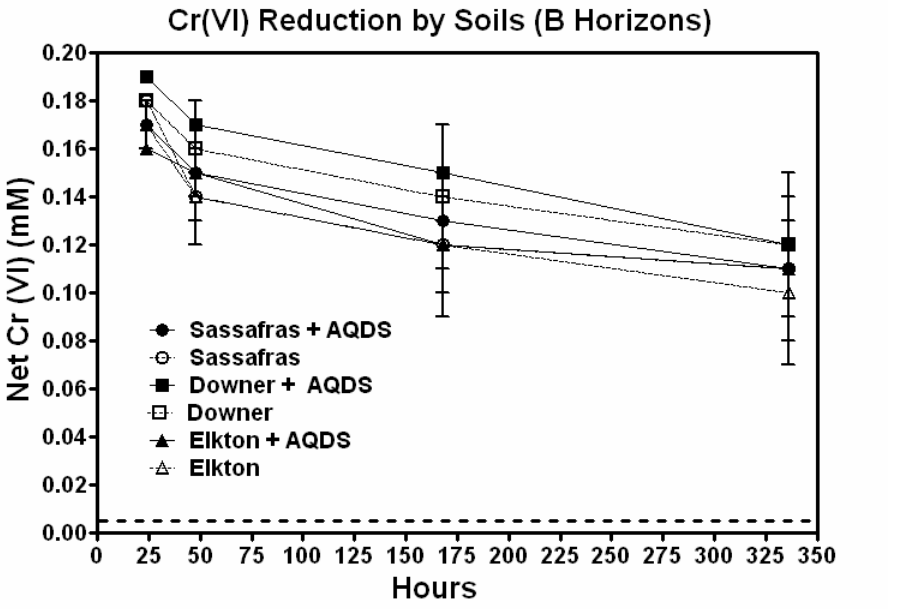


Figure C-2 Reduction of 0.2 mM Cr(VI) for 14 d by B horizons of Beltsville, MD soils, with and without 10 mM AQDS. There was no significant enhancement of reduction after 24 h ($p < 0.05$), but was significant by 14 d for Elkton soil.

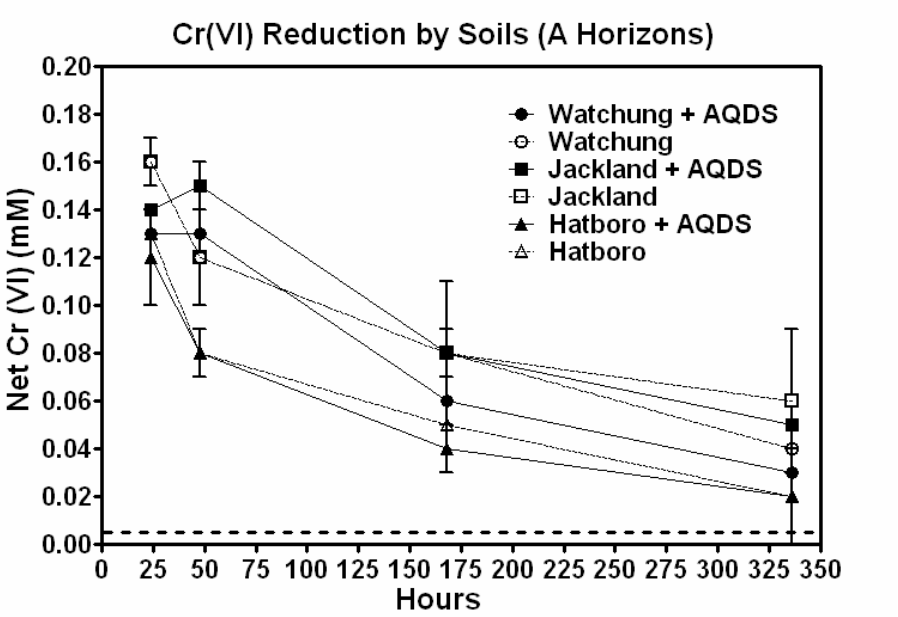


Figure C-3 Reduction of 0.2 mM Cr(VI) for 14 d by A horizons of Boyds, MD soils, with and without 10 mM AQDS. There was a significant enhancement of reduction after 24 h ($p < 0.05$) for all soils, but was only significant by 14 d for Watchung soil.

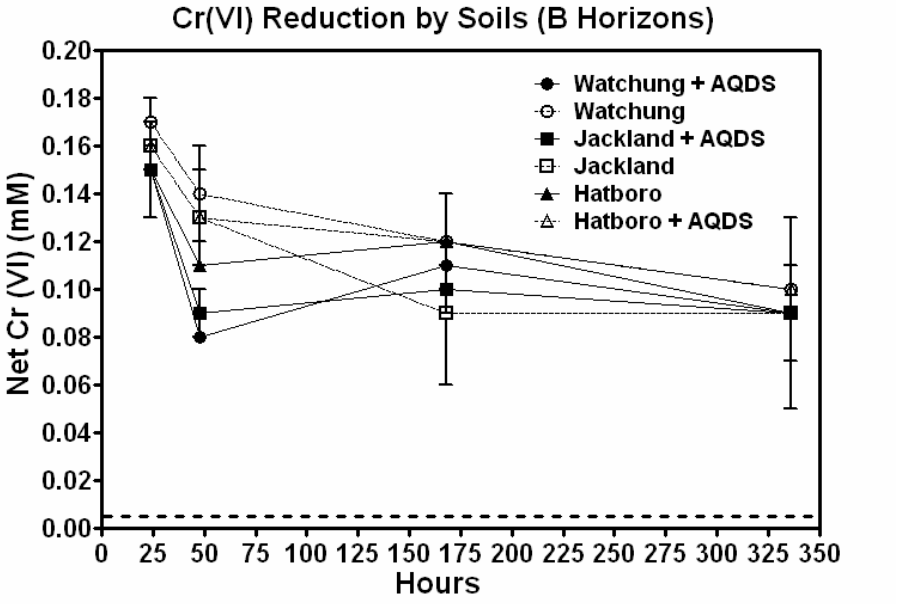


Figure C-4 Reduction of 0.2 mM Cr(VI) for 14 d by B horizons of Boyds, MD soils, with and without 10 mM AQDS. There was significant enhancement of reduction after 24 h ($p < 0.05$) for all soils, but no significant enhancement by 14 d.

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