Environ. Sci. Technol. **2010,** *44,* 9438–9444

Oxidation−Reduction Transformations of Chromium in Aerobic Soils and the Role of Electron-Shuttling Quinones

DOMINIC A. BROSE* AND BRUCE R. JAMES

Department of Environmental Science and Technology, University of Maryland, College Park, Maryland 20742, United States

Received September 9, 2009. Revised manuscript received October 15, 2010. Accepted November 9, 2010.

Oxidation of Cr(III) and reduction of Cr(VI) can occur simultaneously in aerobic soils, but the mechanisms involved are not well-understood, especially how electron shuttling by redox-active organic acids is involved. A and B soil horizons from three topohydrosequences from the Coastal Plain and Piedmont physiographic provinces of Maryland were chosen to investigate oxidation−reduction transformations of Cr under field moist conditions. Reduction of added Cr(VI) to Cr(III) was observed in all 18 samples, and 11 demonstrated enhanced reduction with added anthraquinone-2,6-disulfonate (AQDS) acting as an electron shuttle in 24 h quick tests under aerobic conditions. Oxidation of Cr(III) to Cr(VI) was observed in 12 samples, with 7 demonstrating diminished oxidation with AQDS added. Cr(VI) was undetectable after 11 d of incubation when lactic acid was added as a reductant for Cr(VI) to the Watchung soil A horizon. This reduction occurred in the presence of AQDS and a high salt background to suppress microbial growth, suggesting abiotic reduction was the dominant pathway. The results of this study demonstrate that in field-moist, aerobic soils, the electron shuttle, AQDS, enhanced Cr(VI) reduction and inhibited Cr(III) oxidation. This suggests redox-active organic C amendments and electron shuttles can be important in enhancing rates and extent of Cr(VI) reduction, while inhibiting Cr(III) oxidation in the *in situ* remediation of Cr(VI)-contaminated soils.

Introduction

Chromium (Cr) is a naturally occurring, redox-active, transition metal that may persist as Cr(III) and Cr(VI), or simultaneously undergo oxidation−reduction interconversions in soils, natural waters, and living tissues (*1*). Chromium, in either oxidation state and in a variety of soluble and insoluble compounds, may be added to soils as industrial waste materials, such as chromite ore processing residue (COPR), metal plating waste, tannery and leather offal, pressure-treated lumber, biosolids, and paints (*2*). Hexavalent Cr is a concern as a contaminant due to its carcinogenic properties, principally by inhalation, and due to its high solubility and potential mobility in soils and natural waters as the anionic Cr(VI) species, CrO_4^{2-} and $HCrO_4^-$ (*3*). In contrast, Cr(III), as an organically complexed cation, is an essential human nutrient necessary for the body's metabolism of sugar, protein, and fat (*1*), but it is commonly only sparingly soluble in soils if not complexed by soluble organic ligands.

The reduction of Cr(VI) to Cr(III) is the primary objective in remediation-by-reduction strategies for Cr(VI)-contaminated soils, using both *in-* and *ex-situ* methods (*4*). This goal of soil remediation is complicated by the potential for reoxidation of freshly reduced and precipitated Cr(III) back to Cr(VI). At pH values greater than approximately 5.5, Cr(III) precipitates with OH^- as a sparingly soluble, paracrystalline $Cr(OH)_{3(s)}$, which may gradually dehydrate and crystallize as Cr_2O_3 as thermodynamic equilibrium is approached. If complexed with organic acids, such as fulvic or citric acid, Cr(III) can remain soluble at pH values up to 6.7 or higher depending on pK_a and concentrations of complexing ligands (*5*). Soluble Cr(III) salts and freshly precipitated hydroxides oxidize rapidly to Cr(VI) in the presence of Mn(III,IV)(hydr)oxides (*6, 7*), but Cr_2O_3 and organically complexed Cr(III) species are less likely to be oxidized in soils (*8*).

In the presence of soil organic matter or added organic reducing agents, CrO_4^{2-} and $HCrO_4^-$ can be reduced to Cr(III)(hydr)oxides (*5, 9*). The rate of reduction of Cr(VI) by soil fulvic acids increases with decreasing pH and is best described by an initial rapid reduction period followed by a lower reduction rate as Cr(VI) concentrations decrease (*10, 11*). Peretyazhko and Sposito demonstrated the natural reducing capacities of International Humic Substance Society (IHSS) humic acids and showed that microbial reduction of humic acids increased their reducing capacity for Cr(VI) (*12*).

Phenolic functional groups on quinones and catechols are the main electron donating and accepting moieties in fulvic and humic acids of soil organic matter (*13, 14*). Hydroquinone is capable of donating two electrons and protons as a reductant; however, a one-electron transfer to quinone or a one-electron loss from hydroquinone forms the highly reactive intermediate semiquinone (*15*). Modeling self-exchange electron transfers for AQDS, Rosso et al. calculated a two-electron transfer to anthraquinone-2,6-disulfonate (AQDS) as having a standard electron potential (E°) of 0.253 V (pe = 4.3 at pH 0), relative to the standard hydrogen electrode (SHE), whereas the one-electron transfer to form semiquinone had a calculated E° of −0.055 V (pe = 0.93 at pH 0) (*16*).

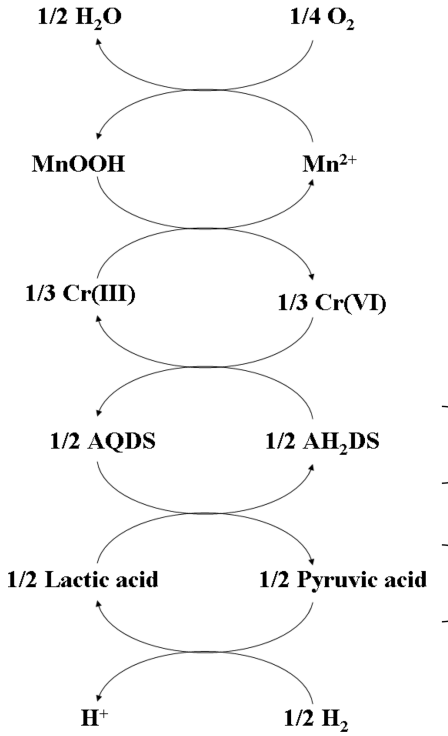
The quinone-hydroquinone couple is the central control of electron transfers in many biological and abiotic redox reactions. AQDS was shown to alter the redox state of arsenic, oxidizing it from As(III) to As(V), making it less mobile and less toxic (*17*). In a microbiological study, *Geobacter sulfurreducens* reduced AQDS to AH_2DS, which then acted as a soluble shuttle for the electrons and reduced Fe(III) in ferrihydrite to soluble Fe(II) (*18*). Additionally, the aerobic reduction of Cr(VI) by *Shewanella oneidensis* was enhanced by the addition of AQDS (*19*). Figure 1 presents a conceptual model for a series of possible electron transferring cycles in soils that can result in the reduction of Cr(VI) by hydroquinone or the oxidation of Cr(III) by a Mn(III,IV)(hydr)oxide.

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 (*20*). The mixed redox potential of an aerobic soil is a function of organic C, pH, redox active species such as O_2, Fe(III), and Mn(III,IV)(hydr)oxides, activity of microbial populations, and soil drainage conditions. Poorly drained soils tend to accumulate organic

* Corresponding author e-mail: dbrose@umd.edu.

10.1021/es101859b

Published on Web 11/24/2010



Reduction Half Reaction	Log K[a]		
	pH 0	pH 5	pH 7
$1/4O_2 + e^- + H^+ \rightarrow 1/2H_2O$	20.8	15.6	13.6
γ-MnOOH + $e^- + 3H^+ \rightarrow Mn^{2+} + 2H_2O$	25.4	14.4	8.4
$1/3HCrO_4^- + e^- + 4/3H^+ \rightarrow 1/3Cr(OH)_3 + 1/3H_2O$	18.9	10.9	8.2
$1/2AQDS + e^- + H^+ \rightarrow 1/2AH_2DS$	4.3[b]	-0.71[c]	-2.7[c]
1/2Pyruvate + $e^- + H^+ \rightarrow$ 1/2Lactate	3.9[c]	-1.2[c]	-3.1
$H^+ + e^- \rightarrow 1/2H_2$	0	-5	-7

a Taken from ref 25 except where indicated otherwise.
b ref 16
c Calculated using tabulated log K values where (reduced species) and (oxidized species) = 10^{-4} M

Electron shuttle surrogate for soluble humic acids in soil

Shuttle-enhanced lactic acid oxidation of α-hydroxy carboxylic acid

FIGURE 1. A redox tree demonstrating the stoichiometric transfer or shuttling of electrons from hydroquinone to Cr(VI), reducing it to Cr(III), and being reoxidized to quinone. Also illustrated is the oxidation of Cr(III) to Cr(VI) by MnOOH.

C more than well-drained soils, and within the same soil profile, an A horizon soil will have different chemical properties from a subjacent B horizon soil. As a result, the chemical behavior of Cr(VI) and Cr(III) will vary depending on soil conditions along a landscape (a toposequence) and in which horizon it is studied.

The objective of this study was to investigate the reduction of Cr(VI) and oxidation of Cr(III), as influenced by the electron shuttle, AQDS, in field-moist samples from A and B horizons of soils with diverse drainage classes along a toposequence. AQDS is regarded as a surrogate for soluble, humic acid-like compounds and is used in this study as an electron shuttle to gain insight into complex soil redox pathways linked to Cr(III) oxidation and Cr(VI) reduction. 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 (*4*).

Materials and Methods

Soils. Two locations in the Coastal Plain and one in the Piedmont physiographic provinces of Maryland were selected for sampling soils that were not known to be contaminated with Cr, other metals, or industrial waste materials. The specific sampling sites comprised soils at the edge of or within temperate hardwood forest vegetation, and they were selected for sampling based on differences in their pedological and chemical properties. Selecting forested sites allowed for sampling of soils that were relatively unchanged in recent decades by the input of lime, fertilizers, or pesticides. The Beltsville location (Coastal Plain) was composed of Ultisols on summits and backslopes and Entisols on the footslopes (*21*). Samples were taken from Christiana, Sunnyside, and Bibb mapping units, which are dominated by kaolinitic clay, are high in Fe(III)(hydr)oxides, and have from 1.7 to 24.7 g organic C/kg soil in the A horizons. The Boyds location (Piedmont) soils were composed of Alfisols on summits and backslopes and Inceptisols on footslopes (*21*). Samples were taken from Watchung, Jackland, and Hatboro mapping units and contain smectitic clay, are high in Mn(III,IV) and Fe(III)(hydr)oxides, and have from 5.2 to 20.2 g organic C/kg soil. The Wye Island location (Coastal Plain) soils were composed of Ultisols along the whole toposequence (*21*). Samples were taken from the Sassafras, Downer, and Elkton mapping units and were chosen because they are sandier than the soils from the other two locations; are low in Al(III), Fe(III), and Mn(III,IV)(hydr)oxides; and contain 1.3 to 14.6 g organic C/kg soil. The Wye Island soils were expected to reduce Cr(VI) or oxidize Cr(III) less than in the other soils, due to lower organic C and Mn(III,IV)(hydr)oxides levels in these soils in contrast to the higher C and Mn contents of the other soils, and especially the piedmont soils dominated by 2:1 phyllosilicate clays with finer textures.

Three soil pits along an approximately 100-m toposequence were dug at each location to depths of approximately 25 to 50 cm, and approximately 20 L samples were taken from the middle of the A and B horizons of each soil profile, resulting in a total of 18 samples. The sampling was done within a three day time period in early summer when soil matric water potentials were approximately −5 to −10 kPa ("field capacity" moisture). The soils were brought into the laboratory, passed through a 4-mm polyethylene sieve, mixed thoroughly by hand, and stored in the dark at 24 °C in plastic buckets lined with 1-mm thick plastic garbage bags to minimize soil drying while maintaining the aerobic status of the soils. The relevant chemical and physical properties of each soil were determined, and collectively, they provide chemical and pedological backgrounds for these sites (Supporting Information Tables S1−S10).

Cr(III, VI) Redox Quick Tests. Triplicate, field-moist samples of the 18 soils, equivalent to 5.0 g oven-dried material (105 °C), were weighed into 50-mL polycarbonate Oak Ridge-type centrifuge tubes. Altogether, four sets of these 54 centrifuge tubes for each soil were prepared. To the first of the four sets, Cr(VI) was added as an aqueous solution of K_2CrO_4 to a final concentration of 0.2 mM, and to another

set, Cr(III) was added, as an aqueous solution of $Cr(NO_3)_3$, to a final concentration of 1.0 mM. To the third set, Cr(VI) and AQDS were added simultaneously, to final concentrations of 0.2 mM and 10 mM, respectively. The fourth set received final concentrations of 1.0 mM Cr(III) and 10.0 mM AQDS. All Cr and AQDS solutions used were made from reagent grade salts and were used within one day of being prepared. Total solution volume was brought up to 25.0 mL with distilled water.

These Cr concentrations reflect approximate levels of Cr contaminated soils with Cr(VI) concentrations of 52 mg/kg soil and Cr(III) concentrations of 260 mg/kg soil (*4*). Due to its redox inertness in aerobic samples, all treatments had 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 and no-soil controls for each of the four treatments were capped with screw tops and shaken continuously on a horizontal shaker at 110 cycles min^{-1} for 24 ± 1 h at room temperature (22 ± 2 °C), at which point, 0.25 mL of a 1 M KH_2PO_4/K_2HPO_4 buffer solution (pH 7.2) was added, and the tubes were recapped and shaken for another hour. The addition of P buffer displaced exchangeable Cr(VI) and ensured that any loss of added or newly formed Cr(VI) was attributable to reduction processes and not as sorption to colloidal surfaces (*6*). All tubes were centrifuged (10 min, 10,000 × *g*, 24 °C), and 1 mL aliquots of centrifugate were diluted to 10 mL.

Diphenylcarbazide (DPC) Analysis for Cr(VI). A UV-1601PC SPC Shimadzu UV−vis spectrophotometer adjusted to a wavelength of 540 nm was used to quantify Cr(VI) concentrations by a modified 1,5-diphenylcarbazide (DPC) spectrophotometric method (*6*). This modified method combines the acidification step (pH 1.5−2) with the redox reaction between added DPC and Cr(VI) and greatly reduces the likelihood for dissolved organic C compounds to reduce Cr(VI) during the analysis. The use of a blank reagent (no DPC, but with acid and ethanol in the reagent) confirmed that the addition of phosphate added to these samples did not interfere with the determination of Cr(VI). In this method, the rapid reduction of Cr(VI) by DPC under acidic conditions is coupled simultaneously with the immediate complexation of newly reduced, unhydrated Cr^{3+} cations by the oxidized form of DPC, diphenylcarbazone (*6*).

Kinetics of Electron Shuttling by AQDS. Two reduction time trials were conducted over the course of 14 days to evaluate the effect of electron shuttling via AQDS on Cr(III) oxidation and Cr(VI) reduction reactions. For the first trial, two sets of field-moist soil samples equivalent to 5.0 g oven-dried soil were weighed out, in triplicate, into 50-mL polycarbonate Oak Ridge-type centrifuge tubes. The first set contained a final concentration of 0.2 mM Cr(VI), and the second contained 0.2 mM Cr(VI) plus 10 mM AQDS. The total volume in each was brought to 25.0 mL with distilled water. All sets had 0.01 M $NaNO_3$ as a background electrolyte and included controls containing the same chemical treatments with no soil added. For this trial, all 18 soils were used, were plugged with foam plugs to allow for gas exchange with the atmosphere, and were shaken vertically on an orbital shaker at 110 cycles min^{-1}. Destructive sampling was performed at 1, 2, 7, and 14 d. The addition of phosphate buffer, centrifugation, and analysis by the DPC method were performed as previously described for the 24 h batch reduction−oxidation trials.

For the second 14 day trial, an A horizon sample (0 to 25 cm in depth) of the Watchung soil series (fine, smectitic, mesic Typic Albaqualfs) was selected because it contains relatively high levels of C and Mn: 20.2 g C/kg soil and 1.27 g total Mn/kg soil. We hypothesized that this soil may demonstrate rapid reduction of Cr(VI) and oxidation of Cr(III) due to the presence of organic C and the presence of Mn(III,IV)(hydr)oxides. For these reasons, it was chosen for a detailed study of electron shuttling processes. Triplicate, field-moist soil samples, equivalent to 5.0 g oven-dried material (105 °C), were weighed into 50-mL polycarbonate Oak Ridge-type centrifuge tubes. All tubes contained 0.2 mM Cr(VI), as K_2CrO_4. Final concentrations of four treatments added to the samples were as follows: 1) 15 mM lactate to serve as an electron source, 2) 15 mM lactate and 10 mM AQDS as an electron source-shuttle combination, 3) 15 mM lactate, 10 mM AQDS, and 0.1 M NaCl to create an osmotic potential equivalent to −30 MPa, to diminish metabolic activity by microorganisms present in this soil (*22*), and 4) no amendments except Cr(VI). A no-soil control contained Cr(VI), 15 mM lactate, 10 mM AQDS, and 0.1 M NaCl.

The soil suspensions were capped with gas permeable foam plugs and shaken vertically on an orbital shaker at 110 cycles min^{-1} and sampled destructively at 1, 3, 6, 10, and 14 d. Solution pH and Eh were measured potentiometrically using glass and Pt combination electrodes, respectively, at each sampling time. Following pH and Eh measurements, the addition of phosphate buffer, centrifugation, and analysis by the DPC method were performed as previously described. Total soluble Fe and Mn concentrations in the supernatant liquids were measured with a Perkin-Elmer 400 flame atomic absorption spectrophotometer.

Results and Discussion

Net Reduction in Cr(III, VI) Redox Quick Tests. All of the A and B horizon soil samples reduced some amount of the Cr(VI) in 24 h, and in 11 of the 18 soils, reduction was enhanced an average of 8.6% by the addition of AQDS (p-values <0.05) as seen in Figure 2. The graphs are labeled as "net Cr(VI)," because as Cr(VI) is being reduced, oxidation of newly precipitated Cr(III) by Mn(III,IV)-(hydr)oxides could take place simultaneously in the same soil suspension.

Of the A horizon soils, Sassafras reduced 4.3% (2.2 mg Cr(VI)/kg soil) without AQDS, the least amount of reduction observed; while Hatboro demonstrated the most with 50% reduction (26.2 mg/kg) without AQDS. Jackland demonstrated the largest enhancement of reduction with a 15% increase in reduction due to AQDS.

Of the B horizons, Elkton reduced 9.3% (5.1 mg/kg) without AQDS, which was the least amount observed, whereas Bibb reduced the most at 24% (10.3 mg/kg). Hatboro B horizon 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 location: Watchung, Jackland, and Hatboro at 30% (15.8 mg/kg), 28% (14.8 mg/kg), and 50% (26.2 mg/kg), respectively. Reduction in B horizons for all the soils, except Sassafras, was lower than their respective A horizons. The Sassafras B horizon reduced 13% (6.7 mg/kg), whereas the A horizon reduced only 4.3% (2.2 mg/kg).

There is an evident trend in the toposequence of the Beltsville location A horizon soils without added AQDS, where reduction increased with progression down the landscape gradient to progressively more poorly drained soils. In this toposequence, Christiana at the summit reduced 21% (11.1 mg/kg), Sunnyside along the back-slope reduced 27% (13.9 mg/kg), and Bibb at the foot-slope (lowest position) reduced the most at 29% (14.9 mg/kg). Greater reduction in lower positions than summit soils ($p < 0.05$) is a pattern also seen in the Boyds and Wye Island toposequences. In the Boyds sequence, Watchung at the summit of the landscape reduced 30% (15.8 mg/kg), and Hatboro at the lowest position reduced 50% (26.2 mg/kg). At Wye Island, Sassafras is the summit soil and reduced 4.3% (2.2 mg/kg), and Elkton at the foot-slope reduced 12% (6.4 mg/kg).

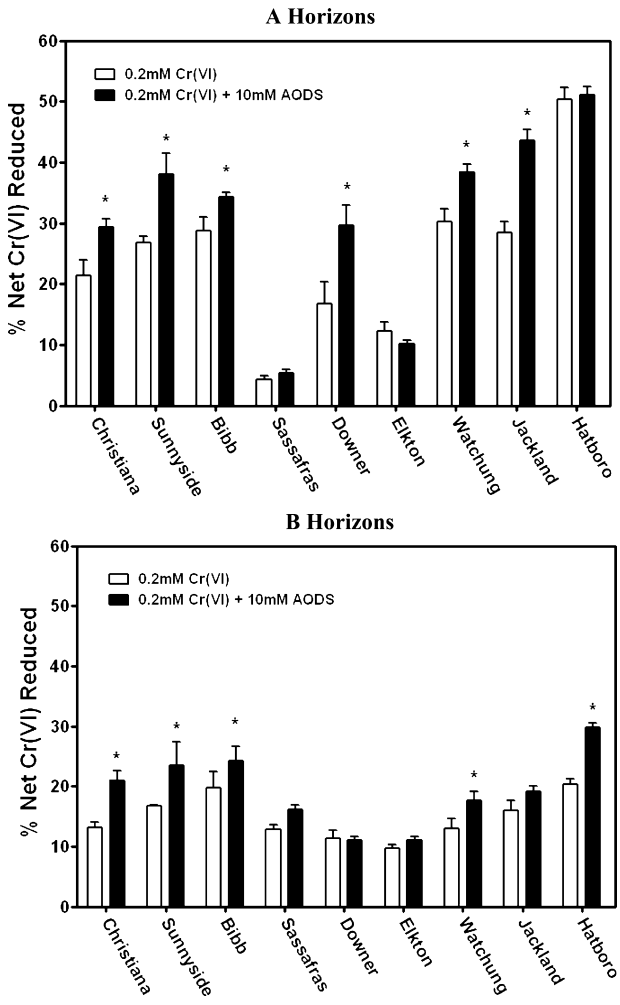


FIGURE 2. Reduction of 0.2 mM Cr(VI) by A horizon and B horizon soils, with or without 10 mM AQDS after 24 h of shaking. Asterisks indicate significant increase in reduction ($p < 0.05$). Bars represent averages of 3 replications ± SEM. The Beltsville location comprised Christiana, Sunnyside, Bibb soils; the Wye Island location Sassafras, Downer, Elkton; and the Boyds location Watchung, Jackland, Hatboro.

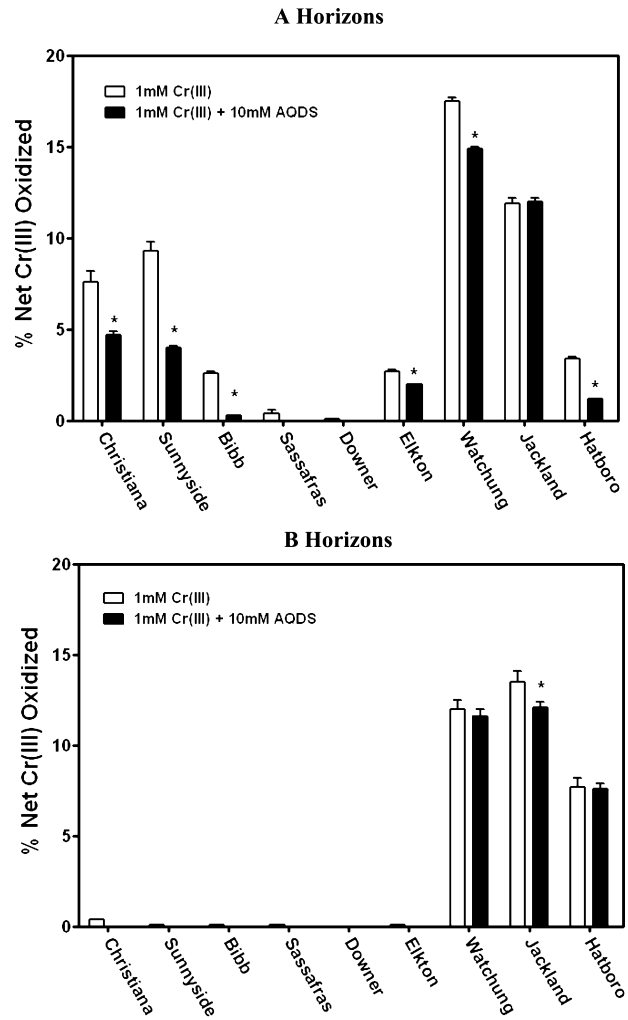


FIGURE 3. Oxidation of 1.0 mM Cr(III) by A horizon and B horizon soils, with or without 10 mM AQDS after 24 h of shaking. Asterisks indicate significant decrease in oxidation ($p < 0.05$). Bars represent averages of 3 replications ± SEM.

These reduction trends may be explained by reducing conditions in the soils as a function of soil organic C. Soils lower on a landscape generally are more poorly drained and tend to accumulate 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 240 g H_2O/kg soil and 25 g C/kg soil, whereas Christiana, the summit series, had 120 g H_2O/kg soil and 12 g C/kg soil. The Elkton soil from Wye Island had 200 g H_2O/kg soil and 15 g C/kg soil, whereas Sassafras, the summit soil, had 7.7 g H_2O/kg soil and 2.8 g C/kg soil. A correlation analysis, however, between Cr(VI) reduction and g C/kg soil for the A horizon soils showed only a weak positive relationship ($p < 0.05$, $r^2 = 0.45$, $n = 9$), indicating that other factors in addition to organic carbon would be needed to fully explain Cr(VI) reduction in these soils.

Net Oxidation in Cr(III, VI) Redox Quick Tests. Of the A horizon samples, 8 of the 9 samples demonstrated oxidation of Cr(III) to Cr(VI) as seen in Figure 3. The Christiana A horizon oxidized 7.6% (19.7 mg Cr(III)/kg soil), Sunnyside A horizon oxidized 9.3% (24.1 mg/kg), and Bibb A horizon oxidized 2.6% (6.8 mg/kg). The Watchung and Jackland were the strongest oxidizers of all A horizons, oxidizing 18% (45.4 mg/kg) and 12% (31.0 mg/kg), respectively. Of the B horizons, only the Watchung, Jackland, and Hatboro (Boyds toposequence) demonstrated oxidation, with 12% (31.3 mg/kg), 14% (35.1 mg/kg), and 7.7% (20.3 mg/kg), respectively. In 7 of the 18 samples, oxidation was significantly inhibited, likely due to increased reduction of newly oxidized Cr(VI) by the addition of the electron shuttle AQDS ($p < 0.05$).

As with the reduction trial, the properties of the A horizon soils resulting from the soil's position on the landscape affected the extent of oxidation of added Cr(III). Oxidation by the Christiana and Watchung summit soils without added AQDS was greater than oxidation by their respective foot-slope soils, Bibb and Hatboro. There is a positive correlation between Cr(III) oxidized and total Mn ($p < 0.05$, $r^2 = 0.78$, $n = 7$). In comparing the Beltsville and Wye Island toposequence data for reduction (Figure 2) with those for oxidation (Figure 3), soils that were strong reducers seemed also to be weak oxiders; however, the Boyds soils were both strong reducers and strong oxidizers, indicating possibly more complex redox pathways in these soils.

Kinetics of Cr(VI) Reduction. *All Soil Samples.* Reduction of Cr(VI) by Christiana, Sunnyside, and Bibb A horizon soils, with and without AQDS, occurred throughout the 14 d equilibration period (Figure 4), but for B horizons, reduction leveled off after a 2-d initial reduction period. The data for the other two toposequences show similar trends for A and B horizons and are presented in Supporting Information Figures S1−S4.

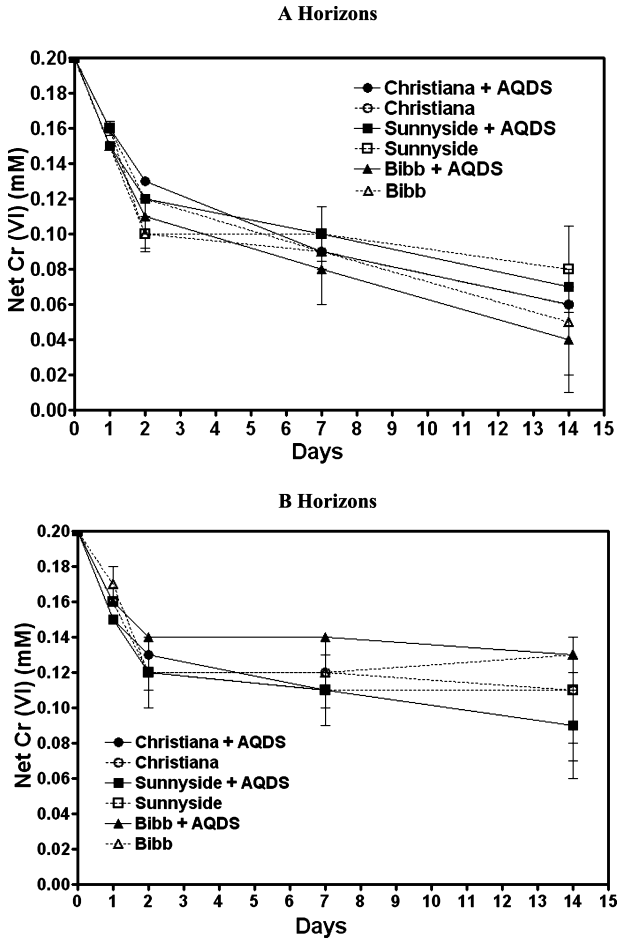


FIGURE 4. Reduction of 0.2 mM Cr(VI) for 14 days by Beltsville toposequence A horizon and B horizon soils, with or without 10 mM AQDS. There was no significant enhancement of reduction after 14 days. Data points represent averages of 3 replications ± SEM.

At 1 d, the Christiana A horizon soil reduced 20% (0.04 mM Cr(VI), 9.4 mg Cr(VI)/kg soil), Sunnyside reduced 25% (0.05 mM, 11.5 mg/kg), and Bibb reduced 25% (0.05 mM, 9.7 mg/kg). These values were not significantly different ($p < 0.05$) from the A horizon values in the batch reduction study (Figure 2). By 14 d, Christiana A horizon reduced 69% (0.14 mM, 31.6 mg/kg), Sunnyside reduced 76% (0.15 mM, 35.1 mg/kg), and Bibb reduced 74% (0.15 mM, 29.2 mg/kg). There was a significant increase in reduction at 1 d with the addition of AQDS for the Christiana B, Sunnyside B, and Bibb B horizons ($p < 0.05$), having 5%, 9%, and 11% increases in reduction, respectively; however, by 14 d, there were no appreciable differences in reduction between soils without or with added AQDS.

The reduction curves for these soils followed pseudo first-order kinetics for the first 72 h. This has been previously described and attributed to electron-rich phenolic groups and fulvic and humic acids in soil organic matter being utilized rapidly before kinetically slower functional groups begin donating electrons for the remainder of the time (*11*). The reduction of Cr(VI) in this study may be attributed to these organic functional groups, because the samples were capped with foam plugs to maintain them aerobically for the 14 d period so that any Fe or S species would be expected to remain as Fe(III) and SO_4^{2-} and unable to reduce Cr(VI).

Watchung Soil. The reduction of Cr(VI) by the Watchung A horizon soil is illustrated in Figure 5. The Watchung soil with no redox-enhancing or -inhibiting amendments reduced 74% (0.15 mM, 39 mg/kg) Cr(VI) over 14 d. The addition of

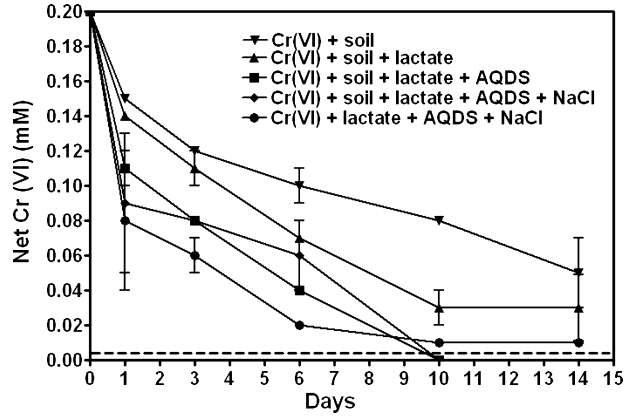


FIGURE 5. Reduction of 0.2 mM Cr(VI) in Watchung A horizon soil and enhanced reduction by addition of 15 mM lactate and 10 mM AQDS, over 14 d. Data points represent averages of 3 reps ± SEM. Dashed line along *x*-axis indicates level of detection.

lactate to the soil increased reduction significantly ($p < 0.05$) to 85% (0.17 mM, 44.2 mg/kg). The addition of the electron shuttle AQDS and lactate to the soil significantly increased reduction to Cr(VI) concentrations below 0.01 mM (detection limit) by 11 d. This AQDS treatment, however, was not significantly different from that which included lactate, AQDS, and 0.1 M NaCl, added to diminish metabolic activity by soil microorganisms. A separate trial investigating lactate as a reducing agent demonstrated no reduction in Cr(VI) after 2 days (50 h) of shaking at lactate concentrations up to 60 mM or 300 times the concentration of Cr(VI).

Microorganisms in marine environments are tolerant of high salt concentrations, but those that exist in fresh water and upland soils are less so (*22*). The addition of the high salt concentration in this treatment created a 1% saline environment, which is approximately equal to an osmotic pressure of −30 MPa (300 bar) (*22*). It would be expected that microbial metabolic activity at this level would be strongly diminished. It was further expected under the mixed, batch conditions of the equilibrations, that any residual metabolic activity due to microorganisms isolated in microenvironments, and not exposed to high salt conditions, would be negligible and would not influence the Cr redox chemistry observed in these trials. Soil sterilization was explored as an option to separate chemical and microbial roles; however, chemical sterilization treatments would also reduce Mn(III,IV) and Fe(III) (hydr)oxides and solubilize humic and fulvic acids (*23*). Autoclaving alters soil structure and surface area of clays, and irradiation can create hydroxyl radicals in the soil (*23*). Creating a hypersaline soil was the method chosen that would least alter the redox condition of the soil, while effectively inhibiting metabolic effects.

The control treatment in this trial composed of Cr(VI), lactate, AQDS, and the high salt concentration, but no soil, reduced 97% (0.19 mM, 49.4 mg/kg) Cr(VI) by 11 d. Equation 1 illustrates the stoichiometric transfer of electrons from lactate to AQDS, thereby reducing it to AH_2DS. Equation 2 shows the reduction of Cr(VI) to a Cr(III)(hydr)oxide by AH_2DS, which is reoxidized back to AQDS, and eq 3 combines eqs 1 and 2 into the full reduction of Cr(VI) by lactate via AQDS

$$AQDS + Lactate(C_3H_6O_3) \rightarrow AH_2DS + Pyruvate(C_3H_4O_3) \quad (1)$$

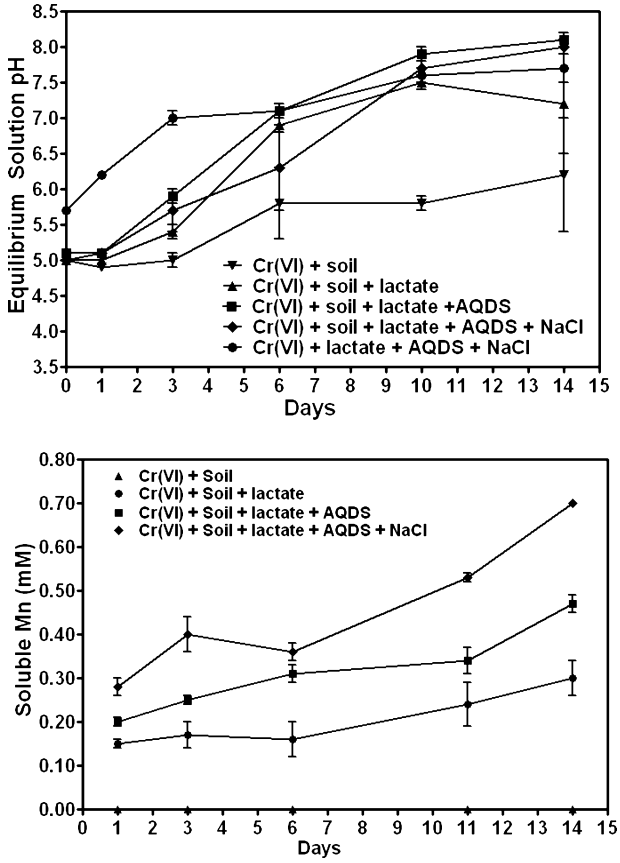


FIGURE 6. Increase in pH over 14 d reduction of 0.2 mM Cr(VI) by Watchung A horizon soil in enhanced reduction by addition of 15 mM lactate and 10 mM AQDS (Top). Increase in soluble Mn over 14 d reduction of 0.2 mM Cr(VI) by Watchung A horizon soil (Bottom). Treatment with soil and no amendments is just above 0 and lies on *x*-axis. Data points represent averages of 3 replications ± SEM.

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

$$HCrO_4^- + 1.5\,AQDS + 3\,Lactate + 2H^+ \rightarrow Cr(OH)_3 + 1.5\,AQDS + 3\,Pyruvate + H_2O \quad (3)$$

The addition of lactate to the sample containing Watchung A horizon soil and no other amendments enhanced reduction by 10%. Using eq 3 as a model, enhanced reduction may be attributed to the reduction of quinone moieties to hydroquinone-containing structures in the soil, which ultimately reduced Cr(VI). Quinone moieties in humic acids can contribute up to 79% of the total electron-carrying capacity, as defined by the reduction of Fe(III)-citrate under anaerobic conditions (*24*).

Equations 2 and 3 show the consumption of protons in the reduction of Cr(VI) to a Cr(III)(hydr)oxide. This is corroborated in Figure 6, which illustrates a pH increase for all treatments over 14 d. The addition of lactate and AQDS to the soil demonstrated the most Cr(VI) reduction and the greatest increases in pH. At 14 d, the lactate-AQDS treatment reached a pH of 8.1, whereas the pH in the soil with no amendments increased to only 6.2. The initial pH for both samples was 5.0, and because the reduction of AQDS by lactate does not consume protons (eq 1), this greater increase in pH can be attributed to the enhanced reduction of Cr(VI) to a Cr(III) via a lactate-AQDS shuttling pathway.

Total Mn levels (assumed to be Mn(II)) in solution after 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 levels to 0.47 mM; more than a 100-fold increase. The presence of soluble Mn indicated that Cr(VI) and Mn(III,IV) competed for reducing equivalents of AH_2DS. Thermodynamically, Fe(III)(hydr)oxides may also be reduced by AH_2DS, but no Fe was detected in solution. It would be expected that under aerobic conditions like these, Fe(II) would rapidly oxidize and possibly precipitate out of solution, thus Fe reduction cannot be dismissed; however, because it is rapidly oxidized in aerobic environments, it likely had little effect on Cr(VI) reduction. The full reduction of Cr(VI) and dissolution of Mn(III,IV)(hydr)oxides in these samples indicate a strong reducing environment that greatly hindered the potential for Mn(III,IV)(hydr)oxides in soils to oxidize Cr(III).

These relationships can be further explored with thermodynamic data. The reduction of MnOOH by AH_2DS has a ΔG_r^o of −122.8 kJ/eq, which is more energetically 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^o of −37.1 kJ/eq. Although this does not speak to the kinetics of reduction, these values corroborate the observation of increased Mn in solution and net reduction of Cr(VI) being favored over oxidation of newly reduced Cr(III) in the presence of lactate and AQDS.

Environmental Significance. The simultaneous reduction and oxidation processes observed in these soils are indicative of the nonequilibrium nature of redox pathways in natural systems. For example, within the same Bibb A horizon, 2.6% of Cr(III) (6.8 mg/kg) was oxidized to Cr(VI), yet 29% of Cr(VI) (14.9 mg/kg) was reduced, and 34% of Cr(VI) (17.9 mg/kg) was reduced with the addition of AQDS. Over the course of 14 d, the Bibb A horizon soil reduced 74% Cr(VI) (29.2 mg/kg). In the pH range of these soils (5−6), Mn(III,IV)(hydr)oxide is a powerful oxidant and the principal naturally occurring, soil-borne oxidant of Cr(III) (*25*).

Mn levels are higher in these A horizons than in B horizons and as seen with correlation analysis, oxidation is directly related to the level of Mn(III,IV)(hydroxides). The pH of these soils range from 5 to 6, which is above the point of zero charge (PZC) for Mn(III,IV)(hydr)oxides, which have been found to be in the range of 1 to 4 (*26*). It is expected that the Mn(III,IV)(hydr)oxides would still be negatively charged under these soil conditions. However, in this pH range, Cr(III) precipitation on the Mn(III,IV)(hydr)oxide surface may inhibit further oxidation reactions (*7*). These reduction and oxidation trials show that although oxidation and reduction occur simultaneously; with time, reduction pathways tend to dominate, resulting in lower levels of Cr(VI). Also, it is clear that A horizons of these soils are more reactive toward reduction and oxidation transformations of Cr than their respective B horizons.

The initial enhancement of reduction from the electron shuttle AQDS, and its diminishing effect 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. 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 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 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 the chemical reduction of quinone

structures on other model shuttling compounds. This would allow for a better understanding of metal cycling in soils, the natural attenuation of a Cr contaminated soil, and possibly the engineering of a more effective remediation by reduction strategy.

Acknowledgments

The authors would like to thank Erin S. Boyle for her assistance and contribution to the current work.

Supporting Information Available

Tables with relevant chemical and physical properties of each soil and figures illustrating 14 day reduction trials not included in the article text. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services. *Toxicological Profile for Chromium*; 2008, DHHS-ATSDR, Atlanta, GA.

(2) Darrie, G. Commercial extraction technology and process waste disposal in the manufacture of chromium chemicals from ore. *Environ. Geochem. Health* **2001**, *23* (3), 187–193.

(3) Burke, T.; Fagliano, J.; Goldoft, M.; Hazen, R. E.; Iglewicz, R.; McKee, T. Chromite ore processing residue in Hudson County, New Jersey. *Environ. Health Perspect.* **1991**, *92*, 131–137.

(4) James, B. R. The challenge of remediating chromium-contaminated soil. *Environ. Sci. Technol.* **1996**, *30*, A248–A251.

(5) James, B. R.; Bartlett, R. J. Behavior of chromium in soils (VII) Adsorption and reduction of hexavalent forms. *J. Environ. Qual.* **1983**, *12*, 177–181.

(6) Bartlett, R. J.; James, B. R. Behavior of chromium in soils (III) Oxidation. *J. Environ. Qual.* **1979**, *8*, 31–35.

(7) Fendorf, S. E.; Zasoski, R. J. Chromium(III) oxidation by delta-MnO_2 (I) Characterization. *Environ. Sci. Technol.* **1992**, *26*, 79–85.

(8) Puzon, G. J.; Tokala, R. K.; Zhang, H.; Yonge, D.; Peyton, B. M.; Xun, L. Y. Mobility and recalcitrance of organo-chromium(III) complexes. *Chemosphere* **2008**, *70*, 2054–2059.

(9) Bartlett, R. J.; Kimble, J. M. Behavior of chromium in soils (II) Hexavalent forms. *J. Environ. Qual.* **1976**, *5*, 383–386.

(10) Kozuh, N.; Stupar, J.; Gorenc, B. Reduction and oxidation processes of chromium in soils. *Environ. Sci. Technol.* **2000**, *34*, 112–1195.

(11) Wittbrodt, P. R.; Palmer, C. D. Reduction of Cr(VI) in the presence of excess soil fulvic acid. *Environ. Sci. Technol.* **1995**, *29*, 255–263.

(12) Peretyazhko, T.; Sposito, G. Reducing capacity of terrestrial humic acids. *Geoderma* **2006**, *137*, 140–146.

(13) Lovley, D. R.; Coates, J. D.; Blunt-Harris, E. L.; Phillips, E. J. P.; Woodward, J. C. Humic substances as electron acceptors for microbial respiration. *Nature* **1996**, *382*, 445–448.

(14) Scott, D. T.; McKnight, D. M.; Blunt-Harris, E. L.; Kolesar, S. E.; Lovley, D. R. Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. *Environ. Sci. Technol.* **1998**, *32*, 2984–2989.

(15) Larson, R. A. *Naturally Occurring Antioxidants*; CRC Press: New York, NY, 1997; p 195.

(16) Rosso, K. M.; Smith, D. M. A.; Wang, Z. M.; Ainsworth, C. C.; Fredrickson, J. K. Self-exchange electron transfer kinetics and reduction potentials for anthraquinone disulfonate. *J. Phys. Chem.* **2004**, *108* (16), 3292–3303.

(17) Jiang, J.; Bauer, I.; Paul, A.; Kappler, A. Arsenic redox changes by microbially and chemically formed semiquinone radicals and hydroquinones in a humic substance model quinone. *Environ. Sci. Technol.* **2009**, *43* (10), 3639–3645.

(18) Straub, K. L.; Schink, B. Evaluation of electron-shuttling compounds in microbial ferric iron reduction. *FEMS Microbiol. Lett.* **2003**, *220*, 229–233.

(19) Lowe, K. L.; Straube, W.; Little, B. Jones-Meehan. Aerobic and anaerobic reduction of Cr(VI) by Shewanella oneidensis: Effects of cationic metals, sorbing agents and mixed microbial cultures. *J. Acta Biotechnol.* **2003**, *23*, 161–178.

(20) Stumm, W.; Morgan, J. J. *Aquatic Chemistry*, 3rd ed.; Wiley-Interscience: New York, NY, 1996.

(21) Soil Survey Staff. Natural Resources Conservation Service. U.S. Department of Agriculture. [*Web Soil Survey*]. Lincoln, Nebraska. Available: http://websoilsurvey.nrcs.usda.gov (accessed March 2008).

(22) Brock, T. D. The poisoned control in biogeochemical investigations. In *Environmental Biogeochemistry and Geomicrobiology*; Krumbein, W. E., Ed.; Ann Arbor Science: Ann Arbor, MI, 1978; Vol. 3.

(23) Trevors, J. T. Sterilization and inhibition of microbial activity in soil. *J. Microbiol. Methods* **1996**, *26* (1−2), 53–59.

(24) Ratasuk, N.; Nanny, M. A. Characterization and quantification of reversible redox sites in humic substances. *Environ. Sci. Technol.* **2007**, *41* (22), 7844–7850.

(25) Bartlett, R. J.; James, B. R. Redox chemistry of soils. In *Advances in Agronomy, Vol 50*; Sparks, D., Ed.; Academic Press, Inc.: 1993; pp 151−208.

(26) Kim, J. G.; Dixon, J. B.; Chusuei, C. C.; Deng., Y. J. Oxidation of chromium(III) to (VI) by manganese oxides. *Soil Sci. Soc. Am. J.* **2002**, *66*, 306–315.

ES101859B