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Authors
Tokunaga, Tetsu K.
Wan, Jiamin
Firestone, Mary K.
et al.

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In-situ Reduction of Cr(VI) in Heavily Contaminated Soils Through Organic Carbon Amendment

Tetsu K. Tokunaga[1]*, Jiamin Wan[1], Mary K. Firestone[2], Terry C. Hazen[1], Keith R. Olson[1], Donald J. Herman[2], Stephen R. Sutton[3], and Antonio Lanzirotti[3]

[1]Lawrence Berkeley National Laboratory, Berkeley, California, 94720
[2]University of California, Berkeley, California 94720
[3]University of Chicago, Chicago, Illinois 60637
*Corresponding author phone: (510)486-7176; fax (510)486-7797; e-mail: tktokunaga@lbl.gov

Abstract

Chromium has become an important soil contaminant at many sites, and facilitating in-situ reduction of toxic Cr(VI) to nontoxic Cr(III) is becoming an attractive remediation strategy. Acceleration of Cr(VI) reduction in soils by addition of organic carbon was tested in columns pretreated with solutions containing 1,000 and 10,000 mg L^{-1} Cr(VI) in order to evaluate potential in-situ remediation of highly contaminated soils. Solutions containing 0, 800, or 4,000 mg L^{-1} organic carbon in the form of tryptic soy broth or lactate were diffused into the Cr(VI) contaminated soils. Changes in Cr oxidation state were monitored through periodic micro-XANES analyses of soil columns. Effective 1st-order reduction rate constants ranged from 1.4×10^{-8} to 1.5×10^{-7} s^{-1}, with higher values obtained for lower levels of initial Cr(VI) and higher levels of organic carbon. Comparisons with sterile soils showed that microbially dependent processes were largely responsible for Cr(VI) reduction, except in the soils initially exposed to 10,000 mg L^{-1} Cr(VI) solutions that receive little (800 mg L^{-1}) or no organic carbon. However, the microbial populations ($\leq 2.1 \times 10^5$ g^{-1}) in the viable soils are probably too low for direct enzymatic Cr(VI) reduction to be important. Thus, synergistic effects sustained in whole soil systems may have accounted for most of the observed reduction. These results show that acceleration of in-situ Cr(VI) reduction with addition of organic carbon is possible in even heavily contaminated soils and suggest that microbially dependent reduction pathways can be dominant.

Keywords: chromium, soil, contamination, remediation, organic carbon, reduction

Abbreviations: APS, Advanced Photon Source; k_{OC}, effective 1st-order rate constant, dependent on organic carbon; k''', Wittbrodt-Palmer rate constant; μ_{Max}, Monod maximum specific reduction rate constant; K_s, Monod half velocity constant; OC, organic carbon; NSLS, National Synchrotron Light Source; SHA, soil humic acid: TSB, tryptic soy broth; X, biomass concentration; X_e, fraction of humic acid oxidized; XANES, x-ray absorption near-edge structure; ΔOC, organic carbon added per mass of soil; $\sum$Cr, total Cr

Introduction

The wide range of applications of chromium has resulted in its occurrence as a common contaminant in soils (Palmer and Wittbrodt, 1991; Proctor et al., 1997). In soils, Cr occurs in two oxidation states having very different behavior (Rai et al., 1989). Hexavalent Cr is generally more soluble, mobile, and toxic. The reduced Cr(III) forms are generally much less mobile and less hazardous. The $p\varepsilon$ of Cr(VI):Cr(III) transformations is in the range of 14 to 5 over the pH range of 4 to 9. Because of this high $p\varepsilon$, availability of reductants, and typically faster rates of Cr(VI) reduction relative to Cr(III) reoxidation, most Cr occurs in trivalent forms in uncontaminated soils. In soils that have been contaminated with Cr(VI), rates of reduction to Cr(III) are of great interest because of the vastly differing toxicities of these two oxidation states.

One general approach to remediating Cr(VI)-contaminated soils involves accelerating in-situ reduction to Cr(III) (Higgins et al., 1998). Enhanced in-situ Cr(VI) reduction is attractive because excavation and waste disposal are very costly. Although Cr(VI) is reduced to Cr(III) to some extent without intervention, rates of natural attenuation can be unacceptably slow. Thus, supplying reductants into soils and groundwaters is becoming attractive for accelerating Cr(VI) reduction. Inorganic treatments include H_2S injection (Thornton and Amonette, 1999; Kim et al., 2001), aqueous Fe(II) injection (Seaman et al., 1999) and use of reduced Fe solids in permeable reactive barriers (Blowes et al., 1997). Proposed organic-based Cr(VI) reduction strategies have included application of various carbon sources such as manure, molasses, and organic acids (Losi et al., 1994; Henny et al., 2001; Higgins et al., 1998; Perlmutter et al., 2001).

The effectiveness of organic carbon (OC) in reducing Cr(VI) depends on the concentration of the contaminant, reactivity of OC, and microbial activity. Higher Cr(VI) concentrations depress microbial activity (Chen and Hao, 1998), thereby diminishing several Cr(VI) reduction processes, including enzymatic reduction (Lovley, 1993), reduction by ferrous iron and sulfide (Fendorf et al., 2000) sustained primarily through the activity of Fe- and S-reducing bacteria (Chapelle, 1992) and reduction by intermediate organic degradation products (Deng and Stone, 1996). Despite the fact that some contaminated soils and groundwaters contain levels of Cr(VI) exceeding 10,000 mg L^{-1} (Palmer and Wittbrodt, 1991; Sturges et al., 1992; Makdisi, 1992), very few laboratory studies have been conducted at concentrations relevant to remediating heavily contaminated environments.

The extent of Cr(VI) reduction in contaminated sediments also depends on the amount of native and added OC. Many forms of OC directly reduce Cr(VI), including phenols (Elovitz and Fish, 1994), organic acids (Deng and Stone, 1996), and humic substances (Wittbrodt and Palmer, 1995, 1996, 1997; Nakayasu et al., 1999). Cr(VI) reduction rate constants for these organic species are highly pH dependent, becoming slow under neutral to alkaline conditions. Chromate reduction by OC also occurs through microbially mediated pathways. Available OC may stimulate activity of some Cr reducing organisms within the native microbial community. Soil microorganisms can also contribute to Cr(VI) reduction through release of ferrous iron, sulfides, and reactive organic intermediates. Soluble organic ligands can kinetically influence the fate of subsurface Cr through Fe(II) and Fe(III) complexes, as well as through complexing Cr(III) in soluble forms (Buerge and Hug, 1998). Some Fe(III) reduction occurs through

microbially produced electron shuttles such as reduced humic acids and hydroquinones (Nevin and Lovley, 2000). Mineral surfaces also catalyze Cr(VI) reduction by OC (Deng and Stone, 1996). This brief survey indicates that Cr reduction in soils occurs through complex interactions of synergistic and competing processes.

In order to understand the dynamics of contamination and in-situ remediation processes, it is essential to include transport influences. One approach to including these effects involves conducting experiments in columns or soil aggregates in which transport can become diffusion-dominated. This approach was used in our previous studies on Cr contamination (Tokunaga et al., 2001, 2003) and is used here to examine OC-based remediation of Cr(VI)-contaminated soils. Competing effects of the initial level of Cr(VI) contamination and the amount of OC added were tested in small columns representing transects into soil aggregates. Our primary goals are (1) to determine the overall effect of OC availability on Cr reduction in soils, (2) to determine the collective importance of microbially-dependent Cr(VI) reduction pathways versus abiotic pathways in highly contaminated soils, and (3) to determine whether or not spatial heterogeneity of Cr develops during OC-stimulated Cr reduction.

Materials and Methods

Small soil columns, similar to those described in Tokunaga et al. (2001), were used to represent transects into subsurface domains where transport is diffusion-limited (Fig. 1). The upper surface of each column represents the exterior of an aggregate, through which components (water, O_2, CO_2, Cr(VI), OC) are exchanged between the system and its environment. Soil from the C horizon of Altamont clay (Aridic Haploxerert) was collected at Altamont Pass (Alameda County, CA). The calcareous (1 to 4% calcite, pH 8.3), clayey (42% clay, 52% silt, 6% sand, 1% organic carbon) soil was crushed, homogenized, and passed through a 250 μm sieve. The native Cr concentration of this soil is 60 mg kg^{-1}, with greater than 95% as Cr(III). The Cr oxidation state was determined by X-ray absorption spectroscopy, as described later. This soil was packed into columns to an equivalent dry bulk density of 1.26 Mg m^{-3}. Soil columns were 11.9 mm diameter, 30 mm long, with porosities and pore volumes of 0.52 and 1.74 mL, respectively. The gravimetric water content of the soil at this stage was 0.074 g g^{-1}. Columns were sequentially treated with 3 different solutions. First, deionized water was added to achieve a soil water content of 0.30 g g^{-1} (equivalent to 70% saturation), by allowing water to infiltrate into columns while the outflow port was open. Using texture and density information, the soil matric potential was estimated to be about -0.3 MPa under these pre-exposure conditions (Campbell, 1985). Columns were incubated at this level of water saturation for 8 days at room temperature to partially reactivate the microbial community while maintaining aerobic conditions. This was followed by ponding 1.10 mL of solutions containing 0, 1,000, or 10,000 mg L^{-1} Cr(VI), added as K_2CrO_4. These solutions were ponded on top of the partially saturated soils while the outflow port was still open, such that inflow continued and drainage of soil water occurred until the chromate pool receded into the soil surface. The outflow port was then sealed for the remainder of the experiment, such that further transport was by diffusion only. At the end of this contamination phase, soils contained either 0, 4.93, or 49.3 μmol of added Cr(VI) per g soil, and the water content was in the range of 0.40 ±0.02 g g^{-1} (95 ±5% saturation). Five days after the initial exposure to Cr(VI) solutions, columns were

ponded with a third solution containing various concentrations of organic carbon. Columns received 0.60 mL (0.345 pore volume) of either tryptic soy broth or sodium lactate solutions as the OC remediation agent. Infiltration and slow evaporation diminished the initial ponding depths (4 to 5 mm) of these solutions, such that distilled water was periodically added in order to maintain about a 1-mm pool for the calomel reference electrode. Solutions with 0 (deionized water control), 800, or 4,000 mg L^{-1} OC were ponded on each column. This amounted to adding 0, 9.3, or 47 µmol of OC per g soil (0, 112, or 560 µg g^{-1}). Tryptic soy broth (TSB) was used as a carbon source representing decomposing plant tissue. Lactate was used in several columns since it is an easily metabolized carbon source, with related compounds now being tested for use in in-situ bioremediation (Lutze et al., 2001; Evans and Koenigsberg, 2001). The ponded surface of each column was capped to minimize evaporation, but vented through a segment of a hypodermic needle to maintain this boundary in equilibrium with atmospheric oxygen. Periodic uncapping for redox potential and pH measurements also help to maintain this aerated boundary condition.

Redox potential profiles were periodically measured through a series of Pt electrodes embedded along columns. Electrodes were set at 0, 2, 4, 6, 10, 15, 20, and 25 mm, relative to the soil surface. For these measurements, a calomel reference electrode was dipped into the surface pool. The reference electrode was rinsed with alcohol and distilled water after removal from each column. Redox measurements presented later are relative to the standard hydrogen electrode, obtained by adding 245 mV to the raw calomel-referenced data. Measurements of pH were also obtained within the surface pool of each column.

Profiles of Cr concentrations and oxidation states in soil columns were obtained at various times after exposure to Cr(VI) solutions, using micro- x-ray absorption near edge structure (micro-XANES) spectroscopy. Micro-XANES measurements (Sutton and Rivers, 1999; Bertsch and Hunter, 2001) were obtained at beamline X26A of the National Synchrotron Light Source (NSLS, Brookhaven National Laboratory, Upton, NY), and the GeoSoilEnviroCARS beamline 13ID-C at the Advanced Photon Source (APS, Argonne National Laboratory, Argonne, IL). Large beam sizes of about 300 µm were used in all measurements in order to average over a large population of mineral grains and pores. The broad beam size also helped minimize x-ray beam-induced Cr(VI) reduction, which was a problem encountered when obtaining micro-XANES data at the brighter GeoSoilEnviroCARS beamline. By using a large spot size and keeping exposure times to less than 15 s per sample location, beam-induced Cr(VI) reduction was kept to less than 6%. No x-ray induced Cr(VI) reduction was detectable in samples measured at beamline X26A. Matrix-matched Cr(III) and Cr(VI) standards were prepared by mixing Cr_2O_3 and K_2CrO_4 into Altamont clays, to obtain Cr concentrations of 10,000 mg kg^{-1}. After collection of Cr K-edge XANES spectra on these Cr(III) and Cr(VI) standards (Fig. 2), soil columns were scanned with the monochromatic beam set at the pre-edge peak energy and at an energy in the range of 150 to 205 eV above the pre-edge absorption energy. The Cr fluorescence in this latter energy range was used to determine total Cr concentrations and to normalize pre-edge peak fluorescence for calculating the Cr(VI)/[total Cr] fraction since it exhibited less than 5% variations regardless of oxidation state. At the GeoSoilEnviroCARS beamline measurements were also obtained at 15 eV below pre-edge energy, for purposes of background fluorescence subtraction. Background

fluorescence subtraction was automatically executed during data collection at X26A. Periodic scanning of the monochromator over the pre-edge peak energy while monitoring fluorescence on a Cr(VI) standard was done to check for drift in energy calibration.

The density and activity of total microorganisms were measured by direct fluorochrome staining (Lloyd and Hayes, 1995) using LIVE/DEAD BAClight viability stain from Molecular Probes Inc. This direct stain was used to determine the total number of cells in a sample and the number that were viable. In order to determine the extent to which microorganisms contributed to Cr(VI) reduction in these soils, a separate set of sterile columns were tested under conditions that covered the range of Cr(VI) and OC additions used in the main experiment. Column sterilization was achieved by autoclaving 3 times; each time for 1 h at 110 kPa and 121°C, with 48 h between each autoclave event. These soils were treated with sterile 1,000 or 10,000 mg L^{-1} Cr(VI), and 0 or 4,000 mg L^{-1} OC (as TSB) solutions. Unlike the soil columns described previously, Cr(VI) and OC were infiltrated simultaneously into these systems. This method of exposure is not expected to yield significantly different results relative to sequentially exposing columns to Cr(VI) and then OC because Cr(VI) reduction rates were very low. Four replicates of sterile soil columns for each of the above combinations were incubated at 27°C. The columns were harvested on day 76. At this time we verified sterility by plating 10^{-2} and 10^{-3} dilutions of soil from each column onto tryptic soy agar plates, incubating the plates at 27°C, and checking for colonies at 24 and 48 h. The extent of Cr(VI) reduction was determined on day 84 by micro-XANES analyses of samples from each of the columns.

Results and Discussion
pH and redox potential measurements

Measurements of pH in water ponded at the surface ranged from 6.9 to 8.2, with typical values ranging from 7.2 to 7.9. Redox potential measurements in the Cr(VI)-contaminated columns remained relatively high throughout the study, showed little if any spatial trends, and showed no significant response to OC additions (Fig. 3). Values were typically in the range of +250 to +400 mV (standard H electrode referenced). For comparison, soils that were not exposed to Cr(VI) supported wider ranges in redox potentials, and a significant shift to lower potentials with addition of OC (Fig. 3, left panel). Redox potential data for soils not exposed to Cr(VI) come from a +0 OC control column monitored during this study, and from a +800 mg L^{-1} OC column (prior to Cr(VI) addition) tested in an earlier study (Tokunaga et al., 2001). The uncontaminated soils did show significant redox gradients, being more oxic at the surface and more reducing at great depths. The high redox potentials in the Cr(VI)-contaminated soils provided an indirect indication that microbial activity was low in each of these systems, since higher respiration rates combined with diffusion-limited oxygen supply would result in lower Eh with depth. Such sustained high redox potentials and inferred low microbial activity suggest that the initial exposure of soils to very high (1,000 and 10,000 mg L^{-1}) Cr(VI) concentrations resulted in long-term suppression of microbial communities. These high redox values also indicate that concentrations of 2 potentially important inorganic reductants, ferrous iron and sulfide, remained very low throughout the experiment.

Microbial

Analyses of microbial populations on day 130 showed low cell counts, ranging from 9.5 x10^4 to 2.1 x10^5 per g soil. Most of these soils were exposed to 1,000 mg L^{-1} Cr(VI) solutions, with varying levels of added OC. Based on their very low rates of Cr reduction, even lower cell counts are expected in the soils initially exposed to 10,000 mg L^{-1}. However, since these latter systems are being maintained for long-term studies, they have not yet been analyzed for their microbial characteristics. The highest cell count of 2.1 x10^5 per g soil was obtained from a control (with neither Cr(VI) or OC added). These low populations indicate that microbial communities exposure to Cr(VI) solutions did not grow significantly in response to later addition of OC. The densities and activity were significantly higher in the bottom half of the columns vs. the top half of the column in all of the columns exposed to OC, although these differences were not large enough to generate gradients in Cr profiles (following section). The control soils (no Cr(VI) added) and the soils with 1,000 mg L^{-1} Cr(VI) and no OC did not show significant differences in density or activity between the top and the bottom of the columns. No colonies were observed on any of the plates of sterile soils, indicating less than 10^2 culturable organisms per g sterile soil.

Total Cr and Cr(VI) profiles in soil columns

Total chromium ($\sum$Cr) concentration profiles were relatively uniform within individual columns. Temporal trends in the distribution of $\sum$Cr between Cr(VI) and Cr(III) showed net reduction over time. Representative micro-XANES maps from the soil column exposed to 1,000 mg L^{-1} Cr(VI), then treated with +4,000 mg L^{-1} OC are presented in Figs. 4A and 4B. Results from other soil columns are shown in later graphs in terms of average values of Cr(VI) concentrations relative to initial values, since intra-column gradients in Cr concentrations and oxidation states were not significant. Reduction of Cr(VI) occurred more rapidly with higher amounts of OC additions for both levels of initial Cr(VI), although the more highly contaminated soils (10,000 mg L^{-1} initial Cr(VI) solutions) generally require longer times to achieve low Cr(VI)/$\sum$Cr ratios. The relatively uniform distribution of Cr(VI) at any given time within individual systems indicated spatially uniform reduction rates. If significantly higher Cr(VI) reduction occurred within certain regions, these locations would exhibit locally lower Cr(VI)/$\sum$Cr ratios, as well as locally higher $\sum$Cr. The latter enrichment would occur through Cr(VI) diffusion into the region supporting a higher reduction rate. The reaction-limited condition observed in these columns is in strong contrast to the diffusion-limited cases examined in our previous experiments (Tokunaga et al., 2001). The previous study involved Cr(VI) diffusion into microbially active aggregates with previously established reducing conditions. Such conditions favored very localized Cr(VI) reduction and Cr(III) precipitation. In the present work, Cr(VI) solutions were rapidly infiltrated into aerobic, unsaturated soil columns having relatively low microbial activity. Low microbial activities were probably maintained throughout this study because of Cr(VI) toxicity.

Cr(VI) reduction rates

Time trends in ratios of Cr(VI) concentrations relative to initially added Cr(VI) concentrations were fit to first-order kinetic equations to permit comparisons among the different soil columns and with other studies (Figs. 5A and 5B). Each data set was fit to

$$[Cr(VI)] = [Cr(VI)]_0 \exp(-k_{oc}t)$$

[1]

where $[Cr(VI)]_0$ is the initial Cr(VI) concentration (mg kg^{-1}), the effective 1st-order rate constant k_{oc} (s^{-1}) is assumed to depend linearly on the amount of OC added, and t is time (s). Since Cr(VI) reduction in soils occurs via numerous interrelated processes, parameters from such expressions only approximately describe net rates. Individual Cr(VI) reduction pathways may better be described by other rate laws, as noted later for reactions with humic substances. A unique time zero for applying the above expression does not exist since the organic carbon solutions were diffused into the soils starting 5 days after infusion of Cr(VI) solutions. For simplicity, the time at which OC solutions were added was taken as time zero for fitting. For each of the 2 initial Cr(VI) concentrations, the 3 sets of Cr(VI) versus time data were fit simultaneously using Eq. [1] and

$$k_{oc} = a + b[\Delta OC]$$

[2]

where $[\Delta OC]$ is the amount of OC added per mass of soil (0, 112, or 560 µg g^{-1}), and a and b are fitting parameters. For each set of data consisting of a common initial Cr(VI) level and 3 values of $[\Delta OC]$, the parameters a and b were adjusted to globally minimize the root mean-square difference between data and Eq. [1]. The largest root mean-square difference (6%) obtained by this fitting procedure is within the measurement uncertainty. The k_{OC} values, ranging from 1.4×10^{-8} to 1.5×10^{-7} s^{-1} (Table 1), are all so low that Cr(VI) reduction in these systems is reaction rate limited rather than diffusion-limited (Thiele, 1939; Tokunaga et al., 2001). The lower values of k_{OC} in the higher Cr(VI) soils indicate that such high levels of Cr contamination have inhibitory effects on chemical and/or microbial Cr reduction capacities.

Comparisons between measured reduction rates in these soils and rate constants for specific reactions can help evaluate the importance of various pathways. Here, 2 relevant pathways are considered: (i) direct reduction by the indigenous microbial community and (ii) reduction by aqueous Fe(II). Rates of Cr(VI) reduction in mixed cultures obtained from soils and sediments were characterized at stationary (no growth) phase by Schmieman et al. (1998). They used a Monod approach to characterizing microbial Cr(VI) reduction,

$$-\frac{d}{dt}[Cr(VI)] = \frac{\mu_{Max}[X][Cr(VI)]}{K_s + [Cr(VI)]}$$

[3]

where μ_{Max} is the maximum specific rate constant (mg Cr(VI) (g dry cells)$^{-1}$ h^{-1}), K_s is the half velocity constant (mg L^{-1}, equal to the substrate concentration at which the reaction rate is half that of its maximum), and $[X]$ is the biomass concentration expressed as (g dry cells L^{-1}). The μ_{Max} values they obtained were in a fairly narrow range, from 1 to 3.3 mg Cr(VI) (g dry cells hour)$^{-1}$. Since our experiments were done with initial Cr(VI) concentrations of 1,000 and 10,000 mg L^{-1}, while Schmieman et al. (1998) obtained K_s

values less than 2 mg L^{-1}, effectively zero-order microbial reduction of Cr(VI) is predicted over most of the experiment with Eq. [4]

$$-\frac{d}{dt}[Cr(VI)] = \mu_{Max}[X] \qquad [4]$$

Although our trends are closer to 1st-order than zero-order (Figs. 5A and 5B), we continue with Eq. [4] in order to determine whether or not direct reduction of Cr(VI) by the microbial community is likely to be significant. For this purpose, we will use the highest value of μ_{Max} reported by Schmieman et al. (1998) of 3.3 mg Cr(VI) (g dry cells hour)$^{-1}$ = 0.92 μg Cr(VI) (g dry cells second)$^{-1}$, combined with the highest microbial cell counts of 2.1×10^5 cells (g soil)$^{-1}$ obtained in our soil columns. Since the soil:water ratio in the columns is 2.55 g mL^{-1}, this population is equivalent to about 5.4×10^5 cells (mL pore water)$^{-1}$ = about 5.4×10^8 cells L^{-1}. Assuming an average live cell volume of 5 μm^3 (a high estimate), the total live cell volume is 2.7×10^9 μm^3 L^{-1} = 2.7×10^{-3} cm^3 L^{-1}. Assuming a wet cell density of 1.1 g cm^{-3} and a solids content of 0.4 g g^{-1} (Paul and Clark, 1996), [X] = 1.2×10^{-3} (g dry cells) L^{-1}. Finally, combining these μ_{Max} and [X] values, yields a constant Cr(VI) reduction rate of -1.1×10^{-6} mg L^{-1} s^{-1} = -0.094 mg L^{-1} day^{-1}. Such a zero-order rate could account for only 0.4% to 3% of the measured Cr(VI) reduction by day 200 (Figs. 5A and 5B).

Aqueous Fe(II) is another potentially important reductant, especially under reducing conditions where its concentrations become appreciable. Chromate reduction at much lower concentrations of aqueous Fe^{2+} expected under the suboxic to oxic conditions of these soils was estimated through calculating Fe^{2+} concentrations in equilibrium with soluble iron hydroxides and oxyhydroxides, then applying Fe^{2+}-dependent rate expressions from the literature. Aqueous Fe^{2+} concentrations in equilibrium with ferric hydroxide were calculated over a range of suboxic and oxic pε, for pH 7 to 8, using the equilibrium relation at unit water activity (Sposito, 1994)

$$\log(Fe^{2+}) = 16.4 - p\varepsilon - 3pH \qquad [5]$$

Solubility products for relatively soluble Fe oxyhydroxides, ferrihydrite, amorphous ferric hydroxide, and soil ferric hydroxide from several other sources (Lindsay, 1979; Macalady et al., 1990; NIST, 1997) yield (Fe^{2+}) values that range within 1.5 log units of Eq. 5. These (Fe^{2+}) were then used to calculate effective 1st-order Cr(VI) reduction rate constants, k, based on the results of Sedlak and Chan (1997) and Pettine et al. (1998). The calculated Fe^{2+} concentrations are plotted in Fig. 6A. Calculated Fe^{2+}-based k values are shown in Fig. 6B, along with ranges of Pt electrode-based pε (from Fig. 3) and k_{OC} (from Fig. 5) representative of the nonsterile soils. Redox measurements obtained with Pt electrodes have been reported to characterize the Fe(II)/Fe(III) couple reasonable well (Macalady et al., 1990; Matia et al., 1991). However, it should be noted that in our case the Pt electrode-based pε values assigned to the soil columns are very approximate since they come from measurements on mixed, nonequilibrium systems (Lindberg and Runnells, 1984). Furthermore the relatively high redox potentials are indicative of Fe^{2+} concentrations that are too low to permit quantitative interpretation (Macalady et al., 1990; Stumm and Morgan, 1996). Nevertheless, the calculations summarized in Fig. 6B indicate that redox-predicted rate constants for Cr(VI) reduction by Fe^{2+} are consistent with much of the observed Cr(VI) reduction (Fig. 6B).

Several other factors are likely to contribute to the observed Cr(VI) reduction rates. These include redox reactions with soil organic matter, and catalytic influences of oxide surfaces (Deng and Stone, 1996; Wittbrodt and Palmer, 1996; Buerge and Hug, 1999) and organo-Fe(II) complexes (Buerge and Hug, 1998). However, detailed comparisons with these previous studies were precluded by lack of information on concentrations of reactive organic functional groups and catalytic surface areas of specific minerals in our soils. Nevertheless, the strong influences of OC concentration and microbial activity on Cr(VI) reduction were demonstrated through comparisons with sterile soils, as discussed next.

Reduction of Cr(VI) in sterile versus nonsterile soils
The extent to which various microbially-dependent pathways collectively contribute to Cr(VI) reduction in these soils was estimated through comparisons with reduction in sterile Altamont soils (Fig. 7). These comparisons are presented in terms of the unreduced Cr(VI) fraction remaining at day 84 since the sterile soils were analyzed at that time. Values for the nonsterile soils were obtained from evaluating 1st-order fits at day 84. Comparisons between the various sterile soils appeared to show slightly greater Cr(VI) reduction in response to OC addition (significant at   = 0.20 and 0.07 for initial Cr(VI) concentrations of 1,000 and 10,000 mg L^{-1}, respectively). Nonsterile soils exposed to 1,000 mg L^{-1} Cr(VI) had about 3 to 4 times greater reduction than their sterile counterparts, indicating that microbially-dependent pathways were dominant. In the systems exposed to 10,000 mg L^{-1} Cr(VI) without additional OC, differences between sterile and nonsterile soils were not significant, indicating negligible microbial influences at these extremely high Cr concentrations. However, these extremely Cr(VI) contaminated systems did exhibit significantly enhanced reduction upon adding OC (1-tail T-test   =0.01).

Organic carbon is assumed to be directly responsible for the observed Cr(VI) reduction in the sterile soils since addition of OC appeared to slightly increase the extent of reduction (Fig. 7) and since the autoclaving procedure decreases the concentration of another potentially important reductant, extractable Fe (Wolf et al., 1989). Wittbrodt and Palmer (1995, 1996, 1997) found that Cr(VI)-OC redox reactions are not adequately described by either 1st- or 2nd-order rate expressions and developed a new model to describe the kinetics of Cr(VI) reduction by humic substances. The rate of Cr(VI) reduction by soil humic acids depended on $[HCrO_4^-]^{1/2}$ and on the inverse of the fraction of humic acid oxidized (X_e)

$$\frac{d}{dt}[Cr(VI)] = -k'''[Cr(VI)]^{0.5} X_e^{-1}$$

[6]

In their experiments, reactions were conducted in batch solution reaction vessels so that all concentrations are relative to the aqueous phase, and the coefficient k''' was found to depend on pH and the initial soil humic acid concentration, $[SHA]_0$.

We applied the Wittbrodt-Palmer approach to the data on Cr(VI) reduction in sterile soils in order to estimate (i) the fraction of the native soil organic carbon pool that has a similar reactivity as TSB, (ii) effective rate constants for abiotic reduction in these soils, and (iii) the relative importance of microbially-dependent versus abiotic pathways.

Details of the model are given in Wittbrodt and Palmer (1995, 1996, 1997) so only major differences from conditions of their original studies will be noted here. Since the soils in our study had pH values in the 7.2 to 7.9 range, Cr(VI) in solution largely occurs as CrO_4^{2-}. In contrast, since the pK_{a2} of chromic acid is 6.5, $HCrO_4^-$ is the dominant Cr(VI) species under the more acidic conditions tested by Wittbrodt and Palmer (1995, 1996, 1997). Since our study was done in soil columns, the native humic substances are largely associated with the solid phase, not the more accessible solution phase. On the other hand, the soil matrix facilitates Cr(VI)-OC redox reactions through catalytic influences of Fe(III) and other oxides (Deng and Stone, 1996) that were not nearly as abundant in the batch suspensions analyzed by Wittbrodt and Palmer (1995, 1996, 1997). Thus, magnitudes of k''' values in our sterile soils are anticipated to differ from those of acidic suspensions of humic substances.

An integral function of Eq. [6] that is linear with respect to time (Wittbrodt and Palmer, 1995, 1996, 1997) was used to obtain values of $[SHA]_0$ and k''' in the following manner. This function has the form

$$z = -[SHA]_0 k''' t$$

[7a]

where

$$z = \frac{2}{3} \left\{ 3[Cr(VI)]_0 [Cr(VI)]^{1/2} - [Cr(VI)]^{3/2} - 2[Cr(VI)]_0^{3/2} \right\}$$

[7b]

and the subscript zeroes denote initial concentrations. The variable z is simply the product of $[SHA]_0$ times the variable y of Wittbrodt and Palmer (1996). Thus z/t gives $[SHA]_0 k'''$.

In the sterile soils, $[SHA]_0$ is assumed to represent the sum of added OC and the available portion of native soil OC having an equivalent reactivity as TSB. Thus, a plot of the dependence of z/t on added OC has an x-axis intercept at the negative of the equivalent native soil OC concentration. This procedure is illustrated in Fig. 8A, using the data from both levels of $[Cr(VI)]_0$ and with the organic carbon added expressed per unit soil mass. Although the intercept values for the two $[Cr(VI)]_0$ lines should be identical, they are ±26% relative to their average value. Using the average intercept, we estimate the concentration of OC in the original soil having the equivalent reactivity as TSB to be 340 ±90 mg kg^{-1}. This amounts to only 3.4% of the total soil OC concentration, reflecting the likelihood that dissolved TSB is much more available and reactive than most of the native soil OC. The Wittbrodt-Palmer rate constants for the sterile cases were then obtained from their associated z/t and $[SHA]_0$ values (Eq. [6a]). These rate constants are presented in Fig. 5 and Table 1.

An alternative estimate of the native soil OC fraction having similar reactivity as TSB can be obtained based on fitting of 1st-order rate expressions for Cr(VI) reduction in the nonsterile soils. Plots of the dependence of k_{OC} on added OC for all nonsterile systems are shown in Fig. 8B. The linearity of each relation simply reflects the use of Eq. [2]. Note the convergence of each line on the x-axis (at k_{OC} equal to zero) to a fairly common OC value. These intercept values of -332 ±55 µg OC per g soil are similar to those obtained based on the Wittbrodt-Palmer model applied to the sterile soils. Thus,

using 2 very different approaches, the portion of the native soil OC inventory having an equivalent reactivity as TSB is estimated to be 3%.

Time trends for the abiotic component of Cr(VI) reduction in the nonsterile soil columns were then calculated using Eqs. [7a] and [7b], with values of $[SHA]_0$ and k''' appropriate for specific combinations of $[Cr(VI)]_0$ and OC treatment. The calculated abiotic reduction trends are shown with the previously discussed nonsterile soil Cr(VI) reduction trends in Fig. 5. These comparisons between measured nonsterile and estimated sterile reduction trends underscore the importance of (indirect and direct) microbially dependent Cr(VI) reduction pathways. Moreover, when abiotic Cr(VI) reduction by OC occurs through a continuum of reactive carbon groups, abiotic reduction efficiency is predicted to diminish over time since the remaining carbon pool becomes more refractory. Thus, viable microbial communities are predicted to become relatively more important in reducing Cr(VI) at longer times.

Summary

Addition of organic carbon accelerates chromate reduction in soils through abiotic and microbially-dependent processes. Ferrous iron, native humic substances, and added OC support abiotic Cr(VI) reduction, which occurs at initial Cr(VI) concentrations as high as 10,000 mg L^{-1}. Under the slightly alkaline-oxidizing conditions of these experiments, microbially-dependent pathways dominated over abiotic reduction in all of the 1,000 mg L^{-1} initial Cr(VI) systems and in the OC-amended 10,000 mg L^{-1} initial Cr(VI) system. However, microbial populations in our soils were so low that direct microbial (enzymatic) reactions were not likely to have contributed significantly to the overall Cr(VI) reduction. Aqueous phase Cr(VI):Fe(II) redox reactions may be significant if Fe^{2+} concentrations are in equilibrium with relatively soluble, ferric hydroxide-like phases. Overall, synergistic interactions between microbial activity, OC degradation, Fe^{2+}, and mineral surfaces determine net rates of Cr(VI) reduction in these soils. The demonstrated accelerated reduction of very high levels of Cr(VI) by addition of OC indicate that this approach to in-situ remediation may be promising. However, similar studies on different soil and sediment types are needed. Finally, information on long-term stability of artificially reduced Cr(VI) is needed.

Acknowledgments

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Treatment	rate constant units	1,000 mg L^{-1} initial Cr(VI)	10,000 mg L^{-1} initial Cr(VI)
Nonsterile, +0 mg L^{-1} OC	s^{-1}	6.4 x10^{-8}	1.4 x10^{-8}
Nonsterile, +800 mg L^{-1} OC	s^{-1}	8.2 x10^{-8}	1.9 x10^{-8}
Nonsterile, +4,000 mg L^{-1} OC	s^{-1}	1.5 x10^{-7}	4.1 x10^{-8}
Sterile, +0 mg L^{-1} OC	M$^{0.5}$s^{-1}	1.0 x10^{-11}	2.3 x10^{-10}
Sterile, +4,000 mg L^{-1} OC	M$^{0.5}$s^{-1}	1.2 x10^{-11}	2.6 x10^{-10}

Table 1. Summary of Cr(VI) reduction rate constants.
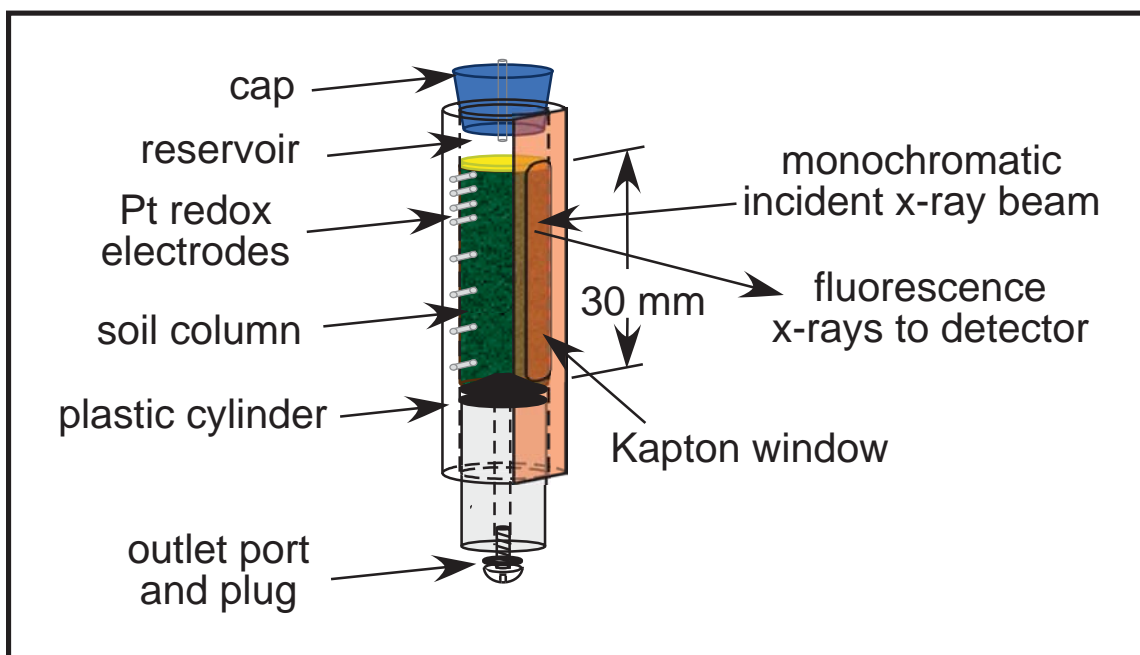
Figure 1. Soil column design.
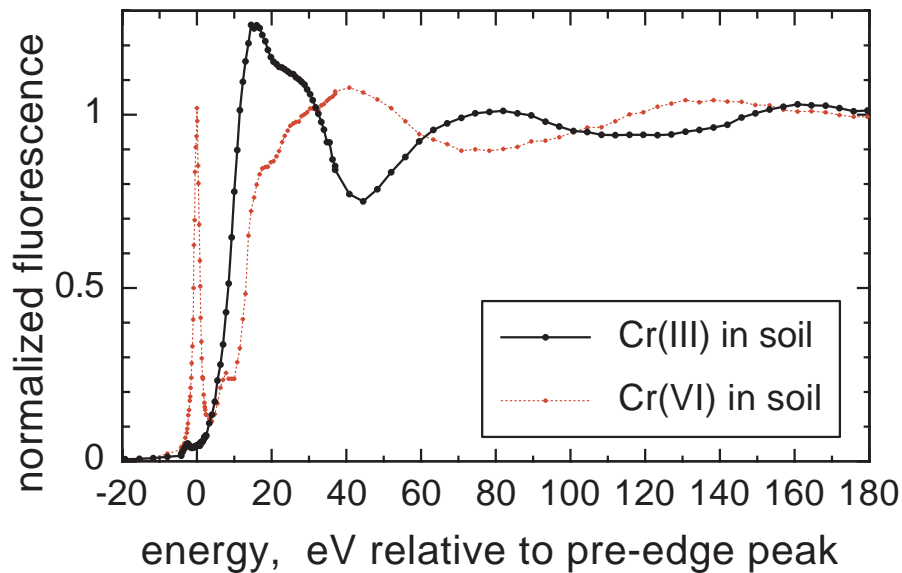
Figure 2. K-edge XANES spectra of Cr(VI) and Cr(III) in a matrix of Altamont soil.
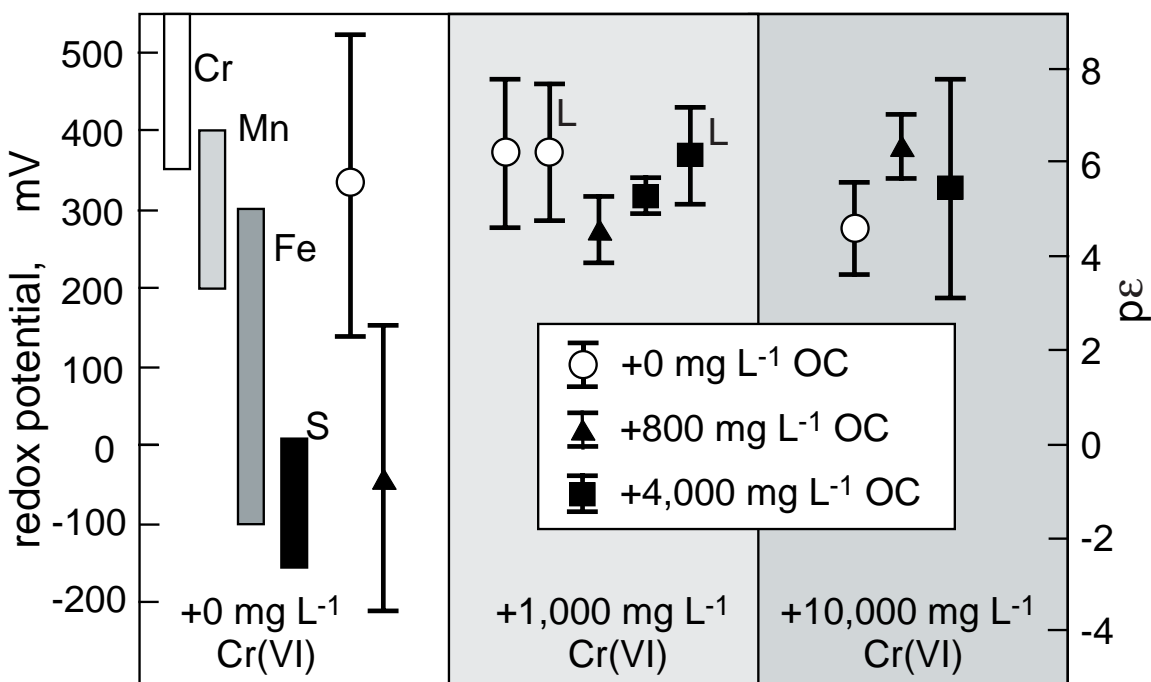
Figure 3. Redox potentials in soil columns (referenced to the standard H electrode). Symbols denote average redox potentials in the +0, +800, and +4,000 mg L^{-1} OC-amended columns, and bars denote ranges encompassed by ±2 standard deviations. No +4,000 mg L^{-1} OC treatment was included in the control (no Cr(VI)) soils. The cases where lactate served as the OC treatment are indicated by and "L" next to the data. Also shown are ranges of redox potentials associated with Cr(VI)/Cr(III), Mn(IV)/Mn(II), Fe(III)/Fe(II), and S(VI)/S(-II) transformations.
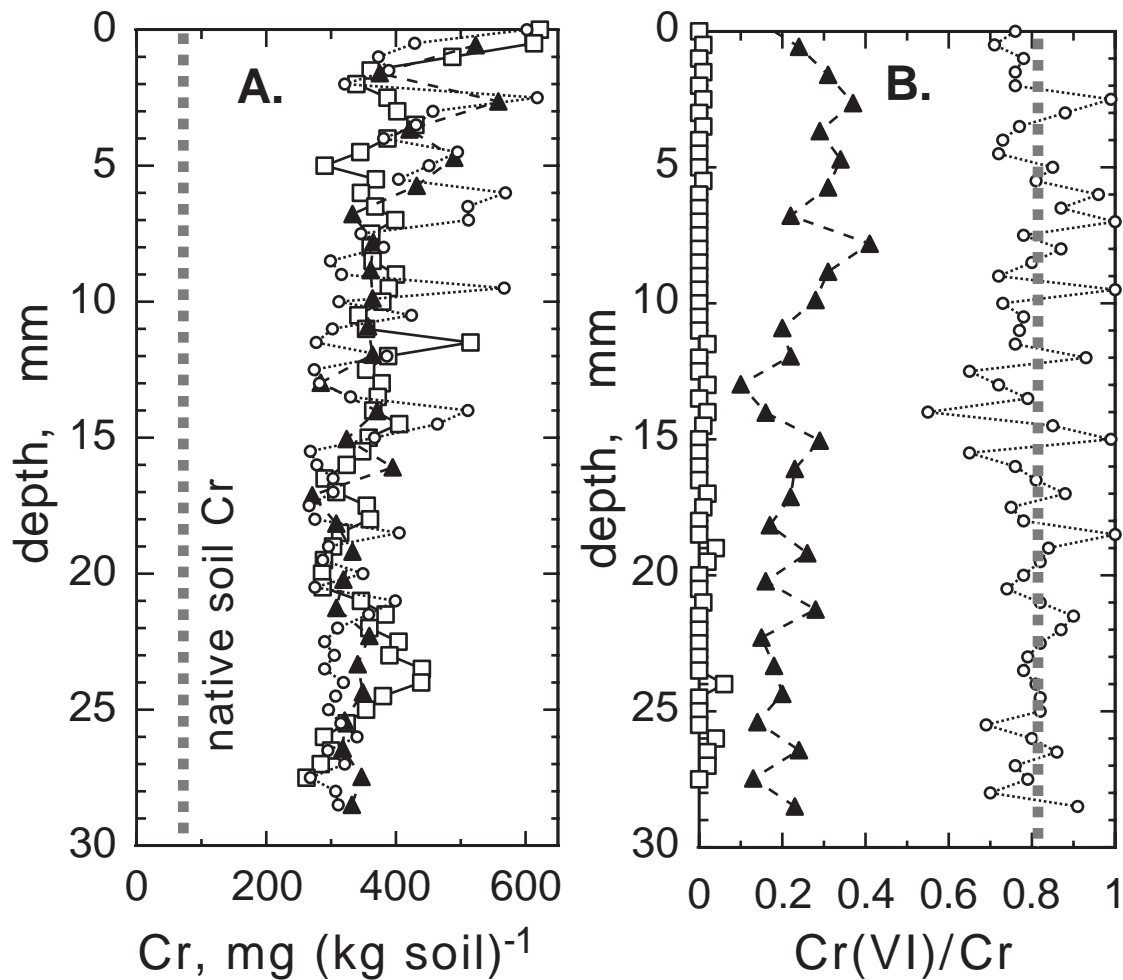
Figure 4. Profiles of Cr in soils contaminated with 1,000 mg L^{-1} Cr(VI) solutions, then exposed to 4,000 mg L^{-1} OC solutions (Days 5 (circles), 84 (solid triangles), and 205 (squares), relative to addition of OC). (**A**) Measured profiles of total Cr concentrations. (**B**) Measured profiles of Cr(VI) to total Cr concentration ratios, with the predicted profile at the initial addition of Cr(VI) shown as a dashed line.
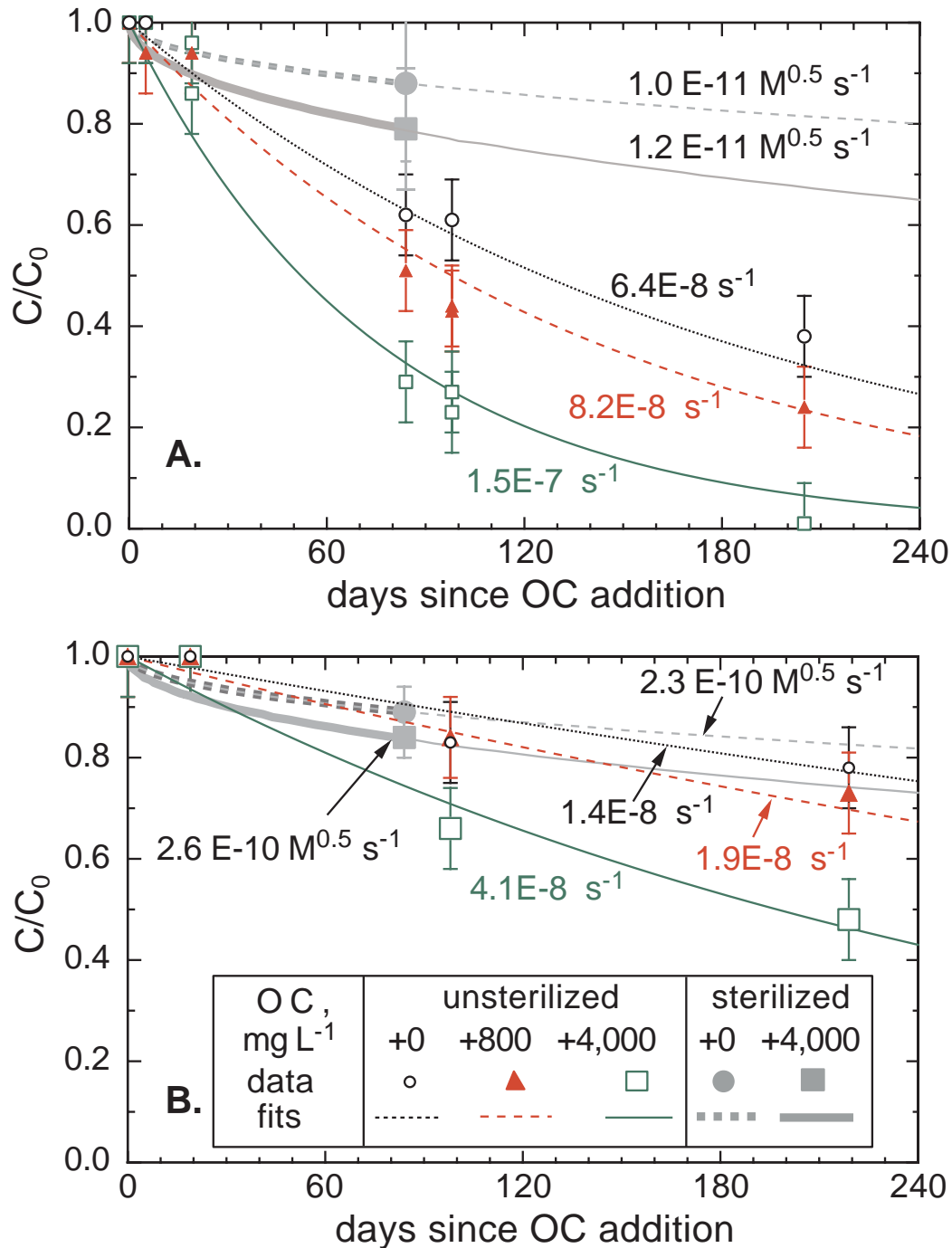
Figure 5. Comparisons between measured (points) and modeled (curves) Cr(VI) reduction trends for columns initially exposed to (**A**) 1,000 mg L^{-1} Cr(VI), and (**B**) 10,000 mg L^{-1} Cr(VI). Data from nonsterile soils were fit for 1st-order Cr(VI) reduction (finer lines, using equation 1) with effective rate constants shown. The sterile soil data (Day 84 only) were fit with Eqs. [5] and [6] (heavier lines, with k''' values shown). Extrapolations of these latter fits beyond Day 84 are shown as fine lines.
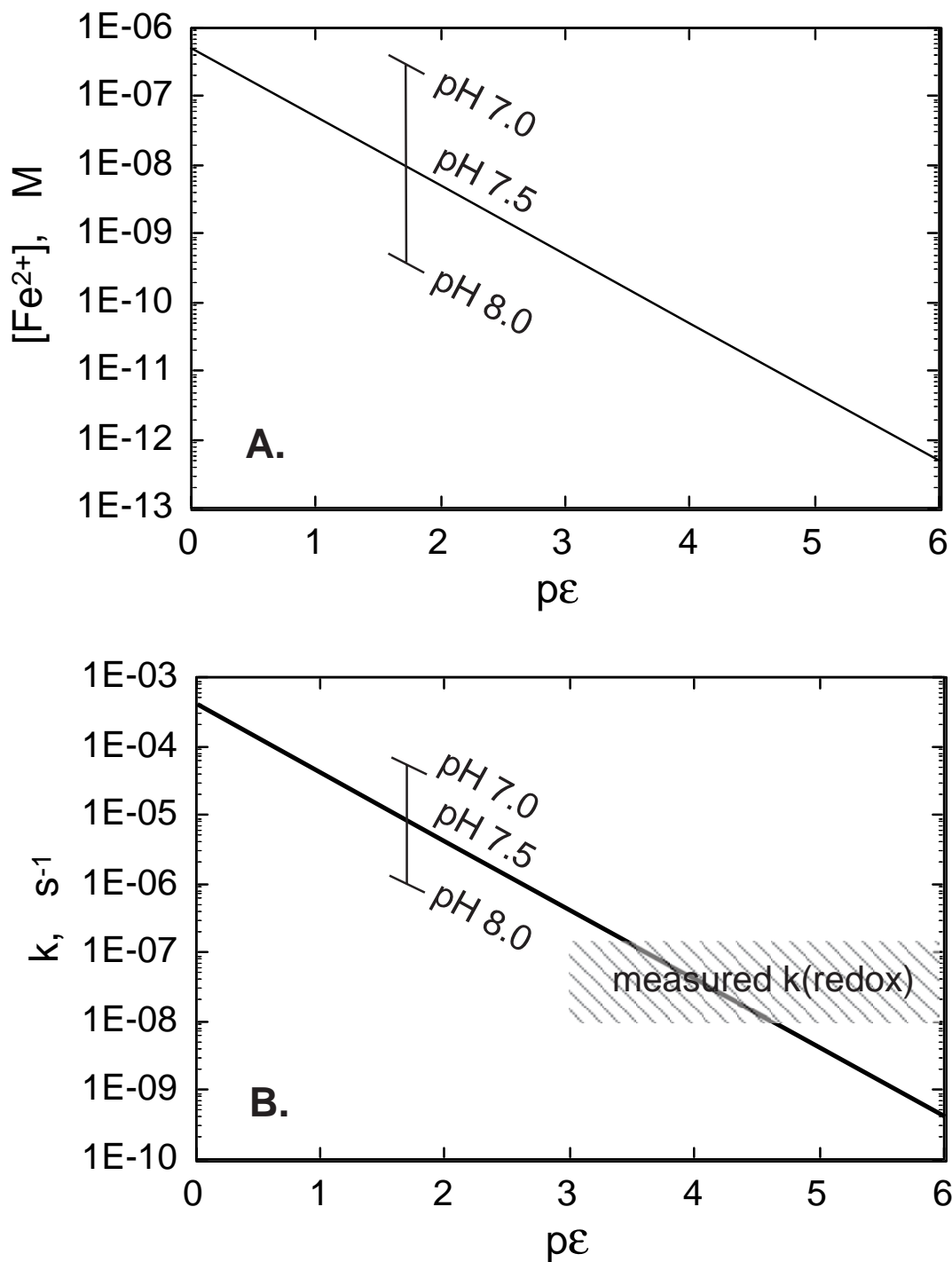
Figure 6. (**A**) Plots of aqueous Fe(II) concentrations for equilibrium with ferric hydroxide versus pε. Lines are for pH 7.5 (characteristic of the experiment), and range bars indicate shifts in Fe(II) concentrations for pH 7 and 8. (**B**) Effective 1st-order rate constants for Cr(VI) reduction by Fe^{2+} as functions of pε, for equilibrium with ferric hydroxide. Lines are for pH 7.5, and range bars indicate shifts in rate constants for pH 7 and 8. Also shown are ranges of measured k and estimated pε from the nonsterile soils.
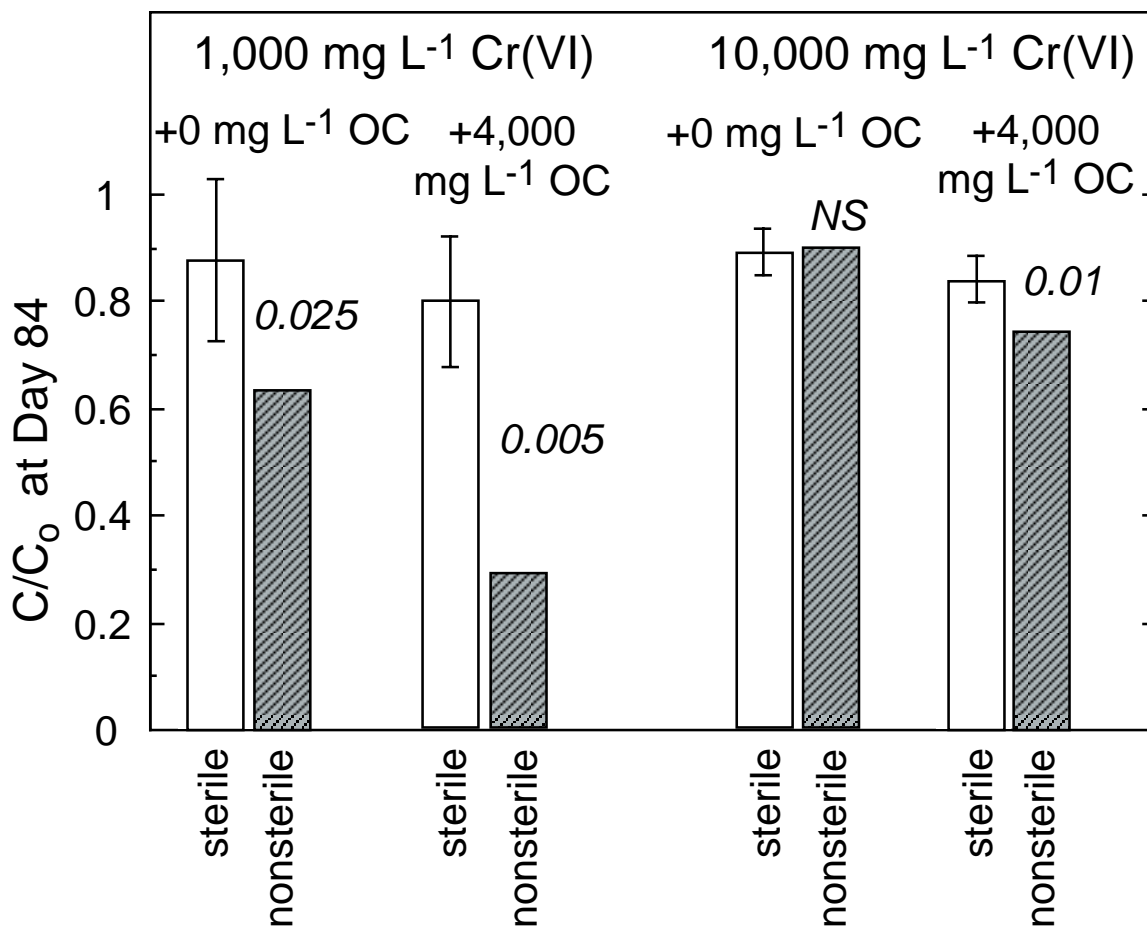
Figure 7. Comparison of Cr(VI) reduction at day 84 for sterile and nonsterile soils. Values for unreduced Cr(VI) fractions at day 84 for nonsterile soils were obtained 1st-order fits (see Fig. 5). Alpha values from T-tests of significance of further Cr(VI) reduction in nonsterile soils relative to their sterile counterparts are indicated in italics (*NS* = not significant).
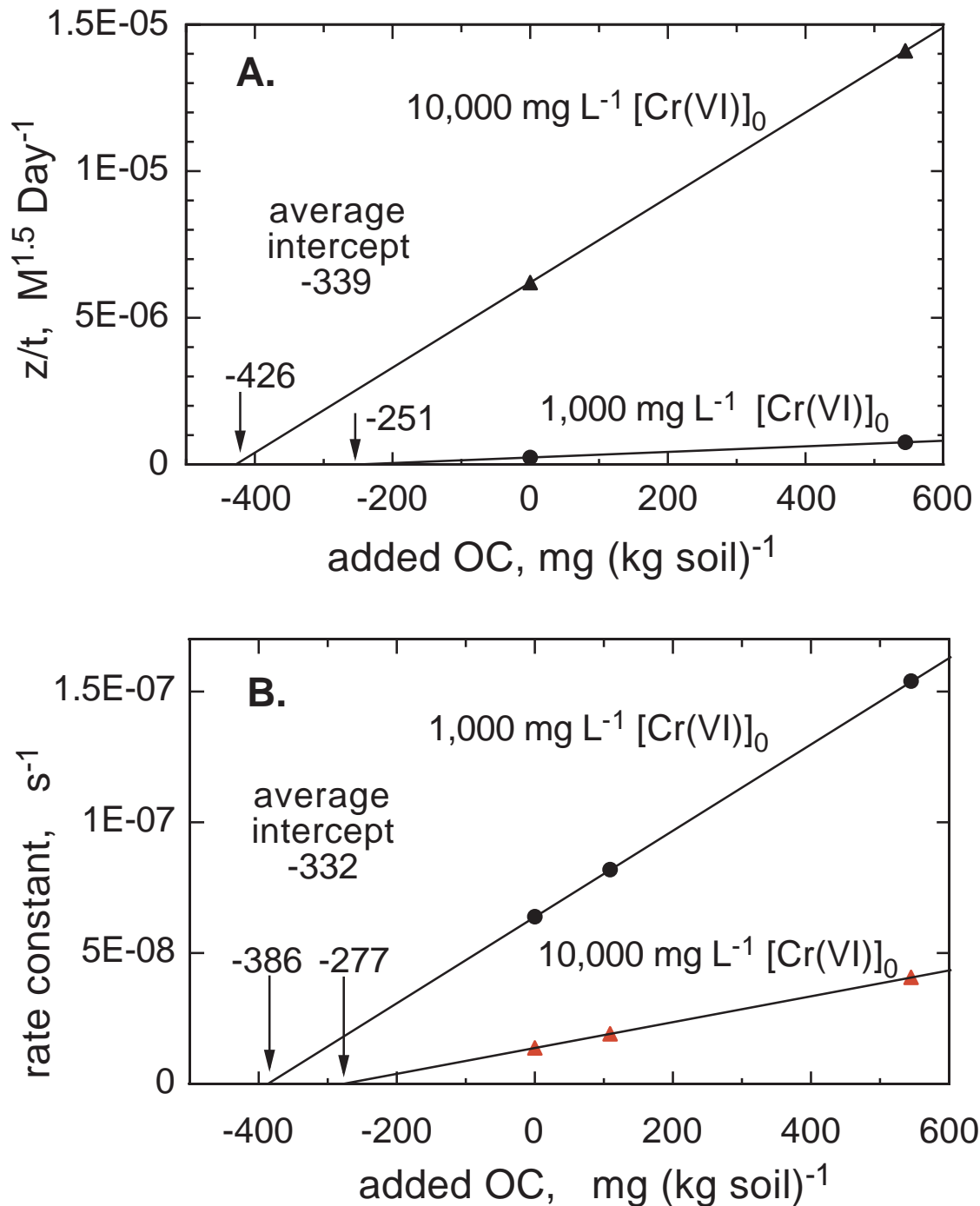
Figure 8. Estimating the concentration of the native soil OC that has an equivalent reactivity to Cr(VI) as tryptic soy broth. (**A**) Sterile soils, based on Eqs. [5] and [6]. This graph indicates that the native soil contains 339±88 mg kg^{-1} of OC having the equivalent reactivity of tryptic soy broth. (**B**) Nonsterile soils, based on Eqs. [1] and [2]. This graph indicates that the native soil contains 332±55 mg kg^{-1} of OC having the equivalent reactivity of TSB.