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Effects of Contaminant Concentration, Aging, and Soil Properties on the Bioaccessibility of Cr(III) and Cr(VI) in Soil*

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Contaminated soils at numerous U.S. Department of Defense, Department of Energy, and other industrial facilities often contain huge inventories of toxic metals such as chromium. Ingestion of soil by children is often the primary risk factor that drives the need for remediation. Site assessments are typically based solely on total soil-metal concentrations and do not consider the potential for decreased bioaccessibility due to metal sequestration by soil. The objectives of this research are to investigate the effect of soil properties on the bioaccessibility of Cr(III) and Cr(VI) as a function of contaminant concentration and aging. The A and upper B horizons of two well-characterized soils, representative of Cr-contaminated soils in the southeastern United States, were treated with varying concentration of Cr(III) and Cr(VI) and allowed to age. The bioaccessibility of the contaminated soils was measured over a 200-d time period using a physiologically based extraction test (PBET) that was designed to simulate the digestive process of the stomach. The sorption of Cr(III) and Cr(VI) varied significantly as a function of soil type and horizon, and the oxidation state of the contaminant. Solid phase concentrations with Cr(III) were significantly greater than Cr(VI) for any given initial Cr concentration. This is consistent with the mechanisms of Cr(III) vs. Cr(VI) sequestration by the soils, where the formation of Cr(III)–hydroxides can result in the accumulation of large mass fractions of contaminant on mineral surfaces. Overall, Cr bioaccessibility decreased with duration of exposure for all soils and at all solid phase concentrations, with aging effects being more pronounced for Cr(III). The decrease in Cr bioaccessibility was rapid for the first 50 d and then slowed dramatically between 50 and 200 d. In general, the effects of Cr solid phase concentration on bioaccessibility was small, with Cr(III) showing the most pronounced effect; higher solid phase concentrations resulted in a decrease in bioaccessibility. Chemical extraction methods and X-ray Adsorption Spectroscopy analyses suggested that the bioaccessibility of Cr(VI) was significantly influenced by reduction processes catalyzed by soil organic carbon. Soils with sufficient organic carbon had lower Cr bioaccessibility values (~10 to 20%) due to an enhanced reduction of Cr(VI) to Cr(III). In soils where organic carbon was limited and reduction processes were minimal, the bioaccessibility of Cr(VI) dramatically increased (~60 to 70%).

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INTRODUCTION

Chromium is used in many industrial processes, including electroplating, leather tanning, pulp production, and wood preservation, and, consequently, can be found throughout the environment (Nriagu and Nieboer, 1988). There are two main oxidation states of chromium found in the environment, anionic Cr(VI) and cationic Cr(III). The two forms of chromium have distinct behaviors in subsurface environments. The anionic Cr(VI) is considered to be highly mobile in soils, while the Cr(III) cation is believed to be significantly less mobile (Chung *et al*., 1994; Fendorf *et al.,* 1997; Jardine *et al.,* 1999). In regards to human health, the two forms of Cr also have major differences, with Cr(VI) considered carcinogenic and mutagenic even at low concentrations, while Cr(III) is considered potentially harmful only at high concentrations (Levis and Bianchi, 1982).

Human health is the usual risk driver that motivates the likelihood of remediation at Cr- contaminated sites. The exposure pathway of concern is usually the ingestion of contaminated soil, especially by children who traditionally have greater hand-to-mouth contact (Paustenbach, 1989; Davis *et al.,* 1990; Sheehan *et al.,* 1991; Skowronski *et al.,* 2001). U.S. EPA soil action levels for Cr(III) and Cr(VI) are 78,000 and 390 mg/kg, respectively, which are protective of soil-ingestion exposures for children in residential sites. However, certain states within the U.S. have designated action levels as low as 310 and 0.2 mg/kg for Cr(III) and Cr(VI), respectively (Proctor *et al.,* 1997). When regulators establish clean-up criteria for chromium-contaminated soils, the ubiquitous metal-sequestering properties of the soils are typically not taken into account (Proctor *et al.,* 1997). Instead, the standards are generally universal for all soils and are usually based on that of a soluble salt of the metal and the assumption that 100% of metal present will be absorbed into the body (Ruby *et al.,* 1999). In order to accurately access the health risk posed by metal-contaminated sites, an improved understanding of the influence of soil sequestration on the bioaccessibility of Cr is needed, where bioaccessibility is defined as that amount of contaminant, which is soluble due to gastric function and has the potential to cross the intestinal wall (Hamel *et al.,* 1998).

Chromium adsorption in soil occurs under different conditions based on the oxidative state of the Cr ion. The Cr anion, Cr(VI), generally adsorbs to positively charged mineral surfaces via electrostatic attraction. Thus, conditions of decreasing pH result in enhanced adsorption of Cr(VI) (Zachara *et al.,* 1989). Surfaces with proton specific sites, particularly iron oxides, are mostly responsible for Cr(VI) adsorption (Davis and Leckie, 1980; Zachara *et al.,* 1987, 1988). Factors interfering with Cr(VI) adsorption include the presence of SO_4^{2-}, the presence of dissolved inorganic carbon (DIC), and Al substitution for Fe in oxides. With a limited number of positive surface sites in soil, there is often competition from SO_4^{2-} and DIC for those sites (Leckie *et al.,* 1980; James and Bartlett, 1983; Zachara *et al.,* 1987, 1988, 1989). Ainsworth *et al.* (1989) concluded that Al substitution in oxides reduces the amount of chromate adsorbed due to the difference in the charge characteristics of the surface sites.

Another important mechanism of Cr(VI) sequestration by soils is the reduction of Cr(VI) to sparingly soluble Cr(III). Electron donors such as organic matter and Fe(II) are capable of reducing Cr(VI). Organic matter and surface bound organics are extremely effective at reducing Cr(VI) to Cr(III) under acidic conditions (Bartlett and Kimble, 1976b; Jardine *et al.,* 1999) Likewise, Fe(II) bearing minerals are known to rapidly reduce Cr in soils (Anderson *et al.,* 1994; Peterson *et al.,* 1997). Low soil pH facilitates the reduction reaction through the release of Fe(II) from soils (Eary and Rai, 1991). Iron sulfides also have the ability to rapidly reduce Cr(VI) to Cr(III), suggesting that complete dissolution of Fe(II) does not have to occur before the Cr can be reduced (Patterson *et al.,* 1997). These results imply that the reduction is taking place at the solid-solution interface making, FeS an effective reductant of Cr(VI).

Cationic Cr(III) also sorbs to soil through a variety of mechanisms. The pH of the soil has a strong influence on Cr(III) adsorption because changes in pH affect the variable charge on minerals and organic matter. Conditions of higher pH creates more negative surface sites on soil mineral surface and organic matter to which Cr(III) can sorb (Sparks, 1995). Further, at pH conditions above 5.5, Cr(III) rapidly precipitates from solution and forms hydroxides on the soil surface (Bartlett and Kimble, 1976a). These hydroxides have low solubility and therefore are not likely to dissolve and reenter the soil solution (Losi *et al.,* 1994).

With all the highly variable factors influencing chromium's ability to sorb to the soil surface, blanket clean-up regulations that ignore the importance of individual soil properties may not be accurate with regard to human health risk. The objective of this research was to investigate the effect of soil properties on the bioaccessibility of Cr(III) and Cr(VI) as a function of contaminant concentration and aging. We show that soils can strongly sequester both anionic and cationic forms of Cr, which, under certain circumstances, dramatically decreases toxic metal bioaccessibility.

METHODS

Soil Type and Characterization

The A and upper B horizons of two soils were obtained from the Melton Valley and Walker Branch watersheds on the Oak Ridge Reservation (ORR) in eastern Tennessee. The soils are representative of Cr-contaminated sites common to the southeastern U.S. Selected physical and geochemical properties of these soils are listed in Table 1. The Melton Valley soil is an acidic Inceptisol derived from interbedded shales and limestone (Kooner *et al.,* 1995; Jardine *et al.,* 1999; Driese *et al.,* 2001). The soils are extensively weathered and devoid of carbonates. Illites dominate the < 2 µm clay fraction, and the clays are heavily coated with amorphous Fe – oxides and goethite. The pH and cation exchange capacity (CEC) of these soils range from 4 to 7 and 10 to 20 $\mathrm{cmol_c\ kg^{-1}}$, respectively (Jardine *et al.,* 1989). Walker Branch soils are an acidic Ultisol that has been weathered from the Knox Group (Arnseth and Turner, 1988), a dolostone sequence with occasional interbeds of limestone and shale. The soils are also extensively weathered and devoid of carbonates. Kaolinite dominates the < 2 µm clay fraction, and the clays are heavily coated with hematite and maghemite. The pH and cation exchange capacity (CEC) of these soils range from 4 to 6 and 4 to 6 $\mathrm{cmol_c\ kg^{-1}}$, respectively (Jardine *et al.,* 1989). All soils were dried in an oven at 40°C and gently crushed with a mortar and pestle to pass a 250-µm sieve.

Contaminant Addition to Soil

Ten grams of the soil and 100 ml of chromium solution were placed in a 200-ml glass centrifuge vessel, shaken, and allowed to equilibrate for 2 days. The spiking concentrations (dose rates) for Cr(VI), as K_2CrO_4, were 1000, 250, and 50 ppm at a pH of 6.0 and for Cr(III), as $CrCl_3$, were 500, 200, and 50 ppm at a pH of 4.0. After a 2-d equilibration period, the slurries were centrifuged and the supernatant was discarded. The soils were then rinsed with double deionized (DDI) water three times to remove chromium in the pore water and allowed to air dry. Once the soils were dry, they were gently crushed, homogenized, and then rewetted with DDI water to 30% moisture. The soils were kept in a container out of direct light and maintained at 30% water content in a moisture-saturated environment.

In Vitro Bioaccessibility

A physiologically based extraction test (PBET) was adapted from Ruby *et al.* (1996, 1999; Ruby, 2000, personal communication) to assess the *in vitro* bioaccessibility of Cr(III) and Cr(VI) from contaminated soils in humans. Sampling was conducted on the treated soils that had been allowed to age in the storage container for 1, 21, 50, 100, and 200 d after the initial treatment and subsequent wetting of the treated soils. Triplicate moist samples (~0.3 g dry weight) were placed in 50-ml polyethylene tubes to which 30 ml of 0.4 *M* glycine at pH 1.5 and 37°C was added. The slurries were quickly placed in a rotating water bath at 37°C and agitated at 30 ± 2 rpm for 1 h. The method was designed to simulate the stomach digestive system in humans and has also been used by Skowronski *et al.*

Table 1 Select soil physical and geochemical properties

	Particle size analysis							
	Sand (%)	Silt (%)	Clay (%)	Organic matter content (%)	pH (5mM $CaCl_2$)	pH (DDI)	Fe (g/kg)	Mineralogy of <2 μm clay fraction *
Melton A	56.2	30.0	13.8	3.55	6.91	7.18	10.68	
Melton B	30.8	50.4	18.8	0.42	4.23	4.87	22.07	$I_{45}IS_{20}V_{10}K_9VC_6M_5Q_3F_1$
Walker A	34.9	58.9	6.2	1.89	6.01	6.61	7.71	
Walker B	32.2	44.2	23.6	0.10	4.30	5.17	19.55	$K_{27}V_{27}VC_{14}Q_{13}I_{10}IS_5G_3F_1$

* K = kaolinite; V = vermiculite; VC = chloritized vermiculite; I = illite (soil mica); IS = interstratified 2:1; Q = quartz; G = gibbsite; M = montmorillonite; F = feldspar. Subscripts refer to the percent by weight of each mineral.

(2001) to assess Cr bioaccessibility in a sandy and a clayey soil. Supernatant was separated from the solid via centrifugation. The pH of the supernatant was measured to ensure that the final pH was within ± 0.5 pH units of the initial pH. This scenario held for all cases. Bioaccessibility was calculated as:

$$\%\ \text{Bioaccessibility} = \left(\frac{\text{Cr in PBET supernatant } (\mu\text{g / mL}) \times 30.0 \text{ mL } \div \text{ 0.3g dry soil}}{\text{Cr on soil surface } (\mu\text{g / g})} \right) \times 100$$

Standard deviations on computed %Cr(III) and Cr(VI) bioaccessibility values following triplicate PBET analyses ranged from 0.03 to 2.01 with the average standard deviation of all values being 0.52.

Chromium Analysis

The PBET supernatant was measured for Cr(VI) and Cr total (Cr_T). Cr(VI) was measured using a modified *s*-diphenylcarbohydrazide colorimetric method (Bartlett and James, 1979) with a UV-VIS spectrophotometer at a wavelength of 540 µm (HP model 8453, Palo Alto, CA). Analysis of Cr(VI) was performed immediately on rapidly cooled PBET solutions to avoid possible reduction of Cr(VI) to Cr(III) by glycine (Jardine *et al.,* 1999). Independent studies revealed that Cr(VI) reduction by glycine at 37°C and 1 h was insignificant. Total chromium was measured on a Perkin Elmer AAnalysist 800 atomic absorption spectrophotometer (Wellseley, PA). All standards used were made from an atomic absorption chromium standard (EM Industries, Hawthorne, NY). Cr(III) was calculated as the difference between Cr_T and Cr(VI).

Determination of Chromium on Soil

Total chromium on the soil was determined using a modification of EPA method 3052. The soil was digested in a CEM microwave, model MDS-81D, with hydrofluoric and nitric acid. Boric acid was added before sample analysis in order to facilitate the removal of hydrofluoric acid from solution through the formation of fluoroboric acid. Soils from the National Institute of Standards, with known concentrations of solid phase Cr, were also analyzed with each block of analyses. Samples were stored and analyzed for total chromium using Inductively Coupled Plasma.

Chromium Solid Phase Speciation

X-ray Adsorption Spectroscopy (XAS). Solid phase Cr was speciated using X-ray adsorption near-edge structure (XANES) spectroscopy, which was conducted at the Stanford Synchrotron Radiation Laboratory (SSRL) under dedicated running conditions. Scans were recorded from –200 to 300 eV around the K-edge of chromium (5989 eV), with 0.2-eV steps across the white-line and main-edge region. Energy selection was accomplished with a Si(*220*) double-crystal monochromator, with a 1-mm (h) x 20-mm (w) beam. Adsorption was measured by a proportional fluorescent X-ray production using a 13-element Ge detector (Cramer *et al*., 1988). Mass fractions of Cr(III) and Cr(VI) were determined for each soil using XANES spectroscopy by placing the soil in a 4- × 4- × 40-mm slot cut in an acrylic plate that was sealed with Kapton. The proportion of Cr(VI) relative to total chromium was then determined by the ratio of the white-line amplitude to the total atomic cross section and comparison to standard curves as described by Patterson *et al*. (1997).

Chemical Extraction. In an effort to indirectly quantify Cr(VI) reduction processes on the soils, sorption isotherms were constructed and the solid phase extracted with SO_4^{2-}. Because SO_4^{2-} competes well for Cr(VI) sorption sites, but does not compete well for Cr(III) sorption sites, an indirect measure of the reduction of Cr(VI) to Cr(III) should be possible. Approximately 1 g soil was placed in preweighed centrifuge tubes, and the soils treated with 15 ml of varying concentrations of Cr(VI) in 5 m*M* $CaCl_2$ that were adjusted to the pH of the soil. Samples were allowed to equilibrate on the shaker for 48 h. Soils were centrifuged and supernatant was saved for analysis. The Cr(VI) was extracted from soils with three sequential washings of 0.05 *M* Na_2SO_4. The equilibrium solutions and extraction solutions were analyzed for both Cr(VI) and Cr(III). The chromium extract was corrected for pore water Cr of the equilibration step.

RESULTS AND DISCUSSION

Influence of Soil Properties on Cr Sorption

As expected, soils treated with solutions containing Cr(III) adsorbed 2 to 10 times more Cr than those treated with Cr(VI) (Table 2). This results from a larger cation exchange capacity vs. anion exchange capacity and the propensity for Cr(III) to precipitate on mineral surfaces at pH values above 5.5. The adsorption of both Cr species became more similar on the WB B-horizon soil because acidic conditions and abundant Fe-oxides provided positive surface charges, thereby enhancing Cr(VI) sorption. Thus, mineral phases, particularly iron oxides, with proton-specific surface sites may effectively adsorb Cr(VI) at low to medium soil pHs (Zachara *et al.,* 1987, 1988, 1989; Leckie *et al.,* 1980; Davis and Leckie, 1980; Mayer and Schick, 1981). The A-horizon soils had a higher pH and organic matter content, creating an environment that was not conducive to Cr(VI) adsorption.

Table 2 Mass loadings of Cr(III) and Cr(VI) on soil (mg/kg) for various Cr treatment concentrations (ppm)

Cr(III)	500 ppm	200 ppm	50 ppm
Melton A	4479.42	1823.26	426.42
Melton B	2002.91	1430.68	452.02
Walker A	2421.67	1779.01	451.00
Walker B	1276.05	1070.32	445.20

Cr(VI)	1000ppm	250ppm	50ppm
Melton A	386.47	199.21	91.28
Melton B	269.14	219.86	150.00
Walker A	391.83	244.24	100.22
Walker B	423.48	330.53	218.25

In the case of Cr(III) the patterns of adsorption were reversed, where the A-horizon soils typically adsorbed more Cr than the B-horizon soils. The A-horizon soils characteristically had higher pH creating an environment that favored Cr(III) adsorption. Deprotonation of oxides and organic matter occurs in soils with higher soil pH values, which results in more negatively charged sites that attract cations such as Cr(III). Also, when the soil pH is above 5.5, as with the two A-horizon soils used here, the Cr(III) most likely precipitates from solution as hydroxides creating a surface coating on a variety of soil mineral surfaces (Bartlett and Kimball, 1976a). This suggests that larger solid phase concentrations of Cr(III) can often be expected in soils with higher pH and abundant inorganic and organic carbon as shown by Stewart *et al.* (2003).

Influence of Aging on Cr Bioaccessibility

Chromium bioaccessibility, as measured by the PBET method, decreased with time for all soils tested and at all solid phase concentrations (with the exception of the 50 ppm Cr(VI) treated MV-A soil), with aging effects being most pronounced for Cr(III) (Figures 1 and 2). Standard deviations on computed % Cr bioaccessibility values were on average 0.52, which were too small to show error bars on the triplicate-measured values of Figures 1 and 2. Analysis of variance (ANOVA) t-test on day 1 vs. 200 for each of the four soils, two contaminants (Cr(III/VI)), and

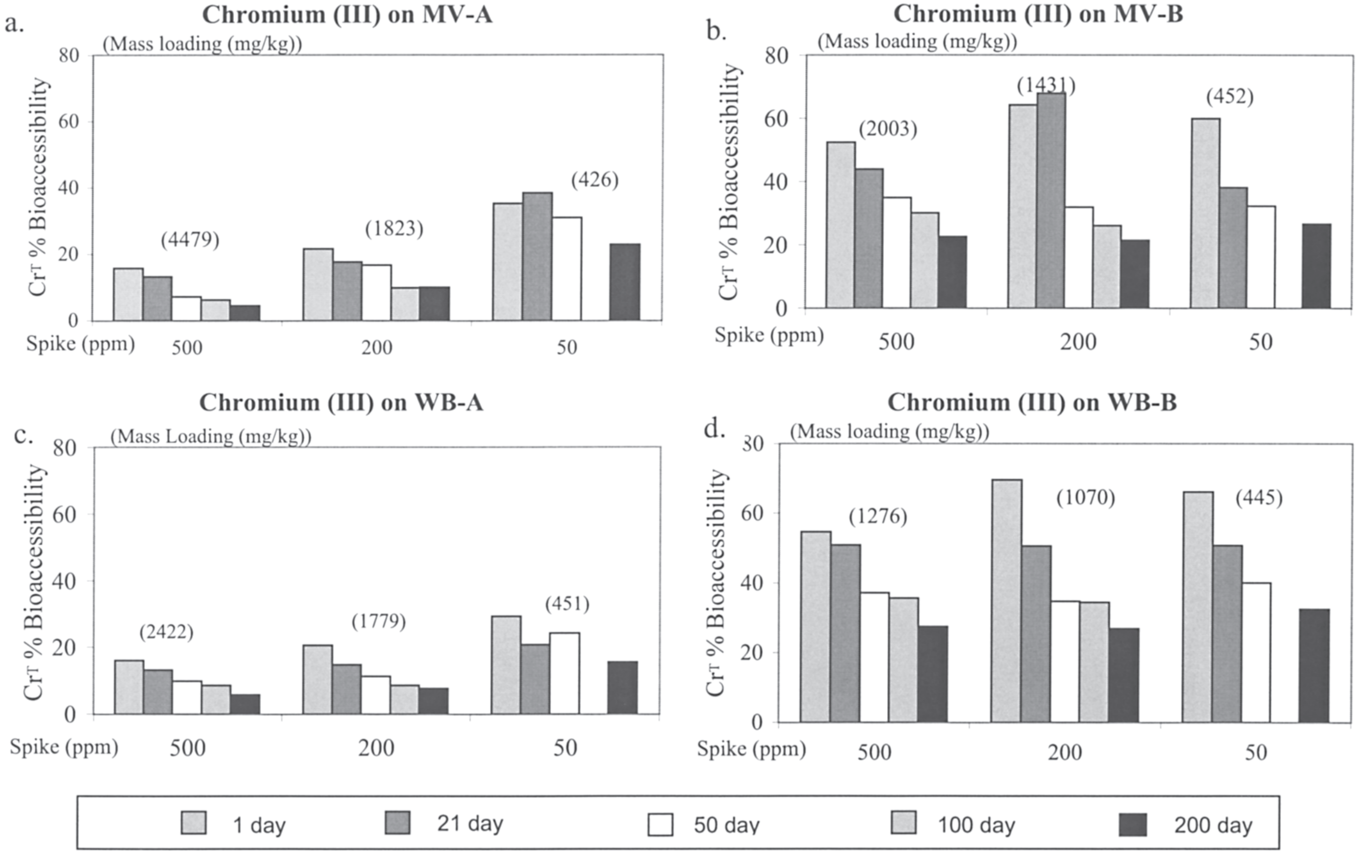

FIGURE 1

Aging and solid phase concentration effects on the percent Cr bioaccessibility for soils treated with varying concentrations of Cr(III) (50, 200, and 500 mg/L). (a) Melton Valley A-horizon soil, (b) Melton Valley B-horizon soil, (c) Walker Branch A-horizon soil, and (d) Walker Branch B-horizon soil.

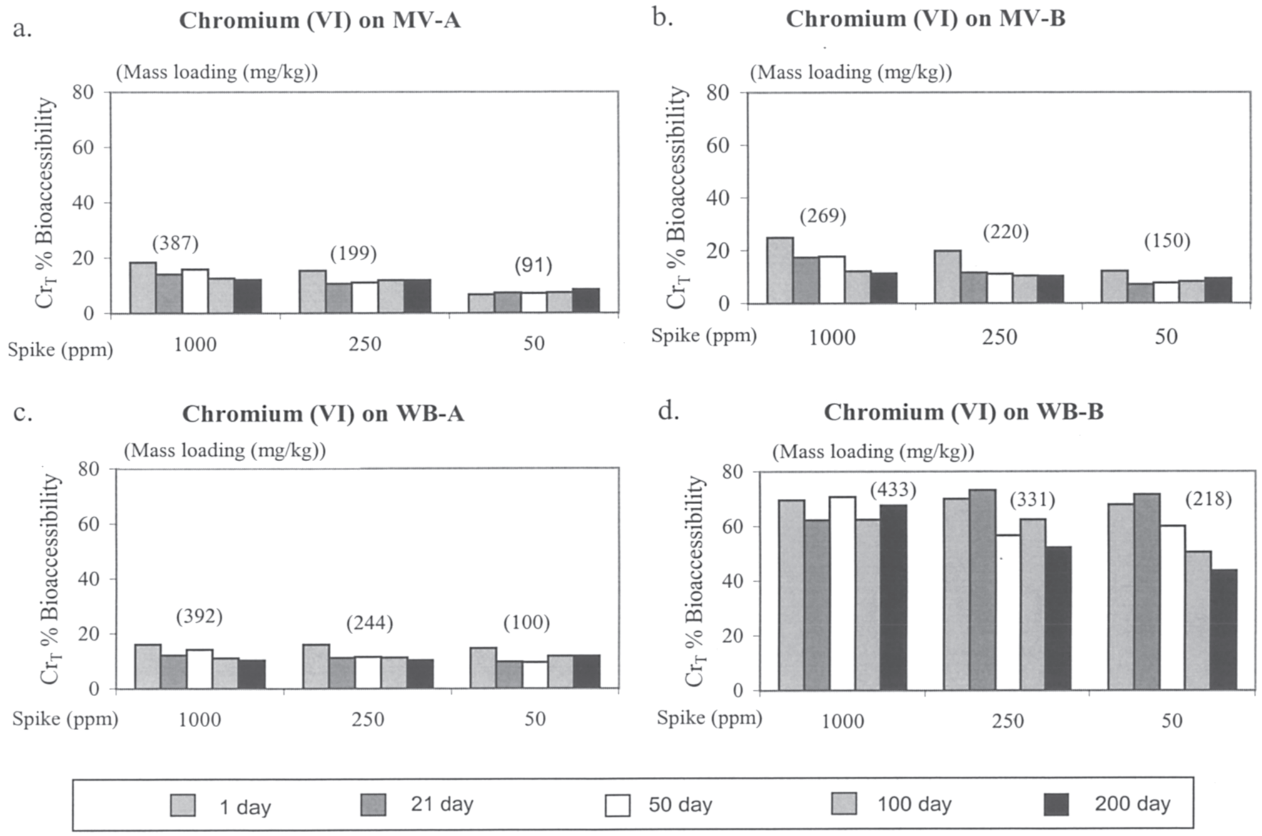

FIGURE 2

Aging and solid phase concentration effects on the percent Cr bioaccessibility for soils treated with varying concentrations of Cr(VI) (50, 250, and 1000 mg/L). (a) Melton Valley A-horizon soil, (b) Melton Valley B-horizon soil, (c) Walker Branch A-horizon soil, and (d) Walker Branch B-horizon soil.

three treatment concentrations (dose) confirmed that the aging effect was significant at the 95% level (i.e., most p values <0.0001) and was most pronounced on Cr(III)- treated soils (results not shown). The decrease in bioaccessibility was rapid for the first 50 d and slowed dramatically as the aging period approached 200 d. As the soils age, they most likely approach a state of equilibrium between the solution phase Cr and the surface of the soil. The aging effect is related to the enhanced stability of Cr on the soil surface with time. Structural reorientation of Cr surface bonds or, in the case of Cr(III), slow precipitation reactions can account for the stronger sorption of Cr at longer times. The greater aging effect observed for Cr(III) vs. Cr(VI) is most likely related to the time-dependent formation of solid phase $Cr(OH)_3$, which is not easily dissolved under the acidic conditions of the PBET.

Influence of Solid Phase Concentration on Cr Bioaccessibility

In general, the effect of Cr solid phase concentration (dose effect) on bioaccessibility was small, with Cr(III) showing the most pronounced effect. A comprehensive ANOVA test, discussed later in the manuscript, confirmed that the dose level exerts only a minor influence on Cr bioaccessibility (see Table 6). No obvious trends were noted for Cr(VI), whose bioaccessibility remained relatively constant at different solid phase concentrations on any given soil (Figure 2). For the Cr(III) system, particularly for A-horizon soils, higher bioaccessibility was noted for soils that were treated with 50 ppm Cr(III) relative to the higher concentration treatments. This is most likely related to the fact that at low surface coverage (< 20%) adsorption is the dominant process where Cr(III) forms inner-sphere complexes with the soil, while at higher surface coverages (> 20%) surface precipitation occurs and becomes the dominant process (Fendorf *et al.,* 1994; Fendorf and Sparks, 1994). The soils that were treated with 50 ppm Cr(III) have significantly lower Cr on the soil than the other soils treated with higher concentrations. Thus, the mechanism of Cr sequestration has a higher proportion of inner-sphere bonds related to precipitated phases, which most likely causes a higher percent of Cr(III) that is bioaccessible at lower solid phase concentrations.

Influence of Soil Properties on Cr Bioaccessibility

The bioaccessibility of Cr(III) and Cr(VI) varied significantly as a function of soil type and horizon, and the oxidation state of the contaminant. Statistical analysis using the ANOVA t-test confirmed that Cr bioaccessibility was significantly influenced by these effects at the 95% level with p values typically <0.0001 (results not shown). In general, A-horizon soils exhibited less Cr bioaccessibility relative to B-horizon soils. In the Cr(III) system, the higher organic matter content and higher pH of the A-horizon soils are probably the main factors responsible for this difference. The Walker Branch B-horizon (WB-B) soil is a good example of how soil properties effect the degree of bioaccessibility because it is the most acidic of the soils and has the lowest organic carbon content, and consequently it shows the highest percent of Cr(III) bioaccessibility (Figure 1d). Both the Melton Valley A-horizon (MV-A) and Walker Branch A-horizon (WB-A) soils have a high pH and high organic carbon content and an equally low Cr(III) bioaccessibility. These results are consistent with observations in Stewart *et al*. (2003) that showed that Cr(III) bioaccessibility was limited in systems with high levels of inorganic and organic carbon. Skowronski *et al*. (2001) also noted that Cr(III) bioaccessibility was lower on an organic-rich sandy soil vs. a clay soil that had significantly less organic carbon.

In the Cr(VI) system, the two A-horizon soils and the Melton Valley B-horizon (MV-B) showed statistically significant lower Cr bioaccessibility than WB-B for all treatment concentrations and aging times. Although the WB-B soil adsorbed the most Cr(VI), its tendency to release Cr under the acidic conditions of the PBET is due to the soil's inability to maintain the weak bond between the Cr and the surface. The Cr(VI) ion is probably electrostatically bound to mineral oxides through outer sphere complexes, which are unstable during the conditions of the PBET. This leads to the question of why is it that both A-horizon soils and even the Melton Valley Inceptisol B horizon soils (MV-B) have such low Cr(VI) bioaccessibility when soil properties are such as to discourage strong sorption?

To address the above question, both direct and indirect solid phase Cr speciation methods using X-ray Absorption Spectroscopy (XAS) and a chemical extraction technique, respectively, were employed. Analysis with XAS of the 250 and 1000 ppm Cr(VI) treated soils after 200 d aging suggested that all soils, except the WB-B soil, had Cr surface coverages that were > 95% Cr(III) (Table 3). The 250 and 1000 ppm Cr(VI) treated WB B-horizon soils contained only 30 and 53% surface bound Cr(III), respectively. Thus, the bioaccessibility of Cr(VI) was significantly influenced by the reduction of Cr(VI) to Cr(III). Skowronski *et al*. (2001) also suggested that Cr(VI) bioaccessibility in their soils was influenced by oxidation-reduction processes. In order for reduction to occur, there needs to be a source of electrons. Both organic matter and the Fe(II)-bearing minerals are able to supply electrons to catalyze the reduction of Cr(VI) to Cr(III). Because the soils used in this study were highly oxidized and most likely devoid of Fe(II)-bearing minerals, the reduction of Cr(VI) to Cr(III) was most likely catalyzed by soil organic matter or surface-bound organic carbon (Adriano, 1986; Sparks, 1995; Deng and Stone, 1996; Jardine *et al.,* 1999). Thus, extensive reduction processes for the A-horizon soils and the MV B-horizon soils are most likely related to the ample supply of organic carbon in these soils (Table 1). Even the WB B-horizon soil showed Cr(VI) reduction to Cr(III) with a solid phase carbon mass of 0.1%. Jardine *et al*. (1999) showed that in acidic soils the availability of even small amounts of surface-bound natural organic carbon (0.05% w/w on the solid) can result in significant reduction

Table 3 Percentage of soil solid phase Cr(III) and Cr(VI) quantified by X-ray Adsorption Spectroscopy (XAS) *

	Soild phase Cr(VI)	Solid phase Cr(III)
250 ppm	~~~~~~~ % ~~~~~~~	
MV-A	< 5	> 95
MV-B	< 5	> 95
WB-A	< 5	> 95
WB-B	70	30
1000 ppm		
MV-A	< 5	> 95
MV-B	< 5	> 95
WB-A	< 5	> 95
WB-B	47	53

* 200 d aged samples

of Cr(VI) to Cr(III). Therefore, Cr(VI) reduction decreases Cr bioaccessibility because the Cr(III) product is more tightly bound to the solid phase. The Cr(III) probably adsorbs to the surface through strong covalent bonds or precipitates as hydroxide complexes on mineral surfaces. Thus, the percent of Cr that is bioaccessible decreases during the PBET.

The XAS data are in agreement with aqueous Cr speciation measurements on the PBET solutions (Table 4). A significant portion of the total bioaccessible Cr was found to be Cr(III), with the WB B-horizon soil having the lowest total amount of extractable Cr(III) as indicated by the high Cr(VI) in Table 4. For all soils except WB-B, the trends in the data suggest an increasing percentage of Cr(VI) in the PBET extraction solution up to ~100 d followed by an abrupt decrease with continued Cr-soil aging to 200 d. These trends are consistent with the enhanced reduction of Cr(VI) to Cr(III) by the A-horizon soils and the MV-B soil relative to the WB-B soil. Using the 200-d aqueous speciation data coupled with the XAS solid speciation results (analyzed on 200 d aged soils), one can calculate the mass fraction of Cr(III) and Cr(VI) that are bioaccessible in each soil (Table 5). In all soils, the bioaccessibility of surface-bound Cr(VI) was significantly greater than that for Cr(III). Between 42 and 108% of the total adsorbed Cr(VI) was bioaccessible when compared with total adsorbed Cr(III), which was only 3 and 14% bioaccessible. Although Cr(III) may dominate total Cr in the PBET, surface-bound Cr(VI) is significantly more bioaccessible. Thus, the reduction of Cr(VI) to Cr(III) by soil

Table 4 Percentage of Cr(VI) in PBET extractant for soils treated with 250 and 1000 ppm Cr(VI)

	1 day	21 day	50 day	100 day	200 day
250 ppm	~~~~~~~~~~~~~~		%		~~~~~~~~~~~~~~
MV-A	28.73	61.90	78.02	64.91	46.26
MV-B	21.64	59.09	71.92	67.75	32.88
WB-A	16.54	22.86	57.28	28.61	26.38
WB-B	76.92	81.16	99.10	76.07	94.27
1000 ppm					
MV-A	3.81	15.29	6.31	44.09	21.89
MV-B	14.59	13.26	22.09	90.46	24.07
WB-A	4.23	8.15	4.31	31.53	21.25
WB-B	60.28	84.72	63.57	73.00	78.91

organic matter significantly decreases total Cr bioaccessibility. These results are important from a human health perspective because Cr(VI) is believed to be much more toxic than Cr(III), with even sub-ppm levels considered lethal. Thus, under certain circumstances, soils that contain sufficient organic carbon or Fe(II)-bearing minerals may be capable of decreasing Cr bioaccessibility through reduction of labile Cr(VI) to the more sparingly soluble Cr(III) species.

An indirect chemical extraction method was also used to show that Cr(VI) was being reduced to Cr(III). Chromium (VI) was adsorbed onto the soils using different treatment solution concentrations, allowed to equilibrate for 2 d, and then the solid phase was treated with 0.05 *M* Na_2SO_4 to remove the Cr(VI) (Figure 3 a-d). The SO_4^{2-} anion should be a sufficient competitor for surface sites occupied by $HCrO_4^-$ because the latter is typically sorbed to the solid phase through weak outer-sphere electrostatic bonds. Thus, if Cr reduction processes are minimal, the SO_4^{2-} should be able to recover nearly all of the initial adsorbed Cr(VI). The extractant Na_2HPO_4 was also utilized on select soils since the HPO_4^{2-} anion can aggressively compete for Cr(VI) that is bound to the soil by either inner- or outer-sphere surface complexes. The results compared favorably with the SO_4^{2-} system; however, the HPO_4^{2-} results were somewhat more erratic for reasons unknown to the authors, and thus the SO_4^{2-} system was preferred. In this study, the chemical extraction method can only be qualitatively compared with the XAS results because the latter technique was employed on 200 d aged samples, whereas the extraction method

Table 5 Percentage of total surface bound Cr(III) and Cr(VI) that was bioaccessible after 200 d aging

	MV-A		MV-B		WB-A		WB-B	
	Cr(VI) treatment conc. (ppm)							
	250	**1000**	**250**	**1000**	**250**	**1000**	**250**	**1000**
% Cr(III) bioaccessible	6.0	9.9	7.1	8.0	7.8	8.3	3.2	14.2
% Cr(VI) bioaccessible	101.0	51.5	65.5	48.1	52.5	42.3	71.1	107.5

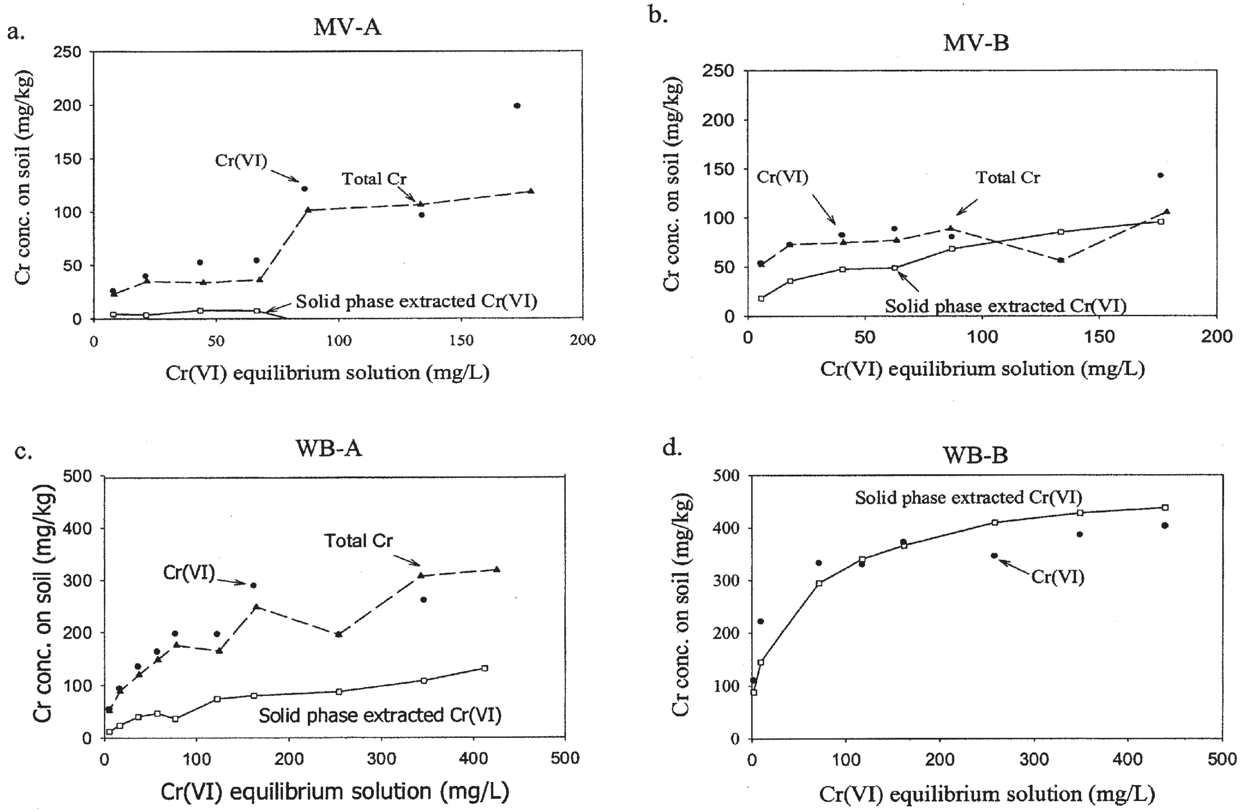

FIGURE 3

Adsorbed and SO_4^{2-} extractable Cr(VI) on soil as a function of solution concentration. (a) Melton Valley A-horizon soil, (b) Melton Valley B-horizon soil, (c) Walker Branch A-horizon soil, and (d) Walker Branch B-horizon soil.

was employed on samples aged for only 2 d. Jardine *et al*. (1999) previously measured a half-life of 85 h for Cr(VI) reduction by organic carbon, so samples analyzed after 200 d of aging should have more Cr(III) product than samples analyzed after 2 d of aging. Nevertheless, the chemical extraction method agreed well with the XAS results and the quantity of organic C in the soils. With the exception of the WB B-horizon soil, the quantity of Cr(VI) extracted from the solid phase was significantly lower than the initial Cr(VI) sorbed, implying that Cr(III) is being formed and remains sorbed to the soil (Figure 3 a-d). A-horizon soils had significantly more Cr(III) production when compared with B-horizon soils, which is consistent with the larger organic carbon content of the former. The WB B-horizon, which had as little as 0.1% organic carbon, showed no Cr(VI) reduction after 2 d (Figure 3b). The low organic content of this soil does not lend itself to the rapid reduction of Cr(VI) or is the source of iron, hematite (Fe_2O_3), and maghemite (γFe_2O_3) conducive to Cr(VI) reduction. The presence of Fe(III) suggests that the iron is already oxidized and therefore not in the correct state to facilitate the reduction of Cr(VI). This further explains why the percent of Cr that is bioaccessible in the WB-B soil remains so high compared with the other three soils examined. These results are consistent with the XAS findings that showed Cr(VI) reduction was nearly complete on all soils after 200 d, with the exception of the WB-B-horizon soil.

Factors Influencing Cr Bioaccessibility

The entire data set was analyzed using an ANOVA model that incorporated three qualitative factors (oxidation state, soil type, and dose level) and one quantitative factor (age). The original dose amounts were converted to low (50 ppm Cr(III) and Cr(VI)), medium (200 ppm Cr(III) and 250 ppm Cr(VI)), or high (500 ppm Cr(III) and 1000 ppm Cr(VI)) categories to simplify the statistical analysis. The complete four-factor ANOVA model explained more than 95% of the variance in bioaccessibility ($r^2 = 0.952$, $F = 127.74$, $p < 0.0001$) with a summary of the ANOVA results shown in Table 6. The oxidation state, soil type, and dose main effects were all significant as were the two-way and three-way interactions among these effects. Age and its interactions with oxidation state and soil type were also significant. However, age and its interactions with dose were only marginally significant. Thus, it is thought that these marginally significant results indicate that the dose level exerts only a minor influence on the relationship between age and bioavailability. It is also important to realize that some of the significance noted in Table 6 is driven by the high analytical precision of the bioaccessibility results. Thus, in certain cases it may be difficult to tease out statistical significance from geochemical and physical significance.

Table 6. Summary of analysis of variance results showing the significance of various factors on the bioaccessibility of Cr in soils.

Factor	SS	df	MS	F	Prob.
Oxidation	1630.7	1	1630.7	73.76	<0.0001
Soil	87926.5	3	29308.8	1325.70	<0.0001
Oxidation-Soil	21324.3	3	7108.1	321.51	<0.0001
Dose	264.4	2	132.2	5.98	0.0028
Oxidation-Dose	3459.0	2	1729.5	78.23	<0.0001
Soil-Dose	1091.5	6	181.9	8.23	<0.0001
Oxidation-Soil-Dose	1317.9	6	219.6	9.94	<0.0001
Age	9378.9	1	9378.9	424.23	<0.0001
Age-Oxidation	1753.8	1	1753.8	79.33	<0.0001
Age-Soil	2956.7	3	985.6	44.58	<0.0001
Age-Oxidation-Soil	621.8	3	207.3	9.38	<0.0001
Age-Dose	126.4	2	63.2	2.86	0.0589
Age-Oxidation-Dose	224.1	2	112.0	5.07	0.0068
Age-Soil-Dose	355.5	6	59.2	2.68	0.0151
Age-Oxidation-Soil-Dose	297.7	6	49.6	2.24	0.0391
Error	6632.4	300	22.1		

SS = sum of squares, df = degrees of freedom, MS = mean squares, F = F-value statistic, Prob. = probability that one obtains the F-value other than by chance.

ENVIRONMENTAL SIGNIFICANCE

This study has shown that the metal-sequestering properties of soil significantly lower the percent of Cr(III) and Cr(VI) bioaccessible after ingestion. The percent of bioaccessible Cr is largely independent of the initial solid phase concentration of Cr prior to the PBET simulated digestion. Sorption and bioaccessibility of Cr(III) and Cr(VI) vary significantly as a function of soil type and horizon, and the oxidation state of the contaminant. Soils with higher pH and abundant inorganic and organic carbon can often be expected to have higher solid phase concentrations of Cr(III), while for Cr(VI) the patterns are reversed, with Cr(VI) adsorption favored by lower soil pH and soil minerals with amphoteric charge. Aging effects show Cr bioaccessibility decreases after the first 50 d, and this is related to the enhanced stability of Cr on the soil surface followed by stable bioaccessibility to 200 d. Bioaccessibility of Cr(III) can be significantly reduced by its ability to bind strongly to organic matter and also to Cr – hydroxide precipitates on the soil surface, even under the conditions present in the PBET. Soil sequestration of Cr(VI) significantly lowers its bioaccessibility. Organic-rich soils and/or soils with Fe(II)–bearing minerals present enhance Cr(VI) reduction to Cr(III), with the latter being strongly adsorbed and less bioaccessible. This is important from a human health perspective because Cr(VI) is believed to be much more toxic than Cr(III).

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