

***QUANTIFYING THE BIOAVAILABILITY OF TOXIC  
METALS IN SOIL***

**CU 1166**

Strategic Environmental Research and Development Program  
Final Report

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## PREFACE

This report describes the final results of the Strategic Environmental Research and Development Program (SERDP) Project CU-1166, *Quantifying the Bioavailability of Toxic Metals in Soil*. Technical questions regarding this report may be addressed to Mark Barnett, Department of Civil Engineering, 238 Harbert Engineering Center, Auburn University, AL, +1 (334) 844-6291, [barnettm@eng.auburn.edu](mailto:barnettm@eng.auburn.edu); Phil Jardine, Environmental Sciences Division, Oak Ridge National Laboratory, P. O. Box 2008, Oak Ridge, TN 37831-6038; +1 (865) 574-8058, [jardinepm@ornl.gov](mailto:jardinepm@ornl.gov); or Scott Fendorf, Department of Geological and Environmental Sciences, Stanford University, Stanford, CA 94305, +1 (650) 723-5238, [fendorf@stanford.edu](mailto:fendorf@stanford.edu).

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## 1.0 PROJECT BACKGROUND

The primary objective of this research was to investigate the relative bioavailability of the toxic metals lead (Pb), cadmium (Cd), arsenic (As), and chromium (Cr) in soils, particularly in relation to the human health risk posed by soil ingestion, which often controls the degree of clean-up required at metal-contaminated sites. These metals were selected because they are the metals of greatest concern at Department of Defense (DoD) facilities (Exponent, 2001).

The research was motivated by the following hypotheses:

Hypothesis 1 The ubiquitous metal-sequestering properties of soil will significantly lower the relative bioavailability of ingested metals when compared to the 100% default relative bioavailability values currently used in risk assessments.

Soils are strong sequestering agents for metals, as aggressive digestion procedures are usually necessary to recover total metals from soils for analytical purposes. Metals may be sequestered in soils by a variety of mechanisms: adsorption to a variety of variable-charge mineral surfaces and coatings, particularly Fe- and Mn- oxyhydroxides; adsorption to fixed charge sites on clays; sorption to natural organic matter; and the formation of secondary solid metal phases such as carbonates, apatites, and sulfides. Many of these mechanisms will resist metal solubility in the mildly acidic (compared to acid digests used to recover total metals), short residence times of the GI tract. Thus, the generic nature of soil-metal interactions will lower the bioavailability of metals relative to soluble metal species, without regard to macroscopic speciation.

Hypothesis 2 Key soil physical and chemical properties [particle size, CEC, pH, Fe, Mn, and natural organic matter (NOM) concentration] will be good predictors of soil-metal bioavailability.

Soils sequester metals through a variety of mechanisms as discussed above, and soil characteristics should reflect potential metal sequestration. For example, the presence of large amounts of Fe and Mn oxyhydroxides and significant cation exchange capacity (CEC) should result in increased soil sequestration. Soils with different properties have been shown to differ significantly in their ability to sequester organic compounds (Chung and Alexander, 1998). Similarly, the *in vitro*-measured bioavailability of Pb and Hg was strongly related to soil properties, with fine-textured soils with high CEC exhibiting the lowest absolute bioavailability (Sheppard et al., 1995). However, there has been no systematic investigation of the bioavailability of Pb, Cd, As and Cr in soils and the quantitative influence of soil properties.

Hypothesis 3 Metal bioavailability is controlled by molecular-level speciation, where metal sequestration and solid phase stability are enhanced by increased soil-metal residence time.

Metal speciation in soils is dynamic, with increasing metal sequestration occurring over time due to the diffusion of metals into remote sites, the formation of secondary metal phases, and the incorporation of metals into re-crystallized solids (Ainsworth et al., 1994). These changes in molecular-level speciation control the bioavailability of metals in the GI tract, with acid-soluble and exchangeable metal species dissolving preferentially to more recalcitrant species. Direct detection of these changes in speciation will be

possible with synchrotron-based X-ray absorption spectroscopy (XAS), which can assess the chemical nature and bonding mechanism of metals on soil in undisturbed samples.

A series of hypotheses-driven tasks was undertaken to test these hypotheses. One of the biggest challenges in investigating metal bioavailability is in selecting a measure of bioavailability. Since the bioavailability of a contaminant is often receptor-dependent (e.g., the bioavailability of a metal in soil may be different to an earthworm than to a plant), fundamental (as opposed to site-specific) research requires general indicators of bioavailability. In this research, soil-metal bioavailability was measured with an *in vitro* protocol, a physiologically based extraction test (PBET) to estimate the bioavailability of soil-bound metals in the human gastrointestinal tract. The PBET (Ruby et al., 1996) is a batch-leaching procedure designed to simulate the solubility limitations in the human GI tract.

The bioavailability of Pb, Cd, As, and Cr was measured as a function of time in metal-spiked soils with a wide range of soil properties. Metal-spiked soils were used because the initial metal concentration and speciation could be controlled and changes in bioavailability and molecular-level speciation from the initial soluble metal could be followed with time. In addition, beginning with soluble metals provided insight into the capacity of soils themselves to limit metal bioavailability, without regard to any unique site-specific speciation, allowing the development of multivariable linear regression models to predict soil-metal bioavailability on the basis of soil properties. This research also featured the use of a powerful technique, synchrotron-generated X-ray absorption spectroscopy (XAS), to monitor unaltered molecular-level speciation to provide a better fundamental understanding of the relationship between bioavailability and speciation.

## 2.0 OBJECTIVE

The overall objective of this investigation was to examine the relative bioavailability of the toxic metals Pb, Cd, As, and Cr in soils, particularly in relation to the human health risk posed by ingestion of these metal-contaminated soils. Specific objectives of this investigation were to:

1. Measure changes in relative bioavailability over time of the SERDP priority toxic metals Pb, Cd, As, and Cr in a wide range of soil types that may be encountered at DoD sites within the U.S;
2. Develop a predictive capability to quantify toxic metal bioavailability on the basis of soil properties;
3. Investigate the fundamental relationship between molecular-level speciation and bioavailability to enhance our understanding of and capability to predict the fate of these toxic metals in soil.

### 3.0 TECHNICAL APPROACH

A series of multidisciplinary, multi-institutional tasks was implemented to test the hypotheses presented above. Specific methodologies are outlined in the peer-reviewed publications resulting from this project (Appendices A-G). The general methodology is described below.

Soil selection and characterization In order to examine the range of metal-sequestering properties of soils commonly found at DoD sites, we selected thirty-six uncontaminated soils (Table 1) from the seven major soil orders within the United States (principal regions shown in parentheses): Inceptisols (Northeast), Mollisols (Great Plains), Alfisols (Midwest), Ultisols (Southeast), Aridisols (Southwest, Mountain West), Spodosols (New England), and Entisols (Central). These soils covered a wide range of soil properties (particle size, CEC, pH, Fe, Mn, and NOM concentration), which we hypothesized to be important in controlling soil-metal bioavailability. These soils were provided courtesy of Mr. Warren Lynn of the National Resource Conservation Service (NRCS). The soils were moist sieved through a Teflon-coated 2 mm screen to remove large roots, rocks, etc. Select physical and chemical properties of the soils were supplied by the NRCS or measured by us using standard soil characterization techniques.

Toxic metal sequestration Metal-spiked soils, rather than actual metal-contaminated soils, were used for the majority of the research, because 1) the initial metal concentration and speciation could be controlled; 2) changes in bioavailability and molecular-level speciation from the initial soluble metal could be followed with time, leading to an increased understanding of the nature of metal sequestration by soils; and 3) beginning with soluble metals provided insight into the ability of soils themselves to limit metal bioavailability, without regard to any unique site-specific speciation. Reliance upon actual metal-contaminated soils would have limited the general applicability of the results as the presence of a particular metal species at one site may lower the bioavailability at that site while being of limited relevance to another site where that species is not present. By focusing on the bioavailability of initially soluble metal species in soils, we produced results that are dependent only on the generic metal-sequestering properties of the soils and so are applicable to a wide range of soils and metals.

The metals were added from stock solutions in a concentrated spike to a well-mixed soil slurry (with enough base to neutralize the acidity in the spike and maintain a constant soil pH). The metals were added to achieve a final concentration of 1000 mg/kg for Pb and Cd and 100 mg/kg for Cd and As. This concentration range reflects the general concentration of these metals in soil at DoD sites (Exponent, 2001), and the effect of concentration on metal bioavailability was also examined. Forty-eight hours after metal addition, the soil slurry was centrifuged and decanted and then washed three times with distilled water to remove any traces of the original soluble metal spike. After the last distilled water rinse was decanted, the moist soils were stored in the dark at room temperature. The soils were maintained in contact with the atmosphere to mimic the conditions of surface soils in the field and remoistened periodically. Bioavailability was measured initially after this 48 hour period, and then at 1 month, 3 months, and 6 months.



**Table 1 Some Physical and Chemical Properties of Soils Used in Study**

Soil	Soil Type	Series	pH	CEC (cmol <sub>c</sub> /kg)	Organic Carbon (%)	Inorganic Carbon (%)	Particle size (%)			Fe (g/kg)	Mn (g/kg)
							Clay	Silt	Sand		
1	Alfisol	Angola-A	5.29	6.70	3.72	0.96	32.1	56.1	11.8	23.28	1.23
2		Angola-B	7.86	4.50	0.09	0.25	25.6	52.9	21.5	5.83	0.19
3		Crider-A	6.57	5.60	0.55	0.39	22.5	75.8	1.7	13.34	0.72
4		Crider-B	5.26	5.40	0.21	0.13	30.9	67.2	1.9	13.38	0.3
5		Lenberg-A	5.92	7.90	3.41	1.01	49.1	44.5	6.4	12.94	1.37
6		Lenberg-B	4.35	5.50	0.36	0.25	64.7	29.5	5.8	15.69	0.12
7		Lawrence-A	4.97	5.80	0.91	0.59	19.5	48.5	32.0	11.17	1.35
8	Aridisol	Doakum-A	6.94	6.90	0.28	0.08	10.8	24.8	64.4	4.74	0.19
9		Doakum-B	6.84	7.00	0.39	0.18	29.3	15.0	55.7	6.86	0.16
10		Kzin-A	7.74	13.30	3.27	1.35	22.2	44.2	33.6	4.07	0.29
11		Kzin-B	7.8	10.00	3.4	1.88	27.0	38.5	34.5	3.26	0.18
12		Oricto-A	8.72	13.70	0.09	0.94	10.2	34.7	55.1	2.92	0.34
13		Oricto-B	9.01	8.60	0.16	1.1	23.2	27.5	49.3	3.16	0.29
14		Stoneham-A	6.42	10.10	1.45	0.71	16.2	41.4	42.4	3.4	0.26
15		Stoneham-B	6.8	7.80	0.66	0.32	21.4	23.2	55.4	2.2	0.2
16	Entisol	Wakeland-A	5.86	6.10	0.92	0	23.8	64.7	11.5	8.82	0.71
17		Wakeland-B	5.77	5.70	0.56	0.25	21.1	66.4	12.5	9.18	0.8
18	Inceptisol	Berks-A	3.65	9.10	2.72	1.01	15.7	46.6	37.7	13.18	0.15
19		Melton-A	6.91	8.00	3.55	0.62	6.0	69.0	25	10.68	1.42
20		Melton-B	4.23	14.00	0.42	0.26	19.0	42.2	38.8	22.07	0.17
21		Rockaway-A	3.86	10.60	3.54	1.49	12.4	34.8	52.8	14.03	0.52
22		Rockaway-B	4.1	3.70	0.21	0.18	12.6	32.1	55.3	17.34	0.16
23		Weikert-A	4.44	13.30	3.97	2.37	24.4	56.2	19.4	21.41	6.47
24		Weikert-B	4.28	8.00	2.01	1.15	23.9	54.3	21.8	28.98	5.42
25	Mollisol	Dennis-A	5.82	8.70	1.32	0.89	15.9	66.1	18	15.11	0.6
26		Dennis-B	4.77	4.40	0.38	0.41	29.7	57.5	12.8	24.29	0.59
27		Sibley-A	6.36	7.10	1.06	0.49	23.5	69.7	6.8	8.23	0.67
28		Sibley-B	6.36	6.80	0.72	0.52	26.9	68.0	5.1	9.11	0.59
29	Spodosol	Charlton-A	3.15	11.90	2.3	0.4	2.9	28.7	68.4	1.33	0
30	Ultisol	Allen-A	4.59	7.70	1.55	0.56	8.7	29.5	61.8	6.95	0.31
31		Allen-B	4.3	1.30	0.19	0.09	14.9	28.4	56.7	18.96	0.1
32		Cecil-A	4.04	5.80	1.64	0.39	10.2	23	66.8	6.01	0.06
33		Cecil-B	4.44	1.60	0.29	0.21	44.8	15.5	39.7	32.56	0.11
34		Lawrence-B	4.28	3.70	0.11	0.1	25.8	38.3	35.9	17.53	0.29
35		Walker-A	6.01	6.00	1.89	0.99	6.1	58.9	34.9	7.71	1.51
36		Walker-B	4.3	4.00	0.1	0.07	23.6	44.2	32.2	19.55	0.16
		Min	3.2	1.3	0.1	0.0	0.4	15.0	1.7	1.3	0.0
		Max	9.0	14.0	4.0	2.4	64.7	75.8	70.6	32.6	6.5
		Mean	5.6	7.4	1.3	0.6	21.0	42.5	36.9	11.9	0.8
		Std. deviation	1.5	3.2	1.3	0.5	16.9	21.8	8.0	8.0	1.3

Metal bioavailability A streamlined version of the physiologically based extraction test (PBET) (Ruby et al., 1996), which was designed to measure the bioavailability of metals in the GI tract, was used to measure “bioavailability.” Strictly speaking, the leachability of a metal in an *in vitro* test is termed the “bioaccessibility,” which is a surrogate for oral bioavailability (Ruby et al., 1999). The PBET has been evaluated by comparison to several animal studies for Pb and As (Ruby et al., 1996) and has been widely used in research (Williams et al., 1998; Rodriguez et al., 1999; Basta and Gradwohl, 2000; Hettiarachchi et al., 2000; Skowronski et al., 2001). To further increase the accuracy of the *in vitro* results, all bioavailability measurements were made on a relative (as opposed to an absolute) basis. Thus, soil-metal bioavailability was measured *in vitro* relative to the bioavailability of a soluble species of the same metal measured in the same manner. In this way, systematic differences between *in vitro*-measured bioavailability and true bioavailability were eliminated as long as any errors in the *in vitro* method could be assumed to be the same for the soluble metal and the metal in soil. As an example, if an *in vitro* methodology systematically underestimated absolute bioavailability by 20% and the true absolute bioavailability of a soluble metal salt and the same metal in soil were 80% and 40%, respectively, and the relative bioavailability (metal in soil to soluble metal salt) was 50% (40/80), then the measured absolute bioavailability of the soluble salt and the metal in the soil would be 64% and 32%, respectively (20% below the true values). However, the *in vitro*-measured relative bioavailability would yield the correct value of 50% (32/64). Thus, by measuring the relative bioavailability of metals in soils to soluble metals salts (on which the EPA reference dose (RfD) is based), we eliminated any systematic proportional errors in the bioavailability protocols. Where possible, bioavailability was measured relative to the exact form and conditions of the metal in the study used for determining the RfD.

Bioavailability statistical modeling After the bioavailability of each metal had been measured in each of the soils, hypothesis 2 was tested by formulating a model to predict the steady-state bioavailability from soil properties using multivariable linear regression and analysis of covariance. Separate models were developed for each metal. Within the multi-regression analyses, both Type I and Type III sum of squares analyses were performed to address the importance of soil chemical and physical properties on the variability in metal bioavailability. Type I analysis is a step-wise analysis which indicates the significance of adding more independent variables to explain the variability in the dependent variable (bioavailability). Type III analysis considers the importance of an independent variable to explain variability in bioavailability when other variables are in the model. Residual analysis was also conducted in order to assess the completeness of the model for describing the variability in metal bioavailability. We used the resulting models (one for each metal) to produce point estimates of steady-state bioavailability and the error associated with these estimates as a function of soil properties.

These models were validated in two ways. First, soil-metal bioavailability was measured in characterized soils not used in the model development and those measurements were compared to the predicted values (i.e., the models were tested using data that was not part of the original calibration data set). Secondly, we validated the results of our research by measuring the molecular speciation and bioavailability of some actual metal-contaminated soils. The bioavailability of each soil was measured using the

same protocols used for the metal-spiked soils. The molecular speciation for some of the contaminated soils was also investigated with synchrotron-generated XAS, as discussed below. The bioavailability results for the actual contaminated soils were used to validate the models' accuracy in estimating soil-metal bioavailability from soil properties. The criterion for validation was whether the measured bioavailability was less than or equivalent to (within model-estimated uncertainty limits) the bioavailability estimate. The measured bioavailability may be less than estimated because of the presence of unusual site-specific species. However, the model was still considered valid if it produced a conservative, yet better estimate of bioavailability than the 100% default value.

Toxic metal speciation To test hypothesis 3, solid-phase speciation on some samples was monitored with synchrotron-generated X-ray absorption spectroscopy (XAS), a powerful tool for probing the chemical and structural state of a contaminant. This task investigated the relationship between molecular speciation and bioavailability, focusing on samples with unusually high or low bioavailability or those registering significant changes in bioavailability over time. The greatest advantage of using synchrotron-generated XAS was the ability to analyze samples in their moist state. Synchrotron-generated XAS is one of the few atomic techniques for obtaining molecular level-information that can be conducted in unaltered samples, which is crucial for examining the true *in situ* molecular-level speciation of these metals. Drying soil samples fundamentally alters the solid-phase speciation by eliminating hydrated electrical double layers and potentially precipitating otherwise soluble metals species that were not part of the speciation of the original sample. By tuning the incident energy of the synchrotron beam, we could obtain element-specific excitation which provided both electronic and structural information about the element.

In the near-edge (XANES) region of XAS, the fingerprints of the chemical state of the element (electronic and some structural information) were obtained and used to quantify the abundance of specific phases within the sample. In the extended spectral (EXAFS) region, quantitative structural information was obtained about the element (the identity, distance, and number of atoms coordinating the x-ray absorber). This data provided key insights into the molecular-level speciation of these elements within soils, how they changed over time, and how molecular speciation influenced the resulting bioavailability. This portion of the research was conducted at the Stanford Synchrotron Radiation Laboratory (SSRL), located at Stanford University, CA.

## 4.0 SUMMARY

In our original proposal, we proposed to produce two products that would be directly beneficial to DoD site managers and risk assessors:

1. A scientifically defensible, peer-reviewed, publicly available methodology for predicting relative bioavailability of metals in soils, which could be used to provide both point and probabilistic estimates of relative bioavailability;
2. An improved understanding of the links between molecular speciation and bioavailability, the mechanisms by which bioavailability changes over time, and the soil properties that promote lower bioavailability.

These deliverables are documented in peer-reviewed publications described in Section 5.0 PROJECT ACCOMPLISHMENTS. Publication in peer-reviewed archival journals was accomplished to both disseminate the results and document their credibility. These results will provide site managers and risk assessors with better tools for making initial estimates of site risk and environmentally acceptable endpoints (EAEs) than the currently available 100% relative bioavailability default values. Although site-specific data will always need to be considered in making final clean-up decisions, these results can be used to prioritize sites and to justify more definitive site-specific bioavailability studies such as detailed soil speciation investigations and *in vivo* studies. They will also contribute to DoD's goal of mission readiness by avoiding unnecessary diversion of DoD funds for unwarranted site clean-up.

## 5.0 PROJECT ACCOMPLISHMENTS

The results of our project have been documented in a significant number of peer-reviewed papers published in leading journals. Peer review is important to disseminate the results and to lend credibility to the results from both a scientific and a regulatory perspective. These papers are included as appendices to this report (in no particular order) and are summarized briefly below.

**APPENDIX A Yang, J. K., M. O. Barnett, P. M. Jardine and S. C. Brooks (2003).** “Factors controlling the bioaccessibility of arsenic(V) and lead(II) in soil.” *Soil and Sediment Contamination* 12(2): 165-179. Soluble As(V) and Pb(II) added to soil exhibited reduced bioaccessibility (i.e., <100%). To our knowledge, this was the first paper to document the reduced bioaccessibility of metals in soil due strictly to soil-metal interactions as opposed to site-specific metal speciation. Since these results are due to generic soil-metal interactions rather than metal-specific speciation, they are valid as long as the major soil properties do not change. This paper also documents and explains (1) the significant and opposite pH dependence in Pb(II) bioaccessibility as compared to As(V) and how this is another source of uncertainty that should be considered in risk assessments; (2) the validity of the implicit, yet important assumption of concentration-independent metal bioavailability used in risk assessments; and (3) the effect of aging on As(V) and Pb(II) bioaccessibility in soils.

**APPENDIX B Yang, J. K., M. O. Barnett, P. M. Jardine, N. T. Basta and S. W. Casteel (2002).** “Adsorption, sequestration, and bioaccessibility of As(V) in soils.” *Environmental Science and Technology* 36(21): 4562-4569. Soluble As(V) added to thirty-six soils relevant to 21 DoD sites was not 100% bioaccessible in most soils, and in many cases was drastically reduced. A model was developed to estimate As(V) bioaccessibility from soil properties (pH and Fe oxides). This model was able to independently estimate the bioaccessibility of As(V) in five soils from different soil orders within a root mean square error of ~10%. This model was also able to predict the bioavailability of As in nine soils used in swine-dosing bioavailability studies within a root mean square error of ~10%. Such estimates can be used to prioritize DoD clean-up sites and justify more detailed site-specific bioavailability (e.g., animal feeding) studies.

**APPENDIX C Williams, L. E., M. O. Barnett, T. A. Kramer and J. G. Melville (2003).** “Adsorption and transport of As(V) in experimental subsurface systems.” *Journal of Environmental Quality* 32(3): 841-850. This research, which was partially supported by SERDP, documented the extreme effect of phosphate (and other physical and chemical conditions) on As(V) adsorption and transport. These results indicate phosphate amendments applied to soil to immobilize Pb could have a disastrous impact on As(V) mobility and availability.

**APPENDIX D Stewart, M. A., P. M. Jardine, M. O. Barnett, L. D. McKay, T. L. Mehlhorn, S. E. Fendorf and K. Paul (2003).** “Effects of contaminant concentration, aging, and soil properties on the bioaccessibility of Cr(III) and Cr(VI) in soil.” *Soil and Sediment Contamination* 12(1): 1-21. The A- and B-horizons of two well-characterized soils were amended with varying concentrations of Cr(III) and Cr(VI). The sorption of Cr(III) and Cr(VI) varied significantly in the

different horizons of the two soils, with Cr(III) being retained more strongly than Cr(VI). The Cr bioaccessibility decreased with aging over a 200-day period. Chemical extraction methods and XAS revealed that Cr(VI) was reduced by soil organic matter. Thus, soils with sufficient organic matter exhibited limited Cr bioaccessibility (~10-20%), while soils with limited organic matter exhibited higher Cr bioaccessibility (~60-70%).

**APPENDIX E** Stewart, M. A., P. M. Jardine, M. O. Barnett, T. L. Mehlhorn, L. K. Hyder and L. D. McKay (2003). “Influence of soil geochemical and physical properties on the sorption and bioaccessibility of Cr(III).” *Journal of Environmental Quality* 32:129-137. The bioaccessibility of Cr(III) added to thirty-six soils whose chemical and physical properties were relevant to 21 DoD facilities was typically <45% initially and <30% after 100 days of aging. Statistical analysis revealed that Cr(III) bioaccessibility was strongly correlated with the clay content and the total inorganic and organic carbon content of the soils, where higher quantities of inorganic and organic carbon resulted in decreased bioaccessibility. Chemical extraction methods and XAS speciation results confirmed that Cr(III) was not oxidized to the more toxic and bioavailable Cr(VI) species.

The following summaries are from draft manuscripts that are being prepared for publication in leading journals. These are mature drafts, but the results should be considered preliminary until officially published.

**APPENDIX F** Yang, J. K., M. O. Barnett, S.E. Fendorf, and P. M. Jardine. “Oxidation and bioaccessibility of As(III) in soils.” **Draft manuscript.** Soluble As(III) added to thirty-six soils relevant to 21 DoD sites and exhibiting a wide range of properties initially exhibited greater bioaccessibility than As(V). However, these differences decreased dramatically over a six-month period as As(III) was oxidized to As(V). A negligible fraction of As(III) remained after six months of aging and the bioaccessibility decreased correspondingly.

**APPENDIX G** La Force, M. J., G. Li, and S. Fendorf. “Temporal changes in soil partitioning and bioaccessibility of arsenic, chromium, and lead.” **Draft manuscript.** A critical step in evaluating the hazard imposed by trace element contaminants within soils is assessing their ability to migrate into water systems and their availability for biological impact. Their biological impact will be initially constrained by the degree to which a contaminant may dissociate from the soil solids and become available to a target organism—a parameter denoted as bioaccessibility. Here we used a physiologically based extraction test (PBET) to estimate the bioaccessible fraction of arsenic-, chromium-, and lead-amended soil. We investigated soils from the A and B horizons of the Melton Valley series, a soil of importance at the Oak Ridge National Laboratory facility, in order to address temporal changes in bioaccessibility. Additionally, common extractions that seek to define reactive pools of metals were employed and their correlation to PBET levels evaluated. With the exception of Pb amended to the A horizon, all other treatments exhibited a marked decrease in bioaccessibility with incubation time that was well described by an exponential decay. The bioaccessible fraction was less than 0.2 within 30 d incubation for As and Cr in the A horizon and for As and Pb

within the B horizon—Cr in the B horizon declined to nearly 0.3 within 100 d of aging. Neither oxalate nor acid-extractable pools exhibited discernable temporal trends and neither correlated well with PBET levels for As or Cr. The exchangeable fraction, however, did decline with incubation period and, in most cases, was highly correlated with the decline in bioaccessibility. In sum, our results demonstrate limited bioaccessibility in all but one case and the need to address both short-term temporal changes and, most importantly, the soil's physiochemical properties.

At least two additional journal publications will be prepared documenting the results of this investigation: 1) Jardine, P.M., M.A. Stewart, S.E. Fendorf, and M.O. Barnett, "The influence of soil properties on the redox transformation and bioaccessibility of Cr(VI)," manuscript in preparation; and 2) Yang, J.K., M.O. Barnett, S.E. Fendorf and P.M. Jardine, "Adsorption, sequestration, and bioaccessibility of Pb(II) and Cd(II) in soil," manuscript in preparation. These manuscripts will further document the behavior of Cr(VI), Pb, and Cd relative to soil bioaccessibility, with an emphasis on comparing model-predicted results with those from contaminated soils.

Other presentations, abstracts, and proceedings resulting from this work include:

La Force, M.J., S.E. Fendorf, M.O. Barnett, and P.M. Jardine. 2000. "Effects of residence time on contaminant bioavailability." *Soil Science Society of America*. Nov. 5-9, 2000, Minneapolis, Minnesota.

Stewart, M., P.M. Jardine, T.L. Mehlhorn, and M.O. Barnett. 2001. "Physical and chemical controls on the bioaccessibility of Cr(III/VI) in soil." *Soil Science Society of America*. October 21-25, Charlotte, NC.

Stewart, M. and P.M. Jardine. 2002. "Effects of soil geochemical and physical properties on the bioaccessibility of Cr." *SE-GSA Spring Meeting*, April 3-5, 2002. Lexington, Kentucky.

Jardine, P.M. 2002. "Soil chemistry needs and expectations at national laboratory facilities." *Soil Science Society of America*, Nov. 10-14, 2002, Indianapolis, IN (Invited).

Stewart, M.A., P.M. Jardine, T.L. Mehlhorn, M. Barnett, and S.E. Fendorf. 2003 "Quantifying the bioaccessibility of Cr and Cd in soils." *Annual International Conference on Contaminated Soils, Sediments, and Water*. Oct. 21-24, 2003, Amherst, MA.

Stewart, M.A., P.M. Jardine, S.E. Fendorf, and M.O. Barnett. 2003. "Influence of soil properties on the bioaccessibility of Cr(VI) in soils." *Soil Science Society of America National Meetings*. Nov. 2-6, 2003. Denver, CO.

Barnett, M. O., J. K. Yang, J. L. Subacz, P. M. Jardine and S. E. Fendorf, 2003. "Estimating the oral bioavailability of arsenic in soil." Presented at the *U.S. EPA Bioavailability Workshop*, April 15-16, Tampa, FL.

Stewart, M. A., P. M. Jardine, L. D. McKay, T. L. Mehlhorn and M. O. Barnett, 2002. "Quantifying the bioaccessibility of heavy metals Cr, Cu, and Cd in soils." Presented at the *23rd Annual SETAC Meeting in North America*, November 16-20, Salt Lake City, UT.

Barnett, M. O., J. K. Yang, P. M. Jardine, M. Stewart, S. E. Fendorf and M. J. La Force, 2000. "Factors affecting the bioavailability of toxic metals in soils." Presented at *SERDP/ESTCP Partners in Environmental Technology Symposium*, November 28-30, Arlington, VA.

Although all of the results from this research will ultimately be published, some important validation data that has not yet made it into draft manuscript form is described preliminarily here. We collected thirty-six samples of metal-contaminated soils from DoD facilities around the country and measured their major soil properties, total metal concentration, and bioaccessibility of Pb, Cr, Cd, and As (Tables 2 and 3). There was a wide range of metal bioaccessibility, ranging from <1 to 100%. The average bioaccessibility of As (5.5%) and Cr (10.1%) in these soils was considerably less than the average bioaccessibility of Pb (84.4%) and Cd (72.1%), a finding consistent with the results of our data for metal-spiked soils. These results will be documented completely in the additional publications described above, but the validation of these results for As will be described further here. Our earlier results (Yang et al., 2002) (Appendix B) indicated that soil pH and Fe oxide content were the major factors controlling the bioaccessibility of soluble As(V) added to soil. Using this simple model

$$\text{Bioaccessibility} = 11.3 * pH - 30.5 \log Fe \quad (1)$$

the measured pH and iron oxide contents (Table 2) were used to calculate the predicted level of As bioaccessibility for the given soil characteristics. The model results consistently over-predicted the As bioaccessibility measured in these soils (Figure 1), yet provided a better estimate than the 100% default value. This is a very important result, as the default bioavailability estimate for these soils would be 100%. Thus, the model produced in this research was able to consistently provide a more conservative yet better estimate for bioaccessibility than the default 100% value, precisely meeting the validation criterion outlined in our original proposal:

The criteria for validation will be whether the measured bioavailability is less than or equivalent to (within model estimated uncertainty limits) the bioavailability estimate. The measured bioavailability may be less than estimated because of the presence of unusual site-specific species. However, the model will still be considered valid as it produced a conservative, yet better estimate of bioavailability than the 100% default value.



**Table 2 Characteristics of Field Soils Used**

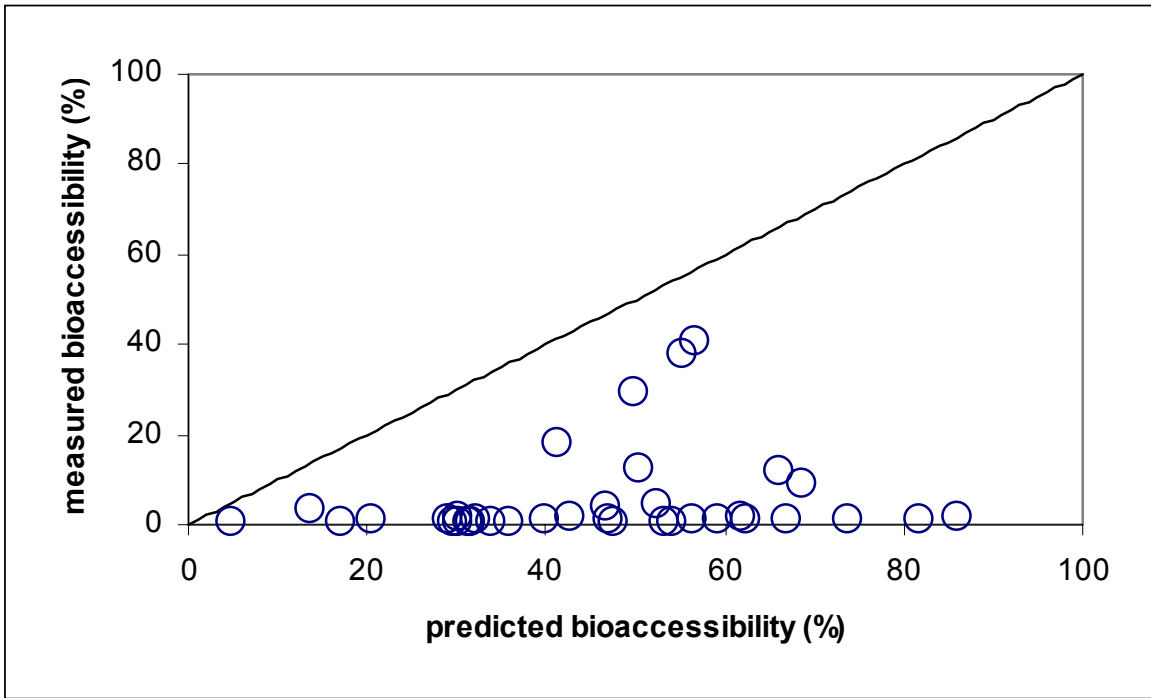
Sample ID	Fe (g/kg)	TC (%)	TOC (%)	TIC (%)	pH	pH	clay (%)	silt (%)	sand (%)	Pb2203	As1890	Cd2265	Cr2843
					(5mM CaCl2)	(DDI)				(mg/kg)			
Bolling AFB #1	12.83	2.95	2.29	0.65	5.83	6.50	8.0	26.0	66.0	104.39	102.48	1.22	59.36
Bolling AFB #2	12.97	2.54	1.94	0.61	4.83	5.47	8.0	26.0	66.0	140.15	101.37	1.22	66.35
McEntire ANG	5.46	0.90	0.80	0.11	6.20	6.85	9.0	14.0	77.0	N/D	93.11	1.17	57.67
Kansas AAP	10.17	2.59	1.55	1.05	7.52	7.82	11.5	16.5	72.0	1884.60	84.48	22.37	57.86
Radford AAP	23.34	0.64	0.49	0.15	4.11	4.63	19.0	20.0	61.0	4657.41	68.71	1.77	103.29
Savanna Depot	23.68	5.56	4.87	0.69	6.70	6.98	6.0	12.5	81.5	57.58	79.54	25.34	61.90
Hill AFB #1	7.47	2.60	2.27	0.33	7.35	7.96	10.0	37.0	53.0	1.44	93.23	2.82	70.97
Hill AFB #2	11.69	4.73	4.21	0.53	7.51	7.96	7.5	16.0	76.5	162.25	82.19	185.71	1143.27
Hill AFB #3	7.73	13.93	16.47	-2.55	7.30	7.43	4.0	26.0	70.0	393.98	149.82	464.75	2812.31
Hill AFB #4	12.25	13.29	15.81	-2.52	7.34	7.70	5.0	20.0	75.0	1057.76	179.77	453.56	2800.73
Hill AFB #5	7.90	7.68	8.02	-0.34	7.43	7.83	5.0	14.0	81.0	1218.88	144.78	806.87	1107.40
Hill AFB #6	13.20	7.21	7.65	-0.44	7.48	7.84	5.0	14.0	81.0	388.75	186.37	958.92	652.16
Travis AFB #1	19.21	1.64	1.44	0.20	6.08	6.52	23.0	33.0	44.0	1068.47	103.99	1.78	65.14
Travis AFB #2	19.28	1.83	1.30	0.53	6.14	6.54	13.0	28.0	59.0	1359.83	126.31	1.97	106.99
Travis AFB #3	18.90	0.97	0.74	0.23	6.21	6.69	24.0	30.5	45.5	219.81	119.98	2.17	73.71
Travis AFB #4	19.29	1.41	1.20	0.20	6.27	6.71	26.0	34.0	40.0	4016.92	126.86	1.92	77.48
Travis AFB #5	17.72	1.17	0.98	0.18	6.54	6.95	26.0	35.0	39.0	5808.71	145.96	2.38	81.82
Deseret CD #1	6.7	2.07	0.42	1.65	8.31	8.95	11.4	38.6	50.0	N/D	566.55	1.02	50.36
Deseret CD #2	7.62	3.74	0.44	3.3	8.24	8.72	16.7	41.4	41.9	N/D	671.00	0.68	14.87
Deseret CD #3	10.27	3.68	0.71	2.97	8.26	8.62	9.1	31.8	59.1	66.55	100.94	7.56	17.63
Deseret CD #4	7.51	4.72	1.13	3.59	8.30	8.44	10.2	30.7	59.1	137.20	128.76	8.74	46.69
Deseret CD #5	89.62	1.78	0.73	1.04	9.06	9.35	10.2	24.9	64.9	316.41	123.13	14.36	95.29
Deseret CD #6	26.41	2.77	0.6	2.17	9.30	9.54	9.2	30.9	59.9	181.42	108.50	4.28	52.07
Dugway PG #1	4.57	5.2	2.28	2.92	8.31	8.58	9.1	9.1	81.8	227.98	127.28	1.30	15.30
Dugway PG #2	3.77	6.88	1.33	5.56	8.79	9.67	11.5	27.1	61.4	11.06	263.48	65.68	16.94
Dugway PG #3	2.09	1.44	0.29	1.15	8.47	8.93	6.3	9.7	84.0	N/D	137.16	0.67	37.47
Dugway PG #4	50.61	5.81	3.69	2.13	8.13	8.37	10.9	19.2	69.9	52.99	121.12	25.40	92.36
Lake City AAP	5.57	9.84	8.28	1.56	7.27	7.60	6.4	14.7	78.9	34.76	96.51	1.10	37.75
Otis AFB	9.86	0.86	0.75	0.1	7.39	7.81	5.2	27.1	67.7	551.07	101.46	3.22	74.03
Aberdeen SS19	11.33	1.49	1.23	0.26	6.99	7.47	6.2	30.6	63.2	4880.25	107.15	59.35	76.57
Aberdeen A79	10.41	1.8	1.33	0.47	6.40	7.01	8.4	55.6	36.0	128.49	414.59	1.64	53.13
Aberdeen B82	6.75	2.25	1.84	0.41	6.39	6.78	8.8	42.2	49.0	117.95	106.50	0.91	42.33
Aberdeen B116	83.91	22.21	36.83	-14.63	6.40	6.79	6.5	14.9	78.6	1427.52	70.07	88.08	584.02
Cherry Point Hor 2	109.72	8.03	9.8	-1.77	8.07	8.40	N/D	N/D	N/D	3478.94	129.80	66.21	250.98
Cherry Point Hor 4	79.76	9.31	11.88	-2.57	7.81	8.06	N/D	N/D	N/D	2954.27	134.19	61.97	325.28
Fort Knox	19.45	0.52	0.33	0.19	4.98	5.91	19.3	59.2	21.5	N/D	113.11	1.53	40.77

**Table 3 Percent of Measured Bioaccessibility for Field Soils**

Sample ID	% Pb Bioaccessibility**	% Cr Bioaccessibility**	% Cd Bioaccessibility**	% As Bioaccessibility**	Predicted As Bioaccessibility
Bolling AFB #1	91.92	1.02	*	1.00	32.08
Bolling AFB #2	77.06	3.02	*	1.23	20.63
McEntire ANG	N/D	3.46	*	0.55	45.32
Kansas AAP	83.91	17.05	28.39	0.66	54.25
Radford AAP		3.09	*	0.74	4.72
Savanna Depot	100.96	2.95	65.75	0.73	33.79
Hill AFB #1	27.82	2.42	*	1.31	56.42
Hill AFB #2	93.79	12.78	88.82	4.31	52.29
Hill AFB #3	64.76	7.11	68.26	37.76	55.40
Hill AFB #4	57.54	10.63	65.33	29.47	49.75
Hill AFB #5	109.75	22.62	71.08	40.60	56.58
Hill AFB #6	99.22	28.73	74.67	12.41	50.35
Travis AFB #1	102.38	3.25	*	0.48	29.56
Travis AFB #2	103.19	1.56	*	0.47	30.19
Travis AFB #3	97.48	2.28	*	0.42	31.24
Travis AFB #4	88.03	2.77	*	0.43	31.65
Travis AFB #5	85.00	0.87	*	0.38	35.82
Deseret CD #1	N/D	4.68	*	9.12	68.71
Deseret CD #2	N/D	6.23	*	11.87	66.21
Deseret CD #3	95.90	49.44	104.31	1.26	62.49
Deseret CD #4	82.05	15.61	103.86	1.33	67.08
Deseret CD #5	54.13	5.73	17.93	1.60	42.83
Deseret CD #6	80.69	5.22	58.90	1.55	61.73
Dugway PG #1	77.64	29.96	*	1.33	73.78
Dugway PG #2		20.20	37.40	1.18	81.75
Dugway PG #3	N/D	4.11	*	1.73	85.95
Dugway PG #4	89.70	16.60	74.68	1.01	39.89
Lake City AAP		6.49	*	1.13	59.40
Otis AFB	78.31	24.50	*	0.38	53.19
Aberdeen SS19	61.21	5.72	102.55	4.10	46.83
Aberdeen A79	81.08	3.16	*	18.30	41.29
Aberdeen B82	102.38	5.83	*	1.26	46.91
Aberdeen B116	70.13	21.93	101.28	3.31	13.64
Cherry Point Hor 2	101.52	4.80	75.37	0.90	28.96
Cherry Point Hor 4	108.13	6.58	87.64	1.79	30.25
Fort Knox	N/D	3.24	*	0.41	16.96
Average	84.49	10.16	72.13	5.46	46.05
Maximum	109.75	49.44	104.31	40.60	85.95
Minimum	27.82	0.87	17.93	0.38	4.72
Standard Deviation	18.87	10.69	25.85	10.23	18.86

\* Denotes samples with less than 5 mg/kg of metal on soil

\*\* Average Bioaccessibility Values



**FIGURE 1 PREDICTED AND MEASURED BIOACCESSIBILITY FOR ARSENIC**

## 6.0 CONCLUSIONS

As outlined in our numerous publications, we have succeeded in realizing the objectives of our original proposal, namely:

1. A scientifically defensible, peer-reviewed, publicly available methodology for predicting relative bioavailability of metals in soils. These models may be used to provide both point and probabilistic estimates of relative bioavailability;
2. An improved understanding of the links between molecular speciation and bioavailability, the mechanisms by which bioavailability changes over time, and the soil properties that promote lower bioavailability.

These results are thus strongly relevant to SERDP and DoD, as they, in the words of our original proposal:

will provide site managers and risk assessors with tools to make better initial estimates of site risk and environmentally acceptable endpoints (EAEs) than using the 100% relative bioavailability default value. Although site-specific data will always need to be considered in making final cleanup decisions, these results can be used to prioritize sites and to justify more definitive site-specific bioavailability studies such as detailed soil speciation investigations and *in vivo* studies. These results will contribute to DOD's goal of mission readiness by avoiding unnecessary diversion of DOD funds for unwarranted site cleanup.

Of course, to be ultimately useful to the DoD, these results must be transitioned to the field, and we have undertaken an aggressive Transition Plan as described below.

## 7.0 TRANSITION PLAN

This research project was inspired by the work of one of the principal investigators on a metal-contaminated Superfund site (Barnett et al., 1995; Barnett et al., 1997). Thus, the genesis of this project was the recognized need for better estimates of risk due to ingestion of metal-contaminated soil. The guiding principle of the research was to produce results that were directly relevant to and useable by risk assessors and site managers. The results of this research have already and will continue to be transitioned to DoD clean-up activities through both broad-based information transfer and site- and agency-specific technology transfer. One of the products of this research was a validated, peer-reviewed model for estimating soil-metal bioavailability in a wide range of soils based on soil properties. These results were made available to the public through presentation at scientific meetings and publications in refereed archival journals (Appendices A-G). These results will be extremely beneficial to risk assessors and site managers as there are currently no methods for estimating bioavailability other than intensive site-specific investigations.

Of course, no technology is useful if it remains on the shelf, and to that end, the following transition activities are underway:

1. Additional funding was provided to ORNL to develop a user-friendly interactive database to allow risk assessors and site managers to easily use generic soil information and the models developed in this project to estimate metal bioavailability. This database, an extension of the original project, is scheduled to be completed in late 2003 or early 2004.

2. One of the PIs (Barnett) is chairing a session on metal bioavailability in soils at the *Annual International Conference on Contaminated Soils, Sediments, and Water*, October 20-23, 2003, in Amherst, MA. The purpose of this session is to establish an open dialog among representatives from the scientific, remediation, stakeholder, and regulatory communities on the bioavailability of metals in contaminated soils and the use of bioavailability adjustments in human health risk assessments.

3. The PIs are active in the scientific community in incorporating the use of these concepts in remediation of contaminated sites. For example, PI Fendorf recently served on a committee of the National Research Council that prepared a report on the use of bioavailability considerations in contaminated soil and sediment management. PI Barnett recently served as a scientific expert on an EPA panel examining metal bioavailability in soils. Through these efforts, the results of this research are being disseminated to the user community.

4. We were fortunate to be funded on an extension of this work, CU 1350 *Decreasing Toxic Metal Bioavailability with Novel Soil Amendment Strategies*, which will allow us to investigate engineered methods to reduce metal bioavailability in soil and further aid in transitioning these results to the field.

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## APPENDIX A

Yang, J. K., M. O. Barnett, P. M. Jardine and S. C. Brooks (2003). "Factors controlling the bioaccessibility of arsenic(V) and lead(II) in soil." *Soil and Sediment Contamination* 12(2): 165-179.



## APPENDIX B

Yang, J. K., M. O. Barnett, P. M. Jardine, N. T. Basta and S. W. Casteel (2002).  
“Adsorption, sequestration, and bioaccessibility of As(V) in soils.” *Environmental  
Science and Technology* 36(21): 4562-4569.

# Adsorption, Sequestration, and Bioaccessibility of As(V) in Soils

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The influence of various soil physical and chemical properties (Fe and Mn oxides, pH, cation exchange capacity, total inorganic and organic carbon, and particle size) on As(V) adsorption, sequestration, and relative bioaccessibility (as a surrogate for oral bioavailability) was investigated in a wide range of well-characterized soils over a 6-month period. Arsenic(V) bioaccessibility was measured using a streamlined version of a physiologically based extraction test (PBET), designed to replicate the solubility-limiting conditions in a child's digestive tract. The soil's dithionite-citrate-bicarbonate (DCB) extractable Fe oxide content was the most important (and only statistically significant) soil property controlling the initial degree of adsorption. Sequestration, as measured by the reduction in bioaccessibility over time, occurred to a significant extent in 17 of 36 (47.2%) soils over the first 3 months. In contrast, only 4 of 36 (11.1%) soils exhibited a significant reduction in bioaccessibility from 3 to 6 months. Soil pH was the most important (and only statistically significant) soil property affecting the decrease in bioaccessibility upon aging for 6 months. Soils with pH < 6 generally sequestered As(V) more strongly over time, whereas those with pH > 6 generally did not. The Fe oxide content and pH were the most important soil properties governing the steady-state bioaccessibility of As(V) in soil. Two multivariable linear regression models of steady-state As(V) bioaccessibility were developed using soil properties as independent variables. Generally, soils having higher Fe oxide content and lower soil pH exhibited lower bioaccessibility. These models were able to account for ~75–80% of the variability in steady-state bioaccessibility and independently predict bioaccessibility in five soils within a root-mean-square error (RMSE) of 8.2–10.9%. One of these models was also able to predict within an RMSE of 9.5% the *in vivo* bioavailability of As in nine contaminated soils previously used in swine dosing trials. These results indicate the bioaccessibility, and thus, potentially the bioavailability of

otherwise soluble As(V) added to soils (i.e., the worst-case bioavailability scenario) is significantly reduced in some soils over time, particularly those with lower pH and higher Fe oxide content. These results also provide a means of estimating As(V) bioaccessibility and bioavailability on the basis of soil properties.

## Introduction

Arsenic is the second most frequently encountered inorganic contaminant (behind lead) at contaminated U.S. Superfund sites (1). Arsenic contamination in soils originates from various anthropogenic sources, such as mining, milling, and agricultural applications, as well as natural geochemical processes that can be exacerbated by degradable organic contaminants at contaminated sites (2). The factors affecting As(V) adsorption to minerals and soils have been relatively well studied (3). However, the effect of As(V)–soil interactions on the cleanup of As-contaminated sites has been less well investigated (1). The ingestion of As-contaminated soils by children, which would have to be considered in addition to the risk from the intake of natural background As (e.g., in drinking water), is typically the risk driver at As-contaminated sites (4). Inorganic As is commonly present in two oxidation states in soil: +III and +V. As(III) is the thermodynamically stable oxidation state in reducing environments, whereas As(V) is the thermodynamically stable oxidation state in oxic surface soils where children typically contact contaminated soil. The risk of ingesting As (and other metals) in soil is often estimated by assuming that soil-bound As is completely absorbed through the human gastrointestinal tract upon ingestion (i.e., 100% bioavailable). This assumption potentially overestimates the risk and the associated cleanup requirements at contaminated sites (5), since soils are known to strongly sequester certain metals. Therefore, As bioavailability should be considered as a potential tool for better decision making in risk assessments and for remediation purposes (1).

The acceptable regulatory inorganic As dose was originally determined on the basis of an epidemiological study of the effects of As in drinking water in Taiwan (6). However, As in drinking water is potentially much more bioavailable than As in soil, because water-soluble inorganic As is rapidly and completely absorbed by humans, whereas As in soil is almost completely eliminated in the feces without being absorbed (7). Compared to drinking water, As in mining soils or aggregates generally exhibits low bioavailability because of the presence of residual metal sulfides, authigenesis, insoluble mineral rinds, etc. (8–10). However, even in nonmining areas, soil itself may lower the bioavailability of otherwise soluble metals due to adsorption to the solid phase and the formation of secondary solid phases.

Much less research has focused on potentially more labile (e.g., adsorbed) forms of As in soil, although such information could be critical for several reasons. First, even in mine tailings, potentially soluble forms of As can be important (11). Second, As in sulfides in surface soils may weather to more labile forms over time (12). Finally, an understanding of the bioavailability of potentially more labile forms of As in soil is important in assessing the risk of As-contaminated soils that originate from processes other than mining. Controlling factors governing the health risk due to ingestion of soils contaminated with As(V) are the pH of both the gastrointestinal fluid and soil, soil metal concentration, soil-to-solution ratio, mineralogy, and particle

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TABLE 1. Some Physical and Chemical Properties of Soils Used in Study

soil	soil type	series	pH	CEC (cmol <sub>c</sub> /kg)	organic carbon (%)	inorganic carbon (%)	Particle size (%)			Fe (g/kg)	Mn (g/kg)	
							clay	silt	sand			
1	alfisol	Angola-A	5.29	6.70	3.72	0.96	32.1	56.1	11.8	23.28	1.23	
2		Angola-B	7.86	4.50	0.09	0.25	25.6	52.9	21.5	5.83	0.19	
3		Crider-A	6.57	5.60	0.55	0.39	22.5	75.8	1.7	13.34	0.72	
4		Crider-B	5.26	5.40	0.21	0.13	30.9	67.2	1.9	13.38	0.3	
5		Lenberg-A	5.92	7.90	3.41	1.01	49.1	44.5	6.4	12.94	1.37	
6		Lenberg-B	4.35	5.50	0.36	0.25	64.7	29.5	5.8	15.69	0.12	
7		Lawrence-A	4.97	5.80	0.91	0.59	19.5	48.5	32.0	11.17	1.35	
8	aridisol	Doakum-A	6.94	6.90	0.28	0.08	10.8	24.8	64.4	4.74	0.19	
9		Doakum-B	6.84	7.00	0.39	0.18	29.3	15.0	55.7	6.86	0.16	
10		Kzin-A	7.74	13.30	3.27	1.35	22.2	44.2	33.6	4.07	0.29	
11		Kzin-B	7.8	10.00	3.4	1.88	27.0	38.5	34.5	3.26	0.18	
12		Oricto-A	8.72	13.70	0.09	0.94	10.2	34.7	55.1	2.92	0.34	
13		Oricto-B	9.01	8.60	0.16	1.1	23.2	27.5	49.3	3.16	0.29	
14		Stoneham-A	6.42	10.10	1.45	0.71	16.2	41.4	42.4	3.4	0.26	
15	entisol	Stoneham-B	6.8	7.80	0.66	0.32	21.4	23.2	55.4	2.2	0.2	
16		Wakeland-A	5.86	6.10	0.92	0	23.8	64.7	11.5	8.82	0.71	
17		Wakeland-B	5.77	5.70	0.56	0.25	21.1	66.4	12.5	9.18	0.8	
18		inceptisol	Berks-A	3.65	9.10	2.72	1.01	15.7	46.6	37.7	13.18	0.15
19			Melton-A	6.91	8.00	3.55	0.62	6.0	69.0	25	10.68	1.42
20			Melton-B	4.23	14.00	0.42	0.26	19.0	42.2	38.8	22.07	0.17
21		mollisol	Rockaway-A	3.86	10.60	3.54	1.49	12.4	34.8	52.8	14.03	0.52
22	Rockaway-B		4.1	3.70	0.21	0.18	12.6	32.1	55.3	17.34	0.16	
23	Weikert-A		4.44	13.30	3.97	2.37	24.4	56.2	19.4	21.41	6.47	
24	Weikert-B		4.28	8.00	2.01	1.15	23.9	54.3	21.8	28.98	5.42	
25	Dennis-A		Dennis-A	5.82	8.70	1.32	0.89	15.9	66.1	18	15.11	0.6
26			Dennis-B	4.77	4.40	0.38	0.41	29.7	57.5	12.8	24.29	0.59
27			Sibley-A	6.36	7.10	1.06	0.49	23.5	69.7	6.8	8.23	0.67
28	spodosol ultisol	Sibley-B	6.36	6.80	0.72	0.52	26.9	68.0	5.1	9.11	0.59	
29		Charlton-A	3.15	11.90	2.3	0.4	2.9	28.7	68.4	1.33	0	
30		Allen-A	4.59	7.70	1.55	0.56	8.7	29.5	61.8	6.95	0.31	
31		Allen-B	4.3	1.30	0.19	0.09	14.9	28.4	56.7	18.96	0.1	
32		Cecil-A	4.04	5.80	1.64	0.39	10.2	23	66.8	6.01	0.06	
33		Cecil-B	4.44	1.60	0.29	0.21	44.8	15.5	39.7	32.56	0.11	
34		Lawrence-B	4.28	3.70	0.11	0.1	25.8	38.3	35.9	17.53	0.29	
35		Walker-A	6.01	6.00	1.89	0.99	6.1	58.9	34.9	7.71	1.51	
36	Walker-B	4.3	4.00	0.1	0.07	23.6	44.2	32.2	19.55	0.16		
min			3.2	1.3	0.1	0.0	0.4	15.0	1.7	1.3	0.0	
max			9.0	14.0	4.0	2.4	64.7	75.8	70.6	32.6	6.5	
mean			5.6	7.4	1.3	0.6	21.0	42.5	36.9	11.9	0.8	
SD			1.5	3.2	1.3	0.5	16.9	21.8	8.0	8.0	1.3	

size of soils (5, 13, 14). However, the influence of soil properties on As(V) bioavailability has not been systematically investigated.

In the study described in this paper, soluble As(V) was added to 36 well-characterized soils with a wide range of soil properties representing seven major soil orders within the U. S. The soils were aged at 30% moisture content for 6 months, with bioaccessibility (a surrogate for oral bioavailability) measured periodically with a physiologically based extraction test (PBET) that was designed to mimic the solubility-limiting conditions in a child's digestive tract. Oral bioaccessibility is defined as the fraction of a contaminant that is soluble and available for systemic absorption in the gastrointestinal environment (5). The soils were spiked with soluble As(V) to elucidate the ability of soil-metal interactions to limit bioaccessibility without reference to any preexisting site-specific solid-phase speciation (e.g., presence in sulfides) that is subject to change over time. Multivariable linear regression was used to identify and quantify the macroscopic soil properties (e.g., Fe and Mn oxide content, pH, cation exchange capacity (CEC), total organic and inorganic carbon (TOC and TIC), and particle size) controlling As(V) adsorption, sequestration, and bioaccessibility. For the purposes of this paper, adsorption was defined as the fraction of As(V) adsorbed to the soil over the initial 48 h contact period and sequestration was defined as the relative change in bioaccessibility over 6 months.

### Experimental Section

**Materials.** All chemicals employed in this research were analytical grade or above, and solutions were prepared with deionized water (18 MΩ-cm) from a reverse osmosis/ion exchange apparatus (Milli-Q Water System). The A and upper-B horizons of soils from seven major U.S. soil orders were collected from the U.S. Department of Agriculture National Resources Conservation Service (NRCS). The A and upper-B horizons from two well-characterized soils from the U.S. Department of Energy Oak Ridge Reservation in East Tennessee were also included, for a total of 36 soils. The soils were gently ground with a mortar and pestle and sieved to <250 μm, representing the soil particles that are more adherent to children's hands and, thus, more likely to be ingested (15). The soil's cation exchange capacity (CEC) and particle size distribution were measured using standard methodologies and reported by the NRCS. The soil pH was measured in a 2:1 solution-to-solid ratio in 5 mM CaCl<sub>2</sub> using a combination pH electrode and meter. Extractable Fe and Mn oxides were measured by extraction with dithionite-citrate-bicarbonate (DCB) (16), and total organic and inorganic carbon (TOC and TIC) was measured by combustion on a total carbon analyzer. The physicochemical properties of the soils are shown in Table 1.

**Soil Spiking.** Soluble As(V) was added to the soils (2 g) from a small volume of a concentrated As(V) stock solution to a 1:10 g/mL soil suspension of 10<sup>-3</sup> M CaCl<sub>2</sub> with a target

soil As(V) concentration of 100 mg/kg (previous research indicated no significant difference in As(V) bioaccessibility over the concentration range of 10–100 mg/kg (14)). To neutralize the NaOH added from the As(V) stock solution, HNO<sub>3</sub> was also immediately added to the soil slurry to maintain the original soil pH (Table 1). After 48 h mixing, the soil suspensions were centrifuged, and the supernatant was decanted. The remaining soil was washed twice with a small volume (~2–3 mL) of distilled water to remove any remaining soluble traces of the original As(V) spike. Negligible As(V) desorption occurred while rinsing the soil with distilled water. The decanted supernatant and rinse water were then combined and filtered through a 0.45- $\mu$ m membrane filter, and the concentration of As in the filtrate was analyzed using an atomic absorption spectrophotometer (AAS) equipped with an electrodeless discharge lamp (EDL). The detection limit for the AAS was ~3  $\mu$ g/L with a relative precision of  $\pm$ 5%. The difference between the amount of As(V) added and the amount of As(V) remaining in the supernatant was used to calculate the adsorbed As(V) concentration. After air-drying, the soils were homogenized by mixing, and a subsample of each soil was taken, which marked the beginning of the aging experiment (i.e.,  $t=0$ ). The remaining soil was placed in a weighing dish and brought to 30% moisture content with deionized water. The soils were then aged in a larger container through which a steady flow of 100% relative humidity air was passed. The moisture content of the soils was monitored periodically by weight, with deionized water added as necessary to maintain a constant moisture content of 30%. Periodically, subsamples were removed and analyzed as described below.

**Extractions.** Bioaccessibility was measured on duplicate soil subsamples over time using a streamlined version of the original PBET (17). This method has been shown to correlate well with in vivo bioavailability for Pb and is currently being validated for As. The streamlined PBET consisted of a sample holder that held 16 wide-mouth, high-density polyethylene bottles (125 mL) and a motor that rotated the sample holder at  $30 \pm 2$  rpm. The sample holder was located in a temperature-controlled water bath. During the 1-h extraction, the water temperature in the bath was maintained at body temperature ( $37 \pm 2$  °C). The extraction solution consisted of 30 g/L glycine (0.4 M), pH-adjusted to 1.5 with HCl. These conditions were designed to replicate the solubility-limiting conditions in a fasting child's stomach. The streamlined procedure was originally designed for 1 g of each soil to be immersed into 100 mL of PBET solution. However, the procedure was modified for 0.1 g of soil and 10 mL PBET solution to conserve soil samples while maintaining the same soil-to-solution ratio. Replicate analyses using both sample sizes indicated there was no significant difference ( $P < 0.05$ ) between the 0.1 and 1.0 g extractions and that good repeatability could be obtained. After extraction for 1 h, a portion of the supernatant was filtered with a 0.45- $\mu$ m filter. The dissolved As concentration in the filtrate was measured as described previously, with the fraction of metal dissolved representing the absolute bioaccessibility. The remaining soil sample was analyzed for As using acid digestion as described below to verify mass balance within  $\pm$ 10%. As described previously (14), the absolute bioaccessibility of a soluble As solution measured in the same manner was  $96.1 \pm 0.1\%$ . Therefore, in this study, the absolute and relative (to soluble As) bioaccessibility were the same. Ruby et al. provide formal definitions of relative and absolute bioavailability and bioaccessibility (5). To verify the mass balance, the residual soil As was determined using a strong acid extraction method (EPA 3050B) after each PBET extraction. After digestion, the samples were filtered using a Whatman filter paper, and the filtrate was measured with AAS to obtain the total As remaining on the soil. Analysis of the soil residues from the

procedure yielded a mass recovery of  $100 \pm 10\%$ . For all soils, blanks were used to correct all data obtained from PBET extractions.

**Multivariable Regression.** Multivariable linear regression was used to identify the significant soil properties affecting adsorption and sequestration (the relative change in bioaccessibility over 6 months) and to derive a model to predict the steady-state bioaccessibility of As(V) in the soils using backward elimination (18) by employing the general equation

$$Y = \beta_0 + \sum_{i=1}^N \beta_i X_i \quad (1)$$

where  $i$  is an index,  $\beta_0$  and  $\beta_i$  are coefficients,  $X_i$  is an independent variable (soil properties), and  $Y$  is the dependent variable (bioaccessibility). Multivariable regression was employed using the eight independent variables shown in Table 1 (only two of the three particle size variables are independent). The least significant variables (as measured by the largest  $P$  value), including the intercept ( $\beta_0$ ) if warranted, were removed one at a time until all the remaining variables were significant at the 95 percentile confidence level ( $P < 0.05$ ). Five soils (one soil chosen at random from each of the five soil orders with more than two samples) were not used in the regression in order to independently validate the resulting model. In addition, the resulting model was used to predict the in vivo bioavailability of As in nine contaminated soils previously used in swine dosing trials (15). The agreement between the measured and model predicted steady-state bioaccessibility/bioavailability were quantified with the root-mean-square error (RMSE),

$$\text{RMSE} = \left[ \frac{1}{n_d - n_p} \sum_{i=1}^{n_d} (B_i - \hat{B}_i)^2 \right]^{1/2} \quad (2)$$

where  $n_d$  is the numbers of data points,  $n_p$  is the number of adjustable parameters (zero when used in a purely predictive manner),  $i$  is an index, and  $B_i$  and  $\hat{B}_i$  are the measured and predicted bioaccessibility at 6 months, respectively. The RMSE, the square root of the mean squared difference between measured and predicted values, is a measure of the average error between the predicted and measured values.

## Results and Discussion

**Adsorption.** Arsenic concentrations in groundwater at contaminated (e.g., U.S. "Superfund") sites are frequently high (2), and the risk of exposure to As from drinking shallow groundwater can be significant (1). Since interactions with the solid phase will govern the transport of As from soil to shallow groundwater at contaminated sites, the soil properties influencing the adsorption of As(V) to these soils over the initial 48 h contact period were examined. There was a wide range in the relative amount of As(V) adsorbed to these soils, ranging from 13 to ~100% with a mean of 81.4% (Table 2). The soil's Fe oxide content was the major factor governing the initial adsorption of As(V) to the soils (Figure 1a). The percentage of As(V) adsorbed increased sharply as the soil's Fe oxide content reached ~5 g/kg, indicating the important role of Fe oxide as binding sites of As(V). Of the eight soils with <5 g/kg of Fe oxides, none adsorbed more than 60% of the added As(V). Above 5 g/kg Fe oxide, 27 of the other 28 soils (96.4%) adsorbed >80% of the dissolved As. An Fe oxide concentration of 5 g/kg and 100 mg/kg of As corresponds to an Fe/As molar ratio of 67 and to a log surface excess ( $\Gamma$ , mole As(V) per mole Fe, calculated from the concentration of each in the soil) of -1.8. This surface excess also closely corresponds to the number of preferred reactions sites (singly coordinated surface hydroxyl groups) on goethite (19, 20).

TABLE 2. As(V) Adsorption and Bioaccessibility<sup>a</sup>

soil	init As(V) adsorption (%)	0 month	1 month	2 month	3 month	6 month	significant aging	
							over 6 months? <sup>b</sup>	between 3 and 6 months? <sup>c</sup>
1	94.8	28.2 ± 3.9	23.3 ± 1.9	26.3 ± 2.2	21.8 ± 1.0	16.6 ± 3.5	yes	no
2	86	45.8 ± 5.8	40.9 ± 0.2	47.7 ± 0.7	44.7 ± 0.1	47.0 ± 2.2	no	no
3	96.5	25.3 ± 0.2	23.2 ± 1.9	23.6 ± 0.4	20.5 ± 0.9	19.4 ± 4.2	no	no
4	98.6	5.4 ± 2.0	15.0 ± 7.7	6.4 ± 1.2	5.8 ± 0.4	4.6 ± 2.0	no	no
5	87.6	50.4 ± 8.4	42.0 ± 4.0	33.6 ± 0.8	31.9 ± 1.0	32.5 ± 2.7	no	no
6	98.6	32.5 ± 0.4	25.7 ± 2.1	24.9 ± 1.3	20.0 ± 0.1	17.0 ± 0.1	yes	yes
7	78.9	60.6 ± 2.3	65.0 ± 4.8	57.3 ± 0.7	52.2 ± 2.6	46.7 ± 2.3	yes	no
8	57.8	57.7 ± 8.9	54.0 ± 5.5	46.8 ± 3.2	49.0 ± 0.9	42.5 ± 7.5	no	no
9	94.4	45.6 ± 17.2	39.9 ± 1.0	26.3 ± 0.5	37.2 ± 4.4	37.5 ± 3.5	no	no
10	37.1	93.0 ± 10.0	98.7 ± 1.3	97.6 ± 2.4	98.8 ± 1.2	100 ± 0.0	no	no
11	43.8	82.2 ± 1.1	90.4 ± 3.2	92.5 ± 4.1	100 ± 0.0	95.8 ± 5.9	no	no
12	13.0	94.0 ± 8.4	100.0 ± 0.0	94.8 ± 5.2	99.2 ± 0.8	100 ± 0.0	no	no
13	17.3	45.0 ± 2.1	75.6 ± 1.0	71.0 ± 3.9	75.3 ± 3.3	73.6 ± 0.6	no <sup>d</sup>	no
14	48.7	63.8 ± 9.4	71.2 ± 3.7	70.5 ± 1.6	71.5 ± 2.2	68.6 ± 8.8	no	no
15	57.5	72.5 ± 5.9	83	60.6 ± 3.2	86.5 ± 3.5	73.2 ± 12.8	no	no
16	93.4	42.6 ± 0.7	39.9 ± 2.0	39.6 ± 0.1	36.6 ± 2.2	37.3 ± 0.7	yes	no
17	97.2	29.2 ± 2.1	24.3 ± 3.7	27.2 ± 2.7	22.6 ± 0.8	23.3 ± 2.1	yes	no
18	92.7	49.1 ± 0.6	52	35.4 ± 1.6	35.9 ± 0.0	28.9 ± 1.1	yes	yes
19	93.8	62.8 ± 1.1	42.4	43.4	30.8 ± 3.1	31.3 ± 0.1	yes	no
20	97.4	51.8 ± 1.1	27.8	27.3	19.6 ± 0.5	16.6 ± 0.4	yes	yes
21	96.0	43.8 ± 4.3	39.0 ± 6.3	31.8 ± 0.5	33.9 ± 1.7	26.2 ± 2.5	yes	no
22	100	16.5 ± 0.9	14.2 ± 0.5	12.3 ± 0.7	12.0 ± 0.8	10.2 ± 0.7	yes	no
23	95.3	29.8 ± 4.1	28.8 ± 4.3	23.6 ± 1.9	19.8 ± 0.4	17.6 ± 0.6	no	no
24	99.2	19.4 ± 2.6	17.7 ± 3.9	14.6 ± 0.1	11.6 ± 0.7	9.4 ± 5.3	no	no
25	92.3	36.1 ± 3.8	25.8	28.0 ± 4.1	26.6 ± 1.2	24.9 ± 0.6	no	no
26	98.6	11.3 ± 0.3	7.6 ± 0.9	6.3 ± 0.6	4.9 ± 1.6	3.6 ± 1.2	yes	no
27	86.9	45.0 ± 0.7	38.8 ± 2.2	36.5 ± 2.7	39.4 ± 1.7	35.9 ± 6.6	no	no
28	96.1	31.4 ± 1.9	32.4 ± 1.2	30.6 ± 2.0	30.0 ± 0.6	26.1 ± 4.1	no	no
29	24.0	100 ± 0.0	51	29.2 ± 9.0	21.4 ± 0.8	14.3 ± 0.7	yes	yes
30	83.0	45.5 ± 3.9	32.4	38.5 ± 2.0	37.1 ± 2.0	34.0 ± 3.7	yes	no
31	97.6	25.3 ± 3.5	22.8 ± 5.5	16.4 ± 0.1	13.8 ± 0.8	11.8 ± 1.5	no	no
32	87.2	54.3 ± 10.1	48.8	38.1 ± 2.4	36.4 ± 0.2	26.6 ± 6.8	yes	no
33	98.2	6.5 ± 3.7	4.0 ± 1.4	6.9 ± 0.8	2.7 ± 1.5	3.4 ± 0.6	no	no
34	99.7	13.6 ± 2.5	9.8	8.6 ± 1.3	8.0 ± 0.1	5.0 ± 1.6	yes	no
35	93.0	47.1 ± 1.6	31.5	33.6	23.9 ± 0.7	24.1 ± 0.7	yes	no
36	99.9	6.6 ± 0.3	5.9	7.8	1.7 ± 0.8	2.6 ± 0.5	yes	no
mean	13.0	43.6	40.1	36.5	35.6	33.0		
min	100.0	5.4	4.0	6.3	1.7	2.6		
max	81.4	100	100	97.6	100	100		
SD	25.6	24.6	25.7	24.3	27.5	27.1		

<sup>a</sup> Errors represent standard deviation ( $n = 2$ ). Some data without errors obtained by single measurement. <sup>b</sup> As measured by paired t-test results with 0 and 6 months bioaccessibility data. <sup>c</sup> As measured by paired t-test results with 3 and 6 months bioaccessibility data. <sup>d</sup> For this soil only, there was a significant increase in bioaccessibility between 0 and 6 months.

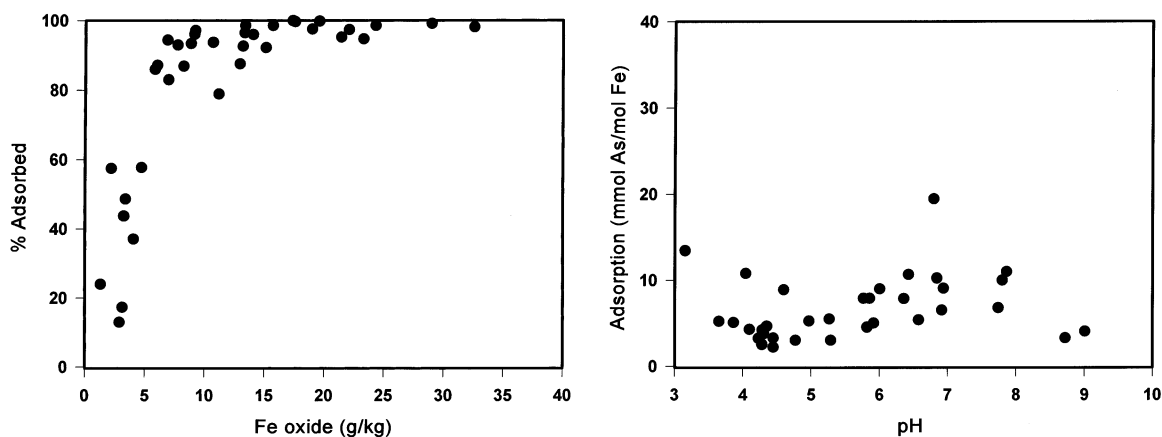


FIGURE 1. (A) Percentage of As adsorbed versus the Fe oxide content. (B) As(V) adsorbed per mole of Fe versus pH.

There were no other readily discernible relationships between As(V) adsorption and the other soil properties. Although As(V) typically exhibits pH-dependent adsorption onto Fe oxides (21), when normalized to the Fe oxide concentration (Figure 1b), there was no evidence of pH-dependent adsorp-

tion. Multivariable regression using backward elimination confirmed that only one variable, log Fe oxide content, significantly ( $P < 0.001$ ) influenced As(V) adsorption over the initial 48 h contact period. The Fe oxide data was logarithmically transformed because of the obvious nonlinearity of

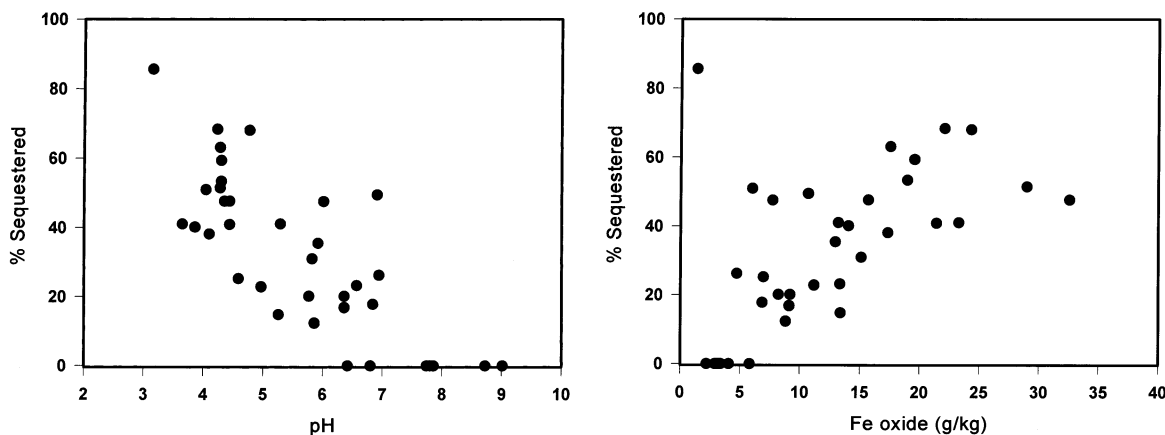


FIGURE 2. (A) Percentage of As(V) sequestered (eq 3) versus pH. (B) Percentage of As(V) sequestered (eq 3) versus the Fe oxide content.

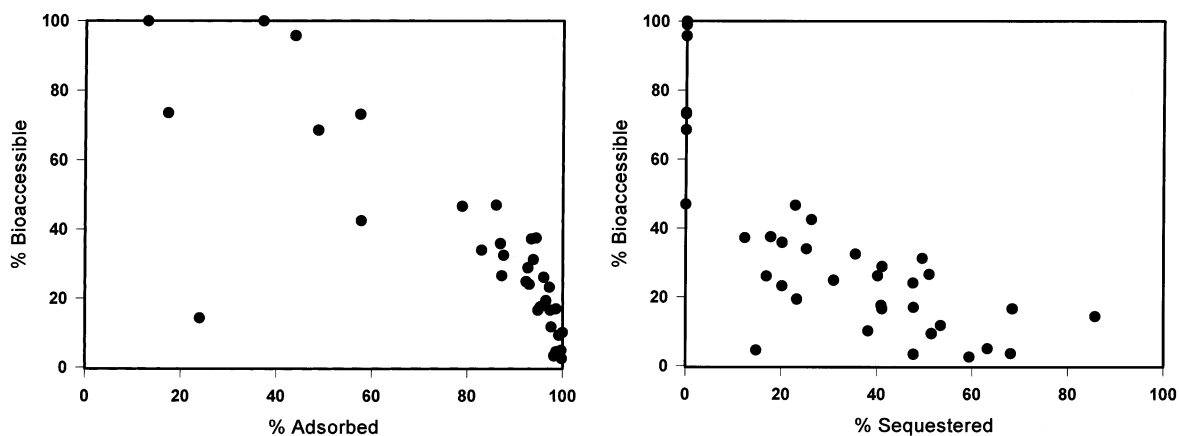


FIGURE 3. (A) Percentage of bioaccessible As(V) versus percent adsorbed. (B) Percentage of bioaccessible As(V) versus percentage sequestered (eq 3).

the relationship between the percentage of As(V) adsorbed and the Fe oxide content exhibited in Figure 1a. The log Fe oxide content explained 56% ( $r^2 = 0.564$ ) of the variability in the relative adsorption of the soils, but all of the variables together explained only 66% ( $r^2 = 0.660$ ) of the variability.

**Reduction in Bioaccessibility with Aging.** The bioaccessibility values as a function of time are shown in Table 2. There was a wide range in the initial bioaccessibility (5.4–100%) as well as the rate of change of bioaccessibility over time, with some soils exhibiting a significant aging effect and the bioaccessibility in the other soils remaining relatively constant. Using a paired  $t$ -test, 17 of the 36 soils (47.2%) exhibited a significant reduction ( $P < 0.05$ ) in bioaccessibility over 6 months. In contrast, only four soils (nos. 6, 18, 20, and 29) exhibited a significant ( $P < 0.05$ ) reduction in bioaccessibility from 3 to 6 months' aging. Thus, reductions in bioaccessibility were largely completed by 3 months. The pH was the major factor affecting the reduction in bioaccessibility over time, with a threshold pH of  $\sim 6$ . Of the 22 soils with  $\text{pH} < 6$ , the bioaccessibility was significantly reduced in 15 (68.2%) of the soils over 6 months. In contrast, of the 14 soils with  $\text{pH} > 6$ , only 2 (14.3%) exhibited a significant reduction in bioaccessibility over 6 months.

The reduction in bioaccessibility was quantified by calculating the percent sequestration, which was defined as the relative change in bioaccessibility over the 6-month study period by the equation

$$\% \text{ sequestration} = \frac{B_0 - B_6}{B_0} \times 100\% \quad (3)$$

where  $B_0$  and  $B_6$  represent the initial and 6-month bioac-

cessibility, respectively. Soils in which the bioaccessibility increased over time were assigned a sequestration of 0% (with one exception, no. 13, these increases were not significant ( $P < 0.05$ )). There was a clear relationship between sequestration and pH ( $r^2 = 0.654$ ), with sequestration consistently increasing at lower pH (Figure 2a). However, the relationship between sequestration and Fe oxide content ( $r^2 = 0.339$ ) was not as clear (Figure 2b). Multivariable linear regression confirmed that the pH was the only significant ( $P < 0.001$ ) variable influencing sequestration. The variability in pH was able to explain 65% ( $r^2 = 0.654$ ) of the variability in sequestration alone, but all the variables together explained only 71% ( $r^2 = 0.715$ ) of the variability. The gradual increase in As(V) sequestration over time at lower pH may be related to changes in the bonding mechanism of As(V) onto the soils. However, because the log Fe oxide concentration was not statistically related to sequestration, the change in bonding at lower pH may involve species other than Fe oxides.

**Steady-State Bioaccessibility.** With the exception of four soils, no further significant ( $P < 0.05$ ) changes in bioaccessibility occurred after 3 months, indicating bioaccessibility had reached a near-steady-state condition. There was a large range in steady-state As(V) bioaccessibility (2.6–100%), with a mean bioaccessibility of 33.0%. There was not a strong linear relationship ( $r^2 = 0.509$ ) between adsorption and bioaccessibility (Figure 3a). The lack of a relationship between adsorption and bioaccessibility is consistent with the generally poor correlation observed between measures of contaminant mobility (e.g., TCLP) and bioaccessibility/bioavailability (22) and reflects the significant differences between conditions in the environment and the human gastrointestinal tract. There was a slightly stronger relationship ( $r^2 =$

**TABLE 3. Multivariable Regression Models**

variable	value	Model 1		VIF <sup>a</sup>
		std error	P value	
	$r^2 = 0.809$		$P < 0.001$	
pH	10.1	0.87	<0.001	1.49
TIC	13.1	4.2	0.004	1.07
log Fe	-32.7	4.5	<0.001	1.65
		Model 2		
	$r^2 = 0.743$		$P < 0.001$	
pH	11.3	0.89	<0.001	1.38
log Fe	-30.5	5.0	<0.001	1.65

<sup>a</sup> The variance inflation factor (VIF) is a measure of collinearity. A VIF of 1 indicates the independent variables have no redundant information.

**TABLE 4. Predicted Bioaccessibility and Measured Bioavailability**

soil no. <sup>a</sup>	pH <sup>a</sup>	Fe Oxide (g/kg)	predicted bioaccessibility <sup>b</sup> (%)	measured bioavailability <sup>a</sup> (%)
1	2.6	125	0	8.62
2	2.6	110	0	4.07
3	3.1	108	0	7.88
4	3.1	88.2	0	19.7
6	7.4	30.4	38.4	38.7
7	7.7	20.2	47.2	42.9
8	7.1	12.9	46.4	39.1
9	7.4	33.4	37.1	42.4
10	7.4	39.5	34.9	21.9

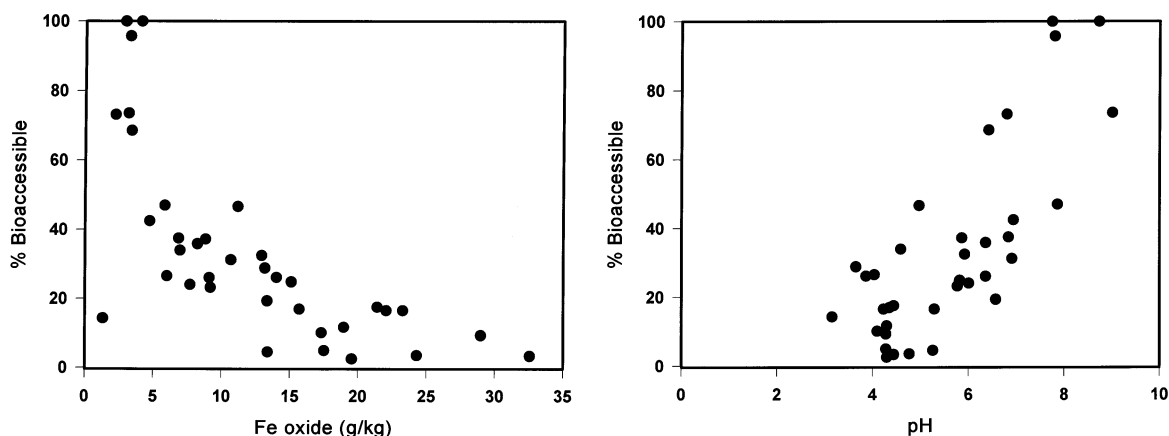
<sup>a</sup> From refs 15 and 28. <sup>b</sup> Predicted using model 2 (Table 3). Predicted values <0% were assigned a value of 0%.

0.590) between sequestration and bioaccessibility (Figure 3b). Sequestration and bioaccessibility are not necessarily related, since sequestration is defined as the relative change in bioaccessibility over 6 months (eq 3). For example, some soils initially exhibited a relatively high bioaccessibility that decreased significantly over time (i.e., a low steady-state bioaccessibility and a high degree of sequestration), whereas other soils initially exhibited a relatively low bioaccessibility that remained relatively constant over time (i.e., low steady-state bioaccessibility and low sequestration).

Using multivariable linear regression, models were developed to describe the steady-state bioaccessibility as a function of soil properties (Table 3). Three variables were shown to significantly ( $P < 0.05$ ) influence steady-state bioaccessibility: log Fe oxide (Figure 4a), pH (Figure 4b), and TIC. These three variables (model 1) were able to describe over 80% of the variability in the measured steady-state

bioaccessibility ( $r^2 = 0.809$ ). Previous molecular-scale observations can be used to interpret the influence of these variables on bioaccessibility. The inverse relationship between bioaccessibility and Fe oxide content may be attributed to the transformation of labile As(V) species to less-soluble mineral phases by complexing with Fe oxyhydroxides. Recent spectroscopic information has revealed that As(V) forms a variety of inner sphere surface complexes with Fe oxides (e.g., mono- and bidentate and mono- and binuclear) (19, 20, 23–26). Soil pH was another important factor governing As(V) bioaccessibility, yielding generally lower bioaccessibility at lower pH. The adsorption of anions such as As(V) is favored at low pH values, where variably charged Fe oxides are generally positively charged (21, 27). However, pH was not a significant variable affecting the initial adsorption of As(V) to the soils, and the Fe oxide concentration was not a significant variable affecting the reduction in As(V) bioaccessibility over time. Values of pH < 6 yielded lower steady-state bioaccessibility by promoting greater sequestration, that is, stronger bonding with the soil over time. There was no evidence that this aging effect at pH < 6 was related to bonding on Fe oxides. The carbonate content of the soil (TIC) also influenced the bioaccessibility of As(V), for which soils with higher TIC exhibited more bioaccessibility. This relationship is most likely the result of a localized pH effect near the carbonate surface, where higher pH discourages sequestration, resulting in greater bioaccessibility. The variance inflation factor (VIF) indicated all three variables were important in their own right, with no indication of collinearity (Table 3). However, removing the TIC as an independent variable from the model only slightly decreases the variance in bioaccessibility explained by the model ( $r^2 = 0.743$ , model 2). Thus, the pH and Fe oxide content can explain nearly 75% of the variability in As(V) bioaccessibility (Figure 5a). The molecular nature of the As(V) interactions in these soils is currently being investigated by synchrotron-generated X-ray absorption spectroscopy (XAS) to correlate these macroscopic observations with molecular-scale speciation.

One of the primary objectives of this study was to develop a simple statistical model to predict the steady-state bioaccessibility of soluble As(V) added to soil. To quantify the ability of the developed model(s) to independently estimate bioaccessibility, 5 (nos. 3, 9, 23, 25, 34) of the 36 soils (one each from the five different soil types with more than two samples) were selected at random and excluded from the multivariable regression analysis. The resulting multivariable regression models were then used to independently predict the steady-state bioaccessibility of As(V) in these five soils. Model 1 was able to predict the steady-state bioaccessibility of these five soils within a RMSE of 8.2% (Figure 5b). Removing the independent variable TIC from the model (model 2)



**FIGURE 4. (A) Percentage of bioaccessible As(V) versus the Fe oxide content. (B) Percentage of bioaccessible As(V) versus pH.**

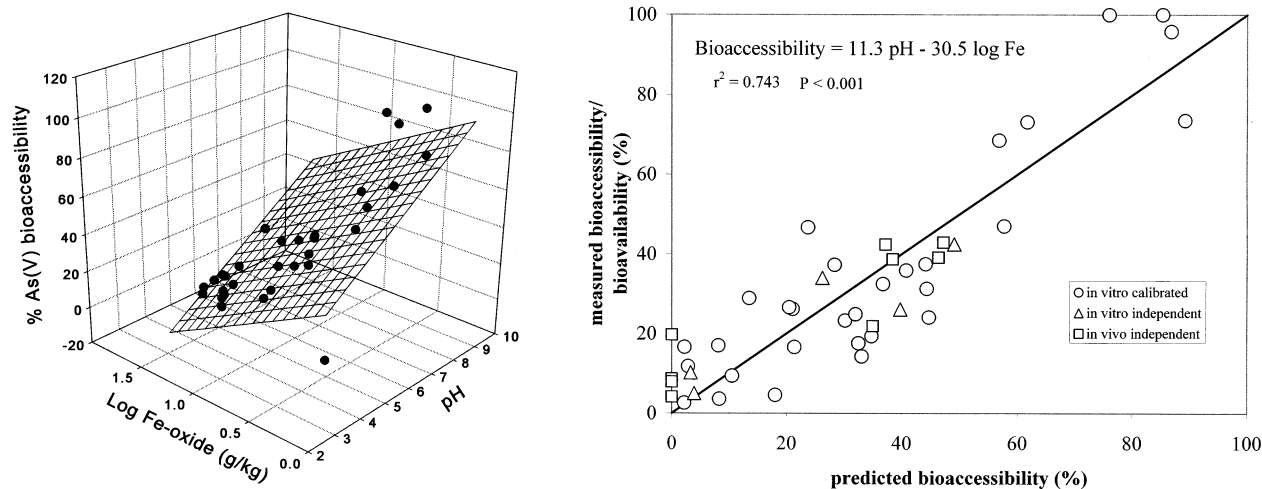


FIGURE 5. (A) Observed (data points) and model-fitted (grid surface) relationship between bioaccessibility, pH, and log Fe oxide content (model 2). (B) Measured bioaccessibility or bioavailability versus predicted bioaccessibility.

increased the RMSE of the predictions only to 10.9%. Previously, Rodriguez et al. (15, 28) reported *in vivo* bioavailability measurements (determined from swine dosing trials) from 15 As-contaminated soils. Subsamples of 9 of these soils (soils 1–4 and 6–10 from ref 15) were obtained from the authors, and the DCB-extractable Fe oxides were measured as described above. Using the pH values reported by Rodriguez et al. (15) and the DCB-extractable Fe oxides measured on the subsamples, the As bioavailability was predicted using model 2 and compared to the values reported by Rodriguez et al. (15, 28). Model 2 was able to predict the *in vivo* bioavailability of these nine soils within an RMSE of 9.5% (Figure 5b). In using model 2, predicted values of bioavailability < 0 (a physical impossibility) were assigned a value of zero.

The results of this investigation have revealed several important factors governing As(V)–soil interactions relevant to contaminated sites. First, there was a wide range in measured As(V) adsorption, sequestration, and bioaccessibility. The Fe oxide content was the most important (and only statistically significant) soil property governing As(V) adsorption over the initial 48 h. Approximately one-half of the soils exhibited a significant reduction in bioaccessibility while being aged for 6 months at 30% moisture content. Further reductions in bioaccessibility had generally been completed by 6 months. The pH was the most important (and only statistically significant) soil property influencing the reduction in bioaccessibility over 6 months, with soils with pH values < 6 generally exhibiting significant reductions over time. In contrast, soils with pH > 6 generally did not sequester As(V) significantly over time. Both Fe oxide content and pH and, to a lesser extent, TIC significantly influenced the steady-state bioaccessibility measured after 6 months.

These results indicate many soils, particularly those with high Fe oxide content and low pH, may reduce As(V) bioaccessibility relative to soluble As(V). Because the allowable inorganic intake of As(V) is based on soluble As in drinking water, the calculated risk to children consuming As-contaminated soils may be overestimated unless site-specific bioavailability is considered. The quantitative models presented here can be used to predict otherwise soluble As(V) bioaccessibility in soil within an RMSE of 6.3–10.9%. In addition, the model was able to predict the *in vivo* bioavailability of As from nine contaminated soils to within a RMSE of 9.5%. Estimates of As(V) bioaccessibility can be used to prioritize sites and justify more-detailed site-specific bioavailability (e.g., animal dosing) studies. Finally, these results demonstrate that fundamental As(V)–soil interactions

may limit otherwise soluble As(V) bioaccessibility without relying on any particular As speciation (e.g., in sulfides) that is difficult to measure and may be subject to change over time. Estimates of bioaccessibility based on soil properties are, by definition, valid unless the major soil properties change. Because most soils have evolved a particular set of physicochemical properties as a result of interactions over a relatively long period of time (e.g., thousands to hundreds of thousands of years), major soil properties are unlikely to change over relatively short time spans (e.g., human lifetimes) unless conditions change dramatically (29). Thus, major soil properties are arguably the most stable aspect of soils, and reductions in metal bioavailability as a result of these soil properties would be correspondingly stable.

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## APPENDIX C

Williams, L. E., M. O. Barnett, T. A. Kramer and J. G. Melville (2003). "Adsorption and transport of As(V) in experimental subsurface systems." *Journal of Environmental Quality* 32(3): 841-850.

# Adsorption and Transport of Arsenic(V) in Experimental Subsurface Systems

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## ABSTRACT

The adsorption and transport of As(V) in a heterogeneous, iron oxide-containing soil was investigated in batch and column laboratory experiments. The As(V) adsorbed rapidly to the soil over the first 48 h, but continued to adsorb slowly over the next several weeks, clearly indicating the potential for rate-limited transport. The equilibrium As(V) adsorption isotherm was markedly nonlinear, further indicating the potential for nonideal transport. A model developed for the adsorption of As(V) to hydrous ferric oxide (HFO) was able to predict the pH-dependent adsorption of As(V) to the soil in batch experiments within 0.116 to 0.726 root mean square error (RMSE). Arsenic(V) was significantly retarded in column transport experiments. The column transport experiments were modeled using the one-dimensional advection–dispersion equation, considering both linear and nonlinear adsorption equilibrium. Although the nonlinear local equilibrium model (NLLE, RMSE = 0.273) predicted the data better than the linear local equilibrium model (LLE, RMSE = 0.317), As(V) breakthrough occurred more rapidly than predicted by either model due to adsorption nonequilibrium. However, due to the presence of an irreversible or slowly desorbing fraction, the peak aqueous As(V) concentration (0.624 mg L<sup>-1</sup>) and the total amount of As(V) recovered (44%) was lower than predicted based on the two equilibrium models (NLLE and LLE). For the conditions used in this study [1 mg L<sup>-1</sup> As(V), pH 4.5 and 9, 0–0.25 mM PO<sub>4</sub>, 0.53–1.6 cm min<sup>-1</sup> pore water velocity], the effect on As(V) mobility and recovery increased in the order pH < pore water velocity < PO<sub>4</sub>.

ARSENIC FROM BOTH anthropogenic and geologic sources is commonly found in the subsurface. Arsenic is a carcinogen and contributes to other health effects such as diabetes and cardiovascular disease. The use of organic and inorganic As as a pesticide in the USA began in the 1850s and peaked in the 1950s, although its use as a wood preservative has recently been increasing (Welch et al., 2000). Historically, the most significant source of As release into the environment is due to the process of mining and smelting metals (Smith et al., 1998). In addition, the presence of naturally occurring As in ground water is a tremendous public health threat to millions of people of West Bengal, India, and Bangladesh (Chowdhury et al., 1999). Although problems in the USA are not as severe, moderate to high (>50 µg L<sup>-1</sup>) naturally occurring concentrations of As are commonly found in ground water throughout the western USA (Welch et al., 2000). The cost of removing As from ground water in the USA may increase to more than \$4.1 billion per year in the near future (Frey et al., 1998). The mobility of As in soil and ground water is

important when evaluating possible environmental and economic effects.

Although As can be found in most natural waters in both organic and inorganic forms with varying oxidation states (+5, +3, 0, -3), As is found predominantly in the inorganic form in oxidation states of +5 or +3 (Sadiq, 1997). In oxidizing conditions, the oxyanion arsenate [As(V)] is the predominate species. A triprotic acid (pK<sub>a</sub> 2.20, 6.97, 11.5), the thermodynamically stable species of As(V) in most normal soil pH ranges (4 to 8) are H<sub>2</sub>AsO<sub>4</sub><sup>-</sup> (pH < 6.97) and HAsO<sub>4</sub><sup>2-</sup> (pH > 6.97). The reactive interaction of As(V) with the subsurface media significantly affects the movement of As in soil and ground water. The equilibrium adsorption of As(V) to pure solid phases and soils has been studied extensively as documented in recent reviews (Sadiq, 1997; Smith et al., 1998). These studies have shown that Fe oxyhydroxides strongly interact with dissolved As(V) and that the degree of As(V) adsorption is extremely pH-dependent. Spectroscopic studies have generated molecular knowledge regarding the adsorption of As(V) to minerals and soils for incorporation in mechanistic models (Rietra et al., 1999; Goldberg and Johnston, 2001). However, the ability to predict As(V) adsorption is still quite limited.

In addition to pH, the presence of other ions also affects the adsorption of As(V). One of the most significant of these ions is PO<sub>4</sub> (Jain and Loepfert, 2000). Phosphate exhibits similar chemical behavior and is often used in fertilizers in agricultural areas where As may have been used as a pesticide or herbicide (Smith et al., 1998). Surface complexation models have been invoked to model the competitive adsorption of As(V) and PO<sub>4</sub> on minerals and soils in batch systems (Manning and Goldberg, 1996a,b). Less attention has focused on the role of PO<sub>4</sub> in promoting As(V) transport through porous media. However, Peryea and Kammereck (1997) concluded that PO<sub>4</sub> greatly enhanced the downward mobility of As(V) in soil columns. Melamed et al. (1995) concluded that PO<sub>4</sub>-amended soils exhibited an increase in mobility of As relative to non-PO<sub>4</sub>-amended soils and noted the potentially important role of physical nonequilibrium on As(V) transport.

Similarly, relatively little attention has focused on adsorption–desorption rates and their effect on As(V) transport, although the adsorption of As(V) to some heterogeneous materials can occur over time spans of weeks or longer (Lombi et al., 1999). Darland and Inskip (1997a,b) demonstrated the significant effects of pore water velocity, pH, and PO<sub>4</sub> on the transport of As(V) through sand columns. However, these phenom-

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**Abbreviations:** AOD, acid ammonium oxalate reacted in the dark; CBD, citrate–bicarbonate–dithionite; HFO, hydrous ferric oxide; LLE, linear local equilibrium; NLLE, nonlinear local equilibrium.

ena have not been well studied in flowing systems with heterogeneous (i.e., multicomponent) porous media.

The objective of this paper is to describe the results of a study of the adsorption and transport of As(V) in a heterogeneous subsurface media. Specific objectives of the investigation included (i) determining the primary physical and chemical parameters that influence As(V) adsorption and transport, with a particular emphasis on those processes that produce nonideal (i.e., nonlinear, rate-dependent) transport, and (ii) determining the ability of commonly used models to predict As(V) adsorption and transport in heterogeneous media.

## METHODS AND MATERIALS

The soil used in this study (Table 1) was acquired from the Melton Branch watershed on the U.S. Department of Energy Oak Ridge Reservation in eastern Tennessee. It was obtained at a depth of 1.5 m from the C horizon of a weakly developed Inceptisol (Montevally series: loamy skeletal, mixed, thermic, shallow Typic Dystrachrept) that has weathered from interbedded shale-limestone sequences. The limestone has weathered to massive clay lenses devoid of carbonates, and the more resistant shale has weathered to a highly fractured saprolite. This soil was chosen because it is heavily coated with iron oxides (Arnseth and Turner, 1988), which are known to contribute to the adsorption and transport of subsurface As(V). The soil was air-dried and gently ground with a mortar and pestle to pass a 2-mm sieve. Particle size was measured with sieves and a hydrometer (Gee and Bauder, 1986). The soil pH was determined using 5 mM CaCl<sub>2</sub> in a 2:1 solution to solid ratio. The pH of the supernatant was measured using an ion analyzer (EA940; Thermo Orion, Beverly, MA) and combination electrode (8102 ROSS; Thermo Orion). The background concentration of As in the soil measured by Method 3050B (USEPA, 1998) was 2.0 mg kg<sup>-1</sup>. Citrate-bicarbonate-dithionite (CBD)-extractable iron and manganese oxides and acid ammonium oxalate (AOD)-extractable iron oxides were also measured (Jackson et al., 1986). Total organic carbon (TOC) was measured by combustion on a total carbon analyzer (CN-2000; LECO Corporation, St. Joseph, MI). The mineralogy of the <2- $\mu$ m clay fraction was identified with X-ray diffraction (DMS 2000; Scintag, Cupertino, CA).

Batch adsorption rate and isotherm experiments at approximately 25°C were conducted in low density polyethylene bottles containing 20 mL of 0.01 M NaNO<sub>3</sub> solution to maintain a constant ionic strength and 0.8 g (rate experiments) or 0.1

to 2.2 g (isotherm experiments) of soil. A 1000 mg L<sup>-1</sup> As(V) stock solution was prepared by adding reagent grade As<sub>2</sub>O<sub>5</sub> to a 4 g L<sup>-1</sup> NaOH solution according to Clesceri et al. (1989). A small volume of the stock solution was added at the beginning of the experiments to achieve the desired initial concentration (10 mg L<sup>-1</sup>) with a small volume of 0.1 M HNO<sub>3</sub> to neutralize the base in the spike and maintain the soil's natural pH of approximately 4.5 (Table 1). The bottles were mixed using slow end-over-end rotation (approximately 4 rpm) and samples were taken from the vessels at desired time intervals and processed as described below.

To measure the influence of pH and the presence of other ions on As(V) adsorption, adsorption envelope [i.e., percent adsorbed as a function of pH at constant As(V) concentration] experiments were also performed. These experiments were also conducted in low density polyethylene bottles at room temperature (approximately 25°C) with 20 mL of 0.01 M NaNO<sub>3</sub> and 0.1 g of soil. A small volume was added from the As(V) stock solution to achieve an As(V) concentration of 1 mg L<sup>-1</sup> followed immediately by the addition of 0.1 M NaOH or 0.1 M HNO<sub>3</sub> to adjust the pH. Additional NaNO<sub>3</sub> (0.1 M final concentration), 10<sup>-3</sup> M CO<sub>3</sub>, or 10<sup>-4</sup> M PO<sub>4</sub> was added as desired to alter the ionic strength or measure the effects of competing ions on As(V) adsorption. Phosphate was chosen as a competing ion because it shares a similar chemical behavior to that of As(V) and it is readily available in many As-contaminated agricultural areas due to land application of PO<sub>4</sub>-amended fertilizers. Carbonate was chosen because the CO<sub>3</sub> system is one of the most important systems in the environment, its effects on other chemical systems can be profound, and recently its effects on the leaching of As into ground water have been noted (Kim et al., 2000). Samples were mixed using slow end-over-end rotation (approximately 4 rpm) for one week and then processed as described below.

Because the batch results indicated a significant potential for nonideal transport, additional column transport experiments were conducted to measure the effects of the most significant variables identified in the batch experiments (pH, time, and PO<sub>4</sub> concentration). These experiments were conducted in a 1-cm-diameter glass column at room temperature and constant ionic strength (0.01 M). Two grams of soil were dry-packed to a depth of 1.7 cm, and the column was flushed from the bottom using a 0.01 M NaNO<sub>3</sub> solution at a pH of 4.5 (the soil's natural pH) or 9.0 until air spaces were no longer visible. A square wave input pulse of 1 mg L<sup>-1</sup> As(V) in a 0.01 M NaNO<sub>3</sub> background matrix was introduced to the column at a pore water velocity of either 0.53 or 1.6 cm min<sup>-1</sup> using high performance liquid chromatography pumps. After a breakthrough period of approximately 875 pore volumes, the inlet solution was switched back to a solution of identical composition but without As(V). Throughout the experiment, effluent samples were collected with a fraction collector and analyzed for As(V) and pH. The column properties were measured physically yielding a porosity of 0.43 and a bulk density of 1.5 g cm<sup>-3</sup>. The dispersion coefficient (*D*) was measured at a pore water velocity of 0.53 cm min<sup>-1</sup> by fitting the one-dimensional advective-dispersive equation to the breakthrough curve of a nonreactive tracer (Cl<sup>-</sup>) by adjusting the parameter *D* using CXTFIT (Parker and van Genuchten, 1984). The nonreactive tracer exhibited a sharp breakthrough yielding a dispersion coefficient of 0.14 cm<sup>2</sup> min<sup>-1</sup> and a column Peclet number ( $vL/D$ , where *v* is the pore water velocity and *L* is the column length) of 6.4, indicating a low potential for nonideal transport due to physical conditions (e.g., preferential flow paths) inside the columns.

The samples were filtered using a 0.45- $\mu$ m syringe filter (Acrodisc 4559; Pall Corporation, Ann Arbor, MI), acidified

**Table 1. Soil properties.**

Property	Value
<b>Particle size, %</b>	
Sand	31
Silt	50
Clay	19
CBD <sup>†</sup> -extractable Mn, g kg <sup>-1</sup>	0.36
CBD-extractable Fe, g kg <sup>-1</sup>	25.8
AOD <sup>‡</sup> -extractable Fe, g kg <sup>-1</sup>	1.1
pH (2:1 v/w 5 mM CaCl <sub>2</sub> )	4.5
Organic matter, g kg <sup>-1</sup>	0.55
<b>Mineralogy of &lt;2-<math>\mu</math>m fraction, %</b>	
Illite	30
Interstratified 2:1	25
Kaolinite	20
Smectite	10
Vermiculite	10
Quartz	5

<sup>†</sup> Citrate-bicarbonate-dithionite.

<sup>‡</sup> Acid ammonium oxalate reacted in the dark.

with 5% HNO<sub>3</sub> and capped pending analysis. The use of a 0.45- $\mu$ m filter may have allowed As(V) adsorbed to colloidal soil material to pass through the filter, potentially overestimating the true dissolved As(V) concentration. Before the samples were acidified, the pH was measured using an ion analyzer (EA940; Thermo Orion) and combination electrode (8102 ROSS; Thermo Orion). Samples were analyzed for As concentration using graphite furnace atomic absorption spectrometry (3110 and HGA-600; PerkinElmer, Wellesley, MA). An electrodeless discharge lamp (EDL System 2; PerkinElmer) was used for As analysis to improve sensitivity and linearity due to the greater spectral purity of their emission relative to a standard hollow cathode lamp. Standards were tested periodically (e.g., every 10 samples) throughout the analysis period to ensure the accuracy of the sample results. The detection limit was determined to be approximately 5  $\mu$ g L<sup>-1</sup> (three times the standard deviation of a blank). Samples were diluted gravimetrically, if necessary, to ensure As measurements of <100  $\mu$ g L<sup>-1</sup>, the maximum linear range of the analysis. Samples were analyzed in triplicate and were considered accurate when the relative standard deviation of the replicates was <5%. The adsorbed As(V) concentration was calculated from the difference in the concentration in solution before and after the experiments. Blank samples were included without soil to verify the initial As(V) concentration and that the As(V) did not adhere to the reaction vessels. Blank samples with no added As(V) were also included to verify that natural background As was not desorbing from the soil.

## MODELING

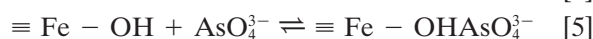
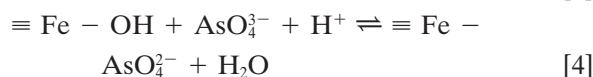
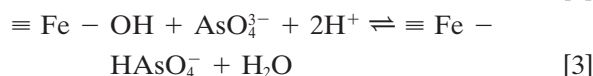
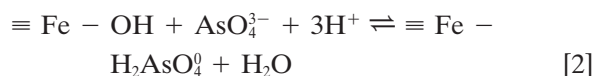
Both equilibrium adsorption modeling and coupled adsorption–transport modeling were conducted. The emphasis on the modeling was to determine the agreement between commonly used predictive models and the experimental results rather than simple curve fitting (i.e., simple isotherms were fit to the data while the remainder of the modeling was predictive). Batch equilibrium isotherms were modeled using two conventional nonelectrostatic models. The experimental data was fit with both linear ( $K_d$ ) and Freundlich isotherms to describe the data quantitatively by the equation:

$$q = KC^n \quad [1]$$

where  $q$  and  $C$  are the equilibrium adsorbed (mg kg<sup>-1</sup>) and aqueous (mg L<sup>-1</sup>) As(V) concentrations, respectively,  $K$  is the adsorption capacity parameter ( $K_d$  for linear and  $K_f$  for Freundlich), and  $n$  is the adsorption intensity parameter. The linear isotherm is a special case of the Freundlich isotherm where  $n = 1$ . The model was fit to the data using linear regression with a linearized version of Eq. [1] with  $K$  (linear and Freundlich isotherms) and  $n$  (Freundlich isotherm only) as fitting parameters. Distribution coefficients ( $K_d$ ) were also calculated for individual experimental points from the ratio of adsorbed to aqueous As(V) concentration.

As this soil is heavily coated with Fe oxides (Arnseth and Turner, 1988), the pH-dependent As(V) adsorption was modeled using the generalized two layer adsorption model developed by Dzombak and Morel (1990) for the adsorption of cations and anions onto hydrous ferric oxide (HFO). The model was used to predict the pH-dependent partitioning of As(V) on the soil by assuming that HFO governed the adsorption of As(V) to these materials. The concentration of HFO in the soils was estimated from both the AOD-extractable (amorphous and poorly crystalline) and CBD-extractable Fe (crystalline) contents (Jackson et al., 1986). The model was chosen because it is widely used by the USEPA (1999) to describe and quantify the adsorption behavior of contaminant

metals and metalloids. This approach has recently been shown to work well for predicting As(V) adsorption to aquifer sediments (Welch and Lico, 1998) and a soil waste (Lumsdon et al., 2001) and for predicting U(VI) adsorption to this same soil (Barnett et al., 2002). The As(V) surface complexation reactions used in the model are:



where  $\equiv \text{Fe} -$  represents surface-bound Fe. Because of the chemical similarity of PO<sub>4</sub> to As(V), the surface reactions used to describe the interactions of PO<sub>4</sub> with surface  $\equiv \text{Fe} -$  groups are analogous to the equations used to describe the interactions of As(V) with  $\equiv \text{Fe} -$  (Eq. [2]–[5]) with P replacing As. The equilibrium constants, however, are different, reflecting the relative strength of the PO<sub>4</sub> and  $\equiv \text{Fe} -$  surface bonds. The mass balance and mass action equations involved in the model were solved using MINTQA2 (Version 4.0) and its standard thermodynamic database, which includes Eq. [2] through [5]. Parameters such as specific surface area and site concentration were taken from Dzombak and Morel (1990).

Column experiments were modeled using the one dimensional advection–dispersive reaction model:

$$\rho_b \frac{\partial q}{\partial t} + \theta \frac{\partial C}{\partial t} = \theta D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} \quad [6]$$

where  $\rho_b$  is the bulk soil density (mass, M, length, L<sup>-3</sup>),  $t$  is the time (T),  $\theta$  is the volumetric water content (L<sup>3</sup> L<sup>-3</sup>),  $C$  is the solute concentration (M L<sup>-3</sup>),  $D$  is the hydrodynamic dispersion coefficient (L<sup>2</sup> T<sup>-1</sup>),  $x$  is the distance along the column (L),  $v$  is the average Darcy velocity (L T<sup>-1</sup>), and  $q$  and  $C$  are as described above.

A common assumption in modeling the transport of reactive contaminants is the local equilibrium assumption, which assumes that the adsorption process is rapid compared with transport via dispersion and advection (Brusseau, 1998). If adsorption equilibrium is obtained, the relationship between the instantaneous rate of change of the adsorbed and aqueous concentrations can be obtained by differentiating Eq. [1] with respect to time to obtain:

$$\frac{\partial q}{\partial t} = nKC^{n-1} \frac{\partial C}{\partial t} \quad [7]$$

Equations [6] and [7] are coupled partial differential equations that represent the reactive transport model assuming local equilibrium. If the adsorption isotherm is linear (i.e.,  $n = 1$ ), Eq. [6] and [7] can be solved analytically and the resulting model is termed the linear local equilibrium model (LLE). If the adsorption isotherm is nonlinear (i.e.,  $n \neq 1$ ), Eq. [6] and [7] must be solved numerically and the resulting model is termed the nonlinear local equilibrium model (NLLE). Both the LLE and NLLE models were applied using the Multireaction Transport Model (MRTM) (Selim et al., 1990), which solves the equations numerically. To validate the correct implementation of MRTM, the numerical solution of the LLE model was compared with an analytical solution.

The fits of the models to the experimental data were quantified by calculating the root mean square error (RMSE) be-

tween the model predicted and experimental data points. The RMSE is calculated from:

$$\text{RMSE} = \left[ \frac{1}{n_d - n_p} \sum_{i=1}^{n_d} \left( \frac{C - \hat{C}}{C_0} \right)^2 \right]^{1/2} \quad [8]$$

where  $n_d$  is the numbers of data points,  $n_p$  is the number of adjustable parameters (zero when used in a purely predictive manner),  $i$  is an index,  $C$  is measured aqueous concentration,  $\hat{C}$  is the predicted aqueous concentration, and  $C_0$  is the initial aqueous concentration (all in  $\text{mg L}^{-1}$ ). The RMSE is a measure of the error between the predicted and measured values expressed as a fraction of the initial concentration (e.g., the closer to zero the RMSE, the better the fit of the model to the data).

## RESULTS AND DISCUSSION

To examine As(V) adsorption dynamics and to determine the time frame when adsorption equilibrium was approached, batch adsorption rate experiments were conducted at the soil's natural pH of 4.5. Long-term (28-d) adsorption rate experiments exhibited a biphasic pattern (Fig. 1), with a rapid period of adsorption occurring over the first 24 to 48 h, followed by a slower period of adsorption over the next several weeks. The As(V) concentration in solution (Fig. 1a) continued to

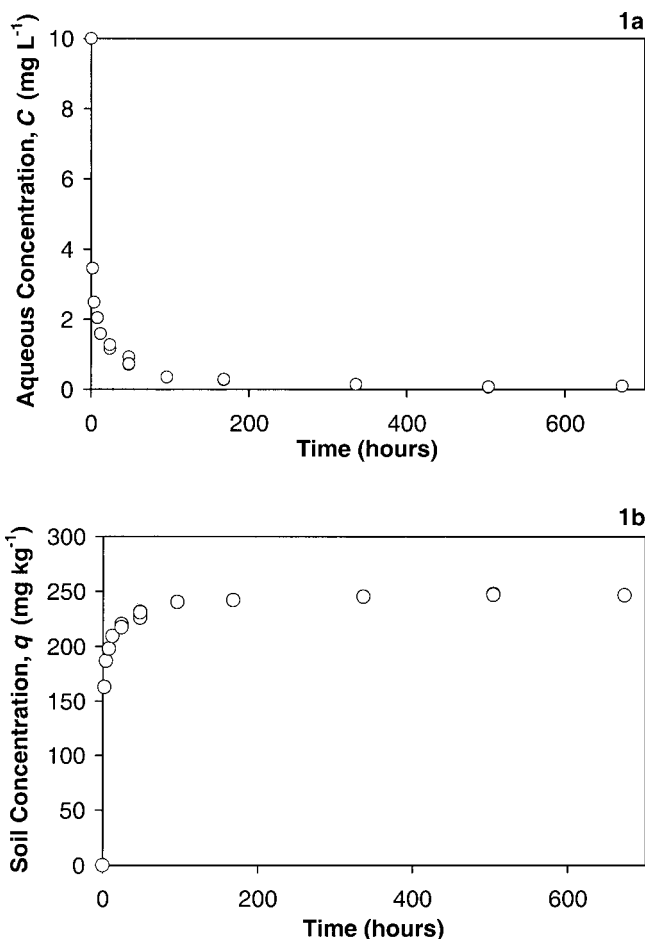


Fig. 1. Adsorption of As(V) as a function of time (40 g soil L<sup>-1</sup>, pH 4.5, 0.01 M NaNO<sub>3</sub>). (a) Aqueous-phase and (b) solid-phase concentration.

decrease for up to three weeks. The distribution coefficient ( $K_d$ , the ratio of the adsorbed to aqueous concentrations) after three weeks was three to four times the value measured after one week, clearly indicating the potential for rate-limited, nonideal transport. This behavior contrasts with that exhibited by As(V) in adsorbing to ferrihydrite and other pure solid oxyhydroxides (Grossl and Sparks, 1995; Raven et al., 1998). However, other investigators have concluded that the rapid adsorption of As(V) onto Fe oxyhydroxides is followed by a slower process of surface diffusion to sites within colloidal aggregates (Fuller et al., 1993) or possibly further surface reactions (Grossl et al., 1997). However, despite the slow decrease in As(V) in solution, its effect on the adsorbed As(V) concentration was minimal after 48 h (Fig. 1b). Therefore, additional batch adsorption equilibrium experiments were conducted after at least 48 h of equilibration time.

After establishing a minimum equilibrium time, a batch adsorption isotherm was measured at the soil's natural pH. Arsenic(V) adsorbed strongly and nonlinearly to the soil at pH 4.5 (Fig. 2). The nonlinear relationship indicates that there is a decrease in the As(V) adsorption capacity of this soil with an increase in the surface coverage. These data also indicate that As(V) subsurface transport would be significantly retarded due to interactions with the solid phase. Although the data were clearly not linear, a linear isotherm was applied to the model because, despite its limitations, linear adsorption is a commonly used approximation in reactive solute transport modeling (Brusseau, 1998; Bethke and Brady, 2000). The linear isotherm was best described with an equilibrium constant  $K_d = 345 \pm 33 \text{ L kg}^{-1}$ . The Freundlich isotherm fit the data more accurately and resulted in the following relationship parameter estimates:  $K_f = 278 \pm 1 \text{ L kg}^{-1}$  and  $n = 0.32 \pm 0.02$ . These parameters were used subsequently in predicting the outcome of As(V) transport experiments.

In addition to time and As(V) concentration, several other parameters have been shown to influence As(V) adsorption, including pH, ionic strength, and the presence of competing anions. Adsorption pH envelopes [percent adsorbed versus pH at constant As(V) concentration] were measured as a function of initial As(V) concentration, ionic strength, and competing ions

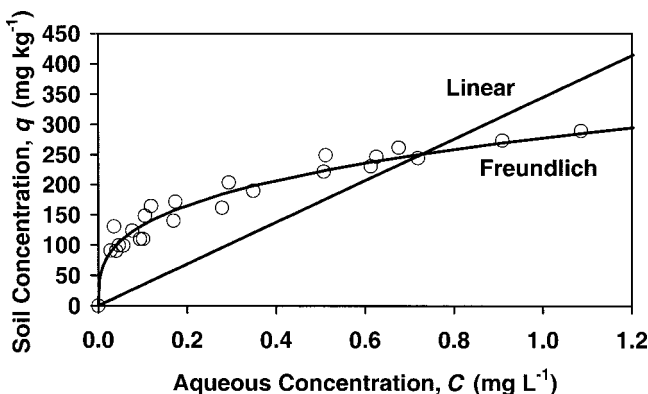


Fig. 2. Adsorbed solid versus aqueous As(V) concentration (pH 4.5, 0.01 M NaNO<sub>3</sub>).

(Fig. 3) to identify the variables that were most likely to influence As(V) transport. The results graphically demonstrate the importance of pH on the adsorption

process (Fig. 3a), with higher adsorption occurring at lower pH. From pH 3 to 7 the percentage of As(V) adsorbed decreased slightly from approximately 95 to

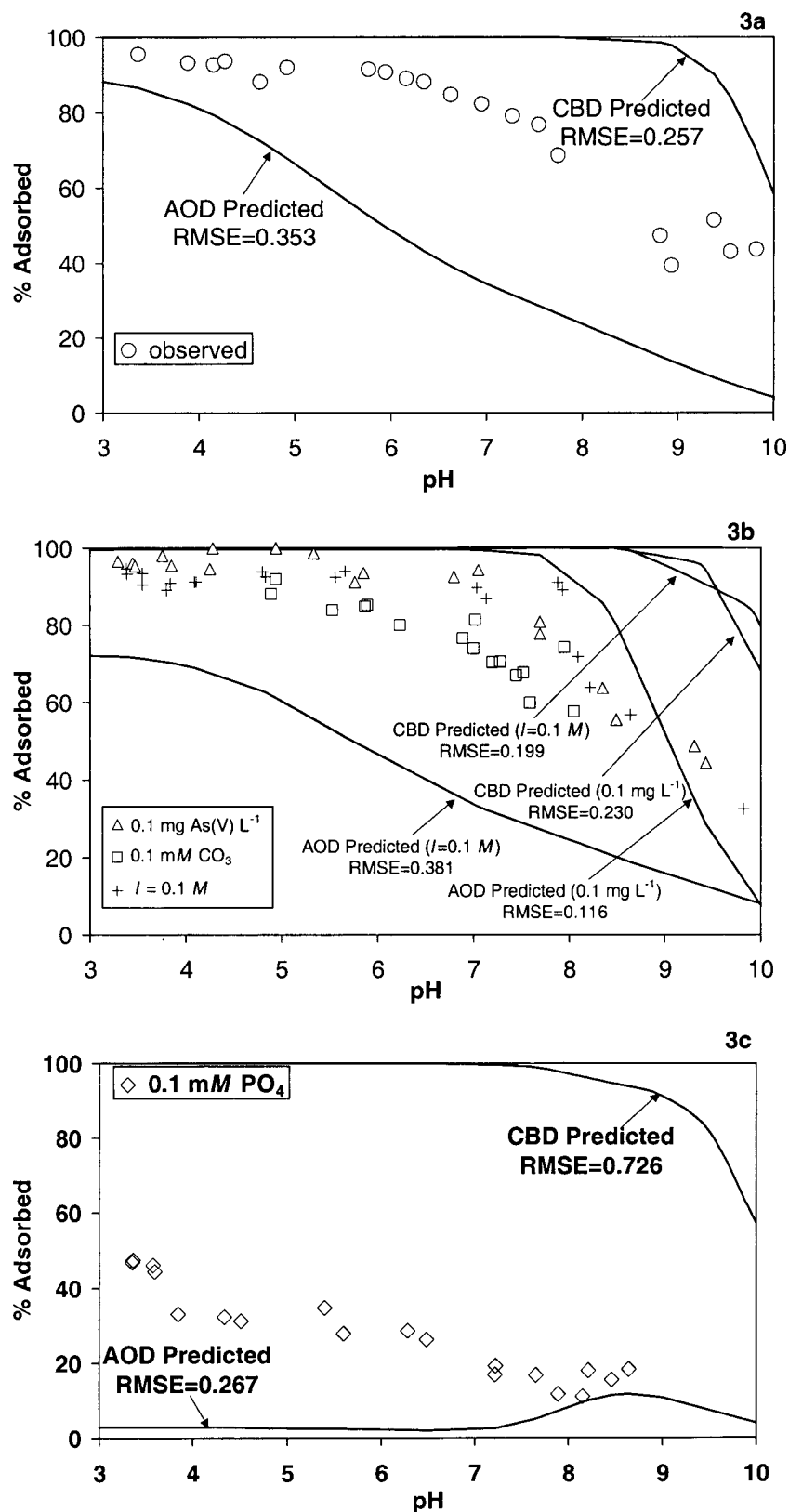


Fig. 3. Adsorption at constant As(V) concentration [5 g soil L<sup>-1</sup>, 0.01 M NaNO<sub>3</sub>, and 1 mg As(V) L<sup>-1</sup> unless otherwise noted]. (a) Effect of pH; (b) effect of ionic strength (I), As(V) concentration, and CO<sub>3</sub>; (c) effect of PO<sub>4</sub>.

85%. As the pH increases from 7 to 10 the percentage of As(V) adsorbed dropped dramatically, decreasing to approximately 40 to 50% between pH 9 and 10. This behavior is typical of anion adsorption onto variably charged surfaces and results from the pH-dependent surface charge and aqueous speciation of As(V). At lower pH values ( $\text{pH} < 7$ ) As(V) exists predominately as an anion in the form  $\text{H}_2\text{AsO}_4^-$  and is attracted to the positively charged soil surfaces (e.g., Fe oxides). At high pH values ( $\text{pH} > 7$ ), As(V) exists as an anion in the form  $\text{HAsO}_4^{2-}$  and the Fe oxide surfaces become increasingly negatively charged. The repelling negative charges between the soil particle and the As(V) ion help explain the decrease in As(V) adsorption with an increase in pH. These results indicate that pH would have a very strong effect on As(V) transport, with distribution coefficients decreasing by almost one order of magnitude in moving from pH approximately 7 to 9. Laboratory measurements of decreased adsorption and increased mobility of As with increasing pH are consistent with observations in As-contaminated ground water (Marianer et al., 1996).

Increasing the ionic strength from 0.01 to 0.1 M did not greatly affect As(V) adsorption (Fig. 3b). However, the increase did extend the 85 to 95% As(V) adsorption range from pH 7 (at 0.01 M ionic strength) to pH 8 (at 0.1 M ionic strength). This is a commonly recognized phenomenon (Manning and Goldberg, 1997; Smith et al., 1999) for the adsorption of oxyanions at high pH onto variably (and increasingly negatively) charged surfaces. The increase in ionic strength lessens the electrostatic repulsion of oxyanions for negatively charged surfaces by shielding the anion from the surface charge (Hiemstra and Van Riemsdijk, 1999). Decreasing the As(V) concentration by an order of magnitude also had a relatively minor effect on As(V) adsorption (Fig. 3b). The decrease in concentration resulted in an increase in the As(V) adsorbed at low pH values. Between pH 3 and 7, adsorption in the system with  $0.1 \text{ mg L}^{-1}$  As(V) was within the range of approximately 90 to 100%, whereas with a system concentration of  $1.0 \text{ mg L}^{-1}$  As(V) the range of adsorption was approximately 85 to 95% for the same pH values. Thus the effect was relatively minor because the degree of adsorption was already relatively strong (approximately 90%) in this region.

The effect of  $\text{CO}_3$  on As(V) adsorption was examined because carbonate is one of the most ubiquitous and important aqueous anions in the environment. The presence of  $\text{CO}_3$  decreased the extent of As(V) adsorption slightly, although the effect was minor in comparison with experimental variability (all replicates shown). Although  $\text{CO}_3$  has been shown to play an important role in mobilizing As from sulfide minerals (Kim et al., 2000), its effect on adsorption in this study was relatively small in keeping with other studies showing the effect of  $\text{CO}_3$  on As(V) adsorption to Fe minerals (Meng et al., 2000).

Phosphate has been previously shown to influence As(V) adsorption (Reynolds et al., 1999), and the addition of  $0.1 \text{ mM PO}_4$  greatly reduced the As(V) adsorption capacity of the soil (Fig. 3c). At low pH values

( $\text{pH} < 7$ ), without the addition of  $\text{PO}_4$ , As(V) adsorption was  $>85\%$  with distribution coefficients  $> 1000 \text{ L kg}^{-1}$ . At low pH values ( $\text{pH} < 7$ ) with the addition of  $0.1 \text{ mM PO}_4$ , As(V) adsorption decreased to approximately 25 to 50% with distribution coefficients ranging from approximately 70 to  $200 \text{ L kg}^{-1}$ . At higher pH values ( $\text{pH} > 7$ ) As(V) adsorption decreased from  $>40\%$  with distribution coefficients of approximately 150 to  $1000 \text{ L kg}^{-1}$  to  $<20\%$  with distribution coefficients of  $<50 \text{ L kg}^{-1}$  on the addition of  $0.1 \text{ mM PO}_4$ . Phosphate was able to effectively compete with As(V) for adsorption sites and significantly decrease adsorption.

As this soil is heavily coated with Fe oxides, the pH-dependent As(V) adsorption was modeled using the generalized two-layer adsorption model developed by Dzombak and Morel (1990) for the adsorption of cations and anions onto hydrous ferric oxide (HFO). This model was used to predict the pH-dependent partitioning of As(V) on the soil by assuming that HFO governed the adsorption of the As(V) on these materials. The model was used to predict the As(V) adsorption envelopes for all of the experimental data sets except for the experiment with  $\text{CO}_3$  (because there are no equilibrium constants for the adsorption of  $\text{CO}_3$  to HFO available in the model). The concentration of HFO in the soils was estimated from both the AOD-extractable (amorphous and poorly crystalline) and CBD-extractable (crystalline) Fe contents (Jackson et al., 1986). For example,  $5 \text{ g soil L}^{-1}$  ( $0.1 \text{ g per } 20 \text{ mL}$ ) with a CBD-extractable Fe concentration of  $25.8 \text{ g kg}^{-1}$  (Table 1) yields  $0.205 \text{ g HFO L}^{-1}$  using the formula  $\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$  for HFO recommended by Dzombak and Morel (1990). Using the AOD-extractable Fe concentration to estimate the HFO content (Fig. 3) resulted in the model typically underpredicting the extent of adsorption. In contrast, using the CBD-extractable Fe concentration to estimate the HFO content (Fig. 3) resulted in the model consistently overpredicting the extent of adsorption and the pH at which adsorption would begin to decrease. These results indicate that the AOD-extractable Fe oxide concentration underestimates the HFO content or that other components of the soil significantly contribute to the adsorption of As(V). These results also indicate that the CBD-extractable Fe oxide concentration overestimates the HFO content of the soil.

Although the model correctly predicted the competitive effect of  $\text{PO}_4$  on As(V) adsorption (Fig. 3c), the model significantly overpredicted the magnitude of the effect when using the AOD-extractable Fe oxide concentration as an estimate of the HFO content and underestimated the magnitude of the effect when using the CBD-extractable Fe concentration to estimate the HFO content. The differences in the effect of adding  $\text{PO}_4$  to the model suggests that the HFO content is somewhere in between the AOD- and CBD-extractable Fe oxide concentration. In most cases, the agreement between the experimental data and model-predicted results is reasonable given the inherent assumptions in the modeling and suggests that the As(V) adsorption capacity for heterogeneous, multicomponent materials may be modeled with knowledge of the soil's Fe oxide content



and pH within the RMSE shown. Overall, the model using the AOD-extractable Fe content (RMSE 0.116–0.381) predicted the pH-dependent adsorption of As(V) to the soil better than when using the CBD-extractable Fe oxide content (RMSE 0.199–0.726) to estimate the HFO content.

The batch data indicate several complicating factors that may influence As(V) adsorption and transport in flowing systems. These include: nonlinear adsorption, adsorption nonequilibrium, and the influence of chemical parameters (pH and the presence of  $\text{PO}_4$ ) on the transport of As(V) in the subsurface. Column experiments were then undertaken to ascertain the effects of these parameters in flowing systems.

At the soil's natural pH of 4.5, the breakthrough curve was asymmetrical (Fig. 4), which is indicative of both nonlinear and rate-limited adsorption (Brusseau, 1998). As predicted by the batch results, the breakthrough curve demonstrates that As(V) exhibits significant retardation due to chemical interactions with subsurface materials. Detectable As(V) concentrations were not observed in the column effluent until almost 200 pore volumes. The transport experiment also illustrated another phenomenon not uncovered in the batch experiments, the presence of an irreversibly adsorbed and/or slowly desorbing fraction. After peaking at a relative As(V) effluent concentration of 0.624 after the completion of the As(V) input pulse, the As(V) concentration began to decrease as the influent concentration was switched to an As(V)-free solution. After >1100 pore volumes of desorption, the relative As(V) concentration was approximately 0.06 and decreasing very slowly, exhibiting a tailing effect. At this point, numerical integration of the effluent curve indicated that the amount of As(V) recovered was only 44% of the total As(V) input. The low recovery suggests that a significant fraction of the initial As(V) has effectively irreversibly adsorbed to the soil. The presence of a irreversible or slowly desorbing fraction has also been detected at As-contaminated sites (Kuhlmeier, 1997a,b).

The As(V) breakthrough was predicted using the measured isotherm (Fig. 2) and two adsorption equilibrium models. Both models assume adsorption equilibrium is instantaneously maintained between the fluid and the solid phase. The LLE model predicted a similar peak As(V) concentration to that of the experimental data. However, the predicted peak breakthrough did not occur until approximately 600 pore volumes later than observed. The model also did not exhibit a tailing effect, as was observed in the experiment breakthrough curve. The model also overpredicted the amount of As(V) available for recovery, as 100% recovery was exhibited in the model-predicted breakthrough curve whereas only 44% recovery was obtained in the experimental breakthrough curve. Quantitatively, the model predicted the experimental data to RMSE = 0.317. The variations between the experimental data and the model-predicted data could be attributed to violations of the underlying assumptions of the model: (i) the nonlinearity of the isotherm and (ii) rate-dependent adsorption equilibrium. This pore water velocity (0.53 cm

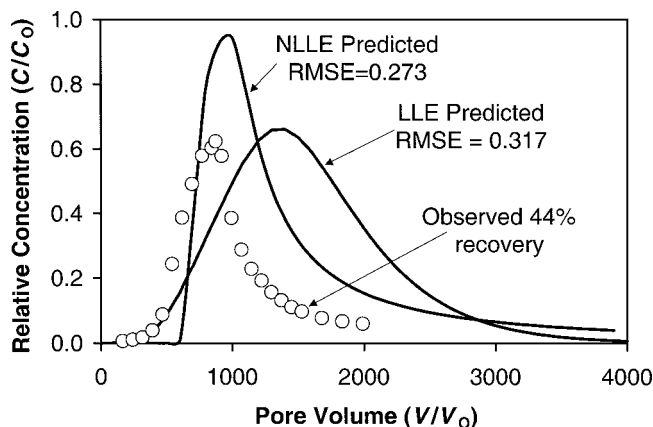


Fig. 4. Relative effluent As(V) concentration versus pore volumes eluted (pH = 4.5, 0.01 M  $\text{NaNO}_3$ ,  $C_0 = 1 \text{ mg L}^{-1}$ , pore water velocity  $0.53 \text{ cm min}^{-1}$ ).

$\text{min}^{-1}$ ) results in a hydraulic residence time of 3.21 min, which is too fast to allow equilibrium between As(V) and the soil based on the kinetic data (Fig. 1). The shortcomings of this model are noteworthy as the model is commonly used in predicting the migration of As(V) (Carrillo and Drever, 1998) and other contaminants in the subsurface (Bethke and Brady, 2000).

The NLE model using the nonlinear Freundlich isotherm parameters ( $K_f = 278 \text{ L kg}^{-1}$  and  $n = 0.32$ ) predicted the data more accurately (RMSE = 0.273) than the LLE model. Again, the experimental breakthrough occurred more rapidly than predicted, though not to the same extent as with the LLE model. The predicted peak relative As(V) concentration was approximately 0.95 and occurred approximately 100 pore volumes after the experimental breakthrough peak. The NLE model thus predicted the time to peak breakthrough (as measured in dimensionless pore volumes) significantly better than the LLE model. Although the NLE model predicted a tailing effect similar to the experimental data, it still overestimated the percentage of As(V) available for recovery. Both the NLE and the LLE models assume complete reversibility of the adsorption reaction. However, the LLE model did not exhibit the same tailing effect that the NLE model and experimental data exhibit. The differences in the two models indicate that the linearity or nonlinearity with which the isotherm data can be described plays a key role in predicting breakthrough curves, even in the absence of rate-dependent adsorption.

The presence of an irreversible or slowly desorbing fraction produced an interesting phenomenon in comparison with the predicted results assuming adsorption equilibrium (Fig. 4). The presence of rate-limited adsorption theoretically promotes greater mobility of subsurface contaminants compared with those that rapidly achieve adsorption equilibrium, as the initial breakthrough curve confirms. Arsenic(V) concentrations appeared in the column effluent faster than predicted by both models. However, due to the presence of an irreversible or slowly desorbing fraction, the peak aqueous As(V) concentration and the total amount of As(V) recovered was lower than predicted based on the two

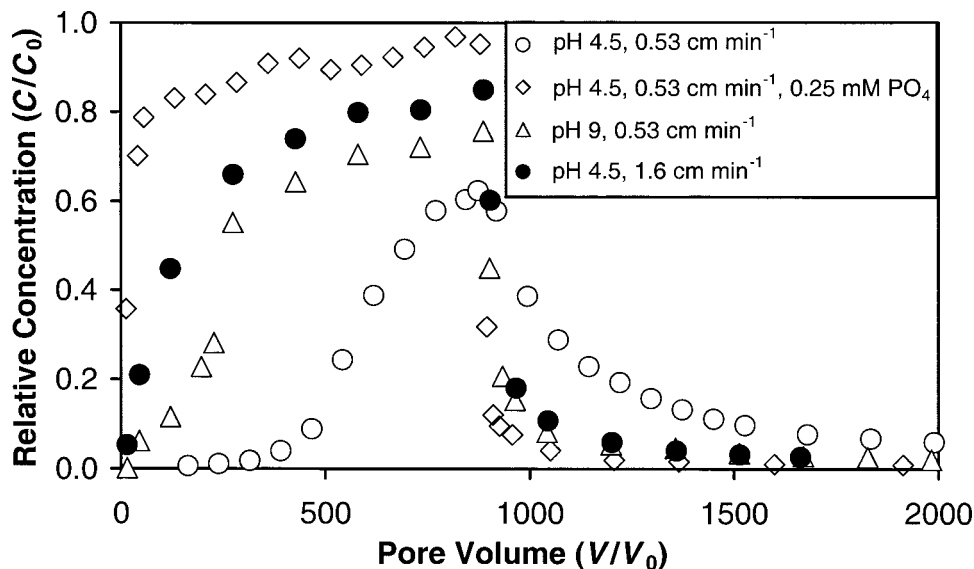


Fig. 5. Effect of varying physical (pore water velocity) and chemical (pH and  $\text{PO}_4$ ) conditions on As(V) transport ( $0.01 \text{ M NaNO}_3$  and  $C_0 = 1 \text{ mg L}^{-1}$ ).

equilibrium models. This may have important implications to subsurface As(V) contamination. Although subsurface As(V) may reach a given point of interest (e.g., a site boundary) faster than predicted by the models, the overall mobility in terms of maximum concentrations and total recovery may be lower than predicted by the models.

Increasing the pore water velocity by a factor of three from  $0.53$  to  $1.6 \text{ cm min}^{-1}$  (decreasing the column residence time from  $3.2$  to  $1.1 \text{ min}$ ) significantly increased As(V) mobility (Fig. 5), further evidence of adsorption nonequilibrium. Detectable As(V) effluent concentrations occurred after approximately 15 pore volumes, an order of magnitude faster than at the lower pore water velocity of  $0.53 \text{ cm min}^{-1}$ . The relative effluent concentration at approximately 875 pore volumes peaked at 0.850. Significantly, the irreversible or slowly desorbing fraction and the associated degree of tailing also decreased. After approximately 1800 pore volumes, the effluent As(V) concentration was below the detection limit and the quantity of As(V) recovered was 76%. A decrease in pore volumes required for breakthrough, an increase in relative peak breakthrough concentration, and an increase in As(V) recovery occurred at higher pore water velocity. The increased pore water velocity also had a marked effect on breakthrough curve asymmetry, which is indicative of adsorption nonequilibrium.

As predicted from the batch data (Fig. 3), an increase in pH from 4.5 to 9.0 significantly increased the mobility of As(V) (Fig. 5) as measured by the initial breakthrough, peak breakthrough concentration, and total As(V) recovery. At the same pore water velocity, As(V) breakthrough commenced at  $<15$  pore volumes at pH 9 compared with  $>200$  pore volumes at pH 4.5. In addition, the peak relative concentration increased to 0.757 and the total recovery increased to  $>65\%$ . The degree of tailing also decreased, as after 2000 pore volumes the effluent As(V) concentration was less than the detection

limit ( $<5 \mu\text{g L}^{-1}$ ). The increased pH results complement the results of the batch experiments in showing that an increase in pH results in a decrease in the total degree of adsorption. These results also extend the results of the batch experiments to show that a decrease in the amount of adsorption results in a corresponding decrease in the amount of irreversible or slowly desorbing As(V).

As in the batch experiments,  $\text{PO}_4$  had the most dramatic effect on As(V) adsorption and the resulting mobility and total recovery of As(V) (Fig. 5). A detectable concentration of As(V) appeared in the effluent almost immediately, and the relative As(V) effluent peak concentration at the end of the input pulse was 0.969 compared with 0.624 in the same experiment without  $\text{PO}_4$ . After the As(V)- $\text{PO}_4$  input pulse, the As(V) rapidly desorbed. After desorbing for approximately 50 pore volumes the relative As(V) effluent concentration was less than 0.10. After 1200 pore volumes the relative effluent As(V) concentration was lower than the detection limit ( $<5 \mu\text{g L}^{-1}$ ) and exhibited no tailing effect. The As(V) recovery of this column was 92%, which is the highest of the column experiments conducted, as the addition of  $\text{PO}_4$  decreased the irreversible or slowly desorbed fraction, which greatly increased the mobility of As(V).

These  $\text{PO}_4$  results are significant. In the absence of  $\text{PO}_4$ , As(V) would be considered a relatively immobile anion. In the presence of  $0.25 \text{ mM PO}_4$ , it would be considered a relatively mobile anion. Although this level of  $\text{PO}_4$  is higher than in many subsurface environments,  $\text{PO}_4$  is ubiquitous in the natural environment. In addition, there are a number of agricultural areas where both As(V) and  $\text{PO}_4$  have been added to the subsurface as a pesticide and fertilizer, respectively (Smith et al., 1998).

## CONCLUSIONS

The results of this paper illustrate several important aspects regarding the mobility of As(V) in the sub-sur-

face. There is a significant potential for both nonlinear and rate-dependent (i.e., nonideal) adsorption-desorption of As(V). Adsorption of As(V) to this iron oxide-containing media continued over a time scale of weeks and the adsorption isotherm was markedly nonlinear ( $n = 0.32$ ). Both of these phenomena could result in greater mobility of As(V) than predicted from simple linear partitioning approaches. However, the presence of an irreversible or slowly desorbing fraction acted to counteract the increased mobility that was otherwise predicted. Chemical parameters such as pH and the presence of  $\text{PO}_4$  also significantly affected As(V) mobility. A commonly used surface complexation model for As(V) adsorption to HFO predicted the pH-dependent adsorption of As(V) to this soil within an RMSE of 0.116 to 0.726, indicating the potential for such models to provide initial estimates of pH-dependent As(V) adsorption in the absence of site-specific data. Using the AOD-extractable Fe concentration as an estimate of the HFO content typically resulted in underpredicting the extent of adsorption, while using the CBD-extractable Fe concentration to estimate the HFO content consistently resulted in overpredicting the extent of adsorption. Arsenic(V) was significantly retarded in soil columns. Although the nonlinear equilibrium model (RMSE = 0.273) predicted the data better than the linear model (RMSE = 0.317), As(V) breakthrough occurred more rapidly than predicted by either model due to adsorption nonequilibrium. For the parameters examined in this study, the effect on As(V) mobility and recovery increased in the order  $\text{pH} < \text{pore water velocity} < \text{PO}_4$ . These results illustrate the complex hydrogeochemical factors (e.g., pH- and rate-dependent adsorption) that would need to be incorporated into any comprehensive modeling approach to describe As(V) transport in the subsurface.

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## APPENDIX D

Stewart, M. A., P. M. Jardine, M. O. Barnett, L. D. McKay, T. L. Mehlhorn, S. E. Fendorf and K. Paul (2003). "Effects of contaminant concentration, aging, and soil properties on the bioaccessibility of Cr(III) and Cr(VI) in soil." *Soil and Sediment Contamination* 12(1): 1-21.

## 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 de-

signed 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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**Key Words:** metal bioavailability, metal sequestration by soil, redox transformations, X-ray absorption spectroscopy.

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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 assess 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  $\text{SO}_4^{2-}$ , 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  $\text{SO}_4^{2-}$  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  $< 2 \mu\text{m}$  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  $\text{cmol}_c \text{kg}^{-1}$ , 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  $< 2 \mu\text{m}$  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  $\text{cmol}_c \text{kg}^{-1}$ , 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- $\mu\text{m}$  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  $\text{K}_2\text{CrO}_4$ , were 1000, 250, and 50 ppm at a pH of 6.0 and for Cr(III), as  $\text{CrCl}_3$ , 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  $30 \pm 2$  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			Organic matter content (%)	pH (5mM CaCl <sub>2</sub> )	pH (DDI)	Fe (g/kg)	Mineralogy of <2 μm clay fraction *
	Sand (%)	Silt (%)	Clay (%)					
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 <sub>45</sub> IS <sub>20</sub> V <sub>10</sub> K <sub>9</sub> VC <sub>6</sub> M <sub>5</sub> Q <sub>3</sub> F <sub>1</sub>
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 <sub>27</sub> V <sub>27</sub> VC <sub>14</sub> Q <sub>13</sub> I <sub>10</sub> IS <sub>5</sub> G <sub>3</sub> F <sub>1</sub>

\* 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  $\pm 0.5$  pH units of the initial pH. This scenario held for all cases. Bioaccessibility was calculated as:

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

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 ( $\text{Cr}_T$ ). 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  $\mu\text{m}$  (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 AAnalyst 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  $\text{Cr}_T$  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- x 4- x 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  $\text{SO}_4^{2-}$ . Because  $\text{SO}_4^{2-}$  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 mM  $\text{CaCl}_2$  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  $\text{Na}_2\text{SO}_4$ . 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)

<b>Cr(III)</b>	<b>500 ppm</b>	<b>200 ppm</b>	<b>50 ppm</b>
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

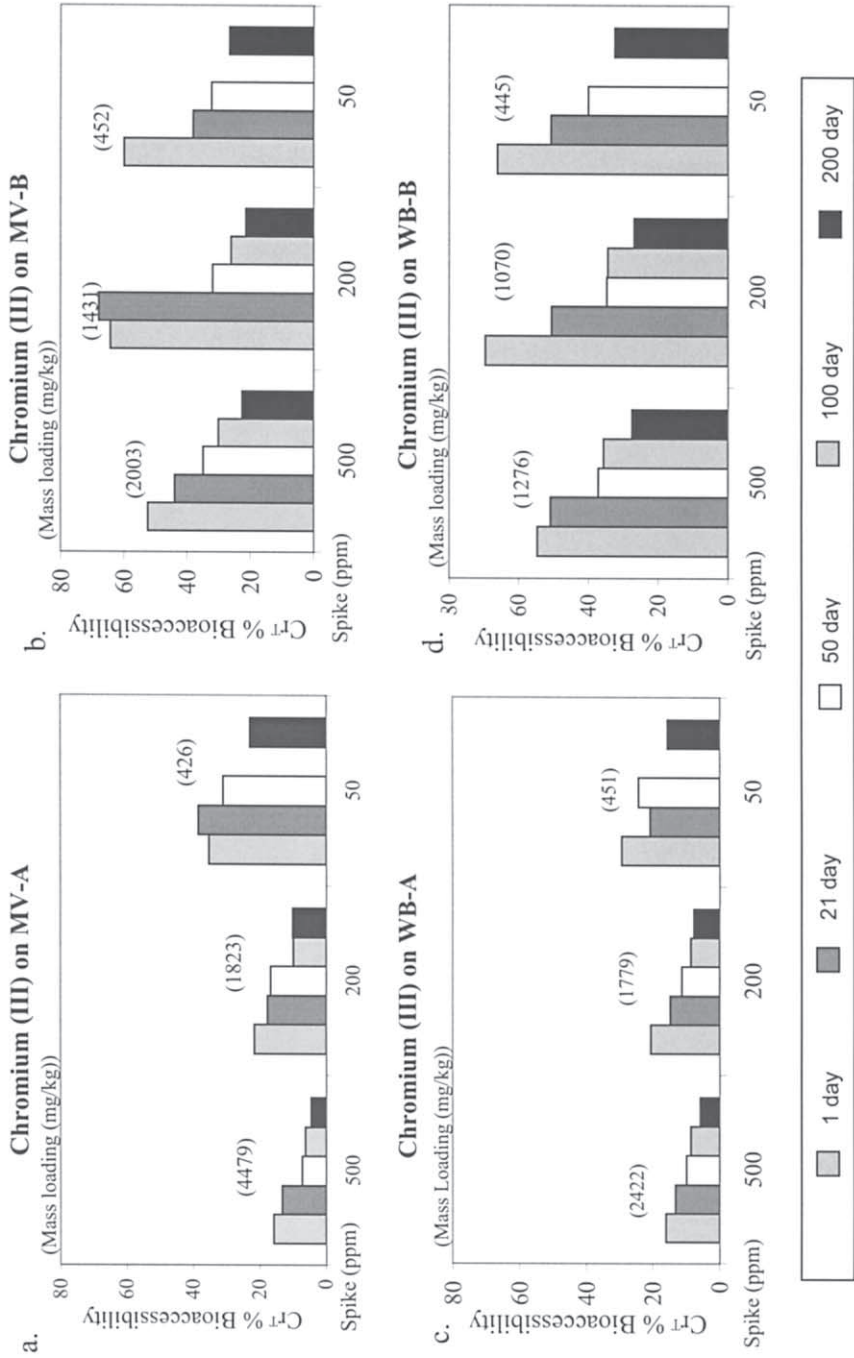
  

<b>Cr(VI)</b>	<b>1000ppm</b>	<b>250ppm</b>	<b>50ppm</b>
Melton A	386.47	199.21	91.28
Melton B	269.14	219.86	150.00
Walker A	391.83	244.24	100.22
Walker B	423.48	330.53	218.25

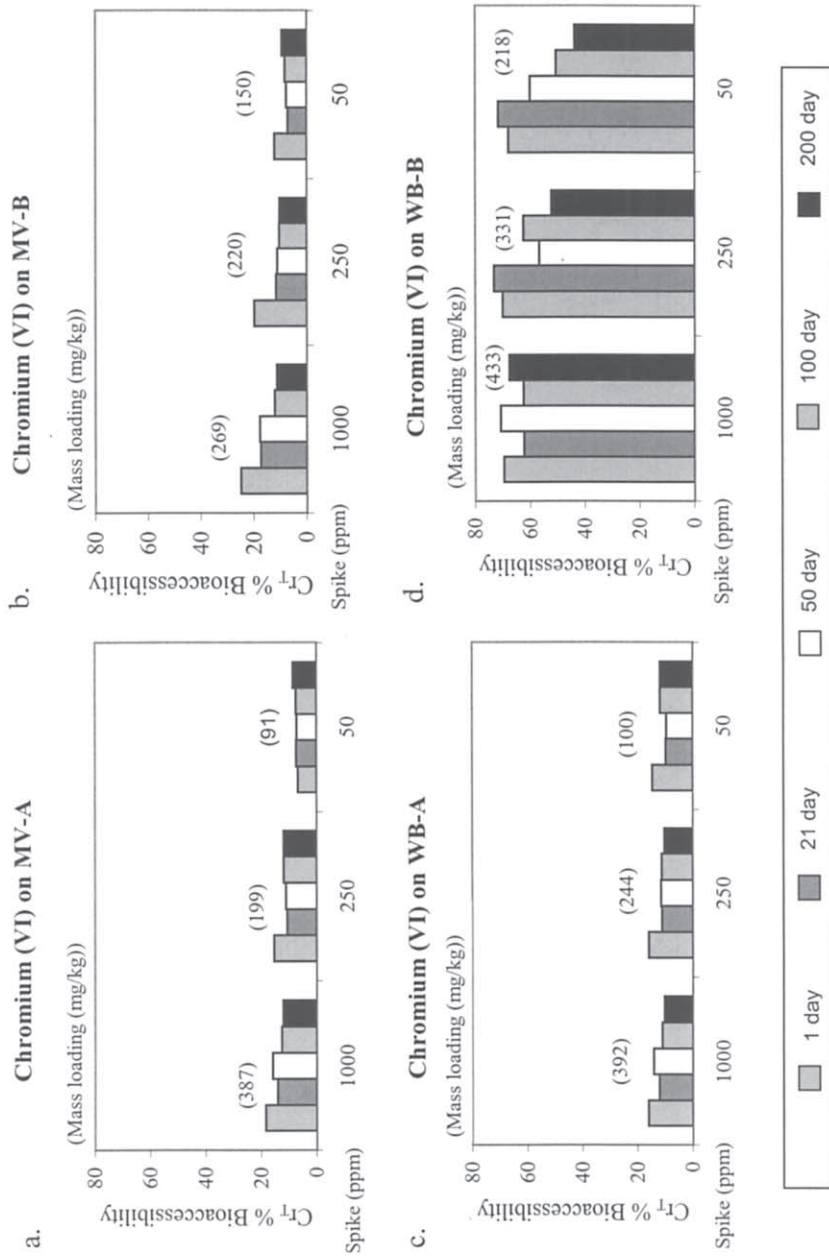
In the case of Cr(III) the patterns of adsorption were reversed, where the A-horizon soils typically adsorbed more Cr than the B-horizon soils. The A-horizon soils characteristically had higher pH creating an environment that favored Cr(III) adsorption. Deprotonation of oxides and organic matter occurs 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)<sub>3</sub>, 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)
	~~~~~%~~~~~	
<u>250 ppm</u>		
MV-A	< 5	> 95
MV-B	< 5	> 95
WB-A	< 5	> 95
WB-B	70	30
<u>1000 ppm</u>		
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)

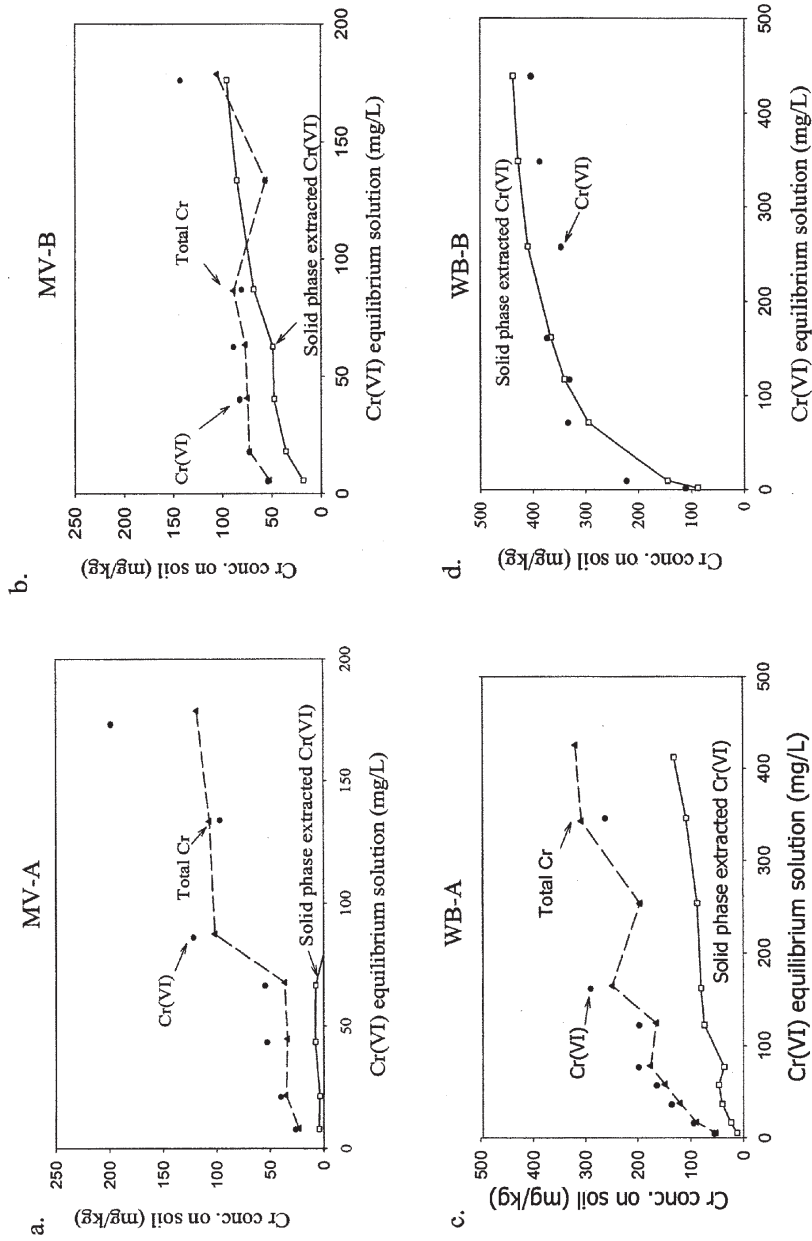
	<u>1 day</u>	<u>21 day</u>	<u>50 day</u>	<u>100 day</u>	<u>200 day</u>
	~~~~~%~~~~~				
<b><u>250 ppm</u></b>					
MV-A	28.73	61.90	78.02	64.91	46.26
MV-B	21.64	59.09	71.92	67.75	32.88
WB-A	16.54	22.86	57.28	28.61	26.38
WB-B	76.92	81.16	99.10	76.07	94.27
<b><u>1000 ppm</u></b>					
MV-A	3.81	15.29	6.31	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<sub>2</sub>SO<sub>4</sub> to remove the Cr(VI) (Figure 3 a-d). The SO<sub>4</sub><sup>2-</sup> anion should be a sufficient competitor for surface sites occupied by HCrO<sub>4</sub><sup>-</sup> 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<sub>4</sub><sup>2-</sup> should be able to recover nearly all of the initial adsorbed Cr(VI). The extractant Na<sub>2</sub>HPO<sub>4</sub> was also utilized on select soils since the HPO<sub>4</sub><sup>2-</sup> 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<sub>4</sub><sup>2-</sup> system; however, the HPO<sub>4</sub><sup>2-</sup> results were somewhat more erratic for reasons unknown to the authors, and thus the SO<sub>4</sub><sup>2-</sup> 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

	<u>MV-A</u>		<u>MV-B</u>		<u>WB-A</u>		<u>WB-B</u>	
	Cr(VI) treatment conc. (ppm)							
	<b>250</b>	<b>1000</b>	<b>250</b>	<b>1000</b>	<b>250</b>	<b>1000</b>	<b>250</b>	<b>1000</b>
% 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 ( $\text{Fe}_2\text{O}_3$ ), and maghemite ( $\gamma\text{Fe}_2\text{O}_3$ ) 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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## APPENDIX E

Stewart, M. A., P. M. Jardine, M. O. Barnett, T. L. Mehlhorn, L. K. Hyder and L. D. McKay (2003). "Influence of soil geochemical and physical properties on the sorption and bioaccessibility of Cr(III)." *Journal of Environmental Quality* 32:129-137.

# Influence of Soil Geochemical and Physical Properties on the Sorption and Bioaccessibility of Chromium(III)

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## ABSTRACT

There are numerous Cr(III)-contaminated sites on Department of Defense (DoD) and Department of Energy (DOE) lands that are awaiting possible clean up and closure. Ingestion of contaminated soil by children is the risk driver that generally motivates the likelihood of site remediation. The purpose of this study was to develop a simple statistical model based on common soil properties to estimate the bioaccessibility of Cr(III)-contaminated soil upon ingestion. Thirty-five uncontaminated soils from seven major soil orders, whose properties were similar to numerous U.S. DoD contaminated sites, were treated with Cr(III) and aged. Statistical analysis revealed that Cr(III) sorption (e.g., adsorption and surface precipitation) by the soils was strongly correlated with the clay content, total inorganic C, pH, and the cation exchange capacity of the soils. Soils with higher quantities of clay, inorganic C (i.e., carbonates), higher pH, and higher cation exchange capacity generally sequestered more Cr(III). The amount of Cr(III) bioaccessible from the treated soils was determined with a physiologically based extraction test (PBET) that was designed to simulate the digestive process of the stomach. The bioaccessibility of Cr(III) varied widely as a function of soil type with most soils limiting bioaccessibility to <45 and <30% after 1 and 100 d soil-Cr aging, respectively. Statistical analysis showed the bioaccessibility of Cr(III) on soil was again related to the clay and total inorganic carbon (TIC) content of the soil. Bioaccessibility decreased as the soil TIC content increased and as the clay content decreased. The model yielded an equation based on common soil properties that could be used to predict the Cr(III) bioaccessibility in soils with a reasonable level of confidence.

THE PRESENCE of chromium (Cr) in the environment is widespread due to its usage in many industrial processes. The metallurgic, tanning, and plating industries are just a few examples of very common applications, large and small, which use Cr on a daily basis (Nriagu and Nieboer, 1988). Chromium itself is thermodynamically stable in two oxidative states: cationic Cr with a valence of three, Cr(III), and anionic Cr with a valence of six, Cr(VI). Chromium(VI) is often considered to be mobile in the environment while the more environmentally stable Cr(III) is considered less mobile (Chung et al., 1994; Patterson et al., 1997). There are several factors that contribute to the decreased mobility of Cr(III) in soil: (i) strong adsorption onto the nega-

tively charged soil surfaces, (ii) the ability to form complex molecules with organics found in the soil, and (iii) the formation of oxides and hydroxides and other insoluble minerals in soil (Fendorf and Zasoski, 1992; Losi et al., 1994; Dragun, 1998).

When assessing the risks posed by Cr(VI) and Cr(III), the exposure pathway of most concern is ingestion by children (Paustenbach, 1989; Davis et al., 1990; Sheehan et al., 1991; Skowronski et al., 2001). Chromium(VI) is considered the most harmful of the oxidative states since it is both a mutagen and a carcinogen at low sub-ppm levels (Levis and Bianchi, 1982). Although Cr(III) is generally considered less harmful to human health than its oxidized counterpart, it may be of concern due to its potential to oxidize to Cr(VI) and its ability to accumulate to very high solid phase concentrations in some soils (Fendorf et al., 1992). The bioaccessibility of organic contaminants in soils has been relatively well studied (Linz and Nakles, 1997); however, the bioaccessibility of soil-bound metals such as Cr has received less attention (Ruby et al., 1996; Rodriguez et al., 1999; Skowronski et al., 2001; Stewart et al., 2003), where the bioaccessibility is defined as that amount of contaminant that is soluble due to simulated *in vitro* gastric functions and has the potential to cross the intestinal wall (Hamel et al., 1998). Typically, calculated health risks are inappropriately based on a reference dose derived from studies that use soluble aqueous metal species. The ubiquitous metal-sequestering properties of soil may significantly lower the bioaccessibility of Cr upon digestion, which, in turn may influence the decision for remediation at contaminated sites. Thus, action levels set by state regulators concerning the bioaccessibility of Cr in soil may need to consider specific soil properties instead of using generic guidelines (Proctor et al., 1997).

The intent of this paper is to show that Cr(III) can be strongly sequestered by soil, which in turn influences its bioaccessibility. We developed a simple statistical model based on measured soil properties to estimate the bioaccessibility of Cr(III)-contaminated soils upon ingestion. We show that common soil properties, which are easily obtainable from the National Resource Conservation Service (NRCS) database, can be used to assess Cr(III) bioaccessibility at contaminated sites.

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**Abbreviations:** DoD, Department of Defense; DOE, Department of Energy; PBET, physiologically based extraction test; NRCS, National Resource Conservation Service; CEC, cation exchange capacity; DDI, double deionized; TOC, total organic carbon; TIC, total inorganic carbon; VIF, Variance Inflation Factor; XAS, x-ray adsorption spectroscopy.

## METHODS

### Soil Type and Characterization

A database of metal-contaminated Department of Defense (DoD) sites was obtained from the U.S. Army Environmental Center, Aberdeen Proving Ground, Maryland. Twenty (20) DoD Army facilities throughout the USA were chosen for consideration based on the high concentration of Cr in their soils and the possible need for remediation (Table 1). Because of the difficulty in obtaining actual contaminated soils from these sites, uncontaminated soils whose properties were similar to the contaminated soils were acquired and treated with Cr(III). The soil series present at the DoD sites of interest were identified using Soil Conservation Survey documents. The USDA-NRCS database was then utilized to locate pedon numbers associated with each soil series. The NRCS was contacted and in most cases 200 g of the A-horizon and the upper B-horizon soil were obtained for each soil series (Table 1). Two additional soils were obtained from the Oak Ridge Reservation in eastern Tennessee, which also had properties similar to DoD sites in the southeast USA. Thirty-five soils were acquired and these encompassed seven major soil orders (Table 1).

Soils were disaggregated with gentle grinding using a mortar and pestle and sieved to provide a soil fraction <250  $\mu\text{m}$ . It is this smaller size material that is more commonly ingested by children since it adheres more readily to the hand (Sheppard et al., 1995; Rodriguez et al., 1999). Soil properties were obtained from (i) the NRCS database and (ii) repeated or additional measurements in our laboratory. Soil properties included pH, cation exchange capacity (CEC), Fe- and Mn-oxide content, particle size distribution, and total organic and inorganic C (Table 2). Repetitive or additional measurements of soil pH, Fe- and Mn-oxide content, and total organic and inorganic C on all soils were performed to verify the quality of, and provide

**Table 1. U.S. Department of Defense Army bases with their associated soil series designations.**

Army bases by soil order	Facility location by state	Soil series
<b>Ultisol</b>		
Holston AAP	Tennessee	Allen
Fort Gillem	Georgia	Cecil
ORNL†	Tennessee	Minvale
<b>Alfisol</b>		
Seneca AD	New York	Angola
Indiana AAP	Indiana	Crider
Bluegrass Facility	Kentucky	Lawrence
Ft. Knox	Kentucky	Lenberg
Lexington Facility—LBAD	Kentucky	Lenberg
<b>Inceptisol</b>		
Letterkenny AD	Pennsylvania	Berks
ARDEC (Picatinny Arsenal)	New Jersey	Rockaway
Letterkenny	Pennsylvania	Weikert
ORNL†	Tennessee	Montevello
<b>Spodosol</b>		
Stratford Army Engine Plant	Connecticut	Charlton
<b>Mollisol</b>		
Kansas AAP	Kansas	Dennis
Lake City AAP	Missouri	Sibley
<b>Aridisol</b>		
Ft. Wingate	New Mexico	Doakum
Tolle Army Depot	Utah	Kzin
Desert Chem. Depot	Utah	Kzin
Dugway	Utah	Kzin
Hawthorne	Nevada	Oricto
Pueblo Chem. Depot	Colorado	Stoneham
<b>Entisol</b>		
Savanna Depot Activity	Illinois	Wakeland

† Department of Energy sites at the Oak Ridge National Laboratory.

missing information to the NRCS database. In general, data generated in our laboratory was in excellent agreement with the NRCS database. Soil pH was determined using double deionized (DDI) water and 5 mM  $\text{CaCl}_2$  in a 2:1 solution/solid ratio. The pH of the clear supernatant was measured with a microprocessor ionalyzer/901 (Orion Research, Beverly, MA) using a combination glass and Calomel electrode (Beckman, Fullerton, CA). Extractable iron and manganese oxides were determined with dithionite-citrate-bicarbonate (DCB) using the methods of Mehra and Jackson (1960). Total organic carbon (TOC) and total inorganic carbon (TIC) were measured by combustion on a Perkin-Elmer 2400 Series II CHNS/O analyzer. Soil TOC was determined on pretreated samples to remove TIC, which involved a near-boiling, 3 M HCl extraction method on agitated samples. Soil TIC was computed from the difference between total soil C (no pretreatment) and TOC.

### Contaminant Addition to Soil

Ten grams of soil was placed in a 200-mL glass centrifuge vessel along with 100 mL of 500 ppm Cr(III) as  $\text{CrCl}_3$ , pH 4.0. The slurry was agitated on a reciprocal shaker for 2 d, centrifuged, and the supernatant decanted for analysis. This was repeated three more times. After the fourth addition of Cr, the soils were washed three times with DDI water and allowed to air dry. Once the soils were dry, they were gently crushed, homogenized, and then wetted with DDI water to achieve a 30% moisture content. The soils were kept in a container out of direct light and maintained at 30% water content in a moisture saturated environment. Soils were incubated in this manner for the duration of the study (i.e., at least 100 d).

### Determination of Chromium on Soil

Total Cr 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 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 analysis. Samples were stored and analyzed for total Cr using inductively coupled plasma.

### In Vitro Bioaccessibility

A physiologically based extraction test (PBET) was adapted from Ruby et al. (1996, 1999; Ruby, personal communication, 2000) to assess the in vitro bioaccessibility of Cr(III) from contaminated soils in humans. The method is designed to simulate the stomach digestive system in humans. The PBET method has been shown to agree with in vivo studies involving Pb-contaminated soils (Ruby et al., 1996) as well as As-contaminated soils (Rodriguez et al., 1999); however, limited data is currently available in the literature that evaluates Cr bioavailability in contaminated soils using in vivo methods (Witmer et al., 1989, 1991; Gargas et al., 1994), and this data does not appear useful for cross-correlating with the results of the current study. Nevertheless, the PBET method can serve as a useful approximation of Cr bioavailability until in vivo studies become available to validate the methods credibility with regard to Cr.

In the current study, triplicate 0.39 g moist samples (0.3 g dry wt) were placed in 50 mL polyethylene tubes to which 30 mL 0.4 M glycine at pH 1.5 and 37°C was added. The slurries were quickly placed in a rotating water bath of 37°C

**Table 2. Select soil chemical and physical properties.†**

	TOC	TIC	Clay	Silt	Fe	Mn	CEC	pH	pH
	%		%		g/kg		cmol/kg	5 mM CaCl <sub>2</sub>	DDI
<b>Ultisol</b>									
Allen A	1.55	0.56	8.7	29.5	6.95	0.31	7.7	4.59	5.05
Allen Ba	0.19	0.09	14.9	28.4	18.96	0.10	1.3	4.30	4.74
Cecil Ap	1.64	0.39	10.2	23.0	6.01	0.06	5.8	4.04	4.47
Cecil Bt1	0.29	0.21	44.8	15.5	32.56	0.11	1.6	4.44	4.48
Minvale Ap	1.89	0.99	6.1	59.0	7.71	1.51	6.0	6.01	6.61
Minvale Bt1	0.10	0.07	23.6	44.2	19.55	0.16	4.0	4.30	5.17
<b>Alfisol</b>									
Lawrence Apl	0.91	0.59	19.5	48.5	11.17	1.35	5.8	4.97	5.27
Lawrence Btl	0.11	0.10	25.8	38.3	17.53	0.29	3.7	4.28	4.91
Angola Ap	3.72	0.96	32.1	56.1	23.28	1.23	6.7	5.29	5.48
Crider Ap	0.55	0.39	22.5	75.8	13.34	0.72	5.6	6.57	6.84
Crider B2lt	0.21	0.13	30.9	67.2	13.38	0.30	5.4	5.27	5.63
Lenberg A	3.41	1.01	49.1	44.5	12.94	1.37	7.9	5.92	6.06
Lenberg Btl	0.36	0.25	64.7	29.5	15.69	0.12	5.5	4.35	4.77
<b>Inceptisol</b>									
Berks A	2.72	1.01	15.7	46.6	13.18	0.15	9.1	3.65	3.91
Rockaway A1	3.54	1.49	12.4	34.8	14.03	0.52	10.6	3.86	3.98
Rockaway B2t	0.21	0.18	12.6	32.1	17.34	0.16	3.7	4.10	4.41
Weikert Ap	3.97	2.37	24.4	56.2	21.41	6.47	13.3	4.44	4.70
Weikert Be	2.01	1.15	23.9	54.3	28.98	5.42	8.0	4.28	4.65
Montevello A	3.55	0.62	6.0	69.0	10.68	1.42	8.0	6.91	7.18
Montevello B	0.42	0.26	19.0	42.2	22.07	0.17	14.0	4.23	4.87
<b>Spodosol</b>									
Charlton A2	2.30	0.40	2.9	28.7	1.33	0.00	11.7	3.15	3.57
<b>Mollisol</b>									
Dennis Ap	1.32	0.89	15.9	66.1	15.11	0.60	8.7	5.82	6.08
Dennis Ba	0.38	0.41	29.7	57.5	24.29	0.59	4.4	4.77	5.28
Sibley A	1.06	0.49	23.5	69.7	8.23	0.67	7.1	6.36	6.66
Sibley B1	0.72	0.52	26.9	68.0	9.11	0.59	6.8	6.36	6.76
<b>Aridisol</b>									
Doakum Ab	0.28	0.08	10.8	24.8	4.74	0.19	6.9	6.94	7.42
Doakum Bt	0.39	0.18	29.3	15.0	6.86	0.16	7.0	6.87	7.39
Kzin A2	3.27	1.35	22.2	44.2	4.07	0.29	13.3	7.74	7.87
Kzin Bk	3.40	1.88	27.0	38.5	3.26	0.18	10.0	7.80	7.88
Oricto A2	0.09	0.94	10.2	34.7	2.92	0.34	13.7	8.72	9.60
Oricto Bt	0.16	1.10	23.2	27.5	3.16	0.29	8.6	9.01	9.60
Stoneham A	1.45	0.71	16.2	41.4	3.40	0.26	10.1	6.43	6.83
Stoneham Bt1	0.66	0.32	21.4	23.2	2.20	0.20	7.8	6.80	7.15
<b>Entisol</b>									
Wakeland Ap	0.92	0.00	23.8	64.7	8.82	0.71	6.1	5.86	6.09
Wakeland Cg1	0.56	0.25	21.1	66.4	9.18	0.80	5.7	5.77	6.07

† TOC, total organic carbon; TIC, total inorganic carbon; CEC, cation exchange capacity; DDI, double deionized.

and agitated at  $30 \pm 2$  rpm for 1 h. Supernatant was separated from the solid via centrifugation. The pH of the supernatant was measured to ensure that the final pH was within  $\pm 0.5$  pH units of the initial pH. This scenario held for all cases. Thus, bioaccessibility was calculated as:

% Bioaccessibility =

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

The PBET pH of 1.5 simulates the most aggressive stomach digestive scenario, which is a condition indicative of human fasting. Conditions of higher pH, as a result of food intake, would most likely decrease Cr bioaccessibility even more profoundly than the results presented in the current study, thus offering a potential avenue for future research. Both Ruby et al. (1996) and Yang et al. (2002) found that soil Pb bioaccessibility was strongly pH dependent with soluble Pb decreasing profoundly over a pH range of 1.5 to 4.0.

### Chromium Analysis

The PBET supernatant, soil spiking solution, and equilibrium solution were measured for Cr(VI) and total Cr (Cr<sub>T</sub>).

Chromium(VI) was measured using a modified *s*-diphenylcarbohydrazide colorimetric method (Bartlett and James, 1979) using a UV-VIS spectrophotometer at wavelength 540  $\mu\text{m}$  (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 Cr was measured on a Perkin Elmer AAnalyst 800 atomic absorption spectrophotometer (Wellseley, PA). Standards were made using an atomic absorption Cr standard (EM Industries, Hawthorne, NY). Chromium(III) was calculated as the difference between Cr<sub>T</sub> and Cr(VI).

### Modeling

A multiple regression technique in the statistical software package SigmaStat 2.0 (Jandel Scientific) was used to derive an expression that related Cr(III) sorption and bioaccessibility to common soil properties. The model was run using forward stepwise regression to determine the most salient soil properties for calculating sorption or bioaccessibility. Multiple linear regression was then employed to determine the linear equation to use when computing the Cr(III) sorption or bioaccessibility based on the important soil properties previously ascertained.

## RESULTS AND DISCUSSION

### Influence of Soil Properties on Chromium Sorption

Chromium sorption (i.e., adsorption and surface precipitation) by the 35 soils varied markedly with values ranging from 736 mg/kg to 17 460 mg/kg (Table 3). Sorption of Cr(III) was independent of horizon type where no distinct trend between A- and B-horizons was evident. The majority of the soils adsorbed between approximately 3000 mg/kg to approximately 6000 mg/kg with four soils as high as approximately 18 000 mg/kg. These four soils were all Aridisols and are noted for their high soil pH and for their high TIC content. Observed Cr(III) loading levels on many of these different soil types were similar to those measured on actual contaminated soils from the DoD sites. For example, actual contaminated Kzin soil (Xeric Torriorthents) from the Desert Chemical Depot contained 27 000 mg Cr/kg soil. Artificially contaminated Kzin soils in this study contained approximately 18 000 mg Cr/kg soil.

The large contrast in Cr(III) sorption by the various

soils can be explained by the differences in soil properties. Multiple linear regression showed that four soil properties were important in determining the amount of Cr adsorbed by the soils: pH, total inorganic carbon (TIC) content, clay content, and cation exchange capacity (CEC). The relationship describing Cr adsorption was:

$$\begin{aligned} \text{Cr(III) (mg/kg on soil)} = & -12\,666.3 + \\ & (113.8 \times \% \text{ clay}) + (364.6 \times \text{CEC}) + \\ & (1743.2 \times \% \text{ TIC}) + (1916.7 \times \text{soil pH}) \end{aligned}$$

Chromium(III) sorption by the soils was strongly correlated with these soil properties ( $r^2 = 0.794$ ) suggesting that nearly 80% of the variability in Cr(III) sorption could be described by pH, TIC, clay, and CEC (Table 4). Incorporating the other measured soil properties from Table 2 (e.g., Fe-oxide content, TOC, etc.) did not improve the model fit. In fact, TIC could have been removed from the model if necessary, since the other three independent soil variables could describe approximately 77% of the variability in Cr(III) sorption. The four-parameter model above was statistically rigorous at the 95% confidence level since P values for the independent variables were all <0.05 (Table 4). Thus, it can be concluded that the independent variables, the soil properties, significantly contribute to predicting the dependent variable, Cr sorption. The Variance inflation factor (VIF) also suggested that collinearity between independent variables was not significant (Table 4). Values for VIF that are 1.0 or slightly larger suggest that the variables do not show multicollinearity and that the parameter estimates are reliable. Collinearity becomes an issue when values of VIF exceed 4.0. This model also passed the Normality Test (indicating that the data was normally distributed) and the Constant Variance Test (suggesting that the variance of the dependent variables was constant). One of the most important criteria of a successful model, however, is the true physical significance of the model parameters. Our model suggests that Cr(III) sorption is enhanced by higher soil pH, more TIC (i.e., carbonates), more clay, and higher CEC. For a sparingly soluble cation, such as Cr(III), these soil conditions should enhance sequestration as the model suggests.

The pH of the soil affects the solubility and form of Cr and therefore affects sorption. As the soil pH increases, the amount of Cr on the soil increases. At low pH, Cr(III) is adsorbed or complexed on soil negative charges; at higher soil pH values, >5.5, Cr precipitates

**Table 3. Chromium(III) solid phase concentrations on the various soils and their corresponding bioaccessibility after 1 and 100 d aging.**

	$C_T$ on soil	1 day % Cr(III) bioaccessible	100 day % Cr(III) bioaccessible
<b>Ultisol</b>	mg/kg		
Allen A	940.32	16.37	8.13
Allen Ba	736.15	31.11	17.98
Cecil Ap	1 342.49	18.84	9.90
Cecil Bt1	2 333.76	41.77	28.34
Minvale Ap	2 261.67	15.88	8.55
Minvale Bt1	1 294.09	54.65	35.52
<b>Alfisol</b>			
Lawrence Ap1	2 586.96	26.03	11.62
Lawrence Bt1	2 359.18	41.48	28.10
Angola Ap	9 408.00	32.40	16.58
Crider Ap	3 719.38	33.90	22.88
Crider B2t	4 247.30	50.30	32.35
Lenberg A	8 169.92	30.27	20.28
Lenberg Bt1	7 254.84	50.89	41.63
<b>Inceptisol</b>			
Berks A	2 275.20	18.77	7.67
Rockaway A1	2 482.08	11.62	6.46
Rockaway B2t	1 525.58	32.96	22.36
Weikert Ap	5 561.77	12.21	5.62
Weikert Be	3 229.97	19.73	10.35
Montevello A	5 925.66	19.03	7.03
Montevello B	2 751.57	47.71	26.23
<b>Spodosol</b>			
Charlton A2	1 721.95	27.65	21.26
<b>Mollisol</b>			
Dennis Ap	3 577.05	19.43	13.67
Dennis Ba	3 521.90	33.61	26.68
Sibley A	4 436.16	29.78	20.50
Sibley B1	4 689.17	36.36	25.37
<b>Aridisol</b>			
Doakum Ab	2 507.82	31.19	16.60
Doakum Bt	5 964.29	39.40	32.77
Kzin A2	16 306.33	17.22	14.00
Kzin Bk	12 452.82	24.03	19.81
Oricto A2	17 460.00	13.66	10.26
Oricto Bt	15 964.28	18.45	16.44
Stoneham A	4 377.44	29.27	18.97
Stoneham Bt1	4 599.44	33.70	24.82
<b>Entisol</b>			
Wakeland Ap	4 262.61	32.33	21.08
Wakeland Cg1	3 802.32	37.68	24.79

**Table 4. Parameter estimates, standard errors, and statistics obtained from a multiple linear regression analysis that related soil properties to Cr(III) sorption.†**

Parameter	Value	SE	P	VIF
Intercept	-12 666.3	1 794.5	<0.001	-
% Clay	113.8	30.4	<0.001	1.119
pH in DDI	1 916.7	250.7	<0.001	1.079
CEC, cmol/kg	364.6	155.7	0.026	1.902
% TIC	1 743.2	850.1	0.049	1.670
$r^2$	0.794		<0.001	

† DDI, double deionized; CEC, cation exchange capacity; TIC, total inorganic carbon; VIF, Variance Inflation Factor.

as hydroxides covering the surface of the soil (Bartlett and Kimble, 1976). It was presumed by Bartlett and Kimble (1976) and James and Bartlett (1983) that the Cr(III) precipitate consisted of macromolecules with Cr ions in six coordination with water and hydroxy groups. Studies by Fendorf et al. (1994) and Fendorf and Sparks (1994), using x-ray adsorption spectroscopy (XAS), showed that with a low Cr(III) surface coverage the principle mechanism was adsorption with an inner-sphere monodentate complex on the silica. With increased surface coverage (>20%), precipitation likely occurred and became the dominant sorption mechanism.

As with pH, TIC or carbonate content in soils enhanced Cr(III) sorption. The mechanism of increased sequestration is most likely a localized pH effect at the carbonate surface, which promotes the formation of Cr(OH)<sub>3</sub> species. The localized pH effect is the most plausible scenario since there was no correlation between soil pH and soil TIC, thus explaining why collinearity was not a problem for these parameters when the model was fit to the Cr(III) sorption data. Several acidic Inceptisols derived from interbedded limey shales and limestone have relatively large residual carbonate contents (Table 2), due to the slow dissolution of local scale dolomite, and this may serve to enhance Cr(III) sequestration in these systems, even though the overall bulk soil pH is acidic.

The model also shows a positive correlation between the amount of Cr adsorbed and the soil clay content and CEC. This was expected since clay minerals tend to be dominated by negatively charged sites on the surface due to isomorphic substitution (Klein and Hurlbut, 1993). These negatively charged sites attract the cation Cr<sup>3+</sup> and a weak, electrostatic bond is formed. The more negatively charged sites that are available (i.e., larger CEC), the greater propensity for Cr(III) sorption. Further, clay minerals typically have a large surface area that is capable of accommodating large quantities of Cr<sup>3+</sup> and Cr(OH)<sub>3</sub> precipitated phases. The more surface area a soil has, the more reactive sites the soil has, and consequently the more Cr that will adsorb to the soil.

### Influences of Soil Properties on Chromium Bioaccessibility

The bioaccessibility of Cr(III), as measured by the PBET method, varied widely as a function of soil type with most soils limiting bioaccessibility to <45% and <30% after 1 and 100 d soil-Cr aging, respectively (Table 3, Fig. 1a–e). Bioaccessibility values were consistently higher for 1 d aging vs. 100 d aging. For all soils the percent bioaccessibility ranged from 3.0 to 54.7% at Day 1 and 1.5 to 35.5% at 100 d (Table 3, Fig. 1a–e). The aging effect is related to the enhanced stability of Cr on the soil surface with time. Structural reorientation of Cr surface bonds or slow precipitation reactions can account for the stronger sorption of Cr at longer times (Karthein et al., 1991). Previous studies by Stewart et al. (2003) have shown that aging effects are insignificant after 100 d and that the 100 d data are most relevant

to actual DoD-contaminated soils. In general, the A-horizon soils had the lowest percent bioaccessible values, even when they adsorb more Cr(III) on the soil vs. the B horizons (Table 3). Bioaccessibility did not appear to be a function of soil order, suggesting that detailed soil series data, as is used in the current study, was necessary for predictive purposes (Table 3, Fig. 1a–e). Chromium(VI) was also measured in the PBET extractant to monitor for oxidation of Cr(III) to Cr(VI). The proportion of bioaccessible Cr that was Cr(VI) was always <1%, suggesting that oxidation reactions were minimal or that any oxidation products of Cr(VI) were tightly held by the soil. These results are consistent with the data presented by Stewart et al. (2003), which showed limited bioaccessibility of Cr(VI) in several soils.

As demonstrated by Stewart et al. (2003), bioaccessibility values leveled off and reached near equilibrium after the first 50 to 100 d. Thus, the 100 d bioaccessibility data is most appropriate for use in the modeling endeavor. Stepwise multiple regression indicated two combinations of variables considered instrumental in predicting the bioaccessibility of Cr(III) in soils: (i) % clay and % TIC and (ii) % clay and % TOC. Using the independent variables from Table 2, the most significant model revealed that the bioaccessibility of Cr(III) on the soils was correlated with clay and TIC of the soil (Table 5). The relationship describing Cr(III) bioaccessibility was:

$$\% \text{ Cr(III) bioaccessible} = 16.02 + (0.426 \times \% \text{ clay}) - (9.56 \times \% \text{ TIC})$$

with an  $r^2$  value of 0.722, which indicated that as much as 72% of the variability in Cr bioaccessibility was explained by the model (Fig. 2). The model was statistically rigorous at the 99% confidence level since P values for the independent variables were well below 0.01, indicating that they all contributed to predicting the % bioaccessible Cr(III) (Table 5). Values for VIF were all nearly 1.000, indicating that there was no redundant information in the other independent variables, i.e., soil properties, and that collinearity between independent variables was not of concern. This indicated that parameter estimates in the model were reliable, which is in agreement with the low standard errors on the estimated values (Table 5). The model also passed the Normality Test and the Constant Variance Test, suggesting that the data was normally distributed around the regression line and that the variance present in the dependent variable is constant. Most important, however, is the true physical significance of the model parameters. The model suggests that Cr(III) bioaccessibility decreases as the TIC content increases and as the clay content decreases. As shown with the Cr sorption data, Cr(III) sequestration is enhanced by soils with high levels of TIC. The presence of TIC promotes the formation of solid phase Cr(III)-hydroxides that are sparingly soluble, even under acidic conditions. These hydroxides [i.e., Cr(OH)<sub>3</sub>] precipitate and cover the surface of the soil and are not easily bioaccessible even in the presence of the low pH in the simulated stomach fluid of the PBET. Conse-



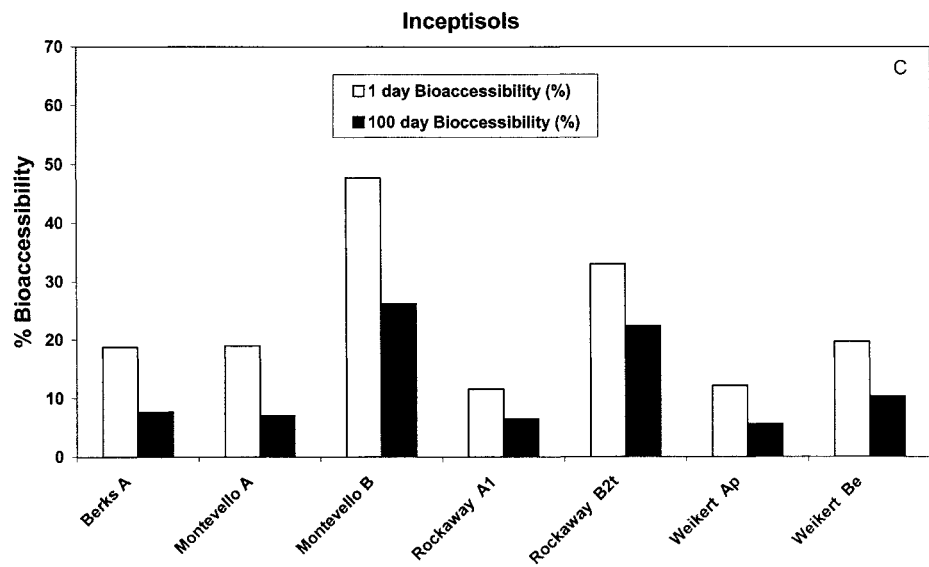
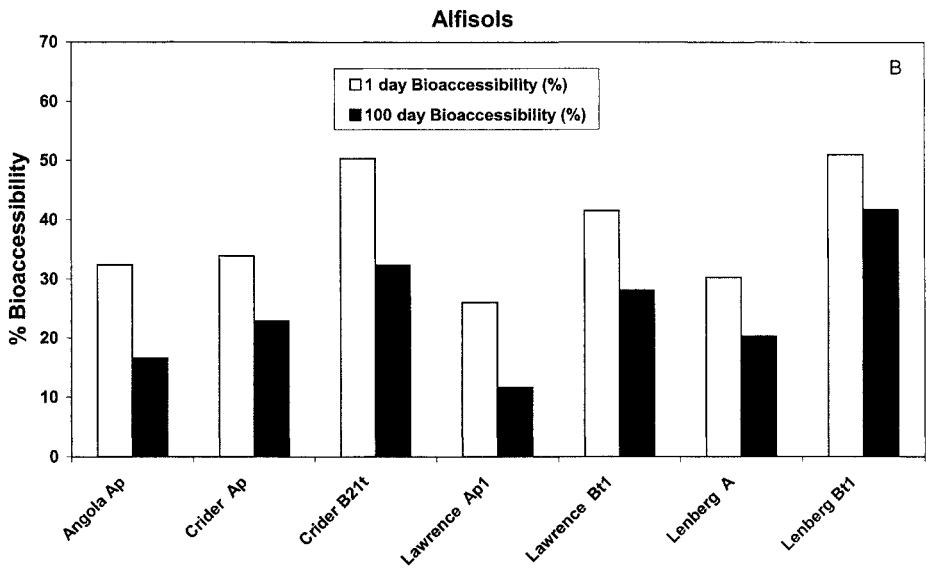
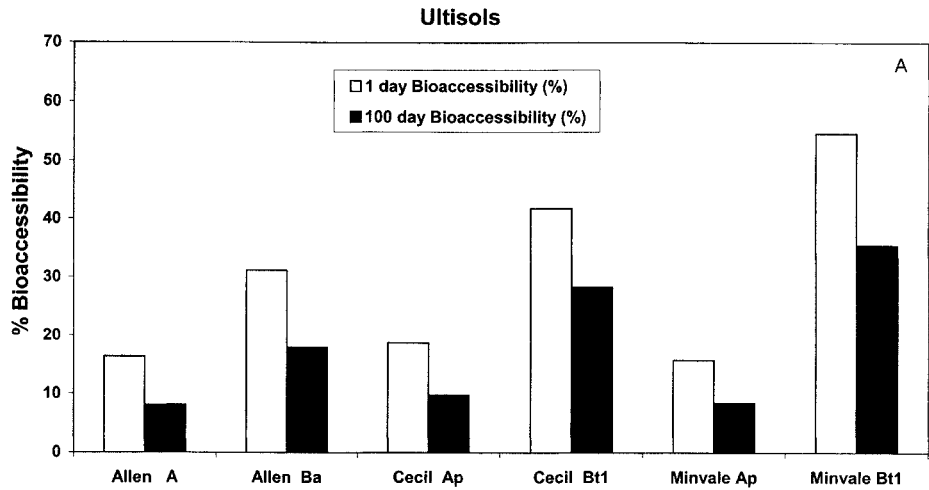


Fig. 1. Percentage Cr(III) bioaccessibility after 1 and 100 d Cr-soil aging for (a) Ultisols; (b) Alfisols; (c) Inceptisols; (d) Spodosols, Mollisols, Entisols; and (e) Aridisols.

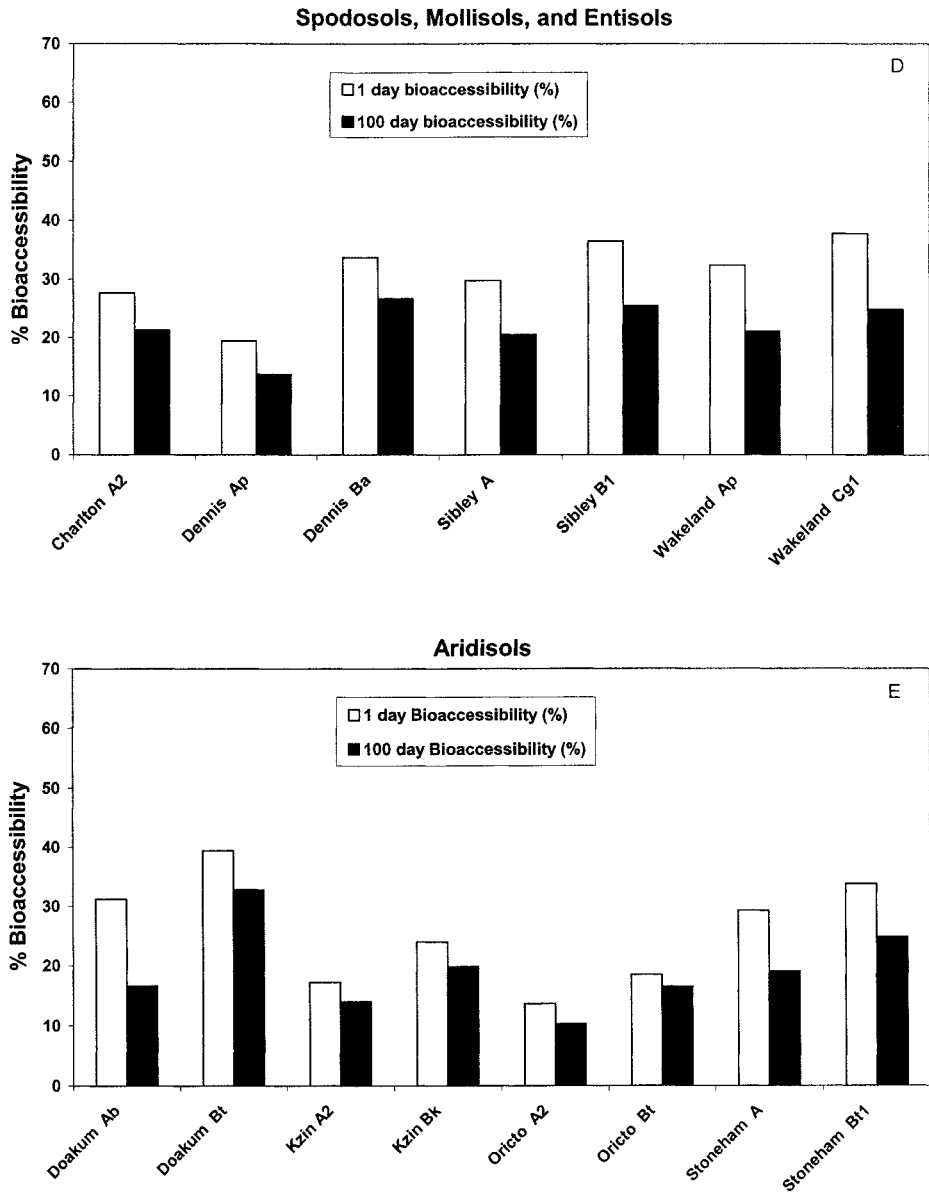


Fig. 1. Continued.

quently as the TIC content increases the bioaccessibility of Cr(III) in soil decreases. As shown with the Cr sorption data the clay content on the soil was also correlated with the amount of Cr sequestration and thus should be important in determining bioaccessibility. The bioaccessibility model suggested that, as the clay content of the soils increased, the percent of Cr on the soil that is bioaccessible also increased. Since the mechanism of Cr retardation on clay minerals is primarily weak electrostatic bonds, these bonds are easily broken under the conditions of the PBET, allowing Cr to desorb from the soil and be released into solution during the simulated digestion.

Stepwise multiple regression analysis also indicated that Cr(III) bioaccessibility was significantly correlated with clay and TOC content of the soil (Table 6). The relationship describing Cr(III) bioaccessibility was:

$$\% \text{ Cr(III) bioaccessible} = 15.54 + (0.408 \times \% \text{ clay}) - (3.78 \times \% \text{ TOC})$$

with an  $r^2$  value of 0.674. This relationship was similar to the clay/TIC model where higher quantities of TIC and TOC resulted in decreased Cr(III) bioaccessibility. When clay, TIC, and TOC were used in the same model, the contribution of TOC was not significant at the 90%

**Table 5. Parameter estimates, standard errors, and statistics obtained from a multiple linear regression analysis that related soil properties (clay and TIC) to percent Cr(III) bioaccessibility.†**

Parameter	Value	SE	P	VIF
Intercept	16.02	1.99	<0.001	-
% Clay	0.426	0.0671	<0.001	1.002
% TIC	-9.56	1.54	<0.001	1.002
$r^2$	0.722		<0.001	

† TIC, total inorganic carbon; VIF, Variance Inflation Factor.

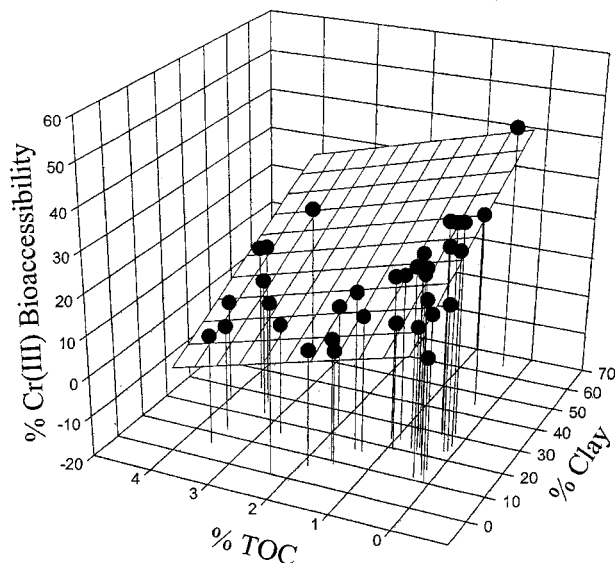


Fig. 2. The observed (data points) and model fitted (grid surface) relationship between the two most significant independent variables (% clay and TIC) and % Cr(III) bioaccessibility using the model: % Cr(III) bioaccessible = 16.02 + (0.426 × % clay) – (9.56 × % TIC) an  $r^2$  value of 0.722.

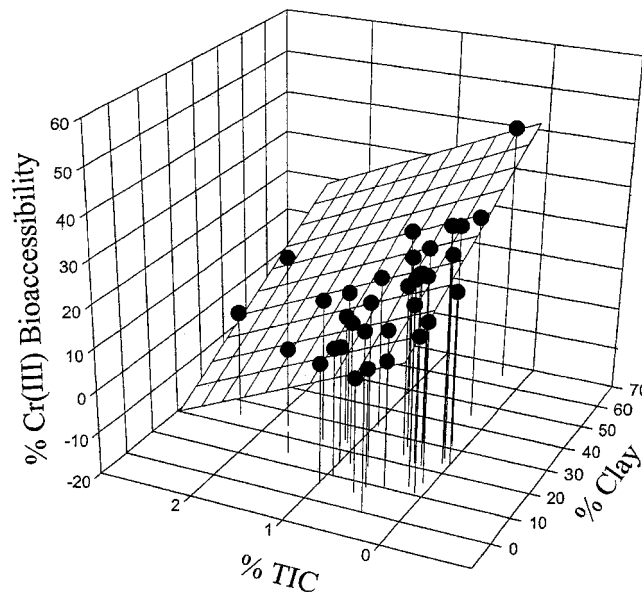


Fig. 3. The observed (data points) and model fitted (grid surface) relationship between the two independent variables (% clay and TOC) and % Cr(III) bioaccessibility using the model: % Cr(III) bioaccessible = 15.54 + (0.408 × % clay) – (3.78 × % TOC) with an  $r^2$  value of 0.674.

confidence level ( $P = 0.115$ ). This scenario may be an artifact of our limited data set, where the most appropriate model, in fact, includes both TIC and TOC along with clay content. A more extensive data set will be necessary to test this hypothesis. Nevertheless, the model using clay and TOC was statistically rigorous at the 99% confidence level since  $P$  values for the estimated parameters were  $<0.01$  and the VIF values are approximately 1.000, indicating that the variables all contribute significantly to the equation and that no multicollinearity was present among the independent variables. This model passed the Normality Test and the Constant Variance Test. The model suggested that as the clay content decreased and the TOC content increased, the % Cr(III) bioaccessible decreased (Fig. 3). The trend regarding clay content is consistent with the previous model and the limited bioaccessibility of Cr in the presence of higher system organic C is conceptually correct. Organic matter found in soil is a major contributor to the overall negative charge in soils and thus is an important sorbent for heavy metal cations (Sparks, 1995). Organic matter has the ability to form strong bonds with the Cr(III) with the metal not readily released during the PBET process. As Cr(III) is considered a Lewis hard acid, it forms stable complexes with the carboxyl group of the organic matter (Sparks, 1995). These bonds are stable

and not easily broken. The current model again explains more than 67% of the variability in Cr(III) bioaccessibility and should be useful for soils low in carbonate (TIC).

### ENVIRONMENTAL SIGNIFICANCE

This study has shown that site assessments of soil metal bioaccessibility based solely on total soil metal concentrations may not accurately reflect the risk posed by the soils. The sequestering properties of soil significantly lower the percent of Cr bioaccessible upon ingestion of the otherwise labile Cr. Chromium(III) can be immobilized as strongly bound species on clay and organic matter, and Cr-hydroxide precipitates on soil mineral surfaces. It has been shown that common soil properties are strongly correlated with Cr(III) bioaccessibility. The availability of these soil properties is commonplace (e.g., NRCS database), which allows the percent bioaccessibility of Cr(III) to be estimated for a variety of contaminated sites whose remediation is pending. The ability to rapidly assess metal bioaccessibility in soils will facilitate decision making strategies regarding the need for more detailed and expensive site-specific bioavailability (e.g., animal feeding) studies, which are designed to assess actual clean-up needs at contaminated DoD sites and other sites to a level safe for human use. Such in vivo studies are lacking with regard to Cr, but research in this area is currently underway (M.V. Ruby, personal communication, 2002).

Table 6. Parameter estimates, standard errors, and statistics obtained from a multiple linear regression analysis that related soil properties (clay and TOC) to percent Cr(III) bioaccessibility.†

Parameter	Value	SE	P	VIF
Intercept	15.54	2.16	<0.001	-
% Clay	0.408	0.073	<0.001	1.010
% TOC	-3.78	0.711	<0.001	1.010
$r^2$	0.674		<0.001	

† TOC, total organic carbon; VIF, Variance Inflation Factor.

### ACKNOWLEDGMENTS

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## **APPENDIX F**

Yang, J. K., M. O. Barnett, S.E. Fendorf, and P. M. Jardine. "Oxidation and bioaccessibility of As(III) in soils." Draft Manuscript.

# **Oxidation and Bioaccessibility of As(III) in Soils**

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## **Abstract**

The influence of various soil physical and chemical properties (Fe and Mn oxides, pH, cation exchange capacity, total inorganic and organic carbon, and particle size) on As(III) adsorption, sequestration, relative bioaccessibility (as a surrogate for oral bioavailability), and oxidation was investigated in 36 well-characterized soils using a physiologically based extraction test (PBET). The effect of soil-metal aging time on the bioaccessibility of As(III) was investigated over a six-month period at PBET pH 1.5 and 1:100 soil to solution ratio. Initial degree of As(III) adsorption was governed by both soil's Fe-oxide content and pH. Average bioaccessibility of As(III) at initial time ( $B_0$ ) in 36 soils was relatively higher than that of As(V) (previous paper), suggesting formation of relatively weaker surface complexes of As(III) with Fe-oxide than As(V). The average bioaccessibility of As(III) and As(V) in 36 soils after one-month were similar resulted from the oxidation of As(III) to As(V). As similar with As(V), both pH and Fe-oxide were the most important soil properties governing the steady-state bioaccessibility ( $B_6$ ) of As(III) in soil. As(III) sequestration (% reduction in bioaccessibility over 6-months) on soil occurred primarily at early aging time and reached near equilibrium at 6-months. From multivariable regression analysis, three parameters, soil's Fe-oxide, pH and DOC, were statistically significant parameters governing the As(III) sequestration. As(III) sequestration was positively related with Fe-oxide content but was negatively related with both pH and TOC. The rapid sequestration of As(III) compared to As(V) with variation of Fe-oxide content was explained by combination effect of different affinity between As(III) and As(V) to Fe-oxide and As(III) oxidation to As(V) during the aging period. As(III) oxidation was rapid at initial aging period for most soils and negligible fraction of arsenite was present after six months in all soils. Rapid As(III) oxidation at initial aging period gives rise to presence of arsenic mostly as As(V), reducing bioaccessibility by forming stronger surfaces complexes with Fe-oxide, suggesting that oxidation of As(III) to As(V) is a kind of remediation skill in the As(III) contaminated soils by reducing arsenic bioaccessibility, mobility, and toxicity.

**Keywords:** bioaccessibility, bioavailability, arsenic, oxidation, soil

## Introduction

Arsenic is generally contaminated in soils due to various anthropogenic sources such as mining activity, discharges of industrial wastes, agricultural application as well as from geochemical reactions (Banerjee 1999; Mariner *et al.* 1996; Davis *et al.* 1996; Bhumbla and Keefer 1994). Although arsenic has multiple oxidation states (+5, +3, 0, and -3), arsenite As(III) and arsenate As(V) are the most common in natural environments. Generally the As(III) is favored in sediment or in aqueous phase under anaerobic conditions while the reverse order of species occurs under aerobic conditions (Faust *et al.* 1987). Speciation and solubility of the inorganic arsenic is sensitive to redox conditions and pH of the environments, affecting bioavailability, toxicity, and mobility of arsenic in soils (Bowell 1994; McGeehan and Naylor 1994). Since As(III) is much more toxic and mobile than As(V), the oxidation of As(III) to As(V) is important in reducing the risk of As-contaminated soils. The mobility and treatability of As is becoming a serious concern as USEPA ordered lowering the drinking water standard from 50 parts per billion (ppb) to 10 ppb by 2006.

Children are often exposed to ingestion of soils contaminated with toxic metals such as arsenic through hand-to-mouth activity in the playground. Therefore the ingestion of soils contaminated with toxic metals is great concern because of their toxicity and threat to human health. This risk has often been estimated by assuming that soil-bound metals were completely absorbed (100% bioavailable) through the human gastrointestinal tract upon ingestion although lower fraction of toxic metals has been known to dissolve from soils (Ruby *et al.* 1992; Davis *et al.* 1992; Freeman *et al.* 1994; Casteel *et al.* 1997). Metal bioavailability in mining soils is generally low because of the presence of low solubility metal sulfides from the ore body or transformation of soluble metal species to solid phase with lower solubility (Barnett *et al.*, 1997). Controlling factors for the dissolution of As(V) and Pb(II) from soils were known as pH of both gastrointestinal fluid and soil, soil metal concentration, soil to solution ratio, mineral form, and particle size (Hamel *et al.* 1998; Ruby *et al.* 1999; Yang *et al.* 2002a).

As mineral surfaces can play an important roles in redox transformation reactions of arsenic, iron- and manganese-(hydro)oxides have been used to oxidize As(III) to As(V) (Oscarson *et al.* 1980, 1981; Scott 1991; Sun and Doner 1998). Scott (1991) reported



that oxidation of arsenite to arsenate in heterogeneous system is related with i) the surface area of mineral form, ii) the surface chemistry, and iii) the energetics of the reactions. The half life of As(III) oxidation with synthetic birnessite was 0.15 and 0.33 hrs at pH 4 and 6.8, respectively (Scott 1991). In addition to these metal-(hydro)oxide, kaolinite and illite surfaces showed heterogeneous oxidation of As(III) to As(V) (Manning and Goldberg 1997).

In this study, labile As(III) was spiked into a wide range of well-characterized soils representing the seven major soil orders within U.S. The As(III) bioaccessibility of 36 soils was measured over six months aging time by employing *in vitro* extraction tests as previously described (Yang *et al.* 2002a, b). The benefits of using labile As(III) are to ascertain the ability of soil themselves to limit As, without regard to any unique site-specific speciation, which is difficult to measure and which is subject to change over time. To determine the macroscopic factors affecting bioaccessibility and the oxidation of As(III), the effect of soil properties such as iron oxide content, manganese oxide, pH, cation exchange capacity (CEC), total organic carbon (TOC), total inorganic carbon (TIC), and particle size were evaluated with multivariable linear regression.

## Experimental Section

**Materials.** All chemicals employed in this research were analytical grade or above, and solutions were prepared with deionized water (18 M $\Omega$ -cm) from a reverse osmosis/ion exchange apparatus (Milli-Q<sup>TM</sup> Water System). The A- and B-horizons of soils from seven major U.S. soil orders were collected, air-dried and passed through a 250  $\mu$ m sieve. The physicochemical properties of soils are shown in Table 1.

**Soil spiking.** As(III) was added to the soils with a target concentration of ~100 mg/kg from a small volume of concentrated As(III) stock solution to a 1:10 g/mL soil solution of 10<sup>-3</sup> M CaCl<sub>2</sub>. To maintain the original soil pH, dilute HNO<sub>3</sub> solution was added to the soil slurry at the same time to neutralize the alkalinity in order to maintain the original soil pH (Table 1). After forty-eight hours mixing, the soil suspension was centrifuged and the supernatant was decanted. The remaining soil was washed twice with distilled water to remove any traces of the original soluble As(III) spike. The decanted supernatant and rinsed water were filtered through 0.45- $\mu$ m membrane filter, and the

concentration of As(III) in the filtrate was analyzed using an atomic absorption spectrophotometer (AAS) equipped with an electrodeless discharge lamp (EDL). The difference between the amount As added and that remaining in the supernatant was used to calculate the initial soil concentration. The As(III) remained in soils was analyzed by EPA Method 3050B to verify a mass balance of  $\pm 10\%$ .

After air-drying the soil and homogenizing by mixing, a portion of sample was taken representing the beginning of the aging experiment (i.e.,  $t = 0$ ) and the remaining soil was placed in a weighing dish and deionized water was added to achieve 30% moisture. The soils were then aged in a larger container in which a steady flow of 100% relative humidity air was passed. The moisture content of the soils was monitored periodically by weight, with deionized water added as necessary to maintain a constant moisture content of 30%. Periodically, sub-samples were removed and analyzed as described below.

**Extractions.** Bioaccessibility of As(III) was measured on duplicate soil subsamples over time by using a modified version of the physiologically based extraction test (PBET) (Ruby *et al.* 1996). During the extraction, the water temperature in the bath was maintained at body temperature ( $37 \pm 2$  °C). The extraction solution consisted of 30 g/L glycine (0.4 M) with the pH adjusted to 1.5 with HNO<sub>3</sub>. Although HCl was generally used to make PBET solution, HNO<sub>3</sub> was used as substitute due to the interference of Cl<sup>-</sup> in the speciation of arsenate and arsenite by ion-exchange column. However, no significant ( $P < 0.05$ ) difference of bioaccessibility was observed by using two different types of PBET solution. Extraction was made using 0.1 gram of soil and 10 mL PBET solution previously equalibrated at  $37 \pm 2$  °C as 1:100 soil to solution ratio. After an hour extraction at  $30 \pm 2$  rpm, a portion of the supernatant was filtered with 0.45  $\mu\text{m}$  filter. Then, the dissolved As concentration in the filtrate was measured with an AAS, with the fraction of As dissolved representing the absolute bioaccessibility. The remaining soil sample was analyzed for As using acid digestion (EPA method 3050B) to verify mass balance within  $\pm 10\%$ .

As described in previous paper (Yang *et al.* 2002a, b), the absolute bioaccessibility (ABA) is the same as the relative bioaccessibility (RBA) because the ABA values of the soluble As(III) were  $96.1 \pm 0.1\%$  (errors represent standard deviation,  $n=3$ ).

**Separation of As(III) and As(V).** In order to quantify As(V) and As(III) in the PBET solution, an anion-exchange method (Wilkie and Hering 1998) was used. The chloride form of the anion-exchange resin (Dowex 1X8-100, sigma) was converted to acetate form in a glass-column (d = 0.8 cm, 1.5 mL resin). Prior to separation of As(III) and As(V), each PBET sample was diluted 10 times and then pH adjusted around 3.5. In this condition, fully protonated As(III) passes through the column while partly deprotonated As(V) was retained. Before analysis of sample, column performance was tested with both As(V) and As(III) dissolved in PBET solution. As(III) recovery was >87% and complete As(V) retention was observed. The total dissolved arsenic concentration before column test and As(III) concentration from column effluent were measured with AAS. The As(V) concentration was then calculated by difference.

## Results and Discussion

**Adsorption.** The fate of As in soils is influenced by interactions with the solid phase, which governs the mobility of As in the aqueous phase. Figures 1a and b show the effects of the Fe-oxide content and pH (based on As adsorbed per mole of Fe) of 36 soils on the adsorption of As(III) over the initial forty-eight hours contact period. Except two soils (#12 and 13), As(III) adsorption was lower than As(V) in most soils (Figure 1a). As(III) adsorption varied from 19% to near 100% with a mean of 55.0% (Table 2), which is significantly lower than that of As(V) (79.8%) on the same set of soils (Yang *et al.* 2002b), and would yield a greater mobility of As(III) than As(V) in natural soil system encountered. From a paired t-test, As(III) adsorption data of the thirty-six soils was different from As(V) ( $P < 0.0001$ ). Due to the non-linear relationship between the percentage of As(III) adsorbed and Fe-oxide content, a linear regression was performed with logarithmically transformed Fe-oxide data. From this regression, log Fe-oxide content significantly ( $P < 0.001$ ) influenced As(III) adsorption and explained 50% ( $r^2 = 0.494$ ) of the variability in the relative adsorption of the soils over the initial forty-eight hour contact period. The dependence of As(III) adsorption to soil's Fe-oxide (slope) was not steeper than that of As(V) below  $10 \text{ g}\cdot\text{kg}^{-1}$ . A few soils (#6, #18, #20, #21, #22, #31) having pH values less than 4.5 show below ~50% adsorption even though Fe oxide

contents of them are greater than  $10 \text{ g}\cdot\text{kg}^{-1}$ . This exceptional trend suggests that pH is another important factor for the As(III) adsorption which will be discussed below.

Figure 1b shows a specific As(III) adsorption ( $\text{mmol-As(III)/mol-Fe}$ ) on thirty-six soils with variation of pH between 3 and 10. As pH increased, the specific As(III) increased except one soil(#29) which was difficult to interpret. This trend was contrast to the previous specific As(V) adsorption with the same soils (Yang *et al.* 2002b) those showed not any specific trend over all pH range investigated. From the linear regression, soil pH significantly ( $P<0.001$ ) influenced As(III) adsorption and explained 68% ( $r^2=0.681$ , w/o considering soil #29) of the variability in the As(III) adsorption. From the adsorption studies of As(III) onto Fe-oxides, Su and Plus (2001) reported maximum As(III) adsorption around pH 9 similar with As(III) adsorption on soils pH ranging from 3 to 9 as observed in this work, suggesting the important role of Fe-oxide as binding sites of As(III). However, in many other previous studies, the pH of maximum adsorption was not consistent but varied with different types of metal(hydro)oxides or soils (Pierce and Moore 1982; Sun and Doner 1998; Singh *et al.* 1988). Manning and Goldberg (1997) also reported different adsorption trend or maxima from studies of As(III) adsorption on kaolinite, illite, montmorillonite, and amorphous aluminium hydroxide ( $\text{am-Al(OH)}_3$ ) as a function of pH and ionic strength.  $\text{Am-Al(OH)}_3$  showed wide range of adsorption maxima pH ranging from 6 to 9. Kaolinite showed adsorption maxima around pH 9.

**Bioaccessibility.** Table 2 shows bioaccessibility values of As(III) in 36 soils as a function of time. A wide range of initial bioaccessibility was observed from 25.6% to 100%. The variation of As(III) bioaccessibility over aging time was greatly dependent on soils. Most soils except five soils showed a significant decrease in bioaccessibility over aging time. From a paired t-test, thirty-one of the thirty-six soils (86.1%) showed a significantly reduced bioaccessibility over six months ( $p<0.05$ ), while eight soils (22.2%) further exhibited a significantly reduced bioaccessibility ( $p<0.05$ ) from three to six months, indicating aging was typically completed after six months. The number of soils exhibiting a significant reduction in As(III) bioaccessibility over six months (86.1%) was greater than that of As(V) (47.2%,  $p<0.05$ ) in the same set of soils (Yang *et al.* 2002b). In contrast, aging effect on the reduction of As(III) bioaccessibility between three and six

months was smaller than that of As(V), indicating rapid sequestration (decrease of bioaccessibility) of As(III) compared to As(V). From this analyses, As(III) sequestration on soil occurred primarily at early aging time and reached near equilibrium at 6-months. To elucidate the effects of nine soil parameters (Table 1) on the 6-months As(III) bioaccessibility, a multivariable regression using a backward elimination was used. From the multivariable regression, both pH ( $P < 0.0001$ ) and log Fe-oxide ( $P < 0.0005$ ) was identified as significant variables on the 6-months As(III) bioaccessibility (Model 1 in Table 3). The variability of these two parameters was 56% ( $r^2 = 0.557$ ) for the 6-months As(III) bioaccessibility. Figure 2 shows 3-dimensional regression of the 6-months As(III) bioaccessibility ( $B_6$ ) with soil pH and log Fe-oxide content.

**Sequestration.** In order to quantify aging of As(III), the relative change in bioaccessibility over the six-months was defined as:

$$\% \text{ Sequestration} = \frac{B_0 - B_6}{B_0} \cdot 100\% \quad [1]$$

where  $B_0$  and  $B_6$  represent the initial and six-months bioaccessibility respectively. Figure 3a shows comparison of % sequestration of As(III) and As(V) on 36 soils over 6-months. Thirty-two of the thirty-six soils (88.9%) showed greater % sequestration of As(III) than As(V), indicating more susceptibility of arsenite than arsenate on the sequestration. From the multivariable regression using a backward elimination, Log Fe ( $< 0.0005$ ), pH ( $P < 0.0001$ ) and TOC ( $P < 0.0005$ ) were identified as significant variables affecting the sequestration of As(III) (Model 2 in Table 3). These three parameters explained the variability of 75% ( $r^2 = 0.754$ ) for the As(III) sequestration. Sequestration of As(III) over six-months was positively related with Fe-oxide content but was negatively related with both pH and TOC. Dependence of As(III) sequestration on Fe-oxide content was greater than of As(V) as shown in Figure 3b. This result can be explained by mixed factors such as different affinity between As(III) and As(V) to Fe-oxide and As(III) oxidation to As(V) during aging period. It is expected that As(III), major oxidation state at initial aging period, is not strongly adsorbed to the soils due to the formation of weak surface complexes with Fe-oxide compared to As(V). Vink (1996) reported that adsorption of

arsenate to iron oxides may produce irreversible mineral Scordite,  $\text{FeAsO}_4 \cdot 2\text{H}_2\text{O}$ , during aging. However, as oxidation fraction increased with aging time, not much difference can be expected in the complexation of arsenic with Fe-oxide, causing a similar bioaccessibility between this (arsenite) and previous work (arsenate). This was supported by greater initial As(III) bioaccessibility (mean of  $B_0$  is 56.5%) than As(V) (mean of  $B_0$  is 43.6%) while reduced difference between 6-months As(III) bioaccessibility (mean of  $B_6$  is 27.8%) and As(V) (mean of  $B_6$  is 33.0%). From a paired t-test, thirty-six  $B_0$  data of As(III) and As(V) was statistically different ( $p < 0.01$ ) but any statistical difference of thirty-six  $B_6$  data between As(III) and As(V) was difficult to identify due to high p-value although this analysis resulted no difference. The greater aging effect observed in As(III) may be related with change in oxidation state of As(III) during aging time. This will be discussed in depth later section.

**As(III) oxidation to As(V).** Figure 4a-d shows variation of As(III) (this work) and As(V) (previous work) bioaccessibility for the four selected soils (#19, 20, 35, 36) with aging. Except #36 soil (Figure 4d), initial bioaccessibility of both As(III) and As(V) measured as total dissolved arsenic were approximately 60%. Greater sequestration of As(V) in #36 soil was observed initially (during 48-hrs spiking period). Although #19 and 35 soils showed a little fraction of  $\text{As}^{3+}$  in extracted solution due to rapid oxidation to As(V), the other soils showed a considerable fraction of  $\text{As}^{3+}$  in initial ( $B_0$ ) extracted PBET solution. During aging period, bioaccessibility of As decreased along with decrease of  $\text{As}^{3+}$  fraction. As similar with these selected soils, for all soils, rapid As(III) oxidation was observed at initial aging period, and little arsenite ( $\text{As}^{3+}$ ) was identified after one month. In order to relate As(III) oxidation with several soil parameters, oxidation data obtained after 7-days were used. Although As(III) oxidation (calculated as % from equation 2) was generally favored at higher soil pH and Mn contents, not any specific trend was identified in overall pH and Mn content in 36 soils as shown in Figure 5a and b.

$$\% \text{ Oxidation} = \frac{\text{As}(\text{total}) - \text{As}^{3+}}{\text{As}(\text{total})} \quad [2]$$

where As(total) and  $As^{3+}$  represent the total arsenic and  $As^{3+}$  concentration, respectively, in the PBET solution. Several factors were known to affect the oxidation of As(III) to As(V) such as pH, mineralogy, manganese oxides and ferric iron. Chiu and Hering (2000) reported that oxidation of As(III) to As(V) was more rapid at pH 4 than at pH 6.3 using a manganite ( $\gamma$ -MnOOH) while little pH dependence on the heterogeneous oxidation of As(III) was reported by Moore *et al.* (1990). Manning and Goldberg (1997) suggested that As(III) oxidation might be enhanced by heterogeneous oxidation on kaolinite and illite surfaces.

There is controversy surrounding the role of Fe(III)(hydro)oxides in As(III) oxidation. Although De Vitre *et al.* (1991) reported As(III) oxidation within 2 days irrespective of pH by Fe(III)(hydro)oxides, Ocarson *et al.* (1981) and Hug *et al.* (2001) reported no oxidation of As(III) by Fe(III)(hydro)oxides. To investigate any possibility of As(III) oxidation by oxidants produced from electron transfer from soil organic species to Fe(III)(hydro)oxides in the ambient light, percent of As(III) oxidation was related with total organic carbon in soils at two different Fe-oxide contents. Figure 6 shows comparison of As(III) oxidation with variation of total organic carbon in soils having a similar Fe-oxide contents (10-20 g/kg). Enhanced As(III) oxidation was observed as TOC content increased, suggesting production of oxidants such as superoxide, hydrogenperoxide, and hydroxyl radicals from photolysis of Fe(III)-complexes. Hug *et al.* (2001) reported light-induced rapid As(III) oxidation in the presence of Fe(II, III) and citrate.

From the relation between percentage of As(III) oxidation and sequestration over six months, it was identified that sequestration of As(III) decreased as initial As(III) oxidation rate increased (Figure 7). This result indicates that rapid As(III) oxidation at initial aging period gives rise to presence of arsenic as mostly as As(V), reducing bioaccessibility by forming stronger surfaces complexes with Fe-oxide as previously discussed.

Several important results were obtained from this work. First, initial As(III) adsorption was governed by both the soil's Fe oxide content and pH value. Second, average bioaccessibility of As(III) at initial time ( $B_0$ ) in 36 soils was relatively higher

than that of As(V) (Yang *et al.* 2002b), suggesting formation of relatively weaker surface complexes of As(III) with Fe-oxide than As(V). The average bioaccessibility of As(III) and As(V) in 36 soils after one-month were similar resulted from the oxidation of As(III) to As(V). As similar with As(V), both pH and Fe-oxide were the most important soil properties governing the steady-state bioaccessibility ( $B_6$ ) of As(III) in soil. Third, As(III) sequestration on soil occurred primarily at early aging time and reached near equilibrium at 6-months. Three parameters, soil's Fe-oxide, pH and DOC, were statistically significant parameters governing the As(III) sequestration. As(III) sequestration was positively related with Fe-oxide content but was negatively related with both pH and TOC. The rapid sequestration of As(III) compared to As(V) with variation of Fe-oxide content was explained by combination effect of different affinity between As(III) and As(V) to Fe-oxide and As(III) oxidation to As(V) during the aging period. Finally, rapid As(III) oxidation at initial aging period gives rise to presence of arsenic mostly as As(V), reducing bioaccessibility by forming stronger surfaces complexes with Fe-oxide, suggesting that oxidation of As(III) to As(V) is a kind of remediation skill in the As(III) contaminated soils by reducing arsenic bioaccessibility, mobility, and toxicity.

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**Table 1. Some Physical and Chemical Properties of Soils Used in Study**

Soil	Soil Type	Series	pH	CEC (cmol <sub>c</sub> /kg)	Organic Carbon (%)	Inorganic Carbon (%)	Particle size (%)			Fe (g/kg)	Mn (g/kg)
							Clay	Silt	Sand		
1	Alfisol	Angola-A	5.29	6.70	3.72	0.96	32.1	56.1	11.8	23.28	1.23
2		Angola-B	7.86	4.50	0.09	0.25	25.6	52.9	21.5	5.83	0.19
3		Crider-A	6.57	5.60	0.55	0.39	22.5	75.8	1.7	13.34	0.72
4		Crider-B	5.26	5.40	0.21	0.13	30.9	67.2	1.9	13.38	0.3
5		Lenberg-A	5.92	7.90	3.41	1.01	49.1	44.5	6.4	12.94	1.37
6		Lenberg-B	4.35	5.50	0.36	0.25	64.7	29.5	5.8	15.69	0.12
7		Lawrence-A	4.97	5.80	0.91	0.59	19.5	48.5	32.0	11.17	1.35
8	Aridisol	Doakum-A	6.94	6.90	0.28	0.08	10.8	24.8	64.4	4.74	0.19
9		Doakum-B	6.84	7.00	0.39	0.18	29.3	15.0	55.7	6.86	0.16
10		Kzin-A	7.74	13.30	3.27	1.35	22.2	44.2	33.6	4.07	0.29
11		Kzin-B	7.8	10.00	3.4	1.88	27.0	38.5	34.5	3.26	0.18
12		Oricto-A	8.72	13.70	0.09	0.94	10.2	34.7	55.1	2.92	0.34
13		Oricto-B	9.01	8.60	0.16	1.1	23.2	27.5	49.3	3.16	0.29
14		Stoneham-A	6.42	10.10	1.45	0.71	16.2	41.4	42.4	3.4	0.26
15		Stoneham-B	6.8	7.80	0.66	0.32	21.4	23.2	55.4	2.2	0.2
16	Entisol	Wakeland-A	5.86	6.10	0.92	0	23.8	64.7	11.5	8.82	0.71
17		Wakeland-B	5.77	5.70	0.56	0.25	21.1	66.4	12.5	9.18	0.8
18	Inceptisol	Berks-A	3.65	9.10	2.72	1.01	15.7	46.6	37.7	13.18	0.15
19		Melton-A	6.91	8.00	3.55	0.62	6.0	69.0	25	10.68	1.42
20		Melton-B	4.23	14.00	0.42	0.26	19.0	42.2	38.8	22.07	0.17
21		Rockaway-A	3.86	10.60	3.54	1.49	12.4	34.8	52.8	14.03	0.52
22		Rockaway-B	4.1	3.70	0.21	0.18	12.6	32.1	55.3	17.34	0.16
23		Weikert-A	4.44	13.30	3.97	2.37	24.4	56.2	19.4	21.41	6.47
24		Weikert-B	4.28	8.00	2.01	1.15	23.9	54.3	21.8	28.98	5.42
25	Mollisol	Dennis-A	5.82	8.70	1.32	0.89	15.9	66.1	18	15.11	0.6
26		Dennis-B	4.77	4.40	0.38	0.41	29.7	57.5	12.8	24.29	0.59
27		Sibley-A	6.36	7.10	1.06	0.49	23.5	69.7	6.8	8.23	0.67
28		Sibley-B	6.36	6.80	0.72	0.52	26.9	68.0	5.1	9.11	0.59
29	Spodosol	Charlton-A	3.15	11.90	2.3	0.4	2.9	28.7	68.4	1.33	0
30	Ultisol	Allen-A	4.59	7.70	1.55	0.56	8.7	29.5	61.8	6.95	0.31
31		Allen-B	4.3	1.30	0.19	0.09	14.9	28.4	56.7	18.96	0.1
32		Cecil-A	4.04	5.80	1.64	0.39	10.2	23	66.8	6.01	0.06
33		Cecil-B	4.44	1.60	0.29	0.21	44.8	15.5	39.7	32.56	0.11
34		Lawrence-B	4.28	3.70	0.11	0.1	25.8	38.3	35.9	17.53	0.29
35		Walker-A	6.01	6.00	1.89	0.99	6.1	58.9	34.9	7.71	1.51
36		Walker-B	4.3	4.00	0.1	0.07	23.6	44.2	32.2	19.55	0.16
		Min	3.2	1.3	0.1	0.0	0.4	15.0	1.7	1.3	0.0
		Max	9.0	14.0	4.0	2.4	64.7	75.8	70.6	32.6	6.5
		Mean	5.6	7.4	1.3	0.6	21.0	42.5	36.9	11.9	0.8
		Std. deviation	1.5	3.2	1.3	0.5	16.9	21.8	8.0	8.0	1.3

**Table 2. As(III) Bioaccessibility of 36 Soils over 6 Months<sup>1</sup>**

Soil	Initial As(III) Adsorption (%)	0-mon	1-mon	2-mon	3-mon	6-mon	Significant aging over six months? <sup>2</sup>	Significant aging between three and six months? <sup>3</sup>
1	83.0	40.0±0.5	19.6±0.2	18.6±2.2	17.6±0.2	17.9±1.1	Yes	No
2	61.3	63.0±5.0	47.0±0.6	32.0±3.1	46.0±3.6	41.8±0.8	Yes	No
3	70.6	59.8±7.4	19.0±1.2	16.9±1.6	16.6±0.1	16.2±0.8	Yes	No
4	86.1	61.6±1.8	30.2±6.2	9.3±0.3	3.8±1.1	4.2±1.5	Yes	No
5	68.1	48.8±4.7	31.4±7.1	26.6±1.0	35.0±2.8	30.6±1.2	Yes	No
6	51.2	49.4±3.1	21.0±0.4	23.8±12.2	15.8±0.5	18.4±2.2	Yes	No
7	74.6	38.0±0.6	19.2±1.8	15.0±4.3	13.7±0.4	13.0±2.8	Yes	No
8	29.9	66.6±3.4	66.3±8.6	50.6±1.5	64.2±1.9	58.1±1.1	No	No
9	50.8	60.9±1.3	47.2±3.0	34.4±0.6	36.8±1.9	38.7±2.4	Yes	No
10	36.8	62.6±0.6	58.8±3.2	63.7±4.8	59.4±0.0	48.7±0.4	Yes	Yes
11	29.9	66.4±2.8	67.0±4.0	63.7±7.5	67.8±0.2	57.7±1.8	No	No
12	28.9	59.4±10.5	47.8±4.7	37.0±11.4	47.6±1.0	37.0±1.1	Yes	Yes
13	30.2	55.8±0.2	53.0±2.8	44.8±8.3	50.3±3.1	42.3±0.5	Yes	No
14	27.5	100.0±0.0	81.7±16.4	58.6±19.4	83.4±2.6	81.4±12.3	No	No
15	31.5	100.0±0.0	88.0±5.3	75.3±2.3	92.5±4.7	92.3±10.3	No	No
16	58.1	60.0±3.2	30.9±2.8	32.3±2.8	31.4±1.1	31.6±1.8	Yes	No
17	71.5	45.6±1.8	22.7±0.4	24.2±1.3	20.4±0.4	21.6±1.3	Yes	No
18	30.8	45.2±1.3	22.0±4.7	20.3±1.0	19.8±1.8	20.6±1.7	Yes	No
19	72.6	65.6±6.6	49.9±4.5	49.8±4.1	51.3±2.1	43.9±1.2	Yes	Yes
20	24.5	59.1±4.2	36.6±3.4	31.7±4.7	29.2±1.8	20.4±1.7	Yes	Yes
21	31.2	60.8±1.2	34.0±1.1	32.2±6.9	27.6±1.8	23.2±4.2	Yes	No
22	49.5	60.6±3.8	33.4	28.9±1.8	22.6±1.7	10.0±0.5	Yes	Yes
23	93.5	28.8±0.6	22.8±2.8	18.3±1.1	20.4±1.0	16.8±1.8	Yes	No
24	96.5	28.0±0.2	13.4±3.7	12.2±3.1	14.7±1.2	13.1±0.3	Yes	No
25	70.3	52.5±1.8	21.2±0.1	20.2±2.0	19.9±0.8	18.2±1.7	Yes	No
26	87.6	45.1±4.0	7.2±0.4	4.8±0.9	4.3±0.1	4.7±0.1	Yes	No
27	52.4	59.0±1.6	29.0±8.6	35.4±1.5	33.2±0.6	32.9±0.4	Yes	No
28	68.8	49.4±5.4	23.6±3.7	24.6±5.6	25.9±1.4	26.6±0.6	Yes	No
29	19.0	25.6±4.3	15.9±1.3	13.4±8.6	13.5±0.5	12.7±1.1	No	No
30	46.7	72.8±7.1	33.2±4.7	24.6±5.7	29.0±2.0	27.6±0.2	Yes	No
31	50.7	53.6±0.8	13.4±0.2	11.2±2.0	8.7±1.6	13.8±2.5	Yes	No
32	20.7	67.8±3.4	47.1±21.6	29.2±1.3	29.0±1.3	22.5±4.0	Yes	No
33	86.1	49.2±5.1	19.4±3.7	4.8±1.5	4.0±0.4	3.7±1.3	Yes	No
34	70.2	57.9±4.2	27.2±6.1	17.2±2.3	9.6±0.5	7.8±0.1	Yes	Yes
35	67.1	52.5±4.5	34.4±3.8	30.9±2.1	30.9±0.6	26.2±0.6	Yes	Yes
36	81.7	62.2±4.3	22.4±4.5	14.6±1.6	6.8±1.4	3.1±0.8	Yes	Yes
Mean	80.8	56.5	34.9	29.2	30.6	27.8		
Min.	19.0	25.6	7.2	4.8	3.8	3.1		
Max	96.5	100	88	75.3	92.5	92.3		
Std. Dev.	23.2	15.6	19.3	17.2	22.0	20.6		

1. Errors represent standard deviation (n=2). Some data without errors obtained by single measurement.

2. As measured by paired t-test results with 0 and 6 months bioaccessibility data.

3. As measured by paired t-test results with 3 and 6 months bioaccessibility data.

**Table 3. Multivariable Regression Models**

<b>Model 1 (Bioaccessibility)</b>	<b><math>r^2=0.557</math></b>	<b><math>P&lt;0.0001</math></b>		
<u>Variable</u>	<u>Value</u>	<u>Standard Error</u>	<u>P-value</u>	<b>VIF<sup>a</sup></b>
pH	8.1	0.8	<0.0001	
Log Fe	-18.4	4.5	<0.0005	
<b>Model 2 (Sequestration)</b>	<b><math>r^2=0.754</math></b>	<b><math>P&lt;0.0001</math></b>		
<u>Variable</u>	<u>Value</u>	<u>Standard Error</u>	<u>P-value</u>	<b>VIF</b>
Intercept	74.3	15.2	<0.0001	
Log Fe	31.1	7.4	<0.0005	
pH	-7.6	1.7	<0.0001	
TOC	-6.9	1.6	<0.0005	

a. The Variance Inflation Factor (VIF) is a measure of collinearity. A VIF of one indicates the independent variables have no redundant information.

**Table 4. Variation of Percent of As(III) Oxidation with Aging Time**

Soil	Soil Type	Series	Fresh	7 days	14 days	1 month	2 months	3 months	6 months
1	Alfisol	Angola-A	55.8	47.9	53	100	100	100	100
2		Angola-B	88.2	83.2	82.9	100	100	100	100
3		Crider-A	52.8	56.1	56	100	100	100	100
4		Crider-B	N/A	41.3	43.3	70.2	100	100	100
5		Lenberg-A	86.6	N/A	68.1	100	100	100	100
6		Lenberg-B	31.8	N/A	51.2	68.1	74.4	N/A	79.3
7		Lawrence-A	62.4	N/A	81	79.7	66.7	N/A	50.8
8	Aridisol	Doakum-A	73.3	79.2	75.7	N/A	81.8	100	100
9		Doakum-B	53	88.4	N/A	90.2	N/A	84.8	83.2
10		Kzin-A	68	88	100	100	100	100	100
11		Kzin-B	53.8	88.8	100	92.7	100	100	89.2
12		Oricto-A	70.2	89.1	99.3	92.7	100	100	100
13		Oricto-B	63.8	87.8	100	100	100	100	100
14		Stoneham-A	91.1	100	92.9	100	100	100	100
15		Stoneham-B	76.9	75.2	98.1	100	100	100	100
16	Entisol	Wakeland-A	N/A	62	62.3	100	100	100	100
17		Wakeland-B	67.8	88	76	100	100	100	100
18	Inceptisol	Berks-A	43.8	72.2	62.4	51.8	61.6	67.2	65.6
19		Melton-A	94.8	93.8	90.4	100	100	100	100
20		Melton-B	41.3	33.7	37.7	51.4	57.4	74.7	100
21		Rockaway-A	79.1	66.7	57.1	78.5	N/A	100	100
22		Rockaway-B	N/A	24.6	42.2	58.7	46	65.5	100
23		Weikert-A	100	100	100	100	100	100	100
24		Weikert-B	100	100	100	100	100	100	100
25	Mollisol	Dennis-A	46.3	70.8	76.6	79.2	79.2	N/A	78.6
26		Dennis-B	32.2	N/A	57.5	100	100	100	100
27		Sibley-A	70.7	67.4	72.2	100	100	100	100
28		Sibley-B	63.2	68.7	76.8	100	100	100	100
29	Spodosol	Charlton-A	29.3	47.7	N/A	42.1	73.1	85.2	N/A
30	Ultisol	Allen-A	77.5	56	65.6	88.2	100	100	100
31		Allen-B	37.3	47.9	58.3	68.7	68.8	55.2	71
32		Cecil-A	66.5	75	47.9	67.3	N/A	100	100
33		Cecil-B	30.3	N/A	44	41.2	100	100	100
34		Lawrence-B	52.3	65.8	42	62.5	50.6	100	100
35		Walker-A	79.8	89.3	100	100	100	100	100
36		Walker-B	27.2	32.7	33.3	36.2	34.9	100	100
		Min	62.6	70.6	70.7	83.4	87.7	94.9	94.8
		Max	27.2	24.6	33.3	36.2	34.9	55.2	50.8
		Mean	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		Std. deviation	21.4	21.2	21.8	20.8	19.5	11.9	11.9

## **APPENDIX G**

La Force, M. J., G. Li, and S. Fendorf. "Temporal changes in soil partitioning and bioaccessibility of arsenic, chromium, and lead." Draft manuscript.



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**Temporal Changes in Soil Partitioning and Bioaccessibility of**

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**Arsenic, Chromium, and Lead**

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**Abstract**

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A critical step in evaluating the hazard imposed by trace element contaminants within soils is assessing their ability to migrate into water systems and their availability for biological impact. The latter consideration will be initially constrained by the degree to which a contaminant may dissociate from the soil solids and become available to a target organism—a parameter denoted as bioaccessibility. Here we used a physiological based extraction test (PBET) to estimate the bioaccessible fraction of arsenic, chromium, and lead amended soil. We investigated soils from the A and B horizons of the Melton Valley series, a soil of importance at the Oak Ridge National Laboratory facility, in order to address temporal changes in bioaccessibility. Additionally, common extractions that seek to define reactive pools of metals were employed and their correlation to PBET levels evaluated. With the exception of Pb amended to the A horizon, all other treatments exhibited a marked decrease in bioaccessibility with incubation time that was well described by an exponential decay. The bioaccessible fraction was less than 0.2 within 30 d incubation for As and Cr in the A horizon and for As and Pb within the B horizon—Cr in the B horizon declined to nearly 0.3 within 100 d of aging. Neither oxalate nor acid extractable pools exhibited discernable temporal trends and did not correlate well with PBET levels for As or Cr. The exchangeable fraction, however, did decline with incubation period and, in most cases, was highly correlated with the decline in bioaccessibility. In sum, our results demonstrate limited bioaccessibility in all but one case and the need to address both short-term temporal changes and, most importantly, the soil physiochemical properties.

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## INTRODUCTION

50 Widespread As, Cr, and Pb contamination occurs as a result of mining and  
51 smelting activities, industrial pollution and paints, pesticide application, metal plating and  
52 tanning processes, anti-knock automobile fuel, and military activities (Nraigu, 1994;  
53 Smith et al., 1998). The toxicity, bioavailability, and mobility of these three  
54 contaminants are governed by their oxidation state (for As and Cr), physicochemical soil  
55 properties, and microbiological activity (Fendorf, 1995; Smith et al., 1998).  
56 Consequently, understanding metal sequestration and reduced bioaccessibility over time  
57 is crucial for accurately predicting contaminant availability and fate in the environment.  
58 Accordingly, this investigation elucidates the temporal changes in As, Cr, and Pb, three  
59 common trace element contaminants, partitioning and bioaccessibility within soils.

60 Arsenic is a redox active element that generally persists in either the +3 or +5  
61 oxidation states within soils; both the toxicity and bioaccessibility of this element are  
62 dependent on its redox state. Arsenate ( $H_xAsO_4^{x-3}$ ), for example, is often considered less  
63 toxic than its counterpart arsenite ( $H_xAsO_3^{x-3}$ ) (Ferguson and Gavis, 1972; Cullen and  
64 Reimer, 1989; Smith et al., 1998). Furthermore, the mobility and availability of arsenic  
65 will be controlled by reactions with soil solids. Phases present in soils and sediments  
66 demonstrated to have influential roles on arsenic include hydrous oxides of Al, Fe, Mn  
67 (Oscarson et al., 1983; Pierce and Moore, 1982; Manning et al. 1998), calcite (Goldberg  
68 and Glaubig, 1988), sulfides (Moore et al., 1988), kaolinite (Manning and Goldberg,  
69 1997a; Foster et al., 1998a), and montmorillonite (Huang, 1975; Manning and Goldberg,  
70 1997b). Moreover, the bioavailability of arsenic is controlled by the stability of the solid-

71 phase complex (Ruby et al., 1996). For example, the bioaccessibility of As (degree of  
72 dissolution in the gastrointestinal tract) within mine waste impacted soil and dust is  
73 limited; however, when it is ingested in a water soluble form it is completely absorbed  
74 into the body (Freeman et al., 1993; Freeman et al., 1995; Ruby 1996; Rodriguez, et al.,  
75 1996).

76         Similar to arsenic, chromium is a redox active soil contaminant, with dramatic  
77 alterations in the toxicity and mobility occurring with changes in oxidation state.  
78 Trivalent Cr is rather benign to most plants and animals and has limited migration within  
79 soils. In contrast, hexavalent Cr is toxic to living cells being a Class A human carcinogen  
80 (Kargacin et al. 1993); because of its anionic nature and inability to form strong chemical  
81 complexes with most soil materials, it is also highly mobile within the surface  
82 environment (Fendorf, 1995; Ball and Nordstrom, 1998). Recent work has demonstrated  
83 that Cr bioaccessibility is a function of soil type and retention time (Stewart et al. 2003).  
84 Lead, in contrast to arsenic and chromium, is a redox-stable divalent cation that has a  
85 high affinity for numerous soil materials (Bargar et al., 1997). An important control on  
86 the residence time, dispersal, and bioavailability of Pb is dependant on the form and  
87 solubility of Pb present within soils (Ruby et al., 1996). Regulatory agencies assume a  
88 fixed bioavailability of ~30% for Pb; however, it appears that Pb bioavailability will  
89 vary depending on its mineralogical form (Ruby et al. 1992, 1993).

90         Trace elements are released into soils and waters through numerous pathways,  
91 many of which are stimulated by anthropogenic effects. Soils often serve as a sink for  
92 metal ions and other contaminants, binding them via various mechanisms—e.g.,  
93 adsorption or absorption—with the strength of retention dependent on the specific

94 mechanism. A fortunate outcome of trace element retention within soils is, generally, a  
95 decrease in the hazard imposed by the contaminant. Natural attenuation will help to limit  
96 the transport of contaminants into surface or groundwater. Additionally, the availability  
97 of contaminants for uptake by biological organisms will be diminished, thereby  
98 decreasing the risk imposed to animals and plants.

99 A number of steps or processes actually define the bioavailability of a  
100 contaminant to a given organism. But, in general, an initial step in the bioavailability  
101 process is the release of a contaminant from the solid phase into either the aqueous or gas  
102 phase. As a consequence, changes in bioavailability will therefore typically be dependent  
103 on the binding mode of the contaminant within soils (Ruby et al., 1992; Davis et al.,  
104 1993). Bioaccessibility is a term encompassing the release step in the bioavailability  
105 process and is defined as the fraction of a contaminant available for absorption in the  
106 gastrointestinal system of an organism (Ruby et al., 1996). Because bioaccessibility  
107 addresses the release of contaminants from the solid-phase, an appreciation of retention  
108 mechanisms within soils is thus crucial for evaluating contaminant risk.

109 A number of mechanisms may be operational in trace element retention, and the  
110 mode of retention may change with (incubation) time. Both cations and anions will  
111 typically adsorb rapidly to soil surfaces forming outer-sphere (electrostatic or physical)  
112 complexes. Following the initial physi-sorption, a secondary (slower) step will follow  
113 leading to the development of an inner-sphere (chemical) complex (Hayes et al. 1986,  
114 Zhang and Sparks, 1989, 1990a,b). Further aging may lead to more extensive changes in  
115 the surface phase (for example, see Aharoni and Sparks, 1991). Surface diffusion within  
116 micro-pores can result in a virtually absorbed phase (Ainsworth et al., 1994; Axe and

117 Trivedi, 2002); it may also give rise to nucleation and the development of a surface  
118 precipitate (for example, see Ford and Sparks, 2000). Finally, deposition of organic or  
119 inorganic material may occlude the contaminant (again the development of an absorbed  
120 phase).

121 At high(er) concentrations, trace elements may form either surface or discrete  
122 precipitates. Kinetic factors usually govern the phase that forms over a short period of  
123 time, which is primarily dictated by the activation energy or the energy barrier of a  
124 reaction. Generally, large well-crystallized particles have a lower  $K_{sp}$  but higher  
125 activation energy. Consequently, amorphous particles are frequently found in soils and  
126 sediments due to their meta-stable conditions. Given sufficient time, these amorphous  
127 phases will transform into more crystalline solids (ripening), which are  
128 thermodynamically more stable (i.e., they have a lower solubility) (Ainsworth et al,  
129 1994). Additionally, existing surfaces often provide a catalytic role in precipitation and  
130 lead to surface (or heterogeneous) precipitates.

131 Recent evidence has revealed the potential for mixed metal phases to form as  
132 precipitates on mineral surfaces, providing the resulting phase has a lower solubility than  
133 the parent substrate. Association of transition metals with unstable aluminosilicate clay  
134 minerals, such as pyrophyllite, may lead to the release of Al from the clay and  
135 incorporation of the transition ion in a solid having a hydrotalcite structure; such phases  
136 have been noted recently for Co (Thompson et al., 1999), Ni (Schiedegger et al., 1996),  
137 and Zn (Ford and Sparks, 2000). Upon aging, silicon appears to be reincorporated into  
138 the precipitate leading to the neoformation of a transition metal-bearing clay mineral  
139 (Ford and Sparks, 2000).

140 A universal theme of the aging processes described above is a decrease in the  
141 potential for contaminant release. That is, the availability of the contaminant should  
142 diminish with soil incubation time (assuming dramatic changes do not ensue that may  
143 destabilize the substrate). In this study, we investigated the changes in arsenic,  
144 chromium, and lead upon exposure and incubation to soils obtained from Oak Ridge, TN.  
145 We evaluated the bioaccessibility of the three contaminants for human receptors using a  
146 physiological based extraction test (PBET) coupled with selective sequential extractions.

147

## 148 MATERIALS AND METHODS

149 **Sample Collection/Surface Loading.** The A and B horizons of Melton Valley soil,  
150 uncontaminated material, collected from Oak Ridge National Laboratory, TN (described  
151 in Stewart et al., 2003) were utilized for this experiment. Soil samples were air-dried,  
152 homogenized, and sieved ( $< 250 \mu\text{m}$  size). The  $< 250 \mu\text{m}$  size fraction was chosen  
153 because predominately smaller particles (i.e.,  $100 \mu\text{m}$ ) are likely to adhere to children's  
154 hands, promoting ingestion, and are subject to wind transport and inhalation (Ruby et al.,  
155 1992). Soils were analyzed for their particle size (hydrometer), organic matter content  
156 (Walkley-Black), pH (saturated paste and  $5 \text{ mM CaCl}_2$ ), Fe concentration (CBD  
157 extractable), and mineralogical content (XRD) using standard soil protocols.

158 Experiments were initiated by placing quintuplicate samples of 20 g of soil into a  
159 250 mL Nalgene reaction vessel. We spiked each soil with 200 mL of  $5 \text{ mg L}^{-1}$  As(III),  
160 Cr(III), and Pb(II) in a  $0.001 \text{ M CaCl}_2$  matrix at pH 3. In addition, two unspiked control  
161 sub-samples per soil horizon were prepared. The soil slurries were placed on an orbital  
162 shaker and allowed to react for 10 h at which time supernatants were collected and

163 analyzed for As, Cr, and Pb. A total of four spikes transpired which resulted in final  
164 contaminant (As, Cr, and Pb) loadings of  $\sim 200 \text{ mg Kg}^{-1}$ . Reaction vessels were  
165 maintained at field capacity ( $\sim 33\%$  moisture content) for the duration of the experiment.  
166 In order to assure homogeneity at the time of sampling, each reaction vessel was  
167 homogenized.

168 **Analytical Procedures.** Trace elements were measured in the aqueous phase using  
169 inductively coupled plasma (ICP) optical emission spectrophotometry (Thermo Jarrell  
170 Ash IRIS ICP-OES; Franklin, MA) with a 10% accuracy range and quality control was  
171 checked every 15 samples. Detection limits were defined by  $3\sigma$  (where  $\sigma$  is the standard  
172 deviation) of 7 blanks. Detection limits were: As  $0.03 \text{ mg L}^{-1}$ , Cr  $0.03 \text{ mg L}^{-1}$ , and Pb  
173  $0.04 \text{ mg L}^{-1}$ . All reaction ware used in the experiment were rinsed in 0.5 M HCl prior to  
174 use.

175 **Physiologically Based Extraction Tests.** The PBET is an *in vitro* leaching procedure  
176 that is used to determine metal concentrations that could be absorbed through digestion in  
177 the human upper GI tract (Ruby et al., 1992; Ruby et al., 1993; Ruby et al., 1996). The  
178 test involves simulating conditions in the stomach and small intestine, using realistic  
179 values for soil-to-solution ratios, stomach mixing, stomach emptying rates, and small  
180 intestine pH and chemistry (Ruby et al, 1993). The PBET is a screening-level test  
181 designed around the GI tract parameters of a 2-3 year old child, considered to be at  
182 greatest risk to metal exposure from accidental soil ingestion (Ruby et al, 1996). The  
183 PBET serves to develop data that correlate well with measures of As and Pb  
184 bioavailability in animal models (Ruby et al, 1996). PBET analyses were conducted on  
185 soils at 0, 14, 30, 60 and 400 d after incubation.



186 The PBET procedure was modified after Ruby et al. (1996) and used 0.5 g of soil  
187 reacted with 50 mL of 1 M glycine at pH 3 for 1 h in a 35.6 °C (96 °F) water bath. The  
188 centrifuge tubes were affixed and submerged on a rotating horizontal armature (12 rpm)  
189 to simulate stomach mixing. The reaction vessels for each sample were centrifuged, and  
190 the supernatant collected and analyzed by inductively coupled plasma optical emission  
191 spectrophotometry (ICP-OES).

192 **Chemical Extractions.** Selective sequential extractions (SSEs), using a series of  
193 increasingly aggressive chemical reagents, allows for general elemental partitioning  
194 patterns to be approximated (for example, see reviews by Pickering, 1981; Chao, 1984;  
195 and Martin et al., 1987). Soil extractions are operationally defined and refer to a reactive  
196 fraction rather than a chemically or mineralogically specific target (Tessier and Cambell,  
197 1991; Kim and Fergusson, 1991).

198 Chemical extractions were initiated on soils using ~1 g (dry weight equivalent) of  
199 homogenized, composite samples. Following each extraction, the soil residue was  
200 washed with double-deionized water. Supernatants were filtered through a 0.2- $\mu$ m  
201 membrane filter and acidified with trace element grade HCl prior to ICP-OES analysis.  
202 Extractions were performed in quintuplicate along with a standard reference material  
203 (SRM 2709) and appropriate blanks. We used common extractions and a series that  
204 seeks to define three reactive pools: exchangeable, ligand dissolvable, and acid  
205 extractable.

206 The first extraction involved using 1 M MgSO<sub>4</sub>, pH 7, and shaking for 1 h; it is  
207 designed to release water soluble and exchangeable As, Cr, and Pb. Next, the soil was  
208 shaken for 4 h with 20 mL of 0.2 M ammonium oxalate (pH 3) in the dark (AOD)—metal

209 (hydr)oxides with less stability (principally short-range order materials) are particularly  
210 susceptible to AOD extraction (Schwertmann, 1973; Jackson et al., 1986). The third  
211 extraction, 12 N HCl, shaken for 12 h, is designed to release constituents from more  
212 recalcitrant phases such as crystalline metal (hydr)oxides (Huerta-Diaz and Morse, 1992).

213

214

## RESULTS

215 **Soil Mineralogical Properties.** Mineralogical properties of the soil are shown in Table  
216 1. The A horizon had a particle size distribution of (56.2%) sand, (30%) silt, and (13.8%)  
217 clay. The B horizon had a lower sand content (30.8%), but a higher silt (50.4%) and clay  
218 (18.8%) content relative to the A horizon. On the other hand, the A horizon had a higher  
219 organic matter content (3.78 %) and pH (6.91) relative to the B horizon (0.73 % and 4.23,  
220 respectively). However, the B horizon had elevated Fe concentrations (22.07 g/kg) as  
221 compared to the A horizon (10.68 g/kg).

222

223 **Physiologically Based Extraction Tests.** The bioaccessibility of arsenic, chromium, and  
224 lead, using the PBET procedure, was evaluated for contaminant spiked A and B horizons  
225 of Melton Valley soil. Alterations in contaminant binding mechanisms with aging may  
226 be a principal factor impacting the bioaccessibility of contaminants. Accordingly, we  
227 measured fluctuations in release of the trace elements with increased incubation time for  
228 each soil (Figure 1). With one exception, the bioaccessibility of the contaminants  
229 decreased with aging—most notably over the first 30 d of incubation. The PBET levels  
230 of Pb within the A horizon of the Melton Valley soil did not decrease with incubation

231 time and, in fact, increased slightly with time; the increase, albeit slight, was linear (Table  
232 2).

233 The decrease in bioaccessibility of the trace elements (with the exception of Pb in  
234 the Melton Valley A soil noted above) is well described by an exponential decay (Figure  
235 1, Table 2). The adequacy of the fits, used to describe changes in PBET levels with time,  
236 were based on  $R^2$  and standard error values. The decay is more rapid, reaching a pseudo  
237 steady-state within the first 14 d, but less extensive for the A as compared to the B  
238 horizon. For the B horizon, the decrease in PBET levels do not diminish until 30 d of  
239 incubation for As and Pb and continue to decline until nearly 100 d for Cr (the value of  
240 which was extrapolated from the data using the exponential fits). Differences in soil  
241 constituents thus appear to impact the degree and rate of diminished bioaccessibility upon  
242 contaminant-soil aging.

243 **Reactive Partitioning.** In order to evaluate the partitioning of the trace elements and  
244 their potential relation to temporal changes in PBET levels, we used a set of common  
245 extractions on the soils. The extractions are not meant to provide definitive information  
246 on the solid-phases but rather to (i) give information on the reactive nature of the  
247 contaminants (defined as reactive pools that are operationally based) and (ii) allow a  
248 relation between common procedures and bioaccessibility to be developed. The easily  
249 exchangeable fraction was estimated using an extractant of  $MgSO_4$ ; oxalate was  
250 employed as a common, natural chelate that may promote contaminant release. Finally  
251 the fraction released by strong acid was assessed using HCl.

252 A discernable trend in the oxalate and acidic decomposition pools with increased  
253 incubation time is not apparent for either the A (Table 3) or B (Table 4) horizons.

254 Moreover, a large fraction of the trace elements remains even after treatment with these  
255 three reactants—a residual pool that is resistant to release by ligand or proton promoted  
256 dissolution over the duration of the procedure. In contrast with oxalate and HCl  
257 extractable pools, the exchangeable fraction ( $\text{MgSO}_4$  extractable), although a small pool,  
258 does decrease with increased incubation time (Figure 2). In fact, the change in the  
259 exchangeable pool is very substantial and appears exponential with time, similar to that  
260 observed for the PBET levels.

261 To further explore the possible relation between the reactive pools and PBET  
262 levels, we performed a correlation analysis (Tables 5 and 6). Consistent with cursory  
263 observation, the exchangeable fraction is highly correlated with PBET levels. However,  
264 the significant ( $P < 0.01$ ) positive correlation between PBET and  $\text{MgSO}_4$  levels holds  
265 only for As and Cr. PBET levels of Pb with time do not correlate with exchangeable Pb  
266 in either the A or the B horizon. While the PBET levels decrease exponentially for Pb in  
267 the B horizon, the exchangeable fraction of Pb was relatively constant with time (Figure  
268 2), leading to a poor correlation between the two.

269

270

## DISCUSSION

271 A spectrum of sorption mechanisms and resulting surface states are possible for  
272 trace elements. The operating mechanism of retention may change with reaction time  
273 and lead to differences in the bioaccessibility of a contaminant. We observe, with a  
274 single exception, that the bioaccessibility of As, Cr, and Pb decrease with increase aging  
275 (reaction time) in soils. An exponential decrease in bioaccessibility through the first 100  
276 d of incubation are accompanied by a highly correlated decrease in the exchangeable

277 fraction of these trace elements, thus suggesting that there is indeed a change in the  
278 operating retention mechanism across this time period. Previously it has been suggested  
279 that the aging processes induced (i) ion substitution for a matrix ion within the lattice of a  
280 mineral, (ii) entrainment of the ion through the aging process, and (iii) diffusion into pore  
281 spaces (Ainsworth et al., 1994). The adsorption of both anions and cations on soil  
282 materials is generally initiated by the rapid formation of outer-sphere (electrostatic)  
283 complexes that gradually undergo a transformation to inner-sphere (chemical) moieties  
284 with increase reaction time (Zhang and Sparks, 1989, 1990a,b). A decrease in the  
285 exchangeable fraction is consistent with a shift from outer-sphere to inner-sphere  
286 complexes and is likely one of the processes responsible for diminished bioaccessibility  
287 with increased incubation time.

288         Alterations in bioaccessibility with time are not, however, restricted to simply a  
289 shift from outer-sphere to inner-sphere complexes. Both the diffusion of ions within  
290 solids having internal porosity, such as hydrated, short-range order iron oxides (Axe and  
291 Trivedi, 2002), and the development of co-precipitates (e.g., Ford et al., 1999) with  
292 constituents of the substrate enhance the stability of contaminants within the solid-phase,  
293 thus restricting their bioaccessibility. Precipitates inclusive of the contaminant may also  
294 become more crystalline with time and thus have a diminished solubility and  
295 bioavailability (Ruby et al., 1993). Such processes would not be expected to impact ion  
296 retention within the first few weeks of incubation but may account for an increase  
297 resistance to release with incubation times > 30 d. A final process that could potentially  
298 alter the susceptibility of contaminants to extraction is occlusion by organic or inorganic  
299 materials. We suspected that under the unsaturated conditions of this study in

300 combination with the low levels of dissolved organic carbon, that such a factor would not  
301 be appreciable on the time scale of these incubations. Moreover, the higher levels of  
302 organic matter within the A horizon would lead one to expect occlusion to have a  
303 greater impact than in the B horizon. However, PBET levels do not decline beyond 14 d  
304 of incubation and thus are consistent with the expectation that occlusion is not a factor in  
305 this study.

306         Examining differences in soil chemical properties is instructive for elucidating  
307 possible retention mechanisms and temporal trends in bioaccessibility. The dominant  
308 differences between the soil horizons are (i) higher pH, (ii) greater organic matter  
309 content, (iii) lower clay (size fraction) content, and (iv) lower Fe (and Al) oxide content  
310 within the A horizon relative to the B horizon (Table 1)—factors consistent with soil  
311 development and diagnostic for horizon designation. Cationic metals such as Cr(III) and  
312 Pb(II) would be expected to partition strongly on organic matter, forming either  
313 electrostatic or chemical bonds with surface functional groups, and to have greater  
314 retention at higher pH (Schindler and Stumm, 1987). Iron oxides also have a  
315 demonstrated affinity for both Cr(III) and Pb(II), with higher pH again favoring sorption  
316 (Dzomback and Morel, 1990). In contrast, arsenic present as the arsenate anion does not  
317 bind appreciably to organic matter and favors adsorption at lower pH—given the broad  
318 adsorption envelope for arsenate on iron oxides (Manning et al., 1998), arsenate retention  
319 on iron oxides should be favorable across the pH range of these horizons.

320         Arsenic and chromium behave in a manner consistent with chemical  
321 considerations of their reactivity and the soil properties. Given the levels of ferric iron  
322 within both the A and B horizons of the Melton Valley soil (Table 1), one would expect

323 appreciable chemical retention and thus resistance to PBET induced release. There is, in  
324 fact, a sizeable limitation in bioaccessibility and the magnitude is inversely proportional  
325 to the ferric oxide content; PBET levels of the B horizon are about half those of the A  
326 horizon and contain about twice the iron oxide content.

327 In contrast to arsenic, the retention of Cr(III) and Pb should be governed  
328 dominantly by pH. The sorption edge for hydrolysable cations, such as Cr(III) and  
329 Pb(II), is directly proportional to their hydrolysis constants with the specific pH based on  
330 the sorbent. Thus, given the high pH of the A horizon (nearly 2.5 units higher) relative to  
331 the B horizon, in combination with appreciable organic matter and ferric oxide content,  
332 one would expect greater chemical retention within the upper horizon. This is indeed  
333 observed for Cr(III)—the PBET levels are significantly lower in the A horizon than the  
334 B. Lead, however, is anomalous in its behavior within the A horizon (it has the greatest  
335 release with the PBET treatment), for which we have no explanation. The chemical  
336 binding, and thus diminished bioaccessibility, for Pb(II) within the B horizon is  
337 consistent with chemical considerations. Lead has a very high chemical affinity for  
338 numerous soil solids (organic matter, iron oxides, etc.) and generally demonstrates even  
339 greater retention than Cr(III) (Dzomback and Morel, 1990).

340 The decrease in bioaccessibility of As and Cr is also more rapid in the A horizon  
341 than within the B. We speculate that the finer texture of the lower horizon may decrease  
342 chemical reaction rates through diffusion limitations. Independent of the mechanism by  
343 which it results, one clearly notes that steady-states in diminished bioaccessibility are  
344 achieved more rapidly within the A than the B horizon. Additionally, only a limited  
345 fraction of the contaminants are generally released by the PBET treatment; Pb within the

346 A horizon is an exception in which nearly all of the added contaminant is released by the  
347 bioaccessibility treatment. Therefore, rather than using single default values for a  
348 bioaccessible fraction within bioavailability models, it is clear from this study that short-  
349 term (100 d of aging) temporal dependence must be considered along with, and more  
350 importantly specific soil properties, both chemical and physical.

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- 488
- 489

489 Table 1. Selective physical and chemical properties of the Melton Valley soil.  
 490

491	Soil type	pH <sup>a</sup>	C	Sand	Silt	Clay	DCB-Fe <sup>b</sup> (g/Kg)
492				%			
493							
494	A-horizon (0-3 cm)	6.9	4.0	56	30	14	10.7
495	B-horizon (3-20 cm)	4.2	0.73	31	50	19	22.1

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498 <sup>a</sup>2:1 5mM CaCl to solid ratio

499 <sup>b</sup>Dithionite/citrate/bicarbonate extractable iron

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501 Table 2. Fitting parameters for temporal changes in PBET values of arsenic, chromium,  
502 and lead.

503

<b>Melton Valley A</b>				
<b>Element</b>	<b><math>y_0</math></b>	<b><math>a</math></b>	<b><math>b</math></b>	<b><math>R^2</math></b>
As <sup>1</sup>	33.1	4.20	0.140	0.997
Cr <sup>1</sup>	22.7	34.1	0.285	0.998
Pb <sup>2</sup>	146	0.157		0.997
<b>Melton Valley B</b>				
As <sup>1</sup>	31.9	12.7	0.122	0.998
Cr <sup>1</sup>	63.8	18.0	0.0278	0.966
Pb <sup>1</sup>	47.6	8.94	0.0708	0.984

504

505 <sup>1</sup> Best fit using an equation of the form:  $y = y_0 + ae^{(-bx)}$ 

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507 <sup>2</sup> Best fit using an equation of the form:  $y = y_0 + ax$ 

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511 Table 3. Selective sequential extraction concentrations as a function of incubation time  
 512 for arsenic, chromium, or lead amended A-horizons of the Melton Valley soil.

513

	Time, d	MgSO <sub>4</sub>	Oxalate	HCl
ARSENIC			mg Kg <sup>-1</sup>	
	0	4.72±0.49	62.0±9.78	9.97±0.83
	14	1.23±0.78	48.3±10.27	10.4±2.46
	30	0.85±0.31	56.8±1.47	11.07±0.53
	60	0.47±0.42	48.2±10.6	11.6±3.37
	400	3.92±0.22	50.4±3.14	17.2±1.15
CHROMIUM	0	21.2±0.86	29.1±6.34	42.3±2.91
	14	3.46±1.01	30.6±6.95	48.3±13.2
	30	2.35±0.11	36.6±1.62	47.7±2.70
	60	1.50±0.32	28.3±5.92	52.7±11.9
	400	1.74±0.08	31.1±2.11	61.7±6.47
LEAD	0	7.20±1.04	103.5±16.84	28.1±1.57
	14	7.64±2.29	88.9±16.7	27.4±9.08
	30	6.00±0.32	113±2.90	28.0±1.84
	60	6.37±1.53	91.0±18.5	28.0±7.09
	400	5.95±0.76	96.6±7.75	31.3±5.48

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518 Table 4. Selective sequential extraction concentrations as a function of incubation time  
 519 for arsenic, chromium, or lead amended B-horizons of the Melton Valley soil.  
 520

	Time, d	MgSO <sub>4</sub>	Oxalate	HCl
ARSENIC			mg Kg <sup>-1</sup>	
	0	6.5±1.85	59.0±15.4	25.7±6.03
	14	1.53±0.75	35.0±24.0	23.9±4.65
	30	1.08±0.54	43.2±6.93	24.7±3.45
	60	0.42±0.31	50.6±4.7	29.3±2.74
	400	3.74±0.06	52.0±7.24	35.5±4.16
CHROMIUM	0	48.9±9.35	12.4±2.59	19.6±4.47
	14	44.5±5.72	11.02±7.60	18.3±3.47
	30	38.86±7.17	15.8±1.22	21.4±1.85
	60	33.2±5.73	19.4±0.90	26.3±2.17
	400	20.6±4.96	25.8±2.56	36.1±2.45
LEAD	0	16.3±4.50	29.6±8.70	15.7±6.29
	14	14.60±0.85	15.9±11.0	10.7±3.42
	30	14.7±2.10	21.4±2.40	11.9±1.58
	60	13.4±1.25	24.5±0.99	28.8±2.12
	400	12.3±0.99	26.0±1.85	13.76±0.84

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524 Table 5. Correlations between SSE and PBET extractions for the A-horizon of the  
 525 Melton Valley soil. Values in bold are significant within 95% confidence; italics are  
 526 within 90% confidence.

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ARSENIC		<b>PBET</b>	<b>MgSO<sub>4</sub></b>	<b>Oxalate</b>	<b>HCl</b>
	<b>PBET</b>	1.0000	<b>0.9780</b>	0.4054	0.1236
	<b>MgSO<sub>4</sub></b>		1.0000	0.5797	0.3260
	<b>AOD</b>			1.0000	<b>0.9485</b>
	<b>HCl</b>				1.0000
CHROMIUM		<b>PBET</b>	<b>MgSO<sub>4</sub></b>	<b>Oxalate</b>	<b>HCl</b>
	<b>PBET</b>	1.0000	<b>0.9981</b>	-0.1482	0.4315
	<b>MgSO<sub>4</sub></b>		1.0000	-0.0963	0.4720
	<b>AOD</b>			1.0000	0.1935
	<b>HCl</b>				1.0000
LEAD		<b>PBET</b>	<b>MgSO<sub>4</sub></b>	<b>Oxalate</b>	<b>HCl</b>
	<b>PBET</b>	1.0000	-0.6081	-0.7525	-0.7579
	<b>MgSO<sub>4</sub></b>		1.0000	0.7299	<i>0.8627</i>
	<b>AOD</b>			1.0000	<b>0.9753</b>
	<b>HCl</b>				1.0000

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533 Table 6. Correlations between SSE and PBET extractions for the B-horizon of the  
 534 Melton Valley soil. Values in bold are significant within 95% confidence; italics are  
 535 within 90% confidence.

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ARSENIC		<b>PBET</b>	<b>MgSO<sub>4</sub></b>	<b>Oxalate</b>	<b>HCl</b>
	<b>PBET</b>	1.0000	<b>0.9873</b>	0.5796	0.3068
	<b>MgSO<sub>4</sub></b>		1.0000	0.7015	0.4514
	<b>AOD</b>			1.0000	<b>0.9292</b>
	<b>HCl</b>				1.0000
CHROMIUM		<b>PBET</b>	<b>MgSO<sub>4</sub></b>	<b>Oxalate</b>	<b>HCl</b>
	<b>PBET</b>	1.0000	<i>0.9101</i>	0.2809	0.7163
	<b>MgSO<sub>4</sub></b>		1.0000	0.6196	<i>0.9409</i>
	<b>AOD</b>			1.0000	0.8043
	<b>HCl</b>				1.0000
LEAD		<b>PBET</b>	<b>MgSO<sub>4</sub></b>	<b>Oxalate</b>	<b>HCl</b>
	<b>PBET</b>	1.0000	-0.0901	0.2436	0.0857
	<b>MgSO<sub>4</sub></b>		1.0000	0.6957	0.8272
	<b>AOD</b>			1.0000	<b>0.9745</b>
	<b>HCl</b>				1.0000

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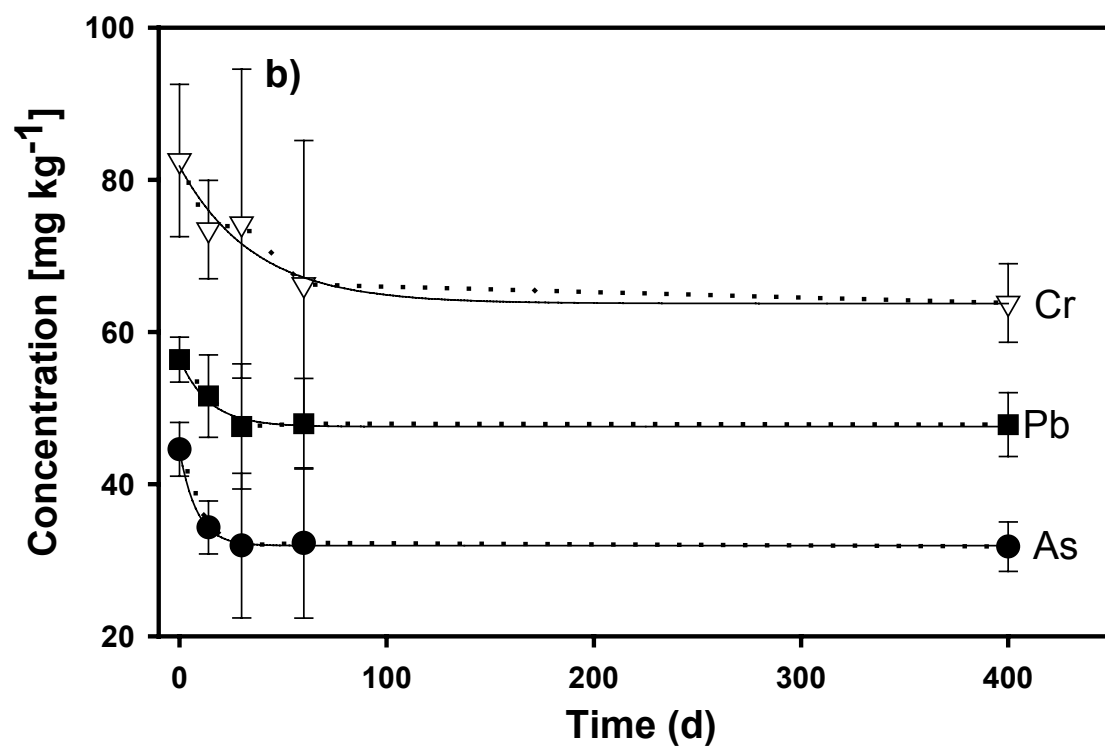
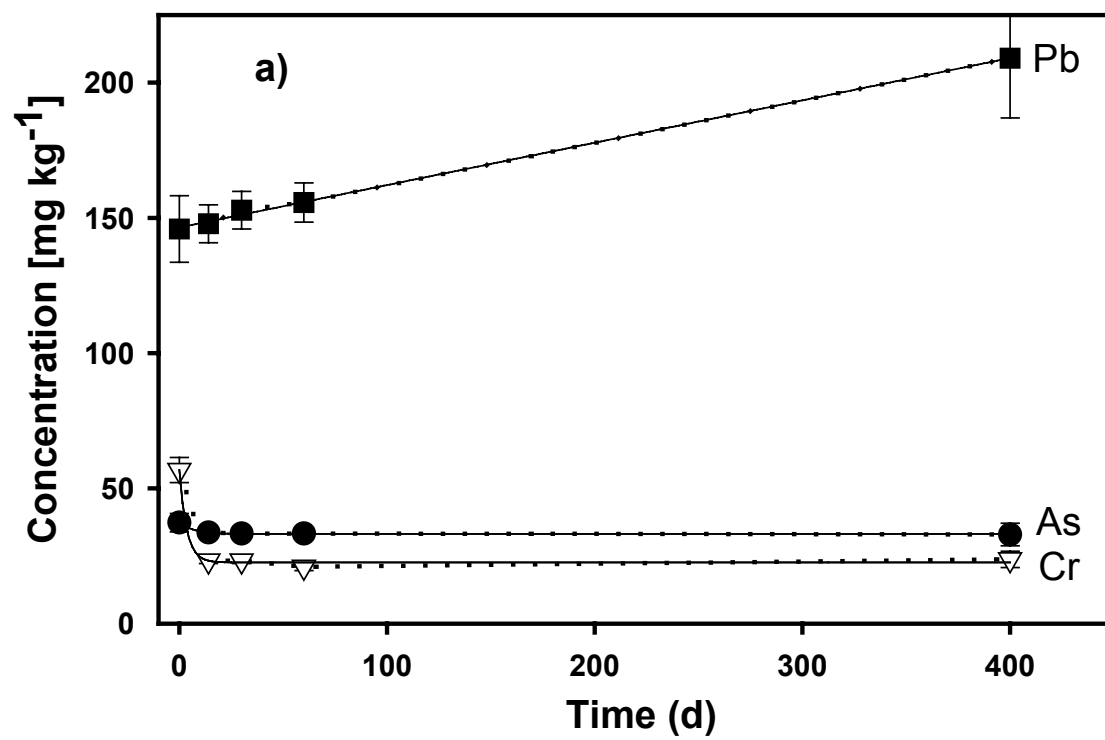
**FIGURE CAPTIONS**

541 Figure 1. Temporal variation in bioaccessible concentrations (evaluated with the PBET  
542 method) of arsenic, chromium, and lead within the (a) A horizon and (b) B  
543 horizon of the Melton Valley soil. Incubation times commence at the point of  
544 contaminant addition.

545 Figure 2. Changes in the exchangeable (MgSO<sub>4</sub> extractable) concentration of metals  
546 with increasing incubation time for the (a) A horizon and (b) B horizon of the  
547 Melton Valley soil.

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Figure 1

