

Fractionation of Fe isotopes by soil microbes and organic acids

Susan L. Brantley*

Laura Liermann

Department of Geosciences, Pennsylvania State University, University Park, Pennsylvania 16802, USA

Thomas D. Bullen

U.S. Geological Survey, Menlo Park, California 94025, USA

ABSTRACT

Small natural variations in Fe isotopes have been attributed to biological cycling. However, without understanding the mechanisms of fractionation, it is impossible to interpret such variations. Here we show that the $\delta^{56}\text{Fe}$ of Fe dissolved from a silicate soil mineral by siderophore-producing bacteria is as much as 0.8‰ lighter than bulk Fe in the mineral. A smaller isotopic shift is observed for Fe released abiotically by two chelates, and the magnitude of the shift increases with affinity of the ligand for Fe, consistent with a kinetic isotope effect during hydrolysis of Fe at the mineral surface. Fe dissolved abiotically without chelates shows no isotopic shift. The $\delta^{56}\text{Fe}$ of the exchange fraction on soil grains is also lighter by $\sim 0.6\text{‰}$ – 1‰ than Fe from both hornblende and iron oxyhydroxides. The kinetic isotope effect is therefore preserved in open systems such as soils. When recorded in the rock record, Fe isotopic fractionation could document Fe transport by organic molecules or by microbes where such entities were present in the geologic past.

INTRODUCTION

Terrestrial and lunar igneous rocks define a bulk Earth-Moon (E-M) Fe isotope composition of $^{54}\text{Fe}/^{56}\text{Fe} = 0.063683 \pm 0.000017$ (Beard and Johnson, 1999). When Fe isotopic compositions of sedimentary samples, $^{56}\text{Fe}/^{54}\text{Fe}_{\text{sample}}$, are compared to the bulk Earth-Moon (E-M) Fe isotope composition, $^{56}\text{Fe}/^{54}\text{Fe}_{\text{E-M}}$, by using the delta notation,

$$\delta^{56}\text{Fe} = \left\{ \left(\frac{^{56}\text{Fe}/^{54}\text{Fe}_{\text{sample}}}{^{56}\text{Fe}/^{54}\text{Fe}_{\text{E-M}}} - 1 \right) \times 10^3 \right\}, \quad (1)$$

Fe isotope compositions vary from $+0.9\text{‰}$ to -1.2‰ (Beard et al., 1999; Beard and Johnson, 1999). Variations in Fe isotopes in ferromanganese nodules ($\delta^{56}\text{Fe} \approx -1.2\text{‰}$ – 0‰ relative to igneous rocks) and between layers in a banded iron formation (range of $\sim 1.2\text{‰}$) have been attributed to biological (vital) fractionation processes by these same authors. Experiments have demonstrated that ferrous Fe in solution is $\sim 1.3\text{‰}$ lighter than ferrihydrite substrate during dissimilatory reduction of Fe by *Shewanella algae* (Beard et al., 1999). However, ferrihydrite precipitated abiotically as a result of oxidation of ferrous iron is $\sim 1\text{‰}$ – 2‰ heavier than aqueous iron (Bullen et al., 1999). Moreover, nonbiological fractionation of >6 per mil has been produced during interaction of Fe with anion-exchange resin (Anbar et al., 2000). The total fractionation observed in the experiment was modeled as a series of small equilibrium steps, each with fractionation factor 1.0001, although this value depends strongly on the interpretation of the elution curve profile. Thus the relative importance of biotic and abiotic fractionation of Fe isotopes in nature is still debated.

Although the capacity for dissimilatory Fe reduction or magnetotaxis is not found in all bacteria, virtually all bacteria assimilate Fe for use in haeme (Hider, 1984), and no researchers have investigated Fe isotope fractionation during assimilation. To scavenge Fe, bacteria often manufacture organic molecules (siderophores, consisting generally of hydroxamates or catecholates) that have large association constants for Fe(III) (Hider, 1984; Schwyn and Neilands, 1987). Catecholate siderophores can dissolve Fe-bearing silicates (Liermann et al.,

2000; Kalinowski et al., 2000). To investigate the isotopic composition of Fe extracted and assimilated by bacteria, we studied the dissolution of Gore Mountain (New York) hornblende in the presence of two catecholate-producing soil bacteria (a putative streptomycete and arthrobacter, both gram positive) (Liermann et al., 2000; Kalinowski et al., 2000), and compared this composition to Fe released abiotically in the presence of various organic molecules, and Fe in Gore Mountain soil.

METHODS

Sterile batch experiments and batch experiments with bacteria were run with and without hornblende in flasks at ambient temperature. All laboratory ware was precleaned (Brantley et al., 2001) and flasks were agitated briefly each day. Each flask contained 60 mL of presterilized MM9 (Liermann et al., 2000), an Fe-free, buffered medium. Hornblende (2 g) was added as precleaned, preautoclaved powder (grain size 250–429 μm , BET surface area 0.17 m^2/g). Bacteria were added by inoculating with 100 μL of 1 week old *Arthrobacter* or *Streptomyces* culture grown in MM9. Three flasks containing hornblende and 24 μM oxalic acid or the siderophore desferrioxamine B mesylate (DFAM) were also prepared without bacteria.

On days 1 and 4, solution samples (10 mL) were collected and filtered (0.2 μm), and unfiltered 100 μL samples (along with inoculum cultures) were plated to count bacteria. After 6 days, bacteria-containing cultures were decanted from the hornblende. All solutions were measured for pH. These supernatants were then centrifuged, filtered, acidified, and stored. Hornblende powder was further separated from bacteria by density-gradient centrifugation (Page and Huyer, 1984) using Percoll (sigma). The cell pellet was rinsed with distilled water (DW) and dried at 60 $^{\circ}\text{C}$ overnight, weighed, then digested. Hornblende powder (unreacted and reacted) was cleaned as described previously (Brantley et al., 2001).

Samples from a hornblende-containing soil at Gore Mountain were extracted for exchangeable Fe and iron oxyhydroxides. Exchangeable Fe was extracted using 30 mL of 1 M MgCl_2 added to 1.2 g soil and equilibrated for 2 h with occasional shaking (Pickering, 1981). The liquid was decanted and treated as described for iron oxides. This procedure does not dissolve minerals, thus no Fe fractionation was ex-

*E-mail: brantley@geosc.psu.edu.

TABLE 1. RESULTS OF Fe ISOTOPE ANALYSIS*

Experiment	In solution at 4 days				In solution at 6 days				Cell Pellet Mass (g)	Mineral Solids $\delta^{56}\text{Fe}$ (‰)	Cell Pellet $\delta^{56}\text{Fe}$ (‰)
	pH	[Fe] (ppb)	log (cells)	$\delta^{56}\text{Fe}$ (‰)	pH	[Fe] (ppb)	log (cells)	$\delta^{56}\text{Fe}$ n(‰)			
Unreacted Hb										-0.25	
Hb only											
3-1	7.29	204			7.34	312		-0.39			-0.21
3-2	7.28	213		-0.32	7.32	302		-0.25			
3-3	7.29	232			7.36	303					
Stm only											
1-1	7.26	Bd	4.9		7.19	Bd	4.7		0.0226		
1-2	7.24	Bd	4.6		7.21	Bd	4.7		0.0275		
Arth only											
2-1	6.55	Bd	7.2		6.69	Bd	7.4		0.0001		
2-2	6.54	Bd	7.3		6.72	Bd	7.7		0.0000		
Hb + Stm											
4-1	7.26	645	Bd		7.39	984	Bd	-0.27	0.0067*	-0.13	
4-2	7.31	777	Bd	-0.98	7.26	1190	5.2	-0.79	0.0688		
4-3	7.33	714	5.1		7.34	1295	5.1	-0.41	0.294		-0.22
Hb + Arth											
5-1	6.98	1940	8.0		7.16	2480	7.7	-1.09	0.0026	0.14	0.08
5-2	6.94	1620	7.9	-0.73	7.09	1980	8.1	-0.71	0.0046		0.14
5-3	6.97	2200	8.0		7.11	2760	7.9	-0.71	0.0045	0.11	
Hb + DFAM											
6-1	c	c			c	c		c			
6-2	7.40	467		-0.88	7.44	546		-0.58		-0.17	
6-3	7.41	527			7.47	652		-0.38			
Hb + oxalic acid											
7-1	7.41	176			7.41	250		-0.49			
7-2	7.39	210		-0.47	c	c		c			
7-3	7.43	224			7.45	296		-0.54		-0.08	
Soil											
Exchangeable											-0.85
Fe oxides											0.16

Note: Hb = Hornblende, Stm = *Streptomyces*, Arth = *Arthrobacter*, Bd = beneath detection, c = flask contaminated.

*This sample showed little to no growth of bacteria.

†Isotopes were measured in blocks consisting of 10 sets of three sequential scans: ^{56}Fe - ^{57}Fe - ^{58}Fe (simultaneously), ^{54}Fe - ^{56}Fe (simultaneously), all on Faraday collectors, and ^{52}Cr on an ion-counting device to correct for occasional minor isobaric interference of ^{54}Cr on ^{54}Fe (assuming $^{54}\text{Cr}/^{52}\text{Cr} = 0.0282$; Matzat and Shiraki, 1978). ^{60}Ni was monitored using the ion counter, but rather than make a correction for isobaric interference of ^{58}Ni on ^{58}Fe , block data were rejected if the signal was >50 cps. Individual sample filaments were analyzed until the standard deviation of the accumulated block average was 0.2‰ or better. Total procedural replicate analyses were made for each sample, and all replicates agreed within 0.15‰. Replicates of analyses of the unreacted hornblende starting material agreed within 0.1‰. All analyses are quoted in per mil notation as described, but using the Icelandic basalt BIR-1 as the standard; positive values indicate relative enrichment in the heavier isotope.

pected. Iron oxides were extracted (Hendershot et al., 1993) by adding 0.5 g of soil to 25 mL of 0.68 M Na citrate solution, with 0.4 M Na dithionite. The mixture was shaken for 17 h, then liquid was decanted, filtered (0.2 μm), and acidified (HNO_3). This procedure extracts all iron oxyhydroxides without extracting silicates (Hendershot et al., 1993), and thus no Fe isotope fractionation was expected.

Solutions, hornblende powders before and after reaction, and bacterial pellets were analyzed for concentrations of Fe, Al, and Si using a Finnigan MAT element high-resolution inductively coupled plasma-mass spectrometer (HR-ICP-MS) at moderate resolution. In addition, these samples and the extracted soil components were analyzed for Fe isotope composition using a Finnigan MAT 261 multicollector thermal ionization mass spectrometer (TIMS). Fe redox state in solution was not measured.

The $^{56}\text{Fe}/^{54}\text{Fe}$ ratios were determined using a ^{57}Fe - ^{58}Fe double-spike amendment to correct for isotope fractionation that may occur during sample purification and the TIMS procedure. The well-established double-spike method (e.g., Russell et al., 1978; Johnson and Beard, 1999) as used here is identical to that described by Johnson et al. (1999) for Se isotope analysis on the same TIMS. Sufficient material to provide 1.25 μg Fe was mixed with a fixed amount of Fe double-spike solution to assure consistent spike:sample ratios ($\pm 5\%$). All samples were evaporated and/or treated with H_2O_2 and dissolved in 6 N HCl. Samples were purified using AG-1-X8 resin, with 6 N HCl as the eluant to strip ions other than Fe and then 0.5 N HCl to strip Fe. Prior to loading into the TIMS, purified Fe samples were mixed with a small

amount of phosphoric acid, treated with H_2O_2 , and deposited onto single Re filaments in a colloidal silica and alumina mixture. Other details of analyses, including precision, are summarized in Table 1.

RESULTS AND DISCUSSION

Cell counts and dissolved Fe ([Fe]) increased faster in the presence of hornblende (Table 1; Fig. 1). Abiotic release rates of Fe ($d[\text{Fe}]/dt$) increased with increasing equilibrium constants for Fe(III)-ligand complexation (water \approx oxalate $<$ DFAM). Fe was released even faster in the presence of bacteria (*Arthrobacter* $>$ *Streptomyces*), consistent with production of catecholate siderophores (Liermann et al., 2000; Kalinowski et al., 2000). Catecholates generally have higher Fe association constants than hydroxamates such as DFAM (Bergeron and McManis, 1991).

The Fe isotope composition of hornblende (Table 1) before and after abiotic dissolution was similar to values (Beard and Johnson, 1999) for igneous rocks and for abiotically leached Fe and iron oxyhydroxides in the soil (Table 1). In contrast, dissolution of hornblende in the presence of the arthrobacter or streptomycete released Fe lighter by as much as 0.8‰ than the Fe in the hornblende, and these values matched the soil Fe-exchange fraction within error. In one experiment (4-1) where insignificant cell growth occurred, the $\delta^{56}\text{Fe}$ was identical to abiotic experiments (Table 1).

Fe released during dissolution with DFAM or oxalic acid was also lighter than hornblende by as much as 0.6‰ and 0.3‰, respectively. Average $\delta^{56}\text{Fe}$ generally decreased with increasing release rate of Fe

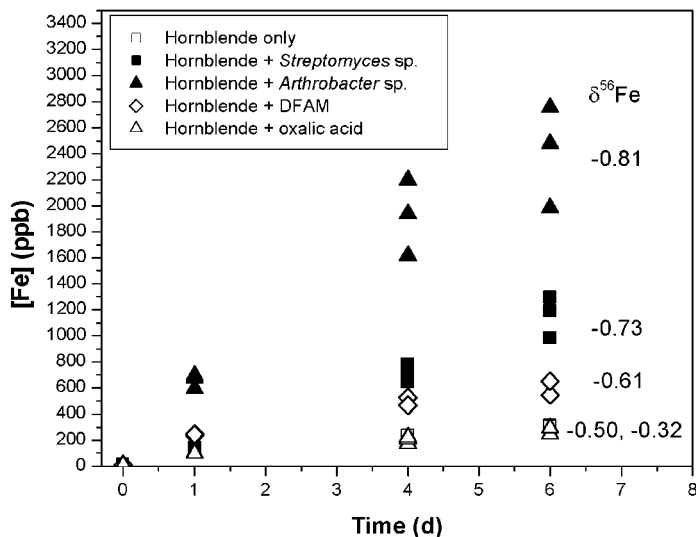


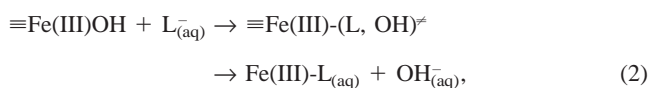
Figure 1. Concentration of Fe in solution (filtered to 0.2 μm) plotted vs. duration of leaching. Average Fe isotope signature (in ‰, referenced to Icelandic basalt BIR-1 standard) averaged over all samples measured at both days 4 and 6 (except data point for *Streptomyces* noted with star in Table 1) is also noted. Fe-release rates for medium only and medium + oxalic acid were indistinguishable, and average isotopic values ($\delta^{56}\text{Fe}$) (-0.32‰ , -0.50‰ , respectively) are noted. Precision for Fe concentrations based on measurements of standards is better than 3%. DFAM is desferrioxamine B mesylate.

(Fig. 1), again consistent with the finding that stronger chelates preferentially extract lighter Fe (Fig. 2).

Released Fe tended to be slightly lighter at day 4 than 6, perhaps suggesting Rayleigh fractionation of a hornblende surface layer. We have shown with X-ray photoelectron spectroscopy (XPS) that the upper ~ 100 Å of the hornblende after reaction with arthrobacter is Fe depleted (Kalinowski et al., 2000). Although XPS was not used here, the ratios of Fe to Si release were larger for biotic than abiotic experiments and larger than the Fe/Si ratio in the hornblende (see Liermann et al., 2000; Kalinowski et al., 2000), consistent with an Fe-depleted surface layer. Kalinowski et al. (2000) showed that some Fe remained in the layer; we infer that this remaining surface Fe is isotopically heavy.

Hornblende after reaction in experiments 5–1 and 5–3 contained isotopically heavier Fe than the hornblende starting material. For experiment 5–1, ~ 100 μg of Fe was released into solution and ~ 10 μg of Fe was in the cell pellet, indicating extraction of $<0.1\%$ of Fe in the reacted hornblende (mass of hornblende powder remained at ~ 2 g during experiment). In contrast, the $\delta^{56}\text{Fe}$ of reacted powder implies leaching of $30\% \pm 20\%$ (5–1) or $40\% \pm 30\%$ (5–3) of the total Fe in the powder, if no precipitation reaction occurred (Table 1). Lack of mass balance may imply incomplete homogenization of hornblende at the start of the experiment, loss of Fe in wash procedures, or precipitation of (isotopically heavy) Fe on the hornblende.

The fact that the Fe in solution is isotopically light compared to hornblende is consistent with a disequilibrium (kinetic) isotope effect (Urey, 1947) during one of several reactions. First, fractionation may be due to a kinetic isotope effect during the reaction:



where the superscript \ddagger denotes the transition state (no charge noted for simplicity) for the Fe-L surface complex, \equiv refers to the surface,

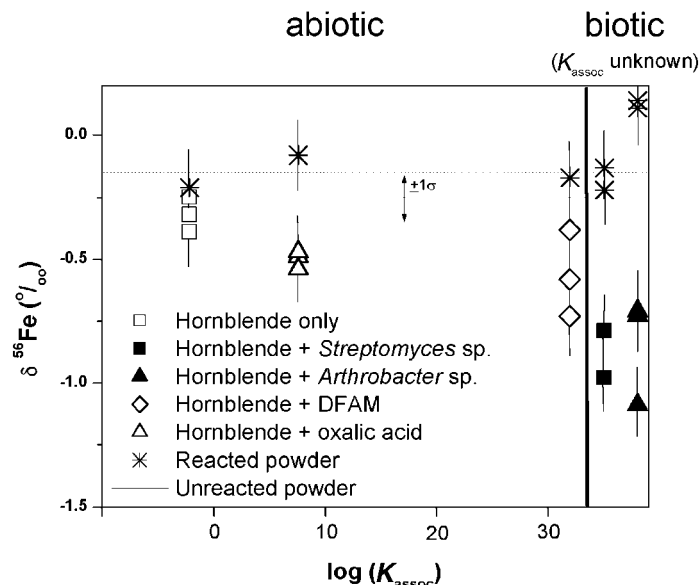


Figure 2. Fe isotope concentration (in ‰, referenced to Icelandic basalt BIR-1 standard) plotted vs. $\log K_{\text{assoc}}$ association constant ($[\text{MOH}^{2+}][\text{H}^+]/[\text{M}][\text{H}_2\text{O}]$, $\log([\text{ML}]/[\text{M}][\text{L}]$, where $\text{L} = \text{C}_2\text{O}_4^{2-}$ (NIST, 1998), or $\log([\text{ML}]/[\text{M}][\text{L}]$, where $\text{M} = \text{Fe(III)}$ and $\text{L} = \text{desferrioxamine}$ (Morel and Hering, 1993) for experiments in medium only, medium + oxalic acid, and medium + DFAM (desferrioxamine B mesylate) respectively. In biotic experiments, both bacteria are known to produce catecholate siderophores, which are reported to have higher association constants than hydroxamate siderophores such as DFAM; however, K_{assoc} is unknown for siderophores in these experiments. Horizontal line indicates average value of Fe in unreacted hornblende starting material ($\pm 0.1\text{‰}$). Stars indicate isotopic concentrations of powder after 6 days of reaction in solutions as noted. One isotopic measurement (4–1) is not plotted because no cell growth occurred (see Table 1).

and L refers to a ligand. According to kinetic theory (Duncan and Cook, 1968), the molecule with the lighter isotope should react faster, and the more strongly bonded the isotopic atom, the larger the effect. This implies that molecules enriched in the lighter isotope should react toward the transition state and product at the greatest rate, although backward reaction at either step would decrease and perhaps reverse the overall fractionation observed as equilibrium is approached. Stronger isotope-ligand bonds in the transition state or product should enhance fractionation by slowing the backward reaction at either step. Thus, stronger ligands (e.g., catecholates) should extract lighter Fe than weaker ligands (e.g., water, oxalic acid, DFAM).

Alternatively, Fe may fractionate during oxidation at the mineral surface. The hornblende has an Fe(II)/Fe(III) ratio of ~ 3.6 (Liermann et al., 2000). If isotopically light Fe(II) is oxidized most rapidly and is preferentially complexed and hydrolyzed as compared to isotopically heavy Fe(II), then dissolved Fe should be isotopically light. Although this hypothesis cannot be disproven, under conditions similar to those of our experiments, Fe oxidizes to Fe(III) immediately upon exposure at the mineral surface (White and Yee, 1985). With complete oxidation of the surface, no fractionation should be observed.

Fe isotope fractionation could also occur during reduction of Fe if Fe was reduced after or during release from the hornblende surface. A few siderophores are known to reduce Fe in solution (Hider, 1984). However, without evidence for Fe(II) in solution, we infer that Fe isotope fractionation probably occurs during hydrolysis of surface complexes. The strength of the Fe-ligand bond in the Fe-siderophore complex is presumed greater than the strength of bonds retaining Fe in the hornblende lattice, possibly accentuating irreversibility of hydrolysis.

If these investigations of scavenging and assimilation of Fe by bacteria can be extrapolated to other systems such as dissimilatory Fe reduction, fractionation probably occurs in one step (cf. Anbar et al., 2000), rather than in small steps, as suggested by Beard et al. (1999).

The observation that the $\delta^{56}\text{Fe}$ of arthrobacter cells is not within error of Fe in solution (Table 1) may indicate contamination of pellets with hornblende, and/or assimilation of heavy Fe by bacteria. In other similar experiments, we concluded that insignificant Fe was entrained in bacterial pellets as powder (Brantley et al., 2001). However, at 6 days, as much as 12 μg of Fe could have been released to solution owing to attack by water rather than siderophore, even in the presence of the arthrobacter, assuming parallel attack by organic ligands and water molecules. If most of this abiotically released Fe was assimilated by the arthrobacter, then the cells would show $\delta^{56}\text{Fe}$ similar to hornblende but dissimilar to Fe in solution.

The largest fractionation was observed for biotic experiments. The bacteria may (1) secrete a stronger siderophore than DFAM, (2) secrete concentrations of siderophore higher than 24 μM , (3) recycle siderophore (e.g., Liermann et al., 2000; Kalinowski et al., 2000), or (4) cause an unknown effect. For example, if bacteria preferentially take up heavy, inorganically complexed Fe as suggested here, the remaining Fe in solution would be fractionated further than in abiotic experiments.

The observation that exchangeable Fe in Gore Mountain soil is $\sim 0.6\%$ lighter than bulk Fe in Gore Mountain hornblende (Table 1) further documents that Fe fractionation occurs in the environment. The exchangeable fraction readily exchanges with Fe in solution and should resemble Fe in solution. Light Fe on exchange sites is consistent with Fe in soil solution fractionated from soil minerals by organic complexation and hydrolysis.

In contrast, Fe in the oxyhydroxide fraction is slightly heavier than bulk Fe in the hornblende and is 1% heavier than the Fe in the exchange fraction. However, ferrihydrite precipitated inorganically from aerobic solutions is $\sim 1\%$ – 2% heavier compared to coexisting aqueous Fe (Bullen et al., 1999). The 1% difference between exchangeable Fe (similar to Fe in solution) and Fe in oxyhydroxide is consistent with these experiments.

Because soils and many other geochemical systems are open systems, the kinetic isotope effect documented here could preserve reservoirs of light and heavy Fe in the rock record, perhaps explaining some observations (Beard et al., 1999; Zhu et al., 2000). Measurement of Fe isotopes should therefore help document contributions from organic complexation of Fe in the rock record, either with or without the presence of bacteria.

ACKNOWLEDGMENTS

The project was funded by National Science Foundation (NSF) grant CHE-9631528, the NASA Astrobiology Institute (NASA Astrobiology Institute Cooperative Agreement NCC2-1057), and the Penn State Biogeochemical Research Initiative for Education (BRIE) sponsored by NSF grant DGE-9972759. Brantley acknowledges the National Research Program, Water Resources Division, U.S. Geological Survey. Reviews by C. Johnson and A. Anbar and discussions with R. Guynn and P. Deines improved the manuscript.

REFERENCES CITED

Anbar, A.D., Roe, J.E., Barling, J., and Nealson, K.H., 2000, Nonbiological fractionation of iron isotopes: *Science*, v. 288, p. 126–128.
Beard, B.L., and Johnson, C.M., 1999, High precision iron isotope measure-

ments of terrestrial and lunar materials: *Geochimica et Cosmochimica Acta*, v. 63, p. 1653–1660.

- Beard, B.L., Johnson, C.M., Cox, L., Sun, H., Nealson, K.H., and Aguilar, C., 1999, Iron isotope biosignatures: *Science*, v. 285, p. 1889–1892.
Bergeron, R.J., and McManis, J.S., 1991, Synthesis of catecholamide and hydroxamate siderophores, in Winkelmann, G., ed., *CRC Handbook of microbial iron chelates*: Boca Raton, Florida, CRC Press, p. 336.
Brantley, S.L., Liermann, L., Bau, M., and Wu, S., 2001, Uptake of trace metals and rare earth elements from hornblende by a soil bacterium: *Geomicrobiology Journal*, v. 18, p. 37–61.
Bullen, T.D., McMahon, P.B., Mandernack, K.W., Bazylnski, D.A., Childs, C.W., and White, A.F., 1999, Using Fe isotopes in biogeochemical studies: Proceed, with caution!: *Eos (Transactions, American Geophysical Union)*, v. 80, p. 479.
Duncan, J.F., and Cook, G.B., 1968, *Isotopes in chemistry*: Oxford, Clarendon Press, 258 p.
Hendershot, W.H., Lalonde, H., and Duquette, M., 1993, Soil reaction and exchangeable acidity, in Carter, M.R., ed., *Soil sampling and methods of analysis*: Boca Raton, Florida, Lewis Publishers, p. 141–145.
Hider, R.C., 1984, Siderophores from microorganisms and plants: Structure and bonding: Berlin, Springer-Verlag, p. 25–88.
Johnson, C.M., and Beard, B.L., 1999, Correction of instrumentally produced mass fractionation during isotopic analysis of Fe by thermal ionization mass spectrometry: *International Journal of Mass Spectrometry*, v. 193, p. 87–99.
Johnson, T.M., Herbel, M.J., Bullen, T.D., and Zawislanski, P.T., 1999, Selenium isotope ratios as indicators of selenium sources and oxyanion reduction: *Geochimica et Cosmochimica Acta*, v. 63, p. 2775–2783.
Kalinowski, B.E., Liermann, L.J., Brantley, S.L., Barnes, A., and Pantano, C.G., 2000, X-ray photoelectron evidence for bacteria-enhanced dissolution of hornblende: *Geochimica et Cosmochimica Acta*, v. 64, p. 1331–1343.
Liermann, L.J., Kalinowski, B.E., Brantley, S.L., and Ferry, J.G., 2000, Role of bacterial siderophores in dissolution of hornblende: *Geochimica et Cosmochimica Acta*, v. 64, p. 587–602.
Matzat, E., and Shiraki, K., 1978, Chromium, in Wedepohl, K.H., ed., *Handbook of geochemistry, elements Cr (24) to Br(35)*: Berlin, Springer Verlag, p. 70.
Morel, F.M.M., and Hering, J.G., 1993, *Principles and applications of aquatic chemistry*: New York, John Wiley and Sons, Inc., 588 p.
NIST 1998, National Institute of Standards and Technology critically selected stability constants of metal complexes database: Gaithersburg, Maryland, U.S. Department of Commerce, 42 p.
Page, W.J., and Huyer, M., 1984, Derepression of the *Azotobacter vinelandii* siderophore system, using iron-containing minerals to limit iron repletion: *Journal of Bacteriology*, v. 158, p. 496–502.
Pickering, W.F., 1981, Selective chemical extraction of soil components and bound metal species, CRC critical reviews in analytical chemistry: Boca Raton, Florida, CRC Press, p. 233–266.
Russell, W.A., Papanastassiou, D.A., and Tombrello, T.A., 1978, Ca isotope fractionation on the Earth and other solar system materials: *Geochimica et Cosmochimica Acta*, v. 42, p. 1075–1090.
Schwyn, B., and Neilands, J.B., 1987, Universal chemical assay for the detection and determination of siderophores: *Analytical Biochemistry*, v. 160, p. 47–56.
Urey, H.C., 1947, The thermodynamic properties of isotopic substances: *Journal of the Chemical Society [London]*, p. 562–581.
White, A.F., and Yee, A., 1985, Aqueous oxidation-reduction kinetics associated with coupled electron-cation transfer from iron-containing silicates at 25 °C: *Geochimica et Cosmochimica Acta*, v. 49, p. 1263–1275.
Zhu, X.-K., O’Nions, K., Guo, Y., and Reynolds, B.C., 2000, Secular variation of iron isotopes in North Atlantic deep water: *Science*, v. 287, p. 2000–2002.

Manuscript received September 5, 2000

Revised manuscript received February 26, 2001

Manuscript accepted March 6, 2001

Printed in USA