

Bacterial effects on the mobilization of cations from a weathered Pb-contaminated andesite

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Received 14 January 1998; accepted 19 February 1999

Abstract

Mobilization of contaminant Pb and other cations from a weathered andesite was examined by conducting water–rock leaching experiments to measure release rates of the major and minor elements found in the bulk rock. Experiments were conducted for over 600 h, at pH 3 and 6.5, as a function of grain size, and in the presence and absence of a common subsurface bacterial species, *Bacillus subtilis*. The acid leaching experiments exhibited enhanced Pb mobilities. The contact area between mineral surfaces and water controlled the amount of most elements leached into solution. Typically, bacteria accelerated the leaching of trace elements. However, major element levels were inversely correlated with bacterial content, and generally lower major element concentrations were observed in the presence of bacteria, relative to the bacteria-free systems. This effect was likely caused by bacterial enhancement of secondary mineral precipitation rates. Extremely low concentrations of Pb were leached into solution during the course of the experiments under conditions most closely approximating natural conditions (i.e., near neutral pH). Moreover, the release rate of Pb relative to other elements was low. Pb was associated with iron oxides precipitated in the rock pore spaces, and the experiments suggest that the iron oxides dissolved to a lesser extent than did other minerals in the rock matrix. The mobility of the Pb associated with these iron oxides was less than that of other heavy metals that were likely associated with the surrounding matrix. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Bacteria; Leaching; Weathering; Dissolved materials; Lead; Biochemistry

1. Introduction

The majority of hazardous waste sites in the US possess aqueous metal contamination, typically Pb

(U.S. Environmental Protection Agency, 1991; Kormianos, 1992). Furthermore, Pb-bearing gasoline exhaust (and smelter) emissions are known to have contaminated soils and sediment worldwide, particularly adjacent to roadways (e.g., Motto et al., 1970; Patterson, 1980; Newsome et al., 1997), though much of it appears to be irreversibly bound to the soil

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matrix (Wang et al., 1995). Irreversible sorption or precipitation of heavy metals onto subsurface mineral surfaces often dramatically reduces metal bioavailability. Irreversibly sorbed contaminants are physically and/or chemically bound to inaccessible pores, or caught up in mineral overcoatings, that are typically very difficult, if not impossible, for microorganisms to influence (see, e.g., the review of Brady et al., 1997). Contaminant inaccessibility makes engineered removal difficult. The net result is that the contaminated sites where sorption is significant (and to an extent irreversible) are often sites where contaminant removal is found to be technically impracticable. The alternative to engineered removal of metals is to stabilize them in situ by adding various chemical binders, or emplacing physical obstacles, such as landfill-type caps or subsurface barriers. These methods are often expensive, and the lingering potential toxicity of the metal remains. Metal release is likely to be stretched out over longer time spans as binders are weathered away, or metals are dissolved and carried around barriers. It is, therefore, of little surprise that the US EPA in 1993 identified removal of metals from soils as the primary objective for new remedial technologies for the Superfund program (U.S. Environmental Protection Agency, 1993).

In recent years, monitored natural attenuation (MNA) has been relied upon to remediate metals-contaminated soils. Natural attenuation is defined by EPA as “the biodegradation, dispersion, dilution, sorption, volatilization, and/or chemical and biochemical stabilization of contaminants to effectively reduce contaminant toxicity, mobility, or volume to levels that are protective of human health and the ecosystem”. For most metals, natural attenuation means dilution, sorption, and the formation of insoluble solids. MNA, or passive remediation, is considered at present only if other remediation approaches are found to be technically impracticable. The difficulties associated with engineered remediation, though, are causing MNA to be considered much earlier in the remediation process. Critical questions which must be answered for both active and passive remediation are: what levels of contaminant reduction are achievable?, and how long will remediation take? The answer to the first question depends on where in the rock matrix the metal resides. The

answer to the second question requires an understanding of the leachability of the primary contaminant host.

Water–rock interactions, in general, and natural attenuation processes in particular, are often controlled by bacteria. Bacteria enhance metal leachability and mineral dissolution by secreting organic molecules that chelate metal cations in solution (e.g., Duff et al., 1963; Webley et al., 1963; McMahon and Chapelle, 1991; Vandevivere et al., 1994; Welch and Vandevivere, 1994; Ullman et al., 1996). Numerous studies have quantified the effects of microbial exudates on the dissolution of minerals (e.g., see Barker et al., 1997, for a recent review), however there are few studies that resolve the overall effects of whole bacteria on metal ion leachability. Quartz grains have been observed to be strongly etched in the presence of sponges (Bavestrello et al., 1995), most likely due to the secretion of ascorbic acid by the organisms. Hiebert and Bennett (1992) performed in situ groundwater experiments to examine the dissolution of quartz and feldspar, and postulated that dissolution rates are enhanced by bacterial secretion of organic acids. Welch and Vandevivere (1994) measured feldspar dissolution in the presence of a range of bacterial exudates, and showed that exudates can either enhance (presumably through ligand-promoted dissolution) or inhibit (through surface site blockage) mineral dissolution rates, depending on the type of organic exudate and fluid conditions. Bacteria also reductively dissolve minerals and mineral coatings of Fe(III) and Mn(III,IV) (hydr)oxides (e.g., Arnold et al., 1988; Nealson et al., 1988; Lovley et al., 1993). Since these (hydr)oxides are often the hosts for contaminant metals, the net effect of dissolution is metal mobilization (see, e.g., Stone, 1987; Stumm, 1995; Zachara et al., 1995). Bacterial surfaces also may enhance mineral precipitation (Urrutia and Beveridge, 1993, 1994; Konhauser et al., 1993). Organic functional groups exposed on bacterial cell walls exhibit a high affinity for aqueous metal cations (Beveridge and Murray, 1976, 1980; Beveridge and Koval, 1981; Crist et al., 1981; Harvey and Leckie, 1985; Gonçalves et al., 1987; Fein et al., 1997; Daughney et al., 1998; Daughney and Fein, 1998). Adsorption of aqueous metals onto bacterial surfaces should, therefore, favor mineral precipitation either through bacterial surface nucleation, or by elevating

metal activities on the bacterial surface (see, e.g., Mann, 1988).

In this study, we examined Pb mobilization from a contaminated, weathered andesite using water–rock leaching measurements. We measured the extent of Pb (and other major and minor cation) dissolution/desorption, and we tested the effect of subsurface bacteria on surface sequestration by measuring the extent of cation leaching both in the presence and absence of a common subsurface bacterial species. There is considerable interest in adapting bioremediation approaches to remove metals and/or radionuclides from contaminated soils, for example, by stimulating soil organisms to reductively dissolve contaminant-sorbing iron hydroxides. One object of the present effort is to understand the natural baseline effect of soil microorganisms on metal availability. Only by first determining the specific effects of subsurface microorganisms on metal mobility in the absence of engineering efforts can any subsequent bioremediation efforts be truly quantified.

2. Experimental procedures

Samples of weathered andesite, contaminated by atmospheric deposition of lead, were taken from a roadcut adjacent to a city street, near Interstate 10 in Tempe, AZ. The bulk composition of the rock, as determined from acid digestion and ICP-MS analysis, is given in Table 1 (details of digestion and analysis procedures are given below). A considerable number of pore spaces within the rock contain iron oxide weathering products (Fig. 1), and a typical microprobe composition determination for these iron oxides is given in Table 2. Pb concentrations in the parent rock are below microprobe detection limits, so Pb is present in the rock due to anthropogenic input, and the Pb is predominantly associated with the secondary iron oxides. Iron oxides have a high affinity to preferentially adsorb Pb (e.g., Lee, 1975; Benjamin and Leckie, 1981; Coston et al., 1995), so presumably, the Pb is adsorbed or precipitated on iron oxide surfaces, or exists as a substitute within the iron oxide mineral structure.

In order to determine the potential mobility of Pb relative to other cations in the rock, we conducted a

Table 1
Bulk rock composition determined by acid digestion

Element	ppm In rock
Mg	918.2
Al	53449.0
Ca	60329.0
Ti	2903.7
V	67.5
Cr	61.7
Mn	466.0
Fe	26860.0
Co	6.5
Ni	18.0
Cu	27.2
Zn	40.1
Cd	0.03
Pb	60.1

series of batch leaching experiments. Specifically, we placed either a 30 g rock fragment, or 1.5 g of rock powder, into a Teflon reaction vessel with a pH-buffered electrolyte solution. The rock fragment experiments were designed to simulate the low rock surface area to water ratio typical of surficial environments dominated by meteoric recharge. The rock powder experiments had high surface area to water ratios, typical of subsurface groundwater systems. Rock fragments were used after the exterior of the rock was rinsed using distilled, deionized water. The rock powder was made by pulverizing washed rock fragments; and the size fraction of 40 to 100 mesh was used for the experiments. Each fragment that was used, either directly or after powdering, was from a single larger rock fragment, and therefore homogeneity of the rock samples was maximized. Experiments were conducted in a 0.1 molal NaNO₃ electrolyte; pH was buffered using 0.1 molal *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid) (HEPES). HEPES offers a high buffering capacity at near-neutral pH values, and does not significantly interact with cations to form aqueous complexes. The desired starting pH value was reached by adding NaOH or HNO₃. Each reaction vessel was placed on a shaking table within a water bath thermostated to 25°C ± 1°C. Aqueous samples were extracted periodically, filtered through 0.45 μm membranes, and the final pH of the solution was measured. Most experiments lasted 620 h. The pH of each experimental

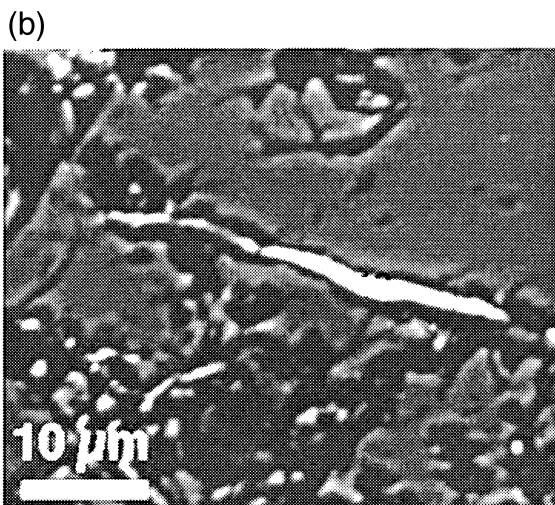


Fig. 1. Backscattered electron images of Pb-enriched Fe(III)-hydroxides in weathered andesite. The white areas are the Pb-enriched iron hydroxides. (A) A typical pore filled by precipitation of clay minerals, amorphous silica, and iron hydroxide. The surrounding host mineral is plagioclase feldspar, with dark areas being more sodium rich. Microprobe analysis indicates a PbO concentration of 0.38% for the iron hydroxide. (B) Lineament iron hydroxide precipitation within a surrounding matrix of quartz. Microprobe analysis of the lineament feature indicates a PbO concentration of 0.88%.

solution drifted higher as mineral dissolution proceeded, and, after each sample was extracted, the pH

was re-adjusted to the desired level through the addition of HNO_3 . The magnitude of the pH variations was not large, and is depicted in Fig. 2 for all experiments.

Bacterial cultures were prepared following the procedure outlined by Fein et al. (1997). Cultures of *Bacillus subtilis* were grown in trypticase soy broth with 0.5% yeast extract to exponential growth phase. Harvested bacteria were washed, rinsed in a 0.01 molal HNO_3 wash, rinsed in a 0.001 molal EDTA wash, and repeatedly rinsed in DDI water to remove solutes present in the growth medium and wash solutions. The wash procedure leaves the cells fully intact and viable (as tested by visual inspection and replating after the wash procedure), but because no readily degradable carbon source is provided, the cells are metabolically inactive during the experiments. Washed cells are whole, have not formed spores, and our previous results have demonstrated the effectiveness of the procedures in cleansing the cell surface of interfering ions (Fein et al., 1997). In each experiment involving bacteria, approximately 2.7 g of bacteria (weighed after 1 h of centrifugation at 7500 rpm to remove excess H_2O) were placed into reaction vessels containing 250 ml of experimental solution. This mass corresponds to approximately $10^{-2.9}$ moles of bacterial surface carboxyl sites per litre of solution (Fein et al., 1997).

Five types of leaching experiments were conducted, each with the same NaNO_3 -HEPES back-

Table 2
Fe-oxide Composition

Component	%
Na_2O	0.58
MgO	3.13
Al_2O_3	18.80
SiO_2	15.69
SO_3	0.08
K_2O	1.47
CaO	1.40
TiO_2	0.73
VO_4	0.08
Cr_2O_3	0.00
FeO	56.30
CuO	0.12
PbO	0.58
Total	98.96

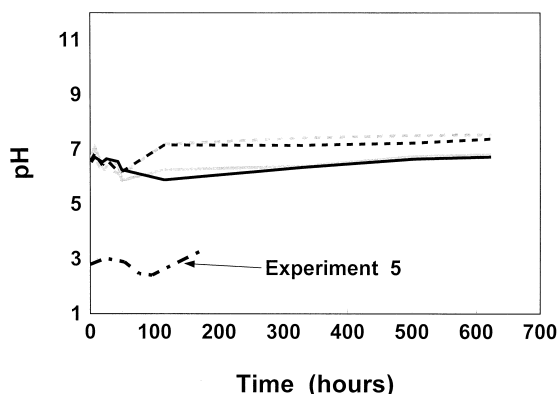


Fig. 2. Measured pH variations as a function of time for the five experiments conducted. Solid black lines represent Experiment 1 data (rock fragment, no bacteria); dashed black lines represent Experiment 2 data (rock fragment, with bacteria); solid gray lines represent Experiment 3 data (rock powder, no bacteria); and dashed gray lines represent Experiment 4 data (rock powder, with bacteria).

ground electrolyte and electrolyte concentration: Experiment 1: Dissolution of a rock fragment in a pH 6.5 solution containing no bacteria; Experiment 2: Dissolution of a rock fragment in the same solution, but containing bacteria; Experiment 3: Dissolution of 1.5 g of rock powder in the same pH 6.5 solution containing no bacteria; Experiment 4: Dissolution experiments identical to (3) but containing bacteria; and Experiment 5: A bacteria-free experiment using a rock fragment, with a starting pH of 3.0.

Experimental samples were analyzed for cation concentrations using a VG Elemental PlasmaQuad model PQII inductively coupled plasma-mass spectrometer (ICP-MS). Single element standard solutions purchased from Inorganic Ventures (Lakewood, NJ) were utilized to prepare calibration and internal standard solutions. Analyses were performed using an external calibration procedure, and internal standards were included for matrix and instrumental drift corrections. For data reduction, the raw intensities were corrected for background counts, instrumental drift, matrix effects, and wherever applicable, for molecular interferences. Procedural blanks were analyzed to check for any contribution from the reagents.

The elemental composition of the bulk rock was determined by digestion of 100 mg of sample powder in a screw-capped Teflon bomb at 100°C, using 2 ml concentrated HNO₃ and 2 ml concentrated HF.

The HF/HNO₃ mixture was then evaporated to dryness, and the residue was dissolved in 2 ml of concentrated HNO₃. The solution was dried and redissolved a second time in 2 ml of concentrated HNO₃ and dried again. The two nitric acid treatments were performed to ensure conversion of fluorides to nitrates. Finally, the residue was dissolved in 2 ml of concentrated HNO₃ and diluted with water to 100 ml to give final total dissolved solids of < 0.1%. The entire procedure was carried out under clean laboratory (class 1000) conditions.

Because of the high concentration of the organic HEPES pH buffer, bacterially-produced organic molecules could not be detected directly using dissolved organic carbon (DOC) analyses. To examine the nature and concentration of the dissolved organic molecules produced by the bacteria during the course of the experiments, we conducted a separate experiment under identical pH (6.5) and ionic strength conditions. This experiment was conducted using the same concentration of bacteria, and pH was maintained using an automated pH-stat apparatus, with inorganic acid and base titrants. Samples were taken periodically and analyzed for DOC concentration using a combustion/non-dispersive infrared gas analysis technique. In addition, some of the samples were analyzed by gas chromatography-mass spectrometry (GC-MS), and by high performance liquid chromatography (HPLC) to determine the mass and functional group characteristics of the aqueous organic molecules in the samples.

3. Results and discussion

The results from the rock leaching experiments are presented in Figs. 3 and 4, which show the concentration of each element for which analyses were conducted as a function of time. Fig. 3 shows element concentration profiles as a function of time for experiments (1)–(4) described above (the pH 6.5 experiments, conducted using either a rock fragment or rock powder, each either with or without bacteria in the system). Virtually all of the elements displayed broadly similar concentration profiles as a function of time. That is, element concentrations tended to increase to a maximum concentration, and

then dropped to lower levels. The maximum concentration typically occurred approximately 100 h after

the beginning of the experiment, and lower concentration plateaus were reached after approximately

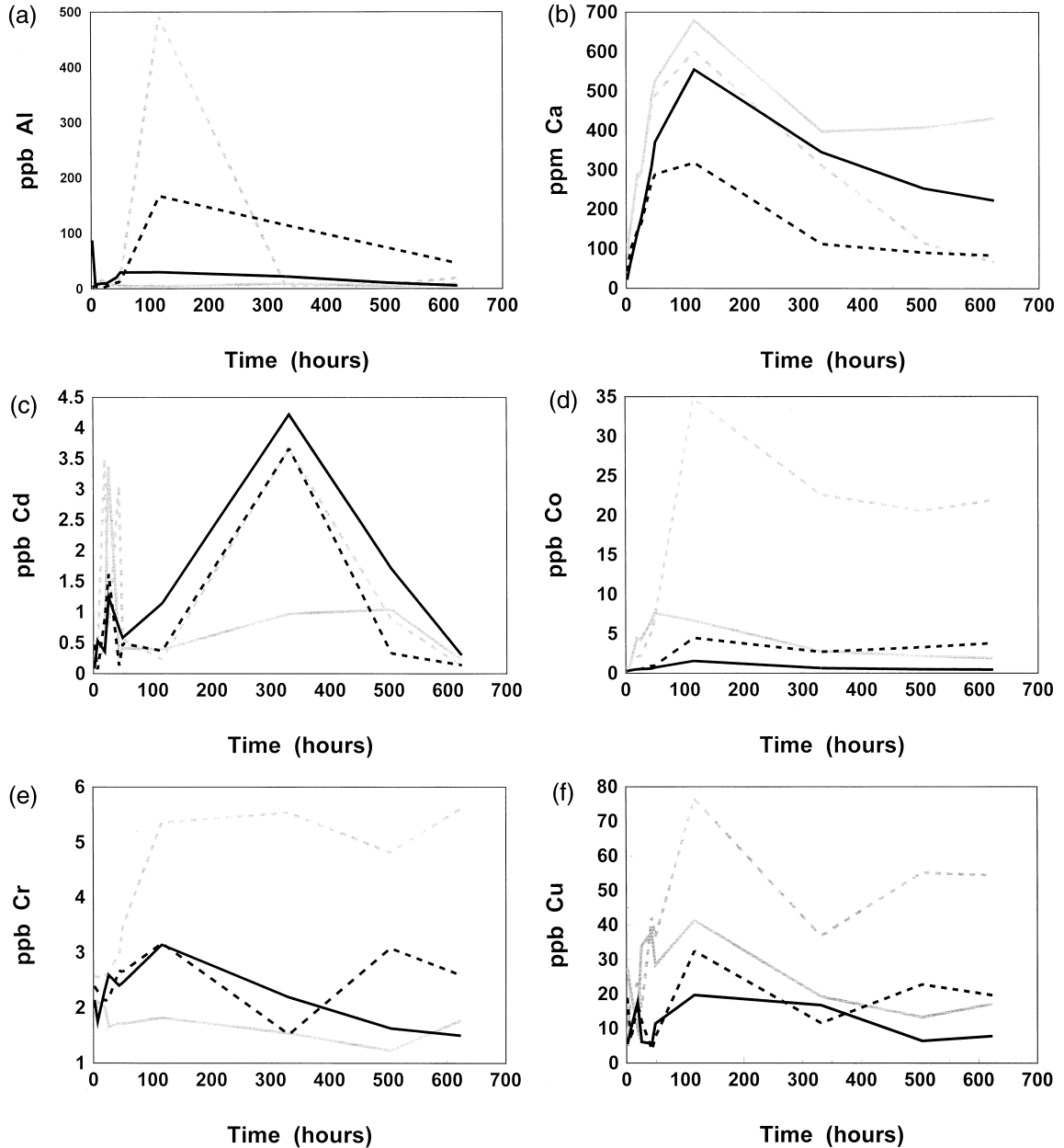


Fig. 3. Elemental compositions of experimental samples as a function of time. Results are plotted in terms of parts per billion of each element (except for Ca, which is depicted in parts per million) as a function of time in hours from the inception of the experiment. Solid black lines represent Experiment 1 data (rock fragment, no bacteria); dashed black lines represent Experiment 2 data (rock fragment, with bacteria); solid gray lines represent Experiment 3 data (rock powder, no bacteria); and dashed gray lines represent Experiment 4 data (rock powder, with bacteria).

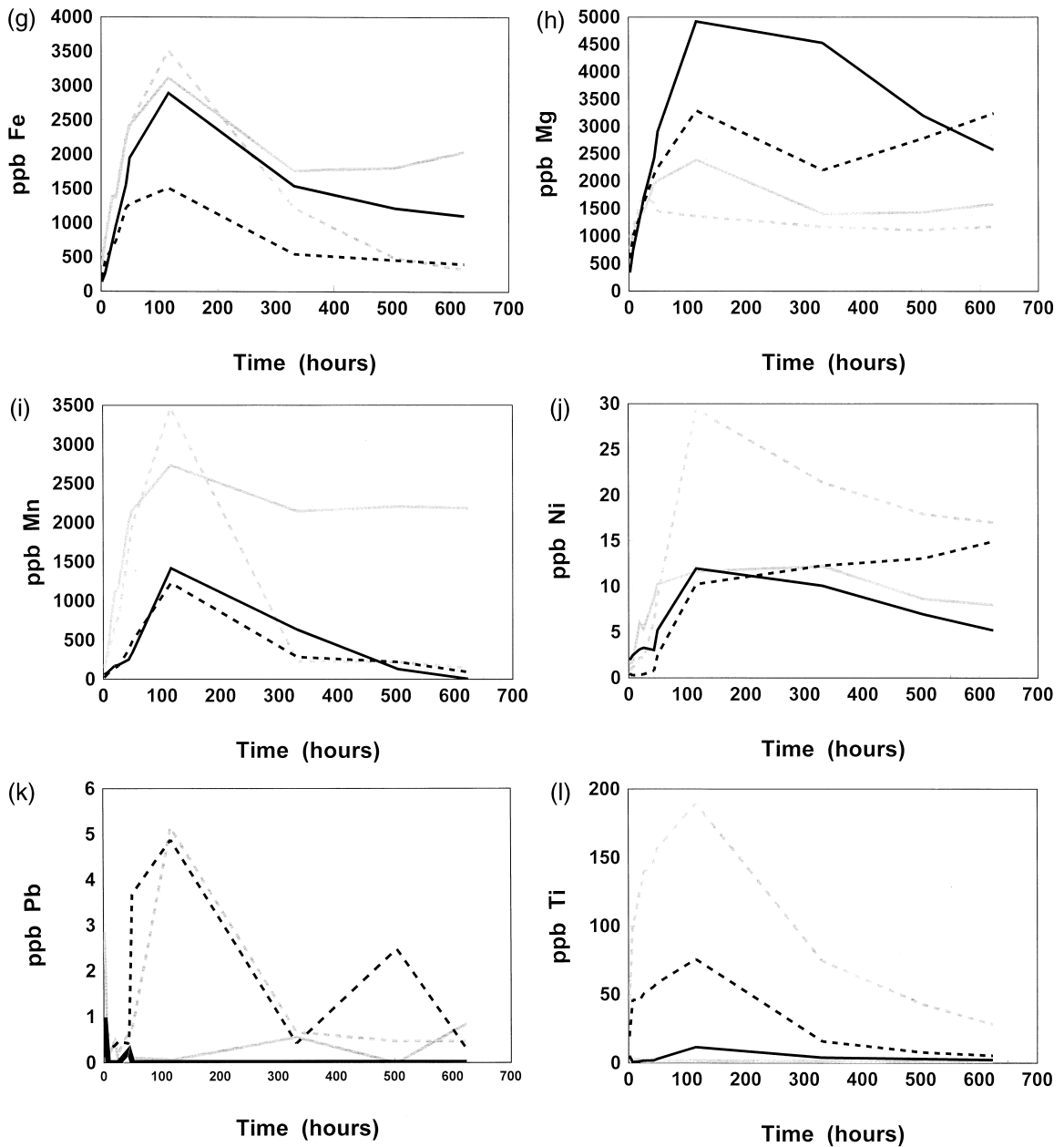


Fig. 3 (continued).

300 h. Note that multiple dissolution and precipitation reactions may have occurred and affected element levels during the course of the experiment. The final concentration plateau may not represent equilibrium in the system, but could be a steady-state between dissolution and precipitation reactions.

3.1. pH Effects

Fig. 4 illustrates the effect of pH on the measured Pb concentrations in the three bacteria-free systems. The pH 3.0 experiment showed enhanced Pb release from the rock matrix relative to that observed for the

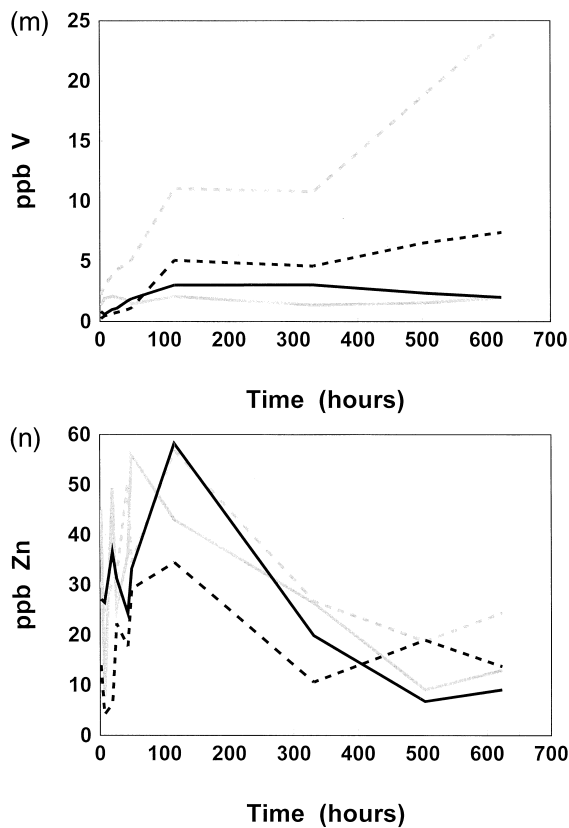


Fig. 3 (continued).

higher pH experiments. This result is not surprising considering that increased solution acidity not only favors increased mineral dissolution rates (e.g., Furrer and Stumm, 1986; Brady and Walther, 1989), but decreased cation adsorption onto mineral surfaces as well (see, e.g., Balistrieri et al., 1981; Stumm and Morgan, 1996). Therefore, whether the Pb was present as an adsorbed species or within a mineral framework, lower pH conditions would have promoted elevated Pb levels in solution. This experiment re-emphasizes the potentially extreme differences in the rate and extent of heavy metal release into solution between acid solutions and solutions with pH conditions more typical of natural environments.

3.2. Surface area effects

Experiments involving the high surface area rock powders exhibited higher concentrations of dissolved

Ca, Co, Cu, Fe, Mn, and Ni than did similar experiments involving rock fragments having relatively low surface areas (Fig. 3). This was true in the presence and absence of bacteria. Although there were minor differences between the pH values of the powder and fragment experiments, the differences never exceeded 0.4 pH units, and therefore were probably not the cause of the release rate differences. It is more likely that the increased surface area of the powder in contact with water promoted faster mineral dissolution rates. For most of the elements considered, this grain size effect appears to have controlled the steady-state element concentrations observed after approximately 300 h. That is, in general, the rock powder experiments displayed higher element concentration plateaus than did the rock fragment experiments. This observation further suggests that the concentration plateaus represent a steady-state balance between competing dissolution and precipitation reactions. Surface area should not affect the final concentration of an element in solution if an equilibrium state is attained, unless it is a sorption equilibrium, in which case it would have an opposite effect than that observed.

Some elements, such as Al, Cd, Mg, Pb, and Zn, did not exhibit consistently higher concentrations in solutions reacted with powders, compared with those reacted with rock fragments. Particularly for Cd, Pb, and Zn (Fig. 3c, k, n), the concentration of each element in solution was broadly similar between the

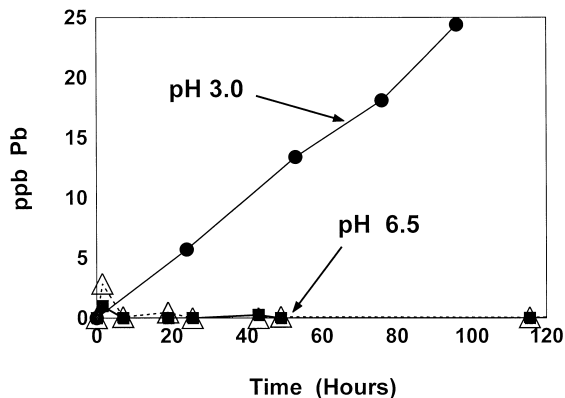


Fig. 4. Measured Pb concentration, in parts per billion, as a function of time for the low pH experiment (circles; pH 3.0), and the two bacteria-free mid-pH experiments (triangles and squares; pH 6.5).

powder and the fragment experiments, suggesting that grain size did not affect whatever processes controlled the release of these elements to solution. For Mg, however, a reverse dependence was observed (Fig. 3h). That is, the concentration of Mg in the rock fragment experiments was elevated relative to that found in the rock powder experiments. This holds whether or not bacteria were present in the system. The Mg concentrations suggest that the overall kinetics of precipitation reactions involving Mg were more rapid than Mg-bearing mineral dissolution reactions when a rock powder was present, perhaps due to an increase in the number of nucleation and/or adsorption sites present in the powder system. For Al (Fig. 3a), there was a switch between the two types of observation as the experiment progressed (see below).

3.3. Pb Release

Perhaps the most important observation that is relevant to natural attenuation of contaminant metals in weathering environments involves the observed Pb concentrations in solution (Fig. 3k). In the bacteria-free experiments, the extent of Pb release was extremely small. The rock fragment experiment produced Pb concentrations of less than 0.3 parts per billion (ppb). The rock powder experiment gave slightly higher Pb concentrations, but Pb levels were still less than 0.9 ppb over the duration. Even in the systems containing bacteria, Pb concentrations were extremely low; maximum observed values were approximately 5 ppb at just over 100 h, and final Pb concentrations after over 600 h were less than 1 ppb. Despite the abundance of anthropogenic Pb in the rocks used in the experiments, the extent of Pb release during leaching, even in the bacterial systems, was significantly below the EPA drinking water standard for Pb concentration of 50 ppb.

3.4. Bacterial effects

The effects of bacteria on the extent of mineral dissolution in the experimental systems can be observed by comparing the solid (no bacteria) to the dashed (with bacteria) curves in Fig. 3. That is, comparing elemental concentrations from experiment 1 (rock fragment, no bacteria), with those from

experiment 2 (rock fragment, with bacteria); and those from experiments 3 (powder, no bacteria) and 4 (powder, with bacteria). For most trace elements (Al, Co, Cr, Cu, Ni, Pb, Ti, and V), the presence of bacteria in the water–rock system significantly increased the concentration of each element in solution. This effect was observed both in the rock fragment and the rock powder experiments. The effect of the bacteria was most dramatic in the rock powder systems, and for many elements, appeared to reach a peak effectiveness at approximately 100 to 200 h. A number of elements (Ca, Cd, Fe, Mg, Mn, and Zn), however, did not exhibit the same increase in aqueous concentration in the presence of the bacteria. Most of these latter elements are major mineral-forming cations in the rock. The mineral-forming cations (Ca, Fe, Mg, and Mn) each displayed an inverse correlation with bacterial content, with generally lower elemental concentrations in the presence of bacteria than were observed in the bacteria-free systems. Cd and Zn exhibited broadly similar elemental concentrations between the two systems, indicating minimal effects of bacteria on their release.

Most of the major rock-forming cations (Ca, Fe, Mg) were present in lower concentrations in the bacteria-bearing systems, while most minor elements (Al, Co, Cr, Cu, Ni, Pb, Ti, V) were solubilized faster in the presence of bacteria. The decrease in major element concentrations between the bacteria-free and the bacteria-bearing systems could have been caused by either bacterial blockage of dissolution sites on the original rock surfaces, or by enhanced secondary mineral precipitation rates. The bacterial blockage scenario is unlikely because only the major rock-forming cations experienced a decrease in concentration in the presence of bacteria. Most elements exhibited higher concentrations in the bacteria-bearing systems, a scenario that is difficult to explain if overall dissolution was diminished due to site blockage on the original rock surfaces.

A more reasonable explanation for the diminished concentrations of the major rock-forming cations is that bacteria enhanced the precipitation rate of secondary minerals containing primary cations. There are few data which quantify the effects of bacteria on the precipitation rates of minerals, but field and experimental studies do indicate that bacterial sur-

faces have the ability to enhance silicate, iron oxide, and carbonate precipitation (Urrutia and Beveridge, 1993, 1994; Konhauser et al., 1993; Ferris et al., 1994; Warren and Ferris, 1998). The experimental solutions are undersaturated with respect to calcite and other carbonate phases (using the EQ3 database; Wolery, 1992). However, bacteria may cause local mineral precipitation on cell wall surfaces even when the bulk solution is undersaturated. Although the exact mechanism has not been determined, a likely first step in the process is adsorption of the elements onto bacterial surfaces. Bacterial surfaces display a high affinity for aqueous metal cations (e.g., Beveridge and Murray, 1976, 1980; Fein et al., 1997; Daughney and Fein, 1998), and adsorption may increase the activity of cations on the cell wall enough to induce surface-catalyzed mineral precipitation. The four elements most affected (Ca, Mg, Mn, and Fe) are also the elements having the highest concentrations in solution (concentrations ranging from hundreds of ppb to hundreds of ppm) and, therefore, are also the most likely to be adsorbed and to be incorporated into secondary mineral precipitates.

The higher concentrations of some elements (Al, Co, Cr, Cu, Ni, Pb, Ti, and V) in the presence of bacteria were probably caused by aqueous complexation between the metals and bacterially-produced organic acids. Adsorption of the metals to bacterial surfaces would not increase the measured metal concentrations because the bacteria were filtered out of solution prior to aqueous analyses. Elevated concentrations may have been due to enhanced mineral dissolution caused indirectly by bacterial adhesion to the original mineral surfaces. However, this process would have dissolved all elements to the same extent. Because the concentrations of the major rock-forming cations were lower in the presence of bacteria, enhanced mineral dissolution was probably not the important mechanism. Bacteria can produce dissolved organic molecules either as exudates that result from bacterial metabolism, or from bacterial lysis and discharge of nucleic acids and proteins. Although binding between aqueous metals and lysis products has not been examined, bacteria can secrete a range of organic acid exudates that are highly efficient aqueous metal complexing agents (e.g., Francis and Dodge, 1990; Welch and Vandevivere, 1994; Ullman et al., 1996). These organic com-

pounds form much more stable aqueous metal–organic complexes with the minor elements than with the more abundant elements such as Ca and Mg (Chen et al., 1995). The thermodynamic stabilities of aqueous Fe–organic complexes are typically similar to those of Al–organic complexes, and therefore one would expect to observe elevated dissolved Fe concentrations in the bacterial experiments. Presumably, the incorporation of Fe into/onto precipitated minerals successfully competes with the dissolved organic ligands for the available Fe. The decrease in minor element concentrations after approximately 100 h may be due to exudate or lysis product degradation, an effect also observed by Ferris et al. (1988).

The presence of high concentrations of bacterially-produced dissolved organic acids was confirmed in our separate DOC experiment. Fig. 5 illustrates the measured dissolved organic carbon profile over the 200 h of the experiment. DOC increased slowly until approximately 100 h, after which more dramatic increases were observed. Over 80 ppm of DOC was measured at 200 h. The timing of the DOC production in these experiments is likely to be radically different than that of the leaching experiments due to markedly different experimental conditions. Although the DOC experiment did not replicate the exact conditions of the rock leaching experiments, the results illustrate, at least qualitatively, that high concentrations of organic acids are produced by *B. subtilis* under non-growth conditions.

HPLC analyses of the DOC experimental solutions showed two prominent peaks, with areas under

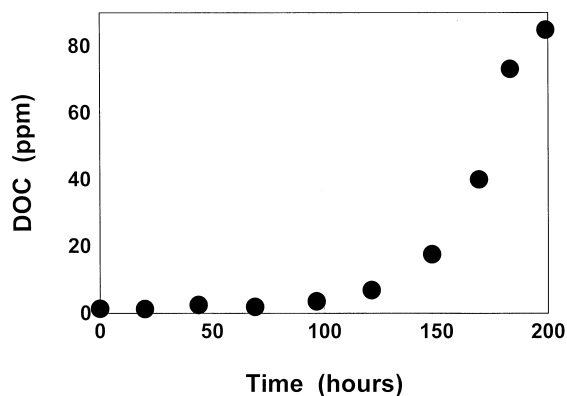


Fig. 5. Dissolved organic carbon experimental results, depicted in terms of ppm of DOC as a function of time.

the peaks representing over 85% of the peak area detected. These molecules exhibited similar retention times to acetic and oxalic acids, suggesting that they are mono- and/or di-functional organic acids. However, the GC-MS analyses indicated that they are significantly heavier than acids such as acetic or oxalic. GC-MS analyses of these samples indicated that over 95% of the DOC in solution was present as relatively heavy molecules (> 100 g/mol), with the largest proportion having molecular weights of 117, 141, and 219 g/mol.

The organic molecules that are produced during the course of the DOC experiment are most likely bacterial exudates. The rate of bacterial lysis for cells suspended in water is slow (Jolliffe et al., 1981). In addition, Ferris et al. (1988) and Urrutia and Beveridge (1993) found that exposure of *B. subtilis* cells to at least 1 μ molar aqueous Fe inhibits autolysis for over 8 weeks. Our bacteria-bearing experiments used similar concentrations of bacteria to those used by Urrutia and Beveridge (1993), and because the experiments rapidly attained aqueous Fe concentrations in excess of 1 μ molar, it is unlikely that cell lysis contributed significantly to the observed increase in DOC.

3.5. Pb Mobility relative to other elements

Although the absolute concentration of leached Pb was extremely low, the Pb content of the bulk rock was low as well. A better indicator of Pb mobility is the Pb:element molal ratios in solution, which normalizes the observed absolute aqueous concentrations to the concentrations of elements in the bulk rock. A lower Pb:element ratio in solution relative to the ratio found in the bulk rock means that Pb is less mobile than the other element in question.

To quantify relative mobilities between Pb and each other element studied, we define a Δ value as follows:

$$\Delta \text{ Value} = \log\left(\frac{m_{\text{Pb}}}{m_{\text{Element}}}\right)_{\text{bulk rock}} - \log\left(\frac{m_{\text{Pb}}}{m_{\text{Element}}}\right)_{\text{leach expt.}} \quad (1)$$

where m represents the molality of the subscripted element. The subscript 'bulk rock' indicates that molalities are from the bulk rock digestion experi-

ment; the subscript 'leach expt.' indicates that molalities are from the rock leaching experiment. The bacteria-bearing systems are not included in this treatment, nor are the results from the rock fragment, no bacteria experiment because many of the observed Pb concentrations from these experiments were below the detection limit of the ICP-MS. For ease of comparison, the leach experiment ratio value is calculated only for the final experimental sampling point (623 h) from Experiment 3 (rock powder, no bacteria). The calculated Δ values are compiled in Table 3. The 623 h data were used because they represent the long-term overall balance of dissolution/precipitation reactions occurring in the experimental systems. Although other sampling points yield slightly different Δ values, the qualitative relationships would be the same as those described below.

The Pb:Cd molal ratio exhibits the largest positive Δ value of any element pair studied ($\Delta = 2.72$ log units), and Mg, Ca, Co, Cu, Ni, and Zn each display large positive Δ values (> 1.4 log units). That is, these elements are found in the solutions of the leaching experiments at a higher level, relative to Pb, than would be predicted based on their elemental abundances within the bulk rock matrix. Mg and Ca are typically exchanged rapidly from alkali silicate surfaces and are replaced by protons (see, e.g., Wogelius and Walther, 1991; Wogelius, 1992). Cu, Ni, and Zn may be released due to oxidation of trace sulfides. Elements such as Fe, Mn, Cr, and V exhibit Δ values indicative of roughly similar release rates

Table 3
Element leachability relative to Pb (Eq. (1))

Element	Δ Value
Cd	2.72
Ca	2.70
Mg	2.09
Cu	1.65
Ni	1.49
Zn	1.47
Co	1.42
Mn	0.76
Fe	0.73
Cr	0.31
V	0.31
Ti	-1.64
Al	-1.95

between Pb and each element; and Ti and Al, with Δ values of -1.5 and -1.8 , respectively, display generally conservative behavior. They were released in less abundance, relative to Pb, than would be expected from complete dissolution of the rock matrix. Clearly, with such a wide range of Δ values, the bulk rock did not undergo complete stoichiometric dissolution, and some components of the rock were dissolving to a greater extent than others. Note that Pb was associated with a phase (or phases) that dissolves (or desorbs) constituent elements to a lesser extent than did most other phases within the rock. Al- and Ti-bearing phases were the only ones to dissolve to a lesser extent than the Pb-bearing phases. For Al, at least, this is not surprising considering the extremely low solubility of Al-bearing oxides, hydroxides, and silicates in the mid-pH range of the experiments.

Pb levels co-varied with those of Fe, Mn, Cr, and V, likely due to its presence on and/or within the iron oxides found in the pore spaces of the bulk rock. Although Table 2 indicates that Cr and V (Mn was not analyzed during the microprobe analyses) are not typically found at elevated concentrations in the iron oxides, several individual microprobe analyses did highlight that some iron oxide grains contain significantly elevated levels of Cr and V. Because their behaviors are markedly different from that of Pb, Fe, Mn, Cr, and V, it is likely that the other heavy metals, Cd, Co, Cu, Ni, and Zn are not associated with the iron oxides, and are either adsorbed or incorporated within the minerals that comprise the bulk rock.

4. Conclusions

This study highlights the importance of bacteria, pH, surface area:water volume ratio, and iron oxides in affecting the extent and rate of metal mobilization from a weathered andesite. The experiments were not designed to directly reproduce natural conditions, but rather to isolate specific factors that affect the natural immobilization of Pb. Bacteria appear to exert two distinct effects on rock-forming cations. The major cations were less concentrated in bacteria-bearing systems, likely due to bacterial en-

hancement of secondary mineral precipitation rates. Conversely, trace cation concentrations were, in general, higher in the bacteria-bearing systems, probably due to aqueous complexation between the cations and bacterial organic acid exudates.

Despite the relatively high concentration of anthropogenic Pb in the bulk rock samples, extremely low levels of Pb were available for dissolution. In addition, Pb release was sometimes nearly two orders of magnitude lower than that of a number of other trace elements (normalized to bulk-rock values). Elements that were released at rock-normalized rates similar to Pb include Fe, Mn, Cr, and V. Pb (and presumably Mn, Cr, and V) is associated with iron oxides precipitated in the rock pore spaces. Iron oxides dissolve to a lesser extent than do the other minerals in the rock matrix. Consequently, Pb associated with these iron oxides was more attenuated than most of the other heavy metals in the system, which are likely associated with the surrounding rock matrix rather than the iron oxides.

Acknowledgements

JBF and PVB appreciate funding from an LDRD grant, 'Mechanistic models for radionuclide desorption from soils', provided by Sandia National Laboratories. The manuscript was greatly improved by thoughtful reviews by Sue Welch, James Drever, and an anonymous reviewer. We thank Peter Wightman, Dennis Birdsell, and Bill Boggess for their help with the organic analyses. This work was supported by the United States Department of Energy under Contract DE-AC04-94AL85000. [JD]

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