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## Microbial mobilization of plutonium and other actinides from contaminated soil



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## ABSTRACT

We examined the dissolution of Pu, U, and Am in contaminated soil from the Nevada Test Site (NTS) due to indigenous microbial activity. Scanning transmission x-ray microscopy (STXM) analysis of the soil showed that Pu was present in its polymeric form and associated with Fe- and Mn- oxides and aluminosilicates. Uranium analysis by x-ray diffraction ( $\mu$ -XRD) revealed discrete U-containing mineral phases, viz., schoepite, sharpite, and liebigite; synchrotron x-ray fluorescence ( $\mu$ -XRF) mapping showed its association with Fe- and Ca-phases; and  $\mu$ -x-ray absorption near edge structure ( $\mu$ -XANES) confirmed U(IV) and U(VI) oxidation states. Addition of citric acid or glucose to the soil and incubated under aerobic or anaerobic conditions enhanced indigenous microbial activity and the dissolution of Pu. Detectable amount of Am and no U was observed in solution. In the citric acid-amended sample, Pu concentration increased with time and decreased to below detection levels when the citric acid was completely consumed. In contrast, with glucose amendment, Pu remained in solution. Pu speciation studies suggest that it exists in mixed oxidation states (III/IV) in a polymeric form as colloids. Although Pu(IV) is the most prevalent and generally considered to be more stable chemical form in the environment, our findings suggest that under the appropriate conditions, microbial activity could affect its solubility and long-term stability in contaminated environments.

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## 1. Introduction

The presence of low levels of plutonium (Pu) in contaminated soils and at remediated sites is a major concern because of its potential for dissolution and mobilization in the environment. Primary worries center on plutonium's toxicity, and the relatively long half-lives of its isotopes ( $87.7$ – $8.0 \times 10^8$  y). Nuclear weapons testing at the Nevada Test Site (NTS) during the 1950s and early 1960s have resulted in the contamination of large area of soil with plutonium and other radionuclides at levels in excess of 40 pCi per gram. The bulk of the activity typically resides within the top few centimeters of the soil; the primary radionuclides of concern are plutonium, uranium, and americium, with lesser amounts of cesium, strontium, and europium (Walker and Liebendorfer, 1998). Studies of the soils at the NTS's Area 11 demonstrated that more than 75% of the total radionuclides are dispersed as particles in the 40-micron soil fractions (Papelis et al., 1996), and/or are partially attached to clay particles. The plutonium particles are relatively

immobile and expected to remain so until disturbed.

Although Pu is considered stable in soil, its transport, albeit at very low concentrations, was observed at several Department of Energy's sites, and at the Mayak Production Association, Urals, Russia (Kersting, 2013). Thus, Pu was present in colloidal form at Los Alamos National Laboratory's (LANL) waste site (Penrose et al., 1990); similarly, at Maxey Flats, a former low-level radioactive waste site, Pu occurred as colloids, as well as soluble tetravalent species complexed with organic ligands (Cleveland, and Rees, 1981). At Mayak, it was bound to iron-oxide colloids (Noviko et al., 2006). The predominant form of Pu in the soil at Rocky Flats, CO, was  $\text{PuO}_2(\text{s})$  (Clark et al., 2006), while, in surface waters, it existed as colloids associated with organic macromolecules (Santschi et al., 2002; Xu et al., 2008). At three other disposal sites, Pu formed similar associations: at the Hanford site, Pu (III/IV) was associated with colloids in the groundwater at the 100 K-Area (Dai et al., 2005); at the Savannah River Site, it was detected in combination with colloids in groundwater samples near the disposal basins in F-Area (Buesseler et al., 2009); and, at the NTS Pu was complexed with mineral colloids (Kersting et al., 1999). Studies at NTS show that the U and the fission products Sr and Cs also are associated with colloids (Utsunomiya et al., 2009).

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Microorganisms were detected in low-level radioactive- and TRU- wastes, in Pu-contaminated soils, and in waste-repository sites under consideration for nuclear-waste disposal (Au and Leavitt, 1982; Barnhart et al., 1980; Johnson et al., 1974; Francis, 1990, 2001, 2007). Microbiological studies at the NTS showed the presence of bacteria and fungi in Area 13 soils (Au and Leavitt, 1982), and bacteria in the subsurface environments at the Rainier Mesa, in oxygenated volcanic tuff, and in groundwater (Amy et al., 1992; Haldeman, and Amy, 1993; Horn et al., 2004). Microbes may affect the solubility and mobility of Pu (Francis, 2001; Neu et al., 2002, 2005; Boukhalfa et al., 2007; Francis et al., 2007, 2008; Renshaw et al., 2009).

Several studies have shown that bacteria and fungi play a major role in the dissolution of PuO<sub>2</sub>, amorphous forms of Pu(IV)OH, and other chemical forms. Some bacteria and fungi grown in the presence of Pu produced extracellular Pu complexes that increased the concentration of Pu in soil-column eluates compared to controls. Elution through soil effectively removed positively charged Pu complexes (Wildung et al., 1987). The increased mobility of Pu in the soil resulted from the formation of neutral and negatively charged Pu complexes. In the presence of known microbial metabolites and the synthetic ligands DTPA, EDTA, and EDDHA, Pu(VI) was reduced to Pu(IV) before complexation, suggesting that the latter valence state would be the dominant one associated with organic complexes in soils (Wildung, and Garland, 1980). Studies show that biologically produced ligands mediate Pu transport, such as cutin-derived soil degradation products containing siderophore-like moieties (Xu et al., 2008).

Although a wide variety of microorganisms were detected at the Pu contaminated sites, very little is known of the effects of microbial activity on the stability and mobility of the actinides in soils and wastes. An increase in moisture content and the availability of metabolizable organic carbon in contaminated soils in arid and semi-arid regions could increase microbial activity. In this study, we investigated the potential effects of indigenous microbial activity on the dissolution of actinides in NTS soil amended with citrate or glucose as carbon sources and incubated under aerobic and anaerobic conditions. Citrate is a naturally-occurring organic compound capable forming stable complexes with actinides and metals. Citrate is readily metabolized soil microorganisms. Glucose an intermediate of cellulose degradation product is metabolized by microorganisms; and under anaerobic and water logged conditions results in the accumulation of low molecular weight organic metabolic products such as acids and alcohols.

## 2. Materials and methods

### 2.1. Soil sample

About 100 g of plutonium-contaminated soil (HP-11) was obtained from Area 11 of the Double Track test shot area at the NTS. Soils at the NTS region are classified as medium-to fine-grained sands (Turner et al., 2003). The predominant vegetation in the area is the shrub species of basin big sagebrush and black sagebrush (Nevada Test Site Annual Site Environmental Report-2002, DOE/NV/11718-842, 2003). One gram of the soil was transferred to each of three platinum crucibles, was digested with concentrated nitric acid and analyzed for the metals Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sr, and Zn by inductively coupled plasma-optical emission spectrometer (ICP-OES).

### 2.2. Actinide analysis

The total activity in “as received” soil was 50 nCi/g. One gram of the soil was digested in a mixture of hydrochloric/nitric acid. The U-

233/234, U-235/236, U-238, Am-241, Pu-238, Pu-239/240 and Pu-241 were separated by anion-exchange column chromatography, followed by liquid scintillation counting (LSC) (EML Procedures Manual, HASL-300, 28th Edition, Volumes I and II. 1970).

### 2.3. Mineralogical analysis

The major mineralogical constituents of the soil are clay, quartz, magnetite, titanomagnetite, and limestone (Hoeffner et al., 2005). The fine fraction consisted of clay, quartz, and feldspar.

The minerals present in the soil were determined by x-ray diffraction (XRD) using beam line X7A at the National Synchrotron Light Source (NSLS). The soil was placed on an Al sample holder, and sealed with Kapton tape. The beamline's energy was 0.69850 Å, and a 2θ scan was obtained from 5 to 68° with a scan rate of 0.02° per second.

The mineralogical association of actinides in the soil was determined using a modified sequential selective-extraction method (Tessier et al., 1985). Briefly, the soil was extracted with (i) 1 M MgCl<sub>2</sub> and 0.05 M citric acid (pH 7) for 1 h (exchangeable fraction), (ii) an acetate buffer prepared by adjusting a 1 M NH<sub>4</sub>-acetate solution to pH 5 with acetic acid, adding 0.05 M citric acid, and then agitating the mix for 5 h (association with carbonate), and, (iii) 25% acetic acid with NH<sub>2</sub>OH·HCl for 6 h (association with Fe-, Mn-oxides). Citric acid was added to the extractant to prevent/minimize actinide precipitation from solution; it had a minimal effect on the extraction of actinides at this pH. The samples were then filtered through a 0.45 μm filter before analysis.

### 2.4. Elemental mapping and actinide association

Approximately 0.5 g soil was placed in an Al sample holder, and sealed with Kapton tape and analyzed by x-ray absorption near edge spectroscopy (μ-XANES), and μ-x-ray diffraction (μ-XRD) at the NSLS's X26A beam line to determine the oxidation state and the mineralogical association of the actinides in the soil. Synchrotron μ-x-ray fluorescence (SXRF) analysis was performed to map the elemental distribution and for determining the association of actinides with the selected elements Ca, Cr, Cu, Fe, Mn, Sr, U, and Zn. The Pu-containing particles in the soil was analyzed Synchrotron scanning transmission x-ray microscopy (STXM) at beam line MES 11.0.2 at the Advanced Light Source (ALS).

### 2.5. Extraction of actinides

To compare the leachability of actinides, the alpha- and beta-emitting isotopes were extracted from the soil by water, citric acid, and nitric acid, as follows. Duplicate one gram samples of soil were transferred to 20 ml serum bottles and ten milliliters of the following solutions added: (i) deionized water, (ii) 0.1 M citric acid, or, (iii) 1 M nitric acid. The soil samples were agitated on a rotary shaker for three hours. An aliquot was removed for analysis of α and β activities in the unsettled suspension, in the settled supernatant and filtered supernatant. The sample was allowed to settle for 1 h and the supernatant was filtered through a 0.45 μm filter (Millipore, MA). The α and β activities determined by liquid scintillation counting (LSC) using a Wallac Guardian 1414 digital spectrum analyzer.

### 2.6. Effect of adding citric acid or glucose on microbial activity and the dissolution of actinides from the soil

To 150 ml Erlenmeyer flasks 40 ml of one of the following solutions (wt. %) were added: (i) deionized water (unamended), (ii) deionized water containing glucose (0.5%) and NH<sub>4</sub>NO<sub>3</sub> (0.015%), or,

(iii) deionized water containing citrate (0.5%) and  $\text{NH}_4\text{NO}_3$  (0.015%). The pH of the solutions were adjusted to 7, the flasks were fitted with cotton plugs and sterilized by autoclaving. One gram of the soil was transferred to each of the flasks and the samples in triplicate were incubated aerobically at  $26 \pm 1$  °C in the dark, on a shaker rotating at 125 rpm. We incubated a second identical set of samples for each treatment, and did not sample them until the end of the experiment for Pu speciation determination.

The anaerobic samples were prepared by transferring 40 ml of the following treatments (wt. %): (i) deionized water (unamended), (ii) deionized water containing glucose (0.5%) and  $\text{NH}_4\text{NO}_3$  (0.015%), and (iii) deionized water containing citrate (0.5%) and  $\text{NH}_4\text{NO}_3$  (0.015%) into acid-washed and autoclaved 125 ml serum bottles. The solutions prior to addition were adjusted to pH 7 and prereduced by boiling while bubbling nitrogen gas through them. One gram of the soil was added to the serum bottles containing the various treatments and were then fitted with butyl rubber stoppers and crimp sealed with aluminum caps. All manipulations were performed inside an anaerobic glove bag filled with nitrogen. The soil samples were incubated in the dark, on a rotary shaker at 125 rpm and  $26 \pm 1$  °C. An identical set of samples from each treatment was incubated but not sampled until the end of the experiment, to determine Pu speciation.

### 2.7. Chemical analysis

Periodically, a 3.0 ml aliquot was withdrawn, the pH was determined, the sample filtered through a 0.45  $\mu\text{m}$  Millex (Millipore, MA) filter, and the total radioactivity in a 1.0 ml aliquot was determined by LSC. Glucose and citrate consumption and the presence of metabolic products were analyzed by HPLC (Shimadzu, LC-10AS) using a refractive index detector to measure glucose and alcohol production, and a UV-vis detector at 210 nm for organic acids. The organic components were separated using an Aminex HPX-87H ion-exclusion column (Bio-Rad, MA) with 0.003 N  $\text{H}_2\text{SO}_4$  as the mobile phase. The solubilization of the alpha-emitting isotopes U-233/234, U-235/236, U-238, Am-241, Pu-238, Pu-239/240, and the beta-emitter Pu-241 were determined by alpha spectroscopy and LSC as described previously.

At the end of the experiment, the pressure in the head space of the undisturbed anaerobic samples was determined with a digital pressure transducer, and the pH and Eh were measured, respectively, with a Beckman  $\Phi$ 350 pH meter with a Beckman 511275-AB combination pH electrode, and with a combination redox electrode (967800, Orion Research, MA). Total and ferrous iron were determined by the *o*-phenanthroline method.

### 2.8. Pu speciation studies

Size fractionation of Pu on all samples was performed after sequential filtration through 0.4, 0.2, 0.03, 0.01, and 0.001  $\mu\text{m}$  Poretics (CA) polycarbonate filters into a weighed LSC vial using an Amicon (MA) ultrafiltration cell.

The sequential solvent-extraction technique was used to determine the oxidation states of Pu in the sample (Nitsche et al., 1988). The samples were filtered through a 0.45  $\mu\text{m}$  Millex filter, and a 0.5 ml aliquot dispensed into a 4 ml silanized glass vial (Sigma-Aldrich, MO) inside a glove bag. To this sample, we added 1.0 ml thenoyltrifluoroacetone (TTA) equilibrated with 1 M HCl, and then extracted the sample for 5 min on a vortex mixer. The organic and aqueous extracts were separated, and each then analyzed for Pu by LSC after adding 8 ml of the LSC cocktail. We repeated the extraction on a separate aliquot of the sample in acetate buffer at pH 4.

**Table 1**  
Alpha activity and concentration in NTS soil.

Isotope	Activity (nCi/g)	Concentration ( $\mu\text{g/g}$ )
U 233/234	<0.18	$<6.1 \times 10^{-2}$
U 235/236	<0.15	$<3.6 \times 10^1$
U 238	<0.14	$<4.2 \times 10^2$
Am 241	$3.8 \pm 0.7$	$1.1 \times 10^{-3}$
Pu 238	$0.44 \pm 0.02$	$2.6 \times 10^{-5}$
Pu 239/240	$69.2 \pm 6.5$	$1.1 \times 10^{-1}$
Total	<75	ND

ND-not determined; Errors represent  $2\sigma$ .

U result is less than the sample detection limit.

## 3. Results

### 3.1. Elemental and actinide analyses

Soil analysis revealed the following major elements (mg/kg soil  $\pm$  1 SEM): Al ( $3890 \pm 100$ ), Ca ( $12,700 \pm 100$ ), Fe ( $6500 \pm 1800$ ), Mg ( $223 \pm 12$ ), Mn ( $223 \pm 23$ ), and Sr ( $133 \pm 4$ ), and others Ba ( $89.5 \pm 1.0$ ), Cd ( $0.2 \pm 0.1$ ), Co ( $2.8 \pm 0.2$ ), Cr ( $3.4 \pm 0.8$ ), Cu ( $4.2 \pm 0.5$ ), Ni ( $<0.1$ ), Pb ( $7.0 \pm 0.6$ ), and Zn ( $22.1 \pm 0.9$ ). The major elements Ca and Fe reflect the predominant mineral phases of the soil which have a high affinity for actinides and regulate their mobility.

Table 1 shows the alpha activity and the concentration in the soil. The individual alpha-emitting isotopes (% total activity): Pu-239/240 (92%), Pu-238 (0.6%), and Am-241 (5%). The total alpha activity in the soil was <75 nCi/g. Gamma spectroscopy of the bulk soil showed the predominant activity was due to the presence of Am-241 (742 nCi/g), Pu-239 (455 pCi/g), and Pu-241 (1.7 nCi/g). Uranium was not detected due to the low specific activity of its isotopes.

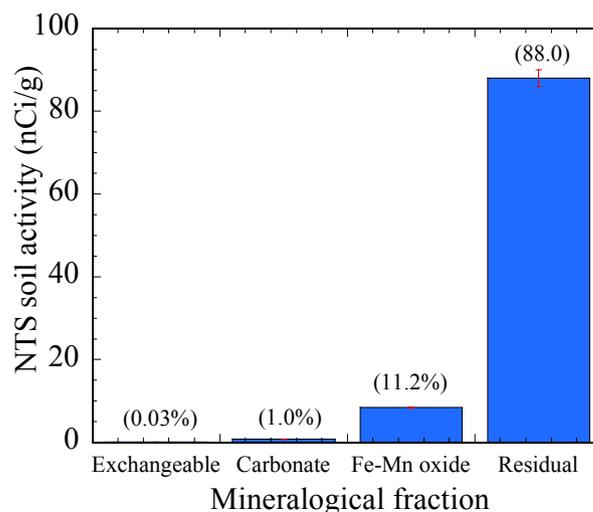
### 3.2. Mineralogical analysis and association of $\alpha$ -activity

Mineralogical analysis of the soil by XRD showed calcite, periclase (MgO), dolomite, magnetite and silicates (quartz, faujasite, labradorite) (ICDD, 2005).

Selective extraction of the soil showed a minimal level of  $\alpha$  activity (0.03%) associated with the exchangeable fraction; about 1% with the carbonate fraction, and 11% with the Fe-, Mn- oxide fraction. The bulk of the activity (~88%) was associated with the residual fraction (Fig. 1).

### 3.3. Mineralogical association of uranium

The  $\mu$ -XRD analysis of separate uranium-containing regions of



**Fig. 1.** Mineralogical association of alpha-emitting fractions in NTS soil.

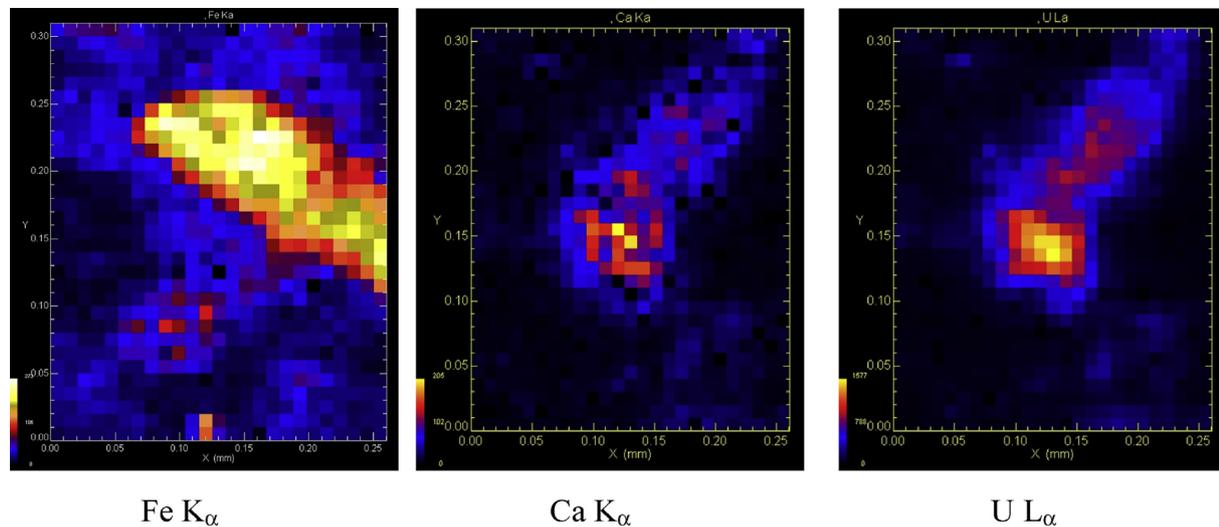


Fig. 2. Synchrotron-based  $\mu$ -x-ray fluorescence map of uranium association with minerals in NTS soil. The Ca-containing phases exhibit the closest correlation.

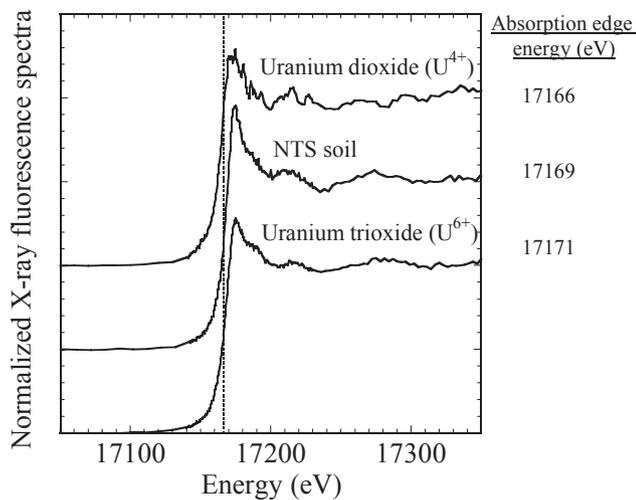


Fig. 3.  $\mu$ -XANES spectrum of uranium in NTS soil. Uranium is present as tetravalent and hexavalent form. The vertical line is set to the absorption edge energy for uranium dioxide.

the soil showed that U was associated with a schoepite phase ( $\text{UO}_3 \cdot 2\text{H}_2\text{O}$ ) (Fig. S1A), and with the carbonate minerals liebigite  $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3 \cdot 10\text{H}_2\text{O}$  and sharpite  $\text{Ca}(\text{UO}_2)_6(\text{CO}_3)_5(\text{OH})_4 \cdot 6\text{H}_2\text{O}$  (Fig. S1B). Elemental mapping by synchrotron  $\mu$ -X-ray fluorescence analysis showed the spatial association ( $10 \mu\text{m}^2$ ) of Ca and Fe with U (Fig. 2). The Ca-containing phases exhibit the closest correlation and no other elements were correlated with U. The normalized  $\mu$ -XANES spectra at the  $\text{U L}_{\text{III}}$  absorption edge for the soil sample at the X-26A beam line (NLS) showed the uranium absorption edge at 17,169 eV, lying between that of tetravalent uranium (17,166 eV) and hexavalent uranium (17,171 eV) (Fig. 3). This indicates that U is in a mixed-valence form, most probably as uranium dioxide and U(VI) compounds.

### 3.4. Mineralogical association of plutonium

Fig. 4A illustrates the synchrotron scanning transmission X-ray microscopy ( $\mu$ -STXM) of the soil particles. The boxed region consisted of Fe- and Mn- oxides and aluminosilicates. Plutonium was observed in this region (Fig. 4B), and was localized to a small area of the sample (500 nm).

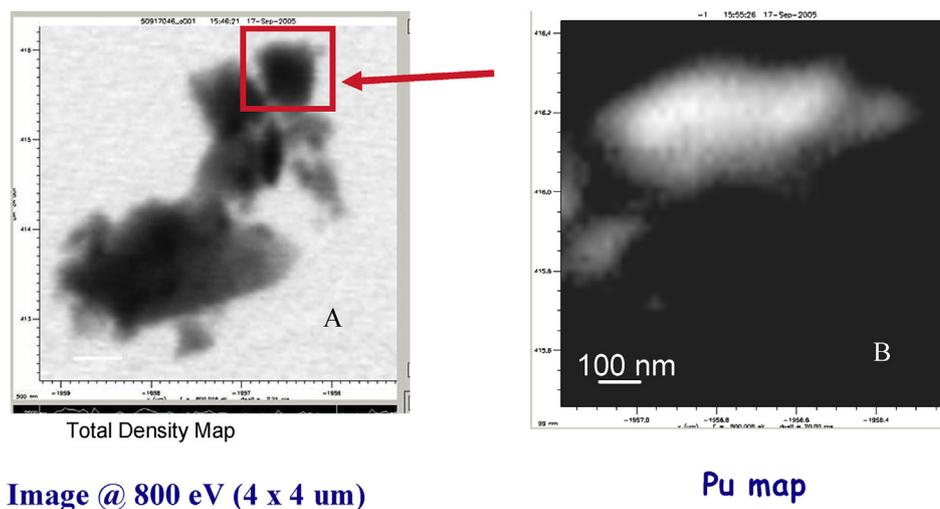


Fig. 4. Scanning transmission x-ray microscopy (STXM) image of NTS soil particles (A). The region boxed in red consisted of Fe and Mn oxides and aluminosilicates. Plutonium was observed in this region (B) and was localized to a small area of the sample (500 nm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 2**  
Extraction of radionuclides from NTS soil.

Treatment	Activity	Suspension	Supernatant	
			Unfiltered	Filtered <sup>a</sup>
kcpm/g soil				
Water	$\alpha$	62.9 ± 0.8	11.7 ± 3.0	0.07 ± 0.01
	$\beta$	30.2 ± 3.7	0.17 ± 0.02	0.04 ± 0.01
Citric acid (0.1 M)	$\alpha$	72.0 ± 9.4	18.7 ± 0.5	7.28 ± 1.86
	$\beta$	23.5 ± 7.5	0.46 ± 0.05	0.36 ± 0.08
Nitric acid (1 M)	$\alpha$	98.0 ± 4.2	27.2 ± 1.6	10.8 ± 0.5
	$\beta$	39.8 ± 3.7	0.76 ± 0.03	0.48 ± 0.3

<sup>a</sup> 0.45  $\mu$ m filtered; Error bars represent  $\pm 1$  SEM.

### 3.5. Extraction of actinides

The total alpha and beta activity of radionuclides extracted from the soil by water, citric acid, and nitric acid is shown in Table 2. The unfiltered suspension showed the highest amount of activity in all three treatments with alpha activity (63–98%) being the largest component. The unfiltered supernate following settling showed a marked decrease in both alpha (70–80%) and beta (>98%) activity compared to the suspension. The activity of the supernatant after 0.45  $\mu$ m filtration showed the least amount of activity in solution indicating that the bulk of the activity was associated with soil particulates larger than 0.45  $\mu$ m. The filtered nitric acid extract had the greatest level of alpha activity in the solution (10.8 kcpm/g), followed by citric acid (7.28 kcpm/g) and water (0.07 kcpm/g). Beta activity followed a similar pattern; the filtered nitric acid extract had the most activity (0.48 kcpm/g), then citric acid (0.36 kcpm/g), with the water having the lowest activity (0.04 kcpm/g).

### 3.6. Effect of adding citric acid or glucose on microbial activity and dissolution of actinides from the soil

Table 3 shows the changes in pH, Eh, total iron, and Fe(II) in both aerobic and anaerobic samples, and the changes in pressure in the latter after 36 days of incubation. In unamended samples incubated aerobically, the initial pH of the soil was ~8.2 at the start of the experiment and it increased to 9.0; and in the anaerobic sample to pH 9.3. Eh, an indicator of the sample's redox potential, was oxidizing (>500) in both the aerobic and anaerobic unamended samples.

In the citrate amended samples incubated aerobically, the pH decreased from 9.0 to 7.7 and the Eh from 607 to 170 (Table 3). There was a similar decrease in pH from 9.3 to 7.4 in the anaerobic samples and the Eh declined markedly to –306 due to the consumption of oxygen and the establishment of reducing conditions. Total iron and ferrous iron were <0.1 mM. No detectable ferrous iron in solution was observed in samples incubated under aerobic and anaerobic conditions. Gas production was not observed in the anaerobic citric acid amended samples.

**Table 3**  
Effect of adding citric acid or glucose on gas production, pH, Eh, and iron in NTS soil incubated for 36 days.

Treatment	Pressure (psig)	pH	Eh (mV)	Total iron (mM)	Ferrous ion (mM)
<b>Aerobic</b>					
Unamended	NA	9.00 ± 0.02	607 ± 11	0.013 ± 0.002	<0.01
Citrate + N	NA	7.70 ± 0.05	170 ± 24	0.051 ± 0.030	<0.01
Glucose + N	NA	5.23 ± 0.28	337 ± 13	0.128 ± 0.045	0.092 ± 0.040
<b>Anaerobic</b>					
Unamended	<1	9.30 ± 0.05	531 ± 3	<0.01	<0.01
Citrate + N	<1	7.43 ± 0.03	–306 ± 24	<0.01	<0.01
Glucose + N	7 ± 1	4.83 ± 0.05	–210 ± 18	1.63 ± 0.14	1.26 ± 0.43

NA—not applicable; Error bars  $\pm 1$  SEM.

In samples incubated aerobically with glucose the pH decreased from 9.0 to 5.2 and the Eh to 337 (Table 3). In the glucose amended anaerobic sample, the pH decreased to 4.8 and the Eh decreased to –210 ± 18, characteristic of highly reducing conditions. The head-space pressure rose to 7 psig due to gas production (CO<sub>2</sub> and H<sub>2</sub>) from the metabolism of glucose. The total and the ferrous iron concentrations in solution increased in the anaerobic sample. This increase in total and ferrous iron reflect the reduction of ferric to ferrous form mostly by the Fe-reducing and/or fermentative bacteria as well as the lower pH of the solution.

Fig. 5 depicts the metabolism of citric acid, changes in pH, and total radioactivity in the solution phase of soil incubated aerobically and anaerobically. In addition to these measurements, we determined the U, Pu, and Am activities, along with the concentrations of Ca, Fe, and Mn in the solution phase of samples incubated anaerobically.

Citric acid was metabolized completely in about 17 and 25 days, in the aerobic and anaerobic samples, respectively. Alpha and Beta activity in samples incubated under both conditions increased in solution up to 18 days, and decreased to below detection levels with time, concomitant with the complete metabolism of citric acid (Fig. 5A and C).

In aerobically incubated samples, the pH decreased slightly from 8.5 to 8.1; and the citric acid was completely metabolized to carbon dioxide and water with no accumulation of metabolic products (Fig. 5B). However, under anaerobic conditions, the pH decreased to 7.8, and acetic acid was the major metabolic product with minor amounts (~0.2 mM) each of propionic acid and butyric acid were detected (Fig. 5D).

The concentrations of Am, Pu, and U, and the metals Ca, Fe, and Mn in the aqueous phase are shown in Fig. 5E and F. Am-241, Pu-239/240, and Pu 241 increased in solution and precipitated out of solution. Pu-239/240 isotopes were the predominant ones with the highest activity in solution. The concentrations of Ca, and Fe increased during the initial days due to complexation reactions and precipitated from solution reflecting citric acid metabolism by the bacteria. Very little dissolution of Mn and U was observed (Fig. 5F).

Glucose was metabolized completely in about 18 days in both aerobic and anaerobic samples (Fig. 6A and C). In the aerobic samples the total activity increased to ~108 cpm/ml at 36 days and the predominant activity was alpha, which remained in solution. The pH decreased from 9.2 to 4.8 with the accumulation of acetic as the major product, followed by butyric and propionic acids (Fig. 6B). In the anaerobic samples the total activity increased similar to aerobic samples with higher alpha activity (Fig. 6C). A slight increase in beta activity was observed as with aerobic samples. The pH of the medium decreased to 5.5; acetic acid was the predominant metabolite, with small amounts of butyric acid (4.5 mM) and propionic acid (1.0 mM) (Fig. 6D).

Pu-239/240 and Pu-241 were the predominant isotopes of Pu in

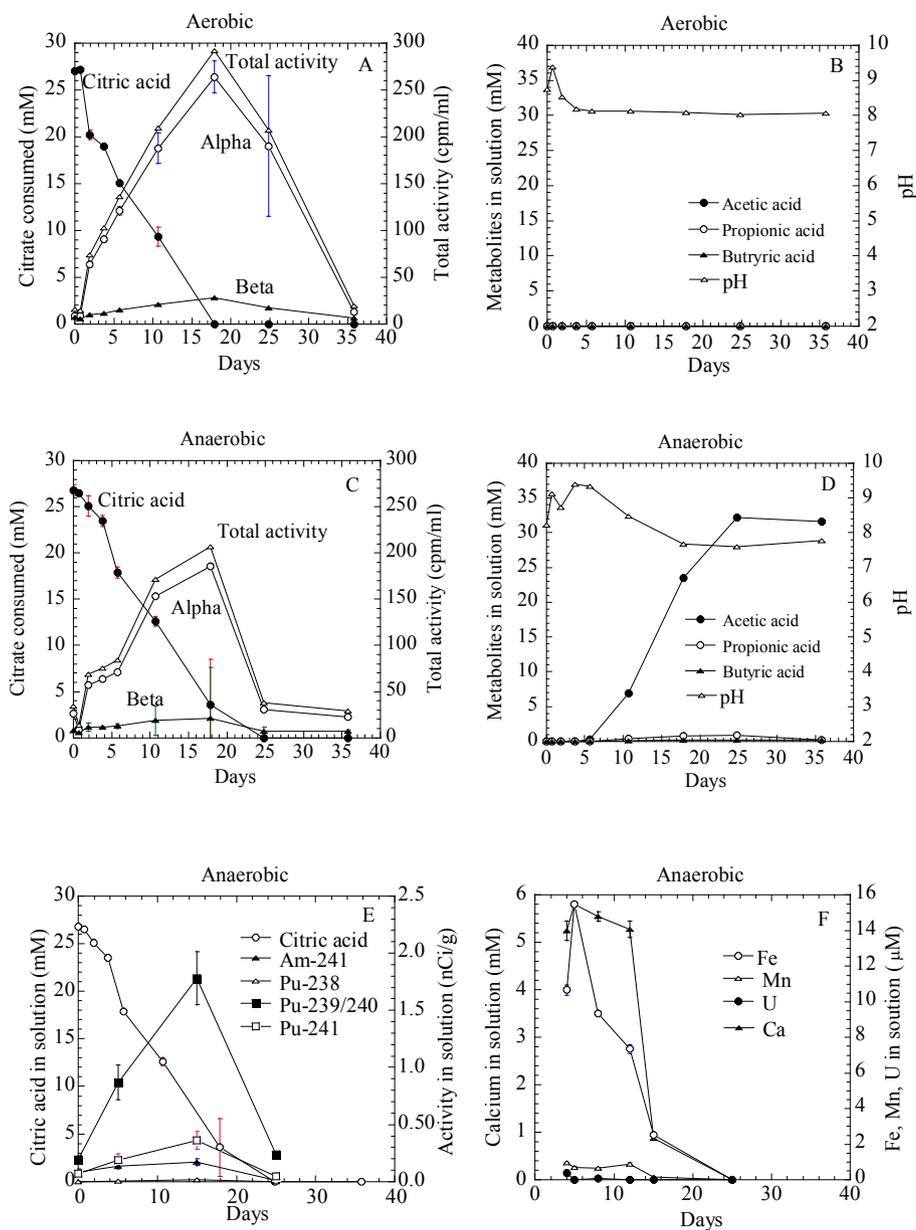


Fig. 5. Effect of adding citric acid on the mobilization of radionuclides and metals by indigenous bacteria incubated under aerobic and anaerobic conditions.

solution followed by small amount of Am-241 were observed under anaerobic conditions. These actinides remained in solution throughout the incubation unlike the citric acid amended sample (Fig. 6E). Uranium increased to ~1.46 μM in solution and it remained almost constant (Fig. 6F). The Ca and Fe concentrations increased to 7.0 mM and 1.29 mM, respectively, and the Mn 77.1 μM in 8 days. Calcium levels started to decrease after 25 days, while a slight change in Mn and no change in Fe in solution were observed. XRD analysis of soil amended with glucose and incubated anaerobically showed fewer peaks compared to the “as received” soil indicating dissolution of some labile mineral phases (Fig. S2). Soil before bacterial activity contained calcite, periclase (MgO), dolomite, magnetite, and silicates (quartz, faujasite, labradorite). Calcite, dolomite, and magnetite phases underwent dissolution after anaerobic bacterial activity.

### 3.7. Pu speciation studies

Samples incubated under anaerobic conditions with glucose showed detectable activity in solution phase. Table 4 shows the effect of sequential filtration on alpha and beta activity. Plutonium activity is high in the unfiltered sample due to the presence of suspended particles containing predominantly Pu-239 (alpha activity) and Pu-241 (beta activity). Sequential filtration of the solution from 0.4 μm to 0.01 μm only marginally attenuated alpha activity (126–106 cpm/ml). However, filtration through the 0.001 μm filter reduced this activity by about 50%, indicating the particles were between 0.01 and 0.001 μm size. No significant difference was observed for beta activity due to its low activity level in solution. This result suggests the presence of a fine colloidal component that the 0.001 μm filter retained and soluble

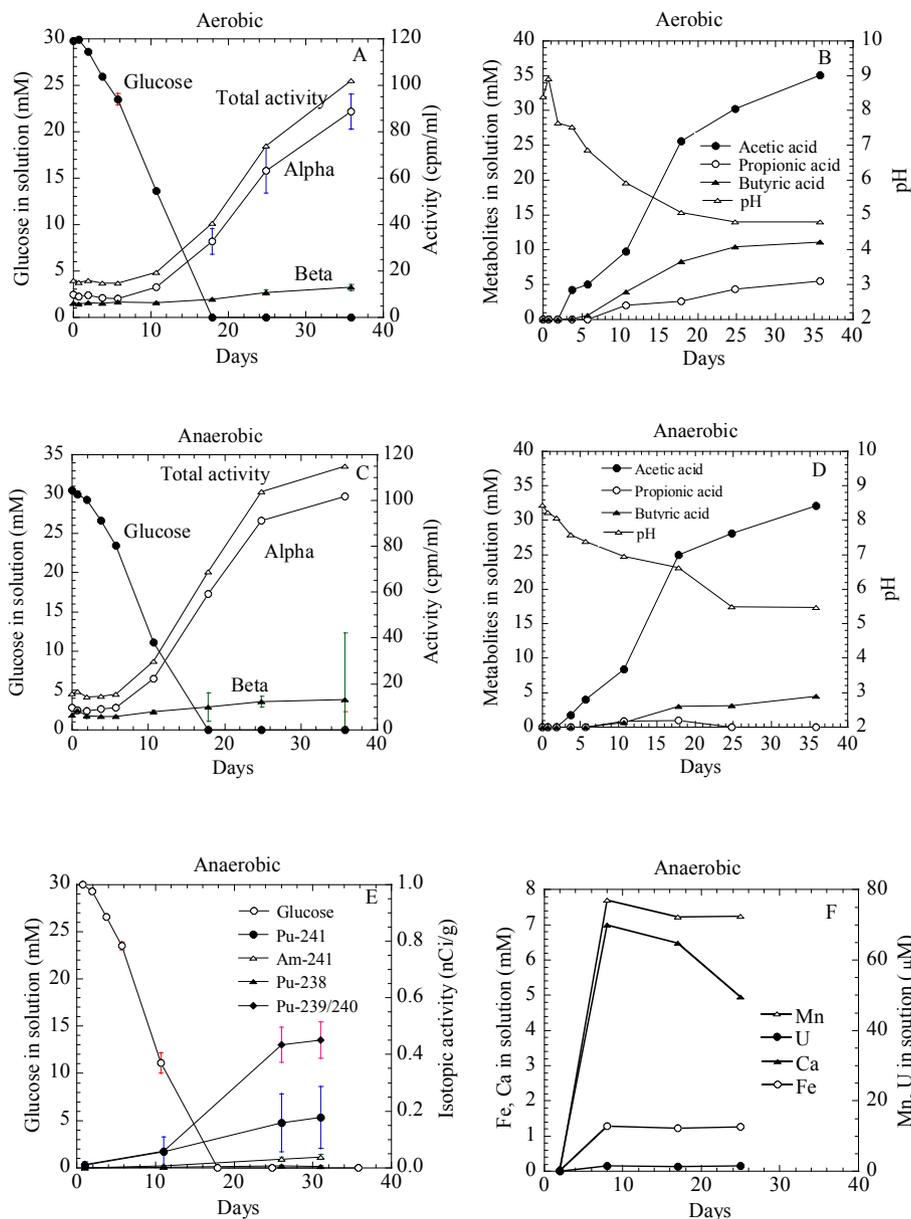


Fig. 6. Effect of adding glucose on the mobilization of radionuclides and metals by indigenous bacteria incubated under aerobic and anaerobic conditions. Error bars are  $\pm 1$  SEM.

**Table 4**  
Sequential filtration of actinides in glucose amended sample incubated anaerobically.

Sample	Activity (cpm/ml)	
	$\alpha$	$\beta$
Unfiltered	3940 $\pm$ 60	86.8 $\pm$ 12.4
0.4 $\mu$ m filtered	126 $\pm$ 3	8.8 $\pm$ 0.1
0.2 $\mu$ m filtered	139 $\pm$ 17	10.1 $\pm$ 0.9
0.03 $\mu$ m filtered	116 $\pm$ 5	9.1 $\pm$ 0.1
0.01 $\mu$ m filtered <sup>a</sup>	106	8.9
0.001 $\mu$ m filtered	69.6 $\pm$ 2.4	7.1 $\pm$ 2.7

<sup>a</sup> Single determination due to filter break-through of replicate. Error bars  $\pm 1$  SEM.

component that the Pu passed through the 0.001  $\mu$ m filter.

We also determined the speciation of Pu by selective solvent extraction and micro-filtration techniques in glucose amended samples that was left undisturbed under anaerobic conditions for 40 days. A TTA extraction of selected 0.45  $\mu$ m filtered samples

showed no discernible difference in the oxidation state of Pu in either the aerobic or anaerobic glucose amended samples (Table S1). However, we observed a decrease in activity in the TTA extraction at pH 4. A brownish colloid was noted in this sample after extraction that might indicate the incomplete separation of the Pu.

#### 4. Discussion

The radioactivity in the NTS soil was predominantly due to the presence of Pu-239/240 (92%), Am-241 (5%), and Pu-238 (0.6%) isotopes. Uranium was not detected due to the low specific activity of its isotopes; however, synchrotron-based  $\mu$ -XRF and  $\mu$ -XRD techniques showed its presence in the soil associated with the minerals schoepite and liebigite. Americium association and the speciation was not investigated however it is known to stick to particles and exist predominantly as Am(III) oxidation state in a stable form in the environment. Plutonium association was

correlated with Fe- and M-oxides and aluminosilicates as particulates. Because of the low concentration of Pu, we could not determine its chemical speciation by XANES, or by the selective solvent-extraction technique. Plutonium can simultaneously coexist in several oxidation states (III, IV, V, VI, VII) and their solution chemistry is very complex. Plutonium (IV) is the most predominant and stable species in contaminated environments. Plutonium has a high ionic charge, and tends to undergo hydrolysis, thereby forming polymers in systems with  $\text{pH} > 2$ . Its chemical speciation is affected by soil pH, organic-matter content, mineralogy, microbial activity, and redox conditions.

The type of carbon source added to the soil affected the extent of solubilization of actinides. Citric acid is a naturally occurring multidentate ligand that forms stable complexes with actinides, and may involve the formation of bidentate-, tridentate-, binuclear-, or poly-nuclear species. For example, it forms 1:1 mononuclear, 1:2 mononuclear biligand, and 2:2 binuclear complexes with Pu(IV); the 1:2 mononuclear biligand complex predominated in the presence of excess citric acid (Francis et al., 2006). The citric acid was rapidly metabolized by *Pseudomonas fluorescens*, common in soils, wastes, and water with generation of a Pu polymer (Francis et al., 2006). Under aerobic and anaerobic conditions both alpha and beta activity in solution increased in citric-acid amended soils; as this organic acid was being metabolized a sudden decrease in both activities as well as Ca, Fe and Mn in solution corresponded to its complete utilization by the bacteria suggesting the precipitation of the actinides and the metals (Fig. 5 A, C, E and F). Previous studies on biodegradation of citric acid soil extracts containing metals have shown the precipitation of the metals (Francis and Dodge, 1998; Francis et al., 2005). Although uranium is known to form strong complexes with citric acid (Rajan and Martell, 1965; Francis et al., 1992), it was not detected in solution suggesting that the mineralogical form of uranium in the soil was not amenable to dissolution by the citric acid concentration used in this study.

Glucose an intermediate product of cellulose degradation is ubiquitous in nature. It is used as an energy source by a wide variety of aerobic and anaerobic microorganisms in soil. In glucose amended samples incubated under aerobic and anaerobic conditions, the metabolism of the carbon source resulted in a decrease in pH due to production of organic acids with an increase in alpha and beta activity in the solution. The predominant mechanism for alpha and beta release into solution is due to direct enzymatic reduction of metals from higher to lower oxidation state and by indirect action due to the production of organic acid metabolites and lowering the pH of the medium. A substantial increase in Pu concentration and a slight increase in Am; but no U was detected in solution.

The increase in concentration of Pu in the solution phase is due to dissolution of the labile Pu chemical species as well as Pu associated with calcium carbonate, and Fe-, and Mn-oxide mineral phases by direct enzymatic reduction and by indirect the action due to organic acids and acidic pH of the medium brought about by the anaerobic bacterial action. An increase Ca in solution due to dissolution of calcium mineral phases by indirect action of the bacteria and an increase in Fe and Mn in solution due to reductive dissolution of Fe- and Mn-oxides by direct enzymatic action of anaerobic bacteria with the concurrent release of their associated actinides is evident (Fig. 6 E and F). XRD analysis of soil amended with glucose and incubated under anaerobic conditions disclosed dissolution of calcite, dolomite, and magnetite phases due to bacterial activity (Fig. S2). Several studies have shown that axenic cultures of bacteria and fungi solubilize  $\text{PuO}_2$ , amorphous Pu(IV)OH and other chemical forms of Pu. Under anaerobic conditions reductive dissolution of amorphous Pu(IV) to Pu(III) by anaerobic bacteria has been reported (Rusin et al., 1994; Neu et al., 2005; Boukhalfa et al., 2007; Ohnuki et al., 2007; Francis et al., 2008).

The speciation of Pu at different contaminated sites varies according to the site and the waste stream (Bondietti, and Tamura, 1980; Cleveland, and Rees, 1981; Penrose et al., 1990; Santschi et al., 2002; Dai et al., 2005; Noviko et al., 2006; Clark et al., 2006). The presence of viable microbial populations in surficial and subsurface environments contaminated with radionuclides clearly suggests that, under appropriate conditions, microbial activity potentially can affect the chemical form and solubility of Pu and other radionuclides in several ways. These include the oxidation–reduction reactions that affect their valence state and solubility; changes in pH that affect the ionic state and their solubility; dissolution, and leaching by microbial metabolites and decomposition products, such as organic acid metabolites or the production of specific sequestering agents such as siderophores (Brainard et al., 1992); the remobilization of coprecipitated Pu. Microbes may contribute to generating and/or destabilizing Pu colloids and that microbial cells may act as colloidal particles and thus facilitate radionuclide transport (Francis et al., 1998).

Dissolution of Pu from contaminated soil by microbes is influenced by the availability and the type of carbon source, moisture, pH, and other environmental factors particularly in nutrient limiting arid or semi-arid environments. Clearly, information is needed on the seasonal variations such as wet and dry cycles that regulate microbial processes and Pu speciation and its interaction in complex soil system to fully understand its long-term fate and transport in the environment.

## 5. Conclusions

Plutonium was the predominant actinide solubilized by indigenous microbial activity in a contaminated soil amended with citric acid or glucose and incubated under aerobic or anaerobic conditions. Citric acid solubilized actinides and metals and soil mineral phases with the formation of actinide- and metal-citrate complexes. Biodegradation of citric acid increased solution pH with concomitant release of the actinides and metals resulting in their precipitation. Glucose metabolism by anaerobic bacteria produced low molecular weight organic acid metabolites and decreased solution pH causing dissolution of carbonate minerals and associated actinides; as well as reductive dissolution of Fe- and Mn-oxide phases and releasing those actinides associated with them. These results suggest that under appropriate environmental conditions microorganisms affect the dissolution of actinides from contaminated environments.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jenvrad.2015.08.019>.

## References

- Amy, P.S., Haldeman, D.L., Ringelberg, D., Hall, D.H., Russell, C., 1992. Comparison of identification systems for classification of bacteria isolated from water and endolithic habitats within the deep subsurface. *Appl. Environ. Microbiol.* 58, 3367–3373.
- Au, F.H.F., Leavitt, V.D., 1982. The soil microbiota of area 13 of the Nevada test site.

- In: Howard, W.A., Dunaway, P.B. (Eds.), *The Radioecology of Transuranics and other Radionuclides in Desert Ecosystems*. U.S. Department of Energy, Nevada Operations Office, Las Vegas, Nevada, pp. 201–242. NVO-224.
- Barnhart, B.J., Campbell, E.W., Martinez, E., Caldwell, D.E., Hallett, R., 1980. Potential Microbial Impact on Transuranic Wastes under Conditions Expected in the Waste Isolation Pilot Plant (WIPP). Los Alamos National Laboratory, LA-8297-PR.
- Bondietti, E.A., Tamura, T., 1980. Physicochemical Associations of Plutonium and Other Actinides in Soils. *Transuranic Elements in the Environment*. U. S. Department of Energy, NTIS, p. 273. DOE/TIC-22800.
- Boukhalfa, H., Icopini, G.A., Reilly, S.D., Neu, M.P., 2007. Plutonium(IV) reduction by the metal-reducing bacteria *Geobacter metallireducens* GS-15 and *Shewanella oneidensis* MR-1. *Appl. Environ. Microbiol.* 73, 5897–5903.
- Brainard, J.R., Strietelmeier, B.A., Smith, P.H., Unkefer, P.J., Barr, M.E., Ryan, R.R., 1992. Actinide binding solubilization by microbial siderophores. *Radiochim. Acta* 58/59, 357–363.
- Buesseler, K.O., Kapalan, D.J., Dai, M., Pike, S., 2009. Source-dependent and source-independent controls on plutonium oxidation state and colloid associations in groundwater. *Environ. Sci. Technol.* 43, 1322–1328.
- Clark, D.L., Janecky, D.R., Lane, L.J., 2006. Science-based cleanup of Rocky Flats. *Phys. Today* 59, 34–40.
- Cleveland, J.M., Rees, T.F., 1981. Characterization of plutonium in Maxey Flats radioactive trench leachates. *Science* 212, 1506–1509.
- Dai, M., Buesseler, K.O., Pike, S.M., 2005. Plutonium in groundwater at the 100K-Area of the U.S. DOE Hanford Site. *J. Contam. Hydrol.* 76, 167–189.
- EML Procedures Manual, HASL-300, 28th Ed., Vol. I and II. N. A. Chieco (Editor): Available from: NTIS, Springfield, VA 22161, Product No. PB97–162549; [www.eml.doe/procman/intro.htm](http://www.eml.doe/procman/intro.htm) (1970).
- Francis, A.J., 1990. Microbial transformation of toxic metals and radionuclides in mixed wastes. *Experientia* 46, 840–851.
- Francis, A.J., Dodge, C.J., 1998. Remediation of soils and wastes contaminated with uranium and toxic metals. *Environ. Sci. Technol.* 32, 3993–3998.
- Francis, A.J., Dodge, C.J., Gillow, J.B., 1992. Biodegradation of metal citrate complexes and implications for toxic-metal mobility. *Nature* 356, 140–142.
- Francis, A.J., 2001. Microbial transformations of Pu and implications for its mobility. In: Kudo, A. (Ed.), *Plutonium in the Environment*. Elsevier Science Ltd, pp. 145–163.
- Francis, A.J., Dodge, C.J., McDonald, J.A., Halada, G.P., 2005. Decontamination of uranium contaminated steel surfaces by hydroxycarboxylic acids with uranium recovery. *Environ. Sci. Technol.* 39, 5015–5021.
- Francis, A.J., Gillow, J.B., Dodge, C.J., Dunn, M., Mantione, K., Strietelmeier, B.A., Pansoy-Hjelvik, M.E., Papenguth, H.W., 1998. Role of microbes as biocolloids in the transport of actinides from a deep underground waste repository. *Radiochim. Acta* 82, 347–354.
- Francis, A.J., Dodge, C.J., Gillow, J.B., 2008. Reductive dissolution of Pu(IV) by *Clostridium* sp. under anaerobic conditions. *Environ. Sci. Technol.* 42, 2355–2360.
- Francis, A.J., 2007. Microbial mobilization and immobilization of plutonium. *J. Alloys Compd.* 444–445, 500–505.
- Francis, A.J., Dodge, C.J., Gillow, J.B., 2006. Biotransformation of plutonium complexed with citric acid. *Radiochim. Acta* 94, 731–737.
- Francis, A.J., Dodge, C.J., Ohnuki, T., 2007. Microbial transformations of plutonium. *J. Nucl. Radiochem. Sci.* 8, 121–126.
- Haldeman, D.L., Amy, P.S., 1993. Bacterial heterogeneity in deep subsurface tunnels at Rainier Mesa, Nevada Test Site. *Microb. Ecol.* 25, 185–194.
- Hoeffner, S.L., Navratil, J.D., Torrao, G., Smalley, R., 2005. Evaluation of Remediation Technologies for Plutonium Contaminated Soil. In: [http://www.netl.doe.gov/publications/proceedings/00/ind\\_part00/emp12.pdf](http://www.netl.doe.gov/publications/proceedings/00/ind_part00/emp12.pdf).
- Horn, J.M., Masterson, B.A., Rivera, A., Miranda, A., Davis, M.A., Martin, S., 2004. Bacterial growth dynamics, limiting factors, and community diversity in a proposed geological nuclear waste repository environment. *Geomicrobiol. J.* 21, 273–286.
- ICDD, 2005. In: McClune, Frank (Ed.), Powder Diffraction File. International Centre for Diffraction Data, 12 Campus Boulevard, Newton Square, Pennsylvania.
- Johnson, J.E., Svalberg, S., Paine, D., 1974. The Study of Plutonium in Aquatic Systems of the Rocky Flats Environs. Final Technical Report Contract No. 41493-F. Dow Chemical Company Rocky Flats Division, Golden, Colorado. Colorado State University, Fort Collins, Colorado.
- Kersting, A.B., 2013. Plutonium transport in the environment. *Inorg. Chem.* 52, 3533–3546.
- Kersting, A.B., Eford, D.W., Finnegan, D.L., Rokop, D.K., Smith, D.K., Thomson, J.L., 1999. Migration of plutonium in groundwater at the Nevada Test Site. *Nature* 397, 56–59.
- Neu, M.P., Icopini, G.A., Boukhalfa, H., 2005. Plutonium speciation affected by environmental bacteria. *Radiochim. Acta* 93, 705–714.
- Neu, M.P., Ruggiero, C.E., Francis, A.J., 2002. Bioinorganic chemistry of plutonium and interactions of plutonium with microorganisms and plants. In: Hoffman, D. (Ed.), *Advances in Plutonium Chemistry 1967–2000*. ANS, La Grange Park Illinois and University Research Alliance, Amarillo, Texas, pp. 169–211.
- Nevada Test Site Annual Site Environmental Report-2002. DOE/NV/11718–11842, Bechtel Nevada, National Nuclear Security Administration, Nev. Site Off., October, 2003. [www.nv.doe.gov/library/publications/NTSER/DOENV\\_25946\\_259\\_AttachA.pdf](http://www.nv.doe.gov/library/publications/NTSER/DOENV_25946_259_AttachA.pdf).
- Nitsche, H., Lee, S.C., Gatti, R.C., 1988. Determination of plutonium oxidation states at trace levels pertinent to nuclear waste disposal. *J. Radioanal. Nucl. Chem.* 124, 171–185.
- Noviko, A.P., Kalmykov, S.N., Utsunomyia, S., Ewing, R.C., Horreard, F., Merkulov, A., Clark, S.B., Tkachev, V.V., Myasoedov, B.F., 2006. Colloid transport of plutonium in the far-field of the Mayak Production Association, Russia. *Science* 314, 638–641.
- Ohnuki, T., Yoshida, T., Ozaki, T., Kozai, N., Sakamoto, F., Nankawa, T., Suzuki, Y., Francis, A.J., 2007. Chemical speciation and association of plutonium with bacteria, kaolinite clay, and their mixture. *Environ. Sci. Technol.* 41, 3134–3139.
- Papelis, C., Jacobsen, R.L., Miller, F.L., Shaulis, L.K., 1996. Evaluation of Technologies for Volume Reduction of Plutonium-contaminated Soils from the Nevada Test Site. DOE/NV/10845–57. US DOE Nevada Operations Office, June, 1996. <http://www.dri.edu/Publications/45139.pdf>.
- Penrose, W.R., Polzer, W.L., Essington, E.H., Nelson, D.M., Orlandini, K.A., 1990. Mobility of plutonium and americium through shallow aquifer in a semiarid region. *Environ. Sci. Technol.* 24, 228–234.
- Rajan, K.S., Martell, A.E., 1965. Equilibrium studies of uranyl complexes: III. Interaction of uranyl ion with citric acid. *Inorg. Chem.* 4, 462–469.
- Renshaw, J.C., Law, N., Geissler, A., Livens, F.R., Lloyd, J.R., 2009. Impact of the F(III)-reducing bacteria *Geobacter sulfurreducens* and *Shewanella oneidensis* on the speciation of plutonium. *Biogeochemistry* 94, 191–196.
- Rusin, P.A., Quintana, L., Brainard, J.R., Strietelmeier, B.A., Tait, C.D., Ekgerg, S.A., Palmer, P.D., Newton, T.W., Clark, D.L., 1994. Solubilization of plutonium hydrous oxide by iron-reducing bacteria. *Environ. Sci. Technol.* 28, 1686–1690.
- Santschi, P.H., Roberts, K.A., Guo, L., 2002. Organic nature of colloidal actinides transported in surface water environments. *Environ. Sci. Technol.* 36, 3711–3719.
- Tessier, A., Rapin, F., Carignan, R., 1985. Trace metals in oxic lake sediments: possible adsorption onto iron oxyhydroxides. *Geochim. Cosmochim. Acta* 49, 183–194.
- Turner, M., Rudin, M., Cizdziel, J., Hodge, V., 2003. Excess plutonium in soil near the Nevada Test Site, USA. *Environ. Pollut.* 125, 193–203.
- Utsunomyia, S., Kersting, A., Ewing, R., 2009. Groundwater nanoparticles in the far-field at the Nevada Test Site: mechanism for radionuclide transport. *Environ. Sci. Technol.* 43, 1283–1298.
- Walker, J.B., Liebendorfer, P.J., 1998. Long-term Stewardship at the Nevada Test Site – (NTS) 1998. <http://www.state.nv.us/nucwaste/nts/steward.htm>.
- Wildung, R.E., Garland, T.R., Rogers, J.E.M., 1987. Plutonium interactions with soil microbial metabolites: effect on plutonium sorption by soil. DOE Symp. Ser. 59, 1–25.
- Wildung, R.E., Garland, T.R., 1980. The relationship of microbial processes to the fate and behavior of transuranic elements in soils, plants, and animals. In: Hanson, W.C. (Ed.), *Transuranic Elements in the Environment*. Technical Information Center/US Department of Energy, Washington, DC, pp. 300–335. DOE/TIC-22800.
- Xu, C., Santschi, P.H., Zhong, J.Y., Hatcher, P.G., Francis, A.J., Dodge, C.J., Roberts, K.A., Hung, C.-C., Honeyman, B.D., 2008. Colloidal cutin-like substances cross-linked to siderophore decomposition products mobilizing plutonium from contaminated soils. *Environ. Sci. Technol.* 42, 8211–8217.