Geochemical association of Pu and Am in selected host-phases of contaminated soils from the UK and their susceptibility to chemical and microbiological leaching

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A B S T R A C T

Understanding the biogeochemical behaviour and potential mobility of actinides in soils and groundwater is vital for developing remediation and management strategies for radionuclide-contaminated land. Pu is known to have a high Kd in soils and sediments, however remobilization of low concentrations of Pu remains a concern. Here, some of the physicochemical properties of Pu and the co-contaminant, Am, are investigated in contaminated soils from Aldermaston, Berkshire, UK, and the Esk Estuary, Cumbria, UK, to determine their potential mobility. Sequential extraction techniques were used to examine the host-phases of the actinides in these soils and their susceptibility to microbiological leaching was investigated using acidophilic sulphur-oxidising bacteria. Sequential extractions found the majority of 239,240Pu associated with the highly refractory residual phase in both the Aldermaston (63.8–85.5%) and Esk Estuary (91.9–94.5%) soils. The 241Am was distributed across multiple phases including the reducible oxide (26.1–40.0%), organic (45.6–63.6%) and residual fractions (1.9–11.1%). Plutonium proved largely resistant to leaching from microbially-produced sulphuric acid, with a maximum 0.18% leached into solution, although up to 12.5% of the 241Am was leached under the same conditions. If Pu was present as distinct oxide particles in the soil, then 241Am, a decay product of Pu, would be expected to be physically retained in the particle. The differences in geochemical association and bioleachability of the two actinides suggest that this is not the case and hence, that significant Pu is not present as distinct particles. These data suggest the majority of Pu in the contaminated soils studied is highly recalcitrant to geochemical changes and is likely to remain immobile over significant time periods, even when challenged with aggressive “bioleaching” bacteria.

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1. Introduction

Plutonium contamination in the environment has arisen from global fallout from nuclear weapons testing, as well as from controlled and accidental discharges of nuclear material (Harley, 1980; Kimber et al., 2011). Americium is often a co-contaminant with Pu due to the formation of 244Am from the beta decay of 244Pu, which is itself formed via successive neutron captures in 239Pu and 240Pu. Even in significantly contaminated environments, Pu and Am isotopes often only exist in low mass concentrations and thus, synchrotron radiation techniques, which provide useful insight into chemical speciation, often cannot be used. However, sequential extraction techniques, in which the sediment or soil is separated into defined, sequentially-dissolved phases via a series of increasingly aggressive chemical extractions, can be used to determine their geochemical associations indirectly. This can
provide useful information on the potential migration of the Pu in soils and under which geochemical conditions, if any, it may be remobilized. The geochemical fractions defined in this study followed the Tessier scheme (Tessier et al., 1979), and were: exchangeable or adsorbed; carbonate-bound; oxide-bound; organically-bound; and residual. In sequential leaching experiments, the actinides associated with the exchangeable phase are taken as being susceptible to mobilization by changes in the compositions of the groundwater. Actinides bound to the carbonate fraction may be susceptible to changes in pH with those bound to the reducible oxide fraction susceptible to changes in Eh. Oxidising conditions may promote the release of organic-bound actinides into solution, whilst actinides associated with the residual fraction are unlikely to be released into solution over a significant time span under most (bio)geochemical conditions.

Associations with several of these components may be affected by microbial respiration, which can alter mineralogy directly through enzymatic redox reactions or indirectly through changes to, for example, pH and Eh (Ehrlich, 1996; Lovley and Phillips, 1988). If the actinides are associated with phases susceptible to dissolution or degradation then microbial activity may result in their mobilization. The potential for Pu and Am mobilization has implications for the management of contaminated land since it can be either a justified benefit in remediation, or increase the risk through migration.

Despite the low Kd values of Pu in soils and sediments (McCubbin et al., 2004; Serne, 2007), the potential remobilization of small quantities of the actinide is still a concern (Kersting et al., 1999; Santschi and Roberts, 2002; Xu et al., 2014). Previous work by the current authors has demonstrated that negligible Pu present in environmental contaminated soils (from the AWE Aldermaston site) is released into solution (<0.1%) under induced-reducing conditions, suggesting that little Pu is mobilized despite the inferred dissolution of oxide minerals and presence of microorganisms linked to Pu reduction (Kimber et al., 2012). Therefore, it seemed unlikely any change in Pu oxidation state occurred as both Pu(III) and Pu(V) are more mobile relative to Pu(IV) which is the dominant Pu species in soils (Begg et al., 2013; Choppin et al., 1997; Kaplan et al., 2004; Powell et al., 2014; Silva and Nitsche, 1995). However, sulphur-oxidising bacteria are effective at leaching metal contaminants and are commonly used in biomining and soil washing (White et al., 1998). Reduced sulphur or iron compounds in the biosphere can be used by sulphur-oxidising bacteria for chemolithotrophic growth under aerobic conditions, leading to production of soluble metal sulphates and sulphuric acid (Norris, 1990). Therefore, biodegradation of Pu and Am through sulphuric acid production could offer a possible mechanism for Pu and Am remobilization or bioremediation.

Particle size and form can also influence the mobility of Pu in the environment; low molecular mass species and colloidal Pu are more mobile than particulate Pu (Kersting et al., 1999; Salbu et al., 2004). Therefore, characterisation of Pu-containing particles can also help to predict its environmental behaviour. Particles can be characterised using autoradiography techniques, in which alpha and gamma radiation can be detected in a soil sample using a storage phosphor imager (Lloyd and Macaskie, 1996). These particles can then be isolated and further characterised using, for example, scanning electron microscopy.

Here, we investigate the biogeochemical behaviour of Pu and Am in contaminated sediments from two sites in the UK with different Pu source terms; weapons manufacturing at Aldermaston and nuclear fuel reprocessing in the Esk Estuary. Our principal aim is to understand better which geochemical conditions could lead to remobilization of the two actinides. To achieve this, we detail the results of sequential leaching of $^{239,240}\text{Pu}$, $^{238}\text{Pu}$ and $^{244}\text{Am}$ to identify and compare the geochemical associations of Pu and Am at the two sites studied. Furthermore, by comparing our data with similar but more historic work on Pu in Esk Estuary soils, our study provides further information on more long term Pu behaviour. In addition, the bioleachable fraction of both actinides was investigated and compared in the Aldermaston soils, using sulphuric acid-producing, sulphur-oxidising bacteria to determine the potential of bioleaching in remobilizing Pu or Am. Finally, further characterization of the Pu in the Aldermaston soils was conducted using autoradiography, with the alpha-emitting particles identified then examined under ESEM.

2. Materials and methods

2.1. Soil and groundwater collection

Soil and groundwater were provided by AWE from their Aldermaston site, Berkshire. Due to the varied industrial history of AWE’s sites, areas of soil and groundwater have become contaminated with both radioactive and chemical substances, including plutonium and americium. Contaminated Land at AWE is regulated by the Office for Nuclear Regulation (ONR) under The Nuclear Installations Act and by the Environment Agency through; Environmental Permits and Environmental Protection Act, Part 2A. Soils and groundwater were collected and stored as described previously (Kimber et al., 2012). The Aldermaston site lies on Quaternary plateau gravels underlain by Tertiary sands and gravels of the Lower Bagshot Beds. In turn, these rest on a layer of London Clay, which acts as a barrier to groundwater flow from the site into the underlying Chalk aquifer. The soils had a pH between 6 and 6.5 with an Eh range between 200 and 250 mV.

Archived sediment samples from the Esk estuary (UK National Grid Reference 088,947), located near the Sellafield nuclear waste reprocessing site, were used. These were collected on 19 March 2008 at low tide. The core sample taken (30 cm depth, approximate age range 1950 to present) was divided into 6 sections. The 10–15 cm horizon was used in this study as the maximum discharges from Sellafield (during the 1970s) are reflected in this horizon (Mccarthy and Nicholls, 1999; Sajih and Livens, 2010). While most Pu released from the Sellafield pipeline into the North Sea is readily adsorbed by silt and hence is deposited on the seabed, resuspension of fine particles can lead to deposition of the radioactive material in intertidal regions of local estuaries (Cambray and Eakins, 1982; Morris et al., 2000). Previous work has identified the bulk soils from the same sample site as being composed of 74.5% silt, 18.6% clay (mostly consisting of illite), and 6.5% sand (Morris et al., 2000). For the 10–15 cm soil horizon, the reducible manganese and iron oxide fractions were between 1.30–1.40 g kg$^{-1}$ and 5.13–6.11 g kg$^{-1}$, respectively, and weight loss on ignition was between 8.6 and 10.5% (Morris et al., 2000).

2.2. Sequential extraction

Sediment samples (10 g) from both Aldermaston and the Esk estuary were subjected to sequential extraction (Tessier et al., 1979). This involved isolating the following fractions: (a) exchangeable, extracted with 100 ml 1 M MgCl$_2$ (pH 7) for 1 h; (b) carbonate-bound, extracted with 100 ml 1 M CH$_3$COONa adjusted to pH 5 with CH$_3$COOH for 5 h; (c) reducible oxide-bound, extracted with 100 ml of 0.04 M NH$_2$OH-HCl in 25% v/v CH$_3$COOH, adjusted to pH 2 with NaOH and stirred at 96 °C for 6 h; (d) organic matter bound, extracted with 30 ml 0.02 M HNO$_3$ and 50 ml of 30% H$_2$O$_2$, adjusted to pH 2 with HNO$_3$ and stirred for 2 h at 85 °C. A further 30 ml 30% H$_2$O$_2$ (pH 2 with HNO$_3$) was then added and stirred as before. Next, 50 ml 3.2 M CH$_3$COONH$_4$ in 20% v/v HNO$_3$ was added, the solution was diluted to 200 ml with distilled deionised water.
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was added with 50 mM of pyrite, FeS₂, provided by Professor Barrie Cr₂(SO₄)₃ KH₂PO₄ (2.5) and Ca(NO₃)₂ (10), CuSO₄ KH₂PO₄, 147 mg CaCl₂ (NH₄)SO₄ (7.5), Na₂SO₄ (Bangor University). All cultures were adjusted to pH 1.8 using sulphuric acid. Microcosms were constructed using 50 g soil and leaching of the actinides, Pu and Am, through the formation of reduced sulphur compounds, to investigate the potential for bio-
ing the contaminated soil from Aldermaston, together with the Aldermaston site as
taining (per liter) 10 g elemental sulfur powder, 20 ml 50× auto-
organic basal salts consisting of (with grams liter⁻¹ in parenthesis) (NH₄)₂SO₄ (7.5), Na₂SO₄ 10H₂O (7.5), KCl (2.5), MgSO₄ 7H₂O (2.5), KH₂PO₄ (2.5) and Ca(NO₃)₂ 4H₂O (0.7) and 1 ml trace elemental solution containing (with grams liter⁻¹ in parenthesis) ZnSO₄ 7H₂O (10), CuSO₄ 5H₂O (1), MnSO₄ H₂O (1), CoSO₄ 7H₂O (1), Cr₂(SO₄)₃ 15H₂O (0.5), H₃BO₃ (0.6), Na₂MoO₄ 2H₂O (0.5), NiSO₄ 6H₂O (1), Na₂SeO₃ 10H₂O (0.1), NaVO₃ (0.1) at a final pH of 2.

2.3. Acidiphile cultures

Acidithiobacillus ferrooxidans was cultured in a liquid medium containing (per litre) 132 mg (NH₄)₂SO₄, 53 mg MgCl₂ 6H₂O, 27 mg KH₂PO₄, 147 mg CaCl₂ 2H₂O, 20 mg FeSO₄ 7H₂O, 0.25 N H₂SO₄. Acidithiobacillus thiooxidans was cultured in liquid medium containing (per litre) 10 g elemental sulfur powder, 20 ml 50× autotrophic basal salts consisting of (with grams liter⁻¹ in parenthesis) (NH₄)₂SO₄ (7.5), Na₂SO₄ 10H₂O (7.5), KCl (2.5), MgSO₄ 7H₂O (2.5), KH₂PO₄ (2.5) and Ca(NO₃)₂ 4H₂O (0.7) and 1 ml trace elemental solution containing (with grams liter⁻¹ in parenthesis) ZnSO₄ 7H₂O (10), CuSO₄ 5H₂O (1), MnSO₄ H₂O (1), CoSO₄ 7H₂O (1), Cr₂(SO₄)₃ 15H₂O (0.5), H₃BO₃ (0.6), Na₂MoO₄ 2H₂O (0.5), NiSO₄ 6H₂O (1), Na₂SeO₃ 10H₂O (0.1), NaVO₃ (0.1) at a final pH of 2.

The bacterial strains were provided by Professor Barrie Johnson (Bangor University). All cultures were adjusted to pH 1.8 using H₂SO₄ and incubated at 30 °C with shaking. Both cultures were grown from frozen cultures for 7 days before cells were harvested.

2.4. Acidiphile microcosms

Sulphur-oxidising bacteria were added to microcosms containing the contaminated soil from Aldermaston, together with reduced sulphur compounds, to investigate the potential for bio-
leaning of the actinides, Pu and Am, through the formation of sulphuric acid. Microcosms were constructed using 50 g soil and 97.5 ml groundwater, obtained from the Aldermaston site as described previously (Kimber et al., 2012), in a 250 ml Pyrex conical flask. A 2.5% v/v inoculum of either A. ferrooxidans or A. thiooxidans was added with 50 mM of pyrite, FeS₂, provided by Professor Barrie Johnson, Bangor University (Bacelar-Nicolau and Johnson, 1999), or 50 mM elemental sulfur powder (Sigma Aldrich, reagent grade) used as the source of reduced sulphur. Optimal growth of A. fer-
rooxidans and A. thiooxidans occurs between pH 2.0 and 2.3, although growth is possible over the wider range of pH 1.0 to 5.0 (Jensen and Webb, 1995; Lizama and Suzuki, 1988). The A. fer-
rooxidans and A. thiooxidans microcosms, when supplied with py-
rite as an energy source, had a similar starting pH of 3.1 and 2.9, respectively. When elemental sulphur was used as an energy source for A. thiooxidans, the starting pH was 4.8, much closer to the upper limit for cell growth. Each microcosm setup was run in duplicate. The microcosms were kept under aerobic conditions at 30 °C and shaken for 28 days with periodic sampling to monitor pH and Eh. At the end of the incubation period, the soil and aqueous fractions were separated via centrifugation (at an RCF of 15,700 for 5 min using an M-24 centrifuge, Boeco) and vacuum filtration. Prior to radiochemical analysis, the supernatant was acidified and stored at 10 °C, and the solid phase was stored at −80 °C for radiochemical analysis. The pH and Eh were measured using a Denver Instrument UltraBasic pH meter UB-10 (Denver Instrument GmbH, Downham Market, Norfolk, UK) in conjunction with a Mettler-Toledo Inlab Redox Micro ORP electrode (Mettler-Toledo Ltd, Leicester, UK).

2.5. Radiochemical analysis

241Am, 239,240Pu and 238Pu activities were measured using gamma- and alpha-spectrometry as described in detail previously (Kimber et al., 2012). Briefly, to determine the 241Am activity the supernatant was evaporated and the residue counted on either a high purity germanium semi-conductor gamma-detector (50% ef-
iciency) (Canberra Ltd.) or a Lo-ax low energy gamma photon semiconductor detector (EG&G), both attached to a signal analyser EG&G Model 919 ADC (analogue-to digital converter) and MCA (multi-channel analyser). To measure the 239Pu and 239,240Pu, 10% v/v HNO₃ and 1 mg KNO₃ per ml of sample were added and the sol-
lutions heated to dryness. The residue was then spiked with a known activity of 244Pu and taken up in 20 ml 9 M HCl, applied to an anion exchange column to isolate a Pu fraction, which was electroplated and counted using alpha spectrometry for 60 h to determine the 239,240Pu and 238Pu activity.

2.6. Autoradiography

A 1 g sample was taken from the soil provided by AWE and spread into a thin layer, then contacted with an uncoated storage phosphor screen. The screen was left for 3 days and then imaged using a GE Typhoon 9410 variable mode imager. The portions with no radioactive particles were discarded, whilst those with radio-
active particles were spread further and the process repeated until the particle were contained within a small quantity of soil suitable for transfer to a carbon adhesive pad used for ESEM analysis. The sample was then analysed using a FEI/Philips XL30 FEG ESEM equipped with an EDAX Genesis EDS system.

3. Results

3.1. Geochemical associations of Pu and Am

The sequential extraction of Pu and Am was performed in duplicate for each soil location. The data is presented as the range of Am or Pu extracted from each soil fraction as a percentage of the total Am or Pu extracted from each sample (Fig. 1). The actual Pu activity measured from each extracted fraction is presented in Table 1.

The 241Am in the Aldermaston soils could not be detected above background activity in the exchangeable fraction, while only 3.1–3.3% of the 241Am was associated with the carbonate fraction (Fig. 1a). The majority of 241Am was distributed between the reducible oxide fraction (26–40%), the organic fraction (46–64%) and the residual fraction (7.3–11%). Similar results were observed for the Esk Estuary samples with less than 0.1% of the 241Am associated with the exchangeable fraction and 3.4–3.9% with the carbonate fraction (Fig. 1b). Again, the majority of the 24Am was associated with the reducible oxide (34–35%) and organic components (58–59%), with a smaller percentage found in the residual fraction (1.9–3.3%).

Little 239,240Pu was found to be associated with the exchange-
able, carbonate and reducible oxide fractions in the Aldermaston samples, ranging from 0.018 to 0.029%, 0.40–0.56% and 3.6–5.7% in each, respectively (Fig. 1c). The most significant associations were found to be with the organic fraction, containing 11–30% of the 239,240Pu and the residual fraction, containing 64–86%. Similar proportions of 239,240Pu were found in the exchangeable, carbonate and reducible oxide phases of the Esk Estuary soils, ranging from 0.015 to 0.020%, 0.75–0.99% and 4.7–7.1%, respectively (Fig. 1d). In contrast to the Aldermaston samples, the Esk Estuary soils were found to have very little 239,240Pu associated with the organic fraction, which contained only 0.028–0.033% of the total 239,240Pu activity. The bulk of activity was again found in the residual fraction, here ranging from 92 to 95%. The sequential extraction data for 239Pu are very similar to those observed for 239,240Pu (Table 1). The bulk of the 238Pu in the Aldermaston samples was also associated...
with the residual component, while the organic fraction was also significant (74–76% and 14–21%, respectively). The $^{238}$Pu association in the Esk Estuary samples also closely mirrored that of the $^{239,240}$Pu, with the majority, 90–91%, again found in the residual fraction. The $^{238}$Pu/$^{239,240}$Pu activity ratios in the duplicate samples are 0.024 and 0.037 for the Aldermaston soils and 0.11 and 0.18 for the Esk Estuary soils. The $^{238}$Pu/$^{239,240}$Pu ratio from global fallout is dependent upon latitude with an average value of 0.034 ± 0.007 from soil cores in Ireland from a 1988 study (Mitchell et al., 1990). The $^{238}$Pu/$^{239,240}$Pu ratio from nuclear fuel reprocessing is much higher, whereas the $^{238}$Pu/$^{239,240}$Pu ratio for weapons-grade plutonium is lower, 0.28 and 0.020 respectively (Holm et al., 1986). When these values are corrected to account for the decay of $^{238}$Pu, the $^{238}$Pu/$^{239,240}$Pu ratios are estimated to be 0.028, 0.23 and 0.016 for fallout, fuel reprocessing and weapons-grade Pu, respectively. The $^{238}$Pu/$^{239,240}$Pu ratios for the Aldermaston soils

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Table 1

Activity of Pu isotopes in various soil fractions (extracted following the Tessier method) in Aldermaston and Esk Estuary soils. Errors are given as 1σ based on counting errors (BDL – below detection limit).

|               | $^{238}$Pu | $^{239,240}$Pu |
|---------------|-----------|----------------|
|               | Activity (mBq) | Percentage (%) | Activity (mBq) | Percentage (%) |
| **Aldermaston 1** |            |                |                |                |
| Exchangeable  | 2.54 ± 18.8 | 0.68           | 4.38 ± 14.45   | 0.029          |
| Carbonate     | 6.25 ± 13.6 | 1.7            | 61.6 ± 5.6     | 0.40           |
| Reducible oxide| 30.0 ± 5.1 | 8.0            | 548 ± 3        | 3.6            |
| Organic       | 51.4 ± 6.1 | 14             | 1600 ± 4       | 11             |
| Residual      | 282 ± 10   | 76             | 13,100 ± 2     | 86             |
| **Aldermaston 2** |            |                |                |                |
| Exchangeable  | BDL        | 0.0            | 3.20 ± 10.49   | 0.02           |
| Carbonate     | 2.50 ± 8.90| 0.37           | 102 ± 2        | 0.56           |
| Reducible oxide| 32.6 ± 8.3 | 4.8            | 1030 ± 5       | 5.7            |
| Organic       | 140 ± 2    | 21             | 5410 ± 2       | 30             |
| Residual      | 501 ± 11   | 74             | 11,500 ± 3     | 64             |
| **Esk Estuary 1** |            |                |                |                |
| Exchangeable  | 2.25 ± 9.84| 0.034          | 8.82 ± 5.17    | 0.02           |
| Carbonate     | 85.0 ± 4.1 | 1.3            | 439 ± 3        | 0.75           |
| Reducible oxide| 545 ± 1    | 8.3            | 2750 ± 1       | 4.70           |
| Organic       | 0.03 ± 18.5| <0.01          | 16.4 ± 0.9     | 0.03           |
| Residual      | 5930 ± 3   | 90             | 55,400 ± 2     | 95             |
| **Esk Estuary 2** |            |                |                |                |
| Exchangeable  | 6.01 ± 8.54| 0.08           | 8.78 ± 7.18    | 0.02           |
| Carbonate     | 82.2 ± 3.1 | 1.0            | 437 ± 2        | 0.99           |
| Reducible oxide| 602 ± 3   | 7.5            | 3130 ± 3       | 7.1            |
| Organic       | 3.11 ± 5.88| 0.04           | 14.6 ± 2.9     | 0.03           |
| Residual      | 7370 ± 3   | 91             | 40,600 ± 2     | 92             |
(0.024–0.037) are similar to those reported previously (McCubbin et al., 2004) and are indicative of ratios from weapons-grade and fallout derived Pu, though the total activity in the soils, 1.5–1.8 Bq/g (Table 2), is too high for the latter to be the source. The $^{238}$Pu/$^{239,240}$Pu ratios for the Esk Estuary soils (0.11–0.18) are broadly similar to those expected for Pu derived from fuel reprocessing, as expected, given the origin of the Pu in discharges from the nearby Sellafield reprocessing site.

3.2. Geochemistry of bioleaching microcosms

In order to investigate further potential differences in the geochemical behaviour of the two actinides, as well as to determine their susceptibility to remobilization under bioleaching conditions, a series of sulphuric acid generating microcosms were constructed. These contained sulphur-oxidising bacteria capable of producing sulphuric acid when supplied with a reduced sulphur source. Microcosms containing either $A$. ferrooxidans with pyrite (FeS$_2$), $A$. thiooxidans with pyrite and $A$. thiooxidans with elemental sulphur powder were used to investigate any differences in the leaching efficiency of different strains grown on various sulphur sources. In all cases, the initial pH (6–6.5) decreased with time, reaching pH 2.4 in both pyrite-containing microcosms and pH 2.7 in the elemental sulphur microcosm.

When supplied with pyrite, the $A$. thiooxidans and $A$. ferrooxidans microcosms had a starting Eh of +423 mV and +392 mV respectively. When elemental sulphur powder was used as the energy source, the $A$. thiooxidans microcosm had a lower starting Eh of +217 mV. At the end of the incubation, day 28, the final Eh of each set of microcosms was very similar, ranging between +494 mV and +506 mV.

3.3. Bioleaching of $^{241}$Am and $^{239,240}$Pu

The most efficient leaching of $^{241}$Am was observed in the microcosms containing $A$. thiooxidans augmented with elemental sulphur powder. Under these conditions, up to 13% of the Am present in the soil was released to solution after 28 days (Table 3). When $A$. thiooxidans was supplied with pyrite as its energy source, up to 3.5% of the $^{241}$Am was released to solution, whereas $A$. ferrooxidans augmented with pyrite, leached up to 6.7% of the $^{241}$Am after 28 days. The most efficient leaching of $^{239,240}$Pu was two orders of magnitude lower than that of $^{241}$Am. A maximum of 0.18% of the $^{239,240}$Pu was leached into solution using $A$. thiooxidans augmented with elemental sulphur, while $A$. ferrooxidans augmented with pyrite gave up to 0.14% $^{239,240}$Pu in solution (Table 3). As was the case for the $^{241}$Am, $A$. thiooxidans augmented with pyrite was the least efficient combination, only solubilizing up to 0.07% of the Pu. Although this bioleaching process is between 2 and 3 orders of magnitude more effective than the biostimulation of native anaerobic bacteria in the contaminated soil (Kimber et al., 2012), the overall solubilization of Pu is still negligible. However, the bioleaching of $^{241}$Am (up to 13%) noted in this study is far more effective than the stimulation of the native-bacteria with glucose investigated previously (Kimber et al., 2012), in which no solubilization of Am was observed, suggesting that $^{241}$Am is more susceptible to mobilization under changing pH as opposed to changing Eh conditions.

3.4. Autoradiography

A small number of alpha-emitting particles were detected, isolated and examined under ESEM. A number of small, high-density particles, containing unusual elements such as tungsten, were detected; however, despite isolation of Pu containing particles by autoradiography, no Pu was detectable in the samples by the EDS system fitted to the ESEM.

4. Discussion

4.1. Plutonium association at the Aldermaston site

The $^{238}$Pu/$^{239,240}$Pu ratios determined using the sequential extraction technique are consistent with different source terms for the Pu at the two sites; weapons manufacturing at Aldermaston and nuclear fuel reprocessing in the Esk Estuary. Consequently, different associations of Pu might be expected. However, the distribution of $^{239,240}$Pu appears to be fairly similar at both sites, with some notable exceptions. Surprisingly, more Pu is associated with the refractory fraction of the Esk Estuary soils than in the Aldermaston soils, despite Pu sourced from weapons manufacturing typically being expected to be more refractory than Pu derived from reactor waste (Choppin and Morgenstern, 2001).

Interestingly, the Pu association at the Aldermaston site differs significantly from that at another former weapons manufacturing site at Rocky Flats, Colorado, where a similar sequential extraction technique was performed (Litaor and Ibrahim, 1996). At Rocky Flats, the majority of the Pu (45–60%) was associated the organic soil fraction, followed by the oxide fraction (20–40%), and with only 10–15% being associated with the residual fraction (Table 4), thus suggesting that the Pu at Rocky Flats may be more susceptible to mobilization than at the Aldermaston site. The high organic C and sesquioxide content of the Rocky Flats soils may offer one explanation as to the high association of Pu with those fractions. However, differences in the form of Pu released into the environment may also be responsible for the variation between their data and ours. Both explanations highlight that although the source term of Pu is important to consider for understanding its geochemical behaviour, other factors such as soil type and the chemical form of Pu are also important in controlling its mobility.

Pu bound in the residual fraction exists in a highly recalcitrant form, associated with primary and clay minerals or existing as insoluble discrete Pu oxide particles. Highly insoluble Pu oxides, such as PuO$_2$, accumulate in soils and sediments (Choppin, 2007) and recent work in size characterization of PuO$_2$ suggests such particles predominantly exist in the sub $\mu$m range (Shinonaga et al., 2012). Although it is difficult to draw many conclusions from the autoradiography and ESEM results regarding the exact form of Pu in the Aldermaston soils, its high recalcitrance is clearly highlighted by the sequential extraction data. This is further supported by our previous work in which no significant Pu was mobilized under anaerobic reducing conditions (Kimber et al., 2012) and also by our present data where less than 0.15% of Pu was mobilized under bioleaching conditions.

4.2. Plutonium association at the Esk Estuary site

Interestingly, our results differ significantly from those of an earlier study on Esk Estuary soils in which the Tessier method was
physically trapped in the particle and hence, display a similar would be expected that 241Am, a decay product of Pu, would be organic fractions. If the Pu existed as discrete, insoluble particles, it both sites, Am association is dominated by the sesquioxide and variation between the association of Am and Pu at each site (Fig. 1). reducible oxide fractions. However, it is interesting to note the large residual fraction makes up the largest host phase for Pu in both our study sites, with the majority of the Am found in the organic and Rocky Flats data is from Litaor and Ibrahim (1996).

Comparison of geochemical associations of Pu from former weapons manufacturing Table 3

Table 3
Activity of 239,240Pu and 241Am in aqueous and solid phases after incubation of Aldermaston soil in acidophilic microcosms. Due to the heterogenous distribution of Pu in these soils, duplicate samples (10 g) were analysed from the solid phase of each microcosm. The percentage leached is calculated using the average activity from the duplicates in each microcosm. The microorganism and its source of sulphur are listed in the first column.

| 239,240Pu activity (Bq) | 241Am activity (Bq) |
|-------------------------|---------------------|
| A. ferrooxidans/pyrite   | A. thiooxidans/pyrite |
| Soluble                 | Organic             |
| 1 0.428 ± 0.009          | 1 0.145 ± 0.008     |
| 2 0.535 ± 0.016          | 2 0.129 ± 0.007     |
| 293 ± 40                 | 502 ± 17            |
| 312 ± 46                 | 138 ± 10            |
| 310 ± 30                 | 869 ± 82            |
| Carbonate                | Oxide               |
| 0.40–0.56                | 499 ± 17            |
| 0.57–7.0                | 370 ± 12            |
| Oxide                   | Residual            |
| 3.50–5.72               | 282 ± 29            |
| 20–40                   | 522 ± 69            |
| Organic                 | 29.9 ± 4.2          |
| 0.031 ± 0.004           | 4.30 ± 1.8          |
| 0.053                   | 42.5 ± 1.8          |
| 0.18                    | 3.5                |
| Residual                | 6.59 ± 0.33         |
| 10.5–63.8               | 46.3 ± 1.84         |
| 10–15                   | 13                 |

4.3. Americium contamination

The geochemical association of 241Am is fairly uniform at both study sites, with the majority of the Am found in the organic and reducible oxide fractions. However, it is interesting to note the large variation between the association of Am and Pu at each site (Fig. 1). Although the Pu is strongly associated with the residual fraction at both sites, Am association is dominated by the sesquioxide and organic fractions. If the Pu existed as discrete, insoluble particles, it would be expected that 241Am, a decay product of Pu, would be physically trapped in the particle and hence, display a similar geochemical association as the parent Pu. As significant differences between the geochemical associations of the two actinide were seen here suggests that a significant proportion of the Pu at both sites does not exist as distinct particles but instead, may be associated with primary and clay minerals of the soil (Litaor et al., 1998).

The high association of Am with sesquioxides and organics would suggest that Am could potentially be mobilized under anoxic, reducing conditions as well as during degradation of organic matter. However, our previous work suggests that dissolution of iron minerals under anoxic conditions does not lead to an increase of Am in solution (Kimber et al., 2012), possibly due to resorption of any Am released onto other mineral phases or the organic fraction.

4.4. Bioleaching of Pu and Am in Aldermaston soils

Our data here shows that under the strong leaching conditions created by the production of sulphuric acid, 3.0–5.3% of 241Am was leached into solution. The lower limit corresponds closely to the percentage of 241Am found in the carbonate fraction (3.1–3.3%) and so may have been leached through the dissolution of Am-bearing carbonate minerals under the low pH conditions in the sulphur-oxidising microcosms. Alternatively, the strong affinity for Am sorption to natural sediments is influenced by pH, with sorption increasing with increasing pH (Das et al., 2011; Moulin et al., 1992). Therefore, desorption of Am under the low pH conditions may also be responsible for its increase in solution.

It is interesting to note that there are significant differences, up to two orders of magnitude, between the leaching of 239,240Pu and 241Am under sulphur-oxidising conditions. As discussed above, if Pu existed as distinct particles, it would be expected that the Am decay product formed in situ, would be physically trapped within the particle and hence, that they would show similar mobilization under bioleaching conditions. As there are large differences between the geochemical association and bioleachability of Pu and Am, it suggests that a significant proportion of the Pu is not present in particulate form. Therefore, although Pu in the Aldermaston and Esk Estuary soils appears to be highly recalcitrant and unlikely to mobilise under most biogeochemical conditions, decay products formed in situ from the Pu, such as 241Am, may be more susceptible to mobilisation.

Table 5

Table 5
Variation in geochemical association of Pu in Esk Estuary soils over time. The 239,240Pu in each soil fraction is given as a percentage of the total 239,240Pu. Data from the present study is as an average of duplicate samples with errors as ±1 SD of the duplicates. Both studies used the Tessier extraction method. Data in column 2 is from Mudge et al. (1988).

| Site            | 2008 10–15 cm | 1987 0–1 cm |
|-----------------|---------------|-------------|
| Soluble         | 0.09–0.22     | 0.15        |
| Exchangeable    | 0.029–0.018   | 0.04–0.08   |
| Carbonate       | 0.40–0.56     | 0.57–7.0    |
| Oxide           | 3.50–5.72     | 20–40       |
| Organic         | 10.5–29.9     | 45–65       |
| Residual        | 85.5–63.8     | 10–15       |

The geochemical associations of Am in the Aldermaston microcosms were investigated using a sequential extraction method based on the Tessier method. The fraction of Pu in each phase is given as a percentage. The Aldermaston data is from the present study and the Rocky Flats data is from Litaor and Ibrahim (1996).
5. Conclusions

This study provides further information on the biogeochemical behaviour of Pu and Am under a range of environmental conditions. Our earlier work demonstrated how Pu is unresponsive to changing (bio)geochanical conditions in anoxic sediments, remaining insoluble during the stimulation of anaerobic microbiological processes, including the production of organic acids during glucose fermentation, and the subsequent activation of Fe(III)-reducing bacteria (Kimber et al., 2012). Here, we have shown that, even under strongly acidic conditions imposed by aerobic sulphur/ sulphide-oxidising bacteria, less than 1% of the Pu was leached into solution. The Am was leached to a greater extent, with up to 13% of the total activity leached into solution. Such acid leaching has been efficient in removing a wide range of heavy metal contaminants from soils and thus, these results suggest a highly refractory nature for Pu in the Aldermaston soils but also that Am may be more susceptible to remobilization. The increased efficiency in leaching Am compared to Pu may suggest that although Am is a product of the 239Pu decay chain, the two actinides develop a different geochemical behaviour over time, with Am more susceptible to mobilization under low pH conditions. The results obtained from sequential extraction of soils from Aldermaston and the EsK Estuary supports this proposition, as clear differences are observed in the associations of each of the actinides. This would suggest that the Pu does not exist as distinct particles in these soils.

The extractions reveal that most of the Pu in the soils is associated with the residual fraction, and metals in this fraction are unlikely to be released over a reasonable time frame under most geochemical conditions. Hence, it is important to consider the recalcitrance of the Pu when assessing its potential environmental impact and in developing management strategies from Pu contaminated land. Its high resistance to dissolution under the most aggressive microbiological leaching regimes indicates it is unlikely to be mobilised in significant quantities under environmental conditions.

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