The biogeochemical behaviour of plutonium and americium in contaminated soils

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Richard Lee Kimber

School of Earth, Atmospheric and Environmental Sciences
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Abstract

The biogeochemical behaviour of plutonium and americium was investigated in contaminated soils from the UK to help determine possible remediation and management options. Stimulating anoxic sediments from Aldermaston, through the addition of a carbon substrate (glucose), induced reducing conditions resulting in a negligible change in Pu mobility. This was despite a substantial shift in the bacterial profile from a diverse community to one dominated by fermentative Betaproteobacteria and Clostridia. The latter group also includes organisms associated with metal reduction, such as close relatives to Clostridium species, reported previously to facilitate the reduction of Pu(IV) to Pu(III). A sequential extraction was performed on soils from Aldermaston and the Esk Estuary to identify which selected fractions the Pu and Am are most strongly associated with. The majority of Pu was associated with the “residual fraction”: 63.8 – 85.5 % and 91.9 – 94.5 % in the Aldermaston and Esk Estuary soils respectively. Metals associated with this fraction are highly recalcitrant and are unlikely to be released into solution over a significant time span under most geological conditions. The Am was more evenly distributed with the “organic fraction” being the most dominant. Degradation of organic matter under oxidising conditions may result in mobilization of metals associated with this fraction. The Aldermaston soil was also subjected to bioleaching using a sulfuric acid producing microbial community, which resulted in a maximum 0.18 % of Pu released into solution. However, up to 12.5 % of Am was found in solution suggesting Am is more susceptible to mobilization than Pu. The potential for Pu mobilization through abiotic oxidative leaching was investigated using permanganate. Even when carbonate was added to act as a potential complexant for the Pu, less than 1 % of the Pu was leached. Greater success was observed when leaching was attempted using citric acid; an estimated 25 – 30 % of Pu was released into solution offering a potential route for remediation of Pu-contaminated soils. These data would suggest that the Pu is highly recalcitrant, and may exist in a small particulate form in the Aldermaston soils, possibly in the oxide form, and is unlikely to mobilize under natural biogeochemical conditions.
Declaration

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Finally, I’d like to thank my family and friends for all their support especially during the last few months of this project.
About the author

The author graduated from the University of Manchester in 2008 with an undergraduate masters degree in Earth Science (MEarthSci). During this time, the author worked on a summer internship project within the Geomicrobiology group of the School of Earth, Atmospheric and Environmental Sciences, under the supervision of Professor Jon Lloyd, Dr Victoria Coker and Dr Richard Cutting. Since October 2008, the author has been engaged in the research reported in this thesis under the supervision of Prof Jon Lloyd and Prof Francis Livens.
Thesis approach

This thesis has been written using the alternative format guidelines, with each chapter presented as an independent body of work. Due to the nature of the alternative format, some amount of repetition can occur in each of the chapters, in particular in the introduction and methodology sections, however this is to enable the submission of each chapter for publication in a scientific journal. Two chapters included in this thesis have already been published and have been included in the format as presented in their respective journal.
Thesis Content, Status of Manuscripts and Author Contributions

Chapter 1 – Introduction and Literature Review

Kimber, R.L.

Chapter 2 – Management of Land Contaminated by the Nuclear Legacy


Kimber, R.L. – Principal author; Livens, F.R. – Manuscript reviewer; Lloyd, J.R. – Manuscript reviewer.

Chapter 3 – Methodology

Kimber, R.L.
Chapter 4 – Biogeochemical behaviour of plutonium during anoxic biostimulation of contaminated sediments


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Kimber, R.L. – Principal author, preparation of soil microcosms, geochemical sampling, radiochemical sampling and analysis, digestion of soil samples, assisted with microbial community analysis; Boothman, C. – microbial community analysis and manuscript reviewer; Purdie, P. – Manuscript reviewer, provided soils from AWE and industrial contact; Livens, F.R. – Manuscript reviewer and supervisor; Lloyd, J.R. – Manuscript reviewer, assisted with microbial community analysis and supervisor.

Chapter 5 – Geochemical association of Pu and Am in selected host-phases of contaminated soils from the UK and their susceptibility to chemical and microbiological leaching


Manuscript in preparation

Kimber, R.L. – Principal author, sequential extraction of soils, preparation of soil microcosms, assisted with culturing of acidophiles, radiochemical analysis, autoradiography and ESEM analysis; Corkhill, C. – culturing of acidophiles and
manuscript reviewer; **Purdie, P.** – Provided soils from AWE and industrial contact; 
**Livens, F.R.** – Manuscript reviewer and supervisor; **Lloyd, J.R.** – Manuscript reviewer and supervisor.

**Chapter 6 – Citric acid and oxidative leaching of Pu from contaminated soils**


Manuscript in preparation

**Kimber, R.L.** – Principal author, citric acid leaching of soil, permanganate leaching of soils and radiochemical analysis; **Livens, F.R.** – Manuscript reviewer, assisted with radiochemical analysis and supervisor; **Lloyd, J.R.** – Manuscript reviewer and supervisor.

**Chapter 7 – Conclusions and Future Work**

Kimber, R.L.
Chapter 1

Introduction and Literature Review

Introduction and review of the current understanding of the biogeochemistry of plutonium and americium in environmental conditions.
1.1 Introduction and project aims

The anthropogenic release of radionuclides is currently of great scientific and public concern. The sources of these releases include nuclear weapons testing, accidental release, controlled discharges and the use of radionuclide-containing munitions. The remediation, decontamination and decommissioning of sites involved with this nuclear legacy is now the focus of governments worldwide. The continued storage and disposal of contaminated equipment and nuclear material, compounded with the contamination of land with radionuclides and co-contaminants, makes this a difficult and expensive undertaking. In the USA, the Department of Energy (DOE)’s 120 sites contain 40 million cubic meters of contaminated soil and debris and 6.4 trillion litres of contaminated groundwater. Contaminants include radionuclides such as caesium-137, plutonium-239, strontium-90, technetium-99, uranium-238, and uranium-235, as well as heavy metals, principally chromium, lead and mercury (McCullough et al., 1999). The associated cleanup cost has been estimated to be in excess of a trillion dollars (Palmisano and Hazen, 2003). The problem also applies to the UK on a smaller but still significant scale with the discounted clean up cost estimated at £44.5 billion (Nuclear Decommissioning Authority, 2010). Understanding the biogeochemical behaviour of radionuclides in the environment is therefore vital in assessing the risks associated with this contaminated land. The potential mobility of radionuclides presents a challenge in keeping the contamination contained without dispersion to the surrounding environment where they may contaminate watercourses and aquifers used for the irrigation of crops or as public drinking water. Conversely, if the mobility of radionuclides can be increased, then it may be possible to leach the contaminants from the soil in a controlled manner, thus remediating the site. The oxidation state of redox-
sensitive radionuclides is a major influence on their solubility and hence potential mobility in the environment. For this reason, the role played by microorganisms in controlling the oxidation state of radionuclides is of particular importance. Plutonium has some of the most complex redox chemistry among the actinides and furthermore, its biogeochemistry is often controlled by its physiochemical properties when released into the environment and so understanding these properties is important for predicting its future environmental behaviour.

To this end, this thesis sets out with the hypothesis that:

“Further understanding of the biogeochemical behaviour of plutonium is needed to underpin remediation and management strategies for radionuclide contaminated land.”

Following this hypothesis, the principal aims of this project were to;

- Investigate the biogeochemical behaviour of plutonium and americium in contaminated soils from the UK with the purpose of evaluating possible bioremediation strategies.
- Investigate the potential for in situ bioremediation of plutonium through biostimulation and bioaugmentation in contaminated soils.
- Investigate radionuclide contamination at sites associated with the nuclear legacy and examine field-tested remediation methods.
- Increase understanding of the physiochemical properties of plutonium and americium in the environment, such as their geochemical association and particle characteristics, to predict better their likely behaviour.
The following sections detail the current understanding of microbial interactions with metals (section 2), environmental radionuclide behaviour focussing on Pu and Am biogeochemistry (section 3), and case studies involving plutonium contamination as well as the characterization of sites studied in this project (section 4).

1.2 Microbial interactions with metals

1.2.1 Microbial metabolism

The ability of microorganisms to reduce metals, including radionuclides, has long been established (LOVELY and PHILLIPS, 1988; LOVELY et al., 1991). During aerobic respiration, electrons are released from the oxidation of organic or inorganic compounds and transferred to oxygen via an electron transport chain, generating energy in the form of ATP (adenosine-5'-triphosphate). Under anaerobic conditions, where no oxygen is present, other reducible chemical species such as nitrate, nitrite, sulfate, ferric iron, manganese oxides and certain organic compounds, are utilised as the terminal electron acceptor. Anaerobic bacteria can be facultative (can adapt to the presence of oxygen) or obligate (cannot grow in the presence of oxygen) anaerobes. If oxygen is available, facultative anaerobes will utilise it as the terminal electron acceptor during respiration, as it produces the most energy. When no oxygen is present, they, along with obligate anaerobes, will reduce alternative electron acceptors in order of greatest energy yield. This can result in a cascade of terminal electron acceptors being utilised during anaerobic respiration, best represented by the ‘redox tower’ (see figure 1).
1.2.2 Fermentation

Fermentation is the catabolic process by which cells conserve energy through oxidation of part of the consumed substrate, coupled to the reduction of the remainder of the substrate, an example being the fermentation of glucose to lactic acid. Therefore, fermentation does not require any external source of terminal electron acceptors; the substrate acts as both the electron donor and acceptor. A number of both anaerobic and facultative bacteria are capable of fermentation, and the process always occurs within the cytoplasm of the cell. Fermentation products, including organic acids produced by metabolic activity, can influence actinide solubility through complexation or displacement of metal cations from mineral surfaces (KONHAUSERA et al., 2002; TABAK
et al., 2005). Although some of these metabolic byproducts may increase actinide solubility, their rapid consumption by other microorganisms is likely to retard their overall impact on actinide dissolution. However, metal ion binding may limit or prohibit the metabolism of some of these byproducts, for example citric acid which forms tridentate complexes with metals has been shown to be resistant to degradation (FRANCIS et al., 1992).

1.2.3 Microbial interactions with iron

Iron is the most abundant element in the Earth and the fourth most abundant in the crust, with an average crustal concentration of 5%. It is a component of many minerals, including olivine, biotite and magnetite, and is consequently found in many sediments and soils. The common oxidation states of iron in nature are +2 and +3. When exposed to oxygenated environments it readily oxidises from its ferrous form (Fe(II)) to its ferric state (Fe(III)). Under reducing conditions it is readily reduced to Fe (II).

As iron is readily oxidised by chemical reaction in aerated environments, it has proved difficult to demonstrate that iron oxidation in aerobic conditions is enzymatic at circumneutral pH. Most evidence of enzymatic oxidation comes from bacteria at pH values below 5, where Fe(II) is more stable. Various acidophilic iron-oxidising bacteria have been characterised including Acidithiobacillus ferrooxidans, an organism associated with acid mine drainage which is also capable of oxidising sulfur (PRONK and JOHNSON, 1992; TEMPLE and COLMER, 1951).

Many microorganisms have the ability to conserve energy through the dissimilatory reduction of iron, where Fe(III) acts either as the dominant or exclusive terminal electron acceptor. In this process, which usually occurs under anaerobic conditions, ferric iron is reduced to its ferrous form. The earliest organisms identified to
grow through iron reduction were *Shewanella oneidensis* (formerly *Alteromonas putrefaciens*) (Lovley et al., 1989) and *Geobacter metallireducens* (formerly strain GS-15) (Lovley et al., 1987). Since then, many strains of bacteria, most of which fall within the family *Geobacteraceae*, which includes the genera *Geobacter, Desulfuromonas, Desulfuromusa and Pelobacter*, have been identified to grow using Fe(III) as a terminal electron acceptor (Lonergan et al., 1996). These organisms are able to oxidise a range of organic compounds (e.g. ethanol, lactate) coupled to the reduction of Fe(III) (Lloyd, 2003). Iron reduction can play an important role in remediation as higher valence contaminants, including radionuclides, have the potential to be reduced via biogenically produced Fe(II) (Lloyd et al., 2000; Reed et al., 2007).

The reduction of iron can further influence the geochemistry of a site through the release of metals bound to Fe(III) minerals (Zachara et al., 2001).

### 1.2.4 Manganese

Manganese has an estimated 0.1% abundance in the Earth’s crust, making it much less plentiful than iron (Ehlrich, 2002). Manganese is a component in over 100 minerals, including oxides, carbonates, and silicates with the II, III and IV oxidation states being environmentally relevant (Ehlrich, 2002). In the II oxidation state it is found as a reduced soluble or adsorbed ion and in the III and IV oxidation states it exists as an insoluble oxide (Gounot, 1994). As is the case with iron, geomicrobiological interactions with this element can influence sediment mineralogy and result in changes in Mn solubility (Lloyd, 2003). The oxidation of Mn(II) is thermodynamically favoured in oxygenated environments at neutral pH. However, the activation energy is high and so the process is usually very slow (Gounot, 1994). Oxidation of Mn(II) by a number of microorganisms has been observed, including bacteria belonging to the
genera *Leptothrix, Pedobacterium, Caulobacter, Bacillus,* and *Micrococcus* (GOUNOT, 1994; TEBO *et al.*, 2005). Mn(IV) can be reduced by a wide range of microorganisms, including many iron reducers, to Mn(II) (LLOYD, 2003). As with iron-reducing microorganisms, Mn-reducing microorganisms can influence sediments through the release of potentially toxic levels of Mn(II) as well as metals that were bound to Mn(IV) minerals (LLOYD, 2003). Many iron-reducing organisms, such as those of the genera *Geobacter, Desulfuromonas, Desulfofusumusa* and *Pelobacter,* are also able to reduce Mn(IV), coupled to the oxidation of various organic compounds or hydrogen (LONERGAN *et al.*, 1996). MnO$_2$ has been suggested to promote oxidation of Pu(IV) to Pu(V) in seabed sediments and hence, potentially provide a mechanism for mobilization of sediment bound Pu (MITCHELL *et al.*, 1995). However, manganese oxides are also able to sorb metals and radionuclides (TEBO, 2004), including Pu(V), which has been suggested to result in oxidation to Pu(VI) by the Mn(IV) (DUFF *et al.*, 1999; KEENIE-KENNICUTT and MORSE, 1985).

### 1.2.5 Microbial interactions with sulfur

Sulfur commonly occurs in the -II, 0, II, IV, and VI oxidation states in its inorganic form with the -II (sulfide), 0 (elemental sulfur), and VI (sulfate) states being the most common in nature (EHLRICH, 2002). Microbes play a key role in controlling the cycling of sulfur in the environment. Many microorganisms are able to conserve energy for growth via the dissimilatory reduction of sulfate (SO$_4^{2-}$) to H$_2$S under anaerobic conditions (LLOYD *et al.*, 2001). Certain sulfate reducers, including *Desulfovibrio desulfuricans,* are able to reduce metal contaminants, such as Cr(VI), Fe(III), U(VI) and Tc(VII), coupled to the oxidation of hydrogen or simple organic compounds in the absence of sulfur (LLOYD *et al.*, 2001). Chemolithotrophic sulfide oxidisers are able to
grow on reduced forms of sulfur, including sulfide, elemental sulfur and in some cases organic sulfur. This group encompasses a wide range of genera including *Acidithiobacillus*, *Achromatium*, *Beggiatoa*, *Thiothrix* and *Thioplaca* (MADIGAN and MARTINKO, 2006). Microbial reactions involved in the natural sulfur cycle provide a potential bioremediation mechanism through an integrated approach using both sulfur-oxidising and sulfate-reducing bacteria (see Figure 2). Sulfuric acid produced by sulfur-oxidising bacteria has been used to bioleach metal contaminants from soils, followed by precipitation of the leached metals as insoluble sulfides using sulfate-reducing bacteria (WHITE et al., 1998).

![Figure 2 - Illustration of integrated approach to bioremediation through application of a sulfur oxidising and sulfur reducing system (WHITE et al., 1997).](image)

### 1.2.6 Heterotrophic Leaching

The production of organic acids and siderophores, and changes to pH during heterotrophic metabolism, can promote the dissolution of metals. Organic acids produced by microorganisms can provide both protons and a metal-chelating anion to complex with the metal cation (GADD, 2004; GADD and POOLE, 1999). Citrate and oxalate can form stable, highly mobile complexes with numerous metals, promoting
their solubility and potential migration. As autotrophic leaching requires low pH conditions for *Acidithiobacillus* species to metabolize, heterotrophic leaching provides a potential solution for the leaching of metals in high pH wastes. However, as fungi require an aerobic environment with a carbon source, their use is often restricted to bioreactors or other very well aerated systems.

1.3 Radionuclide Biogeochemistry

1.3.1 Radionuclides in the Environment

The principal radionuclides of concern are those which have long half-lives and are present in significant quantities, such as the actinides U, Np, Pu and Am as well as $^{99}$Tc and $^{129}$I. Isotopes which are environmentally mobile, such as $^{90}$Sr or $^{137}$Cs, are also important contaminants. For redox-sensitive species, the factor controlling radionuclide migration in environmental systems is the oxidation state and hence, understanding processes which can alter radionuclide speciation is vital to understanding and controlling their mobility. As dioxo cations, in the V and VI oxidation states, the actinides are generally soluble and hence mobile, whereas the III and IV oxidation states commonly form insoluble species. As Pu and Am are most stable as the III and IV oxidation states respectively, they are often immobile in the environment, in contrast to Np and U which, being more stable in the V and VI oxidation states respectively, tend to be soluble (Lloyd and Renshaw, 2005). Local geochemical conditions can influence actinide oxidation states, with the preferred oxidation states reflecting the redox potential of a system (Figure 3).

The mobility of the actinides can also be affected through complexation by various ligands (Dozol et al., 1993) with sorption to colloids and minerals also
contributing factors. U(VI) is reported to sorb to carboxylate functionalities present in the subsurface as humic acids as well as bacterial surfaces (BOYANOV et al., 2007), while Am(III) is reported to sorb strongly to minerals such as hematite (TAO et al., 2006).

Figure 3 - Oxidation state of the actinides at various reduction potentials of a pH 7 system. Redox potentials of common microbial redox processes are included (LLOYD, 2003).

1.3.2 Microbial interactions with actinides

As discussed in section 2.1, microorganisms have the potential to reduce metals, including radionuclides. As the oxidation state of radionuclides is a fundamental factor in determining their mobility, the role that microorganisms can play in controlling the oxidation state is of obvious importance. In environments where oxygen has been depleted, microorganisms have the potential to utilize metals as the terminal electron acceptor during anaerobic respiration, thus reducing the metal. For example, the Fe(III)-reducing bacterium G. sulfurreducens is able to reduce soluble U(VI) to insoluble U(IV)
(Lovley et al., 1991). Stimulating microbial activity in the subsurface can therefore provide a mechanism for reducing metals to more desirable oxidation states, increasing or decreasing their mobility. By increasing their mobility, radionuclides can potentially be leached out of the contaminated soil. Decreasing mobility can help retard radionuclide migration, preventing further contamination of sensitive areas, such as aquifers. As well as redox transformations, microorganisms can also alter actinide mobility through a number of other mechanisms. Biosorption involves the metabolism-independent uptake of metals by microorganisms. This can occur through adsorption, whereby substances (including radionuclides) are accumulated at a surface or interface, and absorption, where the substance penetrates atoms or molecules of one phase to form a solution with a second phase.

Biosorption can occur with both living and dead biomass and the ligands involved include carboxyl, amine, hydroxyl, phosphate, and sulfhydryl groups. Studies have shown that a wide range of microorganisms are capable of biosorbing actinides (Giesy and Paine, 1977; Songkasiri et al., 2002). Metabolism-dependant metal uptake has been demonstrated for most physiologically important metals but has not been studied in detail for radionuclides. Most work undertaken on radionuclide uptake has focussed on the lighter radioelements, such as Cs, Sr and Pb (Kirchner and Dailant, 1998). In the bioaccumulation of actinides, uranium has been the most studied radioelement and has been found to accumulate in bacteria, fungi and algae (Aleissa et al., 2004; Macaskie et al., 1992). These processes have been reviewed extensively elsewhere but a lack of commercial interest has weakened further research into this area (Gadd, 2004; Lloyd et al., 2005; Renshaw et al., 2007).

Radionuclides can form precipitates with ligands generated by enzymatic activity, including phosphates, sulfides and carbonates. Microbial activity can cause a
high local ligand concentration with the surface of the cell acting as a nucleation site for precipitation, allowing for the efficient removal of the radionuclide from solution. U(VI) has been shown to form a precipitate with phosphate hydrolyzed by bacterial strains (Beazley et al., 2007) and so provides an alternative method for the immobilisation of the radionuclide.

1.3.3 Americium

There are numerous isotopes of americium with $^{243}$Am and $^{241}$Am being the most stable with half-lives of 7370 and 432.2 years respectively. In the context of radioactive waste, $^{241}$Am is largely formed through the beta decay of $^{241}$Pu, which is produced by successive neutron capture in $^{239}$Pu and then $^{240}$Pu.

High concentrations of complexing agents are required for the stabilization of Am(IV) with the (V) state prone to disproportionation and the (VI) state easily reduced. This leaves Am(III) as the stable oxidation state in the majority of environmental conditions. The solubility limiting phase is Am$_2$(CO$_3$)$_3$(s), although it may exist as Am(OH)$_3$(s) under low carbonate, high pH conditions. In solution, carbonate complexes, AmCO$_3^+$ and Am(CO$_3$)$_2^-$, and hydroxides, AmOH$_2^+$ and Am(OH)$_2^+$, are the dominant species (Allard et al., 1984). It has been suggested that microbial communities play a role in controlling the solubility of trivalent americium. A correlation was found between minimum porewater concentrations of Am, with maximum microbial biomass at a salt marsh in Cumbria, U.K., which was suggested to be as a result of bioaccumulation (Keith-Roach et al., 2000). Am(III) has been reported to sorb strongly to natural sediments, with sorption often increasing with pH (Das et al., 2011; Moulin et al., 1992).
Transuranic elements, including $^{241}\text{Am}$, were found to migrate rapidly with limited retardation at the Oak Ridge National Laboratory, Tennessee, USA. McCarthy and co-workers postulated that the major releases observed were due to seasonal fluctuations in the water table allowing groundwater to come into contact with actinide-contaminated waste. They suggested that minor releases of the actinides were due to local recharge of stormwater creating transient saturation of the waste trenches above the local water table. The groundwater at Oak Ridge is rich in $\text{CaCO}_3$ due to the dissolution of limestone and hence the $^{241}\text{Am}$ would be expected to exist as a carbonate complex. In both the carbonate and free ionic form, the Am would be expected to adsorb strongly to the silicates and mineral oxides in the Oak Ridge soil (McCarthy et al., 1998). McCarthy and co-workers concluded that the unretarded migration of the Am was due to the formation of highly mobile complexes of Am with natural organic matter (NOM). Although silicates and mineral oxides are reported to have a high affinity for NOM, McCarthy et al. argue that the NOM-binding sites are saturated due to continued contact with groundwater.

1.3.4 Plutonium

The complex radiochemistry and high radiotoxicity of plutonium make it one of the more challenging actinides to study. Of the numerous isotopes of plutonium, $^{239}\text{Pu}$ is the primary isotope used in the nuclear fuel cycle and the production of nuclear weapons. As such, $^{239}\text{Pu}$ which has a half life of 24,110 years is often associated with nuclear legacy contamination (Cantrell, 2009; Gray et al., 1995; International Atomic Energy Agency, 2006; Litaor et al., 1998; McCubbin et al., 2004).

When discussing the environmental behaviour of plutonium, it is useful to consider the source of the contaminant. Choppin has suggested that plutonium species
can be split into two groups: source-dependant and source-independent plutonium (CHOPPIN and MORGENSTERN, 2001). Source-dependant refers to Pu which is not in thermodynamic equilibrium with its surroundings and, as such, its behaviour in natural systems is controlled by the form in which it was released into the environment. An example of such species is the high-fired plutonium oxide formed in a nuclear explosion. This is a refractory, highly insoluble oxide that is likely to accumulate quickly in soils and sediments in lakes, rivers, and oceans. In contrast, source-independent Pu can achieve equilibrium in a system through geochemical processes. Thus, changes in pH, Eh, ligand concentration, and biological activity would be expected to affect the speciation of source-independent Pu. Plutonium released from reprocessing operations would probably exhibit source-independent behaviour. The ratio of Pu isotopes is characteristic of different sources of plutonium in the environment so measuring these ratios in soils can help provide information on the likely origin and hence physiochemical properties of the Pu. Pu originating from global fallout has a $^{238}\text{Pu}/^{239,240}\text{Pu}$ activity ratio dependant upon latitude, with an average value of $0.034 \pm 0.007$ in soil cores taken from Ireland in a 1988 study (MITCHELL et al., 1990). Because much of the $^{238}\text{Pu}$ in global fallout originates from the 1964 SNAP9A spacecraft failure, the $^{238}\text{Pu}/^{239,240}\text{Pu}$ ratio from weapons-grade Pu is slightly lower than this value, whereas the corresponding ratio for Pu originating from nuclear fuel reprocessing is much higher, 0.020 and 0.28 respectively (HOLM et al., 1986). Recalculated values, corrected to 2011 for the decay of $^{238}\text{Pu}$, are given in table 1.

<table>
<thead>
<tr>
<th>Source of plutonium</th>
<th>Global fallout (Ireland)</th>
<th>Weapons-grade</th>
<th>Fuel reprocessing</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{238}\text{Pu}/^{239,240}\text{Pu}$ ratio</td>
<td>0.028</td>
<td>0.016</td>
<td>0.23</td>
</tr>
</tbody>
</table>

**Table 1 - Recalculated values for $^{238}\text{Pu}/^{239,240}\text{Pu}$ ratios to correct for decay of $^{238}\text{Pu}.$**
Plutonium has complex environmental redox chemistry due to its ability to exist in various oxidation states (III, IV, V, VI). The most prominent of these is Pu(IV) which is stable across a wide range of environmental conditions. Pu(IV) is sparingly soluble, especially in the dominant oxide and hydroxide forms and may adhere strongly to particle, colloidal and mineral surfaces (Choppin et al., 1997; Choppin and Morgenstern, 2001; Neu et al., 2005). The formation of amorphous Pu(OH)$_4$ and polycrystalline PuO$_2$, coupled with the strong hydrolysis and sorption of Pu(IV) in natural waters, limits the solubility of Pu, restricting the actinide’s mobility and bioavailability, and results in limited concentrations of dissolved Pu(V) in solution (Choppin and Morgenstern, 2001). The formation of Pu$_2$(CO$_3$)$_3$(s) is also possible under strongly reducing conditions and/or in the presence of high carbonate concentrations. The low solubility of Pu(IV), compounded with its tendency to adhere to organic and mineral surfaces probably restricts the transport of Pu to colloidal and particulate migration. Pu(III) is easily oxidised and is not expected to exist except under strongly reducing conditions (Nash et al., 1988). The higher oxidation states of Pu, (V) and (VI), are favoured under oxic conditions and have the potential to be far more mobile than Pu(IV). The oxidation of Pu(IV) to Pu(V) and then further to Pu(VI) can occur through interactions with minerals, chelators and bacteria (Mitchell et al., 1995; Neu et al., 2005). Pu(V) can also disproportionate, resulting in a combination of Pu(IV) and Pu(VI) species (Neu et al., 2005; Panak and Nitsche, 2001). Due to the susceptibility of the higher oxidation states of Pu to reduction by organic species such as oxalate, citrate, and acetate, they are not expected to be important constituents of Pu in biologically active environments. An overview of processes which can affect the environmental behaviour of Pu can be seen in Figure 4, with these processes discussed in more detail throughout this section.
1.3.5 Potential for Plutonium Remediation

Pu(IV) oxyhydroxide has estimated solubility products of $10^{-56.8}$ and $10^{-57.8}$ whereas Pu(III) hydroxide has a greater, though still small, estimated solubility of $10^{-22.6}$ (FRANCIS, 2007). Therefore, the reduction of Pu(IV) to Pu(III) would be expected to increase the mobility of the actinide. Consequently, the potential for Pu(IV) reduction by microorganism, in environmental systems could be important when developing strategies to remediate and manage Pu contaminated land, and in understanding the long term migration of Pu, for example in a geological repository.

Reduction of the higher valent plutonium species through numerous mechanisms including microbial interactions, inorganic reduction, and disproportionation reactions has been investigated with the formation of either a stable, precipitated Pu(IV) product
or a variety of oxidation states sorbed to cell surfaces (ICOPINI et al., 2009; PANAK and NITSCHE, 2001; REED et al., 2007; RENSHAW et al., 2009). Reduction of the environmentally more relevant Pu(IV) species to Pu(III) has also been investigated in a number of studies. Minor quantities of Pu(III) were produced by the Fe(III)-reducing bacterium Shewanella oneidensis in the presence of Pu(OH)$_4$ (BOUKHALFA et al., 2007a). When EDTA was added, both S. oneidensis and G. metallireducens were able to reduce significant quantities of the Pu(OH)$_4$ (approximately 60 % and 80 % respectively) with the resultant Pu(III) remaining stable under reducing conditions. Another study, this time under aerobic conditions, demonstrated the ability of Bacillus sp. to solubilise approximately 90 % of hydrous PuO$_2$ in the presence of nitrilotriacetic acid (NTA) (RUSIN et al., 1994). Without NTA present, only 40 % of the Pu was solubilised which, along with the study by Boukhalfa et al, would suggest that complexing agents play an important role both in the solubilization of Pu and making it susceptible to bioreduction.

The reductive dissolution of Pu(IV), most likely in the form of Pu(OH)$_4$, using the anaerobic bacterium Clostridium sp. has also been investigated. After 14h of cell growth, 70 % of the Pu passed through a 0.4 μm filter, with 55 % passing through a 0.03 μm filter, suggesting significant solubilization of the Pu (FRANCIS, 2007). The presence of Pu(III) was confirmed using XANES.

There is also the potential for Pu sequestration through siderophore mediated complexation due to the similarity between Pu(IV) and Fe(III) ions. Both ions are hard Lewis acids with high charge densities and have similar hydrolysis products. In cases of iron deficiency, bacteria and fungi may produce iron-chelating siderophores which allow Fe(III) to be sequestered and passed into cells via multi-protein uptake systems. A common aerobic bacterium, Microbacterium flavescens, was found to uptake Pu when complexed with the siderophore desferrioxamine-B (DFOB) (JOHN et al., 2001). The
Pu(IV)-DFOB and Fe(III)-DFOB complexes were found to compete for uptake channels and mutually inhibited accumulation of the other with the rate and cumulative uptake of Pu(IV)-DFOB complexes exceeded by that of Fe(III)-DFOB complexes. Further work into Pu-DFOB complexes indicated that Pu(III) siderophore complexes were 20 orders of magnitude less stable than those formed by Pu(IV), suggesting that, under reducing conditions, stable Pu siderophore complexes are unlikely to exist (BOUKHALFA et al., 2007b).

1.3.6 Plutonium complexation, hydrolysis and sorption

In all oxidation states, plutonium cations are classified as hard Lewis acids and, as such, react with hard Lewis bases including complexants containing oxygen, such as water molecules. The strength of these interactions depends primarily on the charge density of the metal. Thus, the order of increasing complexation strength of the environmentally relevant oxidation states of Pu is:

\[ \text{Pu(IV)} > \text{Pu(VI)} > \text{Pu(III)} > \text{Pu(V)} \]

The tendency for hydrolysis follows the same trend observed for complexation, with Pu(IV) the strongest and Pu(V) the weakest. At pH values between 6 and 9 observed in natural waters, hydrolysis is significant for all Pu states except Pu(V) and results in the removal of Pu(IV) from solution through adsorption to particles and surfaces. This trend is also true for the sorption strength of plutonium as it is determined by the net charge on the Pu cation. The strong hydrolysis of Pu(IV) leads to extensive sorption onto mineral and organic-coated particles, often making sorption the controlling feature of Pu migration (MORSE and CHOPPIN, 1991).
Complexation of Pu with inorganic ligands is not usually considered to be significant in the environment due to the strong tendency of Pu(IV) to hydrolyse and the relatively low concentrations of inorganic ligands in natural systems. Inorganic ligands that may have some relevance for complexation with Pu include carbonate, sulfate, phosphate, chloride, fluoride, nitrate, and silicate. A detailed review of Pu complexation behaviour with many of these inorganic ligands is provided by Clark and co-workers (Clark et al., 2006a).

A wide variety of compounds, including fatty acids, amino acids, carbohydrates, lipids, proteins and humics make up the organic matter present in natural systems. A major component of DOC (Dissolved Organic Carbon) in natural systems is made up of humics and hence they are thought to be the organic ligands which most influence plutonium behaviour. Humic substances, formed via the degradation of organic debris, can be divided into three main fractions; humin (insoluble at all pH), humic acid (soluble at pH > 3) and fulvic acid (soluble at all pH). Little solubilization of plutonium or americium by humic compounds was observed in a study using soils from a plutonium-contaminated area of the Rocky Flats Plant in the U.S. (Cleveland and Rees, 1976). The strong hydrolysis of Pu(IV) probably limits the influence of humics on the speciation of the actinides tetravalent state. Humics may play a role in the elimination of the higher oxidation states of Pu in natural systems, reducing Pu(VI) and Pu(V) to Pu(IV) which then undergoes hydrolysis (Morse and Choppin, 1991; Nash et al., 1981). Under reducing conditions and in the presence of high levels of organics then Pu(III) may exist, complexed to humics (Choppin et al., 1986; Nash et al., 1981).
1.3.7 Colloids

The role of colloids in Pu transport is still not well understood, though it has been argued that models which do not include colloidal transport may significantly underestimate Pu migration (Kersting et al., 1999). Plutonium, as well as the other actinides, can exist as intrinsic colloids, generated by the products of actinide hydrolysis and pseudocolloids, which arise from the sorption of actinide ions or intrinsic colloids onto natural aquatic colloids, examples being PuO$_2$ and PuCO$_3$(OH)$^+$, respectively. The migration of Pu to the far-field through colloidal association has been observed at numerous nuclear facilities (Kaplan et al., 1994; Kersting et al., 1999; McCarthy and Zachara, 1989; Novikov et al., 2006). However, a contrasting study has suggested that colloidal transport of Pu may not be as common as previously suggested (discussed in more detail in section 4.2) (Dai et al., 2002). The tendency of plutonium to form intrinsic colloids is related to the charge on the central atom and follows the trend observed for hydrolysis and complexation. Intrinsic colloid formation mechanisms for plutonium are thought to be similar to those of precipitation and hence, concentrations of Pu which exceed the solubility product for the solid phase are needed for colloid formation. Pu(III), (IV), and (VI) all have oxides and hydroxides which are very insoluble and so they are often the source of intrinsic colloids. The Pu(IV) ion readily hydrolysis forming monomeric hydroxide complexes which, at Pu(IV) concentrations of $> 10^{-6}$ M, go on to form polynuclear species. It is generally assumed that hydrolysis of the Pu(IV) mononuclear species is the first step towards colloid formation, however, the role of Pu(IV) polynuclear complexes is still unclear (Walter et al., 2009). Ageing of the Pu(IV) polymer results in increased resistance to depolymerization and dissolution of the colloid, thought to be due to the conversion of amorphous to crystalline particles.
Plutonium and other actinides are readily sorbed onto groundwater colloids forming pseudocolloids. These colloids are composed of inorganic or organic components, a combination of the two, or microorganisms. The formation mechanism here is thought to be related to the hydrolysis of Pu; thus sorption of Pu(IV) onto colloidal particles is favoured.

1.4 Plutonium contamination case studies and site characterization

1.4.1 Rocky Flats, United States

Between 1958 and 1969, around 196 GBq of plutonium leaked from chemical drums stored in the 903 Pad Area at the Rocky Flats Plant in the U.S. This created a plume of Am and Pu contamination which tracked with the prevailing wind at the site and, prior to remediation efforts, plutonium was present in the soil at over 37 Bq/g in its highest concentration (CLARK et al., 2006b). Most of the radioactive inventory (~ 90%) was located in the top 12 cm of the soil (LITAOR and IBRAHIM, 1996). The oxidation state of plutonium was identified as Pu(IV) via X-ray absorption near-edge structure analysis, with extended X-ray absorption fine structure analysis identifying it as the insoluble hydrous oxide PuO$_2$·xH$_2$O (CLARK et al., 2006b). Although the insoluble oxide of plutonium would generally be considered immobile, a number of studies observe the movement of low concentrations of low-solubility actinides in colloidal and particulate form through wind and groundwater mechanisms (KERSTING et al., 1999; SANTSCHI et al., 2002). As the Pu was largely located in the top soil, the greatest risk to public health during remediation efforts came from wind and water transport of the contaminant. To mitigate this risk, large tents were set up at the 903 Pad to insulate work from wind and water processes. The work involved removal of the mostly highly contaminated horizon
of soil, down to 1 m, with fresh soil then used to replace it. Following remediation efforts, a decrease in actinide migration was observed, with the site now under maintenance and routine inspection by the DOE Office of Legacy Management.

An earlier laboratory scale study also investigated the leachability of $^{239,240}\text{Pu}$ and $^{241}\text{Am}$ from Rocky Flats soils using a combination of a hot solution ($80^\circ\text{C}$) with complexing (citrate) and reducing (ascorbic acid and sodium dithionite) agents (LU et al., 1998). The experiments were run in a series of columns designed to assess the effectiveness of the leaching schemes as a function of particle size. Lu and co-workers found that a combination of sodium citrate with ascorbic acid or sodium dithionite was more effective at leaching total alpha activity than when sodium citrate was used alone, with increased leaching efficiency correlating with increasing particle size.

1.4.2 Savannah River Site, United States

Studies have also been conducted into the migration of Pu at the Savannah River Site (SRS), a former nuclear weapons production site in the U.S. Dai et al. looked into the transport mechanisms and migration of Pu from seepage basins which had received the discharges from the Pu chemical separation area until their closure in 1988 (DAI et al., 2002). Wells upstream of the seepage basin were found to contain the lowest concentrations of $^{239}\text{Pu}$ with the highest levels found in the nearest downstream wells. Concentrations of $^{240}\text{Pu}$ were found to be highest in wells furthest downstream from the basins resulting in an elevated $^{240}\text{Pu}/^{239}\text{Pu}$ ratio, roughly a factor of 200 higher than the estimated $^{240}\text{Pu}/^{239}\text{Pu}$ ratio for SRS (DAI et al., 2002). Dai et al. concluded that this higher concentration of $^{240}\text{Pu}$ originated from the decay of the more mobile $^{244}\text{Cm}$, suggesting that an earlier claim by Kaplan et al., that a plume of $^{240}\text{Pu}$ had migrated $>300$ m from the seepage basin (KAPLAN et al., 1994), was predominately $^{240}\text{Pu}$ formed
in situ from the $^{244}$Cm. In contrast to earlier work at SRS (Kaplan et al., 1994) and work at the Nevada Test Site (Kersting et al., 1999), little Pu was found to be associated with the colloidal fraction, suggesting that colloidal transport may not be as significant as described elsewhere. A possible explanation for the higher colloid-associated Pu observed in earlier studies may be due to colloid generation as an artefact of sampling as suggested by the authors.

1.4.3 Site Characterization and History of AWE, Aldermaston, UK

The Atomic Weapons Establishment (AWE) has been responsible for maintaining the UK’s nuclear deterrent for over 50 years. It operates over two sites, AWE Aldermaston and AWE Burghfield, both located in Berkshire. Aldermaston, a former wartime airfield, is now the company’s headquarters covering approximately 3 square kilometres and encompassing research, design and manufacturing facilities. The Burghfield site, a former munitions factory, covers 1 square kilometre and is responsible for the final assembly, maintenance and decommissioning of the UK’s nuclear warheads. Due to the long and 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. The Pu present has been reported to be sorbed strongly to soils with a $K_d$ ranging from $2 \times 10^6$ to $6 \times 10^6$ (McCubbins et al., 2004). The local geology at the Aldermaston site consists of Quaternary gravels with the underlying Lower Bagshot Beds composed of Tertiary sands and gravels, all resting on a layer of London Clay. The London Clay
formation acts as a groundwater barrier between the site and the underlying Chalk aquifer.

1.4.4 Site Characterization and History of the Esk Estuary

Measurable quantities of radionuclides have been released from the Sellafield nuclear reprocessing site through authorised discharges of low-level radioactive waste into both the local environment and further afield. Most of this activity was released as liquid waste with minor quantities released into the atmosphere (GRAY et al., 1995). The major constituents of these discharges were $^3\text{H}$, $^{90}\text{Sr}$, $^{95}\text{Zr}/^{95}\text{Nb}$, $^{106}\text{Ru}$, $^{134}\text{Cs}$, $^{137}\text{Cs}$, $^{144}\text{Ce}$, $^{238}\text{Pu}$, $^{239}\text{Pu}$, $^{241}\text{Pu}$ and $^{241}\text{Am}$. The largest discharges occurred during the 1970s, when input into the Irish Sea was two to three orders of magnitude higher than contemporary levels. Much of this inventory, including up to 90% of the Pu, is retained rapidly by Irish Sea sediment once discharged (MACKENZIE et al., 1999). Therefore, movement of this sedimentary material is the primary transport process affecting most of the radionuclides discharged from the Sellafield plant. Local sedimentation rates in the Esk Estuary, ~10 km south of Sellafield, have been reported at rates of up to 6.5 cm year$^{-1}$ (ASTON and STANNERS, 1981; HAMILTON and CLARKE, 1984). Various studies have investigated the chemical and physical associations of these radionuclides, in particular Pu, with the soils of the Esk Estuary. By determining the dominant host-phases of the actinides, predictions of their environmental behaviour can be made. For example, radionuclides associated with the exchangeable phase may desorb readily with changing ionic composition in the solution phase. Changing pH and Eh conditions may promote release of radionuclides associated with the carbonate and oxide fractions, respectively, through mineral dissolution. The organic bound fraction may be susceptible to mobilization under oxidizing conditions, which promote degradation of
organic matter. Radionuclides associated with the residual fraction are unlikely to be released into solution over a significant time frame, under most geochemical conditions. Using the McLaren and Crawford extraction method (McLAREN and CRAWFORD, 1973), Livens and Baxter found the majority of Pu in various soil types of the Esk Estuary to be associated with the organic fraction followed by the oxide fraction (LIVENS and BAXTER, 1988). However, using the extraction method developed by Tessier (TESSIER et al., 1979), a study by Mudge and co-workers found the majority of Pu associated with the residual fraction (MUDGE et al., 1988). These differing results highlight the difficulty in interpreting sequential results due to the selectivity of reagents used in the different methods (LITAOR and IBRAHIM, 1996; MAHER, 1984).

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Chapter 2

Management of Land Contaminated by the Nuclear Legacy

Review Book Chapter

Management of Land Contaminated by the Nuclear Legacy

RICHARD KIMBER,* FRANCIS R. LIVENS AND JONATHAN R. LLOYD

ABSTRACT

The widespread spread use of nuclear materials over the past 60 years has lead to anthropogenic release of radionuclides into the environment. The release of such contaminants is currently of great public concern and scientific interest worldwide. Contamination has arisen on sites involved in both military and civilian uses of nuclear material through leakages, spills, controlled discharges and munitions use. The management of this nuclear legacy is a global priority as governments seek to decommission and reclaim land contaminated by the use of nuclear facilities. The scale of contamination presents a serious financial burden with the cleanup of US sites expected to cost up to a trillion dollars. In the UK, the problem exists on a smaller but significant scale with associated cleanup costs estimated to be in the order of £100 billion. A wide range of disciplines are required to understand the behaviour of radionuclides and co-contaminants in these contaminated environments in order for effective remediation techniques to be utilised. Potential remediation strategies cover a range of biological, chemical and physical methods which can be used to treat the complex contamination scenarios found at nuclear sites. A number of these remediation techniques have been trialled at several sites managed by the United States Department of Energy with some success in treating radionuclide contamination.

*Corresponding author

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1 Introduction

The industrial production of nuclear materials and their subsequent use, both militarily and civilian, since the 1940s has left behind a legacy of contamination and hazardous waste. Management of this legacy is now a global priority as many governments seek to decommission and reclaim land once used by their nuclear facilities. However, the storage and disposal of contaminated equipment and spent nuclear material, compounded with the widespread dispersion of radionuclides and co-contaminants at these sites makes this a challenging and expensive undertaking.

Many former nuclear facilities were shut down at the end of the Cold War in the 1990s as the demand for nuclear weapon production decreased. These sites are now the focus of remediation, decommissioning and decontamination efforts for governments and agencies worldwide. Substantial quantities of land, groundwater, and equipment have been contaminated by the former operations of these sites, the details of which will be discussed in this chapter. A number of sites which are still in operation, often dealing with the reprocessing of nuclear material, are also the focus of ongoing remediation efforts. Contamination issues have arisen from various sources including accidental release, the controlled discharge of nuclear waste and the use of radionuclide-containing munitions. The accidental release of radionuclides can occur through the leakage and spills of radioactive material, as well as from incidents (such as explosions) which have occurred on site. Off site contamination is a concern in cases where both on site accidents and natural transport processes have spread radionuclides further afield. Hydraulic flow presents an ongoing problem for the containment of on site contaminants as groundwater plumes threaten to spread contamination to aquifers used in the irrigation of crops or for public drinking water. For this reason, the mobility of radionuclides, heavy metals and toxic organics is a key factor in determining the risk that each contaminant presents to the environment and general public. Understanding the mechanisms which affect contaminant mobility is therefore vital in developing effective remediation strategies. Numerous techniques are available for treating contaminated land and groundwater, generally falling under either biological (bioremediation) or chemical processes. This chapter will outline some of the key techniques available, along with their associated advantages and disadvantages. A number of key case studies relating to former nuclear facilities will also be discussed where a variety of techniques have been applied in field scale studies.

2 Contamination at Worldwide Nuclear Facilities

In 2008, 439 nuclear power plants were in operation in 31 countries accounting for 15% of the world’s electricity production and as of January 1999, a total of 2532 nuclear detonations have been performed. Many sites involved in the production, reprocessing and storage of nuclear materials have contributed to environmental radionuclide release through discharge, spillage, accidents and testing facilities. A summary of such sites and their associated
contamination issues is given in Table 1. The number of nuclear facilities is expected to increase in the near future as governments renew their interest in nuclear power due its reduced CO₂ emissions and the energy security it provides when compared to other forms of electricity generation.

2.1 United Kingdom

The United Kingdom’s nuclear legacy has arisen from a variety of nuclear facilities operated across the country over the past ~ 60 years. These contribute to the production of nuclear material for nuclear reactors or weapons, the use of this material in reactor plants and the re-processing of spent nuclear fuel. Construction on the UK’s first nuclear power plant, Calder Hall, began in 1953 and in 1956 it was connected to the national grid becoming the world’s first commercial nuclear power station. The site was also expanded over the following decades, to result in the present Sellafield site (see section 2.1.1). Between 1953 and 1971 a total of 26 reactors were built at nuclear research and development sites across the UK.³ A substantial part of the UK’s electricity supply has come from the first generation of Magnox nuclear power stations over the past 60 years. Just two of the eleven Magnox stations are still operational.

The Atomic Weapons Establishment (AWE) is responsible for providing and maintaining the UK’s nuclear deterrent and has held this responsibility for over 50 years. AWE operates over two sites, Aldermaston which is a former airfield and Burghfield, a former munitions factory. Although the Aldermaston site is radiologically safe there are areas where soils contain higher than background levels of various radionuclides, including plutonium. Levels of $^{239+240}$Pu have been found to range from 15 to 155 Bq kg$^{-1}$ in certain settled sediments (sludge)$^4$ compared to background levels due to global fallout of 0.02 to 0.7 Bq kg$^{-1}$.$^5$

The Nuclear Decommissioning Authority (NDA), a Non-Departmental Public Body (NDPB), was established in 2005 to manage the decommissioning and clean up of the UK’s civil public sector nuclear legacy sites. The restoration program tasked to the NDA relates to 19 sites covering the length and breadth of the UK with certain sites not expected to reach their planned end state for decades. The discounted lifetime cost for completing their contracted work, the Nuclear Liabilities Estimate (NLE), stands at £44.5 billion.$^3$ A detailed overview of the NDA’s planned approach for decommissioning and clean up is provided in their recent draft strategy published for consultation.$^3$

Although there are a number of sites in the UK where nuclear operations have occurred, the majority of the legacy waste and contamination is located at a few principal facilities. Two key sites (Sellafield and Dounreay) involved in the UK’s nuclear waste inventory and which suffer from the greatest contamination concerns are discussed here in more detail.

2.1.1 Sellafield

Sellafield (formerly Windscale), West Cumbria, is the UK’s largest nuclear complex covering 262 hectares and has supported the nuclear power program
Table 1  Summary of former and current uses of key worldwide nuclear facilities with associated soil and groundwater contamination issues. (Adapted from W. Standring, M. Dowdall and P. Strand, Int. J. Environ. Public Health, 2009, 6, 1).

<table>
<thead>
<tr>
<th>Site</th>
<th>Former uses</th>
<th>Current use</th>
<th>Soil contamination</th>
<th>Groundwater contamination</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sellafield</td>
<td>WW2 explosives production site. Postwar plutonium production, fuel reprocessing, fuel manufacturing, nuclear waste management, nuclear energy generation</td>
<td>Reprocessing, fuel production, waste management and decommissioning</td>
<td>~1600 m$^3$ contaminated with radioactive waste (ILW). ~1 000 000 m$^3$ will require treatment as LLW.</td>
<td>Monitoring results from 2009 • Total alpha activity below WHO safe drinking water limit (0.5 Bq l$^{-1}$) in all but 5 cases • Total beta activity above WHO safe drinking water limit (1 Bq l$^{-1}$) with a highest annual average being 129 000 Bq l$^{-1}$. Groundwater plumes contain $^{90}$Sr, $^{137}$Cs, $^3$H and $^{99}$Tc. Run off water contains: • 0.7 to 44 μBq kg$^{-1}$ dissolved $^{239+240}$Pu • 1.2 to 400 Bq kg$^{-1}$ particle bound $^{239+240}$Pu.</td>
<td>ref. 8, 10, 11</td>
</tr>
<tr>
<td>AWE Aldermaston/ Burghfield</td>
<td>Former RAF site/former munitions site Production/service/ decommissioning of nuclear weapons</td>
<td>Maintenance and decommissioning of nuclear weapons</td>
<td>$^{239+240}$Pu on settled sediment varies from 15–155 Bq kg$^{-1}$.</td>
<td></td>
<td>ref. 4</td>
</tr>
<tr>
<td>Dounreay</td>
<td>RAF site converted to UK’s centre for fast reactor R&amp;D</td>
<td>All reactors closed down, site is in final decommissioning stage (closure expected 2032)</td>
<td>Irradiated fuel particles found on local beaches. $^{137}$Cs contamination on site in small pockets of &gt; 4 Bq g$^{-1}$.</td>
<td>Legacy of irradiated particles discharged into sea during 1960/70s. Particles detected on seabed around Dounreay.</td>
<td>ref. 14, 15</td>
</tr>
<tr>
<td>Site</td>
<td>Former uses</td>
<td>Current use</td>
<td>Soil contamination</td>
<td>Groundwater contamination</td>
<td>Sources</td>
</tr>
<tr>
<td>------</td>
<td>-------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>-------------------------------------</td>
<td>-----------------------------------</td>
<td>---------</td>
</tr>
</tbody>
</table>
| Mayak | Nuclear weapons production, reprocessing nuclear material | Produces radioisotopes and electrical equipment for monitoring, reprocesses fuel, decommissioning | HLW tank exploded in 1957  
- 740 PBq released  
- 90% contaminated immediate vicinity.  
- Parts of the Mayak site have dose rates up to 15 mR h⁻¹. | Open reservoirs on site contain 340 million m³ of radioactive water.  
In 1993:  
- 70 MBq l⁻¹ ⁹⁰Sr and 100 MBq l⁻¹ ¹³⁷Cs in Reservoir 9, (Lake Karachay, R9) were measured  
- groundwater pollution plume from R9 covers 10 km², spreading at 80–100 m y⁻¹. | ref. 20 Standring, 2009 |
| Rocky Flats | Manufacture of nuclear weapons | Cleanup and closure completed in 2005. Most of the land transferred to US Fish and Wildlife Service 2007 for use as reservation | Soils from a contaminated toposequence contain activities of:  
- ²³⁹+²⁴⁰Pu in the range 2220 to 11 460 Bq kg⁻¹  
- ²⁴¹Am in the range 1840 to 8840 Bq kg⁻¹. | ref. 31 |
<table>
<thead>
<tr>
<th>Location</th>
<th>Origin/Activity</th>
<th>Site Management</th>
<th>Uranium and Tc Concentrations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oak Ridge</td>
<td>Separation of uranium for Manhattan Project</td>
<td>National Lab managed by DoE</td>
<td>Sorbed and precipitated uranium concentrations up to 800 mg kg⁻¹, Hg up to 2400 µg g⁻¹ in floodplains along East Fork Popular Creek. Soluble uranium in groundwater plume (up to 210 µM). Leakage from S-3 ponds has created a plume containing uranium (up to 0.2 µM) and Tc (up to 47 nM).</td>
<td>ref. 38, 39</td>
</tr>
<tr>
<td>Hanford</td>
<td>Plutonium production, nuclear reactors</td>
<td>Decommissioning and cleanup</td>
<td>68 out of 149 tanks known or thought to have leaked HLW into sediments beneath them. Pu found in silt layers at up to 9.25 MBq kg⁻¹. Caesium-137 as high as 10⁵ Bq g⁻¹ in contaminated sediments. In 1951, 3.5 × 10⁵ l of highly radioactive waste leaked into subsurface containing an estimated 7000 kg of U, Tritium and ¹²⁹I present in groundwater at above drinking level limits. Tc, U, Pu ⁶⁰Co, ¹³⁷Cs also detected above drinking levels.</td>
<td>ref. 42, 44, 49</td>
</tr>
<tr>
<td>Rifle</td>
<td>Former uranium processing</td>
<td>UMTRA managed site</td>
<td>Uranium concentrations in a contaminated aquifer range from 0.4 to 1.4 µM</td>
<td>ref. 28</td>
</tr>
</tbody>
</table>
since the 1940s with the site containing the world’s first commercial nuclear power station, Calder Hall. Operations at the Sellafield site include spent fuel reprocessing, mixed oxide fuel fabrication (MOX) and nuclear waste storage and management. Discharges into the environment from Sellafield began in 1951 and first became subject to formal authorisation in August 1954 under the “Atomic Energy Authority Act 1954”. Prior to 1954, discharges were subjected to controls derived from consultation with site operators and government departments. Current disposal of radioactive waste is regulated under the “Environmental Permitting (England and Wales) Regulations 2010” (EPR).

During reprocessing, plutonium, uranium and highly radioactive fission products are separated by a series of solvent extractions which results in some of these products being concentrated in aqueous waste. Highly radioactive aqueous waste is added to an acid effluent stream for evaporation and storage and is now being converted into vitrified waste. Low level aqueous waste is discharged into the Irish Sea via pipelines extending 2.5 km from the high water mark. These low level discharges have created an environmental inventory, over the period of 1952–1990, of around $1.1 \times 10^2$ TBq of $^{238}$Pu, $6.1 \times 10^2$ TBq of $^{239,240}$Pu, $1.3 \times 10^8$ TBq of $^{241}$Pu and $9.4 \times 10^2$ TBq of $^{241}$Am (with about $3.6 \times 10^2$ TBq of the americium having been derived from decay of $^{241}$Pu released). Around 90% of the Pu, in its insoluble Pu(IV) state, was retained rapidly by the sediment in the Irish Sea along with the vast majority of the discharged Am. The remaining 10% of plutonium, in the more soluble Pu(V) state, remained in solution and was transported out of the Irish Sea. Since 2006, beach monitoring has detected a number of contaminated sites resulting from the Sellafield discharges, although they are generally less active than those found in Dounreay.

Approximately 1600 m$^3$ of soil around the centre of Sellafield has been contaminated by spillage and reprocessing and will have to be treated as intermediate level waste (ILW). This area overlies an aquifer in the underlying sandstone geology, which is significantly contaminated to the southwest due to leaching of the contaminated soil from above. An estimated 1 000 000 m$^3$ of soil will require treatment as low level waste (LLW). Sellafield is also responsible for the storage of the majority of the UK’s nuclear waste products and, as such, a large inventory of varying levels of radioactive waste is stored on site either awaiting disposal or for the activity to decrease.

Two site investigations have been conducted at Sellafield over the past decade in an attempt to identify and develop conceptual models of below ground contamination. The first phase of the report was completed in 2004 and examined contamination outside of the Sellafield “Separation Area”, where fuel re-processing and fabrication took place, with the second report focussing on contamination within the Separation Area expected to be completed in 2010.

Soil sample records from over 2000 boreholes have demonstrated that radioactively contaminated ground exists beneath and, occasionally, outside the Separation Area. Groundwater monitoring throughout the site has revealed that radioactive contamination is present in distinct plumes in the groundwater which are migrating in the direction of the hydraulic gradient. These contaminants include $^{90}$Sr, $^{137}$Cs, $^3$H and $^{99}$Tc, with actinides also expected.
The maximum activity of the most mobile contaminant, tritium, is around $1.0 \times 10^7$ Bq m$^{-3}$ in contaminated groundwater found in boreholes close to the Separation Area. The activity decreases down the hydraulic gradient towards the River Ehen, until it becomes undetectable (below $1.0 \times 10^5$ Bq m$^{-3}$). Technetium-99, although derived from a different source, becomes a co-contaminant with the tritium in a common plume as they both migrate downgradient. The $^{99}$Tc is known to be a contaminant in the upper strata of the sandstone bedrock and has also been found in monitoring wells as far as the site boundary. The maximum concentration of $^{99}$Tc found in this plume during the phase 1 site investigation was $2.3 \times 10^5$ Bq m$^{-3}$, located near to the site main gate. Strontium-90, which has limited solubility and readily adsorbs to sediments at Sellafield, is detectable in monitoring wells inside the Separation Area where it is mostly contained. Beta activity from the $^{90}$Sr is also detected in two plumes, including the plume contaminated with $^3$H and $^{99}$Tc. Caesium-137, the only other radioactive isotope detected in the groundwater plumes, was found to be present only in very low concentrations and only in filtered solids.

Monitoring of 137 boreholes was conducted for the Sellafield Ltd Groundwater Annual Report and is summarised in Table 2. Although the majority of boreholes contain activity below the WHO drinking standard for total alpha, tritium and technetium activity, there are a significant number of boreholes with total beta activity above the WHO drinking standard. Strontium-90 makes up the bulk of the total beta activity, with caesium-137 also contributing significant activity. However, when both isotopes are examined on an individual

<table>
<thead>
<tr>
<th>Activity analysed</th>
<th>WHO drinking water standard (Bq l$^{-1}$)</th>
<th>Boreholes where WHO standard is exceeded</th>
<th>Location of boreholes</th>
<th>Major isotopes</th>
<th>Highest annual average activity (Bq l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total alpha activity</td>
<td>0.5</td>
<td>5</td>
<td>Within the Separation Area</td>
<td>Uranium isotopes</td>
<td>103</td>
</tr>
<tr>
<td>Total beta activity</td>
<td>1</td>
<td>46</td>
<td>Predominately within Separation Area with several to the south. A minority found close to the River Calder’s west bank</td>
<td>$^{90}$Sr and $^{137}$Cs</td>
<td>129 000</td>
</tr>
<tr>
<td>Tritium</td>
<td>10000</td>
<td>3</td>
<td>Outside south-west corner of Separation Area</td>
<td>$^3$H</td>
<td>39 200</td>
</tr>
<tr>
<td>Technetium</td>
<td>100</td>
<td>1</td>
<td>Between south-west corner of Separation Area and the site main gate</td>
<td>$^{99}$Tc</td>
<td>111</td>
</tr>
</tbody>
</table>
basis, then fewer samples exceed the WHO drinking standard for $^{90}\text{Sr}$ and no samples exceed the $^{137}\text{Cs}$ safe drinking limit. The majority of boreholes with values above the WHO standard are located within the Separation Area with a number also located to its south-west.

The former storage and de-canning facility, known as B-30, houses a pond used for the storage of spent nuclear fuel until its replacement facility, the Fuel Handling Plant, was commissioned in 1986. Although now closed, the storage pond is thought to contain 300 to 450 tonnes of spent nuclear fuel. Fuel was stored in the pond for longer than was anticipated due to an accident at the Magnox reprocessing facility in 1974 causing corrosion of the fuel cans and leakage of radiation into the pond.

### 2.1.2 Dounreay

Another former RAF site, Dounreay, became the centre for the UK’s fast reactor research and development in 1954. Commercial energy production began in 1962 becoming the first fast reactor in the world to supply energy to the grid. However, fast reactor technology proved to be more expensive than was first thought and consequently all fast reactor programs ceased operations in 1994. Reprocessing and fuel fabrication operations ended in 1996 and 2004, respectively. Dounreay is now wholly a decommissioning site owned by the NDA and run by Dounreay Site Restoration Ltd. The site closure program is scheduled to be completed by 2025 at an estimated cost of £2.6 bn. Over the course of decommissioning, Dounreay is expected to generate a lifetime waste of 97 126 m$^3$ of LLW, 3164 m$^3$ of ILW and 0 m$^3$ of high level waste (HLW).$^{12}$ Dounreay has a legacy of irradiated nuclear fuel particles which were discharged into the sea as a result of reprocessing activities during the 1960s and 1970s. These particles have been detected on the seabed around Dounreay with the most hazardous fragments located close to the old discharge point on the seabed. Their disintegration is believed to be the source of smaller, less hazardous particles detected on local beaches. Around 1000 significant ($10^6$ Bq of $^{137}\text{Cs}$). 1000 relevant ($10^5$ to $10^6$ Bq of $^{137}\text{Cs}$) and 3000 minor (< $10^5$ of $^{137}\text{Cs}$) particles are thought to be present within the main particle plume offshore from Dounreay.$^{13}$ Monitoring of the particles is expected to last until 2020s and with a total cost estimated at £18–25 million.

On site, there are pockets of caesium-137 contamination with activities greater than 4 Bq g$^{-1}$, although the majority of contamination over the site is below 0.4 Bq g$^{-1}$. Between 1959 and 1971, solid ILW was disposed of in the Dounreay waste shaft. A purpose built wet silo was constructed in 1971 as an alternative to the shaft after which solid ILW was tipped into the silo, a large underground concrete vault filled with water. Large items too big for a purpose built wet silo continued to be disposed of down the shaft until 1977, when an explosion in the airspace above the water column damaged the shaft cover. There are uncertainties over the exact contents of the shaft, thought to include contaminated equipment, chemicals, natural uranium fuel, radioactive sources and sludges.$^{15}$ A total of 703 m$^3$ of waste is covered by a water column 8 m deep which is below sea level so that groundwater flow is towards the shaft.
2.2 Russia

A large and complicated nuclear legacy has been left behind by the break up of the Soviet Union with numerous nuclear facilities located in Russia and other former Soviet states. Although civilian activities have contributed to this legacy, the majority of contamination issues in the former Soviet Union were created by military nuclear facilities used for the production of nuclear weapons. This problem was exacerbated by a previously relaxed attitude towards environmental issues with regards to nuclear waste disposal. Three nuclear facilities, Chelyabinsk-65, Tomsk-7, and Krasnoyarsk-26 operated in secret in the Ural mountains during the Cold War and were not subject to strict environmental practices. Of these sites, Chelyabinsk-65 (Mayak) is the most publicised regarding its former activities, revealing a long history of accidental release and discharges, contributing to significant environmental contamination which will be discussed in greater detail below.

The worst nuclear power plant accident in history, the only level 7 event on the International Nuclear Event Scale to have occurred, happened on the 26th of April 1986 at Chernobyl, when a test was carried out to determine the ability of a turbine generator to provide power in the event of a station blackout. Serious violations of safety procedures and operating rules resulted in a steam explosion, cutting cooling channels on both sides of the reactor core resulting in a further explosion. The release of $^{137}$Cs from the explosion is estimated to have been around 85 PBq with an estimated 1760 PBq of $^{131}$I, 10 PBq of $^{90}$Sr and 3 PBq of plutonium isotopes also released.

2.2.1 Mayak

Mayak, formerly known as Chelyabinsk-40 and later as Chelyabinsk-65, is one of the biggest nuclear facilities in the Russian Federation housing the former Soviet Union’s first industrial nuclear reactors. The facility was responsible for producing the material for the country’s first atomic bomb beginning in 1948.

Between the commencement of operations in 1948 through to September 1951, 78 million m$^3$ of high-level nuclear waste containing a total of 10$^{17}$ Bq of beta activity was discharged from the radiochemical plant directly into the Techa River six kilometres below its source. A radiation survey in 1951, revealed extensive contamination of the floodplain and bed of the Techa River and consequently excessive exposure to the inhabitants of the region. Of the total radioactivity discharged into the Techa, 99% was deposited in the first 35 km downstream. Four reservoirs were created along the Techa below Lake Kzyzyltash to isolate the most contaminated water. The final reservoir was completed in 1964 and including Lake Kzyzyltash, now contains a volume of 380 million m$^3$ and about 7141 TBq of $^{90}$Sr and $^{137}$Cs.

Discharge of diluted HLW directly into the Techa stopped in 1951 and instead was diverted into Lake Karachay. In 1953, an intermediate waste storage tank was put into operation but the excess supernatant (containing much of the caesium waste) was still discharged into the lake (29.6 PBq was added in 1992). A hot summer, followed by a dry winter in 1967, caused evaporation of
the lake and dust from the shoreline containing around 20 TBq of $^{90}$Sr and $^{137}$Cs (in a 1 : 3 ratio) was blown over an area of 1800 km$^2$ and up to a distance of 75 km.\textsuperscript{21} Up to four million m$^3$ of contaminated groundwater, containing in excess of 185 TBq has migrated 2.5 to 3 km away from Lake Karachay.\textsuperscript{20}

As previously discussed, a waste storage facility became operational in 1953 and consisted of 20 stainless steel tanks utilising an external cooling system involving water flowing through a gap between the tank walls. The cooling system for one of these tanks failed and resulted in the evaporation, heat-up and ultimately explosion of the 70–80 tonnes of highly radioactive nitrate–acetate waste.\textsuperscript{22} Around 740 PBq of activity was ejected from the explosion with approximately 90\% falling out in the immediate vicinity of the accident and the remaining 10\% forming a cloud extending to a height of 1 km. The fallout from this cloud exposed the Chelyabinsk, Sverdlovsk, and Tyumen regions to contamination.\textsuperscript{22} In the immediate area, 1 to 2 km long by 0.5 to 1 km wide, the soil contamination amounted to 5180 TBq km$^{-2}$ with contamination of the wider area, 75 km long and 7 km wide, amounting to around 1 TBq km$^{-2}$.\textsuperscript{21}

\subsection*{2.3 United States of America}

Like the United Kingdom, the United States has accumulated a nuclear legacy from over 60 years of research, production, use and storage of nuclear materials. Over this time frame, nuclear material was produced for use in both civilian power plants and in military weapons. The Department of Energy (DOE)'s 120 sites contain 40 million cubic meters of contaminated soil and debris and 1.7 trillion gallons of contaminated groundwater. Of this, at least 50\% is contaminated with radionuclides, including caesium-137, plutonium-239, strontium-90, technetium-99, uranium-238, and uranium-235, as well as heavy metal contamination including chromium, lead and mercury.\textsuperscript{23} The associated cleanup cost has been estimated to be in excess of a trillion dollars.\textsuperscript{24} Contaminated sites include former uranium ore processing facilities such as Rifle, Colorado and Moab, Utah. The clean-up of these sites was tasked to the DOE under the Uranium Mill Tailings Remedial Action (UMTRA). During the years of operation of the Moab site, approximately 10.5 million tons of tailings and contaminated soils accumulated in an unlined pile 750 feet from the Colorado River.\textsuperscript{25} In 2005, the DOE finalised the remediation strategy to be undertaken at Moab which included active ground water remediation and off-site disposal of the tailings pile and other contaminated materials at the Crescent Junction disposal site.\textsuperscript{26} The Rifle site consists of two old uranium processing plants: Rifle Old Processing Site and Rifle New Processing Site. Tailings and tailings-contaminated material from Rifle were transferred to the Rifle disposal site approximately six miles north of the Rifle New Processing Site and surface remediation was completed in October 1996. Contaminants of concern in the groundwater at both sites include arsenic, molybdenum,
selenium, nitrate, uranium and vanadium, with contamination at New Rifle extending approximately three miles west of the site. Groundwater remediation is being achieved through natural flushing of the groundwater in conjunction with contaminant monitoring. The *in situ* remediation of uranium was examined in a field scale study in 2003 in which acetate was injected into the subsurface over a three month period in order to stimulate microbial reduction of soluble U(vi) to insoluble U(iv) (ref. 28) and is discussed in detail later in this chapter. The Savannah River Nuclear Facility, South Carolina, was used to refine nuclear material for use in the United States defense program. The site used a system of canals and reservoirs to disperse heat from the reactors and consequently, various ponds connected to this system received cooling water discharges from the reactors. One such pond, Pond B, received discharges containing fission products such as $^{137}$Cs, $^{90}$Sr and $^{239}$Pu. Radionuclide input peaked in 1963 and 1964, believed to be caused by the leakage of fuel elements stored in a water-filled basin in the reactor. The vast majority of both $^{137}$Cs (98%) and $^{90}$Sr (85%) were found to be in the pond sediments.

A number of other sites located in the United States have a more complicated environmental legacy left by the nuclear industry and are discussed below in more detail.

### 2.3.1 Rocky Flats

The Rocky Flats Environmental Technology Site, formerly Rocky Flats Nuclear Weapons Plant, is located northwest of Denver, Colorado, and between the years 1952 and 1989 was responsible for the production of components for the United States nuclear weapon program. This involved the use of various radioactive materials such as plutonium and uranium as well as toxic metals and hazardous solvents. Two main events are responsible for the release of plutonium outside the Rocky Flats Plant boundaries. These events were a fire that occurred in the plutonium processing building in 1957 and wind-blown releases occurring mainly during 1968 and 1969 from an outdoor waste storage area called the 903 Area. An estimated 5000 gallons of plutonium-contaminated waste leaked from the waste containers covering an area of 22 500 m$^2$ according to monitoring conducted in 1968. Windstorms in 1968 and 1969 blew the plutonium-contaminated soil off the site thus contaminating a much greater area, with an estimated 66.6 to 518 GBq of $^{239+240}$Pu released to the off-site environment.

Soil samples analysed from a series of pits along a contaminated toposequence at Rocky Flats revealed plutonium contamination ranging from 2220 to 11 460 Bq kg$^{-1}$, with a mean activity of 7250 Bq kg$^{-1}$, and $^{241}$Am contamination ranging from 1840 to 8840 Bq kg$^{-1}$, with a mean activity of 5480 Bq kg$^{-1}$. The activity was primarily located in the uppermost layer of the soil with 90% of the contaminants distributed in the top 20 cm in four of the five pits.

Synchrotron radiation studies revealed the oxidation state of the plutonium in the soils and concrete as Pu(v) and identified its chemical form as the insoluble hydrous oxide PuO$_2$·xH$_2$O (ref. 32) with transport of the plutonium likely confined to fine particle migration.
2.3.2 Oak Ridge

The Oak Ridge Reservation in east Tennessee consists of about 357,250 acres of land and contains the Y-12 Plant which played a historical role in the production of nuclear weapons. Oak Ridge also houses one of the three DOE gaseous diffusion plants (K-25 Plant) used to enrich uranium. The Y-12 facility itself encompasses 324 hectares and is associated with mercury contamination arising from its use in nuclear weapons production through the 1950s and 1960s. An estimated 108,000 to 212,000 kg of mercury was released into the headwaters of the East Fork Poplar Creek over this time frame from the Y-12 plant.

Further studies have showed that 771,800 kg of mercury are contained in the sediments and floodplain soils of a 15 mile stretch of the East Fork Poplar Creek which has its headwaters at Y-12. Some 227 kg are thought to leave this watershed annually. The concentration of mercury in soils from the floodplains ranges up to 2400 μg g⁻¹ with an average of greater than 70% found to be metallic mercury and mercureic sulfide.

Between the years 1951 to 1983, liquid acidic wastes (pH < 2.0) containing metals (including uranium) dissolved in nitric acid were discharged into four seepage pits on the Y-12 complex known as the S-3 Ponds. The ponds were neutralised in 1983 by the addition of limestone, quicklime and sodium hydroxide until the pH reached greater than 9.0 resulting in the precipitation of calcium, iron and aluminium compounds. Leakage from the S-3 ponds has created a contaminated groundwater plume in the underlying shale bedrock which extends more than 2 km both east and west of the ponds. Analysis of soils from this site has revealed sorbed and precipitated uranium concentrations of up to 800 mg kg⁻¹. A summary of groundwater contaminants in the plume extending from the S-3 ponds is given in Table 3.

Uranium and technetium are likely to exist as the mobile U(VI) (as UO₂^{2+}) and Tc(VII) (as TcO₄⁻) forms, although in the low pH conditions and in the presence of high nitrate and sulfate concentrations the uranium may be associated with nitrate (as UO₂NO₃⁻) or sulfate (as UO₂SO₄ or UO₂(SO₄)₂⁻). These conditions present problems for remediation strategies such as in situ bioremediation as the groundwater requires pre-treatment so that conditions are favourable for

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Maximum concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>0.2 mM</td>
</tr>
<tr>
<td>Tc</td>
<td>47 nM</td>
</tr>
<tr>
<td>Al</td>
<td>18 mM</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>100 mM</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>100 mM</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>25 mM</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>8 mM</td>
</tr>
<tr>
<td>Co²⁺</td>
<td>0.02 mM</td>
</tr>
<tr>
<td>Ni²⁺</td>
<td>0.2 mM</td>
</tr>
</tbody>
</table>
microbially mediated reduction, discussed later in this chapter. High nitrate concentrations can potentially present further problems for remediation as in addition to being a competing electron donor to U(VI), it is also an effective oxidant of U(IV), leading to the oxidation and remobilisation of U(VI) through a variety of possible mechanisms.\textsuperscript{40}

2.3.3 Hanford

The Hanford Nuclear Reservation, located on the Columbia River in Washington State, USA, was a plutonium production site which began operation in 1945. As a consequence of its former activities, a number of contamination issues have arisen. It has been estimated that more than 436 TBq of \(^{239}\)Pu, 1065 TBq of \(^{241}\)Am and 2 TBq of \(^{237}\)Np were disposed of as liquid waste to the near surface at Hanford,\textsuperscript{41} with 86\% of the \(^{239}\)Pu, 97\% of the \(^{241}\)Am and 77\% of the \(^{237}\)Np released into the Plutonium Finishing Plant (PFP) zone of the site. Despite the release of large quantities of radionuclides into the Hanford Vadose zone, only negligible amounts have entered the groundwater. Filtered samples from on site wells have so far shown no activity above the DOE-derived guide for \(^{239}\)Pu (1.11 Bq l\(^{-1}\)) and only two unfiltered samples taken in 2006 exceeded this limit (1.3 and 1.5 Bq l\(^{-1}\)).\textsuperscript{41}

Although the majority of plutonium on site is reported to be immobile, there are areas where vertical migration of plutonium and americium has occurred. The Z-9 trench is considered a “worst case” representation of the disposal area due to the acidic (pH 2.5), high salt waste solution containing nitrate (\~5 M), aluminium (\~0.6 M) and organic solvents. As much as 140 kg of plutonium was disposed of in this waste and although \~58 kg of plutonium was recovered in 1978, \(^{239+240}\)Pu has been found to be concentrated (up to 9.25 MBq kg\(^{-1}\)) in silt layers 15–20 m below the surface correlating with the occurrence of co-disposed tributylphosphate (TBP).\textsuperscript{42} Americium-241 was also found to be accumulated at this point, and also concentrated at a second horizon at the bottom of the underlying fine-grained Cold Creek Unit (\~40 m below ground surface) at levels greater than 11.1 MBq kg\(^{-1}\) with no accompanying TBP.\textsuperscript{42} A number of possible conditions may have contributed to this vertical migration including the acidic nature of the waste, formation of soluble complexes and suspension and transport of colloids or nanoparticles.

In order to generate the \(^{239}\)Pu needed to produce nuclear weapons, a very large quantity of uranium (either as metal or UO\(_2\)) was irradiated. The subsequent retrieval of plutonium from the matrix resulted in a large volume of aqueous waste containing high concentrations of uranium. This waste (also containing other fission products) is stored in 177 underground steel tanks in different areas of the Hanford site referred to as “Tank Farms” which are subdivided into Waste Management Areas. A large number (68 out of 149) are known or suspected to have leaked to date with the largest release occurring in 1951 in the 241-BX-Tank Farm. Nearly 3.5 \times 10^7 litres of highly radioactive waste containing more than 7000 kg of uranium was released into the subsurface.\textsuperscript{43} Between the years 1944 and 1988, almost two million cubic meters of
tank waste was generated with subsequent evaporation, discharge, chemical treatment and leakage reducing this volume to 200,000 cubic meters. This makes up to 60% of the current tank waste which contains around $7.03 \times 10^6$ TBq of radioactivity and 170,000 tonnes of chemicals with each cubic meter of tank waste containing nearly 37 TBq of radioactivity.\(^{44}\)

The mobility of uranium in the contaminated sub-surface beneath the tank farms has been shown to vary depending on the surface phases present at different depths. Uranium silicate precipitates were found in relatively shallow sediments, whereas the uranium was found adsorbed to sediment surfaces at intermediate and deeper depths, both in the form of U(\text{vi}).\(^{45}\) Migration of uranium in the shallow sub-surface may therefore be slow as it relies on the slow process of mineral dissolution. In contrast, migration may be relatively fast in deeper conditions as surface desorption processes occur over a faster time frame. Work conducted on sediments taken from boreholes near to the storage tanks at Hanford revealed that the uranium is again predominantly found in the U(\text{vi}) state, with approximately 51% to 63% labile and therefore potentially mobile, with the remaining portion locked up in mobilization-resistant phases.\(^{46}\)

A history of liquid waste disposal activities is provided by Gephart\(^{44}\) and is briefly summarised here. Liquid waste has been dealt with via a number of methods during the operations of the Hanford site. In 1944, during fuel reprocessing, liquids which were only mildly contaminated were dumped into depressions on the ground, contaminating both the sandy sediments and eventually the groundwater. Some of these contaminants were blown downwind thus contaminating an even greater area. When dumping of these liquid wastes was halted they were instead pumped down reverse wells which lead to contaminants being injected closer to, or directly into, the underlying hydrology, bypassing the overlying sediment which could otherwise have acted, via sorption, as a sink. When this process was stopped after only a few months, liquid wastes were pumped directly into shallow buried box structures, gravel-filled tile fields, buried concrete pipes and open trenches later backfilled with gravel.

These processes, compounded by tank leakages, have led to the contamination of up to 28,300 m\(^3\) of soil,\(^{47}\) which along with the contaminated groundwater contains around 8325 TBq of \(^{137}\)Cs, 6660 TBq of \(^{3}\)H, 1924 TBq of \(^{90}\)Sr, 1850 TBq of Pu and 25.9 TBq of \(^{99}\)Tc (ref. 44). Groundwater underlying around 12% of the Hanford site contains carbon tetrachloride, chromium, nitrate, \(^{90}\)Sr, \(^{99}\)Tc, \(^{129}\)I and uranium at levels above the drinking water standard.\(^{48}\) Although the site groundwater is not a source of public drinking water and does not significantly affect off-site water resources, contaminants such as \(^{99}\)Tc and \(^{129}\)I are mobile in groundwater and thus can migrate deep into the vadose zone and could potentially enter aquifers.

Earlier work estimated that up to $4 \times 10^{16}$ Bq of \(^{137}\)Cs had leaked into the vadose zone from the tank farms with measured activity from contaminated sediments as high as $10^5$ Bq g\(^{-1}\).\(^{49}\) The waste stored in these tanks also typically contained a significant concentration of high ionic strength solutions including NaNO\(_3\) (>0.5 mol\text{-1}).\(^{49}\) In the presence of high salt concentrations, caesium
was only found to absorb to high-affinity, frayed edge sites of mica minerals with sodium being an effective competitor for such sites. The high sodium released in the leaked Hanford waste may therefore prevent the retardation of $^{137}$Cs at the site. Borehole data also suggests that caesium is not undergoing significant sorption as peak $^{137}$Cs activities were detected between 20 and 26 m, reaching up to around 40 m, beneath the SX tank farm responsible for the majority of the caesium release.

3 Depleted Uranium

Contamination from depleted uranium (DU) is an issue covering several sites worldwide. The properties of DU, such as its high density (19.05 g cm$^{-3}$) and penetrating strength, have led to its use in a number of civil and military applications including munitions. Such munitions have been used in a number of conflicts over the past few decades with a summary provided in Table 4. Many of these rounds miss their target and can penetrate some distance into the ground.

The experimental test firing of depleted uranium munitions is also responsible for contamination at various firing ranges in both the UK and the USA. The UK Ministry of Defence (MOD) estimates that 15 tonnes of DU rounds were fired at an armour plate at the Eskmeals firing range in Cumbria between 1981 and 1995, with an additional 30 tonnes fired into the Solway Firth at Kirkcudbright, Scotland since 1982 (ref. 52). Experimental firing of DU rounds began in the USA at the Aberdeen and Yuma proving grounds in the early 1970s. More than 70 tonnes of DU have been deposited over 1500 acres at the Aberdeen Proving Ground, Maryland, into the sediments and the aquatic environment.

<table>
<thead>
<tr>
<th>Conflict Zone</th>
<th>Contamination</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iraq and Kuwait (1990–1991)</td>
<td>321 tonnes of DU</td>
<td>United States</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Air Force fired 783,514 rounds of 30 mm DU ammunition</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Army fired 9552 DU tank rounds</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Less than one hundred 120 mm DU rounds</td>
</tr>
<tr>
<td></td>
<td></td>
<td>United Kingdom</td>
</tr>
<tr>
<td>Bosnia-Herzegovina (1994–1995)</td>
<td>3 tonnes of DU</td>
<td>NATO airstrikes</td>
</tr>
<tr>
<td>Kosovo (1999)</td>
<td>10 tonnes of DU spread over 112 sites</td>
<td>• About 10,800 DU rounds</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A-10 antitank aircraft fired ~30,000 rounds (30 mm)</td>
</tr>
<tr>
<td>Iraq (2003)</td>
<td>2 tonnes known 170–1700 tonnes speculated</td>
<td>2 tonnes fired by the UK MOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amount fired by USA forces not yet disclosed but speculative figures range from 170 to 1700 tonnes</td>
</tr>
</tbody>
</table>
Natural and depleted uranium share a similar chemotoxicity but the radio-
toxicity is around 60% higher for the former. The low specific radioactivity
combined with the dominance of alpha emissions means that no acute risk is
associated with external exposure to DU but internal exposure presents serious
health risks. Therefore, the main risk arises from DU dust generated from the
impact of DU munitions on hard surfaces. Re-suspension of settled DU dust
can occur if the particle size is sufficiently small. Traces of $^{236}$U and $^{239+240}$Pu
have been found in DU penetrators collected in Kosovo and trace amounts
of americium, neptunium and $^{99}$Tc are also thought to be present in DU. $^{56}$

A review by the Royal Society $^{57}$ estimates that in a worst case scenario for DU
exposure in the battlefield, a soldier who experiences level I exposure to DU
(exposure dominated by inhalation of aerosols generated by DU impact) has an
increased risk of 1.2 per 1000 of death from lung cancer. However, they cite that
poor data collection on battlefield exposure makes estimating such health risks
very difficult. DU fragments left on the battlefield also pose a concern as a slightly
increased risk of skin cancer is expected from long-term exposure to DU pen-
etrators. This is of particular concern for children who may be attracted to such
objects. DU penetrators remaining in the ground also pose a longer term risk
through potential migration to food sources or into water supplies. The mobility
of the DU from the contaminated “hotspot” depends on a number of factors
including corrosion rates, DU particle re-suspension, and proximity to surface
soils and water sources. Although this form of radionuclide contamination has
been the focus of much recent media interest, there is comparatively little work
published on the scale of the problem, or strategies to decontaminate environ-
ments contaminated by DU munitions. However, the reader is directed to a
recent review on the environmental fate of DU for a more detailed critique. $^{58}$

4 Remediation

A number of different techniques are available for the remediation of both
groundwater and soils and can be categorised into biological, chemical and
physical treatments. This review will look to provide an overview of some of
these key techniques and will then focus on a number of case studies where
these methods have been applied in the field. The advantages and drawbacks of
the major techniques are summarised in Table 5.

4.1 Bioremediation

Biological treatments, referred to as bioremediation, encompass several tech-
niques which can involve the redox transformation, biological accumulation or
breakdown of a contaminant. Chemical speciation (oxidation state and com-
plex form) is one of the primary controls on the mobility of metal contaminants
in the environment, affecting both their solubility and reactivity with surfaces.
For example, the metal chromium is mobile and highly toxic in the Cr(VI) state,
but is both less mobile and up to 1000 times less toxic as the Cr(III) oxidation
state. $^{59}$ The radionuclide, $^{60}$Co can form a stable and mobile complex with
<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotransformation</td>
<td>Can be performed <em>in situ</em> and <em>ex situ</em>.</td>
<td>Potential for re-oxidation and re-mobilisation of metals and radionuclides.</td>
</tr>
<tr>
<td></td>
<td>Relatively low cost compared to physiochemical methods.</td>
<td>Complex groundwater or soil chemistry can complicate or prohibit treatment.</td>
</tr>
<tr>
<td></td>
<td>Highly selective treatment of contaminants.</td>
<td>Regular monitoring required to assess effectiveness.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Can only operate in conditions required for cell growth (i.e. limited pH range).</td>
</tr>
<tr>
<td>Biosorption</td>
<td>Can be performed <em>in situ</em>.</td>
<td>Early saturation can require metals desorption to continue use.</td>
</tr>
<tr>
<td></td>
<td>No additional nutrients required.</td>
<td>No potential for degradation of compounds.</td>
</tr>
<tr>
<td></td>
<td>Relatively low cost compared to physiochemical methods.</td>
<td>Targeting certain contaminants may require the cultivation and introduction of species not natively present.</td>
</tr>
<tr>
<td></td>
<td>Not governed by physiological constraints of living cells.</td>
<td>Very limited commercial application.</td>
</tr>
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<td></td>
<td>No secondary waste produced.</td>
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<td></td>
<td>Metal recovery is possible, especially from process waters.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Specific contaminants can be targeted.</td>
<td></td>
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<tr>
<td>Bioaccumulation</td>
<td>Can be performed <em>in situ</em>.</td>
<td>Requires subsurface conditions favourable for microbial metabolism.</td>
</tr>
<tr>
<td></td>
<td>Relatively low cost compared to physiochemical methods.</td>
<td>Toxicological effect on cell may inhibit cell metabolism or lead to cell death.</td>
</tr>
<tr>
<td></td>
<td>No secondary waste produced.</td>
<td>Targeting certain contaminants may require the cultivation and introduction of species not natively present.</td>
</tr>
<tr>
<td></td>
<td>Specific contaminants can be targeted <em>e.g.</em> Cs⁺ transported by K⁺-uptake processes.</td>
<td>Very limited commercial application.</td>
</tr>
<tr>
<td>Biomineralisation</td>
<td>Relatively low cost.</td>
<td>May only operate over a specific pH range in certain cases.</td>
</tr>
<tr>
<td></td>
<td><em>In situ</em> technique.</td>
<td>Mineral precipitation may clog pore spaces restricting groundwater flow to contaminants further from injection wells.</td>
</tr>
<tr>
<td></td>
<td>Metals can be immobilised in the subsurface so no further treatment of waste is required.</td>
<td></td>
</tr>
<tr>
<td>Phytoremediation</td>
<td>Can be performed <em>in situ</em>.</td>
<td>Treatment is limited to the surface area and depth of the plant roots.</td>
</tr>
<tr>
<td></td>
<td>Cheaper than most other <em>ex situ</em> and <em>in situ</em>.</td>
<td>Possibility of contaminants entering the food chain.</td>
</tr>
<tr>
<td></td>
<td>Plants can be easily monitored to assess effectiveness.</td>
<td>Slow growth and low biomass require long-term commitment.</td>
</tr>
<tr>
<td></td>
<td>Recovery and re-use of valuable metals is possible.</td>
<td>Saturation of contaminants may lead to toxicity affecting plant survival.</td>
</tr>
<tr>
<td></td>
<td>Non-environmentally disruptive.</td>
<td></td>
</tr>
<tr>
<td>Technique</td>
<td>Advantages</td>
<td>Disadvantages</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Chemical</td>
<td>Can be performed <em>in situ</em>.</td>
<td>Non-selective</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Rapid treatment time.</td>
<td>High capital and operating costs.</td>
</tr>
<tr>
<td>Sediment</td>
<td>Ability to treat high concentrations of contaminants.</td>
<td>Most methods operate over a narrow pH range.</td>
</tr>
<tr>
<td>Washing</td>
<td>Closed system allows easier control of geochemical conditions.</td>
<td><em>Ex situ</em> technique.</td>
</tr>
<tr>
<td></td>
<td>Can treat both organic and inorganic contaminants in the same system.</td>
<td>Ineffective in removing metals in the residual phase of sediments.</td>
</tr>
<tr>
<td></td>
<td>Relatively low cost.</td>
<td>Certain chelating agents used present an environmental risk themselves.</td>
</tr>
<tr>
<td>Electrokinetic</td>
<td>Ability to treat organic and inorganic contaminants simultaneously.</td>
<td>Non-selective, problems can arise if target ions are in much lower concentrations than non-target ions.</td>
</tr>
<tr>
<td></td>
<td>Can operate in zones of low hydraulic flow through induction of electric field.</td>
<td>Corrosion of anodes in acidic conditions.</td>
</tr>
<tr>
<td></td>
<td>Effective at removing high concentrations of contaminants.</td>
<td>Contaminants removed may require further disposal.</td>
</tr>
<tr>
<td></td>
<td>Can operate <em>in situ</em>.</td>
<td>Precipitation of metals close to electrode can impede process.</td>
</tr>
<tr>
<td></td>
<td>Contaminants can be removed with electrodes.</td>
<td>Requires continued operational costs.</td>
</tr>
<tr>
<td><em>In situ</em></td>
<td>Can treat organic, inorganic and radionuclide contaminants simultaneously.</td>
<td>Water in soils affects operational time and costs.</td>
</tr>
<tr>
<td>Vitrification</td>
<td>Can be completed <em>in situ</em> with fused glass blocks remaining in place.</td>
<td>Requires special equipment and training.</td>
</tr>
<tr>
<td></td>
<td>Compacts original volume of contamination by up to 20–50%.</td>
<td>High energy input needed.</td>
</tr>
<tr>
<td>Permeable</td>
<td>Ability to treat multiple contaminants simultaneously.</td>
<td>Mineral precipitation may passivate certain reactive media.</td>
</tr>
<tr>
<td>Reactive Barrier</td>
<td>Can be performed <em>in situ</em>.</td>
<td>Groundwater flow must be well characterised.</td>
</tr>
<tr>
<td></td>
<td>Typically low capital and operating costs compared with pump and treat systems.</td>
<td>Mineral precipitation may reduce permeability of barrier and affect groundwater flow.</td>
</tr>
<tr>
<td></td>
<td>Variety of reactive media can be used to target specific contaminants.</td>
<td>Limited to shallow depths (&lt;15.24 metres) due to construction challenges.</td>
</tr>
<tr>
<td></td>
<td>Long-term efficiency can be improved through adsorption from secondary precipitated minerals.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Passive system requiring no ongoing energy input.</td>
<td></td>
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</tbody>
</table>
ethylenediaminetetraacetic acid (EDTA) in the Co(m) state, but is less stable and hence less mobile in the Co(n) state.\textsuperscript{60}

It has long been established that microorganisms are able to reduce metals,\textsuperscript{61,62} with more recent work showing they are able to use such processes to conserve energy for growth. Focussing on reductive transformations, microbes are able to use some metals as the terminal electron acceptor during anaerobic respiration, in environments where oxygen has been depleted. Thus, stimulating their activity in the subsurface can cause the reduction of high oxidation state metal contaminants to less soluble forms and hence retard their migration. The mechanisms involved in microbial metal and radionuclide reduction are described in detail elsewhere.\textsuperscript{59,63} Microorganisms are also able to reduce and degrade some organic contaminants through analogous respiratory processes when supplied with a suitable electron donor. For example, almost 98\% of tetrachloroethylene (PCE) underwent complete reductive dechlorination to ethane when a laboratory column experiment used Rhine river sediment supplied with lactate as an electron donor.\textsuperscript{64} Trichloroethylene (TCE), an industrial solvent and common subsurface contaminant,\textsuperscript{65} was also shown to be degraded by the methanotrophic bacterium \textit{Methylomonas trichosporium} OB3b in a co-metabolic process in a copper deficient medium.\textsuperscript{66} The reader is directed towards a recent review by Pant and Pant, for a detailed account on the microbial remediation of TCE.\textsuperscript{67}

Metal and radionuclide transport can also be restricted through precipitation with enzymatically generated ligands, such as sulfide\textsuperscript{68,69} and phosphate (see Figure 1).\textsuperscript{63} If supplied with an excess of these ligands then most of the metal should be removed from solution. An advantage to this method is that high concentrations of ligand are generated close to the cell surface which can act as nucleation foci for the onset of metal precipitation. An integrated approach to metal remediation using sulfur-cycling bacteria has been demonstrated.\textsuperscript{70}

\textbf{Figure 1} Diagram illustrating an integrated approach to bioremediation of metal-contaminated soils. The conditions and inputs required for bioleaching and bioprecipitation are displayed along with the outline reactions for each stage where $M^{2+}$ = target metal ions (considered as divalent cations). (Adapted from C. White, J. A. Sayer and G. M. Gadd, \textit{FEMS Microbiol. Rev.}, 1997, 20, 3–4).
In this study, a number of metals were leached from artificially contaminated soil through the production of sulfurous acid by sulfur-oxidising bacteria. This leachate was then applied to a bioreactor containing sulfate-reducing organisms where greater than 80% of the metals were precipitated as solid metal sulfides.

The bacterial strains *Rahnella* sp. and *Bacillus* sp. were both shown to be capable of hydrolysing sufficient organophosphate to remove up to 95% of uranium in a simulated groundwater system. The system was most efficient between pH 5.0 and 7.0 with EXAFS spectroscopy identifying the uranyl phosphate precipitate as an autunite/meta-autunite group mineral. This builds on earlier work on a *Citrobacter* (now classified as a *Serratia*) strain which coupled the efflux of phosphate driven by phosphotase-mediated breakdown of glycerol-2-phosphate to efficient uranium precipitation. A case study involving phosphate biomineralisation at the Hanford site is discussed in detail later in this chapter. The biosorption and bioaccumulation of metals may act as a component in metal remediation through sorption of metals to cell surfaces or uptake into the cell. This can occur as a physicochemical, metabolic-independent mechanisms whereby metals sorb onto the surface of biomass or via metabolic-dependent processes in which the metal is taken up into the cell where it may precipitate locally and accumulate. Both processes have been reviewed extensively but a lack of commercial development has weakened continued research into this field.

These techniques can be achieved through several different methods. Biostimulation involves the addition of key nutrients, such as an electron donor and carbon source, to the subsurface to stimulate the native microorganisms, usually done via injection wells. Advantages of such a method include the stimulation of extant bacteria that are already well suited to the environmental conditions and distributed throughout the subsurface. Relying on the local geology and hydrogeology to distribute the nutrients evenly can however, prove to be a disadvantage.

If the native bacteria do not have the metabolic capability to remediate a particular contaminant then bioaugmentation can be employed where by specialised microorganisms are added to the subsurface, along with the required nutrients, in order to remediate the contaminant. A number of reviews are available on the processes involved in bioaugmentation. Both the aforementioned techniques operate in situ but *ex situ* bioremediation is also a possibility. *Ex situ* treatment involves the excavation of contaminated soil or pumping of groundwater into an above ground facility where the biological conditions can be better controlled. Although excavation and pumping is more expensive than *in situ* treatments, benefits include being able to adjust to aerobic or anaerobic conditions as required. The ability to operate in aerobic conditions allows certain bacteria to utilise organic contaminants, such as petroleum hydrocarbon mixtures and polycyclic aromatic hydrocarbons, as their source of carbon and energy thus potentially degrading the contaminants completely to CO₂ and H₂O. A further advantage of *ex situ* remediation is the ability to homogenise and continuously monitor the soil to ensure complete treatment occurs. Numerous studies examining the effectiveness of *ex situ* bioremediation have been performed.
Phytoremediation, which utilises the ability of plants to degrade or accumulate contaminants, can also be employed in the remediation of soil and groundwater. The cost-effectiveness and non-environmentally disruptive nature of phytoremediation offers advantages over other bioremediation techniques. Further advantages include the ability to easily monitor the plants and the possibility of recovering and re-using valuable, accumulated metals. However, there are a number of disadvantages associated with this process which includes remediation being limited to the surface area and depth of the plant roots, the possibility of contaminants entering the food chain and the usually long period of time phytoremediation requires for completion. For further details, the reader is directed to a number of recent reviews.\textsuperscript{82,83}

4.2 Chemical Redox Reactions

This technique is based on the \textit{in situ} delivery of chemical oxidants to the contaminated media to destroy the contaminants by converting them to harmless compounds. Typical oxidants applied in this process include hydrogen peroxide, potassium permanganate, ozone and dissolved oxygen. A common application of this procedure, based on Fenton’s Reagent, involves the addition of hydrogen peroxide and an iron catalyst to the contaminated area, generating a hydroxyl free radical:

\[
H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^* \tag{1}
\]

This free radical is capable of oxidising complex organic compounds, such as TCE, PCE, dichloroethylene (DCE), benzene, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyl (PCBs), with any residual hydrogen peroxide decomposing into water in the subsurface. Fenton’s Reagent oxidation is most effective in acidic environments (pH 2–4) and becomes ineffective under moderate to strongly alkaline conditions.\textsuperscript{84} Ozone can oxidise contaminants directly or through the production of hydroxyl radicals and is also most effective in acidic conditions. Due to its instability and high reactivity, ozone is produced onsite and requires injection \textit{via} closely spaced delivery points. Permanganate is typically provided as a liquid or as solid potassium permanganate (KMnO$_4$), but is also available in sodium, calcium or magnesium salts. Permanganate reactions occur at a slower rate compared to ozone and peroxyde and, depending on the pH, can destroy the contaminant through direct electron transfer or free radical oxidation. An advantage of permanganate use includes an operable pH range of 3.5 to 12.\textsuperscript{84}

This method was used in a field study at the A/M Area of the Savannah River Site where undissolved dense non-aqueous phase liquid (DNAPL), including TCE and PCE, contamination was present. The treatment test operated over six days and used hydrogen peroxide and iron sulfate to generate hydroxyl radicals to destroy approximately 600 pounds of DNAPL-contaminated soil in the target area. After the trial period, 94% of the targeted DNAPL was destroyed\textsuperscript{85} at a total cost of $511k for the project demonstration.
4.3 Permeable Reactive Barrier

The use of a Permeable Reactive Barrier (PRB) involves placement in the subsurface of a barrier consisting of a permanent, semi permanent or replaceable reactive media across the flow path of a contaminated groundwater plume. As the groundwater passes through the barrier under its natural gradient, contaminants are either degraded by, or retained in, the reactive media in a passive treatment system. A typical PRB involves the excavation and back-filling of a continuous trench with a reactive material designed to target particular contaminants. Examples of reactive media used include iron, limestone, calcium phosphate-based minerals, compost and activated carbon, with iron being the most common. A review of the uses of these various reactive media is provided by Thiruvengadachari et al. Zero-valent iron (ZVI) acts as a reactive medium through corrosion/oxidation of the metal in situ and donation of electrons from this process to organic and inorganic contaminants, such as halogenated hydrocarbons, U(VI) and Cr(VI), which are reduced thereby leading to degradation of the organic contaminant or metal immobilization. Consequently, the long-term efficiency of ZVI barriers is heavily dependent on the corrosion of Fe\(^{0}\), as continued use results in authigenic mineral formation which restricts the availability of reactive Fe\(^{0}\). However, the precipitation of ferricydrite clusters found away from the immediate surface of the Fe\(^{0}\) barrier provides an increase in potential sites for metal adsorption thus prolonging the life of the PRB. A PRB containing ZVI was used in a remediation effort at Oak Ridge and is discussed in more detail later in this chapter.

Advantages of using a PRB include the in situ capture of contaminants, alleviating the need to manage the waste generated by pump and treat methods. Additionally, multiple contaminants, such as metals, radionuclides and organics, can be treated simultaneously and both operating and maintenance costs are typically low. A review of the long-term performance of PRBs is presented by Henderson and Demond.

4.4 Sediment Washing

Sediment washing is a relatively simple, typically ex situ technique involving the cleaning of contaminated soils with various reagents. Depending on the nature of the contaminant, a number of additives can be employed in the washing process including acid washing (e.g. H\(_2\)SO\(_4\) and HNO\(_3\)) and chelating agents [e.g. EDTA, diethylenetriaminepentaacetic acid (DTPA) and ethylenediamine-N,N'-disuccinic acid (EDDS)] to assist with the solubilisation and desorption of the metal from the sediment. This technique is useful for weaker bound metals, those associated with the exchangeable, carbonate and reducible oxide fractions of the soil, but is inefficient at removing metals in the residual fraction. Acid washing can be applied through a variety of abiotic and biological ex situ techniques. Chelating agents can be used in soil washing to remove contaminants from sediments through the formation of stable metal chelate complexes which can then be removed in solution. EDTA has been studied extensively for use as a chelating agent for use in soil
washing\(^{96,97}\) and can enhance metal mobilisation via two mechanisms: fast thermodynamically favourable complexation between EDTA and certain cationic metals, as well as slow driven EDTA-dissolution. The former involves the breakdown of some weak soil-metal bonds while the latter can partially disrupt the soil structure thus mobilising metals bound to oxides and organics.\(^{98}\)

### 4.5 Electrokinetic Remediation

Contaminants are treated by electrokinetic remediation through the application of a low intensity electric current between a cathode and an anode placed within the contaminated soil. Through this process, organic, inorganic and radioactive contaminants can be separated and extracted from clay-rich soils, sludges and sediments. Application of the electric field creates an acidic front around the anode, due to an excess of H\(^+\) ions, and an alkaline front at the cathode, due to an excess of OH\(^-\) ions. The electric gradient created initiates the movement of water, charged chemicals and charged particles through the processes of electro-osmosis, electromigration and electrophoresis, respectively, moving anions towards the positive electrode and cations towards the negative. The contaminants can then be removed through electroplating or precipitation at the electrodes, the use of ion exchange resins or pumping the waste to the surface.\(^{99}\) Complexing agents, surfactants and other reagents can be used to increase the efficiency of treatment.\(^{100}\)

A paper by Lageman examines the processes involved in electrokinetic remediation and examines numerous sites where inorganic and organic contaminants have been treated through this technique.\(^{101}\) Work by Cundy examines the use of electrokinetics to generate a Ferric Iron Remediation and Stabilisation (FIRS) barrier. By applying a low, direct electric potential between two or more sacrificial Fe-rich electrodes placed in the contaminated soil, a strong pH\(/\text{E}_{\text{h}}\) gradient can be generated in the soil column. This forces the precipitation of an Fe-rich barrier between the electrodes.\(^{102}\) Soil samples were taken from the Ravenglass estuary, Cumbria, UK, containing artificial radionuclides from the nearby Sellafield plant and placed into a Perspex cell. Cast iron electrodes were embedded into the soil and a potential of 1.5 V was applied between them for 17 days. After this time, a 30\% reduction in \(^{60}\)Co was observed in the anode zone with a 50\% enrichment in the iron band. Manganese, calcium and strontium were also depleted in the anode zone and enriched on, or around, the iron band. Arsenic, which was desorbed at the high pH found in the cathode zone, was found to be 100\% enriched in the iron band. The radionuclides, plutonium and americium, were not found to be significantly mobilised over the this time frame.\(^{102}\) The use of electrodes as a potential electron donor for microorganisms resulted in the removal of U(vi) from solution in a study by Gregory and Lovley. When the electrodes were poised at \(-500\text{ mV}\) in the absence of microorganisms, U(vi) was removed from solution but was returned to solution when the poise at the electrodes was removed. If *Geobacter sulfurreducens* was present on the electrode, then U(vi) did not return to solution suggesting the uranium was reduced from U(vi) to
A review on the electrical stimulation of microbes is provided by Thrash and Coates.\textsuperscript{104}

Electrokinetic remediation offers advantages as a treatment method through: the ability to treat both inorganic and organic contaminants at the same time; being able to treat contaminants in areas of low hydraulic flow by inducing movement of water, ions and colloids through an electric field; and competitive cost and effectiveness. However, the process can be ineffective when target ions are in much lower concentrations than non-target ions, and corrosion of the anodes in acidic conditions presents \textit{in situ} treatment problems.

## 5 Case Studies

A number of the remediation methods described above have been utilised in various field tests at sites suffering with radionuclide contamination. Three of these case studies are discussed below.

### 5.1 Hanford Case Study

Bench- and field-scale studies were performed by the Pacific Northwest National Laboratory along with a number of collaborators in order to test the remediation potential of using polyphosphate injections to reduce uranium concentrations in groundwater beneath the contaminated 300 Area of the Hanford Site. A detailed study is provided in a number of PNNL reports\textsuperscript{105–107} and will be summarised briefly in this section. The concept of polyphosphate injections works by the formation of stable and insoluble uranium phosphate minerals (autunite) and phosphate precipitates (apatite) for uranium sorption.\textsuperscript{71,108} As autunite sequesters uranium as U(\textit{vi}) rather than reducing it to U(\textit{iv}), the issue of re-oxidation and consequent remobilisation is nullified offering a potential advantage over bioreduction methods. Phosphate minerals precipitate when phosphate-containing compounds degrade in water, due to hydrolysis, and hence rapid mineral formation can occur in an aquifer resulting in a reduction in permeability. However, the longer the phosphate chain, the slower the hydrolysis and consequently the use of long-chain polyphosphate compounds results in a lower change in hydraulic conductivity.\textsuperscript{109}

The test site chosen for the field scale study was located in the 300 Area of the site and involved a three-stage approach to the polyphosphate injections. Water was routed from an extraction well located 190 m from the injection well. Sampling pumps were installed in all site monitoring wells, capable of delivering flows up to 7.57 litres per minute. The sample tubing from these wells was routed directly into a mobile laboratory and connected to a sampling manifold which monitored field parameters (Eh, pH, temperature and dissolved oxygen) and collected samples for anion, cation and trace metal analysis.

Based on previous laboratory studies summarised by Vermeul,\textsuperscript{105} a three-phase injection strategy was identified in order to generate both the uranium-bearing autunite and uranium sorping apatite. An initial injection of polyphosphate was delivered to the subsurface to initiate the formation of autunite,
followed directly by an injection of calcium chloride to allow the formation of calcium phosphate, apatite. The process was concluded with a final injection of polyphosphate following on directly from the CaCl₂ injection. A contribution of 25% orthophosphate, 25% pyrophosphate, and 50% tripolyphosphate made up the phosphorous in each polyphosphate injection.

Formation of apatite is affected by the mixing time between the polyphosphate and calcium species which proved to be variable throughout the site. Phosphate data indicated that wells in a radial distance of 23 m from the injection site received between 40% and 60% of the injection concentration. This suggests that a relatively large lateral area could be treated via the formation of autunite although apatite formation may be problematic.

Uranium concentrations in the targeted treatment zone were typically between 60 and 80 µg l⁻¹ prior to the injections. Uranium monitoring data from wells inside the target area showed an initial decrease in the concentration of uranium, to below the drinking water standard of 30 µg l⁻¹, but a significant rebound was observed about two months after the treatment. At a well outside the treatment zone, uranium concentrations were not observed to decrease until one month after treatment and then displayed a slower rebound. This suggests that uranium concentrations were effectively decreased through the formation of uranyl-phosphate mineral phases (autunite) and were then recharged by the uranium plume on site. However, it is also possible that the uranium decrease was partially due to displacement by the injections of large volumes of high ionic strength solutions. It was thought that long-term remediation would occur via the sequestration of uranium through adsorption to apatite and subsequent conversion to stable uranyl-phosphates (autunite) but these data suggest that this is not the case at this site.

### 5.2 Rifle Case Study

As discussed previously, the Rifle UMTRA site in Colorado is an old uranium processing facility which suffers from various contamination issues, including uranium. Uranium is predominantly found in the mobile U(vi) form in the subsurface due to an insufficient supply of electron donors to stimulate anaerobic respiration and/or consume dissolved oxygen. Laboratory studies have demonstrated the potential of microbes to reduce U(vi) to immobile U(iv) in an aquifer system,¹¹⁰ and the in situ treatment of U(vi) using the same method was tested at the Old Rifle site. Contaminated soil has been removed from the site, leaving only groundwater contamination within the local aquifer. Concentrations of uranium in this area range from 0.4 to 1.4 µM, above the maximum UMTRA contamination limit of 0.18 µM.²⁸

The method used in this field-scale test was describe in detail by Anderson et al.,²⁸ and is summarised briefly here. Injection wells were installed in two rows of ten, perpendicular to groundwater flow (which is typically towards the Colorado River). Each well contained three injection points positioned at different depths in the subsurface. A storage tank was filled periodically with native groundwater and was amended with sodium acetate as an electron
donor to stimulate uranium-reducing bacteria and potassium bromide as a 
conservative tracer at concentrations of 100 and 10 mM, respectively. Oxygen 
was removed from the groundwater through nitrogen sparging. During 
operations, the injections were set to provide 1 to 3 ml of the solution from the 
storage tank per minute corresponding to 1 to 3 mM acetate and 100 to 300 μM 
bromide per day. Monitoring wells were installed at intervals downgradient 
corresponding to groundwater travel of approximately 4, 9, and 18 days with a 
further three wells placed upgradient to serve as controls. Acetate was injected 
continuously over a three month period from June to October 2002, with 
groundwater samples collected at regular time intervals from all monitoring 
wells. Groundwater conditions were monitored, including pH, conductivity, 
redox potential and dissolved oxygen, with further samples taken for U(vi), 
anion (bromide, nitrate, and sulfate), Fe(II), sulfide and acetate analysis. A 
second round of acetate injections were made over the same months in 2003, 
after which no further amendments were made.

Bromide, added as a groundwater tracer, was not detected in any of 
the upgradient wells but was detected after 4, 9, and 18 days at each of the 
corresponding downgradient wells confirming the injection solution had 
reached the targeted area. After the first set of injections, U(vi) concentra-
tions were observed to decrease 9 days after the injections began with 
concentrations dropping to or below 0.18 μM within 50 days at some wells. The decrease in U(vi) was concurrent with the accumulation of Fe(II) 
and prior to any sulfate reduction. After 50 days, the U(vi) concentration 
began to increase, coincident with a decrease of Fe(II) and acetate falling to 
non-detectable levels. Bromide levels were still detected at wells where acetate 
levels had fallen suggesting that an increase in consumption of acetate 
was occurring near the point of injection. This correlated with observations 
following the second injection stage of a depletion of reducible iron oxide 
early on, with the greatest enrichment of Geobacteraceae correlated to the greatest 
portion of U(iv) detected. As reducible Fe(III) became depleted and 
sulfide accumulation occurred, the dominance of the Geobacteraceae decreased 
as they were replaced by species related to known sulfate-reducers. After 
the second round of injections in 2003, U(vi) continued to be removed from the 
groundwater for over a year after the cessation of acetate injections. This 
casts doubt on the suggestion following on from the first round of injections 
that U(vi) removal is acetate dependent. Flow-through column experiments 
suggested that the continued decrease in groundwater U(vi) levels could be 
linked to increased sorption to soils in a reduced environment.

This series of field studies suggest that the stimulation of metal-reducing 
bacteria is an effective method for the removal of U(vi) from groundwater. 
However, when the supply of reducible Fe(III) oxides runs out, sulfate-reducers
become dominant and do not appear to be as effective at reducing U(vi) to U(IV). Promising data from the second round of injections indicates that in sufficiently reduced soils, U(vi) removal may continue, without the continued need for acetate injections, via sorption to soils.

5.3 Oak Ridge Case Study

As was discussed previously, a number of contamination issues exist at the Y-12 complex at Oak Ridge. In order to address these concerns, two permeable iron reactive barriers were installed at the Y-12 plant, consisting of two pathways, in 1997. As water flows through the barrier, the reactive medium (in this case FeO) traps or degrades the contaminant. Pathway 1 at the Y-12 complex was designed to capture groundwater in a gravel-filled, high-density-polyethylene-lined trench. The groundwater would then be treated within a vault containing zero-valent iron. The second pathway involved a permeable trench, in a subparallel direction to groundwater flow. The trench, 2 ft wide and 225 ft long, contained a 26 ft long zone of ZVI covered either side by gravel backfilled zones. Groundwater samples from monitoring wells both in, and downgradient, of the iron barrier at Pathway 2 contained only very low concentrations of uranium (<0.05 mg L⁻¹) compared to values found in groundwater samples in upgradient wells. This would suggest that ZVI is effective at immobilising uranium present in groundwater. Uranium concentrations in middle and deep wells located within the iron barrier displayed slightly higher than expected levels of uranium at ~0.2 to 1 mg L⁻¹. These wells are located in the upgradient portion of the iron barrier where upward hydraulic gradients dominate. The higher concentrations seen here may therefore be a result of a higher inflow of untreated groundwater. Some downgradient wells also showed higher than expected uranium concentrations suggesting that treated groundwater is being re-contaminated from the mobilisation of uranium on downgradient soils or that groundwater flows not treated by the barrier are reaching the wells.

Another contrasting field research study was performed using reduction of U(vi) to U(IV) as a method for immobilising the contaminant. Subsurface conditions favourable for bioremediation were established followed by periodic injection of ethanol. The pH of the test area was adjusted to pH 5-6 causing an increase in uranium sorption and resultant decrease in groundwater uranium concentrations from ~300 (at an initial pH of ~3.4) to ~5 μM. Ethanol injections began on day 137 and ended on day 535. Following on from an initial denitrification phase (day 137–184), a period of uranium and sulfate reduction occurred (day 184–535) during which uranium concentrations in the groundwater decreased from 5 to 1 μM. XANES analysis confirmed that between 39% and 53% of the uranium recovered from the sediments after biostimulation was reduced U(IV). The results from this study, where U(vi) reduction correlated with sulfate reduction, contrast to those from a similar study at the Rifle Site (discussed previously), where aqueous uranium concentrations rebounded when sulfate reducing conditions became dominant. It is possible that sulfate-reducing bacteria that are capable of U(vi) reduction were stimulated by the use of an electron donor (ethanol) in this study, in
contrast to the use of acetate at the Rifle site which may have stimulated alternative organisms. Further studies at this site are required to assess the long-term resistance of U(IV) to re-oxidation and remobilisation.

A recent column-flow experiment demonstrated the potential for the remediation of uranium and technetium in low pH, highly contaminated environments, such as Oak Ridge (as discussed previously) through co-precipitation. Luo et al. showed that in conditions found at Oak Ridge, greater than 95% of soluble uranium and 83% of technetium can be co-precipitated with Al-oxhydroxides by raising the pH above 4.5 with the addition of a strong base (NaOH). The precipitated uranium and technetium were found to be stable in the presence of high nitrate concentrations [50 mM Ca(NO₃)₂] and low carbonate concentrations.

6 Conclusions

The successful management of land contaminated by our global nuclear legacy is a major challenge that relies on the successful cooperation across disciplines including chemistry, biology and engineering. The scale and complexity of the problem is apparent from this short review and illustrated by recent estimated costs of $1 trillion for cleanup of legacy wastes in the USA. Cleanup costs in the UK are of the order of £100 billion with the discounted cost recently put at £44.5 billion. However, there is a range of flexible remediation options that have already been trialled, with some success, on land contaminated with radioactive waste, and have the potential to help treat the wide range of highly complex contamination scenarios that are present on nuclear sites worldwide. This is a matter of importance, as widespread public acceptance of nuclear power as an attractive energy source can only be achieved if the successful management of our nuclear legacy wastes can be demonstrated.

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References


Chapter 3

Methodology

Principles of key analytical techniques employed during this research
This section will describe the main analytical techniques employed throughout this research project. The aim is to summarise the principles associated with the techniques used, as opposed to providing a detailed description of each experimental protocol. More detail of methodology can be found in the methods section of each chapter.

3.1 Environmental Scanning Electron Microscopy

Scanning electron microscopes (SEM) consist of an electron column which creates a beam of electrons, a sample chamber where the beam interacts with the sample, detectors which monitor signals resulting from the electron-sample interaction, and a viewing system which constructs an image based on the signals detected. In a conventional SEM, samples must be desiccated, coated to make them electrically conductive, and analysed under high vacuum. The Environmental SEM (ESEM) allows samples to be analysed in their natural state without any preparation and removes the high vacuum constraint. The primary electron beam in the ESEM is able to penetrate the vapour with little scatter and scan the surface of the sample. Interactions between the sample and electrons from the primary beam produce secondary electrons which strike the water vapour molecules, producing further secondary electrons. These are collected by the gaseous secondary electron detector (GSED) with image contrast relating primarily to the topography of the sample. As more of the volume of interaction occurs close to the sample surface, more secondary electrons can escape from a point at the top of a peak in the sample than a point in the bottom of a valley, thus creating a bright image for peaks and dark image for valleys. Sample charging, which can inhibit the image obtained, is limited in an ESEM as the GSED has a strong positive charge which drives water molecules, which are positively charged due to their production of
secondary electrons, towards the sample which is negatively charged owing to the primary electron beam (Figure 1).

![Image](image.png)

**Figure 1 - Water molecules become positively charged due to cascade of secondary electrons produced by the interaction between the electron beam and sample. The positive bias on the GSED forces these water molecules towards the negatively charged sample (ROBINSON, 2003).**

### 3.2 Ion Chromatography

Ion chromatography is a process that separates ions and polar molecules based on the charge properties of the molecules. As such, it is used in the analysis of ionic constituents (analytes) in solution. The sample is injected and passed through a column in which the individual analytes interact with the stationary phase, a solid material with fixed ionic functional groups on its surface. These functional groups are either positively or negatively charged depending on whether the analytes are anionic or cationic, respectively.
Elution of the analyte is achieved through the injection of a mobile phase which contains ions capable of exchanging with the analyte. This results in desorption of the analyte according to equilibrium between the stationary phase, mobile phase and analyte. Adjusting the ionic strength in the mobile phase shifts the equilibrium position and hence, the retention time. This allows separation of multiple analytes with identification achieved through comparison against known standards. Several methods are used to detect the analytes, with conductivity used for ions analysed in this research. Comparison with known standards allows quantification of the analyte concentration.

In this research, organic acids were measured using a Dionex DX120 IC system with a Dionex ICE-AS1 column and a 1 mM octane sulfonic acid mobile phase. Inorganic anions were measured using either a Dionex DX600 system or a Metrohm 761 ‘compact’ IC system, both using a Dionex AS9-HC column and a 9 mM Na$_2$CO$_3$ mobile phase. Both inorganic anions and organic acids were run with a 100 µL sample loop using an autosampler.

3.3 Ferrozine assay

During geochemical analysis of the AWE microcosms, concentrations of Fe(II) were measured using the ferrozine spectrophotometric assay. Ferrozine, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4,.disulfonic acid sodium salt, is able to react with ferrous iron to form a stable magenta complex which is very soluble in water and can be used to determine the concentration of Fe(II) (STOOKEY, 1970). The ferrozine solution is made up of the ferrozine salt and the organic buffering agent HEPES, N-(2-hydroxyethyl) piperazine-N-2-ethanesulfonic acid.
To measure Fe(II) concentrations, samples were added to 0.5 M HCl in a 1:50 dilution which prevents oxidation of Fe(II) and allows the dissolution of biogenic Fe(II) minerals. One hour reaction time is typically sufficient to allow dissolution of these minerals and release mineral-bound Fe(II). Following this, 50 µl of the sample was added to a plastic cuvette containing 2.45 ml of ferrozine solution which was then shaken and the colour allowed to develop for 30 seconds before the sample was placed in a spectrophotometer and its absorbance measured at 562 nm. Fe(II) concentrations are determined by comparing the absorbance to calibration standards containing known concentrations of FeCl₂.

3.4 Autoradiography

Autoradiography involves the use of photographic emulsion and other track detectors to study the presence and distribution of radioactive substances. Storage-phosphor autoradiography, as used in this research, requires an imaging plate containing a fine coating of photostimulable phosphor to be placed over the sample in the dark. Ionizing radiation promotes excitation in the phosphor and the accumulated energy is released as luminescence when the phosphor are excited by a laser beam. This luminescence is digitized producing a quantitative reproduction of the radioactive sample with the relative intensity of the luminescence proportional to the intensity of the original radiation.
3.5 Radiometric Methods

Radionuclide activity can be determined through radiometric techniques which are based upon the detection and measurement of decay processes. These techniques include alpha- and gamma- spectrometry and liquid scintillation counting which can detect both beta and alpha particles. The radionuclides investigated in this research, plutonium and americium, were measured using alpha- and gamma-spectrometry respectively, with both techniques based on semiconductor detectors.

3.5.1 Semiconductor Detectors

During the decay process, alpha and gamma radiation are emitted with discrete energies specific to a nuclear transition. Briefly, the energy from the radiation is dissipated in the intrinsic region of the semiconductor which promotes electrons into the conduction band, leaving holes in the valence band (Figure 2). As alpha particles are poorly penetrating, this region must be produced close to the detector surface and the detector has to be operated under a vacuum to prevent absorption of the alpha particles by the chamber atmosphere. When a voltage is applied, the negative charges (electrons) migrate towards the positive electrode, while the positive charges (holes) migrate to the negative electrode. These migrations create a current pulse which is amplified and converted to a digital signal which is counted in the memory of a multichannel analyzer (MCA). The channel number in the MCA for alpha-spectrometry is proportional to the digital size and hence energy of the incident charged particle (CHOPPIN et al., 2002). In gamma-spectrometry, the size of the current pulse sent from the detector to the amplifier is proportional to the charge deposited in the detector and hence the energy of the gamma ray.
3.5.2 Alpha- and Gamma-Spectrometry Sample Analyses

As alpha particles easily lose energy, the counting source must be as free from other substances as possible to minimize interference. To reduce attenuation of alpha particles, a chemical separation is used to remove other metals such as iron which would otherwise plate out (the separation and plating process is described in detail in Chapter 4). Each peak in the spectrum can be attributed to a certain isotope at the corresponding energy, with the area under each peak proportional to the activity of that isotope. To determine the activity of the sample, an internal standard must be added at the start of the radiochemical process. This standard must be of a known activity and is usually an isotope of the same element but one which is not already present in the sample. By adding a standard of known activity at the start of the procedure, all loses during the chemical separation and electrodeposition processes can be accounted for. The sample activity can then be calculated using the equation:
\[ A(\text{sample}) = \frac{I(\text{sample})}{I(\text{tracer})} \times A(\text{tracer}) \]

where \( A = \) activity and \( I = \) peak integral

Although background counts in an alpha detector are effectively zero, the random process of radioactive decay leads to a degree of uncertainty in any measurement of decay events with time, especially when counts are low. Background counts from potential contamination by alpha-recoils should also be considered. Both the sample and tracer peaks have errors associated with them which must be combined to give an overall counting error. When the half-life of an isotope is sufficiently long, any decay during counting can be ignored. The number of nuclear decay events in a given time interval follows a Poisson distribution, whose standard deviation can be shown to be approximately equal to the square root of the number of counts in the peak integral. Thus, combining the tracer and sample peak errors allows an overall error to be calculated using the following equation:

\[ \text{Total Error} = \left[ \left( A_t \right)^2 \times \left( \frac{1}{I_t} + \frac{1}{I_s} \right) \right]^{1/2} \]

where \( A_t = \) tracer activity, \( I_t = \) tracer peak integral and \( I_s = \) sample peak integral

In contrast to alpha spectrometry, samples counted using gamma spectrometry require little preparation. Soil or groundwater samples can be counted directly without the need for chemical separations. A calibration standard, using a tracer of known activity with the same counting geometry, allows the concentration of radionuclide in the sample to
be determined. The peaks were analysed using the in-house computer program MAESTRO.

An advantage of using alpha-spectrometry includes the ability to obtain acceptable counting statistics for even low activity or low yield samples by just increasing the counting time. Background counts in alpha spectrometry are also typically negligible. Limitations of this technique include the long preparation time involved with chemical separations and electrodeposition as well as the closeness of the alpha energies of $^{239}\text{Pu}$ and $^{240}\text{Pu}$ (5.157 and 5.168 MeV, respectively). The relative ease of sample preparation is an advantage of gamma spectrometry. Many actinides have characteristic gamma energies which overlap the high background part of the gamma spectrum although the gamma emission for $^{241}\text{Am}$ is easily distinguished.

### 3.6 Microbiological Techniques

In this research project, changes in the microbial community over time and under different geochemical conditions were monitored. The various steps involved in this process are outlined here in brief.

#### 3.6.1 DNA Extraction

In the first step, DNA was extracted from environmental samples using a Powersoil DNA Isolation Kit (Cambio, Cambridge, UK). The samples are first mixed with small glass beads which help lyse bacterial cells, releasing their DNA. Sodium dodecyl sulfate (SDS) is added to break down fatty acids and lipids associated with the cell membrane.
which promotes further release of DNA. Decay of genetic material is prevented through the addition of a buffer solution.

The DNA is separated from particulate matter such as cell debris, beads and soil particles through centrifugation. A number of reagents are mixed with the DNA-containing supernatant to precipitate contaminants, which are removed by further centrifugation. The supernatant is transferred to a centrifuge vessel containing a silica membrane which binds the DNA. A series of washes is used to remove remaining contaminants whilst the DNA is bound to the silica membrane. An elution buffer is then added to release the DNA from the membrane, with the resulting solution frozen and stored prior to PCR amplification.

3.6.2 Polymerase Chain Reaction (PCR)

PCR is a molecular biology technique for the amplification of target DNA sequences. Strands of genetic information are unravelled and copied in a repeated cycle of reaction which increases the DNA concentration to that which can later be detected. Initially, denaturing of the DNA double helix occurs, with the hydrogen bonds existing between the base pairs on either DNA strand broken by a temperature rise to 72 °C. Once the DNA strands are separated, short oligonucleotide sequences called primers are added; 8f and 1492r primers were used here (LANE, 1991). These primers, which amplify almost the full 16S RNA gene (~1500 b.p.), bind to complementary strands of the bacterial 16S rRNA gene and are then allowed to anneal with fresh nucleotide base pairs, with the enzyme Taq polymerase used to create a complementary DNA strand between the 8f and 1492r primers. This cycle repeats around 30 times, amplifying the target sequence several million-fold in a few hours. The final product can be visualised on agarose gel after electrophoresis and staining by ethidium bromide.
3.6.3 DNA fragment cloning

In order to identify the relative abundance of the individual species present within the now amplified community, individual DNA strands are isolated from the mixture of PCR product through a cloning reaction. The first step, ligation, involves adding a buffered reaction mix, containing a bacterial vector and ligation enzyme (T4 DNA ligase) to a small quantity of the mixed PCR product. The mixture is then incubated at 14 °C which allows the enzyme to catalyse a reaction in which a DNA fragment from the PCR product is inserted into a target site on a plasmid vector. The vector contains genes which code for ampicillin resistance, allowing selective growth of these bacteria on agar plates containing the antibiotic thus preventing contamination from airborne microorganisms.
The ligation mix can then be added to competent *E. coli* cells, shaken gently and placed on ice for 30 minutes. The competent cells are then heat-shocked via incubation at 42 °C for 30 seconds before immediately being transferred back to ice.

The competent cells are spread over LB agar plates containing ampicillin and X-gal and incubated at 37 °C overnight. The cells containing the ampicillin-resistant vector are able to grow whereas the majority of cells which do not contain the vector are unable to grow on the ampicillin-containing agar. The vector insert site contains the gene necessary for the breakdown of X-gal, which produces a characteristic blue colour. Therefore, colonies which contain the vector will not possess the gene and, being unable to breakdown the X-gal, will appear white.

Following overnight incubation, the white colonies present are picked using sterile pipette tips and re-suspended in sterile water and PCR tubes. Each tube will then contain multiple copies of one insert type, with 96 colonies picked for each microbial library. A sample from these colonies was then subjected to further rounds of PCR. Using primers targeted to the plasmid DNA sequence adjacent to the site of insertion, only the original DNA of interest was amplified.

### 3.6.4 DNA Sequencing

To identify the species of bacteria present in the clone libraries, the specific sequence of nucleotide bases that makes up each of the different PCR products was isolated. This was done via the dideoxynucleotide method (SANGER et al., 1977). Four different deoxynucleotide triphosphates, dATP, dGTP, dCTP and dTTP, make up the growing DNA strand when the sequences undergoes copying during both cell division and PCR reactions. Each nucleotide is added to the 3’-OH group of the last nucleotide added. In sequencing reactions, synthetic dideoxynucleotides are used which lack the –OH at the
3’ carbon atom. Growing DNA strands can incorporate these synthetic dideoxynucleotides but, once this occurs, there is no 3’-OH for the next nucleotide to attach to and so elongation ends. This results in a mixture of DNA fragments of different length with a dideoxynucleotide at their end. These fragments were then size-separated on a high resolution, denaturing polyacrylamide gel. Since each different dideoxynucleotide is labelled with a different fluorescent marker which fluoresces at a different wavelength, the DNA code of the sample can be read by visualising on a gel using a fluorescence detector.

Here, An ABI Prism BigDye Terminator Cycle Sequencing Kit was used combined with an ABI Prism 877 Integrated Thermal Cycler and ABI Prism 377 DNA Sequencer (Perkin Elmer Applied Biosystems, Warrington, UK) to sequence DNA fragments. Sequences were analysed against the NCBI (USA) database using the BLAST program package and matched to known 16S rRNA gene sequences.

3.7 References


Chapter 4

Biogeochemical behaviour of plutonium during anoxic biostimulation of contaminated sediments

Research Paper 1

Biogeochemical behaviour of plutonium during anoxic biostimulation of contaminated sediments

R. L. Kimber1, C. Bootman1, P. Purdie2, F. R. Livens1,3 and J. R. Lloyd1

1 Williamson Research Centre for Molecular Environmental Science and Research Centre for Radwaste and Decommissioning, School of Earth, Atmospheric & Environmental Sciences, University of Manchester, Manchester M13 9PL, UK
2 AWE PLC, Aldermaston, Berkshire RG7 4PR, UK
3 Centre for Radiochemistry Research, School of Chemistry, University of Manchester, Manchester M13 9PL, UK

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ABSTRACT

Understanding the biogeochemical behaviour of actinides in the environment is essential for the long-term stewardship of radionuclide contaminated land. Plutonium is of particular concern due its high radiotoxicity, long half-life and complex chemistry, with these factors contributing to the limited literature available on its environmental behaviour. Here, we investigate the biogeochemistry of Pu in contaminated soil as microbial processes have the potential to mobilize Pu through numerous mechanisms including the reduction of Pu(IV) to the potentially more mobile Pu(III). After the addition of glucose to stimulate microbial activities, there was a substantial shift in the 16S rRNA gene profile of the extant microbial communities between days 0 and 44 with an increase in Clostridium species, known glucose fermenters which have been reported to facilitate the reduction of Pu(IV) to Pu(III). A minor increase in Pu mobility was observed at day 44, returning to initial levels by day 118. The negligible change in Pu mobility, despite the onset of reducing conditions and changing mineralogy, would suggest the Pu is highly refractory. This information is important for developing remediation options for Pu-contaminated soils, suggesting that managing legacy Pu in situ may be preferred to mobilization via the stimulation of metal-reducing bacteria.

KEYWORDS: plutonium, contaminated land, actinides, remediation, microbial interactions.

Introduction

The release of radioactive materials from various anthropogenic sources is currently of great scientific and public concern due to the toxicity and long half-lives of particular radionuclides. Radionuclides are naturally present in soils and groundwater, for example from the uranium and thorium decay series; however, the principal sources of radionuclides in the environment are anthropogenic. These sources include fallout from nuclear weapons testing as well as the continued storage, accidental release and controlled discharge of nuclear material. In the USA alone, there are 120 Department of Energy (DOE) sites which contain 40 million cubic metres of contaminated soil and debris and 6.4 trillion litres of contaminated groundwater (McCullough et al., 1999). At least 50% of this inventory is contaminated with radionuclides including caesium-137, plutonium-239, strontium-90, technetium-99, uranium-238 and uranium-235, as well as heavy metal contamination including chromium, lead and mercury (McCullough et al., 1999), and the associated cleanup cost is in excess of a trillion dollars (Palmisano and Hazen, 2003). The problem also extends to the UK on a smaller
but significant scale with the discounted cleanup cost for the UK’s legacy sites currently estimated at approximately £44.5 billion (Nuclear Decommissioning Authority, 2010). Understanding the biogeochemical behaviour of radionuclides underpins both long-term management of contaminated land and the geological disposal of nuclear waste.

As their chemical behaviour differs significantly from one oxidation state to another, their oxidation state is the single most important geochemical property of the actinides; it controls precipitation, complex formation, colloid formation and sorption, all of which affect the mobility of actinides in environmental systems (Silva and Nitsche, 1995). For this reason, the role that microorganisms play in affecting radionuclide speciation is of particular importance. It has long been established that microorganisms are able to reduce and oxidize metal species, and a wide range of bacteria and archaea are able to utilize these processes to produce energy for growth under anaerobic conditions (Lovley and Phillips, 1988; Lovley et al., 1991). There have been, however, only a limited number of studies published on the interactions and mechanisms involved in reactions between microbes and actinides, especially those with more complex redox chemistry such as plutonium.

The actinide most extensively studied in relation to microbial interactions is uranium, and work carried out over the last two decades has revealed the ability of microorganisms to reduce the element via both direct and indirect mechanisms. The direct enzymatic bioreduction of soluble U(VI) to the insoluble U(IV) was reported for Geobacter metallireducens and Alteromonas putrefaciens (now Shewanella putrefaciens) which were able to use U(IV) as the terminal electron acceptor for anaerobic growth in the presence of various electron donors (Lovley et al., 1991). Further work has suggested enzymatic reduction may not be the only mechanism involved in the transformation of U(VI) to U(IV). Renshaw et al. (2005) showed that the bacterium Geobacter sulfurreducens was able to reduce U(VI) via a single-electron reduction to produce an unstable U(V) intermediate, with the final U(IV) product formed either through further reduction of U(V) or via abiotic disproportionation mechanisms. Alternatively, indirect abiotic Fe(II)-mediated U(VI) reduction mechanisms have also been proposed as significant processes in sediments (Wilkins et al., 2006).

A number of recent studies have examined microbial interactions with other actinides, including Np, where there has been a focus on the microbial reduction of Np(V) and subsequent precipitation of Np(IV) under anaerobic conditions (Iopini et al., 2007; Law et al., 2010b; Lloyd et al., 2000; Rittmann et al., 2002). However, there have been comparatively few studies of microbial interactions with plutonium, which has a far more complex redox chemistry. The principal isotope of plutonium in nuclear waste is $^{239}$Pu, which has a half-life of 24,110 y. The environmental redox chemistry of plutonium is very complex due to its ability to exist in a variety of oxidation states (III, IV, V and VI). The most stable species in most environmental conditions is Pu(IV), which is sparingly soluble, especially in the dominant oxide and hydroxide forms, and may adhere strongly to humates, colloids and mineral surfaces (Choppin et al., 1997; Neu et al., 2005). In the Pu(III) oxidation state, plutonium is generally oxidized and is not expected to exist except under strong reducing geochemical conditions (Neu et al., 2005). However, the reduction of Pu(IV) oxyhydroxide to Pu(III) would be expected to increase its solubility (Francis, 2007). The more mobile higher oxidation states, Pu(V) and Pu(VI), are normally only found in marine or saline environments, or where radionuclide concentrations are high (Neu et al., 2005). However, interactions with minerals, chelating agents, and microorganisms can result in the oxidation of Pu(IV) to Pu(V) and further to Pu(VI) (Mitchell et al., 1995; Neu et al., 2005). A combination of Pu(IV) and Pu(VI) can also arise from the disproportionation of Pu(V) (Panak and Nitsche, 2001; Renshaw et al., 2009). Although the higher oxidation states are potentially more mobile, they are readily reduced by organic anions such as oxalate, citrate and acetate and so are not expected to be major Pu species in biologically active systems (as is assumed to be the case at Aldermaston due to the ubiquitous nature of microorganisms in soils).

The reduction of the higher valent plutonium species by microbial interactions, inorganic reducing agents (Fe$^{2+}$) and disproportionation reactions has been investigated, and results in either the precipitation of a stable Pu(IV) product or a variety of oxidation states sorbed to cell surfaces (Iopini et al., 2009; Panak and Nitsche, 2001; Reed et al., 2006; Renshaw et al., 2009). Further reduction of Pu(IV) to Pu(III) has also
been investigated in a number of pure culture microbiological studies. In the presence of amorphous Pu(OH)$_4$, the metal-reducing bacterium *Shewanella oneidensis* is able to produce minor quantities of Pu(III) (Boukhalfa et al., 2007). With the addition of EDTA, *S. oneidensis* and *G. metallireducens* were able to reduce significant quantities of amorphous Pu(OH)$_4$ (approximately 60% and 80% respectively) and the resultant Pu(III) remained stable in reducing conditions. Under anaerobic conditions, *Bacillus sp.* were able to solubilize 90% of hydrous PuO$_2$ in the presence of nitrolitriacetic acid (NTA). When NTA was not present, only 40% of the Pu was solubilized, suggesting that a complexing agent was required to keep the plutonium in solution (Rusin et al., 1994).

Studies by Francis et al. (2008) on the dissolution of Pu colloids by a *Clostridium sp.* indicated that Pu(NO$_3$)$_4$ added to the bacterial growth medium was initially precipitated as amorphous Pu(OH)$_4$ and after 12 h incubation, 53% was solubilized (passing through a filter <0.03 μm) (Francis et al., 2008). This figure rose to 61% after 36 h of incubation before falling to 37% after 186 h. The presence of Pu(III) was confirmed using X-ray absorption near edge structure (XANES) spectroscopy and it was suggested that direct microbial reduction of Pu(IV) to Pu(III) enhanced the dissolution of plutonium. Leaching and mobilization of $^{239}$Pu and $^{241}$Am from soil is also possible through the use of various leachate solutions (Lu et al., 1998). A combination of citrate and ascorbic acid, used as complexing and reducing agents respectively, was found to remove the greatest amount of Pu and Am from the soil, with over 70% of the total alpha activity removed. A leachate solution containing just citrate was found to remove only 30% of the alpha activity from the soils.

Similar chemical and biological transport properties shared by Fe(III) and Pu(IV), such as their ionic size ratios and hydrolysis properties, suggest that iron-chelating agents could be important in complexing plutonium, thereby potentially increasing its solubility. The common soil aerobic bacterium *Microbacterium flavescentis*, was able to take up both Fe(III) and Pu(IV) complexed with desferrioxamine-B (John et al., 2001). Active cells accumulated both metal-siderophore complexes, with Fe uptake occurring at a faster rate than that of Pu. A higher accumulation was also observed for the former. It was found that the two complexes mutually inhibit uptake, suggesting they compete for shared binding sites. When complexing agents are absent, Pu(IV) has the tendency to hydrolyze to polymeric hydroxide species which results in increased sorption and precipitation, thus potentially increasing its resistance to mobilization in the environment (Bulman, 1983).

The aim of this paper is to investigate further the biogeochemical behaviour of Pu in the environment. Of particular interest is the potential impact of microbial processes on Pu mobilization in order to better understand both the long term transport of the actinide and also the potential development of a bioremediation strategy for Pu contaminated soils. This was achieved by studying a series of microcosms constructed using Pu contaminated soils provided by AWE Aldermaston, Berkshire, and supplemented using a carbon source to stimulate the native microbial community. A broad suite of geochemical markers and terminal electron acceptors were monitored throughout incubation of the microcosms and the Pu in solution was measured at key time points to determine changes caused by microbial activity, during evolution of the biogeochemical conditions.

**Materials and methods**

**Microcosm construction**

Soil samples and groundwater were obtained from the AWE site at Aldermaston, Berkshire; AWE are responsible for maintaining the UK’s nuclear deterrent with the Aldermaston site operating under the Nuclear Installations Act. As such, there are areas of the site where contamination by various radionuclides, including plutonium and americium, has occurred. The plutonium present is strongly sorbed to the soils with previous work indicating $K_d$ values ranging from $2 \times 10^8$ to $6 \times 10^8$ (McCubbin et al., 2004). Americium is stable as Am(III) at most Eh values encountered in environmental systems and is the prevalent form found in the environment. It is readily adsorbed to mineral surfaces in soils and sediments. As $^{241}$Am is formed through the beta decay of $^{241}$Pu, which is produced via successive neutron captures in $^{239}$Pu and $^{240}$Pu, it has the potential to be used as a proxy for estimating Pu activity.

The local geology consists of a layer of made ground, composed predominately of silty soil with some gravel and occasional fragments of brick, underlying a layer of turf and topsoil. Soil
samples were taken in November 2007 using an Atlas Copeco (Cobra) Mark 1 Percussion Drill with a 1 m window sampler, sealed in transport containers and delivered to The University of Manchester. The samples were stored at 4°C until required. Microcosms were constructed using 480 g of soil and 480 ml of groundwater collected from the site, with the addition of 10 mm glucose as a fermentable carbon source and potential electron donor. The head space was flushed with nitrogen to remove any oxygen present. An autoclaved (120°C, 20 min) control was set up in the same manner in addition to a negative control, which contained only the soil and groundwater with no added glucose. Sufficient microcosms were set up for sacrificial sampling at each of 0, 44 and 118 days to monitor Pu solubility. Each set was run in triplicate and incubated at 10°C. A further set of microcosms was set up as described previously, but using only 50 g of soil and 50 ml of groundwater. These smaller microcosms were sampled periodically under aseptic conditions to monitor geochemical parameters such as Fe(ii), glucose, nitrate, nitrite, sulfate and organic acids. The larger microcosms were split into solid and liquid components through centrifugation and vacuum filtration when sacrificed. The liquid samples were made up to a 500 ml, 2% nitric acid solution and stored at 10°C until needed for radiochemical separation, whilst the solid samples were stored at −80°C.

Analytical techniques

Ion chromatography (IC) was used to measure the concentrations of nitrate, nitrite, sulfate and organic acids. Organic acids were measured using a Dionex DX120 Ion Chromatography system with a Dionex ICE-AS1 column and a 1 mm octane sulfonic acid mobile phase. Inorganic anions were measured using either a Dionex DX600 system or a Metrohm 761 'compact' Ion Chromatography system, both using a Dionex AS9-HC column and a 9 mm Na2CO3 mobile phase. Both inorganic anions and organic acids were run using a 100 μl sample loop using an autosampler and conductivity detection.

The Fe(ii) was measured using the ferrozine assay. Briefly, the sample was added to 0.5 m HCl in a 1:50 dilution. After 1 h, 50 μl of the sample was taken and added to a cuvette containing 2.45 ml of ferrozine solution. The ferrozine stock solution used in the assay was made by dissolving 1 g of ferrozine, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4-disulfonic acid sodium salt, and 11.96 g of HEPES, N-(2-hydroxyethyl) piperazine-N-2-ethanesulfonic acid crystals in 1 l of distilled deionized water (DDW). The colour was left to develop for 30 s and the sample was then placed in a spectrophotometer and measured at 562 nm.

The Eh was measured using a Mettler-Toledo Inlab Redox Micro ORP electrode (Mettler-Toledo Ltd, Leicester, UK).

Radiochemical analysis

The 241Am activity in the samples was determined using gamma spectroscopy. The samples were prepared in Pyrex beakers and counted for at least 20 min (dependant on activity) on either a high purity germanium semiconductor gamma-detector (50% efficiency) (Canberra Ltd.) or a Lo-ax low energy gamma photon semiconductor detector (EG&G), both attached to a signal treatment analyser EG&G Model 919 analogue-to-digital converter (ADC) and multi-channel analyser (MCA). To measure the 239,240Pu activity, 400 ml of sample was poured into a Pyrex beaker with 400 mg KNO3 and 40 ml concentrated HNO3 added. The samples were heated to dryness and the residue dissolved in 40 ml concentrated HNO3 and heated to dryness again. The sample was then spiked with a known activity of 242Pu and 20 ml of 9 m HCl and 1 ml of concentrated HNO3 were added. The sample was warmed to 50°C. An ion exchange column was preconditioned by the addition of 5 ml anion exchange resin (Bio-Rad AG1-X8) and 15 ml of 9 m HCl. The sample was poured through and the column washed with 20 ml of 9 m HCl, 20 ml of 8 m HNO3 and finally 20 ml of concentrated HCl. The plutonium was then eluted using a 25 ml solution of 11.5 m HCl and 0.2 m HI and collected in a beaker; 1 ml KHSO4 was added to the sample which was then heated gently to dryness. The sample was dissolved in 3 ml of HNO3, heated to dryness, then dissolved in 3 ml concentrated HCl and heated to dryness again, ready for electrodeposition.

An electrolysis cell consisting of two threaded glass joints (SVL 30) held together by a threaded plastic collar (SVL 30) with a copper cathode base and a platinum anode was prepared. The cell was completed by adding a stainless steel planchet, with a Teflon ring over it, in between the glass tubes. The plutonium sample was dissolved in 15 ml electrolyte solution (4% ammonium oxalate in 0.3 m HCl) and poured
into the cell. The sample beaker was rinsed with 15 ml of distilled-deionized water (DDW), which was also poured into the cell. Electrodeposition was carried out for 2 h at 0.5 A and 20 V. One minute before electrodeposition was finished; 1 ml concentrated ammonia solution was added to the cell. The planchet was removed from the cell, washed with DDW and acetone and left to dry at room temperature.

The planchet was counted using an ion-implanted Si detector for 60 h. The unknown $^{239,240}$Pu activity was calculated from its peak integral relative to the peak integral of the $^{242}$Pu tracer.

**Extraction of $^{239,240}$Pu and $^{241}$Am**

To examine the correlation between $^{241}$Am and $^{239,240}$Pu a series of 10 g (wet weight) soil samples were dried in an oven at 105°C for 1 h, heated at 700°C for 3 h and then heated overnight at 600°C. The sample was gamma counted as described above and the total plutonium in the sample extracted in a series of acid digestions. Briefly, the sample was added to a PTFE microwave vessel with the addition of 14 ml HNO$_3$, 1 ml DDW and 6 ml HF. The solution was then digested in a microwave at $6.9 \times 10^{-5}$ Pa for 40 min. Any remaining solid was then boiled in HCl, filtered (Whatman Grade 5 filter paper) with the resulting solution taken to dryness. To complex any remaining fluoride, 6 g of AlCl$_3$·6H$_2$O was added to the residue, which was then taken into solution using 40 ml concentrated HCl and made up to a 100 ml solution using 8 ml HCl. Finally, 1 ml sub-samples were spiked with a known activity of $^{242}$Pu and treated as described above to determine the $^{239,240}$Pu activity.

**Microbial community analysis**

**DNA extraction and PCR amplification**

The DNA in the sediment samples was extracted using a Powersoil DNA isolation kit (Cambio, Cambridge, UK). The full 16S RNA gene (~1500 base pairs) was amplified from selected samples by PCR using the primers 8F and 1492R. The PCR method was as follows: an initial denaturation at 94°C for 4 min, melting at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min; 35 cycles, followed by a final extension step at 72°C for 5 min. The resulting PCR products were purified using an ExoSap protocol, 2 µl of ExoSap mix (0.058 µl Exonuclease 1, 0.5 µl Shrimp Alkaline Phosphatase and 1.442 µl QH$_2$O) was added to 5 µl of PCR product and incubated at 37°C for 30 min followed by 80°C for 15 min. Purified gene fragments were ligated directly into a cloning vector using a PCR cloning kit (StrataClone, Stockport, UK) containing topoisomerase I-charged vector arms (Agilent Technologies, Wokingham, UK) prior to transformation into E. coli competent cells expressing Cre recombinase (Agilent Technologies, Wokingham, UK). Positive clones (96 per library) were screened by PCR using primers complementary to the flanking regions of the PCR insertion site of the cloning vector, and sequenced using the ABI Prism® BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Life Technologies Corporation, USA). The forward primer, 8F (Eden et al., 1991) was used for the sequencing reaction.

**DNA sequencing and phylogenetic analysis**

Nucleotide sequences were determined by the dideoxy nucleotide method (Sanger et al., 1977). An ABI Prism BigDye Terminator Cycle Sequencing Kit was used combined with an ABI Prism 877 Integrated Thermal Cycler and ABI Prism 377 DNA Sequencer (Perkin Elmer Applied Biosystems, Warrington, UK). Sequences (typically 1000 base pairs in length) were analysed against the NCBI (USA) database using BLAST program packages and matched to known 16S rRNA gene sequences. Sequences have been submitted to NCBI GenBank database (accession numbers JQ663635–JQ663786).

**Results and discussion**

**Fermentation and anaerobic respiration**

To help determine the impact of stimulating anaerobic microbial processes on Pu mobility, glucose was added to the microcosms as a fermentable carbon source which can be metabolized to a wide range of electron donors that support a cascade of anaerobic respiratory processes. Based on previous microcosm studies (data not shown), three time points were chosen for sacrificial sampling of microcosms and measurement of plutonium in solution. These time points at 0, 44 and 118 days, were chosen to coincide with the start of incubation, the end of Fe(III) reduction and the end of sulfate reduction, respectively. The concentrations of various
electron acceptors naturally present in the soils were monitored regularly over the 118 day incubation period of the smaller microcosms. When supplied with the fermentable substrate glucose, the reduction of a wide range of terminal electron acceptors under anoxic conditions could be linked directly to the oxidation of the hexose sugar, or to the oxidation of fermentation products of glucose, including alcohols and short chain fatty acids such as acetic acid (Fig. 1).

Analysis of the samples for organic acid content suggested that fermentation of glucose began to occur in the microcosms during the first week with a 13% decrease in initial glucose concentration observed at day 9. The initial glucose concentration fell by 70% after 15 days and was no longer detected after 29 days. The decrease in glucose concentration was mirrored by an increase in short chain fatty acid concentrations. Both formic and acetic acid were detected at day 15 (<1 mM and 2.4 mM respectively). Acetic acid concentrations continued to rise, reaching 6.5 mM at the end of the incubation period. After day 22, propionic and

![Graph showing concentrations of terminal electron acceptors and geochemical markers in glucose-amended microcosms showing both organic acid (top) and inorganic anions (bottom) concentrations. Results are derived from triplicate samples.](image-url)
n-butyric acid were also detected although concentrations never exceeded 1 mM.

Denitrification was observed between days 2 and 22, indicated by the complete removal of NO₃⁻ from an initial level of 11 mM. This was mirrored by a drop in Eh from +330 mV to +51 mV over the same time period. The denitrification intermediates nitrite was detected during this process, although always at <1 mM and was probably utilized as an electron acceptor. The reduction of Fe(III) followed the denitrification process, as indicated by increasing Fe(II), with concentrations rising slowly to 3.0 mmol Fe kg⁻¹ sediment slurry between day 15 and 37 followed by a rapid increase to 20 mmol kg⁻¹ by day 51. A slow decrease in Fe(II) concentration was then observed until day 118, presumably as the Fe(II) was incorporated in new mineral phases not extracted by the 0.5 M HCl used in the ferrozine assay.

Sulfate reduction also began to occur between days 29 and 37 during the early phase of Fe(III) reduction, suggesting that both processes ran concurrently in the sediments. This observation is at variance with the traditional thermodynamic ladder of terminal electron acceptors in microbial respiration in which sulfate reduction usually occurs after completion of the more energy efficient iron reduction reactions. However, a number of factors may influence which electron acceptor is utilized including mineral form and water chemistry. For example, Fe(III) in goethite loses its energetic advantage over sulfate reduction and so both processes may occur simultaneously (Bethke et al., 2011). The quantities of sulfate respired (approximately 0.6 mmol) were far lower than those of the Fe(III) in the sediments, which corresponded to approximately 20 mmol Fe kg⁻¹ sediment slurry. During iron and sulfate reduction the Eh continued to drop, reaching +4 mV at day 51 and ultimately dropping to −138 mV by day 118.

16S rRNA gene sequencing

The microbial community profiles of the sediment microcosms were examined at 0 and 44 days to observe any changes that could be linked to alterations in soil geochemistry or any plutonium potentially released into solution. The DNA sequencing revealed that a very diverse microbial community was present in the soils at their initial state (t = 0 days) (Fig. 2). Of the 78 clones

![Fig. 2. Changes in microbial community composition after stimulation with glucose, shown at the phylum level from 16S rRNA gene sequencing between day 0 (left) and day 44 (right).](image-url)
sequenced. 74 were unique and 4 were represented twice in the library. Our analysis suggested that the bacterial community in the original, untreated sample was dominated by *Acidobacteria, Sphingobacteria* and *Alphaproteobacteria* represented by 24, 15 and 12% of the clones, respectively. Most of the genes sequenced were closely affiliated with uncultured bacterial species, although organisms known to photosynthesize (*Chloroflexi*), oxidize nitrite (*Nitrospira*) and oxidize Fe(II) (*Gallionellaceae*) were detected as single constituents of the clone library. Approximately 5% of the analysed clones were affiliated with bacteria identified previously in uranium mining wastes (sequences FM866295.1, FM866289.1, and FM877543.1, deposited in the GenBank) and in a low level radioactive waste storage site (Field et al., 2010). Gene sequencing from the sample taken after 44 days of incubation showed a clear shift in the community to include a significant number of anaerobic microorganisms associated with the fermentative metabolism of glucose and more complex carbon substrates, or anaerobic respiration coupled to nitrate or metal reduction (Fig. 2).

Known glucose fermenters detected included organisms most closely related to the class *Clostridia* and also those affiliated with the *Bacteroidetes* (12%). Members of the *Clostridia* class, which were not identified (most probably due to their low number) in the untreated sample, represented 17% of the clones found 44 days after the addition of glucose. This includes 4% of clones which were closely related to *Clostridium* species. Interestingly, *Clostridium* species are also known to reduce metals (Kauffman et al., 1986; Dobbin et al., 1999), including Pu(IV) to Pu(III) (Francis et al., 2008). Other bacteria with the potential to reduce metals were detected, all belonging to the class *Betaproteobacteria*, which increased from 8% of the microbial profile at day 0 to 41% at day 44 which includes an organism most closely related to *Rhodothermus sp.* (GenBank sequence: EF451697.1) (Finneran et al., 2003; Wilkins et al., 2007). Also included are organisms affiliated with *Herbaspirillum* species (GenBank sequence FJ812351.1 and *Aquaspirillum autotrophicum*, now reclassified as a *Herbaspirillum* species), which dominated the library (24% of the sequences detected). The *Herbaspirillum* species, alongside *Janthinobacterium* (4% of the library), have been reported recently to play a major role in anoxic nitrate and metal cycling in sediments collected from the US DOE FRC site at Oak Ridge (Vishniavskaya et al., 2010) and Sellafield (Law et al., 2010a).

### 241Am and 239,240Pu correlation

As determining the 239,240Pu activity in soil samples requires a significant amount of time and the use of very hazardous chemicals (HF), the relationship between the 241Am, which can be easily and quickly counted using gamma spectrometry, and 239,240Pu was examined. A number of 10 g soil samples were counted for 241Am activity and samples representing a range of activities were then digested to extract the 239,240Pu into solution. Overall, some correlation (R2 of 0.63) was observed between the activities of the two actinides, although an outlying result was present, suggesting a degree of heterogeneity between the distributions of the 241Am and 239,240Pu in the soil (Fig. 3). However, the correlation does permit 241Am to be used as a proxy for estimating the 239,240Pu remaining in the solid phase.

### Plutonium mobility

The Pu in solution was monitored at three time points during the incubation of the microcosms: 0 days, 44 days and 118 days. Triplicate microcosms were set up for each time point with the 239,240Pu activity counted in the aqueous phase taken from each. The percentage of Pu in solution was calculated at each time point by gamma counting the remaining solid phase and using the 241Am/239,240Pu correlation to estimate the Pu activity in that phase. An increase in the mobile fraction of Pu of approximately 1.5 times was observed between days 0 and 44. However, even at this increased concentration, the activity in solution is still only a negligible proportion of the activity in the solid phase and >99.9% of the plutonium remained immobile (Fig. 4). The fraction of Pu in solution returned to the initial value after day 118. The marginal increase of plutonium in solution coincided with both a significant change in the microbial profile and with an increase in Fe(II) formed via Fe(III) reduction. The release of Pu into solution could potentially be attributed to reductive dissolution of Pu-bearing Fe assemblages (Sholkovitz et al., 1983), and/or limited Pu(III) formation as a result of reduction via direct enzymatic or abiotic (Fe(II)-catalysed) Pu(IV) reduction. As discussed previously, numerous potential metal-reducing microorganisms were detected at day 44 including...
organisms associated with *Clostridium* species (known to facilitate reduction of Pu(IV) to Pu(III)). The decrease in the mobile fraction of Pu which followed may have been caused by reoxidation to Pu(IV) as available Fe(II) levels decreased and the microbial community and biogeochemical conditions changed as Fe(III) reduction ended. The changing mineralogical conditions, as indicated by the decrease in Fe(II), may have resulted in the formation of new Fe minerals, which may have resorbed any Pu potentially released by the earlier dissolution of Pu-bearing Fe(III) minerals. The mobile fraction of $^{239,240}$Pu observed in the control microcosms was of a similar level to that detected in the glucose amended microcosms, although levels remained constant with no increases detected after 44 days.

Gamma counts for $^{241}$Am in the aqueous phase (data not shown) were not above background,

![Biogeochemical Behaviour of Plutonium](image)

**Fig. 3.** Correlation between the $^{241}$Am and $^{239,240}$Pu in Aldermaston soil samples ($R^2 = 0.6345$). Errors reported as 1σ counting errors.

![Plutonium in solution](image)

**Fig. 4.** Percentage of $^{239,240}$Pu in solution during microcosm incubation stimulated by glucose. Results displayed as average value from triplicate microcosms with errors reported as their standard deviation.
indicating little mobilization of americium during the incubation period. This is to be expected given that americium is stable as insoluble Am$^{3+}$ across the various redox potentials experienced during anaerobic respiration (Lloyd et al., 2002). However, an increase in $^{241}$Am solubility comparable to that of Pu would be difficult to detect by gamma spectrometry.

Despite a substantial shift in the microbial community and a significant change in Fe mineralogy, only a negligible amount of Pu was found to be mobilized. This would suggest that the vast majority of the plutonium at the Aldermaston site is resistant to both redox and mineralogical changes prompted by anaerobic respiration. Plutonium whose behaviour is determined by the form in which it was released into the environment has been described as ‘source-dependent’ (Choppin, 2007). Such plutonium is thought to exist as highly refractory oxides, which remain unaffected by geochemical conditions for long periods of time. The largely unchanging nature of the plutonium observed over the range of geochemical conditions in this study may therefore reflect the presence of plutonium in an inert form, possibly a highly refractory oxide. This study is in agreement with work published on the Rocky Flats Plant, Colorado, USA, which suggests that plutonium, which has contaminated an area of the former weapons site, is largely immobile under natural conditions (Clark et al., 2006; Litaor et al., 1998). Synchrotron radiation studies revealed the oxidation state of the Pu in the soils as Pu(IV) and identified its chemical form as the insoluble hydrous oxide PuO$_2$·$x$H$_2$O (Clark et al., 2006). However, sequential extraction experiments performed on Rocky Flats soil indicates that 20–40% of the $^{239,240}$Pu is associated with sesquioxides (Litaor et al., 1996) and so may be expected to be released into solution under the iron-reducing conditions observed in this study, further suggesting a highly refractory nature for the Pu at the Aldermaston site. The insights into the biogeochemical behaviour of plutonium in this study are important when considering the environmental fate of the actinide in both developing remediation strategies and in predicting its long term migration behaviour in potential waste repositories. The refractory nature of the Pu suggests that it is unlikely to be mobilized under environmental conditions which usually promote metal release, thereby potentially containing the contamination on site. Under such circumstances, the option of managing the contamination in situ may prove favourable to attempted bioremediation. This could allow the contamination to remain untreated in its current form if mobilization is thought to be unlikely but may require routine monitoring of potential contaminant pathways leading off site. The potential for migration through oxidation of the Pu(IV) to the more mobile Pu(V) and (VI) should be considered when devising any treatment strategy. However, the strong hydrolys and tendency for sorption of Pu(IV) (Choppin et al., 2001), as reflected by its refractory nature seen here, would be likely to limit this effect. Due to the tendency of Pu(IV) compounds to sorb to organic and mineral particles, the potential for colloidal migration and particulate transportation must also be acknowledged. Furthermore, it should be noted that these microcosm experiments are of relatively short duration compared to the half-life of plutonium isotopes and that ‘source dependant’ plutonium can eventually become dependant on its surrounding ecosystem. Therefore, although managing the contamination in its current state may potentially be seen as the best option at this time, further characterization may be required in the future to assess any changes to the behaviour of the plutonium.

Conclusions

When supplied with the fermentable substrate glucose, a significant shift in the microbial community of the Pu contaminated soil was observed with known glucose fermentors and metal reducers being detected, including organisms affiliated with Clostridium and betaproteobacterial species. Despite this shift in the microbial community, only a negligible increase in Pu mobility was observed at day 44, possibly as a result of reduction of Pu(IV) to Pu(III) or from the reductive dissolution of iron oxides during Fe(III) reduction. The Pu found in solution decreased to initial levels by day 118 and no increase in $^{241}$Am mobility was detected. The low solubility of the Pu maintained throughout the incubation period, despite an observed increase in metal-reducing microorganisms and changing mineralogy, suggests that it is highly refractory and not susceptible to biogeochemical changes experienced under anaerobic conditions. These data have major implications for (1) the potential to remove the Pu from soils using biological redox processes and (2) the long-term stewardship of the land.
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References


Chapter 5

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

Research Paper 2
5. Geochemical association of Pu and Am in selected host-phases of contaminated soils from the UK and their susceptibility to chemical and microbiological leaching

Richard L. Kimber¹, Claire Corkhill¹, Philip Purdie², Francis R. Livens¹,³ and Jonathan R. Lloyd¹

(1) Williamson Research Centre for Molecular Environmental Science and Research Centre for Radwaste and Decommissioning, School of Earth, Atmospheric & Environmental Sciences, University of Manchester M13 9PL, UK
(2) AWE PLC, Aldermaston, Berkshire, RG7 4PR, UK
(3) Centre for Radiochemistry Research, School of Chemistry, University of Manchester, Manchester M13 9PL, UK

5.1 Abstract

The biogeochemical behaviour of actinides in soils and groundwater controls their environmental mobility. As such, it is important to understand this behaviour in order to develop remediation and management strategies for radionuclide-contaminated land. Here, some of the physicochemical properties of Pu and Am are investigated in contaminated soils from Aldermaston, Berkshire, UK, and the Esk Estuary, Cumbria, UK. Sequential extraction techniques were used to examine the host-phases of the actinides in these soils, and the potential for leaching the contaminants using sulfur-oxidising bacteria was investigated. 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. In both soils, the $^{241}$Am was distributed across multiple phases including the reducible oxide fraction (26.1 – 40.0 %), the organic fraction (45.6 – 63.6 %) and the residual fraction (1.9 – 11.1 %). Plutonium in the Aldermaston soils proved largely resistant to leaching from sulfuric acid, with a maximum of 0.18 % leached into solution, although up to 12.5 % of the $^{241}$Am was leached into solution under the same conditions. Although a number of radioactive particles were detected using autoradiography, we were unable to identify and characterize them under ESEM. These data suggest Pu in the contaminated soils that we have studied, is highly recalcitrant to geochemical changes and is likely to remain immobile over significant time periods.

5.2 Introduction

Plutonium contamination in the environment has arisen from global fallout from nuclear weapons testing, as well as controlled and accidental discharges of nuclear material. The migration of plutonium under environmental conditions is heavily influenced by its source term, in particular its composition and physicochemical properties when it is released into the environment (BUESSELER et al., 2009; CHOPPIN, 2007; SALBU et al., 2004). Americium is often a co-contaminant with Pu due to the formation of $^{241}$Am from the beta decay of $^{241}$Pu, which is itself formed via successive neutron captures in $^{239}$Pu and $^{240}$Pu. Due to their high specific activities, even in significantly contaminated environments, Pu and Am isotopes are often undetectable by conventional techniques so most analyses are carried out through measurement of their activity. At such low mass concentrations, synchrotron radiation techniques, which provide useful insight into chemical speciation, cannot be used. However, a more practical approach to predicting
their environmental behaviour is to determine geochemical associations with a sequential extraction technique, in which the sediment or soil is separated into defined, sequentially-dissolved phases via a series of increasingly aggressive chemical extractions. 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 to be potentially affected 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 geochemical conditions.

Associations with several of these components may be affected through microbial respiration, which can alter mineralogy directly through enzymatic redox reactions or indirectly through changes to 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 possible benefit in remediation, or enhance the risk through migration.

Previous work has demonstrated that negligible Pu (<0.01 %) is released into solution when the native microbial community is stimulated, suggesting that little Pu may be bioavailable (Chapter 4). However, sulfur-oxidising bacteria are effective at leaching metal contaminants and are commonly used in biomining and soil washing (White et al., 1998). Reduced sulfur or iron compounds in the biosphere can be used by
sulfur-oxidising bacteria for chemolithotrophic growth under aerobic conditions, leading to production of soluble metal sulfates and sulfuric acid (Norris, 1990). Effluent containing the soluble metal can then be introduced into a sulfate-reducing system, resulting in the reprecipitation of the metal for disposal or recovery (White et al., 1998).

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 (Salbu et al., 2004). Therefore, characterisation of Pu-containing particles can also help to predict its environmental behaviour. We demonstrate particle characterisation 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.

In this study we aim to provide further information on the biogeochemical behaviour of Pu and Am in the environment. Using Pu- and Am-containing soils provided by AWE Aldermaston, Berkshire, we built on previous work, which suggested that Pu and Am in these soils are highly refractory with very limited susceptibility to mobilization via biological activity (Chapter 4). Here, sequential leaching of $^{239,240}$Pu, $^{238}$Pu and $^{241}$Am was used to further explore their geochemical associations.

It has been suggested that plutonium species can be split into two groups based on the physiochemical form in which it is released into the environment (Choppin and Morgenstern, 2001). Plutonium, which is not in thermodynamic equilibrium with its surroundings, is referred to as source-dependant and as such its behaviour in natural systems is determined by the form in which it was released into the environment. High-
fired plutonium oxides from nuclear explosions are an example of source-dependant plutonium. This form of Pu is a highly refractory, insoluble oxide which is likely to accumulate in soils and sediments. Plutonium, which achieves equilibrium in a natural system through geochemical processes, is termed source-independent. This can include Pu released from nuclear fuel cycle operations, and its speciation may be affected by changes in pH, Eh, ligand concentration and biological activity. To examine any potential difference in the environmental behaviour of differently sourced Pu, sediment samples taken from close to the Sellafield reprocessing plant were subjected to the same sequential extraction method as the Aldermaston samples. In addition, little recent work has been carried out on Pu in the area surrounding Sellafield and hence this study, when compared to more historical work, will contribute to our understanding of longer term Pu behaviour, as well as provide a comparison between the effectiveness of various sequential extraction methods. The samples provided by AWE were also used in experiments with sulfur-oxidising bacteria in order to determine the potential for Pu or Am mobilization under the acidic conditions generated. Finally, further characterization of the Pu in the Aldermaston soils was carried out through autoradiography, with the alpha-emitting particles indentified and examined under ESEM.

5.3 Methods

5.3.1 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 discussed previously (Chapter 4). Archived sediment samples from the Esk estuary 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 it was expected to contain radioactive particles deposited from the peak Sellafield discharges occurring during the 1970s (McCarthy and Nicholls, 1990).

### 5.3.2 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 1M MgCl₂ (pH 7) for 1 hour; (b) carbonate-bound, extracted with 100 ml 1 M CH₃COONa, adjusted to pH 5 with CH₃COOH for 5 hours; (c) reducible oxide-bound, extracted with 100 ml of 0.04 M NH₂OH.HCl in 25% v/v CH₃COOH, adjusted to pH 2 with NaOH and stirred at 96°C for 6 hours; (d) organic matter bound, extracted with 30 ml 0.02 M HNO₃ and 50 ml of 30 % H₂O₂, adjusted to pH2 with HNO₃ and stirred for 2 hours at 85°C. A further 30 ml 30 % H₂O₂ (pH 2 with HNO₃) was then added and stirred as before. Next, 30 ml 3.2 M CH₃COONH₄ in 20% v/v HNO₃ was added, the solution was diluted to 200 ml with distilled deionised water (DDW) and stirred for 30 minutes; and (e) residual, extracted by microwave digestion (100 psi for 40 mins) using 14 ml HNO₃, 4 ml conc. HF and 5 ml DDW.
After each fraction was extracted, the supernatant was separated via centrifugation and vacuum filtration, made up to a 2 % HNO₃ solution and stored at 10°C for radiochemical analysis.

5.3.3 Acidophile 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 liter) 10 g elemental sulfur powder, 20 ml 50x autotrophic basal salts consisting of (with grams liter⁻¹ in parenthesis) (NH₄)SO₄ (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), NaMoO₄.2H₂O (0.5), NiSO₄.6H₂O (1), Na₂SeO₄.10H₂O (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.

5.3.4 Acidophile Microcosms

Microcosms were constructed using 50 g soil and 97.5 ml groundwater, both from the Aldermaston site, 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 sulfur. 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 and vacuum filtration. Prior to radiochemical analysis, the supernatant was made up in 2 % HNO$_3$ 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).

5.3.5 Radiochemical Analysis

$^{241}$Am, $^{239,240}$Pu and $^{238}$Pu activities were measured using gamma- and alpha-spectrometry as described in detail previously (Chapter 4). Briefly, to determine the $^{241}$Am activity the supernatant was evaporated and the residue counted on either a high purity germanium semi-conductor gamma-detector (50% efficiency) (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 $^{238}$Pu and $^{239,240}$Pu, 10 % v/v HNO$_3$ and 1 mg KNO$_3$ per ml of sample were added and the solutions heated to dryness. The residue was then spiked with a known activity of $^{242}$Pu 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 hours to determine the $^{239,240}$Pu and $^{238}$Pu activity.
5.3.6 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 radioactive particles were spread further and the process repeated until the particles 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.

5.4 Results

5.4.1 Geochemical Associations of Pu and Am

The geochemical associations of Pu and Am isotopes were examined in soils from Aldermaston, Berkshire, and the Esk estuary, Cumbria, in a series of sequential extractions. The $^{241}$Am in the Aldermaston soils could not be detected above background activity in the exchangeable fraction, while only 3.1 – 3.3 % of the $^{241}$Am was associated with the carbonate fraction (Fig. 1b). The majority of $^{241}$Am was distributed between the reducible oxide fraction (26.1 – 40.0 %), the organic fraction (45.6 – 63.6 %) and the residual fraction (7.3 – 11.1 %). Similar results were observed for the Esk Estuary samples with less than 0.1 % of the $^{241}$Am associated with the exchangeable fraction and 3.4 – 3.9 % with the carbonate fraction (Fig. 1d). Again, the majority of the $^{241}$Am was associated with the reducible oxide (33.8 – 35.2 %) and organic components (58.9 – 59.3), with a smaller fraction found in the residual fraction (1.9 – 3.3 %).
Little $^{239,240}$Pu was found to be associated with the exchangeable, carbonate and reducible oxide fractions in the Aldermaston samples, ranging from 0.018–0.029 %, 0.40–0.56 % and 3.59–5.72 % in each respectively (Fig. 1a). The most significant association was found to be with the organic fraction, containing 10.5–29.9 % of the $^{239,240}$Pu and the residual fraction, containing 63.8–85.5 %. Comparable proportions of $^{239,240}$Pu were found in the exchangeable, carbonate and reducible oxide phases of the Esk Estuary soils, ranging from 0.015–0.020 %, 0.75–0.99 % and 4.70–7.09 % respectively, as shown in Figure 1c. In contrast to the Aldermaston samples, the Esk Estuary soils were found to have very little $^{239,240}$Pu associated with the organic fraction, which contained only 0.028–0.033 % of the total $^{239,240}$Pu activity. The bulk of activity was again found in the residual fraction, here ranging from 91.9–94.5 %. The sequential extraction data for $^{238}$Pu are very similar to those observed for $^{239,240}$Pu (Table 1). The bulk of the $^{238}$Pu in the Aldermaston samples was also associated with the residual component, while the organic fraction was also significant (72.6–74.4 % and 14.7–22.0 %, respectively). The $^{238}$Pu association in the Esk Estuary samples also closely mirrored that of the $^{239,240}$Pu, with the majority, 89.6–90.7 %, again found in the residual fraction. The $^{238}$Pu/$^{239,240}$Pu activity ratios in the 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 are similar to those reported previously (McCUBBIN et al., 2004) and are consistent with ratios from weapons-grade and fallout derived Pu, though the total activity in the soils, 1.53 – 1.8 Bq/g (Table 1), is too high for the later to be the source. The $^{238}\text{Pu}/^{239,240}\text{Pu}$ ratios for the Esk Estuary soils are 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.

Figure 1 – Association of $^{239,240}\text{Pu}$ and $^{241}\text{Am}$ with various geochemical fractions in soils from Aldermaston and the Esk Estuary. Data are plotted as results from two separate sequential extractions and are presented as a percentage of the total activity in each sample.
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<tr>
<td></td>
<td>0.37%</td>
<td>0.075%</td>
<td>1.02%</td>
</tr>
<tr>
<td></td>
<td>102 ± 2.1</td>
<td>437 ± 2.4</td>
<td>437 ± 2.4</td>
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<tr>
<td></td>
<td>0.56%</td>
<td>0.99%</td>
<td>0.99%</td>
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<tr>
<td></td>
<td>Oxide</td>
<td>Oxide</td>
<td>Exchangeable</td>
</tr>
<tr>
<td></td>
<td>32.6 ± 8.3</td>
<td>602 ± 2.6</td>
<td>6.01 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>4.82%</td>
<td>7.46%</td>
<td>0.075%</td>
</tr>
<tr>
<td></td>
<td>1030 ± 4.7</td>
<td>3130 ± 2.5</td>
<td>8.78 ± 7.2</td>
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<tr>
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<td>5.72%</td>
<td>7.09%</td>
<td>0.020%</td>
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<tr>
<td></td>
<td>Organic</td>
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<td>Exchangeable</td>
</tr>
<tr>
<td></td>
<td>139 ± 2.3</td>
<td>3.1 ± 5.9</td>
<td>82.2 ± 3.1</td>
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<td></td>
<td>20.6%</td>
<td>0.039%</td>
<td>1.02%</td>
</tr>
<tr>
<td></td>
<td>5410 ± 1.8</td>
<td>14.6 ± 2.9</td>
<td>437 ± 2.4</td>
</tr>
<tr>
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<td>29.9%</td>
<td>0.033%</td>
<td>0.99%</td>
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<td>Residual</td>
<td>Residual</td>
<td>Exchangeable</td>
</tr>
<tr>
<td></td>
<td>501 ±11</td>
<td>7370 ± 2.8</td>
<td>6.01 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>74.2%</td>
<td>91.4%</td>
<td>0.075%</td>
</tr>
<tr>
<td></td>
<td>11500 ± 3.3</td>
<td>40600 ± 2.1</td>
<td>8.78 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>63.8%</td>
<td>91.9%</td>
<td>0.020%</td>
</tr>
<tr>
<td></td>
<td>Total activity</td>
<td>Total activity</td>
<td>Total activity</td>
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<tr>
<td></td>
<td>0.372</td>
<td>0 %</td>
<td>6.01 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>15.3</td>
<td>3.20 ± 10</td>
<td>8.78 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>0.024</td>
<td>0.018%</td>
<td>0.020%</td>
</tr>
</tbody>
</table>

Table 1 – Activity of Pu isotopes in various soil fractions in Aldermaston and Esk Estuary soils.

Errors are given as 1σ based on counting errors.
5.4.2 Geochemistry of Bioleaching Microcosms

Microcosms containing Aldermaston contaminated soil were inoculated with sulfur-oxidising bacteria to investigate the potential for bioleaching Pu and Am. A number of acidophilic strains and sulfur sources were used to identify the most efficient setup. The three combinations of acidophiles, with their corresponding energy source used were *A. ferrooxidans* with pyrite, *A. thiooxidans* with pyrite and *A. thiooxidans* with elemental sulfur powder. Optimal growth of *A. ferrooxidans* 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. ferrooxidans* and *A. thiooxidans* microcosms, when supplied with pyrite as an energy source, had a similar starting pH of 3.1 and 2.9 respectively. When elemental sulfur was used as an energy source for *A. thiooxidans*, the starting pH was 4.8, much closer to the upper limit for cell growth. In all cases, the pH dropped over time, reaching pH 2.4 in both pyrite-containing microcosms and pH 2.7 in the elemental sulfur microcosm. This is consistent with microbially-driven oxidation of sulphur species, in the reactions:

\[
2\text{FeS}_2 + 7\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{Fe}^{2+} + 4\text{SO}_4^{2-} + 4\text{H}^+ \quad [1]
\]

\[
\text{S} + 1.5\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{SO}_4 \quad [2]
\]

When supplied with pyrite, the *A. thiooxidans* and *A. ferrooxidans* microcosms had a starting Eh of 423 mV and 392 mV respectively. When elemental sulfur 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.
5.4.3 Bioleaching of $^{241}$Am and $^{239,240}$Pu

In our previous work, biostimulation of anaerobic bacteria native to the contaminated soil resulted in negligible Pu (<0.01 %) released into solution, with no detectable mobilization of Am, despite the potential reductive dissolution of Fe(III) minerals and the formation of mixed organic acid fermentation products. In the current study, sulfur-oxidising bacteria were added to the contaminated soil, together with reduced sulfur compounds, to investigate the potential for bioleaching of the actinides, Pu and Am, through the formation of sulfuric acid. The most efficient leaching of $^{241}$Am was seen in the microcosms containing A. thiooxidans augmented with elemental sulfur powder. Under these conditions, up to 12.5 % of the Am present in the soil was released to solution after 28 days (Table 2). 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 sulfur, while A. ferrooxidans augmented with pyrite gave up to 0.14% $^{239,240}$Pu in solution (Table 2). 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 (Chapter 4), the overall solubilization of Pu is still negligible. However, the bioleaching of $^{241}$Am (up to 12.5 %) is far more effective than the biostimulation method, in which no solubilization of Am was observed (Chapter 4), suggesting that $^{241}$Am is more susceptible to mobilization under changing pH as opposed to changing Eh conditions.
Table 2 - $^{239,240}$Pu and $^{241}$Am in aqueous and solid phase after incubation of acidophilic microcosms.

Due to the heterogenic distribution of Pu in soils, duplicate samples were analysed in each microcosm. The percentage leached is calculated using the average activity in the solid phase from the duplicates.

5.4.4 Autoradiography

In an attempt to understand better the geochemical behaviour of Pu, a number of particle characterisation techniques were used on the Aldermaston soil. Autoradiography was used to isolate individual Pu-containing particles. A small number of alpha-emitting particles were detected, as shown in Figure 2. These particles were isolated and examined under ESEM. A number of small, high-density particles,
containing unusual elements were detected under the ESEM, but, as would be expected from the low mass concentrations present, no plutonium was detected.

Figure 2 - Autoradiography scan of Aldermaston soil with radioactive particles highlighted.

5.5 Discussion

The $^{238}\text{Pu}/^{239,240}\text{Pu}$ ratios 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 and consequently different associations of Pu might be expected. However, the distribution of $^{239,240}\text{Pu}$ appears to be fairly similar at both sites, but with a stronger association of Pu with the organic fraction in the Aldermaston soils. The $^{239,240}\text{Pu}$ at both sites appears to show a much stronger affinity for the residual fraction than at most other nuclear sites studied.
For example, at the Rocky Flats site, Colorado, USA, little of the $^{239,240}$Pu contamination at the former Pu processing site was found to be associated with the residual component (Table 3). The $^{239,240}$Pu associated in the exchangeable and carbonate phases was similar across both the Rocky Flats and Aldermaston soils, but Pu in the reducible oxide and organic phase was much higher at the former, 20 – 40 % and 45 – 65 % respectively (LITAOR and IBRAHIM, 1996). The $^{239,240}$Pu associated with the residual phase at Rocky Flats was only 10 – 15 %. The differences observed over these two comparable sites may have arisen from the variation in the sequential extraction methods used. Alternatively, the source term for the Rocky Flats Pu may differ to that of the Aldermaston Pu. Although both weapons manufacturing facilities, the source term of Pu can still differ depending on which stage of the manufacturing process the Pu contamination arose from. The former would highlight the difficulty in interpreting leaching results from different extraction methods whilst the later would further highlight the importance of source term in determining the environmental behaviour of Pu. Although the sequential extraction results must be interpreted with caution, when taken together with further data including the $^{238}$Pu/$^{239,240}$Pu activity ratio and characterization results then a more confident interpretation of the physiochemical properties of the Aldermaston Pu can be built up. With most of it held in the residual fraction, the Pu would be expected to be highly recalcitrant and resistant to mobilization, typical of Pu which is not in thermodynamic equilibrium with its surroundings. This form of Pu often exists as refractory oxides (CHOPPIN, 2007) and recent work in size characterization of PuO$_2$ suggests such particles predominantly exists in the sub $\mu$m range (SHINONAGA et al., 2012). Our autoradiography and imaging results are consistent with these suggestions.
Interestingly, the sequential extraction results from the Esk Estuary soils presented here differ significantly from similar studies performed on soils from the area over the past 30 years. In this study, the vast majority of the $^{239,240}$Pu (91.2 – 94.1 %) was found in the residual fraction whereas, in previous work, the $^{239,240}$Pu has been distributed amongst the oxide, organic and residual fractions. Work by Livens and Baxter found the majority of $^{239,240}$Pu, 53 – 68 %, to be associated with the organic fraction, with the oxide fraction containing 18 – 31 % and most of the remaining activity, 7 – 17 %, found in the residual fraction (Livens and Baxter, 1988). In another example, soils contaminated with Pu at Dounreay, UK, a former fuel fabrication and reprocessing site, also differ to the samples analysed in the current work. Only 8 % of the Pu was found associated with the residual fraction, with 63 % associated with the organic fraction and 27 % with the reducible oxide fraction (Cook et al., 1984). The $^{238}$Pu/$^{239,240}$Pu ratio in the Esk Estuary soils in the current study is similar to both that seen in the earlier study by Baxter and Livens and the study on Dounreay soils by Cook et al (Table 4). These ratios would suggest the Pu at each site originated from fuel reprocessing and hence,
likely exists as source-independent species. Therefore, it may be expected that the dominant host-phases of the Pu would appear similar in each study. It is therefore surprising that the Pu association in the Esk Estuary soils in the present study displays a greater similarity to the Aldermaston soils than to the more comparable, earlier studies on Esk Estuary and Dounreay soils. A number of factors should be considered when making these comparisons. Firstly, it must be noted that the soil examined here is from a deeper horizon, 10 – 15 cm, than the Dounreay and previous Esk Estuary work, 0 – 7 cm. There are also numerous soil types across the Esk Estuary and so sampling location may also lead to differences in Pu association (Livens, 1985; Livens and Baxter, 1988). Therefore, differences in soil properties with respect to location and depth may play a role in affecting the host-phases of the Pu (Livens, 1985). In gley soil, an increase of Pu in the organic phase was coincident with a decrease in the oxide phase when moving from the 0 – 7.5 cm to 7.5 – 15 cm horizon followed by a reversal of this trend in the 15 – 22.5 cm horizon. Livens suggested that an increase in anaerobic conditions with depth may be responsible for the dissolution of oxide phases, thus releasing Pu which could then be taken up by the organic fraction. As this trend then reverses in the 17 – 22.5 cm horizon, Livens postulated that the effectiveness of the organic extractant may decrease as organic material becomes more humified with further increasing depth. Secondly, the time span between studies should also be taken into account. Over the course of 25 years the more labile Pu fraction may have either migrated away, or since become fixed in the more resistant fractions, thus resulting in an increase in the recalcitrant Pu observed in the current study. However, this would seem unlikely to play a significant role due to the relatively limited amount of Pu considered to be labile (Hamilton-Taylor et al., 1993). Again, the most likely explanation for the differences observed in the Pu association between these sites is
likely due to the variation in extraction methods applied. The study by Baxter and Livens as well as that by Cook, use the McLaren and Crawford technique (McLaren and Crawford, 1973) whereas the Tessier method was applied here. In another earlier study on Esk Estuary soils, using the top 0 – 1 cm horizon, where the Tessier method was also applied, the results suggest the majority of Pu was associated with the residual phase (Mudge et al., 1988), as was observed here, with a much lower fraction of Pu bound to the organic phase than in the studies using the McLaren and Crawford method (Table 4). This would seem to highlight the role played by the extraction method in identifying the dominant host-phases of Pu, with the Tessier method possibly less effective at dissolving the oxide and organic fractions than other methods. The uncertainties associated with sequential extractions processes must also be considered as boundaries between soil components can be somewhat arbitrary. For example, oxides may be coated in, or complexed with, organics resulting in oxides appearing in the organic defined phase, thus potentially causing an underestimation of the oxide fraction and an over estimation of the organic fraction (Maher, 1984). The order of extraction can also influence results with the modified version of the Tessier method which, due to extracting the organic fraction before the oxide fraction, results in an overestimation of the oxide phase due to readsorption (Litaor and Ibrahim, 1996).
Table 4 – Comparison of sequential extraction studies of $^{239,240}$Pu. The Pu in each fraction is given as a %. Data from the present study is presented as average of duplicate samples.

The geochemical association of $^{241}$Am is fairly uniform at both study sites, with the majority of the Am found in the organic and reducible oxide phases. These results contrast with an earlier study at the Rocky Flats site, where $^{241}$Am was predominantly associated with the sesquioxide fraction (39 – 47 %) with the exchangeable, carbonate and residual fractions also important, and the organic fraction being the least significant (IBRAHIM and SALAZAR, 2000). Hence, the $^{241}$Am at Rocky Flats may be most susceptible to mobilization under anoxic conditions in which sesquioxides are unstable, whereas at both sites studied here, anoxic conditions as well as degradation of organic matter would be expected to mobilize the greatest amount $^{241}$Am. However, our previous work suggests that dissolution of iron minerals under anoxic conditions does not lead to an increase of Am in solution (Chapter 4), possibly due to resorption of any Am released onto other mineral phases or the organic fraction. Our data here shows that under strong leaching conditions created by the production of sulfuric acid, 3.02 – 12.5 % of $^{241}$Am was leached into solution. The lower limit corresponds closely to the percentage of $^{241}$Am 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 sulfur-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 fairly significant differences, up to two orders of magnitude, between the leaching of $^{239,240}$Pu and $^{241}$Am under sulfur-oxidising conditions. Although $^{241}$Am in the Esk Estuary may be present from both the decay of $^{241}$Pu and as Am discharged from Sellafield, the $^{241}$Am at Aldermaston is likely only present through $^{241}$Pu decay. The sequential extraction and sulfuric acid leaching results suggest that $^{241}$Am which forms in situ from $^{241}$Pu becomes less recalcitrant than the Pu, showing a stronger affinity for the less stable soil fractions and hence becoming more prone to mobilization.

5.6 Conclusions

This study has helped to clarify further the biogeochemical behaviour of Pu and Am under environmental conditions. Our earlier work demonstrated how Pu is unresponsive to changing (bio)geochemical conditions, remaining insoluble under induced microbiological reductive conditions (Chapter 4). Here, we have shown that, even under strongly acidic conditions, less than 1 % of the Pu was leached into solution. The leaching of Am was slightly more successful, with up to 12.5 % of the total activity leached into solution. Such acid leaching has been successful in removing a wide range of heavy metal contaminants from soils, so these results suggest a highly refractory nature for the two actinides, Am and in particular Pu, in the Aldermaston soils. The
increased efficiency in leaching Am compared to Pu may suggest that although $^{241}$Am is a product of the $^{239}$Pu decay chain, the two actinides develop a different geochemical behaviour over time, with Am 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. The extractions reveal that most of the Pu in the soils is in 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. Comparison with sequential extraction results on similar sites suggests that the results should be interpreted with caution, but, when supported with further data, can be useful in predicting the physiochemical properties, and hence potential migration and/or bioavailability, of actinides. When taken together, the autoradiography, isotopic ratio, and sequential extraction data suggest the Pu in the Aldermaston soils exists as highly recalcitrant, small discrete particles, possibly in the oxide form.

5.7 References


Chapter 6

Citric acid and oxidative leaching of Pu from contaminated soils

Research Paper 3
6. Citric acid and oxidative leaching of Pu from contaminated soils

Richard L. Kimber¹, Francis R. Livens¹,² and Jonathan R. Lloyd¹

(1) Williamson Research Centre for Molecular Environmental Science and Research Centre for Radwaste and Decommissioning, School of Earth, Atmospheric & Environmental Sciences, University of Manchester M13 9PL, UK
(2) Centre for Radiochemistry Research, School of Chemistry, University of Manchester, Manchester M13 9PL, UK

6.1 Abstract

The mobilization of Pu in contaminated soils was investigated using two chemical remediation techniques. Organic acids, such as citrate, are able to form stable, soluble complexes with many metal cations, including Pu (FRANCIS et al., 2007; GADD, 2004; LU et al., 1998). Adding 6 g/L of citric acid to Pu contaminated soils from Aldermaston, UK, resulted in leaching of 27.1 % of $^{239,240}$Pu into solution. Doubling the citric acid concentration did not increase Pu leaching but apparently only leached 0.016 % of $^{239,240}$Pu. A number of factors may be responsible for this unexpected result including interference of citrate in the chemical separation or disequilibrium of the oxidation state of the sample Pu prior to separation. The potential for Pu mobilization through oxidative leaching was also investigated using permanganate which has previously been demonstrated to leach Pu in sludges from Hanford, US (RAPKO et al., 2004; RAPKO and VIENNA, 2002). Only 0.31 % of $^{239,240}$Pu was found in solution after the addition of 50 mM of KMnO₄, suggesting either little oxidation of Pu took place or that any mobilized Pu was resorbed onto other minerals, possibly MnO₂ formed from permanganate
reduction. To mitigate possible re-sorption, 0.5 M Na$_2$CO$_3$ was used to investigate whether carbonate complexes may stabilize mobile Pu species, however, even with the addition of carbonate, only 0.85 % of $^{239,240}$Pu was found in solution. This study suggests that Pu dissolution is unlikely to occur through oxidative leaching in Aldermaston soils but that citric acid may enhance Pu mobility.

6.2 Introduction

The Atomic Weapons Establishment (AWE) has maintained the UK’s nuclear weapons deterrent for over 50 years, operating over the Aldermaston and Burghfield sites in Berkshire. Due to the long and 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 the Environmental Protection Act, Part 2A. Here, we investigate potential chemical remediation options using Pu and Am contaminated soil from an area of the Aldermaston site. Previous studies reported particle-bound and dissolved $^{239,240}$Pu within the water management system at Aldermaston that varied in activity from ~1.2 Bq kg$^{-1}$ to ~400 Bq kg$^{-1}$ and ~0.7 µBq kg$^{-1}$ to ~44 µBq kg$^{-1}$, respectively (McCUBBIN et al., 2004). Remobilization experiments gave Pu $K_a$ values ranging from 2 x $10^6$ to 6 x $10^6$, suggesting the Pu is strongly sorbed to the soils (McCUBBIN et al., 2004).

Plutonium can exist in a variety of oxidation states in the natural environment as both the ‘reduced’ III and IV, and ‘oxidised’ V and VI forms. Pu (III) is generally oxidised and is not expected to exist in the absence of geochemically- or biologically-
driven reducing conditions (Neu et al., 2005). In oxic environments, which are dilute in plutonium and chelating agents, Pu(V) is favoured but it can reduce to Pu(IV) or oxidize to Pu(VI) upon interactions with minerals, bacteria and biogenic chelators (Keeney-Kennicutt and Morse, 1985; Neu et al., 2005). Pentavalent Pu can also undergo disproportionation to form a mixture of Pu(IV) and Pu(VI). Pu(VI) is generally not considered to be significant in the environment but can persist in marine environments or at high radionuclide concentrations in saline environments (Choppin et al., 1997; Neu et al., 2005; Runde et al., 1999). The higher oxidation states of Pu are readily reduced by organic species such as oxalate, citrate and acetate; as well as by microbial and mineral interactions, which results in Pu(IV) being the most common and stable species in biologically active systems such as soils. The sparing solubility of Pu(IV), especially in its dominant oxide and hydroxide forms, and its tendency to adhere strongly to mineral and particle surfaces often ensures Pu(IV) migration is limited to colloidal or particulate transport (Choppin and Morgenstern, 2001; Kersting et al., 1999).

Plutonium, in all oxidation states, acts as a hard Lewis acid and as such, forms strong complexes with O-donor ligands such as carbonate, carboxyl, and hydroxyl ions (Choppin and Morgenstern, 2001; Clark et al., 2006a). As complexation strength is a function of the charge density of a metal, the increasing strength of Pu complexation is as follows:

\[
\text{Pu (IV)} > \text{Pu (VI)} > \text{Pu (III)} > \text{Pu (V)}
\]
Organic acids produced by microorganisms can provide protons and metal-chelating anions capable of complexing metals cations (GADD, 2004; GADD and POOLE, 1999). For example, citrate is able to form stable, mobile complexes with Pu(III) and Pu(IV) (FRANCIS et al., 2007). The ability of citrate to leach plutonium and americium was investigated in contaminated soils from Rocky Flats Plant, Colorado, US. When sodium citrate was used as the sole leaching agent, between 20 and 68 % of the total alpha activity was removed from the soils, with efficiency increasing with particle size (LU et al., 1998). However, when used in conjunction with a reducing agent such as ascorbic acid or sodium dithionite, between 60 and 88 % of the alpha activity was removed. Previous work on Rocky Flats soils indicated that most of the Pu is associated with the organic and oxide fractions of the soil (LITAOR and IBRAHIM, 1996) whereas our previous work suggests the majority of Pu in the Aldermaston soils is associated with the highly refractory residual phase (Chapter 5). Plutonium in this fraction is highly recalcitrant and so may prove more resistant to dissolution through reduction or complexation; as such, mobilization through this mechanism may prove to be site specific.

Plutonium is more mobile in its oxidised (V) and (VI) forms than as the reduced (III) and (IV) states and hence, oxidation of Pu may enhance its mobility. Investigations into leaching Cr from contaminated sludges from the Hanford site, US, found that caustic, oxidative leaching resulted in the dissolution of the co-contaminant, Pu (RAPKO and VIENNA, 2002). A summary of various leaching methods found that permanganate, MnO$_4^-$, in combination with high OH$^-$ concentrations (3M) to be the most efficient, removing up to 50% of Pu from the sludges (RAPKO et al., 2004). Manganese is not stable in the Mn(VII) form under the redox conditions encountered in most natural
environments and so is a potent oxidant. In most natural groundwater systems, equation 1 is the dominant reaction by which permanganate is reduced (PETRI et al., 2011).

\[ \text{MnO}_4^- + 2\text{H}_2\text{O} + 3e^- \rightarrow \text{MnO}_2(s) + 4OH^- \quad 3.5 < \text{pH} < 12 \quad \text{(Eq. 1)} \]

As between 10.5 – 29.9 % of the Pu is found associated with the organic fraction in Aldermaston soils (Chapter 5), the use of permanganate may promote further Pu mobilization through breakdown of organic material. Metal speciation can also be altered during \textit{in situ} oxidation using permanganate through changes to both pH and Eh, with the latter increasing up to +800 mV (PETRI \textit{et al.}, 2011). At pH > 7, the mobility of Pu can be influenced by dissolved carbonate and hydroxyl ions with the mixed hydroxyl-carbonate ligand set one of the most stable complexes of Pu in the environment (SMITH and AMONETTE, 2006). Carbonate complexation forms strong anionic species, which will not sorb to negatively charged mineral surfaces, potentially increasing Pu mobility in carbonate-rich conditions. To mitigate Pu sorption onto \text{MnO}_2, the use of carbonate as a potential complexant was investigated during oxidative leaching. The stability of Pu-carbonate complexes in high pH / Eh conditions typical of oxidative leaching is shown in Figure 1.

The efficiency of Pu leaching on Hanford sludge simulants was found to be the reverse trend of Cr content (NASH \textit{et al.}, 2005). This was postulated to be due to re-adsorption of Pu onto \text{MnO}_2 produced from the oxidation of Cr(III) by the permanganate. The oxidizing properties of \text{MnO}_2 have also been suggested as contributing to the oxidation of Pu(IV) to Pu(V) in marine sediments and so may potentially provide a mechanism for remobilization of sediment bound Pu (MITCHELL \textit{et al.}, 1995). However, manganese
oxides have also been reported to sorb dissolved Pu(V) in synthetic groundwater, thus reducing the concentration of Pu in solution (Duff et al., 1999). In situ micro-X-ray absorption near-edge structure (XANES) spectroscopy measurements indicated the average oxidation state of the sorbed Pu was (V) in one enriched region and (VI) in another. It has been suggested that Mn(IV) may oxidise Pu(V) to Pu(VI), itself becoming reduced to Mn(III) or Mn(II) (Duff et al., 1999; Keeney-Kennicutt and Morse, 1985). Thermodynamic equilibrium calculations indicated the dominant species of Pu in solution as PuO₂CO₃⁻, suggesting sorption to the negatively charged Mn surface would be energetically prohibited. Therefore, it was suggested that speciation of sorbed Pu on surfaces is more influenced by solid-water interfacial processes than by the equilibrium speciation of Pu in solution.

Figure 1 - Eh-pH diagram for plutonium in water containing hydroxide, carbonate, and fluoride ions. Red dots represent “triple points” at which Pu can exist in three different oxidation states (Runde, 2000).
6.3 Materials and Methods

6.3.1 Citric Acid Leach
Soil contaminated with plutonium and americium, provided by AWE (Chapter 4), was added as 12.5% w/v to 100 ml leaching solution in 250 ml conical flasks. Chemical leaching was carried out using citric acid (sigma / monohydrate) in concentrations of 6 g/L and 12 g/L. The conical flasks were autoclaved prior to the addition of citric acid, which was then added aseptically. The flasks were incubated for 24 hours at 30 °C with shaking (120 RPM). The supernatant was separated by centrifugation and vacuum filtration. To ensure any Pu would not pass through the separation column as a complexed species, the supernatant was heated for 6 hours at 600 °C to remove organic species, then 100 mg NaCl was added prior to radiochemical analysis.

6.3.2 Oxidative Leach
10 g of AWE soil was added to a 50 ml centrifuge tube. A 30 ml solution containing either 50 mM KMnO₄ or 50 mM KMnO₄ with 0.5 M Na₂CO₃ was added to the tubes, which were then shaken for 24 hours at room temperature. Samples were centrifuged and vacuum filtered to separate the solid and aqueous phase. A 5 ml subsample was taken to measure pH using a Denver Instrument UltraBasic pH Meter UB-10 (Denver Instrument GmbH, Downham Market, Norfolk, UK).

6.3.3 Radiochemical Analysis
To prepare the supernatants for radiochemical analysis, 10 % v/v HNO₃ and 1 mg KNO₃ per ml of sample were added and the solutions heated to dryness. The residues were spiked with a known activity of ²⁴²Pu, taken up in 20 ml 9M HCl, applied to an
anion exchange column (described in detail in Chapter 4), electroplated and counted using alpha spectrometry for 60 hours to determine $^{239,240}$Pu activity.

6.4 Results and Discussion

6.4.1 Citric Acid Leach

The alpha spectrometry data for both the samples containing 6 g/L and 12 g/L of citric acid are presented in table 1. Although it may be expected that increasing the concentration of citric acid would increase the complexation and dissolution of Pu, it was found that the sample amended with 6 g/L citric acid performed more efficiently, with an estimated 27.1% of $^{239,240}$Pu leached compared to only 0.016% from the sample amended with 12 g/L. These results should be taken with some caution as a high error was associated with the 12 g/L sample with $0.003 \pm 0.009$ Bq of $^{239,240}$Pu detected in solution compared to $5.12 \pm 0.007$ Bq for the 6 g/L sample. The $^{238}$Pu/$^{239,240}$Pu ratio for the 6 g/L sample, 0.04, is also far closer to previously calculated values, ranging from 0.021 – 0.037 (Chapter 5) than the ratio from the 12 g/L sample, 0.41. This would suggest that data from the 12 g/L sample are unreliable. As the $^{242}$Pu tracer count was significant in the 12 g/L sample and as it is added prior to the radiochemical procedure, a low yield chemical separation is unlikely. Therefore, it may be possible that there are other factors which influenced the low activity of $^{238}$Pu and in particular $^{239,240}$Pu. For example, interference of citrate with the chemical separation through chelation of the sample Pu. However, heating the sample to 600 °C and the addition of NaCl prior to chemical separation would likely minimize this effect. Instead, it may be possible that during heating of the sample, the Pu became stuck to the glass as it was attacked by the
citric acid. An alternative explanation may be that insufficient equilibration of the sample Pu oxidation state was achieved prior to chemical separation.

<table>
<thead>
<tr>
<th>Citric acid concentration</th>
<th>$^{242}$Pu</th>
<th>$^{238}$Pu</th>
<th>$^{239,240}$Pu</th>
<th>$^{238}$Pu</th>
<th>$^{239,240}$Pu</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 g/L</td>
<td>125</td>
<td>365</td>
<td>8520</td>
<td>0.219 ± 0.008</td>
<td>5.12 ± 0.007</td>
</tr>
<tr>
<td>12 g/L</td>
<td>1760</td>
<td>28</td>
<td>68</td>
<td>0.001 ± 0.01</td>
<td>0.003 ± 0.009</td>
</tr>
</tbody>
</table>

Table 1 - Alpha spectrometry counts and corresponding activity for Pu isotopes detected in solution following citric acid leaching.

Total digestion of Aldermaston soils has shown they contain an average $^{239,240}$Pu and $^{238}$Pu activity of 1.51 (± 0.31) Bq/g and 0.0434 (± 0.022) Bq/g respectively. Based on these values, the percentage of $^{239,240}$Pu leached through the addition of 6 g/L of citric acid is likely to be in the range 25-30%, whereas the addition of 12 g/L of citric acid resulted in the dissolution of < 0.05% of $^{239,240}$Pu (Table 2).

The 25 – 30 % of Pu leached using 6 g/L (0.03 M) of citric acid here is similar to the total α activity leached (30 %) from Pu- and Am-containing soils from Rocky Flats using 0.1 M citric acid at 20 °C (LU et al., 1998). Increased α activity was leached in the Rocky Flats soils with increasing particle size; <0.053 mm (20%), 0.053-0.075 mm (37%), 0.075-0.25 mm (41%), 0.25-0.50 mm (50%), 0.50-1.0 mm (68%). This may suggest that Pu associated with smaller particles at Rocky Flats is more recalcitrant than Pu associated with larger particles. Synchrotron radiation studies revealed the Pu at Rocky Flats to exist as PuO$_2$, a highly refractory form which often adheres to small mineral particles (CHOPPIN and MORGENSTERN, 2001; CLARK et al., 2006b). As we see
highly recalcitrant Pu in this study, the Aldermaston Pu may also exist in a similar particulate form; this is supported by observations in our previous work (Chapter 5).

Citrate leaching appears to be more promising than previous methods for solubilizing Pu. Stimulating microbial reduction processes and sulfuric acid leaching resulted in only negligible Pu solubilization, < 0.01 and up to 0.18 % respectively (Chapters 4 and 5). However, the reasons for the much poorer performance of the 12 g/L citrate leaching procedure need to be better understood if the technique is to be applied on a large scale.

<table>
<thead>
<tr>
<th>Total Pu after soil digestion (Bq/g)</th>
<th>$^{239,240}$Pu</th>
<th>$^{238}$Pu</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.20</td>
<td>0.0256</td>
<td></td>
</tr>
<tr>
<td>1.53</td>
<td>0.0372</td>
<td></td>
</tr>
<tr>
<td>1.81</td>
<td>0.0675</td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>1.51 ± 0.31</strong></td>
<td><strong>0.0434 ± 0.022</strong></td>
</tr>
</tbody>
</table>

| Activity leached (Bq) 6 g/L | 5.12 | 0.219 |
| Percentage leached 6 g/L | 27.1 | 40.3 |
| Activity leached (Bq) 12 g/L | 0.003 | 0.001 |
| Percentage leached 12 g/L | 0.016 | 0.18 |

Table 2 – Total Pu activity in Aldermaston soils after sample digestion. The average is used to estimate the initial Pu activity of samples used in citric acid leaching.

6.4.2 Oxidative Leaching

The potential for Pu mobilization through oxidative dissolution was investigated using permanganate. When permanganate was added to the Aldermaston soils, 0.31 % of $^{239,240}$Pu was found in solution after 24 hours (Table 3).
The concentration of permanganate was far in excess of that of Pu, which even in relatively active soils is typically present in very low mass concentrations. However, numerous competing species, including organics and other metal species, may limit the oxidation of Pu. Furthermore, when permanganate is used in excess, formation of MnO₂ may catalyze MnO₄²⁻ autodecomposition (Equation 2), thus decreasing its ability to oxidise plutonium.

\[
MnO_2 \quad 2MnO_4^- + H_2O \rightarrow 2MnO_2 + 2OH^- + \frac{3}{2}O_2 \quad \text{(Eq. 2)}
\]

The increase in MnO₂ resulting from an excess of permanganate also increases the number of potential sorption sites for any Pu solubilized through oxidation. Although organics may compete with the Pu for the oxidant, their oxidation may be expected to result in dissolution of organic-bound Pu, as up to 29.9 % of Aldermaston Pu is associated with this fraction. Negligible Pu was found in solution, suggesting that either little oxidation of organic material or Pu took place or that any Pu released during dissolution was re-adsorbed onto another mineral phase, possibly the newly formed MnO₂.
The potential for reducing sorption of Pu to MnO₂ was investigated through the use of carbonate combined with permanganate leaching. Carbonate complexes of Pu(V) and (VI), which do not sorb strongly to mineral surfaces, can form in high Eh / pH conditions. Disproportionation of Pu(V) is retarded when Pu concentrations are low (< 10⁻⁶ M), thus reducing the formation of Pu(IV) and Pu(VI). As Pu(V) complexes are orders of magnitude more soluble than Pu(IV), the concentration of Pu in solution would be expected to be higher in oxidising, carbonate-rich conditions (RUNDE, 2000). The addition of carbonate increased the pH of the system to 10.5, compared with a pH of 7.65 when permanganate was used alone which, along with the high Eh conditions created during oxidative leaching, could allow the formation of such complexes. However, only 0.85 % of the ²³⁹,²⁴⁰Pu was found in solution in the carbonate-amended system, a small increase on the 0.31 % detected in solution when permanganate was used alone, suggesting that, even in the presence of ligands capable of creating sorption resistant complexes, little Pu is mobilized through oxidative leaching. This may be due an insufficient concentration of carbonate required for stabilization of a soluble Pu(V) species or the oxidation of the Pu species may have been unsuccessful due to the wide range of competing species present. Alternatively, sorption of the Pu onto mineral surfaces may be more influenced by mineral-water interfacial interactions than by the Pu species present in solution (DUFF et al., 1999).

6.5 Conclusions

To evaluate the potential for Pu migration in contaminated soils, two possible mechanisms were investigated. The ability of organic acids to mobilize heavy metals has been widely reported (GADD, 2000; GADD, 2004; REN et al., 2009), but their
interactions with radionuclides have not been studied in as much detail. Although earlier work has demonstrated the potential for leaching Pu and Am, the biogeochemical behaviour of Pu is often specific to both conditions present at the site and to the form in which it was released into the environment. Using citric acid, an estimated 25 – 30 % $^{239,240}$Pu was leached out of Aldermaston soils, which has thus far otherwise proved to be extremely recalcitrant. Therefore, this may offer a potential mechanism for the removal of Pu from contaminated soils and opens up further research avenues into the possibility of using biogenically derived organic acids, such as citrate produced by *Aspergillus niger*, in radionuclide bioremediation. The range of Pu leached into solution was similar to results from Rocky Flats soils, in which comparable amounts of Pu were leached from soil particles < 0.075 mm.

The potential for Pu migration in its more mobile, higher oxidation states was investigated through the addition of an oxidising agent, permanganate. Permanganate has been used to oxidize and leach Cr from waste sludges with occasional concurrent oxidation of the Pu co-contaminant (*RAPKO* and *VIENNA*, 2002). As MnO$_2$, formed via the reduction of permanganate, can sorb Pu, carbonate was also added to investigate whether any oxidised Pu could be stabilized as a soluble complex. In both cases, < 1% of Pu was found in solution indicating either little Pu oxidation had taken place or that the higher oxidation states formed were not stable as soluble species, either undergoing reduction to Pu(IV) or sorbing to mineral surfaces, such as MnO$_2$. 
6.6 References


Chapter 7

Conclusions and Future Work

Summary of previous chapters and an overall review of this research project.
7.1 Conclusions

Remediation and decommissioning of sites associated with the nuclear legacy is currently a major focus for governments worldwide. Developing remediation and management strategies for actinide-contaminated land requires knowledge of the actinides’ environmental behaviour. The complex chemistry of plutonium makes its environmental behaviour particularly hard to predict. Its mobility in the subsurface is controlled by its physiochemical properties, with the key factors being its oxidation state and the form in which it is released into the environment. The central hypothesis investigated here is that by developing our understanding of the biogeochemistry of Pu in the environment, we can better predict its behaviour in contaminated land and develop suitable remediation and management options.

A review of the current state of radionuclide-contaminated land worldwide was undertaken to assess a range of issues including the extent of contamination, associated costs and possible remediation options (Chapter 2). Radionuclide contamination is a global challenge, with cleanup costs estimated at a trillion dollars in the USA and £100 billion in the UK. A range of contaminants, including radionuclides and heavy metals, is present in both groundwater and soil at many sites associated with the nuclear legacy. However, numerous remediation options are available, with the potential to treat both groundwater and soil, and field studies have demonstrated the effectiveness of a number of these methods. The efficiency of these techniques often proves to be both site- and contaminant-specific. Where contaminants are highly insoluble and resistant to mobilization, long term management, rather than clean-up, of the contaminated land
may prove to be a more appropriate option. Here, monitoring likely transport pathways may be a more useful solution than attempting costly and inefficient remediation.

In order to understand the biogeochemistry of Pu at the Aldermaston site, the potential for microorganisms to control oxidation state was investigated. Reduction of Pu(IV), the most prominent environmental oxidation state, to the potentially more mobile Pu(III) species has been demonstrated using pure culture of bacteria in controlled laboratory conditions (BOUKHALFA et al., 2007; RUSIN et al., 1994). Here, we investigated the potential for this process to occur under the far more complex conditions found in the environment (Chapter 4). We found that under reducing conditions, stimulated by the addition of a carbon substrate (glucose) to anoxic sediments, only a negligible change in Pu solubility was observed, with < 0.01 % found in solution after 118 days. This was despite a substantial shift in the bacterial profile from a diverse community at day 0 to a community dominated by Betaproteobacteria and Clostridia at day 44. The latter community included organisms associated with metal reduction, such as close relatives to Clostridium species which have been reported previously to facilitate the reduction of Pu(IV) to Pu(III) (FRANCIS et al., 2008). The release of Pu bound to Fe minerals may have been expected, given the likely dissolution of Fe minerals, indicated by the increase in Fe(II) in solution, but again was not observed. This may indicate that any Pu released from mineral dissolution is quickly resorbed onto new or existing mineral phases. These findings suggest that the Pu in the Aldermaston soil is highly resistant to mobilization under microbially-induced reducing conditions.

In order to understand the refractory nature of the Pu and further investigate its biogeochemistry, a sequential extraction was performed on the Aldermaston soil
(Chapter 5). The majority of Pu was found to be associated with the residual, organic and oxide fractions, 63.8 – 85.5 %, 10.5 – 29.9 %, and 3.59 – 5.72 %, respectively. Metals associated with the residual fraction are highly recalcitrant, and likely to remain stable over changing geochemical conditions for a significant period of time. The Aldermaston soil was also subjected to sulfuric acid bioleaching in a sulfur-oxidizing bacterial system with a maximum 0.18 % Pu found to be released into solution. The biogeochemistry of americium, a co-contaminant in the Aldermaston soils, was also investigated. The Am was more evenly distributed across the residual, organic, oxide, and carbonate fractions (7.3 – 11.1 %, 45.6 – 63.6 %, 26.1 – 40.0 %, and 3.1 – 3.3 % respectively), with greater dissolution observed under sulfuric acid bioleaching conditions (up to 12.5 %). This would suggest that $^{241}$Am, formed in situ from $^{241}$Pu decay, is more susceptible to mobilization than Pu, presumably due to the increased tendency for Am(III) to sorb to less stable soil fractions.

The form in which Pu is released into the environment, typically from either nuclear weapons related processes or the nuclear fuel cycle, can play a major role in influencing its biogeochemical behaviour. As such, soil samples contaminated by discharges from the Sellafield reprocessing plant were also subjected to sequential extraction to compare any differences in geochemical behaviour to that of the Aldermaston Pu. These results revealed that a greater proportion of the Sellafield derived Pu was associated with the residual phase (91.9 – 94.5 %) which would suggest it is even more refractory than the Aldermaston Pu. However, these data contrasted with those from similar studies conducted more than 20 years ago using sediments from similar field sites, which found the majority of Pu in the organic fraction (Cook et al., 1984; Livens and Baxter, 1988), highlighting that caution is needed when interpreting sequential extraction
profiles. The order of extraction and variation of reagents used in sequential extraction methods appears to influence results strongly. Alternatively, Pu in the labile fraction may have washed away over time due to continued sediment washing or since become fixed in the recalcitrant fractions. Although it is clear that sequential extraction results should be treated with caution, they can provide useful information when combined with other data. The lack of Pu mobilized during microbial reduction and sulfuric acid production, combined with its association with residual material, strongly indicates that the Pu is highly recalcitrant. This is further supported by the characterization of the Aldermaston Pu through autoradiography and ESEM analysis. Small, alpha emitting particles were detected and isolated through autoradiography but could not be imaged using ESEM, suggesting the Pu exists as discrete particles, too small for detection. Taken together, these data are consistent with the Pu form being source-dependant, that is, a species which is not in thermodynamic equilibrium with its surroundings and as such whose environmental behaviour is controlled by the form in which it was released into the environment. Plutonium in this form often exists as highly refractory oxides (CHOPPIN, 2007), with particle size predominantly in the sub µm range (SHINONAGA et al., 2012).

As the higher oxidation states of Pu are more mobile, the potential migration of plutonium through oxidative leaching was investigated (Chapter 6). Previous studies have suggested that permanganate can promote the dissolution of Pu from contaminated sludges (RAPKO et al., 2004; RAPKO and VIENNA, 2002), although MnO₂ produced as the permanganate oxidises other metals may result in the resorption of Pu from solution (NASH et al., 2005). Carbonate was also added in one experiment (described in this thesis) to examine whether the complexation of Pu oxidised by permanganate under
high pH, carbonate-rich conditions would limit its resorption onto MnO$_2$. However, both with and without the addition of carbonate, permanganate was found to leach $< 1\%$ of the Pu from the soil. Although up to 69\% of Pu was shown to be leached from Hanford waste sludges, in the majority of samples $<1\%$ of Pu was leached (RAPKO et al., 2004; RAPKO and VIENNA, 2002). The most favourable conditions for leaching were at high temperature; 85°C, and high (3M) OH$^-$ concentrations. These data, along with our results suggests that the Pu at Aldermaston is unlikely to be at prone to mobilization through oxidation, even in the presence of inorganic ligands.

Organic chelating agents, such as citric and oxalic acid, have been demonstrated to enhance the solubility of heavy metals significantly (REN et al., 2009). An estimated 25 – 30\% of the $^{239,240}$Pu was leached from the Aldermaston soils using 6 g/L (0.03M) of citric acid. These data are comparable to a similar study at the Rocky Flats site, where 30\% of total $\alpha$ activity was leached using 0.1 M citric acid at 20°C (LU et al., 1998). At Rocky Flats, the leaching efficiency increased with increasing particle size suggesting that Pu associated with smaller particles is more recalcitrant than Pu associated with larger particles. Pu at Rocky Flats has been shown to exist as PuO$_2$, a highly refractory form which often adheres to small mineral particles (CLARK et al., 2006). The highly recalcitrant nature we observe from the Aldermaston Pu may suggest it also exist in a similar particulate form; supported by our particle characterization work. However, these results offer a potentially promising method of removing a significant proportion of Pu from the Aldermaston soils. The potential for increased efficiency using biogenic citric acid, such as is produced commercially by the fungus Aspergillus niger, and concurrent use of reducing agents should be investigated.
Although the potential for Pu remediation has been demonstrated in a number of studies, many of these were conducted under idealised conditions. When investigated under more environmentally relevant conditions or ideally during field studies, the remediation of Pu has proved more problematic, although techniques such as oxidative and citric acid leaching clearly offer some potential for the clean up of Pu contaminated land. However, even these methods appear to be reliant on the presence of specific conditions for both the Pu source term and the site and thus, only by defining the biogeochemical behaviour of the Pu at a particular site can appropriate remediation or management strategies be applied. The development of a generally applicable treatment process seems unlikely. This study has shown the Pu in the Aldermaston soils is highly recalcitrant and unlikely to mobilize under naturally occurring biogeochemical conditions. Indeed, even when more extreme treatments are applied, negligible Pu is mobilized. This would suggest that migration of the Pu is likely to be restricted to physical mechanisms such as particle transport processes.

7.2 Future Work

This research has opened up a number of pathways for further study into the biogeochemistry of Pu which could help develop our understanding behind the complicated environmental behaviour of this actinide.
These areas of interest for potential further work include:

**i. Characterization of Pu particles**

Characterization of Pu particles can help identify the mineral associations, and chemical and structural form of the actinide to provide further insight into its biogeochemical behaviour. In this research, particle characterization was undertaken through autoradiography and ESEM analysis. However, although radioactive particles were identified during autoradiography, we were unable to image them under ESEM, probably due to the particles being too small to image in a soil matrix, especially one containing many other heavy metals. Heavy liquid density separation could potentially allow better separation of the Pu particles which are much denser than the soil matrix, making identifying Pu-bearing particles under ESEM easier and leading to identification of associated minerals.

**ii. Heterotrophic Leaching**

Leaching of Pu using citric acid gave promising results which suggest this may offer a viable method for removing Pu from contaminated soils. The fungus *Aspergillus niger* is widely used for the commercial production of citric acid, and offers potential for the bioleaching of heavy metals via the production of this organic acid, as well as oxalic acid. Using these organic acids to leach Pu is a potential avenue for further research. The leaching efficiency of the biogenic and synthetic citric acid can be compared to investigate whether, for example, bioproduction in situ offers any advantage over the application of the chemically synthesised analogue. Investigation into potential increases in efficiency through using reducing agents in conjunction with chelating
organic acids is also an area for further study. This could potentially offer approaches
that could be optimised for in situ or ex situ Pu remediation.

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