CHAPTER 10. BIOINORGANIC CHEMISTRY OF PLUTONIUM AND INTERACTIONS OF PLUTONIUM WITH MICROORGANISMS AND PLANTS
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CHAPTER 10. BIOINORGANIC CHEMISTRY OF PLUTONIUM AND INTERACTIONS OF PLUTONIUM WITH MICROORGANISMS AND PLANTS.

10.1 Introduction

Nearly all aspects of plutonium chemistry have been described previously in reference books, such as Cleveland’s “The Chemistry of Plutonium”; the chemistry of plutonium in a range of biological systems is one exception. The behavior of plutonium in biological systems is important to understand: 1) to increase our fundamental and general knowledge of such an important element, 2) to improve our understanding and ability to predict the behavior of plutonium in the environment, 3) to enable reasonable risk assessment for human activities—ranging from effects on human health (if any) due to very low concentrations of Pu in groundwaters and due to the handling of large quantities in the nuclear fuel and weapons industries and 4) to develop decontamination methods for mammalian and environmental systems. In this chapter we describe our current understanding of the biological chemistry of plutonium. We begin with a brief description of the general chemical properties of plutonium that are relevant to biological systems, followed by an overview of plutonium bioinorganic chemistry and specific information on the interactions with mammals, microorganisms and plants.

Plutonium nuclides have been produced in significant quantities only in the past 50 years, but its unique and amazing properties have made it one of the most interesting and researched elements. Plutonium from cosmic origin has long since decayed, except for minute quantities of $^{244}$Pu (Hoffman 1971). $^{239}$Pu has been produced in natural reactors in particular geologic formations via neutron capture of $^{238}$U. These natural sources are minimal; nearly all existing Pu is man-made. Plutonium was first produced artificially and isolated in 1941 by Seaborg and coworkers at the Chicago Metallurgical Laboratory. The potential radiotoxicity of Pu was recognized, by analogy to radium, as soon as it was found to be an alpha emitter. Initial rat toxicology studies were performed in 1944. This was even before gram quantities were produced via reactors beginning in mid 1945 (Seaborg and Loveland, 1990). Of the 15 isotopes of Pu, only $^{239}$Pu is important for most general chemical, biological and environmental considerations. The bulk of plutonium produced is usually greater than 93% $^{239}$Pu by mass, with the remainder being $^{240}$Pu, $^{241}$Pu, and $^{238}$Pu (in order of decreasing mass fraction). $^{239}$Pu has a half-life of about 24,000 years, which means one milligram emits about $10^6$ alpha particles per second. Other isotopes are important for special applications and research, such as heat sources based on the decay of $^{238}$Pu (half-life of 87.7 years) and experiments requiring lower radioactivity employing $^{242}$Pu (half-life of 375,000 years). Additionally, a few microbial and plant studies used the isotope $^{237}$Pu (half-life of 45.2 days).

Plutonium displays the most complex chemistry among any of the elements in the periodic table. It exhibits seven discrete crystal metallic phases, is highly reactive, and is known to form compounds or alloys with nearly every other element (Katz, Seaborg and Morss, 1986). Its ionic forms include five oxidation states which can form solution complexes or solid state compounds containing a very few to a dozen chemical bonds with other atoms. In aqueous solution the highly electropositive plutonium atom will readily lose three to seven of its outer electrons to form cations in the formal oxidation states, Pu(III), Pu(IV), Pu(V), Pu(VI), and Pu(VII). The chemistry of each of these ions is distinct, that is, they form different types of compounds with distinct physical and chemical properties.

One of the most challenging and interesting aspects of plutonium chemistry is that significant quantities of more than two oxidation states can coexist. This is unlike nearly all other elements. The relative amounts of the ions present depend on the initial composition (both isotopic and
chemical) and the particular conditions. Because it is only formed under extreme conditions, Pu(VII) is not relevant for biological systems and therefore will not be discussed. Radiolysis tends to reduce Pu(VI) and Pu(V) to Pu(IV) and Pu(III). Acidic conditions generally favor the lower oxidation states, III and IV, and basic conditions generally favor the higher oxidation states, V and VI. The electrochemical potentials relating the ions in acidic, neutral, and basic aqueous solution are given below (Scheme 10-1) and illustrate the relatively low thermodynamic barriers between oxidation states. Plutonium(III) and Pu(IV) can exist in solution as roughly spherical hydrated ions, retaining their overall charge. Whereas, Pu in the V and VI oxidation states are are highly positively charged that in aqueous solution they immediately hydrolyze to form complex ions, referred to as transdioxo or plutonyl species, \( \text{PuO}_2^+ \), and \( \text{PuO}_2^{2+} \), which have estimated effective charges of 2.2 and 3.3, respectively. The number of atoms with which these ions form bonds (coordination numbers) generally varies from six to twelve for Pu(III) and Pu(IV) and two to eight, in addition to the 'yl' oxygens, for Pu(V) and Pu(VI). Generally, the thermodynamic stability of solution complexes and tendency to hydrolyze decrease, and the solubilities of solid compounds increase, following the effective charges: Pu(IV) > Pu(VI) • Pu(III) > Pu(V).

\[ \begin{align*}
\text{Pu(III)} & : +0.982 -0.39 -1.04 \\
& \quad \quad \quad +1.170 +0.70 +0.52 \\
& \quad \quad \quad \quad \quad +0.916 +0.60 +0.16 \\
\text{Pu(IV)} & : +1.043 +0.65 +0.34 \\
\text{Pu(V)} & : +1.076
\end{align*} \]

Scheme 10-1. Electrochemical potentials relating plutonium ions in acidic (1 M HClO₄, top), neutral (pH 8, middle), and basic (1 M NaOH, bottom) aqueous solution (Allard et al., 1980, Cleveland, 1979).

Plutonium ions are 'hard' acids, meaning they have high charge to ionic radius ratios and form strong inner sphere complexes with ligands containing 'hard' donor atoms, such as oxygen. For biological and environmental systems the most stable complexes are formed with oxide, hydroxide, carbonate, fluoride, phosphate and sulfate type ligands. Hydrolysis and the generally low solubilities of the resulting oxide, oxyhydroxide and hydroxide phases limit the amount of plutonium that is bioavailable. At the extreme end of the spectrum is Pu(IV), which hydrolyzes even in pH 1 solutions and can form complicated colloidal hydroxides. For example, at concentrations ranging from \( 0.4 \times 10^{-4} \) to \( 1.2 \times 10^{-2} \) M in 0.1 M HNO₃, 55% of the Pu(IV) will polymerize within 1 hour (Katz, Seaborg and Morris, 1986). These colloidal species can stay suspended in solution for extended periods of time, and once formed, they are inordinately stable leading to Pu(IV) soluble concentrations exceeding the calculated thermodynamic solubilities. The free ion or this colloid can also attach to particles, such as microscopic mineral fragments or microorganisms, to form composite colloids. While the published solubility product of Pu(IV) hydroxide, \( 7 \times 10^{-56} \) (Katz, Seaborg and Morris, 1986); gives a calculated Pu(IV) solution concentration at pH 7 of \( 10^{-28} \) M, observed solubilities are closer to \( 10^{-8} \) to \( 10^{-13} \) M due to interactions with other simple ligands, redox instability, and the formation of colloidal species. (We note that the determination of hydrolysis constants and solubility products of Pu(IV) oxides
and hydroxides is exceedingly difficult due to the uncertainty and variability in exact composition of the solid phases and solution species, the low soluble concentrations and the difficulties of phase separation. Thus, the reported thermodynamic constants for these compounds must generally be considered best possible estimates.)

The formation constant for the first Pu(III) hydrolysis product, Pu(OH)\(^{2+}\), has been reported to be 105.8 and the solubility product of Pu(III) hydroxide has been reported to be 2 x 10\(^{-20}\) (Kraus and Dam, 1949, Katz, Seaborg and Morss, 1986). Plutonium(VI) hydrolysis has not been well studied, but comparison with Pu(III) and U(VI) allows estimation of the first hydrolysis product of ~108.5 to form PuO\(_2\)(OH)\(^+\), or at higher Pu concentration, the corresponding dimer, [PuO\(_2\)(OH)]\(^{2+}\). In contrast with the other oxidation states, the onset of hydrolysis for Pu(V) is not observed until pH~9. Thus, at environmental and physiological pH the 'free ion', PuO\(_2\)^{+}, is the predominant species. (If millimolar carbonate is present, the monocarbonato complex, PuO\(_2\)(CO\(_3\))\(^{-}\), will be formed.)

Biological systems will generally favor Pu in the IV and V and to a lesser extent III oxidation states, similar to environmental waters, which generally are pH 5 to 9, Eh from -300 to +500 mV, low (\(< 1 \text{ M}\)) ionic strength. Because the solution concentrations are generally quite low, the disproportionation of Pu(V) to Pu(IV) and Pu(VI), which governs the chemistry at concentrations generally studied in the laboratory, is greatly reduced. Counteracting this stability of Pu(V), the presence organic acids and proteins favor Pu(IV) through the formation of very stable Pu(IV) complexes. Similarly, Pu(III) will generally be oxidized to Pu(IV). Therefore, Pu(IV) is the oxidation state most important to consider in biological systems.

Another aspect of plutonium chemistry that is significant for biological systems is the chemical similarity of Pu(IV) and Fe(III) ions. Both are hard lewis acids and they have high charge to ionic radius ratios: 0.46 for 6-coordinate Fe(III) and 0.42 for 8-coordinate Pu(IV). On a per hydroxide basis their hydrolysis products are similar, 10\(^{-12.6}\) for Fe(III) and 10\(^{-13.7}\) for Pu(IV) (Martell and Smith). Their formation constants for complexes with organic and biological ligands are also similar (Table I). They also both form colloidal hydroxide species, which may be suspended or precipitate, and form highly insoluble hydroxides and oxides. An important difference for biological considerations is that Fe(II) and Fe(III) have greater affinity than Pu ions for sulfur donor ligands. Under oxic conditions, Fe(II) is typically oxidized to the less-soluble Fe(III); similarly Pu(III) is oxidized to the less soluble Pu(IV). Because of these similarities, the biochemistry of Pu is compared to that of Fe and the in vitro or in vivo behavior of Pu is often described in terms of known Fe processes. For example, we know that Pu is bound to transferrin, the serum iron transport protein in a wide variety of species, and ferritin, the iron storage protein (Harris, 1998, Taylor, 1995 and references therein).

Iron is an essential element for all organisms, from bacteria to mammals, with the exception of a few microbes. Because of the low solubility and bioavailability of iron in the environment, organisms have developed ways to solubilize, sequester, take up and/or store the iron they require. A classic system involves low molecular weight organic ligands, siderophores, which are excreted and used by plants and microbes to chelate iron and transport it across the cell membrane. Siderophores and siderophore-based processes have been used to affect plutonium chemistry and biological behavior. The class of siderophores that have been studied the most for both Fe and Pu are the hydroxamate-containing ferrooxamines. The structures of two desferrioxamines (DFO), which will be discussed later in this chapter are shown in Scheme 10-2.

<p>| Table 10-1. Solution Stability Constants (log β) for Pu and Fe Complexes of a Few Biologically Important Ligands. |</p>
<table>
<thead>
<tr>
<th>Ligand</th>
<th>Complex</th>
<th>Pu$^{3+}$</th>
<th>Pu$^{4+}$</th>
<th>Fe$^{2+}$</th>
<th>Fe$^{3+}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric Acid</td>
<td>ML/M-L</td>
<td>8.9</td>
<td>15.2-15.5</td>
<td>4.4</td>
<td>11.2</td>
<td>1,2</td>
</tr>
<tr>
<td>Oxalic Acid</td>
<td>ML/M-L</td>
<td>8.3</td>
<td>3</td>
<td>7.5</td>
<td>13.6</td>
<td>1,2</td>
</tr>
<tr>
<td></td>
<td>ML$_2$/ML$_2$</td>
<td>9.3</td>
<td>14.9</td>
<td>5</td>
<td>13.6</td>
<td>1,2</td>
</tr>
<tr>
<td></td>
<td>ML$_3$/ML$_3$</td>
<td>9.4</td>
<td>23</td>
<td>18.5</td>
<td>1</td>
<td>1,2</td>
</tr>
<tr>
<td>EDTA</td>
<td>ML/M-L</td>
<td>17.3</td>
<td>25.7</td>
<td>14.3</td>
<td>25</td>
<td>1,2</td>
</tr>
<tr>
<td>NTA</td>
<td>ML/M-L</td>
<td>12.9</td>
<td>8.3</td>
<td>15.9</td>
<td>1,3</td>
<td></td>
</tr>
<tr>
<td>Desferrioxamine B</td>
<td></td>
<td>30.8</td>
<td>9.5</td>
<td>30.6</td>
<td>1,4-6</td>
<td></td>
</tr>
</tbody>
</table>


Scheme 10-2. Chemical structures of two desferrioxamine siderophores (DFO), the linear trihydroxamate produced by *S. pilosus*, Desferrioxamine B (DFB) and the cyclic trihydroxamate produced by *P. stutzeri*, Desferrioxamine E (DFE).

10.2. Plutonium Biochemistry
10.2.1 Pu in Mammals

One area of study that has been extensively researched and well reviewed is the behavior of plutonium in mammalian systems. Experimental studies include: i) quantitative description of distribution and retention, ii) identification of chemical forms transported in blood and excreted, iii) dose-dependent acute and chronic toxicity and iv) intrinsic and promoted removal (Durbin, 1992). The International Commission on Radiological Protection (ICRP) has published excellent comprehensive reviews, for example publications 19 (1972) and 48 (1986). Mammalian metabolism and toxicity have been thoroughly described by others: Thompson 1962, Bair 1974 and 1979, Nenot and Stather 1979, ICRP 1972, 1980, 1986, 1993, Bulman, 1980, Metivier, 1982, Taylor, 1988, and BEIR 1998. There are also substantial reviews of potential human health effects of plutonium, the 1971 Hanford Biology Symposium, edited by Thompson and Bair (1972), the extensive history of radioactivity and health effects compiled by Stannard (1988), and a recent review by Clarke et al. (1996). Given the large number of critical literature reviews, the topics of mammalian distribution, excretion, and toxicity will be only briefly summarized here.

The extent of early investigations on the effect of Pu on mammals is impressive. The first major studies, done on rats, included, oral ingestion, inhalation, and parenteral injection routes of administration and extensive tissue analyses for Pu in oxidation states III, IV, and VI as well as fission products. (Numerous studies have shown that Pu is not absorbed through the skin.) In these studies, absorption, distribution, retention, and excretion were investigated and
autoradiographic studies were carried out on tissue that appeared to most concentrate activity. Another noteworthy aspect of most studies, particularly the early experiments, is that the activity administered ranged from 0.5 to 5.0 microcuries—levels much higher than any modern industrial exposure and vastly higher than any predicted environmental exposure.

Mammalian distribution results from the vast number of studies are consistent, that Pu deposits mostly in the bone and liver and is retained in these tissues for many years. Smaller fractions may be present in blood and the lymph system. In general, monomeric Pu complexes will deposit about 70% of the Pu in the skeleton and about 30% in the liver (Thomas, Healy and McInroy, 1984). Metabolic activity, cell renewal in the liver and tissue reconstruction in the bone, move Pu from old to new binding sites. There is substantial variability in metabolic behavior and toxicity depending on the form of Pu administered, as well as differences between species. Even among human autopsy analyses of occupationally exposed individuals, most of whom were exposed to PuO₂, there was considerable variability in distribution. The liver to skeleton distribution ratios varied from 1:5.2 to 21:1. (Nenot, 1979) This variability may be due to differences in chemical and physical characteristics of the contaminant and the circumstances of exposure, but other biological factors must also play a role. From a Pu chemistry perspective, colloidal species may form on entry into the body, and be distributed very differently from Pu that is complexed with plasma proteins.

When inhalation is the route of intake, particulate Pu is retained in the lungs and more soluble forms are rapidly distributed. When particles are inhaled they become internalized into lysosomes, subcellular organelles that are responsible for the digestion of foreign materials. Just as specific activity affects solution solubility measured in the laboratory, ²³⁸PuO₂ aerosols are retained in the lung for shorter times than are ²³⁹PuO₂ or mixed U and Pu oxide aerosols. (Guilmette et al., 1992) Particle size has also been shown to affect behavior in the lung. Particles of ²³⁸PuO₂ or ²³⁹PuO₂ with sizes >0.025 µm did not result in movement of Pu to blood; (Smith et al., 1977; Stradling et al., 1978) however, smaller particles were shown to leave the lung within minutes. From analyses of plasma samples taken from 2 to 30 minutes after administration, three major fractions were observed, intact 1 nm particles, an intermediate fraction (smaller and perhaps complexed with citrate), and transferrin bound Pu.

Concentration in lymph nodes also occurs, but does not appear to lead to deleterious effects. Fractional uptake from the human gut is as low as 10⁻⁵ for relatively insoluble compounds, such as PuO₂ and still low at 10⁻³ or even 10⁻² for soluble complexes (Clarke, et al., 1996). Excretion of plutonium in urine is minimal, reported excretion amounts are generally 5 to 10 % and only as high as 30% over 20 to 50 years. The forms excreted are thought to be mainly citrate complexes in the urine (Durbin, 1992) and carbonate complexes in bile from the liver (Duffield and Taylor, 1985). While the amount and rate of plutonium absorbed from the lungs or from a wound may be expected to vary, the distribution of absorbed Pu was indistinguishable when Pu(III), (IV), or (VI) was administered to rats and was always consistent with Pu(IV). (Results were found to be consistent with Pu(IV) when they were compared with results from studies performed using trivalent lanthanides, Th(IV), U(VI), and Pu(IV) only.)

Under normal exposure and even in most accident conditions, levels of exposure are very far below levels that would immediately damage tissue. Alpha particles emitted from Pu are highly energetic, but heavy particles that are not very penetrating. The 4-5 MeV alphas emitted from most Pu isotopes have a range of only approximately 40 micrometers, depending on the type of tissue. It has been calculated that about 90% of the ionization in an alpha track is deposited within a cylinder of 0.01 µm radius, with a further 9% deposited from 0.01 to 0.2 µm. (Bleaney, 1973) Energy is deposited at a high rate over a small volume; thus tissue damage is highly
localized. Because of this very small range of energy deposition, the highly localized concentrations, and long half-lives of Pu isotopes long-term, chronic toxicity is much greater than the immediate acute toxicity. A latent period of about 20 years is expected between exposure and potential development of a solid tumor in the lung or bone. Differences in sensitivity between species studied—rodents, dogs, and non-human primates—have been noted, with primates being the least sensitive (Clarke, et al. 1996).

Cancers unequivocally attributable to alpha radiation from plutonium have not been observed in humans (Clarke, et all, 1996). Estimates of the effects of Pu in man have to be obtained indirectly. (The main source of epidemiological information about radiation effects is the study of the survivors of the atomic bombing of Hiroshima and Nagasaki; however, this information relates to gamma radiation at very high dose rates and is not applicable to Pu exposure). The life span of laboratory animals is shortened by doses of $^{239}$Pu (Jee, 1971) While mice suffered no ill effects from doses less than 1/1000 of the acutely toxic dose (~1 µg/kg), a dose of 0.26 µg/kg given to dogs increased the incidence of bone cancer from 1/10,000 to 1/3 and decreased their lifespan 14%. Lung cancer formed in dogs that inhaled ~µg/kg of PuO$_2$, but their lifespan was not significantly shortened. Other studies show that subacute changes in rat livers were observed only at a human dose equivalent of approximately 200 mg Pu administered intravenously, which is approximately 330,000 times the maximum body burden recommended by the ICRP in 1959 (Mahlum and Sikov, 1969).

There is no evidence of detrimental health effects of plutonium in humans who have had low-level exposure due to global nuclear fall out. One may suspect a different case of radiological workers at Pu production and processing facilities. Epidemiological studies are being carried out on Pu workers at the Rocky Flats Site and the Los Alamos National Laboratory in the USA, the Sellafield nuclear processing plant in the UK and the Mayak plant in Russia (Clarke et al., 1996). For example, the report on the Rocky Flats employees was a mortality study of about 5400 white males. When cancer mortality was compared with US national rates, no excess was found for all cancers combined or for cancers in the lungs, skeleton, or liver. When the mortality of employees having greater than 74 Bq was compared with employees with a lower body content, there appeared to be a statistically significant excess for lymphopoietic tumors, but only if a lag time of five years was assumed in the calculations. No excess was seen if other lag times were used. Results from the studies at other sites are similar, with no cause of death significantly elevated among Pu workers and data from some studies for subgroups that received higher doses suggestive of slightly elevated risks of specific cancers. Overall, the history of Pu is remarkably free from cases of injury to occupational workers and the public. The only clear cases of harm were from a small number of criticality accidents. The average intake of Pu by occupational workers is consistently low. With one possible exception, epidemiological studies have not been able to demonstrate adverse health effects in humans (Clarke et al., 1996).

10.2.2 Plutonium Interactions with Biological Molecules

Plutonium retention and distribution are reportedly due mostly to the action of the iron transport protein, transferrin (Tf). Iron in blood serum is carried by transferrin to erythroid cells for hemoglobin synthesis. Excess iron is stored primarily in the liver and spleen as ferritin and hemosiderin. Pu(IV) is also bound by transferrin in serum to form a Pu-transferrin protein complex (Pu-Tf) and is bound by ferritin. However, Pu-transferrin does not bind to blood cells and has not been shown to actively ‘deliver’ Pu to specific sites (Bruenger et al., 1969). It is thought that transferrin affects metabolism indirectly by preventing excretion and by acting as a long-lived Pu buffer in blood. (Durbin, Horovitz and Close, 1972). Early studies reported that
plutonium may also be bound by albumin, alpha-macroglobulin and very weakly, if at all, to collagen (Stevens et al., 1968, Turner and Taylor, 1968). However, data from these studies have been reinterpreted and suggest the formation of high molecular weight Pu hydroxide polymers, rather than additional Pu-protein complexes. And more recent studies using ion exchange and affinity chromatography consistently show that Tf is the only significant protein ligand for Pu in serum (Taylor D.M., et al 1991; Lehman M, Culig H, Taylor DM (1983); Taylor DM, et al 1987; Harris, 1998).

The circulating Pu-Tf protein complex is in equilibrium with reactive low molecular weight Pu complexes that, unlike the Pu-Tf complex, are taken up by cells. The complexes have not been unequivocally identified, but an active hypothesis is that citrate plays a role possibly forming the species Pu(citrate)$_2$(OH)$_2$$^-$ (Duffield, May and, Williams, 1984, Duffield, Raymond, and Williams, 1987).

10.2.2.1. Plutonium Interactions with Transferrin

Transferrin is a glycoprotein of approximately 80,000 MW that transports iron through the blood between sites of uptake, utilization and storage. Harris (1998) recently reviewed the binding and transport of metals by Tf. The protein consists of two distinct lobes, each containing a high affinity metal binding site. Normally the transferrin in serum is only partially saturated with iron (about 30%), so that the protein has vacant binding sites that can sequester other metal ions. Given that the serum transferrin ranges from 25 to 50 µmol/L, normal serum will contain about 50 µmol/L vacant transferrin metal binding sites (Brock, 1985). It is known that the strong binding of a metal ion to apoTf (metal free transferrin) requires the concomitant binding of a synergistic anion, generally carbonate. The metal binding of the first coordination sphere includes the phenolic oxygens to two tyrosine residues, the imidazole of a histidine, and a monodentate carboxylate side chain of an aspartic acid. The fifth and sixth positions in the Fe(III) coordination sphere are occupied by the bidentate carbonate (Harris, 1998 and references therein).

Plutonium injected intravenously into rats was found bound to serum transferrin. (Boocock and Popplewell, 1965) Stover et al demonstrated that plutonium was bound to transferrin in serum obtained from humans and from dogs (1968). And Turner and Taylor demonstrated the binding in vitro of Pu(IV) with horse serum transferrin required the presence of bicarbonate (1968). Pu binding to transferrin in solution is blocked by the addition of Fe(III) (Stover et al., 1968, Turner and Taylor, 1968). In addition, spectroscopic data show that Tf coordinates Pu by the OH groups of tyrosine residues. Taken together all of these studies indicate that Pu(IV) is bound with synergistic carbonate by Tf and in the same sites as Fe(III). A binding constant of log K$_{1}^{*} = 21.2$ has been reported for Pu-Tf (Taylor, 1993). This is slightly higher than the Fe-Tf binding constant. Since it is known that Fe(III) readily replaces Pu(IV) from transferrin and that DTPA, citrate, and other chelators remove Pu from serum transferrin (Stover et al., 1968, Turner and Taylor, 1968, Taylor and Kontoghioghi, 1986), this high Pu-Tf binding constant should be considered preliminary (Harris, 1998). Based on Th(IV) and Hf(IV) data, it is unlikely that the binding of Pu(IV) to apotransferrin will compete effectively with hydrolysis unless there is an excess of apoTf; the hydrolysis and polymerization of Pu may be expected to be dominant reactions near stoichiometric Pu:Tf ratios. (Harris, 1998)

The mechanism by which iron is then internalized into a cell via transferrin is well established. Iron is transferred when the Fe-Tf complex combines with a specific receptor on the hepatocyte plasma membrane and the metal is translocated alone into the cell interior, before being transferred to apoferritin. It is not known whether Pu-Tf complex binds to this receptor.
The fact that the transferrin receptor is widely expressed in growing tissues but plutonium deposition is not widespread and is different from Fe suggests that even if Pu-Tf binds to the receptor then it is not internalized (Crichton, 1991). If Pu followed the Fe(III) bioinorganic pathways, heme-containing cells would be expected to contain Pu, but this is not observed. Taylor has suggested that the transferrin receptor fails to recognize Pu-Tf because Pu(IV) fails to promote the change to the 'closed' conformation characteristic of ferric transferrin (1995). These conformational differences may be a consequence of the larger size and higher preferred coordination number of Pu(VI) versus Fe(III).

The percentage of serum Pu reported to be bound to Tf varies from 80 to 100% (Taylor et al., 1991, Turner and Taylor, 1968; Lehman et al., 1983, Taylor et al., 1987). A kinetic model for plutonium deposition indicates that the low molecular weight fraction of Pu clears from the blood more rapidly than the Pu bound to Tf, so that the fraction of Pu-Tf tends to increase with time and accounts for nearly all of serum Pu 2 hours post-injection (Durbin, et al., 1972). Because Pu-Tf is unable to penetrate the cell membrane, protein binding limits uptake and chemical toxicity.

10.2.2.2. Plutonium Interactions with Liver Cells

There appears to be no significant uptake of Pu directly from Pu-Tf into the liver (Durbin, 1972). In fact, the addition of apoTf almost totally inhibits Pu uptake into hepatocytes (Planas-Bohne and Duffield, 1988), while the addition of diferric Tf has almost no effect (Planas-Bohne and Duffield, 1988, Schuler, Csovcvsics, and Taylor, 1987). It has also been shown that Pu does bind to the cell membranes following exposure to Pu-Tf. Taylor and coworkers (Schuler, Csovcvsics, and Taylor, 1987) studied Pu uptake in liver cells grown as multicellular spheroids rather than as a monolayer and found much higher uptake of Pu from Pu-Tf. However, there was still no competition from Fe-Tf, so it appears that even under conditions leading to higher cellular uptake of Pu, Pu-Tf does not deliver Pu to the cell via the usual Fe-Tf receptors. A possible explanation is that Pu-Tf binds at the normal Fe-Tf receptor, but cannot be internalized and/or released from Tf for incorporation into the cell (Planas-Bohne, Jung, and Neu-Müller, 1985, Planas-Bohne, Taylor, and Duffield, 1985). However, the addition of detergent to solubilize membrane proteins from lymphoblasts shows that the Pu is not present either as Pu-Tf or as the Pu-Tf-receptor complex (Planas-Bohne and Rau, 1990). Instead, the Pu appears to dissociate from Tf and bind to some other membrane protein.

Soluble low molecular weight complexes such as Pu-citrate are taken up in hepatocyte (functional liver cells) cultures, presumably by passive diffusion (Planas-Bohne and Duffield, 1988, Planas-Bohne, Jung, and Neu-Müller, 1985, Planas-Bohne, Taylor, and Duffield, 1983). It has been suggested that transfer to the liver may be through complexation by phospholipids (Bulman, 1980). Although the mechanisms are not completely understood, Pu clearly is transferred to the liver. Plutonium ends up in the cytosol, and then is distributed among various organelles with preferential accumulation in lysosomes and nuclei.

10.2.2.3. Plutonium Interactions with Bone and Bone Proteins

Plutonium will readily bind to any mineralizing tissue, whether it be phosphate or carbonate based (Bulman, 1980). A significant portion of Pu taken into the blood initially localizes on the external and internal anatomical surfaces of mammalian bones. Soluble, monomeric Pu is preferentially taken up by bone and the degree of bone deposition is directly related to bone growth (Durbin, 1975) and blood flow (Howells et al., 1984). Plutonium(IV) is deposited preferentially on resorbing and resting endosteal surfaces surrounded by active red marrow (Durbin, 1992). The specific nature of the initial chemical binding is not yet known. Similar to
the transfer into the liver, the process does not involve transferrin receptors. Instead, it appears that there is a ligand exchange reaction between transferrin and either phospholipids or proteins at the mineralizing surfaces of the bone (Priest and Giannola, 1980). Several bone proteins are reported to have Pu-binding affinities greater than that of apoTf (Chipperfield and Taylor, 1968, 1970). These bone proteins are carbohydrates composed largely of sialic, aspartic, and glutamic acids.

It has been observed that the initial bone deposition site is at the mineral-organic interface and that both the bone proteins and mineral components are important for deposition. The Pu does not appear to substitute for calcium within the mineral component of the bone. Studies with synthetic bone mineral crystals (hydroxypatite) suggest specific, saturable binding sites. Since Pu-phosphate complex formation constants are quite high, $10^{28}$ for Pu(PO$_4$)$_2^{2-}$, (Martel and Smith, 1989), binding by surface phosphate groups would be thermodynamically favored over transferrin or other solution complexes. It has been suggested that initial binding involves two phosphate groups at the mineral surface and hydroxyls of bone protein residues (Durbin, 1992). After initial sorption onto the surface, Pu is taken in to the bone volume due to redeposition and burial of Pu recirculated at low blood concentration. There is some evidence that the distribution between organic and mineral bone components and the rate of incorporation into bone volume depends on the age of the animal (Ramounet, et al., 1998). Because Pu becomes imbedded in the bone matrix, this fraction of Pu body burden is physically inaccessible to serum chelating agents (Raymond and Smith, 1981).

10.2.3. Removal from mammalian systems

While the radiological controls, which have been in effect for decades, have been very successful in preventing excessive exposures, many questions remain unanswered with respect to its detailed biochemistry and behavior in humans. Long-term effects observed in animal experiments (mainly rats and dogs) are malignant changes in the organs in which the material deposits. There changes are mainly in the lung, bone and liver: from inhalation, lung cancer or, less frequently, liver cancer; from injection, bone cancer or, less frequently, liver cancer. (Again, the levels studied, μCi/kg, are hundreds to thousands of times greater than human exposure limits, which in turn are hundreds of times greater than normal occupational exposure.)

Since Pu is distributed to storage sites rather rapidly and eliminated so slowly from mammals, prompt and continuous treatment is suggested to decrease health effects. Therapeutic removal of Pu evolved from substituting a nontoxic metal for Pu bound in blood and tissue to chelating the metal with general or specific decorporation agents. The earliest treatment was intravenous administration of large amounts of zirconium citrate (Taylor, 1995). While administered immediately, excretion from dogs was increased to about 90%. However, when two hours elapsed between Pu and Zr injections, only 10% of the Pu was excreted. It was suggested that the mechanism of removal resulted from the formation of colloidal zirconium hydroxides and phosphates. Other metals behaved similarly, and even worse, Th(IV) and Al(III), which hydrolyze to form large polymers that do not pass through the kidneys, caused increase deposition of Pu in the liver (Raymond and Smith, 1981). Polymeric phosphates were also tested and found to reduce Pu bone absorption, but increase storage in the liver.

Because citrate complexes were suspected to be the major form of Pu naturally excreted, citrate was the first ligand to be tested as a removal agent. The rapid metabolism of citrate and its metal complexes limited its effectiveness, with excretion being significantly increased if administration was within 30 minutes and insignificantly increased if administration was after two hours. Chelators used to remove other toxic metals, such as 2,3-dimercaptopro-1-propanol, were tested. This sulfur donor ligand, as well as others, was found to be ineffective for removing the
oxophilic Pu. The study of Pu decorporation agents then turned to ligands that have greater affinity for Pu and are not metabolized.

To be effective, the chelator should form Pu complexes that are chemically and biologically stable and rapidly and quantitatively excreted via the urine or bile. Further, the chelator should be nontoxic, selective for Pu, able to out compete transferrin for Pu, reach extra- and intracellular storage sites in adequate concentration, and ideally be administered orally. Amino carboxylates, first EDTA, were tested and found to be effective. One disadvantage of these ligands is their tendency to remove essential metals, such as Ca, Mg, and Zn, in addition to Pu. Schubert suggested that since the concentration of serum calcium is much greater than that of other metals, any chelating agent capable of complexing calcium would exist as the calcium chelate in the circulation system. Thus, increasing Pu removal could be accomplished by decreasing the chelator’s affinity for calcium or by increasing its affinity for Pu. (Schubert, 1955) A series of modified EDTA ligands were studied based on this premise, including replacement of carboxyl groups with hydroxyl or phosphate groups and increasing the bridge length between amino carboxyl groups. (Raymond and Smith, 1981) Another approach to improving chelator effectiveness was increasing its lipophilicity. All of these changes resulted in only modest improvements, at best. However, an improvement was found with the addition of another aminocarboxylate group to EDTA—the use of DTPA.

One study showed that DTPA treatment of rats post administration of Pu citrate caused urinary excretion of Pu about four times greater than controls. The authors suggest that chelating agent treatment decreased the skeleton retention by about 15%. It was also reported that treatment by DTPA increased the fraction of Pu in bone volume, suggesting that DTPA was most effective in removing surface bound Pu (Ramounet, et al., 1998). When DTPA is administered repeatedly for a long time after intake of Pu to blood, the net retention half-time in the skeleton is reduced significantly. Presumably, this is due to the fact that the fraction of Pu released from bone, which would ordinarily have redeposited, is diverted to excretion. The Pu released from the liver to the blood is also diverted to excretion. In vitro studies of the exchange of Pu between the Pu-DTPA complex and plasma proteins have shown that little exchange occurred. Because DTPA is non-specific and removes essential metals including Ca and Zn, the current treatment of choice is administration of CaNa₃DTPA followed by ZnNa₃DTPA. The Zn complex is both less toxic and less effective because the high stability of the complex limits the amount of Pu removed. The concentration of DTPA necessary to achieve effective chelation in vitro appears to be at least two orders of magnitude greater than that of Pu (Duffield et al., 1986). Despite DTPA having a Pu solution complex formation constant about 10⁸ greater than the Pu-TF formation constant. (Taylor, 1995) DTPA is ineffective at removing relatively insoluble Pu forms, e.g. PuO₂ from the lung, and is not very effective at removing Pu deposited in the skeleton. The mechanism of removal from these sites relies on slow natural distribution to more tractable forms in equilibrium. Optimal removal from liver would require penetration of hepatocyte membranes.

Although DTPA is effective in increasing the overall rate of Pu excretion, it is neither specific for Pu specific nor effective in removing Pu from the skeleton, and it is most effective when administered intravenously which is undesirable for continuing therapy (Taylor, 1995). These disadvantages prompted research into the evaluation of new chelating agents. Raymond and coworkers have lead this cause and synthesized a number of new ligands containing functionalities present in siderophores: catecholatoles (CAMs or catecholamides), hydroxamates, and hydroxypyridonones (HOPOs) (Raymond and Smith, 1981). Ligands containing these and other functionalities have also been studied for their efficiency in recovering Pu from process waste streams (O’Boyle, 1997).
One of the most promising of the ligands this team has studied is the tetracarboxyatechololate, 3,4,3-LICAM(C) (the tetrasodium salt of N1, N5, N10, N14-tetrais(2,3-dihydroxy-4-carboxybenzoyltetraazetet radecane). Durbin, Raymond, and coworkers, found that when injected within one hour after Pu(IV) citrate injection, this chelator removes about the same fraction of Pu from animals as CaNa3-DTPA. Unfortunately, this ligand removes less inhaled Pu and leaves a Pu residue in the renal cortex. However, the formation constant of the expected Pu-3,4,3-LICAM(C) complexes are orders of magnitude greater than that of Pu-DTPA, and 3,4,3-LICAM(C) is 100-fold more efficient than CaNa3-DTPA for removing Pu from transferrin in vitro (Durbin et al., 1989). 3,4,3-LICAM(C) is more effective than CaDTPA in beagles for chelation of Pu if treatment is prompt, otherwise there is no difference, probably because both are confined to extracellular spaces. Analyses of the animal studies, the solution protonation behavior of the ligand and the solution speciation of the Pu-ligand complexes, led the group to conclude that deprotonation of the catechol ligands prior to metal binding slowed the Pu-binding reaction kinetics and reduced the overall efficiency of the ligand. They also concluded that the stability of the Pu-ligand complex was too low, despite being orders of magnitude greater than that of Pu-DTPA.

The next promising chelators developed contain hydroxypyridinones. The most efficient were the octadentate 3,4,3-LI(1,2-HOPO) and the tetradentate 5-LI(Me-3,2-HOPO) (Durbin, 1998). Based on fundamental chemistry and animal studies, oral administration or infusion of DTPA remains the most effective treatment for inhaled Pu. And the new chelator, 3,4,3-LI(1,2-HOPO) can be appreciably more effective than DTPA if both are administered by intravenous injection and the contamination is from inhalation or wound contamination. The acceptance of 3,4,3-LI(1,2-HOPO) for use in humans depends on toxicity testing, but the evidence available so far is encouraging (Stradling, 1994, Stradling, 1998).

Other types of treatments have also been explored, but none have been found to be as effective as DTPA and the new ‘siderophore analog’ chelators (Taylor, 1995 and references therein). For example, combinations of chelating agents have been studied, e.g. DFO and citrate or DTPA and salicylic acid. The hypothesis was that the chelators would form a Pu mixed ligand complex; but results suggest instead that in fact one ligand may mobilize Pu from tissues, while the other trapped it in the plasma and facilitated urinary excretion. In attempt to increase excretion from more intractable storage sites, more lipophilic agents that may cross more membrane boundaries have been studied (such as esterified DTPA). However, these compounds proved to be much more toxic and not significantly more effective than their more hydrophilic parents. Another approach, based on liposomal encapsulation, has been explored. This involved placing a chelating agent inside a multilamellar lipid sphere which can be taken up into liver cells. DTPA in liposomes had mixed results: it was more effective than DTPA at removing Pu from mice, but was not more effective than DTPA at removing Pu from rats or hamsters (Bulman, 1980). Alternatively, glucan, a polysaccharide found in yeast cell walls and removed by reticuloendothelial cells, was proposed to be associated with lysosomes and could potentially promote Pu removal. All of these approaches have yielded mixed results and generally proved faulty due to poorer than expected Pu removal from intercellular spaces and/or redistribution of bound Pu.

10.2.2.4 Plutonium Interactions with Other Biomolecules.

An interesting use of Pu binding by a protein is the replacement of light metals by Pu to provide a heavy element for structural probes. For example, Pu has useful X-ray absorbance and scattering and neutron scattering properties. Trewhella and coworkers used $^{240}$Pu(III) to determine distances between Ca(II) binding sites in calmodulin using neutron resonance
scattering (Seeger et al., 1997). This study provided accurate and precise structural information on the metal binding sites of a major Ca binding protein. But it also raised new questions regarding health effects from Pu, including impact on cellular regulation and Pu in vivo transport mechanisms.

Plutonium mammalian toxicity is reportedly exclusively radiological. However, recent studies show that Pu(IV) can also catalyze reactions that induce hydroxyl radicals under conditions at which chemical radical generation was expected to exceed radiolytic generation by 10^5 (Claycamp and Luo, 1994). Oxidative DNA damage could be induced from Pu-catalyzed reactions of hydrogen peroxide and ascorbate. Reactions of this type induce oxidative stress, a major factor in carcinogenesis. Hydrolysis of RNA by Th(IV) further suggests that Pu could also have chemical toxicological affects (Kuimelis et al., 1995).

10.3 Plutonium and Microorganisms

Plutonium can interact with microorganisms by affecting their growth and activity and in turn, microorganisms can affect Pu speciation and mobility. Microbial activity is generally affected by the availability of electron donors, electron acceptors (Fe^{3+}, Mn^{4+}, NO_3^-, SO_4^{2-}, organic compounds), nutrients (nitrogen, carbon, phosphorus), and environmental factors (pH, Eh, temperature, moisture). The biotransformation of Pu and viability of microbes will be affected by these factors as well as the chemical form (e.g. free ions, organic or inorganic complexes, solid phases) and solubility of Pu. The solubility, bioavailability, and mobility of Pu can be altered by the direct enzymatic or indirect non-enzymatic actions of microorganisms. Bacteria can cause (i) bioreduction or oxidation of plutonium or other metals that interact with plutonium; (ii) changes in pH, Eh, or anion availability; (iii) changes in plutonium chelation by the production of specific sequestering agents, such as siderophores and extracellular polymers; (iv) biosorption of plutonium by surface binding to biomass and biopolymers or bioaccumulation by active cation/metal uptake systems; (v) biocrystallization (precipitation and mineral formation); (vi) biotransformation of Pu complexes with synthetic and naturally occurring organic compounds by changes in or by catabolism of chelating agents, and (vii) biocolloid formation. The redox potentials of common microbial electron acceptor couples overlap with the redox potentials of the actinides (Figure 10-2). A few of these microbial processes that can affect the chemistry of Pu and other metals are shown schematically in Figure 10-1. Although the microbial transformations of actinides have not generally been examined in detail, U has been comparatively well studied while we have only very limited information for Pu. A review of actinide interactions with microbes in subsurface environments has been recently published (Banasak et al., 1998). There are a few reviews of the possible interactions of microbes with nuclear waste and nuclear waste components, but these did not focus on the chemistry of plutonium (Francis, 1998; Experientia, 1990 & 1991; West et al., 1985, Little 1996, Means, 1981). The complex chemistry of plutonium, as described in the introduction to this chapter, makes understanding plutonium-microbe transformations even more interesting and challenging than that of other metals.

Figure 10-1. Schematic representation of some of the mechanisms by which microorganisms can interact with Pu and other metals. Plutonium may be complexed by extracellular polymers and/or cell wall components. It may also be taken into the cell via the siderophore system.
These chelators, as well as metabolites can change the oxidation state, speciation, and/or solubility of Pu.

10.3.1 Effects of Plutonium on Microorganisms and Microbial Activity

The effects Pu has on microorganisms depends on the quantity, specific activity and form(s) of the Pu, and also on the availability of nutrients and other bacterial growth requirements, incubation time, and the microbe type. Studies on the effects of Pu have examined both pure and mixed cultures of bacteria and soil microorganisms, including both actinomycetes and fungi. They have focused on cell viability, cell growth, plutonium chemical form, and toxicity effects.

Plutonium has been shown to affect cell growth in liquid cultures in a concentration range from 0.015 to 10.0 μCi/mL (Table 3). Cells of Salmonella typhimurium exposed to 239Pu citrate solution showed cell death and induction of mutations exponentially related to radiation dose (Gafieva and Chudin, 1988). The loss of cell viability of the NTA-degrading Chelatobacter heintzii occurred at 239Pu NTA concentrations from 0.24 to 2.4 ppm 239Pu. (Reed, 1999). In this case, it was believe that the degredation of the Pu-chelating NTA led to the bioassociation of the plutonium with the cells, leading to alpha particle induced radiotoxicity... Dinococcus radiodurans showed regular growth at 120 ppm and no growth at 160 ppm for 239Pu NTA (Neu et al., 2000). The initial growth rate of a halophilic mixed culture consisting of Haloarcula sinaliensis, Alteromonas sp, Marinobacter sp, and an unclassified gamma Proteobacterium sp was affected by 239Pu-EDTA at concentrations of 1.0x10⁻⁵ M (2.4 ppm or 0.15 μCi/mL), but this concentration had no effect on the growth rate of a pure culture of the halophilic bacteria Halomonas sp (Pansoy-Hjelvik et al., 1996; Francis et al., 1998). However, both Halomonas sp and the halophilic mixed culture exposed to Pu concentrations > 1.0x10⁻⁶ M (0.24 ppm or 0.015 μCi/mL) showed decreases in cell numbers in late log phase growth. (Pansoy-Hjelvik et al., 1997, Francis, 1998).

Plutonium has been shown to inhibit soil bacteria in a concentration range of 0.05 to 10 μCi/g of soil (Wildung and Garland, 1982). Aerobic spore-forming and anaerobic bacteria were significantly affected by soil Pu levels as low as 1 μCi/g when Pu was added as the hydrolyzable 239Pu nitrate (solubility <0.1% of initially applied Pu). Spore-forming anaerobic organisms were affected at soil Pu levels of 10 μCi/g. Fungi were affected at higher soil levels of 239Pu, 180 μCi/g. The effect of Pu solubility on toxicity was also demonstrated for fungi. Using colony-forming unit (CFU) counts, Wildung and Garland showed that 239Pu-DTPA was an order of magnitude more toxic than Pu nitrate at the same mass level to microorganisms in soils.

The toxicity of Pu is believed to be primarily due to radiological effects rather than to chemical effects. Studies were performed to differentiate between the two effects of Pu by exposing microorganisms to two plutonium isotopes, one with higher activity. Wildung and Garland (1982) showed that in soils receiving 10μCi/g of 239Pu or 238Pu (0.6μg/g of 238Pu-DTPA or 180μg/g of 239Pu-DTPA, a concentration difference of over 300) fungal colony forming units (CFU) were similarly significantly reduced, relative to controls. The analogous comparison of 238Pu nitrate and 239Pu nitrate treated soil at the 10μCi/g level showed the same effect, but toxicity at both mass levels was not as pronounced as in the DTPA treated soils, again reflecting the importance of solubility (Wildung and Garland, 1982). Cells of Cheletobacter heintzii exposed to 239Pu(IV) and 242Pu(IV) complexed with NTA (nitritotriacetic acid) showed differences in loss of viability which were attributed to differences in alpha activity rather than
chemical effects (Reed et al., 1999). At the same Pu concentration, loss of viability was much greater for 239Pu than 242Pu. And no difference was noted between the 10-5 M (2.4 ppm) 242Pu and 10-6 M (0.24 ppm) 239Pu samples. While the Pu toxicity to Cheletobacter heinzii appeared to be radiological, the level of ionizing radiation from the Pu was a small fraction of the radiation tolerance of this organism based on gamma irradiation (~165 Gy). It was therefore suggested that bioassociation of plutonium with cells is a necessary step in the observed loss of viability in order for there to be direct alpha-particle interaction with the cells. Interestingly, although Pu concentrations 1.0x10^{-6} M (0.24 ppm) caused a decrease in cell number of Halomonas sp in late log phase growth, 243Am concentrations 5 x 10^{-6} M (1.2 ppm) of which has 60% more a-radiological activity than the Pu used in these experiments, caused no decrease in cell numbers, suggesting that the toxicity cannot be purely alpha-based radiological, although differences in cellular association could account for this difference. (Pansoy-Hjelvik et al., 1997)

One of the most interesting species to consider regarding actinide toxicity is the astonishingly radiation resistant Dinococcus radiodurans. The NTα complexes of U(VI) and Pu(IV) inhibited growth of D. radiodurans at 100-160 ppm and 120-160 ppm for U(VI) and Pu(IV), respectively (Neu et al., 2000a). These concentrations correspond to radiation dose equivalents orders of magnitude lower than what has been reported to be toxic based on gamma irradiation studies (5000 ~30,000 Gy or 0.5 to 3 Mrad) (Minton, 1994). Thus, the toxicity due to alpha radiation must be much lower than that due to gamma radiation, or be chemical in nature. The similar toxicity of depleted U and 239Pu and the comparison with toxicity ranges of other metals, Ba(II) (200-500ppm), As(IV) (30-50ppm), Pb(II) (>50ppm), suggest metal chemistry and not radioactivity is responsible for growth inhibition.

Chemical toxicity of Pu may be due to the similarity of plutonium with iron. Siderophore mediated uptake of Pu by the siderophore auxotroph, Aureobacterium flavescens (JG-9) has been demonstrated (Neu et al., 2000b) Figure 10-2. Initial bacterial uptake studies show that 239Pu-DFB and Fe-DFB have similar uptake profiles. The bacteria can take up the DFB-complexed metals, whereas the metals without DFB cannot be taken up. Heat-killed bacteria cannot take up any metals, regardless of the presence of DFB, eliminating the possibility that observed uptake is due to surface binding and suggesting a metabolically active process. Fe uptake appears to be faster than Pu by 2 orders of magnitude, and the uptake appears to stop sooner, possibly due to the bacteria obtaining sufficient iron. Metal uptake inhibition experiments suggest that Pu-DFB and Fe-DFB interact with the same transport channels. When 239Pu-DFB and Fe-DFB are both added to cultures of Aureobacterium flavescens JG-9 Fe uptake is inhibited; it is reduced by approximately 50% relative to when Fe-DFB alone is added. The similarity between Fe-DFE and Pu-DFE may explain why bacteria take up Pu-DFFB complexes. Recent single crystal x-ray diffraction studies of DFE show the free ligand, Fe(III) complex and the Pu(IV) complex of DFE have interesting similarities which may explain why they are taken up by the microorganism via the same mechanism (Figure 10-3, Neu, et al., 2000c). The Pu(IV) is nine coordinate, bound by DFE in approximately one hemisphere and three waters in the other with a slightly distorted tricapped trigonal prismatic geometry (three bound waters and three oximate oxygens form trigonal planes and three carbonyl oxygens cap the prismatic faces) while the Fe is six coordinate and octahedral. However, the conformation of the PuDFE is cis-cis, the same as Fe-DFB, and the Fe(III) and Pu(IV) complexes have nearly identical molecular footprints (Figure 10-3).

Figure 10-2. Uptake of 55Fe and 239Pu by the siderophore auxotroph, Aureobacterium flavescens (JG-9).
Figure 10-3. Single crystal X-ray diffraction structures of the siderophore desferrioxamine E (DFE) and the Fe(III) and Pu(IV) complexes.

In addition to inhibiting growth, Pu may induce physiological changes in microorganisms. Production of unique extracellular chelating agents resembling siderophores was observed for a *Pseudomonas aeruginosa* when it was grown in the presence of U and Th (Premuzic et al., 1985). Cell morphology changes have also been observed. *Halomonas* sp exposed to Pu showed cells that were rods initially becoming shorter and more coccoid in appearance (Pansoy-Hjelvik, et al.1997). Growth in the presence of plutonium may also change microbial populations. In environments containing low levels (nanocuries) of alpha and beta activity, radiation-resistant microbes are constantly being enriched (Barnhart et al., 1980). Bacterial isolates from TRU waste storage sites have shown increases in levels of radiation resistance. (Barnhart et al., 1979). Bacterial isolates (*E. coli* and *bacillus*) from areas affected by the Chernobyl fallout have been shown to have increased resistance to X-rays, UV and 4-Nitroquinoline-1-oxide (a DNA damaging agent) compared with isolates of the same strains from control sites unaffected by the fallout. (Zavilgelsky, 1998).

The effects of Pu on microorganisms clearly vary from species to species and may even vary strain to strain. Because so few species have been studied it is not possible to make general conclusions, such as fungi are more Pu resistant than other microbes. In addition the few studies reported have used different approaches to define toxicity and viability; some are based on growth rates and others are based on CFU counts, and all appear to be performed at different stages of growth. For comparisons between species to be possible, a standard approach would need to be adopted, including phase of growth, forms of Pu used, initial and final media conditions (such as pH and ionic strength, which effect plutonium solubility and speciation), and definitions of toxicity and viability.

Table 10-3. Inhibitory Concentrations of Pu to Microorganisms.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Pu form</th>
<th>Conc. (ppm)</th>
<th>Activity Gy, μCi/g, μCi/mL</th>
<th>Toxicitya</th>
<th>Method of toxicity determination</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liquid Culture Studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>239Pu citrate</td>
<td>NA</td>
<td>19-35 Gy</td>
<td>ND</td>
<td>LD37 = 34.8 Gy; mutation doubling dose = 19 Gy.</td>
<td>1</td>
</tr>
<tr>
<td><em>Chelatobacter heintzi</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>239Pu NTA</td>
<td>.024</td>
<td>0.0015</td>
<td>Rad.</td>
<td>slight CFU counts decrease, 5-12 days.</td>
<td>2</td>
</tr>
<tr>
<td><em>Chelatobacter heintzi</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>239Pu NTA</td>
<td>.24</td>
<td>0.015</td>
<td>Rad.</td>
<td>CFU counts decrease, 5-12 days.</td>
<td>2</td>
</tr>
<tr>
<td><em>Chelatobacter heintzi</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>239Pu NTA</td>
<td>2.4</td>
<td>0.15 (2.7Gy /week)</td>
<td>Rad</td>
<td>significant CFU counts decrease, 5-12 days.</td>
<td>2</td>
</tr>
<tr>
<td><em>Chelatobacter heintzi</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>242Pu NTA</td>
<td>2.4</td>
<td>0.015</td>
<td>Rad</td>
<td>CFU counts decrease, 1 5-12 days, hrs</td>
<td>2</td>
</tr>
<tr>
<td><strong>Dinococcus radiodurans</strong></td>
<td>239Pu NTA</td>
<td>bewteen 120-160</td>
<td>7-10</td>
<td>Chem.</td>
<td>No growth at 24hr compared to control.</td>
<td>3</td>
</tr>
<tr>
<td>---------------------------</td>
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</tr>
<tr>
<td><strong>Halomonas sp</strong>&lt;br&gt;(WIPP-1A, halophile)</td>
<td>239Pu EDTA</td>
<td>&gt;2.4</td>
<td>&gt;0.15</td>
<td>ND</td>
<td>No growth rate decrease, &lt;3 days</td>
<td>4</td>
</tr>
<tr>
<td><strong>Halomonas sp</strong>&lt;br&gt;(WIPP-1A, halophile)</td>
<td>239Pu EDTA</td>
<td>0.24</td>
<td>0.015</td>
<td>ND</td>
<td>CFU count decrease, 4-10 days.</td>
<td>4</td>
</tr>
<tr>
<td><strong>Mixed culture of Halophiles</strong>&lt;br&gt;c</td>
<td>239Pu EDTA</td>
<td>2.4</td>
<td>0.15</td>
<td>ND</td>
<td>Growth rate decrease, &lt;3 days</td>
<td>4</td>
</tr>
<tr>
<td><strong>Mixed culture of Halophiles</strong>&lt;br&gt;c</td>
<td>239Pu EDTA</td>
<td>0.24</td>
<td>0.015</td>
<td>ND</td>
<td>CFU count decrease, 4-10 days.</td>
<td>4</td>
</tr>
<tr>
<td><strong>Soil Culture Studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Soil Aerobes</strong></td>
<td>239Pu NO₃</td>
<td>10.0</td>
<td>0.50</td>
<td>ND</td>
<td>CFU counts decrease, 2-10 days</td>
<td>5</td>
</tr>
<tr>
<td><strong>Soil Aerobe, Spore formers</strong></td>
<td>239Pu NO₃</td>
<td>1.0</td>
<td>0.050</td>
<td>ND</td>
<td>CFU count decrease, ~30 days</td>
<td>5</td>
</tr>
<tr>
<td><strong>Soil Anaerobes</strong></td>
<td>239Pu NO₃</td>
<td>1.0</td>
<td>0.050</td>
<td>ND</td>
<td>CFU counts decrease, ~30 days</td>
<td>5</td>
</tr>
<tr>
<td><strong>Soil Anaerobe, Spore formers</strong></td>
<td>239Pu NO₃</td>
<td>10.0</td>
<td>0.50</td>
<td>ND</td>
<td>CFU counts decrease, ~30 days</td>
<td>5</td>
</tr>
<tr>
<td><strong>Soil Fungi</strong></td>
<td>239Pu NO₃</td>
<td>180</td>
<td>10.0</td>
<td>Rad</td>
<td>CFU counts decrease, &gt;20 days</td>
<td>5</td>
</tr>
<tr>
<td><strong>Soil Fungi</strong></td>
<td>239Pu DTPA</td>
<td>10</td>
<td>0.50</td>
<td>Rad</td>
<td>CFU counts decrease, 25-95 days</td>
<td>5</td>
</tr>
<tr>
<td><strong>Soil Fungi</strong></td>
<td>238Pu NO₃</td>
<td>0.6</td>
<td>10</td>
<td>Rad</td>
<td>CFU counts decrease, 11-95 days</td>
<td>5</td>
</tr>
<tr>
<td><strong>Soil Fungi</strong>&lt;br&gt;d</td>
<td>238Pu DTPA</td>
<td>0.6</td>
<td>10</td>
<td>Rad</td>
<td>CFU counts decrease, 4-95 days</td>
<td>5</td>
</tr>
<tr>
<td><strong>Soil Actinomycetes</strong></td>
<td>239Pu NO₃</td>
<td>1.0</td>
<td>0.05</td>
<td>ND</td>
<td>CFU counts decrease, &lt;30 days</td>
<td>5</td>
</tr>
</tbody>
</table>

NA - not available; ND - not determined

a) Toxicity by radiological or chemical effect, on not determined.
b) CFU count decrease for 2.4 ppm 242Pu same as 0.24 and 239Pu.
CFU counts were almost an order of magnitude less at 5 days for 2.4 ppm 239Pu, and over 2 orders of magnitude less by 12 days.
c) Mixed culture consisted of *Haloarcula sinaiensis*, *Alteromonas sp*, *Marinobacter sp*, and Unclassified *Gamma Proteobacterium* (new).
d) The effect soluble of $238\text{Pu}\text{DTPA}$ complex was much more pronounced than that of $238\text{Pu}(\text{NO}_3)_4$.


10.3.2. Effect of Microorganisms on Plutonium

The solubility, bioavailability, and mobility of Pu can be altered by the direct enzymatic or indirect non-enzymatic actions of microorganisms. Microbiologically mediated redox changes and
complex formation (with microbial metabolites and organic degradation products) are the key processes of which affect the solubility and are therefore most environmentally significant. Fundamental understanding of the mechanisms of the microbial (im)mobilization of Pu will be useful in the long-term management of contaminated sites and nuclear waste repositories as well as potentially in the development of strategies for decontaminating soils and materials.

10.3.2.1. Dissolution and Redox Changes of Plutonium

Plutonium can be dissolved by direct enzymatic and indirect actions of microorganisms which may involve: 1) the production of mineral acids, such as the production of sulfuric acid from the oxidation of sulfide minerals by autotrophic bacteria, 2) the production of organic acids from the metabolism of organic compounds, such as oxalic, isocitric, citric, succinic, hydrobenzoic, and coumaric acids which can form complexes with Pu via their carboxylic and phenolic groups, 3) the production of oxidizing, chelating, and metal-sequestering agents, such as dicarboxylic acids, polyhydroxy acids, and phenolic compounds, such as protocatechuic acid and salicylic acid, and siderophores, which are all effective chelating agents, and 4) the lowering the pH of the medium. Often a combined effect is important; for example, organisms may secrete organic acids that can increase Pu dissolution by lowering pH and by complexing Pu.

One example of direct action is the reductive dissolution of Pu(IV) solids. The mobility and bioavailability of Pu(IV) is limited by its low solubility whereas the solubility of Pu(III) hydroxide is much greater (Ksp for Pu(IV) oxyhydroxide ~ 10^-56 vs Ksp for Pu(III) hydroxide = 10^-20.) Consequently, the reduction of Pu(IV) oxyhydroxides to Pu(III) is expected to increase the solubility of plutonium in the environment. The similarity in electrochemical potential for Pu(IV) oxyhydroxide reduction to Pu(III) and for Fe(III) oxyhydroxide (ox-FeOOH) reduction to Fe(II) suggests that microbes capable of reducing Fe might also reduce Pu under similar conditions and, thus, reductively solubilize Pu. The persistence of Pu(III) would then be determined by the redox stability and speciation of the ion. Rusin et al (1994) showed that an iron-reducing bacteria Bacillus sp solubilized up to 90% hydrous PuO_2(s) to Pu(III) under anaerobic conditions in the presence of nitritolriacetic acid (NTA). Solubilization presumably occurred via the formation of a Pu(III)-NTA complex because in the absence of NTA only 40% of Pu was solubilized. Little PuO_2 dissolution was observed in sterile culture media or in the presence of a non-iron-reducing bacteria such as Escherichia coli.

Indirect action has been demonstrated by the dissolution of Pu by extracellular metabolites. Microorganisms grown in the presence of Pu produced complexing agents capable of dissolving Pu in soils. These compounds may also facilitate transport of Pu into the cells (Wildung and Garland, 1980; Wildung et al., 1987; Beckert and Au, 1976). Several bacteria and fungi grown in the presence of Pu produced extracellular Pu complexes that increased the concentration of Pu in soil column eluates relative to controls by a factor of 1.6 to 310. Positively charged Pu complexes were effectively removed by elution through soil. Increased Pu mobility in soil resulted from the formation of neutral and negative charged Pu complexes, which differed with organism type. In the presence of known microbial metabolites and synthetic ligands (DTPA, EDTA), Pu(VI) is reduced to Pu(IV), (Al Mahamid et al., 1996) supporting the current prediction that Pu(IV) is the dominant oxidation state associated with organic complexes in soils. Aspergillus niger solubilized PuO_2 that was in the form of 0.3 μm spheres (Au, 1974). Although the agent responsible for solubilizing PuO_2 was not identified, it is mostly likely citrate, which is produced by the fungus. Cultures from Pu-resistant fungi grown in the presence of Pu DTPA were shown to contain several Pu-containing cellular and extracellular components that differed from the initial Pu DTPA complex. Some components were negatively charged and an isolated
globular protein had an equivalent molecular weight of < 3000 (Robinson et al., 1975, Robinson et al., 1977). These studies show that microorganisms have the ability to change the chemical form of highly stable Pu compounds.

Another type of ligand exuded by microorganisms are Fe(III)-binding siderophores. The ubiquity of siderophore producing microbes in nature and the chemical and biochemical similarities between Pu(IV) and Fe(III) suggest that these iron-sequestering agents could be important in the solubilization and complexation of Pu. This hypothesis has been tested by experiments where siderophores alone and siderophore-producing microorganisms were combined with Pu ions and Pu(IV) solids and the solution phase was monitored. Brainard et al. (1992) determined rate constants for the solubilization of hydrous PuO$_2$(s) by the siderophores enterobactin and desferrioxamine B and selected carboxylate, amino polycarboxylate, and catecholate ligands. The measured rate constants for solubilization of PuO$_2$(s) show that siderophores are extremely effective in solubilizing actinides on a per molecule basis. Enterobactin was observed to be ~10$^3$ times more effective than the other ligands studies. Notably, ferric siderophore complexes were found to be more effective in solubilizing actinide oxides than the same siderophores in the absence of iron. Other experiments of a longer duration suggest that not all siderophores are effective at solubilizing Pu. The desferrioxamine siderophores were found to be far less effective at solubilizing Pu(OH)$_4$ and PuO$_2$ in buffered neutral solution than simple organic chelators such as EDTA, citrate and tiron (Ruggiero, et al., 2000). Pu(IV) hydroxide can be slowly solubilized by EDTA, tiron, and citrate at rates of approximately 2μM, 0.9 μM, and 0.3 μM/day per day reaching 260 μM, 49 μM and 106 μM after 130 days, respectively. However, the siderophores DFE and DFB are 100 times slower than EDTA with rates of 0.02 μM/day for both DFB and DFE. (Studies were done at pH = 7.5, 10 mM chelator.) These results are unexpected given thier thermodynamic solution Pu(IV) complex formation constants (log $\beta$ DFE = 32; DFB = 30, EDTA = 26, Citrate = 15, Tiron ~18). In fact, the solubilization of Pu(OH)$_4$ by EDTA was inhibited by pre-treating the plutonium with desferrioxamine B. These surprising results suggest that the desferrioxamine siderophores are passivating the surface. Evidence for this type of surface passivation by hydroxamates was reported by Casey and Holmen (1996) where they found acetohydroxamate dissolves an iron oxide at a rate an order of magnitude lower than oxalate (Zinder et al., 1988).

Christy, edit to fit figures & level of detail for other sections--its close Please also provide refs for dissolution and this redox part. Although DFO siderophores only very slowly solubilize Pu(IV) solids, they rapidly stabilize the (IV) oxidation state in neutral pH solution (Ruggiero et al., 2000b). If Pu(III) is mixed with DFB or DFE in air, the Pu(IV) complex quickly forms. DFE and DFB also rapidly and irreversibly reduce Pu(VI) to Pu(V), and eventually to Pu(IV) (Figure 10-5). Up to 12 equivalents of Pu(VI) can be reduced to Pu(V) per DFB/DFB instantaneously, so long as there is greater than 1 equivalent DFO per six equivalents plutonium. The reduction is slower at lower ratios. At a ratio of 1 equivalent DFO to 12 equivalents plutonium, the initial reduction to Pu(V) takes over an hour. In this case, the initial formation of a Pu(VI)-DFO complex is indicated by absorbance spectroscopy. Based on optical absorbance and NMR spectroscopy, it appears that the DFO is oxidized and cleaved during the Pu(VI) reduction, in a process that leaves some DFO intact unless a full 12 equivalents of Pu are reduced. The higher the ratio of DFO to plutonium, the faster both reduction steps occur. Both are pH and [DFO] dependent. Excess DFO in solution acts as a thermodynamic driving force for the formation of Pu(IV)-DFO from the Pu(V). At pH greater than 6, the reduction of Pu(VI) directly to Pu(IV) is instant. At lower pH the reduction of Pu(V) to Pu(IV) takes months.
Figure 10-4. Solubilization rates of Pu(IV) hydroxide by common chelating agents and desferrioxamine siderophores.

Figure 10-5. Reduction of Pu(VI) to Pu(V) by desferrioxamine siderophores. Conditions are mM Pu and pH 2.

Figure 10-6. Reduction of Pu(VI) to Pu(IV) by desferrioxamine siderophores and neutral pH.

10.3.2.2. Immobilization of Plutonium
Microorganisms may immobilize Pu through bioreduction, bioaccumulation and biosorption, bioprecipitation and mineral formation. Although there is no direct evidence for the microbial reductive precipitation of Pu(VI) to Pu(IV), microbial activity may reduce Pu(VI) to Pu(IV) at the cell or exopolymer surface or may indirectly reduce Pu(VI) by altering the redox potential.

Bioaccumulation and biosorption of Pu by microbes has received much attention because of the potential use of these processes for bioremediation of radionuclide contaminated sites and waste-streams. Although the biochemistry of the interactions of toxic metals and uranium with bacterial cell walls, extracellular biopolymers, and microfossil formations have been extensively studied, limited information is available on the nature of Pu association with microorganisms (McLean et al., 1996; Macaskie et al., 1996; Francis 1998). Of particular concern in all these systems is the long-term stability of the biosorbed metal, which may be remobilized.

Beveridge and coworkers have described numerous different charged groups on the cell surface, which potentially can bind metals (Beveridge, et al. 1982). For example, B. licheniformis produces a γ-polyglutamic acid (PGA) exopolymer with a molecular weight of 800 kDa (2700 glutamate residues). Pu(IV) binds to the polymer as a soluble complex up to a 1 Pu to 20 glutamate residue ratio at pH=4, but forms a flocculant at fewer residues per Pu atom (Johnson et al., 2000). Uncomplexed Pu(IV) forms colloidal species as the pH is increased; however, when a complex of Pu to Glu residue of 1:100 is prepared at pH=1, the pH can be raised to 12 and lowered again without observation of colloid formation. This is presumably due to encapsulation of the metal center by the polymer and inhibition of colloid formation. It was determined that 0.30 μmol, 0.36 μmol, and 0.45 μmol of U(VI), Fe(III), and Pu(IV), respectively, were bound per mg of exopolymer (Johnson et al., 2000, McLean et al. 1990). The metal binding strength of the polymer was determined by competition experiments. When tiron or NTA is titrated into the metal PGA complex solution (pH=4 with a ratio of 1:100 metal to Glu residue), Fe(III), Pu(IV) and U(VI) all form metal tiron or NTA complexes, suggesting that the actinide polymer complex has a binding constant log K_i < 10. This is consistent with the binding strength of other exopolymers, humic and fulvic acids (Lester et al. 1984 and Chen et al. 1995).

Metal binding to B. licheniformis whole cells was also studied to determine if other exterior functionalities (in addition to the exopolymer) enhance metal binding (Johnson et al., 2000). When Pu(IV) was added to the whole cells at pH=6 in saline, greater than 90% of the metal added was bound by the whole cell (including the exopolymer) compared with about 50% binding by the exopolymer only (Figure 10-7). As we expected, the increase in the number of binding sites increased the amount of metal bound.
Figure 10-7. Pu(IV) Binding by Bascillus lichenformis and the Isolated Polyglutamate Exopolymer Produced by Bascillus lichenformis

Bacteria isolated from soil that had been contaminated with Pu by fallout for more than 40 years in Japan accumulated low concentrations of Pu(IV) during growth. The association of Pu varied with type of bacterial isolate indicating differences in the mechanisms of binding of Pu (Kauri, et al. 1991). Studies on the interactions between $^{239}$Pu and sulfate-reducing anaerobic bacteria showed that living bacteria accumulated more Pu than the dead bacteria. The higher uptake of Pu by living bacteria was observed between pH 6.83-8.25, while no visible pH effect was observed for the dead bacteria (Kudo, et al 1997). Suspended pure and mixed halophilic bacterial cells ($10^6$ to $10^9$ cells ml$^{-1}$) in a medium containing NaCl or MgCl$_2$ brine, at various growth phases showed that 10-18 - 10-21 mol Pu was associated cell$^{-1}$ (calculated) and it varied with the bacterial culture. The sizes of the bacterial cells ranged from 0.54 x 0.48 µm to 7.7 x 0.67 µm (Francis et., al., (1998). In these studies it was not determined whether the plutonium associated with these organisms was incorporated into the cells or was simply sorbed onto the external cell surface.

The bioavailability of Pu and the extent of uptake by the fresh-water bacterium Aeromonas hydrophila and by the alga Scenedesmus obliquus was affected by the naturally-occurring organics compounds in water (Giesy et al (1977). Naturally occurring organic matter was concentrated from Skinface Pond, near Aiken, South Carolina, and separated into 4 nominal diameter size fractions (F I >0.0183; F II >0.0032; F III >0.009; F IV <0.009 µm) by membrane ultrafiltration. Each fraction was introduced into Scenedesmus obliquus and Aeromonas hydrophila cultures at concentrations equal to those found in nature to determine their effects on $^{237}$Pu uptake. Plutonium uptake was determined in log phase cultures after 6 h incubations. The initial Pu concentrations in each flask was 1.1 x 10$^{-4}$ µCi/mL $^{237}$Pu nitrate. Fractions I and II reduced $^{237}$Pu uptake by S. obliquus, F IV increased uptake, and F III had no effect. $^{237}$Pu uptake by A. hydrophila was no different in the presence of F I, F II, or F III than tryptic broth medium alone, whereas F IV increased $^{237}$Pu uptake.

Beckert and Au (1976) showed $^{238}$Pu was taken up by the fungus Aspergillus niger. Plutonium, when added to the culture medium as dioxide microspheres, nitrate, or citrate, was transported to the spores, and an almost linear relation existed between transport and concentration. Raising the pH of the culture medium from 2.5 to 5.5 generally increased transport of Pu to spores for all 3 chemical forms. At Pu concentrations of 224 pCi/g in the culture media, and for both pH 2.5 and 5.5, transport of Pu to spores was approximately three-fold as high from the nitrate or citrate form as from the dioxide microspheres. The specific activities of the spores grown at pH 5.5 were generally at least twice those of the spores grown at pH 2.5. The transport factors derived from these experiments indicated that Pu was concentrated in the mycelium and further transported to the aerial spores of this fungus. These findings suggest that the common soil fungus Aspergillus niger is capable of solubilizing soil-deposited Pu and rendering it more bioavailable for higher plants and animals. Biosorption of Pu by the fungus Rhizopus arrhizus has been reported (Li, et al. 1995; Dhami,et al. 1998). Alpha-energy spectrum analysis of the Rhizopus arrhizus biomass in combination with SEM observation showed that the absorption occurred mainly on the cell walls, with flocculation.

Numerous studies have shown that bacteria can form 'biocolloids' that affect the mobility of metals. Throughout our discussion of bioaccumulation the affect of immobilizing Pu has been implied or explicitly stated. However, depending upon the nature of the interaction(s) between Pu and the microorganism and the physiology and motility of the microorganism, bioaccumulation may either retard or enhance mobility. For example, some microbes produce
copious amounts of exopolymers and tend to flocculate, other strongly sorb to mineral surfaces to acquire nutrients. Plutonium interacting with these microorganisms would tend to be immobilized and these species may be considered for bioremediation applications. In contrast, there are species and individual strains of microorganisms that are more hydrophobic. Plutonium associated (perhaps internalized) by these organisms will tend to be mobilized. In addition, even for microorganisms that adhere to mineral surfaces, there is some evidence that only a small fraction of the population is present at the mineral surface with the remainder suspended in solution acquiring nutrients liberated by the surface bound fraction. Generally, bacterial cells fall within the colloidal size range (typically 0.5 to 1.0 μM in length) that can be mobile in subsurface environments. Colloid facilitated transport has been recognized as a potentially important phenomenon affecting the long term storage of plutonium-bearing wastes. Therefore, bioaccumulation on cell surfaces can dramatically affect plutonium mobility, actually enhancing it rather than immobilizing plutonium in the environment. Bacteria are known to affect soil Kd (distribution coefficient) values for Pu.(Sanchez et al., 1982) chrisy next sent. does not make sense to me--just me? In a study examining the behavior of 239Pu interacting bentonite contaminated by sulfate reducing bacteria, there was more than one order of magnitude difference in Kds between non-sterilized and sterilized bacteria for an environment where bacteria could live comfortably (Kudo, 1998). Column transport experiments have shown that bacteria affect transport of actinides (Champ et al., 1982, Yelton et al., 1996). Increase, decrease, variable affect on transport? This increased Pu mobility in the presence of microorganisms has been demonstrated in field studies. Environmental surveys of 239,240Pu in Nagasaki found that the mobile Pu was produced in various environments, although at a very low level, and that production of the mobile Pu depended strongly on natural organic materials, bacterial activities, aerobic and anaerobic conditions.(Mahara and Kudo, 1996) It was also found that the Kd of plutonium to living bacteria is 20 times greater than the dead bacteria under anaerobic conditions, indicating that mobile plutonium in soil and sediment may be affected most by the number of living anaerobic bacteria.(Mahara and Kudo, 1999)

10.3.2.3. Biotransformation of Plutonium-Organic Complexes

Naturally occurring and microbially produced complexing agents organic complexing agents can affect the mobility of Pu in the environment. Chelating agents are present in wastes because they are widely used for decontaminating nuclear reactors and equipment and separating radionuclides. These organic complexing agents are carboxylic acids, such as citric, hydroxyacetic, oxalic, and tartaric acids, and amino-carboxylic acids such as EDTA, DTPA, NTA, and N-hydroxyethylene-diaminetriacetic acid (HEDTA). The metal complexes of these ligands are either poorly biodegraded aerobically, or undergo little anaerobic biodegradation. Their biodegradation should cause the precipitation of released ions, thereby retarding metal migration. Although the biodegradation of synthetic chelating agents complexed with toxic metals has been investigated, little is known of the biotransformation of Pu complexed with natural organic compounds and synthetic chelating agents.

10.3.2.4. Effect of Microorganisms in Plutonium-containing Wastes.

Many reviews on possible impacts of microorganisms on nuclear waste disposal and storage have been compiled (Experientia, Vol 76, pp 777-851, 1990 and Vol 77, pp507-584, 1991, West et al. 1985) Possible effects of microorganism include: 1) radionuclide mobilization by microbi ally produced complexing agents, such as siderophores, exopolymers, and the byproducts of microbial metabolism (organic acids and alcohols) (Birch and Bachofen, 1990); or by the formation of biocolloids (Francis, et al 1998). 2) microbial corrosion of storage materials and vessels (bitumen, concrete, cement, steel, aluminum) (Little and Wagner, 1996); 3) Gas
pressurization of radioactive waste storage vessels or enclosures as the result of microbially produced gases such as CO₂, CH₄, N₂O, and N₂. 4) Biodegradation of organic materials in waste, such as cellulose, lignin, and bitumen, and organic ligands used in decontamination or reprocessing, providing additional nutrient sources from microbial growth (Francis 1990a). The degradation of cellulose and lignin is of particular concern because it releases large macromolecular dissolved organic carbon compounds capable of chelating high molar ratios of metal ions. Seventy-percent of the TRU waste destined for the WIPP repository is cellulosic material. Biodegradation of cellulose under hypersaline, repository relevant conditions with the concomitant production of gases such as CO₂ and CH₄, could have significant ramifications for the long-term stability of the repository (up to 10,000 years).

Experimental evidence shows that the effects of microorganisms on the long-term stability of radionuclides in radioactive waste could be significant. Degradation of actual and simulated wastes showed that microorganisms produce far more gas than that produced from other mechanisms including corrosion processes (Molecke, 1979). Increased solubility and/or migration of radionuclides by chelating agents have been observed at waste burial grounds. (Means and Alexander 1978; Cleveland and Rees, 1981). For example, in trench leachate samples collected from the low-level radioactive waste disposal sites at Maxey Falls, KY, and West Valley, NY plutonium and a variety of organic chelating compounds, reflecting both the nature of the waste and the byproducts of biodegradation, were detected (Husain et al. 1979, Weiss et al. 1979; Cleveland and Rees, 1981). Several aerobic and anaerobic bacteria were isolated from the leachate samples; including Bacillus sp., Pseudomonas sp., Citrobacter sp., and Clostridium sp. The radioactivity and the organic chemicals present in the leachate were not toxic to the bacteria. The bacteria metabolized the organic compounds and produced 3H and 14C methane (Francis et al. 1980a,b; Francis et al. 1990b). Viable, metabolically active microbes were also detected at the Los Alamos National Laboratory (LANL) TRU waste burial site containing 239Pu contaminated soil and flammable waste (Barnhart et al., 1980).

10.3.3. Bioremediation of Plutonium Contaminated Soils and Wastes

There is considerable interest in the use of microorganisms to remediate contaminated soils, materials, and waste streams. Bioremediation of contaminant metals is a rapidly advancing field. Numerous techniques and technologies exist for the concentration and removals of metals contaminants from soils and waters; Many reviews have been published: see BOOKS>>> Bioremediation, etc. and reviews. . Many of the technologies that are being applied to other metals could be adapted for use with plutonium. Some of the processes that are being investigated for plutonium include biosorption and bioprecipitation of Pu and removal and recovery of Pu by extraction with siderophores and other microbial products such as citric acid, and bioreduction. The following descriptions and examples illustrate some approaches being investigated.

10.3.3.1. Biosorption/Bioaccumulation of Plutonium. Biosorption is process where the biomass provides a reactive surface upon which metals bind by ligand interactions or by ion exchange. Living and dead microorganisms, possess abundant functional groups such as carboxyl, hydroxyl, and phosphate on their surface that bind metal ions. Polymers secreted by many metabolizing microbes also immobilize metals. Desorption and recovery of the biosorbed radionuclides is easy. In contrast, bioaccumulation is a metabolically active process wherein metals are taken up into living cells and sequestered intracellularly by complexation with specific metal-binding components or by precipitation. Intracellular accumulation of metals occurs among all classes of microorganisms, usually by an energy-dependent transport system.
Localizing the metal within the cell permits its accumulation from bulk solution although the metals cannot be easily desorbed and recovered. Radionuclide-binding to cell surfaces and polymers is a promising technology for remediating contaminated waters (Voilesky and Holan, 1995; McLean et al., 1996; Macaskie et al., 1996, Gadd, 1990, (Mareev, 1993) ADD IN SOME OF THOSE RECENT metal/microbe and/or bioremediation books)

Viable cells of *Pseudomonas aeruginosa*, in stable association with fine-spun polypropylene web concentrated significant amounts of PuO₂ or PuCl₄ from aqueous media suggesting that such a system may be useful for the removal of Pu from aqueous waste streams. Removal of an average of 34% of suspended PuO₂ particles and 94% of soluble PuCl₄ was observed under these conditions (Meyer, et al. 1979). In a subsequent study Tengerdy, et al. (1981) demonstrated that the immobilized *P. aeruginosa* on polypropylene web removed 95% of a 1.7 nCi PuCl₄ activity from a nuclear plant wastewater in a batch operation. Actinide atom-enriching microorganisms and the use of some strains such as Bacillus and Arthrobacter capable of selective enrichment of U, Th, and Pu and their application for concentration of actinides in used nuclear fuels as well as recovery of actinides from radioactive wastewater has been proposed (Sakaguchi 1997). The fungus *Rhizopus arrhizus* showed maximum biosorption of ²³⁹Pu at pH 6-7. Under optimal conditions, 99% of the ²³⁹Pu was removed from the wastewater, and the recovery of ²³⁹Pu was 95%. This biomass is a promising sorbent for the treatment of radioactive effluents from nuclear industry (Li, et al. 1995; Dhami, et al. 1998).

10.3.3.2. Bioprecipitation. Many heavy elements, including actinides, form insoluble precipitates with ligands such as inorganic phosphate. This can be generated biochemically using the activity of a phosphatase enzyme produced by *Citrobacter* sp. The metals can be removed efficiently from dilute solution since the continuous production of a high localized concentration of phosphate allows the solubility product of the metal phosphate to be exceeded. Application of this approach to the removal of U, Am, Pu, and Np from acid mine drainage waters has been proposed based on laboratory studies with test solutions containing Am, Pu, Np (Macaskie et al 1996). Techniques for removing metals having phosphates of low water solubility, such as plutonium, with polyphosphate accumulating microorganisms, have been patented. (Dick & Macaskie, 1994 patent). Removal by microbially-enhanced chemisorption of heavy metals (MECHM), which is combines bioaccumulative and chemisorptive mechanisms has been demonstrated in laboratory studies and has been proposed for Pu removal. Pu was not effectively removed from solution by bioprecipitation by phosphate ligand produced by *Citrobacter* sp. Removal of Pu was enhanced by prior exposure of the biomass to La in the presence of organophosphate substrate to form cell-bound LaPO₄. Polyacrylamide gel-immobilized cells removed little Pu per se, but preloaded LaPO₄ promoted the removal of Pu upon subsequent challenge in a flow-through column (Macaskie and Basnakova, 1998).

10.3.3.3 Bioreduction:
Bioreduction of uranium

mention potential for all the iron reducers, bioleaching, etc. Lovley stuff....

10.3.3.4. Other technologies:
The removal of Pu (and other metals) from soils is challenging because of the strong sorption of the metal by soils and limited metal solubility. A biological process for recovering plutonium
from soils using an iron-reducing bacterium, *Bacillus circulans* NRRL B-21037 has been proposed (Rusin, 1995). The use of siderophores and citric acid to selectively extract plutonium from wastes and soils is attractive, but the practicality of such methods has not been evaluated. Metalloprotein affinity chromatography (MAMC) has been proposed for the separation of actinides from other metals in liquid waste streams. The separation of trivalent lanthanides and thorium and a model actinide has been tested using column or batch treatment with ovotransferrin immobilized to sepharose. (Cannel and Vincent, 1995). The use of high gradient magnetic separation to remove paramagnetic metal ions precipitated on the surface of microorganisms from waste suspensions has been tested with plutonium. (Watson and Ellwood, 1998)

Clearly, these studies are preliminary and much work is required to determine the feasibility of these processes. Potentially, any of the ways microorganisms can interact with plutonium could be exploited into an effective bioremediation technology. However, much work would need to be done in order to understand the fundamental chemistry, geochemistry, and microbiology of the interactions of microbe and microbial populations with plutonium. This would be a prerequisite to the order to rational and logical development of any cost effective plutonium bioremediation technologies.

10.4. Plutonium and Plants

Plutonium can interact with plants just as it can interact with microorganism—plutonium can affect plant growth and plants can affect plutonium geochemistry. Although numerous studies have addressed plant uptake of plutonium in relation to the potential hazards to humans and plant uptake as an indicator of environmental contamination levels, there is limited information on the chemistry of this interaction. Few studies have examined the plant physiology and biochemistry that effects and is affected by plutonium in detail. Also, relatively few studies have addressed the implications of plant-plutonium interactions on the environmental fate (the biogeochemistry) of plutonium.

The interaction of plants and plutonium is complex. Like microbes, the interactions of plants and plutonium will depend on the chemical form of the plutonium, the availability of oxygen, nutrients, electron donors and acceptors, environmental factors, soil chemical and physical properties, plant species, and even the microbial ecology. Like microbes, plants can either directly or indirectly effect plutonium solubility, bioavailability and mobility. Like microbes, these processes have substantial environmental significance and could potentially be exploited for plutonium remediation.

10.4.1. Accumulation of Plutonium By Plants
10.4.1.1. Aquatic Plant Accumulation of Plutonium.

Numerous studies have shown that plutonium is strongly concentrated by aquatic plants (green, brown algae, kelp, seaweed, phytoplankton). Concentration factors for aquatic plants are many order of magnitude greater than soil plants, often greater than $10^3$ or even $10^5$ (recent studies: Skwarcz, 1997; Ikahelimonen, 1997; Ibrahim, 1989; Fisher, 1985). A significant portion of filterable Pu in surface waters was shown to be associated with phytoplankton cells. (Fowler, 1983) These studies indicate that surface absorption of plutonium is the major method of uptake from aqueous environments—most plutonium is found in the outer layers and uptake depends mostly on surface area. (Wong, 1972; Hodge, 1974, Folsom, 1975; Gromov, 1976; Noshkin,
The vast experimental variability (media, time, cell density, cell treatment, Pu source, Pu concentration, oxidation state, pH, light, uptake measuring and reporting method etc) makes it impossible to do any detailed comparison of the various studies; however some biochemical information is available. Studies have indicated that there is no significant oxidation state or isotope effect, although uptake is dependent on plutonium concentration (Tkacik 1977; Fisher, 1983a). Pu(VI) may be reduced to Pu(IV) by the cell wall, which may account for the lack of isotope effect (Tkacik, 1979). At least for seaweeds, it has been suggested that sulphated polysaccharides, which compose part of the cell wall, are responsible for Pu binding (Hodge, 1974). Uptake also seems to be an order of magnitude lower in fresh water phytoplankton than in marine plants (Livingston, 1977), and higher in artic waters than in temperate waters (Fisher, 1999). For brown algae, metabolic inhibitors such as CN- did inhibit uptake, whereas NH4Cl enhanced uptake (Zlobin, 1970 & 1971). This may be a result of cell wall changes rather than an indication of the necessity for metabolic activity for efficient uptake. In fact, for unicellular algae, it has been shown that live & dead cells accumulate at same initial rate, indicating that initial uptake is passive (Fisher, 1980). However, Pu uptake in rapidly dividing culture is greater than late log or senescent cultures, which has been explained as a phenomenon of a difference in surface properties, although with little evidence (Fisher, 1980). In one study it was shown that organic constituents in media (either added EDTA, organic materials not destroyed by UV, or cultures where exometabolites have accumulated), decrease uptake; however, other studies have shown that fluvic or humic acids, DOM, or diatom exudates do not decrease uptake, and in fact, may increase uptake by complexing competing transition metals (Fisher 1983a & b). Cells don’t easily lose Pu, but it is easier to remove after short term than long-term exposure (Fisher, 1980). Plant-associated plutonium is generally believed to be the major source of plutonium in higher aquatic (Livingston, 1977).

10.4.1.2. Lichen, Mosses, Mushrooms.

Most studies with lichen, mosses, and mushrooms have used them as bioindicators of air deposition of plutonium. There have been many recent studies (Bogoeva, 1992; Jia, 1997 a&b; Paatero, 1998; Roos, 1994; Thomas, 1995; Ferris, 1995; Triulzi, 1996). In some moss species, Pu vertical profiles corresponded to eras of increased fallout, indicating no mobility in the moss (Testa, 1998). Other mosses show an exponential decrease of Pu with the height. (Jia, 1997a) Still, in other mosses and lichens there seemed to be no vertical profile, indicating mobility (Testa, 1998). Studies have estimated the biological half-life of plutonium in lichen to be 1-3 years (Paatero, 1998; Ellis, 1987). Chitin from mushrooms has been shown to be a strong sorbent for plutonium from solution, which may higher Pu uptake in mushrooms than in other plants (Hoshi, 1994; Yamamoto, 1995).

10.4.1.3. Plant Accumulation of Plutonium

Plants can accumulate plutonium via passive processes, primarily foliar deposition and entrapment of resuspended particulate matter, or from active biochemical processes, primarily root uptake and adsorption & translocation of foliar deposited plutonium. Although in the past, foliar deposition has been estimated to be orders of magnitude more important to the plutonium content in plants than other processes, only releases from nuclear facilities and wind resuspended contaminated soil are likely to significantly contribute to air-borne contamination now; therefore, it is believed that the foliar depositions pathways will become increasingly less important, and root uptake more important. Foliar deposition in plants has been reviewed (Romney, 1977; Dahlman, 1977a&b; Cataldo, 1975, 1977, 1980a&b, 1981; Simmonds, 1982). Here, the emphasis is on the biochemistry of plant uptake.

Studies with plants have typically documented the transfer of plutonium to plants from soils as a function of environment, isotope concentration, plant species, and growth conditions.
Many reviews have been published (Price, 1973; Francis, 1973; Hakonson, 1975; Bulman, 1978; Mullen, 1976; Dahlman 1976; Thomas, 1976; Wallace, 1979; Adriano, 1980). Most studies have focused on transfer of plutonium within agricultural systems; far fewer have dealt with indigenous plants. Typically, plant concentration ratios have been reported in these studies. Concentration ratios are also known as concentration factors or distribution ratio, which are units of plutonium per weight plant tissue divided by units of plutonium per dry weight of soil. Transfer factors, sometime used interchangeably, relate specifically to the uptake of activity from soil into the plant, with direct deposition and translocation processes eliminated (Green, 1997). Concentration ratios (CRs) have been related to fresh, dry, or ash weight of plants, and been reported in varying units. It has been proposed to use curies per gram of plant dry weight per curies per gram of dry soil as a standard for comparison (Pimpl, 1981), and to use CRs based on a uniform distribution of activity in soil to a depth of 150mm (Bilthoven, 1989), although other units are still used. CRs will, of course, depend on chemical, physical, biological, and environmental factors. These conditions are not always determined or made clear in many studies making comparisons difficult and may be one reason that concentration ratios can vary widely. For Pu, CRs from 10^-9 to 10^-3 have been reported for the same plant (Pimpl, 1981). Additionally, the methods of harvesting and treating plants and determining uptake can impact the apparent Pu uptake.

With those uncertainties in mind, in normal soils, studies have shown that in general root absorption of Pu from soil is relatively low, at least over the short term, especially compared to other metals/radionuclides, indicating that environmental plutonium is relatively unavailable for physiological incorporation in plants (Romney, 1981). For the transuranics, the relative plant availability is Np > U > Cm > Am > Pu (Watter, 1980; Romney, 1981; Dahlman 1976; Garten, 1981 & 1987). Typical reported concentration ratios are 10^-4 or less, although concentration ratios as high as 20 and as low as 10^-10 have been reported (Hanson, 1980; Garland, 1987; Adams, 1974; Romney, 1982, Schulz, 1977). In marsh environments, CR are similar low (10^-5 - 10^-6) however, CRs many orders of magnitude higher if compared to the soil solution (Livens, 1994). In field studies, it has been estimated that contamination of plants by direct deposition of Pu on surfaces and resuspension of soil particles is higher by up to three orders of magnitude than the contamination resulting from soil/root uptake (Pimpl, 1981; McLeod, 1984; Adriano, 1982; Romney, 1982). In spruce trees, more than 80% is believed to be from adsorption of Pu from air, resuspended soil or dead vegetation Pu (Hartmann, 1988; McLeod, 1980). Direct deposition of plutonium has also been used to explain why pot experiments typically have lower uptake than field by 10-1000 X, however, it has not been clearly shown that conditions for uptake are comparable between pot and field studies. Typically there are differences in root size and distribution due to growth in pots, which may affect uptake. Also, plutonium concentrations are typically higher in pot studies, which can affect CRs (Pimpl, 1981; Dahlman, 1976). Foliar deposited Pu can be absorbed and transported into the plant, possibly of equal magnitude to soil-root uptake (Cataldo, 1977 & 1980b). Soil adherence to vegetation may account for the majority of apparent absorbed Pu in some plants (Beresford, 1991; Pinder, 1988 & 1990; Fedorov, 1986). Higher than typical concentration ratios in at least one study were due to the direct accumulation of soil on the surface of the plant (Romney, 1975). In fact, more recent studies have used uptake of plutonium from contaminated soil as an indicator of mass loading of soil particles on plant surfaces, assuming that plant uptake was negligible in comparison (Pinder, 1989).

For actual uptake, the concentration of plutonium in soils seems to affect plant absorption. In general it is believed that CRs for metals that mimic essential plant nutrients follow a non-linear curve model and are highest at low soil concentrations, whereas non-essential elements or elements that are not physiologically regulated by plants typically follow a linear
curve model (CR of element is constant regardless of substrate concentration). Some studies have included U, Sr, Cs in the first group, and Am and Pu in the second group (McLeod, 1981). However, numerous studies have shown that plutonium uptake by plants does not increase linearly with increasing in soil contamination—CRs are higher at lower plutonium soil concentration. (Pimpl, 1981; Wildung, 1974). This could be due to plutonium toxicity affecting the plant or necessary bacteria (Wildung, 1979). It has been shown that when plutonium is added to soil complexed with excess DTPA, CRs are not affected by soil Pu concentrations (Garland 1987). This can be explained by assuming soil has a limited number of natural ligands available to solubilize plutonium, and once they are saturated, uptake (CRs) would decrease with increasing Pu concentration.

Evidence does indicate that the predominant factor in plutonium uptake from soil by plants depends on the chelation of the plutonium in order to increase plutonium solubility (Harris, 1989; Watters, 1980; Garland, 1981; Dahlman, 1976; Brown, 1978; Cataldo, 1983; Schulz, 1977, Romney, 1982). The application of chelating agents to either soil or hydroponic solutions greatly increases uptake (Delaney, 1978; Francis, 1973; Hale, 1970). Compounds such as citric acid and/or other similar chelating agents released from the plant roots may chelate plutonium and significantly enhance uptake Synthetic chelators (EDTA, DPTA) have been shown to increase uptake of plutonium up to 1300 times (Lipton, 1976). The ranking of various ligands and counterions for enhancing uptake of plutonium has been shown to be nitrate < acetate < glycolate < oxalate < citrate < EDTA < DTPA (Vyas, 1983; Pimpl, 1981). Concentration factors as high as 20 have been achieved in laboratory cultures with high concentrations of chelating agents (Garland, 1987).

Plutonium isotope, chemical form, and oxidation state may influence uptake. In alkaline soil containing lime, it was found that uptake of plutonium added as the nitrate salt was greater than uptake of plutonium added as PuCl₃, whereas there was no difference non-lime containing acid or neutral soils. It was suggested that the higher stability constant for Pu(IV) with carbonate led to increase uptake in carbonate-containing soils (Schulz, 1976). In another study, PuO₂⁺ uptake from clay was greater than Pu(IV) or Pu(III) uptake, which agrees with the lower sorption to clay for Pu(VI) (Jackobson, 1948). With bush beans, it was also shown that Pu(VI) uptake is 6-8X greater than Pu(IV), although both end up as Pu(IV) in xylem (Delaney, 1978; Garland, 1981). Another study showed plant uptake followed the valence order 5 > 6 = 3 > 4 (Bondietti, 1977). In other studies, there is no difference in Pu(VI) and Pu(IV) uptake (Garland, 1981 & 1987). There is some evidence that there is isotopic discrimination in uptake, with uptake of ²³⁷Pu > ²³⁸Pu > ²³⁹Pu > ²⁴⁰Pu (Hersloff, 1978; Hakonson, 1972; Fleisher, 1975; Brown, 1979; McLendon, 1976). This directly relates to isotope specific activity, although the mechanism of how specific activity effects uptake or even solubility is not clear. Other studies do not show clear isotopic differences (Fresquez, 1998; Nishita, 1981; Schulz, 1981). It has been reported that increased plutonium particle size decreases uptake (Pimpl, 1981). But, another study has indicated that larger colloids are taken up more than smaller colloids (Lipton, 1976); however, the study also indicated that colloid size mattered more for more shallowly buried plutonium and more shallowly buried plutonium has greater uptake, which could be simply an indication of soil resuspension of Pu particles near the surface, with more plutonium resuspended when larger particles are used.

The influence of soil type is not clear. Conflicting and often scientifically ambiguous effects of pH, organic matter content and cation exchange capacity have been reported (Schulz, 1977). Many studies have shown that radionuclide uptake is higher in soils that contain more sand and less organic matter (OM) and less clay, which are believed to act as a binding agent (Green, 1997; Vyas, 1981). Other studies show that added organic materials or nitrogen/
sulfurous fertilizers could have no effect on CRs or even increase CR by an order of magnitude (Pimpl, 1981). In another study, Pu increased when soil was amended with carbon and nitrogen to provide maximum microbial activity (Garland, 1974). The addition of Rhizobia bacteria to alfalfa or soybeans was shown to significantly increase growth yield, but decrease CR values (Garland, 1981 & 1987). Glyphosate (N-(Phosphonomethyl)glycine, a herbicide which can chelate metals), increases uptake Am but not Pu (Nisbet, 1990). Increased cationic exchange capacity decreases uptake (Pimpl, 1981). In many crop species, CRs were shown to increases when pH decreases, i.e. uptake from acid soil is greater than uptake from alkaline or calcareous soil (Francis, 1973; Price, 1973). However, in a pot study, uptake was greatest from an alkaline/lime soil. Increased OM in the acid soil used in this study could have overridden pH influences, or carbonate in the calcareous soil could have increased plutonium solubilization (Schulz, 1976). One possible reason for apparently conflicting results is that the magnitude of the effect of soil type on uptake has been estimated to be no more than a factor of 10 (Romney, 1972).

In some studies, plant uptake of Pu has been shown to increase over time and with successive plantings. (Mullen, 1976; Price, 1973; Francis, 1973; Pimpl, 1981; Adriano, 1986; Romney, 1970). There are many possible reasons for this: geochemical changes that could occur after plutonium has been in contact with the soil environment for an extended period of time; physical changes in the plant such as increased root development; physiological changes in the plant as it ages such as increased production of nutrient transport agents, or chemical changes in the soils that result from plant growth (such as pH changes, production of acids, production of chelators, production of organic matter etc.). In other studies with crops, plant CRs increased, simply varied or decreased during successive planting. No clear explanation has been found. The influences of climatic conditions have not been sufficiently investigated.

Plant physiology has a major effect on plutonium uptake. Radionuclide uptake depends on plant species (Dahlman, 1976, Seel, 1995); for example, lettuce has been shown to have higher CRs than turnip greens, broccoli or cabbage (McLeod, 1984). Grasses typically have higher uptake than shrubs or trees (Pimpl, 1981). Dicotyledonous plants (corn, wheat, sorghum) accumulate more plutonium than monocotyledonous plants (bean, tomato) (Vyas, 1983; Cline, 1976). Other studies showed legumes accumulated more than grasses (Schreckhise, 1980). These differences may be due to the higher relative surface area of some species (Hakonson, 1975 McLeod, 1984). Pu uptake can vary up to 1000 X over plant parts in a single species. Non-edible plant tissues tend to accumulate more radionuclides than the fruiting bodies of the same species and lowest actinide content in the whole plant, by up to three orders of magnitude, occurs in the seeds (Fresquez, 1998, Menzel, 1965; Barber, 1964; Romney, 1960; Seel, 1995; Menzel, 1954; Nisbet, 1994; Pimpl, 1981; Romney, 1981). In harvested and milled cereals, the bran seems to contain more plutonium than the flour (Bunzl, 1987). Plutonium uptake into plant roots is often highest (Wildung, 1979). This may be due to surface absorption of plutonium. In one study, 93% of Pu uptake was found in the surface layer of many subterranean crops (carrots, potatoes, turnips), only 7% remained after the crops were peeled. Of the 'surface' bound Pu, half could be removed by physical scrubbing. This is different than radionuclides such as 137Cs and 226Ra, where internal uptake predominated and surface absorption was negligible (Corey, 1983). However, at least in potatoes, if plutonium was applied to leaves instead of via root uptake, most of the transfer Pu ended up in the flesh (Bulman, 1993). Plant age can affect uptake. Maximum plutonium uptake has been shown to occur when the plant had attained maximum growth (Cataldo, 1975; Nisbet, 1994).

Plutonium seems to be mobile in some parts of some plants at some times. Studies have shown that plutonium spreads regularly over the entire root system of plants, but can be found an
order of magnitude less concentrated in the shoot (Garland, 1981). In some studies, concentration of Pu was found to be inversely related to the distance from the roots (Pimpl, 1981). In other studies, Pu concentrations were found to be higher in plant leaves than in shoots (Garland, 1981; Pimpl 1981). In soybeans, the distribution was found to be 84% root (28% of it soluble), 0.6% stem (54% soluble), 15.3% leaves (67% soluble), 0.01% seeds (14% soluble), and plutonium seemed to be freely transported through the xylem during growth but not subject to remobilization on flowering (Cataldo, 1987; Garland, 1981). The distribution of Pu is characteristic of xylem transport, dependent on the flux of water. Lack of accumulation in stem tissues indicates Pu stays mobilized and soluble in plants. In some trees, Pu concentration was found to be higher in sapwood than in heartwood, and was not correlated with fallout deposition, indicating Pu is mobile in the tree (Barci-Funel, 1995). In others tree studies, Pu seemed to follow fallout deposition, although a fraction still seemed to be somewhat mobile (Mahara, 1995 & 1996).

For plutonium to be transported and distributed in plants and to remain highly soluble, it must remain in some complexed form or be in a non-hydratable valence state (V or VI). However, plants reduce Pu(VI) upon uptake; various studies have shown that Pu is present only as Pu(IV) (> 90%) in plant xylem, even if supplied as Pu(VI) (Delaney, 1978). This reduction process may be similar to the purported requirement for reduction of Fe(III) to Fe(II) prior to root membrane translocation. (Delany, 1978; Garland, 1981) They also indicate the Pu is transported as an anionic organic complex that is different than the form supplied to root bathing solutions (Garland, 1987). In hydroponic Pu uptake studies in soybeans using Pu(IV) nitrate, xylem exudates analysis of samples with Pu added both in vivo and in vitro (xylem exudates collected then Pu added) using anion and cation exchange columns and thin-layer electrophoresis showed that both Fe and Pu are present primarily as organic acid complexes (instead of amino acid or peptide), with some of the same species in both in vivo and in vitro samples, but some unique to in vivo studies (>85% Pu soluble, three anionic, one cationic species for in vivo, three anionic for in vitro). Ni(II) and Cd(II) were present primarily with components of the amino acid/peptide fraction; Fe(III) was also present with amino acid fractions (Cataldo, 1988b). The form of Pu transported in the xylem, based on the organic acid fraction, appears to change with plant age, and concentrations parallels essential ion concentrations, suggesting that Pu complexation patterns track the normal production of plant ligands used for metal uptake and growth (Garland, 1987). Indeed, it has been suggested that after absorption in the plant, trace contaminants are translocated, metabolized and stored generally analogously to nutrient elements (Cataldo, 1983). Of the soluble plutonium fractions in soybean roots and leaves, 90% appears to have a MW of 10,000 or more; however, for the soluble fraction in stems, there is a greater distribution of MW, with over 20% MW 500, principally the organically complexed transport forms found in the xylem (Cataldo, 1987). Pu(IV) nitrate added in vitro to potato juice was shown to be complexed by citrate and phytate (Cooper, 1984a&b), naturally incorporated Pu was found to be in at least three fractions, possibly a phytate complex, another low MW fraction but not a citrate complex, and a high MW (>10kDa) fraction (Bulman, 1993). The physical and molecular weight distribution of plutonium in plants seems to indicate that the complexation of plutonium must change throughout the plant and plant part physiological must either encourage or inhibit plutonium transport and storage in vivo. Plant species and the presence of nutrient elements, especially Fe, Zn and Cu, that compete for reaction sites on plant organic ligands also affect the chemical form of Pu (Garland, 1987).

For foliar deposited plutonium, the retention and leaching can be affected by chemical form, solubility, residence time on the foliage, particle size, and environmental conditions. Small particles (submicron) are significantly retained on plant foliage and can be
translocated in to plants (Cataldo, 1981). The translocation depends on many of the same factors as uptake: plant age and development stage at deposition, solubilization of plutonium via rainfall or other vector, particle size, chemical form and presence of chelating ligands (Pu-nitrate > Pu-citrate > aged oxide > fresh oxide) (Cataldo, 1976, 1977, 1980a&b, 1981; Romney, 1977). Interestingly, it is generally believed that for materials to move from mature leaves into plant roots or seeds, phloem transport is needed. However, it is also believed that Pu is not transported in the phloem to any appreciable extent (Cataldo, 1983). Because phloem transport is also the essential pathway for transport of nutrients and contaminants to fruits and seeds, the low Pu uptake typically observed in fruits and seeds can be attributed to poor phloem transport; however, the low uptake could also be a result of Pu being made into some immobile form after uptake from roots and translocation into other plant parts.

As with aquatic systems, it is generally believed that plants are the vectors of plutonium to herbaceous animals. However, there is no evidence for biomagnification by animals of terrestrial or aquatic food chains (Dahlman, 1976). CRs are used in model systems to estimate human risk, however, as has been shown, CRs are highly variable. Initial models for Pu accumulation have been developed (Cowan, 1983), but for successful modeling, all the factors that have significant influence on plant uptake, foliar deposition, and retention of plutonium need to be taken into account.

10.4.2. Plants Effects on Plutonium.

Plants can indirectly effect plutonium by excreting organic acids, phytosiderophores, amino acids, peptides, or other biogenic chelating or reducing agents into the soil and can change soil chemical potential and oxygenation; plant leaf litter, decaying plant matter, and microbial degradation of plants can greatly increase organic matter content of soil. These modifications to soil chemistry can influence plutonium speciation, mobility and bioavailability. Plant exudates and plant matter could chelate, reduce, oxidize, solubilize, mobilize, or immobilize plutonium. Plant exudates could from ternary or higher order complexes with plutonium and other soil components (fulvic and humic acids, mineral surfaces, clays), which could have a variety of effects on plutonium environmental behavior. Plant can also dramatically influence microbially communities, which can affect Pu chemistry.

Very few studies have been done directly examining how plants affect plutonium in the environment. It has been demonstrated that trees produce significant amounts of organic carbon, a small fraction of which consists of low molecule weight amino acids, peptides, and organic acids that can complex radionuclides and could effect their mobility and solubility (Cataldo, 1988). Bush beans in a hydroponics study were shown to reduced external Pu(VI) to Pu(IV) in the growth solutions (Delaney, 1978). Plants can translocate plutonium from aerial deposits into the soil. There is some evidence of leaf deposited plutonium being mobilized to roots, however the majority stays immobilized on the leaves (Cataldo, 1976; Bulman, 1978; Garland, 1987; Wildung, 1979; Vyas, 1980). In potatoes, the average transfer of Pu from leaves to tubers was 0.2 – 0.4% (Bulman, 1993; Cooper, 1985). Although this may be a small percentage, it is a significant vector for Pu to enter subsurface environments. Tree crowns have been shown to intercept Pu fallout and thereby give higher Pu concentrations in the organic matter and soil under trees (Adriano, 1977).

Plants can directly affect plutonium chemical properties. In one study, a monocellular algae was shown to change the properties of plutonium. After being incorporated into the biological cycle of the algae, there was an increase in smaller Pu particles and a change in the sorption characteristics to clay (non-sorbed fractions sharply decreased and sorption onto clay increases.) (Gromov, 1976). In a study with rats, more Pu was incorporated from alfalfa pellets made from
plants grown in plutonium than from pellets simply soaked in plutonium, indicating that plant
growth effects bioavailability (Sullivan, 1980).

10.4.3. Phytoremediation of Plutonium Contaminated Soils and Wastes.

We need to truly understand the interactions of plutonium and plants for not only accurately
predicting the long term stability, mobility, and bioavailability of plutonium in the environment
and for better estimates of animal and human risk, but also to most efficiently and effectively
develop any bioremediation and phytoremediation strategy. There have been no studies directly
examining the possibilities for phytoremediation of plutonium; In fact, in a recent summary of
remediation options for plutonium-contaminated soils, not even bioremediation options were
mentioned. (Ijaz, 1999). There have been many studies examining the possibilities for
phytoremediation of uranium. For uranium phytoremediation, the key to increasing accumulation
was to increase the soil solubility of uranium by the addition of chelators such as citrate or
EDTA (Huang, 1998). If the typically low plant uptake of plutonium is only a result of low
plutonium solubility in soil rather than plant discrimination against plutonium at the membrane
level, this would surely be true for any plutonium phytoremediation system.

Algae and seaweeds, with their binding capacity and efficient concentration of aqueous
plutonium, have been proposed as a possible method for removing Pu from water. Plants with
filamentous root systems have been suggested for use in Pu removal from aquatic systems since
plants grown in aquatic systems rapidly accumulate large amounts of plutonium (McFarlane,
1978). The use of plant based biosorbents for plutonium has been investigated, just as with
microbial biosorbents. Chitin from cell walls of higher fungi is an extremely fast and efficient
biosorbent for plutonium. Distribution coefficients (Kd) for 10 to 105 have been measured
(Gorovoj, 1996 & 1997; Kosyakov, 1997a&b), with uptake was shown to be dependent on pH
and counter ions present.

10.5. Conclusions

The interesting and complex biochemistry and biological behavior of plutonium stems from
the unique properties and rich fundamental chemistry of this element. While a couple of have
been studied extensively, distribution, excretion, and health effects in mammals, chemistry in
serum, and undefined association with plants and microorganisms, most biochemical processes
are poorly understood and very little is known at the microscopic and molecular levels.

10.5.1. Biochemistry/Bioinorganic Chemistry and Health Effects

Biological mechanisms by which plutonium is moved between and within tissues and cells
Mechanisms of deposition and retention—chemical and physiological specifics.
Details of binding of plutonium to blood proteins
Retention in bone—exact nature of inorganic or organic binding
Understanding of dose-effect relationships. Current standards have generally been set from
linear extrapolations done on rats and dogs at much higher levels. And there is much research
which suggests the relationship is far from linear and supports the notion of threshold doses for
particular effects.

10.5.2. Plutonium and Microorganisms (ROUGH!!!)

The study of plutonium interactions with microorganism is clearly in its infancy. We have a some understanding of what will affect these interactions based on fundamental chemical
and microbiological principles (Microbial activity will be affected by electron donors, electron
acceptors, nutrients, pH, Eh, temperature, moisture, etc.; bioavailable and solubility of Pu will be affected by redox state, concentration, pH, Eh, presence of free ions, organic or inorganic complexants, solid phases); and we have an understanding of the possible mechanisms of microbial interactions that could effect Pu solubility, mobility, and bioavailability from studies with numerous other toxic/non-essential metals (oxidation-reduction reactions, chelation, or the production of specific sequestering agents, or catabolism of metal chelating agents, biosorption/bioaccumulation/biosequesterization by biomass and biopolymers, biocrystallization (precipitation and mineral formation). But, we have little knowledge of the chemical detail of the interactions, especially with chemically-complex plutonium, and little ability to predict how microbes will affect and be affect by plutonium in dynamic environments.

The effects of microorganisms on Pu clearly vary with environmental and experimental conditions, and likewise, the effects of Pu on microorganisms clearly vary from species to species and may even vary strain to strain. Because so few conditions and species have been studied it is not possible to make general conclusions or predictions yet.

Clearly, more rigorous studies need to be performed, with particular care taken to note all conditions that can effect the results, such as phase of microbial growth, forms of Pu used, initial and final media/environmental conditions (such as pH and ionic strength, which effect plutonium solubility and speciation), and definitions of toxicity and viability.

Numerous questions remain to be answered:

1. Under what conditions will microbes increase plutonium mobility in the environment, either through biocolloid formation, or dissolution of solids, or chelation of plutonium from other environmental sources?

2. What forms of plutonium are bioavailable, and under what conditions? For examples, can microbe out-compete other environmental chelators for plutonium, such as humates, clays, mineral oxide surfaces?

3. Is plutonium chemical toxic to microbes, or predominately radiation toxic? If chemically toxic, do organisms mediate the toxicity in the same way as other metals (excretion, block uptake, or sequester)?

3. By what mechanisms can microbes and microbial exudates solubilize plutonium oxides and hydroxides?

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4. By what mechanisms can microbes and microbial exudates solubilize plutonium oxides and hydroxides?

etc…

The potential benefits of understanding these interactions, and being able to predict how microbes with interact with plutonium, both for the possible remediation uses and for accurately accessing environmental risks, makes undertaking these studies worthwhile.

10.5.3. Plutonium and Plants

These findings suggest that the common soil fungus Aspergillus niger is capable of solubilizing soil-deposited Pu and rendering it more bioavailable for higher plants and animals. If a similar process occurs in Pu-contaminated soils, it could be an important link in the transfer of soil-deposited Pu to man, and would also explain the apparent time-dependent increases in the uptake rate of Pu by plants grown on contaminated soils (Au, et al. 1976).
Although there have been numerous studies on plant uptake of plutonium, the processes that influence the transfer Pu from soils to plants, the basic biochemical and bioinorganic processes that affect uptake, and the chemical fate of Pu in plants is not well understood. There is even less understanding on how plant matter and plant exudates effect plutonium in soils. We need some key information in order to gain a better picture of the role plants play in plutonium biogeochemistry:

1. Plant Absorption of Plutonium: What effects bioavailability? What chemical/geological/microbiological conditions allow Pu to reach the root of plants? Is bioavailability to plants predominately controlled by biogenic or anthropogenic chelates increasing plutonium solubility? Is uptake truly limited only by the solubility of plutonium in soil, so that as long as the plutonium is solubilized, the plant roots do not discriminate against it? Can leaves significantly absorb plutonium that is deposited from air or aerosoled soil? How does this absorption depend on chemical form of plutonium? For algae and plankton, what cell component chelates plutonium? How is the plutonium bound? can it easily be remobilized?

2. Translocation across root/leaf membrane: What chemical form plutonium is translocated across the root? What anthropogenic or biogenic chelators, if any, can be translocated? What role, if any, do microbes play in plutonium uptake by plants? What membrane channels are used to translocate the plutonium—are they the same as iron? Do plant roots reduce plutonium? Does Ferric Chelate Reductase, a plant enzyme that reduces iron, reduce plutonium? What chemical form plutonium is translocated across leaves?

3. Mobilization: What form and oxidation state is plutonium in after it crosses the root/leaf membrane? What is its form and concentration in the xylem & phloem? What chemical or physiological features controls translocation? What role do organic acids, amino acids, peptides, metallothelin, nicotianamine or other known metal-chelating species play in mobilization?

4. Final storage or efflux of Pu: What is the chemical form of Pu in leaves/stems/seeds? What forms are toxic to plants/how is toxicity mediated? How can plutonium in plants be remobilized? How does Pu form in the plant affect future bioavailability (by both human/animal uptake and by plant composting into soil)? Do plants efflux plutonium?

5. Plant effects on plutonium: Do plants exudates contain chelating agents for plutonium? If so, how much do they produce? How will these chelating agents affect plutonium mobility, solubility, and bioavailability? How does decaying plant matter interact with plutonium?

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