SA

PNL-2416

UC-11

The Relationship of Microbial Processes to the Fate and Behavior of Transuranic Elements in Soils, Plants, and Animals

---

**R. E. Wildung
T. R. Garland**

October 1977

**Prepared for the
U.S. Department of Energy
under Contract EY-76-C-06-1830**

Battelle

Pacific Northwest Laboratories

PNL-2416

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

The views, opinions and conclusions contained in this report are those of the contractor and do not necessarily represent those of the United States Government or the United States Department of Energy.

PACIFIC NORTHWEST LABORATORY
operated by
BATTELLE
for the
UNITED STATES DEPARTMENT OF ENERGY
Under Contract EY-76-C-06-1830

Printed in the United States of America
Available from
National Technical Information Service
United States Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22151

Price: Printed Copy \$.....*: Microfiche \$3.00

*Pages	NTIS Selling Price
001-025	\$4.50
026-050	\$5.00
051-075	\$5.50
076-100	\$6.00
101-125	\$6.50
126-150	\$7.00
151-175	\$7.75
176-200	\$8.50
201-225	\$8.75
226-250	\$9.00
251-275	\$10.00
276-300	\$10.25

3 3679 00051 7922

PNL-2416
UC-11

THE RELATIONSHIP OF MICROBIAL PROCESSES
TO THE FATE AND BEHAVIOR
OF TRANSURANIC ELEMENTS
IN SOILS, PLANTS, AND ANIMALS

R. E. Wildung

T. R. Garland

October 1977

BATTELLE
Pacific Northwest Laboratories
Richland, Washington 99352

SUMMARY

This review considers the influence of soil physicochemical and microbial processes on the long-term solubility, form, and bioavailability of plutonium and other transuranic elements important in the nuclear fuel cycle. Emphasis is placed on delineation of the relationships between soil chemical and microbial processes and the role of soil microorganisms in effecting solubilization, transformation and plant/animal uptake of elements considered largely insoluble in soils strictly on the basis of their inorganic chemical characteristics.

Soluble, diffusible Pu in soils (usually less than 0.1% of total) appears to be largely present as particulates of hydrated oxide, but several lines of evidence suggest that microorganisms may influence the solubility of Pu and that the nonparticulate plant-available fraction is stabilized in solution by inorganic or organic ligands of limited concentration in soil. The role of soil microorganisms in influencing the solubility, form and plant availability of the transuranics is discussed on the basis of the (1) known chemistry of organic ligands in soils, (2) effects on the soil microflora, and (3) principal microbial transformation mechanisms, including direct alteration (valence state, alkylation), indirect alteration (metabolite interactions, influence on the physicochemical environment), and cycling processes (biological uptake and release on decomposition of tissues).

The toxicity of Pu to microorganisms depends on Pu solubility in soil. However, soil microorganisms are generally resistant to Pu, with toxicity apparently due to radiation rather than chemical effects. Highly resistant bacteria, fungi, and actinomycetes have been isolated from soil, and these organisms have been shown to be capable of transporting Pu into the cell and altering its form in the cell and in solution. The resulting soluble Pu complexes tend to be of higher molecular weight than simple complexes (Pu-DTPA) and negatively charged. The form of Pu, although not well-defined, is dependent upon organism type, carbon source, and time of Pu exposure during growth. These factors, in turn, are a function of Pu source, soil properties, and soil environmental conditions. Knowledge of the relative influence of these factors serves as a valuable basis for predicting the long-term behavior of Pu and other transuranic elements in soils. There is growing evidence that these phenomena also markedly influence plant and animal availability.

Preliminary studies indicate that Pu present in solution as an organic complex is readily assimilated by the plant. Evidence to date indicates that organometal complexes serve mainly to deliver the metal to the root membrane, and the ligands are not taken up by the plant stoichiometrically with the metal. After passing the root membrane, Pu is transported to the shoots in the xylem through formation of a number of complexes with plant ligands. The form of Pu differs in leaves and stems, but greater than 90% was not present as the ionic Pu(IV) originally added to soil on which the plants were grown. A reevaluation of plant-to-animal transfer coefficients

may be required as Pu incorporated in plant tissues is markedly more available to animals than Pu(IV) gavaged in the inorganic Pu solutions which have been used for previous measurements. Differences in gastrointestinal transfer of Pu in stems and leaves of alfalfa are related to differences in Pu solubility in these tissues. Thus, the form of Pu in soils and plants may be closely related to Pu availability to animals.

Although information leading to an understanding of the complex interrelationships that exist between soils, plants and animals is rapidly developing, these phenomena are not sufficiently understood, at present, to be described by simple models.

CONTENTS

SUMMARY	iii
INTRODUCTION	1
TRANSURANIC CHEMISTRY IN SOIL	3
Chemical Reactions Influencing Plutonium Behavior	3
Chemical Reactions Influencing the Behavior of Other Transuranic Elements	10
Organic Complexation Reactions	11
Humic Substances	11
Nonhumic Substances With Potential For Metal Complexation . .	12
MICROBIAL TRANSFORMATION OF THE TRANSURANIC ELEMENTS IN SOIL	13
Potential Mechanisms of Transformation	13
Microbial Alteration of Solubility in Soil	14
Effect on the Soil Microflora	16
Effect on Microbial Types, Numbers and Activity	17
Mechanism of Effect	19
Isolation of Resistant Organisms	20
Microbial Transformations	21
Direct Transformations	21
Indirect Transformations	22
Cycling During Decomposition	29
MICROBIAL INFLUENCE ON THE AVAILABILITY AND FORM OF TRANSURANIC ELEMENTS IN PLANTS AND ANIMALS	31
Plants	31
Animals	34
RECOMMENDATIONS FOR FUTURE RESEARCH	35
LITERATURE CITED	39
ACKNOWLEDGMENTS	45

THE RELATIONSHIP OF MICROBIAL PROCESSES TO THE
FATE AND BEHAVIOR OF TRANSURANIC ELEMENTS
IN SOILS AND PLANTS

INTRODUCTION

In the terrestrial environment, soils represent the principal repository of the transuranic elements over geologic time. The major factor governing transuranic availability to plants in soils will be the solubility of the transuranics associated with the solid phase, since in order for root uptake to occur a soluble species must exist adjacent to the root membrane for some finite period. The form of this soluble species will have a strong influence on its stability in soil solution, mobility in soils and on the rate and extent of uptake, and perhaps, mobility and toxicity in the plant. Furthermore, the results of preliminary studies to be discussed in this review suggest the concentration and chemical form of the element in the plant plays a major role in influencing its availability to animals on ingestion. Thus, any assessment of the long-term behavior of the transuranics in the terrestrial environment must be based on determination of the factors influencing solubility and form of soluble species in soil. These factors are illustrated in Fig. 1 and include the concentration and chemical form of the element entering soil, the influence of soil properties on the elemental distribution between the solid and liquid phase, and the effect of soil processes, such as microbial activity, on the kinetics of sorption reactions, transuranic concentration, and the form of soluble and insoluble chemical species.

Fig. 1. Factors influencing transuranic behavior in the terrestrial environment.

The transuranic elements of principal importance in the nuclear fuel cycle (Pu, Am, Cm, Np) may enter the soil through several avenues (Vaughan *et al.*, 1976) including (1) fallout from atmospheric testing, (2) possible escape of airborne particulates and liquid effluents during reprocessing of spent fuels and fuel fabrication and (3) leaching from waste storage facilities. The major sources of the transuranics may be classified according to expected initial solubility in soil (Fig. 2). Particulate oxides of the transuranics initially may be expected to be largely insoluble in the soil solution. Ultimately, solubility is expected to be a function of the composition, configuration and equivalent diameter of the particle as well as soil properties and processes. Oxide particles of the highest specific activity and containing the highest concentrations of impurities in the crystal lattice may exhibit greatest solubility. The combination of configuration and equivalent diameter as reflected in surface area exposed to solution will be the other main factor governing oxide solubility. Once solubilized, the transuranic elements will be subject to the chemical reactions governing soluble salts. Hydrolyzable transuranics entering the soil in acid solutions sufficiently concentrated to maintain soluble ions may be expected to be rapidly insolubilized due to hydrolysis on dilution and subsequent precipitation on particle surfaces. These include Pu(III, IV, and VI), Am(III, IV), Cm(III, IV), and Np(III, IV, VI). Conversely, transuranics not subject to marked hydrolysis may be initially more soluble. These include Pu(V) and Np(V). Immobilization of these chemical species (PuO_2^+ or NpO_2^+) may occur through cation exchange reactions with particulate surfaces. Complicating this situation, disproportionation and complexation reactions may occur concurrently.

INSOLUBLE SOURCE TERMS

SOLUBLE SOURCE TERMS

HYDROLYZABLE

NONHYDROLYZABLE

ORGANIC COMPLEXES

Fig. 2. Influence of source term on transuranic behavior in soil. Transuranic elements are represented by M, and L represents inorganic and organic ligands capable of reacting with transuranic elements and forming soluble or insoluble products.

Transuranics entering the soil as stable organocomplexes as might occur in the vicinity of a spent fuel separation facility may be initially highly soluble (Wildung and Garland, 1975). The duration of solubility and mobility in the soil will be a function of the stability of the complex to substitution by major competing ions, such as Ca and H (Lahav and Hochberg, 1976; Lindsay, 1972; Norvell, 1972) and the stability of the organic ligand to microbial decomposition (Wildung and Garland, 1975). The disruption of the complex may lead to marked reduction in transuranic solubility through hydrolysis and precipitation reactions as described for acid solutions on dilution. A portion of the ion released may react with other, perhaps more stable, ligands in soil. The mobility of the intact complexes, in turn, will be principally a function of their chemical and microbiological stability and the charge on the complex which will govern the degree of sorption on soil particulates.

Further generalizations of transuranic behavior on the basis of source terms are complicated by the overwhelming importance of soil properties and processes in influencing transuranic behavior on a regional and local basis. This review considers, in detail, the influence of soil properties and abiotic and biotic processes on the long-term solubility of the transuranics entering soils. Consideration is also given to the implications of these processes in terms of transuranic plant and animal availability. Principal emphasis is directed toward the role of soil microorganisms in this phenomenon. Microorganisms, in intimate association with soil particles, are known to play an important role in effecting solubilization of elements considered insoluble in soils strictly on the basis of their inorganic chemistry. To date, studies of the microbiology of the transuranic elements have been limited principally to Pu. This review will emphasize Pu, but, where possible, the available information is used as a framework for broader discussions encompassing the long term behavior of other transuranic elements.

TRANSURANIC CHEMISTRY IN SOIL

Chemical Reactions Influencing Plutonium Behavior

The principal chemical reactions likely influencing Pu behavior in soil are summarized in Fig. 3. Plutonium ions may commonly exist in aqueous solution in valence states III, IV, V(PuO_2^+) and VI(PuO_2^{+2}). Other valence states are known (II, VII) and predicted (VIII) but these occur under unique conditions (Cleveland, 1970). Disproportionation reactions are common, and due to kinetic factors, Pu is unique among the chemical elements in that it may simultaneously exist in all of the common valence states. The tendency of Pu to hydrolyze in aqueous solutions of low acidity follows the order $\text{Pu}^{+4} > \text{PuO}_2^{+2} > \text{Pu}^{+3} > \text{PuO}_2^+$ (Cleveland, 1970). Hydrolysis, which occurs in a stepwise fashion, is likely the major mechanism whereby Pu is insolubilized in the environment. At high (g/l) Pu concentrations, hydrolysis of Pu^{+4} , may lead to the formation of a colloidal Pu polymer. At these concentrations, the polymer is characterized by a distinct absorption spectrum. Although the polymer has not been fully characterized, it is generally thought to be an intermediate

- FOUR OXIDATION STATES

- DISPROPORTIONATION

- HYDROLYSIS

- COMPLEX FORMATION

Fig. 3. Chemical reactions influencing plutonium behavior in soil.

hydrolysis product of Pu^{+4} containing oxide or hydroxide bridges, with an absorption spectrum different than $\text{Pu}(\text{OH})_4$. However, studies by Lloyd and Haire (1973) indicated that the polymer may be aggregates of small, discrete, amorphous or crystalline, primary particles of 5 to 20 Å in diameter. It is of interest that x-ray diffraction patterns of the polymeric Pu and that of $\text{Pu}(\text{OH})_4$ (Ockenden and Welch, 1956) both showed a pattern characteristic of the cubic PuO_2 lattice, suggesting that the polymer and the hydroxide of Pu^{+4} may be hydrated PuO_2 with differences occurring in primary particle size and crystallinity (Lloyd and Haire, 1973). The formation of the hydrated PuO_2 is likely directly related to Pu^{+4} concentration and inversely related to the acid concentration.

Plutonium also tends to form many complexes with a range of stabilities. The strongest complexes are generally formed by reaction of organic ligands with Pu^{+4} . However, many inorganic complexes and organic complexes of all valences may be stable under appropriate conditions. The presence of organic ligands in soils likely influences the equilibrium and concentration form of Pu in solution through complexation and subsequent inhibition of hydrolysis, polymerization, or disproportionation. These reactions, in various highly complex combinations resulting from differences in source term, soil properties and processes, govern Pu solubility in soil and availability to plants.

Soil chemical reactions are important in governing the behavior of the various forms of Pu entering soil. Initially soluble forms entering soil have the potential for undergoing a range of chemical transformations (Fig. 1-3). Insoluble Pu, such as high-fired oxide, entering soil likely will be solubilized with time, provided soluble, stable complexes are formed (Fig. 3). However, regardless of the form of Pu entering soil, its ultimate solubility will be controlled by its aqueous chemistry and by

soil factors. Soil physicochemical properties may be expected to have complex, interdependent effects on Pu solubility. The long-term behavior of Pu in soil will be a function of the kinetics of these reactions.

On the basis of research with other trace metals, recently summarized by Keeney and Wildung (1977), and limited information on the transuranic elements it may be concluded that the soil physicochemical parameters most important in influencing the solubility of the transuranics include: (1) solution composition, Eh and pH, (2) type and density of charge on soil colloids, and (3) reactive surface area. These phenomena will in turn be dependent upon soil properties, including particle size distribution, organic matter content, particle mineralogy, degree of aeration and microbial activity. The delineation of the influence of these factors on Pu solubility is difficult due to the complex chemistry of Pu.

A reasonable approach to the study of the chemistry of Pu in soil is to direct initial attention to the factors influencing its solubility in soil. However, it is difficult to define Pu solubility in soil because solubility will depend upon the method of measurement and because solubility must be arbitrarily evaluated due to the sorption of Pu on sub-micron clay particles or to the formation of submicron particles of hydrated Pu oxide which are difficult to centrifuge and may pass membrane filters. These effects may be illustrated by comparison of the differences in the solubility of Pu in soils [100 days after amendment as $\text{Pu}(\text{NO}_3)_4$] as determined by water extraction and subsequent membrane filtration using membranes of different average pore sizes (Table 1). The major fraction of the Pu added was sorbed on the soil, as a maximum of 10% of the extracted Pu passed through the 5 μ membrane. Successive filtration through membranes with decreasing pore size resulted in decreases in Pu concentration in the filtrate. Thus, Pu in the aqueous extract appeared to be in a wide range of particle sizes. Although membranes with pore sizes of 0.45 μ are commonly used to separate soluble from particulate matter, Pu in these filtrates may be in colloidal forms. The Pu in the 0.0010 μ filtrate appeared soluble, was stable in solution, and approximated the quantity of Pu taken up by plants (Wildung and Garland, 1974). Of the soluble Pu forms likely to enter soils (previous section), $\text{Pu}(\text{NO}_3)_4$ and Pu-DTPA represent, in their respective chemistries, the range in soil behavior likely to occur. The water solubility (< 0.01 μ) of ^{238}Pu and ^{239}Pu amended to a Ritzville silt loam (organic C content 0.7%, pH 6.2) in the $\text{Pu}(\text{NO}_3)_4$ and Pu-DTPA forms differs markedly (Wildung and Garland, 1975). The DTPA complexes of both isotopes were water-soluble in soil and appeared to be stable over the first 40 days of incubation (Fig. 4). After 7 days of incubation, the ^{238}Pu -DTPA appeared to be slightly less soluble than the ^{239}Pu -DTPA. After 95 days of incubation, both isotopes, initially added as the complex, appeared to decrease in solubility; perhaps as a result of microbial degradation of the organic moiety and the development of new chemical equilibria.

Equilibrium concentrations of soluble Pu added as the nitrate were not obtained until 7 to 10 days. The solubility of ^{238}Pu and ^{239}Pu added to the soil as nitrates was much lower than the DTPA complexes, likely

Table 1. Solubilities of plutonium in water extracts of a Ritzville silt loam as determined by filtration with membranes of different pore sizes (Garland and Wildung, 1977).

Membrane Pore Size, μm	Plutonium Solubility, pg/g^*
5	60,000
0.45	20,000
0.01	4,000
0.0015	1,000
0.0012	300
0.0010	50

*Plutonium added at a level of 620,000 pg/g of soil.

Fig. 4. Solubility of plutonium with time after addition to soil as the nitrate and the DTPA complex (Wildung and Garland, 1975).

reflecting hydrolysis to the largely insoluble hydrated oxide. It is clear that organic ligands have a pronounced effect on Pu solubility in soil. The rate of decrease in solubility of each isotope added as the nitrate was similar. However, in contrast to the slightly lower solubility of the ^{238}Pu -DTPA compared to the ^{239}Pu -DTPA, ^{238}Pu added as the nitrate was a consistent factor of 2 to 3 times more soluble than ^{239}Pu initially added as the nitrate. This difference probably resulted from the formation of larger hydrated oxide particles at the higher Pu concentration (^{239}Pu), but it may also have reflected the presence of soil components such as organic ligands, which stabilized Pu in solution but were present in limited concentrations and became important only at lower Pu concentrations.

The water solubility of ^{238}Pu , when incorporated in relatively large Pu oxide particles ($>1\ \mu$), would be expected to be greater than the solubility of ^{239}Pu oxide particles of similar size due to crystal damage and radiolysis arising from the greater specific activity of the ^{238}Pu (an approximate factor of 270). However, the behavior of the two isotopes in soil on solubilization of the oxide might be expected to follow a course similar to that exhibited by the nitrates (Fig. 4).

Equilibrium solubility after 6 days of incubation (Garland *et al.*, 1976) of Pu, added as $\text{Pu}(\text{NO}_3)_4$, in soils of different properties occurred after approximately 20 hours (Fig. 5). The quantities of Pu soluble at equilibrium in water and 0.01 M CaCl_2 differed with soil type. In the CaCl_2 solution, solubility was lowest in the Muscatine soil which exhibited higher silt and clay content than the other soils. Importantly, at equilibrium there was more Pu extracted by water than 0.01 M CaCl_2 in the Muscatine soil. The Hesson and Ritzville soils did not exhibit this property. This may be related to a difference in the dispersibility of fine colloids in this soil and/or the presence of higher concentrations of stabilizing ligands. However, the lack of a proportional dilution effect (not shown in Fig. 5) in the water extractability of Pu at lower solution to soil ratios in this soil, as compared to the Ritzville and Hesson soils, provided presumptive evidence for the presence of a dispersible ligand in higher concentration in the Muscatine soil.

Applying diffusion principles to characterization of mobile Pu species in soils, Garland and Wildung (1977) estimated the concentrations and molecular weight of mobile Pu in five surface soils representing a range in particle size distributions, pH (4.4 to 6.2) organic C (0.7 to 12.5%) and cation exchange capacities (14 to 45 meq/100 g). Diffusion coefficients were calculated from measurements of the rate and extent of Pu migration from soil through an agar matrix. The diffusion coefficients calculated for the most mobile species in the five soils varied from 1.5 to $3.0 \times 10^{-6}\ \text{cm}^2/\text{sec}$ (Table 2). Estimated concentrations and molecular weights of the most mobile Pu components in the five soils ranged from 9 to 55 pg/g and from 5000 to 21,000 g/mole, respectively. Thus, estimated concentrations of the most mobile Pu species were of the same order of magnitude as those observed by water extraction and subsequent ultrafiltration through the 0.0010 μ membrane (Table 1). This membrane retained Pu-DTPA (molecular weight 1700). Hypothetical globular peptides of molecular weights less than 500 would pass

Fig. 5. Quantity of soluble plutonium removed from three soils by 0.01 M CaCl_2 (Garland *et al.*, 1976).

through this membrane. However, if the molecule was a hydrated PuO_2 sphere of similar dimensions, it would have a molecular weight between 10,000 and 25,000 approximating the molecular weights of the most mobile Pu species as determined from diffusion coefficients. This fraction, therefore, likely consisted of small particles of $\text{Pu}(\text{OH})_4$ or hydrated oxide.

The estimated diffusion coefficients for the least mobile Pu components ranged from 2.3 to $3.1 \times 10^{-7} \text{ cm}^2/\text{sec}$ with corresponding soil concentrations of 150 to 1200 pg/g (Table 2). This concentration of Pu in soil approximated the quantity of water-soluble Pu passing the 0.0015μ ultrafiltration membrane (Table 1). Hypothetical globular proteins in this size range would have average molecular weights $< 10,000$. Particles of $\text{Pu}(\text{OH})_4$ or hydrated oxides would have molecular weights of 200,000 to 500,000. Estimated molecular weights for these least mobile species calculated from diffusion coefficients were between 600,000 and 900,000. Thus, it would appear, as in the case of the most mobile species, that the least mobile species of Pu were particulate $\text{Pu}(\text{OH})_4$ or hydrated oxides.

The comparison of filtration and diffusion data indicates that the mobile Pu in incubated soils was in the form of hydrated oxide of hydroxide in a continuum of sizes. If it can be assumed that Pu in particulate form was not available to plants, it is possible that the small fraction of Pu taken up by plants was present in soil as reaction or dissolution products with insufficient stability and/or concentration to be detected by the methods employed. Insight into this possibility was not provided by comparison of

Table 2. Estimated concentrations and molecular weights of mobile plutonium in soils from measured diffusion coefficients (Garland and Wildung, 1977).

Treatment	Most Mobile Species			Least Mobile Species		
	Diffusion Coefficient $\times 10^{-6}$	Molecular Weight, g/mole	Soil Concentration, pg/g*	Diffusion Coefficient $\times 10^{-7}$	Molecular Weight, g/mole	Soil Concentration, pg/g*
<u>Control</u>						
Pu-DTPA	5.8	1,700		53	1,700	
<u>Soils</u>						
Ritzville	3.0	5,000	24	2.3	0.9×10^6	150
Quillayute	2.5	7,200	47	2.7	0.7×10^6	1,200
Hesson	2.4	8,100	9	2.7	0.6×10^6	330
Salkum	1.5	21,000	55	2.3	0.8×10^6	340
Muscateen	1.9	13,000	36	3.1	0.5×10^6	170

*Plutonium added at a level of 620,000 pg/g of soil.

Pu behavior in different soils, as might be expected, because the estimated concentrations and molecular weights of the mobile species were not related to the soil properties measured.

Several conclusions may be drawn from studies of the soil chemistry of Pu which have important implications in terms of the potential role of the soil microbiota in influencing Pu behavior in soil. Definition of Pu solubility by filtration or diffusion alone is complicated by Pu chemistry, but, in conjunction, the measurements suggest that mobile Pu is largely particulate. However, a fraction of the mobile Pu is available to plants.

This material is obviously not particulate, but is present in insufficient concentration for characterization using current methods. The question remains--what is the form of the small quantity of Pu available to plants? This information is essential to understanding the mechanisms whereby Pu may be resupplied to solution from the solid phase in a range of soils and to predictions of the long-term availability of Pu to plants. From investigations of Pu valence state in a neutral, 0.0004 M NH_4HCO_3 solution equilibrated with PuO_2 microspheres and in burial ground leachates, Bondietti and Reynolds (1976) concluded that Pu(VI) may be stable in significant quantities in solution and suggested that monomeric Pu(VI) and its complexes may be important in Pu mobilization. In the present studies, several lines of evidence were presented suggesting that Pu ions are stabilized in soil solution by inorganic or organic ligands for subsequent uptake by the plant. Furthermore, equilibration of weathered Pu-contaminated soil with chelating resins has been shown (Bondietti *et al.*, 1975) to result in significant desorption of Pu from the solid phase. It is known that organic ligands result in the most stable Pu complexes. Soluble organic ligands in soil are generally derived from microbial processes.

Chemical Reactions Influencing the Behavior of Other Transuranic Elements

Other transuranic isotopes of concern in the nuclear fuel cycle include ^{241}Am , ^{243}Am , ^{242}Cm , ^{243}Cm , ^{244}Cm , and ^{237}Np . Although detailed studies of their interaction with soils are lacking, some information has become available in recent years. Furthermore, the aqueous chemistries of these elements have been fairly well established (Katz and Seaborg, 1957). The most stable ions of Am and Cm in aqueous solutions are the cations (III); Np is most stable as the oxyion (NpO_2^+). Disproportionation is not common with these elements. Thus, major differences in their environmental behavior as compared to Pu, would be expected. Hydrolysis reactions may still be a primary factor governing the environmental behavior of Am and Cm but greater mobility and plant availability in soils might be predicted on the basis of greater solubility of the hydroxides in comparison to $\text{Pu}(\text{OH})_4$. The Np oxycation should not be subject to significant hydrolysis at environmental pH values (Burney and Harbour, 1974). The environmental behavior of Np has been least studied of the transuranics but because of its chemical characteristics it may be the most available to the biota. A comparison of Pu, Am, and Np sorption in several soils (Routson *et al.*, 1975) indicated sorption in the order $\text{Pu} > \text{Am} > \text{Np}$. The chemistry of Cm should be very similar to Am if present at equal mass concentrations.

Organic Complexation Reactions

Research to date on the chemistry of the transuranic elements in soil has pointed to the importance of understanding transuranic organic complexation reactions in soil; particularly in surface soils and aquatic sediments where organic matter content is generally highest or in subsoils where the transuranics may be dispersed in conjunction with synthetic complexing agents. Very little information is available concerning the interaction of the transuranic elements with the soil organic fraction. However, despite the difficulties in characterization of soil organic complexes, much is known both theoretically and experimentally regarding the interactions of metals with functional groups of soil organic matter (Keeney and Wildung, 1977). Much of this information concerns micronutrients of greatest agronomic importance (B, Co, Cu, Fe, Mn, Mo, Se, Zn) and this research has been the subject of a number of excellent reviews over the last two decades (Mitchell, 1964, 1972; Mortensen, 1963; Hodgson, 1963; Stevenson and Ardakani, 1972). Earlier studies generally emphasized metal interactions with intact soil or with the higher molecular weight humic components of soil whereas recent studies emphasize the more soluble components of soil.

It is practical to categorize metal complexes in soil in terms of their solubility since, in general, it is this factor, as previously noted, which most influences their mobility and plant availability. Three principal categories have been proposed (Hodgson, 1963) although the complexity of the soil system results in considerable overlap between categories. These include (1) the relatively high molecular-weight humic substances containing condensed aromatic nuclei in complex polymers derived from secondary syntheses which have a high affinity for metals but are largely insoluble in soil, (2) low molecular weight organic acids and bases, classified as nonhumic substances, and derived largely from microbial cells and metabolism which demonstrate relatively high solubility in association with metals, and (3) soluble ligands which are precipitated on reaction with metals.

Humic Substances. Humic substances are generally divided into three categories based on their solubilities (Felbeck, 1965). The humin (alkali and acid insoluble) fraction is soluble only under drastic conditions and is apparently of the highest molecular weight. The humate (alkali soluble, acid insoluble) and fulvate (alkali and acid soluble) fractions of soil may constitute up to 90% of the soil organic fraction (Kononova, 1966). The humates and fulvates are characterized, in part, by a high charge density due to acidic functional groups (Stevenson and Ardakani, 1972; Felbeck, 1965). This property leads to a high degree of reactivity and these materials exhibit a strong pH-dependent affinity for cations in solution and are likely strongly bound to soil minerals and other organic constituents in soil (Greenland, 1965). The acidic functional groups consist principally (in general order of acidity) of carboxyl, hydroxyl (phenolic, alcoholic), enolic, and carbonyl groups (Broadbent and Bradford, 1952; Felbeck, 1965; Schnitzer *et al.*, 1959). Total acidity has been estimated to range between 500 to 900 and 900 to 1,400 meq/100 g for humic acids and fulvic acids, respectively (Stevenson and Butler, 1969). The acidic H of humic acids was differentiated by Thompson (1965) into three groups at 100 to

200, 500 to 700, and 1,000 to 1,200 meq/100 g using nonaqueous titration methods. Basic functional groups, likely amides and heterocyclic nitrogen compounds (Bremner, 1965), probably also contribute to retention of metals but are of much less importance than acidic groups at most soil pH values.

In batch equilibration studies (Bondietti, 1974), Ca-saturated humates removed greater than 94% of the Pu(IV) from pH 6.5 aqueous solutions (compositions not given). It is unclear whether the humates represented a surface for precipitation of hydrolyzed species or were involved in complexation of Pu. However, in studies of Pu desorption from humates and reference clays, citrate removed 10 to 30% of sorbed Pu from the clays but less than 1% from the humic acids. Ligands forming stronger complexes with Pu (DTPA and EDTA) were required to remove significant quantities (up to 30%) of the Pu from the humate complex.

Although humic and fulvic acids likely account for most of the metal immobilization attributed to the soil organic matter, (e.g., Hodgson, 1963; Stevenson and Ardakani, 1972), they have the potential for formation of soluble complexes with metals, particularly in dilute solutions. Small quantities of metal fulvates, thought to be of lower molecular weight than the humates, may be present in soil solution. A nondialyzable material with infrared absorption spectra and elemental analyses similar to fulvic acids was isolated from a dilute salt (0.01 M KBr) extract of a mineral soil by Geering and Hodgson (1969). The material exhibited a concentration equivalent to 2.5% of a dialyzable fraction but was more effective in complexing Cu and Zn.

Nonhumic Substances With Potential For Metal Complexation. Lower molecular weight biochemicals of recent origin have been implicated in metal complexation and solubilization in soil. These materials represent (1) components of living cells of microorganisms and plant roots and their exudates and (2) the entire spectrum of degradation products which ultimately serve as the building units of the soil humic fraction. The quantity and composition of these materials will vary with soil, vegetation, and environmental conditions (Alexander, 1961, 1971). Readily decomposable wastes disposed to soil under conditions appropriate for microbial growth may, for example, result in immediate and marked increases in organic materials identified in (1) and longer term increases of materials in (2). Conversely, toxic materials may have the opposite effects. The specific compounds produced will be dependent upon the properties of the waste and soil environmental conditions after disposal (Routson and Wildung, 1969).

Although the concentration of the transuranics and other metals soluble in the soil solution or in mild extractants is low, often near minimum detectable levels, the major portion of Cu and Zn were shown to be associated with low molecular weight components. Most of the titratable acidity of this fraction was attributed (Geering and Hodgson, 1969) to aliphatic acids (< pH 7.0) and amino acids (> pH 7.0).

The production, distribution, and action of organic acids in soil was reviewed by Stevenson (1967). A wide range of organic acids are produced by microorganisms known to be present in soil. These include (1) simple acids such as acetic, propionic, and butyric, produced in largest quantities by bacteria under anaerobic conditions, (2) carboxylic acids derived from monosaccharides, such as gluconic, glucuronic, and α -ketogluconic acids produced by both bacteria and fungi, (3) products of the citric acid cycle such as succinic, fumaric, malic, and citric acid, which are common metabolic excretory products of fungi, and (4) aromatic acids such as *p*-hydroxybenzoic, vanillic, and syringic acids thought to be fungal decomposition products of plant lignins. A variety of organic acids have also been reported in root exudates.

Amino acids are the other important group of compounds identified in significant quantities in the soil solution by Geering and Hodgson (1969) which may be expected to exhibit strong affinity for metals. The qualitative and quantitative aspects of amino acids and other nitrogenous components in soils have been reviewed by Bremner (1967). It was concluded that soil acid hydrolysates do not differ greatly in amino acid composition but quantitative differences may occur with differences in soil, climatic, and cultural practices. A number of acidic and basic amino acids have been reported in soil. However, it appears that the major portion of amino acid-N that is present in hydrolysates is in (1) the neutral amino acids glycine, alanine, serine, threonine, valine, leucine, isoleucine and proline, (2) the acidic amino acids, aspartic acid and glutamic acid, and (3) the basic amino acids, lysine and arginine. Most of the amino acids detected in soil hydrolysates have also been shown to exist free in small quantities in soils with levels seldom exceeding 2 $\mu\text{g/g}$. In the soil solution (Geering and Hodgson, 1969), neutral amino acids also appeared to predominate. Basic amino acids were not detected although two acidic amino acids (aspartic and glutamic acids) were present.

Stevenson and Ardakani (1972) concluded that organic acids and amino acids, while present only in small quantities in soil, were present in sufficient quantities in water-soluble forms to play a significant role in solubilization of mineral matter in soil. Small quantities of a number of other complexing agents, such as nucleotide phosphates, polyphenols, phytic acid, porphyrins, and auxins, also exist in soil (pertinent references summarized by Mortensen, 1963). However, it is unclear at present, whether these materials would be present in sufficient quantities in the soil solution under most soil conditions to affect transuranic solubility over the long-term.

MICROBIAL TRANSFORMATION OF THE TRANSURANIC ELEMENTS IN SOIL

Potential Mechanisms of Transformation

From the results of limited studies of soil chemistry, microbiology and plant availability of transuranics in soils, and by inference from studies of complexation of other trace metals in soils it may be concluded that the

soil microflora will play a significant role in transformations governing the form, and ultimately, the long-term solubility and behavior of transuranic elements in soil. There are four general mechanisms whereby microorganisms may alter the form of trace metals in soil (Alexander, 1961; Wood, 1974). These include (1) indirect mechanisms resulting from metal interactions with microbial metabolites, or changes in pH and Eh, (2) direct transformations such as alkylation and alteration of the valence state through microbial oxidation (use of the metal as an energy source) or microbial reduction (use of the metal as an electron acceptor in the absence of oxygen), (3) immobilization by incorporation into microbial tissues, and (4) release of metals on decomposition of organic residues.

All of these mechanisms may be operational in transformations of transuranic elements in soils. However, on the basis of present knowledge, it is not possible to draw conclusions as to their relative importance in affecting the long-term behavior of the transuranic elements. Since there is a paucity of information available, these mechanisms will be addressed around a framework of current information, limited principally to Pu.

Microbial Alteration of Solubility in Soil

To provide a preliminary assessment of the potential for microbial alteration of Pu solubility in soil under aerobic conditions, Wildung *et al.* (1973, 1974) measured soil respiration rate (an index of soil microbial activity), microbial types and numbers and Pu water solubility in sterile (gamma irradiation) and nonsterile soils, which contained 10 μCi Pu/g of soil [added as $\text{Pu}(\text{NO}_3)_4$]. Carbon dioxide evolution was utilized as a measure of soil respiration rate. To measure Pu solubility, the soil was subsampled at intervals during incubation over a 30 day period, and the subsamples (1 g) suspended in 1 liter of distilled water. After a 4 hr equilibration period, an aliquot of the soil suspension was filtered through 5, 0.45 and 0.01 μ filters. The Pu in the 0.5 and 0.01 μ filtrates was designated water soluble although it was recognized that Pu likely was present as fine colloids (previous section).

Changes in the soil respiration rate and Pu solubility during the initial 30 day incubation are shown in Fig. 6. The concentration of Pu in the 0.45 μ filtrate during the incubation period ranged from approximately 0.5 to 1.5% of the Pu initially applied. Solubility was initially higher in the sterile soil than in the nonsterile soil but was relatively constant with time in the sterile soil. The initial increase in solubility in the sterile soil was anticipated in view of the known increases in soluble organic matter which result from gamma irradiation of soil.

The Pu solubility ($< 0.45 \mu$) in the nonsterile soil, while initially lower, increased by a factor of 3 with incubation time to 14 days and remained significantly higher than the sterile soil during the incubation period. This increase generally followed the accumulative CO_2 curve, and maximum solubility occurred at the end of logarithmic growth for all classes of organisms. The concentration of Pu in the 0.01 μ filtrate, which represented a Pu level less than 0.2% of that applied, did not change significantly with treatment.

Fig. 6. Changes in soil respiration rate and solubility of applied plutonium with time of soil incubation (Wildung *et al.*, 1973, 1974). Inoculation of sterile soil with the incubated plutonium-treated nonsterile soil resulted in a factor of 2 increase in plutonium solubility after 4 days.

In an ancillary experiment, incubation was continued for 65 days until the CO₂ evolution rate reached a constant level. The Pu-containing sterile soil was then inoculated with the incubated Pu-treated nonsterile soil and the respiration rate and solubility of Pu in the inoculated soil measured for a period of 30 days. When the sterile soils were inoculated with nonsterile soil, CO₂ evolution increased at a much more rapid rate without a lag phase, and this was accompanied by a factor of 2 increase in water solubility (< 0.45 μ) of Pu after only 4 days of incubation, suggesting the development of a microbial population particularly capable of alteration of Pu solubility. Again, there was no change in the < 0.01 μ fraction which amounted to approximately 10% of the Pu present in the < 0.45 μ fraction.

At least under the conditions of this study, the evidence strongly suggested that the solubility of Pu in soil was influenced by the activity of the soil microflora. The potential mechanisms effecting the change in solubility include mechanisms (1) and (2) described above, i.e. indirectly, through the production of organic acids that may complex Pu or the alteration of the solution pH and/or Eh near the soil colloid (or Pu polymer) without measureable effects on the overall soil pH; or directly, through the transformation of Pu. Microbial effects may have occurred at the colloidal level since detectable increases in solubility did not occur in the < 0.01 μ filtrates. This appears to implicate the indirect mechanisms

since direct changes generally occur at the molecular level and these would be reflected in changes in Pu concentration in the $< 0.01 \mu$ filtrates provided polymerization did not occur during extraction. Of course, a combination of the mechanisms is possible, i.e., alteration of affinity for organic ligands through a change in valence state. If the mechanism of solubilization was indirect, the results might be applicable to other transuranic elements, e.g., from consideration of the aqueous chemistry, a reduction in pH would be expected to increase the solubility of the other transuranic elements as well as Pu.

Increased water solubility of Pu on incubation under optimum conditions for microbial activity may be expected to increase Pu uptake by plants provided the limiting factor is not discrimination at the root membrane. In order to determine if the increased solubility on incubation resulted in increased Pu uptake by plants, the incubated soils were planted to barley and cultured using a split-root technique which allowed measurement of the uptake, sites of deposition and chemical forms of Pu in plant shoots and roots (Wildung and Garland, 1974). The results were compared to the results of similar plant studies in which the soils had not been incubated.

Prior incubation, which in microbial studies was shown to increase the solubility of Pu in soil, increased Pu uptake by shoots compared to the unincubated controls (Table 3). The effect was greatly accentuated in the case of the soil-free roots and incubation increased the soil to plant concentration ratios by up to 37 times relative to the unincubated control, depending upon Pu soil concentration level. Thus, plant uptake measurements tend to verify measurements of increased Pu solubility in the $< 0.45 \mu$ fraction in the incubated soil. However, if, as previously discussed, Pu in particulate form (i.e., $> 0.01 \mu$) was not available to plants, and if increases in the concentration of Pu in the 0.01 to 0.45μ fraction resulting from microbial activity were not due to procedural artifacts arising from polymerization, the colloidal particles may be serving as a reservoir of plant-available Pu manifested only when the equilibrium concentration of Pu in solution is reduced by plant uptake.

Effect on the Soil Microflora

Soil microorganisms may be exposed to relatively high transuranic concentrations even when total transuranic soil concentration is low. Soil organisms may be expected to be present at highest levels in the immediate vicinity of soil colloids (Alexander, 1961) where, from the aqueous chemistry of the transuranics and on the basis of recent information on transuranic chemistry in soil (previous section), the transuranic elements are likely to be concentrated. It is therefore necessary to determine the toxicity of the transuranic elements to soil microorganisms, as microorganisms exhibiting resistance to the chemical effects of the transuranics may have the highest potential for participating in alteration of transuranic form. However, the transuranic series does not contain stable isotopes and organisms chemically resistant to these elements must exhibit a degree of radiation resistance which is dependent, in large part, upon the radiochemistry of the isotope. Resistance to the chemical effects of transuranics may occur

Table 3. Uptake of plutonium and americium in barley as a function of prior incubation in a Ritzville silt loam soil (unpublished).

Plant Component	Concentration Ratio*			
	Unincubated		Incubated**	
	Plutonium	Americium	Plutonium	Americium
Shoots	0.019	0.042	0.071	0.12
Roots (soil-free)	0.060	0.13	2.2	3.6

* $\mu\text{Ci Pu/g}$ oven-dry plant tissue per $\mu\text{Ci Pu/g}$ oven-dry soil. Initial concentration of ^{239}Pu and ^{241}Am were $1 \mu\text{Ci/g}$ and $0.03 \mu\text{Ci/g}$ respectively, on a dry weight basis.

**Soil was incubated 30 days after amendment with carbon and nitrogen to provide optimal microbial activity.

by three general mechanisms including (1) inability of the transuranics to produce a toxic effect on cell metabolism at the cytoplasmic or exocytoplasmic levels (2) inability of organisms to transport the transuranics or (3) ability of the organisms to convert transuranics, by the direct and indirect mechanisms discussed in a previous section, to a form that is either less capable of entering the cell or is not toxic to the cell. It is the latter mechanism which is likely most important in alteration of transuranic form in soil.

Effect on Microbial Types, Numbers and Activity. The effect of soil Pu concentration on the soil microflora has been measured as a function of changes in microbial types and numbers and soil respiration rate (Wildung *et al.*, 1973, 1974a). A noncalcareous Ritzville silt loam (pH 6.7) was amended with $^{239}\text{Pu}(\text{NO}_3)_4$ at levels of 0.05, 0.5 and $10 \mu\text{Ci/g}$ and with starch, N and water to provide optimal microbial activity. Subsamples of soil were periodically removed to determine the changes in types and numbers of soil microflora with time. During this period, soil respiration rate was monitored by continuous collection of soil-evolved CO_2 .

The growth curve of fungi (Fig. 7) was generally typical of the growth response for other classes of microorganisms. Total microbial numbers were compared at the end of logarithmic growth. The organisms generally reached this stage after 8 to 14 days of incubation. Growth rates were compared over the intervals of maximum microbial growth for each organism at each Pu concentration. The results are summarized in Table 4.

The Pu did not generally affect the rate of growth but decreased the total numbers of all classes of microorganisms at levels as low as $0.05 \mu\text{Ci/g}$ or $7 \mu\text{g/g}$. The fungi were the exception, differing from the controls only at

Fig. 7. Influence of plutonium concentration on the growth of fungi in soil (Wildung *et al.*, 1974a). Arrows denote time intervals at which growth rates and total numbers were compared with other microbial types (Table 4).

Table 4. Summary of the effects of plutonium at several soil concentration levels on the distribution of microorganisms in soil relative to controls (Wildung *et al.*, 1973, 1974).

Microbial Type	Effect (p < 0.05)* of Plutonium on					
	Growth Rate at Plutonium Concentrations (µCi/g) of			Total Numbers at Plutonium Concentrations (µCi/g) of		
	0.05	0.5	10.0	0.05	0.5	10.0
<u>Bacteria</u>						
Aerobic and Microaerophillic						
Nonspore Formers	0	0	0	+	+	+
Spore Formers	0	0	0	+	+	+
Anaerobic and Facultative Anaerobic						
Nonspore Formers	0	+	0	+	+	+
Spore Formers	0	0	0	+	+	+
<u>Fungi</u>	0	0	0	0	0	+
<u>Actinomycetes</u>	0	0	+	+	+	+

*A positive sign denotes a significant effect. A zero indicates that there was no significant effect.

a Pu concentration of 10 $\mu\text{Ci/g}$ or 144 $\mu\text{g/g}$. Thus, the Pu did not affect the maximum generation rate but rather affected the lag period or onset of the stationary phase, limiting microbial numbers.

The accumulative CO_2 curve generally corresponded to the growth curve of the fungi. In the case of the other classes of organisms, maximum logarithmic growth occurred before the rate of CO_2 evolution reached minimum levels. The rate of CO_2 evolution and cumulative CO_2 over the incubation period were significantly reduced only at the 144 $\mu\text{g/g}$ level of Pu amendment, although numbers of all classes of organisms except the fungi were depressed below this level (Table 4). This is in marked contrast to the results of studies with a number of other heavy metals (Drucker *et al.*, 1973) such as Ag and Hg, in which respiration rate was a sensitive measure of metal effect at levels as low as 1 $\mu\text{g/g}$ in soil. Differences in the effects of the metals may be related to differences in soil solubility as well as toxicity. It should also be noted, however, that the effect on respiration rate was dependent upon the magnitude of the soil respiration rate in Pu-treated soil relative to untreated controls, which, in turn, was dependent upon the initial level of microorganisms in soil. In soils exhibiting a higher CO_2 evolution rate, the reduction of respiration rate due to Pu amendment was more pronounced.

Studies of the toxicity of other transuranic elements to soil microflora have not been conducted.

Mechanism of Effect. To understand the long-term effects of microorganisms on transuranic form, it is important to distinguish, where possible, chemical and radiation effects of the transuranics on soil microorganisms. Pronounced initial chemical toxicity, may result in the development of special pathways of detoxification leading to alteration of transuranic form. The lack of chemical toxicity may imply chemical modifications of the transuranic elements through interaction with cell metabolites. In contrast, radiation resistance is associated with an enhanced ability to repair radiation damage to key macromolecules without development of new biochemical pathways leading to alteration of transuranic form. However, the possibilities for indirect alteration of transuranic form would be higher for a radiation resistant organism than for an organism that did not exhibit either radiation or chemical resistance since, due to competitive advantage, these resistant organisms may be expected to be present in larger numbers in the vicinity of transuranic colloids than less resistant organisms.

The effects of Pu on soil microorganisms may be due largely to radiation damage. Schneiderman *et al.* (1974) measured the effects of Pu form and solubility on soil metabolic activity and on the types, numbers, and resistance of soil fungi and actinomycetes in soil separately amended with ^{239}Pu (1 to 144 $\mu\text{g/g}$) and ^{238}Pu (0.6 $\mu\text{g/g}$) in soluble nitrate and DTPA complex forms, and with C, N, and water to provide optimal microbial activity. Subsamples of soil were removed over a 95-day aerobic incubation period to determine changes in numbers of fungi and actinomycetes and

relative water solubilities ($< 0.01 \mu$) of the Pu forms. Comparisons of soil fungal numbers in the presence of ^{238}Pu and ^{239}Pu at common radioactivity levels, but at different mass concentrations, indicated that Pu toxicity was due to radiation rather than chemical effects (Fig. 8). Solubility of Pu in soil influenced Pu toxicity to microorganisms with the more soluble Pu-DTPA forms resulting in greatest reductions in numbers. Similar studies have not been conducted with other transuranic elements.

Isolation of Resistant Organisms. Although much information is available regarding organic ligands in soil (previous section), an organometal complex has never been isolated intact from soils. A logical approach to the study of microbial transformations of the transuranic elements is to isolate, from soil, resistant organisms most likely to alter transuranic form, study the transformation *in vitro* and validate the results in the soil system using techniques specifically tailored to metabolites identified from the simpler *in vitro* systems.

Fig. 8. Effect of different isotopes of plutonium on survival of soil fungi (Schneiderman *et al.*, 1975).

Application of enrichment techniques to the isolation of Pu resistant fungi, which have been demonstrated (previous section) to be the most resistant class of microorganisms, and actinomycetes from soil using starch as a C source (Schneiderman *et al.*, 1974) resulted in the isolation of 14 fungal cultures and 13 cultures of actinomycetes distinct in colonial morphology. Of these, 7 of the actinomycetes and 5 of the fungal isolates were capable of growth at 100 µg/ml Pu as the soluble DTPA complex. There appeared to be a succession of actinomycetes types in the soil during incubation as indicated by the different colony morphologies obtained from enrichments after 4 and 25 days incubation. Although this phenomenon may have resulted from changes in the soil arising from the production of metabolites or chemical degradation products, it may also have resulted from a response to the presence of Pu. Only one actinomycete isolate was found which was common to enrichments from both incubation periods and this organism was present at all Pu concentrations in the media. In contrast, the fungal isolates exhibited 6 common morphological types regardless of incubation period.

Subsequent enrichment studies by R. A. Pelroy, Battelle-Northwest (1976) have resulted in the isolation of 30 distinct cultures of bacteria from soil. Of these, 11 were resistant to Pu at concentrations as high as 100 µg/ml. These studies also indicated that C source as well as soil Pu concentration will play a role in determining the types and numbers of Pu resistant microorganisms present in soil, providing presumptive evidence that microbial metabolites, which will differ with C source, may play a role in Pu resistance. This subject will be discussed in the next section. The presence of Pu resistant organisms is apparently related to factors that may be expected to vary with soil type and environmental conditions. Again, similar studies have not been conducted with other transuranic elements.

Microbial Transformations

Several means exist whereby microorganisms may transform trace metals in soil. These may be generalized to (1) direct mechanisms such as alteration in valence state or alkylation (2) indirect mechanisms such as interactions with normal metabolites or microbial alterations of the physicochemical environment and (3) cycling mechanisms such as uptake during cell growth and release on cell decomposition. In the latter case, any combination of indirect and direct methods of alteration may be operational. Although there have been no studies conducted to date which would allow the unequivocal separation of these mechanisms, studies have been conducted which demonstrate the alteration of Pu form *in vitro* by soil microorganisms and provide evidence for transformation of Pu.

Direct Transformations. The potential for direct transformation of the transuranic elements through alteration of valence state or alkylation is difficult to assess. Although, the transuranics have the potential for existing in aqueous solution in several valence states, information is not available to assess the role of soil microflora in direct alteration of valence. More information is available regarding the mechanism of metal alkylation.

Alkylation of metals involving the alkyl donor methyl cobalamine and other alkyl cobalamines has been clearly demonstrated for Hg, As, and Pt (Wood *et al.*, 1968; McBride and Wolfe, 1971; Taylor and Hanna, 1977). The methyl derivative of Hg may be present in significant quantities in soils (Beckert *et al.*, 1974). Wood (1974) suggested that methylated derivatives of Hg and As are important in governing their behavior in the environment. McBride (1977) also suggested that these reactions occur abiotically. The process of biochemical methylation of metals may be described as an overlap between the chemistry of methyl cobalamine (an intermediate in methane synthesis by anaerobic bacteria and methionine synthesis in aerobic bacteria) and the chemistry of the metals. In the case of the transuranics, particularly Pu, it is the complexity of the aqueous chemistry that has limited research into alkylation phenomena.

It is unknown whether an ionic species of Pu is capable of reacting *in vitro* with an alkyl cobalamine. Further, if a mechanism for biological alkylation of Pu, similar to the Hg, As, Pt alkylation reaction did exist, it would be of importance in influencing environmental behavior only if the alkylated molecule exhibited stability (Wood, 1974), i.e., a half-life in soils and sediments of hours rather than seconds. Considering the coordination chemistry of the actinides, Marks (1976) noted that U- and Th-C linkages are formed in organic solvents and the complexes are relatively stable thermally though highly sensitive to oxygen. Meaningful microbial studies await the development of an understanding of the chemical speciation of transuranics in aqueous solutions at environmental concentration levels.

Indirect Transformations. The potential for indirect transformation of the transuranic elements may be greater than for direct transformation. The potential for Pu interaction with microbial cells and metabolites has been demonstrated and many of the other transuranics form stable complexes with known microbial metabolites.

Beckert and Au (1976) demonstrated the uptake of ^{238}Pu , applied initially to malt agar in nitrate, citrate and dioxide forms, by a common soil fungus, *Aspergillus niger*. Using a specialized spore collection method, the Pu was shown to be present in the fruiting bodies. Subsequent washing to remove external contamination indicated that the major portion of the ^{238}Pu was incorporated into the spores. Similar results were obtained in preliminary studies with U, Th, and Po in mine tailings. The order of uptake was related to pH and expected solubility of the Pu added with Pu in the initially soluble nitrate and citrate forms exhibiting a factor of 2 to 3 greater uptake than the dioxide (Table 5). Availability to microorganisms of the Pu in citrate and nitrate might be expected to be considerably higher than the oxide from solubility considerations at the pCi/ml level. The relatively high microbial availability of Pu as the oxide is highly significant, and further studies are warranted to determine the mechanisms of solubilization and uptake and the significance of microorganisms in recycling processes.

There is a growing literature on organic acids and bases, capable of complexing heavy elements, which are produced directly or by secondary

Table 5. Plutonium transport to the spores of *Aspergillus niger* (Beckert and Au, 1976).

Chemical Form	pH	Specific Activity of Culture Medium (dry), pCi/g	Specific Activity of Spores* pCi/g	Transport Factor
Dioxide	2.5	3.34×10^3	4.7	1.4×10^{-3}
		3.34×10^4	32	0.96×10^{-3}
		3.34×10^5	383	1.1×10^{-3}
	5.5	3.39×10^3	9.4	3.4×10^{-3}
		3.39×10^4	101.3	2.98×10^{-3}
		3.39×10^5	976	2.88×10^{-3}
Nitrate	2.5	1.67×10^3	3.8	2.3×10^{-3}
		3.34×10^3	11	3.3×10^{-3}
		6.69×10^3	15	2.24×10^{-3}
	5.5	1.69×10^3	8.3	4.9×10^{-3}
		3.38×10^3	26	7.7×10^{-3}
		6.76×10^3	23	3.4×10^{-3}
Citrate	2.5	1.67×10^3	7.1	4.3×10^{-3}
		3.34×10^3	11	3.3×10^{-3}
		6.69×10^3	18.5	2.8×10^{-3}
	5.5	1.69×10^3	6.7	4.0×10^{-3}
		3.38×10^3	26	7.7×10^{-3}
		6.76×10^3	56	8.3×10^{-3}

*Arithmetic mean of 3 samples.

syntheses by a variety of microorganisms. Their concentration and form in soils will be dependent upon the environmental factors influencing microbial metabolism, such as C source and their residence time will be dependent upon subsequent chemical and microbiological stability.

In preliminary (unpublished) studies by the authors and others, mixed cultures of soil organisms, isolated from soil on the basis of C requirements and Pu resistance, were analyzed as to their ability to transport Pu into cells and to alter Pu form in the cellular and exocellular media. In addition, an experiment was conducted to distinguish complexation reactions resulting from Pu interactions with metabolites arising from normal metabolic processes and Pu interactions with metabolites arising from Pu resistance. To make this distinction, Pu was added at the stationary growth phase of soil microorganisms isolated from soil in the absence of

Pu and the transport and complexation were compared to microbial cultures isolated from Pu-containing soil and grown in the presence of Pu.

After growth for 96 hr, the cultures were separated into cellular and exocellular fractions. The cell fraction was, in turn, homogenized into intracellular soluble and cell debris fractions. The results of studies in which Pu was added at the stationary growth phase of cultures of fungi or bacteria grown on mixed organic acids or sugars are summarized in Table 6. These cultures, selected only on the basis of their ability to grow on either of two C sources, differed to a first approximation in their interactions with Pu. In general, the majority of Pu was associated with the exocellular fraction, but significant quantities were insoluble and associated with the cell wall and membrane fractions. However, the distribution of Pu between fractions was dependent upon microorganism type and C source. In the case of fungi, the exocellular fraction of organisms grown on the organic acid C source contained less Pu than when mixed sugars were utilized as a C source. The reverse of this relationship occurred with the bacteria.

Differences in Pu distribution as a function of C source used in enrichment were also found in cultures grown in the presence of Pu throughout incubation (Table 7). The fungal cultures grown on mixed organic acids exhibited larger concentrations of Pu both in the exocellular fraction and bound to the cell debris fraction; the cultures grown on mixed sugars contained a higher fraction of added Pu in the intracellular soluble fraction. In the bacterial cultures, the situation was somewhat different, in that higher concentrations of Pu occurred in the exocellular fraction of the culture grown in organic acids; less Pu was associated with the cell debris fraction as compared to cells grown on sugars.

In general, the continuous presence of Pu during growth did not have pronounced effects on the distribution of Pu in the cultures (compare Tables 6 and 7). Rather, the metabolic properties of the mixed cultures as determined by C source appeared to be the major factor resulting in the observed differences. Under both sets of culture conditions, there was a high concentration of Pu bound to cell wall and membrane fractions and thus insoluble. As these materials are degraded by lytic enzymes, e.g., proteases and chitinases, soluble Pu compounds may be formed.

Preliminary characterization, using gel permeation chromatography, of the mixed culture of fungi isolated from soil and grown in sugars indicated that Pu form was altered during fungal growth (Fig. 9). The exocellular and intracellular soluble fractions obtained from organisms exposed to Pu in a single exposure and in continuous exposure contained a majority of Pu in compounds of molecular size greater than Pu-DTPA, which was used as the source of soluble Pu. Furthermore, there appeared to be a difference in Pu chemical form comparing Pu complexes formed on simple interaction of Pu with metabolites (single exposure) and Pu complexes formed on interaction after continuous Pu exposure of the culture. This suggests either that the culture grown in the continuous presence of Pu contained metabolites capable of interacting with Pu which were different chemically from those

Table 6. Distribution of plutonium in mixed microbial cultures exposed to plutonium at stationary growth phase and grown on different carbon sources (unpublished).

Fraction	Distribution* of Plutonium (%) in Cultures			
	Fungi		Bacteria	
	Mixed Sugars	Organic Acids	Mixed Sugars	Organic Acids
Exocellular Medium	75	42	39	89
Intracellular Soluble	0.49	0.068	8.3	2
Cell Debris	10	42	28	8.7

*Cultures were not replicated. Analytical precision was $< \pm 10\%$ (1σ).

Table 7. Distribution of plutonium in mixed microbial cultures continuously exposed to plutonium and grown on different carbon sources (unpublished).

Fraction	Distribution* of Plutonium (%) in Cultures			
	Fungi		Bacteria	
	Mixed Sugars	Organic Acids	Mixed Sugars	Organic Acids
Exocellular Medium	29	54	46	88
Intracellular Soluble	4.2	0.24	2.7	4
Cell Debris	29	39	31	3.5

*Cultures were not replicated. Analytical precision was $< \pm 10\%$ (1σ).

Fig. 9. Separation of soluble plutonium complexes in microbial cultures by gel permeation chromatography (unpublished).

produced by the culture grown in the absence of Pu or that the culture grown in the presence of Pu contained different organisms capable of adaptive response to the element leading to the synthesis of compounds relatively specific to detoxification of Pu.

Further chemical characterization using thin-layer chromatography and electrophoresis verified differences in Pu form. Several solvents of different polarities and pH values were employed to provide a range of

chemical conditions for separation. Solvent systems included: A, butanol-pyridine, a system used in resolution of amino acids; D, pentanol-formic acid, a system used in separation of sugars and sugar acids; and G, water-acetic acid, a solvent utilized in resolving keto-acids and sugars. These systems were used to resolve Pu as Pu-DTPA, and Pu in the soluble exocellular and soluble intracellular fractions of the above cultures (Fig. 10). Thin-layer chromatography using solvent A indicated that the exocellular fraction contained one component of chromatographic mobility different than the added Pu-DTPA but the complex remained present in detectable quantities. The intracellular soluble fraction contained a component of lesser chromatographic mobility but there was no evidence of Pu-DTPA. Solvents D and G did not provide good resolution. Solvent D did not mobilize Pu-DTPA or other possible complexes, Solvent G mobilized Pu-DTPA and indicated the presence of immobile Pu components in the exocellular and intracellular fractions but these were not resolved.

Application of thin-layer electrophoresis (pH 6.6, pyridine-acetate buffer system; cellulose support) indicated the presence (Fig. 11) of a relatively large amount of material of greater negative charge than Pu-DTPA in the exocellular fraction along with Pu-DTPA. The Pu ligands in the intracellular fraction were either neutral in charge in this buffer system or were of a molecular size too large to migrate under the conditions of electrophoresis. Similar alterations of Pu form by a single Pu-resistant fungus exposed continuously to Pu during growth have also been reported (Robinson *et al.*, 1977).

Several phenomena may have been responsible for the observed changes in chemical form of Pu. The organism may have synthesized compounds which either bind Pu-DTPA or bind Pu more tightly than DTPA, thereby successfully competing for Pu in the presence of DTPA. Alternatively, the organism may degrade or modify the DTPA moiety allowing Pu transfer to ligands arising from microbial synthesis and degradation.

The number of known compounds with the potential to bind Pu more strongly than DTPA appears to be quite limited although hydroxamate derivatives, (Emergy, 1974) catechol derivatives, (Tait, 1975) and tetrapyrrole ring systems (Balker, 1969) may exhibit this property. If modification of the Pu-DTPA occurred prior to ligand transfer, then a myriad of microbially-produced compounds, e.g., phenolic acids, peptides, and carboxylic acids have potential for binding Pu (see previous section; also Alexander, 1971). In either case, the Pu was not in the form initially added. Thus, applications of gel permeation chromatography, thin-layer chromatography and thin-layer electrophoresis indicate that soil microorganisms were capable, through simple expression of metabolic potential, of changing the chemical form of Pu-DTPA with the resulting formation of Pu complexes exhibiting a range in chemical properties. Differences in Pu distribution in microbial systems and in Pu form resulted from both simple interaction with metabolites and perhaps, more specific processes. These differences were dependent on organism type, metabolism and Pu resistance.

Fig. 10. Thin-layer chromatographic behavior in three solvent systems of plutonium complexes separated by gel permeation chromatography (unpublished).

Fig. 11. Thin-layer electrophoretic behavior of plutonium separated by gel permeation chromatography (unpublished).

Although published information on transformation of transuranic elements other than Pu is limited, it is likely that transformations similar to Pu will occur. The extent of these transformations will be dependent upon the solubility of the element, its availability to microorganisms, its toxicity to microorganisms and its potential for complexation. While microbial interactions remain to be elucidated, the solubility and potential for complexation may be preliminarily assessed from known chemistry (Table 8). It is evident that the transuranic elements form DTPA complexes with stabilities similar in magnitude to Pu-DTPA over environmental pH ranges. It may be concluded that complexation with organic ligands produced by soil microflora is highly probable and investigations to identify and characterize the indirect processes and the ligands responsible for complexation of Pu in soil are equally applicable to other transuranic elements.

Cycling During Decomposition. A final process whereby the soil microflora may play a role in transformation of the transuranic elements involves the biological uptake (plants, microorganisms) of the elements and subsequent release on decomposition. Several studies have demonstrated plant uptake of Pu and Am and incorporation into above ground tissue. These tissues, deposited on soil either through litter fall or agricultural incorporation of crop residues, will be subject to microbial decomposition. Furthermore, recent studies (Wildung and Garland, 1974) have indicated that barley roots (uncontaminated with soil particles), contained 3 to 8 times more Pu

Table 8. Stability of DTPA complexes with the transuranic elements (Hafez, 1969).

Complex*	Stability Constant	Stable pH Range
<u>Neptunium</u>		
Np(III)	—**	—**
Np(IV) DTPA	10^{24}	0.5 - 5.8
[Np(IV)] ₂ DTPA ₃	10^{20}	>5.8
<u>Plutonium</u>		
Pu(IV) DTPA	10^{24}	1.0 - 5.8
[Pu(IV)] ₂ DTPA ₃	10^{18}	5.8 - 8.5
Pu(IV) DTPA ₂	10^{14}	>8.5
<u>Americium</u>		
[Am(III)] DTPA	10^{20}	1.8 - 6
Am(III) DTPA	10^{23}	>6

*Curium may be expected to form complexes of stabilities similar to americium.

**Unstable in oxygenated solutions.

than the shoots. The roots of plants are in intimate contact with the soil and may be expected to decompose rapidly (weeks) under appropriate conditions of temperature and moisture, even in arid regions (Wildung *et al.*, 1975). Relatedly, microorganisms, due to their distribution in soil and large absorptive surface, compete efficiently with plants for ions in soil (Alexander, 1961). Studies described in a previous section demonstrated the association of Pu with microbial cells. Growth of microbial cells, a significant portion of the soil biomass, may therefore represent an important mechanism for biological incorporation of the transuranic elements. Decomposition of microbial cells generally proceeds at a more rapid rate than plant tissues.

Little is known of the form of the transuranics in plant or microbial tissues; of the form, rate, and extent of the transuranics released on decomposition of these tissues; or of the chemical reactions governing transuranic solubility after decomposition. However, considering the known products of microbial metabolism of organic substances, including a number of strong complexing agents and the susceptibility of a number of the transuranic elements to complexation, it may be concluded that the transuranics, initially immobilized through biological uptake, may be at least as soluble and perhaps more soluble on decomposition.

In preliminary studies (Wildung and Garland, unpublished), Pu-amended soil containing largely undecomposed roots from a previous barley crop was leached with water and Pu solubility compared to a fallow soil containing Pu at similar levels. The results indicated that soluble Pu was initially immobilized by incorporation into roots, decreasing by a factor of 10 after root growth. Root decomposition studies are in progress. Previously observed (Romney *et al.*, 1970) increases in Pu uptake from soils by plants with increased time, generally attributed to increased root development, may have been due to increased availability through a recycling process on decomposition of plant roots. The importance of the process will be dependent upon transuranic availability to different plants and microorganisms, the turnover rate of this tissue in soils under different conditions and the stability, chemistry and biological availability of transuranic metabolites. Until this information is developed, the long-term effects of recycling processes will remain unknown.

MICROBIAL INFLUENCE ON THE AVAILABILITY AND FORM OF TRANSURANIC ELEMENTS IN PLANTS AND ANIMALS

Plants

The results of investigations to physicochemically characterize the mobile forms of Pu in soils (< 0.1% of total Pu) suggested that mobile Pu was largely particulate (Garland and Wildung, 1977; previous section). The nonparticulate or soluble fraction was present in insufficient quantities, at any single point in time, to separate from soil and chemically characterize using present methods. However, Pu must pass through the solution phase to be available to plants and the quantities taken up by plants exceeded those present in the soil solution. Thus, in plant studies, Pu was being resupplied to solution from the solid phase. Since Pu(IV) ions would hydrolyze in solution precipitating on soil surfaces, it is likely the plant available fraction was stabilized in soil solution by complexation with inorganic or organic ligands and/or was present in a different, more soluble, valence state.

Circumstantial evidence suggested (Wildung and Garland, 1975; previous section) that inorganic or organic ligands present in limited concentrations in soil stabilized Pu in soil solution. Furthermore, dissolution of Pu from the solid phase has been shown to be accelerated by complexing agents (Bondietti *et al.*, 1975; previous section). Organic ligands, which form the most stable Pu complexes, are generally derived from microbial processes in soil and previous studies (Wildung *et al.*, 1973; previous section) have shown that Pu solubility in soil and availability and distribution in plants are influenced by microbial activity, although mechanisms other than complexation may have been responsible. However, a synthetic ligand (DTPA) was shown to maintain Pu essentially soluble in soil for extended periods. Thus, it is likely that organic ligands of microbial origin, which differ markedly in their form, concentration and stabilizing Pu in solution for subsequent uptake by plants. A key question is whether the relatively low uptake exhibited by plants from Pu-amended soils (reported concentration ratios of 10^{-2} to 10^{-7} , ERDA, 1975)

is due to limited solubility in soil as a result of sorption on particulate surfaces or to discrimination by the plant. If discrimination is not at the plant level, then the potential role of the soil microbiota in increasing Pu availability from the solid phase (oxide particles from the nuclear fuel cycle or soil particles) becomes very important in influencing the long-term availability of Pu to plants.

The role played by organic ligands in facilitating plant uptake of ions, particularly hydrolyzable ions, has long been a subject of controversy. The question has been whether the complex simply serves as a means of delivering the ion to the root membrane, supplying the ion to the root by dissociation, or is taken up intact by the plant. Perhaps the most meticulous investigation of this phenomena has been the work of Tiffin and coworkers (summarized by Tiffin 1972, 1977). At least in the case of Fe the evidence, derived in part from direct analyses of xylem exudates, strongly supports the role of complexors in increasing uptake by plants but indicates that the complex serves mainly to deliver the metal to the root membrane and the ligand is not taken up by the plant stoichiometrically with the metal. Recently, Malzer and Barber (1976) concluded that less than 16% of several Ca and Sr chelating ligands was removed from nutrient solution by corn (*Zea mays*), whereas over 90% of the Ca and Sr was taken up by the plant. Both studies employed several physicochemical as well as radiochemical measures of chelate concentration in aqueous solution. It should be noted, however, that detailed, exhaustive procedures are required to purify metal chelates, particularly in the case of the transuranic elements (Swanson *et al.*, 1975). Without this effort, it is possible that studies employing only ^{14}C analysis as a measure of chelate concentrations in plants may over estimate uptake since low molecular impurities containing ^{14}C might account for the ^{14}C present in the plant, particularly where chelate uptake rates are low.

To aid in distinguishing soil sorption and plant root discrimination and to evaluate the role of complexes in the plant uptake of Pu, hydroponically grown soybeans (*Glycine max*) were placed in μM ^{238}Pu -DTPA solutions and permitted to accumulate Pu for up to 48 hr (Garland, Cataldo and Wildung, unpublished). Concentration ratios ($\mu\text{Ci/g}$ plant per $\mu\text{Ci/ml}$ nutrient solution) for shoot tissues were found to be 6×10^{-3} and 0.3 after 1 and 24 hr, respectively. This would suggest that plants do possess the potential to effectively accumulate Pu with much of the apparent discrimination in soil-plant studies resulting from the effect of soil sorption in reducing the quantity of Pu available to the plant. In a preliminary effort to determine the form of mobile Pu in the plant, several plants were decapitated, xylem exudates collected in 1 ml aliquots at intervals over a 48 hr period, and the solutions subjected to thin-layer electrophoresis to resolve Pu-containing components. The electrophoretic mobilities of Pu in the medium, in the control exudate spiked with $\text{Pu}(\text{NO}_3)_4$, and in the exudate collected from decapitated plants grown in 0.1 μM Pu-DTPA solution over a 24 hr period are illustrated in Figure 12. The Pu-containing components in the exudate from plants grown in the absence

Fig. 12. Thin-layer electrophoretic behavior of plutonium in soybean xylem exudates (Wildung *et al.*, 1977).

of Pu-DTPA, but spiked with Pu(IV) or Pu(VI), indicated the presence of ligands capable of binding Pu and forming stable complexes. Similarly, several anionic and cationic Pu complexes were evident in exudates from plants grown in the presence of Pu-DTPA. A major anionic component with an electrophoretic mobility similar to Pu-DTPA reached maximum concentration in the second aliquot after decapitation then decreased in concentration with time. Application of several solvent systems to separation subsequently indicated that this component was not the Pu-DTPA complex supplied in the growth media. These data suggest that plants do possess the ability to effectively accumulate soluble Pu and transport the Pu to shoots in one or more organic complexes. Furthermore, from the high concentration ratios for Pu supplied as Pu-DTPA and the apparent lack of uptake of the complex, it may be concluded that much of the apparent discrimination in soil-plant studies results from the effect of soil sorption in reducing the quantity of soluble Pu available to the plant. The form of Pu in alfalfa fed to animals was also characterized, providing insight into observed differences in gastrointestinal absorptions.

Animals

The accepted value for the gastrointestinal transfer ratio of Pu from food-to-man is 3×10^{-5} (WASH-1535, 1974). This value is based on the gastrointestinal absorption of inorganic Pu(IV) in animals, administered by gavage (Weeks *et al.*, 1956), and the apparent assumption that food-stuffs would contain primarily inorganic Pu(IV). However, the study demonstrated that gavaging Pu(VI) and complexed forms of Pu(IV) resulted in higher absorption rates e.g., 500 times greater for Pu(VI). Until recently, it was not possible to test the assumptions because plant tissues with Pu concentrations sufficient to measure uptake were not available. However, studies with alfalfa indicated that tissues containing up to 400,000 d/m/g could be obtained under a sequential harvesting regime (Garland, Cataldo and Wildung, unpublished). These tissues, used in conjunction with a sensitive analytical method (Wessman *et al.*, 1971) for measurement of Pu at levels of < 1 d/m, allowed preliminary investigations of the availability to animals of Pu in alfalfa tissues grown on Pu-containing soil. In these studies gut absorption of Pu gavaged in inorganic solution and Pu fed in alfalfa tissue was compared for rats (nonherbivorous) and guinea pigs (herbivorous). In both rats and guinea pigs absorption through the gastrointestinal tract of Pu incorporated in alfalfa tissue was greater (7.7 to 53 times) than by gavaging inorganic Pu(IV) solutions (Sullivan and Garland, 1977). Uptake by rats fed alfalfa containing the Pu exceeded that from gavaging Pu(IV) nitrate or Pu(IV) citrate, but was less than that resulting from gavaging Pu(VI) nitrate (Table 9). Uptake was higher when stems and leaves were fed than when leaves only were fed. Several variables, including animal species, duration of feeding, and types of plant tissues fed were evaluated, but due to the small number of animals which could be utilized, the limited quantity of tissues and variability, further studies are required to evaluate the statistical significance of individual variables.

Table 9. Gastrointestinal transfer of plutonium in rats as a function of plutonium source.

Source	Fraction of Administered Dose in Bone Plus Liver*	Literature Cited
Pu(IV) Nitrate	0.000012	Weeks <i>et al.</i> , 1956
Pu(VI) Nitrate	0.0175	Weeks <i>et al.</i> , 1956
Pu(IV) Citrate	0.00026	Weeks <i>et al.</i> , 1956
Pu in Alfalfa	0.00086	Sullivan and Garland, 1977

*Analyzed 4 days after a single administration.

Preliminary chemical characterization (Garland, McFadden and Wildung, unpublished) of the form of Pu present in the alfalfa tissue fed to the rodents indicated that the Pu was more soluble in stems than in leaves, perhaps by a factor of 2. Thus, the differences in the gastrointestinal absorption between leaves and stems appeared to reflect differences in relative solubility. The results of preliminary studies indicated that (1) > 90% of the Pu in either stems or leaves was not ionic Pu(IV) and (2) up to 45% (stems) of the soluble Pu was strongly complexed Pu(IV) with the remaining Pu in the Pu(V) or Pu(VI) valence state, or that all of the Pu was present as Pu(V) or Pu(VI), with reduction occurring to Pu(IV) during chemical characterization. These studies are continuing in an effort to evaluate the importance of the ingestion pathway for transuranic uptake by man and to determine the factors that influence the rate and extent of biological uptake.

RECOMMENDATIONS FOR FUTURE RESEARCH

A broad range of soil-plant concentration ratios (10^{-2} to 10^{-7}) has been reported for the transuranic elements (ERDA, 1975). If this variability is attributed to the usual unexplained, experimental and environmental parameters, the information would be of little use in predicting the long-term behavior of these elements. However, a closer examination shows that although the ratios ranged over many orders of magnitude, they encompassed, and were often dependent upon, different source terms, soil types, plant species, plant components, climatic conditions, extent of foliar contamination and kinetic factors. Consideration of these variables and use of rapidly accumulating information on transuranic behavior at the chemical, microbiological and physiological levels should allow reduction of the level of unexplained variability by many orders of magnitude and provide a valuable basis for prediction of transuranic behavior over a broad range of conditions.

There is a need to continue to develop a basic understanding of the processes influencing the fate of transuranics in soils and plants over a range of soil types and experimental conditions representing those likely to be encountered in the environment. Research should emphasize those elements that are (1) expected to be present in soil in the highest concentration, (2) most soluble in soil, (3) mobile in the plant, (4) transported to edible tissues, and (5) most toxic to plants and/or animals. Source terms receiving future emphasis should be those likely to result from the nuclear fuel cycle. From previous discussions of the soil chemistry of the transuranic elements, which illustrate the marked differences in soil behavior and plant availability resulting from different source terms, sources due to fallout and local nuclear testing, useful for initial approximations, cannot be taken as fully representative for validation purposes.

Research should emphasize determination of (1) source term physicochemical characteristics (composition, mineralogy, particle size, valence) as a function of source (fallout, reactor operation, reprocessing, burial leachates), (2) physical redistribution processes (erosion, resuspension), (3) biological redistribution processes (litter incorporation, root decomposition), (4) processes of solubilization and/or transformation of refractory materials entering soil and the factors influencing the form and equilibria between relatively insoluble forms and soluble chemical species, (soil type, soil solution composition, pH, temperature, redox conditions, diffusion and microbial action), (5) the capacity of representative plant species to assimilate soluble chemical species, plant alteration of chemical form and translocation to edible plant components as a function of plant growth stage, form and concentration in soil and the presence of competitive ions in the soil solution, and (6) the nature and extent of the above processes on a regional basis, as influenced by soil, plant and climatic factors and land use practices. This information is currently being accumulated in laboratory studies and initial investigations are underway in several geographic regions to selectively validate the findings in the field.

The role of the soil microflora must be viewed as only one contributory factor among a number of highly important physicochemical and biological phenomena influencing the overall behavior of the transuranic elements in terrestrial environments. However, evidence is increasing that organic ligands resulting from microbial activity will play an important part in influencing the behavior and plant availability of hydrolyzable species, such as Pu, in soils. Future studies in this area should involve a systematic investigation of the major classes of soil organisms exhibiting highest transuranic resistance and representing different metabolic types, determination of their ability to alter transuranic form in soil, and evaluation of the soil and environmental factors influencing the rate and extent of alteration. In view of the relatively high concentrations of transuranics associated with roots and other organic components in soil, particular emphasis should be placed on determining the role of the microflora in recycling and redistribution processes. It is possible that

microbial processes responsible for alteration of metal form may function in a like manner for metals exhibiting similar chemical properties, particularly for organisms exhibiting cross-resistance to these metals. Thus, model systems may be available for those organisms likely to be most responsible for alteration of metal form in soil over the long-term, i.e., those organisms capable of growth and reproduction at higher metal concentrations in the immediate vicinity of refractory oxides or soil colloids with surface deposits of the transuranics.

A key integrating factor, in all studies of the transuranic elements and in interpretation of environmental phenomena, is a knowledge of the chemical form of the element. With this information, studies conducted under broadly different conditions in various substrates and biological media can be compared and toxicological interpretations made on a common basis.

Investigations which are currently underway in several institutions across the country should ultimately provide a realistic evaluation of the role of microbial processes in influencing the long-term behavior of the transuranic elements in soil. Furthermore, the studies should provide a basis for evaluation of the availability of transuranic metabolites to plants and insight into the potential for entrance of these elements into food-stuffs for a broad geographical region over the long-term.

LITERATURE CITED

1. Alexander, M. 1961. *Introduction to Microbiology*. John Wiley and Sons, Inc., New York, NY.
2. Alexander, M. 1971. "Biochemical Ecology of Microorganisms". *Annual Review of Microbiology* 25:361-392.
3. Balkar, E. W. 1969. "Porphyrins". In: *Organic Geochemistry*. G. Eglinton and M. T. Murphy (Ed.). Springer-Verlag, New York, NY. pp. 464-497.
4. Beckert, W. F., and F. H. F. Au. 1976. "Plutonium Uptake by a Soil Fungus and Transport to its Spores". In: *Transuranium Nuclides in the Environment*. IAEA SM 199/72.
5. Beckert, W. F., A. A. Moghissi, F. H. F. Au, E. W. Bretthauer, and J. C. McFarlane. 1974. "Formation of Methylmercury in a Terrestrial Environment". *Nature* 249:674.
6. Bondietti, E. A. 1974. "Adsorption of Plutonium (IV) and Thorium (IV) by Soil Colloids." In: *Agronomy Abstracts*. ASA, SSSA and CSSA Annual Meetings, November 10-15, 1974. Chicago, IL. p. 23.
7. Bondietti, E. A., S. A. Reynolds, and M. H. Shanks. 1975. "Interaction of Plutonium with Complexion of Substances in Soils and Natural Waters. In: *Transuranium Nuclides in the Environment*, IAEA SM 199/51.
8. Bondietti, E. A., and S. A. Reynolds. 1976. "Field and Laboratory Observations on Plutonium Oxidation States". In: *Proceedings of an Actinide Sediment Reactions Working Meeting*, Seattle, WA, February 10-11, 1976.
9. Bremner, J. M. 1965. "Organic Nitrogen in Soils". In: *Soil Nitrogen*. W. V. Bartholmew and F. E. Clark (Ed.). Am. Soc. of Agron. Madison, WI. pp. 93-149.
10. Bremner, J. M. 1967. "Nitrogenous Compounds". In: *Soil Biochemistry*. A. D. McLaren and G. H. Peterson (Ed.). Marcel Dekker, New York, NY. pp. 19-66.
11. Broadbent, F. E., and G. R. Bradford. 1952. "Cation-exchange Groupings in the Soil Organic Fraction". *Soil Sci.* 74:47-457.
12. Burney, G. A., and R. M. Harbour. 1974. *Radiochemistry of Neptunium*. NAS-NS-3060. NTIS, Springfield, VA.
13. Cleveland, J. M. 1970. *The Chemistry of Plutonium*. Gordon and Breach Science Publishers, New York, NY.

14. Drucker, H., R. E. Wildung, T. R. Garland, and M. P. Fujihara. 1973. "Influence of Seventeen Metals on Microbial Population and Metabolism in Soil". In: *Agronomy Abstracts*. ASA, SSSA and CSSA Annual Meetings. November 11-16, 1973. Las Vegas, NV. p. 90.
15. Emergy, T. 1974. "A Model for Carrier-Mediated Iron Transport". *Biochemica et Biophysica Acta*. 363:19-225.
16. ERDA. 1975. Environmental Research for Transuranic Elements. In: *Proceedings of the Workshop on Environmental Research for Transuranic Elements*. November 12-14, 1975, Seattle, Washington, NTIS, Springfield, VA.
17. Felbeck, G. T., Jr. 1965. "Structural Chemistry of Soil Humic Substances". *Adv. Agron.* 17:27-368.
18. Garland, T. R., and R. E. Wildung. 1977. "Physiochemical Characterization of Mobile Plutonium Species in Soils". In: *Biological Implications of Metal in the Environment*. H. Drucker and R. E. Wildung (Ed.). ERDA Symposium Series. Fifteenth Annual Hanford Life Sciences Symposium. September 29-October 1, 1975. Richland, WA. (In Press).
19. Garland, T. R., R. E. Wildung, and R. C. Routson. 1976. "The Chemistry of Plutonium in Soils. I. Plutonium Solubility." In: *Pacific Northwest Laboratory Annual Report for 1975*. BNWL-2000, pt. 2. Battelle-Northwest, Richland, WA. pp. 23-26.
20. Geering, H. R., and J. F. Hodgson. 1969. "Micronutrient Cation Complexes in Soil Solution: III. Characterization of Soil Solution Ligands and their Complexes with Zn^{2+} and Cu^{2+} ". *Soil Sci. Soc. Am. Proc.* 33:54-59.
21. Greenland, D. J. 1965. "Interactions between Clays and Organic Compounds in Soils. Part I. Mechanisms of Interaction between Clays and Defined Organic Compounds". *Soils Fert.* 28:415-425.
22. Hafez, M. B. 1969. *Spectrophotometric Study of the Complexes of Cerium and Uranides with DTPA Acid*. UCRL Trans.-10366. Lawrence Livermore Laboratory, Livermore, CA.
23. Hodgson, J. F. 1963. "Chemistry of the Micronutrient Elements in Soils". *Adv. Agron.* 15:119-159.
24. Katz, J. J. and G. T. Seaborg. 1957. *The Chemistry of the Actinide Elements*. Wiley and Sons, New York, NY.
25. Keeney, D. R. and R. E. Wildung. 1977. "Chemical Properties of Soils". In: *Soils for Managment and Utilization of Organic Wastes and Waste Waters*. L. Elliott and F. J. Stevenson (Ed.) American Society of Monograph Series. (In Press).

26. Kononova, M. M. 1966. *Soil Organic Matter*. Pergamon Press, New York, NY.
27. Lahav, N. and M. Hochberg. 1976. "A Simple Technique for Characterizing the Stability of Metal Chelates in the Soil". *Soil Science* 121:58-59.
28. Lindsay, W. L. 1972. "Inorganic Phase Equilibria of Micronutrients in Soils". In: *Micronutrients in Agriculture*. J. J. Mortvedt, P. M. Giordano and W. L. Lindsay (Ed.). Soil Sci. Soc. Am., Madison, WI. pp. 41-57.
29. Lloyd, M. H., and R. G. Haire. 1973. In: *Proceedings of the XXIVth IU PAC Congress, September*. Hamburg, F. R. Germany. CONF 730927-2 (ORNL).
30. Malzer, G. L., and S. A. Barber. 1976. "Calcium and Strontium Absorption by Corn Roots in the Presence of Chelates." *Soil Sci. Soc. Am. J.* 40:727-731.
31. Marks, T. J. 1976. "Actinide Organometallic Chemistry". *Accounts of Chemical Research* 9(6):223-229.
32. McBride, B. L. 1977. "Biochemistry of Metal Transformation by Microbes". In: *Biological Implications of Metals in the Environment*. H. Drucker and R. E. Wildung (Ed.). ERDA Symposium Series. Fifteenth Annual Hanford Life Sciences Symposium. September 29 - October 1, 1975. Richland, WA. (In Press).
33. McBride, R. L., and R. S. Wolfe. 1971. "Biosynthesis of Dimethylarsine by *Nethanobacterim*". *Biochemistry* 10:4312-4317.
34. Mitchell, R. L. 1964. "Trace Elements in Soils". In: *Chemistry of the Soil*. F. E. Bear (Ed.). Reinhold Publishing Corp., New York, NY. pp. 320-368.
35. Mitchell, R. L. 1972. "Trace Elements in Soils and Factors that Affect their Availability". *Geol. Soc. Am. Bull.* 83:1069-1076.
36. Mortensen, J. L. 1963. "Complexing of Metals by Soil Organic Matter". *Soil Sci. Soc. Am. Proc.* 27: 179-186.
37. Norvell, W. A. 1972. "Equilibria of Metal Chelates in Soil Solutions". In: *Micronutrients in Agriculture*. J. J. Mortvedt, P. M. Giordano, and W. L. Lindsay (Ed.). Soil Sci. Soc. Am., Madison, WI. pp. 115-138.
38. Ockenden, D. W., and G. A. Welch. 1956. "The Preparation and Properties of Some Plutonium Compounds. Part V. Colloidal Quadrivalent Plutonium." *J. Chem. Soc.* 653: 3358.

39. Robinson, A. V., G. S. Schneiderman, R. E. Wildung, and H. Drucker. 1977. "Microbial Transformation of Plutonium". In: *Biological Implications of Metals in the Environment*. H. Drucker and R. E. Wildung (Ed.). ERDA Symposium Series. Fifteenth Annual Hanford Life Sciences Symposium. September 29 - October 1, 1975. Richland, WA. (In Press).
40. Romney, E. M., H. M. Mock, and K. H. Larson. 1970. "Persistence of Plutonium in Soil, Plants, and Small Mammals". *Health Physics* 18:487-491.
41. Routson, R. C., and R. E. Wildung. 1969. "Ultimate Disposal of Wastes to Soil". In: *Water-1969*. L. Cecil (Ed.). Chem. Eng. Progr. Symp. Ser. Am. Inst. Chem. Eng., New York, NY. pp. 19-25.
42. Routson, R. C., G. Jansen, and A. V. Robinson. 1975. *Sorption of ^{99}Tc , ^{237}Np and ^{241}Am on Two Subsoils from Differing Weathering Intensity Areas*. BNWL-1889. Battelle, Pacific Northwest Laboratories, Richland, WA.
43. Schneiderman, G. S., T. R. Garland, H. Drucker, and R. E. Wildung. 1974. "Plutonium-resistant Fungi and Actinomycetes in Soil. I. Mechanisms of Plutonium Toxicity". In: *Pacific Northwest Laboratory Annual Report for 1974*. BNWL-1950, Pt. 2. Battelle-Northwest, Richland, WA. pp. 25-28.
44. Schnitzer, M., D. A. Shearer, and J. R. Wright. 1959. "A Study in the Infrared of High-molecular Weight Organic Matter Extracted by Various Reagents from a Podzolic B. Horizon". *Soil Sci.* 87:252-257.
45. Stevenson, F. J. 1967. "Organic Acids in Soil". In: *Soil Biochemistry*. A. D. McLaren and G. H. Peterson (Ed.). Marcel Dekker, New York, NY. pp. 119-146.
46. Stevenson, F. J., and J. H. A. Butler. 1969. "Chemistry of Humic Acids and Related Pigments". In: *Organic Geochemistry*. G. Englinton and M. T. Murphy (Ed.). Springer-Verlag, New York, NY. pp. 534-557.
47. Stevenson, F. J., and M. S. Ardakani. 1972. "Organic Matter Reactions Involving Micronutrients in Soils". In: *Micronutrients in Agriculture*. J. J. Mortvedt, P. M. Giordano, and W. L. Lindsay (Ed.). Soil Sci. Soc. Am., Madison, WI. pp. 79-114.
48. Sullivan, M. F., and T. R. Garland. 1977. "Gastrointestinal Absorption of Alfalfa-Bound Plutonium-238 by Rats and Guinea Pigs." In: *Pacific Northwest Laboratory Annual Report for 1976*. BNWL-2100, Pt. 1, Battelle-Northwest, Richland, WA.
49. Swanson, J. L., T. R. Garland, and R. E. Wildung. 1975. "Preparation and Chemical Characterization of Plutonium-DTPA Complexes." In: *Pacific Northwest Laboratory Annual Report for 1974*. BNWL-1950, Pt. 2. Battelle-Northwest, Richland, WA. pp. 21-23.

50. Tait, G. H. 1975. "Identification and Biosynthesis of Siderochromes Formed by *Micrococcus dentrificans*. *Biochem. J.* 146:191-204.
51. Taylor, R. T., and M. L. Hanna. 1977. "Methylation of Platinum by methylcobalamine". In: *Biological Implications of Metals in the Environment*. H. Drucker and R. E. Wildung (Ed.). ERDA Symposium Series. Fifteenth Annual Hanford Life Sciences Symposium. September 29-October 1, 1975. Richland, WA. (In Press).
52. Tiffin, L. O. 1972. "Translocation of Micronutrients in Plants." In: *Micronutrients in Agriculture*. J. J. Mortvedt, P. M. Giordano, and W. L. Lindsay (Ed.) Soil Sci. Soc. Am., Madison, WI pp.79-114.
53. Tiffin, L. O. 1977. "The Form and Distribution of Metals in Plants. An Overview." In: *Biological Implications of Metals in the Environment*. H. Drucker and R. E. Wildung (Ed.). ERDA Symposium Series. Fifteenth Annual Hanford Life Sciences Symposium. September 29-October 1, 1975. Richland, WA (In Press).
54. Thompson, S. O. 1965. *Comparative Properties of Plant Lignins and Soil Humic Materials*. Ph.D. Thesis. University of Wisconsin, Madison, WI.
55. Vaughan, B. E., R. E. Wildung, and J. J. Fuquay. "Transport of Airborne Effluents to Man Via the Food Chain". In: *Proceedings of Symposium on Controlling Airborne Effluents from Fuel Cycle Plants*. August 5-6, 1976. USERDA CONF 760806. (In Press)
56. WASH-1535. 1974. *Proposed Final Environmental Statement for the Liquid Metal Fast Breeder Reactor Program*. Vol. II, p. II.G-31, NTIS, Springfield, VA.
57. Weeks, M. H., J. Katz, W. O. Oakley, J. E. Ballou, L. A. George, L. O. Bustad, R. C. Thompson, and H. A. Kornberg. 1956. "Further Studies on the Gastrointestinal Absorption of Plutonium." *Radiation Research* 4:339.
58. Wessman, R. A., W. J. Major, K. D. Lee, and L. Leventhal. 1971. "Commonality in Water, Soil, Air, Vegetation, and Biological Sample Analysis for Plutonium." In: *Proceedings of Environmental Plutonium Symposium*, E. B. Fowler, R. W. Henderson, M. F. Milligan, (Ed.). LA-4756. NTIS, Springfield, VA pp 73-79.
59. Wildung, R. E., T. R. Garland, and H. Drucker. 1973. "The Potential Role of the Soil Microbiota in Increasing Plutonium Solubility in Soil and Plutonium Uptake by Plants." In: *Agronomy Abstracts*. ASA, SSSA and CSSA Annual Meetings. November 11-16, 1973. Las Vegas, NV. p. 190.

60. Wildung, R. E., and T. R. Garland. 1974. "Influence of Soil Plutonium Uptake and Distribution in Shoots and Roots of Barley". *J. Agr. Food Chem.* 22:836-838.
61. Wildung, R. E., T. R. Garland, and H. Drucker. 1974. "Potential Role of the Soil Microbiota in the Solubilization of Plutonium in Soil. In: *Pacific Northwest Laboratory Annual Report for 1973*. BNWL-1850, Pt. 2. Battelle-Northwest, Richland, WA. pp. 21-22.
62. Wildung, R. E., T. R. Garland, H. Drucker, and G. S. Schneiderman. 1974a. "Influence of Plutonium on the Soil Microflora". In: *Pacific Northwest Laboratory Annual Report for 1973*. BNWL-1850, Pt. 2. Battelle-Northwest, Richland, WA. pp. 19-21.
63. Wildung, R. E., T. R. Garland, and R. L. Buschbom. 1975. "The Interdependent Effects of Soil Temperature and Water Content on Soil Respiration Rate and Plant Root Decomposition in Arid Grassland Soils". *Soil Biology and Biochemistry* 7:373-378.
64. Wildung, R. E., and T. R. Garland. 1975. "Relative Solubility of Inorganic and Complexed Forms of Plutonium-238 and Plutonium-239 in Soil". In: *Pacific Northwest Laboratory Annual Report for 1974*. BNWL-1950, Pt. 2. Battelle-Northwest, Richland, WA. pp. 23-25.
65. Wildung, R. E., T. R. Garland, K. M. McFadden, D. A. Cataldo, H. Drucker and M. F. Sullivan. 1977. "Transuranic Complexation in Soil and Uptake by Plants." In: *Pacific Northwest Laboratory Annual Report for 1976*. BNWL-2100, Pt. 2. Battelle-Northwest, Richland, WA.
66. Wood, J. M., F. S. Kennedy, and C. G. Rosen. 1968. "Synthesis of Methyl-Mercury Compounds by Extracts of a Methanogenic Bacterium". *Nature* 220:173-174.
67. Wood, J. M. 1974. "Biological Cycles for Toxic Elements in the Environment". *Science* 183:1049-1052.

ACKNOWLEDGMENTS

Appreciation is extended to H. Drucker for his technical contributions and advice during the conduct of the PNL microbiological studies and to F. H. F. Au for review of portions of this manuscript.

This paper is based on work performed under U.S. Energy Research and Development Administration Contract E(45-1):1830.

DISTRIBUTIONNo. of
CopiesNo. of
CopiesOFFSITE

1 A. A. Churm
DOE Patent Group
9800 South Cass Avenue
Argonne, IL 60439

27 Technical Information Center

1 R. L. Watters
Division of Biomedical and
Environmental Research
U.S.DOE
Washington, DC 20545

1 R. E. Franklin
Division of Biomedical and
Environmental Research
U.S.DOE
Washington, DC 20545

1 R. C. Dahlman
Division of Biomedical and
Environmental Research
U.S.DOE
Washington, DC 20545

1 J. Swinebroad
Division of Biomedical and
Environmental Research
U.S.DOE
Washington, DC 20545

1 D. N. Edington
ANL
9700 South Cass Avenue
Argonne, IL 60439

1 L. J. Johnson
LASL
P.O. Box 1663
Los Alamos, NM 87545

1 J. W. Healy
LASL
P.O. Box 1663
Los Alamos, NM 87545

1 J. C. Rodgers
LASL
P.O. Box 1663
Los Alamos, NM 87545

1 F. O. Hoffman
ORNL
P.O. Box X
Oak Ridge, TN 37830

1 A. Wallace
Nuclear Medicine and Radiation
Biology
UCLA
900 Veteran Avenue
Los Angeles, CA 90024

1 L. F. Elliott
USDA-ARS
Department of Agronomy and Soils
Washington State University
Pullman, WA 99164

ONSITE

1 R. P. Larsen
ANL
9700 South Cass Avenue
Argonne, IL 60439

1 W. C. Hanson
LASL
P.O. Box 1663
Los Alamos, NM 87545

1 DOE Richland Operations
Office
H. E. Ransom

1 Rockwell Hanford Operations
K. R. Price

48 Battelle-Northwest

T. W. Ambrose
W. J. Bair
D. A. Cataldo
D. B. Cearlock
J. F. Cline
H. Drucker
T. R. Garland
W. T. Hinds
S. Marks
D. E. Olesen
D. Paine
D. Rai
L. A. Rancitelli
W. H. Rickard
L. E. Rogers
R. G. Schreckhise
G. A. Sehmel
R. J. Serne
N. M. Sherer
W. L. Templeton
W. Wiley
B. E. Vaughan
R. E. Wildung (20)
Technical Information Files (5)
Technical Publications