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The Relationship of Microbial Processes to the Fate of Transuranic Elements in Soil

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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 solubility of the transuranics associated with the solid phase, since a soluble species must exist adjacent to the root membrane for some finite period for root uptake. The form of this soluble species will have a strong influence on its stability in soil solution and on the rate and extent of uptake, and perhaps, mobility and toxicity in the plant. Furthermore, it is the solubility and form of the element which largely governs mobility in soil. 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 in soil. These factors are illustrated in Fig. 1 and include the concentration and chemical form of the element entering soil, soil properties, as these influence the elemental distribution between the solid and liquid phase,

and soil processes, as these influence the kinetics of sorption reactions, transuranic concentration, and the form of soluble and insoluble chemical species.

The transuranic elements (Pu, Am, Cm, Np) may enter the soil through several avenues including fallout from atmospheric testing, particulates reprocessing and leaching from waste storage facilities. The major sources of the transuranics may be classified according to expected initial solubility in soil. 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 the highest concentration of impurities in the crystal lattice are expected to 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 such as Pu, Am, and Cm entering the soil as soluble salts in acid concentrations exceeding 2M may be expected to be rapidly insolubilized due to hydrolysis on dilution and subsequent precipitation on particle surfaces. Conversely, Np is not subject to marked hydrolysis and may be initially more soluble. Immobolization (NpO_2^+) may occur through cation exchange reactions with particulate surfaces.

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, primarily Ca and H (Lahar, and Hochberg, 1976, Lindsay, 1972, Norvell, 1964) and the stability of the organic ligand to microbial decomposition (Wildung and Garland, 1975). The distruption of the complex will lead to marked reduction in transuranic solubility through hydrolysis and precipitation reactions as described for acid solutions on dilution. The mobility of the complex, in turn, will be principally a function of its chemical and microbiological stability and the charge on the intact complex which will govern the degree of sorption of the complex on soil particulates.

Further generalizations of transuranic behavior on the basic 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 will consider, in detail, the influence of soil properties and abiotic and biotic processes on the long-term solubility of the transuranics entering soils. Principal emphasis will be directed toward the role of soil microorganicsms in this phenomenon. Microorganisms, in intimate association with soil particles, are known to play an important role in affecting solubilization of elements considered insoluble in soils from strictly inorganic chemical considerations. However, 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 will be used as a framework for broader discussions encompassing the long term behavior of other transuranic elements.

Plutonium

The principal chemical reactions likely influencing Pu behavior in soil are summarized in Table 2. 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 $Pu^{+4} > PuO_2^{2+} > Pu^{+3} > PuO_2^+$ (Cleveland, 1970). Hydrolysis, which occurs in a stepwise fashion, is likely the major mechanism whereby Pu is insolublized in the environment. At high (g/ℓ) Pu concentrations, hydrolysis of Pu^{+4}, may lead to the formation of a collodial Pu polymer. At these concentrations, the polymer is characterized by a distinct adsorption spectrum. Although the polymer has not been fully characterized, it is generally thought to be an intermediate hydrolysis product of Pu^{+4} containing oxide or hydroxide bridges, with an absorption spectrum different than $Pu(OH)_4$. However, studies by Lloyd and Haire, 1973, have indicated that the polymer may be aggregates of small, discrete, amorphous or crystalline, primary particles 5 to 20 A° in diameter. It is of interest that x-ray diffraction patterns of the polymeric Pu and that of $Pu(OH)_4$ (Ochenden and Welch, 1956) both show a pattern characteristic of the cubic PuO_2 lattice, suggesting that the polymer and the hydroxide of Pu^{+4} may both be hydrated PuO_2 with differences occurring in primary particle size and crystallinity. A similar conclusion was reached by 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 form of Pu through complexation and subsequent inhibition of hydrolysis, polymerization, or disproportionation. It is these reactions in various highly complex combinations resulting from differences in source term, soil properties and processes that govern Pu solubility in soil and availability to plants.

Soil chemical reactions are of manifest importance 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 tranformations (Fig. 1,2). Insoluble Pu, such as high-fired oxide, entering soil likely will be solubilized with time, provided soluble, stable complexes are formed (Fig. 2). However, regardless of the form of Pu entering soil, its ultimate solubility will be controlled by its aqueous chemistry and by soil factors. The long-term behavior of Pu in soil will be a function of the kinetics of these reactions. Soil physiochemical properties may be expected to have complex, interdependent effects on Pu solubility.

On the basis of research with other trace metals recently summarized by Keeney and Wildung (1976), 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 delination of the influence of these factors on Pu solubility is complicated by the complex chemistry of Pu.

Perhaps the simplest 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 submicron clay particles or to the formation of submicron particles of hydrated Pu oxide which may pass membrane filters and are difficult to centrifuge. These effects may be illustrated by comparison of the differences in the solubility of Pu in soils (100 days after amendment as Pu $(NO_3)_4$ 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, it is evident that Pu in these filtrates may be in collodial 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),

$Pu(NO_3)_4$ and Pu-DTPA likely represent, in their respective chemistries, the range in soil behavior likely to occur. The water solubility (defined as described above) of ^{238}Pu and ^{239}Pu amended to a Ritzville silt loam (organic C content 0.7%, pH 6.2) in the $Pu(NO_3)_4$ 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. 3). 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 be decreasing 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 7-10 days. The solubility of ^{238}Pu and ^{239}Pu added to the soil as nitrates was much lower than the DTPA complexes likely reflecting hydrolysis to the largely insoluble hydrated oxide. 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 2-3 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 but it may also have reflected the presence of a soil component such as an organic ligand, which stabilized Pu in solution but was 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 μ), 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 on solubilization of the oxide might be expected to follow a course similar to that exhibited by the nitrates.

Equilibrium solubility after 6 days of incubation (Garland et al. 1976) of Pu added as $Pu(NO_3)_4$, in soils of different properties occured after approximately 20 hours (Fig. 4). 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 a marked increase in the quantity of Pu extracted by water relative to 0.01 M $CaCl_2$ in the Muscatine soil relative to the Hesson and Ritzville soils. This may be related to a difference in the dispersibility of fine colloids in this soil and/or the presence of higher concentration of stabilizing ligands. However, the lack of a proportional dilution effect (not shown in Fig. 4) in the water extractabilitity 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 (1976) estimated the concentrations

and molecular weight of mobile Pu in five surface soils representing a range in particle size distributions, pH (4.4-6.2) organic C (0.7 to 12.5%) and cation exchange capacities (14 to 45 me./100 g).

The diffusion coefficients calculated for the most mobile species in the five soils varied from 1.9 to 3.0 x 10^{-6} cm^2/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, 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 1600). Hypothetical globular peptides of molecular weights less than 500 would pass 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 $Pu(OH)_4$ or hydrated oxide.

The estimated diffusion coefficients for the least mobile Pu components ranged from 2.3 - 3.1 x 10^{-7} cm^2/sec with corresponding soil concentrations of 150 - 1200 pg/g (Table 2). This concentration of Pu in soil approximated the quantity of water-soluble Pu passing the 0.0015 μ ultrafiltration membrane (Table 1). Hypothetical globular proteins in this size range would have average molecular weights < 10,000. Particles

of $Pu(OH)_4$ 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 $Pu(OH)_4$ 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 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 $\underline{M}$ NH_4 HCO_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 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 (Bontietti 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. Most soluble organic ligands in soil are derived from microbial processes. The organic complexation reactions and the microbiological factors potentially influencing Pu behavior in soil will be discussed in subsequent sections.

Other Transuranic Elements

The other principle transuranic elements of concern in the nuclear fuel cycle are isotopes of americium, curium, and neptunium. Principle isotopes of other transuranics 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 the interaction of these elements with soils are lacking, some information has become available in recent years. Furthermore, the aqueous chemistries of these elements have been fairly well established (Burney and Harbour, 1974). The most stable ions of Am and Cm in aqueous

solutions are the +3 cations; Np is most stable as the +1 oxyion ($N_pO_2^+$). Disproportionation among valence states of these elements is also minor. Thus, major differences in the environmental behavior of these elements, as compared to Pu, would be expected. Hydrolysis reactions may still be a primary factor governing in 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 $Pu(OH)_4$. The Np oxycation should not be subject to significant hydrolysis at environmental pH values. 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, 1975) indicated sorption in the order Pu > Am > Np. The chemistry of Cm should be very similar to Am if 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 transuranics 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, 1976). 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 & Ardakani, 1972). In general, earlier studies emphasized metal interactions with intact soil or with the higher molecular weight humic components of soil whereas more recent studies have emphasized 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 & 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 me./100 g for humic acids and fulvic acids, respectively (Stevenson & Butler, 1959). 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 me./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 repesented 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 10-30% of sorbed Pu from the clays but less than 1% from the humic acids. Ligands forming stronger complexes with Pu (DPTA 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 & 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 potential 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). 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 category (1) and longer term increases of materials in category (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 have been shown to be associated with low molecular weight components. Most of the titratable acidity of this fraction has been attributed (Geering & Hodgson, 1969) to aliphatic acids (< pH 7.0) and amino acids (> pH 7.0).

The production, distribution, and action of organic acids in soil has been 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 a ρ-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.

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 are the amino acids. 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 amd 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 μg/g. In the soil solution (Geering & 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 have been 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 (as discussed above) it may be concluded that the soil microflora may play a significant role in transformations governing the form, and ultimately, the long-term solubility and behavior of transuranic elements in soil. There are five general mechanisms whereby microorganisms may alter the form of trace metals in soil (Alexander, 1961; Wood, 1974). These include (1) indirect transformations resulting from metal interactions with microbial metabolites, or changes in pH and Eh (2) direct transformations such as alkylation, (3) 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), (4) immobilization by incorporation into microbial tissues, and (5) release of metals on decomposition of organic residues.

All of these mechanisms may be operational in transformations of transuranic elements in soils, and there is insufficient information to unequivocably rule out any transformation mechanism. However, on the basis of present knowledge, it is possible to draw some tentative

conclusions as to their relative importance in affecting the long-term behavior of the transuranic elements. Since there is a paucity of information available, rather than discuss these mechanisms individually they will be addressed around a framework of current information, limited principally to Pu.

Microbial Alteration of Transuranic Solubility in Soil

Investigations have been conducted to determine the potential for microbial alteration of Pu solubility in soil and availability to plants.

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 μ Ci Pu/g of soil, (added as $Pu(NO_3)_4$). 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 (1g) suspended in 1 liter of distilled water. After a 4 hr equilibration period, an aliquot of the soil suspension was filtered through 5, 0.5 and 0.01 μ millipore 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 colliods (previous section).

In an ancillary experiment, incubation was continued for 65 days until the CO_2 evolution rate reached a constant level. The Pu-containing sterile soil was then inoculated with the Pu-treated nonsterile soil and the respiration rate and solubility of plutonium in the inoculated soil measured for a period of 30 days.

Changes in the soil respiration rate and plutonium solubility during the initial 30 day incubation are shown in Fig. 5. The concentration

of Pu in the 0.45 μ filtrate ranged from approximately 0.5 to 1.5% of the Pu applied during the incubation period. 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 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.

When the sterile soils were inoculated with nonsterile soil, CO_2 evolution increased at a much more rapid rate without a lag phase, and this was accompanied by an additional factor of 2 increase in water solubility 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 mechanism (1)-(3) described above i.e. the direct alteration of Pu form such as modification of the Pu polymer or plutonium valence state, indirect alteration through the production of organic

acids which may complex Pu or the alteration of the pH of the soil solution in the immediate vicinity of the colloid without measurable effects on the overall soil pH. If the mechanism of solubilization was indirect, the results might be applicable to other transuranic element, e.g., from consideration of the aqueous chemistry described in a previous section, 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 plant is not able to exclude the increased metal. In order to determine if the increased solubility on incubation resulted in increased Pu uptake by plants, the soils, incubated as previously described, 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 solubility of Pu in soil, increased Pu uptake by shoots compared to the unincubated controls. 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 incubated soil.

Effect of Transuranics on the Soil Microflora

Soil organisms may be expected to be present at highest levels in the immediate vicinity of soil colloids (Alexander, 1961). From the aqueous chemistry of the transuranics and on the basis of recent knowledge

(previous section) it is likely that the major fraction of the transuranic elements in soil will also be associated with colloids. Thus, soil microorganisms may be exposed to relatively high concentrations of the transuranics in soil even when total soil concentration is low. 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 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 incapable 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, 1974).

A noncalcareous Ritzville silt loam (pH 6.7) was amended with $^{239}Pu(NO_3)_4$ at levels of 0.05, 0.5 and 10 μCi/g and with starch, nitrogen

and water to provide optimal microbial activity as previously described. 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 the fungi (Fig. 6) 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 3.

It is apparent that 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 μCi/g or 1 μg/g. The fungi were the exception, differing from the controls only at a Pu concentration of 10 μCi/g or 180 μg/g. Thus, the Pu did not affect maximum generation rate but rather affected the log 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, the 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 10 μCi/g level of Pu amendment, although numbers of all classes or organisms except the fungi were depressed below this level (Table 3). 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 Tl, in which respiration

rate was a sensitive measure of metal effect at levels as low as 10 μg/g in soil. It should 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, were dependent upon the initial level of microorganisms in soil. In soils exhibiting a lower 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 chemical toxicity, as noted above, 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 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 which did not exhibit either radiation or chemical resistance since, due to competitive advantage, these 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. (1975) 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 145 μg/g) and ^{238}Pu (0.6 μg/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 μ) of the Pu forms. Comparisons of soil microbial 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. 7). 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. 5.3.3 Isolation of resistant organisms

Isolation of Resistant Organisms. The study of complexation of trace metals in soils is extremely difficult due to the complexity of the soil system (Keeney and Wildung, 1976). In fact, 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.

Application of enrichment techniques to the isolation of Pu resistant fungi, which have been demonstrated (see previous section) to be the most resistant class of microorganisms, and actinomycetes from soil using starch as a C-source (Schneiderman et al., 1975) 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 isolated at all Pu concentrations in the media. In contrast, the fungal isolates exhibited 6 common morphological types regardless of incubation period.

Subsequent enrichment studies (Pelroy, 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. It is apparent that the presence of Pu resistant organisms is related to metabolic potential and thus may be expected to vary with soil type and environmental conditions. Again, similar studies have not been conducted with other transuranic elements.

Microbial Transformations

As previously discussed, there are several means 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 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 from in vitro by soil microorganisms and provide evidence for both direct and indirect transformation of Pu.

Direct Transformation. The potential for direct transformation of the transuranic elements through alteration of valence state or alkylation is difficult to assess. Although, as previously discussed, 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.

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, 1976). It has been suggested that methylated derivates of Hg and As are important factors governing their behavior in the environment (Wood, 1974). It has also been suggested (McBride and Wolf, 1971) that these reactions occur abiotically. The process of biochemical methylation of metals may be described as an overalp between the chemistry of methyl cobalamine (and intermediate in methane synthesis by anaerobic bacteria, 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 latter which 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 biologic alylation 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. As in the case of valence alteration, the lack of experimental information precludes discussion of alkylation phenomena. Meaningful microbial studies await the development of an understanding of the chemical speciation of transuranics in aqueous solutions at environmental concentration levels.

Indirect Transformation. 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 transported into the fruiting bodies. Subsequent washing to remove external contamination indicated that the major portion of the ^{238}Pu was incorporated into tissues. The order of uptake was related to expected solubility of the Pu added with Pu in the initially soluble nitrate and citrate forms exhibiting a factor of 3 greater uptake than the dioxide (Table 4).

There is a growing literature on organic acids and bases produced directly on or by secondary syntheses by a variety of microorganisms and which are capable of complexing heavy elements. These products may be expected to be present in soils (discussed in detail in a previous section). Their concentration and form will be dependent upon the environmental factors influencing microbial metabolism, such as C-source,

(previous section), and their residence time will be dependent upon subsequent chemical and microbiological stability.

In preliminary (unpublished) studies by the senior authors, 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. To distinguish complexation reactions resulting from Pu interactions with metabolites arising from normal metabolic processes and Pu interactions with metabolites arising from Pu resistance soil microorganisms were in the first case isolated from soil in the absence of Pu and Pu added at the stationary growth phase of an enriched culture and, in the second case, isolated from Pu containing soil and grown in the presence of Pu.

After growth for 96 hr, the cultures were separated into cellular 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 5. 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 exocellar 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 6). 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 culture grown in organic-acids; less Pu was associated with the cell debris fraction as compared to cells grown on sugars.

In general, the presence or absence of Pu during growth had little effect on the distribution of Pu in the cultures. Rather, the metabolic properties of the mixed culture 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 fractions of 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. 8). The exocellular and intracellular soluble fraction of organisms exposed to Pu in a single exposure and in continuous exposure exhibited a majority of material of molecular size greater then 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 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; butanolpyridine, 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-DPTA, and Pu in the soluble exocellular and soluble intracellular fractions of the above cultures (Fig. 9). Thin-layer chromotography 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-DPTA or other possible complexes, Solvent G mobilized Pu-DPTA 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. 10) of a relatively large amount of material of greater negative charge than Pu-DPTA, in the exocellular fraction along with some Pu-DPTA. 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 continously to Pu during growth have also been reported (Robinson, et al, 1975).

Several phenomena may have been responsible for the observed changes in chemical form of Pu. The organism may synthesize compounds which either bind Pu-DPTA 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 (Bulker, 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 (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 cultures of soil microorganisms were capable, through simple expression of the metabolic potential of microorganism present in soil, of changing the chemical form of Pu-DPTA with the resulting formation of a number 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 the nature of microbial metabolism and Pu resistance.

Although published information on transuranic elements other than Pu is not available, it is likely that similar transformations will occur. The extent of these transformation 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 (discussed in a previous section) and potential for complexation may be preliminarily assessed from known chemistry (Table 7). 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 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 transuranics 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 the roots of barley, (uncontaminated with soil particles), contain 3-8 times more Pu than the shoots. The roots of plants are in intimate contact with the soil and may be expected to decompose rapidily (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 from plant tissues.

Little is known of the form of the transuranics in plant or microbial tissues; 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 (previous section) and the susceptibility of a number of the transuranic elements to complexation (previous section), it may be concluded that the transuranics, initially immobilized through biological uptake, may be at least as soluble and perhaps more soluble on decomposition.

Preliminary studies (Wildung and Garland, unpublished), in which 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, 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 the recycling process will remain unknown.

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Table 1. Solubilities of Plutonium in Water Extracts of a Ritzville Silt Loam as Determined by Filtration with Membranes of Different Pore Sizes

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.

Table 2. Estimated Concentrations and Molecular Weights of Mobile Plutonium in Soils from Measured Diffusion Coefficients

Treatment	Most Mobile Species			Least Mobile Species		
	Diffusion Coefficient x 10^{-6}	Molecular Weight, g/mole	Soil Concentration, pg/g	Diffusion Coefficient x 10^{-7}	Molecular Weight, g/mole	Soil Concentration, pg/g
Control						
$Pu_2(DTPA)_3$	5.8	1,700		53	1,700	
Soils						
Ritzville	3.0	5,000	24	2.3	0.9 x 10^6	150
Quillayute	2.5	7,200	47	2.7	0.7 x 10^6	1,200
Hesson	2.4	8,100	9	2.7	0.6 x 10^6	330
Salkum	1.5	21,000	55	2.3	0.8 x 10^6	340
Muscateen	1.9	13,000	36	3.1	0.5 x 10^6	170

Table 3. Summary of the Effects of Plutonium at Several Soil Concentration Levels on the Distribution of Microorganisms in Soil Relative to Controls

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
Bacteria						
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	+	+	+
Fungi	0	0	0	0	0	+
Actinomycetes	0	0	+	+	+	+

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

Table 4. Plutonium Transport to the Spores of *Aspergillus niger**

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}

*After Beckert and Au (1976).

†Arithmetic mean of 3 samples each.

Table 5. Distribution of Plutonium in Mixed Microbial Cultures Exposed to Plutonium at Stationary Growth Phase and Grown on Different Carbon Sources.

	Distribution* of Plutonium (%) in Cultures			
	Fungi		Bacteria	
Fraction	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 is < ± 10% (1σ).

Table 6. Distribution of Plutonium in Mixed Microbial Cultures Continuously Exposed to Plutonium and Grown on Different Carbon Sources.

	Distribution* of Plutonium (%) in Cultures			
	Fungi		Bacteria	
Fraction	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 is < ± 10% (1σ).

Table 7. Stability of DTPA Complexes with the Transuranic Elements*,†

Complex	Stability Constant	Stable pH Range
Neptunium		
Np^{+3}	-**	-**
Np(IV) DTPA	10^{24}	0.5 - 5.8
$[Np(IV)]_2DTPA_3$	10^{20}	5.8 - 8.9
$[Np(IV)]_2DTPA_3$	10^{18}	>8.9
Plutonium		
Pu(IV) DTPA	10^{24}	1.0 - 5.8
$[Pu(IV)_2DTPA_3$	10^{18}	5.8 - 8.5
$Pu(IV)\ DTPA_2$	10^{14}	>8.5
Americium		
[Am(III)] DTPA	10^{20}	1.8 - 6
Am(III) DTPA	10^{23}	>6

* After Hafez, 1969.

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

**Unstable in oxygenated solutions.

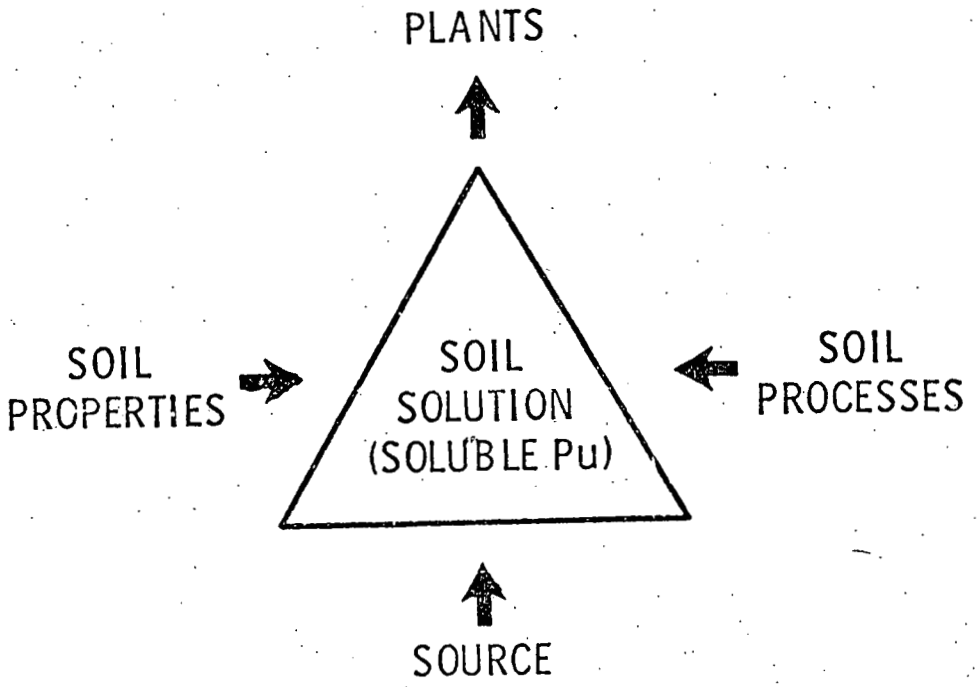


Fig. 1. Factors influencing plutonium behavior in the terrestrial environment.

- FOUR OXIDATION STATES

  Pu^{+3}, Pu^{+4}, PuO_2^{+1}, PuO_2^{+2} (Pu^{+2}, Pu^{+7})

- DISPROPORTIONATION

  $$Pu^{+4} + PuO_2^{+1} \rightleftharpoons Pu^{+3} + PuO_2^{+2}$$

- HYDROLYSIS

  $$Pu^{+4} + 4H_2O \rightleftharpoons Pu(OH)_4 + 4H^+ \quad (K_{sp} \approx 10^{-56})$$

- COMPLEX FORMATION

  $$2Pu^{+4} + 3\ DTPA \rightleftharpoons Pu_2DTPA_3 \quad \log K \approx 26$$

Figure 2. Chemical reactions influencing plutonium behavior in the terrestrial environment.

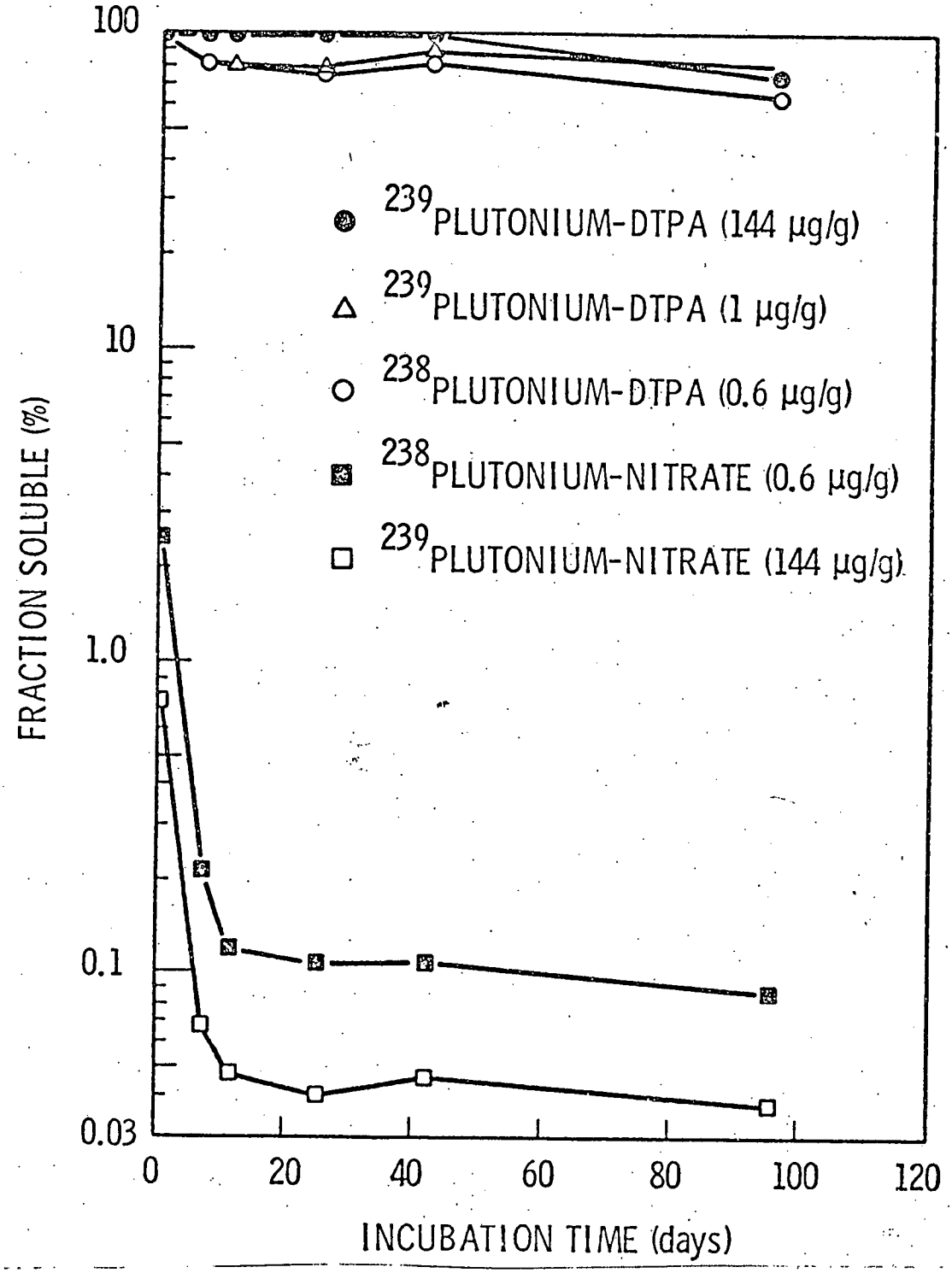


Fig. 3. Solubility of plutonium with time after addition to soil as the nitrate and the DTPA complex.

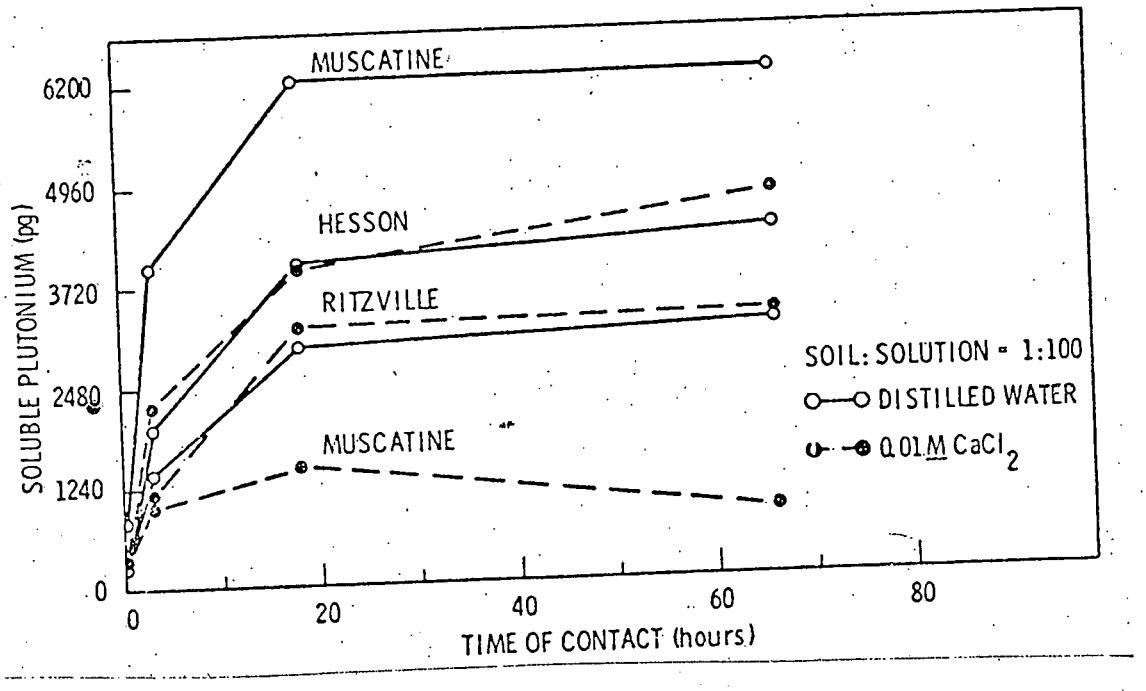


Fig. 4. Quantity of soluble plutonium removed from three soils by water and 0.01 M $CaCl_2$.

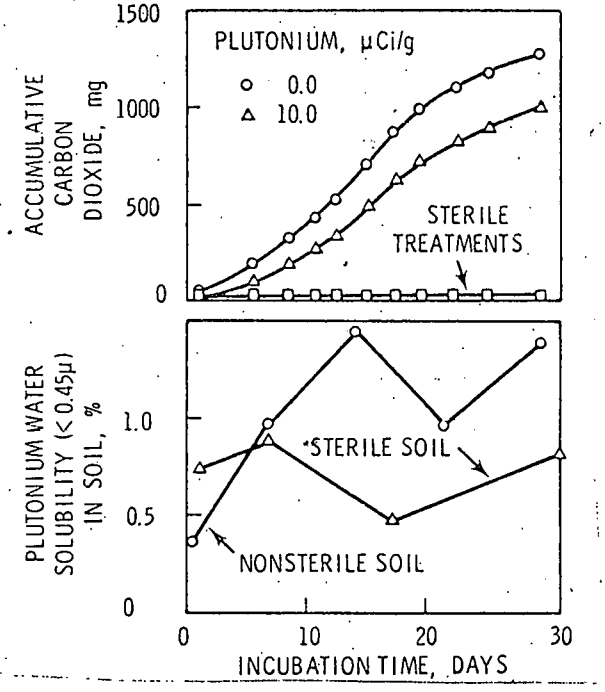


Fig. 5. Changes in soil respiration rate and solubility of applied plutonium with time of soil incubation.

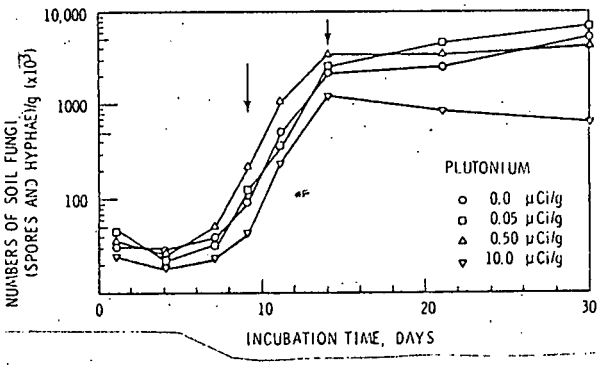


Fig. 6. Influence of plutonium concentration on the growth of fungi in soil. Arrows denote time intervals at which growth rates and total numbers were compared with other microbial types (Table 3).

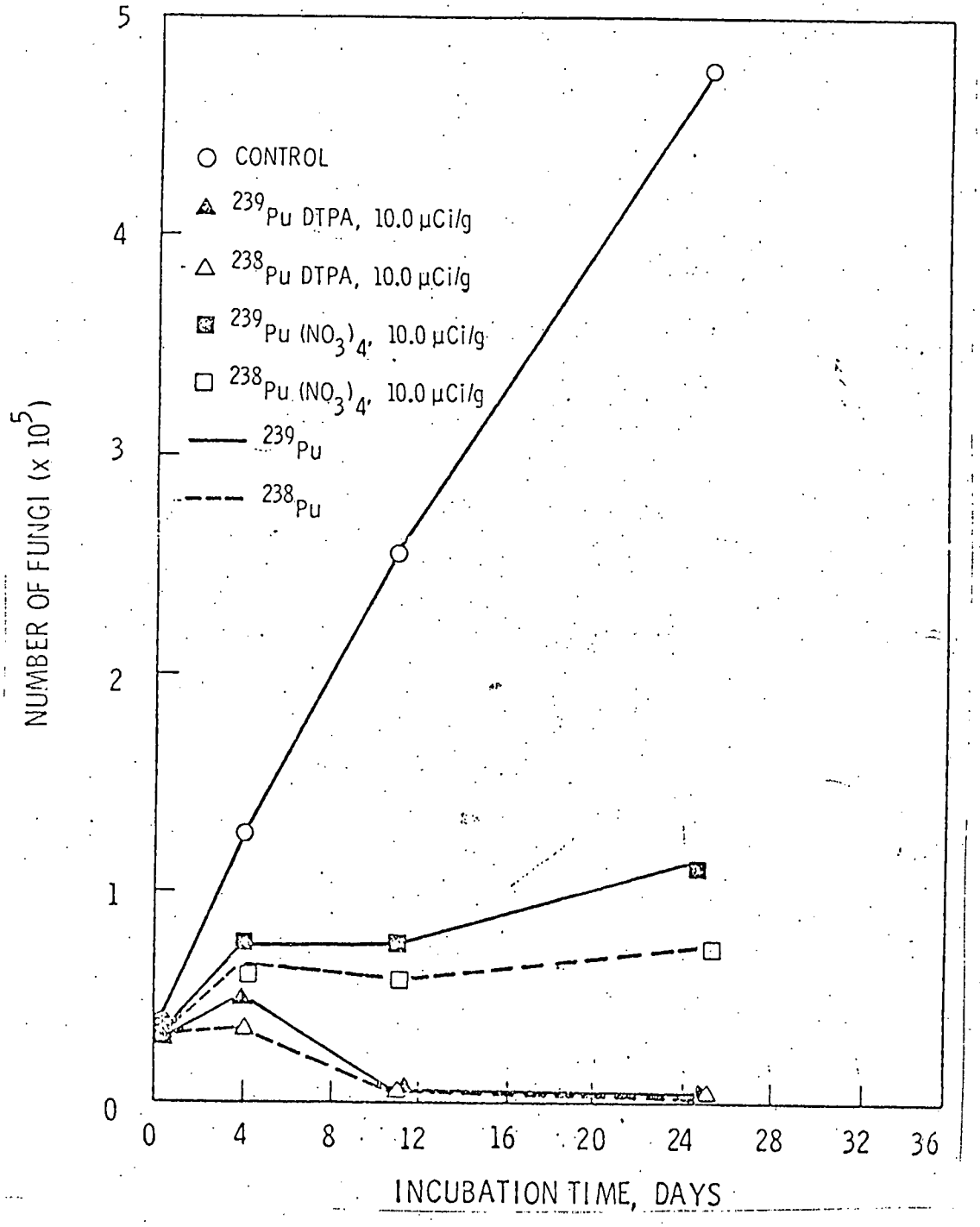


Fig. 7. Effect of different isotopes of plutonium on survival of soil fungi.

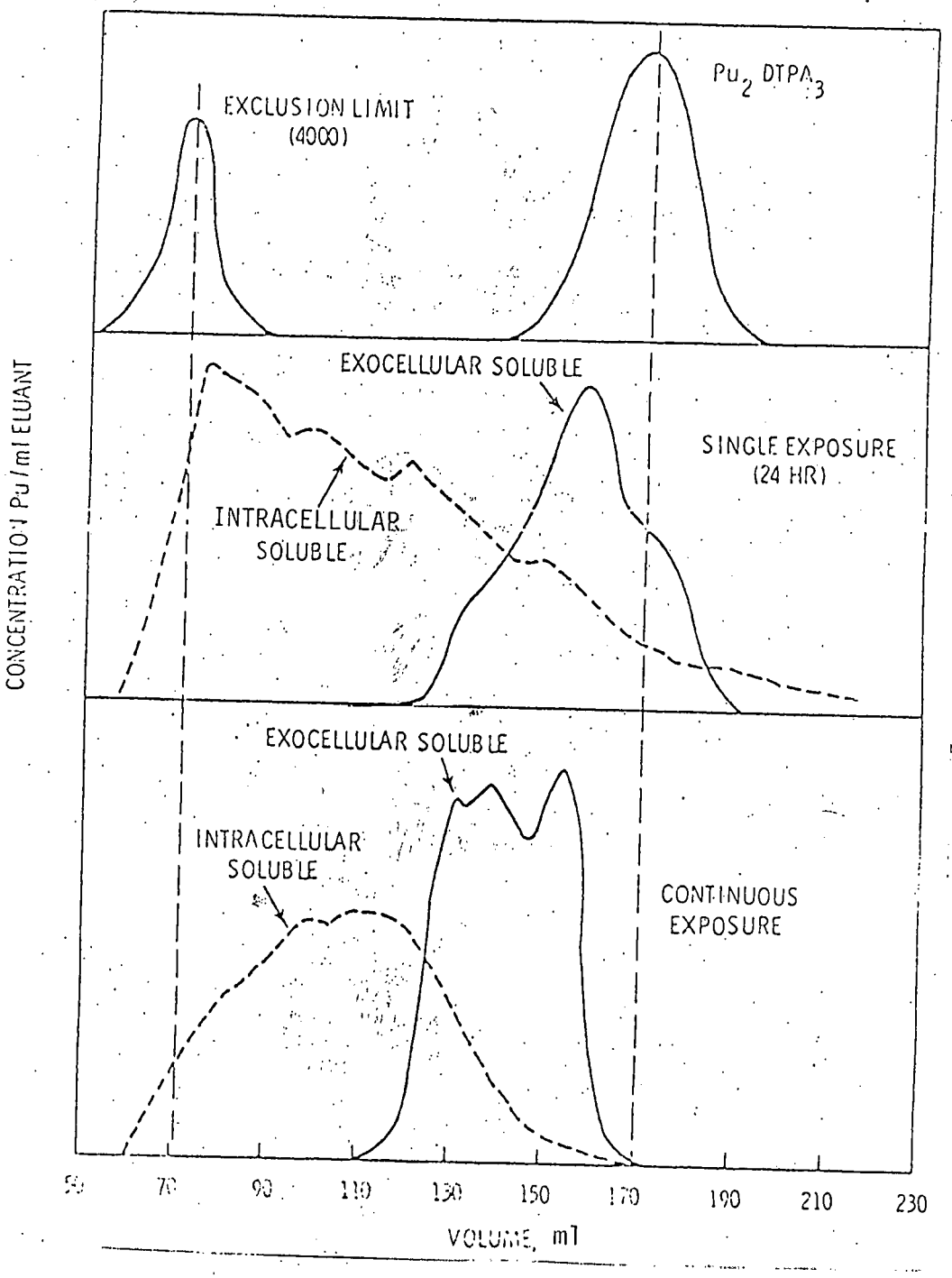


Fig. 8. Separation of soluble plutonium complexes in microbial cultures by Sephadex gel chromatography.

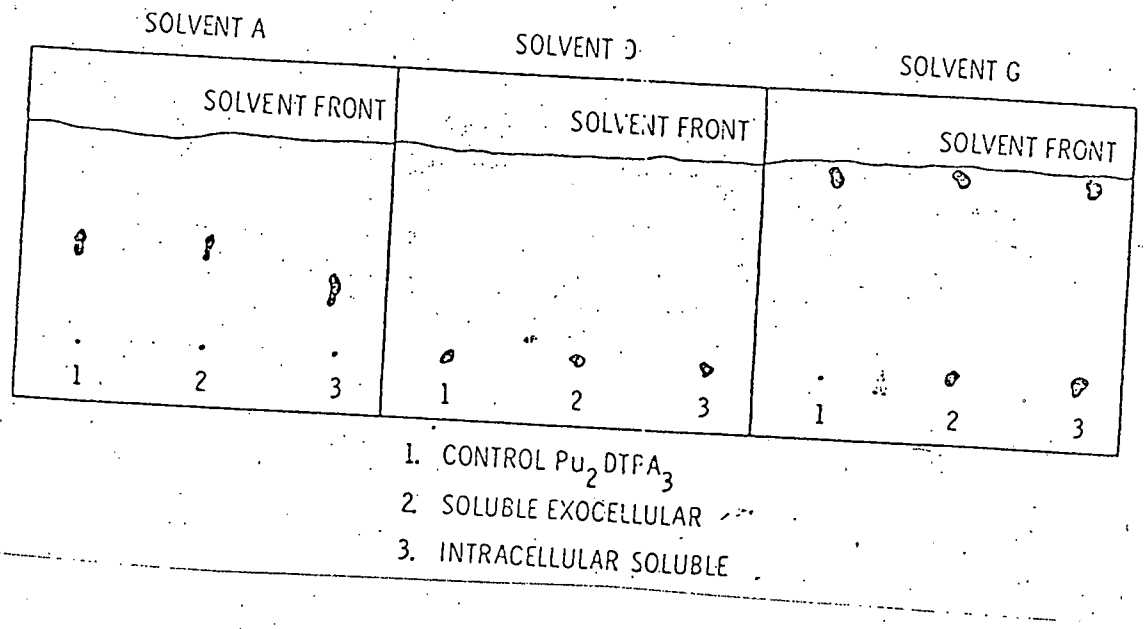
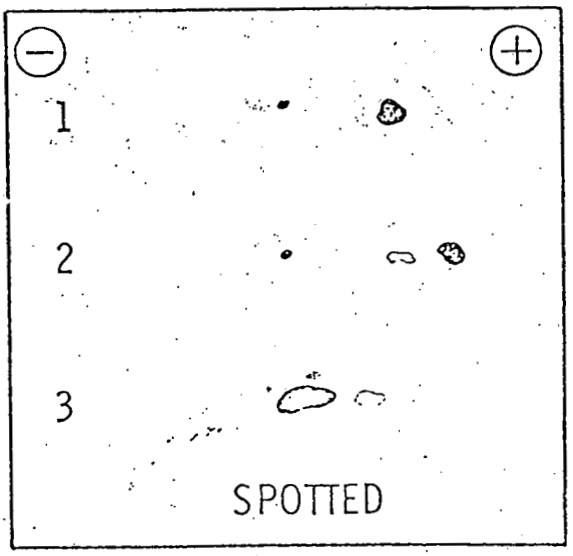


Fig. 9. Thin-layer chromatographic behavior of plutonium complexes separated by Sephadex gel chromatography in three solvent systems.



1. Pu_2DTPA_2 CONTROL
2. SOLUBLE EXOCELLULAR
3. INTRACELLULAR SOLUBLE

Fig. 10. Thin-layer electrophoretic behavior of plutonium separated by Sephadex gel chromatography.

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