• Transuranic Behavior in Soils and Plants

Principal Investigators: R. E. Wildung, T. R. Garland, D. A. Cataldo, J. E. Rogers, K. M. McFadden, V. M. McNair and R. G. Schreckhise

The principal objectives of these investigations are to determine 1) the potential for alteration of transuranic solubility through formation of transuranic complexes in soil and the role of the soil microflora in this process, 2) the extent of uptake and translocation by plants and the sites of plant deposition of transuranics or their complexes, 3) the bond types and chemical forms of transuranics or their metabolites in microbes, plant tissues and soils, 4) the influence of soil properties, environmental conditions and cropping on these processes and 5) the retention of airborne pollutants by plant foliage and their subsequent absorption by leaves and transport to seeds and roots.

Soil, Plant and Animal Studies

Soil, plant and animal investigations have resulted in the development of a model for the chemistry or biochemistry of plutonium in the environment. Present investigations have emphasized the mechanistic approach for developing an understanding of the kinetic aspects of the model controlling long-term chemical behavior and bioavailability. In addition, investigations have been extended to apply this knowledge to other actinide elements.

Modification of the Chemical Form of Complexed and Hydrolyzed Plutonium by Soil Fungi

Microbial studies have been directed toward determining the mechanism for previously observed complexation of plutonium and alteration of its mobility in soil. These research efforts are being continued in an effort to determine the mechanisms of plutonium transport by fungi and the exact function of pH in this process; i.e., is the fungal membrane being affected or is the stability of DTPA or organic ligands of fungal origin being altered? Fifty-nine pure fungal cultures isolated from soil, on the basis of carbon source and resistance to plutonium and other metals, have been tested for their ability to modify the chemical form of plutonium diethylenetriamine pentacidic acid (Pu-DTPA) and $Pu(OH)_n$. Of these cultures, 56 of the fungi reduced the pH of the culture medium from 6.2 to a value less than 3.4. Of the remaining three, two did not alter the pH and the third reduced the pH to a level of 4.1.

The observed changes in pH were strongly correlated with the final solubility (<0.01) of Pu-DTPA in solution. Of the 56 fungi which reduced the pH to less than 3.4, 51 reduced the solubility of Pu-DTPA to less than 5% of the controls at the end of growth. The remaining three fungi did not alter the solubility of Pu-DTPA. A similar but less pronounced pH effect was observed with $Pu(OH)_n$.

The effect of pH was further investigated by electron microscopy autoradiography to determine if the decrease in solubility was caused by 1) transport of plutonium across the fungal membrane at low pH, 2) binding of plutonium to the membrane surface or 3) formation of particulate plutonium. Four fungi were examined in this study: two from the high pH group (Drechsleria-352 and Drechsleria-369) and two from the low pH group (Trichoderma-380 and Fusarium-427). These studies were conducted in liquid culture using a standard mineral base media with glucose as the carbon source. The plutonium level was 0.02 µg/ml for both chemical forms. After completion of growth, samples of mycelia were removed, fixed, embedded and prepared for autoradiographic analysis. During this process, essentially no plutonium was lost to the solution when added as the DTPA complex. However, when $Pu(OH)_n$ was used, substantial quantities of plutonium were lost to the fixing and embedding solutions. This suggests that the $Pu(OH)_n$ was loosely associated with the fungal mycelia.

Autoradiographs of Trichoderma-380 and Fusarium-427 inoculated with Pu-DTPA (Figure 1) indicated that essentially all of the plutonium tracks were associated with

A

B

C

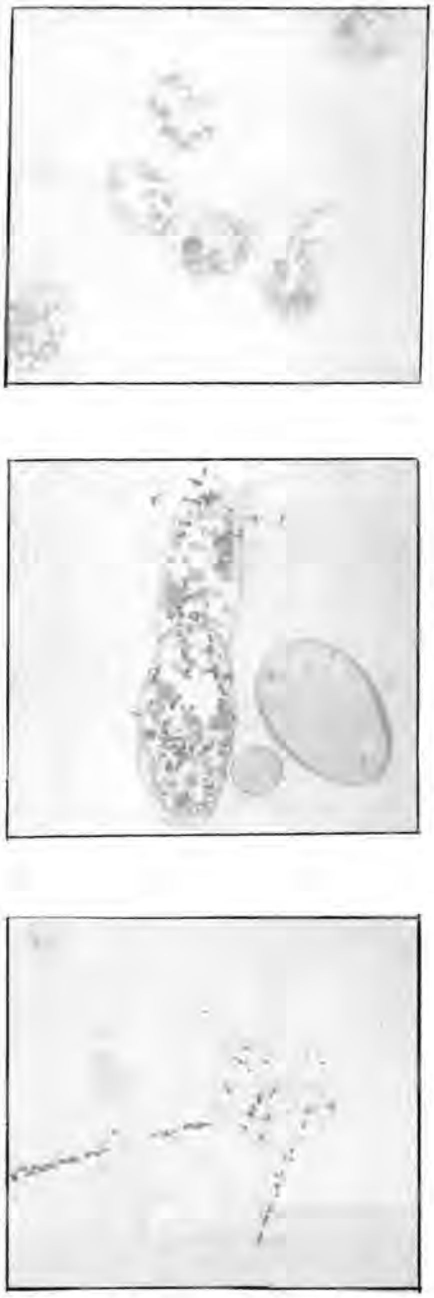

FIGURE 1. Electronmicroscopic autoradiographs of Fusasium-427 Incubated in the Absence of Plutonium (a), Pu-DTPA (b) and $Pu(OH)_n$ (c)

the intracellular portion of the mycelia. Similar results were observed with both species when incubated with $Pu(OH)_n$. However, in the latter case, significant quantities of plutonium were associated with the surface membrane. As expected, the autoradiographs of both Drechsleria spp. (352 and 369), when incubated with Pu-DTPA, showed very few plutonium tracks. Those present were associated with the surface membrane. The results with Drechsleria-369 and 352, when incubated with $Pu(OH)_n$, were in sharp contrast, however; numerous plutonium tracks and stars (particles) were observed. Essentially all the plutonium was either associated with the surface membrane or, to a small degree, with extracellular amorphous material. These results suggest that the final pH of the culture medium may play an important role in plutonium uptake by different fungi.

Incorporation and Transport of Plutonium in Plants and Animals

Our plant studies have concentrated on 1) the factors controlling plutonium absorption by plant roots; 2) plutonium mobility within the plant following either foliar or root absorption; 3) the chemical form of plutonium while in transport (plant xylem) and following deposition in leaves and seeds and 4) evaluation of the influence of plutonium incorporation in plants on gut absorption and subsequent food chain transfer.

Studies have recently been completed using ^{238}Pu-DTPA(^{14}C) to evaluate the factors controlling root absorption of plutonium. Analysis of $^{238}Pu/^{14}C$ ratios in the root zone and xylem exudates indicates an independent plutonium uptake and not absorption of the intact Pu-DTPA complex. Chemical characterization of plutonium forms in exudates clearly shows that the plutonium ligands are not those of DTPA. This is further evidence for the lack of an intact Pu-DTPA complex following root absorption. This data supports the view that organoplutonium complexes in soils serve mainly to deliver the metal to the root membrane, and that the ligands are not taken up by the plant stoichiometrically with the metal.

While plutonium mobility in plants has been shown to be acropetal (root $\gg$ stems $>$ leaves $>$ seeds), previous studies at PNL, involving both foliar absorption and soil studies using split-root techniques, have suggested the presence of a relatively plant-mobile component capable of basipetal transport (leaves to roots). Recent studies, using bifurcated soybean roots, have shown that $\sim$0.2% of the shoot plutonium is transported basipetally to the unlabeled root. These results and those relating to transport form of plutonium demonstrate the importance of organic plutonium complexes in controlling root absorption and ultimate distribution within the plant.

Previous studies have shown plant-incorporated plutonium to be substantially more available for gut absorption than either plutonium nitrate or Pu-DTPA following ingestion. To identify the relationship between the fate of plutonium in plants and its bioavailability after ingestion, soybean plants which had accumulated Pu^{238} for various periods of time were subjected to chemical fractionation procedures. This was done to determine the chemical fate of plutonium. Leaf, stem and root tissues were homogenized and centrifuged, and soluble components were further characterized by ultrafiltration and gel filtration. After homogenization and centrifugation, 64, 56, and 31% of the plutonium was associated with the soluble fraction of leaves, stems and roots, respectively. Ultrafiltration of the solubles show 94% of the soluble leaf and root plutonium to be associated with proteins having molecular weight >10,000, while stems have a substantial fraction (32%) of the solubles associated with ligands of <500 molecular weight. Gel filtration of the soluble fractions of leaves and roots demonstrate the presence of five stable plutonium-containing ligands varying in molecular weight from 1,000 to >150,000. The data thus far obtained on the chemical behavior of plutonium in soybean plants indicates that the metabolic incorporation of plutonium into plant proteins is not unlike the behavior of many nonnutrient heavy metals, for which mobility within the plant (exudate and stem data) is dependent on the presence and formation of organic complexes of low molecular weight.

An important aspect of these data is the difference between the chemical form of plutonium in stem tissue and in leaf tissue. This difference explains results from animal feeding studies, in which gut absorption values were found to be substantially higher for stem tissues than for leaf tissues. This would appear to be a function of the high percentage of low molecular weight components in stem tissues, and their increased bioavailability for gut absorption. Studies are currently underway to determine the chemical fate of plutonium accumulated in soybean seeds and its comparative availability following ingestion.

Incorporation and Transport of Uranium and Thorium in Plants and Animals

Previous investigations have indicated that plutonium added to soil in the VI valence state behaved as Pu(IV) in plant uptake, indicating that the Pu(VI) was probably reduced before plant uptake. In an effort to observe the effect of valence without the concurrent problems of oxidation or reduction, uranium and thorium were used as models for the VI and IV valence, respectively. Both valences are relatively stable in aqueous aerobic conditions.

Short-term studies (<24 hr) of the plant uptake of U^{232} from nutrient solutions containing DTPA, $CO_3^{=}$ or Cl^- showed a nearly quantitative removal of uranium from solution to the roots but little translocation to shoot tissues. Analysis of the xylem exudates indicated that the primary activity transported to the shoots was not U^{232}, but Th^{228} and daughters. Actual concentrations of U^{232} in the tissues are currently being determined. The associated anion had no effect on root uptake or translocation to the shoot tissues.

Studies of the chemical form of uranium in the nutrient solutions using thin-layer electrophoresis and chromatography indicated that the stability of any complexes formed with uranium was too low to remain intact under the conditions of the analysis. Related studies are underway to incorporate sufficient uranium and thorium into alfalfa for longer studies of animal uptake.

Foliar Studies

Evaluations of the interactions of submicronic (0.02-2 μm, count mode) particles with foliage of terrestrial plants have been completed. These studies have shown that the behavior of particles submicronic particles differ markedly from the established behavior of particles 1 to 100 μm. They have also shown that the size aspect for airborne pollutants significantly affects retention on foliar surfaces and the subsequent foliar absorption and transport of surface contaminants to other plant parts such as roots and seeds. The results show the foliar route of plutonium transport to be at least as effective as the soil route for acute exposure (less than one year) situations, if not more so. In the long-term chronic situation, where the soil is the cumulative repository for aerial input, the soil route should become the more important transport route with time.

Among the parameters shown to be important in foliar absorption are the chemical form of the contaminant, relative humidity, rainfall events and their timing and, of course, particle size. Based on the principles developed for submicronic particles, facilities are currently being developed to enable evaluation of a broader range of physical parameters and compound types.