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PLUTONIUM UPTAKE BY PLANTS FROM SOIL CONTAINING PLUTONIUM 238 DIOXIDE PARTICLES

Environmental Monitoring and Support Laboratory
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PLUTONIUM UPTAKE BY PLANTS FROM SOIL CONTAINING PLUTONIUM-238 DIOXIDE PARTICLES

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ABSTRACT

Three plant species--alfalfa, lettuce, and radishes--were grown in soils contaminated with plutonium-238 dioxide ($^{238}PuO_2$) at concentrations of 23, 69, 92, and 342 nanocuries per gram (nCi/g). The length of exposure varied from 60 days for the lettuce and radishes to 358 days for the alfalfa. The magnitude of plutonium incorporation as indicated by the discrimination ratios for these species, after being exposed to the relatively insoluble PuO_2, was similar to previously reported data using different chemical forms of plutonium.

Evidence indicates that the predominant factor in plutonium uptake by plants may involve the chelation of plutonium contained in the soils by the action of compounds such as citric acid and/or other similar chelating agents released from the plant roots.

FOREWORD

Protection of the environment requires effective regulatory actions which are based on sound technical and scientific information. This information must include the quantitative description and linking of pollutant sources, transport mechanisms, interactions, and resulting effects on man and his environment. Because of the complexities involved, assessment of specific pollutants in the environment requires a total systems approach which transcends the media of air, water, and land. The Environmental Monitoring and Support Laboratory-Las Vegas contributes to the formation and enhancement of a sound integrated monitoring data base through multidisciplinary, multimedia programs designed to:

- develop and optimize systems and strategies for monitoring pollutants and their impact on the environment
- demonstrate new monitoring systems and technologies by applying them to fulfill special monitoring needs of the Agency's operating programs

This report describes the plutonium transfer between soil and plant systems. The purpose is to better predict and understand the behavior of plutonium in plant-soil systems. Radiobiologists should find this report of value. If further information is needed on this subject, the Pollutant Pathways Branch of the Monitoring System Research and Development Division, U.S. Environmental Protection Agency's, Environmental Monitoring and Support Laboratory, Las Vegas, Nevada, should be contacted.

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INTRODUCTION

The use and production of transuranic elements by the expanding nuclear power industry, along with the release of many of these elements into the biosphere during periods of atmospheric nuclear testing, have prompted the need for assessing their behavior in biological systems. Of the transuranic elements being utilized and produced in these energy- and weapons-related programs, many investigators, including McKay (1961), Saenz and Ramos (1973), and Fraser (1967), consider plutonium, because of its long half-life, to be one of the most radiologically and biologically toxic radioelements. Seaborg (1970) estimated that industrial and medical applications may utilize from 60 to 80 tons (54 to 73 metric tons) of plutonium-239 and up to 6 tons (5.4 metric tons) of plutonium-238 by the end of this century. The small portion of this plutonium which escapes containment may pose a serious threat to human health.

A review of the pertinent literature concerning the uptake and translocation of plutonium within biological systems by Mullen and Mosley (1976) showed that the amount of plutonium-239 deposited worldwide on the first few centimeters of surface soils from past weapons tests and nuclear accidents alone would comprise approximately 1 part per trillion (ppt) of the soil volume. They further reported that air samples collected from numerous locations around the world in 1963 had plutonium-239 concentrations of 10^{-15} Ci/m^3. This was higher than the concentration of 10^{-16} to 10^{-17} Ci/m^3 in air samples collected in 1965 at various locations in the United States.

The critical pathway of plutonium from its source to man is generally considered to be by inhalation. The environmental dissolution of plutonium particulates, which may become accidentally incorporated into man's metabolic system either by inhalation or ingestion, necessitates determining the retention and absorption characteristics of plutonium from sites of deposition. One such study, an *in vitro* investigation to determine the solubility of plutonium-238 and plutonium-239 dioxides, was conducted by Raabe *et al.* (1973). They found that after an exposure to a serum simulant, similar in chemical composition to blood serum, small amounts of the plutonium dioxides were dissolved and carried off in the serum stream. Although the plutonium-238 and plutonium-239 dioxide particles were relatively insoluble, the solubility rate was about two orders of magnitude higher for the plutonium-238 dioxide particles than for the plutonium-239 dioxide particles. If this difference in solubility occurs in environmental plutonium contaminants, there are important problems facing radiobiologists. For instance, contamination predictions must be modified to reflect different plutonium isotopes. Also, many pathway studies using plutonium-238 will be invalid in predicting plutonium-239 behavior.

The pathway of plutonium to man by his ingestion of vegetative material is considered to be a much smaller risk than inhalation. However, in 1955, Rediske et al. indicated that of the radioactive isotopes that become incorporated into biological systems, most have entered initially via plants. Investigations concerning this mode of biological entry have been primarily designed to determine the pathways and rates of radionuclide transfer among the many soil, soil-plant and plant components of both native and agricultural ecosystems. Many of these studies have been initiated in the field following accidental releases or during continual releases of radioactive pollutants into the environment, while others have been conducted in the laboratory under controlled conditions. Both types of investigations have added to our understanding and knowledge of the complexity of transuranic pollutants pathways.

The available literature identifying both the physical and biological parameters for assessing the amount of plutonium assimulated by plants via a soil rooting media is limited. In most controlled studies, plutonium is uniformly mixed in soil. As a result, the data from these experiments would not necessarily be correlatable to data collected from native vegetation growing in soils where the plutonium is deposited on the soil surface. Investigations by Romney et al. (1970) have indicated that plutonium is normally quite immobile and tends to remain in the upper few centimeters of the soil; therefore, it is not readily available for plant uptake. Nevertheless, the results of laboratory investigations where plutonium is uniformly mixed in the rooting media are valuable in understanding the contamination of cultivated vegetation grown on plowed lands and in identifying the mechanisms which control its uptake and distribution in plants.

The use and/or the production of the common oxide of plutonium, plutonium dioxide (PuO_2) in fast breeder reactors (Pigford, 1974), as fuel for the nuclear power system (SNAP devices) for space explorations (Adams and Fowler, 1974), and the results of investigations by a small number of researchers such as Dr. O. G. Raabe were instrumental in the initiation of this investigation. This study was designed to obtain information regarding the differences in isotopic uptake by plants. However, only the portion dealing with plutonium-238 is complete and is reported in this paper.

CONCLUSIONS

The results of this study have shown that plutonium in the form of plutonium-238 dioxide is taken up and translocated to the aerial portions of three commonly cultivated plant species. The magnitude of assimilation and translocation of this chemical form of plutonium by these plants appears to be in about the same proportion as the incorporation of other chemical forms of plutonium by a variety of other plants, including both aquatic and terrestrial species. The long-term exposure of the alfalfa did not show any increase in the specific activity of plutonium in the plant tissue, even though the root mass increased. This increase in root mass would normally enhance the probability of a contaminant assimilation as the chance of physical contact with the soil-borne pollutants would increase.

As the behavior of $^{238}PuO_2$ in soils, as indicated in this study, parallels other chemical forms of plutonium as far as plant assimilation, the rate and means of uptake may be largely determined by the effect of root exudates. A number of investigators, including Romney et al. (1970), Schultz et al. (1976), Rhodes (1957), and Price (1972), have indicated that the biological availability of plutonium is largely governed by its solubility and also to the numerous chemical reactions which occur in soils. These reactions, which are enhanced by the soil microflora, as shown by Au (1974), involve and affect soil pH and the rates of natural and/or induced chelation. As such, the effects of chelation, additions of various soil dressings and a changing soil pH on the availability of plutonium transfer from soils to plants are important factors that merit further study.

METHODS AND MATERIALS

This investigation was designed to determine the extent and magnitude of plutonium assimilation by plants growing in soils. The chemical form and the isotopes of plutonium selected for this study, $^{238}PuO_2$ and $^{239}PuO_2$, were based primarily on the observations made by Dr. O. G. Raabe and his research associates in 1973. However, only sized particles of $^{238}PuO_2$ were available at the scheduled start of this study.

Monodisperse $^{238}PuO_2$ particles were obtained from the Inhalation Toxicology Research Institute, Lovelace Foundation, located in Albuquerque, New Mexico. The particles had a geometric mean diameter of 0.32 micrometers (μm) and were prepared initially in December of 1973. They were stored dry on stainless steel foil inside a screw-capped plastic centrifuge tube. The amount obtained for this study consisted of 2.6 mCi of ^{238}Pu with a trace amount of ytterbium-169 (850 nCi as of November 15, 1974). The specific alpha activity of these particles was 13.6 Ci/g. The chemical composition by mass was 97% PuO_2 and 3% ytterbium trioxide ($^{169}Yb_2O_3$). The plutonium contained 90% ^{238}Pu and 10% ^{239}Pu by mass. The particles were further identified as being from segment number 16, LAPS soil number 1, production run number 73337.

The soil selected for the rooting media was a silty loam consisting of 57.6% sand, 36.8% silt, and 5.6% clay. It has a pH of 7.9 and a cation exchange capacity of 12.23 milliequivalents (meq)/100 g. This soil which had been sieved through a 0.417-mm standard sieve and the plutonium dioxide were shipped to the Nuclear Chemistry Division at the Naval Weapons Center, White Oak, Maryland, for mixing.

The initial procedure for preparing the rooting media was to remove the particles from the foil and suspend them in a suitable solution. This procedure was previously described by Raabe et al. (1975). Basically this method involves adding 50 ml of a 0.02% surfactant solution (Triton® X-100) to the centrifuge tube, thereby submerging the stainless steel foil. The centrifuge tube was then placed into an ultrasonic water bath to dislodge the plutonium particles from the stainless steel foil. After a 4-hour period of ultrasonic agitation, the foil was removed.

Because of the necessity of dry mixing to obtain a homogeneously mixed rooting media, aliquots of the surfactant solution containing the plutonium particles were added to a slurry of talc, $H_2Mg_3(SiO_3)_4$. The talc, which readily absorbed the liquid, was then dried under an infrared lamp. The talc mix, which dried into a brittle conglomerate, was transferred in toto to a 16-quart (30-liter) capacity Patterson-Kelley Twin Shell ® blender. The mixing action of the soil particles broke down the talc conglomerate into a fine powder.

The homogeneity of the soil-plutonium mix was determined over a 20-hour blending period by taking soil samples from the blender at various times and analyzing for the ^{169}Yb content using a gamma scintillation detector. Four different soil-plutonium concentrations (mixes) were made for this study, each consisting of approximately 5,100 g. After mixing, each of the four soil mixes was divided into six nearly equal portions, put into 1,000-g volume plastic bottles, and then placed into a 2R type radioactive material shipping container.

The transfer of the potting soil into specially designed 5-inch (127-millimeter) greenhouse pots was completed at the Las Vegas Laboratory. This procedure was accomplished in a standard radiation glovebox. Before transferring the soil, 25 g of vermiculite was added to each of the 24 plastic bottles to prevent excessive soil compaction during plant growth. The bottles, which contained approximately 850 g of the plutonium-contaminated soil, were capped and then rotated by hand for approximately 5 minutes to mix the vermiculite into the soil. After mixing, all the soil from one of the bottles was poured into one of the 5-inch pots. This procedure was duplicated until all 24 pots were filled. The pots were transferred from the glovebox into a self-contained environmental growth chamber. As previously stated, the pots were specially designed as shown in Figure 1. The pots were designed to contain the plutonium over an extended period. To prevent loss of the plutonium particles by leaching, a nylon reinforced Acropor ® filter with a pore size of 0.20 μm was cemented over the drain holes. To protect the Acropor filter from damage by roots and to prevent it from being plugged by soil particles, a Microsorban ® filter was placed in the bottom of the pot to act as a prefilter. To reduce the loss of the plutonium particles by upward migration via capillary action, the soil surface was covered by a Whatman ® filter that had been impregnated with seeds. The Whatman filter was then covered with a 3.0-cm deep layer of 0.3-cm diameter polystyrene beads.

Further safety precautions included the construction of a fiberglass-lined wooden tank measuring 115 x 115 x 18 cm which was placed in the growth chamber to hold the pots. Once the pots were placed in the tank, handling of the contaminated material was eliminated except during harvesting. Also, an automatic irrigation system, as shown in Figures 2 and 3, was designed. Features of this system included the recycling of the evapotranspired water, exterior controls, and a safety float installed in the fiberglass-lined tank. The safety float was installed to shut off the pump, timer, and solenoid if an excessive amount of water occurred in the tank. To ensure a fairly even distribution of water to each pot, four manifolds were used, each distributing water to six pots (Figure 3). Each pot was irrigated with approximately

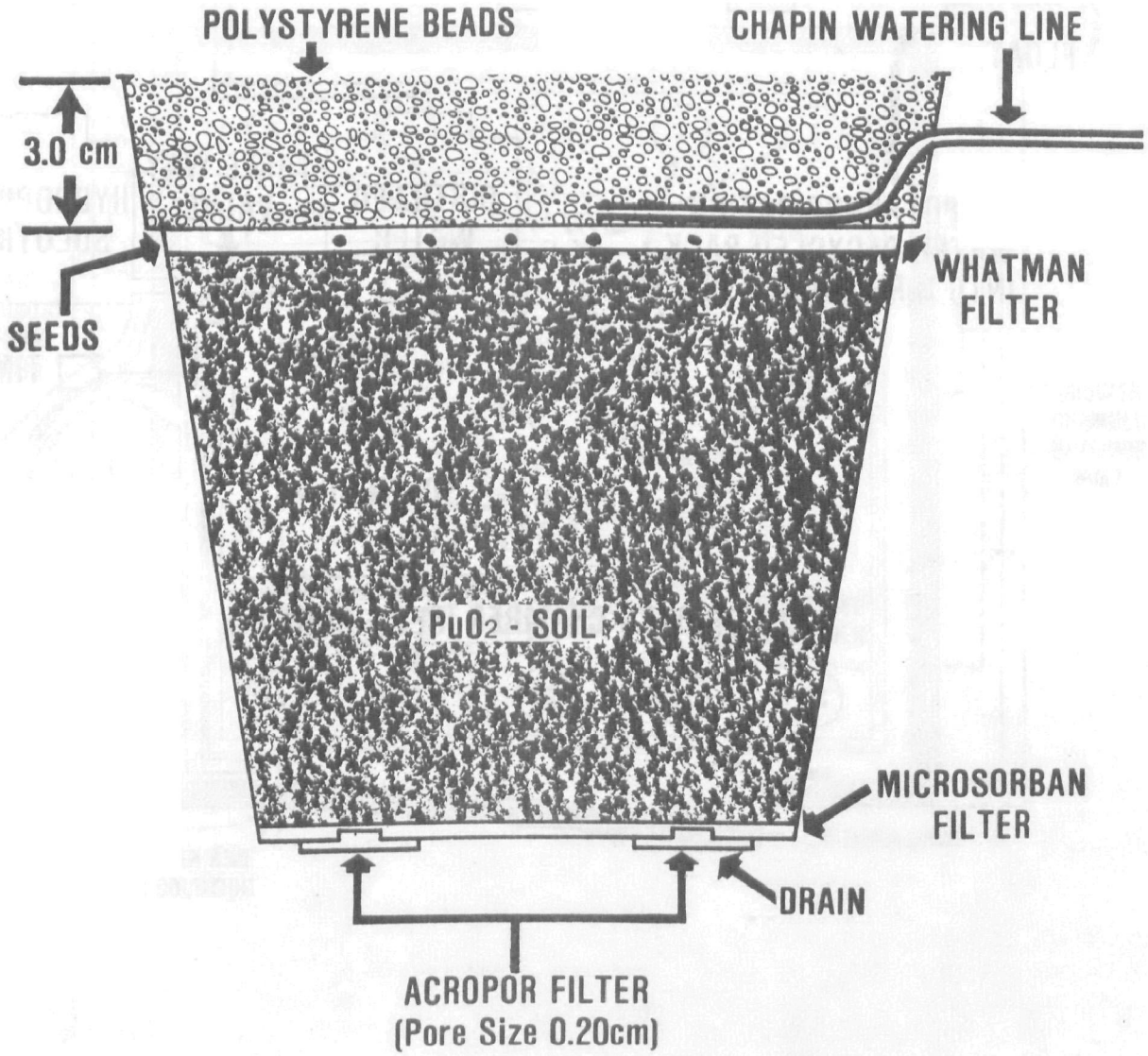

Figure 1. The design of five-inch plastic greenhouse pots used to hold the $^{238}PuO_2$ contaminated soil.

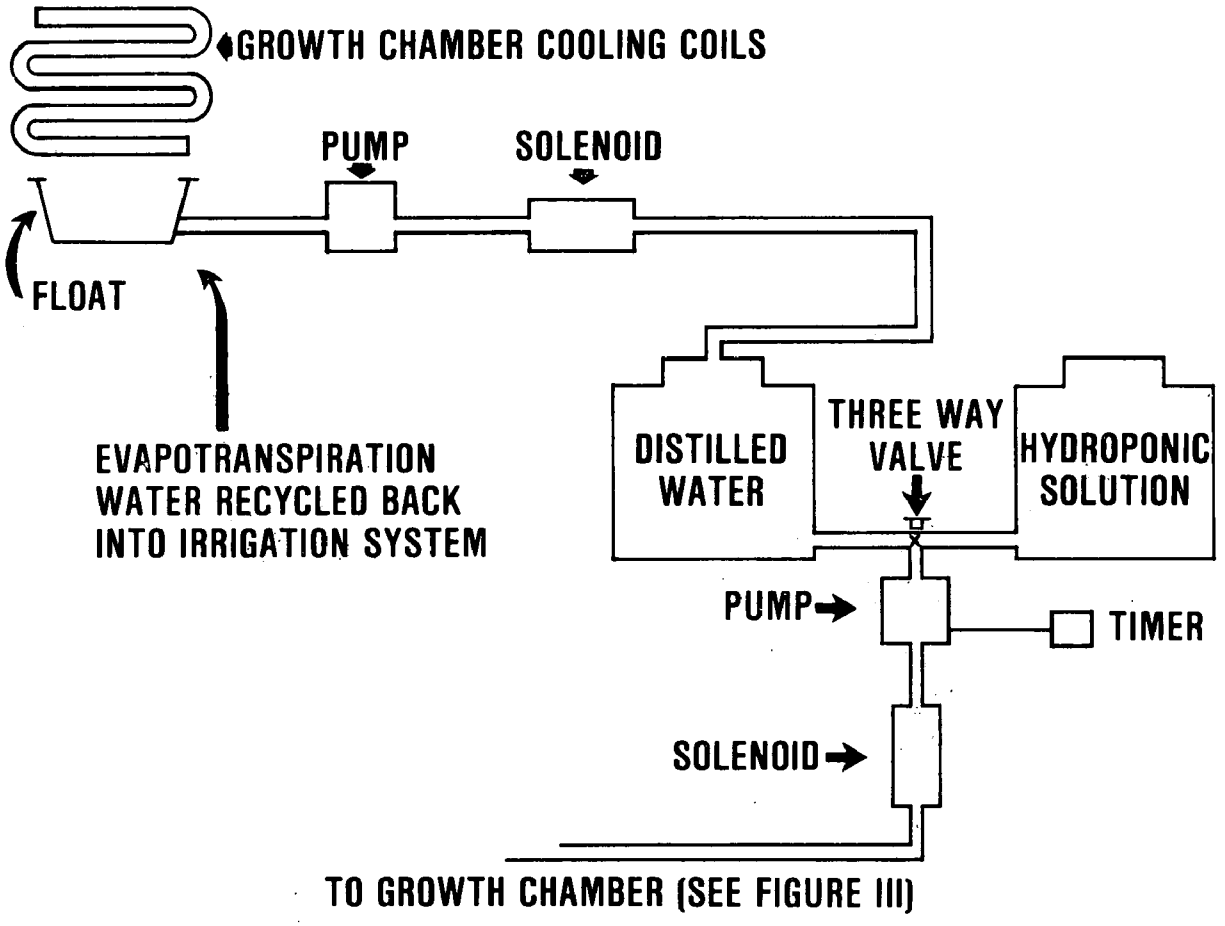

Figure 2. Schematic of the irrigation system used to recycle evapotranspired water, add nutrient solutions, and water plants growing in $^{238}PuO_2$ contaminated soil.

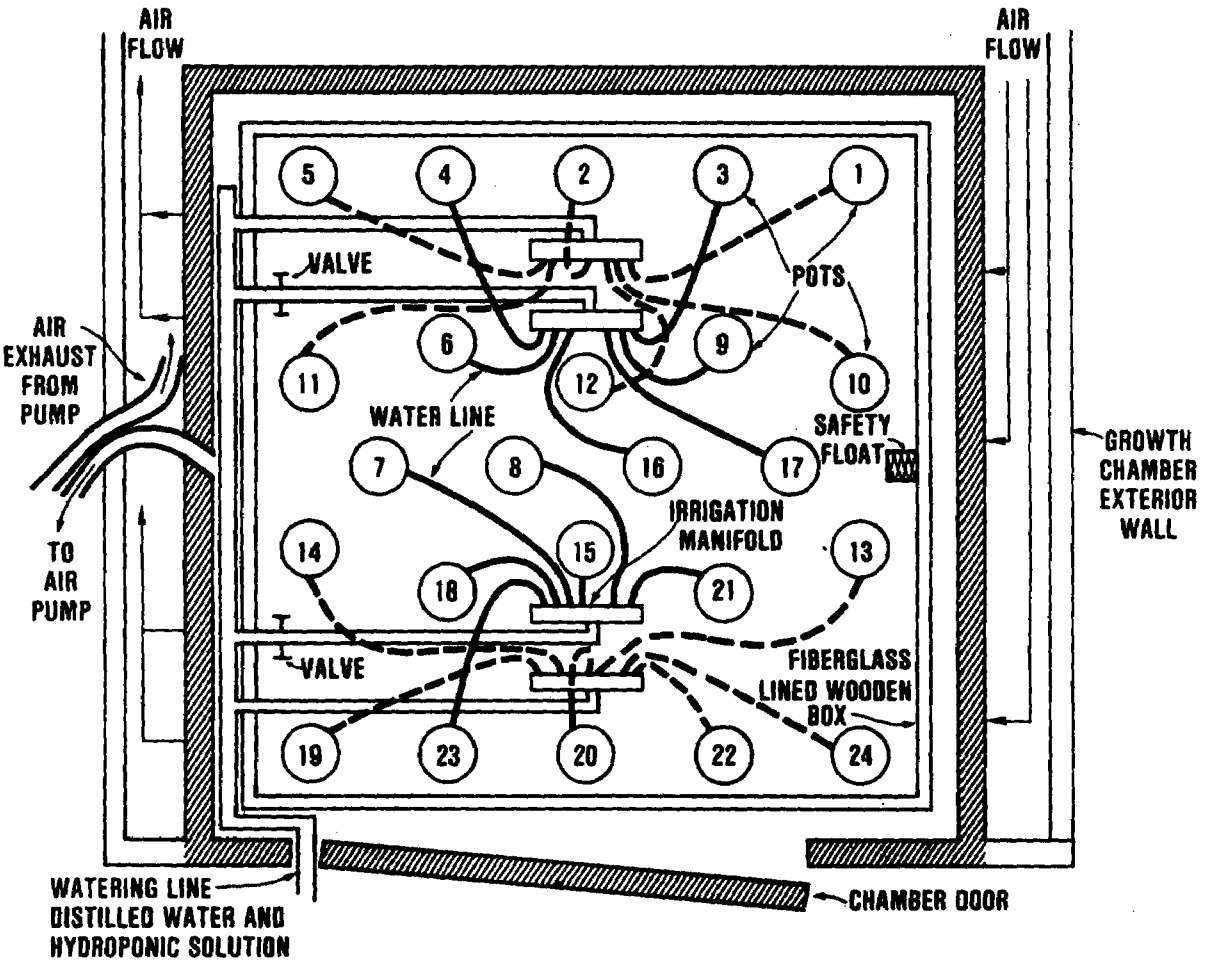

Figure 3. Design of the interior portion of the growth chamber showing the irrigation system, pot emplacement and direction of chamber air flow.

180 ml of water per day. Plant nutrients were provided by irrigating once every six weeks with a modified Hoagland solution as described by Berry (1971).

An air sampling system was constructed as a safety precaution to sample the chamber air. The air was pumped from the chamber through a Millipore® membrane filter having a pore size of 0.1 μm and then exhausted back in the chamber. Figure 3 shows the intake and exhaust ports of this system along with the direction of the air flow in relation to the pot placement within the chamber. The chamber air was sampled for approximately 6 hours at a flow rate of 12 liters per minute (lpm) This sampling was conducted 24 to 36 hours prior to entering the chamber. After the decay of naturally occurring radon, the filter was counted to determine if any of the plutonium-238 had become airborne.

The environmental growth chamber used for this study was specially constructed to conduct soil-plant kinetic studies involving selected chemical forms of radioisotopes. As a result, the chamber was virtually airtight with all the controls on the exterior. Throughout this investigation a chamber photo-period of 16 hours was maintained. The light-dark temperatures were kept at 25° C and 20° C, respectively. Carbon dioxide (CO_2) was automatically injected into the chamber atmosphere to maintain a uniform daytime concentration of 350 parts per million (ppm).

SAMPLES AND SAMPLE ANALYSIS

Soil samples were collected from each pot 2 days after the pots were placed in the chamber. This 2-day delay was to ensure that all of the soil in the pots was damp due to irrigation. Samples were collected by inserting a 10-cc disposable syringe, which had the bottom (needle attachment) end cut off, into the soil. The syringe was then withdrawn containing a 7- to 10-g (dry weight) core of soil. Using the syringe plunger, the soil core was removed and placed into an aluminum can. To avoid cross-contamination, 24 different syringes were used. The wet weight of each soil sample was determined and then they were dried in an oven at a temperature of 100° C to determine the dry weight. The cans were then sealed and sent to the Eberline Instrument Laboratories for plutonium-238 analysis.

The initial plant samples were collected on April 28, 1975. They were collected by clipping with scissors and then were placed into a small preweighed paper bag. The samples were weighed and then dried at a temperature of 75° C. The bags were individually sealed in aluminum cans and sent to the Eberline Instrument Laboratory for plutonium-238 analysis. These samples were not removed from the paper bag but were dissolved *in toto*. This procedure eliminated additional handling of plant material and therefore increased the precision of analysis. No attempt was made to separate the various plant organs, for example, stems from leaves. Following a period of regrowth, usually from 5 to 6 weeks, the plants were reharvested.

Basically, the analytical technique included decomposition of the soil and plant material by potassium fluoride fusion and/or acid dissolution. After decomposition, plutonium-236 was added as a tracer followed by the separation of the plutonium by ion exchange or solvent extraction. The

plutonium was electroplated and then counted by alpha spectroscopy.

RESULTS AND DISCUSSION

Two plant species, alfalfa, Medicago sativa, and lettuce, Lactuca sativa, were originally planted. Twelve pots, three replications of each of the four plutonium soil mixes, were sown with each species. All of the alfalfa germinated; however, only 3 of the 12 pots of lettuce germinated. As a result, after harvesting the lettuce, all 12 of the pots originally planted with this species were resown with radish, Raphanus sativus.

As previously stated, different plutonium concentrations constituted the four treatments used in this study. The soils were mixed in batches, divided into individual pots, and then subsampled for analysis. The results of the soil analysis for the soil plutonium-238 concentration are shown in Table 1. In soil treatments 1 and 2, the standard deviations were 13% and 12% of the means, and only 3% in treatments 3 and 4. These soil concentration values compare closely with the soil concentrations calculated and analyzed by gamma counting the ^{169}Yb. The results based on the ^{169}Yb concentration, as analyzed and reported by the Naval Ordnance Laboratory, for the four treatments were 24, 62, 94, and 310 nCi/g, respectively. The use of the ^{169}Yb as an analytical tool for determining the plutonium-238 concentration in the treatment 1 and 2 soils indicated that much less variation existed in each of these two mixes when compared to the soil plutonium-238 analyses, as the standard deviations were calculated to be only 7% and 6% of the means. It is assumed that the larger variability (13 to 12%) was the result of the ^{238}Pu analysis rather than in the soil preparation techniques. The variation in the treatment 3 and 4 soils was nearly identical after the two analyses.

TABLE 1. PLUTONIUM-238 CONCENTRATION IN SOILS

Treatment	^{238}Pu Concentration (nCi/g)
1	23 ± 3*
2	69 ± 8
3	92 ± 3
4	342 ± 10

*Standard deviation, σ, of six soil analyses.

Investigations involving the transfer of plutonium from soils to plants via root assimilation have shown that a large discrimination ratio (DR)

(DR) = $\frac{\text{Plutonium concentration in the plant nCi/g dry}}{\text{Plutonium concentration in the soil nCi/g dry}}$ exist. This ratio is presented in Table 2 for each of the three species. The high coefficient of variability in the alfalfa (about 47%) is thought to be the result of imprecision in plant analysis rather than variability of plant absorption. This conclusion was based on observing similar variability in plutonium analysis of aliquots of the same samples.

The magnitude of plutonium uptake appeared to be greater in both the lettuce and radishes than it did in the alfalfa as indicated by the discrimination ratios shown on Table 2. However, the ratios calculated for these two species are in most cases based on a single observation and are considered to be inconclusive as an indication of species differences or trends in plutonium incorporation.

TABLE 2. PLUTONIUM DISCRIMINATION RATIOS FOR ALFALFA, LETTUCE, AND RADISH PLANTS GROWN IN SOILS CONTAMINATED WITH $^{238}PuO_2$ SPHERES

Soil Concentration (nCi/g)	Discrimination Ratios		
	Alfalfa	Lettuce	Radish
23	$7.4 \pm 3.0^{*} \times 10^{-5}$	2.6×10^{-5}	3.4×10^{-4}
69	$7.5 \pm 4.0 \times 10^{-5}$	1.7×10^{-4}	1.2×10^{-4}
92	$8.8 \pm 4.0 \times 10^{-5}$	1.8×10^{-4}	4.2×10^{-5}
342	$7.4 \pm 3.8 \times 10^{-5}$		2.7×10^{-4}

*Standard deviation, σ, of ten discrimination ratios

Francis (1973) summarized the available literature and reported that the discrimination ratio generally falls between 10^{-4} and 10^{-6}. This large discrimination against plutonium absorption by plants has more recently been confirmed by Hansen (1975) and again summarized by Bernhardt and Eadie (1976). The alfalfa discrimination ratios shown in Table 2 are similar in magnitude to those previously reported. It is somewhat surprising to find that these values, which represent the absorption and translocation of plutonium from soils contaminated with discrete spheres of relatively insoluble PuO_2, are similar to those in which ionic and chelate-complexed plutonium were applied as the contaminant. Even experiments reported by McFarlane *et al.* (1976), in which plant roots were treated with plutonium in solution cultures, showed discrimination ratios against plutonium in the same general magnitude. This suggests that the chemical reactions which occur at the root surface predominate the kinetics of plutonium uptake and translocation in plants. Plants are known to exude organic compounds such as citric and humic acids which form strong chelates with plutonium. Based on the results of this study and the results from other investigations (Romney *et al.*, 1970), it seems possible that the release of citric acid and/or other similar chelating

compounds may be responsible for plutonium uptake.

If the absorption of plutonium by plants is dependent on the release of a particular compound or on the formation of some chemical complex at the root surface, it would explain why plutonium solubility in soil, water movement in plants, growth rate, and root contact potential appear to have little impact on plant assimilation of plutonium. This would also explain the tight grouping of discrimination ratios for dissimilar experiments where the chemistry of the contaminating plutonium was extremely different.

The growth rate (dry matter synthesis) of the alfalfa in this study increased with each successive cutting. This increase is evidence that the rooting systems were increasing in size, therefore, coming in physical contact with more potentially absorbable plutonium. A proportionate increase in total plutonium uptake and translocation was associated within this increased plant growth rate. However, the specific activity in the plant tissue remained unchanged and was apparently independent of time or root exposure. At one point in the experiment the growth chamber overheated due to a mechanical failure. Severe wilting occurred followed by the harvesting of the alfalfa stems and leaves. Subsequent growth was suppressed and damage to the root system was suspected. Despite this stress, no detectable change occurred in the rate of plutonium uptake, the specific activity, nor in the discrimination ratios.

For a number of reasons, a relatively few laboratory plant kinetics studies involving plutonium are conducted over an extended period of time. However, one study conducted and reported by Dr. Romney and his research associates in 1970, was in many aspects similar to this investigation. In their study, Nevada Test Site (NTS) soils contaminated with various chemical forms of plutonium were used as the rooting media. Although the specific chemical composition of plutonium in Dr. Romney's study was not known, Bretthauer et al. (1974), reported that NTS soils contain plutonium dioxide, silicates and organic particles of plutonium having diameters of less than 0.5 μm. Close similarities between the two investigations were that both were cropping studies conducted over a considerable length of time, and they both utilized plant species belonging to the leguminosae family.

Dr. Romney et al. (1970) reported that the Ladino clover that they grew and cropped over an extended 5-year period had plutonium discrimination ratios that varied from 1.9×10^{-5} to 14.0×10^{-5}. They also reported that a trend of increasing plutonium uptake by this plant species occurred with time. Their data support the hypothesis that plutonium incorporation by plants may be primarily dependent upon the release of chemical compounds from plant roots and/or from microbial action. Even though the magnitude of plutonium incorporation by the alfalfa was similar to that taken up by the Ladino clover as shown by the discrimination ratios, no similar increase of plutonium uptake by the alfalfa as a function of time was evident. The absence of this trend may have been due to the comparatively short duration of the alfalfa exposure which would reduce the amount of soil chemical formation.

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16. ABSTRACT

Three plant species--alfalfa, lettuce, and radishes--were grown in soils contaminated with plutonium-238 dioxide ($^{238}PuO_2$) at concentrations of 23, 69, 92, and 342 nanocuries per gram (nCi/g). The length of exposure varied from 60 days for the lettuce and radishes to 358 days for the alfalfa. The magnitude of plutonium incorporation as indicated by the discrimination ratios for these species, after being exposed to the relatively insoluble PuO_2, was similar to previously reported data using different chemical forms of plutonium.

Evidence indicates that the predominant factor in plutonium uptake by plants may involve the chelation of plutonium contained in the soils by the action of compounds such as citric acid and/or other similar chelating agents released from the plant roots.

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