Influence of Soil Plutonium Concentration on Plutonium Uptake and Distribution in Shoots and Roots of Barley

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A split-root (soil–nutrient solution) plant culture technique was employed to determine the effect of soil plutonium concentration on the uptake and distribution of Pu in the shoots and roots of barley. Although the absolute concentrations of Pu in plant tissues (micro-Curies of Pu/gram of plant tissue) decreased with decreased soil Pu concentration, the concentration factors (micro-Curies of Pu/gram of plant tissue per micro-Curies of Pu/gram of soil) were markedly higher below a soil Pu level of 10 μCi/g which was used previously to establish soil-plant concentration factors. This effect may have resulted from a concentration-dependent change in Pu availability to plants or from a toxic effect of Pu on the plants and subsequent inhibition of uptake. The concentration of Pu in roots exceeded the shoots by factors of 3 to 8 depending on soil Pu concentration, and Pu was shown to be translocated downward in the roots after uptake from the soil. The results indicate the advisability of further delineation of Pu uptake by plants at environmental levels of Pu with particular emphasis on root crops. Furthermore, studies should be undertaken to establish the role of roots in the transport of Pu in soil and the solubility, chemical form, and availability to plants of Pu released on decomposition of plant roots.

With increased production and use of plutonium which will accompany increased world dependence upon nuclear energy (Langham, 1971), the potential for environmental dispersion of Pu isotopes may increase. It is, therefore, essential that the potential for Pu entrance into the food web at the soil-plant level be assessed. However, investigations of the fate of Pu in soils and plants have been very limited (Price, 1973), likely due to major problems in handling and study of an isotope which is among the most toxic substances known to man and the results of early investigations which indicated that the solubility of Pu in soils is relatively low (Rhodes, 1957) limiting Pu mobility in soils and uptake by plants (Jacobson and Overstreet, 1948; Wilson and Cline, 1966).

An inherent difficulty in early laboratory studies of the plant uptake of Pu added to soils was the necessity of using relatively high soil levels of Pu (approximately 10 μCi/g) in order to have sufficient quantities of Pu in soils and plant tissues to be measurable by the techniques employed. At these levels there is a risk of chemical or radiation damage to the roots as has been observed for other radioelements in nutrient solution (Jacobson and Overstreet, 1948; Cline, 1967). If the effect of root damage was to limit plant uptake, previously accepted soil-plant concentration factors for Pu might be unrealistically low.

Measurements of Pu uptake by plants have emphasized the above-ground portions of plants because of the importance of the tops in food supplies for the crops investigated and the difficulties inherent in separating Pu-containing soil particles from roots in soil cultures or in separating adsorption from uptake in nutrient cultures. Knowledge of the concentration of Pu in roots is, however, essential because the occurrence of significant quantities of Pu in roots would necessitate (i) new emphasis on assessment of Pu in root crops consumed by man, (ii) further attention to solubility, chemical form, and subsequent availability to plants of Pu released on decomposition of roots, and (iii) assessment of root growth as a potential mechanism for movement of Pu in the soil profile. For these reasons preliminary studies using a split-root (soil–nutrient solution) technique were undertaken to determine the effect of Pu soil concentrations of 10 μCi/g and less on the distribution of Pu in barley shoot and root tissues.

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EXPERIMENTAL SECTION

A Ritzville silt loam surface (0–15 cm) soil was utilized for the plant studies. After sampling in sufficient quantity, the soil was screened (50 mm), air-dried (approximately 8% moisture), thoroughly mixed, and stored at 4°. Subsamples were removed for physicochemical characterization and subsequent plant studies. The soil was noncalcareous, exhibited a pH of 7.0, and contained 0.7% organic C. All results are reported on the basis of oven-dry (110°) soil.

A standard solution of $Pu(NO_3)_4$ prepared in 2.0 *M* HNO_3 was employed for soil amendments. The total α activity of the standard solution was due primarily to ^{239}Pu, but in addition the solution contained other isotopes which contributed to total α activity, including ^{240}Pu (21.2%), ^{238}Pu (5.8%), and ^{241}Am (4.0%). The Pu isotopes constituted 99.9% of the radioisotopes present in solution on a weight basis.

A subsample (50 g) of the air-dry soil to be employed at each treatment level (800 g) was removed and mixed with (i) sufficient $CaCO_3$ (less than 0.4 g) to neutralize the HNO_3 to be added in the Pu standard solution and (ii) the standard solution to levels of 0.05, 0.5, and 10 μCi of ^{239}Pu/g of soil. The amended soil was dried (4 hr, 60°) and thoroughly mixed (4 hr) with the original air-dry soil in a V-blender. After dry mixing, the soil was mixed with sufficient H_2O to bring the soil to 22% H_2O. Triplicate subsamples of soil (100 g) at each treatment level were subsequently placed in containers used for plant uptake studies.

Barley (*Hordeum vulgare* var. Vangard) was employed in all studies. Modified Neubauer (Wilson and Cline, 1966) and split-root (Brown *et al.*, 1959) methods were used in plant culture. In the modified Neubauer method, the amended soil (100 g) was placed in a crystallizing dish (12.5 cm diameter, 6.5 cm depth) and planted (100 seeds). Following planting, the soil was covered with washed quartz sand (50 g) and the entire unit covered with a glass plate to maintain humidity during germination. The glass plates were removed after the plants reached 3 cm in height. Sufficient distilled H_2O was added on a daily basis to maintain the original water content. To ensure maximum growth, NH_4NO_3 was added (0.2% w/w) as a fertilizer in the irrigation water when the plants were 5 cm in height. The plants were cultured in a growth chamber under constant conditions of temperature (27°, light; 20°, dark), light (14 hr light, 4000 ft-candles; 10 hr dark),

Table I. Influence of Soil Pu Concentration on the Yield of Barley Shoots Cultured by Different Methods and on Uptake of Pu by Barley Shoots and Roots

			Plant uptake of Pu[a,c]					
			Shoots					
	Yield of plant shoots, g[a,b]		Neubauer method		Split-root method		Roots	
Soil Pu concn, μCi/g	Neubauer method	Split-root method	Concn, μCi/g × 10^{-4}	Concn factor[d]	Concn, μCi/g × 10^{-4}	Concn factor[d]	Concn, μCi/g × 10^{-4}	Concn factor[d]
10.00	1.5	4.9	5.5	0.55	5.4	0.54	36	3.6
0.50	1.3	5.1	1.1	2.1	0.95	1.9	3.0	6.0
0.05	1.5	3.9	0.28	5.6	0.07	1.4	0.55	11

[a] Based on oven-dry (60°) weight. [b] Yield of controls 1.0 and 4.3 g for Neubauer and split-root methods, respectively. [c] Mean standard errors were ±13, 39, and 52% (n = 3) for soil Pu levels of 10, 0.50, and 0.05 μCi/g, respectively. [d] (μCi of Pu/g of oven-dry plant tissue per μCi of Pu/g of oven-dry soil) × 10^{-4}.

and humidity (40–45%). The plant tops were harvested after 30 days of growth, dried (24 hr, 60°), and subjected to radiochemical analysis.

The split-root technique involved culture of plants in a closed container (7 cm diameter) which contained soil over a nutrient solution (Hoagland and Arnon, 1950) each in separate compartments separated by a layer of quartz sand, a stainless steel screen, and a small air space. The amended soil (100 g) was planted with 33 seeds and covered with washed quartz sand (30 g) and the entire unit covered with a glass plate. The glass plates were removed after the plants reached 3 cm in height. The nutrient solutions were changed at 3-day intervals and analyzed for Pu. No Pu was ever detected in the nutrient solutions. The plants were cultured and the plant tops were harvested and treated as previously described. The root mass was dipped twice in distilled water, allowed to drain, and then cut below (2.5 cm) the screen level and treated in the same manner as the tops.

The plant tissues from both experiments were digested (85°) in a 2:1 solution of $HClO_4$ (70%) and HNO_3 (70%), and the solution was evaporated to near dryness. The moist residues were redissolved in HNO_3 (2 M). Total α activity in this solution was determined by evaporation of an aliquot on stainless steel planchets and counting in a 2π gas flow proportional counter. Plutonium was obtained by correction of the total α activity for α activity due to ^{241}Am. The ^{241}Am was determined by counting (60 keV, γ) an aliquot of the solution in a NaI(Tl) well crystal. The lower detection limit for Pu was 5×10^{-7} μCi/g of dry plant tissue.

RESULTS AND DISCUSSION

Previous studies on a calcareous silt loam soil containing Pu at a level of 10 μCi/g and using the modified Neubauer plant culture technique (Wilson and Cline, 1966) indicated that the concentration of Pu in barley shoots was of the order of 4×10^{-4} μCi/g. The Neubauer method as employed may be generally considered to give a near optimum uptake of Pu from soil due to fertilizer supplements and the high ratio of root biomass to soil. Uptake of Pu by barley shoots from noncalcareous silt loam soil measured using the modified Neubauer method in the present studies amounted to 5.5×10^{-4} μCi/g of dry plant tissue and thus agreed very well with earlier studies (Table I). Furthermore, although the use of the split-root method resulted in higher yields of plant shoots due to the additional supply of nutrients available in the rooting media, Pu uptake measured by this method agreed with the Neubauer method, particularly at soil Pu levels of 10 and 0.50 μCi/g (Table I). Thus, the comparative studies suggest that the split-root method as employed would serve as a suitable replacement for the modified Neubauer method resulting in near optimum uptake by shoots of Pu applied to soil. In addition, the split-root technique has the advantage of providing roots free of soil for subsequent analyses. Because conditions for plant uptake are optimized, the methods likely serve to estimate the potential for uptake in the field, and the results cannot be taken as representative of uptake under field conditions.

Plant yield was unaffected by soil Pu concentration (Table I). Although variability within treatments increased with decreased soil Pu concentration, it was evident that the concentration of Pu in barley shoots and roots decreased with decreased soil Pu concentration regardless of the culture method employed (Table I). However, the concentration factor (micro-Curies of Pu/gram of tissue per micro-Curies of Pu/gram of soil) increased with decreased Pu concentration. This effect may have resulted from a concentration-dependent change in Pu availability or from a toxic effect of Pu on the plant which had the effect of reducing uptake. In view of the known toxicity of Pu to yeast cells (Bair and Hungate, 1958) and the lack of known specific chemical or microbiological mechanisms to effect an alteration of Pu solubility in soil, the latter explanation would appear the most likely. However, it should be noted that the potential exists for microbial alteration of Pu form and availability in soil and the observed effects could be explained on the basis that microbes which participated in solubilization were susceptible to Pu at the higher soil concentration levels. Investigations to determine the effect of Pu on the soil microflora and to evaluate their potential role in solubilization of Pu are presently underway.

Regardless of the mechanism for the observed increase in Pu concentration factors at the lower soil concentration levels, it must be emphasized that most estimates of Pu hazard to man (*e.g.*, Langham, 1971) are based on concentration factors of approximately 5×10^{-5} derived largely from studies at the higher soil Pu levels (*e.g.*, Wilson and Cline, 1966). The results therefore suggest that new emphasis be placed on determination of Pu uptake by plants from soils containing Pu at environmental levels and re-evaluation of previously accepted concentration factors.

The Pu levels in barley roots differed markedly from the shoots with levels of Pu exceeding the tops by factors of 3 to 8, depending upon soil Pu concentration (Table I). Autoradiographs indicated that in contrast to the shoots where Pu was concentrated near the crown, Pu was distributed over the entire length of the root. In the system employed, Pu was not added to the nutrient solution in which the roots were grown nor was Pu detected in the nutrient media. Thus, the Pu in the roots originated from the soil and was translocated downward from the soil in the root system. At the high root level of Pu, less than 3% of the total Pu associated with the roots would have been detected if released to the nutrient solution. The lack of detectable quantities of Pu in the nutrient solution therefore suggests that Pu was bound strongly to the root tissue. The implications of these findings are also important

in terms of evaluation of Pu hazards in the environment because (i) root crops directly consumed by man may contain Pu at levels exceeding those found in other crop plants in which the tops are consumed, (ii) Pu, considered largely immobile in soil (Francis, 1973), may be distributed much further down the soil profile than previously expected due to its mobility in the plant root system, and (iii) the potential exists that decomposing roots may represent a significant source of Pu of different solubility and plant availability than the Pu directly entering the soil environment. The possibility exists that observed (Romney *et al.*, 1970) increases with time in uptake of Pu by successive crops of ladino clover grown on soil contaminated with fallout resulted from this latter phenomenon. In order to provide a better understanding of the fate and hazard of Pu in the environment, it is essential that research be directed toward determination of (i) the uptake of Pu by a broad range of plants from representative soil types containing Pu at environmental levels with emphasis on root crops, (ii) the potential for recycling of Pu present in plant roots, and (iii) the form and behavior of Pu in soils and plants.

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Major Alkaloids of a *Claviceps* Isolated from Toxic Bermuda Grass

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Submerged cultures of a *Claviceps* isolated from toxic *Cynodon dactylon* (L.) Pers. (common Bermuda grass) produced an alkaloid fraction consisting of greater than 45% ergot-type alkaloids (colorimetrically determined as ergonovine malate). Multidevelopment thin-layer chromatography of the alkaloid extract from the nutrient medium revealed ergonovine and ergonovinine as the major alkaloids produced by *Claviceps* sp. strain 178 (30 and 22%, respectively). Penniclavine and chanoclavine I were also identified. Evidence presented might implicate *Claviceps* in the etiology of the nervous disorder, "Bermuda grass tremors," which occurs in cattle grazing both common and coastal Bermuda grass.

Over the past 20 years in several southern states, a toxic syndrome has occurred in cattle grazing common and coastal Bermuda grass, *Cynodon dactylon* (Atwood, 1953; Gibbons, 1953; Fichte, 1972; Porter *et al.*, 1973b; Whitehair *et al.*, 1951). Commonly known as "Bermuda grass tremors," the disease is characterized by a general nervousness in cattle which varies from a slight twitching or palsy of the muscles in the shoulders and flank regions, to an inability to stand or walk because of an apparent posterior paralysis (Kingsbury, 1964).

Examination of toxic *Cynodon dactylon* for fungi resulted in the isolation of two unidentified strains (174 and 178) of *Claviceps* sp. (Porter *et al.*, 1973b). Analyses have shown strain 178 to be an ergot producer (Porter *et al.*, 1973a), and the effects of ergot alkaloids on humans and ruminants are well documented (Bové, 1970; Mantle, 1969). Ergotism may be divided into two broad categories: a nervous or convulsive form and a gangrenous form (Bové, 1970). Nervous or convulsive ergotism is a syndrome analogous to that found in cattle with Bermuda grass tremors (Brown and Ranck, 1915; Kingsbury, 1964; Nicholson, 1971).

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The present investigation was prompted by the importance of *Cynodon dactylon* as a forage crop in the South, and its subsequent importance to the commercial production of beef and dairy products.

MATERIALS AND METHODS

Organism. The *Claviceps*, strain 178, used in this study was originally isolated from sclerotia obtained from common Bermuda grass, *Cynodon dactylon* (L.) Pers., and was maintained on potato–dextrose agar slants in closed screw cap tubes at 2–4°.

Growth of Cultures and Preparation of Inocula. The general procedure for submerged cultivation of strain 178 was a modification of a three-stage fermentation method (Pacifici *et al.*, 1963). Flasks (500 ml with three baffel indentions and stainless steel caps) containing 100 ml of medium were inoculated with 10-day-old mycelium from a potato–dextrose agar slant and incubated in the dark at 24–26° on a gyratory shaker, 200 rpm (1-in. stroke) for 12 days. The medium (PS) for this first stage consisted of potato–dextrose broth (Difco), 24 g; succinic acid, 10 g; mannitol, 20 g; and concentrated ammonium hydroxide to pH 5.2 and 1000 ml of distilled water. Ten milliliters of this submerged culture, prepared as described, was used to inoculate a second shaking flask containing 100 ml of PS medium. After the second submerged culture had grown for 6 days, 2 ml was used as inoculum for alkaloid