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Terrestrial Plant Species"

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PHYTOREMEDIATION OF RADIONUCLIDE CONTAMINATED SOILS – FIELD, GREENHOUSE, AND LABORATORY STUDIES

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Introduction

For the past three years, our laboratory has been involved in a collaborative project with the U.S. Department of Energy (USDOE) and MSE Technology Applications, Inc., focusing on a range of issues related to the phytoremediation of heavy metal- and radionuclide-contaminated soils. While much of our research has been fundamental in nature, involving physiological and molecular characterizations of the mechanisms of hyperaccumulation in plants, we are also investigating more practical issues related to phytoremediation. A central issue in this latter research has been the identification of amendments capable of increasing the bioavailability and subsequent phytoextraction of radionuclides. The results described below detail these efforts for uranium and ^{137}Cs . We also conducted a study on a ^{137}Cs -contaminated site at Brookhaven National Laboratory (BNL), which allowed us to apply our laboratory and greenhouse results to a field setting.

Laboratory and greenhouse studies on the bioavailability and uptake of uranium in plants

The development of a phytoremediation strategy for U-contaminated soil has proven to be a challenging problem, as the complex, pH-dependent speciation of this element in soils makes the study of U uptake by plants difficult. U is present in the soil primarily (80-90%) in the +VI oxidation state as the uranyl (UO_2^{2+}) cation. While soil conditions influence the formation of soluble U carbonate, hydroxide, or phosphate complexes^{1,2}, the free uranyl cation typically sorbs to soil solids and organic matter, reducing its bioavailability^{3,4}. Preliminary

research in our laboratory has shown, however, that the uranyl cation, which predominates only at pH <5.5 (Fig. 1), is the form of U most readily accumulated by plants⁵ (Fig 2).

In terms of phytoremediation, the initial implication of these results was that for U contaminated soils with pH values greater than 6, acidification may be required in order to facilitate U phytoextraction. Furthermore, due to the sorption of the free uranyl cation to soil particles, a soil amendment capable of both solubilizing and chelating U may be required as well. To test this, samples of soil obtained from a U-contaminated site in Ashtabulah, OH, were incubated for 24 days in the dark in the presence of compounds which decreased the soil pH (HCl, elemental S, ammonium sulfate) or with chelates that could solubilize and bind U (HEDTA, citric acid, oxalic acid). The results indicated that soil acidification did not increase U solubility significantly. In contrast, citric acid was highly effective in desorbing U, solubilizing 270 mg U kg⁻¹ soil (~30% of the total U) after only 3 days of incubation⁶ (Fig. 3). However, the increase in U solubility was transient with U solubility decreasing to levels comparable to the control soil by the end of the experiment (Fig. 4). This decrease in citrate was most likely due to the microbial degradation of citrate over the 24 days of the experiment.

A subsequent soil experiment was conducted to examine in greater detail the effect of pH and citric acid concentration on the solubilization of U and to determine whether the degradation of citric acid over time resulted in a decrease in U solubility. A 4x4 factorial design was used, consisting of four soil pH values (4, 5, 6, 6.8) and four citrate buffer concentrations (0, 10, 50, and 100 mM). It was found that U solubilization was directly related to citrate concentration and, to a lesser extent, soil pH. After only 6 hours of incubation at pH 5.0, for example, the 100 mM citrate treatment solubilized >750 mg U kg⁻¹ soil, corresponding to ~83% of the total U in the soil² (Fig. 5). This experiment also confirmed that the duration of increased solubility was primarily related to the rate of citrate decomposition (Fig. 6). In the pH 5 soil, there was >95% degradation of citrate (10 mM citrate) after 96 hours. Concurrent with this decrease in citrate was a sharp drop in soluble U.

A subsequent pot experiment was conducted to determine if application of citric acid to U-contaminated soil increased U phytoextraction along with U solubility. Beets (*Beta vulgaris*) were grown for eight weeks in a U-contaminated soil. One week prior to harvest, the soil was amended with either citric acid (10 mg citrate kg⁻¹ soil) or, for comparative purposes, HEDTA (2.5 g kg⁻¹ soil). As in the soil incubation experiments, the addition of HEDTA did not increase U solubility significantly (Fig 7). Likewise, HEDTA had no effect on shoot biomass (Fig. 8) or U accumulation (Fig 9). Citric acid application, however, significantly increased U solubility (Fig. 7) and induced U hyperaccumulation in plant shoots, increasing the shoot U concentration from 15 to 209 mg U kg⁻¹ d.w. (Fig. 9). The U bioaccumulation ratio (shoot [U]/soil[U]) of 0.67 after citrate treatment was 10-100-times greater than previous values in the literature^{4,7-9}.

Bioavailability and phytoremediation of ¹³⁷Cs from contaminated soil: From the laboratory to the field

The phytoremediation of radionuclides such as ¹³⁷Cs and ⁹⁰Sr represents a fundamentally different problem from heavy metal and uranium contamination in that the nature of the contaminant to be remediated differs. For metals and uranium, the goal of remediation is to decrease the *total* level of the contaminant to below the regulatory limit. For ¹³⁷Cs and ⁹⁰Sr, the goal is to decrease the level of the *radionuclide* to below the regulatory limit. In the latter case, the target of phytoremediation is the radioisotope, which is present against a background of naturally-occurring, non-radioactive isotopes of Cs and Sr and high concentrations of their respective plant nutrient analogs, K and Ca.

A hydroponic experiment demonstrated that plants grown in a nutrient solution supplemented with 1 μM CsCl were capable of accumulating significant levels of Cs¹⁰ (Table 1). The observed bioaccumulation ratios, ranging from 40 to 165, were comparable to those reported in another study¹¹. These results suggested that plants can readily take up and translocate ¹³⁷Cs, provided that the Cs⁺ cation is available in the soil solution. We have subsequently found that there was a significant species-dependent variability in the ability of plants to accumulate ¹³⁷Cs

from contaminated soil. A pot study using soil from a contaminated site at Brookhaven National Laboratory (BNL) demonstrated that the accumulation of ^{137}Cs by the shoots of the plants species followed the order: cabbage (*Brassica oleracea*) > tepary bean (*Phaseolus acutifolius*) > Indian mustard (*Brassica juncea*) > reed canarygrass (*Phalaris arundinacea*) (Fig 10).

As with U, the phytoremediation of ^{137}Cs is limited by its availability in the soil. ^{137}Cs can become selectively bound within the crystal lattice of layer silicate minerals such as smectitic clays¹². This sorption greatly reduces the solubility and uptake of ^{137}Cs into plant roots, particularly in soils that have been contaminated with ^{137}Cs for long periods of time¹³. We have screened a variety of soil amendments for their ability to desorb ^{137}Cs from the BNL soil and have found that the ammonium salts are the most effective, solubilizing 10-25% of the total ^{137}Cs in the soil¹⁰ (Table 2). Further experiments have shown that the desorption of ^{137}Cs is dependent on the NH_4^+ concentration (Fig. 11). We have also shown that the addition of NH_4NO_3 to contaminated soil elicited a 2-12-fold increase in the accumulation of ^{137}Cs in the shoots of the four species listed above¹⁰ (Table 3).

Following these studies, a field trial was conducted to investigate the phytoremediation potential of three plant species on a ^{137}Cs -contaminated site at BNL. From the 1940's to the 1950's, this site received ^{137}Cs from stored activated experimental parts and spent fuel rods. As a result, the ^{137}Cs activity in this highly acidic (pH=3.8) loamy-sand soil ranges from 80 to 900 pCi g⁻¹ soil. The field trial involved a random split plot design, utilizing three species and three amendment concentrations (0, 0.1, and 0.2 M NH_4NO_3). The four replicate blocks each consisted of nine 0.25 m² cells. The three species used were red root pigweed (*Amaranthus retroflexus*), Indian mustard, and tepary bean. The results of this field study demonstrated that *Amaranthus* accumulated approximately 40-fold more ^{137}Cs in its shoots than did Indian mustard or tepary bean¹⁴. This greater ^{137}Cs phytoextraction by *Amaranthus* was due to both a higher concentration of ^{137}Cs in the shoot and a greater production of shoot biomass (Fig. 12). The bioaccumulation ratio of ~3.0 obtained for this species (Fig. 12), achieved after only 9 weeks of growth, is several orders of magnitude greater than most values reported in the literature¹⁵⁻²⁰.

The amount of ^{137}Cs removed from the soil by the shoots of *Amaranthus retroflexus* represented ~3% of the total ^{137}Cs . Based on this removal rate and assuming a sustained rate of phytoextraction, it was projected that with two croppings of pigweed per year, remediation of this site could be feasible within 15 years¹⁴.

Conclusions

The results presented above suggest that phytoremediation may be a viable remediation option for radionuclide-contaminated soils. Using the amendments described above, it will be possible to greatly enhance the phytoextraction of U and ^{137}Cs . The use of NH_4NO_3 to increase ^{137}Cs solubility clearly needs to be optimized, as indicated by the results obtained from the field study at BNL.

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Table 1. Accumulation of cesium in shoots of plants grown on a nutrient solution containing $1 \mu\text{M}$ CsCl.

Plant Species	Shoot mean Dry wt. (g)	[Cs] in shoots $\mu\text{g g}^{-1}$ dry wt.	Bioaccumulation ratio ^a	Total Cs accumulated in shoot (μg)
Broccoli	0.062	14	105	0.87
Cabbage	0.075	22	165	1.65
Cauliflower	0.065	14	105	0.91
Colonial bent grass	0.043	6	45	0.26
Hairy vetch	0.071	15	113	1.11
Indian mustard	0.075	16	120	1.22
Reed canarygrass	0.060	6	45	0.36
Red fescue	0.028	5	38	0.14
Tepary bean	0.079	19	143	1.50

^a $[\text{Cs}]_{\text{shoots}}/[\text{Cs}]_{\text{solution}}$

Table 2. Effect of several chemical compounds on ^{137}Cs desorption from contaminated soil.

	Ammonium salts					Potassium salts			Organic acids					Mineral acids		Reducing agents	
	NO_3^-	Cl^-	SO_4^{2-}	Acetate	Acetate-EDTA	NO_3^-	Cl^-	SO_4^{2-}	Formic	Acetic	Oxalic	Malic	Citric	HCl	HNO_3	Na-Dithionite	Hydroxylamine-HCl
	% ^{137}Cs desorbed from the dry soil ^a																
0.05 M	15	12	15	- ^b	-	7	8	6	0	0	0	0	0	1	2	1	3
0.5 M	24	25	22	-	-	15	14	14	0	0	0	0	0	3	2	1	7
1.0 M	-	-	-	17	18	-	-	-	-	-	-	-	-	-	-	0	-

^a ^{137}Cs activity in the BNL soil was ~400 pCi g dry soil.

^b Data not available.

Table 3. Effect of soil treatment on ^{137}Cs accumulation^a in shoot of four plant species.

Soil treatment	Indian mustard ^b		cabbage		Tepary bean		reed canarygrass	
	pCi pot ⁻¹	%	pCi pot ⁻¹	%	pCi pot ⁻¹	%	pCi pot ⁻¹	%
Control (no NH_4NO_3)	37	100	69	100	28	100	1	100
40 mmols NH_4NO_3	61	165	110	159	100	357	18	1200
80 mmols NH_4NO_3	64	173	140	203	118	421	12	1800

^a Determined as: $[\text{Cs}]_{\text{shoot}} \times \text{shoot biomass}$

^b Each pot contained two plants from each species

Figure 1. U speciation in a modified Johnson's nutrient solution in the absence of P, as predicted by GEOCHEM-PC.

Figure 2. Uranium concentration in roots and shoots of peas (cv. 'Sparkle') exposed to 5 μMU for seven days at 3 different pH values.

Figure 3. Soluble U in the Ashtabula, OH., U-contaminated soil after three days of incubation in the presence of soil amendments. “Am sulfate” refers to the addition of ammonium sulfate. “HCl+citric” and “HCl+oxalic” refer to combined treatments of HCl with citric or oxalic acid. Total soil U = 909 mg kg⁻¹.

Figure 4. Soluble U in the Ashtabula, OH., U-contaminated soil after 24 days of incubation in the presence of soil amendments. "Am sulfate" refers to the addition of ammonium sulfate. "HCl+citric" and "HCl+oxalic" refer to combined treatments of HCl with citric or oxalic acid. Total soil U = 909 mg kg⁻¹.

Figure 5. U solubilization after 6 hours of incubation as a function of soil pH and initial citrate buffer concentration.

Figure 6. Relationship between citrate degradation and uranium solubility at pH 5.0.

Figure 7. Soluble U in pots of U-contaminated soil amended with either HEDTA or citric acid. Total soil U = 340 mg kg⁻¹.

Figure 8. Shoot biomass for beet grown for five weeks in pots of either unamended U-contaminated soil or soil amended with HEDTA or citric acid.

Figure 9. Shoot U concentration for beets grown for five weeks in U-contaminated soil amended one week prior to harvest with either HEDTA or citric acid. Total soil U = 340 mg kg^{-1} .

Figure 10. Effect of application of NH_4NO_3 solution to contaminated soil on ^{137}Cs bioaccumulation ratio in shoots of 40-day-old plants. Data point represent means and standard errors of four replicates.

Figure 11. Concentration-dependent desorption of ^{137}Cs from aged contaminated BNL soil. Soil was incubated in NH_4NO_3 , NH_4Cl , or KNO_3 solution at the concentrations shown. After 2 h, the slurry was filtered and gamma activity in the filtrate measured. Data points represent means and standard errors of four replicates.

Figure 12. ^{137}Cs concentration in shoots (A), shoot biomass (B), and ^{137}Cs bioaccumulation ratios in shoots (C) of three plant species grown in HWMF/BNL soil treated with 0, 0.1, or 0.2 M NH_4NO_3 .

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