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**Title: Phytoremediation of a radiocesium-contaminated soil. Field evaluation of ^{137}Cs
bioaccumulation in the shoots of three plant species**

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ABSTRACT

A field study was conducted to investigate the potential of three plant species for phytoremediation of a ^{137}Cs -contaminated site. From the contaminated soil, approximately 40-fold more radiocesium was removed in shoots of red root pigweed (*Amaranthus retroflexus* L.) compared with those of Indian mustard (*Brassica juncea* (L.) Czern) and tepary bean (*Phaseolus acutifolius* A. Gray). The greater potential for ^{137}Cs removal from the soil by *Amaranthus* was associated with both high concentration of radiocesium in shoots and high shoot biomass production. Approximately 3% of the total ^{137}Cs was removed from the top 15 cm of the soil (which contained **most** of the soil radiocesium) in shoots of three-month-old *Amaranthus* plants. Soil leaching tests conducted with 0.1 and 0.5 M NH_4NO_3 solutions eluted as much as 15 and 19%, respectively, of the soil ^{137}Cs . Addition of NH_4NO_3 to the soil, however, had no positive effect on ^{137}Cs accumulation in shoots in any of the species investigated. It is proposed that either NH_4NO_3 solution quickly percolated through the soil before interacting at specific ^{137}Cs binding sites or radiocesium mobilized by NH_4NO_3 application moved below the rhizosphere becoming unavailable for root uptake. Further research is required to enhance the phytotransfer of the NH_4NO_3 -mobilized ^{137}Cs . With two croppings of *Amaranthus* per year and a sustained rate of extraction, phytoremediation of this ^{137}Cs -contaminated soil appears feasible in less than 15 years.

INTRODUCTION

^{137}Cs is a long-lived byproduct of nuclear fission. Soils have become contaminated with ^{137}Cs following nuclear testing, accidental release or nuclear energy production. Because of its slow decay ($t_{1/2}$ 30.2 years), radiocesium poses a serious threat to populations inhabiting the contaminated environment. This concern is exacerbated by low mobility of ^{137}Cs in the soil even under high rainfall. Because of the high cost, decontamination of large areas polluted with ^{137}Cs by conventional engineering methods remains an intractable problem. In addition, these methods negatively affect physicochemical properties of the soil and drastically disturb the landscape and ecosystems. Phytoremediation is emerging as an alternative technology to high-cost energy-intensive conventional methods. At the core of this novel approach is the cultivation of higher plants capable of accumulating in shoots high levels of contaminants from polluted soils. Pollutants are subsequently removed by harvesting above ground plant tissues. Because it is cost efficient and decontaminates soil in-situ without disturbing the ecosystem, phytoremediation has been cited as the method of choice particularly for the clean up of large areas polluted with moderate levels of contaminants (Baker et al., 1994). Although the potential for phytoremediation of some heavy-metal polluted soils has been previously documented (Baker et al., 1994; Brown et al., 1994; Mc Grath et al., 1996; Ebbs et al. 1997), little has been published regarding the use of plants to remediate radionuclide-contaminated soils.

Several earlier studies have reported that higher plants possess the ability to accumulate ^{137}Cs in shoots (Dahlman et al., 1975; Salt et al., 1992; Entry et al., 1993; Demirel et al., 1994; Nisbet and Shaw, 1994). Following entry into symplasm, cesium is known to be highly mobile

1 within internal plant tissues (Resnik et al., 1969). In support of this, bioaccumulation ratios
2 ($[^{137}\text{Cs}]_{\text{shoot}}/[^{137}\text{Cs}]_{\text{solution}}$) significantly greater than 1 were reported in shoots of grasses (Smolders
3 and Shaw, 1995; Lasat et al., 1997) and a variety of dicot species (Lasat et al., 1997) grown in
4 hydroponic culture. However, accumulation of Cs from the soil into plant shoots is usually quite
5 limited. Frequently, bioaccumulation ratios considerably lower than 1 have been reported for
6 plants grown in radiocesium-contaminated soil (Dahlman et al., 1975; Nisbet and Shaw, 1994;
7 Varskog et al., 1994). A major factor limiting radiocesium uptake into roots is its strong retention
8 to soil particles (Cremers et al., 1988). The extent of cesium fixation to the clay minerals was
9 shown to depend upon the physicochemical properties of the soil (Francis and Brinkley, 1976;
10 Kirk and Staunton, 1989). Previous results (Jackson et al., 1965; Lasat et al., 1997) have shown
11 that soil-fixed radiocesium can be desorbed to some extent by the monovalent cation NH_4^+ and to
12 a lesser extent by K^+ . Subsequently, however, these cations might compete with cesium for
13 uptake into roots (Shaw and Bell, 1991).

14 In a field trial, we investigated the potential for ^{137}Cs extraction by three plant species
15 grown in a contaminated soil at the Hazardous Waste Management Facilities (HWMF) at
16 Brookhaven National Laboratory (BNL). The effect of ammonium nitrate application on ^{137}Cs
17 accumulation from soil into shoots was also investigated. In addition, because the potential for
18 phytoremediation could be affected by radiocesium movement down the soil profile, leaching
19 studies were conducted to investigate whether ^{137}Cs is mobilized when water or ammonium
20 nitrate solution percolates through the soil.

MATERIALS AND METHODS

Field study

Some of the physicochemical properties of the radiocesium contaminated soil at HWMF/BNL are shown in Table 1.

Lime (0.6 kg/m^2) and N:P:K (16-16-16) fertilizer (Green Charm/Walmart) (28 g/m^2) were applied to the soil surface and incorporated by rototilling to a depth of 15 cm. The experimental site was fenced and divided into four replicate blocks of nine plots each. Plots were $0.5 \text{ m} \times 0.5 \text{ m}$ in size and distanced at 0.5 m apart. Each of the three species tested: Indian mustard (*Brassica juncea* (L.) Czern), red root pigweed (*Amaranthus retroflexus* L.) and tepary bean (*Phaseolus acutifolius* A. Gray), were grown in three plots within each block. Indian mustard was directly seeded into the assigned plots ($250\text{-}300 \text{ seeds/m}^2$). At the same time two-week-old *Amaranthus* and tepary bean seedlings were transplanted into the field at a density of 60 plants/m^2 . The site was irrigated to maintain a soil moisture of about 80% of field capacity. Seven weeks after planting, in each block of the three plots cultivated with the same species, one set received 4 L of water, the second 4 L of a $0.1 \text{ M NH}_4\text{NO}_3$ solution, and the third 4 L of a $0.2 \text{ M NH}_4\text{NO}_3$ solution. These treatments were repeated two weeks later. After 10 days, the number of plants in each plot was determined, shoot tissues were harvested by cutting the stems approximately 5 cm above the soil surface, dried and weighed. Soil and plant material were analyzed for ^{137}Cs using an intrinsic Ge gamma-detector with a Canberra multichannel analysis system. Eight samples of 20 g each were collected from the upper 15-cm-layer of the soil from each plot and combined. The composite samples were counted in 4.8 cm diameter plastic cups and were standardized

1 against NBS standard number SRM-4350B (a river sediment). Dried plant material was counted
2 in plastic bags pressed flat into a 19.5 cm diameter plastic holder. It was standardized with the
3 NBS soil standard and a secondary standard (DOE-EML QAP44(9603)) consisting of powdered
4 plants.

5 The design of the field experiment was a two factorial replicated in four completely
6 randomized blocks. Field data were subjected to ANOVA with soil NH_4^+ applications (0, 0.1 and
7 0.2 M) and plant species (*Amaranthus*, Indian mustard and tepary bean) as the two experimental
8 factors. The differences in ^{137}Cs phytoextraction between treatments were compared using
9 Fisher's least significant difference test ($p < 0.01$).

11 *Soil leaching tests*

12 Four Plexiglas columns, each measuring 3.1 cm in inside diameter and 4.7 cm in length,
13 were set up for a flow-through leaching experiment. In each column, about 50 g of HWMF/BNL
14 soil were packed. The soil used in this test contained 80 pCi/g of ^{137}Cs and had a moisture
15 content of 12.4%. The columns were connected to a Gilson Minipuls peristaltic pump with
16 Tygon tubing. One column received distilled water, one received 0.5 M NH_4NO_3 and two
17 columns received 0.1 M NH_4NO_3 solution. **The inlet was at the bottom of each column and**
18 **the effluent was collected and weighed periodically.** All columns ran for 10 days. Volumes of
19 effluent differed somewhat, particularly for column #2 (0.1 M NH_4NO_3). However, this did not
20 appear to affect the results. Subsamples of the effluent were analyzed for ^{137}Cs on an intrinsic Ge
21 gamma detector. The columns were run until ^{137}Cs activity in the effluent became very low (< 0.1
22 pCi mL^{-1}).

1 To assess whether additional incubation time would result in greater mobilization of ^{137}Cs
2 from the soil, following the 10-day-leaching-experiment, the columns were saturated with the
3 specific ammonium solution. After 14 weeks, the solution was then pumped through the column
4 and samples collected over a 2-day-period.

6 RESULTS AND DISCUSSION

7
8 The extent of ^{137}Cs removal from the soil depends on the ability of the plant to concentrate ^{137}Cs
9 in shoots and to produce high shoot biomass. Radiocesium concentrations in shoots of the tested
10 species are shown in Fig 1A. The greatest level of ^{137}Cs (1,023 pCi g⁻¹ dry wt) was concentrated
11 in shoots of *Amaranthus* followed by Indian mustard (140 pCi g⁻¹ dry wt) and tepary bean (50
12 pCi g⁻¹ dry wt). In addition to concentrating higher levels of ^{137}Cs in shoots, *Amaranthus* also
13 produced significantly more shoot biomass compared to the other two species; approximately 5-
14 and 3-fold more biomass was produced by *Amaranthus* compared with Indian mustard and tepary
15 bean, respectively (Fig 1B). Because of its ability to concentrate high ^{137}Cs in shoots and produce
16 high biomass, *Amaranthus* removed 30- to 60-fold more radiocesium than either Indian mustard
17 or tepary bean (Table 2).

18 As we have previously shown in pot studies that application of NH_4^+ ion to ^{137}Cs
19 contaminated soil increases its bioavailability and subsequently its accumulation in shoots (Lasat
20 et al., 1997) we investigated the effect of the same ion on ^{137}Cs shoot accumulation in the field
21 trial. Results shown in Fig 1A suggest that at least for *Amaranthus* and tepary bean, the addition
22 of ammonium nitrate produced a slight (but statistically insignificant) increase in the level of

radiocesium in shoots. It is likely, however, that this small increase in ^{137}Cs shoot concentration was caused by the higher initial soil ^{137}Cs level in the plots to which ammonium treatment was randomly assigned. In support of this, greater amounts of ^{137}Cs were removed in shoots of both *Amaranthus* and tepary bean grown in plots with higher ^{137}Cs levels regardless of NH_4NO_3 application (Fig 2). These results agree with previously published results which showed that accumulation of radiocesium in shoots was dependent upon initial radiocesium concentration in the soil (Shaw and Bell, 1989 and Demirel et al., 1994). To eliminate the confounding effect of spatial variation in soil ^{137}Cs concentration, we compared the abilities of the three species to accumulate ^{137}Cs in shoots based on their bioaccumulation ratios ($[\text{}^{137}\text{Cs}]_{\text{shoot}} / [\text{}^{137}\text{Cs}]_{\text{soil}}$) (Fig 1C). The ^{137}Cs bioaccumulation ratio was significantly greater for *Amaranthus* (2.2 to 3.2) than for Indian mustard (0.4-0.5) and tepary bean (0.2-0.3). Addition of ammonium at either 0.1 or 0.2 M did not increase the value of bioaccumulation ratio in any of the species investigated. This result is somewhat unexpected, because we previously reported in a pot study that application of NH_4NO_3 solution to the soil significantly increased radiocesium concentration in shoots of several species (Lasat et al., 1997). Three hypotheses could account for the absence of a positive effect of ammonium application on radiocesium accumulation in shoots. First, prior to planting, the whole site was treated with NH_4^+ as a part of N-P-K soil fertilization. It is possible that a readily exchangeable ^{137}Cs fraction was released from the soil at this time rendering subsequent NH_4NO_3 application ineffective. Secondly, because of significant rainfall during the field trial and irrigation, it is possible that the applied ammonium nitrate solution quickly percolated through the soil without allowing time for NH_4^+ interaction at specific radiocesium binding sites. Finally, it is possible that although NH_4NO_3 application might have induced ^{137}Cs desorption

1 from soil particles, the bulk of this released radiocesium could have leached below the
2 rhizosphere before being absorbed by roots. In a pot study, comparable rates of ammonium
3 nitrate caused a significant reduction in shoot biomass, presumably due to ammonium toxicity
4 (Lasat et al., 1997). In the present study, however, no reduction in shoot biomass was observed in
5 plants grown in ammonium-treated plots (Fig. 1B). These results suggest that in the field, NH_4^+
6 solution quickly percolated through the soil before reaching concentrations in the rhizosphere
7 that were inhibitory to plant growth.

8 The effect of water or NH_4^+ application on the mobility of ^{137}Cs from the HWMF/BNL
9 soil was investigated in a set of leaching studies. These studies provided information on the
10 availability of ^{137}Cs in the soil under controlled conditions. Results of these studies are shown in
11 Fig 3. The two soil columns leached with 0.1 M NH_4NO_3 eluted 15.3 and 14.5% of the ^{137}Cs in
12 the soil, respectively, while the column treated with 0.5 M solution eluted 19.5% of the soil ^{137}Cs .
13 No counts above background were detected in the effluent of the column that received distilled
14 water (data not shown). In a previous soil extraction study (Lasat et al., 1997), 25 and 21% of the
15 soil ^{137}Cs were mobilized with a 0.5 and 0.1 M NH_4NO_3 solution, respectively. From these
16 studies, it was not clear whether additional time would increase ^{137}Cs mobilization from the soil.
17 To assess this, we allowed the soil columns to incubate in the same ammonium solutions for an
18 additional 14 weeks before determining total ^{137}Cs eluted. If ^{137}Cs mobilization were kinetically
19 limited, there should have been a pulse of radiocesium in the effluent. No such pulse was
20 observed for any of the columns (data not shown). This indicates that the fraction of ^{137}Cs that
21 had been removed during the initial part of the experiment was all that could be eluted with these
22 solutions.

1 The results of the leaching test demonstrate that NH_4^+ has the ability to desorb a significant
2 fraction of the soil-bound radiocesium. Because ^{137}Cs in soil solution is readily absorbed into
3 roots, it is possible that the absence of a NH_4^+ -induced increase in radiocesium shoot
4 concentration is caused by ^{137}Cs movement below the rhizosphere before uptake into root tissues.
5 In support of this, Shaw and Bell (1989) showed that in wheat roots, the Michaelis-Menten
6 model provides an accurate description of Cs uptake. Consequently, radiocesium uptake in roots
7 would be limited by the value of the kinetic constant $-V_{\max}$ - of the transport system. Radiocesium
8 in excess of this capacity would leach down the soil or possibly rebind to soil particles. Results
9 reported in this study cannot elucidate whether the lack of an increase in ^{137}Cs shoot
10 accumulation following application of ammonium nitrate solution is due to NH_4^+ leaching from
11 the contaminated soil layer or due to displacement of bioavailable ^{137}Cs below rooting zone.

12 The amount of ^{137}Cs removed in *Amaranthus* shoots represents approximately 3% of total
13 contamination of the uppermost 15 cm of the soil (where most of the radiocesium contamination
14 resides). Because plants were grown in field for only three months, it is reasonable to assume that
15 two croppings of *Amaranthus* could be obtained per year. This suggests that in the first year,
16 *Amaranthus* could remove as much as 6% of the soil ^{137}Cs . This projection assumes however
17 that the rate of radiocesium phytoextraction could be sustained over successive croppings.
18 Previous results indicate that after the removal of a more available ^{137}Cs fraction which is less
19 tightly bound to soil minerals, subsequent phytotransfer of ^{137}Cs from the soil would decline ().
20 **This difference in the uptake of different soil ^{137}Cs fractions is particularly noticeable in**
21 **young radiocesium contaminated soil. However, because at this site, radiocesium**
22 **contamination is aged and therefore fixation of ^{137}Cs to soil minerals is complete, we**

anticipate little change on the rate of ^{137}Cs phytoextraction over time. In addition, there might be other means to sustain the rate of ^{137}Cs phytoextraction from the soil. For example, small increases in ^{137}Cs transfer overtime were reported for cabbage and barley grown in organic soils (Nisbet and Shaw, 1994). This is in agreement with earlier reports indicating a significant increase of ^{137}Cs biotransfer from soils rich in organic matter (Barber and Mitchell, 1963; van Bergeijk et al., 1992).

CONCLUSIONS

The results of this study indicate that *Amaranthus* is a plant with high potential for extraction of ^{137}Cs from polluted soils. This capacity seems to be outstanding compared to other plant species. Although ammonium application has the potential for desorbing ^{137}Cs from the soil, it did not enhance radiocesium accumulation in shoots. It is possible that this mobilized radiocesium leached below the rooting zone. Clearly, more research is needed to improve NH_4^+ application practices which in turn could enhance the biotransfer of this available ^{137}Cs fraction from the soil. Currently, research in our laboratory are also focused on the effect of organic amendments on bioavailability of ^{137}Cs -aged contaminated soil.

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Table 1. Some physicochemical characteristics of the HWMF/BNL soil.

Moisture	Gravel	Sand [†] (> 0.074 mm)	Silt [†] (0.005-0.074 mm)	Clay [†] (<0.005 mm)	pH
%	%	%	%	%	
10	0.5	68	17	15	3.8

[†]Percentage of mineral fraction only.

Table 2. Radioactivity in soil (upper 15 cm soil) and total[†] ¹³⁷Cs removed from 0.25 cm² plots in shoots of three plant species. Data represent means and standard errors of 4 replicates.

	<u>Indian mustard</u>			<u>Amaranthus</u>			<u>tepary bean</u>		
	<u>NH₄NO₃</u>			<u>NH₄NO₃</u>			<u>NH₄NO₃</u>		
	0	0.1 M	0.2 M	0	0.1 M	0.2 M	0	0.1 M	0.2 M
¹³⁷ Cs in plots	301±60	221±62	-	274±102	390±208	422±205	192±75	332±51	390±122
¹³⁷ Cs removed in shoots	8±1	8±4	-	339±177	241±105	461±381	6±2	7±3	8±3

[†] Calculated as [¹³⁷Cs]shoot x shoot biomass

FIGURE LEGENDS

Figure 1. ^{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 grown in the presence or absence of ammonium nitrate.

Figure 2. Effect of ^{137}Cs soil concentration on radiocesium accumulation in shoots of *Amaranthus* (●) and tepary bean (○). The values on the Y-axis represent ^{137}Cs accumulation in shoots of plants grown on 0.25 m² cell. The values on the X-axis represent the average of 8 measurements of soil ^{137}Cs taken from individual 0.25 m² cell (upper 15 cm layer).

Figure 3. Column desorption of ^{137}Cs from HWMF/BNL soil by 0.1 or 0.5 M ammonium nitrate. Column through which distilled water was flowed did not yield detectable levels of ^{137}Cs and is not shown. Solution containing 0.1 M NH_4NO_3 was passed through two of the columns (○, ●) while the third soil column received 0.5 M NH_4NO_3 (■).

Fig 2. Lasat et al. Accumulation of ^{137}Cs