Phytoremediation of Uranium-Contaminated Soils: Role of Organic Acids in Triggering Uranium Hyperaccumulation in Plants

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Uranium phytoextraction, the use of plants to extract U from contaminated soils, is an emerging technology. We report on the development of this technology for the cleanup of U-contaminated soils. In this research, we investigated the effects of various soil amendments on U desorption from soil to soil solution, studied the physiological characteristics of U uptake and accumulation in plants, and developed techniques to trigger U hyperaccumulation in plants. A key to the success of U phytoextraction is to increase soil U availability to plants. We have found that some organic acids can be added to soils to increase U desorption from soil to soil solution and to trigger a rapid U accumulation in plants. Of the organic acids (acetic acid, citric acid, and malic acid) tested, citric acid was the most effective in enhancing U accumulation in plants. Shoot U concentrations of *Brassica juncea* and *Brassica chinensis* grown in a U-contaminated soil (total soil U, 750 mg kg^{-1}) increased from less than 5 mg kg^{-1} to more than 5000 mg kg^{-1} in citric acid-treated soils. To our knowledge, this is the highest shoot U concentration reported for plants grown on U-contaminated soils. Using this U hyperaccumulation technique, we are now able to increase U accumulation in shoots of selected plant species grown in two U-contaminated soils (total soil U, 280 and 750 mg kg^{-1}) by more than 1000-fold within a few days. Our results suggest that U phytoextraction may provide an environmentally friendly alternative for the cleanup of U-contaminated soils.

Introduction

Uranium contamination of surface soils has resulted from the development of the nuclear industry, which involved the mining, milling, and fabrication of various U products. Uranium contamination poses significant health risks to both humans and animals and limits the future use of many sites formerly used for U production and processing. Because there are large areas of U-contaminated soils in the world, engineering-based remediation methods such as excavation require millions of tons of soils to be disposed of as low-level radioactive waste. This process is expensive, fills up scarce landfill space, and requires additional site restoration. The remediation of U-contaminated soils represents a significant expense to many industries and governmental agencies. The development of a cost-effective method to remove U from contaminated soils could accelerate the cleanup process and reduce remediation costs.

Plants have a remarkable ability to absorb and accumulate metals and organic compounds from soil, water, and air. Over the last 10 years, there has been increasing interest in developing a plant-based technology (phytoremediation) to remediate soils contaminated with heavy metals and radionuclides (*1–4*). Recently, extensive research has been carried out to demonstrate the use of phytoextraction technology for the cleanup of contaminated soils (*5–8*). The low solubility of heavy metals and radionuclides in the soil is often a limiting factor in metal extraction by plants. Increasing metal solubility in soil and the bioavailability of metals to the plants are important to phytoextraction of heavy metals and radionuclides from contaminated soils.

Chelating compounds have been used in soils and nutrient solutions to increase the solubility of metals in plant growth media (*9–11*). Recently, synthetic chelates have been used to trigger Pb hyperaccumulation in a number of plant species grown on Pb-contaminated soils (*6–8*). Chelating compounds have also been used to enhance soil U desorption in soil washing experiments (*12*). However, there is little information in the literature concerning the use of these compounds to enhance U accumulation in plants.

The use of phytoextraction to remove heavy metals and radionuclides from contaminated soils provides a low-cost alternative to currently employed remediation procedures. First, plant cultivation and harvesting are relatively inexpensive processes as compared to traditional engineering approaches that involve intensive soil manipulation. Second, in phytoextraction, a minimum amount of secondary waste is generated as compared to heap leaching or soil washing that produces large amounts of heavy metal laden leachate. It is very expensive to dispose of the leachate as a secondary waste. Furthermore, phytoextraction allows in-situ treatment and greatly decreases the burden on existing hazardous or radioactive waste landfills.

Multiple crops of metal accumulating plants can be grown, harvested, and dried during a single growing season. The dried plant materials then may be combusted or composted to reduce mass, and the residue may be vitrified or otherwise stabilized and deposited in a radioactive waste disposal facility. Commercial application of phytoextraction provides the opportunity to develop a new agriculture-based industry and a new application of crop plants that markedly reduces the cost of treating and reclaiming heavy metal contaminated soils at many of the world's hazardous waste sites.

To develop a plant-based remediation technology for the cleanup of U-contaminated soils, we have investigated the biogeochemical and physiological basis for U phytoextraction and developed techniques to trigger rapid U hyperaccumulation in a number of plant species grown in U-contaminated soils.

The objectives of this study were (i) to identify U hyperaccumulating plant species with high biomass production, (ii) to identify soil amendments that increase U desorption from soil to soil solution and to trigger U hyperaccumulation in plants, and (iii) to study the physiological aspects of U accumulation in selected plant species.

Materials and Methods

Soil Characterization. Uranium-contaminated soils were collected from an industrial site in northern Ohio. The soil

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TABLE 1. Physical and Chemical Characteristics of the U-Contaminated Soils Used in This Study

soil properties	U−soil 1	U−soil 2
sand (%)	40	42
silt (%)	40	39
clay (%)	20	19
soil texture	loam	loam
organic mater content (%)	4.2	4.5
soil pH (1:1 soil/water ratio)	7.3	7.7
total soil U (mg kg^{-1})	280	750

was screened to pass through a 1.0 cm sieve and thoroughly mixed before use. The following procedures were used to characterize the soil. Soil pH was measured using 1:1 soil/water ratio; total soil U was determined by the EPA-3050 method (*13*), organic matter content was measured by the Walkley Black method (*14*); and particle size was measured by the hydrometer method (*15*). The selected physical and chemical properties of the U-contaminated soils are presented in Table 1.

Screening for Soil Amendments. To identify soil amendments that enhance U desorption from soil to soil solution, the U-contaminated soils were treated with a number of selected soil amendments (synthetic chelates, inorganic and organic acids, sodium and potassium bicarbonates) at levels ranging from 0.5 to 20 mmol kg^{-1} of soil. The effectiveness of soil amendments in enhancing soil U desorption was determined by examining U concentrations in soil solutions in response to the added soil amendments. The treated soil was watered to field capacity and kept at room temperature for 24 h before extracting the soil solution. The soil solution was extracted from the soil with a moisture content at field capacity by centrifugation (*8, 16*). The soil solution was passed through a 0.45-μm membrane filter during the centrifugation, and the U concentration in the soil solution was analyzed by ICP−AES (Fisons Accuris, Fisons Instruments, Inc., Beverly, MA).

Plant Culture. For each experiment, 500 g of the U-contaminated soil (Table 1) was placed in a pot (12 cm diameter). Seeds of selected plant species were sown in the soil, and the seedlings were grown in a growth chamber with a 16 h, 22 °C/8 h, 18 °C day−night regime. Plant species/cultivars tested were the following: Amaranth (*Amaranth cruentus* L.), *Brassica juncea* (cv. 18293, 21100, 426308, and 531268), bush bean (*Phaseolus vulgaris* L.), Chinese cabbage (*Brassica chinensis* L.), Chinese mustard (*Brassica narinosa* L.), corn (*Zea mays*), cow pea (*Pisum sativum* L.), field pea (*Pisum sativum* L.), sunflower (*Helianthus annuus* L.), and winter wheat (*Triticum aestivum* L.). The soil moisture content was maintained at field capacity by adding water as needed. A liquid fertilizer was supplied weekly to the plants at the following level (in mg kg^{-1} soil) N, 130; P, 30; and K, 40. Unless otherwise specified, plants were grown for 4 weeks before applying selected soil amendments.

Before amendment application, a stock solution for each soil amendment was prepared: 0.5 M organic and inorganic acids, 0.1 M sodium and potassium bicarbonate and sodium acetate. The appropriate amount of each stock solution was added to the pot to achieve the desired rate. Plants were harvested 1 week after the application of soil amendments. At harvest, the plants were cut 1 cm above the soil, and the shoots were washed with deionized water. The plant samples were dried in an oven at 70 °C and ground in a stainless steel Wiley mill to pass through a 0.85-mm diameter screen.

Plant Sample Analysis. Subsamples of ground plant material (500 mg) were digested in a mixture of concentrated HNO$_3$/HClO$_4$. The digested samples were brought to a constant volume with deionized water, and the digests were
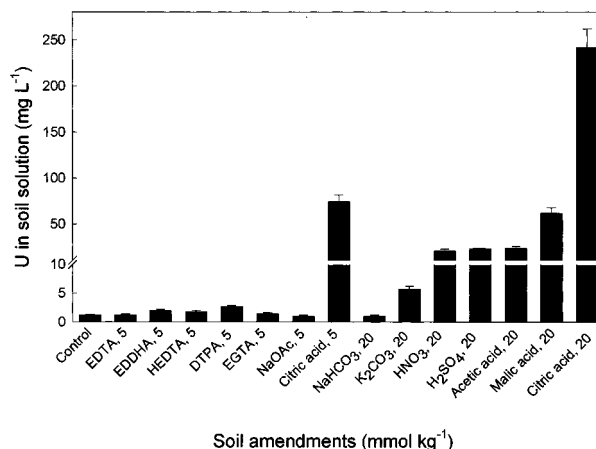
FIGURE 1. Effects of adding synthetic chelates (as sodium form), inorganic and organic acids, sodium acetate, sodium bicarbonate, and potassium carbonate on U concentrations in soil solution. The number next to the soil amendment is the concentration (mmol kg^{-1}) of the amendment used. After mixing with the various soil amendments, the contaminated soil (total soil U, 280 mg kg^{-1}) was watered to field capacity and allowed to stand at room temperature for 24 h before extracting soil solution by centrifugation. The soil solution was passed through a 0.45-μm filter during the centrifugation. Control denotes the U concentration of the soil solution from the contaminated soil in the absence of soil amendment. Error bars represent ± SE (*n* = 4).

analyzed for U by ICP−AES. Data reported in this paper were analyzed using Statistical Analysis System (*17*). A probability of 0.05 or less was considered to be statistically significant.

Results

Identification of Soil Amendment To Increase Soil U Desorption. To test the effects of various soil amendments on soil U desorption, we examined U concentrations in soil solution after the addition of synthetic chelates, inorganic acids, and organic acids to the contaminated soil. The efficiency of soil amendments in enhancing soil U desorption was determined by examining U concentration in the soil solution. Among the amendments tested, citric acid was the most effective in enhancing U desorption from soil to soil solution (Figure 1). For simplicity, the data for the amendment concentrations of 0.5, 1, and 10 mmol kg^{-1} were not shown. We did the initial screen for soil amendments using U-soil 1 (Table 1). The pattern of the U concentration in soil solution in response to the application of soil amendments for U-soil 2 was identical to that of U-soil 1. Therefore, only the results from U-soil 1 are presented in Figure 1. Applications of citric acid (20 mmol kg^{-1}) increased U concentrations in soil solutions from 1.2 to 240 mg L^{-1}, representing a 200-fold increase (Figure 1). Addition of citric acid to the contaminated soil transiently reduced the soil pH by 0.5 to 1.0 unit. The application of nitric acid and sulfuric acid at the same concentration as citric acid also reduced the soil pH by a similar magnitude, but the increase in soil U desorption was significantly less as compared to that with citric acid (Figure 1).

Triggering Rapid U Hyperaccumulation in Plants. On the basis of the results of soil U desorption, we investigated the potential of applying organic acids (acetic acid, malic acid, and citric acid) to the contaminated soils in U phytoextraction. We also included the treatment with nitric acid, sulfuric acid, and sodium bicarbonate, which have been used in soil washing for U-contaminated soil (*18*). Of the soil amendments tested, citric acid was the most effective in enhancing U hyperaccumulation in plants (Figure 2).
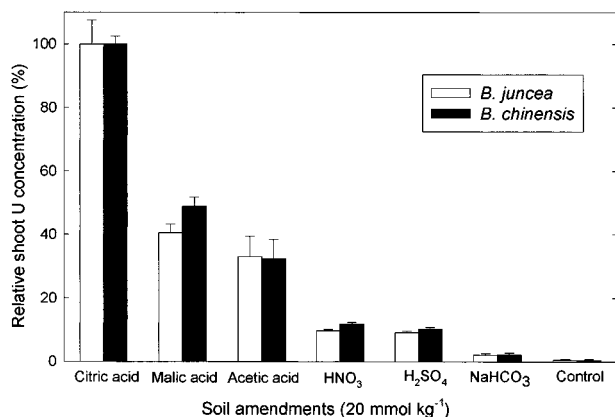
FIGURE 2. Relative efficiency of six soil amendments in enhancing U accumulation in shoots of *Brassica juncea* and *Brassica chinensis* grown on a U-contaminated soil with a total soil U of 750 mg kg^{-1}. Plants were grown in the U-contaminated soil for 4 weeks before applying the soil amendments and were harvested 1 week after the soil amendment application. Control denotes the plants grown in the U-contaminated soil in the absence of soil amendment. For this and subsequent figures, the *B. juncea* cultivar 426308 was used unless specified. Error bars represent ± SE (*n* = 3).
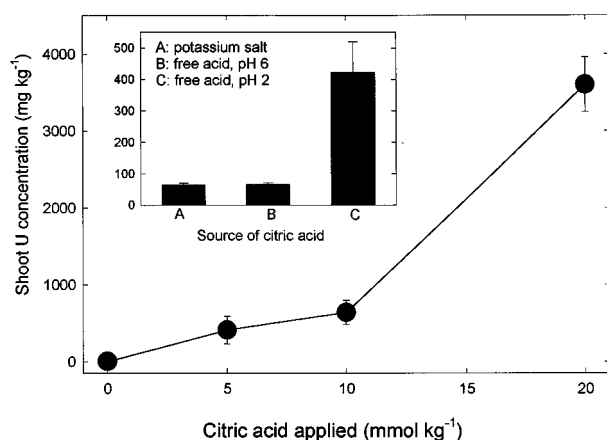
FIGURE 3. Uranium accumulation in shoots of *Brassica juncea* in response to the level of citric acid (free acid form) added to an U-contaminated soil (total soil U, 750 mg kg^{-1}). Insert, shoot U accumulation in response to different sources of citric acid (10 mmol kg^{-1} of soil); A, potassium citrate; B, citric acid as free acid with the solution pH adjusted to 6; C, citric acid as free acid without pH adjustment, pH = 2. Plants were grown in the U-contaminated soil for 4 weeks before the citric acid treatments and were harvested 1 week after the citric acid treatments. Error bars represent ± SE (*n* = 3).

There was a concentration-dependent response to citric acid-triggered shoot U accumulation (Figure 3). The rate of shoot U accumulation increased slowly for the citric acid application at a level less than 10 mmol kg^{-1}. In contrast, the rate of shoot U accumulation increased more than 5-fold for a 1-fold increase in the rate of citric acid application at the level of 10 mmol kg^{-1} or higher (Figure 3). We also compared the effects of different forms of citric acid on shoot U accumulation. The following three sources of citric acid were tested: (A) a solution made from citric acid as potassium salt, (B) a solution made from citric acid as free acid with the solution pH adjusted to 6.0 with KOH, and (C) a solution made from citric acid as the free acid without pH adjustment (pH = 2). Of the three sources of citric acid tested, source C yielded the highest shoot U concentrations (Figure 3, insert). These results indicate that the combination of lower pH and chelation contributed to citric acid-triggered U hyperaccumulation in plants.
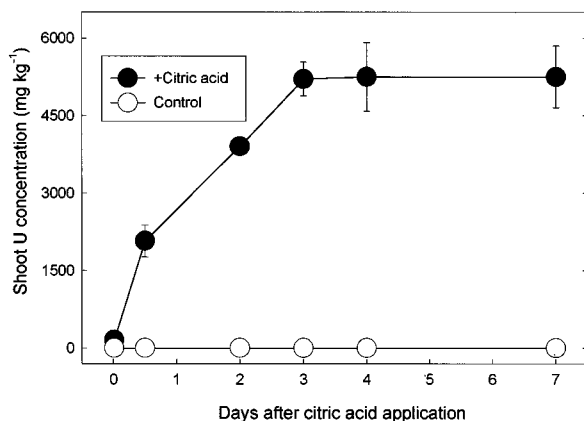
FIGURE 4. Time-dependent U accumulation in shoots of *Brassica juncea* after the application of citric acid (20 mmol kg^{-1}) to the U-contaminated soil (total soil U, 750 mg kg^{-1}). Plants were grown in a U-contaminated soil in a growth chamber for 4 weeks before applying citric acid and were harvested at the time indicated. The control denotes the plants grown in the U-contaminated soil in the absence of citric acid treatment. Error bars represent ± SE (*n* = 3).
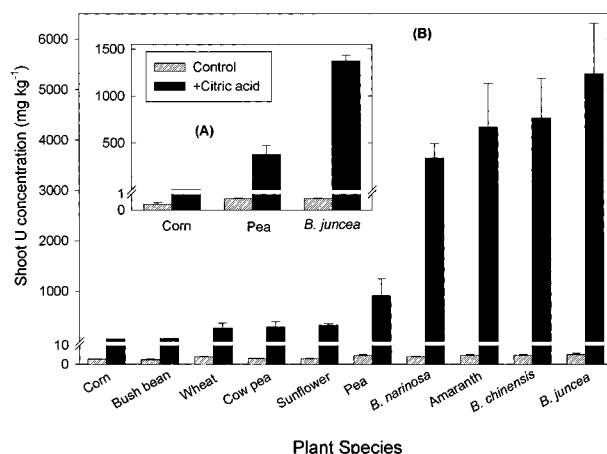
FIGURE 5. Uranium accumulation in shoots of 10 plant species grown on two U-contaminated soils with total soil U of 280 mg kg^{-1} (A) and 750 mg kg^{-1} (B) in response to the addition of citric acid [20 mmol (kg of soil)$^{-1}$]. Plants were grown in the U-contaminated soil for 4 weeks before applying citric acid and were harvested 1 week after the citric acid application. The control denotes the plants grown in the U-contaminated soil in the absence of citric acid treatment. Error bars represent ± SE (*n* = 3).

Time-Dependent Kinetics of U Accumulation in Plants. Time-dependent U accumulation was examined by assaying shoot U concentration at various times after applying citric acid. Uranium hyperaccumulation was observed within 24 h after citric acid treatment, and the shoot U concentration reached a steady state 3 days after citric acid application (Figure 4). At steady state, shoot U concentrations for plants treated with citric acid were more than 1000-fold higher than control plants.

Species Variation in U Accumulation. We examined U uptake and translocation in more than 30 plant species/ cultivars, including agronomic crops, weeds, and known metal hyperaccumulators. Figure 5 shows shoot U accumulation in 10 selected species grown in U-contaminated soils with or without citric acid treatment. Of the 30 plant species/cultivars tested to date, four plant species (*B. juncea*, *B. chinensis*, *B. narinosa*, and amaranth) demonstrated significant potential in U accumulation in shoots (Figure 5). Shoot U concentrations in these four species increased by more than 1000-fold in response to the application of citric acid to the contaminated soil.
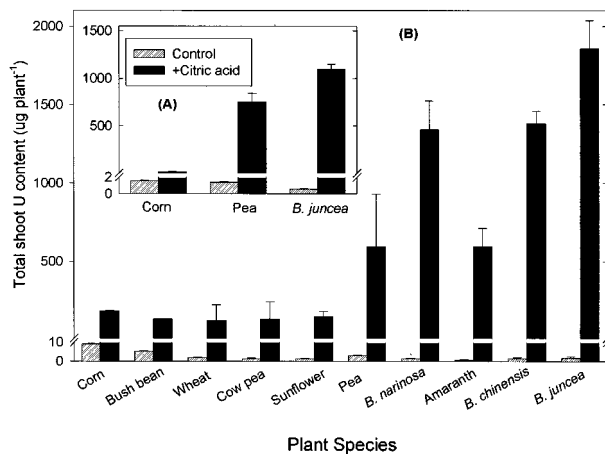
FIGURE 6. Effects of adding citric acid to two U-contaminated soils with total soil U of 280 mg kg^{-1} (A) and 750 mg kg^{-1} (B) on total U accumulation in shoots of 10 plant species. Plants were grown in the U-contaminated soil for 4 weeks before applying citric acid and were harvested 1 week after the citric acid application. The control denotes the plants grown in the U-contaminated soil in the absence of citric acid treatment. Error bars represent ± SE (n = 3).
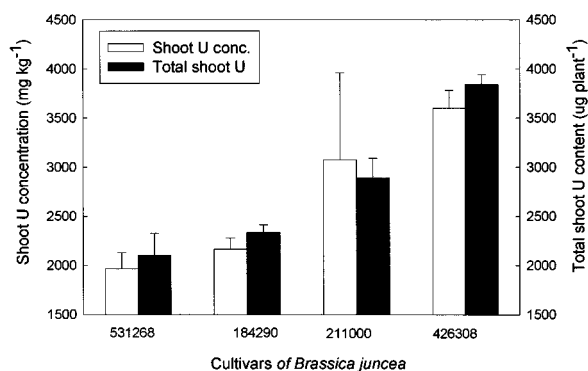

FIGURE 7. Uranium accumulation in shoots of four cultivars of *Brassica juncea* grown on a U-contaminated soil with a total soil U of 750 mg kg^{-1} in response to the citric acid treatment. Plants were grown in the U-contaminated soil for 4 weeks before applying citric acid and were harvested 1 week after the citric acid application. Error bars represent ± SE (n = 3).

It is important to point out that, without citric acid treatment, shoot U concentrations in any species tested were less than 5 mg kg^{-1} (Figure 5). The results indicate that a screening for U hyperaccumulating plants should be performed in combination with soil amendment application. We also examined total U accumulation in shoots of selected plant species/cultivars (Figure 6). The pattern of total U content for the selected plant species was similar to that of shoot U concentration with the exception of amaranth (Figures 5 and 6). The amaranth grew relatively slowly during the 4-week experimental period, yielding an amaranth biomass about 30% that of *B. chinensis* (data not shown). The results indicate that both biomass production and shoot U concentration are important parameters for U phytoextraction.

The highest shoot U concentration and total U accumulation in *B. juncea* lead us to further examine the variation of U accumulation by different cultivars of *B. juncea* (Figure 7). The four cultivars (cultivar 531268, 18293, 21100, and 426308) tested in this study were previously shown to have the ability to accumulate significantly higher Pb in shoots as compared to other cultivars of *B. juncea* (19). Cultivar 426308 displayed the highest shoot U accumulation and also

had the highest shoot Pb accumulation (19). These results demonstrate that there are significant variations in plant species and cultivars in U accumulation.

Discussion

Uranium phytoextraction is the use of plants to extract U from contaminated soils. This technology may offer several advantages over engineering-based processes (such as excavation or soil washing) for the remediation of U-contaminated soils. Soil washing has shown some promise in removing U from contaminated soils (23); however, it also creates secondary problems. One of the problems in soil washing is the generation of large volumes of liquid waste containing high levels of Al, Ca, Fe, and Mg mixed with U. The treatment of such liquid waste is difficult and expensive. Another secondary problem in soil washing is the drastic alteration of soil physical and chemical properties that makes vegetation establishment very difficult for the treated soil (23). The soil usually requires additional restoration treatment following soil washing before revegetation can be established. In contrast, phytoextraction allows in situ treatment and does not generate liquid waste. Furthermore, the U-contaminated site is covered by plants during phytoextraction that could prevent migration of U-contaminated soil by wind and rain.

Results from this study demonstrate that citric acid is an efficient soil amendment in enhancing U desorption from soil to soil solution and triggering U hyperaccumulation in plants. The form of citric acid used is critical to trigger shoot U hyperaccumulation. Comparing to the potassium citrate, the free acid form of citric acid was more effective in triggering U hyperaccumulation in selected plant species (Figure 3). As mentioned earlier, the application of citric acid (free acid form) transiently reduced the soil pH by 0.5 to 1.0 unit depending on the original soil pH. The application of nitric acid and sulfuric acid at the same concentration also reduced the soil pH by a similar magnitude; however, soil U desorption and shoot U accumulation were much less as compared to the citric acid treatment (Figures 1 and 2). The results indicate that the reduction in soil pH contributed to only part of the enhanced soil U desorption and shoot U accumulation. The driving force for the citric acid-enhanced soil U desorption was probably the chelation between U and citric acid. Another mechanism of citric acid-enhanced soil U desorption may be that citric acid at low pH effectively removes coatings of amorphous iron and aluminum sesquioxide from solid-phase U particles, thus, enhancing the dissolution and extraction of U from soil to soil solution. We found that Fe and Al concentrations in soil solution increased dramatically in response to the citric acid treatment and that there was a positive correlation between U and Fe or U and Al in the soil solutions after the citric acid treatment (data not shown). These results support our speculation of citric acid-enhanced soil U desorption.

There are several advantages of using citric acid as soil amendment for U phytoextraction. First, citric acid is biodegradable, rapidly degrading to carbon dioxide and water (24−26). Huang et al. (26) found that at pH 8−9, more than 99% of U−citric acid complex was rapidly biodegraded. Furthermore, Dodge et al. (25) reported that when exposed to visible light, U−citrate complex could rapidly photodegrade to acetic acid and carbon dioxide, with the precipitation of U as uranium trioxide. The rapid degradation of citric acid and U−citrate makes citric acid an ideal soil amendment for U phytoextraction. For the levels of citric acid used in this research, there was no residual effects on continuous cultivation of crops. We have conducted long-term experiments to study the sustainability of continuous application of soil amendments and plant cultivation. Using two U-contaminated soils differing in total soil U levels, eight

crops have been harvested from each soil with the plants displaying normal growth for each crop grown in the soils either untreated or treated by soil amendments. These results suggest that U phytoextraction can be used continuously to extract U from contaminated soils. The rapid degradation of citric acid also helps prevent the possible movement of U–citric acid complexes across the soil profile. Another advantage of using citric acid is its relatively low cost, and it may be obtained as an industrial byproduct. This will help make U phytoextraction a cost-effective method for the remediation of U-contaminated soils.

Although citric acid is rapidly biodegradable, it is important to point out that the addition of citric acid to soils has to be done in a carefully controlled manner so that there is no enhanced movement of U into groundwater. The use of certain irrigation systems, which can quantitatively control water movement in plant root zone, may be important in specific instances. It is also important to avoid the citric acid application during heavy precipitation events. Since the citric acid triggered U hyperaccumulation in plants is rapid, the citric acid can be applied to the root zone after the vegetation is well-established on the contaminated sites. We are currently concentrating on the development of the best management practice of citric acid assisted U phytoextraction at U-contaminated sites.

Plant species and cultivars differ significantly in response to citric acid-triggered U hyperaccumulation in shoots (Figures 5–7). It is important to note that the dramatic species variation in U accumulation is observed only when soil amendments are applied. This result suggests that screening for an ideal plant species for U phytoextraction should be carried out in conjunction with soil amendment applications.

Of the species and cultivars tested, four species demonstrated significant potential in citric acid-triggered U hyperaccumulation. Three of these species (*B. juncea*, *B. narinosa*, and *B. chinensis*) are members of *Brassicaeae* family. There are a number of species from the *Brassicaeae* family that have demonstrated the ability to hyperaccumulate heavy metals such as Zn, Pb, and Cd under natural conditions (*5, 20–22*). Initial field trials to examine heavy metal removal from soils by known Zn and Cd hyperacumulators indicated that the remediation potential may be limited by the low quantities of biomass produced by these species (*2, 5*). To date, there is no known U hyperaccumulating plant reported in the literature. Using soil amendments to trigger U hyperaccumulation in certain plant species opens up the opportunity of using plant species with higher biomass production for phytoremediation of U-contaminated soils. This technique has also been used in phytoextraction of Pb from contaminated soils (*6–8*).

It is important to note that citric acid-triggered U hyperaccumulation in plants is rapid. The induced U hyperaccumulation in shoots can be observed within 24 h after the application of citric acid, and shoot U concentration reaches a maximum in 3 days (Figure 4). In this form of U phytoextraction, the plant would contain very low U concentration for most of its life. After the application of citric acid, U accumulation in plant shoots would increase rapidly, and the plants could be harvested a few days after the citric acid application. Using this technique, we are now able to increase U accumulation in shoots of selected plant species by more than 1000-fold within a few days. This strategy has advantages in reducing the risk that might have been present by having plants with high U levels in the field for long periods of time. Applying this technique in the field will speed up the removal of U from contaminated soils and provide a cost-effective soil decontamination strategy. Most impor-

tantly, this technology represents an environmentally friendly alternative for the cleanup of U-contaminated soils.

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