Published in final edited form as:

Environ Sci Technol. 2018 November 20; 52(22): 13089–13098. doi:10.1021/acs.est.8b02724.

Effect of Calcium on the Bioavailability of Dissolved Uranium(VI) in Plant Roots under Circumneutral pH

Eliane El Hayek^{*,†}, Chris Torres[‡], Lucia Rodriguez-Freire[§], Johanna M. Blake^{||}, Cherie L. De Vore[⊥], Adrian J. Brearley[#], Michael N. Spilde[#], Stephen Cabaniss[†], Abdul-Mehdi S. Ali[#], and JoséM. Cerrato^{*,⊥}

[†]Department of Chemistry and Chemical Biology, MSC03 2060, University of New Mexico, Albuquerque, New Mexico 87131, United States

[‡]Department of Chemical and Biological Engineering, MSC01 1120, University of New Mexico, Albuquerque, New Mexico 87131, United States

[§]Department of Civil and Environmental Engineering, New Jersey Institute of Technology, Newark, New Jersey 07102, United States

^{||}U.S. Geological Survey, 6700 Edith Blvd. NE, Albuquerque, New Mexico 87113, United States

[⊥]Department of Civil Engineering, MSC01 1070, University of New Mexico, Albuquerque, New Mexico 87131, United States

[#]Department of Earth and Planetary Sciences, MSC03 2040, University of New Mexico, Albuquerque, New Mexico 87131, United States

Abstract

We integrated field measurements, hydroponic experiments, microscopy, and spectroscopy to investigate the effect of Ca(II) on dissolved U(VI) uptake by plants in 1 mM HCO₃[−] solutions at circumneutral pH. The accumulation of U in plants (3.1–21.3 mg kg^{−1}) from the stream bank of the Rio Paguete, Jackpile Mine, New Mexico served as a motivation for this study. *Brassica juncea* was the model plant used for the laboratory experiments conducted over a range of U (30–700 μg L^{−1}) and Ca (0–240 mg L^{−1}) concentrations. The initial U uptake followed pseudo-second-order kinetics. The initial U uptake rate (V_0) ranged from 4.4 to 62 μg g^{−1} h^{−1} in experiments with no added Ca and from 0.73 to 2.07 μg g^{−1} h^{−1} in experiments with 12 mg L^{−1} Ca. No measurable U uptake over time was detected for experiments with 240 mg L^{−1} Ca. Ternary Ca–U–CO₃ complexes may affect the decrease in U bioavailability observed in this study. Elemental X-ray mapping using scanning transmission electron microscopy–energy-dispersive spectrometry detected U–P-bearing precipitates within root cell walls in water free of Ca. These results suggest that root interactions with Ca and carbonate in solution affect the bioavailability of U in plants. This study contributes relevant information to applications related to U transport and remediation of contaminated sites.

^{*}Corresponding Authors Phone: (001) (505) 277-0870; fax: (001) (505) 277-1918; jcerrato@unm.edu. ^{*}Phone: (001) (505) 582-1362, elhayek@unm.edu.

Notes

The authors declare no competing financial interest.

Graphical Abstract

INTRODUCTION

The bioavailability of uranium (U) in contaminated environments has received growing attention because of potential ecological and human health risks from mining, nuclear energy generation, and weapons manufacturing. Improper uranium mine waste disposal remains a global concern because of U contamination of water, soils, and various ecosystems.^{1–3} The accumulation of U and other toxic metals in plants has been commonly studied as a potential pathway for human exposures^{1,4–6} and for the development of remediation approaches in contaminated sites.^{7–9}

This study was conducted in the context of the Jackpile Mine, Laguna Pueblo, New Mexico, which is an example of a mine site affected by U mining legacy. Mining operations at the Jackpile Mine were active from 1953 to 1982.^{10,11} Concentrations of U in surface waters from Rio Paguete near the Jackpile Mine have been recently detected above the U.S. Environmental Protection Agency (USEPA) Maximum Contaminant Level (MCL) of 30 $\mu\text{g L}^{-1}$.^{12,13} Given the water chemistry at Rio Paguete (e.g., pH and Ca, U, and carbonate concentrations), aqueous ternary uranyl-carbonate complexes (Ca-U-CO_3) may be an important factor decreasing U interaction with sediments collocated in the stream bed and bank of Rio Paguete.¹⁴ However, the effects of these Ca-U-CO_3 complexes on U bioavailability in plants at the Jackpile Mine are unknown.

Water chemistry has a major influence on U bioavailability. It is generally understood that an increase in pH, water hardness, alkalinity, and organic colloids reduces U uptake and toxicity.^{15–17} Such effects are attributed to either competitive binding–uptake mechanisms at the cell surface (e.g., between the free UO_2^{2+} and H^+ , Ca^{2+}) or U speciation through aqueous complexation.^{18,19} Most studies consider that the free uranyl ion^{20,21} (UO_2^{2+}) and U hydrolysis species²² are key to U uptake by living systems (e.g., terrestrial plants, invertebrates, algae). Binary and ternary uranyl-carbonate complexes are also relevant in natural waters in which Ca and carbonate are present.^{23–26} However, the importance of these uranyl carbonate complexes has been largely overlooked in the literature for U bioavailability, and the effect of Ca on U toxicity in contaminated waters is not well-understood.²⁷ A recent study²⁸ reported that uranyl-carbonate complexes can be an indicator of U uptake rates in invertebrates but that ternary complexes (e.g., $\text{CaUO}_2(\text{CO}_3)_3^{2-}$) decreased rates of uptake. Both $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ and $\text{CaUO}_2(\text{CO}_3)_3^{2-}$ complexes can act as

nontoxic species in contaminated waters.^{29–31} Limited studies have addressed Ca impact on U bioavailability in plants.^{18,21,32–34}

The mechanisms for U uptake in plants largely depend on aqueous chemical speciation.^{22,35–37} The ion UO_2^{2+} can highly adsorb and/or accumulate in plant roots at low pH and low sulfate and phosphate concentrations.^{33,38} Complexation of UO_2^{2+} with carbonate and citrate was found to increase root-to-shoot translocation but to decrease total U accumulation in plants.³³ Inside the plant roots, UO_2^{2+} can precipitate with the endogenous phosphorus as U(VI)-phosphate,^{39–41} whereas U citrate can be accumulated as U carboxylate.³⁹ Under favorable conditions for iron-reducing bacteria, U can be found mainly associated with phosphorus on plant roots in the oxidized U(VI) and the reduced U(IV) forms.^{42,43} However, the effects of water chemistry at environmentally relevant conditions (e.g., circumneutral pH, the presence of complexing agents such as carbonate and/or major elements such as Ca and Mg) on the bioavailability and the uptake mechanisms of U by plants are still largely unknown. Information on the mechanisms governing U uptake in plants is important for predicting U mobility and long-term remediation strategies.

The main objective of this study was to assess U bioavailability integrating field measurements, hydroponic experiments, microscopy, and spectroscopy analyses. The accumulation of U was measured in plants collected from the Jackpile Mine, along the Rio Paguate. Hydroponic experiments were conducted in systems containing 1 mM HCO_3^- at pH 7.5, with U and Ca concentrations relevant to water chemistry in the Rio Paguate. *Brassica juncea* (*B. juncea*) was selected as the model organism as it is a known U hyper-accumulator plant.^{8,9} Our findings contribute relevant information to better understand the influence of Ca and carbonate on U bioavailability in abandoned mine wastes and other natural sites affected by elevated U concentrations.

MATERIALS AND METHODS

Plant Sampling Locations.

Plant samples (shoots and roots) were collected from the stream banks of the Rio Paguate near and within the wetland area up to 5 km downstream of the Jackpile Mine, situated in Figure S1 between sites 1 and 10. The sampling sites of plants were colocated with water and stream bank sediment samples that were studied by Blake et al. during the same time period (Table S1).¹⁴ Regional plants (grass, willow (*Salix*), and cattail (*Typha latifolia* L.)), which are abundant at the site and may be used as cattle fodder or for ceremonial traditions, were sampled (Table S1). Detailed information for the sites and laboratory plant preparation is presented in the Supporting Information.

Growth and Preparation of *Brassica juncea*.

After germination, the seedlings of *B. juncea* were allowed to grow in a hydroponic system supplied with NPK liquid fertilizer under 21–25 °C day and night temperature in 12 h/12 h light cycle. Two-month-old seedlings were acclimatized to the exposure conditions for 5 days by placing each one of them separately in 500 mL of Nalgene polypropylene (PP) bottles containing the same elemental composition of the exposure solution but without U.

To avoid any possibility of improper U complexation or precipitation (e.g., complexation with phosphate) in the uptake experiments, the exposure solutions were made in ultrapure water containing only simplified Hoagland nutrients (MgSO_4 , 0.5 mM; NH_4NO_3 , 2 mM; KCl, 1 mM; NaHCO_3 , 1 mM; and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 3 mM). The measured pH of the acclimatized solution was in the range of 5.8–6.2. More details are presented in the Supporting Information.

Exposure of *Brassica juncea* to U.

The effect of Ca on U bioavailability was tested by studying U uptake kinetics at three Ca concentrations (0, 12, and 240 mg L^{-1}) in water containing 1 mM HCO_3^- (alkalinity 50 mg L^{-1}) with pH maintained at 7.5 using 2 mM HEPES buffer. Laboratory controlled experiments were conducted using *B. juncea* as a model system for research investigating U bioaccumulation in plants.^{9,44,45} The tested U, Ca, and pH levels correspond to Jackpile Mine conditions¹⁴ (Table S1) and to other environments where binary and ternary uranyl-carbonate complexes are prevalent.²³ Uptake experiments were performed in freshly made solutions prepared in ultrapure water with the same simplified Hoagland nutrients but with changing Ca concentrations (0, 12, and 240 mg L^{-1}) and adding 30, 100, 300, or 700 $\mu\text{g L}^{-1}$ U concentrations as $\text{UO}_2(\text{NO}_3)_2$. All the experiments were performed in triplicate. Aliquot volumes were collected at various time points (from 4 to 336 h) to assess U uptake kinetics. We also exposed other plant seedlings to 700 $\mu\text{g L}^{-1}$ U at the same conditions described above to assess the kinetics of U uptake during the first hours of U exposure from 0.6 to 24 h. Detailed descriptions about seedling preparation, the composition of exposure solutions, and methods used for U analysis are presented in the Supporting Information.

Solid and Solution Analyses.

Total U for hydroponic solutions and acid-digested plant samples were analyzed using inductively coupled-plasma mass-spectrometry (ICP-MS). We used Visual MINTEQ^{46,47} to assess U aqueous speciation using inputs based on the experimental conditions used for this study. Statistical analyses were conducted to analyze the significance of the effects of U, Ca, and $\text{U} \times \text{Ca}$ treatments on U uptake by plant roots using the software XLSTAT.⁴⁸ Nonparametric tests were performed because of the non-normality of our data sets as determined using the Shapiro–Wilk test. The statistically significant level was set at $\alpha = 0.05$ ($p < 0.05$) for all the statistical tests. Additional details about these statistical methods are presented in the Supporting Information.

Dried root samples were analyzed using scanning electron microscopy (SEM), electron microprobe analysis (EPMA), and focused ion beam-transmission electron microscopy (FIBTEM) techniques. Additional details describing solution and solid analyses can be found in the Supporting Information.

Kinetic Analyses of Aqueous U.

The quantity of U removed in micrograms per gram of dry weight of roots at time t was calculated from the mass balance between the initial concentration in the exposure solution and the measured concentration at different time intervals. Note that the uptake of U by the plant was also confirmed by measuring U content in the digested plant samples at the end of

the experiments. Pseudo-second-order kinetic analyses were found to best mathematically represent the uptake of U by *B. juncea* roots in comparison with several kinetic models (first- and second-order and pseudo-first-order) tested on the experimental data. The kinetic equation is

$$dq_t/dt = k_2(q_e - q_t)^2 \quad 1$$

where k_2 ($\text{g } \mu\text{g}^{-1} \text{ h}^{-1}$) is the rate constant of pseudo-second-order of metal uptake^{49,50} and q_e and q_t represent the mass of U per gram of root tissue ($\mu\text{g g}^{-1}$) at equilibrium and time t , respectively. Solving and rearranging eq 1 gives

$$t/q_t = 1/V_0 + 1/q_e t \quad 2$$

where V_0 is the initial uptake rate ($\mu\text{g g}^{-1} \text{ h}^{-1}$) and is equal to the following equation:

$$V_0 = k_2 q_e^2 \quad 3$$

The values of V_0 , k_2 , and q_e can be experimentally obtained by plotting t/q_t versus t . Uranium uptake kinetics applied in this study follow a similar approach to the model used for adsorption–absorption mechanisms and metal bioaccumulation by plants and bacteria.^{49,51–54} Metal uptake can occur by various mechanisms, which were reported to include extracellular accumulation and adsorption–absorption on cell surfaces or precipitation.^{49,55}

RESULTS AND DISCUSSION

Uranium Content in Plant Samples along Rio Pagueate.

The accumulation of U was detected in plant samples collected from the field. Samples were collected along the Rio Pagueate either near the abandoned mine wastes (sites 1, 5, and 7) or 5 km away in and near the wetland area (sites 8–10) (Table S1). Among the studied species, the highest U accumulation was found in grass plants. Uranium concentrations in grass root samples were 2.6–18-fold higher ($3.1\text{--}21.3 \text{ mg kg}^{-1} \text{ U}$) compared to those in willow and cattail. As for shoots, grass samples had 5.2–24-fold higher ($2.6\text{--}11.7 \text{ mg kg}^{-1} \text{ U}$) contents compared to willow and cattail shoots. The variation of U concentrations in grass samples did not show a correlation with the sediments and water properties along the stream bank of Rio Pagueate (e.g., water pH, Ca, and U concentrations). However, the highest observed U accumulation in grass roots (21.3 mg kg^{-1}) was at the wetland site, which coincides with the highest U concentration in sediments as measured by Blake et al.¹⁴ This observation is consistent with previous studies where the rhizosphere in wetland sediments naturally contributes to the immobilization of U.^{42,56} However, the range of U concentrations in grass roots and shoots are still lower than those reported for other plants studied at various U mine sites.^{57,58} Although plant samples collected from the field had detectable U content measured by ICP-MS, it was difficult to use X-ray spectroscopy analyses to understand the

mechanisms affecting U accumulation given that the concentrations measured were below the detection limit ($<100 \text{ mg kg}^{-1}$). Another limitation is that the effects of plant maturation, the U exposure duration, and the typical seasonal variation of sediment and water properties (e.g., U, Ca, and carbonate concentrations)¹⁴ at the site on the processes of U accumulation in these plants are still unknown. Thus, hydroponic experiments were conducted under laboratory- controlled conditions using *B. juncea*, a hyper-accumulator model plant, to investigate the kinetics of U uptake and how aqueous Ca and U concentrations at circumneutral pH could affect U bioavailability. Although the mechanisms of U accumulation by *B. juncea* may not be directly transferrable to species from the field, *B. juncea* was used in this study for testing the mechanisms of U bioaccumulation as a research model species.^{9,44,45}

Hydroponic Experiments for Uranium Uptake.

B. juncea was observed in other studies to be able to hyperaccumulate U in the roots but also in the shoots under high U concentrations that have limited environmental relevance for various abandoned mine sites.^{7,8,59} In this study, we selected U and Ca concentrations (Table S1) representative of the Rio Paguete surface water concentrations and assessed U uptake in *B. juncea* from carbonate water. The uptake of U by plants was calculated based on changes in aqueous U concentrations over time. The highest U uptake was observed in experiments conducted with no added Ca where aqueous U concentration significantly decreased over time ($p < 0.05$). However, high Ca concentrations showed a significant ($p < 0.05$) inhibitory effect on U uptake from carbonate water (Figures 1 and S2). Fast U uptake was observed with 8% of the total U concentration removed from solution within 20 min of roots exposure to $700 \mu\text{g L}^{-1}$ U for experiments with both 0 and 12 mg L^{-1} Ca (Figure S2). However, after 2 h, U uptake was 2.2-fold higher in water free of Ca compared to that containing 12 mg L^{-1} Ca. The experiments conducted for 14 days showed that U uptake occurred mainly in the first 24 h for experiments with 0 and 12 mg L^{-1} Ca (Figure 1). For example, at 0 mg L^{-1} Ca, an uptake of 17% was observed for $700 \mu\text{g L}^{-1}$ U and 47% was observed for $30 \mu\text{g L}^{-1}$ U. However, only 7–18% of U was removed for experiments with 12 mg L^{-1} Ca, and no measurable uptake was detected in solutions at 240 mg L^{-1} Ca over time. Uranium uptake was limited after 24 h as evidenced by the non-noticeable change in U concentration in the later period of the experiment.

Higher percentages of U uptake (60–80%) were observed within 24 h in a previous study with plants exposed to similar U concentrations as those used in our experiments.⁶⁰ However, the pH during the exposure period and water chemistry (i.e., hard elements, carbonates) were not reported. Another study has shown 24% U uptake by sunflowers at pH 7³⁴ but in the presence of phosphate, which is known to preferentially bind U with respect to the other complexing agents (i.e., carbonates) over a pH range of 4.5–9.³⁵ Although our study was conducted using different experimental conditions and in the absence of phosphorus in the water, we obtained comparable U uptake (7–18% for experiments with 12 mg L^{-1} Ca), which can be related to the circumneutral pH used in both studies. On the basis of chemical equilibrium analyses (Table S2), UO_2^{2+} should not be predominant, and phosphate- and carbonate-U complexes are neutrally to negatively charged at pH 7.5. The binding of these negative or neutral complexes with plant roots was previously reported to

be lower compared to UO_2^{2+} ,^{61,62} most likely because of the negatively charged compounds that compose the vegetal cell walls and membranes.^{33,39}

Uranium Accumulation in *Brassica juncea*.

Uranium accumulated in *B. juncea* roots, with no detectable root-to-shoot translocation. For example, when exposed to $700 \mu\text{g L}^{-1}$ U at 0 mg L^{-1} Ca, $924 \pm 447 \text{ mg kg}^{-1}$ U was measured in the digested samples of plant roots, whereas only $0.7 \pm 0.3 \text{ mg kg}^{-1}$ U was measured in the shoots (Figure S3). As shown in Figure S3, the content of U in plant roots that was obtained by calculation was found to be significantly similar ($p > 0.05$) to that measured in the digested root samples for both experiments with 0 and 12 mg L^{-1} Ca. It is worth noting that the measured concentrations of U in *B. juncea* roots for experiments with 240 mg L^{-1} Ca correspond to only ~1% of U uptake, which could not be detected during the measurements of U uptake in solution over time. The undetectable root-to-shoot translocation is consistent with a previous study where the measured U concentration in leaves was $\sim 1.64 \text{ mg kg}^{-1}$ in plants exposed to lower than $6.4 \mu\text{mol L}^{-1}$ U at pH 7.5.⁶³ However, at higher U concentrations ($> 25 \mu\text{mol L}^{-1}$, which are seldom observed in the environment), considerable translocation of U to the shoots was typically observed and attributed to the mobility of uranyl-carbonate complexes^{33,63} at pH 7.5 compared to uranyl cations formed at pH 4. Future studies should focus on a greater understanding of the effect of Ca and U concentrations on U translocation. In addition, the measured concentrations of U in *B. juncea* roots for experiments with 240 mg L^{-1} Ca ($27.4 \pm 8.1 \text{ mg kg}^{-1}$) were close to U contents in the grass roots at the wetland site in the Jackpile Mine where plants were exposed to natural Ca concentrations in surface water ($7.7\text{--}278 \text{ mg L}^{-1}$), similar to our experimental conditions (Table S1).

The initial U and Ca concentrations in the exposure solution significantly ($p < 0.05$) affect the accumulation of U in plant roots (Table S3). For example, when initial U concentrations increased from 30 to $700 \mu\text{g L}^{-1}$, U uptake ($\mu\text{g g}^{-1}$) significantly increased ($p < 0.05$) 36-fold at 0 mg L^{-1} Ca and 8-fold at 12 mg L^{-1} Ca (Table S3). A linear relationship was obtained for experiments using 0 and 12 mg L^{-1} Ca with coefficient of correlation (R^2) values of 0.9866 and 0.89 respectively (Table S3). These results are in agreement with another study that showed a linear relationship between the accumulation of U in sunflowers and its initial concentration in soil solution.³⁴

Kinetic Analyses of Aqueous U.

Kinetic analyses were conducted to evaluate the differences in initial uptake rates (V_0) of U into plant roots, as a function of aqueous U and Ca concentrations in the carbonate water at pH 7.5 under environmentally relevant conditions. Significant increase in U uptake rate ($p < 0.05$) was observed when waters contained the highest U concentration ($700 \mu\text{g L}^{-1}$) with no Ca (Table 1). In all the cases, pseudo-second-order was the most appropriate fitting model (Figure 2) generating R^2 values of 0.9382 (Table 1) for the plots of t/q_t versus t , except at 240 mg L^{-1} Ca where no detectable uptake was measured over time. The values of V_0 and q_e were determined following eq 3 and are presented in Table 1 for all U initial concentrations. A high linear relationship ($R^2 = 0.9994$) exists between all the experimental and the calculated values of q_e (Figure S4). The uptake of U by *B. juncea* roots was found to

follow pseudo-second-order kinetics, presenting a fast U uptake in the first 24 h of exposure until approaching equilibrium between the remaining aqueous U and the accumulated U in roots (Figure 2). The approaching equilibrium can be observed by the linear relationship between the remaining aqueous U and U uptake for experiments with 0 mg L⁻¹ Ca ($R^2 = 0.9931$) and 12 mg L⁻¹ Ca ($R^2 = 0.7161$) (Figure S5). The pseudo-second-order model was reported in other studies attributing metal uptake in plants and bacteria to adsorption–absorption mechanisms.^{52,53,64,65} These mechanisms were also reported in studies investigating U biological uptake where U adsorption and precipitation were proposed to represent the main mechanisms of U uptake onto root cell walls.^{33,60} Gerber et al. attributed the fast phase of U uptake by a Gram-negative bacteria to the biosorption of U onto the cell membrane, which was confirmed by TEM.⁶⁶

The initial uptake rate (V_0) of U was found to be highly affected by Ca concentration in carbonate water at circumneutral pH. The increase of Ca from 0 to 12 mg L⁻¹ in carbonate water at pH 7.5 reduced V_0 by at least 6-fold for all initial U concentrations (Table 1). For instance, at 700 µg L⁻¹ U, V_0 significantly decreased ($p < 0.05$) from 62.75 ± 5.54 to 2.07 ± 0.21 µg g⁻¹ h⁻¹. The inverse relationship between the uptake rate of U and Ca concentration in solution is consistent with the inhibiting effect of Ca on U bioavailability. This effect may be due to the following possibilities: (1) a direct effect of Ca²⁺ on U uptake by inducing a competitive mechanism with UO₂²⁺ or (2) the complexation of UO₂²⁺ with Ca²⁺ and carbonate to form strong ternary Ca–U–CO₃ complexes that are less bioavailable to plants than other aqueous species.

Chemical equilibrium modeling based on the experimental conditions used in this study suggests that the distribution of U aqueous species was greatly affected by the presence of Ca and carbonate at pH 7.5. For instance, in the absence of Ca at 700 µg L⁻¹ U, (UO₂)₂CO₃(OH)₃⁻ is predicted to highly occur (91.1%) with the presence of 7.6% of binary uranyl-carbonate complexes U–CO₃ (mainly UO₂(CO₃)₂⁻² and UO₂CO₃) (Table S2). In water containing 12 mg L⁻¹ Ca, the fraction of (UO₂)₂CO₃(OH)₃⁻ decreases to 79% with a simultaneous 12.6% formation of ternary Ca–U–CO₃ (mainly CaUO₂(CO₃)₃⁻² and Ca₂UO₂(CO₃)₃). For experiments with 240 mg L⁻¹ Ca, Ca–U–CO₃ aqueous species highly occur (about 99%). Among all exposure solutions, the presence of free UO₂²⁺ was not detected, and only 0.1–4.9% of UO₂–OH were predicted to be present. These observations are consistent with those reported in many other studies.^{28,67}

The negligible presence of the positively charged uranyl cations in all solutions suggests that the charge competitive mechanism between U aqueous species and Ca²⁺ uptake likely was not the main reason for U uptake inhibition. Croteau et al. have reported that U does not exclusively use Ca membrane transporter during its bioaccumulation in invertebrates.²⁸ Alternatively, Ca may exert an indirect effect by decreasing the U bioavailable fraction through the formation of Ca–U–CO₃ complexes. When neutral Ca₂UO₂(CO₃)₃ became the dominant species for experiments with 240 mg L⁻¹ Ca, U uptake decreased (Figure S6). This is consistent with the study of Croteau et al., which showed that UO₂(CO₃)₂⁻² aqueous species represent the best predictor for U uptake rate and suggested that the Ca–U–CO₃ aqueous species are less bioavailable to invertebrates than other U species.²⁸ In our study, the rate constant of pseudo-second-order k_2 presented a highly linear relationship (R^2

0.9486) with the distribution of U–CO₃ species (Figure S7) as a function of the total U concentration at 0 and 12 mg L⁻¹ Ca. When U–CO₃ species predominate, the calculated k_2 in this study ($k_2 = 0.0103 \pm 0.0003 \text{ g } \mu\text{g}^{-1} \text{ h}^{-1}$) was found to be higher than those reported in other studies for U uptake by bacteria⁶⁸ or for the uptake of other metals by various plants (Table S4).^{53,69} Other studies reported that neutral Ca₂UO₂(CO₃)₃ can inhibit U toxicity in porcine proximal kidney cells³⁰ and microbiological U(VI) reduction.^{67,70} However, the effect of Ca on U bioavailability in plants through the formation of Ca–U–CO₃ species has been not recognized in the literature. For example, Markich related the mechanisms of U bioaccumulation by macrophyte to a competitive mechanism between Ca²⁺ and UO₂²⁺ and did not consider the influence of uranyl-carbonate species despite their predominance in the exposure media.²¹ Thus, the findings presented in this study provide relevant insights about the bioavailability and the effect of U–CO₃ and Ca–U–CO₃ in plants. Further microscopic and spectroscopic analyses were conducted to *B. juncea* roots to better understand the mechanisms of U uptake.

Solid Analysis.

The accumulation of U on the surface of plant roots that were exposed to 700 $\mu\text{g L}^{-1}$ U at low Ca concentration (0 and 12 mg L⁻¹) was confirmed by SEM-EDS and electron microprobe X-ray mapping analyses (Figures 3 and S8). Images obtained using SEM–backscattered electron (BSE) show U-bearing particles (0.3–0.7 μm) randomly distributed on the root surface for experiments using 0 mg L⁻¹ Ca, which were confirmed by microprobe mapping (Figure 3). The EDS spectra of these particles showed the presence of P, S, U, Ca, and K. These elements have an important structural and functional role in plant tissues.⁷¹ Some clusters of U-bearing particles (~2.2 μm) with similar elemental compositions were also detected on root surfaces. Because of the low accumulated U concentration (<100 mg kg⁻¹) in roots for experiments using 12 mg L⁻¹ Ca, fewer clusters of U-bearing particles were detected in these samples (Figure S8).

To identify if U has penetrated the epidermis cells, a FIB section prepared from across the cell walls was analyzed by scanning transmission electron microscopy/energy-dispersive X-ray spectrometry (STEM/EDS) X-ray mapping. Bright precipitates enriched with U were identified in the cell walls (Figure 3). The EDS spectra of these U-bearing precipitates contained P, U, K and Fe (Figure 3). According to previous studies, U could be highly retained on cell walls forming precipitates with endogenous P, especially when the U is not complexed with organic acids.^{7,39} Furthermore, the coexistence of a high Fe peak in the same area where U-bearing precipitates (Figure 3) occur can also have implications for the mechanisms of U bioaccumulation. For instance, although cell walls are known to contain essential elements like P, K, and Fe, Berthet et al. have shown that U accumulation in plant roots can have significant effect on Fe and P homeostasis.⁷²

Other studies have reported that U accumulation mechanisms can differ depending on its aqueous speciation and suggested that U accumulation in root cell walls can be attributed mostly to the following electrostatic interactions: (1) high electrostatic attraction between the positively charged UO₂²⁺ and the cell walls, resulting in high root accumulation with no root-to-shoot translocation, and (2) high electrostatic repulsion between the negatively

charged uranyl-carbonate and the cell walls, resulting in lower U root accumulation with higher root-to-shoot translocation.^{33,39} Plants exposed to uranyl-carbonate (100 μM U in solutions with 10 mM carbonate at pH 7) by Laurette et al. have shown U entrapped with P in the cell walls of leaves, but the accumulation sites for U in the roots could not be detected.³³ However, in that study, plants were exposed to 100 times higher U concentration than in this study. Thus, a direct comparison between the results from this study and those from Laurette et al. is not possible because U toxicity may also contribute to the uptake mechanisms at higher concentrations.⁴⁰ Future investigations should assess the impact of U concentrations on uranyl-carbonate uptake in the root and root-to-shoot translocation.

Although other studies have reported that electrostatic repulsion occurs between uranyl-carbonate complexes and the root cell walls, our study suggests that the exposure of plants to negatively charged uranyl-carbonate can still lead to the accumulation of U on the cell walls of roots. However, the surface complex form in which U was bound and accumulated in the roots is not yet identified or confirmed. The spectroscopic and microscopic results of this study agree with observations made in a study by Gorman-Lewis et al. in which U was found to be adsorbed on the negatively charged bacterial surface after being exposed to the negatively charged uranyl-carbonate complexes.⁷³ More information is necessary to understand the specific mechanism affecting the interaction and the accumulation of negatively charged U aqueous species in the roots.

Environmental Implications.

Uranium uptake by *B. juncea* was found to be greatly affected by Ca in carbonate circumneutral water over a range of environmentally relevant U concentrations. The effect of Ca on U bioavailability was assessed as a function of U speciation in the presence of uranyl-carbonate and ternary uranyl-calcium-carbonate aqueous species, unlike most previous studies, which have focused on investigating solutions in which UO_2^{2+} are predominant.^{21,74,75} Kinetic analyses indicate that higher uptake rates are obtained when uranyl-carbonate complexes are present in water. However, the U uptake in plants was inhibited in the presence of Ca and carbonate solutions used in this study, likely due to interactions with neutrally charged ternary uranyl-calcium-carbonate complexes. Given the crucial physiological role of Ca^{2+} in cells, more information is necessary to assess if Ca^{2+} ions affect the uptake of uranyl-carbonate complexes (i.e., Ca^{2+} channels and other membrane proteins). A rapid equilibrium following a pseudo-second-order approach was observed for U in solution at low Ca concentrations (e.g., lower than 12 mg L^{-1} Ca). This is a relevant observation that has implications in semiarid environments characteristic of the southwestern United States in which short rain events can enhance U dissolution¹⁴ and thus U exposure to plant roots. Analyses conducted with STEM and SEM/EDS detected U precipitates (U–P–K-bearing minerals) on the root cell walls after plants were reacted under the conditions selected for this study. The detection of these U-bearing precipitates on root cell walls suggests that negatively charged uranyl-carbonate complexes can interact with the negatively charged cell wall components (e.g., phosphate). Further investigations are necessary to understand how these insoluble U phases could affect the mechanisms of U toxicity in plants. The accumulation of U in plants is relevant as an exposure pathway or as a potential remediation alternative in mining sites. Therefore, more research should focus on

better understanding the mechanisms that affect U uptake at environmentally relevant conditions, especially as these relate to the following aspects: (1) determining in which chemical form U surface complexes bind to the root cell walls in carbonate water at weakly basic pH and (2) the influence of U and Ca concentrations on the accumulation and root-to-shoot translocation of U for plants exposed to uranyl-carbonate complexes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

Funding for this research was provided by the National Science Foundation (Grants NM EPSCoR #HIA-1301346, CREST 1345169, and CAREER 1652619), the National Institute of Health Centers of Excellence on Environmental Health Disparities Research (Grant Numbers 1-P50-ES-026102-01 and US-EPA 836157-01), and the National Institute of Environmental Health Sciences Superfund Research Program (Award 1 P42 ES025589). Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. Electron microprobe, focused ion beam sample preparation, and transmission electron microscopy were performed in the Electron Microbeam Analysis Facility, Department of Earth and Planetary Sciences and Institute of Meteoritics, University of New Mexico, a facility supported by the State of New Mexico, NASA, and the National Science Foundation. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. All data generated or analyzed during this study are included in the main text of this publication and in the supporting information.

REFERENCES

- (1). Winde F; Erasmus E; Geipel G Uranium contaminated drinking water linked to leukaemia- Revisiting a case study from South Africa taking alternative exposure pathways into account. *Sci. Total Environ* 2017, 574, 400–421. [PubMed: 27639476]
- (2). Ferrari CR; Do Nascimento HDAF; Rodgher S; Almeida T; Bruschi AL; Do Nascimento MRL; Bonifácio RL Effects of the discharge of uranium mining effluents on the water quality of the reservoir: An integrative chemical and ecotoxicological assessment. *Sci. Rep* 2017, 7 (1), 13919. [PubMed: 29066833]
- (3). Avasarala S; Lichtner PC; Ali A-MS; González-Pinzón R; Blake JM; Cerrato JM Reactive transport of U and V from abandoned uranium mine wastes. *Environ. Sci. Technol* 2017, 51 (21), 12385–12393. [PubMed: 29017012]
- (4). Schreck E; Dappe V; Sarret G; Sobanska S; Nowak D; Nowak J; Stefaniak EA; Magnin V; Ranieri V; Dumat C Foliar or root exposures to smelter particles: Consequences for lead compartmentalization and speciation in plant leaves. *Sci. Total Environ* 2014, 476–477, 667–676.
- (5). El Hayek E; El Samrani A; Lartiges B; Kazpard V; Benoit M; Munoz M Potential of *Opuntia ficus-indica* for air pollution biomonitoring: A lead isotopic study. *Environ. Sci. Pollut. Res* 2015, 22 (22), 17799–17809.
- (6). El Hayek E; El Samrani A; Lartiges B; Kazpard V; Aigouy T Lead bioaccumulation in *Opuntia ficus-indica* following foliar or root exposure to lead-bearing apatite. *Environ. Pollut* 2017, 220, 779–787. [PubMed: 27816295]
- (7). Huang JW; Blaylock MJ; Kapulnik Y; Ensley BD Phytoremediation of uranium-contaminated soils: Role of organic acids in triggering uranium hyperaccumulation in plants. *Environ. Sci. Technol* 1998, 32 (13), 2004–2008.
- (8). Duquène L; Vandenhoove H; Tack F; Meers E; Baeten J; Wannijn J Enhanced phytoextraction of uranium and selected heavy metals by Indian mustard and ryegrass using biodegradable soil amendments. *Sci. Total Environ* 2009, 407 (5), 1496–1505. [PubMed: 19054545]
- (9). Eapen S; Suseelan KN; Tivarekar S; Kotwal SA; Mitra R Potential for rhizofiltration of uranium using hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor*. *Environ. Res* 2003, 91 (2), 127–133. [PubMed: 12584014]

- (10). Moench RH; Schlee JS Geology and uranium deposits of the Laguna district; US Government Printing Office: NM, 1967.
- (11). Nash JT Uranium deposits in the Jackpile Sandstone, New Mexico. Econ. Geol. Bull. Soc. Econ. Geol 1968, 63, 737–750.
- (12). U. S. Geological Survey, 08349800 Rio Paguete below Jackpile Mine near Laguna, NM; https://waterdata.usgs.gov/nwis/inventory?agency_code=USGS&site_no=08349800, 2017 (accessed Januray 14, 2017).
- (13). USEPA. Technical report on technologically enhanced naturally occurring radioactive materials from uranium mining volume 1: Mining and reclamation background; U.S. Environmental Protection Agency, Office of Radiation and Indoor Air; EPA 402-R-08–005, 2008.
- (14). Blake JM; De Vore CL; Avasarala S; Ali A-M; Roldan C; Bowers F; Spilde MN; Artyushkova K; Kirk MF; Peterson E; Rodriguez-Freire L; Cerrato J Uranium mobility and accumulation along the Rio Paguete, Jackpile Mine in Laguna Pueblo, New Mexico. Environ. Sci. Process. Impacts 2017, 19, 605–621. [PubMed: 28352908]
- (15). Goulet RR; Fortin C; Spry D Uranium. In Homeostasis and toxicology of non-essential metals; Wood CM, Farrel AP, Brauner CJ, Eds.; Academic Press, 2012; pp 391–428.
- (16). Markich SJ; Twining JR Radioecology of tropical freshwater ecosystems: Mechanisms and kinetics of bioaccumulation and the importance of water chemistry. In Tropical Radioecology; Amsterdam, 2012; pp 233–82.
- (17). Trenfield MA; McDonald S; Kovacs K; Leshner EK; Pringle JM; Markich SJ; Ng JC; Noller B; Brown PL; Van Dam RA Dissolved organic carbon reduces uranium bioavailability and toxicity. 1. Characterization of an aquatic fulvic acid and its complexation with uranium [VI]. Environ. Sci. Technol 2011, 45 (7), 3075–3081. [PubMed: 21351802]
- (18). Fortin C; Denison FH; Garnier-Laplace J Metal-phytoplankton interactions: modeling the effect of competing ions (H^+Ca^{2+} and Mg^{2+}) on uranium uptake. Environ. Toxicol. Chem 2007, 26, 242–248. [PubMed: 17713211]
- (19). Tran D; Massabuau JC; Garnier-Laplace J Effect of carbon dioxide on uranium bioaccumulation in the freshwater clam *Corbicula fluminea*. Environ. Toxicol. Chem 2004, 23, 739–47. [PubMed: 15285368]
- (20). Campbell PGC Interactions between trace metals and aquatic organisms: A critique of the free-ion activity model. In Metal Speciation and Bioavailability in Aquatic Systems; Tessier A, Turner DR, Eds.; John Wiley and Sons: New York, 1995; pp 45–102.
- (21). Markich SJ Water hardness reduces the accumulation and toxicity of uranium in a freshwater macrophyte (*Ceratophyllum demersum*). Sci. Total Environ 2013, 443, 582–589. [PubMed: 23220392]
- (22). Du L; Feng X; Huang Z; Liu B; Jin Y; Fang Z; Zhang D; Liu N; Wang R; Xia C The effect of U speciation in cultivation solution on the uptake of U by variant *Sedum alfredii*. Environ. Sci. Pollut. Res 2016, 23 (10), 9964–9971.
- (23). Taylor HE; Peart DB; Antweiler RC; Brinton TI; Campbell WL; Garbarino JR; Roth DA; Hart RJ; Averett RC Data from synoptic water-quality studies on the Colorado river in the grand canyon, Arizona, November 1990 and June 1991. USGS Open-File Report 96–614; 1996.
- (24). Dong W; Brooks SC Determination of the formation constants of ternary complexes of uranyl and carbonate with alkaline earth metals ($Mg^{2+}Ca^{2+}Sr^{2+}$ and Ba^{2+}) using anion exchange method. Environ. Sci. Technol 2006, 40 (15), 4689–4695. [PubMed: 16913125]
- (25). Yan S; Boyanov MI; Mishra B; Kemner KM; O'Loughlin EJU(VI) Reduction by biogenic and abiotic hydroxycarbonate green rusts: Impacts on U(IV) Speciation and stability over time. Environ. Sci. Technol 2018, 52, 4601–4609. [PubMed: 29630355]
- (26). Wen H; Pan Z; Giammar D; Li L Enhanced uranium immobilization by phosphate amendment under variable geochemical and flow conditions: Insights from reactive transport modeling. Environ. Sci. Technol 2018, 52, 5841–5850. [PubMed: 29648808]
- (27). Riethmuller N; Markich S; Van Dam R; Parry D Effects of water hardness and alkalinity on the toxicity of uranium to a tropical freshwater hydra (*Hydra viridissima*). Biomarkers 2001, 6, 45–51. [PubMed: 23886056]

- (28). Croteau MN; Fuller CC; Cain DJ; Campbell KM; Aiken G Biogeochemical controls of uranium bioavailability from the dissolved phase in natural freshwaters. *Environ. Sci. Technol* 2016, 50 (15), 8120–8127. [PubMed: 27385165]
- (29). Muller D; Houpert P; Cambar J; Henge-Napoli MH Role of the sodium-dependent phosphate co-transporters and of the phosphate complexes of uranyl in the cytotoxicity of uranium in LLC-PK(1) cells. *Toxicol. Appl. Pharmacol* 2006, 214, 166–77. [PubMed: 16476458]
- (30). Carrière M; Avoscan L; Collins R; Carrot F; Khodja H; Ansoborlo E; Gouget B Influence of uranium speciation on normal rat kidney (NRK-52E) proximal cell cytotoxicity. *Chem. Res. Toxicol* 2004, 17 (3), 446–452. [PubMed: 15025516]
- (31). Prat O; Vercouter T; Ansoborlo E; Fichet P; Perret P; Kurtio P; Salonen L Uranium speciation in drinking water from drilled wells in Southern Finland and its potential links to health effects. *Environ. Sci. Technol* 2009, 43 (10), 3941–3946. [PubMed: 19544911]
- (32). Goulet RR; Thompson PA; Serben KC; Eickhoff CV Impact of environmentally based chemical hardness on uranium speciation and toxicity in six aquatic species. *Environ. Toxicol. Chem* 2015, 34 (3), 562–574. [PubMed: 25475484]
- (33). Laurette J; Larue C; Mariet C; Brisset F; Khodja H; Bourguignon J; Carrière M Influence of uranium speciation on its accumulation and translocation in three plant species: Oilseed rape, sunflower and wheat. *Environ. Exp. Bot* 2012, 77, 96–107.
- (34). Rodríguez PB; Tomé FV; Fernández MP; Lozano JC Linearity assumption in soil-to-plant transfer factors of natural uranium and radium in *Helianthus annuus* L. *Sci. Total Environ* 2006, 361 (1–3), 1–7. [PubMed: 16182341]
- (35). Ebbs SD; Brady DJ; Kochian LV Role of uranium speciation in the uptake and translocation of uranium by plants. *J. Exp. Bot* 1998, 49 (324), 1183–1190.
- (36). Boghi A; Roose T; Kirk GJD A model of uranium uptake by plant roots allowing for root-induced changes in the soil. *Environ. Sci. Technol* 2018, 52 (6), 3536–3545. [PubMed: 29466669]
- (37). Edayilam N; Montgomery DA; Ferguson BO; Maroli AS; Martinez N; Powell BA; Tharayil N Phosphorus stress-induced changes in plant root exudation could potentially facilitate uranium mobilization from stable mineral forms. *Environ. Sci. Technol* 2018, 52 (14), 7652–7662. [PubMed: 29722538]
- (38). Saenen E; Horemans N; Vanhoudt N; Vandenhove H; Biermans G; Van Hees M; Wannijn J; Vangronsveld J; Cuypers A Effects of pH on uranium uptake and oxidative stress responses induced in *Arabidopsis thaliana*. *Environ. Toxicol. Chem* 2013, 32 (9), 2125–2133. [PubMed: 23737149]
- (39). Laurette J; Larue C; Llorens I; Jaillard D; Jouneau PH; Bourguignon J; Carrière M Speciation of uranium in plants upon root accumulation and root-to-shoot translocation: A XAS and TEM study. *Environ. Exp. Bot* 2012, 77, 87–95.
- (40). Misson J; Henner P; Morello M; Floriani M; Wu TD; Guerquin-Kern JL; Fevrier L Use of phosphate to avoid uranium toxicity in *Arabidopsis thaliana* leads to alterations of morphological and physiological responses regulated by phosphate availability. *Environ. Exp. Bot* 2009, 67, 353–62.
- (41). Gunther A; Bernhard G; Geipel G; Reich T; Rossberg A; Nitsche H Uranium speciation in plants. *Radiochim. Acta* 2003, 91, 319–328.
- (42). Li D; Kaplan DI; Chang HS; Seaman JC; Jaffé PR; Koster Van Groos P; Scheckel KG; Segre CU; Chen N; Jiang DT; Newville M; Lanzirotti A Spectroscopic evidence of uranium immobilization in acidic wetlands by natural organic matter and plant roots. *Environ. Sci. Technol* 2015, 49 (5), 2823–2832. [PubMed: 25634067]
- (43). Chang HS; Buettner SW; Seaman JC; Jaffe PR; Koster Van Groos P. G.; Li D; Peacock AD; Scheckel KG; Kaplan DI Uranium immobilization in an iron-rich rhizosphere of a native wetland plant from the savannah river site under reducing conditions. *Environ. Sci. Technol* 2014, 48 (16), 9270–9278. [PubMed: 25051143]
- (44). Straczek A; Duquene L; Węgrzynek D; Chinea-Cano E; Wannijn J; Navez J; Vandenhove H Differences in U root-to-shoot translocation between plant species explained by U distribution in roots. *J. Environ. Radioact* 2010, 101 (3), 258–266. [PubMed: 20080323]

- (45). Qi F; Zha Z; Du L; Feng X; Wang D; Zhang D; Fang Z; Ma L; Jin Y; Xia C Impact of mixed low-molecular-weight organic acids on uranium accumulation and distribution in a variant of mustard (*Brassica juncea* var. *tumida*). J. Radioanal. Nucl. Chem 2014, 302 (1), 149–159.
- (46). Gustafsson JP Visual MINTEQ ver. 3.1 <https://vminteq.lwr.kth.se/download/>.
- (47). Hu S; Lu Y; Peng L; Wang P; Zhu M; Dohnalkova AC; Chen H; Lin Z; Dang Z; Shi Z Coupled kinetics of ferrihydrite transformation and As (V) sequestration under the effect of humic acids: A mechanistic and quantitative Study. Environ. Sci. Technol 2018, 52 (V), 11632–11641.
- (48). Le Menn N; Marchand S; De Revel G; Demarville D; Laborde D; Marchal RN,S,O-Heterocycles in aged champagne reserve wines and correlation with free amino acid concentrations. J. Agric. Food Chem 2017, 65 (11), 2345–2356. [PubMed: 28110533]
- (49). Keskinan O; Goksu MZL; Basibuyuk M; Forster CF Heavy metal adsorption properties of a submerged aquatic plant (*Ceratophyllum demersum*). Bioresour. Technol 2004, 92 (2), 197–200. [PubMed: 14693453]
- (50). Hossain A; Bhattacharyya SR; Aditya G Biosorption of cadmium by waste shell dust of fresh water mussel *lamellidens marginalis* Implications for metal bioremediation. ACS Sustainable Chem. Eng 2015, 3 (1), 1–8.
- (51). Bai HJ; Zhang ZM; Yang GE; Li BZ Bioremediation of cadmium by growing *Rhodobacter sphaeroides* Kinetic characteristic and mechanism studies. Bioresour. Technol 2008, 99 (16), 7716–7722. [PubMed: 18358716]
- (52). Reddad Z; Gerente C; Andres Y; Le cloirec P Adsorption of several metal ions onto a low-cost biosorbent: Kinetic and equilibrium studies. Environ. Sci. Technol 2002, 36, 2067–2073. [PubMed: 12026994]
- (53). Maleva MG; Malec P; Prasad MNV; Strzałka K Kinetics of nickel bioaccumulation and its relevance to selected cellular processes in leaves of *Elodea canadensis* during short-term exposure. Protoplasma 2016, 253 (2), 543–551. [PubMed: 25985854]
- (54). Limcharoensuk T; Sooksawat N; Sumarnrote A; Awutpet T; Kruatrachue M; Pokethitiyook P; Auesukaree C Bioaccumulation and biosorption of Cd²⁺ and Zn²⁺ by bacteria isolated from a zinc mine in Thailand. Ecotoxicol. Environ. Saf 2015, 122, 322–330. [PubMed: 26300116]
- (55). Veglio F; Beolchini F Removal of heavy metal ions by biosorption: A review. Hydrometallurgy 1997, 44, 301–316.
- (56). Kaplan DI; Xu C; Huang S; Lin Y; Toli N; Roscioli-Johnson KM; Santschi PH; Jaffé PR Unique organic matter and microbial properties in the rhizosphere of a wetland soil. Environ. Sci. Technol 2016, 50 (8), 4169–4177. [PubMed: 27091553]
- (57). Butler AD; Wynter M; Medina VF; Bednar AJ Depleted uranium toxicity, accumulation, and uptake in *Cynodon dactylon* (Bermuda) and *Aristida purpurea* (Purple Threeawn). Bull. Environ. Contam. Toxicol 2016, 96 (6), 714–719. [PubMed: 27016940]
- (58). Favas PJC; Pratas J; Mitra S; Sarkar SK; Venkatachalam P Biogeochemistry of uranium in the soil-plant and water-plant systems in an old uranium mine. Sci. Total Environ 2016, 568, 350–368. [PubMed: 27314898]
- (59). Chang P; Kim KW; Yoshida S; Kim SY Uranium accumulation of crop plants enhanced by citric acid. Environ. Geochem. Health 2005, 27 (5–6), 529–538. [PubMed: 16237609]
- (60). Lee M; Yang M Rhizofiltration using sunflower (*Helianthus annuus* L) and bean (*Phaseolus vulgaris* L var. *vulgaris*) to remediate uranium contaminated groundwater. J. Hazard. Mater 2010, 173 (1–3), 589–596. [PubMed: 19783370]
- (61). Dushenkov S; Vasudev D; Kapulnik Y; Gleba D; Fleisher D; Ting KC; Ensley B Removal of uranium from water using terrestrial plants. Environ. Sci. Technol 1997, 31 (12), 3468–3474.
- (62). Yang M; Jawitz JW; Lee M Uranium and cesium accumulation in bean (*Phaseolus vulgaris* L var. *vulgaris*) and its potential for uranium rhizofiltration. J. Environ. Radioact 2015, 140, 42–49. [PubMed: 25461514]
- (63). Saenen E; Horemans N; Vanhoudt N; Vandenhove H; Biermans G; Van Hees M; Wannijn J; Vangronsveld J; Cuypers A The pH strongly influences the uranium-induced effects on the photosynthetic apparatus of *Arabidopsis thaliana* plants. Plant Physiol. Biochem 2014, 82, 254–261. [PubMed: 25014646]

- (64). Vijayaraghavan K; Arun M; Joshi UM; Balasubramanian R Biosorption of As (V) onto the shells of the crab (*Portunus sanguinolentus*): Equilibrium and kinetic Studies. Ind. Eng. Chem. Res 2009, 48, 3589–3594.
- (65). Mikutta R; Baumgärtner A; Schippers A; Haumaier L; Guggenberger G Extracellular polymeric substances from bacillus subtilis associated with minerals modify the extent and rate of heavy metal sorption. Environ. Sci. Technol 2012, 46 (7), 3866–3873. [PubMed: 22443088]
- (66). Gerber U; Zirnstein I; Krawczyk-Bařsch E; Lünsdorf H; Arnold T; Merroun ML Combined use of flow cytometry and microscopy to study the interactions between the gram-negative betaproteobacterium *Acidovorax facilis* and uranium (VI). J. Hazard. Mater 2016, 317, 127–134. [PubMed: 27262280]
- (67). Brooks SC; Fredrickson J. k.; Carroll SL; Kennedy D. w.; Zachara JM; Plymale AE; Kelly SD; Kemner KM; Fendorf S Inhibition of bacterial U (VI) reduction by calcium. Environ. Sci. Technol 2003, 37 (9), 1850–1858. [PubMed: 12775057]
- (68). Xie S; Yang J; Chen C; Zhang X; Wang Q; Zhang C Study on biosorption kinetics and thermodynamics of uranium by *Citrobacter freundii*. J. Environ. Radioact 2008, 99 (1), 126–133. [PubMed: 17765369]
- (69). Keskin O; Goksu MZL; Basibuyuk M; Forster CF Heavy metal adsorption properties of a submerged aquatic plant (*Ceratophyllum demersum*). Bioresour. Technol 2004, 92 (2), 197–200. [PubMed: 14693453]
- (70). Stewart BD; Amos RT; Fendorf S Effect of uranium (VI) speciation on simultaneous microbial reduction of uranium (VI) and iron (III). J. Environ. Qual 2011, 40 (1), 90. [PubMed: 21488497]
- (71). George EF; Hall MA; De Klerk GJ Plant propagation by tissue culture, 3rd ed.; 2008; pp 65–113.
- (72). Berthet S; Villiers F; Alban C; Serre NBC; Martin-Laffon J; Figuet S; Boisson AM; Bligny R; Kuntz M; Finazzi G; Ravanel S; Bourguignon J *Arabidopsis thaliana* plants challenged with uranium reveal new insights into iron and phosphate homeostasis. New Phytol 2018, 217 (2), 657–670. [PubMed: 29165807]
- (73). Gorman-Lewis D; Elias PE; Fein JB Adsorption of aqueous uranyl complexes onto *Bacillus subtilis* Cells. Environ. Sci. Technol 2005, 39 (13), 4906–4912. [PubMed: 16053091]
- (74). Soudek P; Petrova T; Benesova D; Dvorakova M; Vanek T Uranium uptake by hydroponically cultivated crop plants. J. Environ. Radioact 2011, 102 (6), 598–604. [PubMed: 21486682]
- (75). Soudek P; Petrová Š; Buzek M; Lhotský O; Van T Uranium uptake in *Nicotiana sp* under hydroponic conditions. J. Geochem. Explor 2014, 142, 130–137.

Figure 1.

Concentration of U ($\mu\text{g L}^{-1}$) as a function of reaction time in solutions containing 1 mM HCO_3^- at pH 7.5 at different levels of initial U_i (30 , 100 , 300 , and $700 \mu\text{g L}^{-1}$) and Ca (0 , 12 , and 240 mg L^{-1}) concentrations. The standard deviation was determined from triplicate experiments. Different letters indicate significant difference ($p < 0.05$) in the variation of aqueous U concentration (as indicated by Kruskal–Wallis test followed by Dunn’s test) between solutions with different Ca treatments at the different levels of initial U_i concentration.

Figure 2. Uranium uptake in plant roots ($\mu\text{g g}^{-1}$) at different initial U_i and Ca concentrations in solutions containing 1 mM HCO_3^- at pH 7.5. The uptake rate followed a pseudo-second-order kinetic model. The standard deviation was determined from triplicate experiments. Different letters indicate significant difference ($p < 0.05$) in U uptake (as indicated by Kruskal–Wallis test followed by Dunn’s test) between solutions with different Ca treatments at the different levels of initial U_i concentration.

Figure 3.

Backscattered electron (BSE) SEM images, microprobe mapping, and energy dispersive spectroscopy (EDS) spectra for root surface in water free of Ca at $700 \mu\text{g L}^{-1}$ U indicating the accumulation of U on root cell walls and the formation of particles containing U and P: (a) BSE image corresponding to the microprobe mapping; (b) microprobe mapping showing the distribution of U on root surface; (c and d) BSE images and EDS spectra of a cluster of U–P bearing particles; (e) EDS spectra for the FIB cross section across the cell walls (CW) of root surface identifying the co-occurrence of U, P, and Fe in the cell walls; (f) secondary electron (SE) SEM extraction of the FIB section across the cell walls of root surface; (g and h) STEM images of FIB cross section showing U precipitates in the cell wall of root; and (i) STEM X-ray map for U in the FIB cross section confirming U precipitation in the cell wall of epidermal cells.

Table 1.**Kinetic Parameters for U Uptake by Brassica juncea Roots^a**

U concentration ($\mu\text{g L}^{-1}$)	Ca (mg L^{-1})	V_0 ($\mu\text{g g}^{-1} \text{h}^{-1}$)	k_2 ($\text{g } \mu\text{g}^{-1} \text{h}^{-1}$)	q_e ($\mu\text{g g}^{-1}$)	R^2
30	0	$4.44 \pm 1.9\text{abcd}$	$0.0103 \pm 0.0003\text{a}$	$24 \pm 6.3\text{bc}$	0.9949
100	0	$25.94 \pm 3.3\text{abc}$	0.003ab	$95.5 \pm 5.7\text{abc}$	0.9888
300	0	$29.17 \pm 5.9\text{ab}$	0.0004bc	$264.8 \pm 29.6\text{a}$	0.9743
700	0	$62.75 \pm 5.54\text{a}$	$0.00011 \pm 0.00009\text{c}$	$868 \pm 344\text{a}$	0.9527
30	12	$0.73 \pm 0.15\text{d}$	$0.003 \pm 0.001\text{ab}$	$17 \pm 0.7\text{c}$	0.9787
100	12	$1.05 \pm 0.5\text{d}$	0.002abc	$20 \pm 7.8\text{bc}$	0.9406
300	12	1.82cd	0.00017bc	99.01ab	0.9382
700	12	$2.07 \pm 0.21\text{bcd}$	$0.00012 \pm 0.00002\text{c}$	$109.3 \pm 45.7\text{ab}$	0.9535

^aThe following parameters are represented as the calculated average value with the corresponding standard deviation (SD) for triplicate experiments: the initial uptake rate, V_0 ($\pm$ SD); the rate constant of pseudo-second-order, k_2 ($\pm$ SD); and the mass of U per gram of root at equilibrium, q_e ($\pm$ SD). Different letters indicate significant differences ($p < 0.05$) between different U and Ca treatments in each column as indicated by the Kruskal–Wallis test followed by Dunn's test.