Role of uranium speciation in the uptake and translocation of uranium by plants

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Received 10 November 1997; Accepted 16 February 1998

Abstract

Uranium (U) uptake and translocation by plants was characterized using a computer speciation model to develop a nutrient culture system that provided U as a single predominant species in solution. A hydroponic uptake study determined that at pH 5.0, the uranyl (UO$_2^{2+}$) cation was more readily taken up and translocated by peas (Pisum sativum) than the hydroxyl and carbonate U complexes present in the solution at pH 6.0 and 8.0, respectively. A subsequent experiment tested the extent to which various monocot and dicot species take up and translocate the uranyl cation. Of the species screened, tepary bean (Phaseolus acutifolius) and red beet (Beta vulgaris) were the species showing the greatest accumulation of U. In addition to providing fundamental information regarding U uptake by plants, the results obtained also have implications for the phytoremediation of U-contaminated soils. The initial characterization of U uptake by peas suggested that in the field, a soil pH of <5.5 would be required in order to provide U in the most plant-available form. A pot study using U-contaminated soil was therefore conducted to assess the extent to which two soil amendments, HEDTA and citric acid, were capable of acidifying the soil, increasing U solubility, and enhancing U uptake by red beet. Of these two amendments, only citric acid proved effective, decreasing the soil pH to 5.0 and increasing U accumulation by a factor of 14. The results of this pot study provide a basis for the development of an effective phytoremediation strategy for U-contaminated soils.

Key words: Uranium, citrate, phytoremediation, uptake, speciation.

Introduction

The US Department of Energy (USDOE) has for decades been the organization most heavily involved with the nation’s uranium (U) resources. Unfortunately, emissions and accidental spills have led to soil U contamination at a number of USDOE research laboratories and commercial mining facilities. More than 50% of USDOE facilities involved in reactor operations, weapons research, nuclear fuel production, and waste reprocessing reported that U was the most frequent radionuclide contaminant in groundwater and surface soils (Riley et al., 1992). U-contamination at the former Feed Materials Production Center at Fernald, OH., led to the addition of this area to the US Environmental Protection Agency’s National Priorities List as a ‘Superfund’ site. Remediation of these contaminated soils is a high priority within the USDOE primarily because these soils represent a potential source of groundwater contamination (Lee et al., 1993).

As is the case for heavy metals, the current remediation technology for U-contaminated sites most often involves identification of the contaminated soils, mass excavation of these areas, and packaging and removal of the soil to disposal facilities. However, this technique is expensive and requires long-term monitoring. As a result, USDOE has initiated restoration projects such as the ‘Uranium in Soils Integrated Demonstration’ (USID) to evaluate and compare the versatility, efficiency, and economics of various technologies for the removal of U from contaminated soils (Lee and Marsh, 1992). Phytoremediation, the use of terrestrial plants to remove, or phytoextract, contaminants from polluted soil, is one technology being evaluated.

The development of a phytoremediation strategy for any contaminated site requires, among other things, an understanding of the geochemical behaviour of the contaminant in the soil, knowledge of the chemical species of the contaminant most readily accumulated by plants, the identification of a plant species capable of both taking up and translocating that chemical species, and the development of agronomic and/or amendment strategies designed to maximize the availability and uptake of the chemical species of interest. For heavy metals such as Cd,
Ni, and Zn, current research in the field of phyto-
remediation has provided much of this information.
Unfortunately, such fundamental information is lacking
for U.

The pH-dependent speciation of U in soil and aqueous
systems is an area that has been extensively studied. U is
present in the soil primarily (80–90%) in the + VI oxida-
tion state as the uranyl (UO$_2$$^+$) cation (Bondietti and
Sweeton, 1977; Sheppard, 1980; Sheppard and Evenden,
1988; Allen et al., 1994; Mortvedt, 1994), despite the fact
that the contamination at sites like the Fernald, OH.,
USDOE facility originated as tetravalent UO$_2$ (Allen
et al., 1994). Under acidic, reducing conditions, UO$_2$$^+$
is the predominant U species in the soil (Hostetler and
Garrels, 1962; Langmuir, 1978; Mortvedt, 1994). Hydrox-
ide complexes, such as UO$_2$OH$^+$, (UO$_2$)$_2$(OH)$_3$$^+$,
(UO$_2$)$_3$(OH)$_4$$^-$, and (UO$_2$)$_4$(OH)$_5$$^-$; and phosphate
complexes such as UO$_2$(HPO$_4$)$_2^-$ and UO$_2$(HPO$_4$)$_2$$^-$, form under
near neutral conditions (Langmuir, 1978), while carbon-
ate complexes such as UO$_2$CO$_3$$^-$, UO$_2$(CO$_3$)$_2$$^-$, and
UO$_2$(CO$_3$)$_3$$^-$ predominate under alkaline conditions
(Langmuir, 1978; Lee et al., 1993; Mortvedt, 1994). The
stability of these complexes vary (Mortvedt, 1994), but
UO$_2$(HPO$_4$)$_2$$^-$ is the most stable complex from pH 4–7.5
(Langmuir, 1978; Lee et al., 1993).

There is little information relating U speciation to plant
uptake. The form(s) of U taken up by plants and the
mechanism by which this occurs have yet to be identified.
Most of the work to date has focused on either the U
content of native plants growing on U-contaminated soils
(Ibrahim and Whicker, 1988; Saric et al., 1995) or U
accumulation by field and garden crops of importance to
animals and humans (Sheppard et al., 1984; Lakshmanan
and Venkateswarlu, 1988; Sheppard et al., 1989). These
studies do not, however, provide the information
necessary to develop a phytoremediation strategy for
U-contaminated soils.

The present study was undertaken to provide funda-
mental information regarding the uptake and transloca-
tion of U by plants and to utilize that information to
improve the phytoextraction of U from a contaminated
soil. The first part of this study used computer speciation
modelling and hydroponic experiments to determine the
form of U most readily accumulated in plant shoots. This
was accomplished by buffering nutrient solutions at vari-
ous pH values so as to expose plants to a single predomi-
ant U species, rather than a mixture of U species. This
allowed for an assessment of both the uptake and the
toxicity of U species. The results indicated that the free
uranyl (UO$_2$$^+$) cation, which predominates at a pH of
5.0–5.5, was the form of U most readily accumulated by
plants. With this established, numerous plant species were
then screened hydroponically for the ability to both take
up and translocate the uranyl cation. A variety of species
were tested, including both monocot and dicot species.

Among those included were Brassica juncea and Brassica
rapa, two species which has recently been shown to
accumulate heavy metals such as Cd, Cu, Ni, Pb, and Zn
(Kumar et al., 1995; Ebbs et al., 1997). Corn and oats
were included as representative high-biomass monocot
species. Finally, using one of the plant species identified
by the screening experiments and a U-contaminated soil
obtained from a contaminated site near Ashtabulah, OH.,
a pot experiment was conducted to assess the extent to
which red beet was capable of phytoextracting U from
contaminated soil. This research involved the use of weak
organic acids that could both lower soil pH to convert
most of the U to the uranyl cation, and increase the
bioavailability of U for plant uptake.

Materials and methods

Design of hydroponic culture system

The modified Johnson’s nutrient solution used in the hydropro-
nic experiments described below consisted of the following: 3.0 mM
KNO$_3$; 2.0 mM Ca(NO$_3$)$_2$; 0.5 mM MgSO$_4$; 25 µM KCl;
12.5 µM H$_2$BO$_3$; 1.0 µM MnSO$_4$; 1.0 µM ZnSO$_4$; 0.5 µM
CuSO$_4$; 0.1 µM H$_2$MoO$_4$; and 0.1 µM NiSO$_4$. Iron (5 µM)
was supplied to dicots as Fe-EDDHA (N,N,N’,N’-ethelene-
bis(2-hydroxyphenyl-glycine)) and to grass species as
Fe-HEDTA (N-hydroxyethylthelylenediaminetriacetic acid).
Using this nutrient composition as a basis, GEOCHEM-PC
(Parker et al., 1995) was used to model nutrient solutions which
provided a good separation of U species. The normal P
source for this nutrient solution, 0.1 mM NH$_4$H$_2$PO$_4$, was
excluded from the model to preclude the formation of uranyl
phosphate complexes. The goal was to develop a hydroponic
system that provided U predominantly as either the free uranyl
cation, uranyl hydroxides, or uranyl carbonates.

Uptake of individual U species

Seeds of peas (Pisum sativum cv. ‘Sparkle’) were germinated on
moistened filter paper for 4 d and transferred to 21 pots
containing the nutrient solution described above. Three pH
treatments were imposed by addition of 2 mM 2-[N-morpholinol]-
ethane-sulphonic acid (MES) (Sigma, St Louis, MO.), titrated
to pH 5.0 or 6.0, or the addition of 1 mM HClO$_4$ (pH 8.0).
There were four replicates of each treatment. Plants were pre-
cultured in a growth chamber with a 16 h photoperiod for 10 d
in the presence of 0.1 mM NH$_4$H$_2$PO$_4$. After this time, plants
were removed from the solution, the roots rinsed in deionized
water and the plants transferred to solutions containing nutrient
solution of the same composition except that P was excluded
and 5 µM UO$_2$(NO$_3$)$_2$ was included. The control treatment
consisted of nutrient solution lacking both P and U. Plants
were grown for an additional 7 d before roots and shoots were
harvested. Roots were rinsed five times in deionized water.
Root and shoot tissues were dried and digested with nitric acid
at 180 °C for more than 2 h followed by 1:1 nitric/perchloric
acid at 220 °C until the sample was completely digested. The
ash was resuspended in 5% nitric acid and analysed using an
inductively-coupled argon plasma Emission Spectrometer
(ICAP-ES) (Model 61E, Thermo Jarrell Ash, Franklin, MA).
Statistical analyses of the results from this and all subsequent
experiments utilized the Student’s t-test at the 0.05 level of
significance.
Effect of P on the uptake of UO$_2^{2+}$

Pea seedlings were grown hydroponically as in the previous experiment for 10 d in the presence of 100 µM P. After the preculture period, plants were transferred to one of four treatments: control (no-U, no-P); 5 µM U no-P; no-U + 5 µM P; and 5 µM U + 5 µM P. Each treatment was replicated four times. Plants were grown for an additional 7 d, with roots and shoots harvested and analysed as in the previous experiment.

Screening of plant species for U accumulation

The following species were screened for the ability to accumulate U: peas (Pisum sativum L. cvs ‘Sparkle’ and ‘E107’); two varieties of Indian mustard (Brassica juncea L.), accessions 426308 (USDA-ARS Plant Introduction Center, Iowa State University) and cv. ‘RH–30’ (Mycogen Plant Sciences, Madison, WI); turnip (Brassica rapa L., accession 163496) (USDA-ARS Plant Introduction Center, Iowa State University); red beet (Beta vulgaris L. cv. ‘Sweetheart’, Johnny’s Seeds, Albion, ME); alfalfa (Medicago sativa L., cv. Germain WL512); common vetch (Vicia sativa L.) and hairy vetch (Vicia villosa L.) (cvs unknown, F. and J. Seed Service, Woodstock, IL.); crown vetch (Coronilla varia L., cv. ‘Chemung’, Granite Seed, Lehi, UT); tepary bean (Phaseolus acutifolius L., var. L567, U.C. Riverside Legume Collection, Riverside, CA), oats (Avena sativa L., cv. ‘Otana’, Treasure St. Seed, Fairfield, MT), and corn (Zea mays L., var. 3377, Pioneer Seed, Johnston, IA). Nomenclature for the above species follows Terrell et al. (1986). Seedlings were grown as in the first experiment, a 10 d preculture period in the presence of P followed by exposure to 5 µM U in the absence of P, with four replicate plants for each species. Plants were harvested and analysed in the same manner as in previous experiments.

Uptake of U from contaminated soil

A pot study was conducted to assess the extent of U uptake by red beet. U-contaminated soil was provided by RMI U-hydroxyl precipitates would form rather than the carbonate complexes that had been predicted in the absence of P. Annise et al., unpublished results). The soil was sieved through a 5 mm steel sieve and stored in plastic barrels at a moisture level close to that at which the soil had been collected.

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Beet seeds were germinated for 2–3 d on moistened filter paper. Beets were grown in potting mix for an additional 7 d before being transplanted to pots containing 500 g of U-contaminated soil. Seedlings were watered with the same nutrient solution used in the hydroponic experiments, buffered to the soil pH of 6.8 using 1 mM N-2-hydroxyethylpiperezine-N’-2-ethanesulfonic acid (HEPES). To prevent the development of P deficiency, plant shoots were sprayed twice each week with 10 mM KH$_2$PO$_4$ adjusted to pH 6.0 with KOH. During foliar P application, the soil surface was covered to prevent the introduction of P into the soil. Care was also taken to ensure that the foliar P solution did not flow down the stem. The beets were grown for an additional 5 weeks.

During the last week of growth, subsets of pots (5 replicates per treatment) were treated with one of two soil amendments. The treatments consisted of the addition of 50 ml of either 0.09 mM HEDTA (trisodium salt) or 0.25 M citric acid, followed by a second application of the same solutions 3 d later. These treatments raised the soil concentration of HEDTA to 5 g kg$^{-1}$ soil and the citric acid concentration to 10.5 mg kg$^{-1}$ soil. This concentration of HEDTA is comparable to that found in studies artificially inducing hyperaccumulation from a heavy metal-contaminated soil (Huang and Cunningham, 1996; Huang et al., 1997). The citric acid concentration used in this study was based upon results obtained from other experiments investigating the kinetics of U solubilization using this U-contaminated soil mixture (Ebbs et al., unpublished results). Deionized water was added to a third subset of pots as a control. After this additional week of growth, plant shoots were harvested and analysed as in the previous experiments.

Soil samples were taken from each pot at harvest. Soil pH was measured to determine if the amendments lowered the pH to the target value of 5.0–5.5. The soil samples were extracted with water to determine the effect of the amendments on U solubility.

Results

Hydroponic culture system development

GEOCHEM-PC modelling indicated that the best resolution of U species was achieved at pH values of 5.0, 6.0 and 8.0. At pH 5.0, nearly 80% of the U was present in solution as the free uranyl (UO$_2^{2+}$) cation (Fig. 1). At pH 6.0, 94% of the U was present as hydroxide complexes, while at pH 8.0, nearly 100% of the U was present as carbonate complexes. As expected, the modelling also indicated that in the presence of P, uranium phosphate complexes would be stable over the pH range from 4.5 to 9.0 (Fig. 2). The formation of the U-phosphate complexes was predicted to reduce the level of free uranyl cation and uranyl hydroxides by approximately 10%. At pH >7.0 in the presence of P, the model indicated that U-hydroxyl precipitates would form rather than the carbonate complexes that had been predicted in the absence of P.

![Fig. 1. U speciation in a modified Johnson’s nutrient solution in the absence of P, as predicted by GEOCHEM-PC.](image-url)
The uptake of U into shoots of peas was influenced by pH, presumably because of the differences in the form of U in solution. The greatest shoot U concentration and accumulation occurred at pH 5.0 when U was present predominantly as the free uranyl cation (Figs 4, 5). Uptake at pH 6.0 was less than 20% of that at pH 5.0 while uptake at pH 8.0 was about 5% of that at pH 5.0. These results suggest that the cationic uranyl ion is the species most readily taken up and translocated by peas. As a desorption procedure was not carried out, concentrations of U in roots may include U adsorbed to root cells walls as well as what was taken up. Root U concentrations...
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were generally higher than those for shoots. Uptake/adsorption by roots was greatest at pH 6.0 and 8.0, with a lower concentration at pH 5.0 (Figs 4, 5).

Effect of P on the uptake of \( \text{UO}_2^{2+} \)

In the absence of U, there was no significant difference in root or shoot dry weights for pea plants grown for 7d in the presence or absence of P at pH 5.0 (Fig. 6). Shoot P concentrations for control plants also did not differ between the two treatments (data not shown), suggesting that the plant had sufficient P reserves after the preculture period to allow for normal growth and development during the exposure to U.

In the presence of \( 5 \mu\text{M U} \), in the absence of P, growth of both roots and shoots was severely inhibited compared to the –U-grown control plants. In solutions containing both U and P, root and shoot dry weights did not differ significantly from the –U control plants, suggesting that P had largely overcome the toxic effects of U in solution, most likely due to complexation of the U with phosphate.

Complexation may have also reduced the bioavailability of U to peas, as there was a >50% reduction in the U concentration of roots and shoots at pH 5.0 (Fig. 7). Roots of peas in solution containing both U and P still displayed symptoms of toxicity, but the dry weights were similar. This may have been due to the fact that lateral roots for the plants grown in the presence of both U and P were stunted and thickened, compensating for the reduction in lateral root length.

Screening of plant species for U accumulation

On a concentration basis, all the species screened in this experiment exhibited a greater accumulation of U than peas. The highest concentrations were found in beet and crown vetch (Fig. 8). On a per plant basis total U accumulation was greatest for beet and tepary bean (Fig. 9). Species with small seeds showed symptoms of P deficiency during the seventh day of treatment, suggesting that the P reserves within the plant had been exhausted. This deficiency, however, could be overcome through foliar P fertilization. This method could be used to provide P to developing plants without the need to include P in the growth solution.
not increase U uptake by beet (Fig. 12). However, the addition of citric acid dramatically stimulated shoot U accumulation by beet, by increasing U concentration 14-fold (from 15 to 209 mg U kg\(^{-1}\) DW). The bioaccumulation ratio (shoot [U]/soil [U]) for beet (0.67) was at least one order of magnitude greater than many of the values previously reported in the literature for U (Ibrahim and Whicker, 1988; Lakshmanan and Venkateswarlu, 1988; Sheppard and Evenden, 1988, 1992; Sheppard et al., 1989).

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Discussion

The results presented here clearly indicate that the plant-available form of U is the uranyl cation. Since this U species is present in solution only at pH 5.5 or less, U-contaminated soil with neutral to alkaline pH values may require acidification in order for the phytoextraction of U to be successful. Geochemical studies of U-contaminated sites such as the Fernald Environmental Management Project suggested that U is present in the soil chiefly as the anionic carbonate species (Lee and Marsh, 1992). For several years, alkaline, carbonaceous materials were added to this soil for erosion control and road construction activities. Geochemical modelling has shown, however, that the carbonate complexes that subsequently formed are not only stable but potentially mobile, posing a threat to groundwater. Thus, the addition of these carbonaceous materials was counter-productive. Similar management practices at other U-contaminated sites could make phytoextraction more difficult since the carbonate species do not appear to be taken up and translocated to an appreciable extent. The results presented here also indicate that citric acid can greatly facilitate U bioavailability, even in a soil with a neutral pH. This effect is due primarily to the solubilization and complexation of the uranyl cation by this organic ligand and, to a lesser extent, by the change in pH (Ebbs et al., unpublished results).

One point that has not been established previously is whether subsequent uptake of U into the roots of plants involves transport of the U-citrate complex or just the uranyl cation. The results of the hydroponic experiments reported here suggest that it may be the latter species. Furthermore, Munier-Lamy and Berthelin (1987) suggested that during the dissolution of U from soil, transient complexes with simple molecules like organic acids may form, preceding the formation of more stable polyanionic or polycationic complexes. In a U-contaminated soil following the addition of citric acid, there would be extensive formation of these transient organic complexes, more so than would be observed under normal soil conditions. As these complexes break down in the soil, most likely due to microbial degradation of the organic acid, the dissociation of the U-citrate complex could release large amounts of the uranyl cation. If this were to occur in the rhizosphere, plant uptake of U could occur before the uranyl cation has an opportunity to sorb to soil particles and organic matter, or form additional complexes in the soil solution.

Further research will be required, however, in order to develop this information into an effective remediation strategy for U-contaminated soils. The mechanism by which citric acid increases U solubility in the soil and uptake by plants needs to be studied in order to refine addition of this compound to soil and to maximize U bioavailability. The effect of citric acid on the solubilization of U from additional U-contaminated soils, varying in soil type, organic matter content, pH, and level of U contamination, also needs to be examined to understand more fully the role of other soil factors in U solubility as they relate to phytoextraction.

Another aspect that requires investigation with respect to the addition of citric acid to U-contaminated soil is the long-term impact of this amendment on U speciation in soil. The U-citrate complex, by its nature, is transient, due to microbial and photodegradation. Francis et al. (1992) and Dodge and Francis (1994) have shown, however, that degradation of U-citrate complexes can lead to the formation of insoluble, non-leachable compounds such as uranium trioxide. While this may facilitate U recovery from aqueous systems, the conversion of soil U to this form could hamper subsequent attempts to phytoextract U. The U chelated by citric acid following the initial addition of this compound to U-contaminated soil may originate in a pool of bound but potentially exchangeable U. Dissociation of the U-citrate complex following microbial or photodegradation could shift this U into a pool that is less available than the pool from which the U originated (i.e. to uranium trioxide). Thus, while the first few croppings following citric acid addition may effectively remove U from the soil, the long-term effect of this addition may be to convert the U into a form that does not respond to citric acid addition and cannot be phytoextracted. Thus, the use of citric acid may be effective in the short term, but may also create a longer term problem by fixing U in the soil as uranium trioxide. Given the problems that have developed from the addition of alkaline, carbonaceous materials to U-contaminated soil, further attempts at amend these sites should be well planned so as not to compound the existing problem.

Acknowledgements

The authors would like to thank Jay Cornish of MSE Technology Applications Inc., Butte, MT, for contacting RMI Environmental Services (Ashtabula, OH) and securing the U-contaminated soil used in this study. We would also like to thank Dr Wendell Norvell of the US Plant, Soil, and Nutrition Laboratory, USDA-ARS, for advice concerning the speciation modelling and the pot study. This research was supported by a grant from the United States Department of Energy- Division of Energy Biosciences (Interagency Agreement DE-A102-95ER21097).

References


