EMPS Annual Report

Project Number: 86687

Project Title: Phytosiderophore Effects on Subsurface Actinide Contaminants: Potential for Phytostabilization and Phytoextraction

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1. Research Objectives

This project seeks to understand the influence of phytosiderophore-producing plants (grasses, including crops such as wheat and barley) on the biogeochemistry of actinide and other metal contaminants in the subsurface environment, and to determine the potential of phytosiderophore-producing plants for phytostabilization and phytoextraction of actinides and some metal soil contaminants. Phytosiderophores are secreted by graminaceous plants such as barley and wheat for the solubilization, mobilization and uptake of Fe and other essential nutrients from soils. The ability for these phytosiderophores to chelate and absorb actinides using the same uptake system, as for Fe is being investigated through characterization of actinide-phytosiderophore complexes (independently of plants), and characterization of plant uptake of such complexes. We may also show possible harm caused by increased chelation of actinides, which may increase actinide mobilization & migration in the subsurface environment. This information can then be directly applied by either removal of harmful plants, or can be used to develop plant-based soil stabilization/remediation technologies. Such technologies could be the low-cost, low risk solution to many DOE actinide contamination problems.

2. Research Progress and Implications

This report summarizes work after ~1.75 year of a three-year project (~55% of year-2 budget spent).

Since the beginning of this project in September 2002, we have made significant progress in examining uranium and nickel uptake into plants, and examining Pu-coordination by phytosiderophores.

One component of our experimental process was to obtain phytosiderophores in order to identify and define their association with plutonium, uranium, and nickel. Mugineic acid, the principal form of phytosiderophore produced by barley plants, can be isolated from barley root exudates. Isolating mugineic acid from barley root exudates required germinating and growing barley plants, and establishing an iron-deficient environment to optimize phytosiderophore production. Root washings were then collected every morning for a period of two weeks, and the combined exudates were concentrated down to approximately 500 mL. We are in the process of purifying these exudates. We have also obtained an isolated and purified mugineic acid sample for use in coordination chemistry experiments.

- We have tested how Fe status affects the uptake of Uranium into Barley plants at 3 different solution pH conditions (pH =5, 6, 8, 1000ppm U), with and without the addition of citrate (40

mM citrate added or no citrate added), at Fe concentrations of 0, 7.5 and 100 μM (no Fe, low Fe and high Fe, respectively). With no added Fe, Barley plants are extremely chlorotic and have decreased growth. With low or high Fe, plants grow well. Roots of plants under all conditions had significantly higher uranium absorption than shoots, often by more than two orders of magnitude. Roots of plants grown without citrate absorbed significantly more U than roots of plants grown with citrate, from 2-3 times more at pH 6 and 8, to an order of magnitude more at pH 5. This is probably due to citrate keeping the uranium in a soluble form (>90% soluble), while less than 10% is soluble without citrate.

On the other hand, shoots of plants treated with citrate generally had at least an order of magnitude higher U uptake than plants without citrate. With citrate, shoot uptake was highest at pH 6 under all Fe conditions; without citrate, shoot uptake was highest at pH 5 for low and high Fe plants. Importantly, at high pH, Fe status may have a slight effect on uranium uptake into the shoots, with the most uranium uptake when no Fe was added. And this effect was more dramatic without citrate added. There are two possible explanations: one, phytosiderophores, known to be produced under these 'alkaline/ calcareous-like' soil conditions, may contribute to plant uptake of uranium. Or two, at least, that high Fe status interferes with uranium uptake into plants in alkaline conditions. Under our conditions, concentration factors ($\mu\text{g U /g plant dry weight}$) / ($\mu\text{g U/g solution}$) of 0 – 1 were obtained for shoots without citrate, and 0-1.7 for shoots with citrate. For roots grown without citrate, concentration factors were 45-480, while concentration factors of 20-30 were obtained for roots with citrate. The overall uptake of Uranium into plants shoots is very low, and would be difficult to adapt into a truly effective phytoextraction strategy, even when citrate is added. However, the root absorption of these plants is very high, suggesting that plants could be used in water treatment or phytostabilization technologies. Importantly in this case, citrate actually dramatically decreases the uranium associated with the plant roots, and increases solubility, suggesting that it could lead to contaminant mobilization.

- We have begun to examine the root absorption and shoot uptake of Pu into plants in similar tests to those we have completed with uranium. We expect to see more dramatic effects of Fe status on Pu uptake because Pu is chemically more similar to Fe than is uranium, so should be more affected by the phytosiderophore production/plant Fe status.

- We have begun to examine the root absorption and shoot uptake of Nickel into plants in similar tests to those we have completed with uranium. Nickel at 100 ppm was toxic to the plants under all conditions. Leaves became yellow and dry within two days after nickel addition, Fe-deficient plants being especially small and unhealthy. At pH 6, the more rapid death of the iron-deficient plants makes assessing the effect of Fe on Ni uptake difficult. However, this suggests that Fe helps the plants deal with Ni toxicity or, possibly, that a lack of Fe increases initial Ni uptake leading to rapid toxic effects. Overall effects of pH, Fe, and citrate can be seen in figures below. At pH 6, nickel remained soluble (> 98%) in solution (even without citrate), while at pH=8, on average only 60-70% of the nickel was soluble at the end of the experiment (regardless of citrate). At pH 6 in low and high Fe treated plants, shoot uptake was approximately an order of magnitude less with citrate than without citrate. On average, root uptake was also slightly higher without citrate. Citrate did not increase nickel solution solubility, but did decrease nickel uptake into the plants, suggesting that citrate somehow interferes with the nickel uptake mechanisms. At pH 8, plants without Fe generally had higher Ni shoot uptake than those grown with higher Fe levels. Additionally, citrate either slightly decreased or had little effect on uptake of nickel.

Shoot uptake was expected to be lower due to decreased solubility of nickel at pH 8. This was true at low and high Fe with no added citrate, where pH 6 plants has significantly higher uptake. However, uptake was similar under all other conditions. At pH 8, roots had ~ 3 times more Ni than when grown at pH 6, presumably due to decreased nickel solubility. For shoots, concentration factors were 2.5-3 with citrate and 5-20 without citrate. For roots, concentration factors were 40-80 with citrate and 40-120 without citrate.

At 50 ppm nickel, plants can survive the toxic effects of nickel longer, perhaps allowing us to better interpret the effects of Fe, citrate and pH. We have conducted uptake experiments at pH 6 and pH 8 with variable Fe levels and are in the process of analyzing the results.

Overall, the results of the Uranium and Nickel experiments suggest that:

1. Citrate, often suggested as an additive to help Pb and other metal uptake into the shoots of some plants, may not significantly help (or may even decrease) the overall adsorption of U and Ni into grasses and could promote metal solubilization and mobilization.
2. Fe may help plants deal with toxic metals, but the effect on uptake of Ni and U, metals that are unlike Fe, is small.
3. Concentration factors that may be usable for phytoremediation may be achievable with nickel, if toxicity can be avoided. However, adding chelators to improve the concentration ratios of uranium uptake into plants seems unlikely to allow for the dramatic improvements needed to make uranium phytoextraction a viable technology, at least with barley grass. However, uranium adsorption on plant roots can lead to significant uranium removal from solutions.

Figure 1:
Nickel concentrations in plants. Plants were grown in nutrient solution with 0, 7.5 μ M, or 100 μ M Fe, with or without added citrate. Bars show data for individual plants, and lines show averages.

• We have examined the chelating properties of Pu(IV) by the phytosiderophore mugineic acid (MA) by UV-Vis spectroscopy. The MA-Pu(IV) complex is spectroscopically nearly identical to the EDTA-Pu(IV) complex, and is a stable complex over the pH range 1-10 (Figure 3). This suggests that phytosiderophores will behave in the environment very similarly to EDTA, forming strong stable complexes under most environmental conditions, which could be highly mobile. We are attempting to obtain crystals of the MA-Pu(IV) complex in order to determine its exact structure. Titration of the bacterial siderophore Desferrioxamine B (DFO) into a solution of Pu(IV)-MA indicates that DFO cannot displace the MA, suggesting that the MA-Pu(IV) has a solution stability constant at least as high as DFO-Pu(IV), or that the exchange rate between these complexes is kinetically slow. Either way, this may suggest that MA-phytosiderophore complexes could contribute to actinide solubility and mobility in the environment, if bacteria do not break them down. We are beginning to set up actinide solubility experiments to determine the solubilization rates of Pu-hydroxide by bacterial siderophores, phytosiderophores, EDTA, citrate, and other common chelators. We are also attempting to obtain electrochemical data on the Pu-MA complex.

Figure 2: UV-Vis spectra of Pu(IV)-mugineic acid (MA) and Pu(IV)-EDTA showing similar spectral features as pH increases. Drawings show proposed changes in Pu(IV)-EDTA complex as pH increases, and possible parallel changes that could occur in Pu(IV)-MA complex.

3. Planned Activities

Presently, our priority is to obtain more purified phytosiderophores in order to determine the extent and type of uranium, plutonium and nickel chelation and solubilization. In the next year, we will determine the rate and amount of actinide solubilization from solid actinide forms (hydroxides, oxides) and from soil-sorbed species, in comparison to the solubilization ability of known common chelators, such as acetohydroxamic acid, EDTA and citrate.

Hydroponic plant uptake experiments have already begun with plutonium. We expect to publish results from experiments with uranium and nickel in fall of 2004, while we are obtaining new results with Pu uptake. These experiments will give us insight as to the ability of barley plants to adsorb, absorb or mobilize plutonium from solution and if it is correlated to plant-Fe-status and phytosiderophore production. We will also perform direct phytosiderophore utilization experiments. These experiments will continue into fiscal year 2005 with different variables and controls, such as comparisons between barley and Indian mustard uptake and solution amendments (additional phytosiderophore, acetohydroxamic acid, citrate, EDTA). The timeline for this set of experiments is one year.

While we are obtaining results from hydroponic experiments, other uptake experiments will be performed with synthetic soils that are amended with uranium, nickel or plutonium. These will be performed using the same hydroponic set-up with the soils in nylon or dialysis bags in order to prevent soil particle adherence to the plant roots. This will allow us to determine whether the plants can extract the metals directly from soils (phytoextraction). We will also perform modified column-type experiments to determine if phytosiderophore mobilize metals out of the plant root zone (phytostabilization). These experiments will take place between January 2005 and September 2005.

4. Information Access:

- Plutonium Futures: The Science 2003: Poster Presentation: <http://www.lanl.gov/pu2003> or <http://proceedings.aip.org/proceedings/confproceed/673.jsp?jsessionid=2678361087964579129>
- ARQ (Actinide Research Quarterly) Actinide Research Quarterly, 3rd/4th quarter 2003, pg 29-34. <http://www.lanl.gov/orgs/nmt/nmtdo/AQarchive/AQhome/AQhome.html>
- Nickel and Uranium Uptake into Grasses: Effects of Citrate and Fe. C.E. Ruggiero, E. Deladurantaye, S. Twary. Manuscript in preparation, to be submitted to *J. Environ. Quality*.