EMPS Annual Report- 2005

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 though characterization of actinide-phytosiderophore complexes (independently of plants), and characterization of plant uptake of such complexes. We may also show possible harm caused by these plants through increased chelation of actinides that increase in 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 ~2.5 year of a three-year project (~55% of year-3 budget spent; remainder being carried over for 1 year unfunded extention.)

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 purifiying these exudates. We have also obtained a isolated and purified mugineic acid sample for use in coordination chemistry experiments.

•We have tested how Fe status effects 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 (4M citrate added or no citrate added), in duplicate runs. Under our conditions, concentration factors (ug U / g plant dry weight / ug 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, concentration factors of 20-30 were obtained for roots with citrate. Important for DOE, 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, again suggesting that plants could be used in water treatment or phytostabilizaiton technologies. [For the highest removal result (pH= 5, all Fe levels, no citrate) , ~10% of the total uranium in the solution was absorbed by the plant root, which is significant level of removal; however, in all other conditions, less than 1% of the total Uranium available was removed by the plant, which is not a useful amount of remediation.] 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 mobilzation.

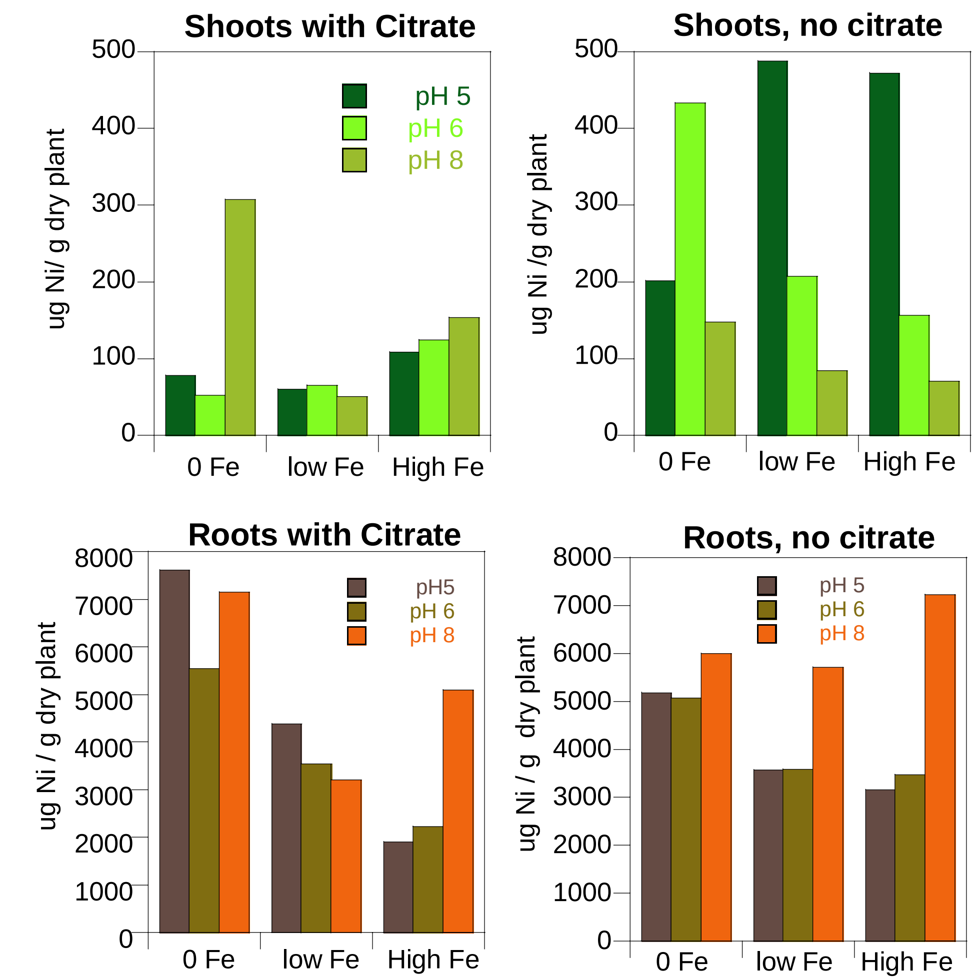
• We have begun to examine the root absorption and shoot uptake of Th and Pu into plants in similar tests to those we have completed with uranium. We expect to see more dramatic effects of Fe status on Pu uptake because Pu is chemically more similar to Fe than is uranium, so should be more effected by 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. While nickel, at 100ppm, was extremely toxic to the plants under all conditions, especially low Fe plants, 50ppm nickel allowed plants to grow. Over all effects of pH, Fe, and citrate can be seen in Figure 1 below. At pH = 5 and 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. As can be seen from the graphs below, citrate did not increase nickel solution solubility, but DECREASED or had no significant effect on nickel uptake into the plants or on nickel absorption onto roots at almost all conditions tested, suggesting that citrate somehow interferes with the nickel uptake mechanisms. The effects of Fe were variable, depending on conditions. We need to obtain more plant data in order to analyze the statistical significance, if any, of the variations we see. Under out conditions, concentration factors of 1-6 were obtained for shoots with citrate (average 2), and 1-10 for shoots without citrate (average 5). For roots grown with citrate, concentration factors were 40-150 (average 90); for roots grown without citrate, concentration factors were 60-140 (average 95). Again, as with Uranium, the actual uptake of nickel into plants is low (< 1% total Nickel removed), and toxicity becomes a significant issue at higher nickel solution concentrations, where higher concentration factors are expected.

<u>Overall, the results of the Uranium and Nickel experiments suggest that:</u>

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 effects on uptake of Ni and U, metals that are unlike Fe, are small.
3. Concentration factors that may be usable for phytoremediation may be achievable with nickel, if toxicity can be avoided, but true hyperaccumulators (rather than chelator assistance) may be a far better and safer option. With Uranium, even using added chelators to improve the

concentration ratios of Uranium uptake into plants seems unlikely to allow for the dramatic improvements needed to make uranium phytoextraction, at least with barley grass, a viable technology. However, uranium adsorption on plant roots can lead to significant uranium removal from solutions.



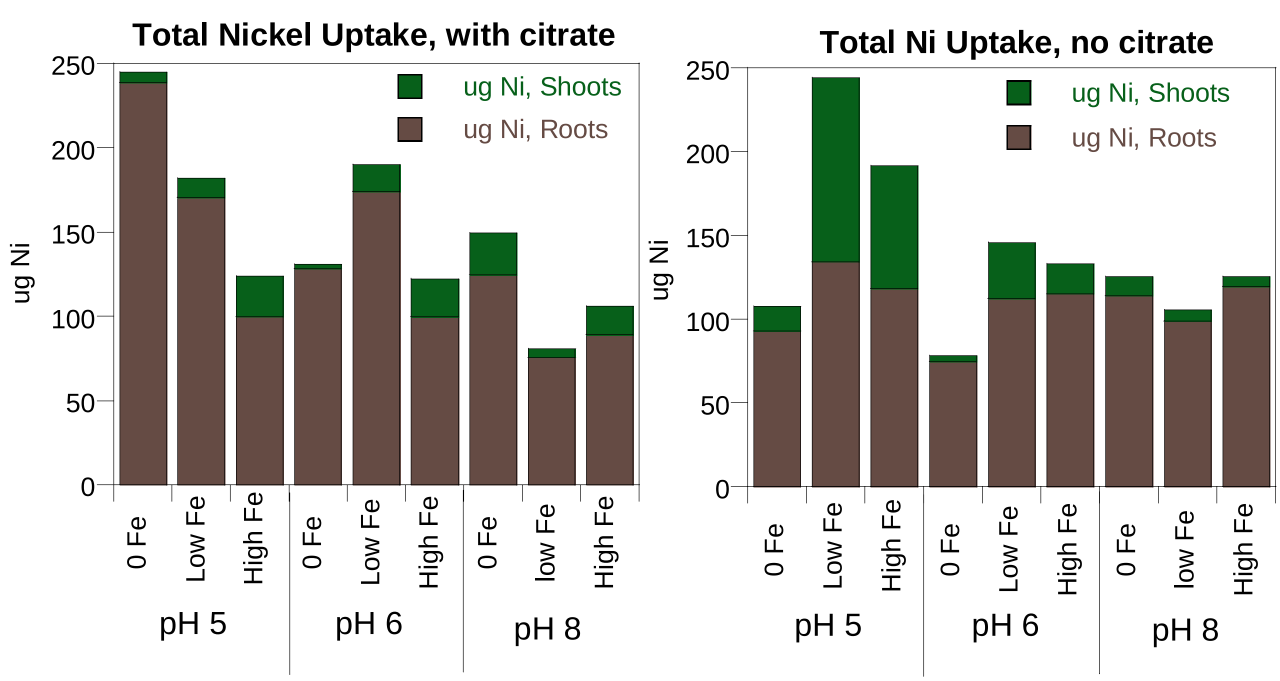


Figure 1: Nickel concentrations (µg Ni / g plant dry weight) in plants, and total nickel uptake into plants (total µg) Plants were grown in hydroponic solution with 50 ppm nickel (total 15mg) and 0, 7.5µM, or 100 µM Fe, with or without added citrate. Bars show averages for four plants.

• We have examined the chelation 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, that 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 they are not broken down by bacteria. 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.

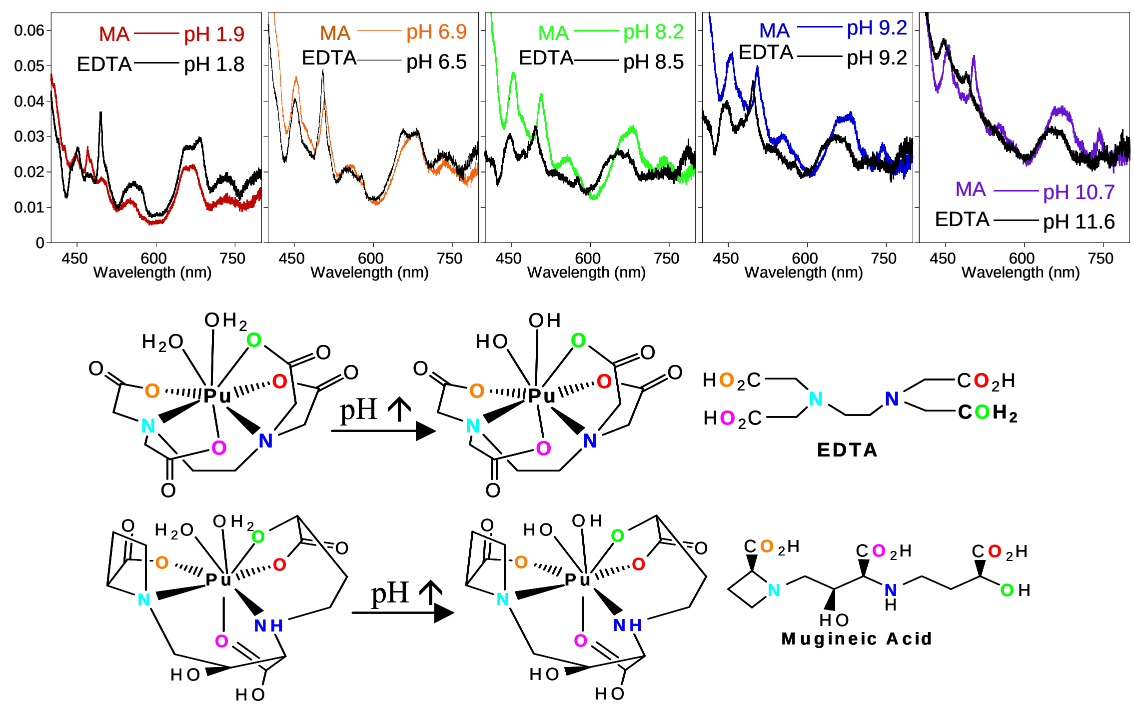


Figure 2: UV-Vis spectra of Pu(IV)-mugineic acid (MA) and Pu(IV)-EDTA showing similarity in spectral feacturea as pH increases. Drawings show proposed changes in Pu(IV)-EDTA complex as pH increases, and possible parallel changes that could occur in Pu(IV)-MA complex.

3. Planned Activities

Presently, our priority is to obtain more purified phytosiderophores in order to determine the extent and type of uranium, plutonium chelation and solubilization. In the next year, we will determine of the rate and amount of actinide solubilization from solid actinide forms (hydroxides, oxides) 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 or results from experiments with uranium and nickel in fall of 2005, while we are obtaining new results with Pu and Th 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.

While we are obtaining results are obtained from hydroponic experiments, other uptake experiments will be performed with synthetic soils that are amended with 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).

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, G. Purdy, S. Twary. Manuscript in preparation, to be submitted to *J. Environ. Quality*.