# PHYTOSIDEROPHORE EFFECTS ON SUBSURFACE ACTINIDE CONTAMINANTS: POTENTIAL FOR PHYTOSTABILIZATION AND PHYTOEXTRACTION

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EMSP
Basic research to solve cleanup needs

# Key Site Specific and Environmental Management Issues

- **Radionuclide and metal contamination prevalent at many DOE sites:**

  - Basic science problem: lack of scientific understanding of factors that govern metal and radionuclide contaminant stability, mobility and long term fate in the environment.
  - Few cost–effective and safe methods for remediation or stabilization of metal contaminants, particularly actinide contaminants
  - Need for basic research to provide new knowledge/technologies to support DOE's mission to clean up or stabilize these sites.

# Key Site Specific and Environmental Management Issues

- **Basic Science Needs:**
  - Biological properties of the subsurface & how they control contaminant fate & transport.
  - Mechanisms of biological processes that could be exploited for new stabilization /containment/remediation approaches OR that could be used to understand the long term reversibility of chemical and biological stabilization methods.

- **We will address these needs by assessing the impact of plant exudates on contaminant stability and mobility:**
  - How do plants and rhizosphere microbes affect the fate and transport of contaminants?
  - Can these processes be exploited or controlled to improve clean up/stabilization technologies?

# Phytoremediation: Use of Plants to Clean Up the Environment

- Phytostabilization:
  Use of vegetation to prevent erosion of soil, control groundwater movement through transpiration, and immobilize contaminants. (Hydraulic Control)

- Phytoextraction:
  Uptake of metals/contaminants into plant roots, accumulation into above ground parts, Harvest/removal of plants. (A solar driven pump)

**Benefits:**

Lower cost
Accelerate regulatory approvals
Increase public confidence
Low risk (in situ treatment)
Form of Natural Attenuation
Reduce waste volumes
Unlike bioremediation, easy removal from soils
Already commercially available
Proven design principles and cost analyses can be directly applied to new metal/plant systems.

**Drawbacks:**

SLOW
Need to dispose of plants
Plants commonly studied/used are not able to grow everywhere
No hyperaccumulating plants known for some metals
Climate Factors
Success depends on many environmental factors

# Chelate-Enhanced Phytoremediation or "Induced" Phytoextraction

- Add metal chelators & other soil amendments which solubilize metals to facilitate plant uptake:

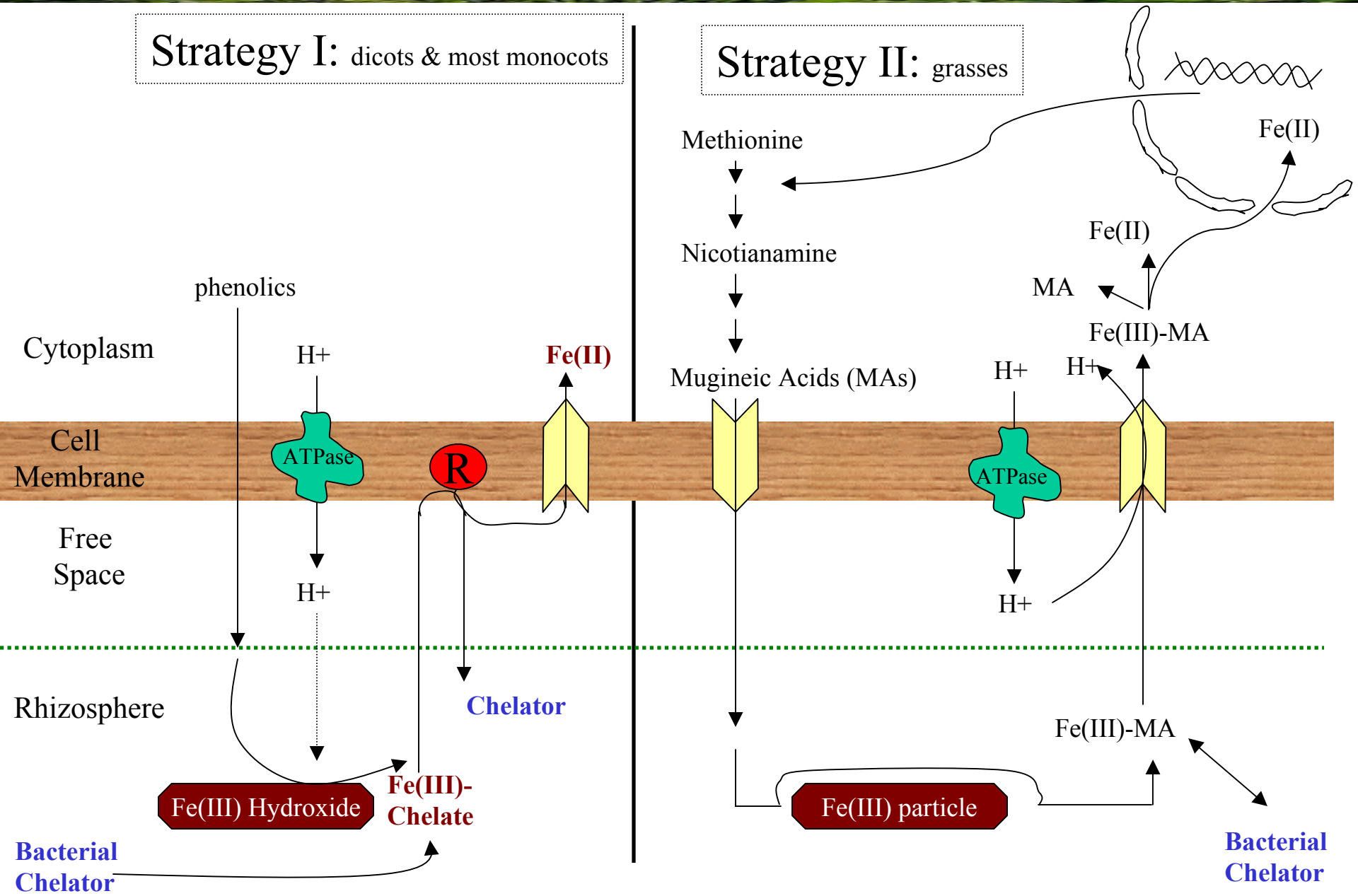
  ✚ Increased bioavailability of metals, which can dramatically improve uptake (200-1300X).

  − MOBILIZATION RISK:
  May not increase uptake and translocation to above ground parts. Studies have shown added chelators can increase soil solubility more than they increase plant uptake and can increase mobilization faster than root uptake; some have even demonstrated decreased plant uptake.*

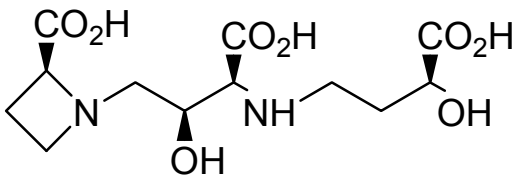
- NEEDED:Natural chelators that can be utilized directly by plant, are biodegradable, can chelate metals we want to remove, and can facilitate root to shoot transport as well as root uptake.

* Römkens *et al*. Environ. Pollution, **2002,** *116*, 109.

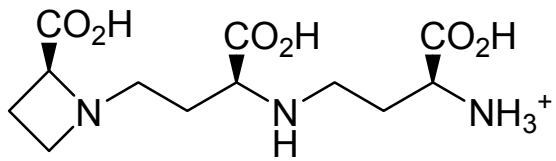
# Phytosiderophores-Natural Fe Chelators



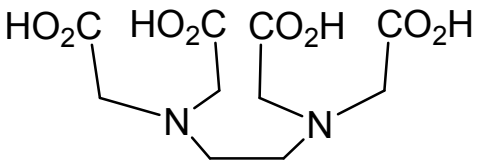
# The Mugineic Acid Phytosiderophores



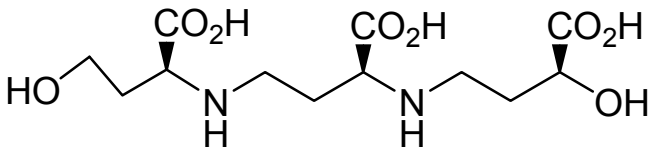

**Mugineic Acid Phytosiderophore**



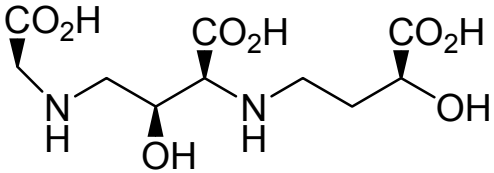

**Nicotianamine Phytosiderophore Precursor**



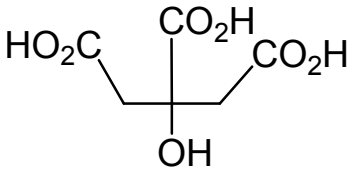

**EDTA**



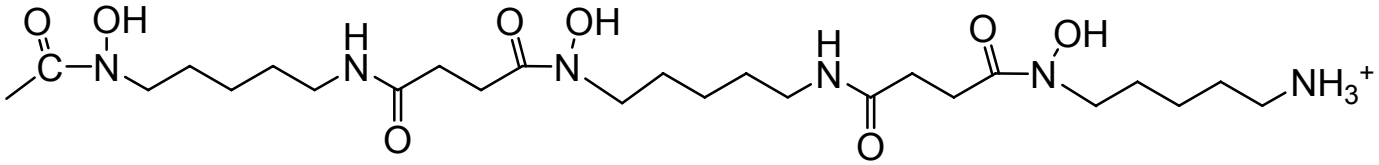

**Avenic Acid A Phytosiderophore**




**Distichonic Acid Phytosiderophore**



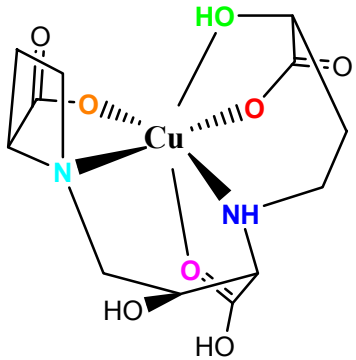

**Citric Acid**



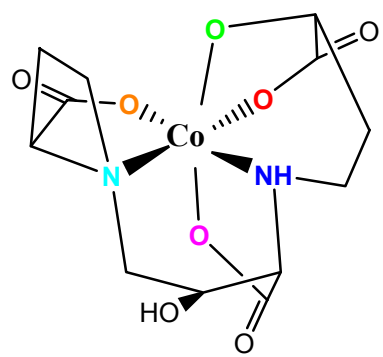

**Desferrioxamine B- Bacterial Siderophore**

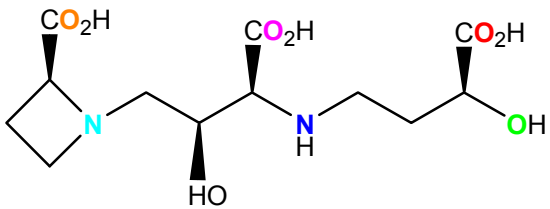
# Phytosiderophore-Metal complexes

**Cu(II)-Mugineic acid***




**Co(III)-Mugineic acid***




**Mugineic Acid**

**Stability constants (log *K*) of Phytosiderophores and other chelators with various metal ions.**

| Chelator | Mn(II) | Fe(II) | Co(II) | Zn(II) | Ni(II) | Cu(II) | Fe(III) | Pu(IV) | U(VI) |
|---|---|---|---|---|---|---|---|---|---|
| Nicotianamine | 8.8 | 12.8 | 14.8 | ~15 | 16.1 | 18.6 | 20.6 | | |
| Mugineic acids* | 8.0– 8.3 | 10.0-10.5 | | 12.4-12.8 | 14.4- 14.9 | 18-18.7 | 32-33§ | | |
| Desferrioxamine B | | 7.2 | 10.3 | 10.1 | 10.9 | 14.1 | 30.6 | 30.8 | 18 |
| Citrate | 3.8 | 4.6 | 5.0 | 5.0 | 5.4 | 5.9 | 11.4 | ~12 | 7.4 |
| EDTA | 13.8 | 14.3 | 16.3 | 16.4 | 16.5 | 18.8 | 25.1 | 25.6 | 7.4 |

*Murakami *et al. Chem Letters*, **1989**, 2137. Von Wiren, *et al.*, *Plant Physiol*. **1999,** *119,* 1107; Hiridate & Inoue, *Soil Sci. Am. J.* **1998**, *62*,159; Mino *et al.*, *Inorg. Chem*. **1981**, *20*, 3440.

§ the anionic complex forms with Fe(III), so value is for $K_{FeLH-1}$, not $K_{FeL}$

# Phytosiderophores: Better for Phytoextraction

- Designed for metal uptake in complex & competitive soil environment.
- More efficient for metal uptake.
- May have more general metal uptake properties beyond Fe.
- Phytosiderophore biosynthesis genetics largely known. Making hyper-phytosiderophore producers/accumulators is possible.
- We gain basic science knowledge: one genetic/physiological/chemical factor that may affect uptake and transport of heavy metals in a number of plants. Science basis for developing phytoremediation at DOE sites.

* Romheld & Marschner, *Plant Physiol*, **1986**, 80-175; Takagi, **1993**, in *Iron chelation in plants and soil microorganisms*, 111; Mihashi & Mori, *Biol. Metals*, **1989**, *2,* 146; Ebbs & Koch, *Environ. Sci. Tech* **1998**,*32*,802; Romheld & Awad, **2000**, *23*, 1857.

# Grasses: Better for Phytoremediation

- Grasses grow almost everywhere! Annuals & perennial species exist.
- Crops, such as Barley, Oats, Wheat, Rice are GRASSES. We have agricultural equipment to plant & harvest.
- Many grasses are not nutrient/water intensive.
- Grasses grow fast, have dense root systems & high biomass. High biomass can compensate for lower uptake.
- Some grasses have been shown to be more tolerant to high levels of metals than known hyperaccumulators.*
- Grasses have been used commercially for phytostabilization of Cu, Pb, Zn.
- Grasses known to take up Pu, U, and may have higher uptake levels than other plants*

* Ebbs & Kochian, *Environ. Sci. Tech*, **1998**, *32*, 802. *Advances in Plutonium Chemistry*, D. Hoffman, Ed. **2002**.

# Key Objectives

Science basis for developing phytoremediation for DOE sites:

- **Fundamental Science:**
  - Do phytosideropores affect metal fate and transport?
  - How do phytosiderophores affect metal biogeochemistry?
  - Are phytosiderophores metal solubilizers?
  - Do phytosiderophores contribute to plant uptake of actinides & other metals?
  - Are phytosiderophore actinide complexes trapped in root zone?
  - Are phytosiderophore actinide complexes mobile in soils?
- **Applied science:**
  - Can phytosiderophore producing plants be used for metal phytoextraction?
  - Can phytosiderophore producing plants be used for long-term phytostabilization?
    - At what contamination levels?
  - Do they compete with uptake levels of known 'hyperaccumulators' for certain metals, such as Pu and U?
  - Do phytosiderophore-producing plants cause any harm?

# Research Approach

Controlled basic experiments

Phytosiderophore solubility studies (minerals, soils).
Phytosiderophore inorganic chemistry with actinides, other metals.

Ability of phytosiderophores produced by plants to contribute to uptake of metals- hydroponic studies.

Extent of plant uptake (concentration ratios).
Conditional Factors (concentration of contaminants, other additions).

Ability of phytosiderophores produced by plants to contribute to uptake of metals on soils & field samples. -hydroponic studies.

Ability of plants that produce phytosiderophores to uptake metals, stabilize metals in soils or to contribute to migration. -pot/column experiments

Lysimeter studies

Applied Resarch

# Research Plan: Solubility & Inorganic Chemisty

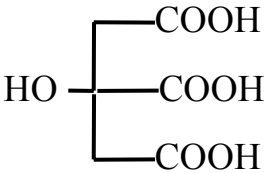
- Do phytosiderophores solubilize Pu(IV) hydroxides?
  - At what rate?
  - Comparable to EDTA/DTPA?
  - What about $PuO_2$?
- Do phytosiderophores mobilize Pu sorbed onto soils?
  - At what rate?
  - What about aged or real field samples instead of spiked soils?
  - Do other metals present in soil interfere?
- What are stability constants of Pu(IV), Th(IV), U(VI) - phytosiderophore complexes?
- What are structures of Pu(IV), Th(IV), U(VI)-phytosiderphores complexes? similar to Fe(III), Cu(II), Co(III)??
- Do phytosiderophore promote Pu(VI) & Pu(V) reduction?

# Results with Bacterial Siderophores

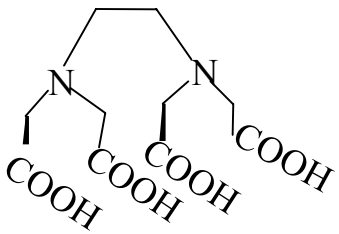
**Pu(IV) hydroxide can be solubilized by organic chelators, although slowly:**

**EDTA >> citrate > tiron >> DFE > DFB**

Pu(IV) solution stability constants (log β)

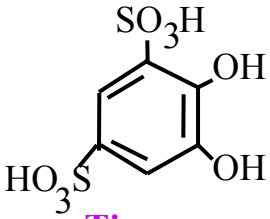



**Citric Acid**
**15.6**

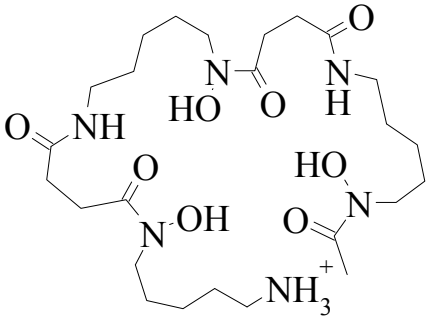



**EDTA**

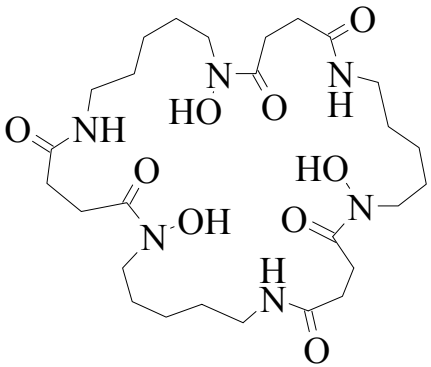
**25.8**




**Tiron**
**>16 (est.)**

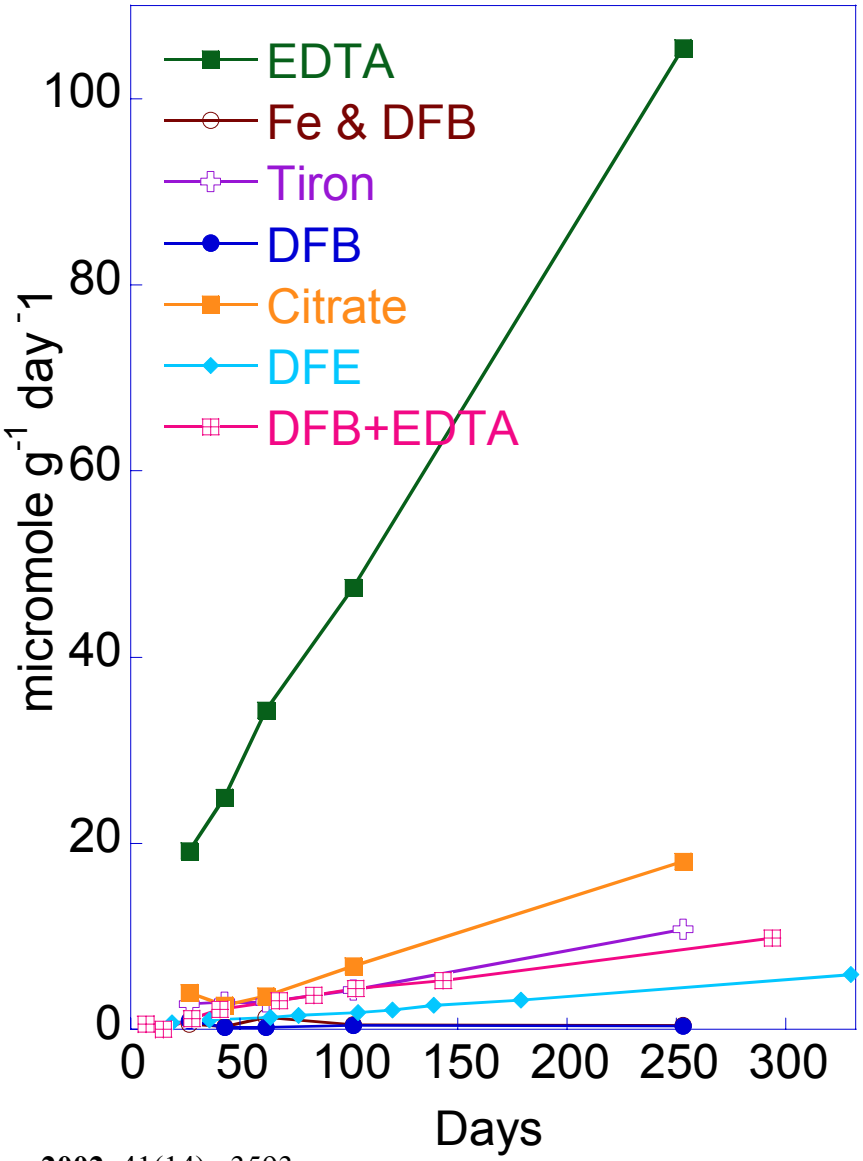



**Desferrioxamine B**
**30.8**

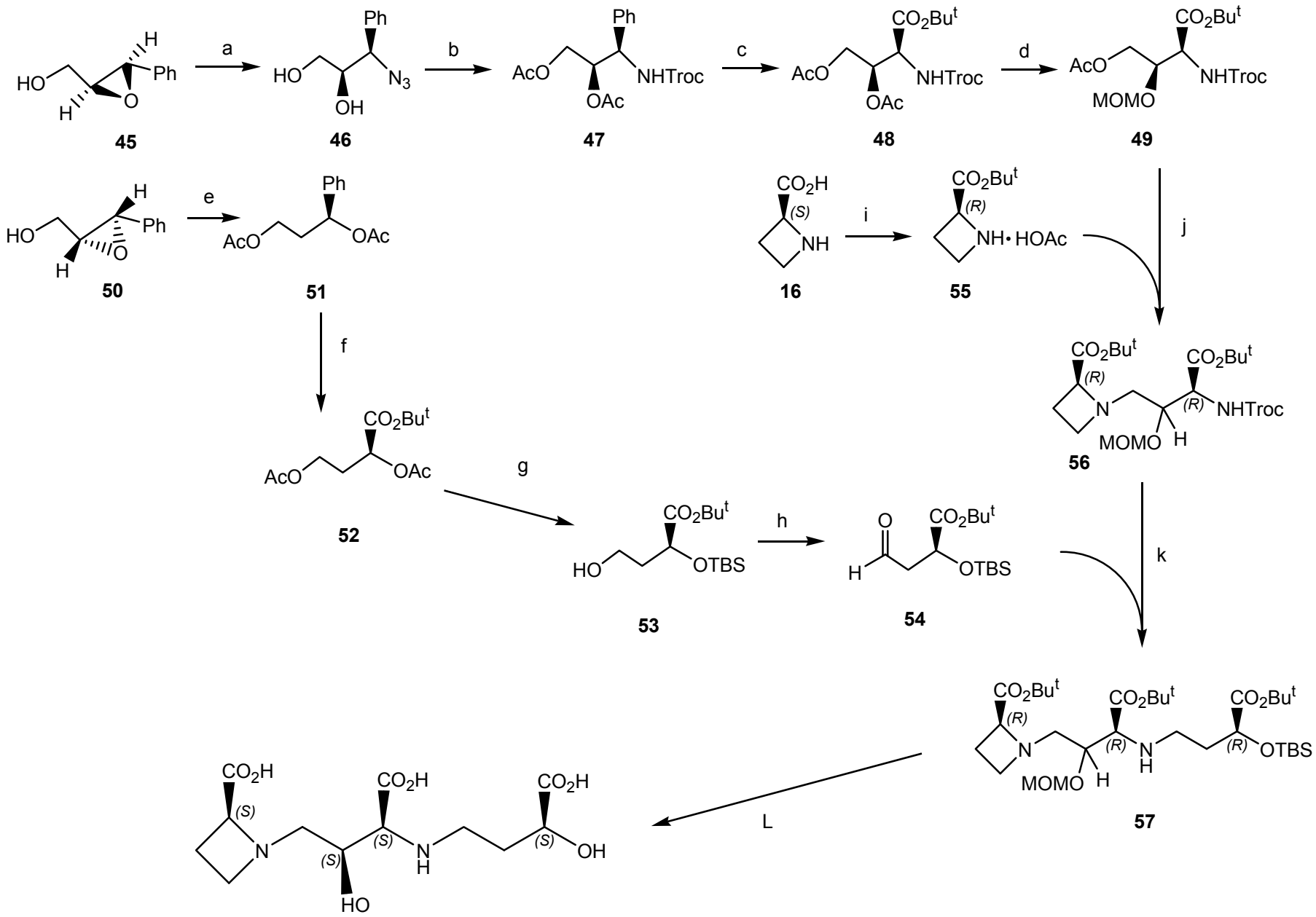



**Desferrioxamine E**
**>31**

**Pu(IV) Hydroxide Solubilization Rate**




Ruggiero, Neu, Reilly, *Inorganic Chemistry*, **2002**, 41(14), 3593.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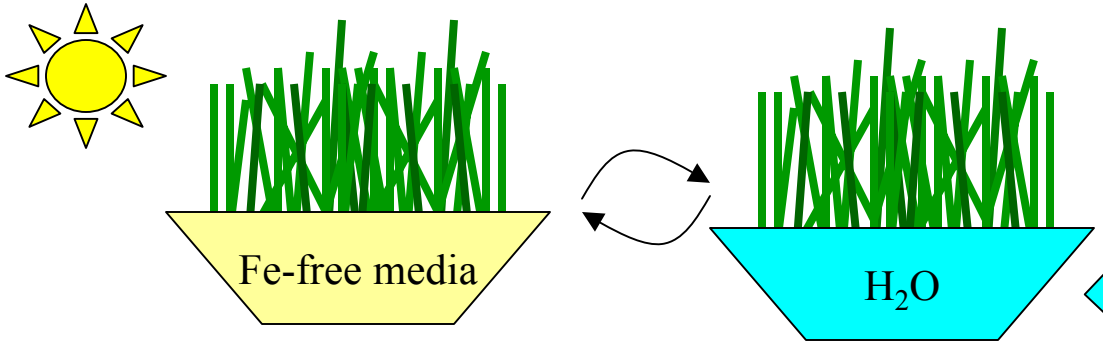
Mugineic Acid

# Phytosiderophore Isolation



Phytosiderophore-rich root washings

Chromatography/HPLC

Purified phytosiderophore

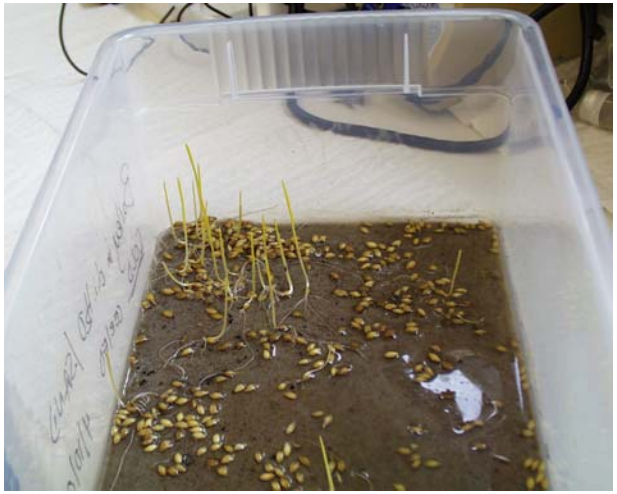
# Plant Uptake of Actinides: Hydroponics

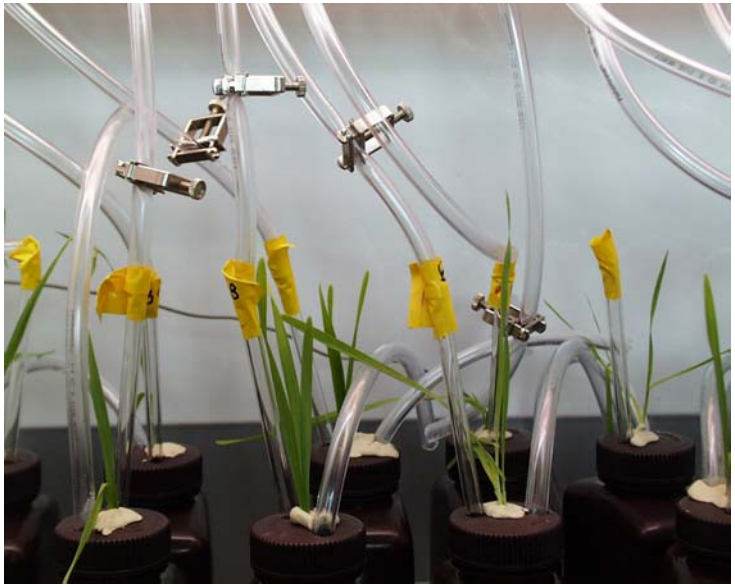
- Do plants take up more Pu/U/Th/Ni when grown in Fe-deficient media? Is the effect greater in phytosiderophore producing plants?

  Compare Barley to Indian Mustard; ICP or LSC analysis of roots & shoots. Both pure solution & soil-in-a-bag studies.

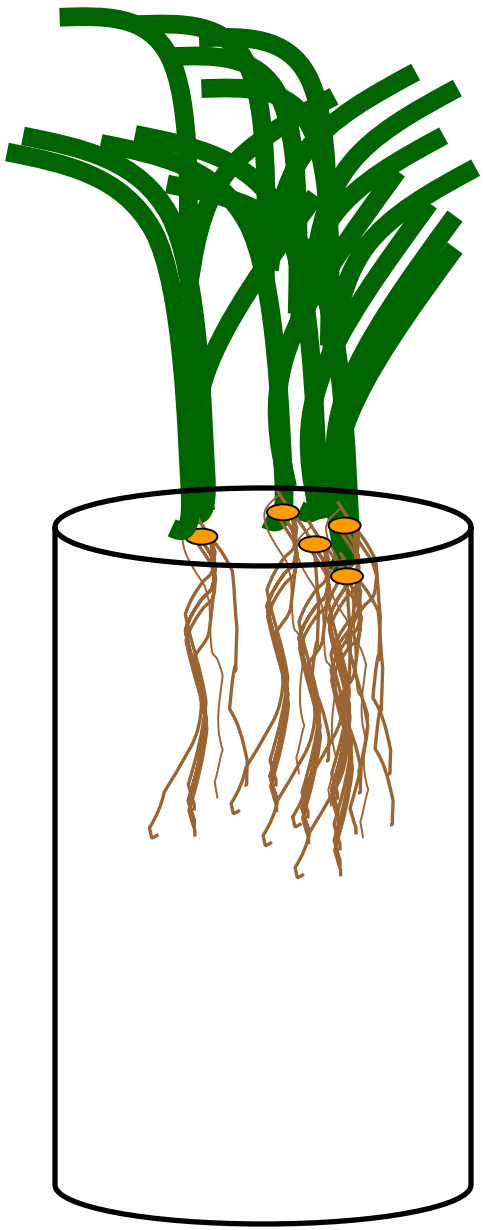
- Does adding extra phytosiderophore/root washings increase uptake and translocation of metals? Is the effect greater than by adding EDTA or other synthetic chelator?
- How much metal can be taken up/extent of uptake?
- How do concentration ratios relate to starting contaminant concentration?
- Do other solution additives or conditions influence uptake?
- How does actinide/metal uptake rate compare to Fe uptake rate?

# Plant Uptake of Actinides: Hydroponics





# Plant Uptake of Actinides: Soils



- Do plants growing in best/worst possible conditions for metal uptake cause metal mobilization down a soil column?
- Are phytosiderophore-actinide complexes degraded before they can migrate?
- Can phytosideriohore-producing plants stabilize metals in soils in their root zone?
- What happens to metals in roots if plant dies or is harvested?

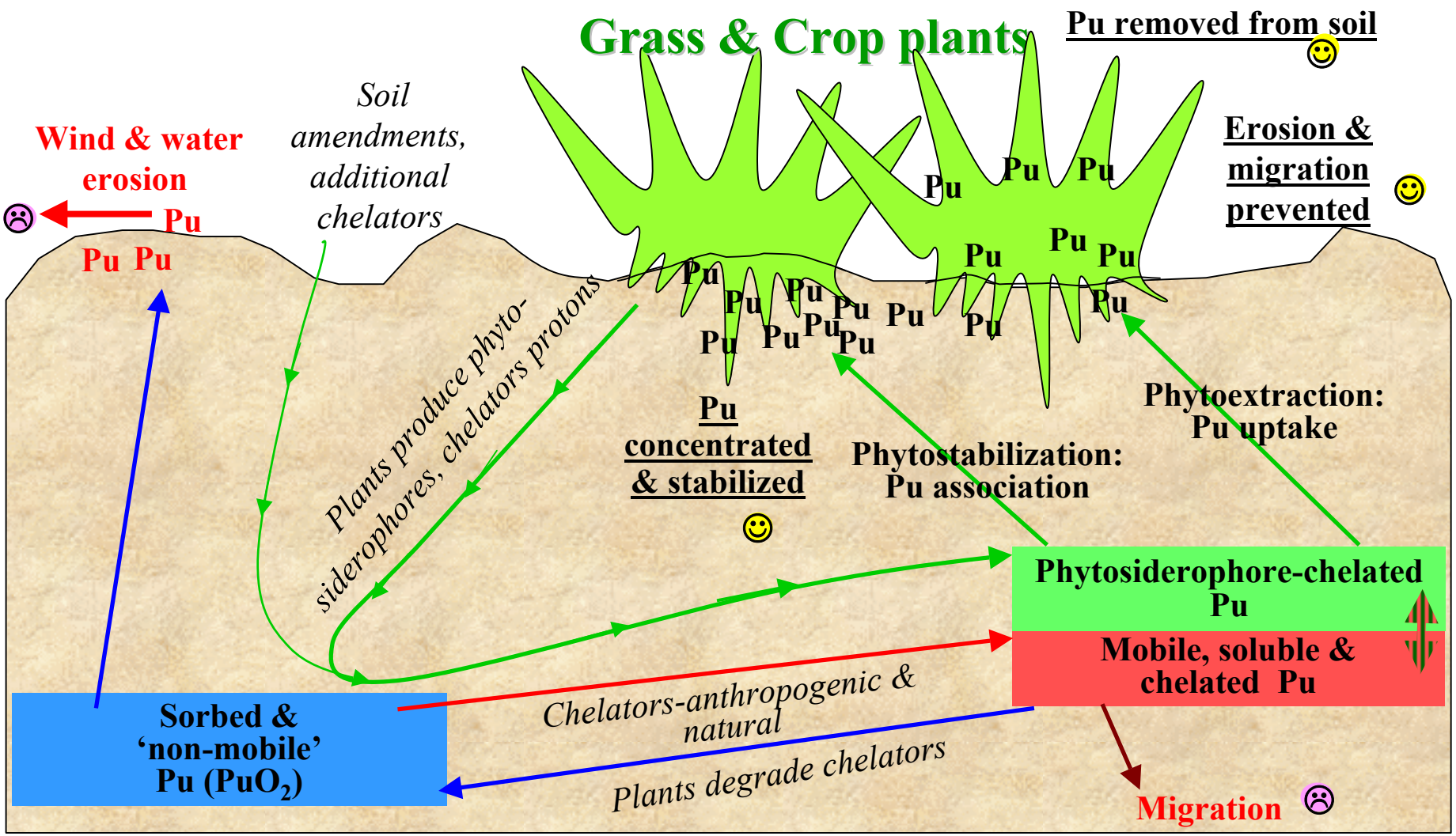
# Site Samples Needs to Enhance Applicability and Impact of Research

<u>Ideally soil or groundwater samples from sites:</u>

- where long-term stabilization/remediation is an option.
- where grasses grow well.
- with Pu, U, Th, or other 'hard' metals (Cr, Al, Ni).
- that are well characterized with respect to metal concentrations/ radioactivity levels.
- with predominately shallow (< 2 meter) metal contamination (root zone).
- that have large soil areas with low or moderate contamination levels.
- that have low acidity or low Fe bioavailability.

# Potential Research Applications

Contaminant reduction → Contaminant stabilization



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