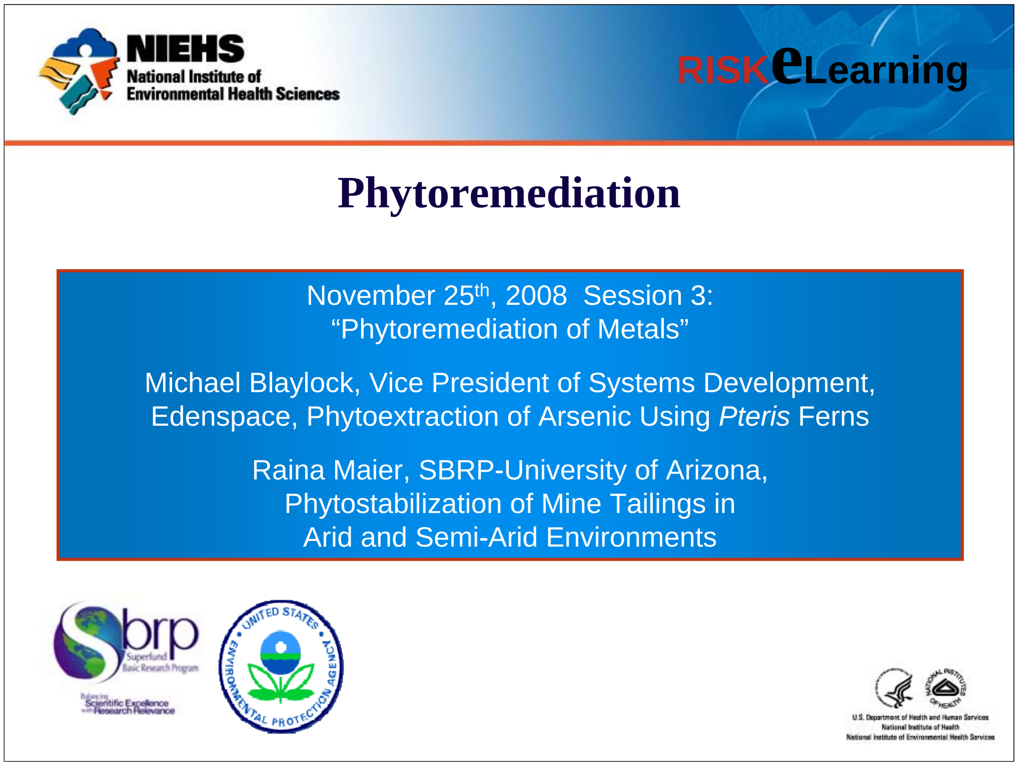


NIEHS National Institute of Environmental Health Sciences

RISKeLearning

Phytoremediation

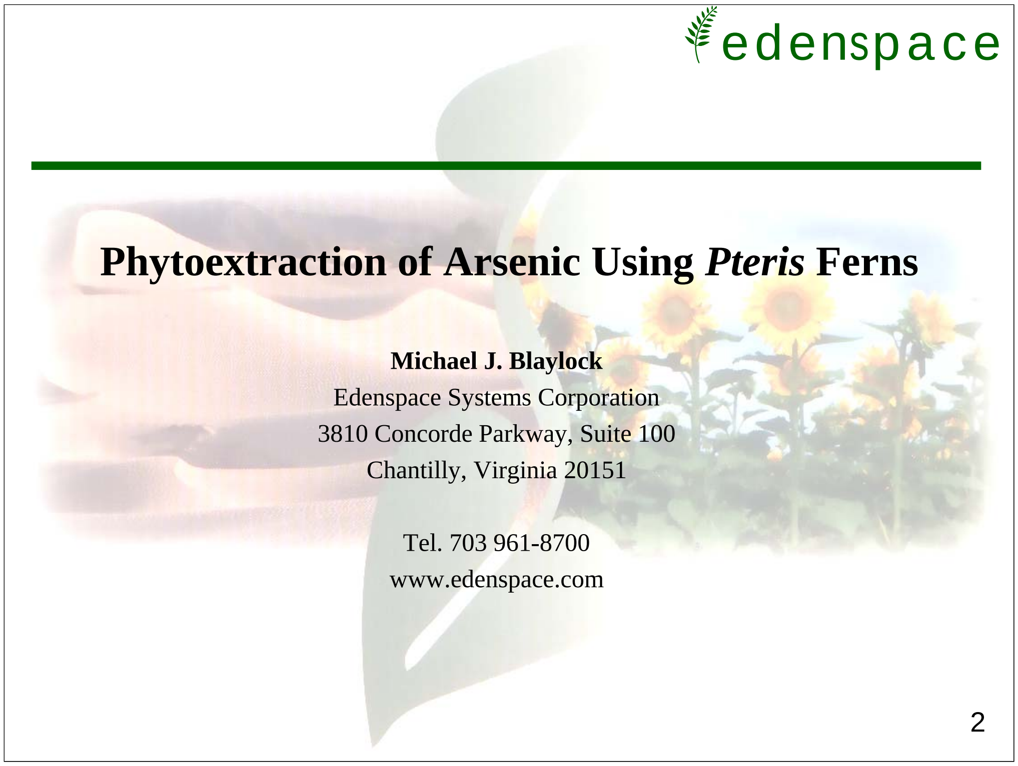
November 25th, 2008 Session 3:
“Phytoremediation of Metals”

Michael Blaylock, Vice President of Systems Development,
Edenspace, Phytoextraction of Arsenic Using *Pteris* Ferns

Raina Maier, SBRP-University of Arizona,
Phytostabilization of Mine Tailings in
Arid and Semi-Arid Environments

sbrp Superfund Basic Research Program
Balancing Scientific Excellence with Research Relevance

U.S. Department of Health and Human Services
National Institutes of Health
National Institute of Environmental Health Sciences



edenspace

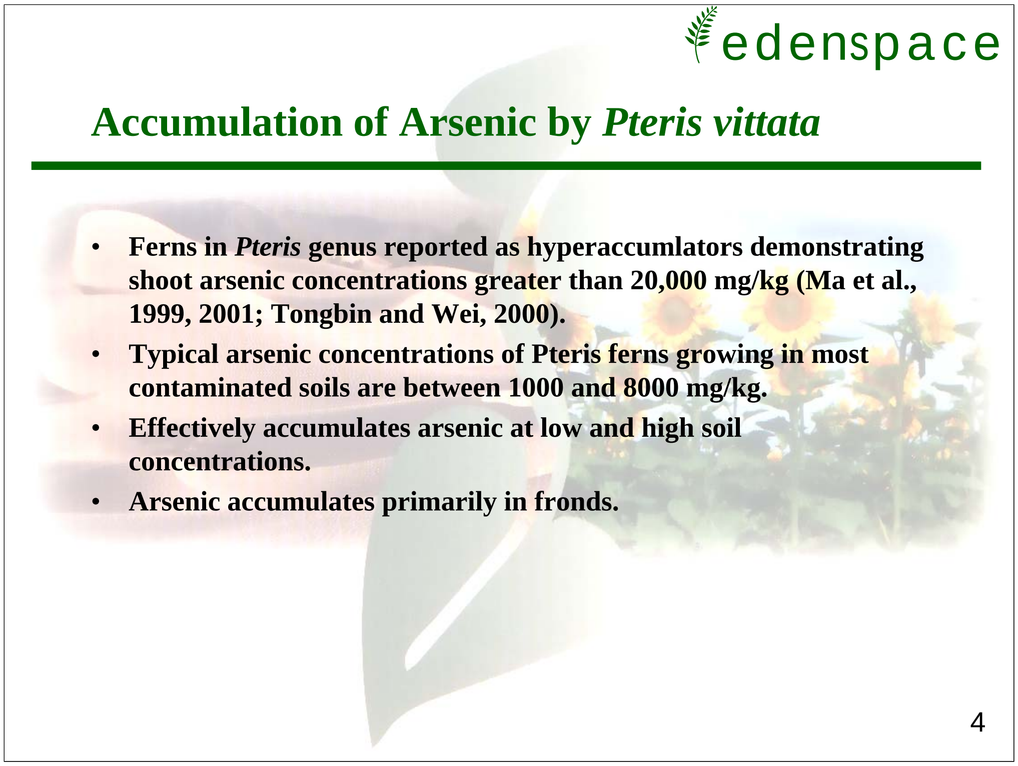
Phytoextraction of Arsenic Using *Pteris* Ferns

Michael J. Blaylock
Edenspace Systems Corporation
3810 Concorde Parkway, Suite 100
Chantilly, Virginia 20151

Tel. 703 961-8700
www.edenspace.com

Arsenic Phytoremediation

- Soil arsenic is increasingly receiving more attention due to housing expansion on former agricultural lands.
  - Apple and citrus orchards
  - Large acreages, low concentrations
- Excavation and disposal is effective for small soil volumes but cost prohibitive for large areas
- Arsenic exists in oxidized soils primarily as an anion (arsenate).
  - Low solubility and mobility
  - Primarily surface soil (0-15 cm) contamination.
  - Relatively low regulatory limits (US) ranging from less than 1 mg/kg to approximately 20 mg/kg
  - Typical soil concentrations (pesticide and CCA afffected soils) are less than 200 mg/kg



edenspace

Accumulation of Arsenic by *Pteris vittata*

- Ferns in *Pteris* genus reported as hyperaccumlators demonstrating shoot arsenic concentrations greater than 20,000 mg/kg (Ma et al., 1999, 2001; Tongbin and Wei, 2000).
- Typical arsenic concentrations of Pteris ferns growing in most contaminated soils are between 1000 and 8000 mg/kg.
- Effectively accumulates arsenic at low and high soil concentrations.
- Arsenic accumulates primarily in fronds.

edenspace

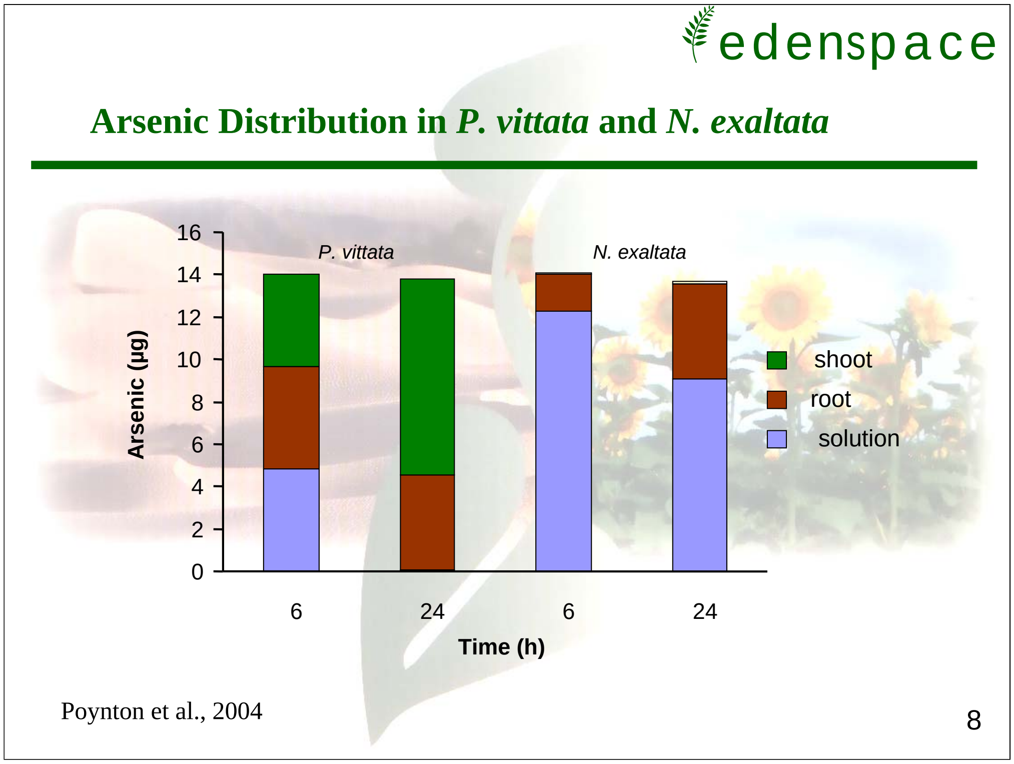
Pteris Characteristics

- **Indigenous to tropical and semi-tropical climates.**
- **Vigorous growth under suitable conditions.**
- **Grown as a perennial in Zones 8 and above.**
- **Can be grown as an annual in Zones 7 and below.**
- **Mature plants tolerate air temperatures to approx. 26 ºF (-3 ºC).**

Pteris vittata

Pteris cretica mayii

Pteris multifida



edenspace

Arsenic Distribution in *P. vittata* and *N. exaltata*

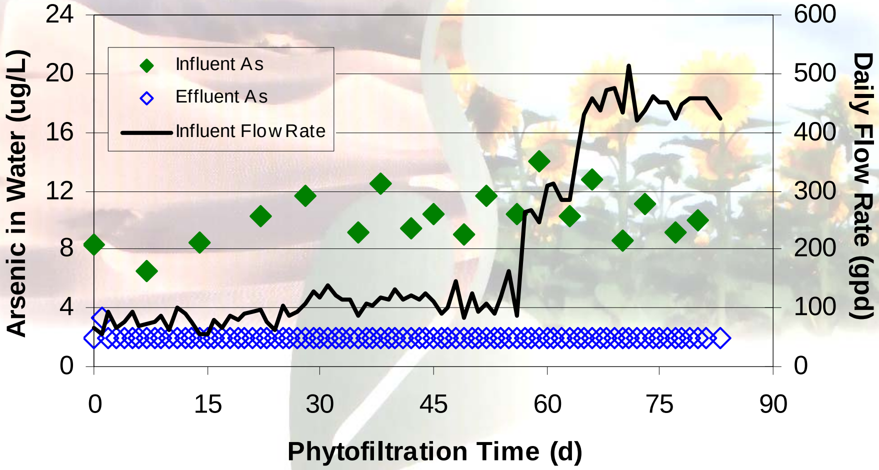
Poynton et al., 2004

8

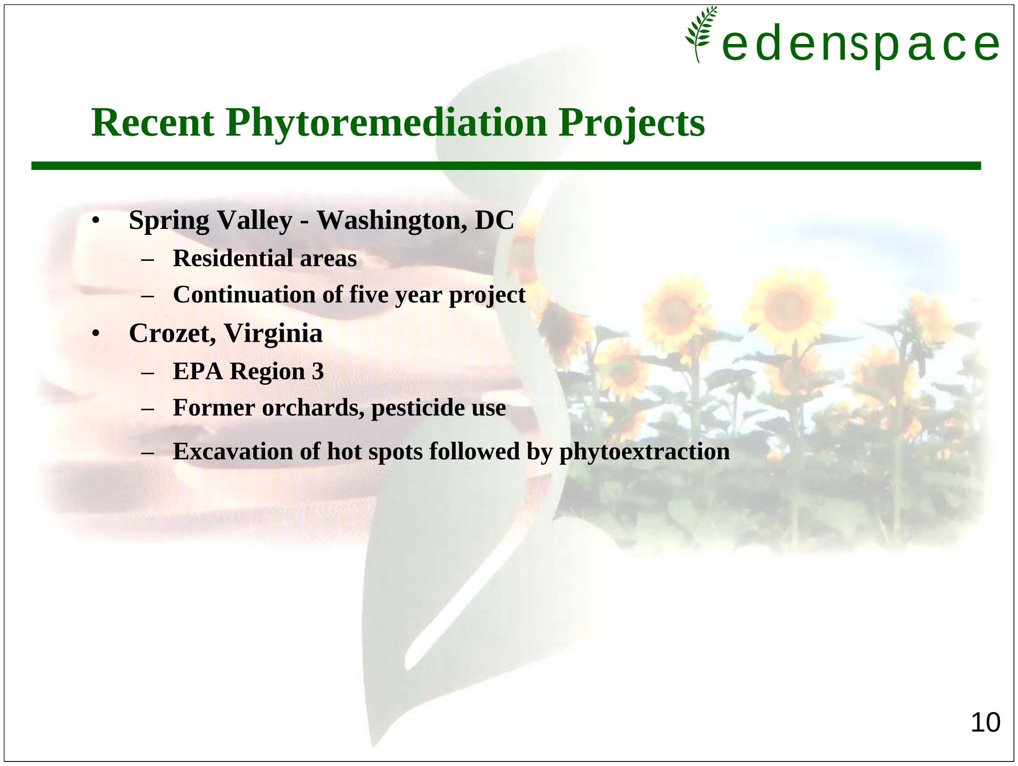
The experiment started with 15 ug As in 75 mls of solution. The control (no plant) values were 14.8 ug at both 6 and 24 hours. In the mass balance 95% and 93% of the total As could be accounted for after 6 and 24 hours respectively.

edenspace

Arsenic Continuously Removed from Drinking Water



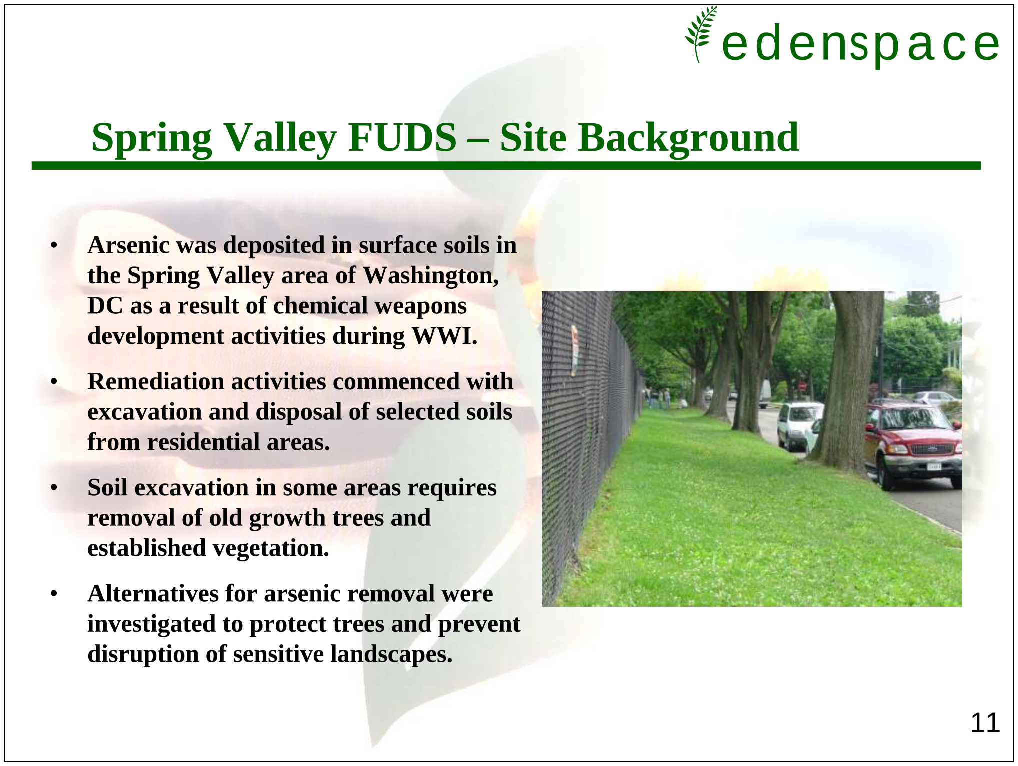
Elless et al., 2005



edenspace

Recent Phytoremediation Projects

- **Spring Valley - Washington, DC**
  - **Residential areas**
  - **Continuation of five year project**
- **Crozet, Virginia**
  - **EPA Region 3**
  - **Former orchards, pesticide use**
  - **Excavation of hot spots followed by phytoextraction**



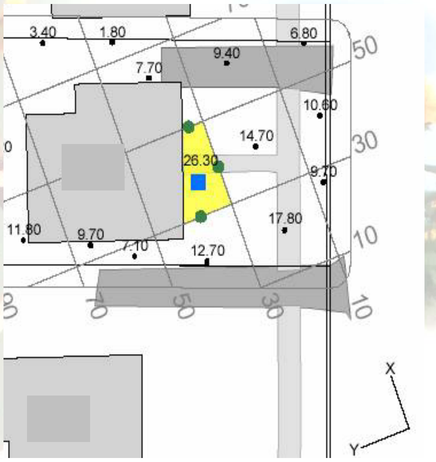
edenspace

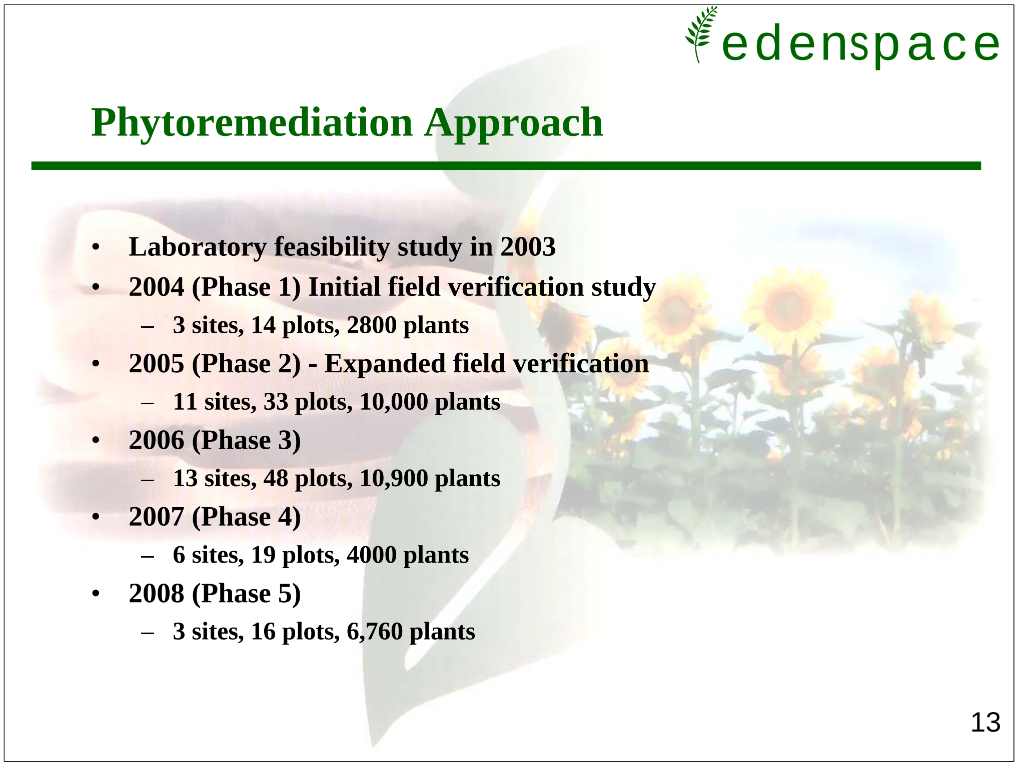
Spring Valley FUDS – Site Background

- Arsenic was deposited in surface soils in the Spring Valley area of Washington, DC as a result of chemical weapons development activities during WWI.
- Remediation activities commenced with excavation and disposal of selected soils from residential areas.
- Soil excavation in some areas requires removal of old growth trees and established vegetation.
- Alternatives for arsenic removal were investigated to protect trees and prevent disruption of sensitive landscapes.

Plot Identification

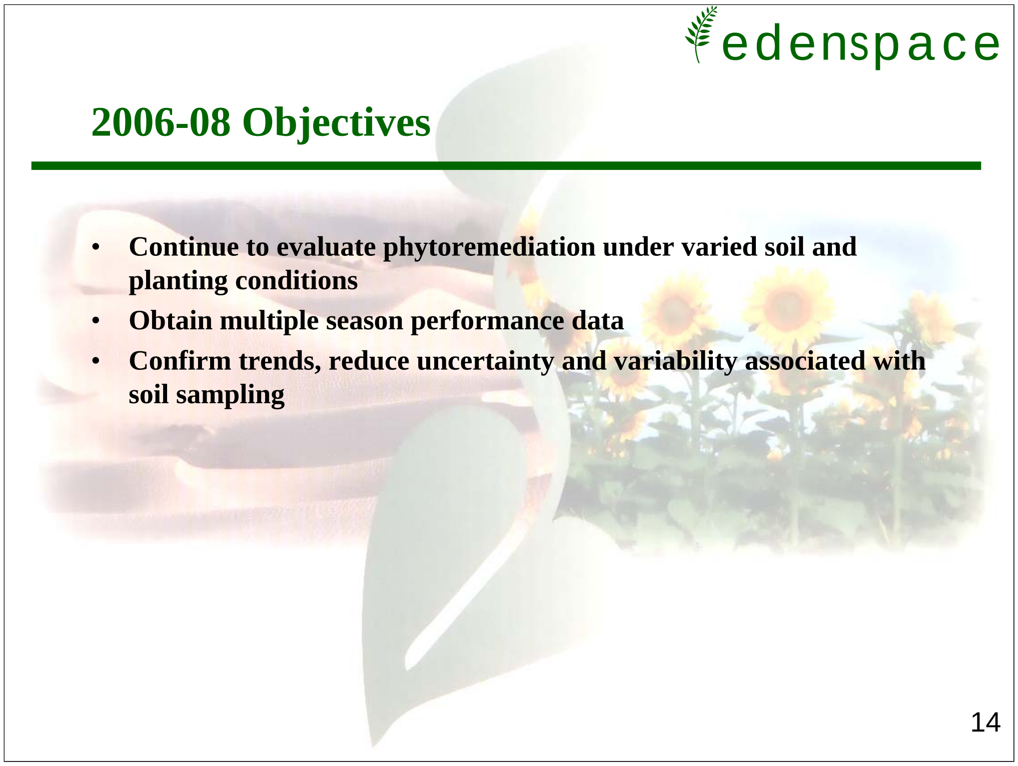
- Areas sampled on a 20’ x 20’ grid spacing
- Areas selected based on arsenic concentration
- Targeted cleanup level of 20 mg/kg (43 mg/kg in special circumstances)
- Presence of trees, landscape features
- Homeowner willingness





Phytoremediation Approach

- Laboratory feasibility study in 2003
- 2004 (Phase 1) Initial field verification study
  - 3 sites, 14 plots, 2800 plants
- 2005 (Phase 2) - Expanded field verification
  - 11 sites, 33 plots, 10,000 plants
- 2006 (Phase 3)
  - 13 sites, 48 plots, 10,900 plants
- 2007 (Phase 4)
  - 6 sites, 19 plots, 4000 plants
- 2008 (Phase 5)
  - 3 sites, 16 plots, 6,760 plants



edenspace

2006-08 Objectives

- Continue to evaluate phytoremediation under varied soil and planting conditions
- Obtain multiple season performance data
- Confirm trends, reduce uncertainty and variability associated with soil sampling

Assessing Phytoextraction Performance

- Phytoextraction is an *in situ* technology – requiring comparison of concentrations in before and after soil samples
- Determine appropriate sampling scheme
  - Assess site status
  - Evaluate phytoremediation performance
- Effects of soil variability on performance assessment
  - Analytical variability (Method 3050)
  - Sampling variability
- Expected magnitude of effect over the short term (one season) is often less than expected analytical or sampling error.

2004-05 Field Verification Soil Sampling

- **Initial and final five point composite and six point composites**
- **Initial and final grab sample from grid sampling location**

edenspace

2004 Soil Arsenic

Plot	Sample Type/Location	2001Grid Sample As (mg/kg)	Pre Plant Soil As (mg/kg)	Post Harvest Soil As (mg/kg)
Lot 15 A	Grid Sample Core	156	**139.3**	**53.2**
	Surface Composite		62.4	40.5
Lot 15 B	Grid Sample Core	122	68.2	75.0
	Surface Composite		57.2	45.3
Lot 15 C	Grid Sample Core	126	**109.3**	**74.9**
	Surface Composite		39.1	33.5
Lot 15 D	Grid Sample Core	64	**95.6**	**122.7**
	Surface Composite		39.6	37.2
Lot 15 E	Grid Sample Core	70	129.7	154.0
	Surface Composite		61.2	52.4
Lot 15 F	Grid Sample Core	163	137.7	118.7
	Surface Composite		38.9	49.5
Lot 15 G	Grid Sample Core	64.7	94.2	51.9
	Surface Composite		**24.7**	**38.3**
Lot 15 H	Grid Sample Core	76.8	72.0	77.7
	Surface Composite		40.1	37.0
Lot 15 I	Grid Sample Core	250	128.3	150.0
	Surface Composite		44.5	41.8

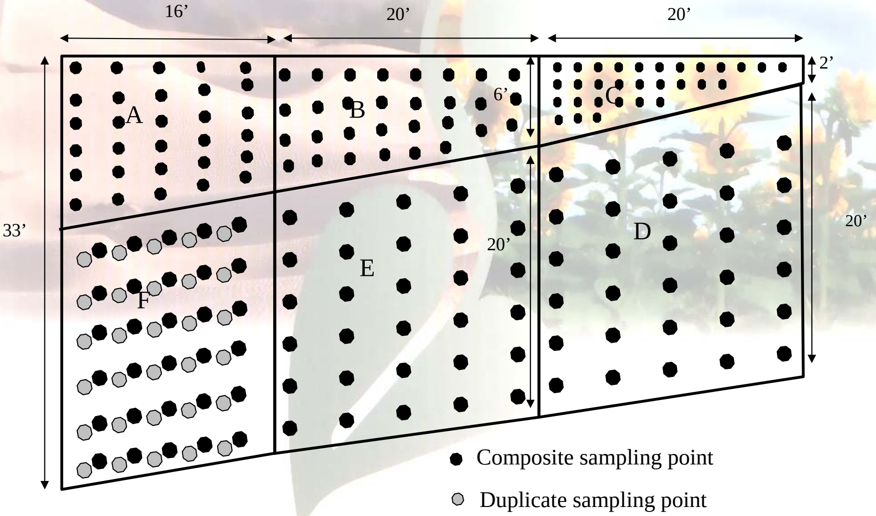
edenspace

2006-08 Soil Sampling and Analysis

- The composite sampling scheme was adjusted to use thirty-point composites from each grid.
  - Thirty one-inch cores collected from 0 to 6 inch depth
  - Samples composited in the field to yield a 2-3 kg sample
- A duplicate composite sample was collected from at least 10% of the grids.
- Air-dried, sieved to 2 mm
- A 1 kg subsample was milled using a ball mill until the soil passed 1 mm sieve
- A 200 g subsample was submitted for arsenic analysis

edenspace

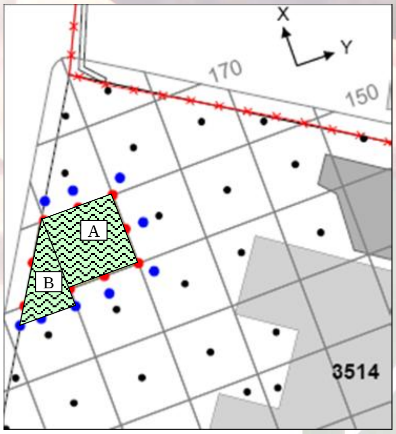
Thirty-Point Composite Sampling Scheme

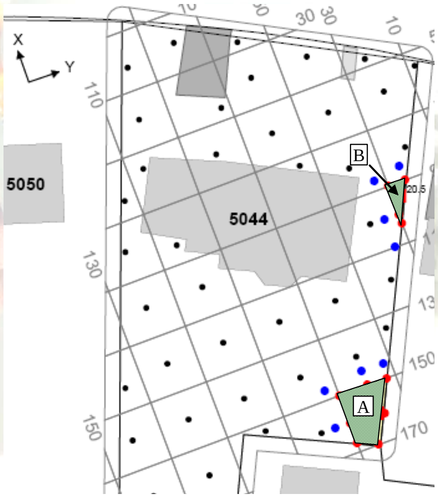


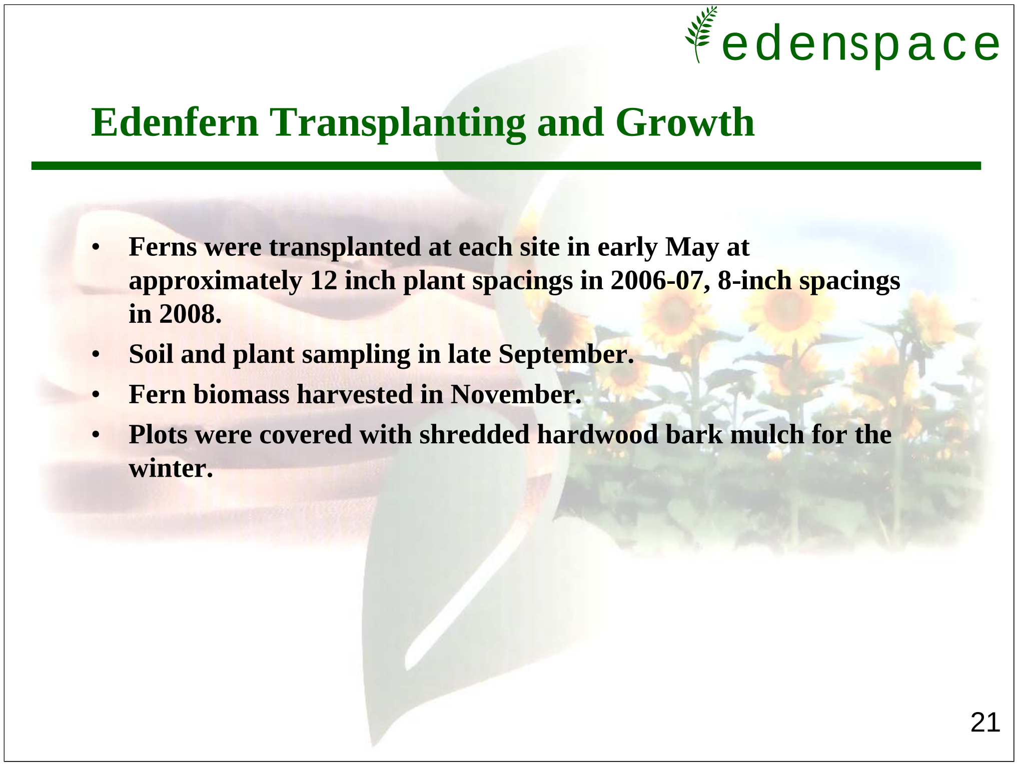
● Composite sampling point

○ Duplicate sampling point

Example Plot Layouts







Edenfern Transplanting and Growth

- **Ferns were transplanted at each site in early May at approximately 12 inch plant spacings in 2006-07, 8-inch spacings in 2008.**
- **Soil and plant sampling in late September.**
- **Fern biomass harvested in November.**
- **Plots were covered with shredded hardwood bark mulch for the winter.**

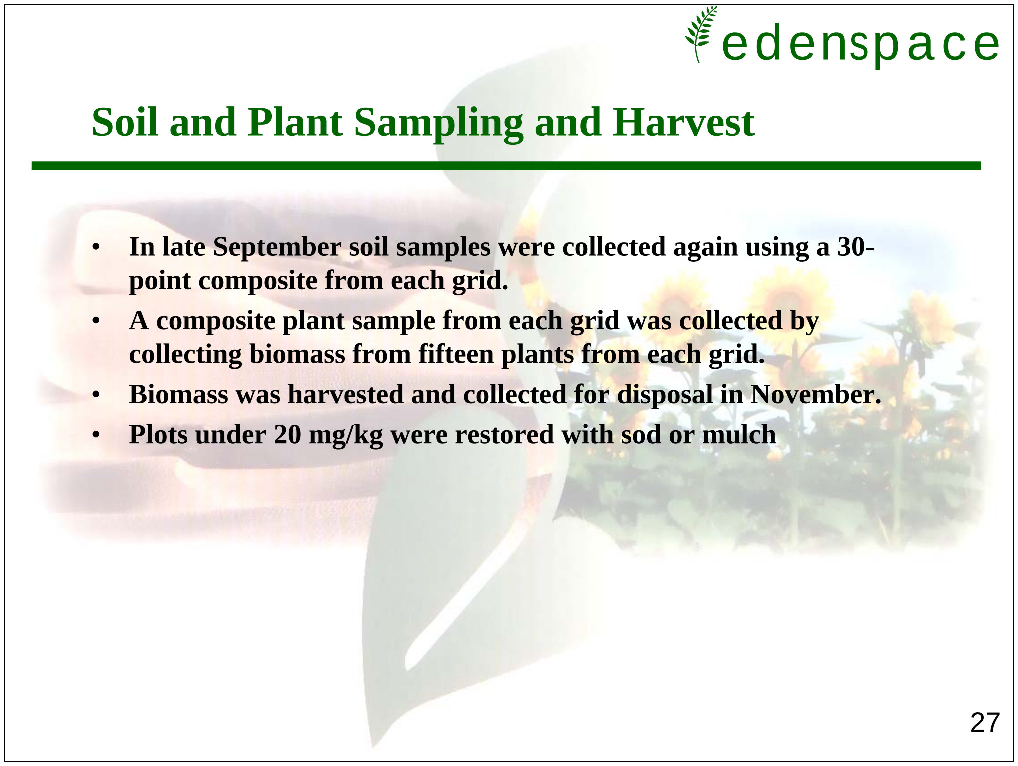
edenspace


23

edenspace

Transplanting


26



edenspace

Soil and Plant Sampling and Harvest

- In late September soil samples were collected again using a 30-point composite from each grid.
- A composite plant sample from each grid was collected by collecting biomass from fifteen plants from each grid.
- Biomass was harvested and collected for disposal in November.
- Plots under 20 mg/kg were restored with sod or mulch

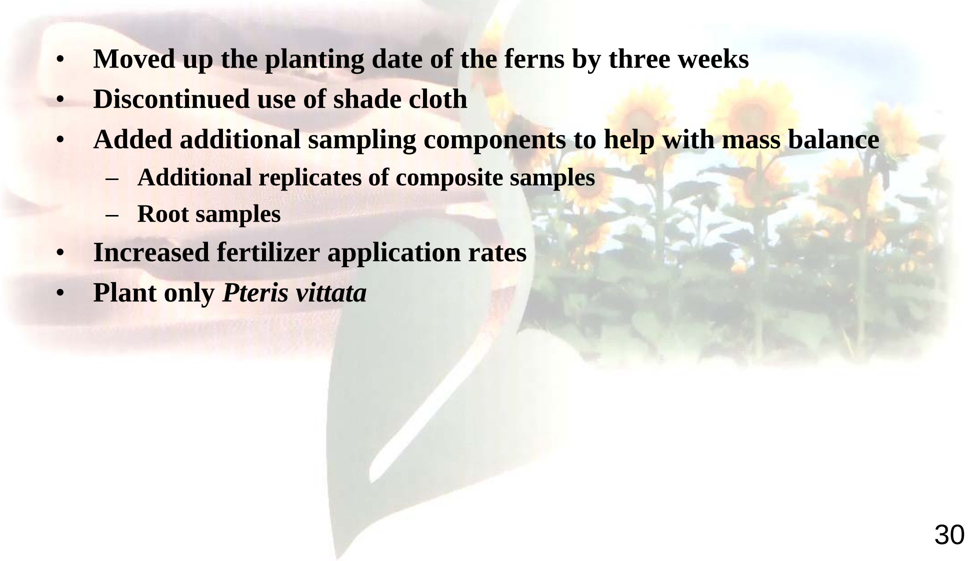
Improved Sampling Precision

- Improved precision through composite sampling and particle size reduction of soil samples
  - 60% of duplicates collected using five point composites in 2005 exceeded 10% RDP, ranging from –104% to 58%
  - In 2006, only one duplicate, exceeded 10% RDP, duplicates ranged from –9% to 21%
  - Unplanted control plots were not useful

2006 Summary

- **Decreases in soil arsenic concentrations were observed in 63% of the planted grids.**
- **Although analytical variability still hinders assessment of changes in soil concentrations in some cases, the trend of decreasing arsenic concentrations has continued for the third consecutive year.**
- **Plant biomass yields and arsenic concentrations were significantly lower than in previous years.**
- **Seasonal variations in species performance supports continued evaluation, although Victory (*P. vittata*) appears to be the most promising.**
- **The thirty point composite and sample homogenization was effective in improving precision and confidence.**

2007-2008 Modifications



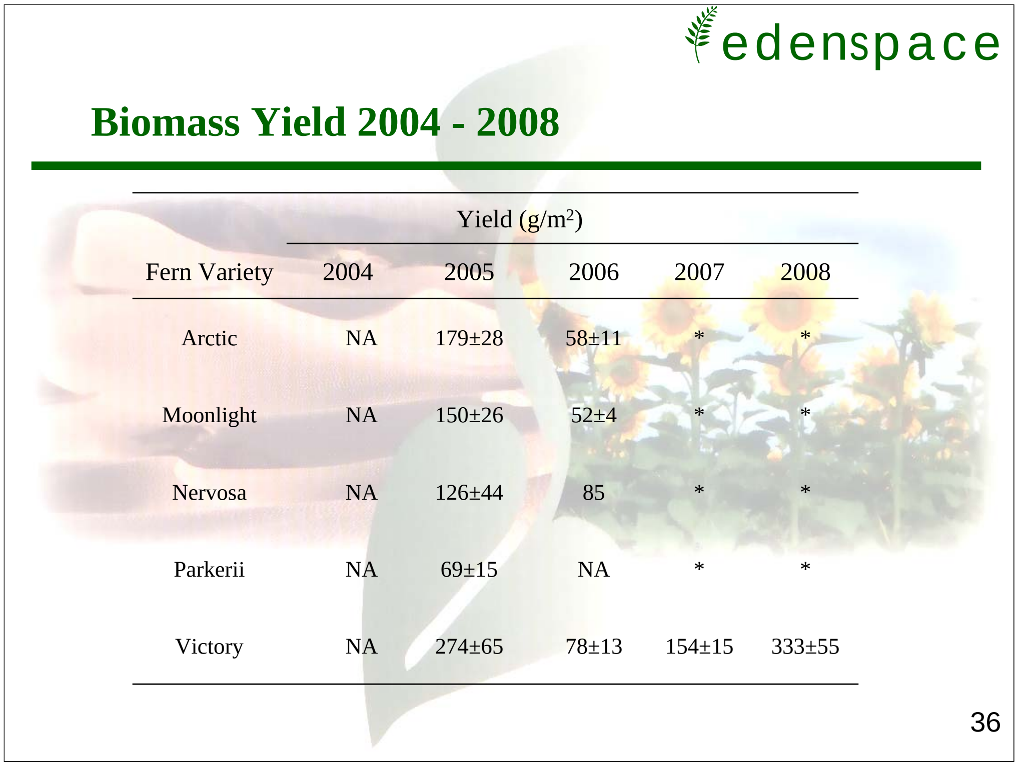
- Moved up the planting date of the ferns by three weeks
- Discontinued use of shade cloth
- Added additional sampling components to help with mass balance
  - Additional replicates of composite samples
  - Root samples
- Increased fertilizer application rates
- Plant only *Pteris vittata*


34

edenspace

Arsenic Uptake 2004 - 2008

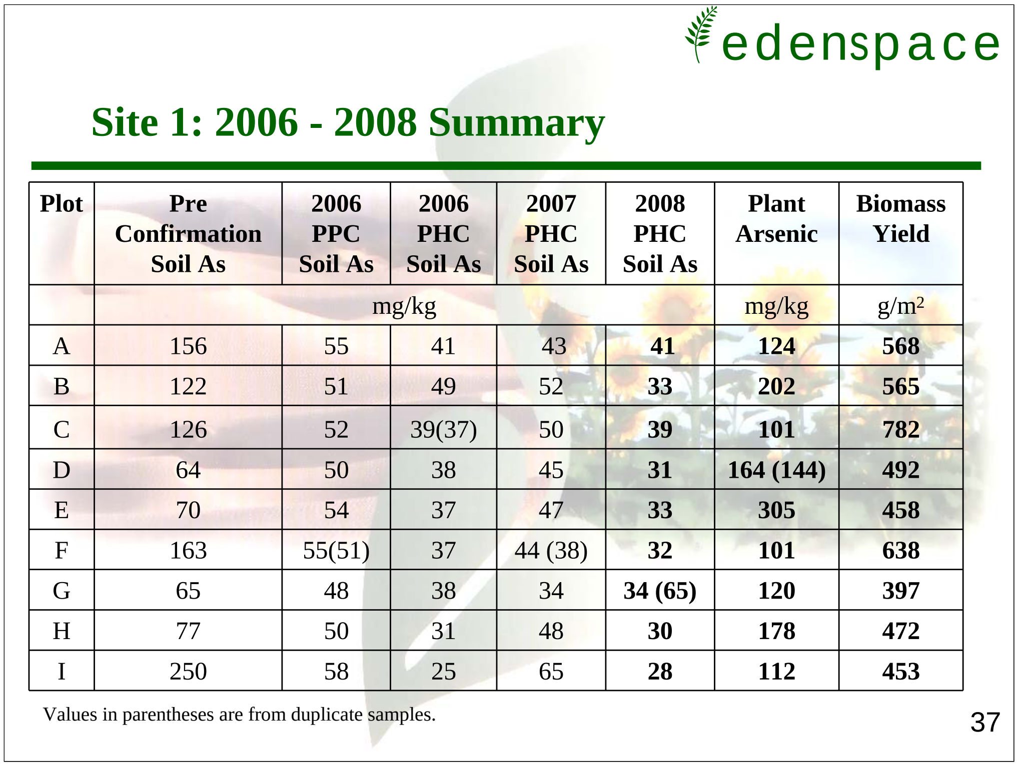
Fern Variety	Frond Arsenic (mg/kg)				
	2004	2005	2006	2007	2008
Arctic	386±107	376±72	20±4	*	*
Moonlight	451±57	209±44	125±20	*	*
Nervosa	NA	169±47	40	*	*
Parkerii	NA	44±15	NA	*	*
Victory	547±163	533±164	119±61	531±63	237±46



edenspace

Biomass Yield 2004 - 2008

	Yield (g/m²)				
Fern Variety	2004	2005	2006	2007	2008
Arctic	NA	179±28	58±11	*	*
Moonlight	NA	150±26	52±4	*	*
Nervosa	NA	126±44	85	*	*
Parkerii	NA	69±15	NA	*	*
Victory	NA	274±65	78±13	154±15	333±55

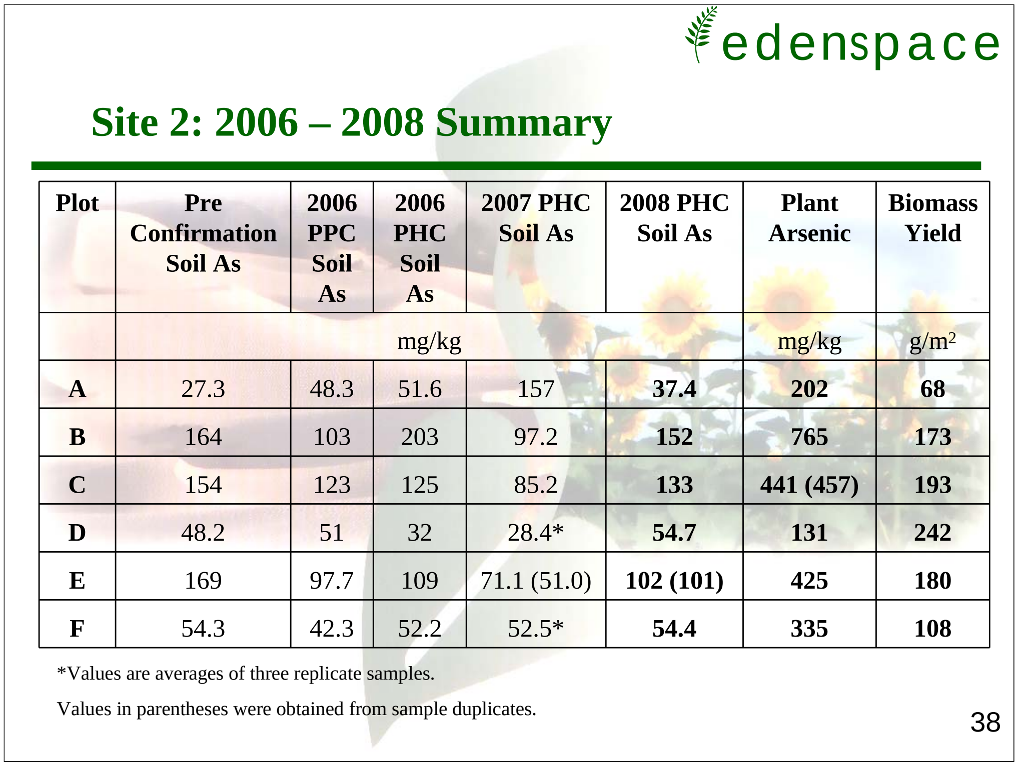


edenspace

Site 1: 2006 - 2008 Summary

Plot	Pre Confirmation Soil As	2006 PPC Soil As	2006 PHC Soil As	2007 PHC Soil As	2008 PHC Soil As	Plant Arsenic	Biomass Yield
	mg/kg					mg/kg	g/m^2
A	156	55	41	43	**41**	**124**	**568**
B	122	51	49	52	**33**	**202**	**565**
C	126	52	39(37)	50	**39**	**101**	**782**
D	64	50	38	45	**31**	**164 (144)**	**492**
E	70	54	37	47	**33**	**305**	**458**
F	163	55(51)	37	44 (38)	**32**	**101**	**638**
G	65	48	38	34	**34 (65)**	**120**	**397**
H	77	50	31	48	**30**	**178**	**472**
I	250	58	25	65	**28**	**112**	**453**

Values in parentheses are from duplicate samples.



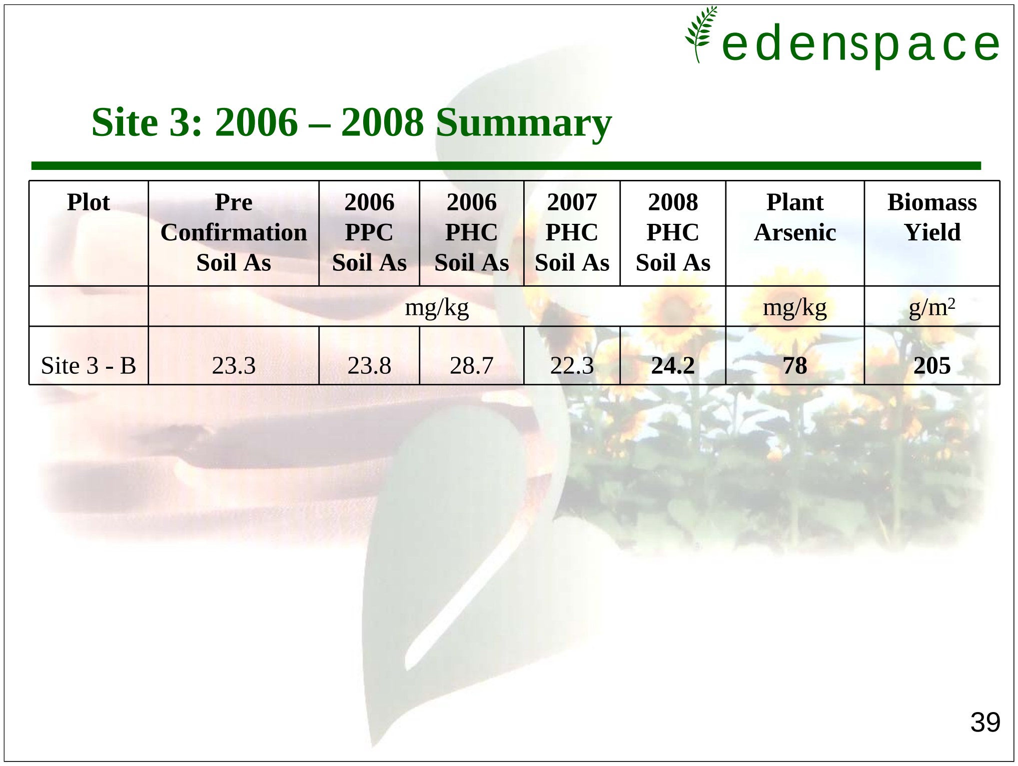
edenspace

Site 2: 2006 – 2008 Summary

Plot	Pre Confirmation Soil As	2006 PPC Soil As	2006 PHC Soil As	2007 PHC Soil As	2008 PHC Soil As	Plant Arsenic	Biomass Yield
	mg/kg					mg/kg	g/m^2
A	27.3	48.3	51.6	157	**37.4**	**202**	**68**
B	164	103	203	97.2	**152**	**765**	**173**
C	154	123	125	85.2	**133**	**441 (457)**	**193**
D	48.2	51	32	28.4*	**54.7**	**131**	**242**
E	169	97.7	109	71.1 (51.0)	**102 (101)**	**425**	**180**
F	54.3	42.3	52.2	52.5*	**54.4**	**335**	**108**

*Values are averages of three replicate samples.

Values in parentheses were obtained from sample duplicates.



edenspace

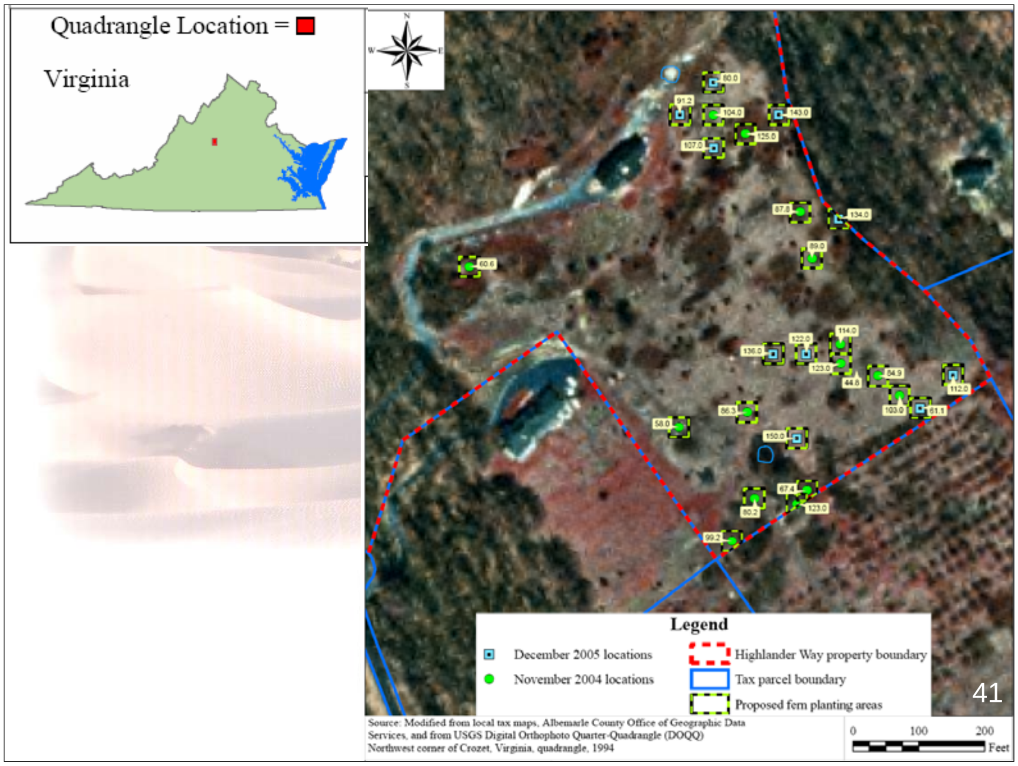
Site 3: 2006 – 2008 Summary

Plot	Pre Confirmation Soil As	2006 PPC Soil As	2006 PHC Soil As	2007 PHC Soil As	2008 PHC Soil As	Plant Arsenic	Biomass Yield
	mg/kg					mg/kg	g/m^2
Site 3 - B	23.3	23.8	28.7	22.3	**24.2**	**78**	**205**

edenspace

Summary – Spring Valley

- Changes in management practices has improved growth substantially
- *P. vittata* exhibited a strong correlation in growth with exposure to sunlight, growth improves with earlier planting dates.
- Soil fertility and irrigation management is important for maximum growth.
- After four consecutive years, we continue to observe decreases in the soil arsenic.
- Soil/sample variability continues to pose challenges for establishing confidence in the data at some locations.



Source: Modified from local tax maps, Albemarle County Office of Geographic Data Services, and from USGS Digital Orthophoto Quarter-Quadrangle (DOQQ) Northwest corner of Crozet, Virginia, quadrangle, 1994

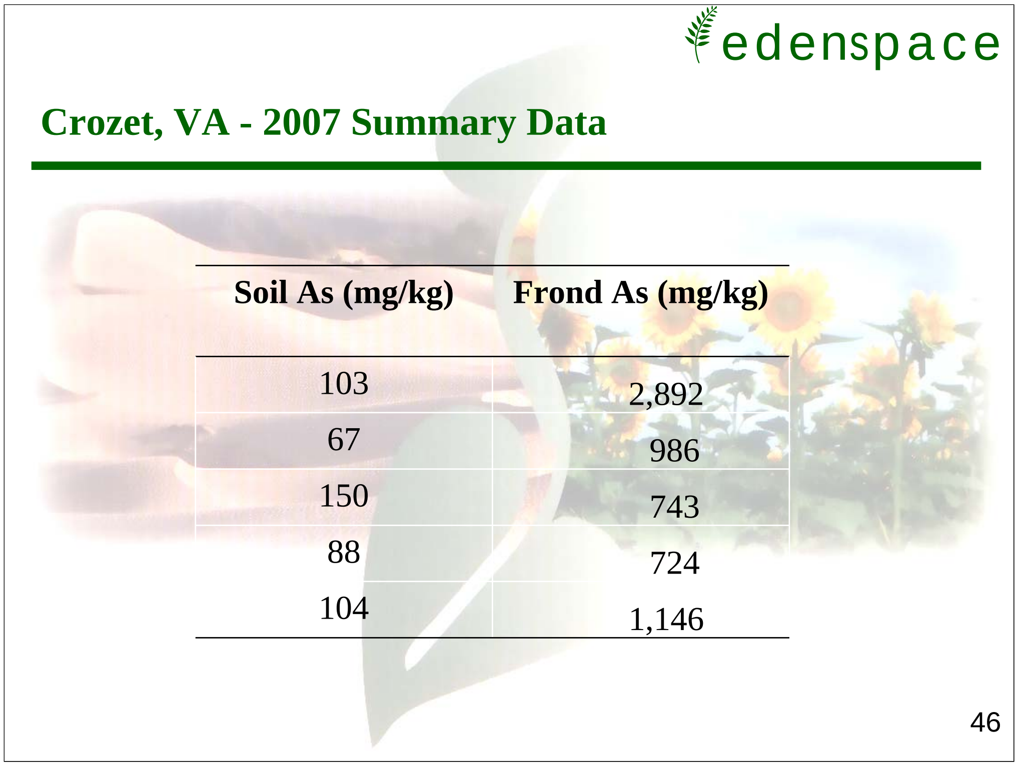
edenspace

Gravity-Flow Drip Irrigation


43

44

45



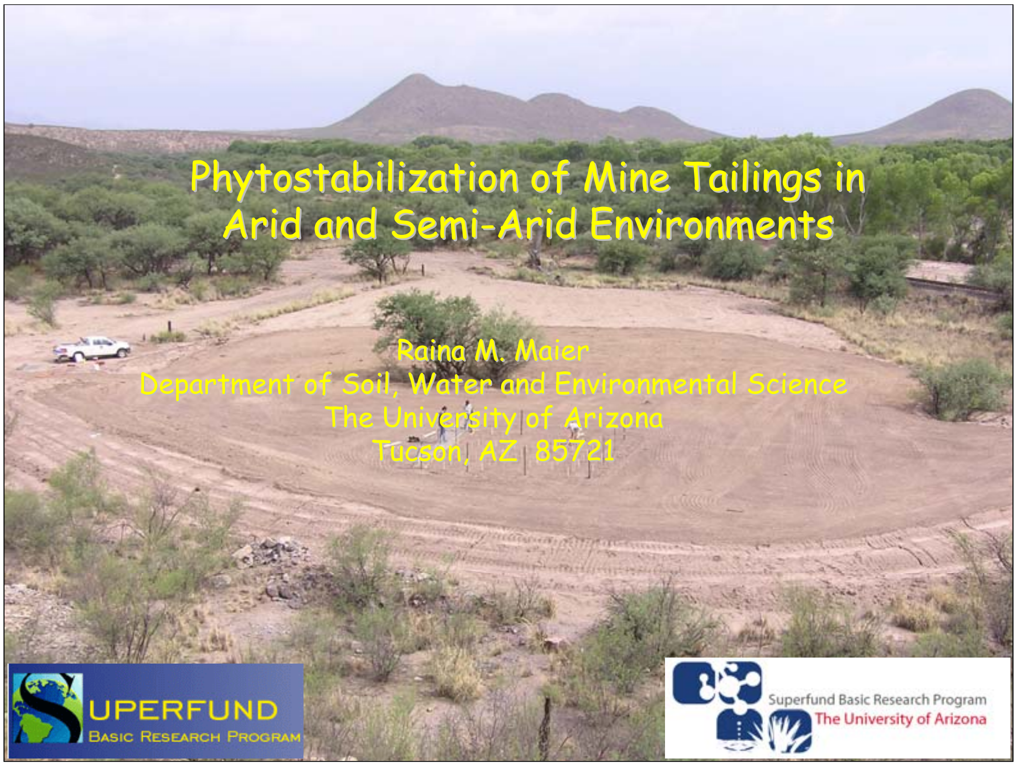
edenspace

Crozet, VA - 2007 Summary Data

Soil As (mg/kg)	Frond As (mg/kg)
103	2,892
67	986
150	743
88	724
104	1,146

Conclusions

- Phytoremediation of arsenic continues to develop as an alternative for remediation of surface soils
- Large scale projects are providing data to validate the performance at multiple sites over multiple years
- Managing, maximizing biomass growth is one of the primary limiting factors affecting performance



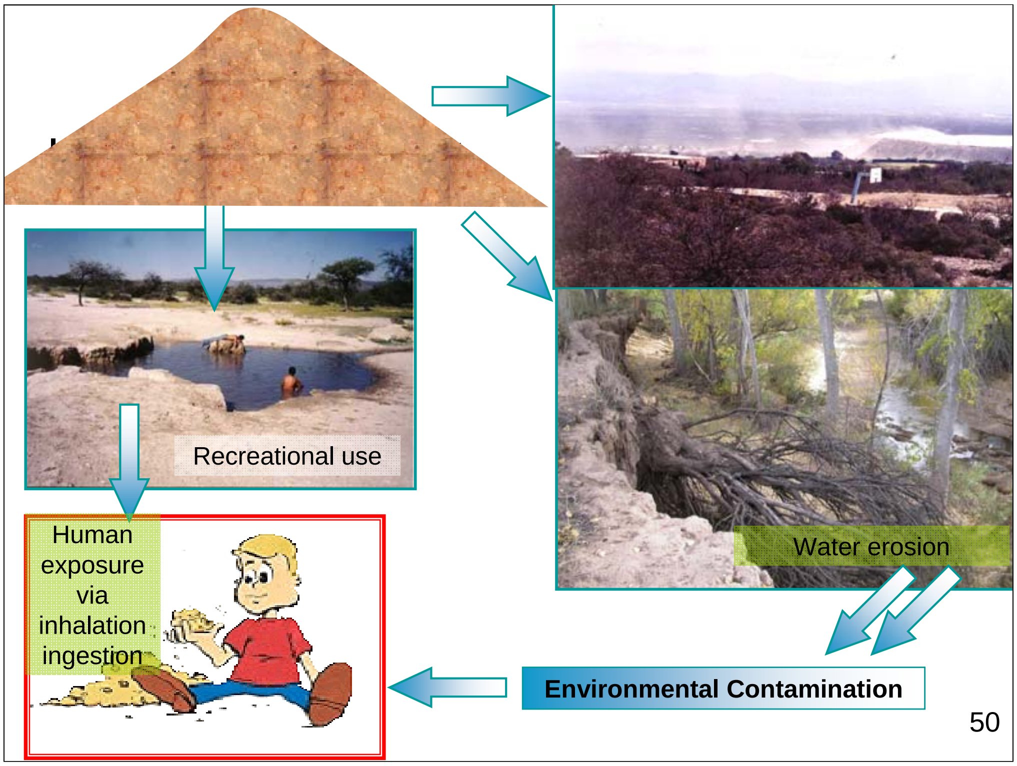
Phytostabilization of Mine Tailings in Arid and Semi-Arid Environments

Raina M. Maier
Department of Soil, Water and Environmental Science
The University of Arizona
Tucson, AZ 85721

SUPERFUND
Basic Research Program

Superfund Basic Research Program
The University of Arizona

~ 750,000 dumpsites worldwide



Recreational use

Human exposure via inhalation ingestion

Water erosion

Environmental Contamination

Superfund Basic Research Program
The University of Arizona

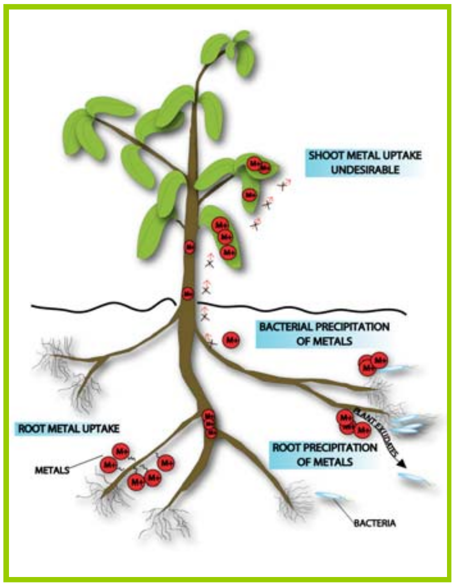
Phytostabilization

BENEFITS:

- Aesthetic vegetative cap
- Economic
- Long-term stabilization
- Reduces metal exposure
- Self-sustainable
- Ecological succession

CHALLENGES:

- Plant selection
- Amendment impacts
- Evaluation criteria



Mendez, MO. 2008. EHP

Plant Selection Criteria

#1 – Drought and salt tolerant

- Arid and semi-arid tailings are generally dry and saline
- Need pioneer, perennial species
- Need good germination

Atriplex canescens
(fourwing saltbush)

Atriplex lentiformis
(quailbush)

Prosopis velutina
(velvet mesquite)

Halophytes - salt accumulation does not = metal accumulation

Plant Selection Criteria

Superfund Basic Research Program
The University of Arizona

#2 - Native plants

Native colonizers?

Common Name	Scientific Name
Alkalai sacaton	*Sporobolus airiodes*
Buffalo grass	*Buchloe dactyloides*
Inland saltgrass	*Distichlis stricta*
Velvet mesquite	*Prosopis velutina*
Quailbush	*Atriplex lentiformis*
Sand dropseed	*Sporobolus cryptandrus*
Wright's (Big) sacaton	*Sporobolus wrightii*
Fourwing saltbush	*Atriplex canescens*
Winterfat	*Ceratoides lantana*
Indian rice grass	*Achnatherum oryzopsis hymenoides*
Mormon tea	*Ephedra trifurca*
Creosote	*Larrea tridentata*
Desert willow	*Chilopsos linearis*
Deer grass	*Muhlembergia rigens*
Desert salt grass	*Atriplex polycarpa*

Survived in Sonoran desert tailings

Did not survive

Plant Selection Criteria

#3 - Metallophytes

Plant accumulation factors should be << 1

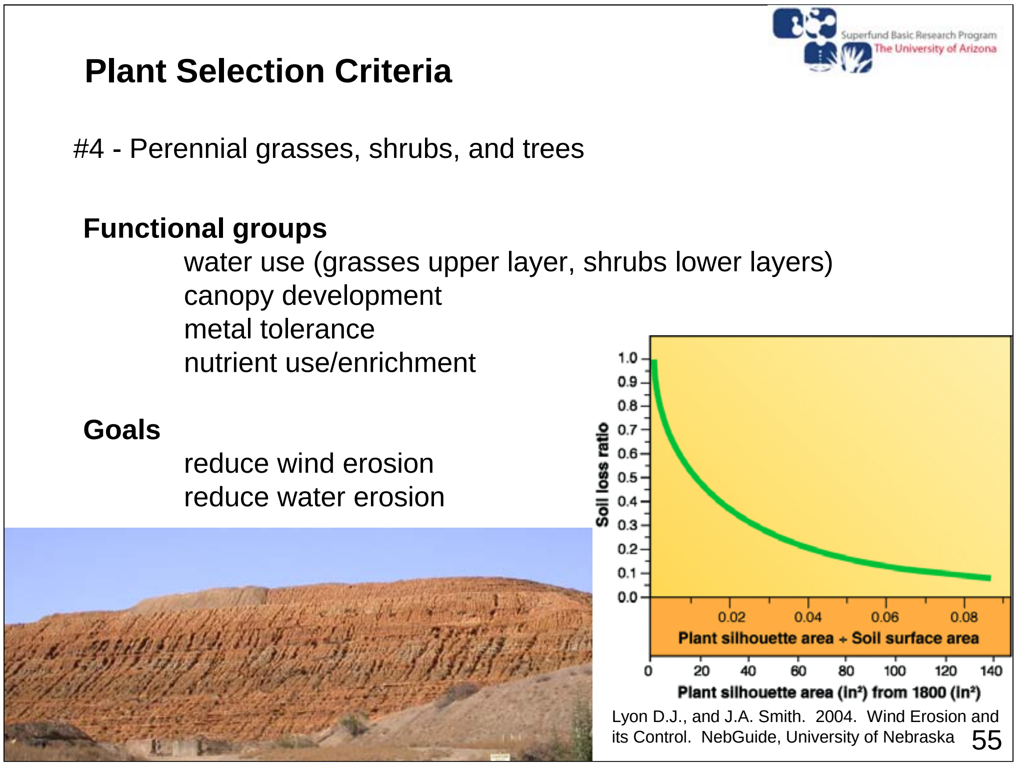
Bioconcentration Factor (BF) or Accumulation Factor (AF)	$\frac{\text{Total element}_{\text{shoot}}}{\text{Total element}_{\text{mine tailings}}}$
Translocation Factor (TF) or Shoot:Root (S:R) ratio	$\frac{\text{Total element}_{\text{shoot}}}{\text{Total element}_{\text{root}}}$

Metal toxicity

Soil→ Soil Plant Toxicity Limits (SPL)

Plant→ Plant leaf tissue toxicity limits

Animal→ Domestic Animal Toxicity Limits (DATL)



Plant Selection Criteria

#4 - Perennial grasses, shrubs, and trees

Functional groups

water use (grasses upper layer, shrubs lower layers)
canopy development
metal tolerance
nutrient use/enrichment

Goals

reduce wind erosion
reduce water erosion

Lyon D.J., and J.A. Smith. 2004. Wind Erosion and its Control. NebGuide, University of Nebraska

Amendments

Shorter term effects

Longer term effects

Organic (biosolids/compost)

Increases pH of acidic mine tailings

Improves physical structure

Slow-release nutrient source

Complexation of heavy metals

Shorter term effects

Inorganic

NPK fertilizers: Increase nutrient contents

Lime: Increases pH of acidic mine tailings

Phytostabilization Evaluation Criteria

#1 - Plant Criteria

- Biomass and percent cover
- Self-propagation
- Native colonizers
- Metal shoot concentrations < DATL’s
- Plant survival and productivity > 10 – 20 years

#2 - Microbial Criteria

- Increased heterotrophic bacterial and fungal counts
- Decreased autotrophic iron- and sulfur-oxidizers
- Increased diversity and activity

#3 - Soil Criteria

- Improved soil aggregation
- Reduced erosion and runoff
- Decreased metal bioavailability and mobility

University of Arizona Examples

Klondyke Site: Greenhouse Studies

- Aravaipa Creek, AZ
- Pb and Zn ore (1948 - 1958)
- Acidic (pH 2 – 7)
- Pb and Zn (up to 20 g/kg)
  - As, Cd, Cu (elevated)

Boston Mill Site: Field Studies

- San Pedro River, AZ
- Ag and Au ore (1879-1887)
- Alkaline (pH 8.3 - 9.0)
- Up to Pb (16) As (0.8), Hg (0.5), Mn (50), Cu (3), Zn (8) g/kg

Klondyke Site: Greenhouse

Atriplex lentiformis (quailbush)

3 month study

Compost amended samples

- K4 (pH 2.7)
- K6 (pH 5.7)
- Offsite control

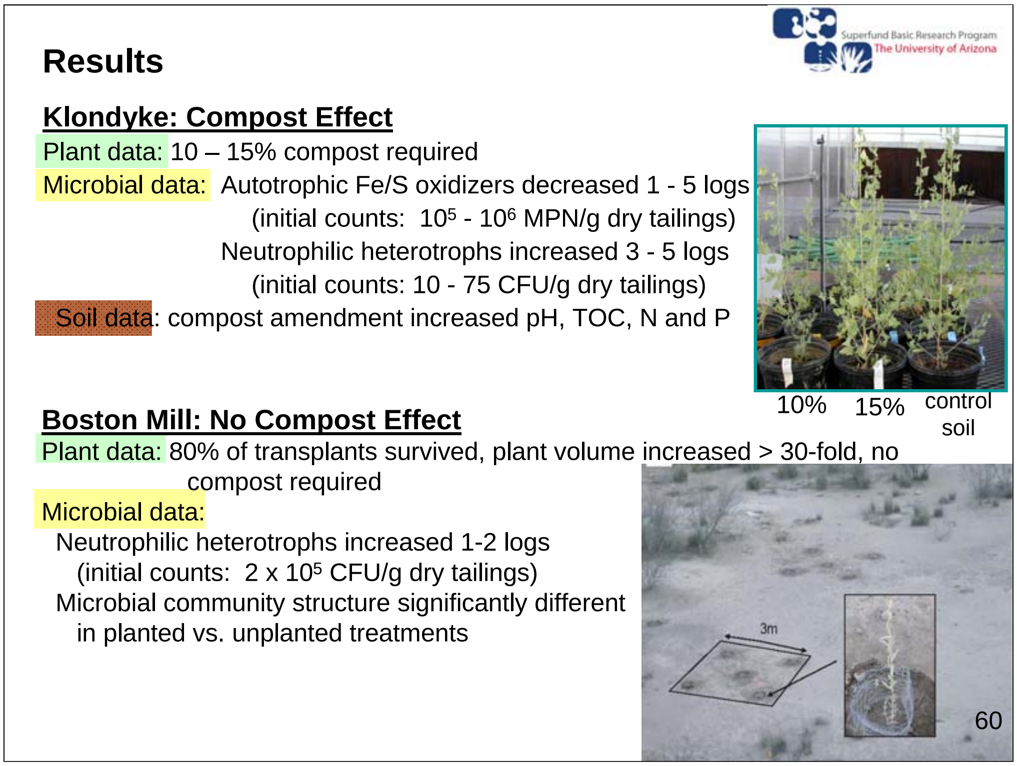
} Mine tailings

Boston Mill Site: Field

Atriplex canescens (fourwing saltbush)

18 month study

Compost amended



Superfund Basic Research Program
The University of Arizona

Results

Klondyke: Compost Effect

Plant data: 10 – 15% compost required

Microbial data: Autotrophic Fe/S oxidizers decreased 1 - 5 logs
(initial counts: 10^5 - 10^6 MPN/g dry tailings)
Neutrophilic heterotrophs increased 3 - 5 logs
(initial counts: 10 - 75 CFU/g dry tailings)

Soil data: compost amendment increased pH, TOC, N and P

Boston Mill: No Compost Effect

Plant data: 80% of transplants survived, plant volume increased > 30-fold, no compost required

Microbial data:
Neutrophilic heterotrophs increased 1-2 logs
(initial counts: 2 x 10^5 CFU/g dry tailings)
Microbial community structure significantly different in planted vs. unplanted treatments

Slide 60

Results

Klondyke

Quailbush: Phytostabilization potential

Metal tolerant: Mn, Pb, Zn (exceeded PTLs)

Primarily root concentrated: Fe, Cu, and Pb

Shoot accumulated nutrients: K, Mn, Na, and Zn

Boston Mill

Fourwing saltbush: Phytostabilization candidate?

Metal tolerant: As, Cd, Mn, Pb

Pb levels were up to 3-fold DATL (100 mg/kg)

What we have learned:

Four categories of tailings are encountered:

pH	metal concentration	legacy
low (acidic)	high	historical
high (neutral – alkaline)	high	historical
low (acidic)	low	modern
high (neutral – alkaline)	low	modern

Successful plant growth depends on factors other than pH and metals:

Klondyke: 10-15% compost required
Acidic (pH 2 – 5)
Metals (high Pb, Zn, some As, Cd, Cu)
TOC < 0.4 g/kg
Total N (<0.2 g/kg)

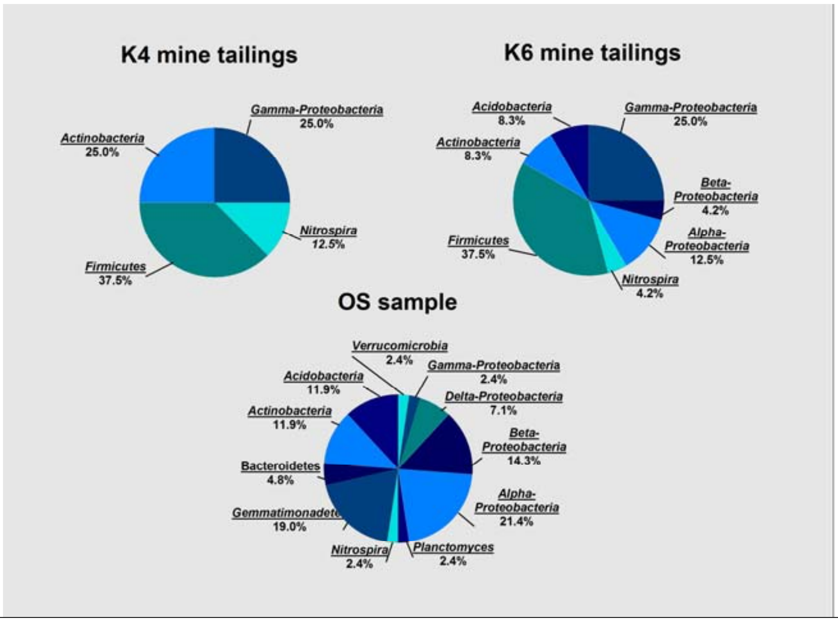
Nacozari Tailings: 5-10% compost required
Acidic (pH 3.7)
Metals (low)
TOC (0.25 g/kg)
Total N (0.02 g/kg)

Boston Mill: no compost required
Alkaline (pH 8.3 – 9.0)
Metals (high Pb, some As, Hg, Mn)
TOC (8 – 21 g/kg)
Total N (0.2 -0.8 g/kg)

Unnamed: 5-10% compost required
Neutral (pH 7)
Metals (low)
TOC (0.2 g/kg)
Total N (0.02 g/kg)

What we have learned:

Complex diversity analysis can provide detailed information on tailings impact level

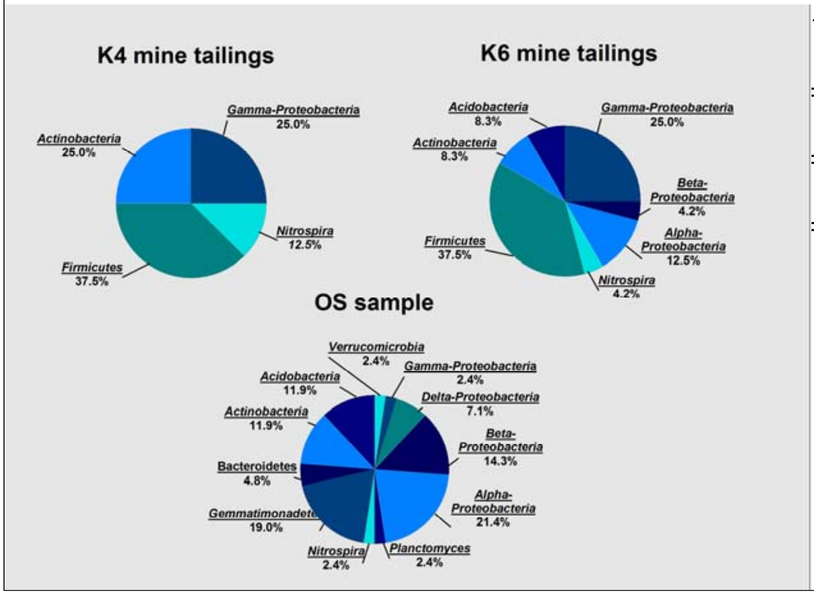


What we have learned:

Superfund Basic Research Program
The University of Arizona

Complex diversity analyses provide detailed information on tailings impact level

But a simple neutrophilic heterotrophic count may be enough for a practitioner



trophilic counts

= 10 CFU/g

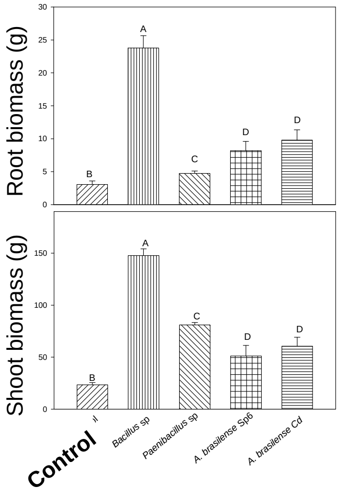
= 75 CFU/g

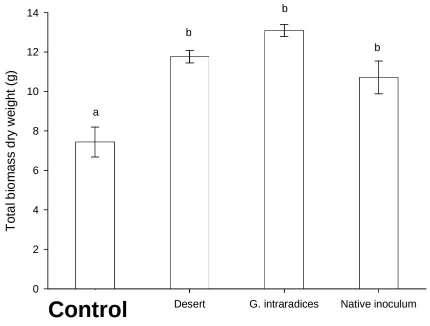
= 8 x 10^4 CFU/g

Klondyke: 10-15% compost required Acidic (pH 2 – 5) Metals (high Pb, Zn, some As, Cd, Cu) TOC < 0.4 g/kg Total N (<0.2 g/kg)	< 100 CFU/g
Boston Mill: no compost required Alkaline (pH 8.3 – 9.0) Metals (high Pb, some As, Hg, Mn) TOC (8 – 21 g/kg) Total N (0.2 -0.8 g/kg)	2 x 10^5 CFU/g
Nacozari Tailings: 5-10% compost required Acidic (pH 3.7) Metals (low) TOC (0.25 g/kg) Total N (0.02 g/kg)	< 100 CFU/g
Unnamed: 5-10% compost required Neutral (pH 7) Metals (low) TOC (0.2 g/kg) Total N (0.02 g/kg)	8 x 10^3 CFU/g

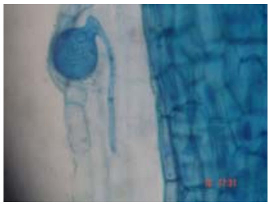
Final Point: Plant growth-promoting microbes

Revegetation possible at low end of compost range with increased biomass





Alginate-encapsulated bacterial inocula



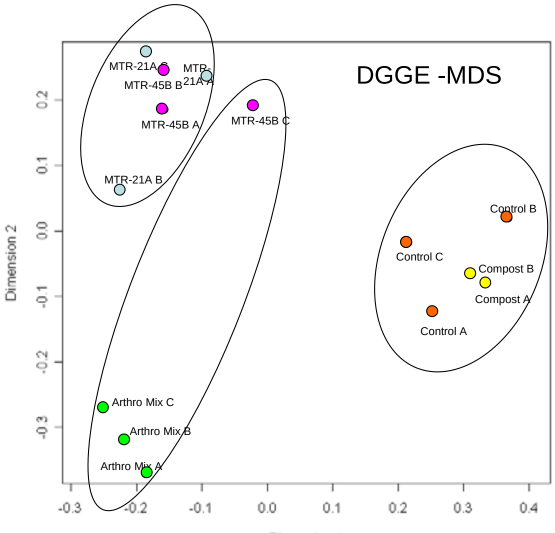
Mycorrhizal fungi

Final Point: Plant growth-promoting microbes

- Inocula seem to direct or influence community development

- Inoculated rhizosphere communities significantly different than non-inoculated

- Non-inoculated rhizosphere communities dictated by compost community



Conclusion

Phytostabilization has excellent potential as a cost-effect remediation strategy for mine tailings in arid and semi-arid environments

Major information gaps

Are vegetative caps viable over the long-term?

Is this a technology that can eventually be self-sustaining?

Do normal successional plant processes take place? Does metal uptake into shoot material occur in successional plants?

Do tailings transition into soils?

Does speciation of tailings metals in the rhizosphere change in the short- or long-term?

What impact might this have on metal mobility and bioavailability?

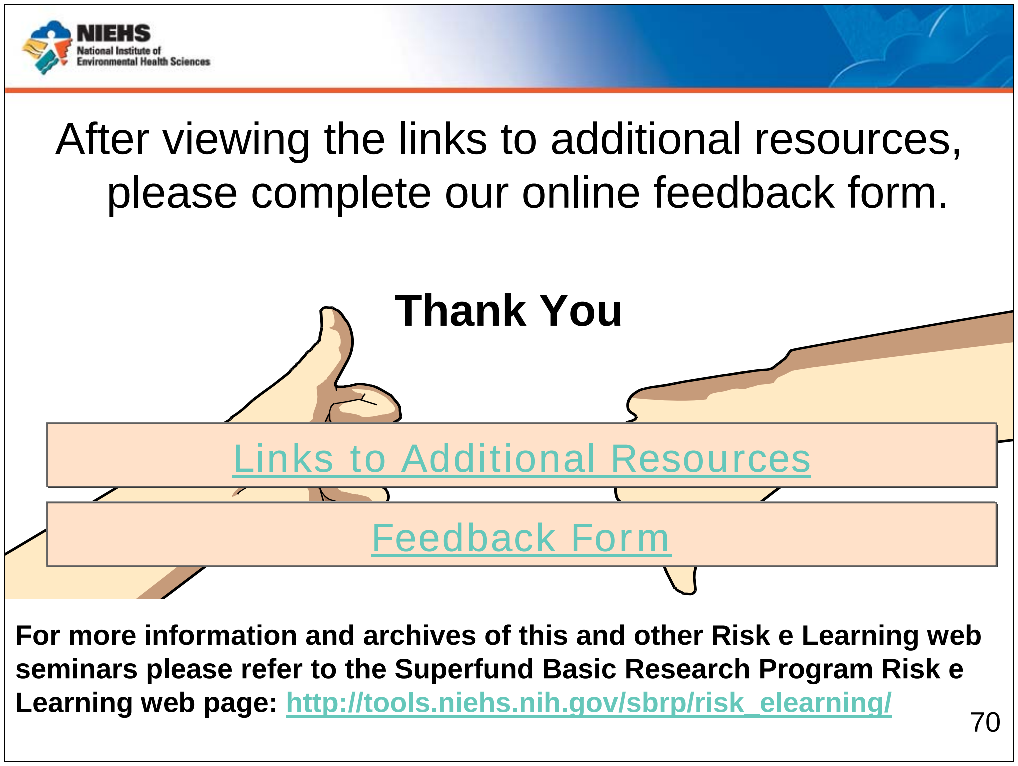
Superfund Basic Research Program
The University of Arizona

Acknowledgments

Colleagues
Yoav Bashan
Luz de-Bashan
Jon Chorover
Tom Thompson
Christopher Grandlic
Sarah Hayes
Sadie Iverson
Karyna Rosario
Monica Mendez
Julie Neilson
Fernando Solis-Dominguez
Michael Steward
Angelica Vasquez
Scott White

Funding
NIEHS SBRP Grant P42 ES04940
Bureau of Land Management
US-Mexico Binational Center

SUPERFUND
BASIC RESEARCH PROGRAM



After viewing the links to additional resources, please complete our online feedback form.

Thank You

Links to Additional Resources

Feedback Form

For more information and archives of this and other Risk e Learning web seminars please refer to the Superfund Basic Research Program Risk e Learning web page: http://tools.niehs.nih.gov/sbrp/risk_elearning/