Commercialization of Phytoextraction and Phytostabilization of Metal Rich Soils.

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PHYTO-EXTRACTION CONCEPT

- Polluted soils require remediation
- Geochemically enriched soils exist
- Soil Removal/replacement is very expensive.
- Hyperaccumulator species exist in nature.
- An industry exists to recycle metals from biomass.
- For Ni/Co, we domesticated a “new crop”.
  - Farm Phytoextraction Crop to remove metals from soil; make “hay” using traditional farm practices.
  - Developed all soil and plant management practices needed to produce high yields of phytomining crop.
  - Incinerate or pyrolyze to recover biomass energy and ash.
  - Recover metals from ash; MARKET Ni/Co.

Crop Models for Ni Phytomining: Hyperaccumulators are necessary!

Assume soil contains 2500 ppm Ni = 10,000 kg Ni/ha-30 cm

Species	Yield	Ni in the Crop			Ni in Ash
	t/ha	mg/kg	kg/ha	% of Soil	%
Corn (50% YD)	10	100	1	0.01	0.2
Wild *Alyssum*	10	20,000	200	2.0	20 - 40
Alyssum cultivar	20	30,000	600	6.0	25 - 50

Natural Hyperaccumulation

- Plant accumulates metal from rhizosphere, transports to shoots, tolerates for whole growth cycle.
- Only usual fertilizers and soil amendments required for whole process.

Induced Hyperaccumulation

- Addition of chelating agent is required to dissolve metal and induce root leakage; plant rapidly injured/killed after addition of chelator.
- Requires added EDTA; at 10 mmol/kg soil as published by Blaylock et al., it would cost $30,000 per ha-yr using truckload lots of EDTA.
- Leaching of Pb-EDTA prevents acceptance.

ccumulation in crop Plants is unable
o give annual removal rate needed
or cost effective technology.

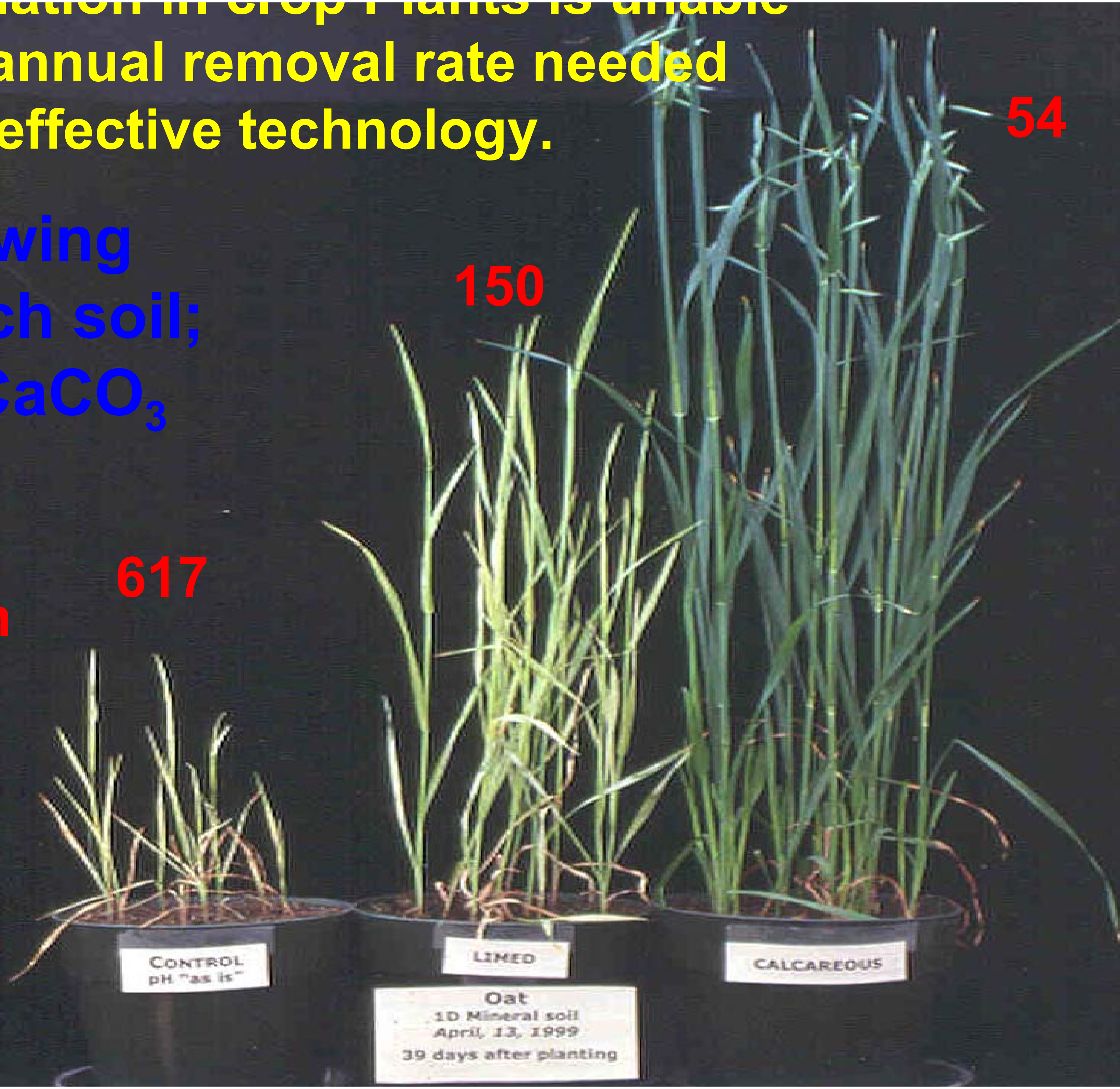
at growing
n Ni rich soil;
aried $CaCO_3$

Shoot
Ni, ppm

617

150

54



Chaney, R.L., J.S. Angle, A.J.M. Baker and Y.-M. Li. 1998.

Method for Phytomining of Nickel, Cobalt and Other Metals From Soil.

US Patents 5,711,784 and 5,944,872; others pending.

Licensed to Viridian Resources LLC in relation to CRADA to develop Ni & Co Phytomining Technologies.

Estimated Economics of Ni phytomining.

If we use "Low End" assumptions:

- 400 kg Ni/ha (20 t biomass/ha with 2% Ni in biomass)
- $8.97/kg Ni (1984 - 1994 average value)
- 75% of Ni value for company which does phytomining.

The value of an annual phyto-mining crop ($/ha) is:

20000 kg biomass/ha • 0.020 kg Ni/kg biomass

= 400 kg Ni/ha in biomass.

400 kg Ni/ha • $8.97/kg Ni • 0.75 of Ni value

= $2619 gross return/ha

-($250/ha production costs and $200/ha land rental cost)

= $2241 net/ha = $908 net/acre

The annual value of alternative normal crops on infertile serpentine soils is about $125/ha or $50/acre.

Steps to Develop Ni/Co Phytomining-1

- Select promising species for development
- Collect seeds of Ni/Co Hyperaccumulator genotypes from different sites where they occur naturally in order to obtain wide genetic diversity to aid breeding cultivars.
- *Alyssum murale* and *Alyssum corsicum* occur across Southern Europe. Seed collected when mature but before dispersed in field. Alan Baker, Roger Reeves, Alex Koulikov, Van Volk et al. did collection.


9/96A-J5

Steps to Develop Ni/Co Phytomining-2

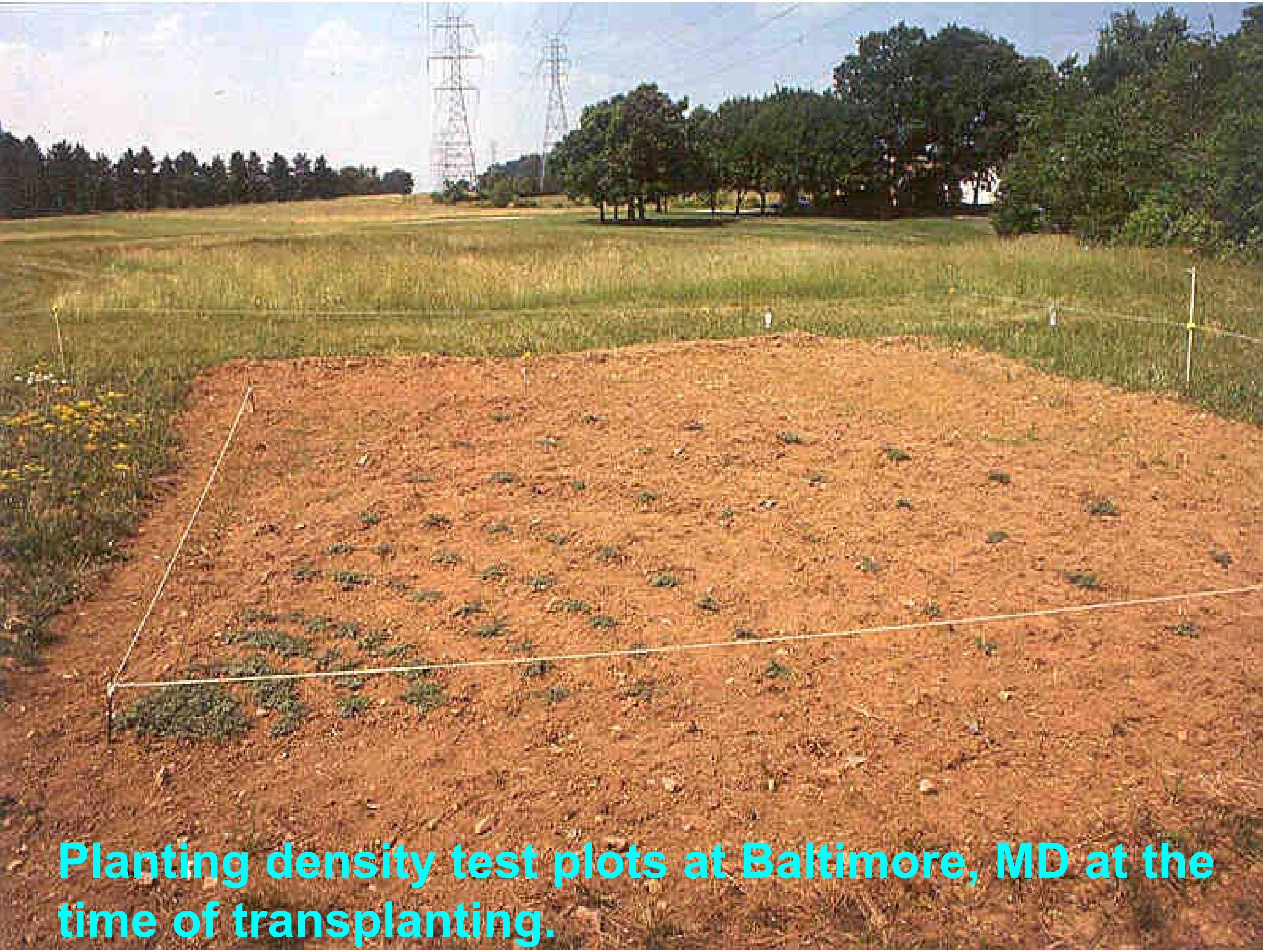
- Evaluate germplasm in both controlled environment and in contaminated soils and serpentine soils.
  - Test uptake of Ni and biomass yield.
  - Use pH and fertilizers based on lab tests.
- Identify methods to breed phyto-extraction crop:
  - Characterize inheritance of metal uptake, translocation, metal tolerance, yield, growth habit. *Alyssum species* self-incompatible.
  - Improve by recurrent selection based on combination of yield and Ni level.


PHYTOMINING SOIL NICKEL USING *ALYSSUM MURALE*
Field test site used to evaluate genotypic differences in Ni hyperaccumulation by new crop for serpentine soils in SW Oregon.
Average test genotype contained 2.0% Ni in shoot biomass. .

Yin Li making crosses between *Alyssum murale* genotypes in Field, 2000.

Steps to Develop Ni/Co Phytomining-3

- **Identify production practices to maximize profit:**
  - Fertilizer, pH, seed depth, herbicide management.
  - Annual *vs.* perennial culture; planting density; irrigation; planting and harvesting schedule.
- **Evaluate methods to maximize metals in biomass just short of phytotoxicity for these plants:**
  - Adjust soil pH; fertilization; maintain Ca level.
  - Management of Ca, N, P, and K fertility.

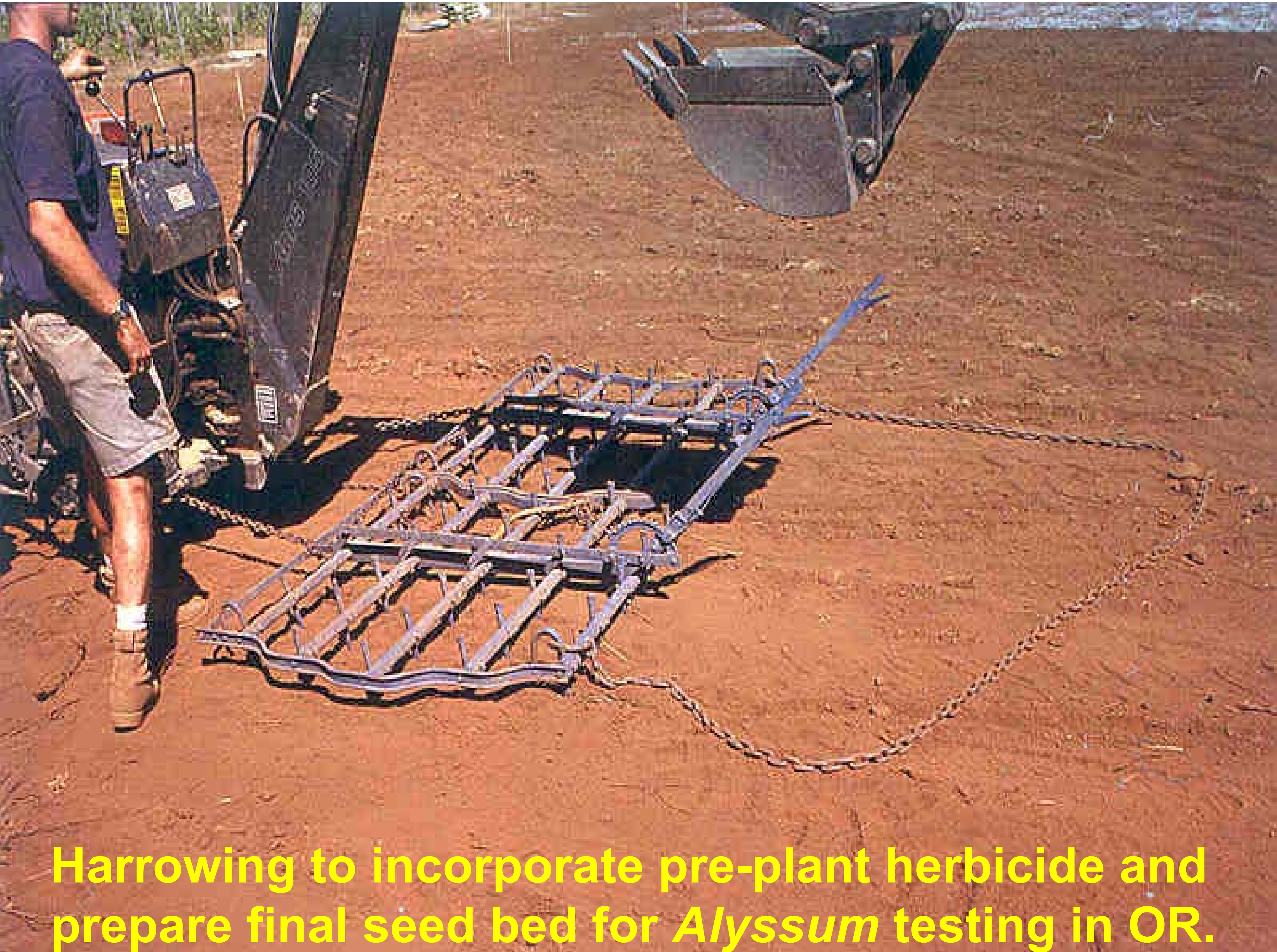


Planting density test plots at Baltimore, MD at the time of transplanting.

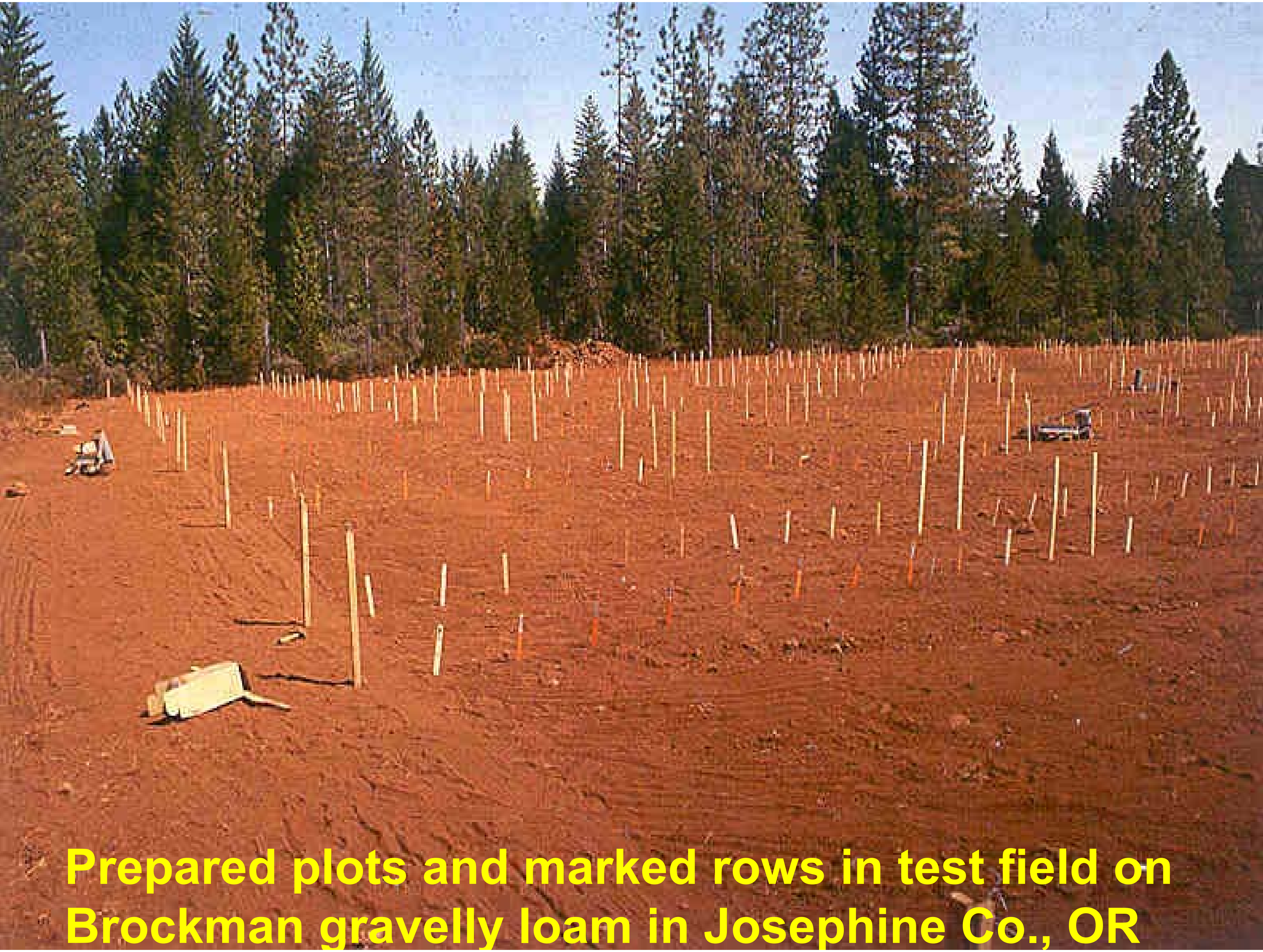

Planting density test at Baltimore, MD, at the time of
Harvest about 8 months after transplanting.

Steps to Develop Ni/Co Phytomining-4

- Field demonstrate improved phytomining crop
  - Mine tailings; ultramafic soils; contaminated land.
  - Different classes of potential production fields.
- Optimize recycling of metals + energy from biomass.
  - Recover energy and ash during burn.
  - Recovery metals from biomass ash by electrowinning.
- Develop farming systems for phytomining.
  - Identify soils where phytomining would be profitable.
  - Contract with farmers?
  - Lease mineralized soils?
  - Mining claims on public lands?



Harrowing to incorporate pre-plant herbicide and prepare final seed bed for *Alyssum* testing in OR.



Prepared plots and marked rows in test field on Brockman gravelly loam in Josephine Co., OR

Brockman gravelly loam plots being irrigated to aid In establishing *Alyssum* genotypes for testing.

Mowing *Alyssum murale* using farm equipment


OSU

Methods used in Greenhouse Pot Studies of Ni Phytomining by *Alyssum* species from serpentine Soils and Smelter-Contaminated Soils.

Soils were collected from field, sieved < 5 mm, and kept moist during preparation for cropping.

Required nutrients for the biomass to be grown were added (N, P, K, Ca, Mg, B, Mo, S). Pots contained 4 L soil.

When pH was acidified, nitric acid was added and soluble nitrates were then leached before fertilized.

Seedlings were transplanted into treatments (4 replications) for 60 and 120 days; harvested shoots were washed to remove soil, dried, dry-ashed, and analyzed by ICP and AAS.

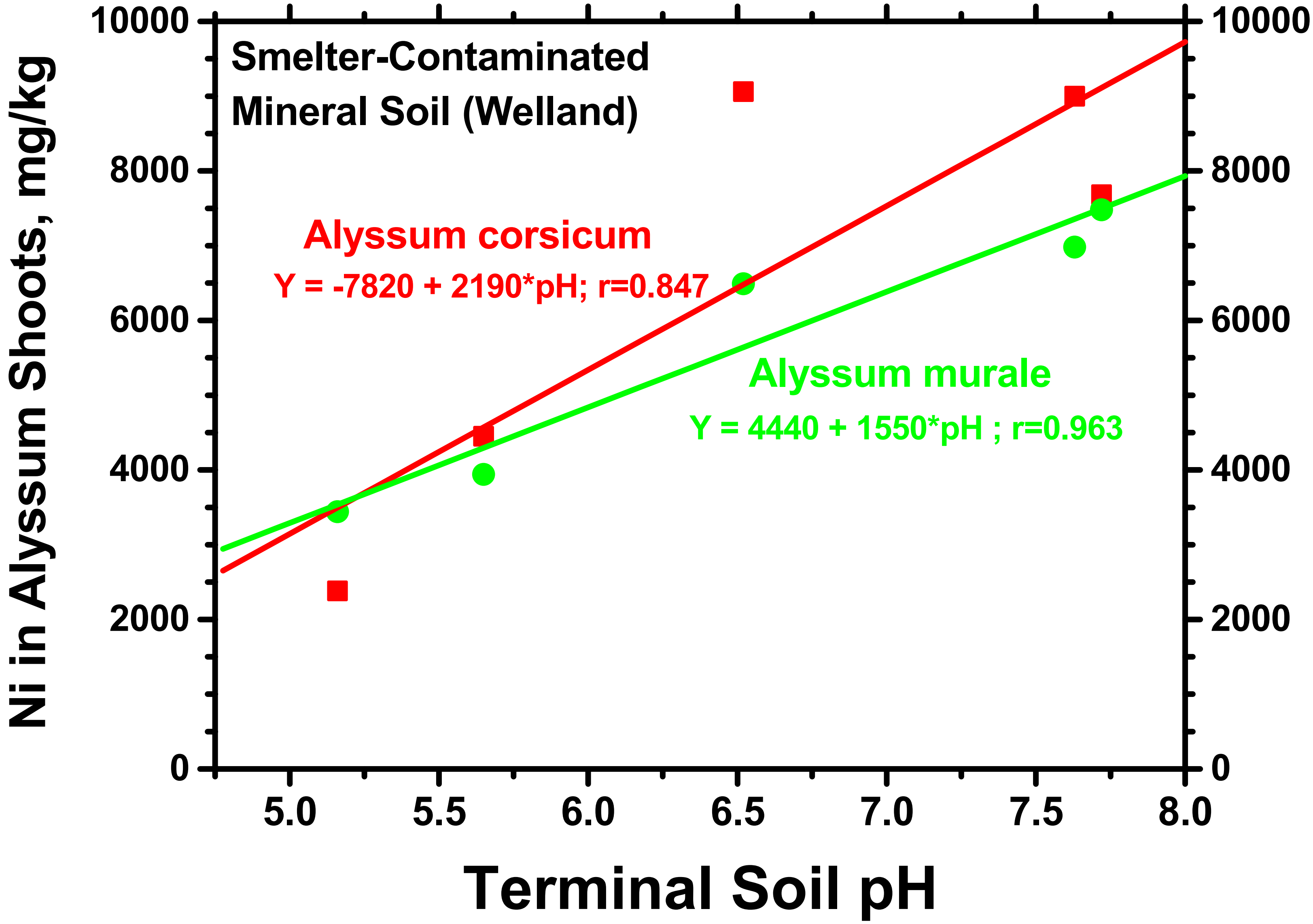
When soil pH was raised, a mixture of fine powdered Ca and Mg carbonates were mixed with the soil and allowed to react before planting.

Effect of Phosphate Fertilization on Ni Phytomining By *Alyssum* Species From Serpentine and Smelter-Contaminated Soils.

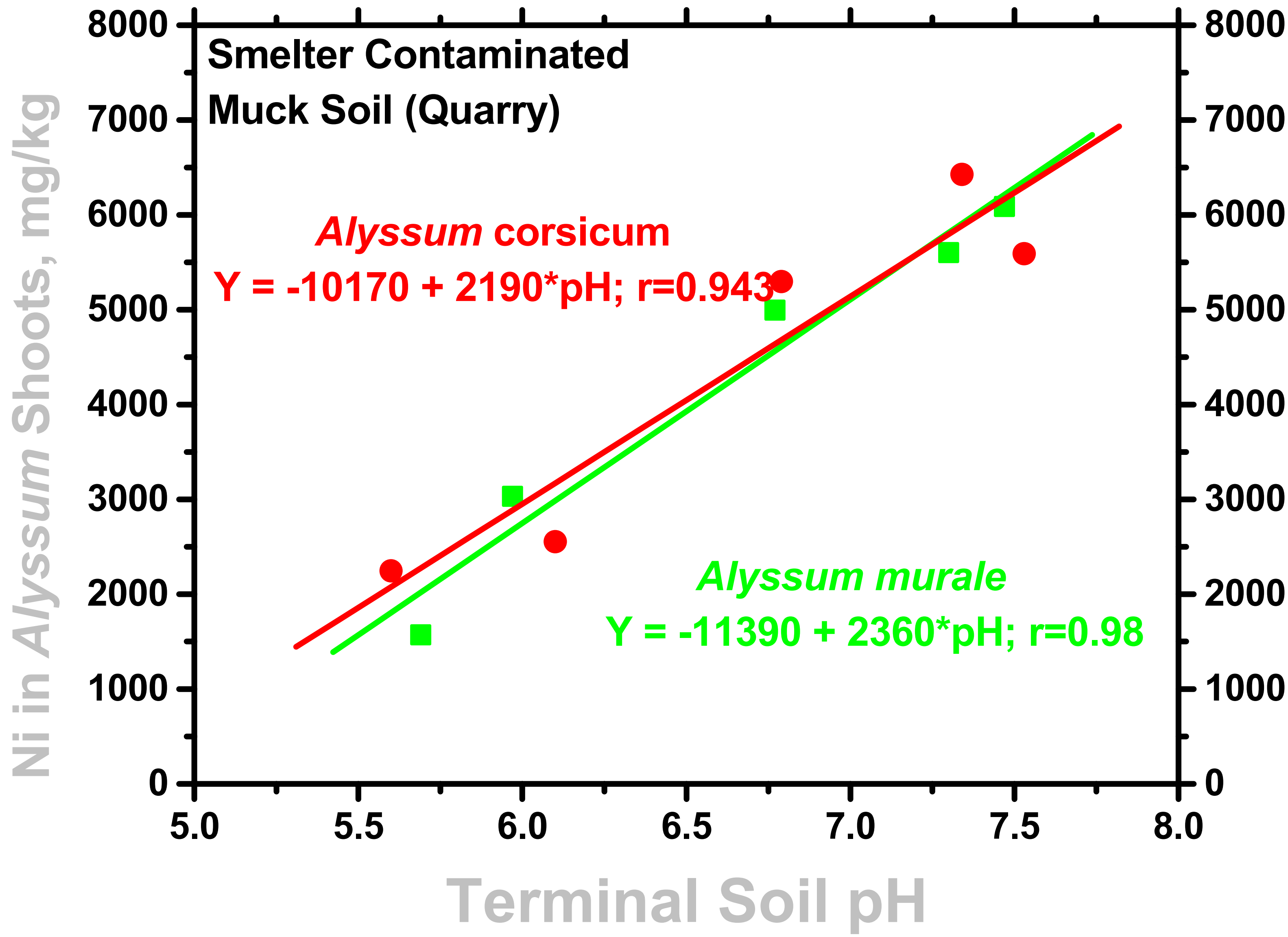
Phosphate Treatment	Yield	Composition of Biomass: P	Ni	Mn	Ca	Mg
kg/ha	g/pot	g/kg	------- mg/kg -------		----- g/kg -----	
Serpentine soil:						
None	4.1 c	1.04 e	14700 a	56.5	18. ab	4.1 d
0	1.6 d	0.61 f	6250 cd	62.3	18. ab	6.6 a
100	24.5 a	2.16 cd	6270 cd	60.9	17. ab	6.2 bc
250	23.2 ab	3.00 b	6810 bc	65.2	19. a	6.5 bc
500	26.5 a	3.59 a	5690 d	67.2	18. ab	6.4 bc
Smelter-Contaminated Mineral Soil:						
None	10.9 e	3.2 ab	8130. ab	39. e	23. a	1.5 c
0	32.0 bcd	2.0 f	7580. bc	56. cd	22. a	1.6 c
100	36.2 ab	2.4 de	7340. bc	60. cd	23. a	1.4 c
250	34.6 abc	3.1 abc	7370. bc	57. cd	24. a	1.5 c
500	40.3 a	3.3 a	6500. cd	53. cde	21. a	1.4 c

Effect of Ca and/or Mg Fertilization on Ni Phytomining by *Alyssum* species from serpentine and smelter-contaminated soils.

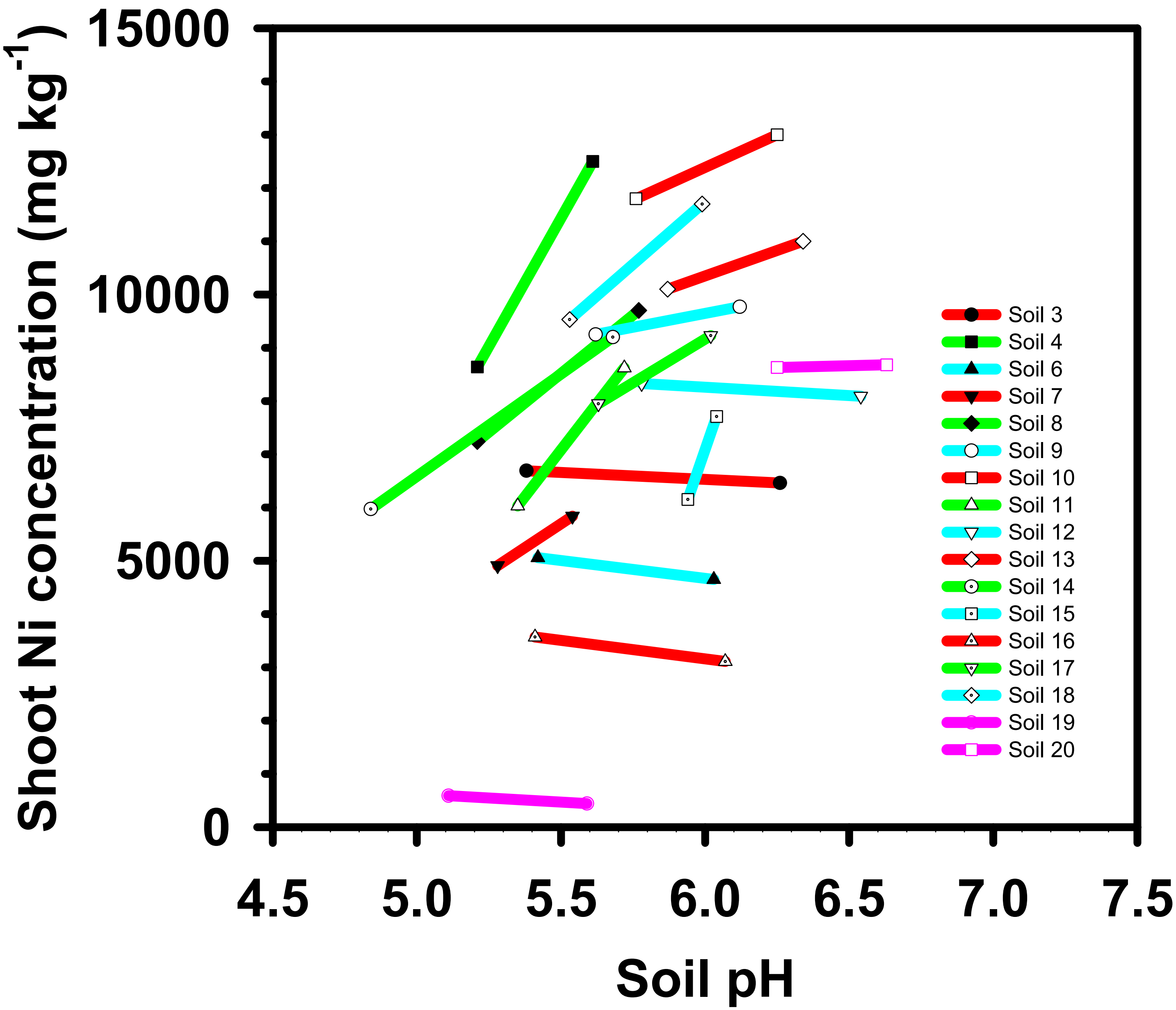
Ca or Mg Treatment	Yield	Composition of Biomass: P	Ni	Mn	Ca	Mg
t/ha	g/pot	g/kg	------- mg/kg -------		----- g/kg -----	
Serpentine soil:						
None	4.1 c	1.04 e	14700 a	56 e	23 a	1.5 c
0.0	19.3 b	2.43 c	7860 b	62 cde	15 b	5.7 bc
1.0	24.5 a	2.16 cd	6270 cd	61 cde	17 ab	6.2 bc
2.5	25.2 a	2.10 d	6050 cd	65 cde	18 ab	6.7 b
5.0	24.2 a	1.94 d	5630 d	78 cde	16 ab	6.3 bc
Smelter-Contaminated Mineral Soil:						
None	10.9 e	3.2 ab	8130 ab	39 e	23 a	1.5 c
1.0 Ca	29.5 cd	2.8 bcd	7320 bc	65 c	24 a	1.7 c
0.0	36.2 ab	2.4 de	7340 bc	60 cd	23 a	1.4 c
2.5 Mg	34.9 abc	2.4 de	6750 cd	49 de	24 a	2.1 b
5.0 Mg	32.9 bcd	2.3 ef	5710 d	55 cd	24 a	2.7 a



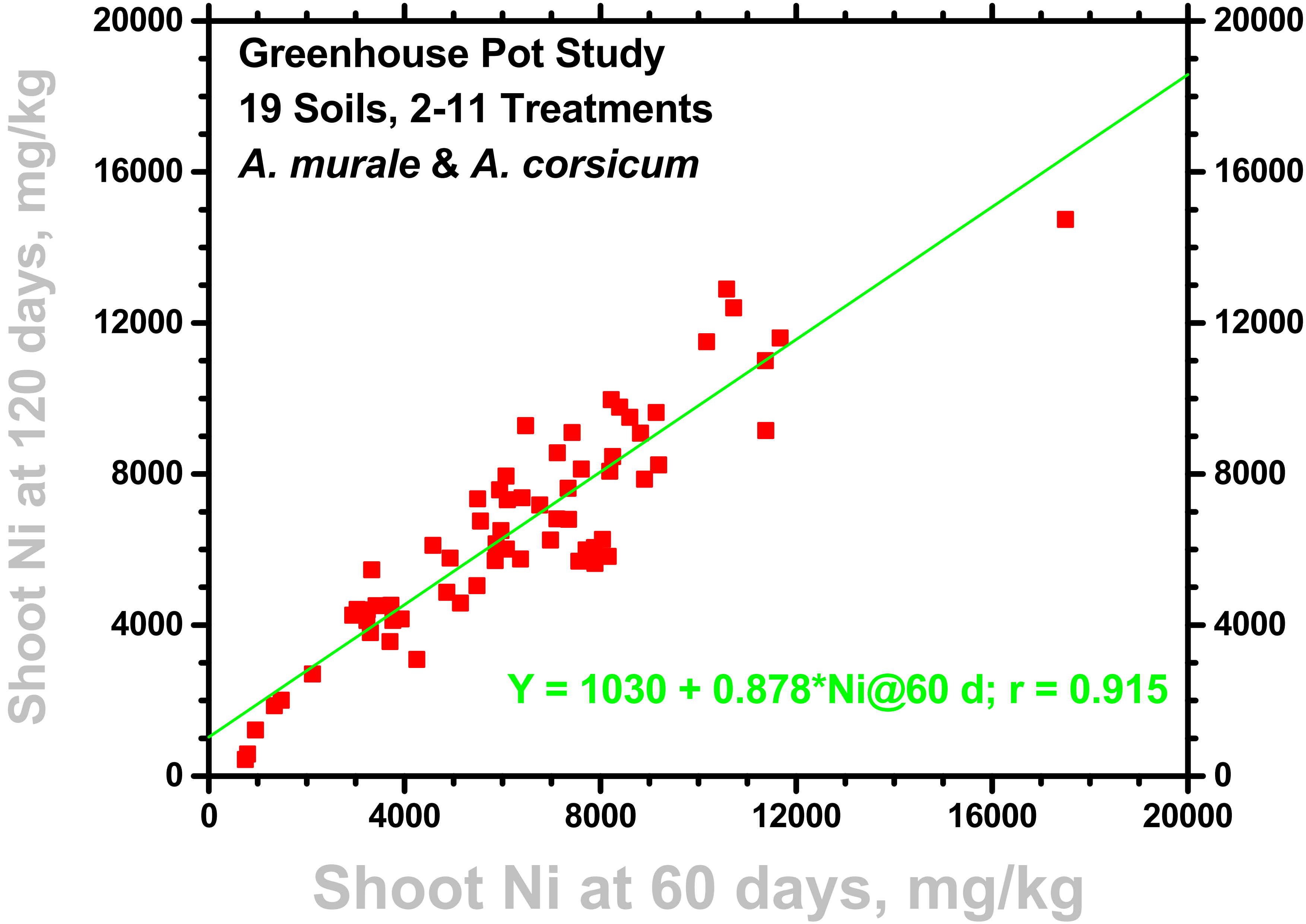
Effect of soil pH on Ni phytoextraction by two *Alyssum* species grown on Welland loam for 60 days.



Effect of soil pH on Ni phytoextraction by *Alyssum* grown on Quarry muck for 60 days.

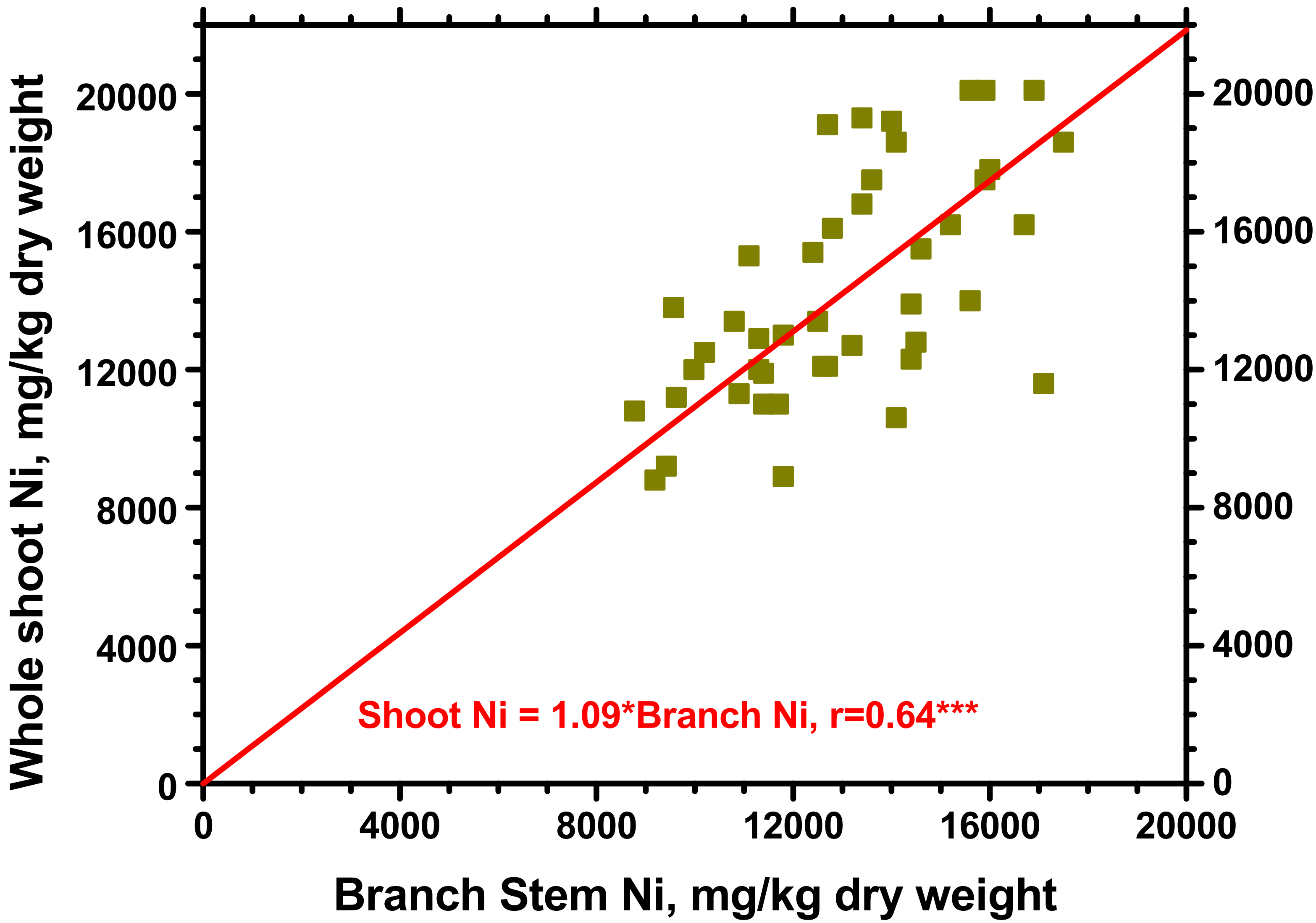


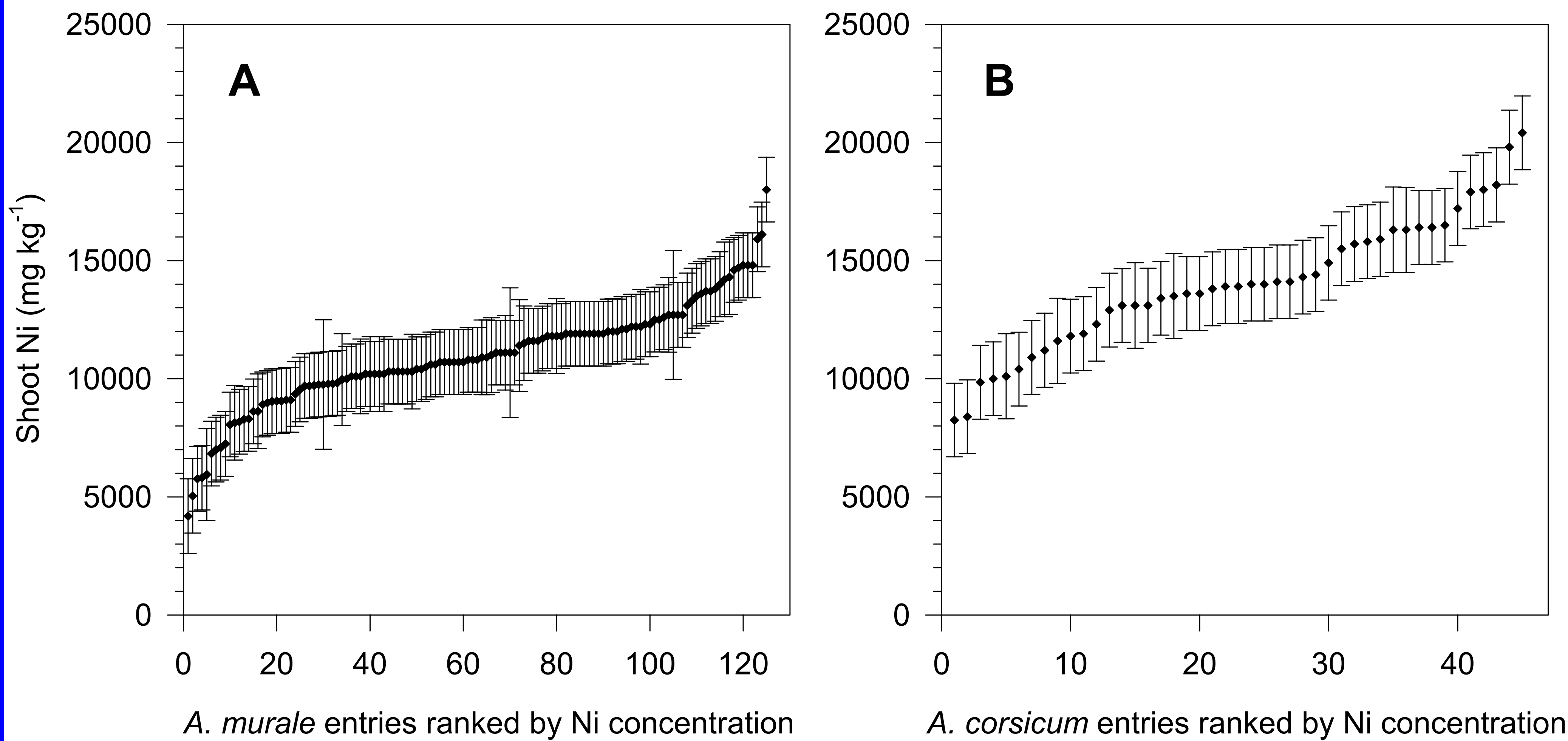
Soil pH effect on *Alyssum murale* accumulation of Ni from different Oregon serpentine soils



Correlation of *Alyssum* Hyperaccumulator Shoot Ni after 60 days with Shoot Ni after 120 days.

Concentration of Ni in branch stems in Spring compared to whole shoots in Fall, 1998; colluvial soil contained only 660 mg total Ni/kg.





Mean shoot Ni concentration, with SE bars:

(A) 125 *Alyssum murale* entries

(B) 45 *Alyssum corsicum* entries

Grown on an Oregon Brockman gravelly loam serpentine soil, Ni = 5500 mg/kg. [From Li et al., 2003, ICOBTE Proc.]

Selected properties of the soils

Soil Name	Total Ni	Total Co	Total Fe	pH	OC	Sand	Clay
	----mg/kg---		%		------ % ------		
Serpentine							
Brockman	4700	350	22.2	6.30	3.0	58	21
“Mineral”							
Welland	3200	48	1.76	5.24	8.7	14	31
“Muck”							
Quarry	2150	25	1.05	5.66	36.3	-	-

Soil Treatments and Resulting pH

Amendment	Brockman	Welland	Quarry
Acidified	5.8		
None	6.1-6.4	5.1-5.2	5.6-5.7
Limed		5.6-5.7	6.0-6.1
Limed	6.8-7.0	6.5	6.8
Calcareous	7.8	7.6-7.7	7.3
Calcareous + HFO†		7.7	7.5

† HFO=Hydrous Ferric Oxide

(2.5% Fe by weight - Quarry, 1.4% Fe by weight - Welland)

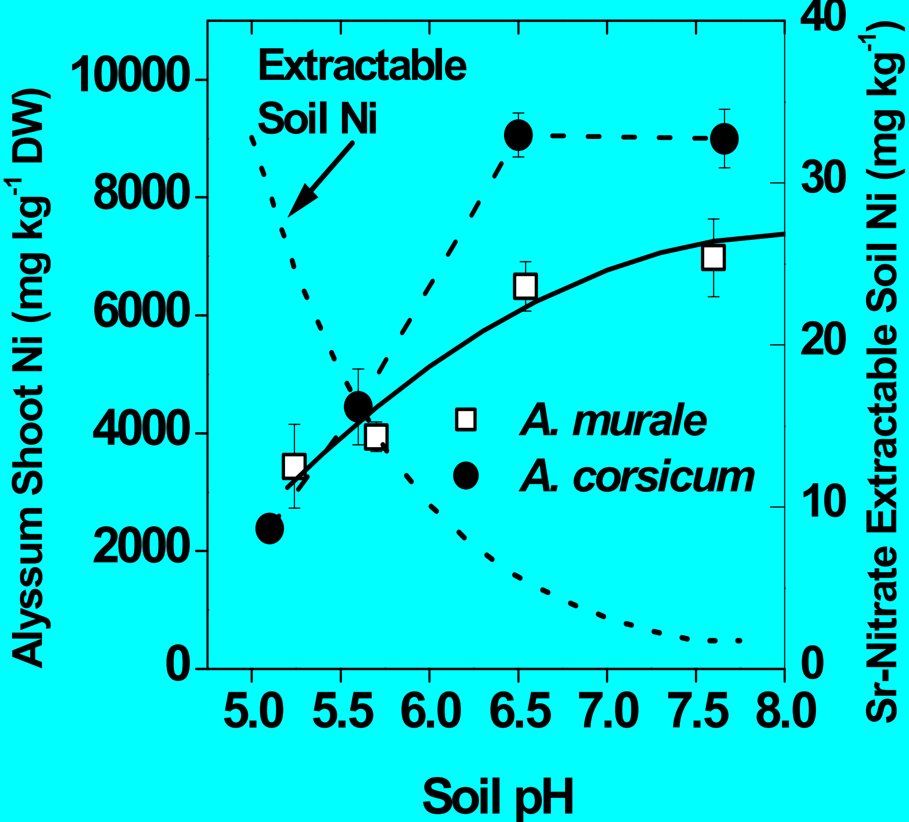


Figure 1. The effect of soil pH on Sr-nitrate extractable soil Ni and Ni levels in *Alyssum* shoots.

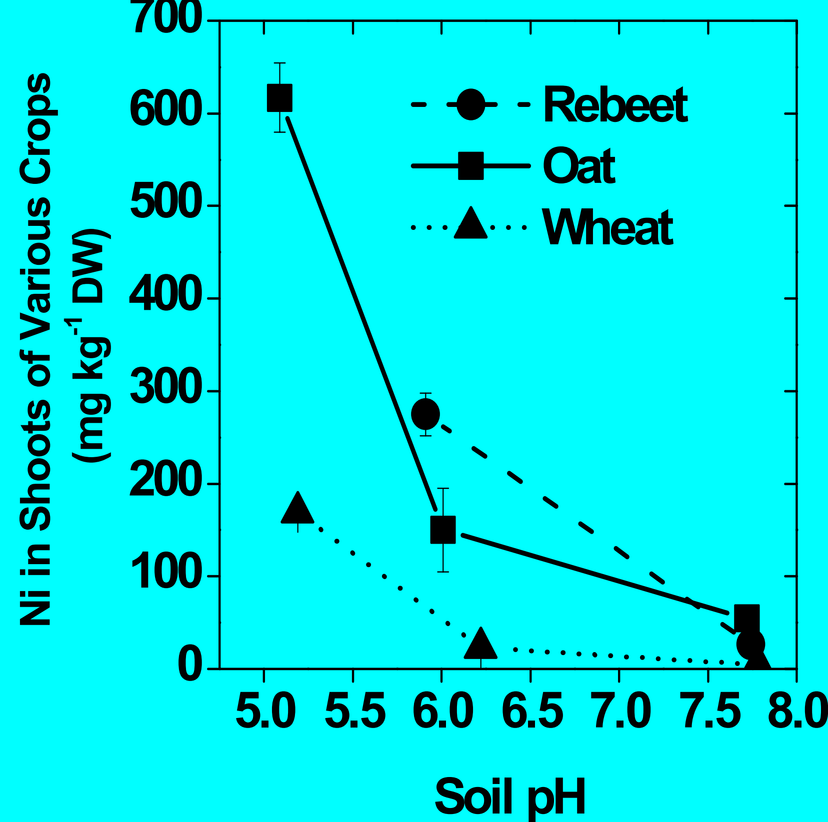
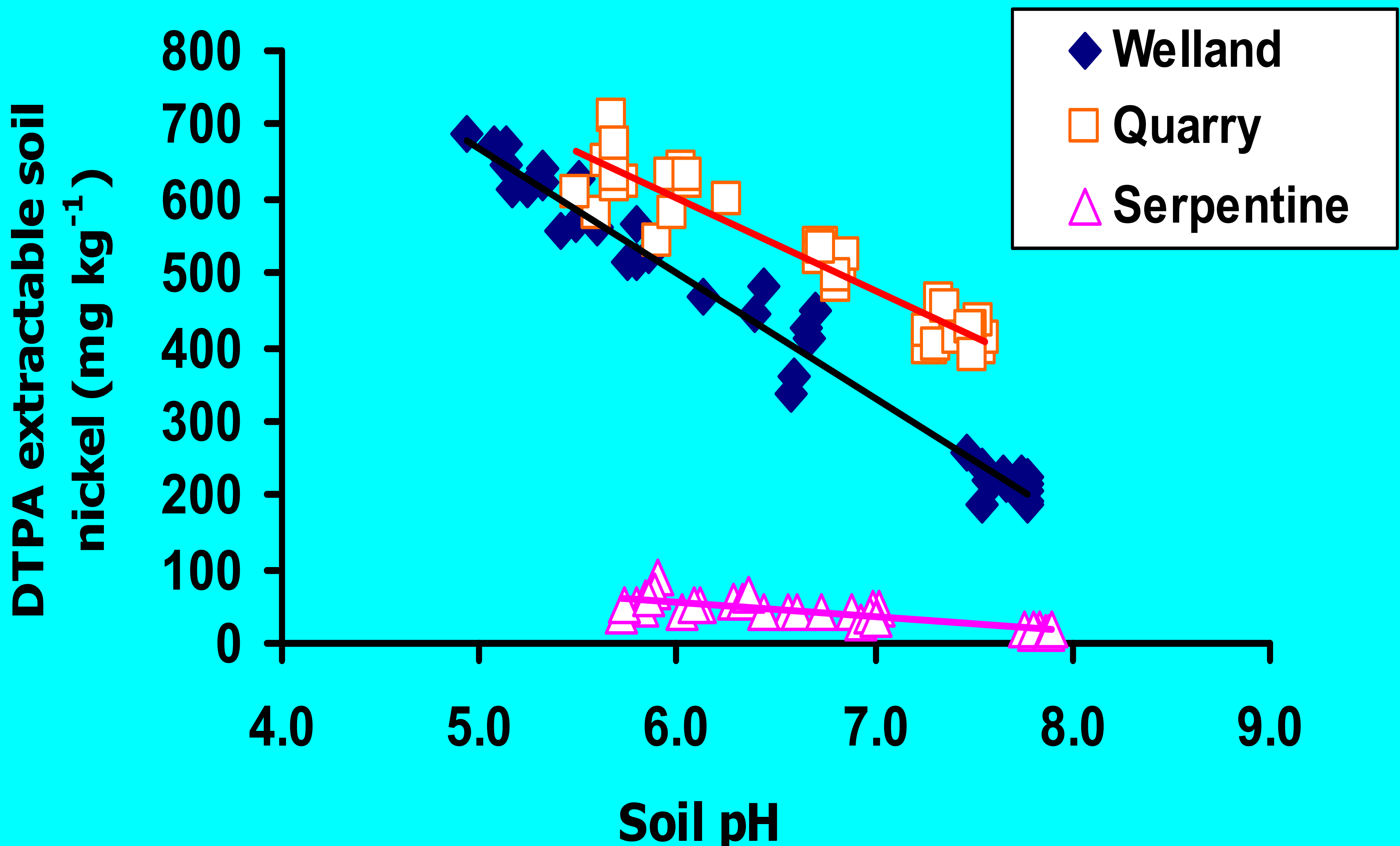
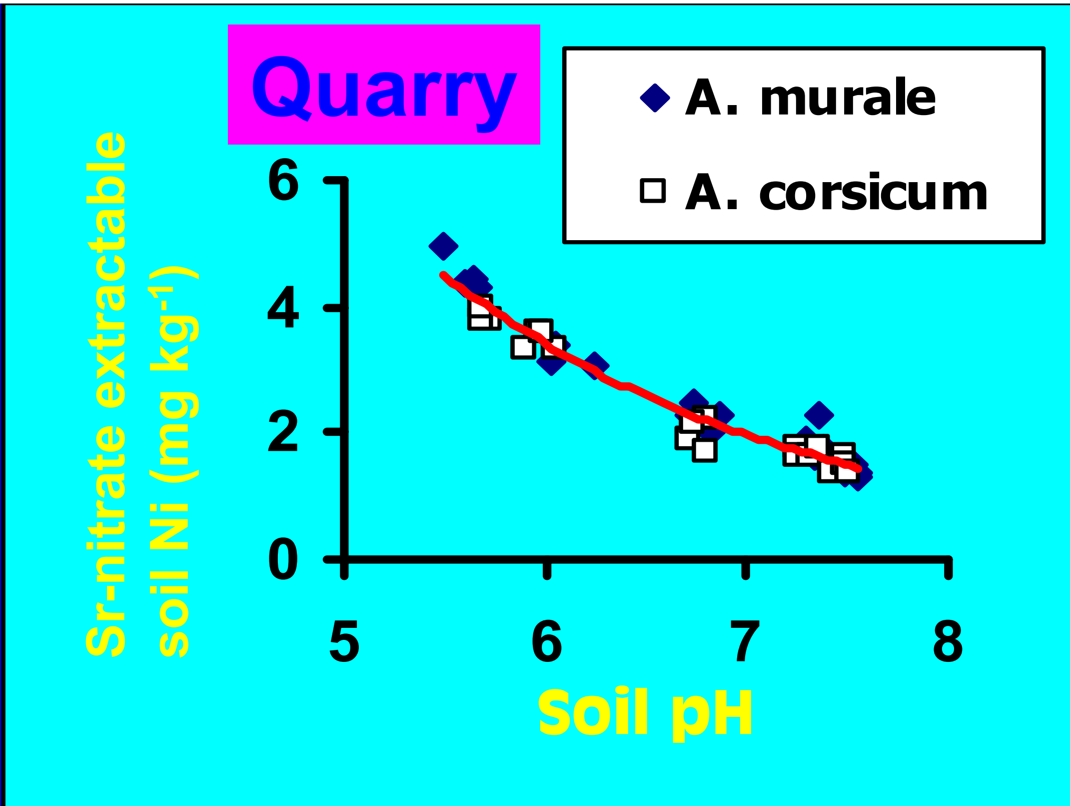
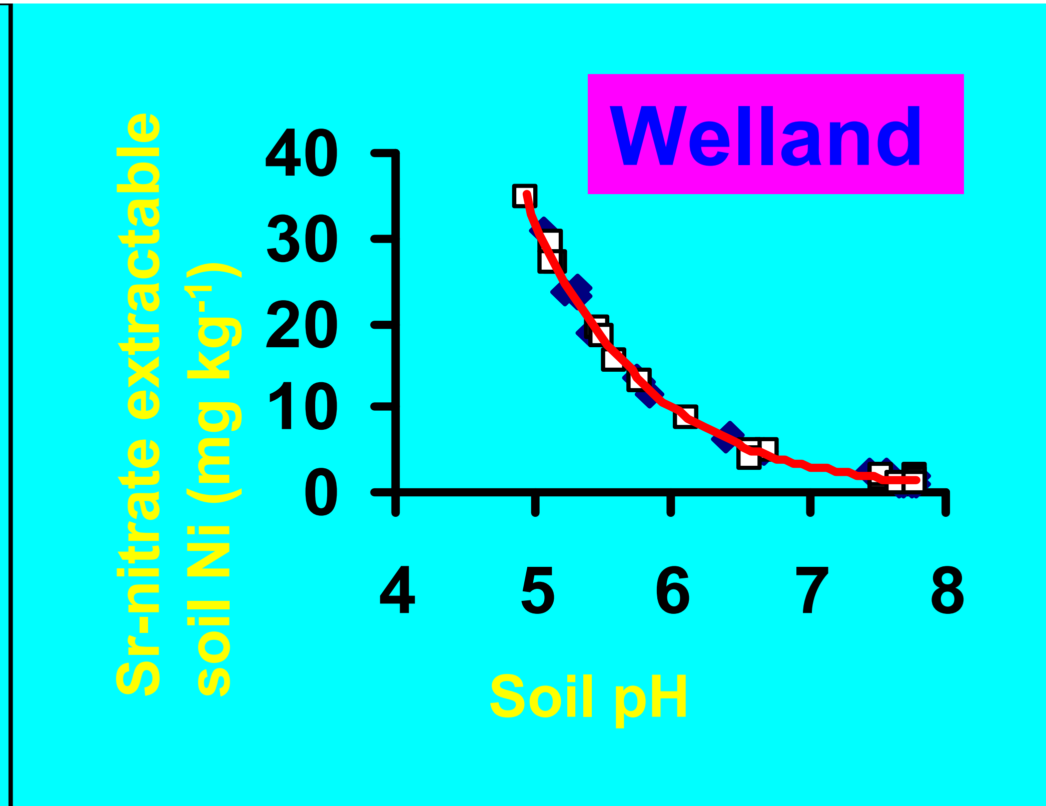


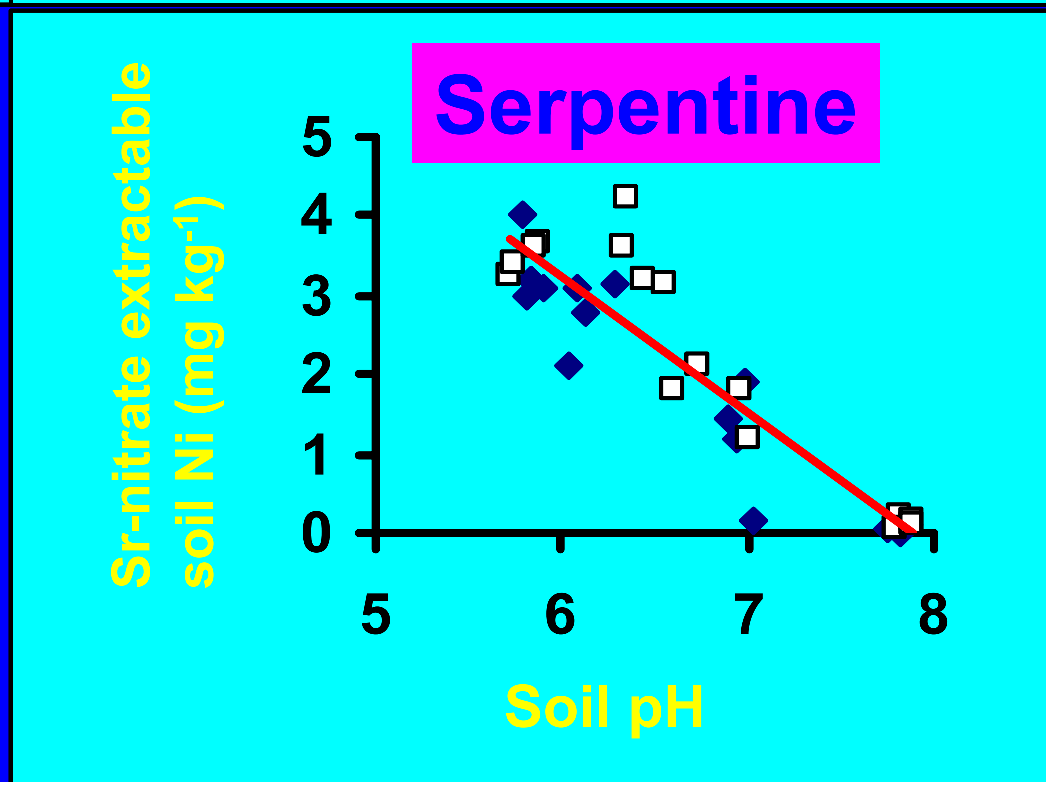
Figure 2. The effect of soil pH on the Ni levels in shoots of agricultural crops.

Increasing soil pH decreased DTPA-extractable Ni.









Increasing soil pH decreased Sr-nitrate extractable soil Ni.

Alyssum shoot yield and Ni uptake - Brockman soil.

Treatment	*A. murale*			*A. corsicum*		
	Yield	Ni uptake		Yield	Ni uptake	
	g/pot	mg/pot	% ‡	g/pot	mg/pot	%
Acidified	12.1 a†	147. ab	0.70	12.2 b	132. b	0.63
"As is"	18.4 b	162. a	0.77	22.9 a	177. a	0.84
Limed	19.5 b	126. b	0.60	21.4 a	173. a	0.82
Calc.	16.1 b	76. c	0.36	14.4 b	78. c	0.37

Alyssum shoot yield and Ni uptake - Welland soil.

Treatment	*A. murale*			*A. corsicum*		
	Yield	Ni uptake		Yield	Ni uptake	
pH range 5.1 - 7.7	g/pot	mg/pot	% ‡	g/pot	mg/pot	%
"As is"	22.0 a†	76 a	1.17	22.4 a	53 a	0.82
Limed - 1	20.3 a	80 a	1.24	23.5 a	102 a	1.58
Limed - 2	21.0 a	135 b	2.09	20.5 a	188 b	2.91
Calc.	24.3 a	168 b	2.61	24.6 a	222 b	3.43
Calc.+HFO	20.1 a	151 b	2.33	23.5 a	180 b	2.79

† Means within column followed by the same letter are not significantly different according to Duncan's multiple range test ($P<0.05$).

‡ Ni uptake expressed as % of the total Ni present in the soil in the pot.

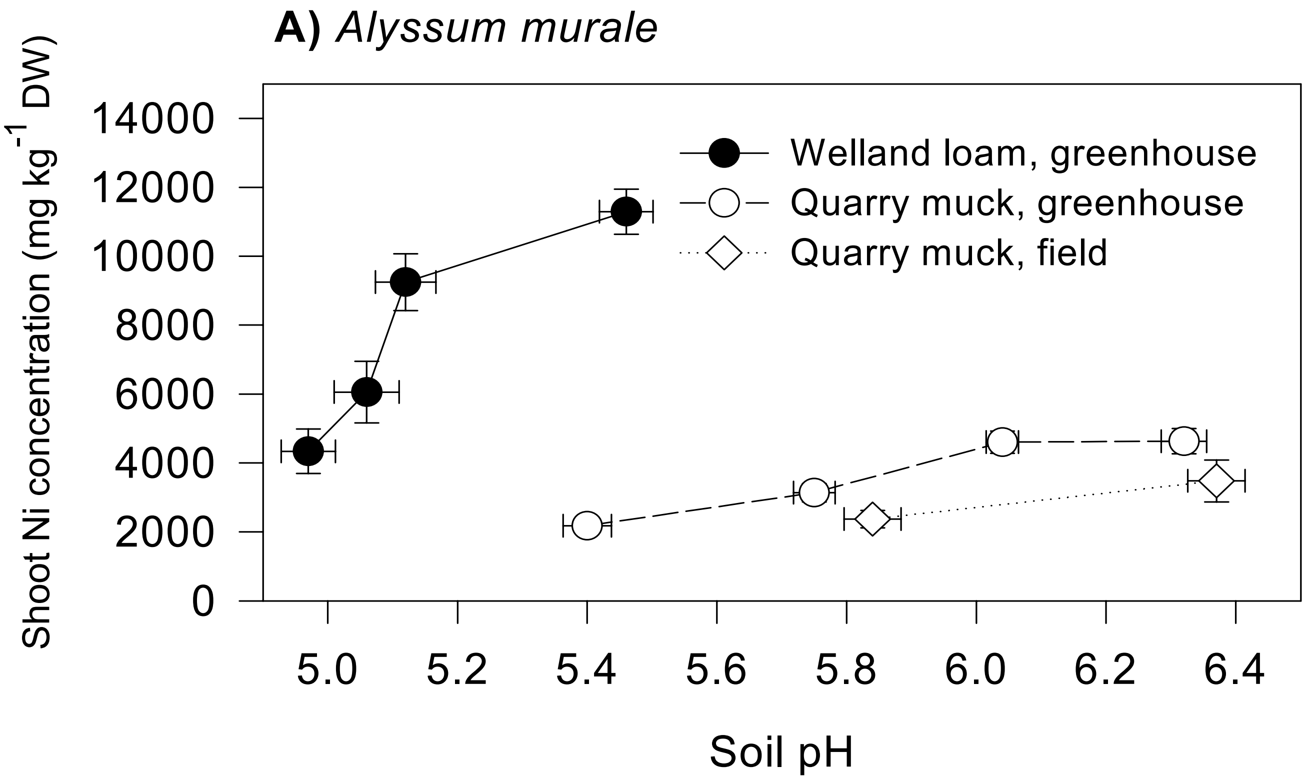
Alyssum shoot yield and Ni uptake - Quarry soil.

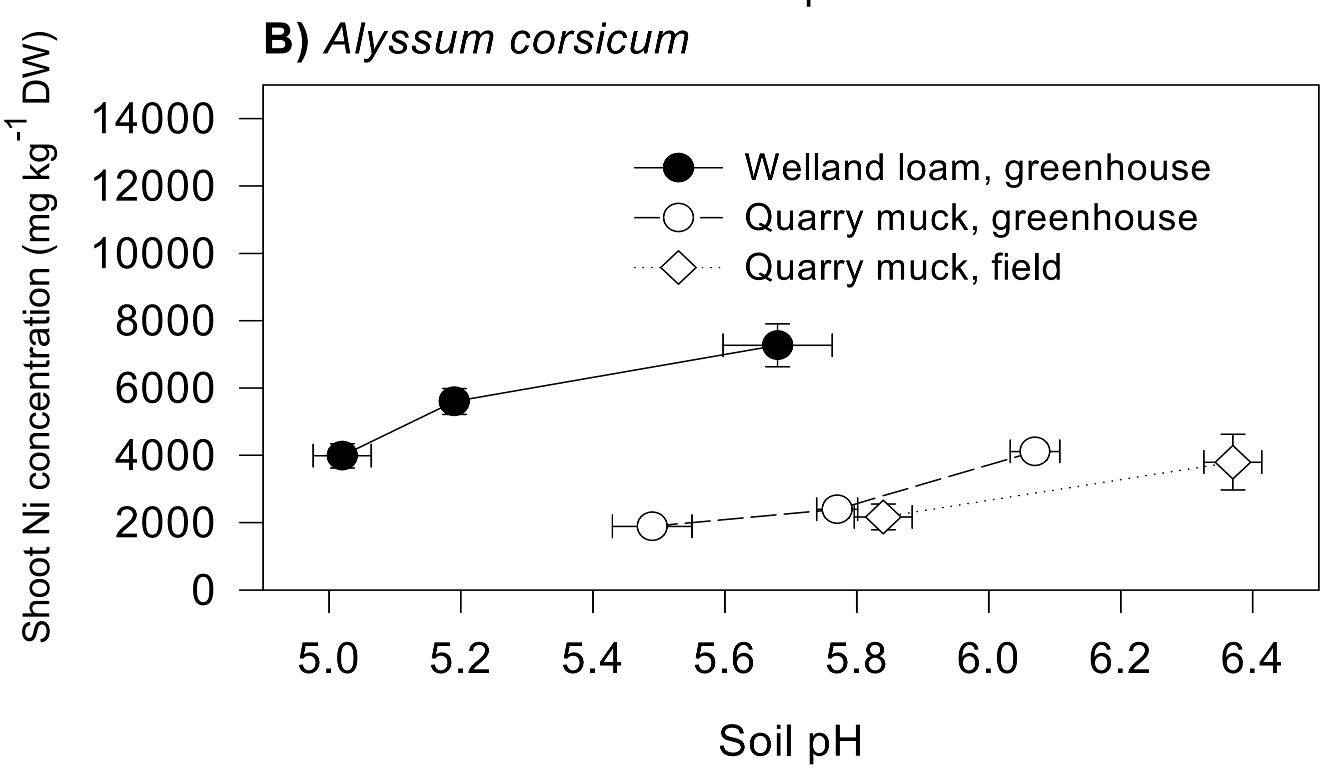
Treatment	*A. murale*			*A. corsicum*		
	Yield	Ni uptake		Yield	Ni uptake	
pH range 5.6 - 7.5	g/pot	mg/pot	%‡	g/pot	mg/pot	%
"As is"	21.3 a	35 a	1.29	23.9 a	56 c	2.10
Limed - 1	20.3 a	41 a	1.52	25.0 a	64 c	2.41
Limed - 2	21.7 a	107 b	4.03	23.1 a	122 b	4.57
Calc.	22.6 a	125 b	4.69	25.3 a	169 a	6.33
Calc.+HFO	20.7 a	126 b	4.72	26.2 a	146 ab	5.49

The effect of soil pH on trace metal levels in the whole shoots of *A. corsicum* grown in Welland soil.

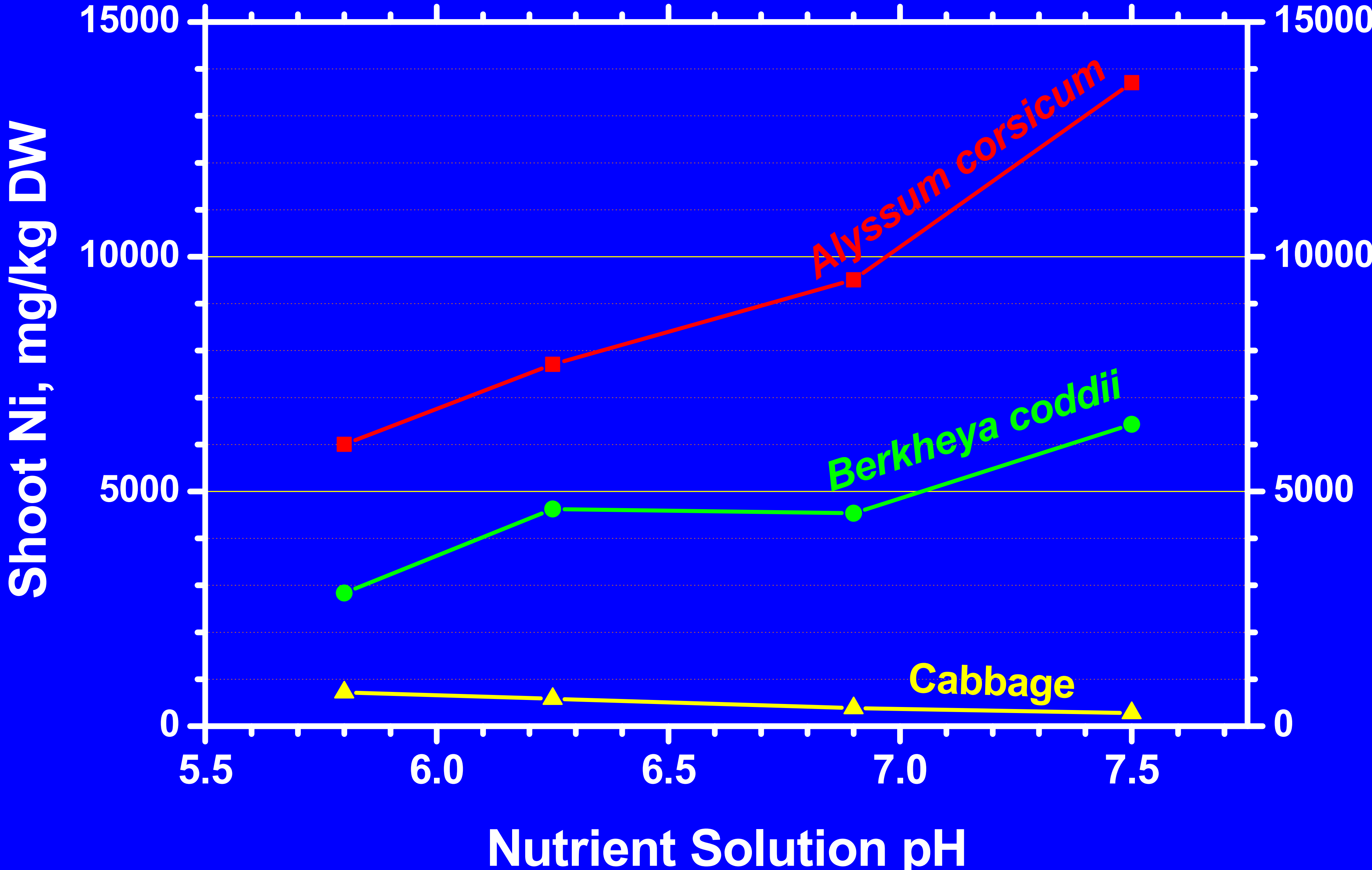
pH	Co	Mn	Fe	Zn	Cu
	----------------------------	mg kg^{-1}	----------------------------		
5.1	49.0 a †	130. a	46.5 a	238. a	24.1 a
5.6	25.4 b	78.4 b	69.0 a	283. a	25.1 a
6.5	12.2 c	27.4 c	54.0 a	165. b	21.1 a
7.7	12.2 c	83.6 b	53.3 a	84. c	24.1 a

† Means within column followed by the same letter are not significantly different (Duncan's multiple range test ($P<0.05$)).





Effect of soil pH on shoot Ni levels in Shoots of two *Alyssum* species

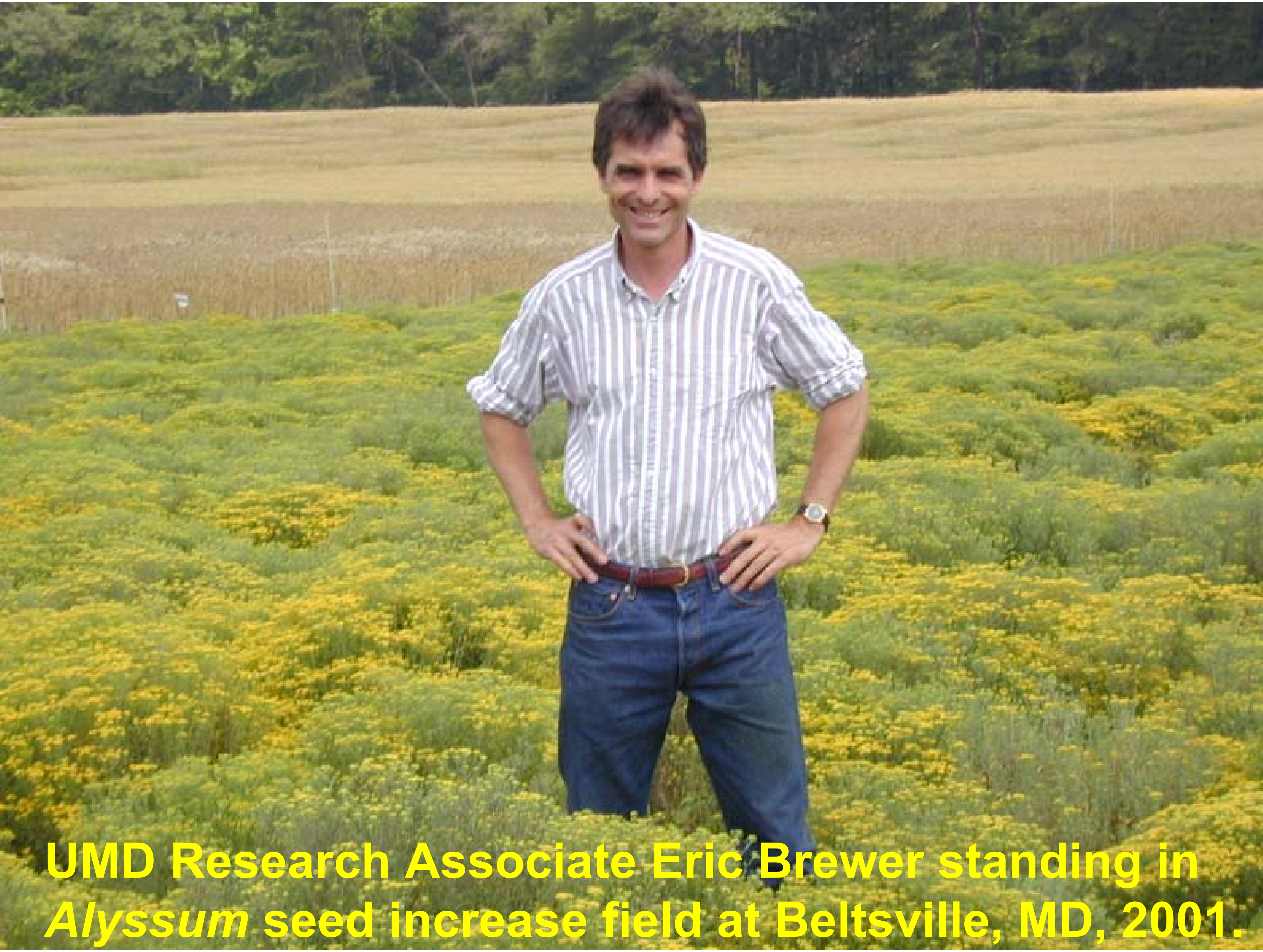


Effect of Nutrient Solution pH on Ni accumulation in Shoots of three species after 30 days (Peters et al.).

How does one go about commercial production?

- Labor costs and irrigation water availability control selection of planting method.
  - Direct seeding (shallow)
  - Transplant
- Access to land for phytomining:
  - Contract with company needing remediation-Inco
  - Lease land from land-owners.
  - Contract with farmers to grow biomass as crop.
  - Purchase land where available
  - Make mining claims – cost effective method.
- Viridian has been very creative

Mechanically transplanting *Alyssum* seedlings



UMD Research Associate Eric Brewer standing in *Alyssum* seed increase field at Beltsville, MD, 2001.

Viridian Chief Geneticist, Yin-Ming Li, in *Alyssum murale* seed Increase field at Beltsville, MD, 2001

Muck site, Port Colborne, Ontario, Oct. 30, 1999. *A. murale* grew well with some plants as large as 2 ft in diameter. *A. corsicum* (on right) was smaller and had a poorer stand (Li et al., 2000).

On April 3, 2000, the *Alyssum* at the Muck site was just starting its spring growth. *A. murale* survived the winter well at this site, but many *A. corsicum* plants (on left in foreground) died.

On May 22, 2000, *A. murale* had grown well on much of the Muck site, with many plants about 2 feet tall.

Muck site, May 22, 2000. *A. murale* 'AJ 9.'

Muck site, May 22, 2000. Some areas of the Muck site were poorly drained. The plants here were smaller, and as wet weather persisted into the early summer, plants in these areas began to die.

Muck site, June 21, 2000. This 2.5 ft. tall *A. murale* 'AJ 9' plant has started to wilt because of wet soil conditions.

Muck site, June 24, 2000. In a poorly drained part of the plot, these *A. murale* plants died after flowering.

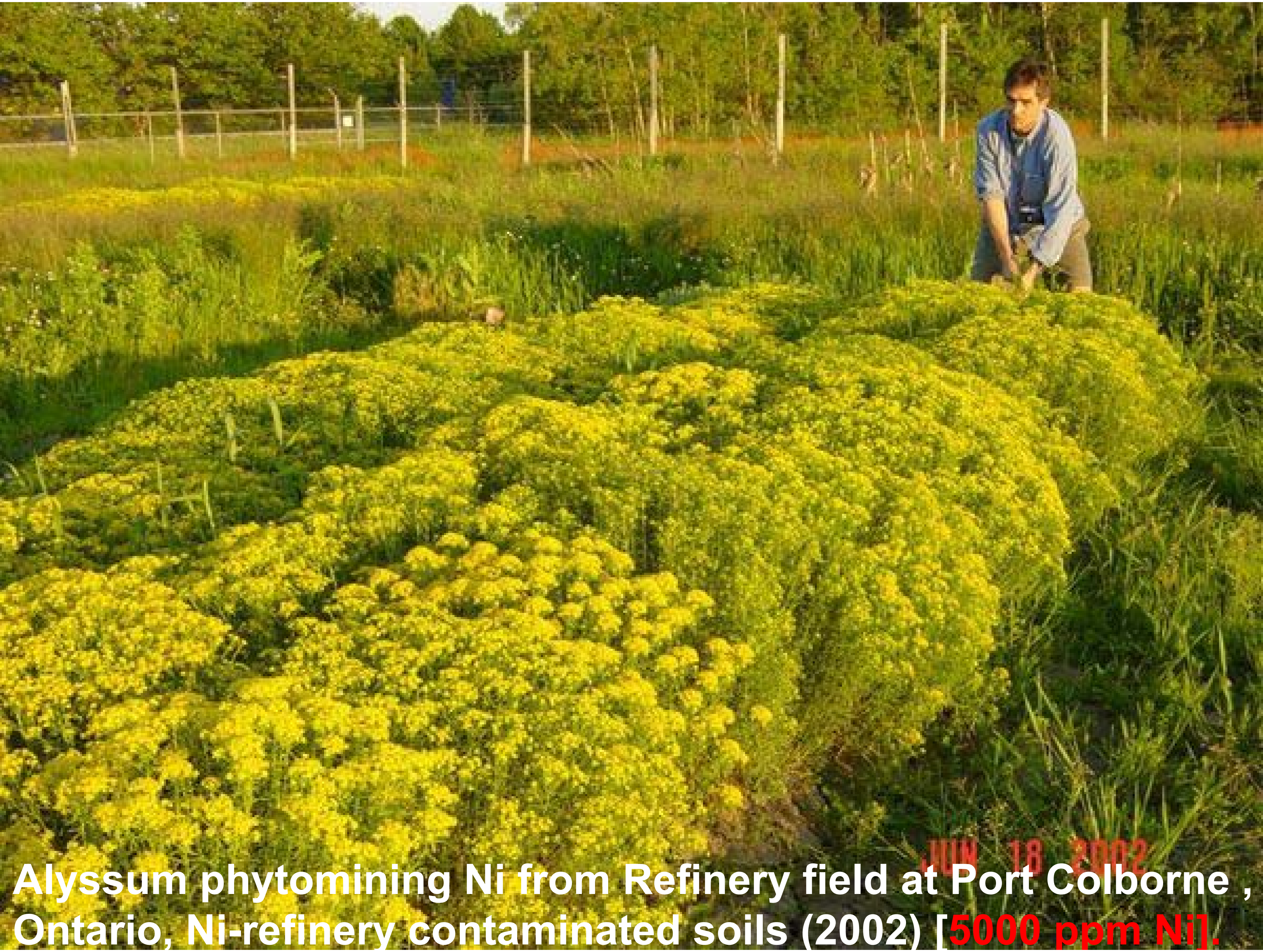
Agronomy of Alyssum Phytomining

- **Must adapt Alyssum agronomic management to the nature of contaminated soil and environment.**
- **Port Colborne has poorly drained soils which are very wet in most springs.**
- **Alyssum is adapted to Mediterranean climate and dry soils.**
- **In 2001, too wet soils for too long caused death of some of the fields of *Alyssum* at Port Colborne.**
- **We decided to test "ridge" tillage of the plow layer highly contaminated soils; plow layer ridged and *Alyssum* seeded on top of ridges using herbicides and fertilizers previously identified for *Alyssum* production. Ridge production worked very well!**

Alyssum phytomining Ni from Hruska field at Port Colborne , Ontario, Ni-refinery contaminated soils (2002) [3000 ppm Ni].

Alyssum phytomining Ni from Hruska field at Port Colborne , Ontario, Ni-refinery contaminated soils (2002) [3000 ppm Ni].

Alyssum phytomining Ni from muck field at Port Colborne , Ontario, Ni-refinery contaminated soils (2002) [3000 ppm Ni].



Alyssum phytomining Ni from Refinery field at Port Colborne , Ontario, Ni-refinery contaminated soils (2002) [5000 ppm Ni].

Viridian is Commercializing Ni Phytomining in 2002-2003

- Increased seed of improved genotypes in 2001-2002 to allow commercial plantings.
- Solicited land owners/farmers to contract for growing *Alyssum* biomass in SW Oregon in 2002-2003; payment based on Ni delivered in biomass.
- Planted over 700 Acres; growing now.
- Serpentine derived or colluvial soils rich in Ni; land had been farmed in past; low slopes.
- Also working with Inco Ltd. When decision made regarding remediation of soils at Port Colborne and other locations which Inco controls land.

Dr. Scott Angle (Univ. Maryland) Holding ash from burning High-Ni *Alyssum murale* grown on field plots on OR serpentine soils.

Demonstration Addition Of Viridian *Alyssum* Ash To a C.C. Smelter Bulk Converter

Processing of 500 kg Ni-rich *Alyssum* Phytomining Biomass Ash, July 9, 2002

Cooperation with Inco Ltd

Sudbury, Ontario, Canada


04m 50s 00f - 06m 55s 00f
500 kg revert bag
of Viridian ash

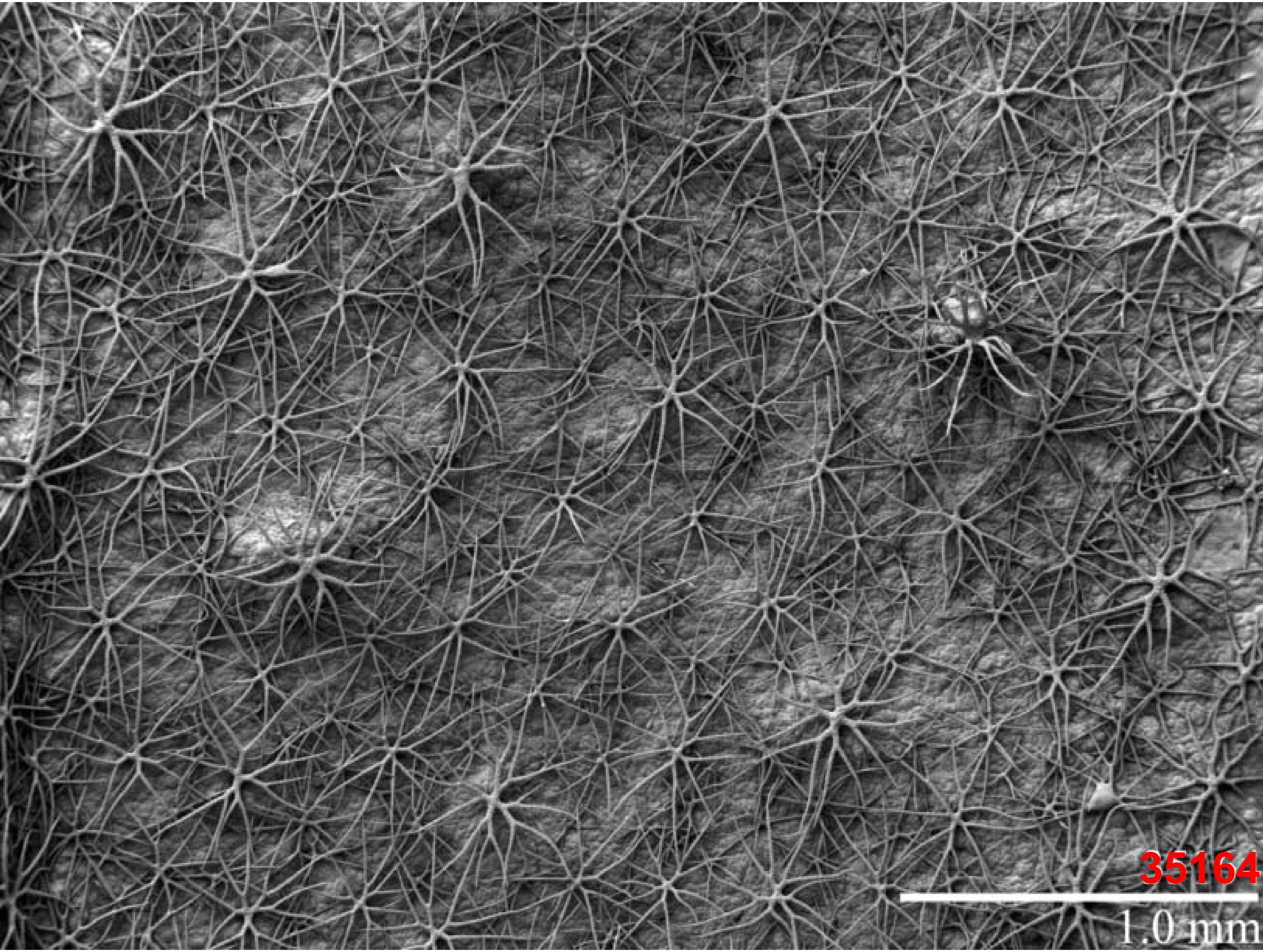

07m 47s 00f - 08m 24s 00f
Viridian
Nickel Ash


18m 51s 00f - 20m 10s 00f

Localization of Ni within leaves of *Alyssum murale*

- Grew plants with varied Ni added to Pro-Mix.
  - Higest Ni supply caused phytotoxicity-chlorosis.
- Examined fully developed young leaves using SEM and EDX; analyzed similar leaves by ICP.
- Treatments were: Leaves (mg Ni/kg)

Treatments	Leaves (mg Ni/kg)
0 mmol/kg = 0 ppm	5.
5 mmol/kg = 289 ppm	3,540.
10 mmol/kg = 578 ppm	10,200.
20 mmol/kg = 1156 ppm	15,000.
40 mmol/kg = 2312 ppm	17,200
80 mmol/kg = 4614 ppm	32,300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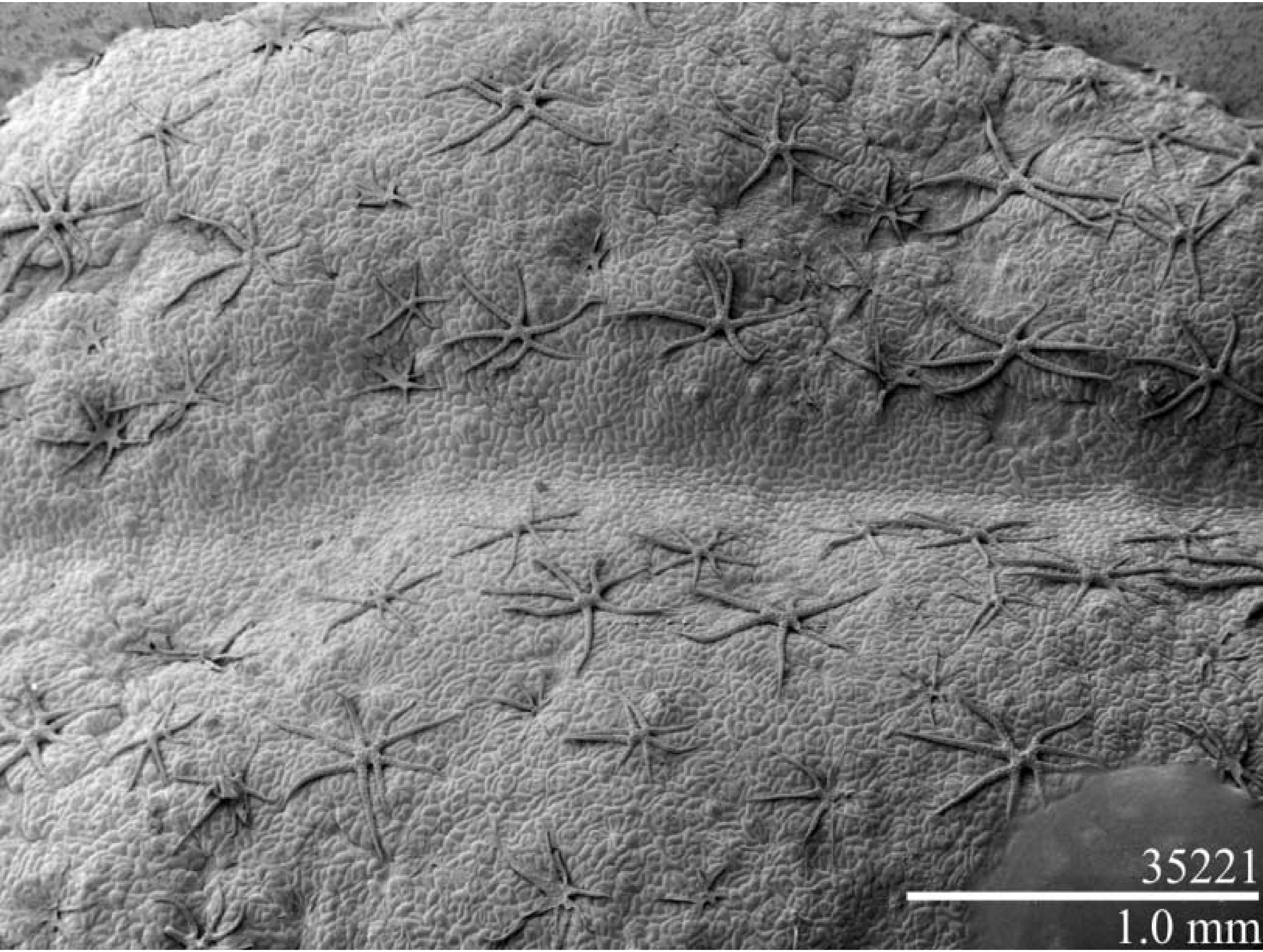
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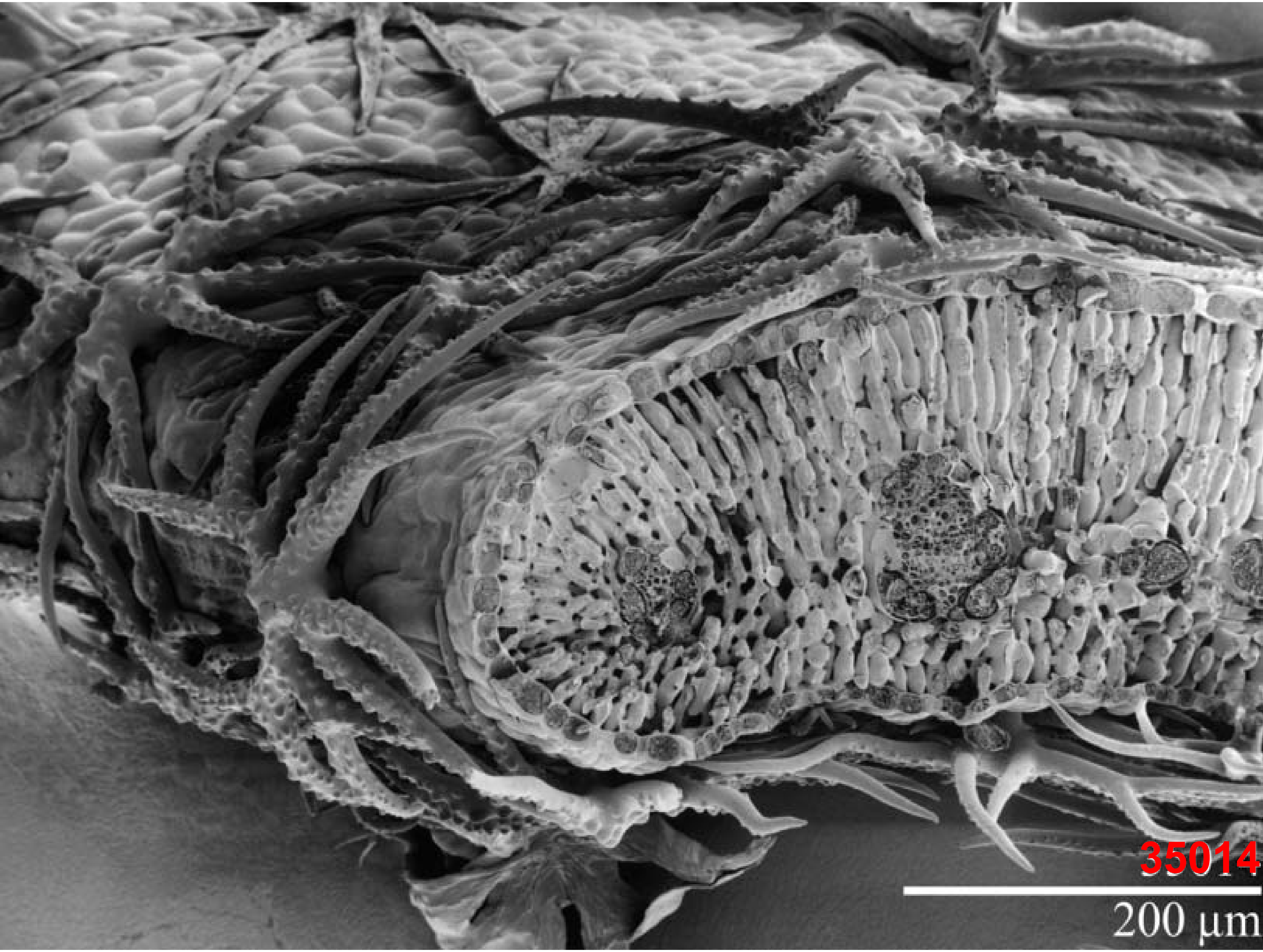
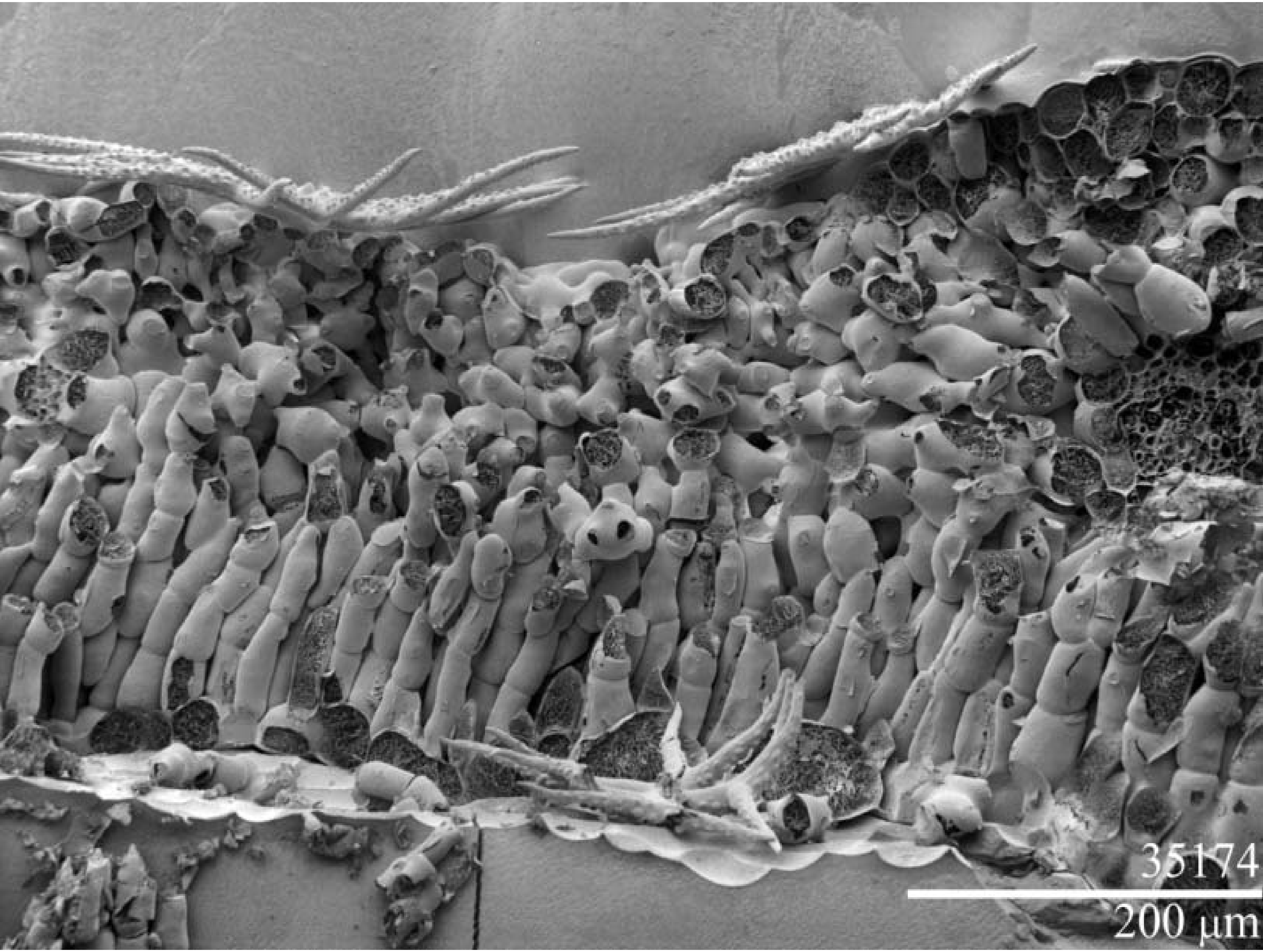

35014
200 µm


35174
200 µm

VIRIDIAN

RESOURCES L.L.C.

Phytoextraction Associates, LLC

Formed in 2003 to license our inventions for Cd+Zn phytoextraction. Angle, Baker, Reeves have equity, but I am not allowed to be owner or USDA won't let me do any of the research. CRADA formed between Phyextraction Associates LLC, University of Maryland, and USDA-ARS to allow licensing of existing patents and all new work.