

Phytomining Ni in the USA

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Beltsville, MD, USA**

**ARPA-E Video Conference on
Ni Phytomining in the USA**

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Field of flowering *Odontorrhena chalcidica* and *O. corsica* (seed increase field) near Cave Junction, Oregon, USA, on June 13, 2003. Soil was derived from serpentine parent materials and is rich in Ni. Mountain in background shows typical native poor forest cover.

Phytomining is Farming!

- **Select/Test useful phytomining plant species.**
- **Identify US locations with Ni rich arable soils.**
- **Identify needed fertilizers and soil conditioners.**
- **Breed improved cultivars (yield of shoot Ni).**
- **Establish harvest and biomass processing system to recover biomass Ni for market.**
 - **Incinerate and “smelt” ash to obtain Ni.**
 - **Release Ni from biomass—pressure; chemistry**
 - **Even with burning biomass, much CO₂ release is smaller than traditional Ni ores mining and processing.**

Before Phytomining Concept

- **Researchers studied:**
 - **Botanical prospecting (hyperaccumulators):**
 - Se, Ni, Zn, Cu etc.
 - Se hyperaccumulators found U deposits – cold war.
 - **Biogeography**
 - **Geobotany**
 - **Metal tolerant plants**
 - Revegetation of mine and smelter sites.
 - What was the biochemistry for metal tolerance?
 - **Accumulator and Hyperaccumulator species**

Combining Information to Form Idea of Phytomining.

- Could use for soil decontamination.
- Hyperaccumulators might be ‘farmed’ to remove problem metals from soils; = **agronomy!**
- Angle, Baker, Li and I cooperated on Cd phyto.
- I started Ni phytoextraction studies with *Dicoma*.
- Alan Baker and I met at 1979 Conference and discussed Cd phytoextraction using *Noccaea*
- I published the phytoextraction idea in 1980 in a report to the US-EPA; then in 1983 book chapter.
- At the Serpentine Ecology Conference in Davis, CA, in 1991, I discussed phytomining Ni and the idea spread rapidly.

We began Ni Phytomining Research to Obtain Funding for R&D in CRADA

- My research group included a plant breeder.
- Chaney, Angle and Li recognized that the only element for which there were lots of hyperaccumulators, and much soil rich in that metal (in US), with high metal \$ value, was nickel.
 - We obtained seeds from Bulgaria.
 - We grew *O. chalcidica* on soils at the Serpentine area near Reisterstown, MD (1996-1997).
 - Looked at pH effect, and tested reproduction system.
 - We conducted research/breeding to justify **Ni patent**.
 - With Baker, spent next 2 years seeking \$.
 - In 1997, finally signed CRADA to start R&D in Oregon.

Phyto/Agro-Mining Concept

- **Geochemically enriched soils=untapped resource**
- **Hypernickelophore (>1%) species exist in nature.**
- **An industry exists to recycle metals from biomass.**
- **Need to domesticate a “new crop”.**
 - **Farm hypernickelophore (>1%) crop to remove Ni from soil; make “hay” using traditional farm practices.**
 - **Need to develop all soil and plant management practices needed to produce high Ni yields of phytomining crop.**
 - **Improved cultivars, fertilizers, weed control, harvest.**
 - **Incinerate or pyrolyze to recover biomass energy and ash; energy could pay crop production costs.**
 - **Recover metals from ash; MARKET.**
 - **Current studies on obtaining Ni from wet biomass?**

120°W

110°W

100°W

90°W

80°W

70°W

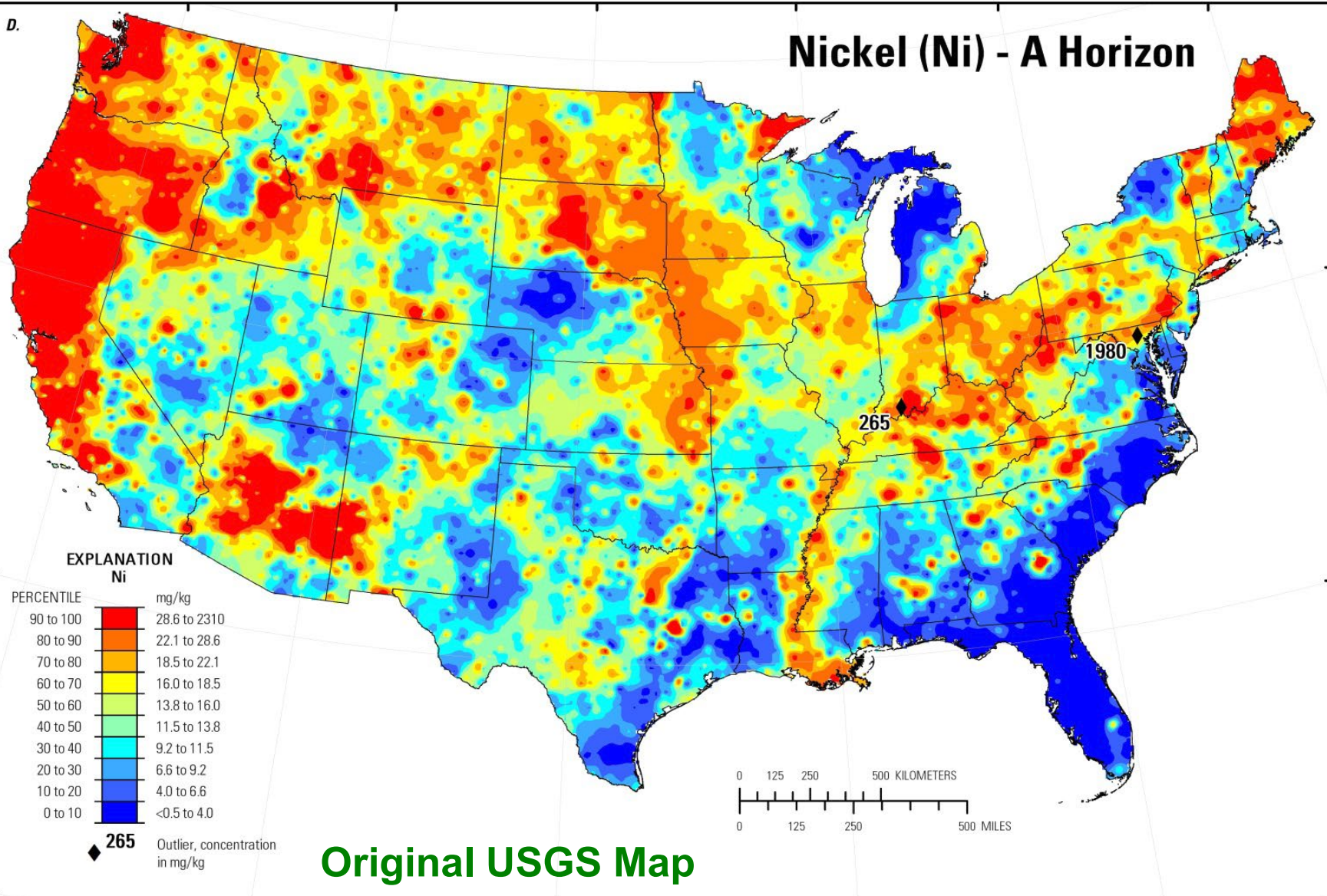
D.

Nickel (Ni) - A Horizon

40°N

30°N

20°N



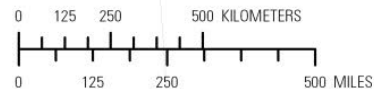
EXPLANATION

Ni

PERCENTILE

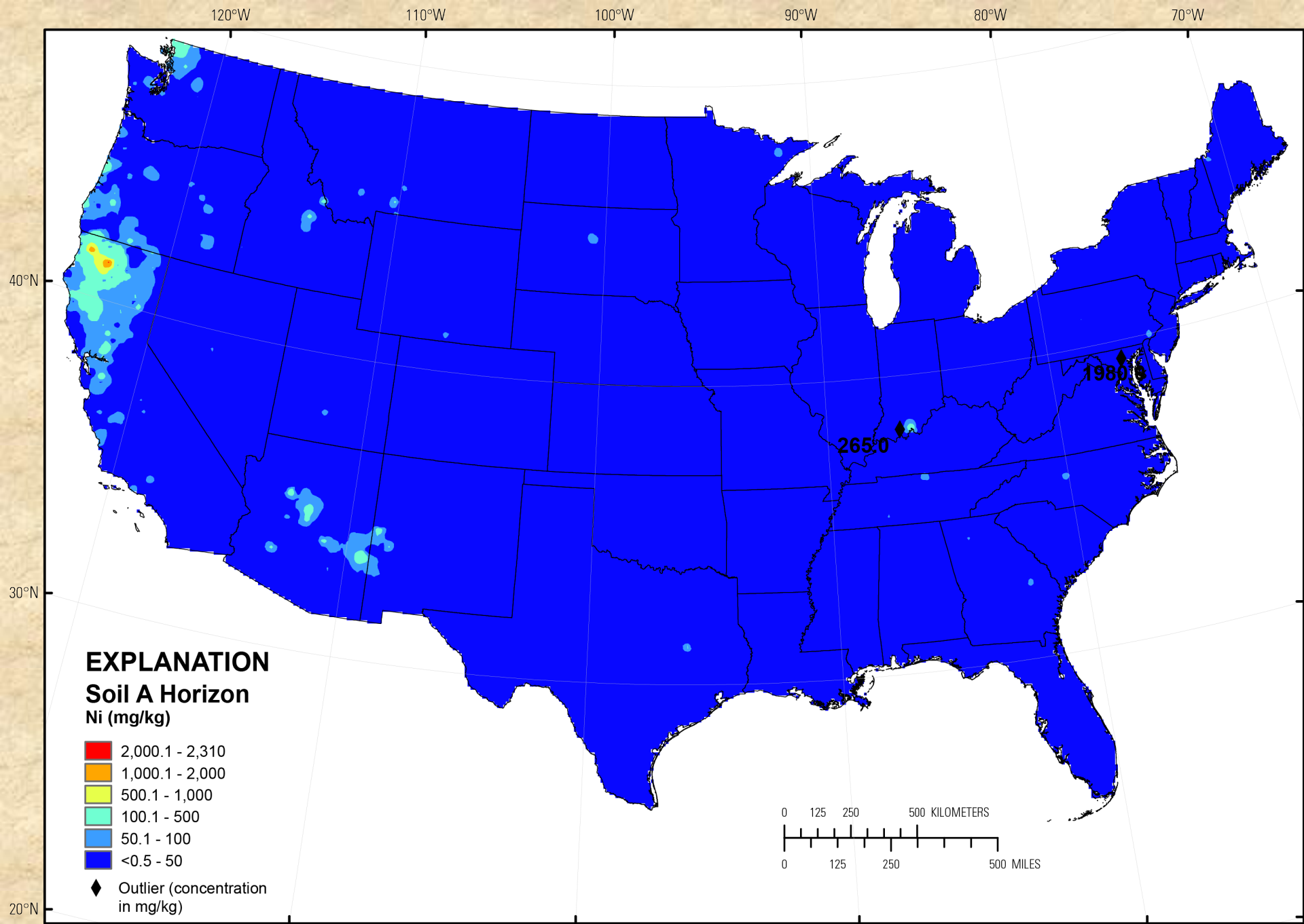
PERCENTILE	Color	mg/kg
90 to 100	Red	28.6 to 231.0
80 to 90	Orange	22.1 to 28.6
70 to 80	Yellow-Orange	18.5 to 22.1
60 to 70	Yellow	16.0 to 18.5
50 to 60	Light Green	13.8 to 16.0
40 to 50	Green	11.5 to 13.8
30 to 40	Cyan	9.2 to 11.5
20 to 30	Blue-Cyan	6.6 to 9.2
10 to 20	Blue	4.0 to 6.6
0 to 10	Dark Blue	<0.5 to 4.0

◆ 265 Outlier, concentration in mg/kg

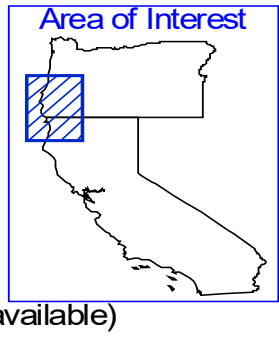


Original USGS Map

Base map from U.S. Geological Survey digital data
 Lambert Conformal Conic projection
 Standard parallels 33°N and 45°N
 Central Meridian 96°W
 Datum NAD 1983



Oregon Ultramafic Geology & Soils



Coos Co.
(digital soil survey not available)

Douglas Co.

0 10 Miles

	OR um geology
	CA um geology
Oregon	
	Curry um soils
	Douglas um soils
	Josephine um soils
California	
	Klamath um soils
	6 Rivers um soils

Curry Co.

Josephine Co.

Jackson Co.

Del Norte Co.

Siskiyou Co.

**-Great ultramafic soil/
geology relationship**

Extent of **Potentially Arable Ultramafic Soils** in Josephine Co.

Unit Acres Soil Series Name Slope (20%; arable)

4	2173	Banning l	<3
11B	2018	Brockman cl	2-7
11C	1914	Brockman cl	7-12
12B	2459	Brockman cob cl	2-7
12D	3694	Brockman cob cl	7-20
13	1728	Brockman variant vgrl	<5
18A	700	Copsey c	3-7
18B	666	Copsey c	3-7
19D	1122	Cornutt-Dubakella	7-20
		Cornutt cob. cl	
		Dubakella v. cob. cl	
31D	2747	Eightlar ext. st. c	5-20
38A	3682	Foehlin gr l	0-3
38C	1226	Foehlin gr l	3-12
52	4645	Kerby l -	
73	8011	Takilma cob. l <	3
74	1647	Takilma var. ext. cob. l	<3

Sum 38432 All < 20% slope ultramafic-derived soil series

Bioengineer a new crop?

- The main alternative to domestication of a wild hypernickelophore species as a phytomining crop plant is to bioengineer a hyperaccumulating cultivar of a high yielding crop plant.
- Have to find **all functional and regulatory genes** involved and transfer them to the crop plant.
- Many are working under the assumption that their work will lead to a GMO hyperaccumulator.
- One team bio-engineered *Thlaspi arvense* to be a winter cover crop rather than a poisonous weed.
 - Increased seed size; stopped shattering; etc.
 - Arabidopsis sequence helped find genes needing revision.
- A sterile hybrid system could prevent dispersal.
- Each nation could require different crop species.

Ni Hyperaccumulator Crops: A New Commercial Farming Opportunity

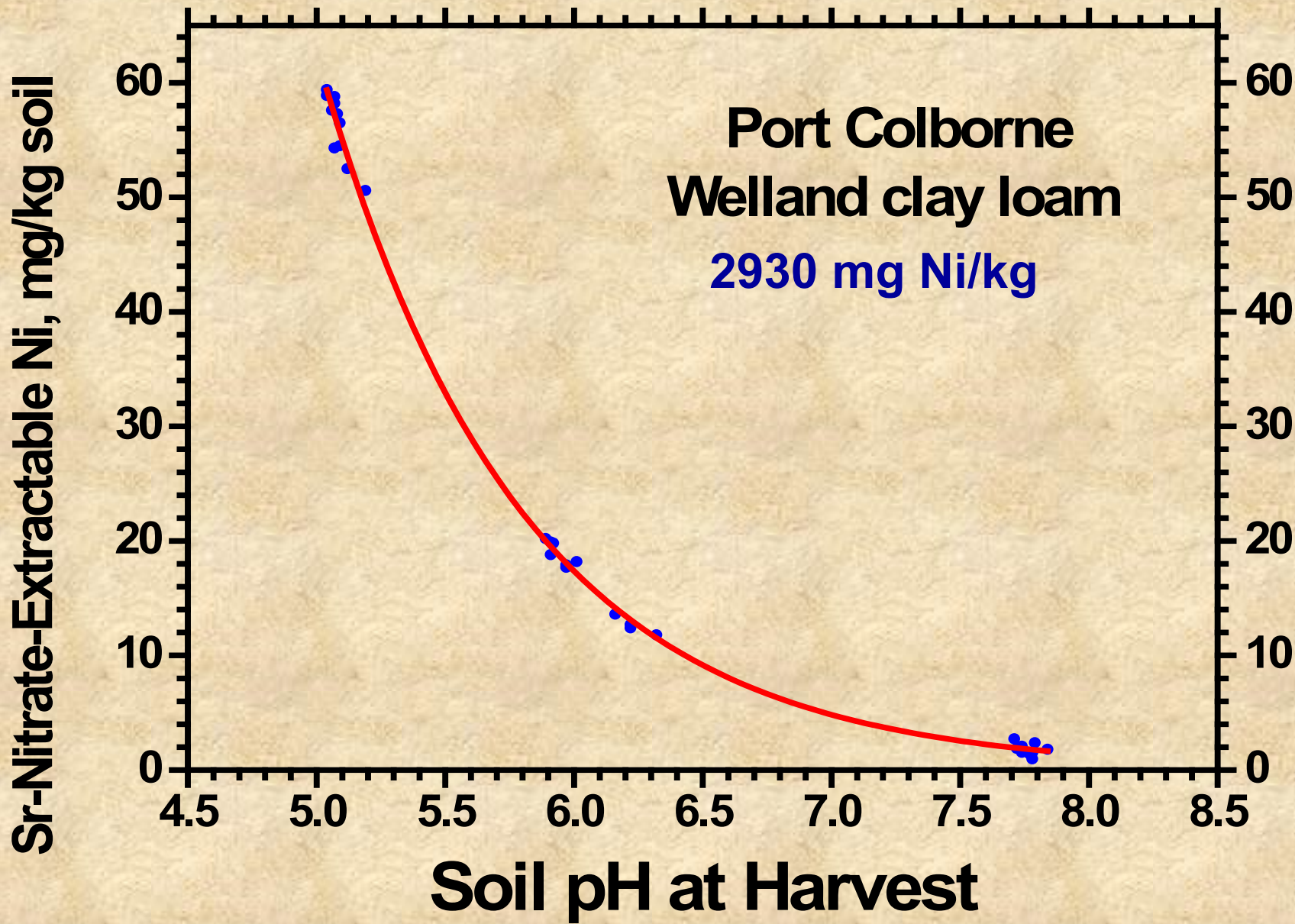
- Ni is a valuable metal
- We have domesticated a large wild plant species that “hyperaccumulates” over 2% Ni in shoots at harvest.
- The US currently has one Ni mine and needs to import or recycle nearly all of this strategic metal.
- The US has Ni mineralized natural soils in several states where farmers could grow the Ni hyperaccumulator plants as a commercial crop.
- **Ni ore** would be produced along with biomass energy.
 - Ash is higher than 20% Ni.
 - Wet processing is potentially available

Commercialization of Ni Phytomining

- The patents and technology our team developed were licensed to Viridian LLC, the firm which supported our R&D under a CRADA (1998-2002).
- Viridian employed Dr. Li and Mr. Brewer who continued cultivar development and field demonstration at varied Inco locations in Canada, the UK, and Sulawesi, Indonesia
- Viridian was not able to commercialize the technology despite clear demonstration of the value that our team developed. **Patents expired in 2015-2018.**
- **Alyssum declared noxious weed in Oregon.**

Phytoremediation of Soil Ni

- **No real Ni contaminated soils in the US reported to need remediation.**
- **Worked with INCO at Port Colborne, Ontario, to characterize Ni phytotoxicity of their contaminated soils and methods for practical remediation of the high Ni soils.**
- **Liming and other agronomical techniques achieved effective diverse revegetation on even high Ni.**



The effect of soil pH on 0.01 M $\text{Sr}(\text{NO}_3)_2$ -extractable soil Ni.

Ni Accumulation in Crop Plants is unable to give annual removal rate needed for cost effective technology.

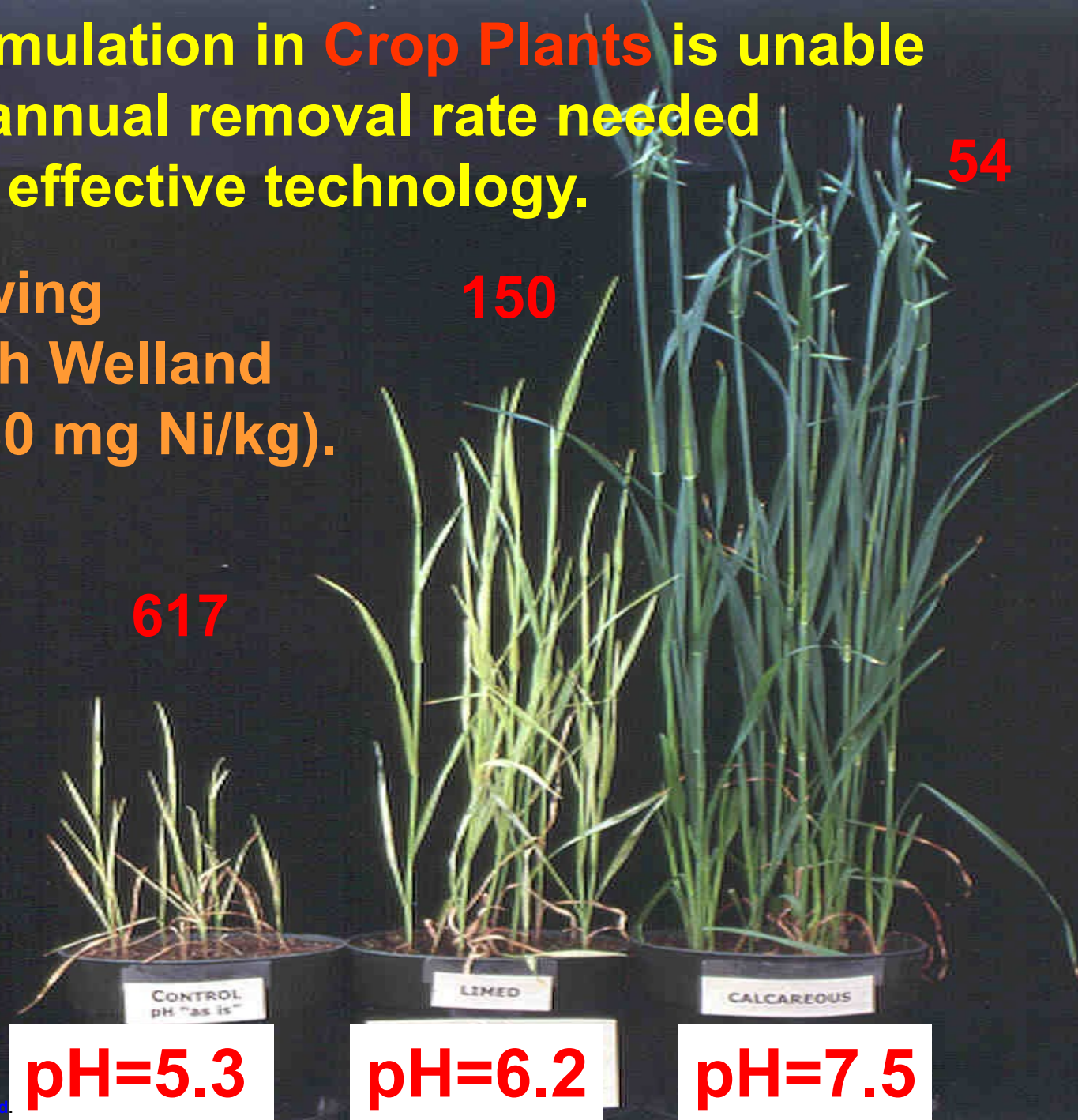
54

Oat growing on Ni rich Welland Soil (2930 mg Ni/kg).

150

Shoot Ni, ppm

617



pH=5.3

pH=6.2

pH=7.5

Crops vary in tolerance of soil soluble Ni.

Highly Ni susceptible
Tomato.



Highly Ni resistant Corn/Maize.



Crops Can't Achieve Ni Phytomining

Hypernickelophores are ESSENTIAL!

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

Species	Yield t/ha	Ni in Shoot Biomass		Ash-Ni	
		<u>mg/kg</u>	<u>kg Ni/ha</u>	<u>% of Soil</u>	<u>%</u>
Maize (Control)	20	1	0.02	0.01	0.002
Maize (50% YD)	10	100	1	0.01	0.2
<i>O.c. in pasture</i>	2	10,000	20	0.20	10-15
Wild <i>O.c. crop</i>	5	15,000	75	0.75	20-30
<i>O.c. + Agronomy</i> ¹	10	20,000	200	2.00	20-30
<i>O.c. cultivar</i> ^{1,2}	15	25,000	375	3.75	25-35

¹Agronomic practices: NPK S Ca B Mo fertilizers; herbicides; planted.

²Cultivar bred to attain higher biomass with higher Ni concentration.

³Estimate: Crop removes 200 kg Ni per harvest; means 200/10,000 kg/ha or 2% removed/yr, or 50 yr resource **ignoring deeper subsoil access by roots.**



Drying *Odontarrhena* biomass to produce ash for smelter test

Pogradec, Albania 2000 -- Shallari/Volk

This meeting is being recorded.



Burning *Odontarrhena* Biomass for Smelter Test:
Pogradec, Albania 2000 - Shallari/Volk - USDA Grant

This meeting is being recorded.

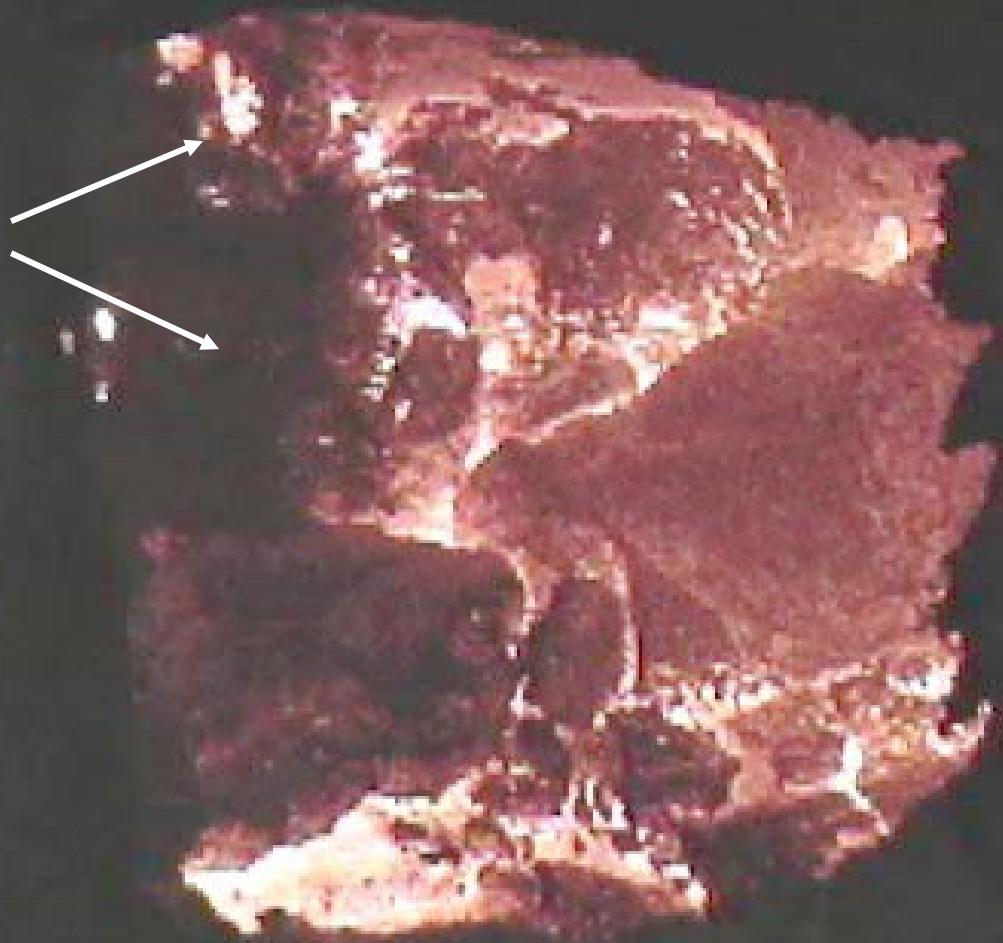
04m 50s 00f - 06m 55s 00f

500 kg **Alyssum**
ash in revert bag

Odontarrhena ash produced in Albania by Shalleri
Viridian-Inco test in Sudbury, Ontario, Canada.

07m 47s 00f - 08m 24s 00f

Viridian
Odontarrhena
Ash



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



Steps to Develop Ni Phytomining-1

- **Select Ni hyperaccumulator species with phenotype needed for phytoextraction.**
 - **Height; Yield; Perennial; High accumulation.**
- **Collect seeds of Ni/Co Hyperaccumulator genotypes from different sites where they occur naturally in order to obtain wide genetic diversity to aid breeding improved cultivars.**
- ***O. chalcidica* and *O corsica* which occur across Southern Europe were selected for development. Seed collected when mature but before dispersed in field.**
 - Alan Baker, Roger Reeves, Alex Koulikov, Seit Shallari, et al. did seed collection in EU.**

Alternative US Native Ni Hyperaccumulators

- Few US native Ni hyperaccumulators
 - *Noccaea* v. *montanum* v. *siskiyouense*
 - *Streptanthus polygaloides*
- They are **small, short**.
- Most Ni rich biomass very near soil.
 - Very difficult to harvest mechanically.
- They are not excellent in Ni hyperaccumulation.
- None were “promising” for US.



Thlaspi montanum v. *montanum* v. *siskiyouense* adjacent to
“vineyard” near O’Brien, OR. Aug. 11 1998 (Reeves)

This meeting is being recorded.



***Thlaspi montanum* v. *montanum* in Teanaway River Valley, WA. Aug. 2, 2002. (Reeves)**



***Streptanthus
polyagiloides***
**In California
(Reeves).**



***S. polygaloides* near China Camp, CA (Nicks and Chambers)**

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Blepharidium guatemalense in southern Mexico and Guatemala.

Likely strong climate limitations in OR and CA.



9/9GA-J5

Collecting seed from *Odontarrhena chalcidica* in southern Bulgaria, 1996.

Steps to Develop Ni Phytomining-2

- **Evaluate germplasm in field and controlled environment in Ni rich soils**
 - Test uptake of Ni and biomass yield.
 - Oregon serpentine soils.
 - Port Colborne Ni-Refinery contaminated soils.
 - Use pH and fertilizers based on greenhouse and growth chamber tests.
- **Identify methods to breed phytomining crop:**
 - Characterize inheritance of metal uptake, translocation, metal tolerance, yield, growth habit.
 - *Odontarrhena chalcidica* is Self-Incompatible; = recurrent selection required in breeding!





PHYTOMINING SOIL NICKEL USING *Odontarrhena chalcidica/corsica*
Field test site used to evaluate genotypic differences in Ni
hyperaccumulation by new crop for serpentine soils in SW Oregon.
Average test genotype contained 1.2% Ni in shoot biomass.

Acknowledgements: Scientists Who Played Major Roles in our Team's Development of a Valid Ni Phytomining Technology

Original PI's:

Rufus Chaney (ARS)

Scott Angle (UMD)

Alan Baker (ECUS)

Yin-Ming Li (ARS)

OR State Univ. Co-PI:

Richard Roseberg

ECUS Support (NZ):

Roger Reeves

UMD and Viridian LLC:

Eric Brewer

ARS-Beltsville:

Carrie Green (Lab)

Oregon Viridian:

Mark Wiest

Graduate Students:

Cari Peters

Keri Zdimal

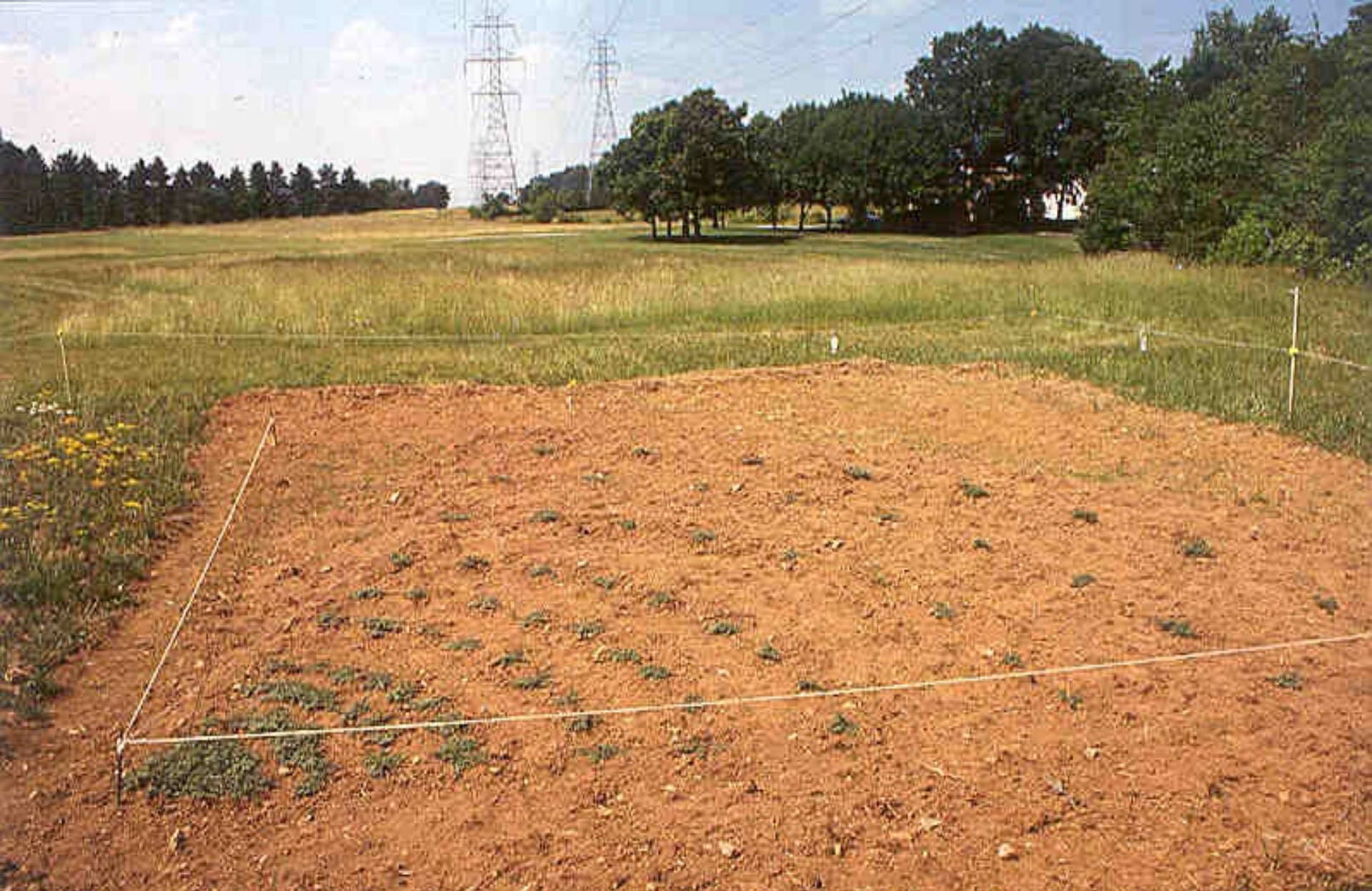
Poland visitors:

Urszula Kukier

Grzegorz Siebielec

Steps to Develop Ni Phytomining-3

- **Identify production practices to maximize profit:**
 - Fertilizers, pH, seed depth, herbicide management.
 - Annual vs. perennial culture; planting density; irrigation; planting and harvesting schedule.
- **Use plant breeding methods to combine high Ni accumulation and high harvestable yield.**
 - Breed and patent improved phytomining cultivars.
 - *Odontarrhena chalcidica* is self-incompatible.
 - Mediterranean climate species for US serpentines.
- **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. This meeting is being recorded.

Steps to Develop Ni 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 or thermal processing.
- **Develop farming systems for phytomining.**
 - Identify soils where phytomining would be profitable.
 - Contract with farmers?
 - Lease mineralized soils?
 - Pay for Ni in biomass delivered by growers?



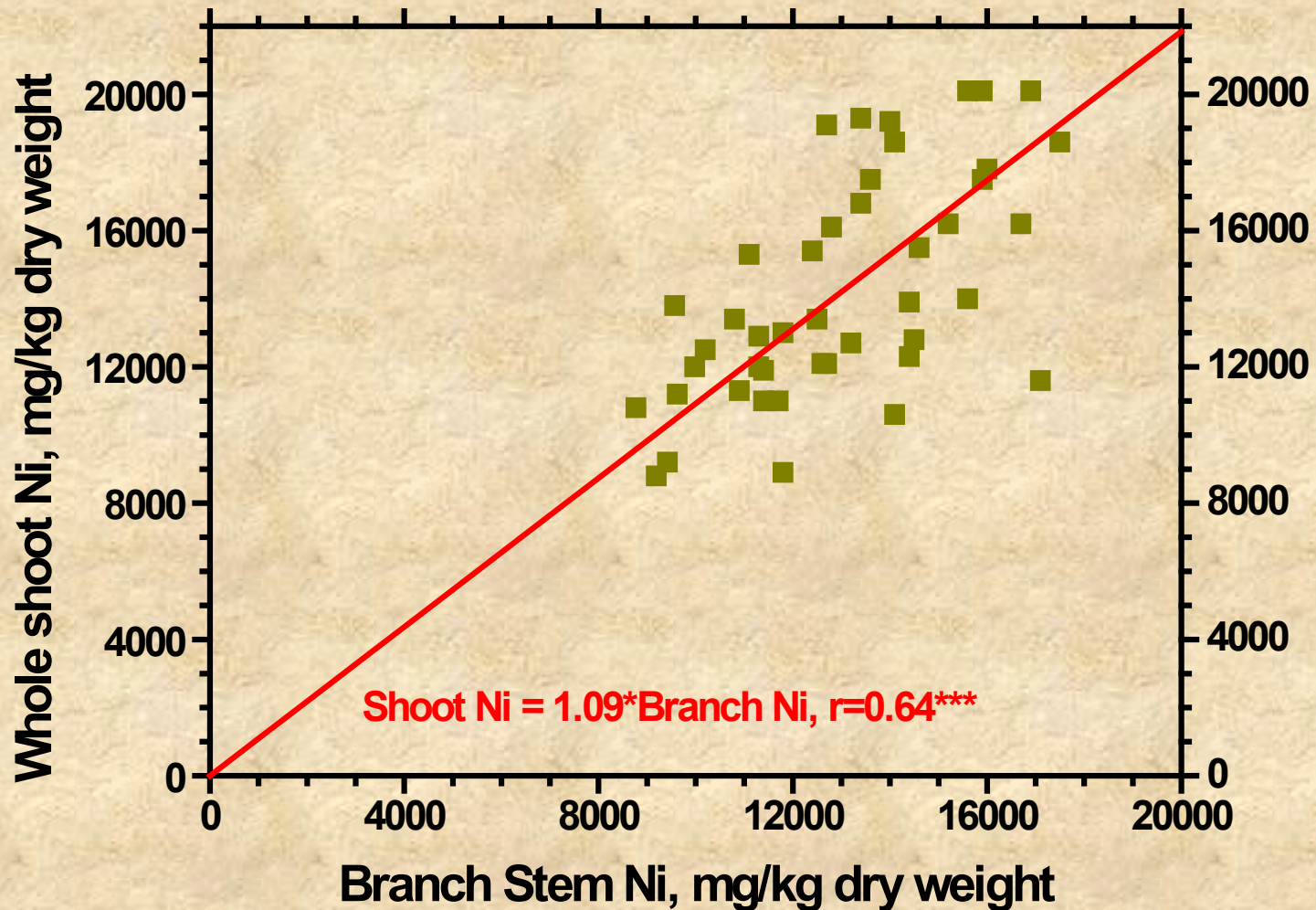
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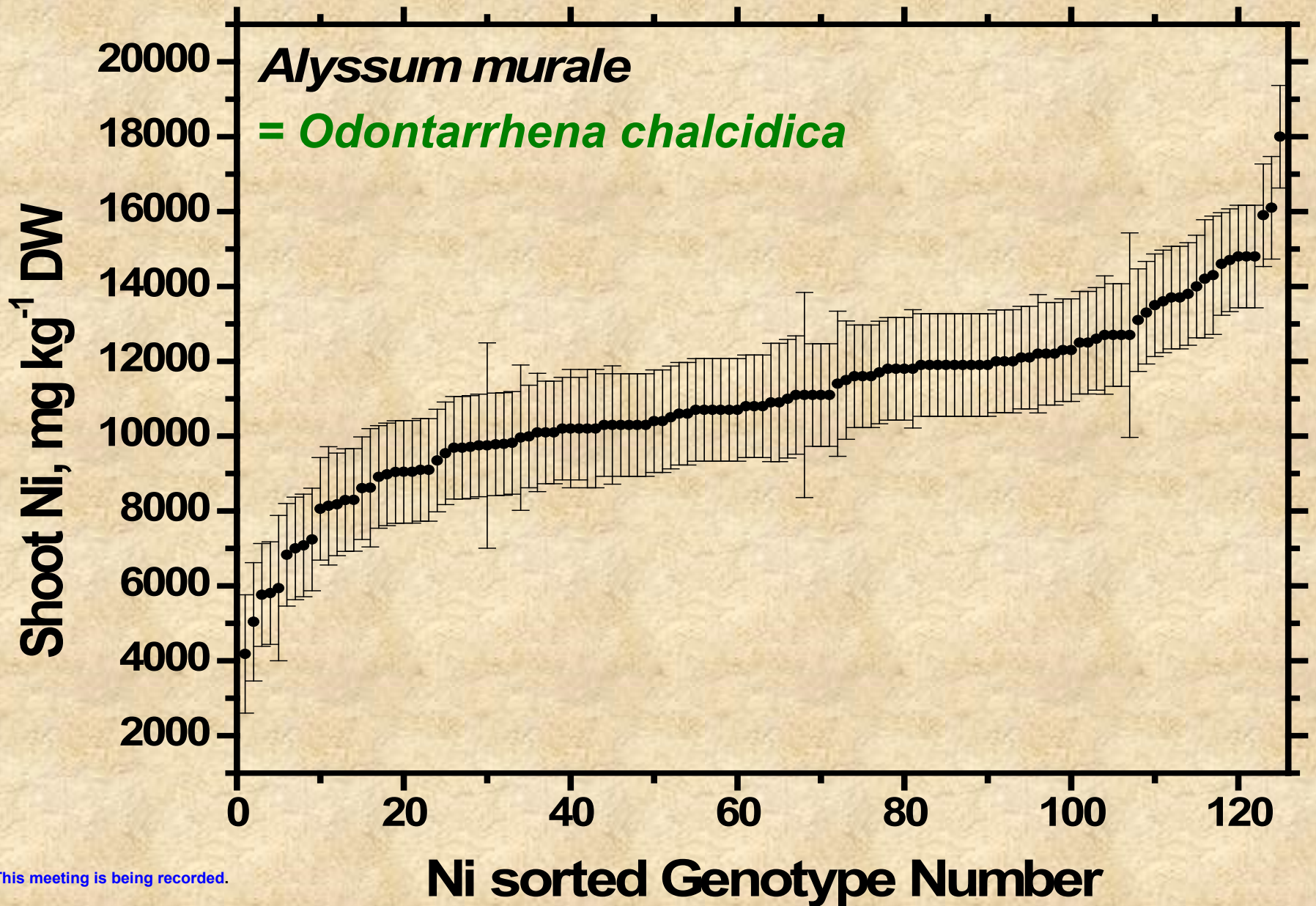
Mowing *Alyssum murale* using farm equipment





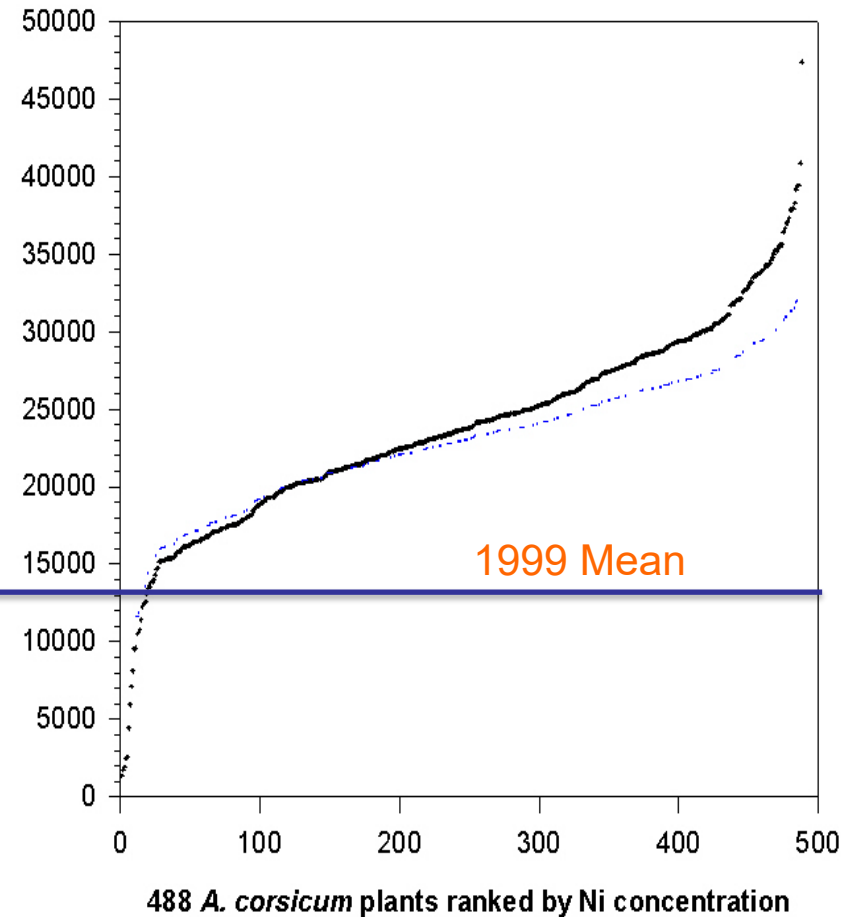
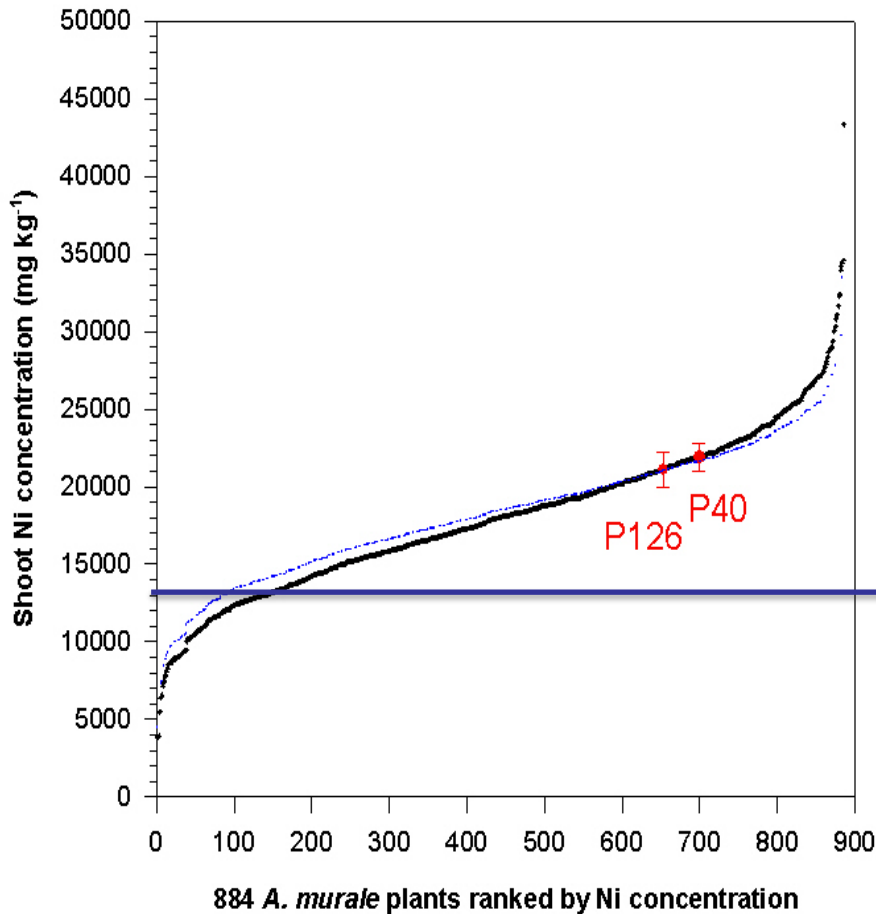
Concentration of Ni in flowering branch stems in Spring 1999 compared to whole shoots in June. Colluvial soil contained only 660 mg total Ni/kg.





Grown on an Oregon Brockman variant serpentine soil, with 5500 mg Ni/kg. [From Li et al., Plant & Soil, 2003]

Results of third round of selection in *Alyssum* breeding program, Vineyard Site 1, Cave Junction, Oregon, 2004-2005

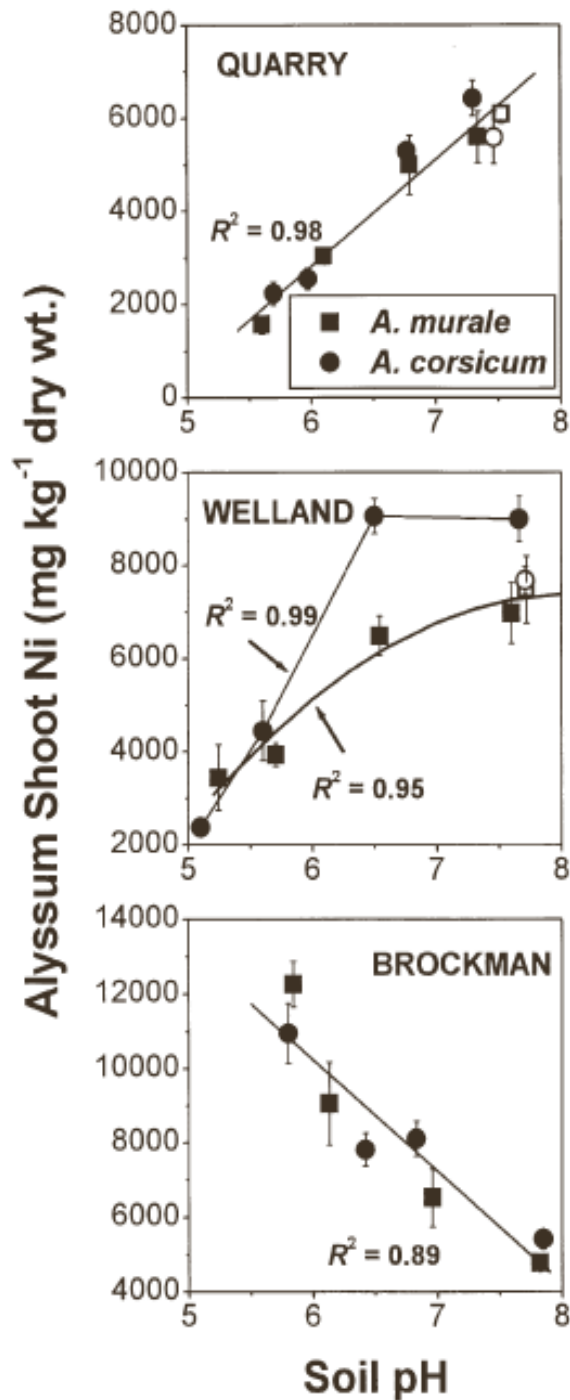


Viridian LLC Data; high Ni plant biomass incorporated

Effect of phosphate, pH and Ca:Mg treatments of Soil-3 (Navarro Backfield) serpentine soil on geometric mean yield and element concentrations in Shoots of two species of *Alyssum* after 120 days growth. [Greenhouse]

Treatment	Final pH	GM-Yield g/pot	GM-Ni g/kg	GM-Co -----	GM-Mn mg/kg DW	GM-Zn -----	GM-Fe -----	Cu -----
1 None	6.56 [#]	4.1 c	14.7 a	34.3	56	63	154	3.0
Phosphate Treatments:								
3 0 P	5.82	1.6 d	6.25 cd	19.4	62	118	273	2.8
2 100 P	6.24	24.5 a	6.27 cd	19.9	61	59	112	3.6
4 250 P	6.14	23.2 ab	6.81 bc	22.6	65	60	104	4.2
5 500 P	6.16	26.5 a	5.69 d	18.1	67	55	92	4.0
pH Treatments:								
6 Lo pH	5.42	27.4 a	6.15 cd	224.	462.	63	144	4.4
7 MLo pH	5.69	26.2 a	6.80 bc	50.4	132.	68	117	4.6
8 MHi pH	5.89	27.0 a	5.99 cd	28.8	73.	58	96	3.6
2 As is pH	6.24	24.5 a	6.27 cd	19.9	61.	59	112	3.6
Ca:Mg Treatments:								
9 0.0 Ca	6.10	19.3 b	7.86 b	21.1	56.	49	87	3.1
2 1.0 Ca	6.24	24.5 a	6.27 cd	19.9	61.	59	112	3.6
10 2.5 Ca	6.04	25.2 a	6.05 cd	18.4	58.	59	87	3.8
11 5.0 Ca	6.03	24.2 a	5.63 d	24.4	78.	63	93	3.6

[#]Means followed by the same letter are not significantly different (P < 0.05 level) according to the Duncan-Waller K-ratio t-test.

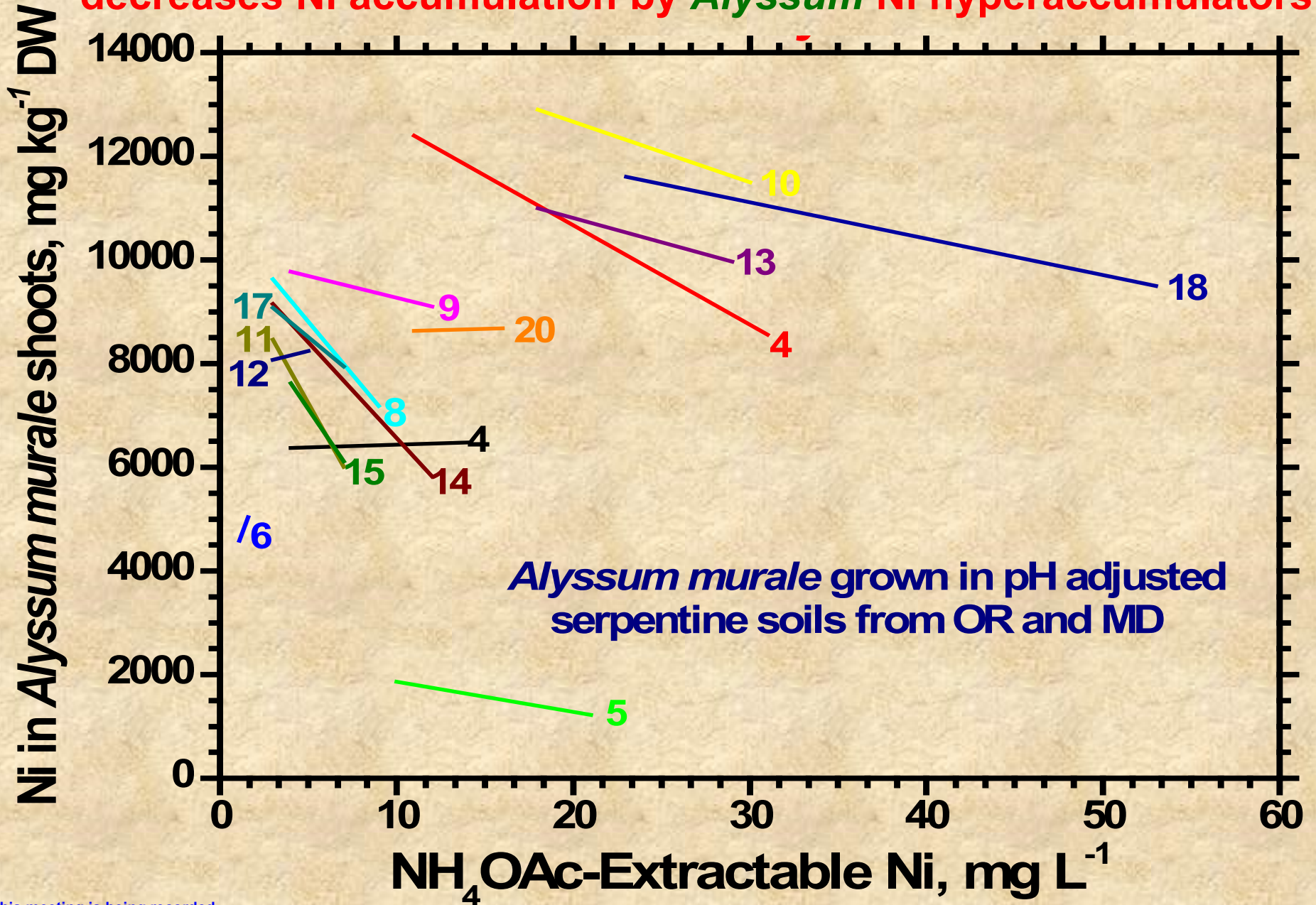


Kukier et al., 2004.

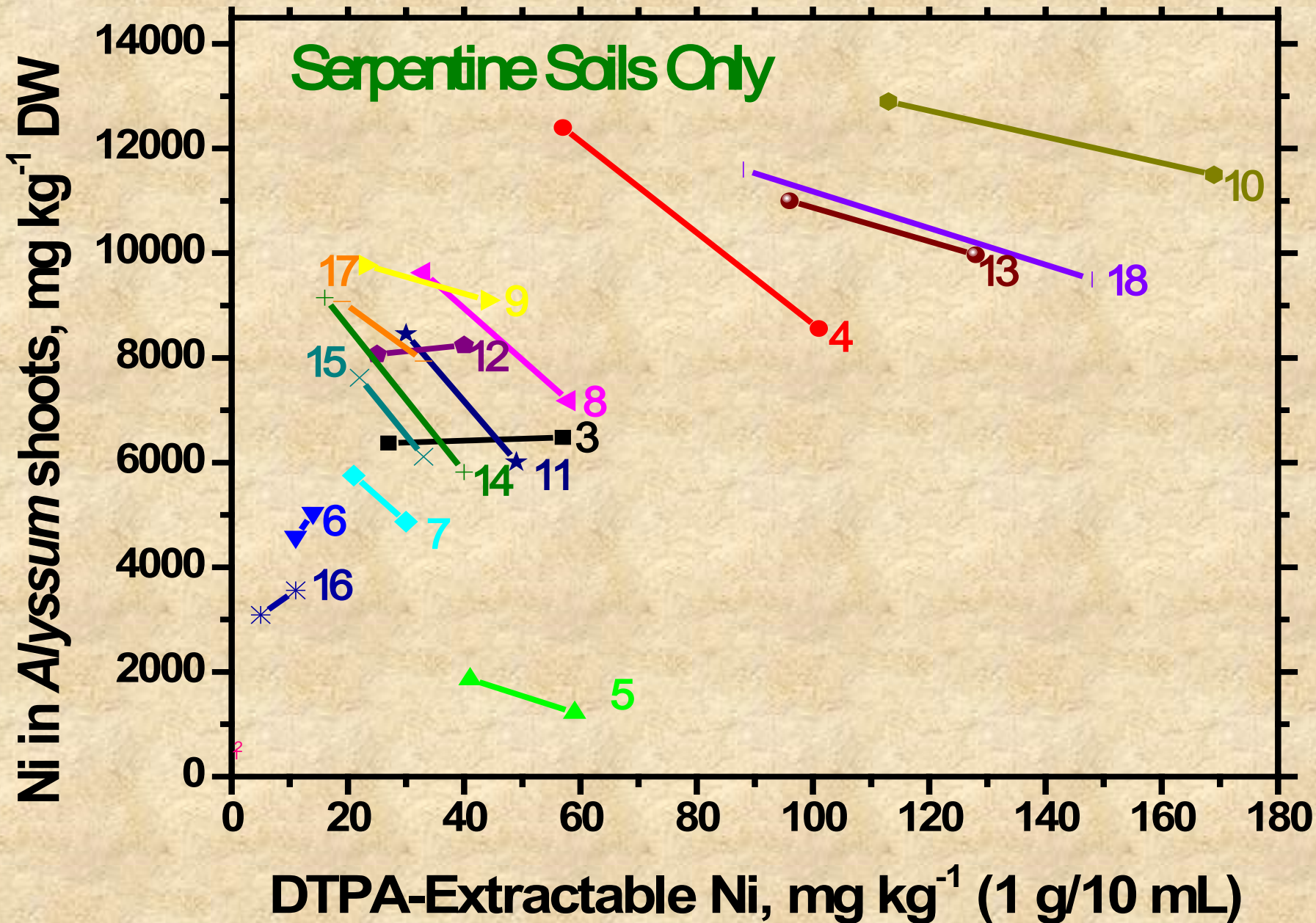
Effect of soil pH on Ni accumulation in shoots of *Alyssum* species.

Brockman soil has 22% Fe.

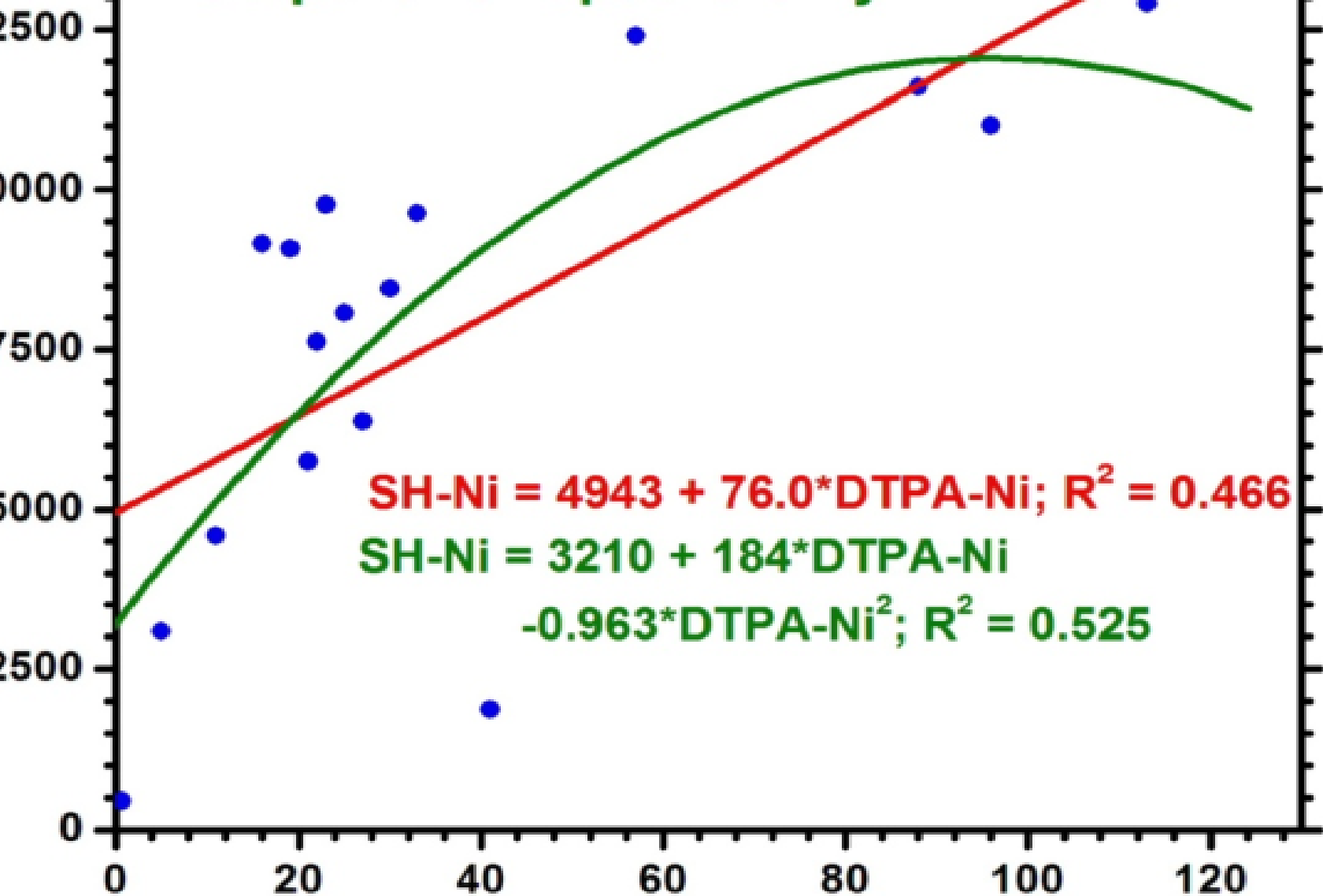
Soil acidification increases NH_4 -Acetate-extractable Ni, but decreases Ni accumulation by *Alyssum* Ni hyperaccumulators



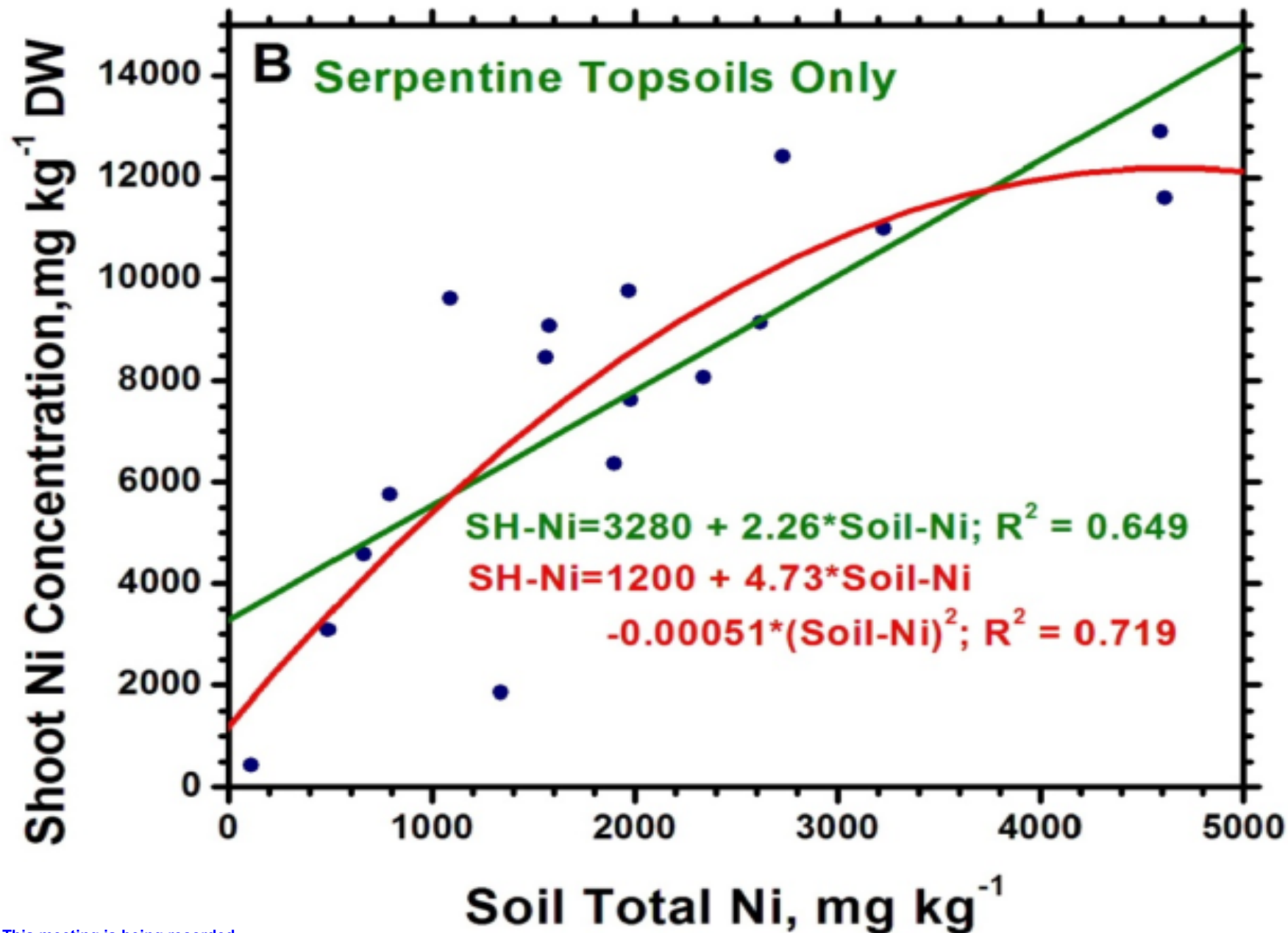
Lower Soil pH Increases DTPA-Ni, but Decreases Alyssum Ni



A Serpentine Topsoils Only



DTPA-Extractable Ni, mg kg⁻¹



Agronomy of Alyssum Phytomining

- Must adapt *Alyssum* agronomic management to the nature of soil and environment where grown.
- Port Colborne has some very poorly drained soils which are too 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 O.c. at Port Colborne.
- We tested “ridge” tillage of the plow layer highly contaminated soils; plow layer ridged and O.c. seeded on top of ridges using herbicides and fertilizers previously identified for *Alyssum* production. Ridge production worked very well!
- But the soil Ni was only in the topsoil; low shoot Ni.



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).

This meeting is being recorded.



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



JUN 19 2002

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

Accumulation of Ni from Soil Solution

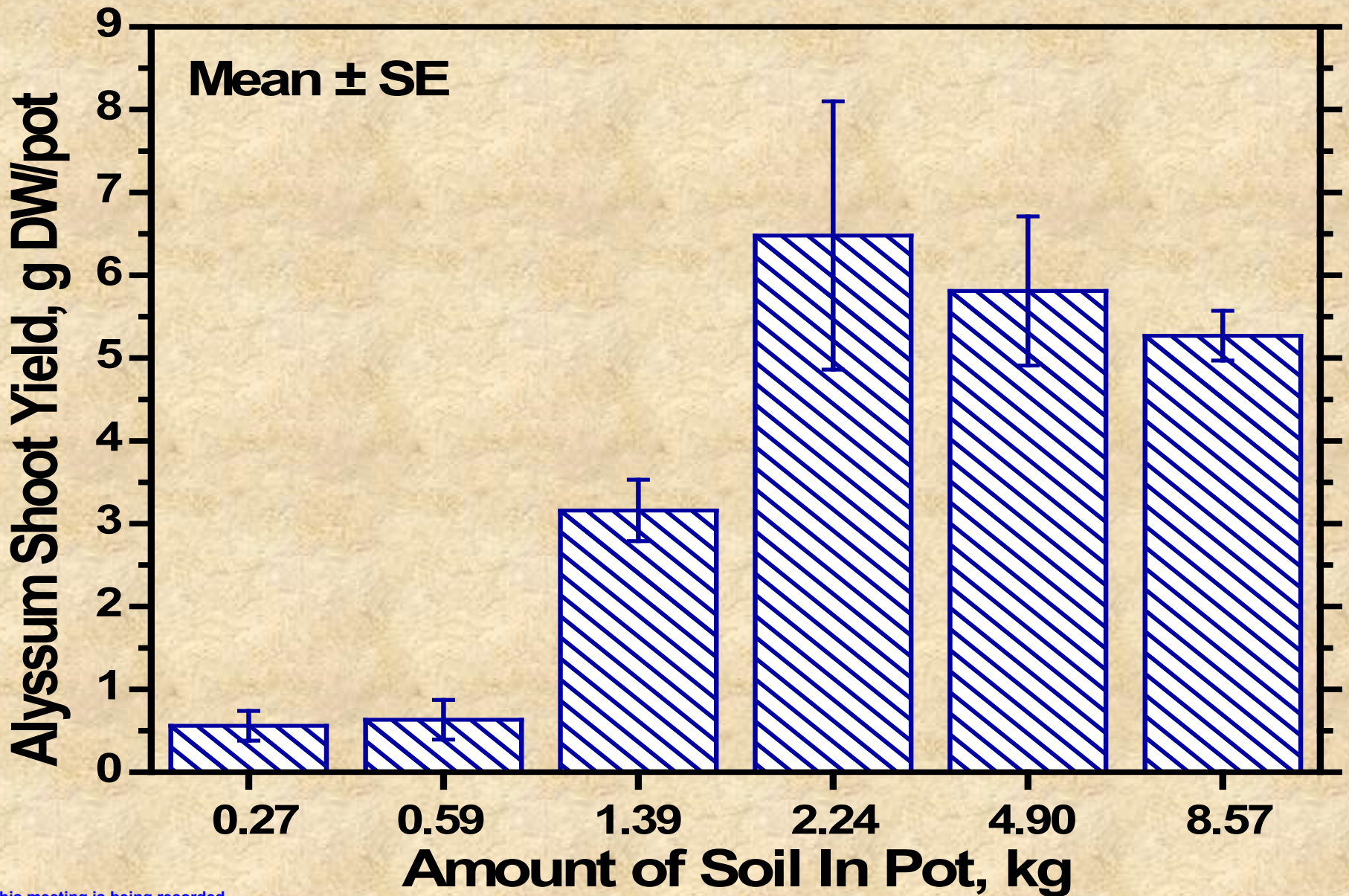
- Shoots contain $\geq 15,000 \mu\text{g Ni/g}$ dry matter
- Water use is $\sim 250 \text{ g water/1 g shoot dry matter}$
- If **convection** of soil solution carried Ni to roots for uptake, the soil solution would have to contain $15,000 \mu\text{g}/250 \text{ mL} = 60 \mu\text{g Ni mL}^{-1}$.
- But soil solution of Brockman variant soil with $4700 \text{ mg Ni kg}^{-1}$ was $0.8 \mu\text{M}$ or $0.047 \mu\text{g Ni/mL}$.
- Thus **diffusion** must supply nearly all Ni accumulated by *Alyssum* from serpentine soils.
- And thus **root length or root surface area** are key to high Ni accumulation – **Large soil volume in testing is key to valid hypernickelophore research in lab or field. (Subsoil?)**

Pot Size Affects *Odont. corsica* Growth



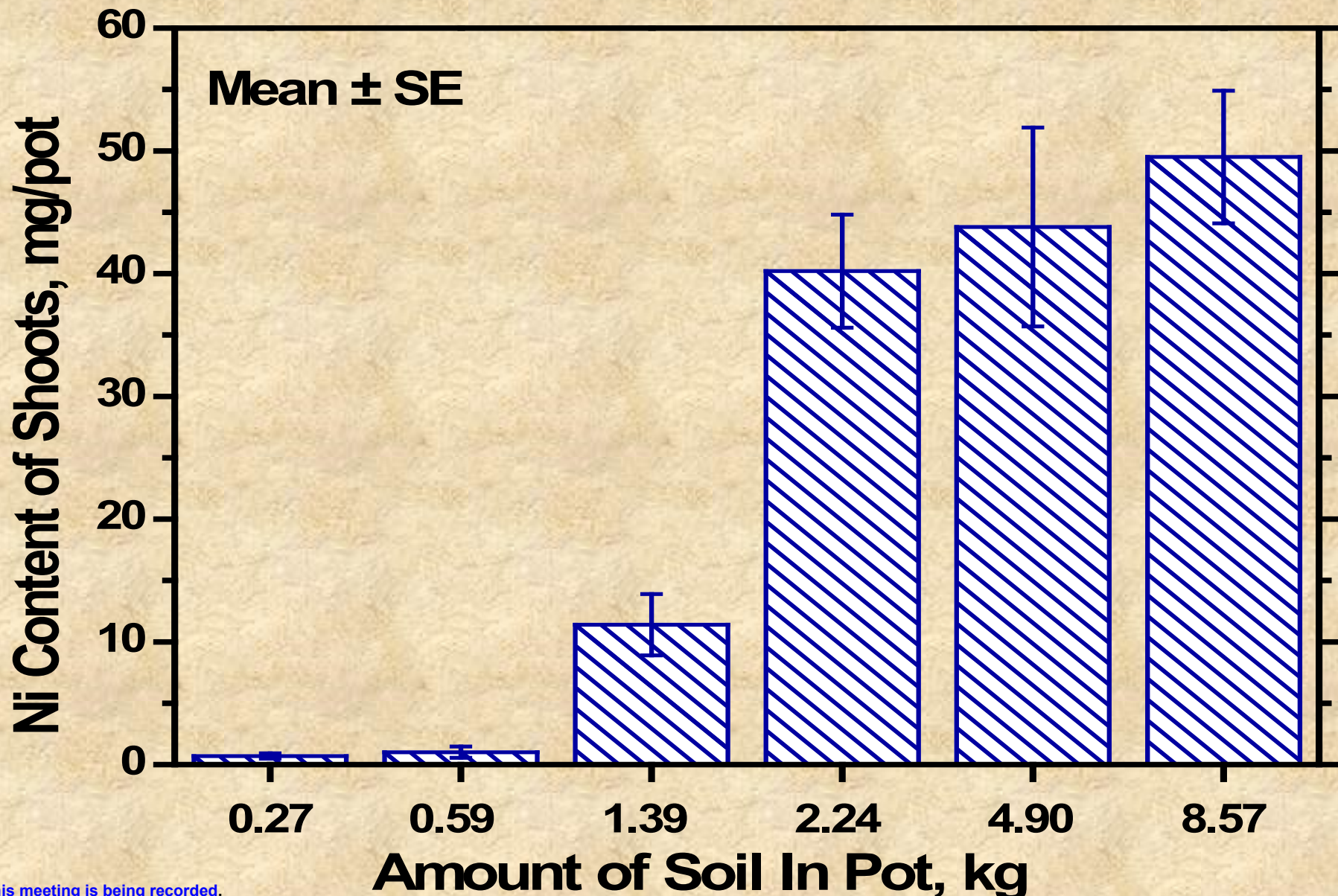
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Effect of pot size on growth of *N. chalcidica* in “Vineyard” serpentine soil (4700 mg Ni kg⁻¹). From smallest to largest, the weight of soil in the pots were: Smallest , 274 g; Small, 593 g; 4 inch, 1.39 kg; 6 inch, 2.24 kg; 9 inch, 4.90 kg; and 10 inch, 8.57 kg.



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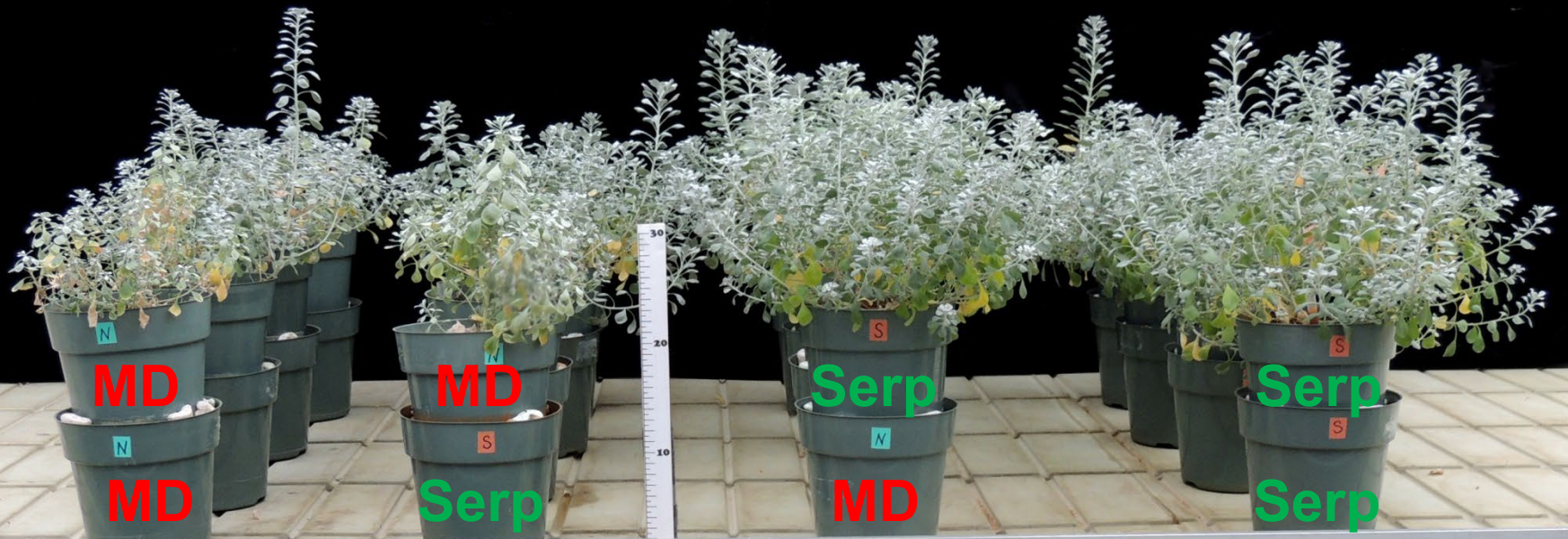
Effect of Soil Volume on Shoot Yield of *Alyssum corsicum* grown on serpentine soil



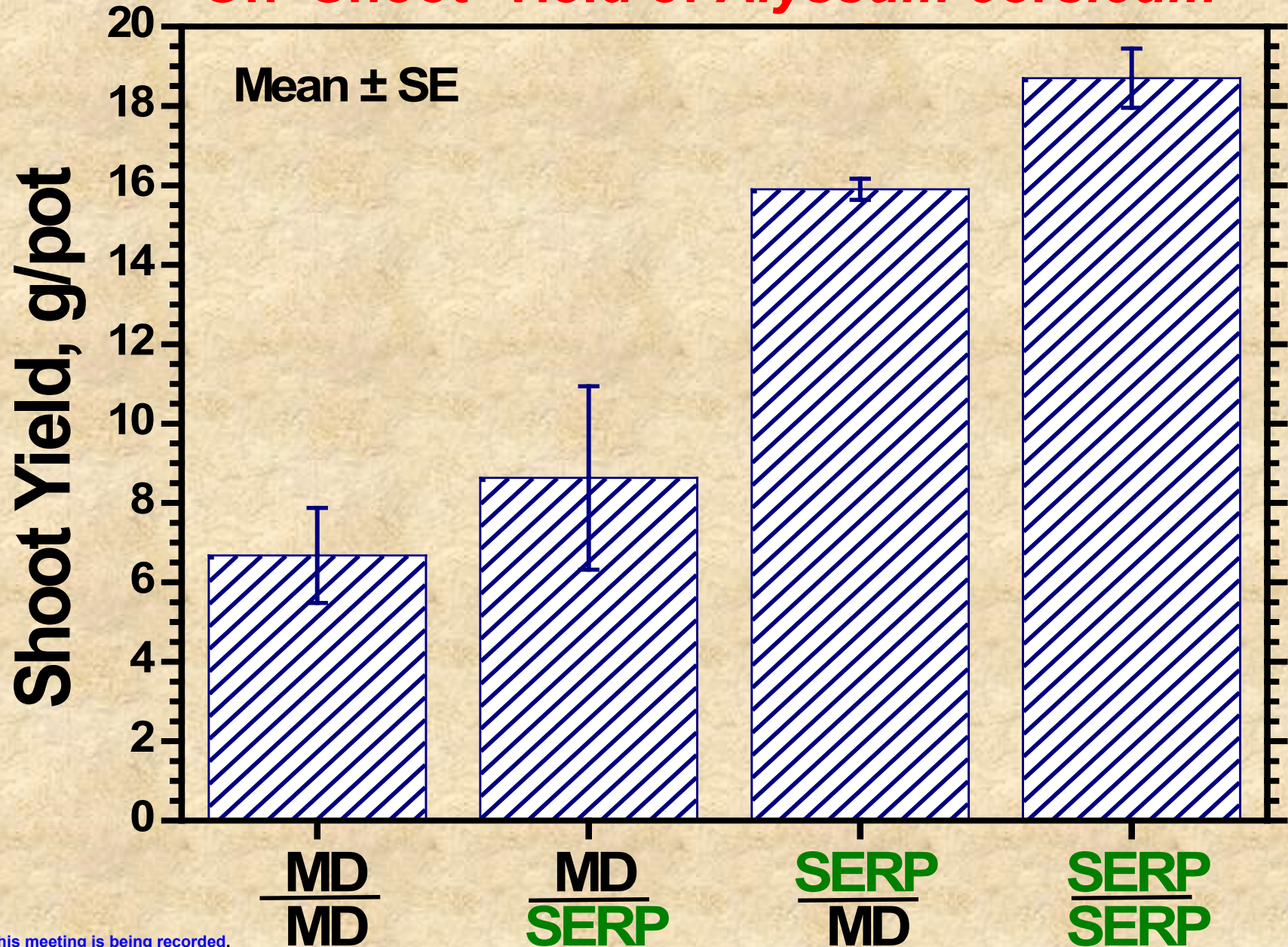
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**Effect of Soil Volume on Quantity of Ni
in Shoots of *Alyssum corsicum***

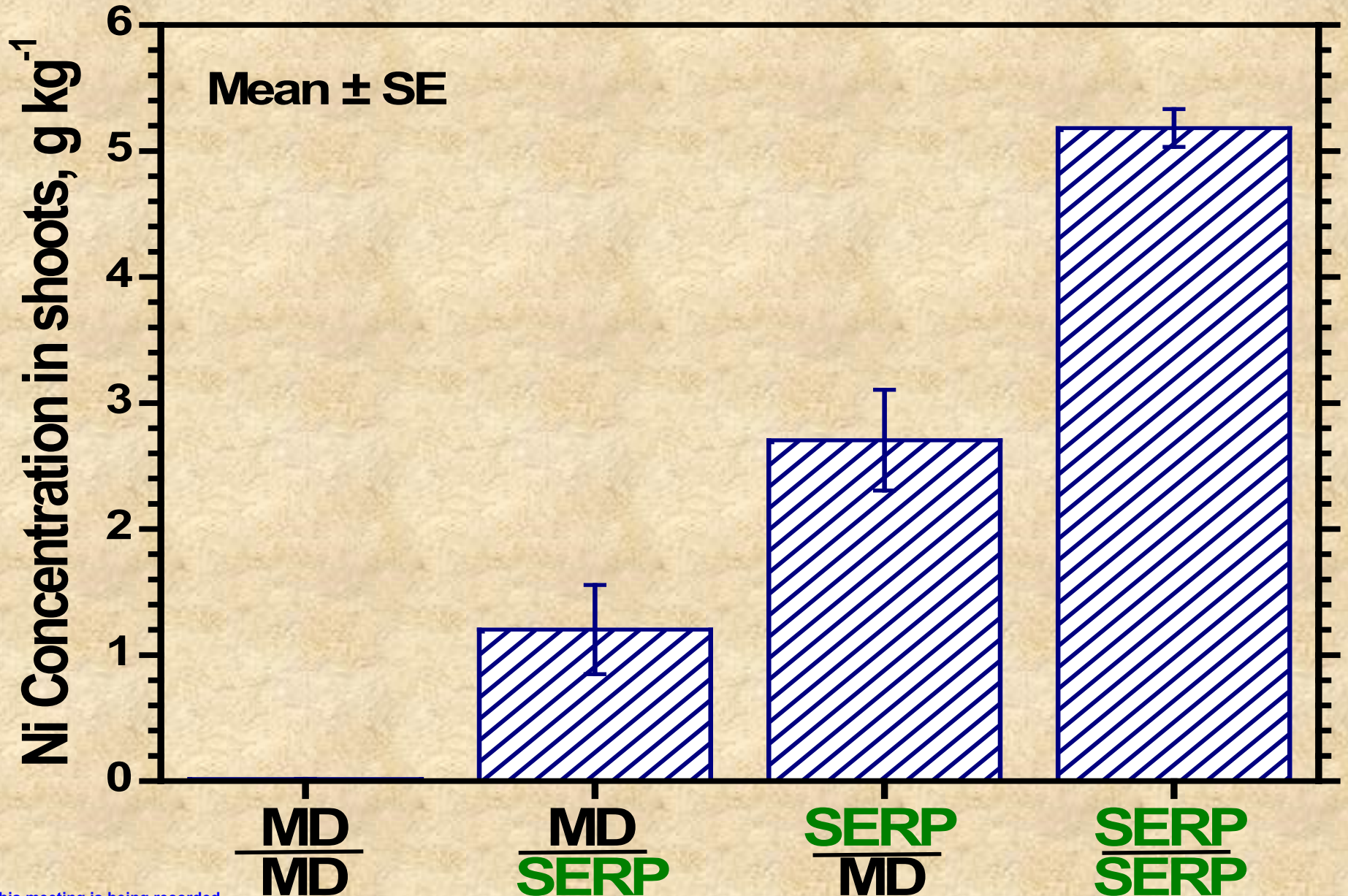
Effect of MD **Serpentine** or MD Farm Soil as Topsoil or Subsoil on Growth of *Odontarrhena chalcidica*



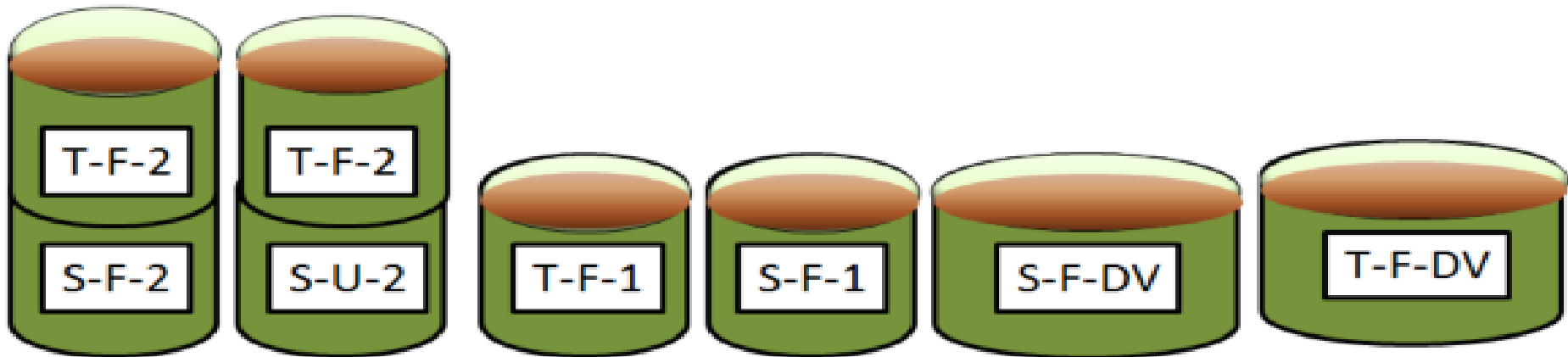
Effect of Serpentine Topsoil or Subsoil On Shoot Yield of *Alyssum corsicum*



Effect of Serpentine Topsoil or Subsoil on Ni Concentration in Shoots of *Alyssum corsicum*



Strategy of treatments to test the effect of topsoil, access to subsoil, and double volume of Maryland serpentine soil on Ni phytoextraction by *Odontarrhena corsica* (Adrian Paul).



T = Topsoil

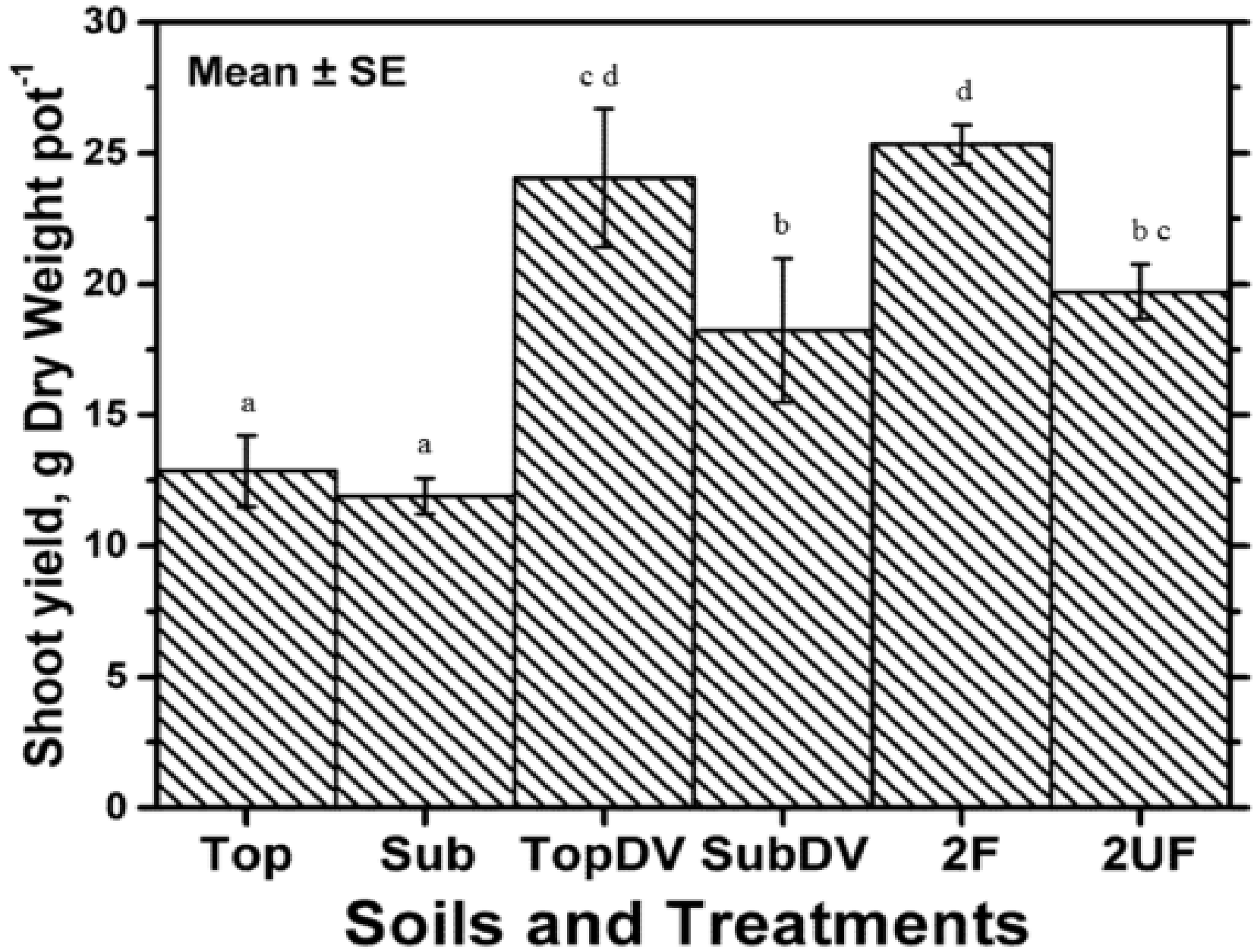
F=NPK

DV=Double Volume

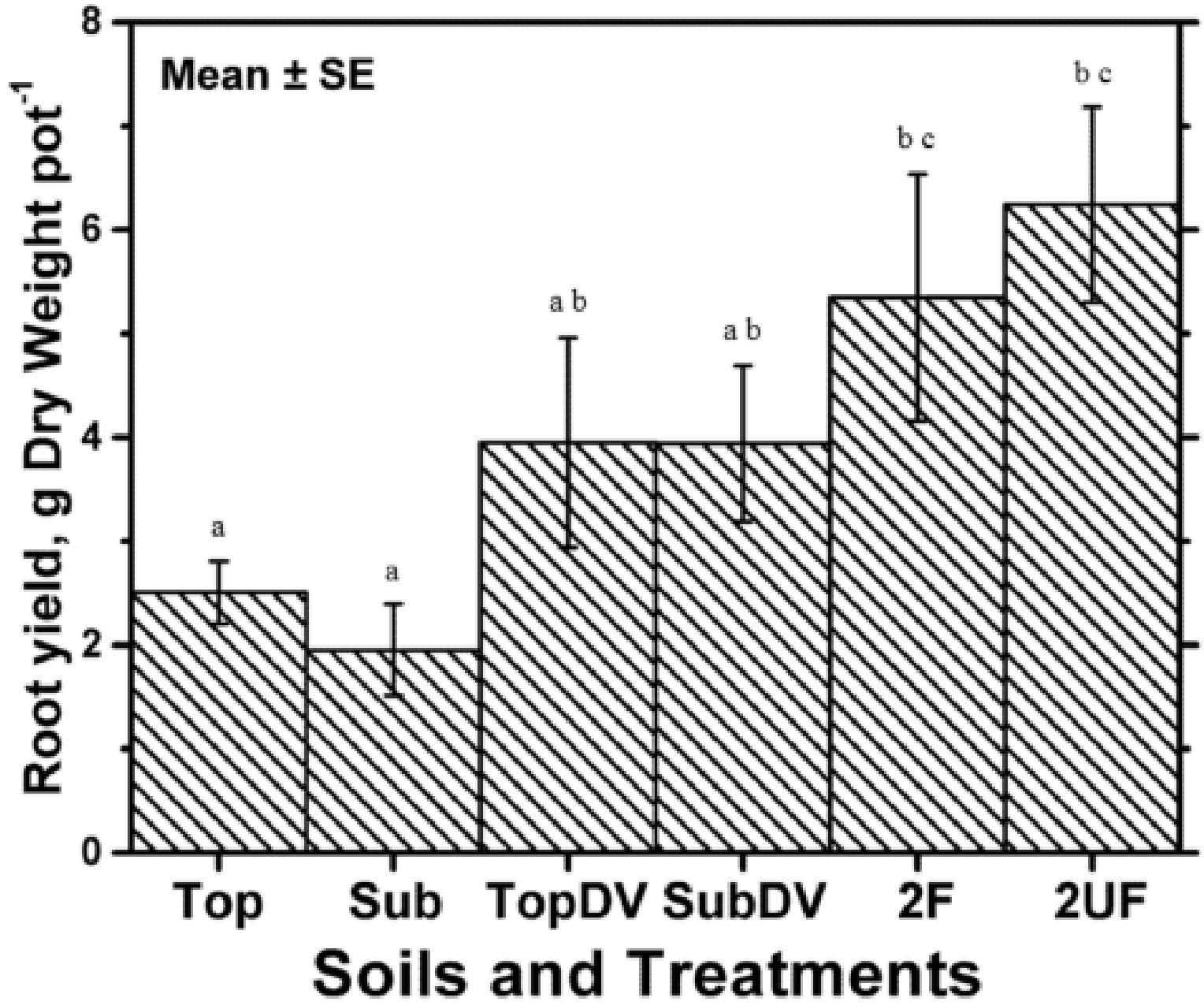
S = Subsoil

U=None

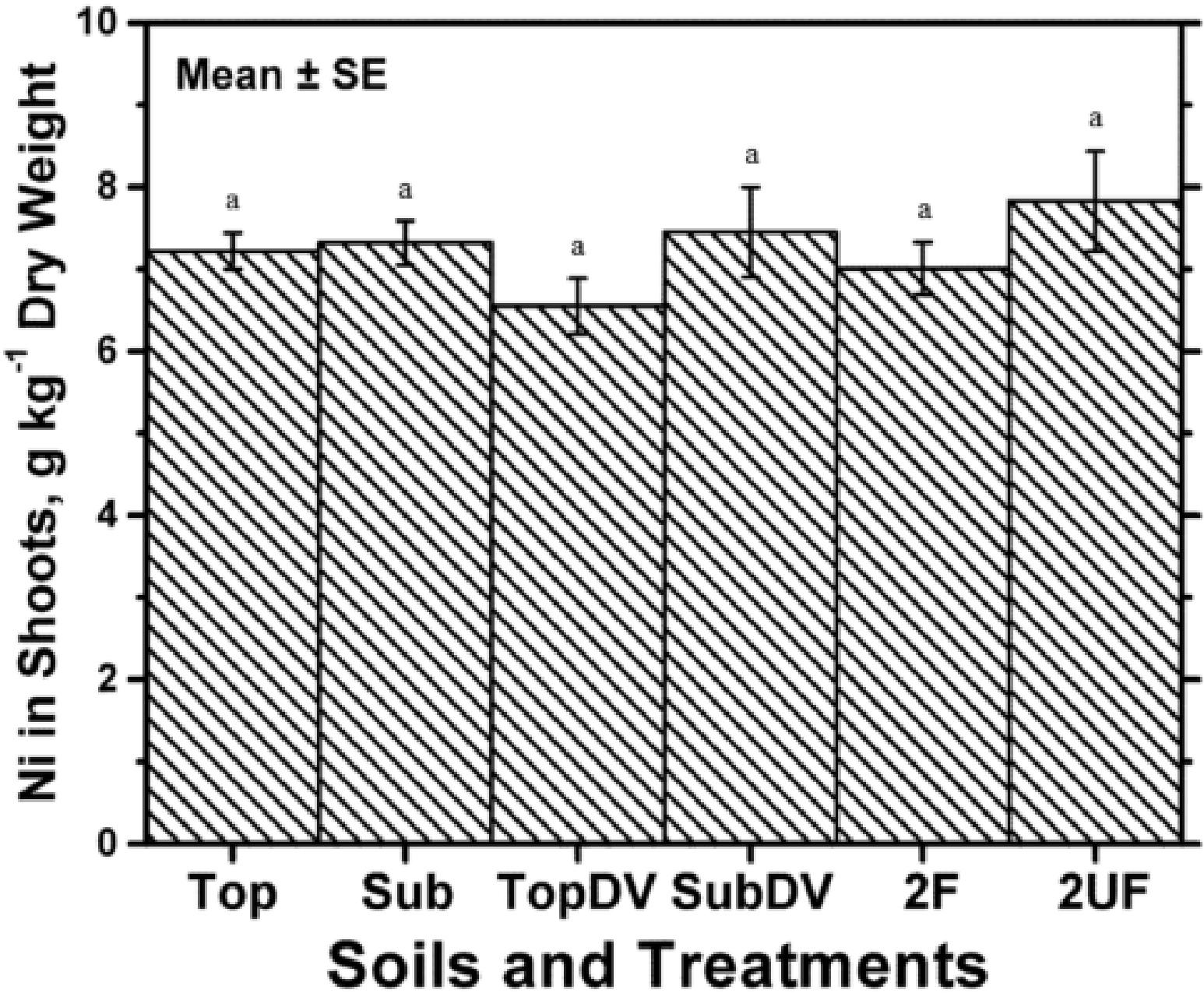
Odontarrhena corsica grown in Maryland serpentine soil.



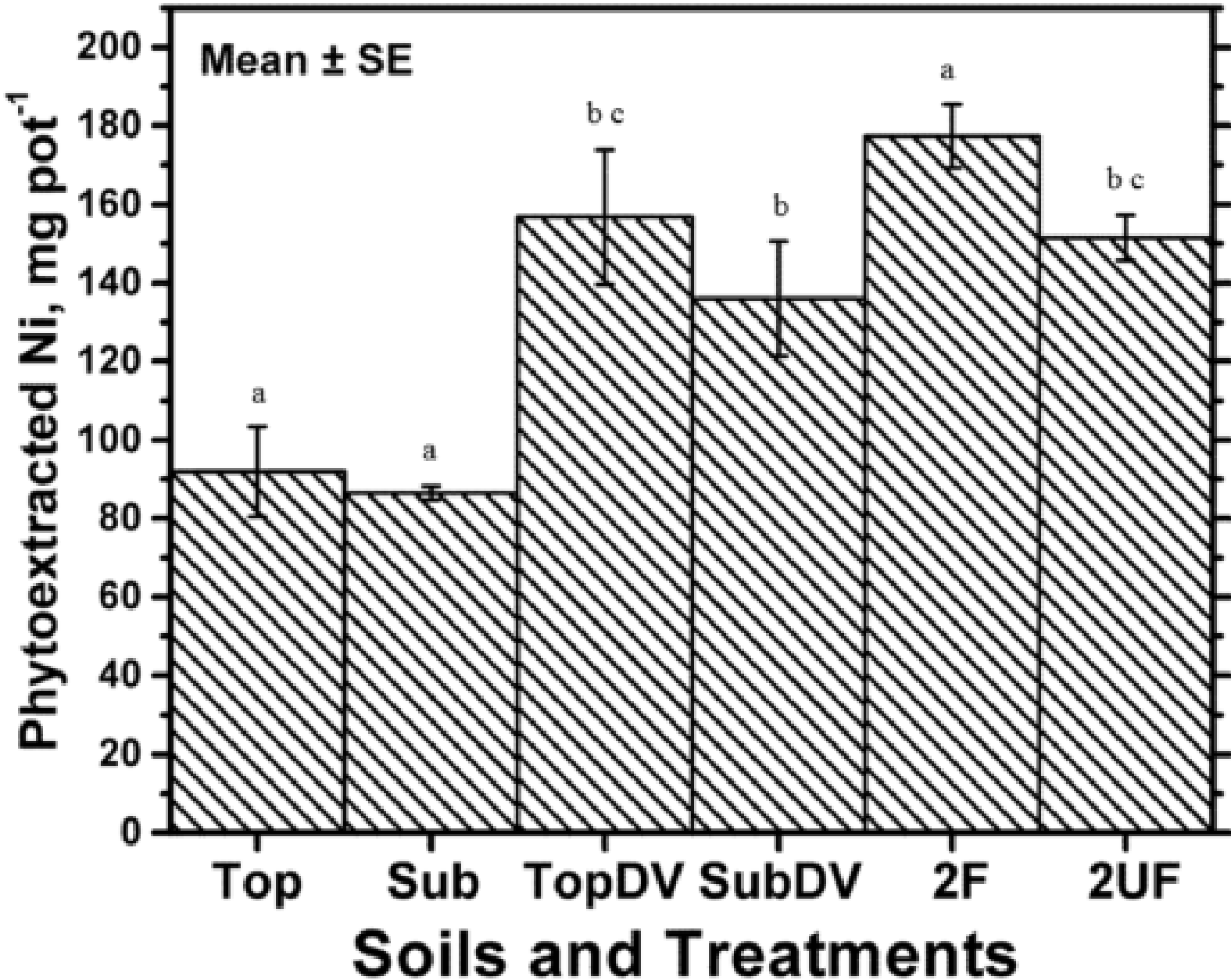
***Odontarrhena corsica* grown in Maryland serpentine soil.**



Odontarrhena corsica grown in Maryland serpentine soil.



Odontarrhena corsica grown in Maryland serpentine soil.



Field Agronomic Studies in Oregon.

Treatment	Soil pH	Branch Ni	Stand
<i>Odontarrhena chalcidica</i>		g/kg	%
Basal-NPK	6.72	14.7 bc	73
0.5 P	6.75	12.8 c	78
Gypsum	6.78	11.9 c	84
2X-N ((Split)	6.67	14.0 c	85
Limestone	7.35	12.9 c	78
Sulfur	6.00	8.2 d	13
<i>Odontarrhena corsica</i>			
Basal-NPK	6.72	21.5 a	49
0.5 P	6.75	15.0 bc	49
Gypsum	6.78	20.1 ab	59
2X-N (Split)	6.67	21.5 a	61
Limestone	7.35	21.7 a	68
Sulfur	6.00	14.1 c	11

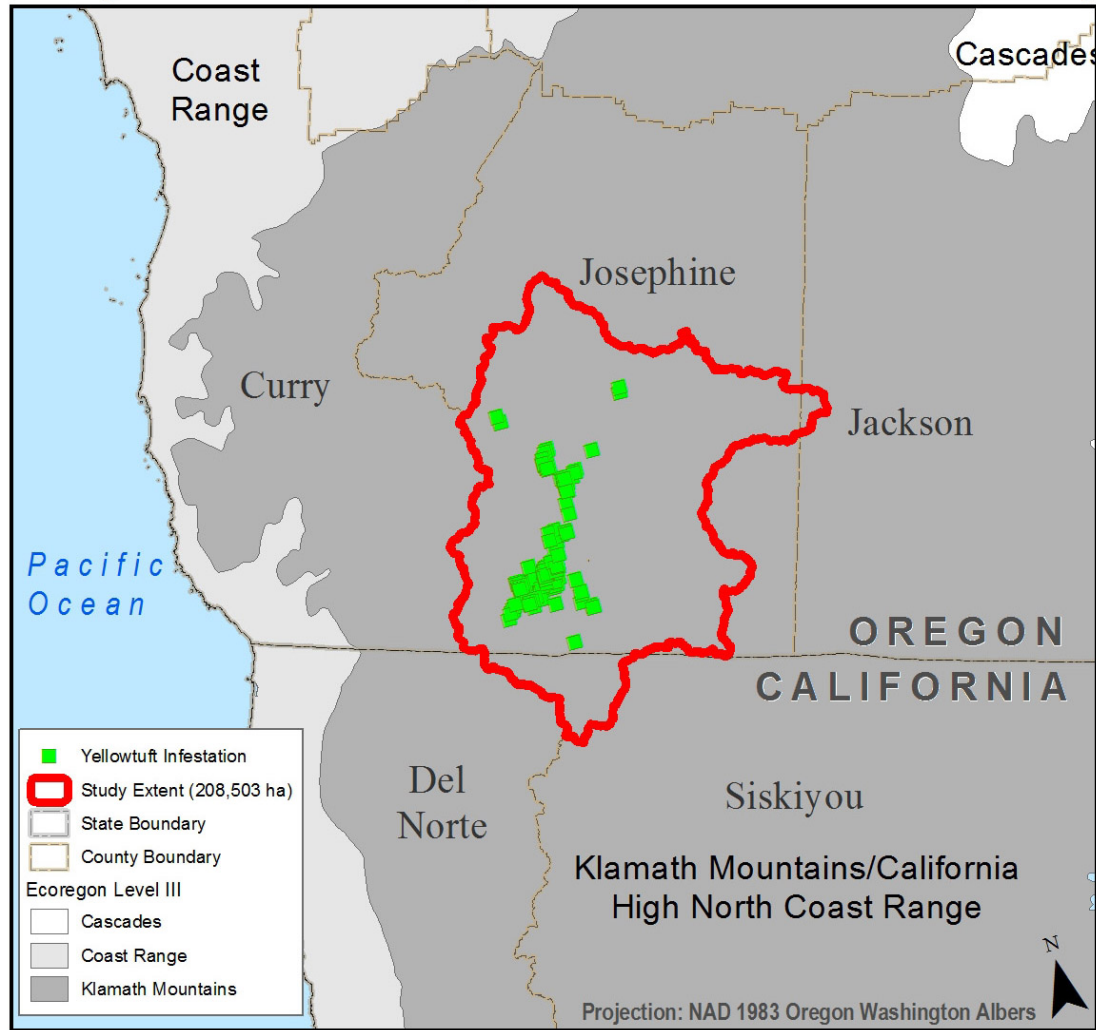
Unimproved Genotypes!

This meeting is being recorded.

Branch samples at late Spring harvest to select parents.

Strawn, K.E. 2013. Unearthing the habitat of a hyperaccumulator: Case study of the **invasive plant** yellowtuft (*Alyssum*; Brassicaceae) in Southwest Oregon, USA. *Manag. Biol. Invas.* 4:249–259.

Study extent: Yellotuft predicted suitable habitat study



Alyssum declared Noxious weed in Oregon



Project Terminated by State.

This meeting is being recorded.



**“Parents of Phytomining”: Reeves, Bani, Baker, Chaney, Morel; in Albania.
More recent “parents”: Angle, Li, Roseberg, Brewer, Echevarria, van der Ent.**

This meeting is being recorded.



This meeting is being recorded.

First Tropical Phytomining Farm in Sabah at Planting



**First Tropical Agromining Farm in Sabah
After one year of growth**

***Phyllanthus
rufuschaneyi***
in Sabah, Malaysia

**from Antony
van der Ent
1-2 m tall**

