Equilibrium Environmental Inc.

For a balanced environment…

Finalization of Research and Preliminary Selenium Soil Quality Guideline Derivation

Remtech 2022
Banff, Alberta

Vicky Winter (Equilibrium Environmental, Canada)
Darlene Lintott (Element Materials Technology, Canada)
Anthony Knafla (Equilibrium Environmental, Canada)

Acknowledgements

- PETROLEUM TECHNOLOGY ALLIANCE CANADA (PTAC)
  Project champion (Cenovus Energy):
  - Shawn Glessing

- INNOTECH ALBERTA
  - Jay Woosaree
  - Dr. Dani Degenhardt

- ELEMENT MATERIALS TECHNOLOGY
  - Darlene Lintott

- EQUILIBRIUM TEAM

- ADDITIONAL CONTRIBUTING SCIENTISTS
  - Tyler Prediger

What is Special about Selenium (Se)?

- Non-metal located between sulfur & tellurium: Se
  4 valence states (0, II, IV, VI);
  forms a number of compounds in environment

- Naturally sources:
  sedimentary rocks, coal, fossil fuels
- Anthropogenic sources:
  coal plants, wastewaters, copper refining, drilling
- Commercial use:
  glass, pigments, metallurgy, electronics

- Essential micronutrient for humans, animals, beneficial for some plants
  'double-edged sword' (Brozmanova et al. 2010)
  'essential toxin' (Lenz and Lens 2009)

- Narrow range between the dietary deficiency (<0.05-0.1 mg/kg) and
  toxic over-consumption (5-15 mg/kg) for animals (Mayland et al. 1989)

Toxicity vs Deficiency

- **Humans**
  - **Deficiency: cardiovascular disease, muscular dystrophy, arthritis, liver necrosis, anemia, etc.**
  - **Toxicity: selenosis (vomiting, diarrhea, hair and nail losses)**
    - *Se toxicity in humans is far less spread than Se deficiency*

- **Wildlife & Livestock**
  - **Deficiency: muscle weakness, ‘ white muscle disease’, reduced appetite, growth, and fertility**
  - **Toxicity for livestock: alkali disease (chronic), blind stagger (acute)**
    - *2 mg/kg is a maximum safe level for ruminants (NRC, 1980)*
  - **Toxicity for small rodents: 1.5-3.0 mg/kg body weight (lethal)** *(NRC 1983)*

- **Plants**
  - **Benefits: acts as an anti-oxidant at low doses, increases yield, protect from pathogens and herbivores, delays senescence, helps resist drought**
  - **Toxicity: acts as a pro-oxidant at high doses, reduces yield, causes chlorosis and black spots**

High Level Summary

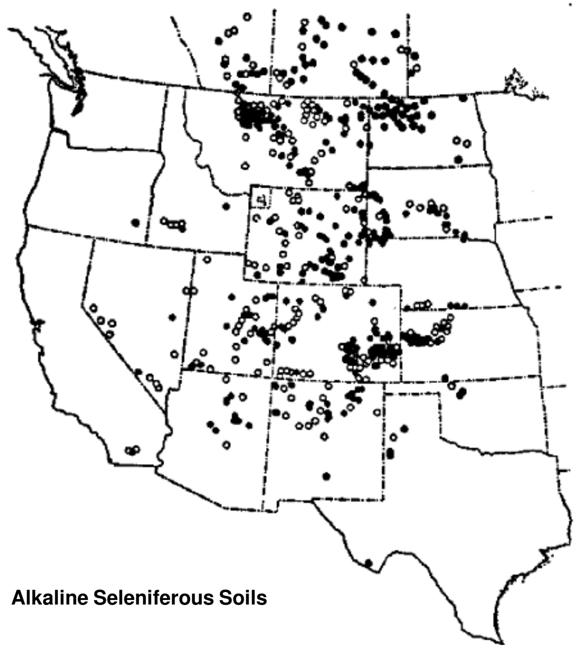
- Tier 1 soil quality guideline is 1 mg/kg
  - Based on a limited toxicology database
  - Doesn't address elevated soil sulphate
  - Doesn't address bioconcentration / bioaccumulation

- Natural Se in background <1.0 to 2.3 mg/kg in Alberta and up to 4.7 mg/kg in Canada
  - Exceeds Tier 1 guideline

- Se in oil and gas drilling sumps at > 1 mg/kg

- Toxicity observed in livestock and other grazing species historically at natural levels
  - First evidence of alkali disease in 1500s
  - In 1800s in North Dakota and Nebraska, cavalry horses had hair loss and hoof sloughing
  - Called alkali disease as associated with alkali seeps

Selenium Distribution in Canadian and USA Soils

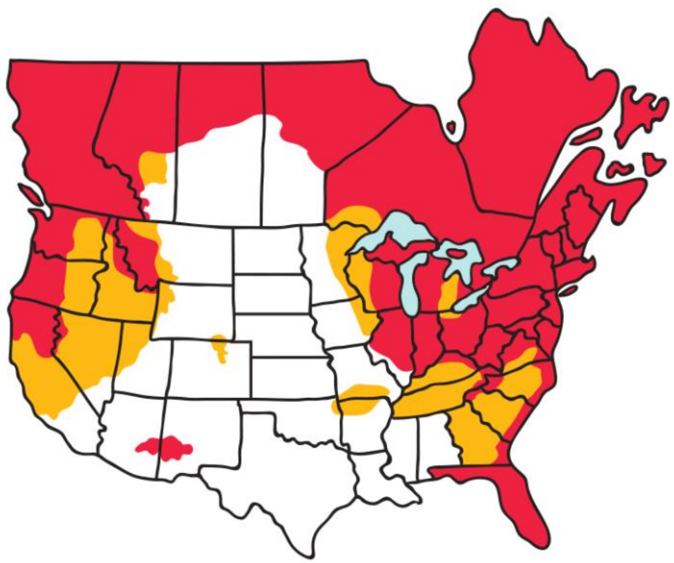


Alkaline Seleniferous Soils

Open dot — plant specimen containing 50-500 mg/kg Se

Solid dot — plant specimen containing >500 mg/kg Se

(Map adapted from Rosenfield & Beath 1964)



Selenium in Forage and Grain

Red — 80% of all forage contain <0.10 mg/kg Se

Yellow — 50% of all forage and grain contain >0.1 mg/kg Se

White — 80% of all forages and grain contain >0.1 Se

(Map adapted from Ontario Ministry of Agriculture website & Horse Journals)

Overview

Toxicity Testing
(Plants, Invertebrates)

Sulphate Effect

Selenate vs Selenite

Reassessment
of Selenium
Guidelines

Bioconcentration

Statistical Analysis & Modeling:
BMDS, SSD Curve, Easy Fit

Alkaline Seleniferous Soils

- Typical soils selenium concentrations range up to 2.3 mg/kg in Alberta (Penny, 2003)
  - can range up to 4.7 mg/kg in the Western Sedimentary Basin (CCME 2009)
  - Alberta Tier 1 Guideline of 1 mg/kg

- Alkaline seleniferous soils have been known in Alberta, Saskatchewan, and parts of SW Manitoba since the early 1900s
  - Soil Concentrations are typically > 1 mg/kg
  - Agricultural uses has lead to livestock losses
  - Plants concentrations were typically > 50 mg/kg and up to > 500 mg/kg (livestock dietary limit is 2 mg/kg plant; McDowell et al. 2005)
  - Some of the accumulator species are not preferred by livestock until there is an absence of ‘tasty’ forage
  - Total selenium concentrations in soil not well correlated with plant levels due to multiple forms of selenium, based on oxidation-reduction potential and pH

  - Not always clear whether a particular soil concentration will be associated with toxicity or not

Oxidation States

- Four common oxidation states of selenium
- Important to know which states are relevant for different environments of Alberta and Saskatchewan

Hydrogen Selenide	[-2]	Reduced conditions, relatively insoluble, can be volatile, found in poorly aerated acidic soils
Elemental	[0]	Not common in natural environments, except in poorly aerated acidic soils, relatively insoluble
Selenite	[+4]	Well-drained soils, present in mildly oxidizing & reducing conditions, less soluble in water (83 g/200 g water), bioavailable
Selenate	[+6]	More soluble in water (170 g/200 g water), more mobile in groundwater under neutral and alkaline pH, present in oxidizing conditions and alkaline soils, weakly adsorbed to soil at alkaline pH, BIOAVAILABLE

Selenium Oxidation States, Potentials, & pH

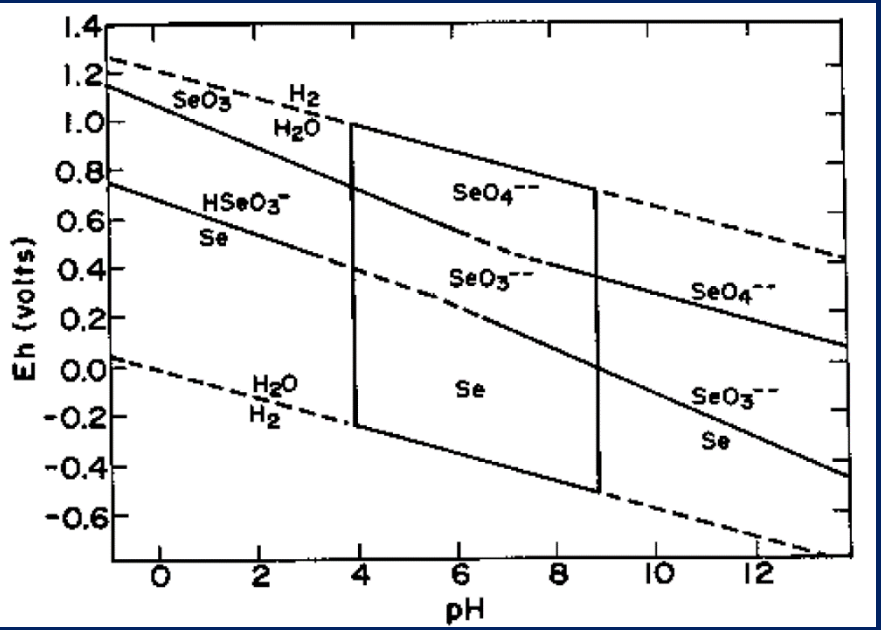
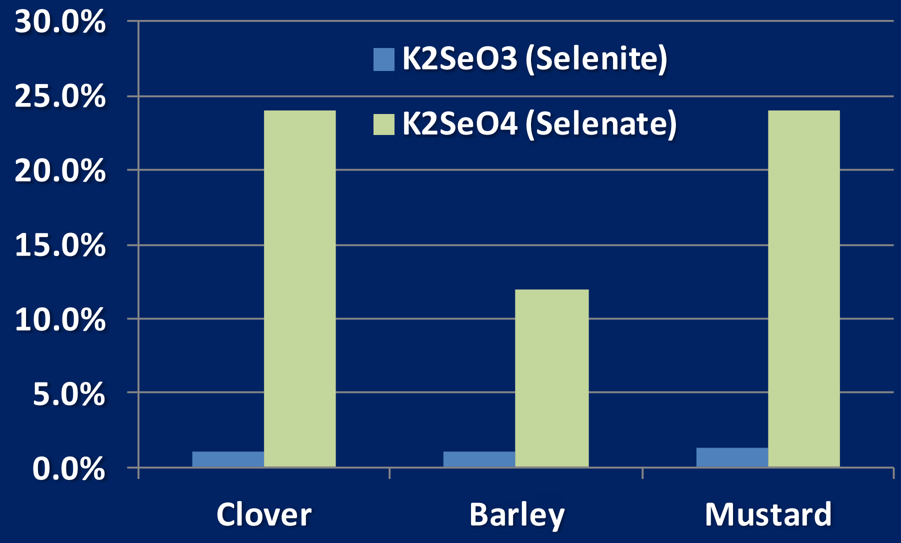


Figure adapted from Mayland et al. 1983

Alkali Slough (pH > 8.5)

Plant Uptake

- Differences in plant uptake
  - Selenite preferentially bound to soils and less available for uptake
  - Selenate readily absorbed
  - This may partially explain the lower toxicity of selenite towards livestock consuming plants grown in clays

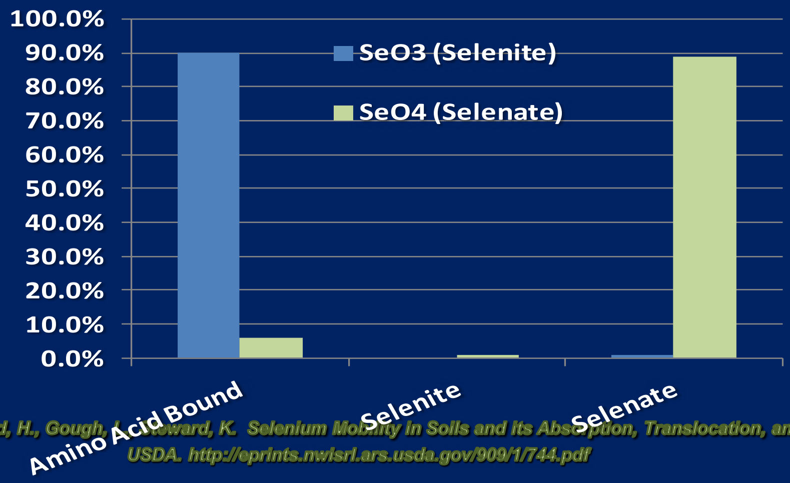


Percent uptake into plant tissue at equivalent concentrations added to loamy sand soil

Mayland, H., Gough, L., Steward, K. Selenium Mobility in Soils and its Absorption, Translocation, and Metabolism in plants. USDA. http://eprints.nwisrl.ars.usda.gov/909/1/744.pdf

Plant Uptake & Metabolism

- Selenate readily absorbed and translocated into plant tissue; found in xylem sap as selenite
- Selenite slowly absorbed & rapidly metabolized to organo-selenium compounds in minutes and detected as seleno-amino acids in xylem sap; <1% may remain as selenite
  (This may partially explain the lower toxicity of selenite towards livestock consuming plants)

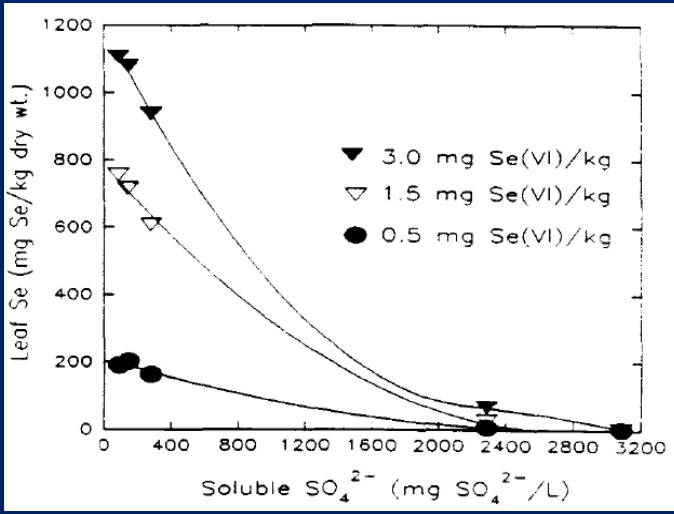


Percent uptake into plant tissue at equivalent concentrations added to soil

Mayland, H., Gough, L., Steward, K. Selenium Mobility in Soils and its Absorption, Translocation, and Metabolism in plants. USDA. http://eprints.nwisrl.ars.usda.gov/909/1/744.pdf

Sulphate Reduction of Plant Uptake

- Study with alfalfa at three selenate soil concentrations and three sulphate concentrations, Se plant tissue decreased
- Could lead to a reduction in plant toxicity or livestock toxicity from consuming plants



Mayland, H., Gough, L., Steward, K. Selenium Mobility in Soils and its Absorption, Translocation, and Metabolism in plants. USDA. http://eprints.nwisrl.ars.usda.gov/909/1/744.pdf

Weight-of-Evidence Approach

- Development of EcoContact Soil Selenium (Se) Guideline (PTAC funded project)

Research Participants

- Equilibrium Environmental Inc. responsibilities
  - identification of data gaps
  - experimental design and scope developed for additional toxicity testing work to support guideline development
  - processing of commissioned toxicity testing results to develop a defensible guideline

- InnoTech Alberta
  - toxicity testing work with plants and invertebrates species

- Element Materials Technology
  - toxicity testing work with plants
  - bioconcentration studies

Toxicity Testing: Plants

- Started in 2012 (T. Prediger, A. Knafla)
  Artificial soils, Alfalfa, 32 treatments:
  4 SO_4 concentrations (150 – 1,600 mg/kg) × 8 Se concentrations (0.5 – 15 mg/kg)

- Continued in 2014-2017 (Equilibrium & InnoTech Alberta)
  Coarse and fine sandy loam soils (collected in Alberta), 6 test species:
  Alfalfa, Barley, Carrot, Cucumber, Northern Wheatgrass, Red Fescue

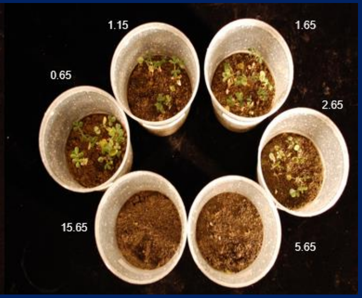
- 2014: 6 different treatments
  6 Se conc. (0.3 – 16 mg/kg) × 1 SO_4 conc. (10-12 mg/kg), 6 replicates
  Selenium in form of selenate (SeO_4)

Plant Growth Endpoints

Percent (%) seedling emergence (day 7)

Root and shoot length and dry weight

Alfalfa in coarse soils

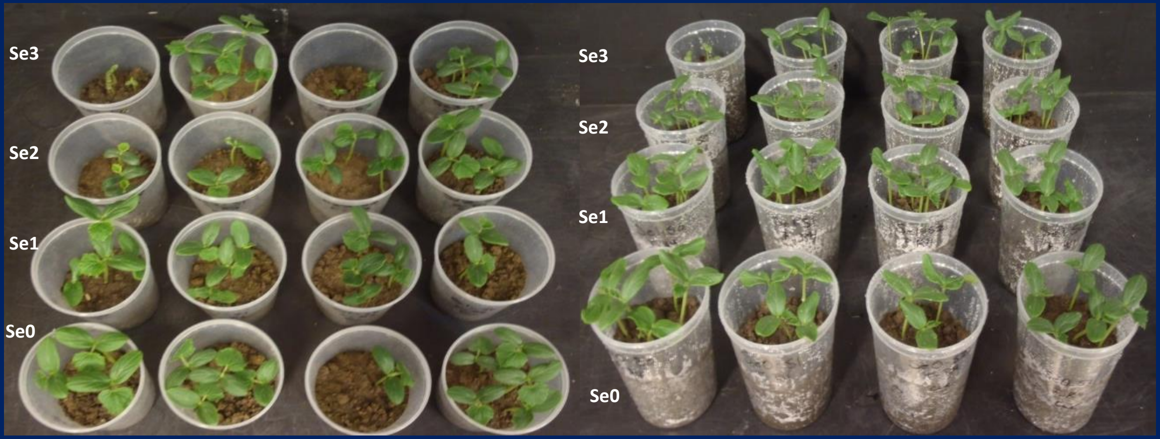


Alfalfa in fine soils

Barley in Coarse soil

Toxicity Testing: Plants

- 2015: 16 different treatments (14 to 21 days)
  4 Se conc. (0.3 – 9.5 mg/kg) × 4 SO_4 conc. (40 – 1,500 mg/kg), 4 replicates



SO4-Control SO4-Low SO4-Mid SO4-High SO4-Control SO4-Low SO4-Mid SO4-High

Cucumber in coarse soils Cucumber in fine soils

- 2017: additional test, 4 treatments
  4 Se conc. (0.4 – 5.8 mg/kg) × 1 SO_4 conc. (~ 680 mg/kg)

Toxicity Testing: Invertebrates

Coarse and fine soils, 2 test species (28 to 56 days)

Springtails

Earthworms

Live adults (day 28 for springtails and earthworms)
Juveniles (day 28 for springtails; day 56 for earthworms)
Dry weight of juveniles (earthworms only, day 56)

- 2016-2017: 20 different treatments
  5 Se conc. (0.6 – 30.6 mg/kg) × 4 SO_4 conc. (90 – 1,430 mg/kg),
  3 replicates for springtails, 10 replicates for earthworms

- 2017: additional test, 4 treatments
  4 Se conc. (0.4 – 5.8 mg/kg) × 1 SO_4 conc. (680 mg/kg)

Selenium with Increase Sulphate Doses

Selenium is chemically similar to sulphur, and may be taken up by the sulfate transporter in the root plasma membrane, and compete for the same binding site (Terry, 2000; Wu et al., 2003)

Several studies reported that selenate uptake by plants can be inhibited by sulfate, but only limited studies are quantifying selenate toxicity to plants in the presence of sulfate

Complicated interaction:

- Selenium is toxic at relatively lower doses compared to sulphate
- Sulphate is toxic at relatively higher concentrations
- Sulphate can inhibit the toxicity of selenium
- Some data suggests low levels of selenium may increase the resistance of plants to sulphate toxicity

Dose-Response Analysis

Statistical Analysis and Dose-Response Curve:
(US EPA) Benchmark Dose Software (BMDS), Version 2.7 to 3.2

BMDS assesses the dose-response curves by determining P-values for four different tests.

Hill model is a preferred model in BMDS. The Hill Model is a sigmoidal dose-response curve consistent with receptor-mediated responses (US EPA 2016).

Based on P-values for Tests 1-4 and visual goodness of fit for the curves (V2.7) and model comparison (V3.2), it was concluded that the Hill Model was the best representation of the data for the control sulfate dose.

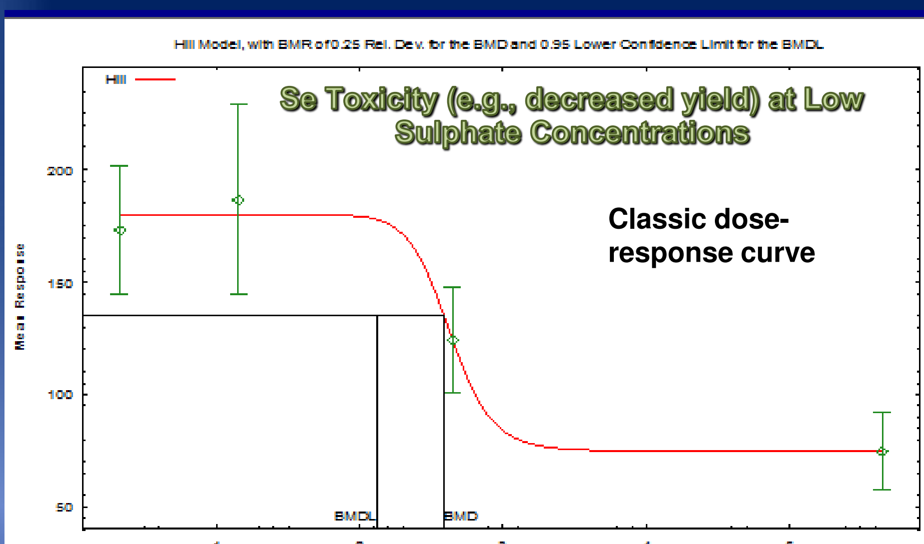
EC_{25} value, calculated by BMDS (Hill model) is used to estimate guidelines

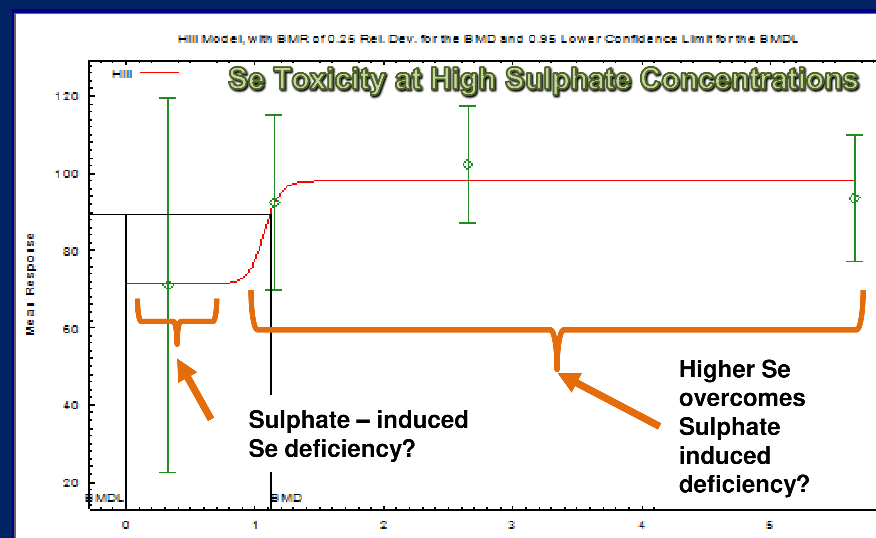
Response Pattern & Statistical Analysis

- Analysis of species-specific and sulphate concentration-specific Se dose response curves – sulphate toxicity dose-response curves also analyzed
  - US EPA BMDS V2.6.0.1 - assesses dose-response curves
  - P-values for four different tests & evaluates best model fit
- Hill model typically best
- EC25 values calculated by BMDS (Hill model) for each species and each sulphate level, at variable Se concentrations

$$\mu\,(X) = \gamma + \frac{\nu \times X^{n}}{k^{n} + X^{n}}$$

Where γ = *intercept (control)*
k = *dose with half-maximal change (must be positive number)*
n = *power (must be a positive number ≤ 18; if n is restricted, the number must be > 1)*
v = *maximum change*





Species Sensitivity Distribution for Selenium Toxicity

Species Sensitivity Distribution Curve is based on rank percentile, derived from EC_{25} values, versus concentration of the chemical. The 25^{th} percentile of the rank distribution, identified as the "estimated species sensitivity distribution – 25^{th} percentile" ($ESSD_{25}$), is used as the basis for soil contact guidelines for the agricultural and residential/parkland land uses (CCME, 2006).

To avoid redundancy, EC_{25} values for the root weight and length, and for the shoot weight and length were calculated as geometric mean and later combined into a single composite response concentration for dry matter. The same way, EC25 for juveniles # and dry weight were geomeaned into a single composite response from earthworms reproduction.

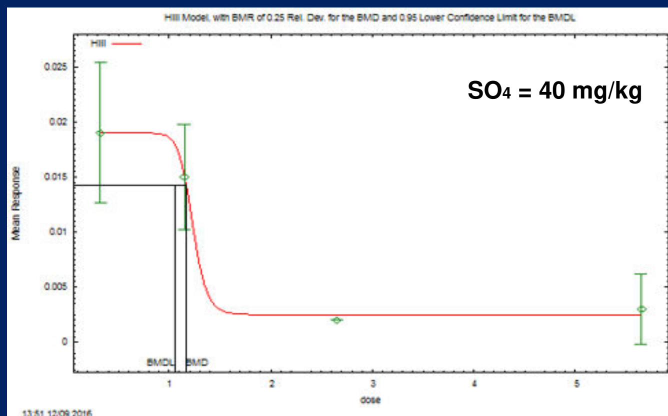
The resulting data were ranked, and rank percentiles determined for each data point. Rank percentiles was be calculated using the equation:

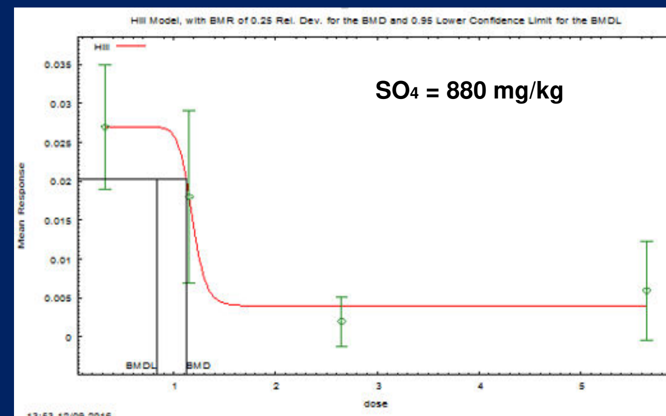
$$j = i/(n-1) \times 100$$

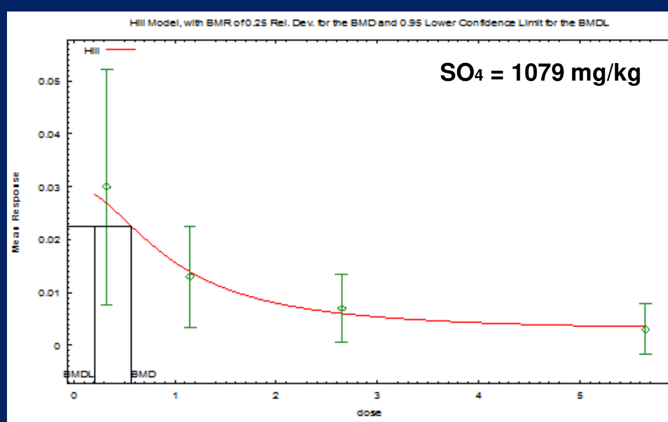
where,
j = rank percentile
i = rank of the data point in the data set
n = total number of data points in the data set

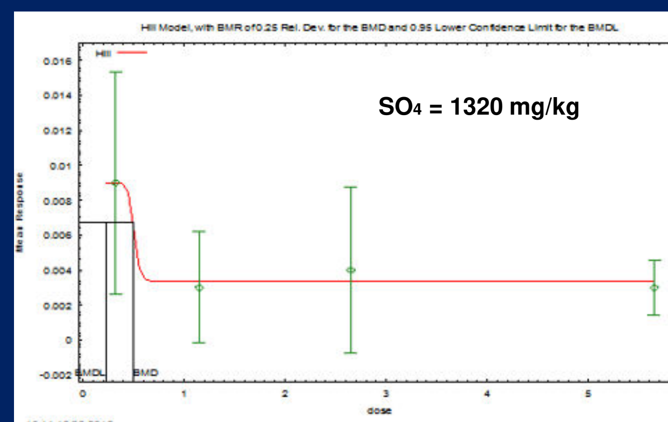
Alfalfa, coarse soils, root weight

No sulphate ameliorative effect, although magnitude of Se toxic effect is decreased at higher sulphate concentrations

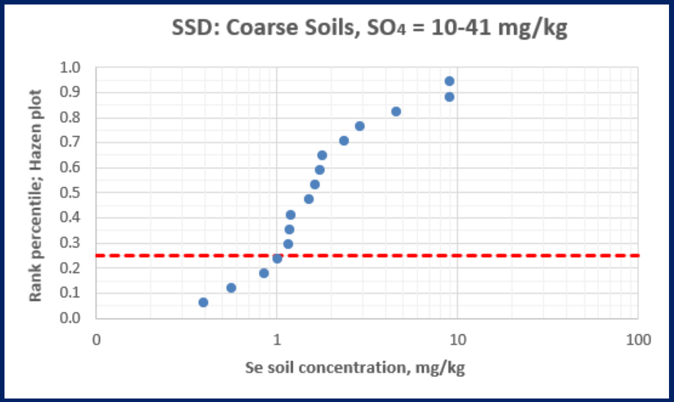


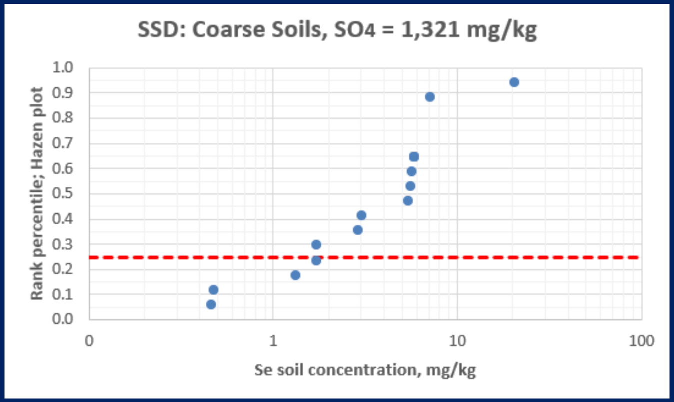


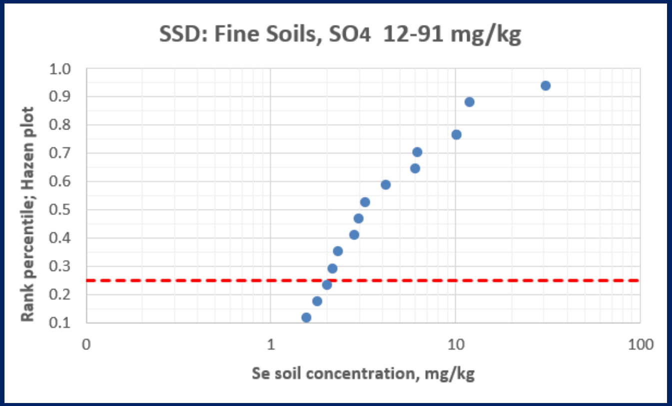


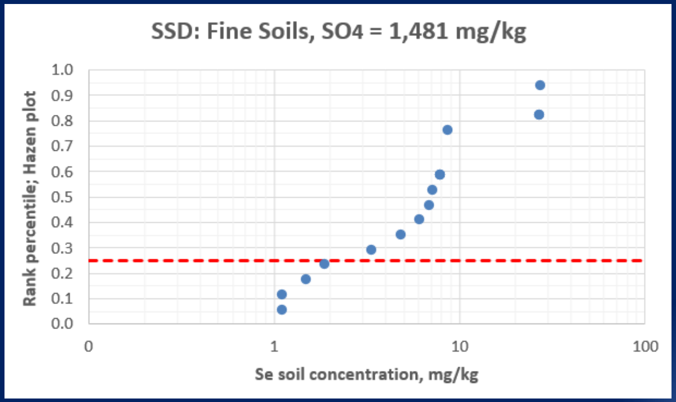


SSD Curve Examples, with Increasing SO_4







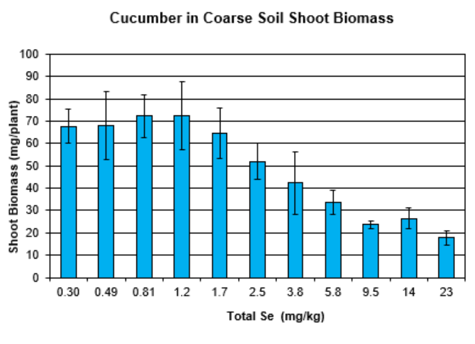


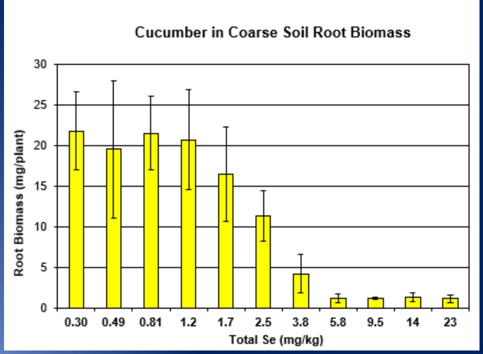
Recent Toxicity Testing: Plants

By: Element Materials Technology

Cucumber, Coarse

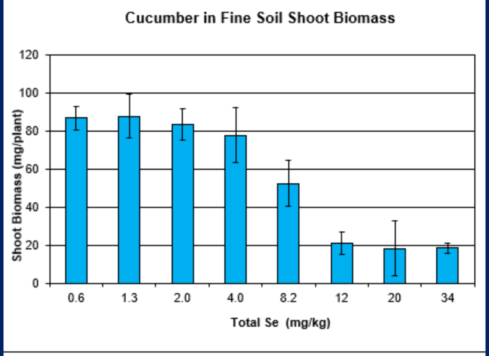
Se range 0.3 – 23 mg/kg
11 doses, 6 replicates

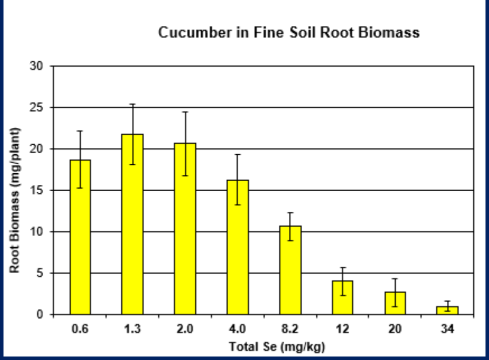




Cucumber, Fine

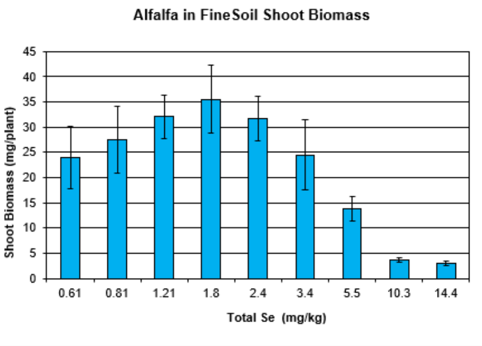
Se range 0.6 – 34,5 mg/kg
8 doses, 6 replicates

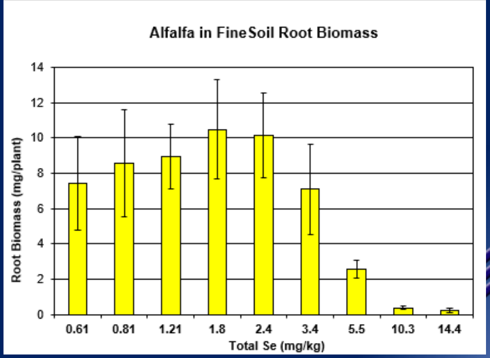




Alfalfa, Fine

Se range 0.6 – 21.6 mg/kg
9 doses, 6 replicates





Alfalfa, Fine Soils

Treatment	Total Se mg/kg
0	0.61
1	0.81
2	1.21
3	1.8
4	2.4
5	3.4
6	5.5
7	10.3
8	14.4
9	21.6

Dose-Response Analysis

(US EPA) Benchmark Dose Software (BMDS), Version 3.2

Cucumber, Coarse, Shoot biomass

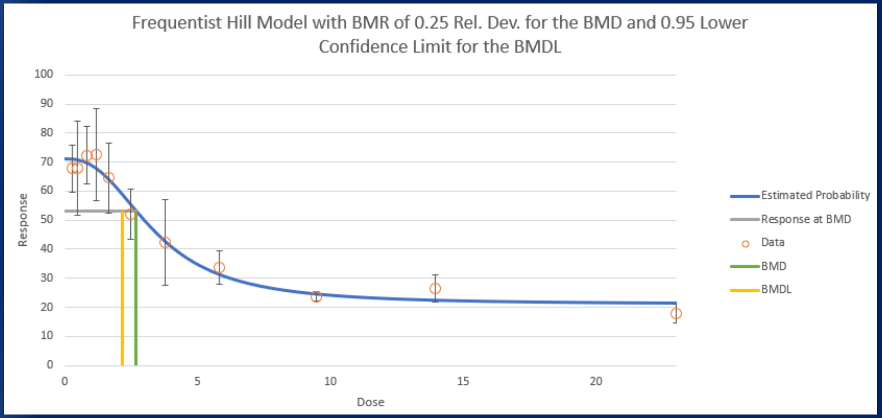
EC_{25} = 2.68 mg/kg

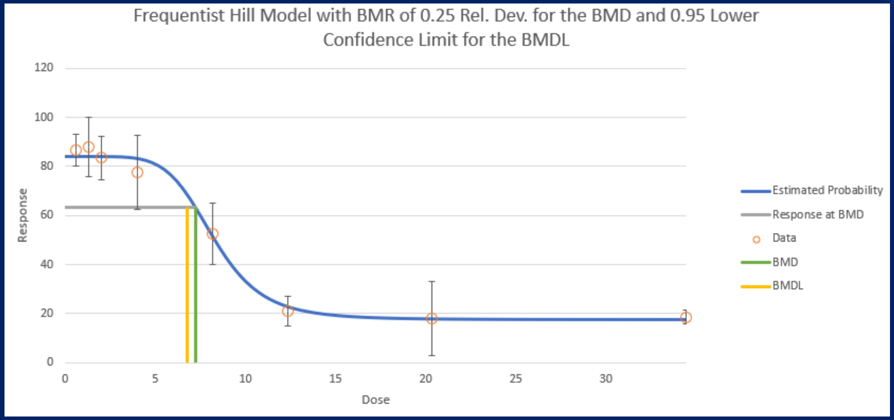
SO_4 (control) = 14 mg/kg

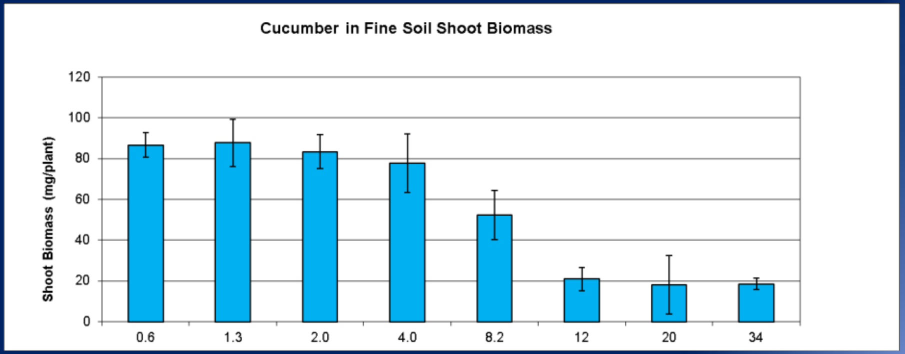
Cucumber, Fine, Shoot biomass

EC_{25} = 7.24 EC_{25}

SO_4 (control) = 53 mg/kg







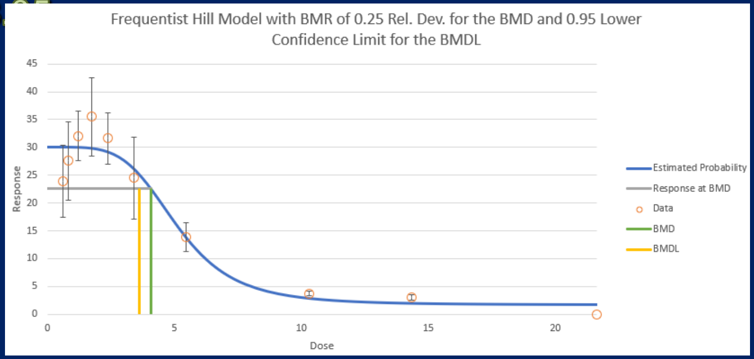
Dose-Response Analysis

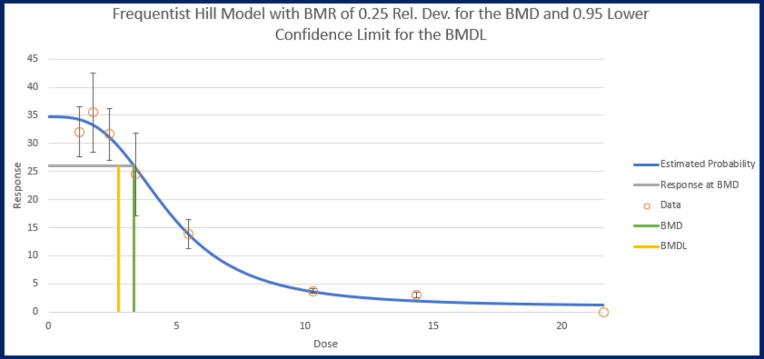
(US EPA) Benchmark Dose Software (BMDS), Version 3.2

Alfalfa, Fine, Shoot biomass, SO_4 (control) = 83 mg/kg

EC_{25} = 4.07 EC_{25} =

3.

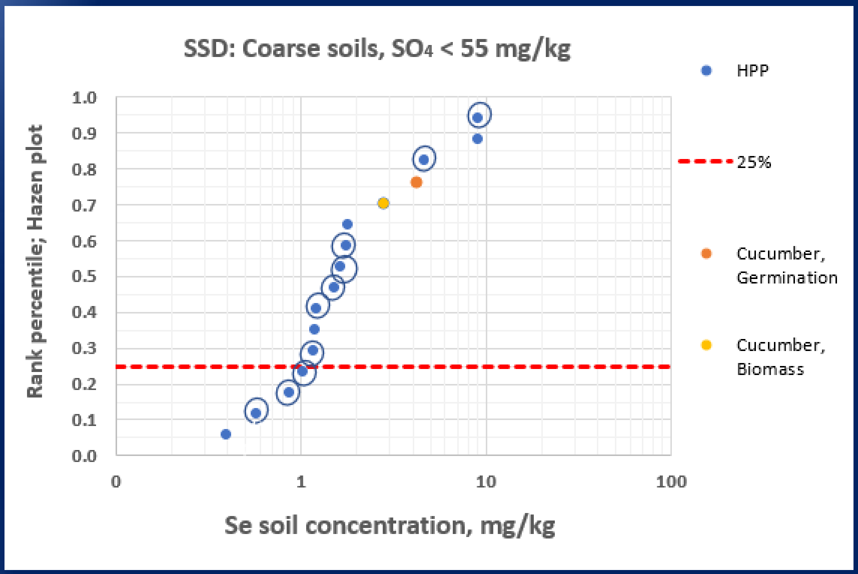


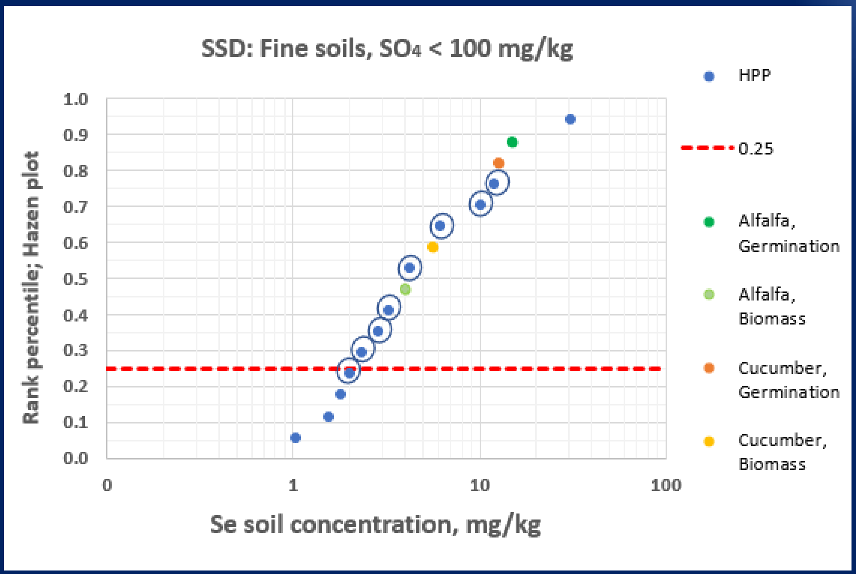


Two lowest Se doses are taken out (potential nutrient deficiency)

Not accounting to the nutrient deficiency may lead to less conservative toxicological effect level and in turn impact the SSD

How new data will affect an SSD curve?



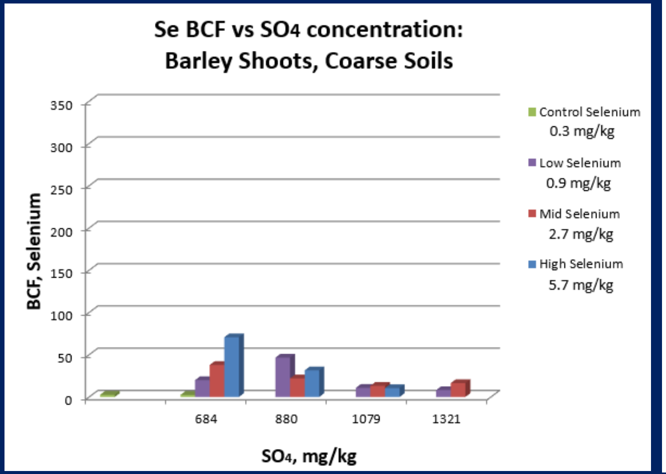


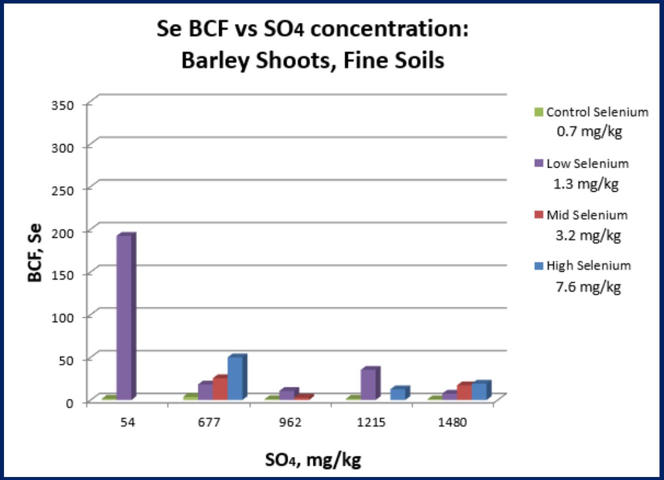
Slight increase in coarse soils

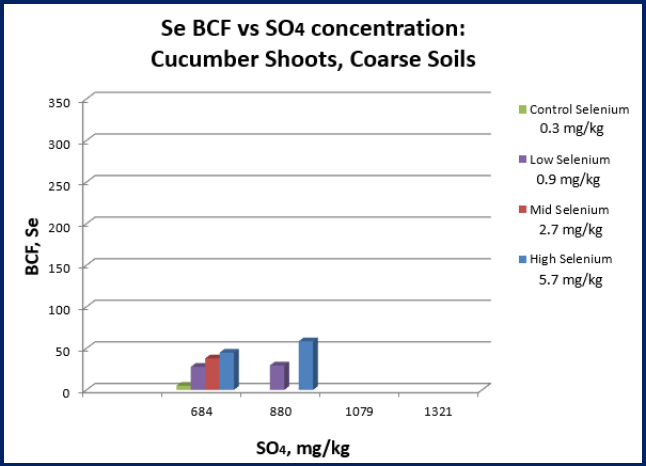
Noticeable increase in fine soils

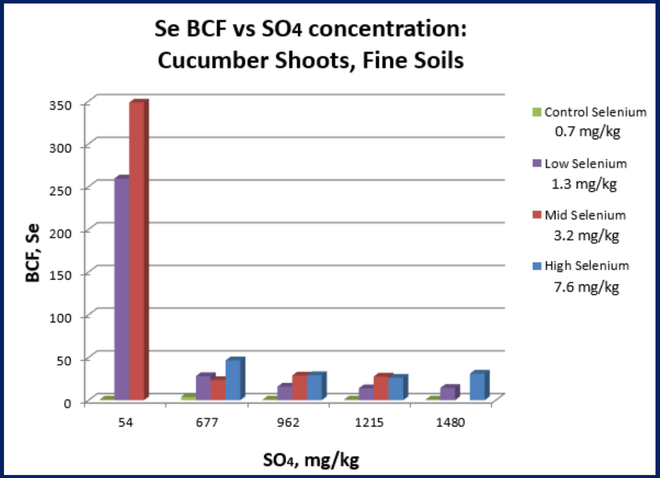
More plant tests will be added (all circled dots)
Potential increase of Se guidelines to 2 mg/kg for fine soils with low sulphate (< 100 mg/kg)

Bioconcentration



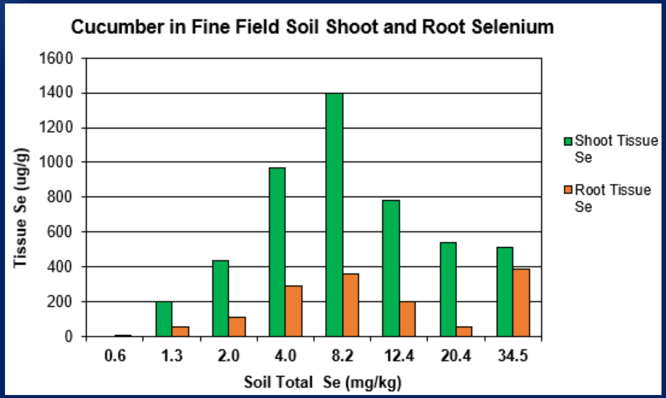


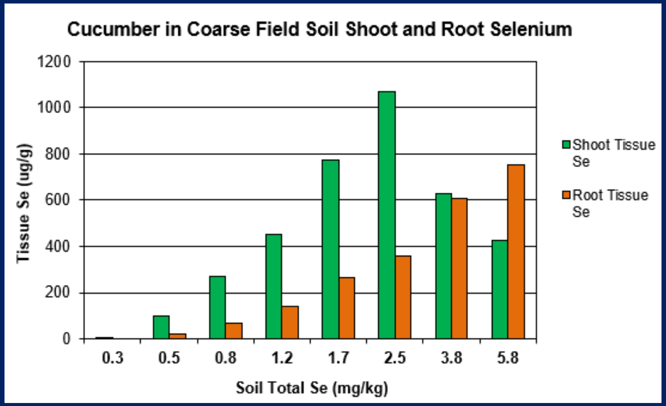


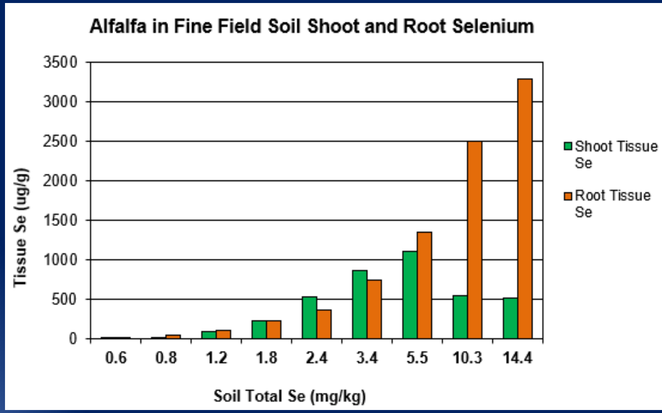


In 2015-2017 studies, the highest Se bioconcentration was observed in SO_4 control groups

2020-2021 Tissue Chemistry







Max. accumulation in shoot for cucumber (14 day test):
at 8.2 mg/kg in fine soils
at 2.5 mg/kg in coarse soils

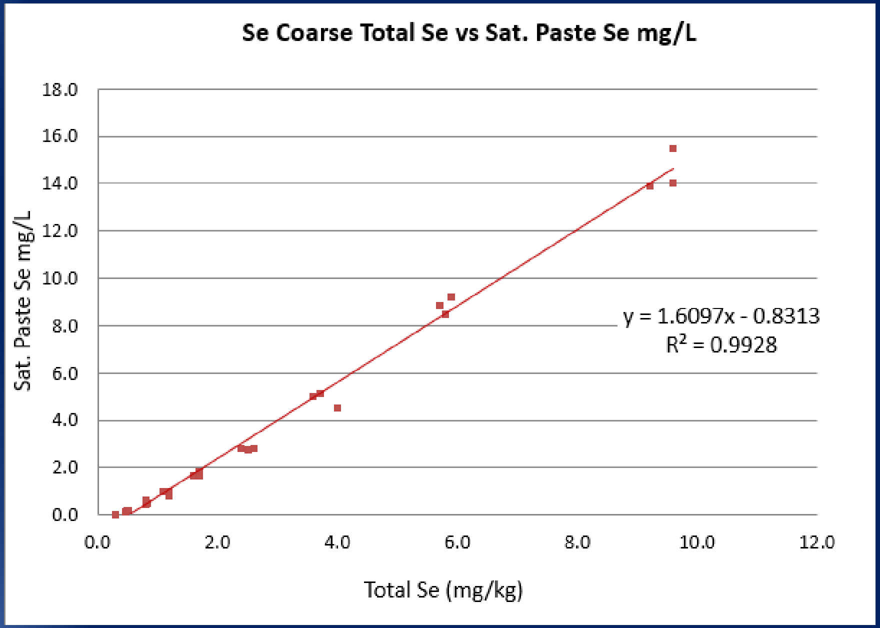
Max. accumulation in root for alfalfa (21 day test):
at 14.4 mg/kg in fine soils

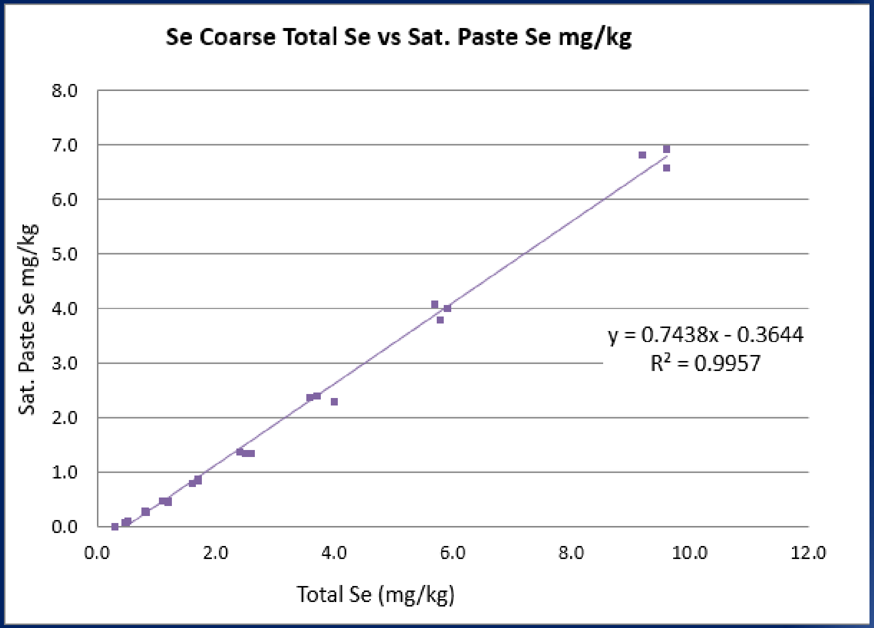
~1,000 ug/g Se accumulated in
4.0-5.5 mg/kg (fine soils)
2.5 mg/kg (coarse soils)

- Would it change for mature plants?
- Would it change if SO_4 added?

Saturated Paste vs Acid Extractable Se

To assess Se bioavailabilty, select soil samples were analyzed for the saturated paste
The sat. paste method was chosen for the more representative measure of the amount of Se in soils available for plants under typical field conditions (Gartley, 2011).





~ 4 mg/kg of total Se is equal to ~ 2.3 mg/kg of sat. paste Se

May be applicable to selenate
Whereas acid digestion method works with selenite

Preliminary Selenium Guidelines

There is a mathematical relationship

- Final models are evaluated as best fit (Easy Fit), as a function of SO_4 concentrations, ranging up to 1,500 mg/kg

- Guidelines with no considerations of SO_4 levels:
  1 mg/kg for coarse soils
  2 mg/kg for fine soils

- Guidelines with consideration of SO_4 levels may range to:
  2 mg/kg for coarse soils
  5 mg/kg for fine soils

Final statistical analyses and guideline developments need to be completed, with additional considerations:

- Bioconcentration factor data
- Wildlife risk assessment: will be using the surrogate species as per AEP of the vole and deer
- Protecting vegetation and wildlife near alkali sloughs: the new guidelines are not expected to be applied near alkali sloughs