Equilibrium Environmental Inc.

For a balanced environment...

Recent Research Results for Selenium and Sulphate Ecotoxicology and Methods for Livestock Risk

October 2024

The Remediation Technology Symposium

Banff, Alberta

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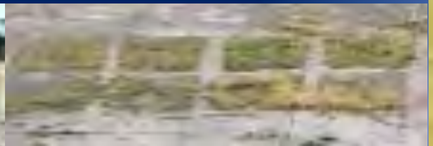

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- ADDITIONAL CONTRIBUTING SCIENTISTS
  - Tyler Prediger

Overview

Toxicity Testing
(Plants, Invertebrates)

Sulphate Effect

Selenate vs Selenite

Reassessment of Selenium Guidelines

Bioconcentration/
Livestock Risk

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

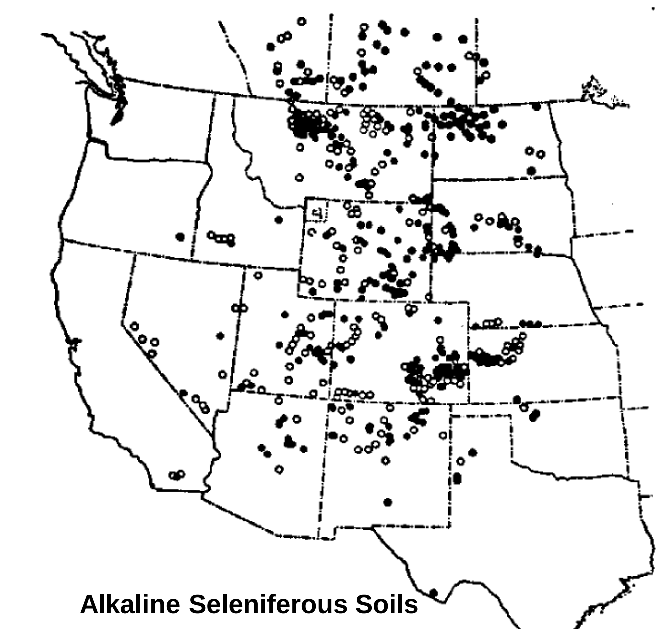
What is Special about Selenium (Se)?

- Non-metal located between sulfur & tellurium: Se$^{34}_{78.96}$

4 valence states (0, II, IV, VI); forms a number of compounds in environment

- Essential micronutrient for humans, animals, beneficial for some plants

- 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)

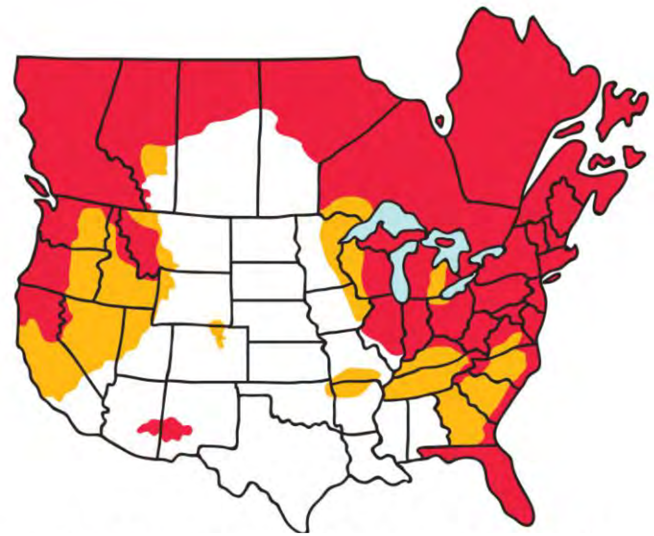


Alkaline Seleniferous Soils

Open dot plant containing 50-500 mg/kg Se

Solid dot plant containing >500 mg/kg Se

(Map adapted from Rosenfield & Beath 1964)



Selenium in Forage and Grain

Red	80% forage contain <0.10 mg/kg Se
Yellow	50% forage contain >0.1 mg/kg Se
White	80% forage contain >0.1 Se

(Map adapted from Ontario MoA website & Horse Journals)

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 (dry wt) 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

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

Plant Uptake and Metabolism

- Differences in plant uptake
  - Selenite preferentially bound to soils and less available for uptake

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

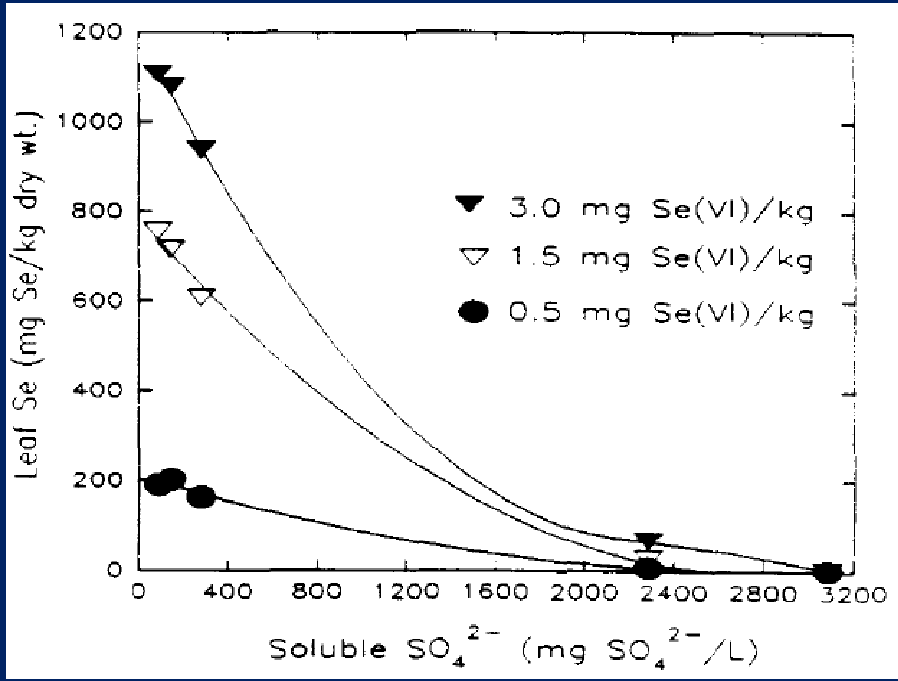
– This may partially explain the lower toxicity of selenite towards livestock consuming plants

  - Selenate readily absorbed and translocated into plant tissue; found in xylem sap as selenite

- This may partially explain the lower toxicity of selenite towards livestock consuming plants grown in clays

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

Selenium with Increase Sulphate Doses

Selenium is chemically similar to sulphur, and may be taken up by the sulphate 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 sulphate, but only limited studies are quantifying selenate toxicity to plants in the presence of sulphate

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

Guidelines & Questions

- The current Soil Selenium (Se) Guideline is 1 mg/kg (Canada and Alberta)
  - limiting pathway is ecological direct soil contact
- Guideline Limitations:
  - background concentrations can be greater than Tier 1 guidelines (Se ranging from 2.3 to 4.7 mg/kg in Canada; CCME 2009)
  - based on two studies (1991 and 1979) using selenate (SeO_4) – studies that were limited in scope
  - may be over-conservative if sulphate is present - sulphate (SO_4) is wide-spread across Alberta and competes with Se uptake
- Redefining the Se guideline would benefit environmental performance through reduced unnecessary remediation efforts on impacts that have a low probability for an unacceptable risk of adverse effects

Weight-of-Evidence Approach

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

  Research Participants

  - Equilibrium Environmental responsibilities
    - identification of data gaps
    - experimental design for additional toxicity testing work
    - toxicity testing work with plants
    - processing results to develop a defensible guideline
    - bioconcentration studies for guideline protection analyses/ livestock risk

  - InnoTech Alberta
    - toxicity testing work with plant and invertebrate species

  - Element Materials Technology
    - toxicity testing work with plants

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

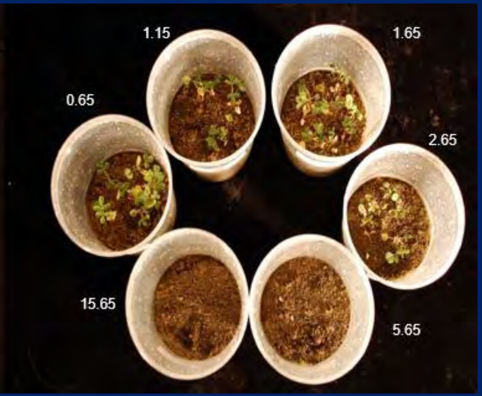
- 2014: 6 different treatments
  6 Se conc. (0.3 – 16 mg/kg) × 1 SO_4 conc. (10-12 mg/kg), 6 replicates
  Selenium in form of selenate (SeO_4)

Plant Growth Endpoints

Percent (%) seedling emergence (day 7)

Root and shoot length and dry weight

Alfalfa in coarse soils

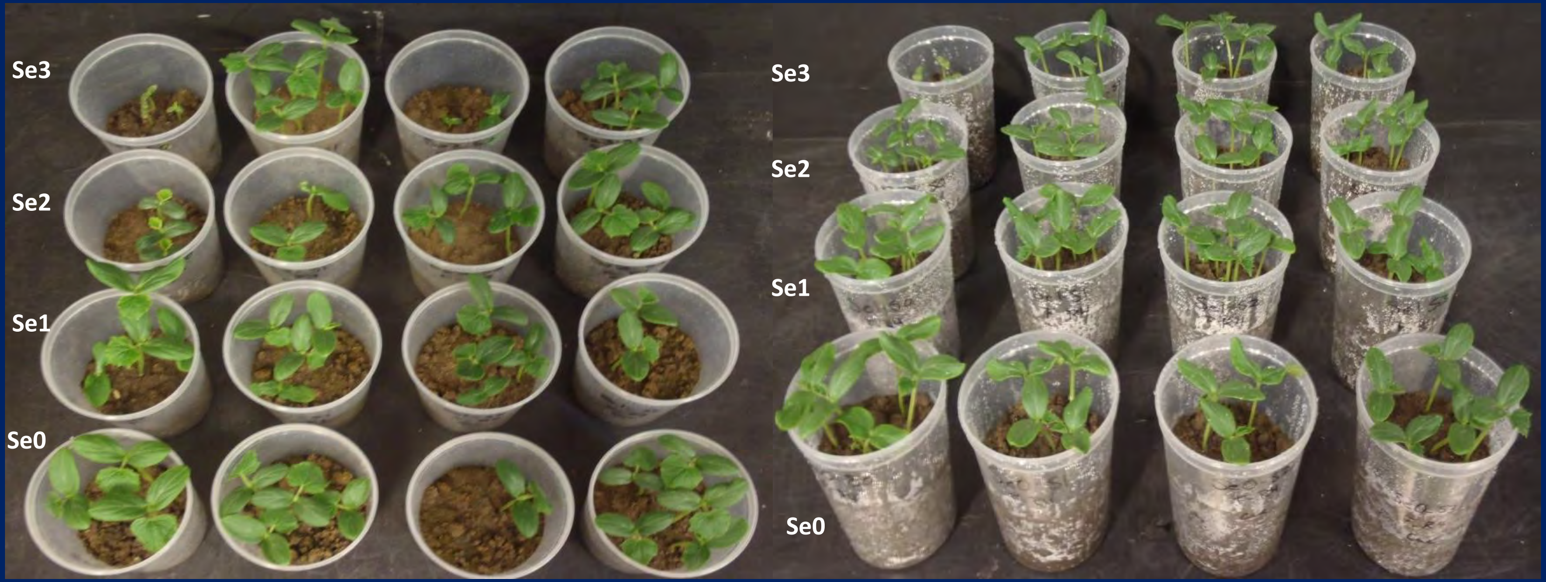


Alfalfa in fine soils

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

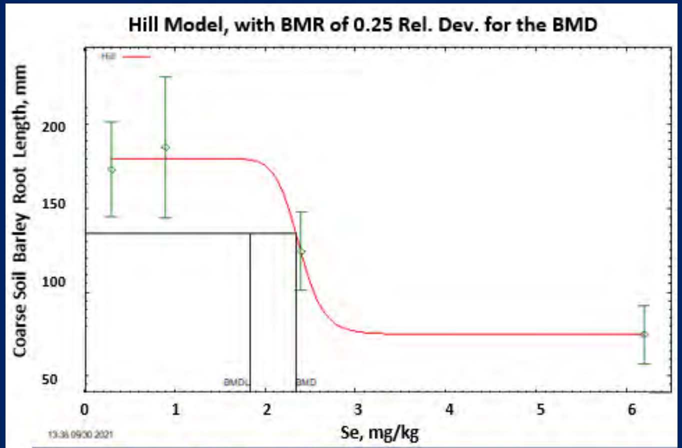
Earthworms

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)

Dose-Response Analysis

Se toxicity; no SO_4 effect



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   Test 4          1.34828          1          0.2456

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the the data


        Benchmark Dose Computation

Specified effect =          0.25

Risk Type        =     Relative deviation

Confidence level =           0.95

             BMD =         2.34641

            BMDL =        1.83765
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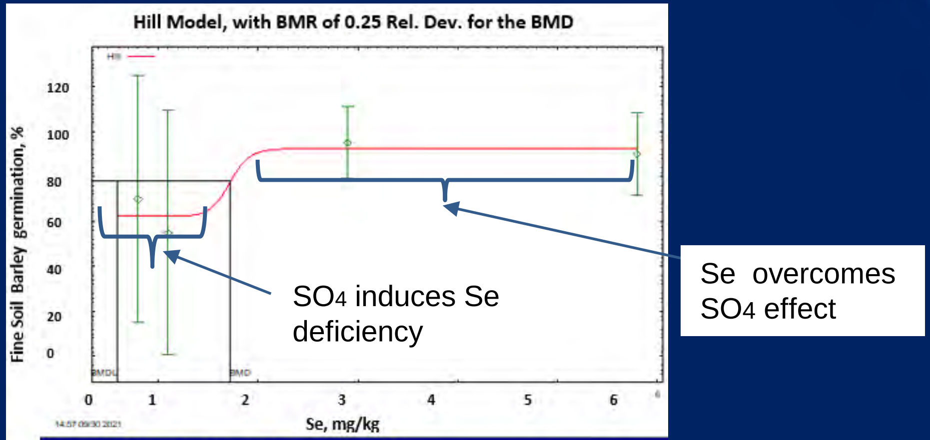
Se toxicity with SO_4 outcompeting effect



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The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =          0.25

Risk Type        =     Relative deviation

Confidence level =           0.95

             BMD =        1.7239

            BMDL =       0.594266

            BMDU =        290000
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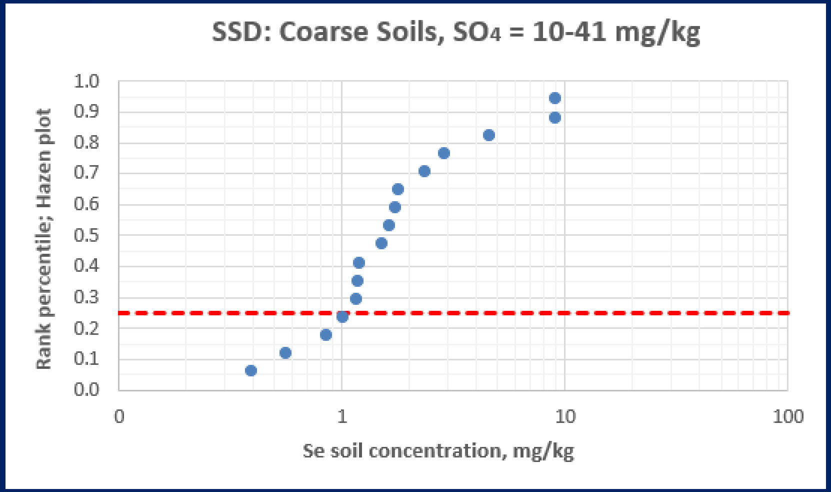
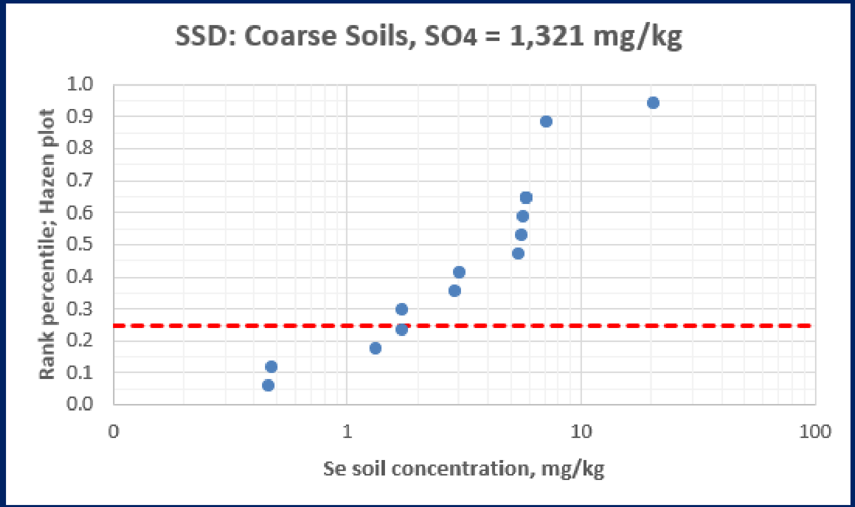
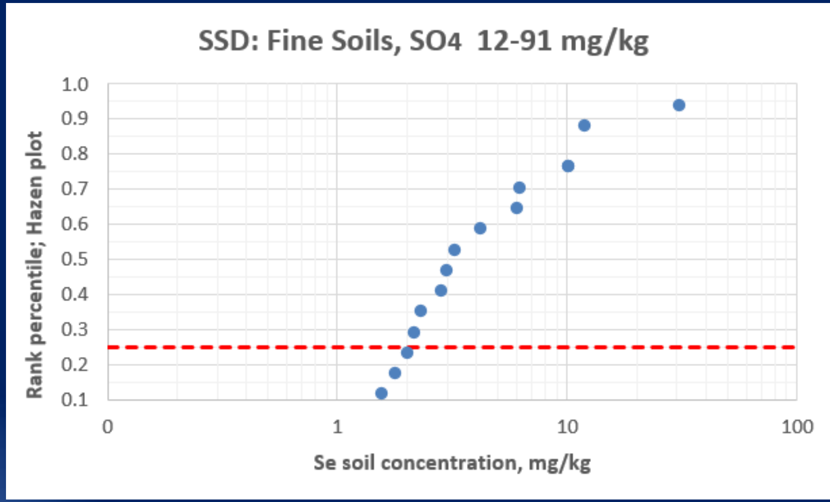
Statistical Analysis and Dose-Response Curve: (US EPA) Benchmark Dose Software (BMDS), Version 2.7 to 3.3

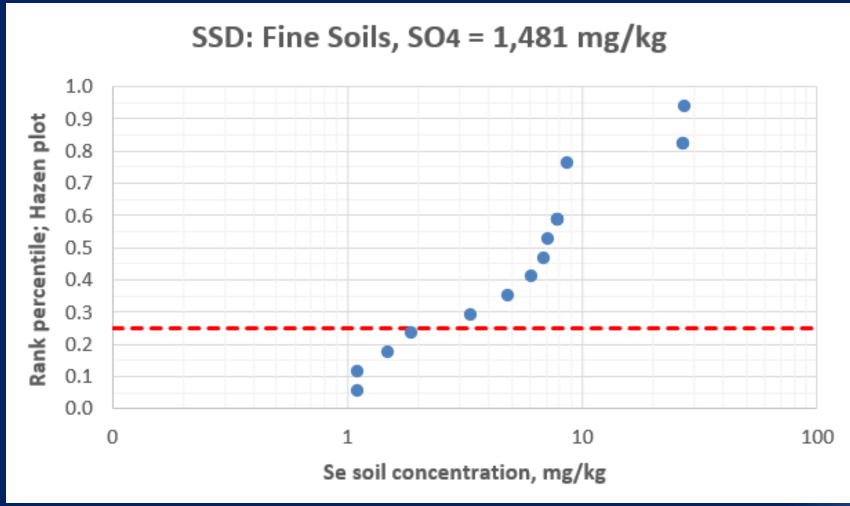
Species Sensitivity Distribution

SSD Curve Examples, with Increasing SO_4









2014-2017 Studies Limitations

If no effect observed, EC25 is assumed to be equal to the highest Se dose

- Conservative approach; the highest Se dose was 9.5 mg/kg for plants
- Actual EC25 may be higher
- Sulphate toxicity (SO_4 EC25 lower than the highest SO_4 treatment observed in 50% of endpoints in coarse soils, and in 20% in fine soils
- Data gap between control SO_4 (15-90 mg/kg) and ~1,000 mg/kg concentrations

2017 experiment (with SO_4 concentrations of 680 mg/kg) may have significant interference due to various amount of artificial light; overall, 9% and 5% of 2015 to 2017 data were excluded from the coarse and fine soil data pools, respectively

Toxicity Testing: Plants

- Definitive tests: Element Materials Technology (2020-2023)
  Confirmed the range of potential Se toxicity:
  up to 20 mg/kg for fine soils, and up to 15 mg/kg for coarse 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

Toxicity Testing: Plants

- $Se\text{-}SO_4$ interaction: Equilibrium Environmental (2024 – on-going)

2024: 16 different treatments (14 to 21 days)

  - 4 Se concentrations (control, 5 mg/kg, 10 mg/kg, and 15 mg/kg) for coarse soils
  - 4 Se concentrations (control, 5 mg/kg, 10 mg/kg, and 20 mg/kg) for fine soils
  - 4 SO_4 concentrations (control, 250 mg/kg, 500 mg/kg, and 1,000 mg/kg) for all soils
  - 4 replicates per treatment

- 5 plant species: Alfalfa, Barley, Cucumber, Northern Wheatgrass, Red Fescue

- Extensive soil preparation work; preliminary mixing trials to understand optimal spiking proportions

- Germination pre-test to evaluate seed quality and pick up the most suitable plant species variety

Soil Preparation

- Soil testing for the water holding capacity and desired moisture content
- Fume hood dried soils (not oven baked, to save potentially beneficial organisms), to 5% moisture in coarse soils and 10 % moisture in fine - this way, soils will adsorb the water solution volume with target salt concentrations
- Sieved with 4 mm – helps in homogenization
- Salt solubility testing - to ensure that the salts added for the sulphate doses ($CaSO_4$ + $MgSO_4$ +Na_2SO_4) are not associated with precipitation that would affect the experimental results
- Salt solution (equal distilled water for control samples) was added to the fume hood dried, sieved soils and carefully mixed until crumbly appearance with aggregates around 2 mm in diameter.

Toxicity Testing: Barley (Cowboy var.)

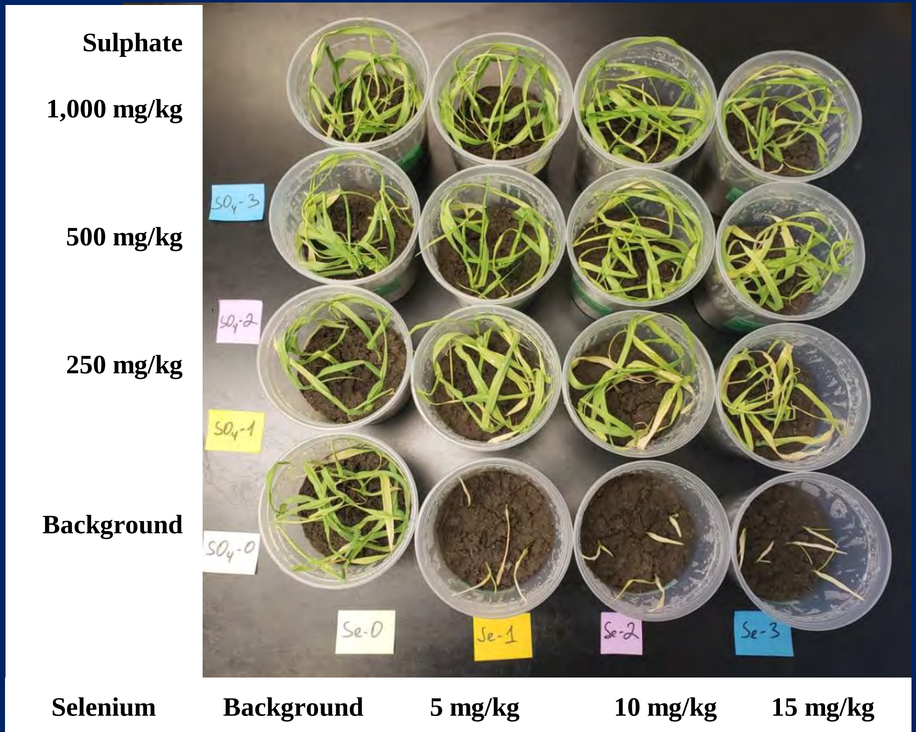
- Barley (Cowboy var., acquired at Warburg Seeds), has been tested first

– 100% germination on emergence pre-test in all type of soils: artificial, fine, and coarse

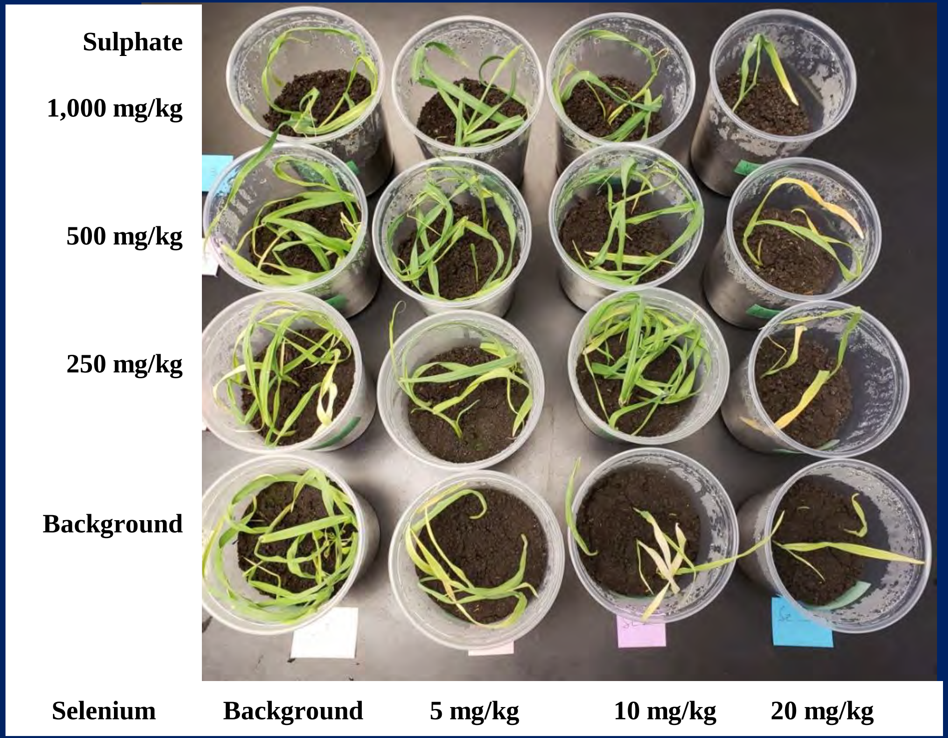
Barley Cowboy, Coarse

Barley Cowboy, Fine

Toxicity Testing: Barley (Cowboy var.)



Barley Cowboy, Coarse



Barley Cowboy, Fine

Dose-Response Analysis

Barley, Coarse, Shoot and Root Length

Se = 15 mg/kg

SO_4 = 15 mg/kg

Se = 15 mg/kg

SO_4 = 1,000 mg/kg

Barley, Fine, Shoot and Root Length

Se = 5 mg/kg

SO_4 = 50 mg/kg

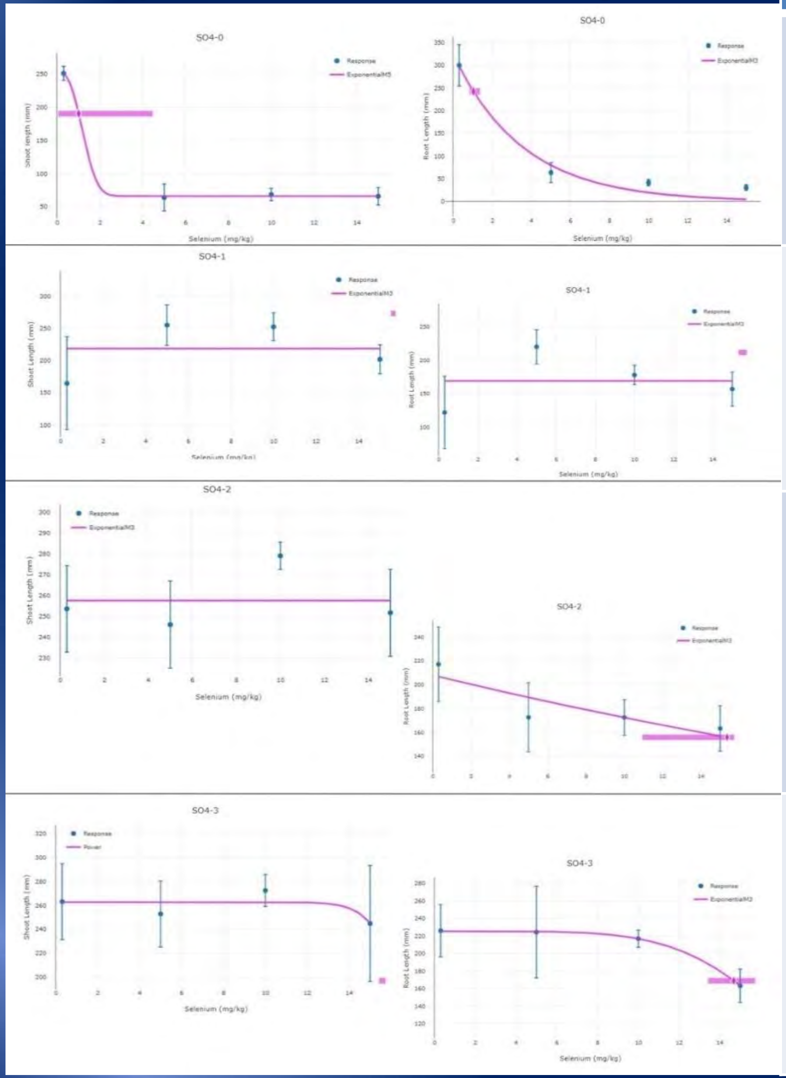
Se = 5 mg/kg

SO_4 = 250 mg/kg

Dose-Response Analysis

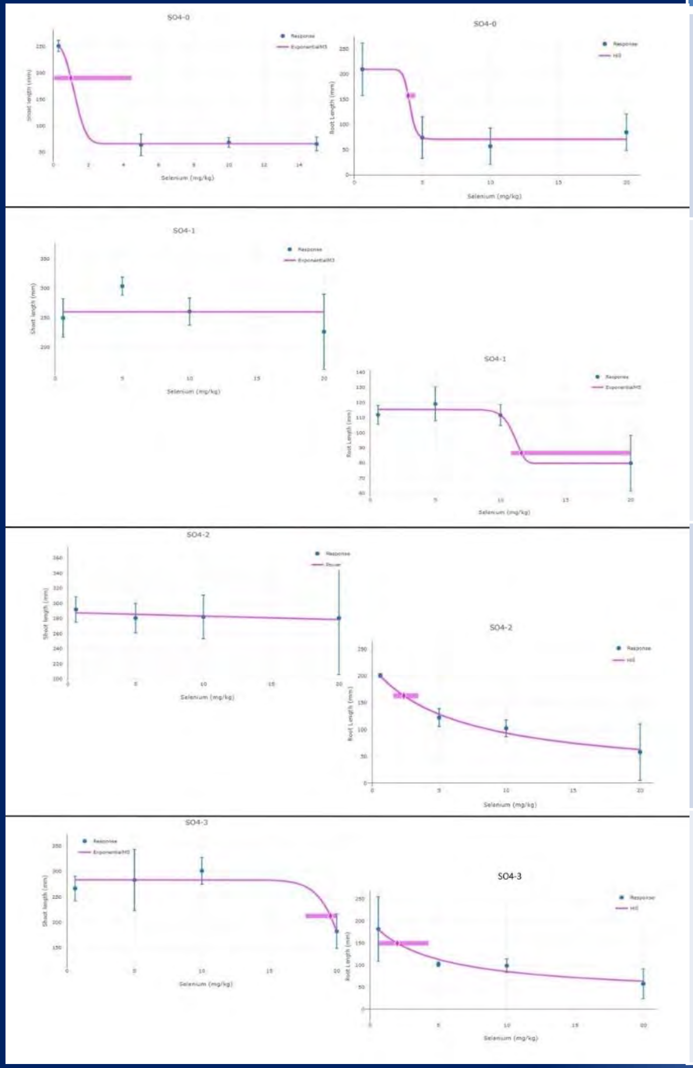
(US EPA) Benchmark Dose Software (BMDS), Version 3.3

Barley Coarse, Shoot and Root Length EC25



SO_4, mg/kg	Se, mg/kg
15	1.0
250	15.4
500	15.9
1,000	15.4

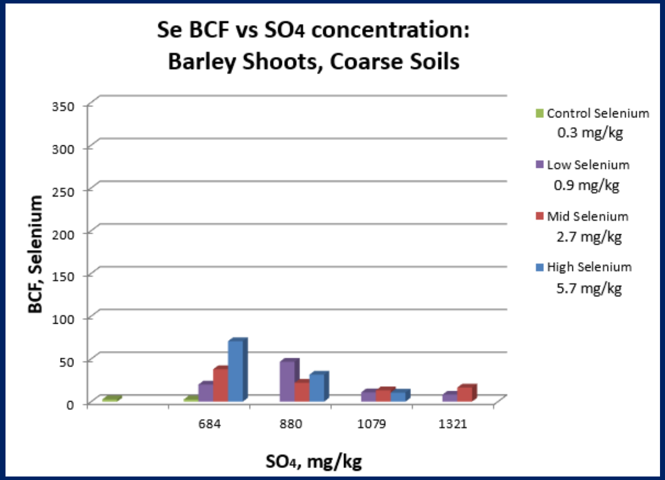
Barley Fine, Shoot and Root Length EC25

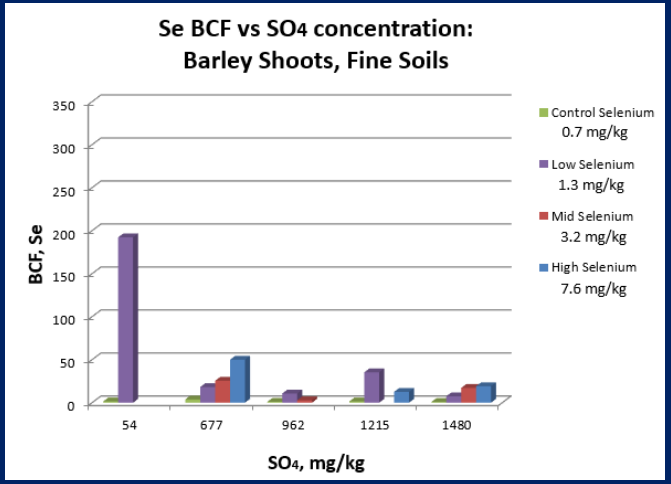


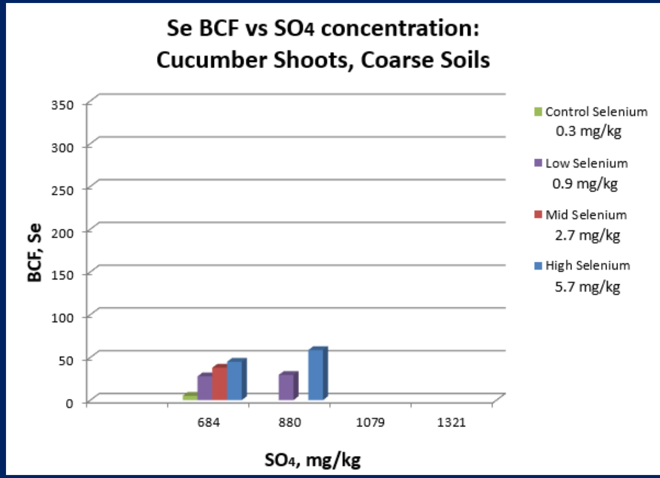
SO_4, mg/kg	Se, mg/kg
50	4.1
250	20.9
500	7.1
1,000	6.3

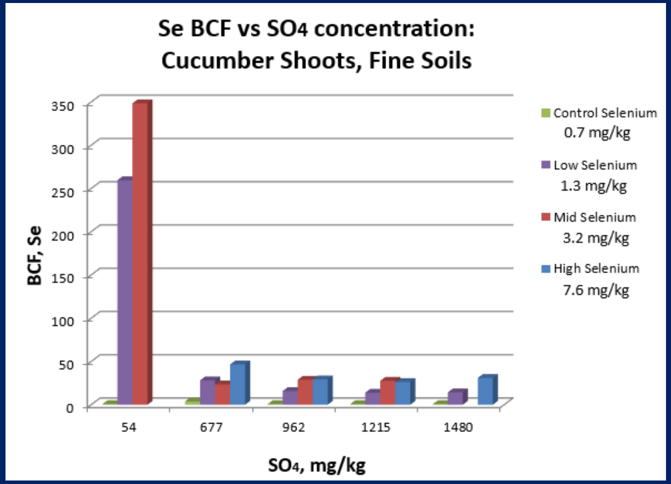
Bioconcentration Studies for Livestock Protection

Short-Term (14 days) Plant Tissue





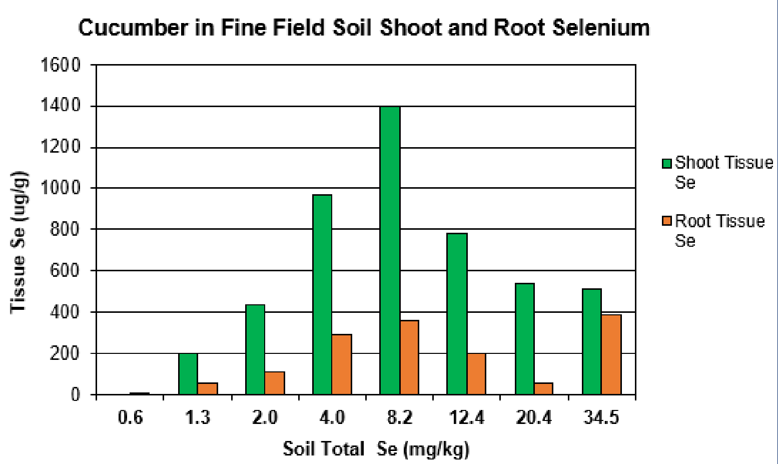


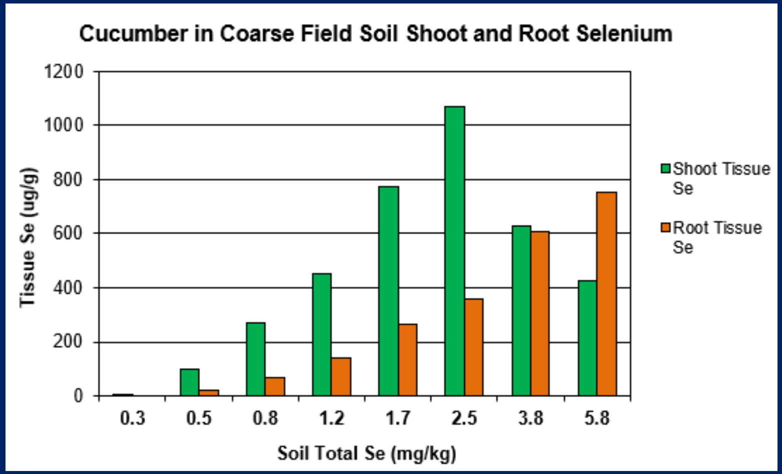


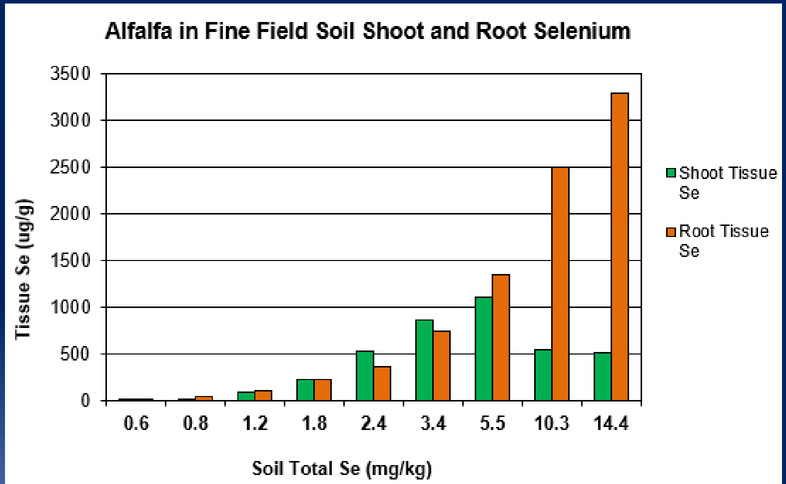
In 2015-2017 studies, the highest Se bioconcentration was observed in SO_4 control groups: - very low sulphate concentrations

2020-2021 Tissue Chemistry

Short-Term (14-21 days) Plants Grown in Soils with Very Low Sulphate







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 (i.e., 4 months test duration)?
- Would it change in soils with higher sulphate concentrations?

Preliminary Selenium Guidelines

There is a mathematical relationship

- Final models will be evaluated as best fit (Easy Fit), as a function of SO_4 concentrations (potential range to 1,000 mg/kg)

- Guidelines with no considerations of SO_4 levels may be shifted to:
  - 1 mg/kg for coarse soils
  - 3 mg/kg for fine soils

- Guidelines with consideration of SO_4 levels may range to:
  - 2 mg/kg for coarse soils (more plants to be tested)
  - 5 mg/kg for fine soils (more plants to be tested)

Final statistical analyses and guideline developments will be based on the final dataset for all tested species (historical information combined with 2024 plant toxicity studies) with additional considerations:

- Bioconcentration factor data (long-term bioconcentration studies)

- Wildlife risk assessment: will be using the surrogate species as per AEP of the vole and deer

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