Equilibrium Environmental Inc.

For a balanced environment...

Finalization of Research for Selenium and Sulphate Ecotoxicology

October 2025
The Remediation Technology Symposium
Banff, Alberta

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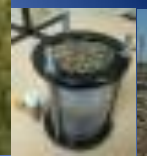







Acknowledgements

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

- EQUILIBRIUM TEAM
  - Anthony Knafla, Vicky Winter, Jenny Zaiser, Danielle Schmidt, Oleg Korotin

- ALS ENVIRONMENTAL

- ADDITIONAL CONTRIBUTING SCIENTISTS
  - Tyler Prediger
  - Jay Woosaree
  - Dr. Dani Degenhardt
  - Darlene Lintott

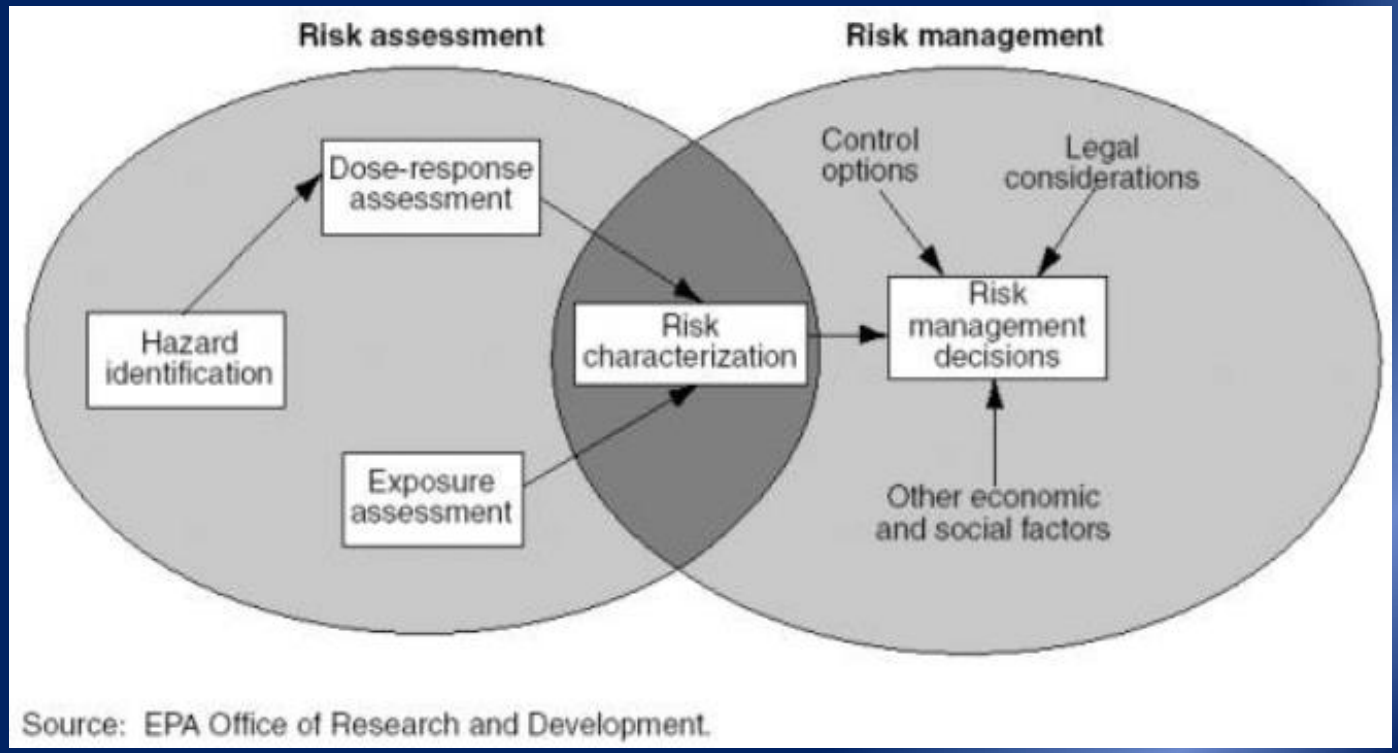
What Drives Risk Assessment, Risk Management, & Guideline Development

- Exposure Assessment
  - Typically modified when calculating Tier 2 guidelines
  - Variables include land use, soil type, groundwater characteristics, climate, etc.
  - Sometimes we use different mathematical fate and transport models
- Dose response (Toxicity) Assessment
  - Typically not modified when calculating Tier 2 guidelines
  - Complicated – data needs, chemical interactions

  - This project addresses toxicity side of equation for ecological receptors

  - Just two chemicals (Se & SO_4)
  - But highly complicated interactions

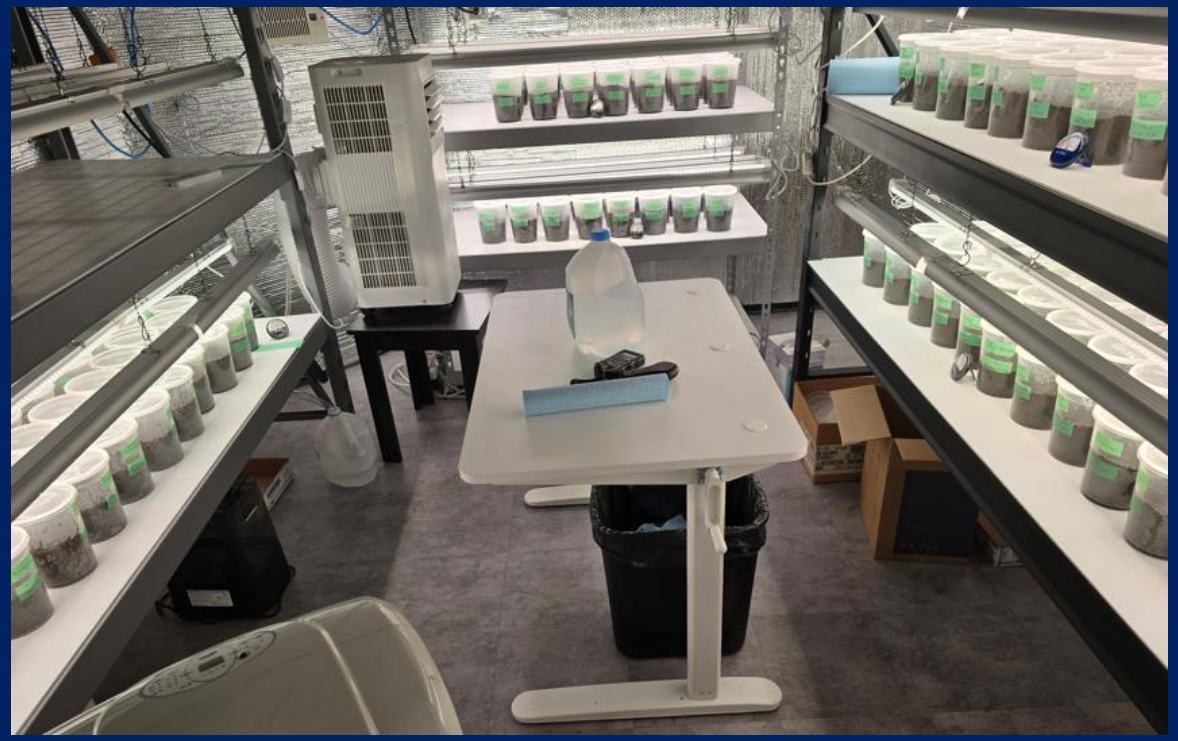
  - Nutrient effects, inhibitory effects, potential cumulative toxic effects



Source: EPA Office of Research and Development.

Ecotoxicology

- Equilibrium developed an ecotoxicology lab for invertebrates and plants
  - Progressing through National accreditation process
  - Predict long-term adverse health consequences in complex ecosystems
  - natural area, agricultural, native prairie
  - Work can be used to develop Ecotoxicological Benchmarks (EBs), classed under the term Toxicity Reference Value (TRV)
  - These can be used by regulators to incorporate into guideline updates



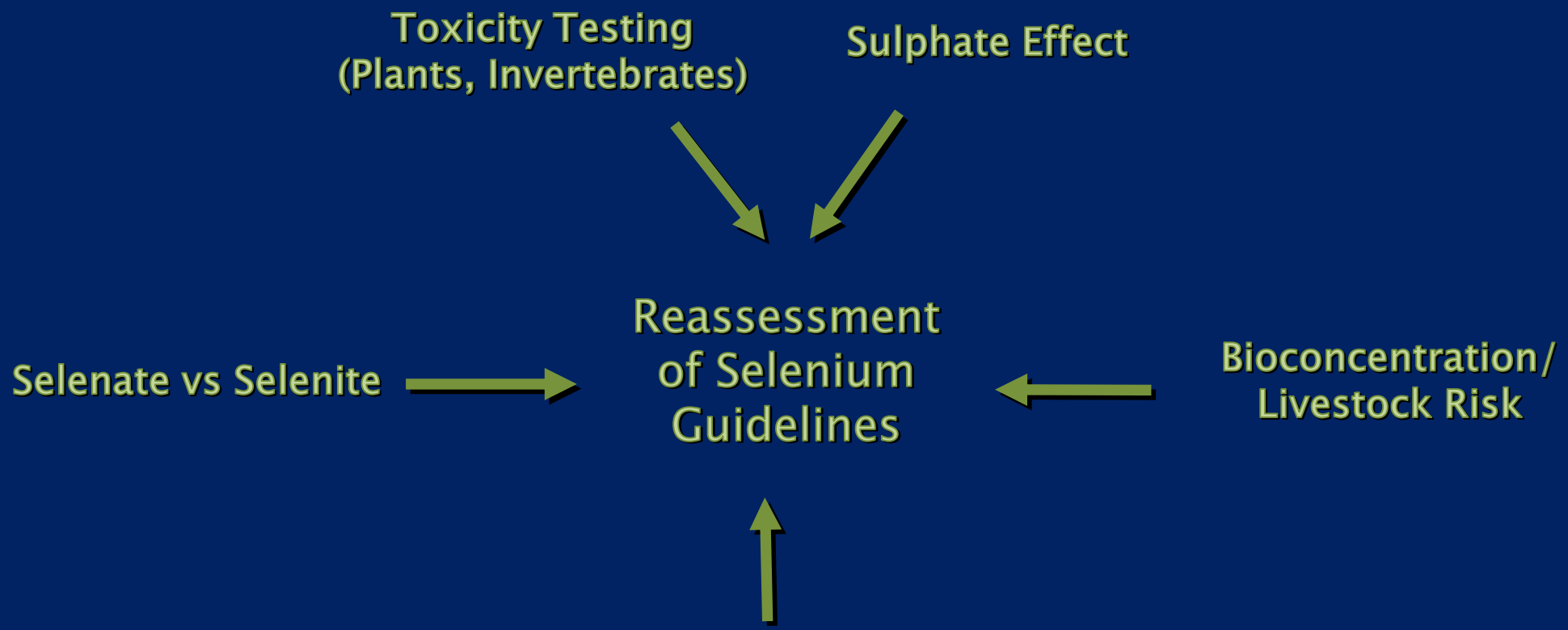
Ecotoxicology

- How do you apply this kind of testing at the site-specific level?
  - There is a process in BC for completing this kind of approach

- In Alberta, you can potentially implement EcoTRVs on a site-specific basis if:
  - Conducting a SSRA on a **substance with no guideline available** in Table 2 of Alberta Environment and Parks 2022 '*Supplemental guidance on site-specific risk assessments in Alberta*' (ISBN 978 1 4601 5490 8) policy doc
  - **Cannot readily apply these Table 2 sources as TRVs**
  - There are paths to trigger these clauses and develop site-specific EcoTRVs
  - Examples might include, weathered crude oil (or F2 fraction hydrocarbons) with methylated PAHs not in AB T1, solvents, or metals, where the difference in toxicity of weathered versus unweathered with simultaneous exposure to metals, solvents, etc. may produce a toxic response not reflected in Tier 1
  - The are four approaches for selecting/developing site-specific EcoTRVs, one of which is site-specific toxicity testing such as the work present herein
  - National guidance for ecotoxicity testing - CCME & Environment Canada

  - '*The appropriate regulator must be consulted regarding the development and/or use of EcoTRVs other than those values used in Tier 1 guidelines and Alberta surface water guidelines*' (EPA, 2022)

Guideline Development



What is Special about Selenium (Se)?

- Non-metal located between sulfur & tellurium: $^{34}_{78.96}Se$
  4 valence states (0, II, IV, VI); forms several 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)

- Typical soils selenium concentrations range up to 2.3 mg/kg in Alberta
  Can range up to 4.7 mg/kg in the Western Sedimentary Basin (CCME 2009)

  - Alberta Tier 1 Guideline of 1 mg/kg

- 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

Selenium - Sulphate Interaction

- Selenium is chemically similar to sulphur, and may compete for the same binding site
- Several studies reported that selenate uptake by plants can be inhibited by sulphate
- Could lead to a reduction in plant toxicity or livestock toxicity from consuming plants
- 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 Guideline (PTAC funded project)

  Research Participants

  - Equilibrium Environmental Inc. responsibilities
    - identification of data gaps (started in 2012)
    - experimental design and scope developed for additional toxicity testing work to support guideline development (started in 2013)
    - toxicity testing work with plants (2024-2025)
    - processing of commissioned toxicity testing results to develop a defensible guideline (2025-2026)
    - bioconcentration studies (2026)
  - InnoTech Alberta
    - toxicity testing work with plants and invertebrates (2015-2017)
  - Element Materials Technology
    - toxicity testing work with plants (2020-2023)

Previous Toxicity Testing

- Started in 2012 (T. Prediger, A. Knafla)
  Artificial soils, Alfalfa; Se-SO_4 interaction


- Continued in 2014-2017 (Equilibrium & InnoTech Alberta)
  Coarse and fine sandy loam soils (collected in Alberta)
  Se-SO_4 interaction
  6 plants: Alfalfa, Barley, Carrot, Cucumber, Northern Wheatgrass, Red Fescue
  2 invertebrates : Springtails, Earthworms


- 2020-2023 (Equilibrium & Element Materials Technology)
  Coarse and fine sandy loam soils (collected in Alberta)
  Definitive tests to confirm the range of potential Se toxicity to plants:
  up to 20 mg/kg for fine soils, and up to 15 mg/kg for coarse soils

2014-2017 Studies Limitations

If no effect observed, EC_{25} is assumed to be equal to the highest Se dose

- Conservative approach; the highest Se dose was 9.5 mg/kg for plants
- Actual EC_{25} may be higher
- Sulphate toxicity (SO_4 EC_{25}) 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 added 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

Se-SO_4 Most Recent Studies

Main plant test by Equilibrium Environmental (2024 – 2025)

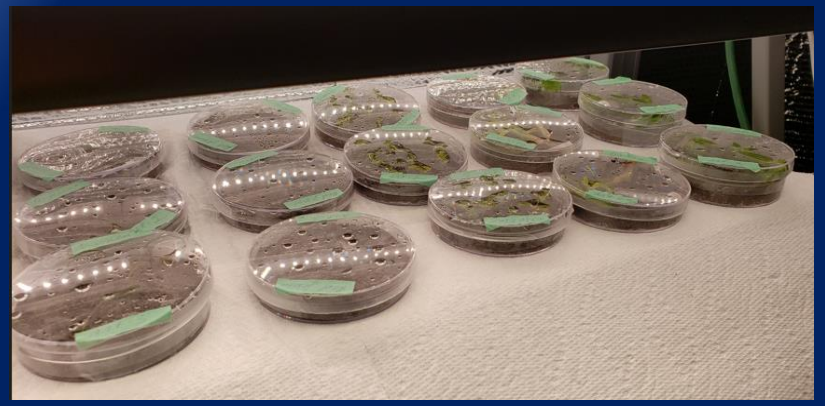
5 plant species: Alfalfa, Barley, Cucumber, Northern wheatgrass, Red fescue
2 soil types: natural coarse and fine soils for main test; artificial soil negative control
14 to 21 days test duration

16 different treatments
- 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

Biological endpoint
- Germination %
- Growth (Root and shoot length, dry root and shoot weight)

2024-2025 Plant Testing in Ecotox Lab

Emergence Pre-test



Barley Cowboy

Cucumber Marketmore 76

Main Test, General View

Cucumber – Test End

Root and Shoot Measurement

Dose-Response: Barley (Cowboy var.)

Coarse

Fine

Se = 15 mg/kg

SO_4 ~ 15 mg/kg

Se = 15 mg/kg

SO_4 = 1,000 mg/kg

Se = 5 mg/kg

SO_4 ~ 90 mg/kg

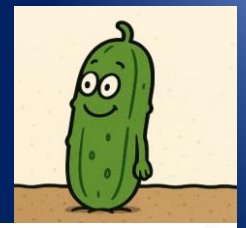
Se = 5 mg/kg

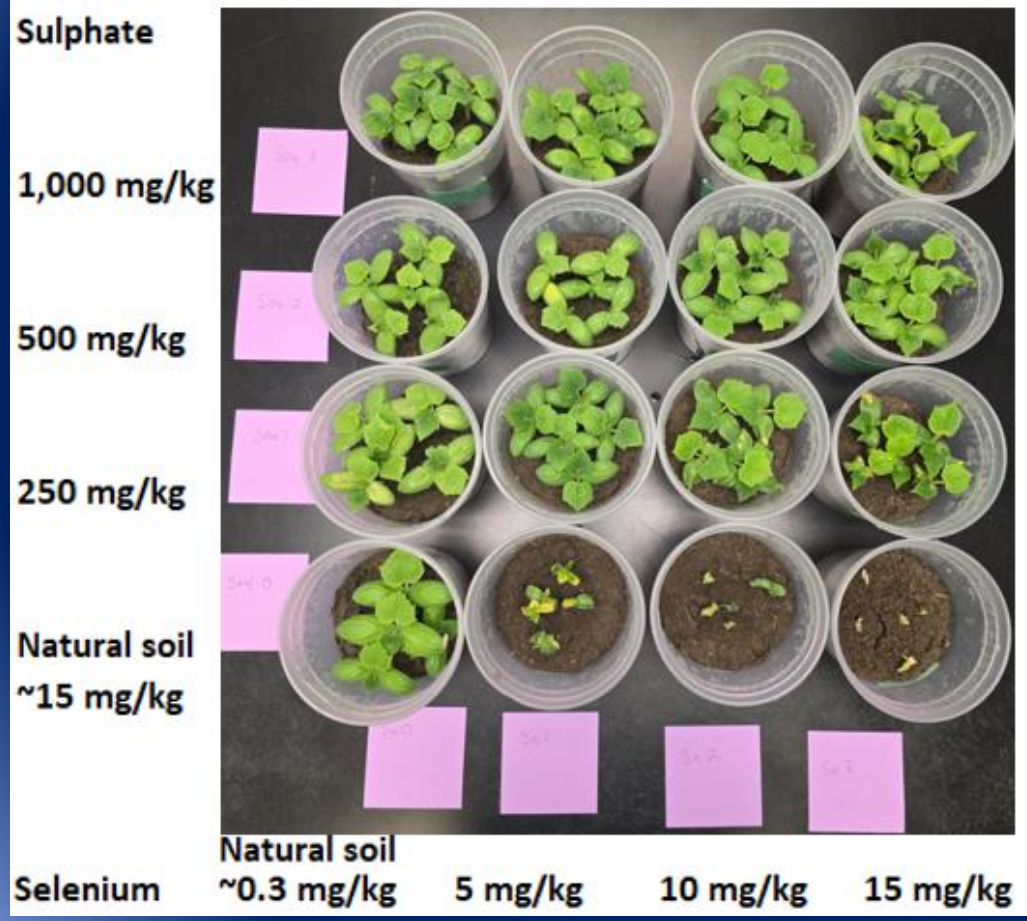
SO_4 = 250 mg/kg

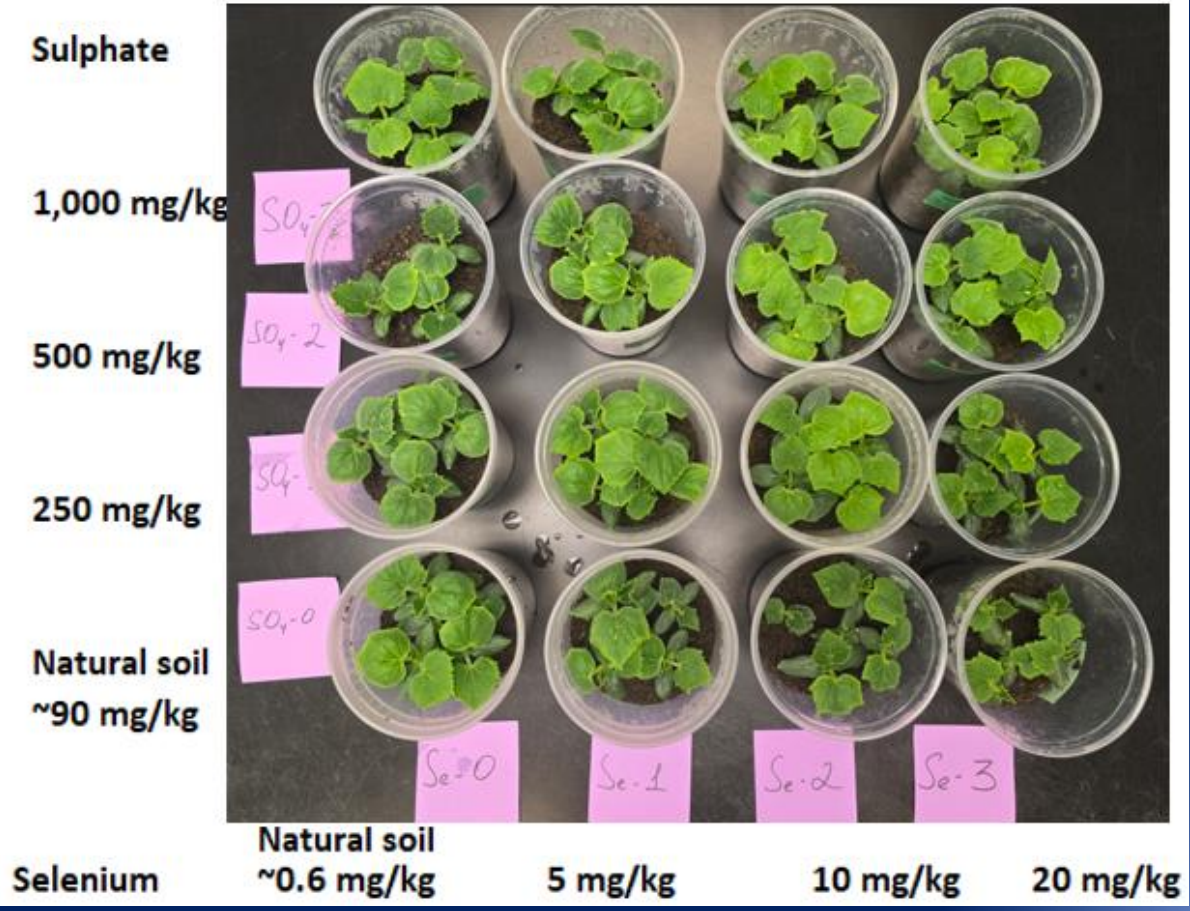
Dose-Response: Cucumber (Marketmore 76 var.)

Coarse

Fine

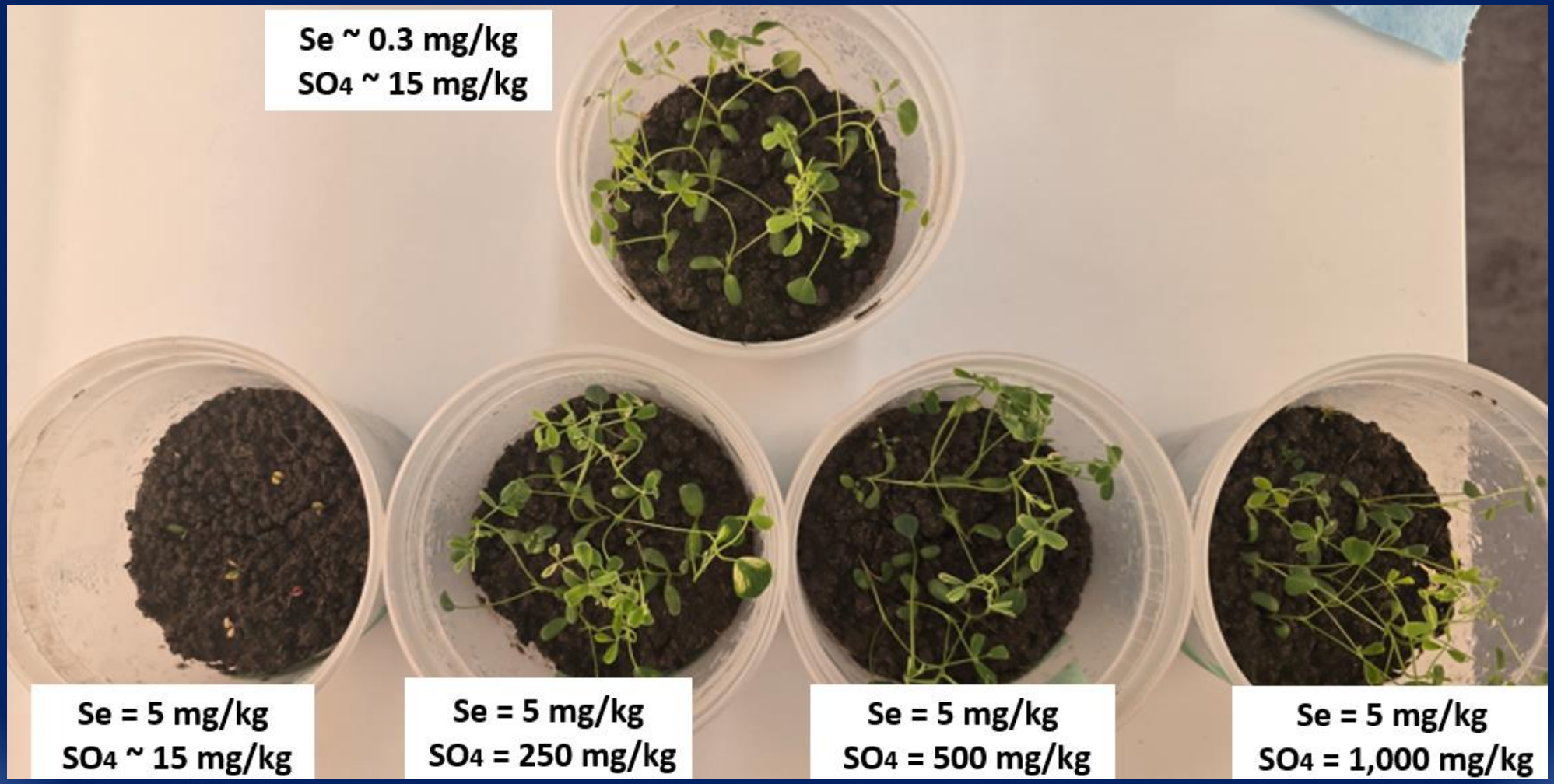






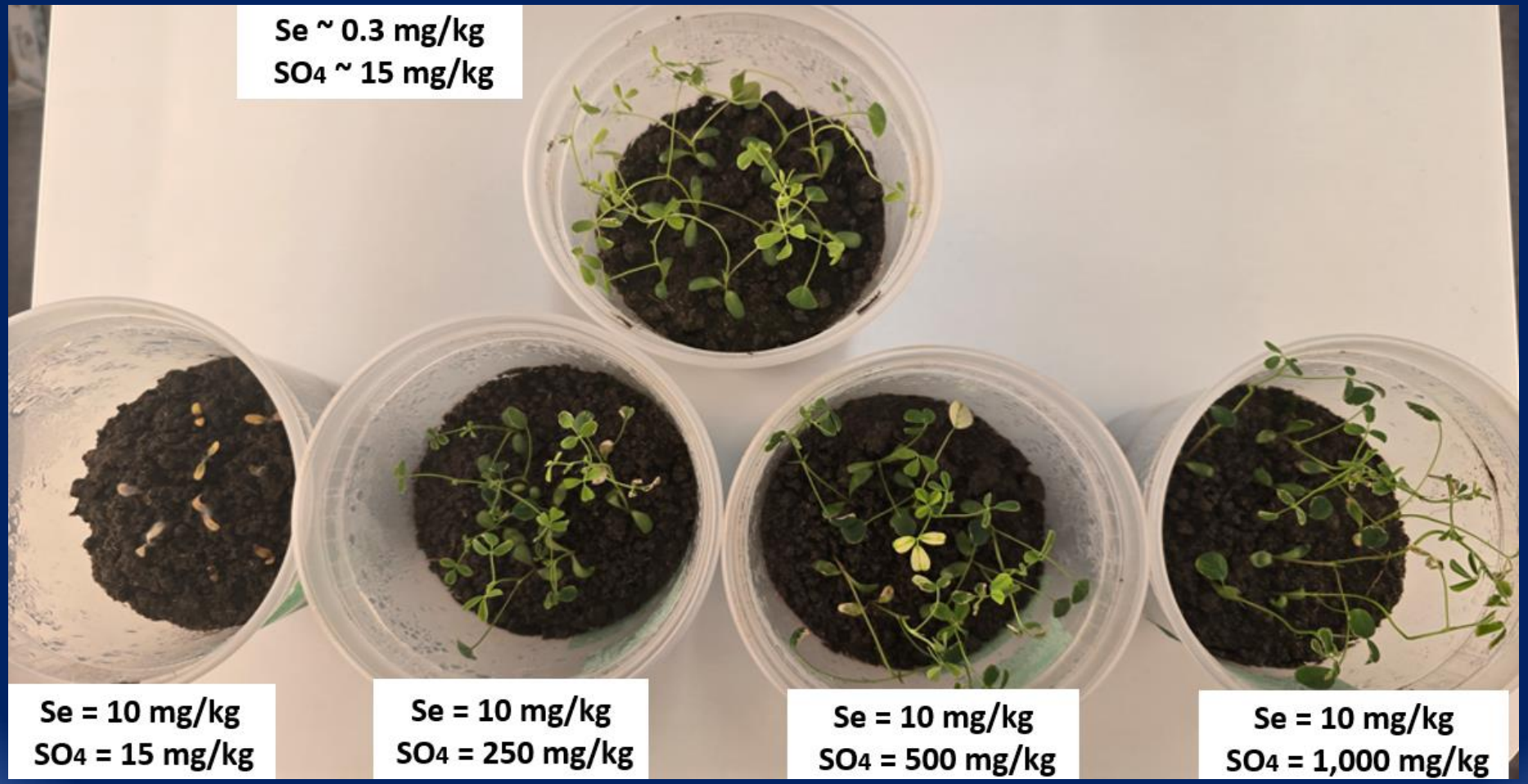
Dose-Response: Alfalfa (Common var.)

250-1,000 mg/kg of SO_4 may help plants to survive 5 mg/kg Se in coarse soils



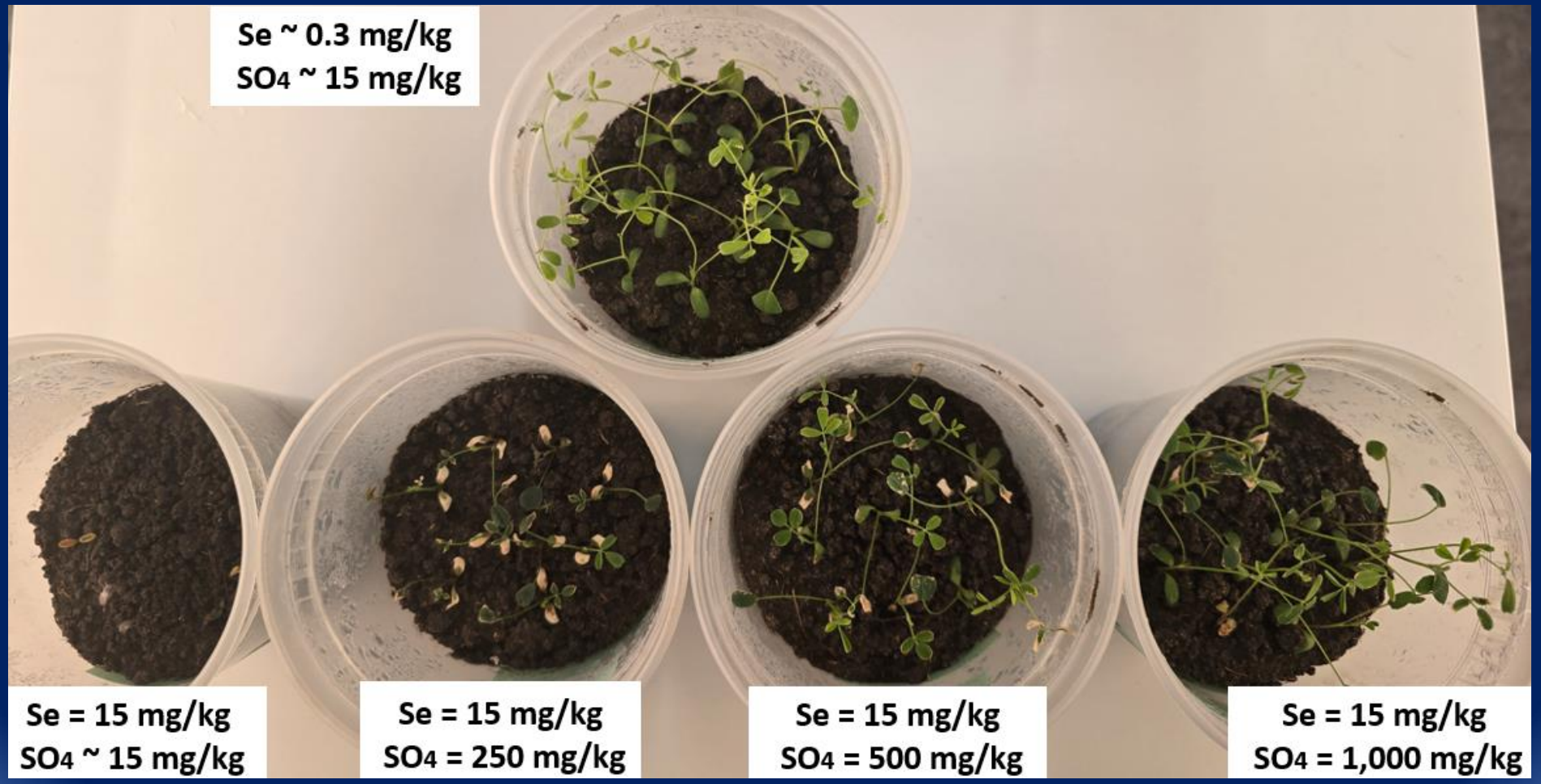
Dose-Response: Alfalfa

500-1,000 mg/kg of SO_4 may likely help plants with 10 mg/kg Se in coarse soils
250 mg/kg is not enough



Dose-Response: Alfalfa

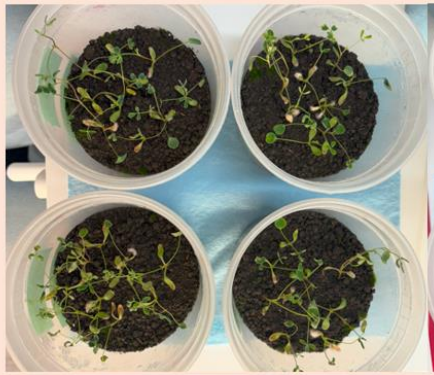
1,000 mg/kg of SO_4 have better effect on 15 mg/kg Se in coarse soils
500 mg/kg is not enough



Dose-Response: Alfalfa

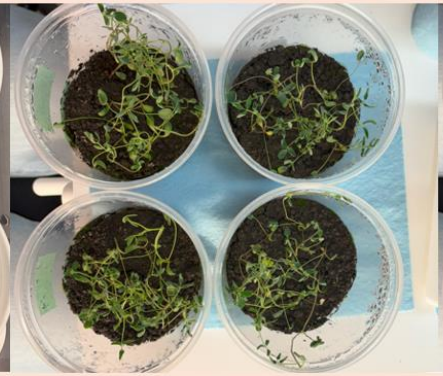
250-1,000 mg/kg of SO_4 may help plants to survive 5 mg/kg Se in fine soils

Se ~ 0.6 mg/kg
SO_4 ~ 90 mg/kg

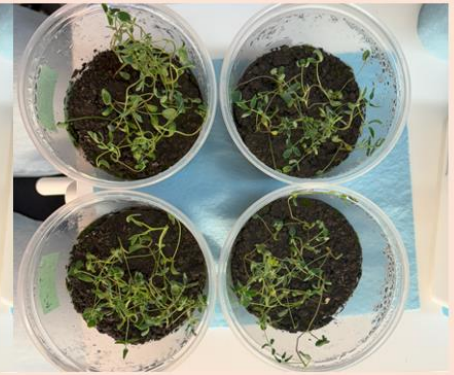


Se = 5 mg/kg
SO_4 ~ 90 mg/kg

Se = 5 mg/kg
SO_4 = 250 mg/kg



Se = 5 mg/kg
SO_4 = 500 mg/kg

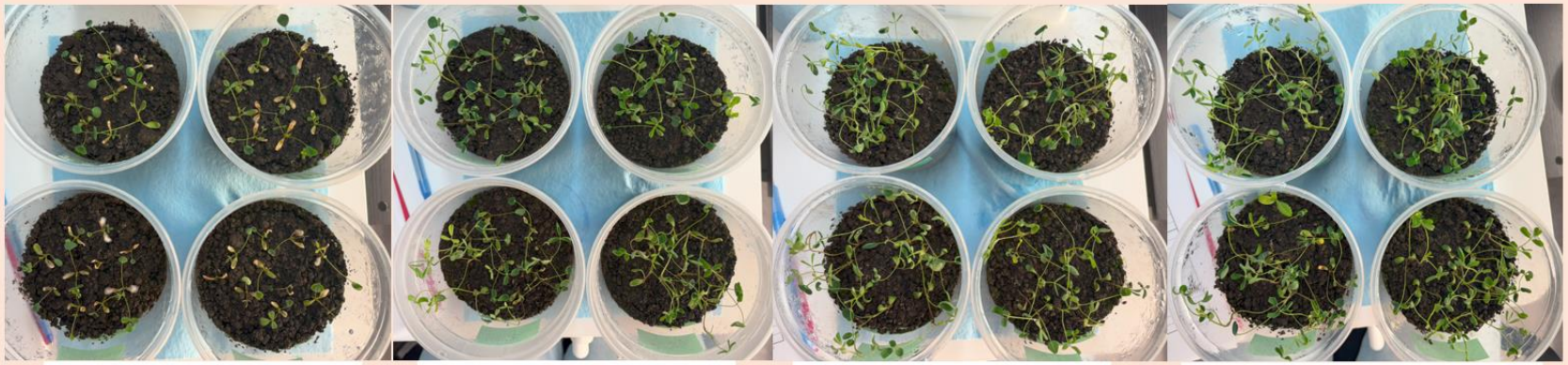


Se = 5 mg/kg
SO_4 = 1,000 mg/kg

Dose-Response: Alfalfa

500 -1,000 mg/kg of SO_4 may help plants to survive 10 mg/kg Se in fine soils
250 mg/kg - may be

Se ~ 0.6 mg/kg
SO_4 ~ 90 mg/kg



Se = 10 mg/kg
SO_4 ~ 90 mg/kg

Se = 10 mg/kg
SO_4 = 250 mg/kg

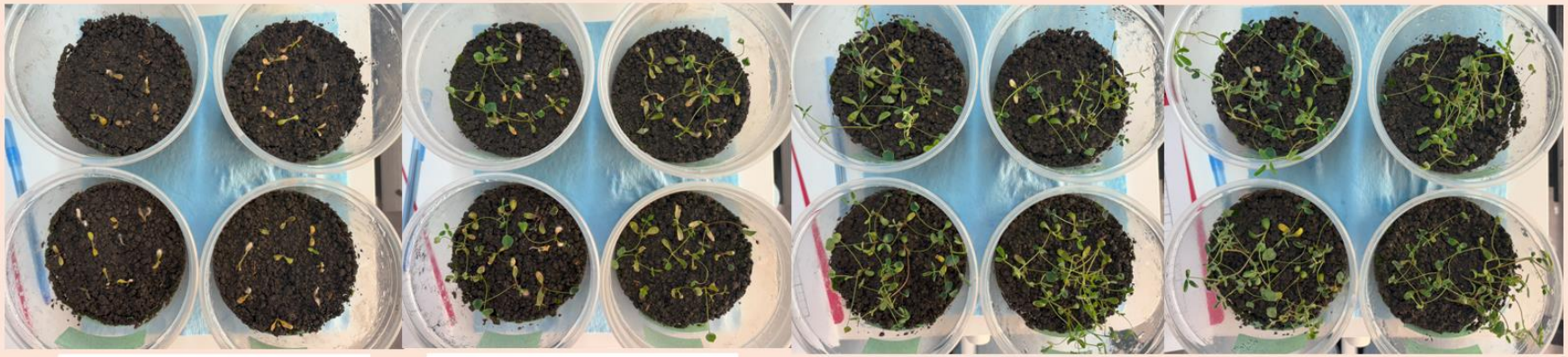
Se = 10 mg/kg
SO_4 = 500 mg/kg

Se = 10 mg/kg
SO_4 = 1,000 mg/kg

Dose-Response: Alfalfa

500 – 1,000 mg/kg of SO_4 may help plants to survive 20 mg/kg Se in fine soils
250 mg/kg – not enough

Se ~ 0.6 mg/kg
SO_4 ~ 90 mg/kg



Se = 20 mg/kg
SO_4 ~ 90 mg/kg

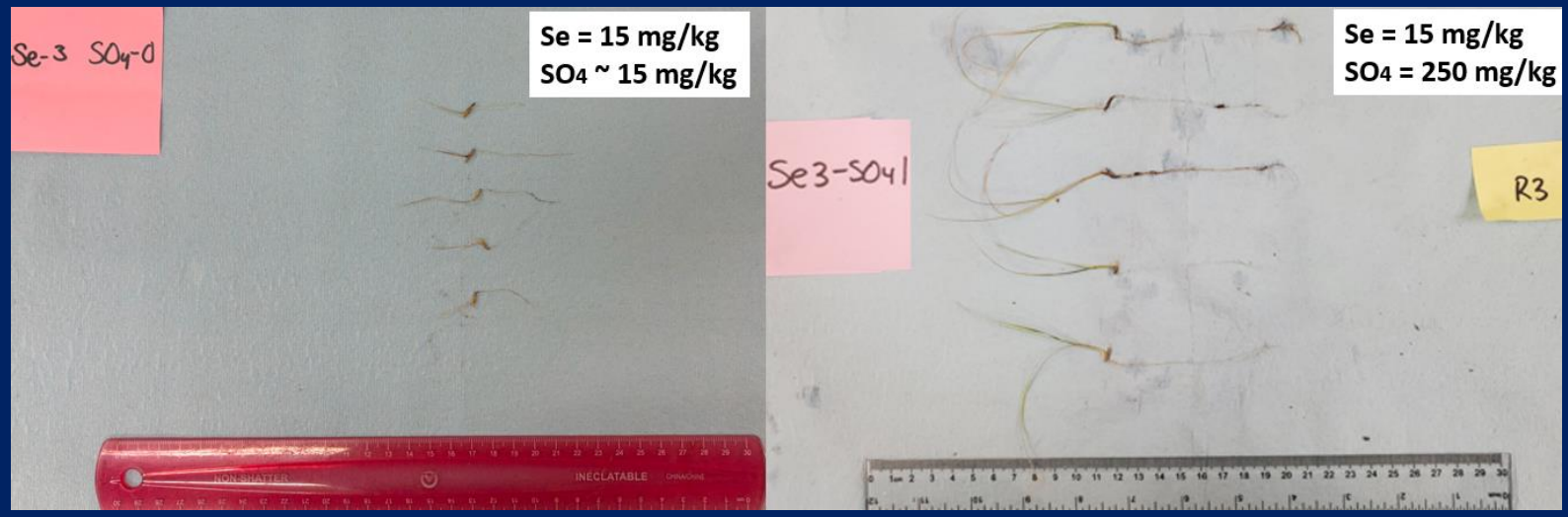
Se = 20 mg/kg
SO_4 = 250 mg/kg

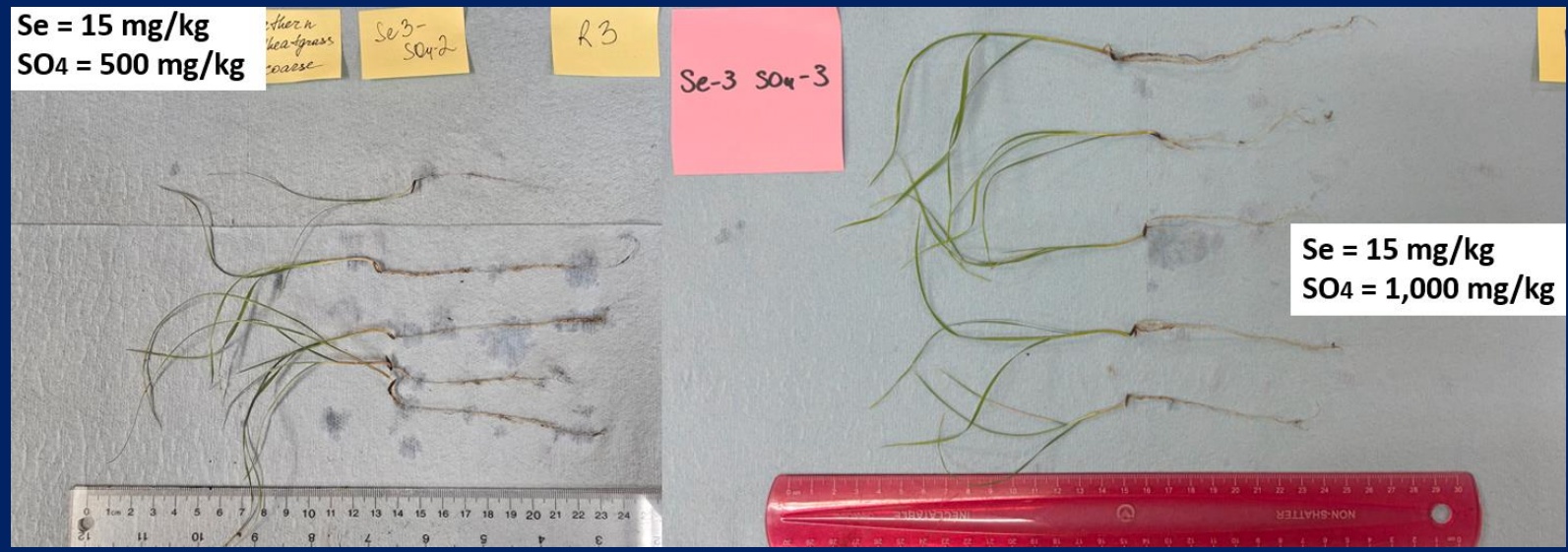
Se = 20 mg/kg
SO_4 = 500 mg/kg

Se = 20 mg/kg
SO_4 = 1,000 mg/kg

Dose-Response: Northern Wheatgrass

Coarse





Dose-Response : Red Fescue

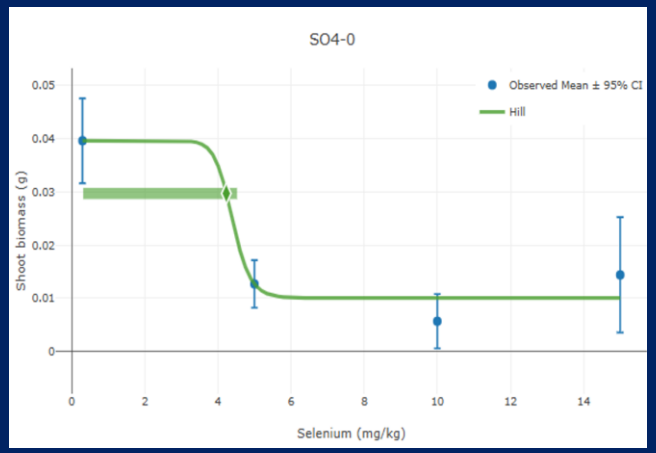
Fine

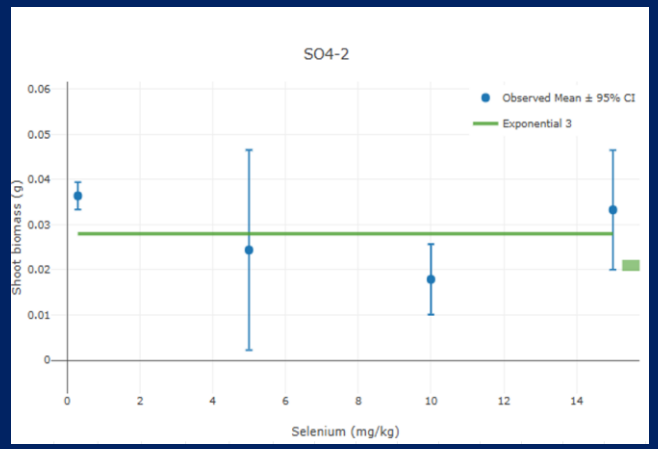
Dose-Response Analysis

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

Cucumber, Coarse, Shoot biomass

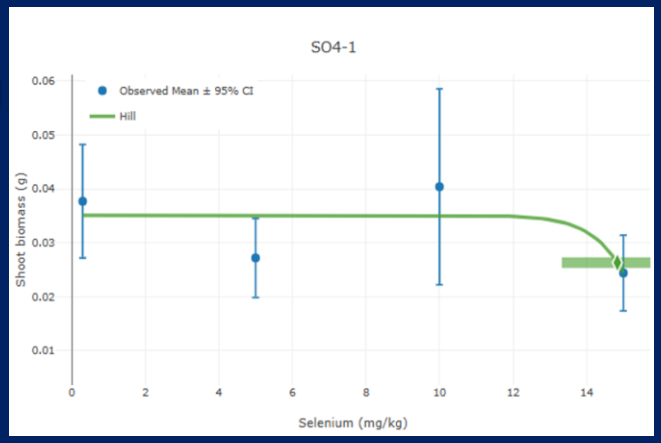
EC25 = 4.2 mg/kg
SO_4 ~15 mg/kg

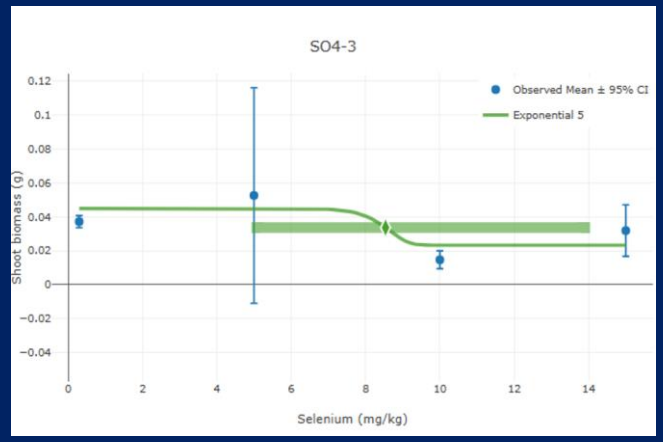




EC25 = 15.25 mg/kg
SO_4 = 500 mg/kg

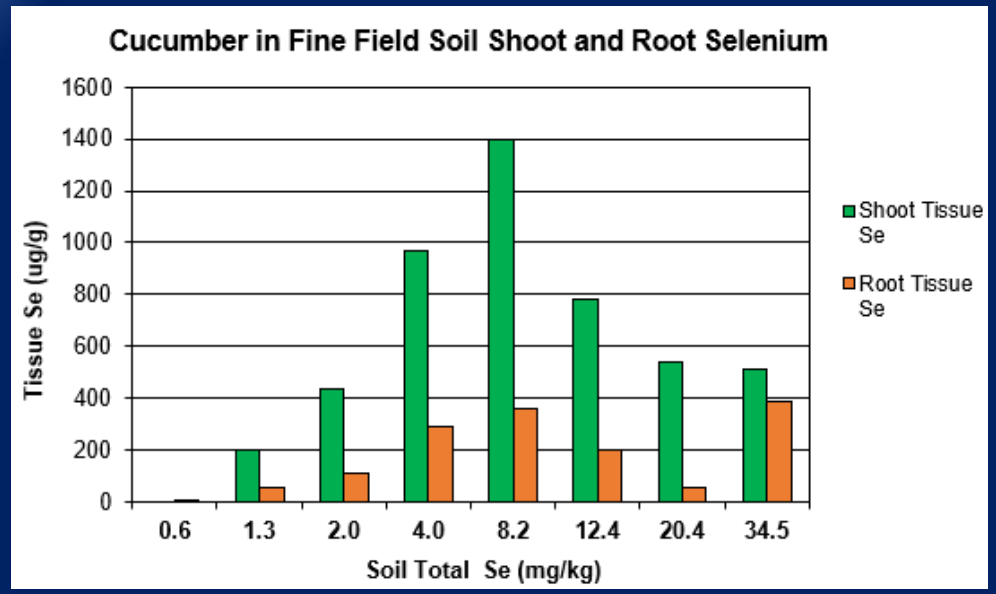
EC25 = 14.8 mg/kg
SO_4 = 250 mg/kg

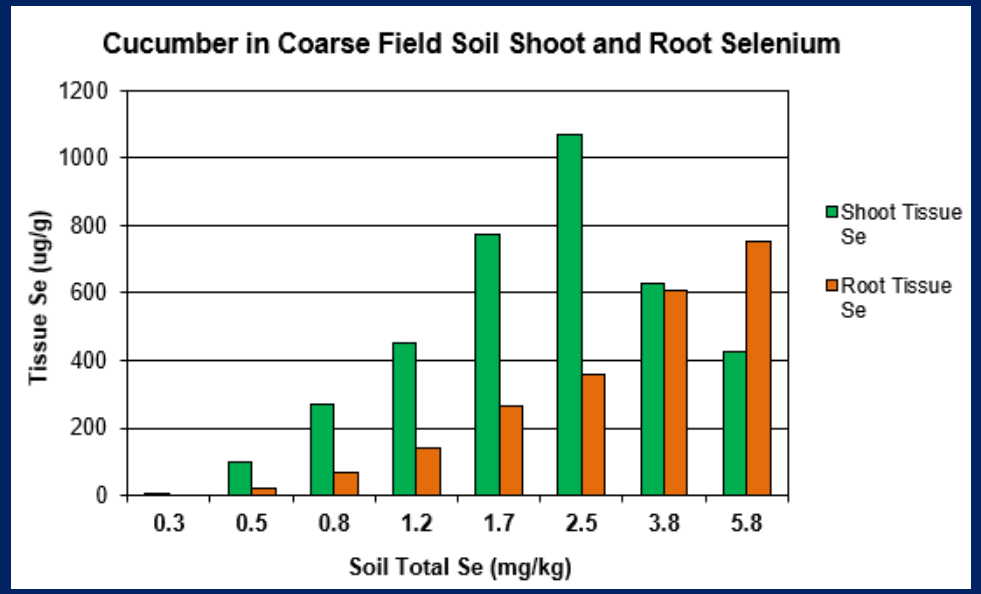




EC25 = 8.5 mg/kg
SO_4 =1,000 mg/kg

2020-2021 Tissue Chemistry



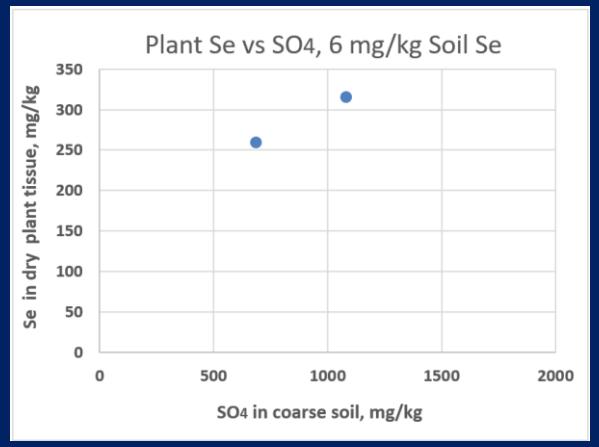


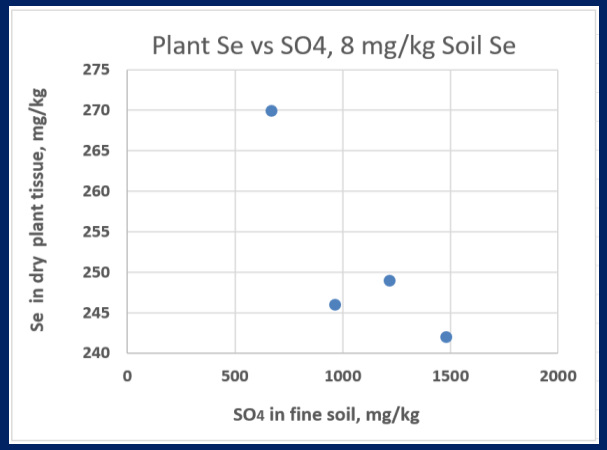
Max. accumulation in shoot for cucumber (14 day test):
at 8.2 mg/kg in fine soils
at 2.5 mg/kg in coarse soils

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

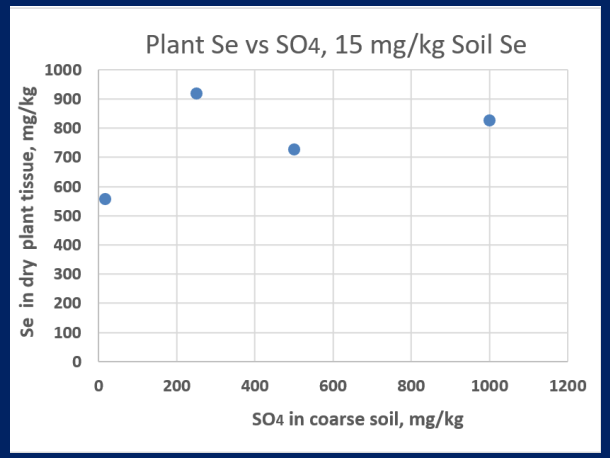
Se in Plant Tissue: Cucumber

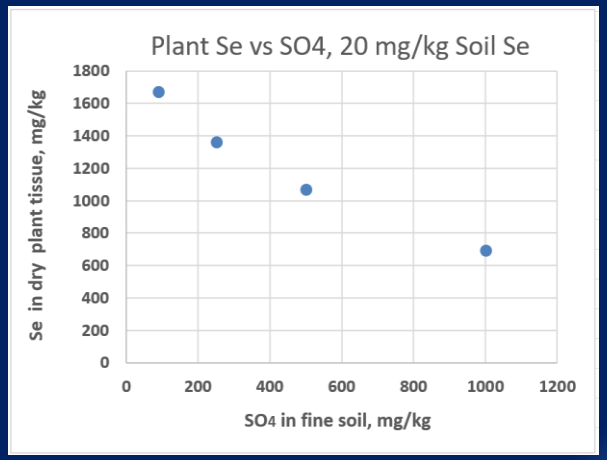
2015-2017 14-day test:
Se in dry cucumber shoots from the highest Se treatment





2024-2025 14-day test:
Se in dry cucumber shoots from the highest Se treatment





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 (long-term studies to be added)
  5 mg/kg for fine soils (long-term studies to be added)

Final statistical analyses and guideline developments will be based on the final dataset for all tested species (historical information combined with 2024-2025 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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