Investigating the effects of selenium on fungal growth and mineral production

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Introduction

Selenium (Se) contamination and toxicity has become more prevalent due to anthropogenic activities. Se toxicity affects animal and human health, causing neurological disturbances, bone deformations, and nervous system abnormalities. As of now, regulations exist for Se in water but there are no such regulations for Se in soil. Soils can act as a source of Se to sensitive ecosystems. Microorganisms, such as bacteria and fungi, are known to transform Se compounds, are known to transform Se compounds, although their mechanisms and environmental impact are poorly understood. These organisms can reduce mobile, dissolved selenium (Se (VI), Se (IV)) into its solid, less mobile form (Se(0), Se(-II)). The role of fungi in aerobic environments is particularly understudied, although their impact is likely important. Here we show experimental results of Se impacts on fungal growth in an aerobic environment.

Image 1. Fish deformation and sterilization from Se exposure (Lemly and Skorupa, 2012).

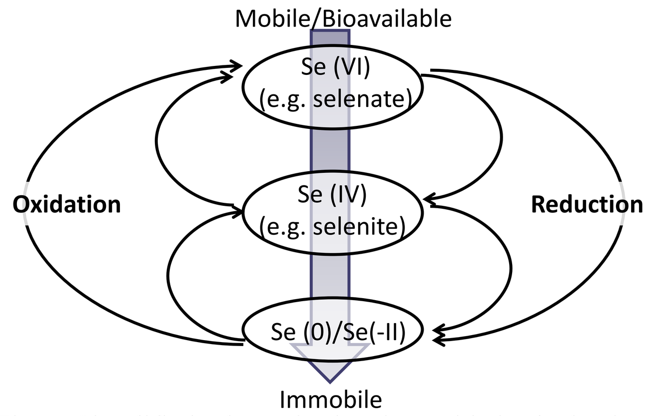


Fig. 1. Simplified schematic showing oxidation/reduction Se transformations in soils and expected mobility.

Objectives

- Measure growth and tolerance of common soil fungi to selenium and observe the effects of different Se concentrations and forms (selenite vs. selenate)
- Identify species with the ability to transform selenium to less soluble forms

Methods

- Use isolated cultures of six common soil fungi species: *Alternaria alternata, Acremonium strictum, Paraconiothyrium sporulosum, Plectosphaerella cucumerina, Pyrenochaeta sp.,* and *Stagonospora sp.*
- Measure growth in solid and liquid AY media in varying concentrations of selenium, both selenite (Na_2SeO_3) and selenate (Na_2SeO_4): 0mM, 0.001mM, 0.01mM, 0.1mM, 0.5mM, 1.0mM, and 10mM
- Periodically assess (quantitatively and visually) fungi for approximately 30-40 days in order to determine growth rate and effect of selenium on fungi
- Perform X-ray diffraction (XRD) analysis to determine crystallographic properties of Se nanoparticles produced by fungi
- Conduct scanning electron microscope (SEM) analysis of liquid culture fungi following fixation in 2.5% gluteraldehyde to identify appearance and form of precipitated Se particles and further examine effects of Se on growth

Interpretations

- Se effects both morphology and growth rate, frequently stunting or halting growth
- *A. alternata* and *A. strictum* were most resilient to higher Se concentrations and showed greatest Se reduction
- Four of six species showed signs of Se reduction
- Selenite (Na_2SeO_3) more likely to stunt growth, selenate (Na_2SeO_4) more likely to stop growth; selenite is more readily reduced than selenate
- SEM imaging suggests extracellular precipitation of Se nanoparticles
- XRD analysis indicates either amorphous nature or small size of Se nanoparticles

Future Work

- Complete SEM work on remaining three fungi species
- Identify additional features on pictures obtained from stereoscope and SEM
- Test different strains and species of fungi
- Introduce more complex variables, including higher nutrient concentrations and other forms of media
- Employ other techniques in order to determine structure of Se precipitates
- Obtain and run similar tests on fungi species from known Se-rich environments

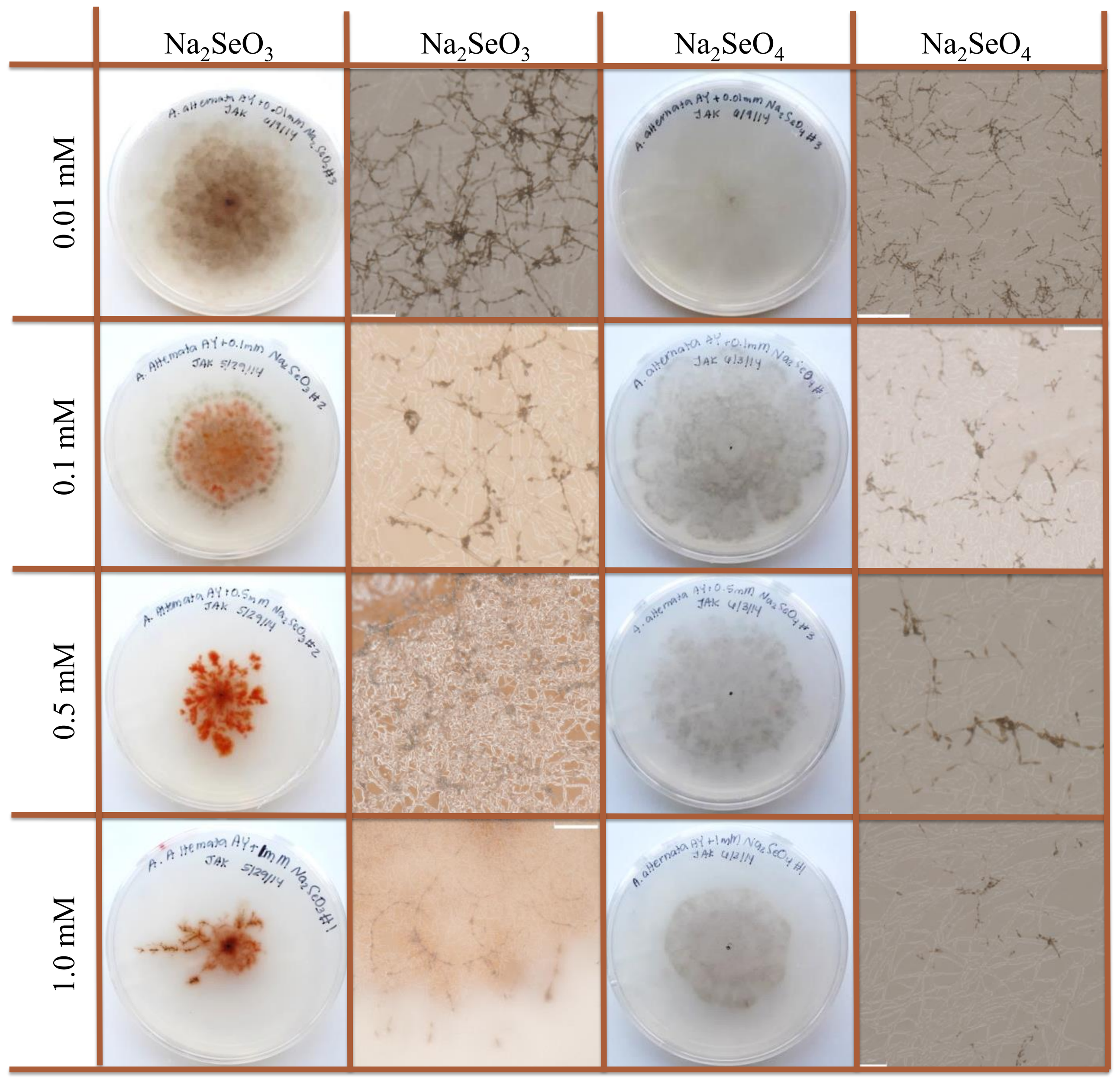
Results

Effects on fungal morphology

Fig 2 (left). *A.alternata,* 0mM Se

Fig 3 (below). *A. alternata* grown with AY media on 90mm pitre dishes. Image on right of each image-pair shows close-up of fungal hyphae.

- Pink color is indicator of Se reduction
- Gray color is pigment



SEM analysis

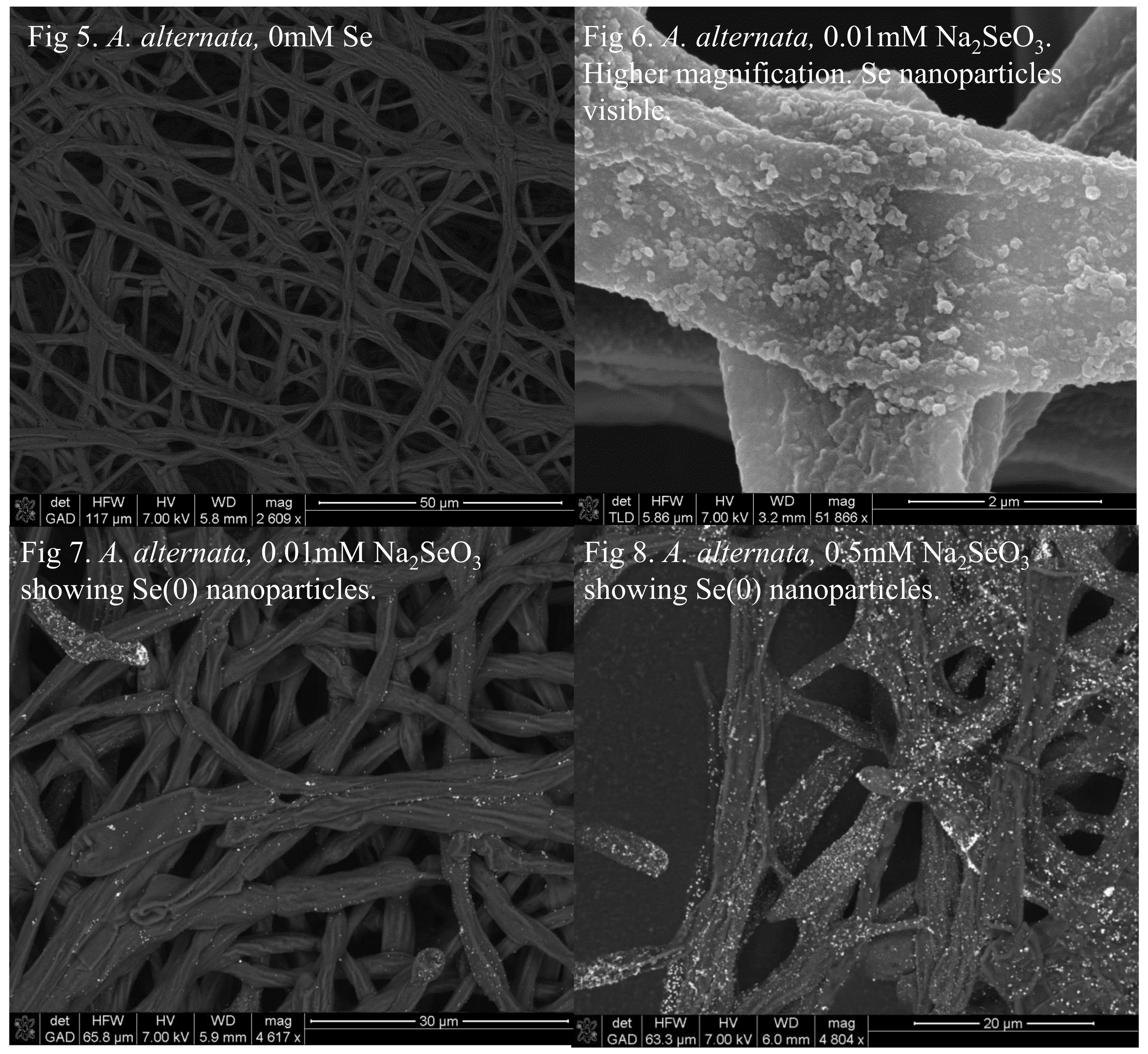


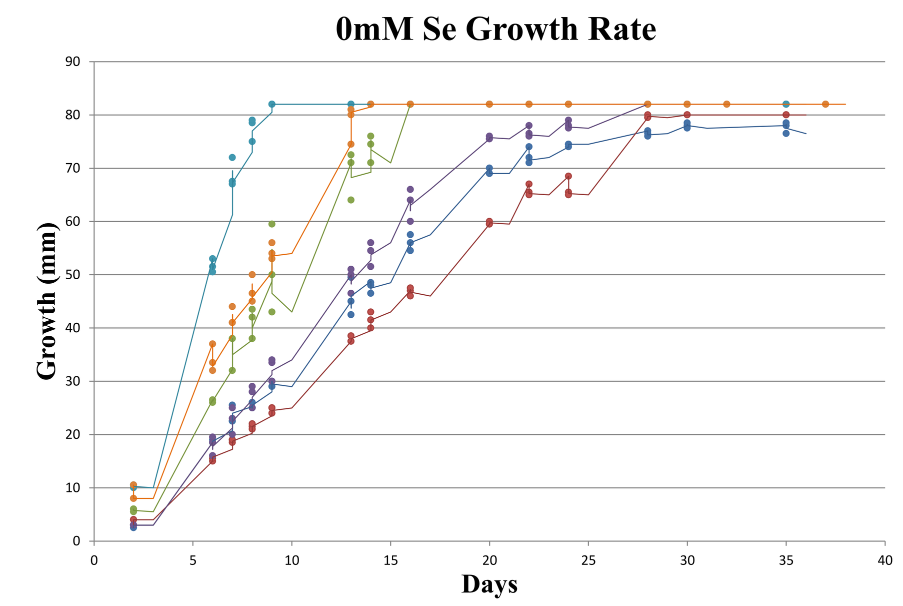
Fig 5. *A. alternata,* 0mM Se

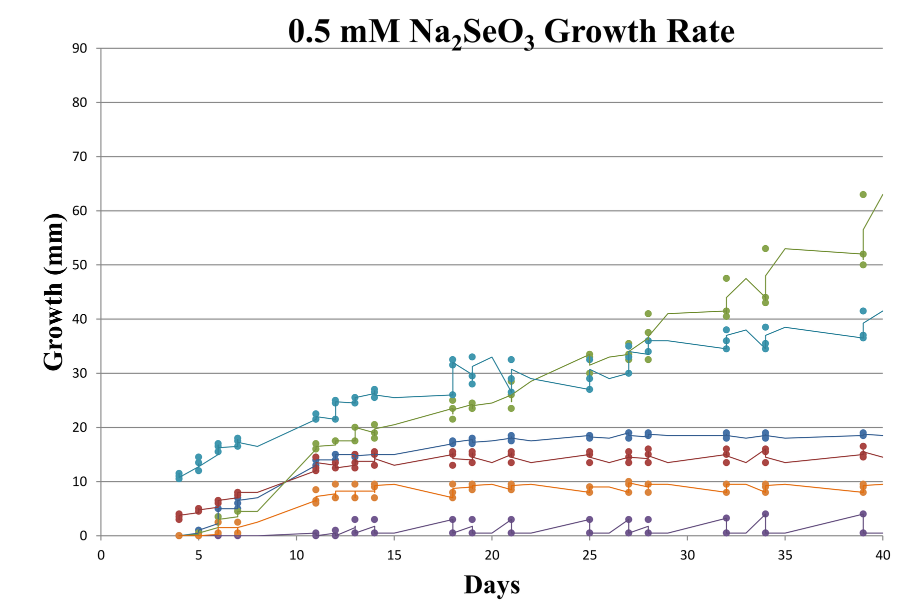
Fig 6. *A. alternata,* 0.01mM Na_2SeO_3. Higher magnification. Se nanoparticles visible.

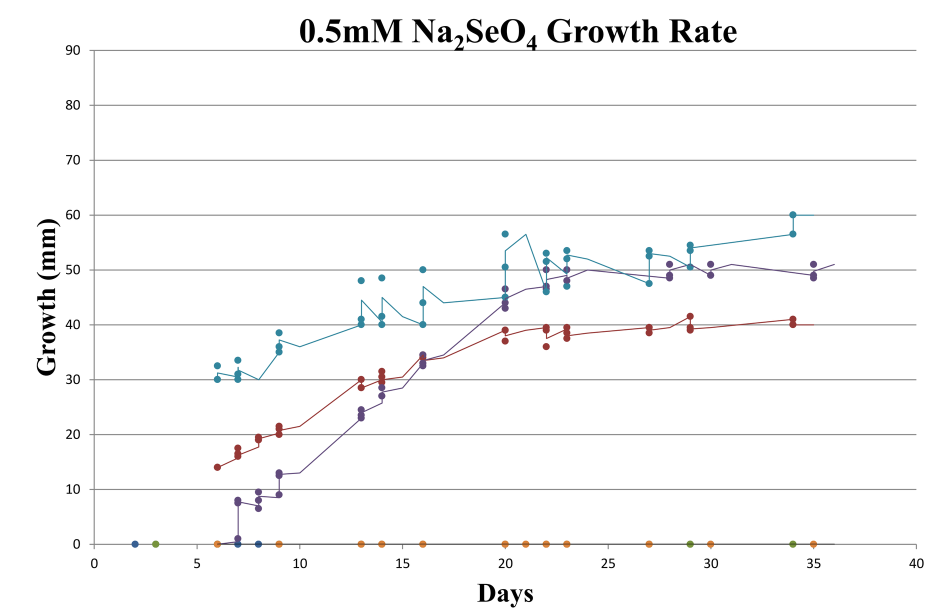
Fig 7. *A. alternata,* 0.01mM Na_2SeO_3 showing Se(0) nanoparticles.

Fig 8. *A. alternata,* 0.5mM Na_2SeO_3 showing Se(0) nanoparticles.

Changes in growth rate







- *Pyrenochaeta sp.*
- *A. strictum*
- *P. cucumerina*
- *Stagonospora sp.*
- *A. alternata*
- *P. sporulosum*

Fig 4. The six tested fungi compared across a no selenium control, a 0.5mM Na_2SeO_3 concentration, and a 0.5mM Na_2SeO_4 concentration. All scale proportions are identical.

Pyrenochaeta sp.			
Concentration	Growth rate (mm/day)	Max growth (mm)	Days before leveling off
Control (no Se)	3.64	77.67	28
0.01mM Na_2SeO_3	2.50	60.83	28
0.1mM Na_2SeO_3	1.78	46.17	32
0.5mM Na_2SeO_3	1.23	19	21
1mM Na_2SeO_3	1.00	14	32
10mM Na_2SeO_3	0	0	0
0.01mM Na_2SeO_4	3.28	75.5	28
0.1mM Na_2SeO_4	0.80	21.33	36
0.5mM Na_2SeO_4	0	0	0
1mM Na_2SeO_4	0	0	0
10mM Na_2SeO_4	0	0	0

Table 1. Growth rates, max diameter, and days of growth for *Pyrenochaeta sp.* in various Se concentrations and forms.

X-Ray Diffraction

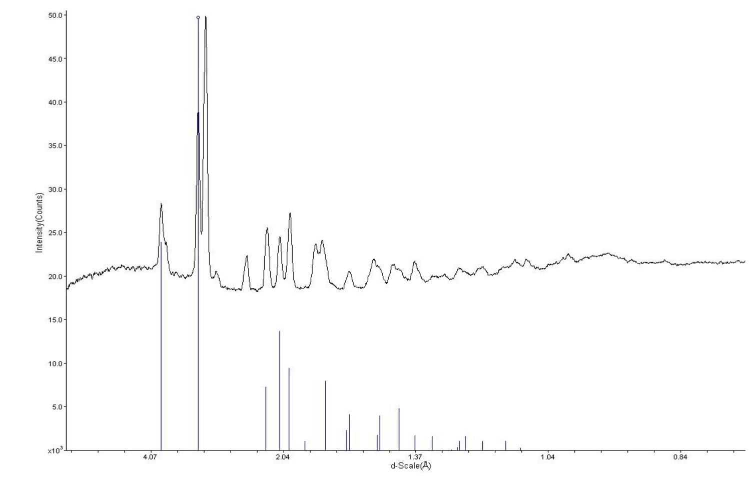


Fig 9. Synthetic selenium.

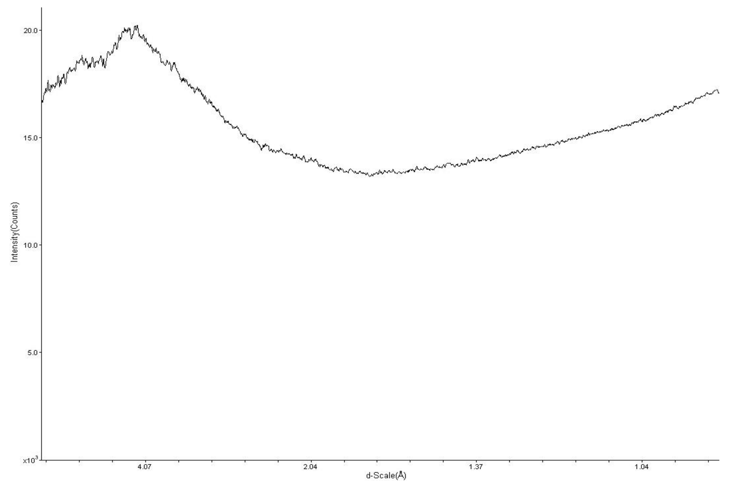


Fig 10. *P. cucumerina* 0.01mM Na_2SeO_3.

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