

Smithsonian Institution



Manganese Oxide Precipitation Induced by Fungal Species

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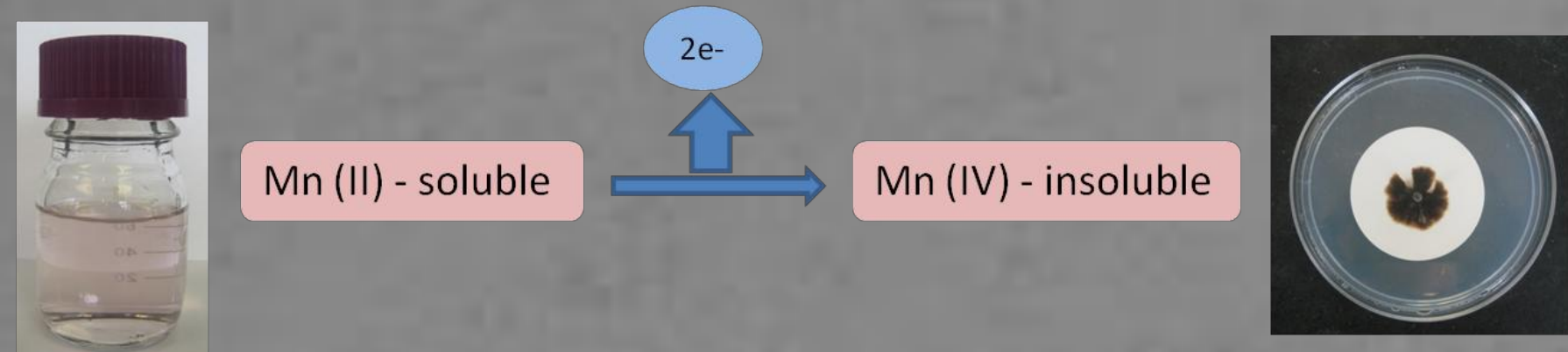
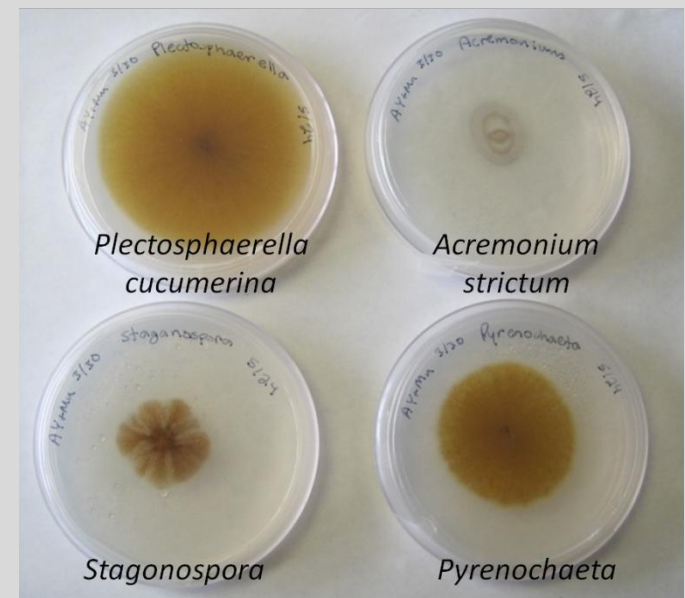
Background



Abandoned coal mines can emit water laden with high concentrations of dissolved metals, resulting in serious environmental contamination known as acid mine drainage. One of the commonly released toxins is the soluble metal, manganese (Mn). Previous research has isolated bacteria from within existing acid mine drainage treatment systems that oxidize Mn (II) into insoluble Mn (III) and Mn (IV) oxide minerals (Bargar et al. 2005). However, it has recently been discovered that certain fungal species are also present that precipitate Mn oxides (Santelli et al. 2010). By converting the metal into its insoluble form, it is removed from the water and can also promote the removal of other metal contaminants via adsorption. Because of its mineral structure, Mn oxides are able to easily adsorb, or incorporate into their structure, other dissolved metals (Post 1999). Therefore, fungal species isolated from these drainage sites could potentially be an important component of acid mine drainage remediation efforts. However, in order for fungi to be used effectively in remediation, more needs to be known about their tolerance to different levels of dissolved Mn. In addition, it is important to find out the mineral structures of the Mn oxides precipitated by the different fungal species. Ideally, the mineral structure should be able to adsorb other minerals and also be relatively stable so that, once the Mn is precipitated out, it will not dissolve again.

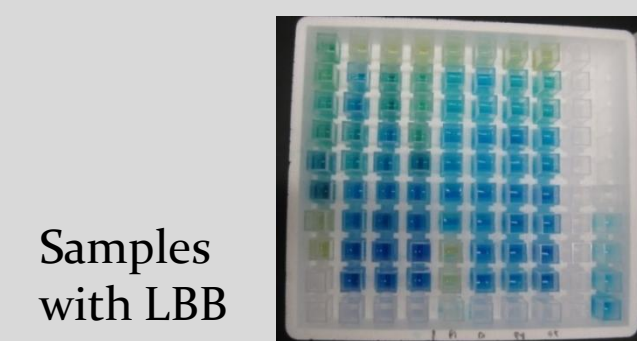
In these experiments, we used four fungal species isolated from a coal mine drainage treatment site in Pennsylvania: *Plectosphaerella cucumerina*, *Pyrenochaeta* sp., *Acremonium strictum*, and *Stagonospora* sp. We observed their growth and oxidation in both liquid and solid media containing different initial Mn (II) concentrations. We attempted to correlate fungal biomass with amount of Mn oxide precipitated. We then tried to determine the mineral structure of the Mn oxides precipitated under different growth conditions.

Fungal Species



Liquid Experiment

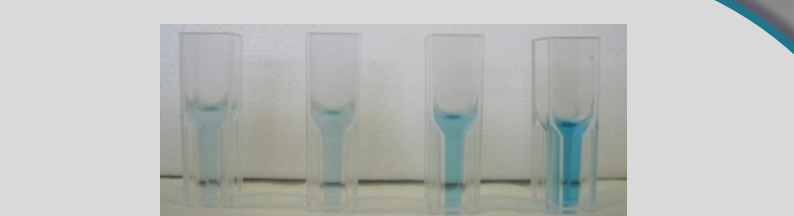
- Each fungal species was grown in liquid media of 8 different Mn (II) concentrations: 0 mM, 0.2 mM, 0.5 mM, 1.0 mM, 1.5 mM, 2.0 mM, 2.5 mM, 5.0 mM
- Grown in dark for 12 days
- Used LBB (leucoberbelin blue), a solution that turns dark blue in the presence of Mn (III) or Mn (IV), to test each flask for Mn oxidation



- Analyzed samples that precipitated Mn oxide with XRD (x-ray diffraction), an instrument used to determine mineral structure, and SEM (scanning electron microscope) to take close-up pictures and affirm the identity of the precipitate.

Solid Experiment

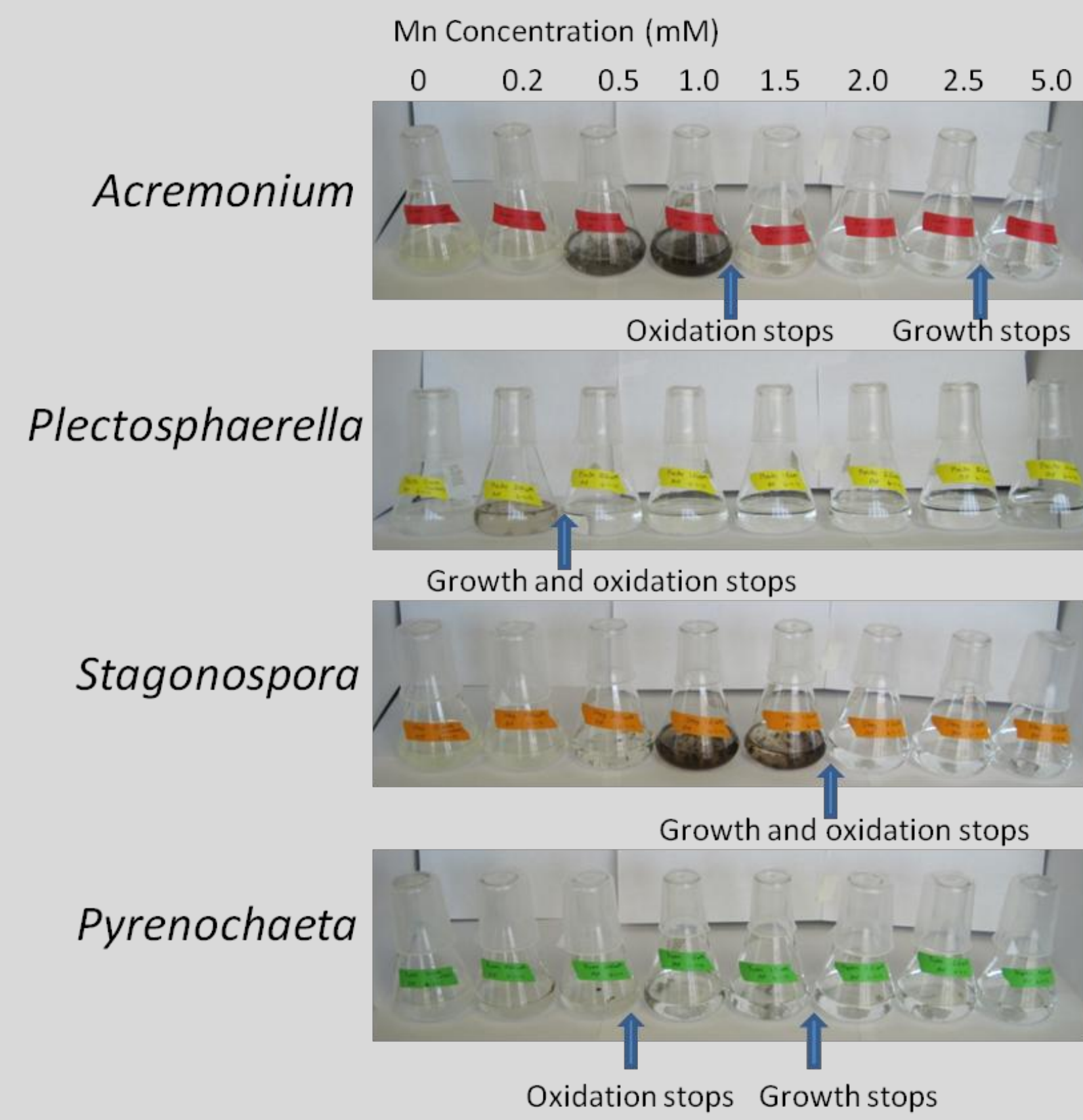
- Each fungal species was grown on filters on top of solid media in 4 different Mn (II) concentrations: 0.2 mM, 0.5 mM, 1.0 mM, 2.5 mM
- Each species was sampled 3 times and each time:
 - 2 filters of each concentration were dried in an oven and then weighed
 - 1 filter saved for XRD and SEM
- Used the colorimetric method to determine amount of oxidized Mn in each dried sample:
 - LBB added to each sample
 - Used a spectrophotometer, an instrument that measures light absorbance, to determine color intensity, and therefore amount of oxidized Mn present



LBB standards of increasing Mn (IV) concentrations used for colorimetric assay.

Growth Results

Liquid



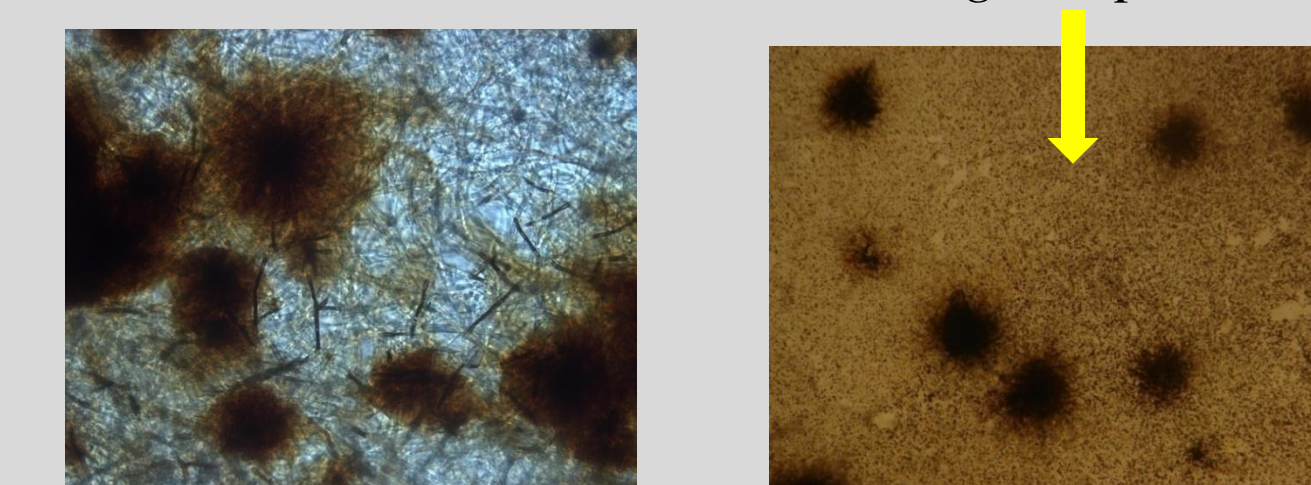
- Different fungal species oxidized and grew best at different initial Mn (II) concentrations.

- In *Stagonospora* and *Pyrenochaeta*, oxidation stopped when growth stopped, but in *Acremonium* and *Plectosphaerella*, growth continued into higher concentrations than did oxidation.

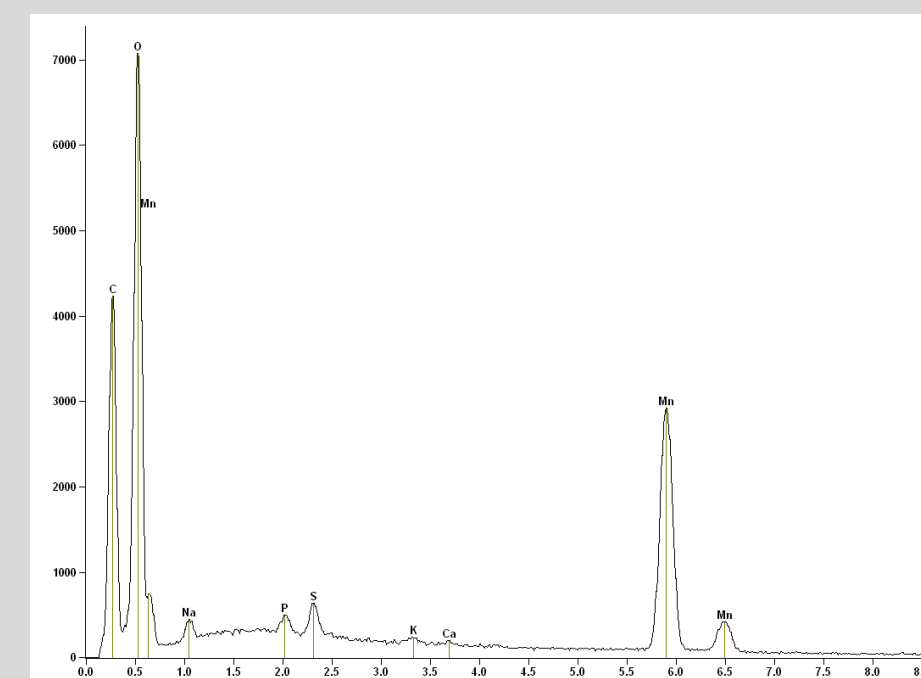
- Oxidation looked very different between species.



Acremonium and *Stagonospora*

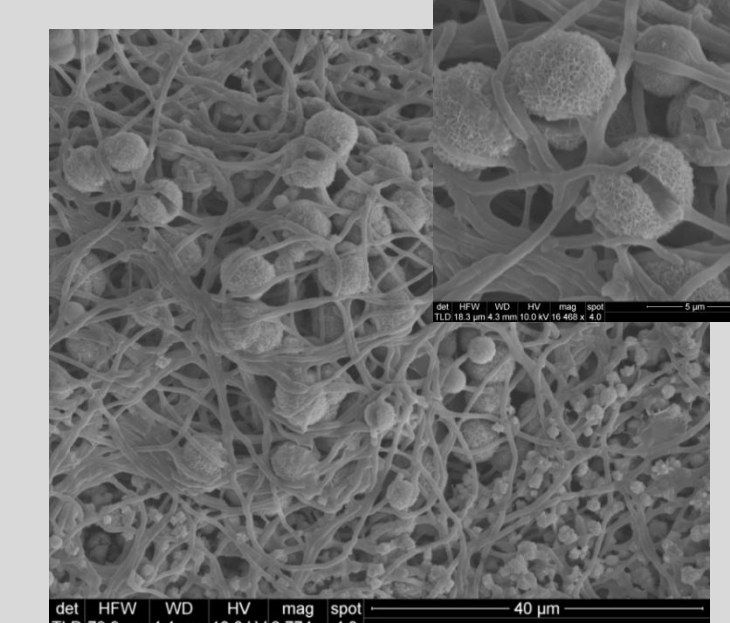


Pyrenochaeta had Mn oxidation along hyphae and fruiting bodies (spores). *Stagonospora* had clumps of Mn oxides, perhaps associated to proteins it excretes.



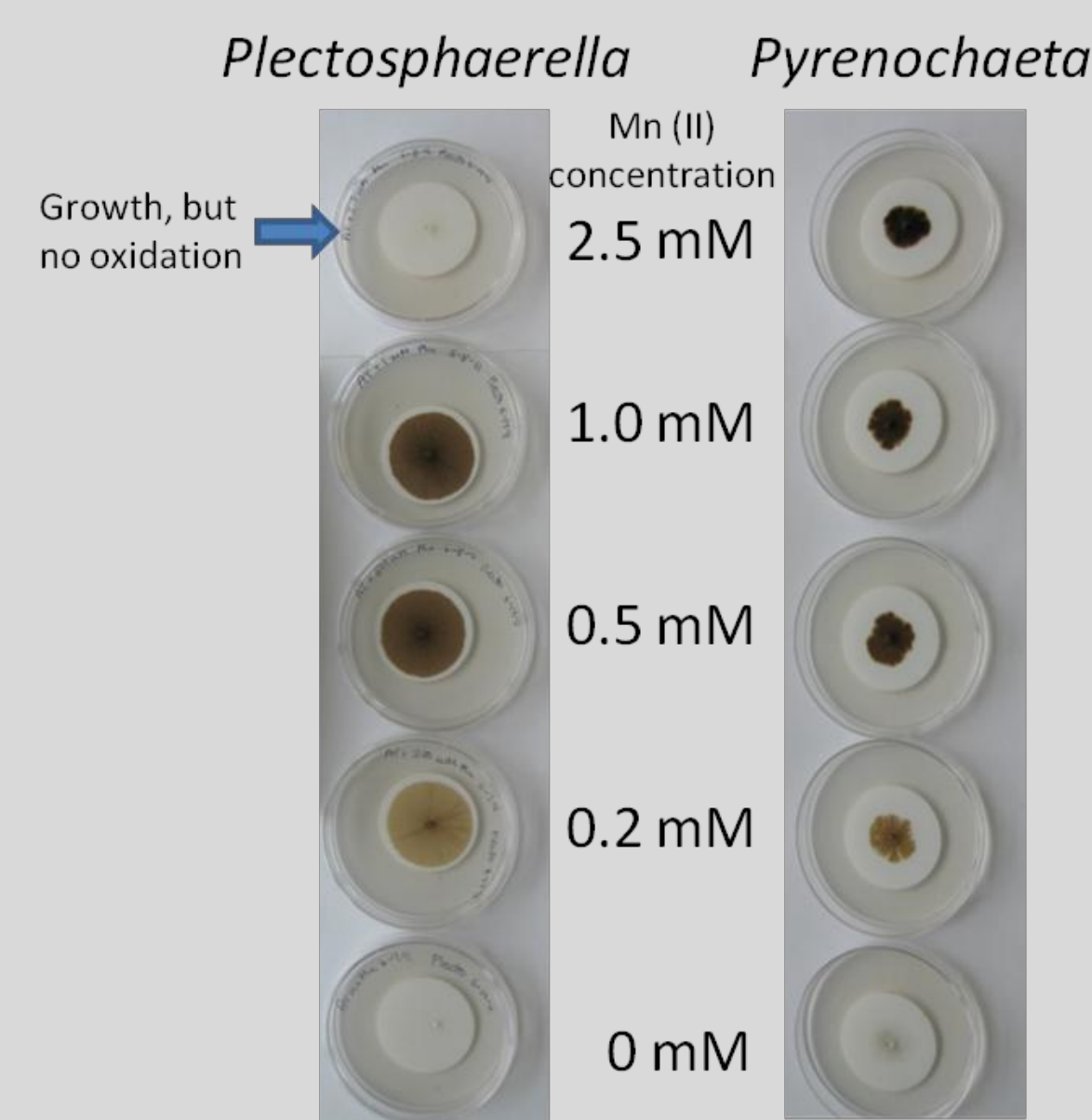
Element	Wt.%	Compound	Wt.%
C	18.07	CO2	66.22
O	56.22	---	---
F	1.41	F	1.41
Na	0.55	Na2O	0.74
P	0.28	P2O5	0.64
S	0.57	SO3	1.42
K	0.11	K2O	0.14
Ca	0.07	CaO	0.1
Mn	22.71	MnO	29.33

The EDS (Energy-dispersive X-ray spectroscopy) spectrum of the elemental composition of *Acremonium* grown in 1.0 mM Mn (II) liquid media. High carbon content is due to the fungal hyphae, and high Mn content is due to precipitated Mn oxides.

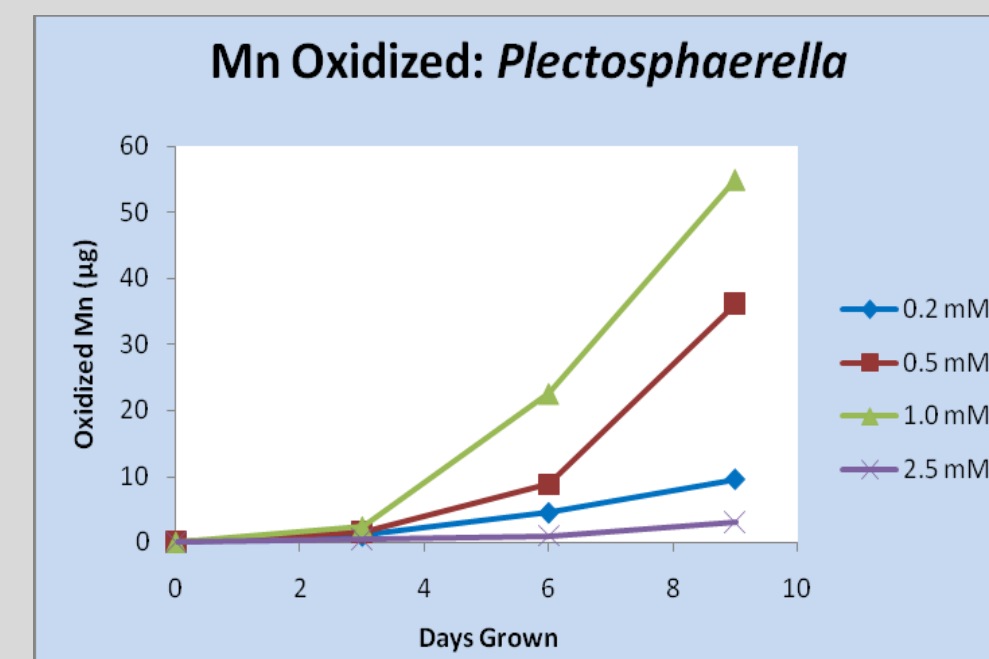


SEM pictures of *Acremonium* grown in 1.0 mM Mn (II) liquid media. The "strings" are fungal hyphae and the "balls" are Mn oxides.

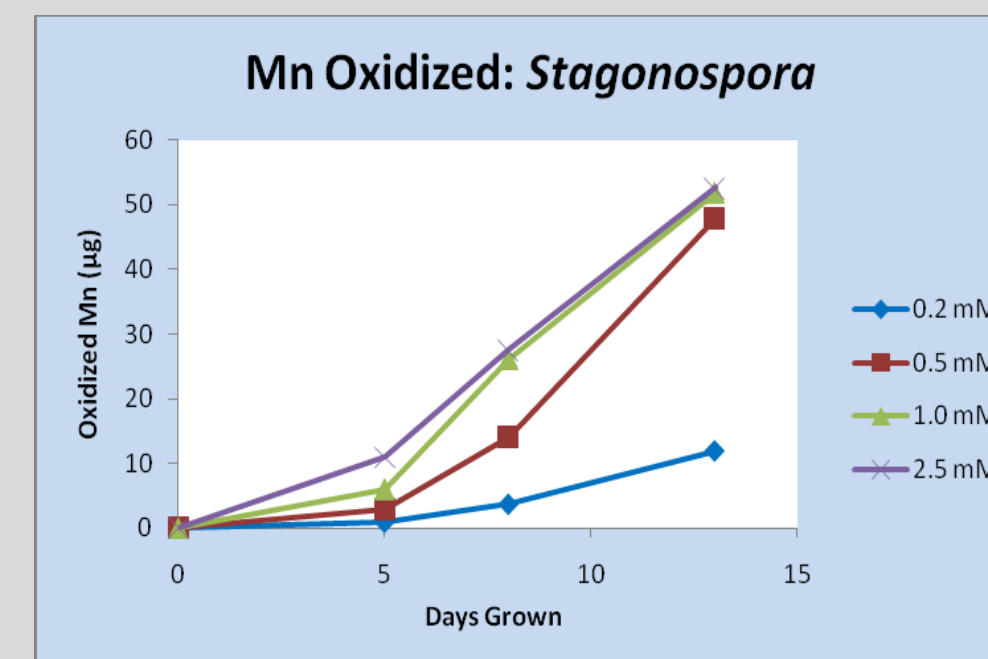
Solid



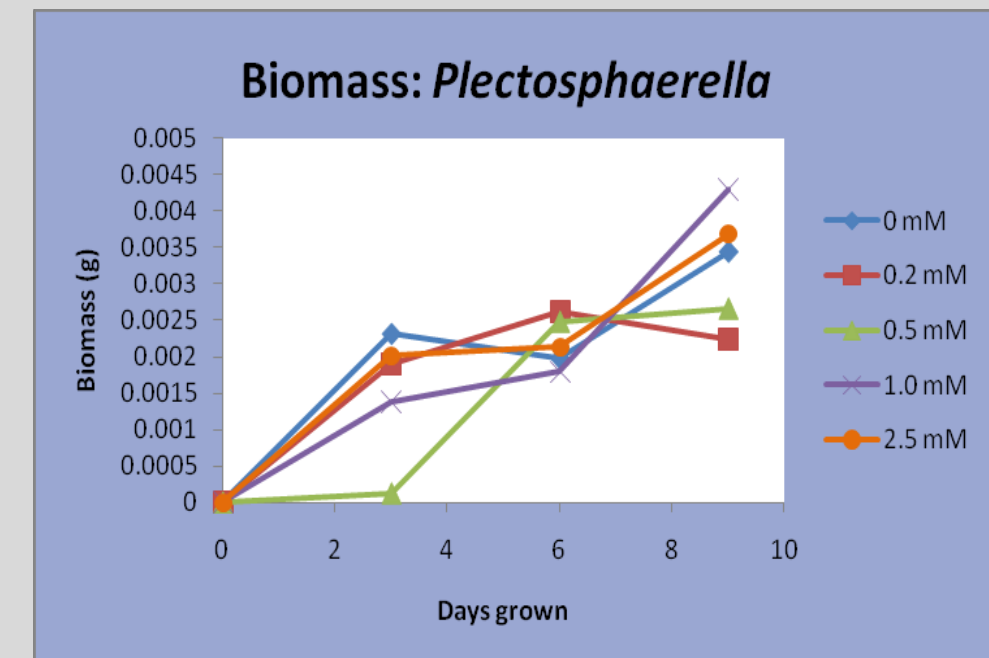
Oxidation was observed in all species at all concentrations except *Plectosphaerella*- there was growth at 2.5 mM, but no oxidation. It appears as though high Mn (II) concentrations inhibit Mn oxide precipitation in this species.



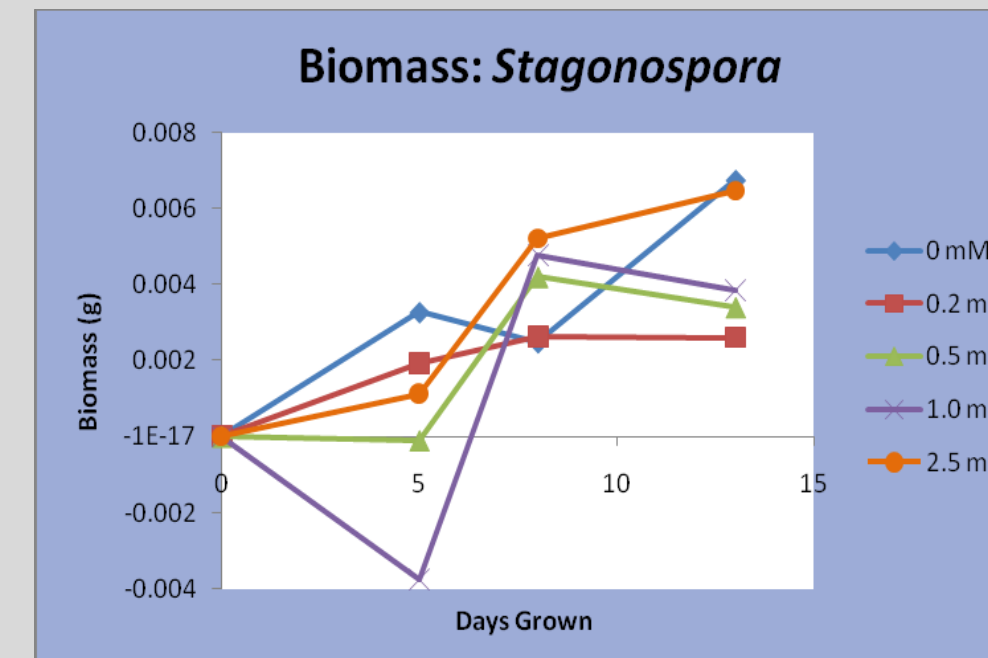
In this species (and *Pyrenochaeta*), the higher the Mn (II) concentration, the more Mn oxidized (except 2.5 mM with no oxidation).



This species (and *Acremonium*) seems to reach a point of maximum Mn oxidation, despite the differing initial Mn (II) concentrations.



As the fungi grew, they tended to increase in mass. The final biomass appears to vary with the initial Mn (II) concentration. (Massing filter paper proved tricky, hence the negative values in *Stagonospora*. We adjusted our protocol for *Plectosphaerella*.)



Discussion

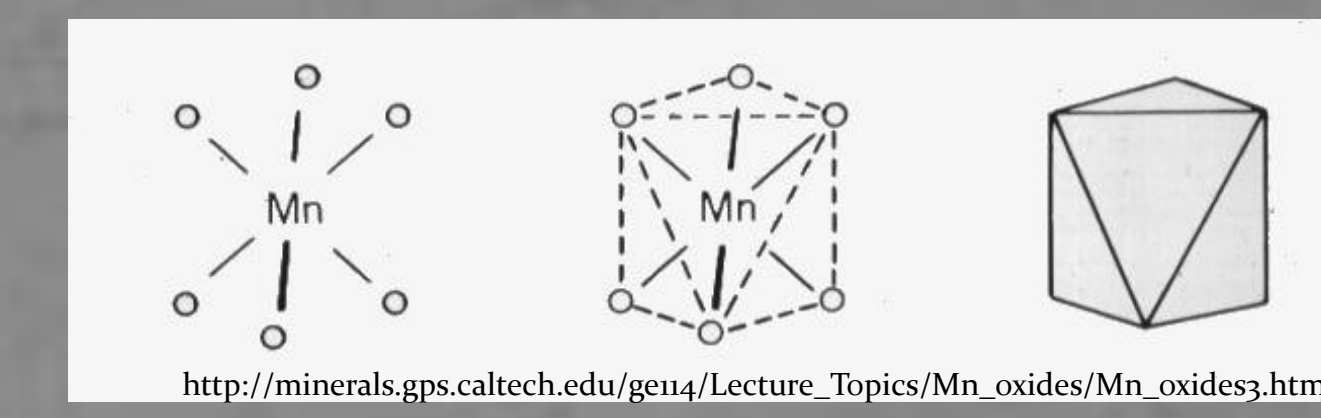
The four species of fungi have different tolerance levels to dissolved Mn, and each has an optimum concentration(s) for oxidation. However, some species grew and oxidized differently in liquid media than in solid media of the same Mn (II) concentrations. This is an interesting result and will require follow-up experiments.

In the liquid samples, the mineral structure observed in all Mn concentrations is birnessite. This is different than what was observed in bacteria: Past research has shown that Mn oxides produced by bacteria in solutions over 0.5 mM usually have a more crystalline structure, feitknechtite. Below this concentration, the layered structure, birnessite, is observed (Bargar et al. 2005). However, in our experiments, birnessite was observed even in concentrations over 0.5 mM, meaning the fungi produce Mn oxides of a different mineral structure than bacteria. We are currently executing another experiment in which we keep the Mn (II) concentrations steady as the fungi grow by continually adding more Mn. This will hopefully determine whether birnessite is still the predominant structure when the concentrations do not steadily decrease as the fungi precipitate the Mn out of solution.

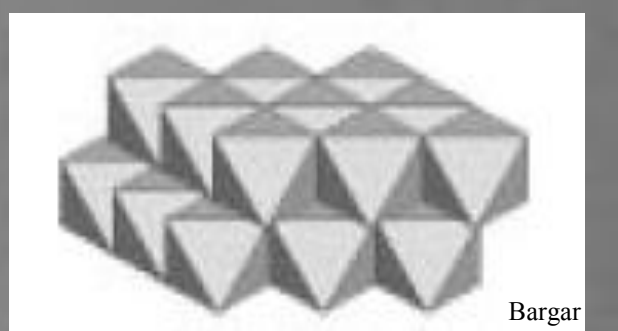
Birnessite is the ideal mineral structure for acid mine drainage remediation. It is a relatively stable structure, so will hopefully prevent the Mn from going back into solution. It also readily adsorbs other dissolved metals (Cu, Zn, Pb), taking them out of the water. Once we learn even more about the fungi and their oxidation products, there is a great potential for fungi to be used in acid mine drainage treatment systems to help clean acidic waters.

Mineralogy Results

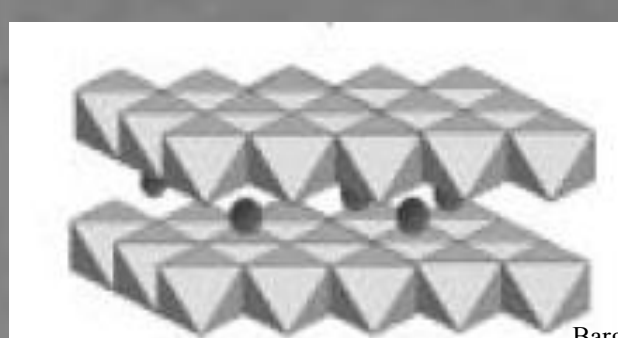
- XRD revealed that the precipitated Mn oxides were birnessite in all samples.



Mn oxide octahedron (MnO₆) - "building blocks" of mineral structure



Feitknechtite- not found in samples



Birnessite- found in samples. The black circles are structural interlayer cations.

Acknowledgements

I would like to thank my advisor, Cara Santelli, for all her help, guidance, and patience during the course of this project. I would also like to thank Ryan Nell for teaching me about lab work and making the lab a fun place to be. Thanks to Gene Hunt, Liz Cottrell, Virginia Power, and the NHRE program. This project was funded by the NSF.



References

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