Remediation potentials of Pb, Zn, and Mn in mining impacted water by a novel Mn-oxidizing fungus from Tar Creek Superfund Site, Oklahoma

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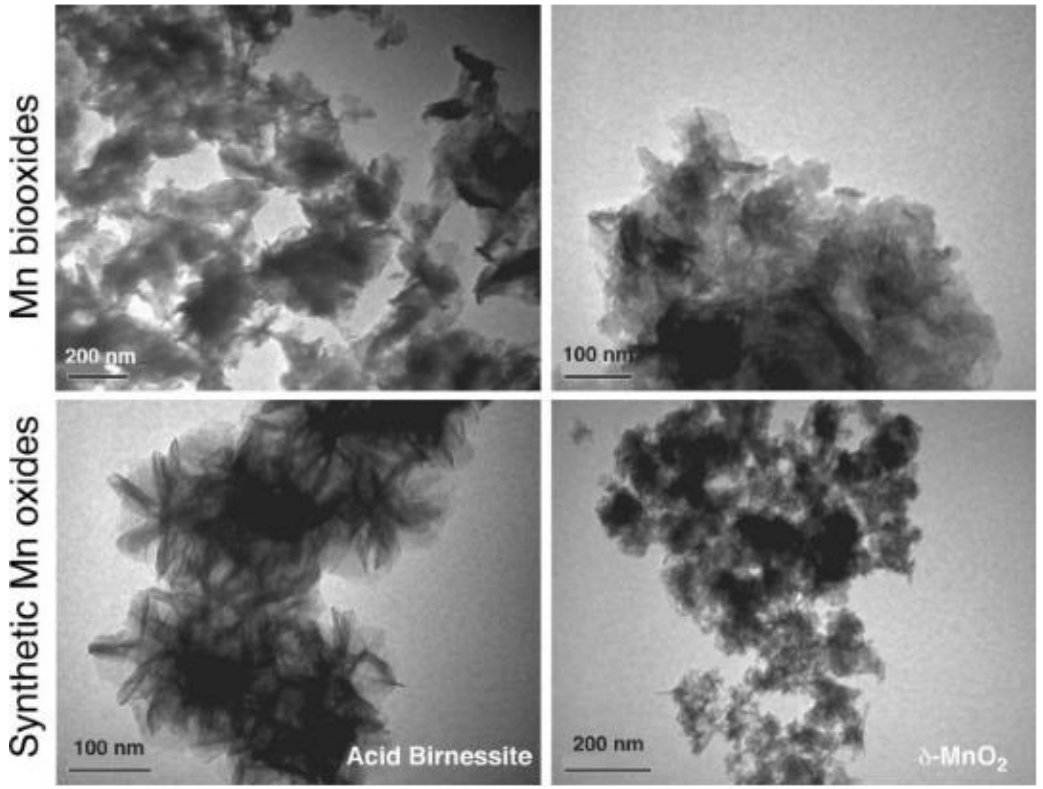
Mn oxides

Mn (III/IV) oxides



Villalobos et al. (2003)
Geochimica et Cosmochimica Acta

Adsorb

Incorporate

Trace elements

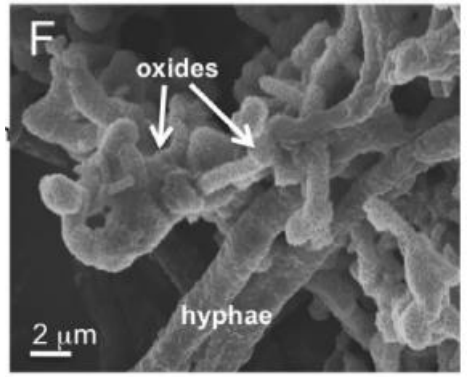
Cu, Co, Cd, Zn, Ni, Sn, Pb, Ca, Fe, Ra, Hg, U, Pu, Po, As, Se, and Th…

Biogenic Mn oxides: important minerals in nature

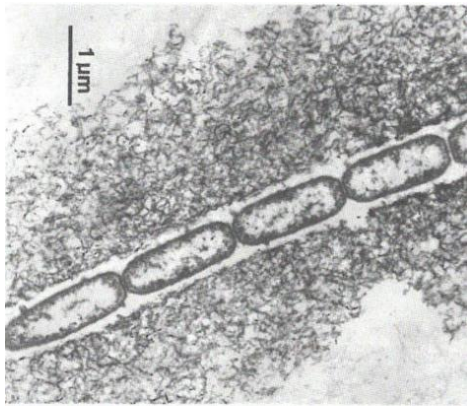
Mn (II) aq

Microbial activities

Mn (III/IV) oxides

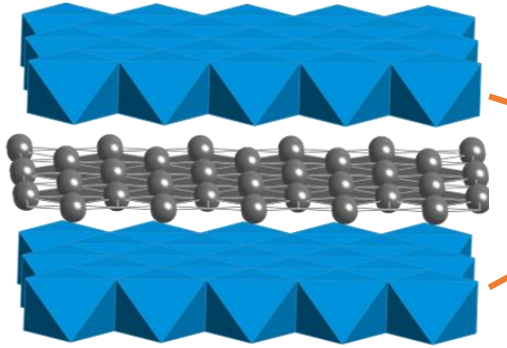


Stagonospora sp. SRC1lsM3a
Santelli et al. (2011) GCA

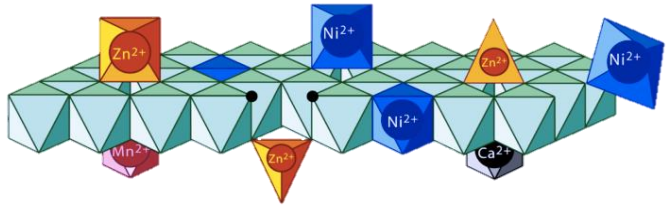


Leptothrix spp.
Tebo et al. (1997) Geomicrobiology

Hydrated interlayers



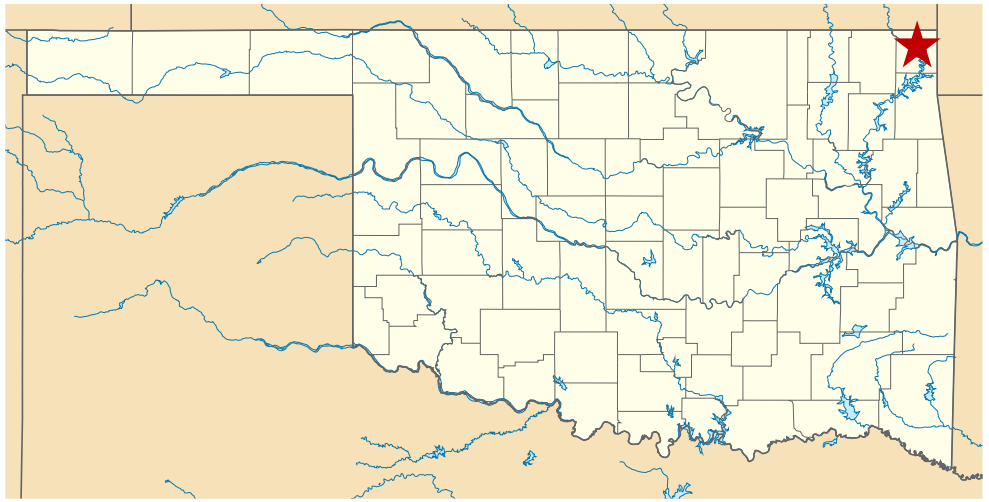
Negatively charged sheets



Manceau et al. (2007) GCA

Mn oxides can incorporate and adsorb micronutrients and metal pollutants at vacancies and particle edges

Study Site: Tar Creek superfund site, Oklahoma



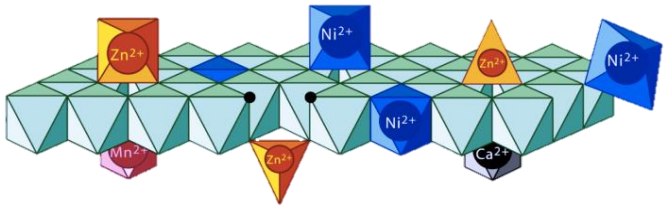
- **Major concern of metals:**

Elements	Tar Creek	EPA drink water
Lead (Pb)	60 ppb	15 ppb
Zinc (Zn)	10 ppm	5 ppm
Manganese (Mn)	2 ppm	0.05 ppm

- **Cost-effective remediation treatments needed!**

Objectives

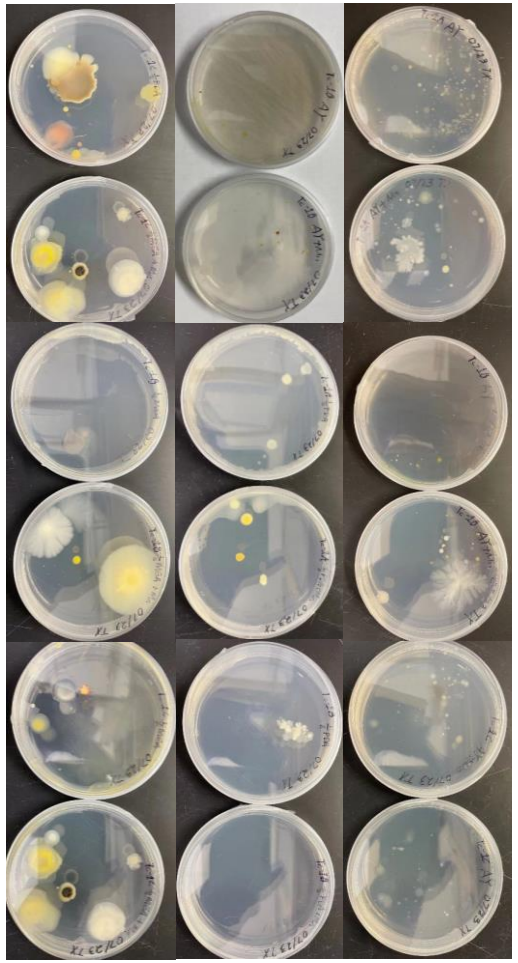
Metal-rich water



Metal-free water

- Need to find a fungus that can oxidize Mn(II) and remove metals.
- What will affect the formation of Mn oxides and metals removal rates?
- Why can Mn-oxidizing fungus oxidize Mn(II)?
- What's the main pathway of the myco-genic Mn(II) oxidization process?

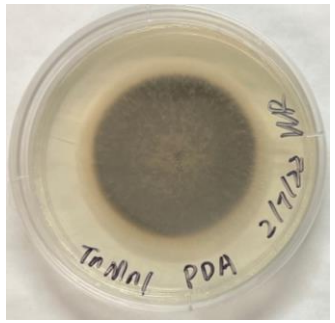
Isolate Mn-oxidizing fungi from Tar Creek superfund site, Oklahoma

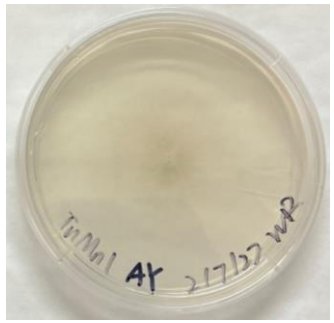


Media
AY+200 µM $MnCl_2$
½ PDA+200 µM $MnCl_2$
½ MEA +200 µM $MnCl_2$

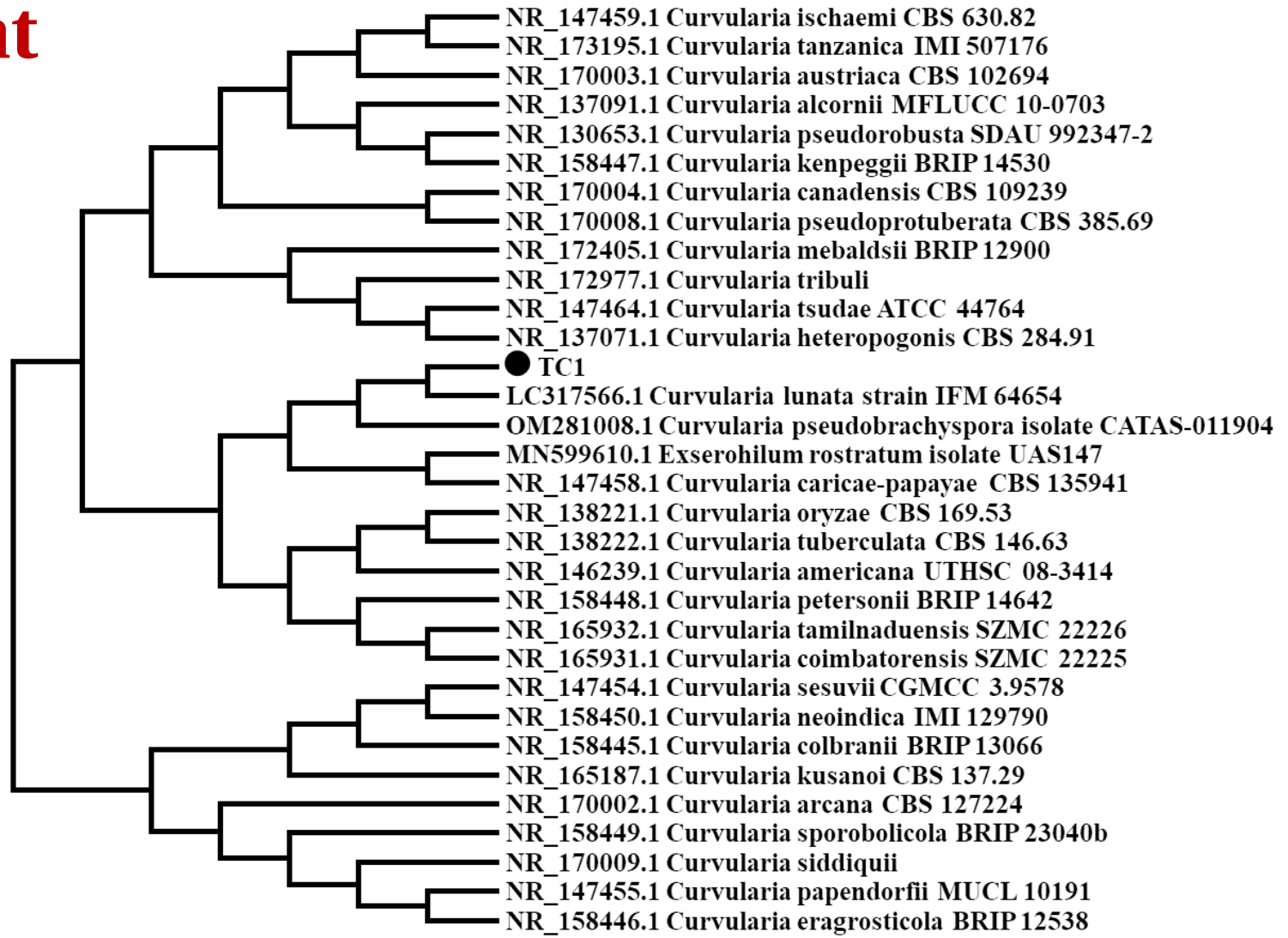
Leucoberbelin Blue (LBB) assay
Pick fungi that formed brown particle to do LBB assay.

Found a new fungus that can oxidize Mn(II)





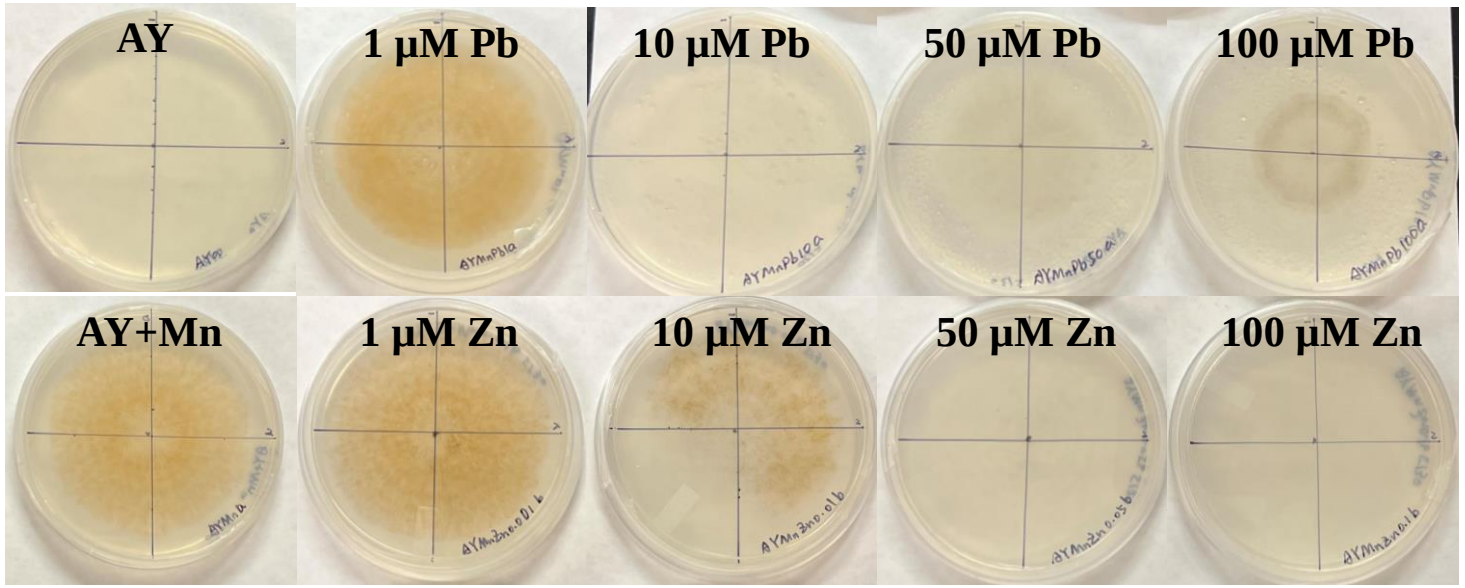
TC1: *Curvularia lunata* (OP531858)



Experimental Setup

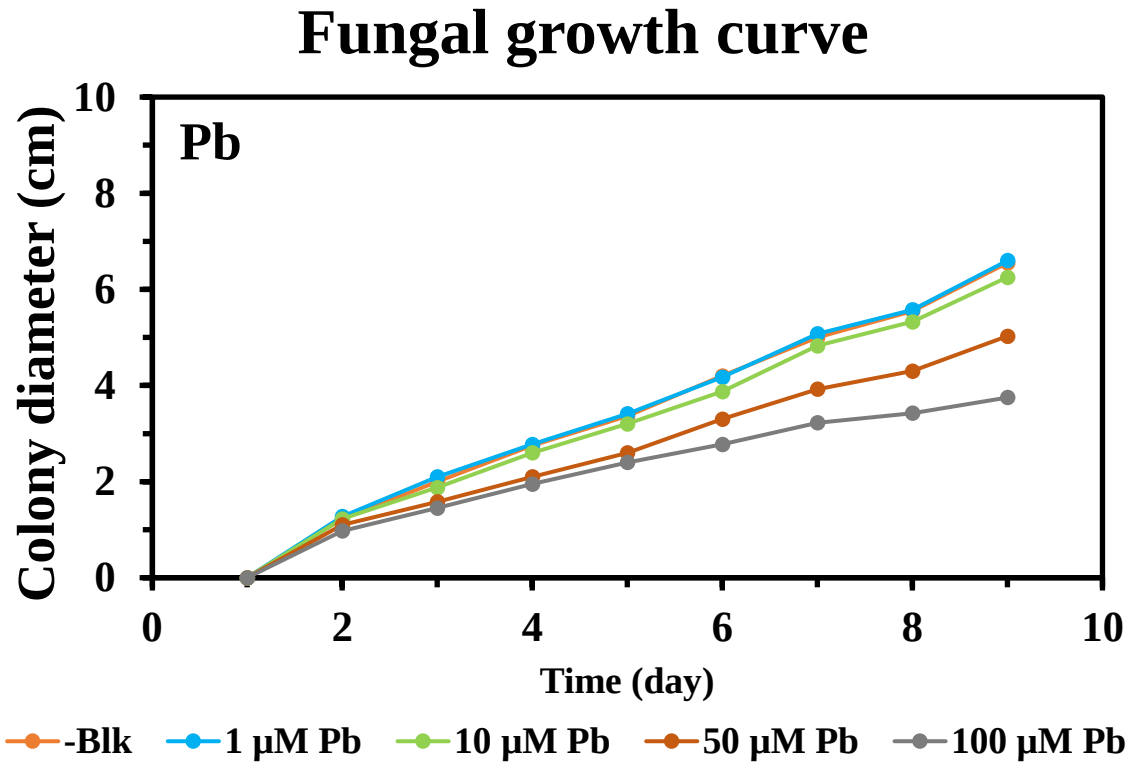
- **Target fungi:** TC1
- **Qualitative analysis of Mn(II) oxidation capacity in solid media**
  - 0 or 200 µM Mn(II)
  - Spike Pb(II) and Zn(II) of different concentration
- **Quantitative analysis of Mn(II) oxidation capacity in liquid media**
  - 200 µM Mn(II)
  - Spike Zn(II) of different concentration
- **Growth media:**
  - AY media (HEPES buffer, pH 7)
- **The Mn(II) oxidizing pathway (on going)**
  - Enzyme/Extracellular superoxide

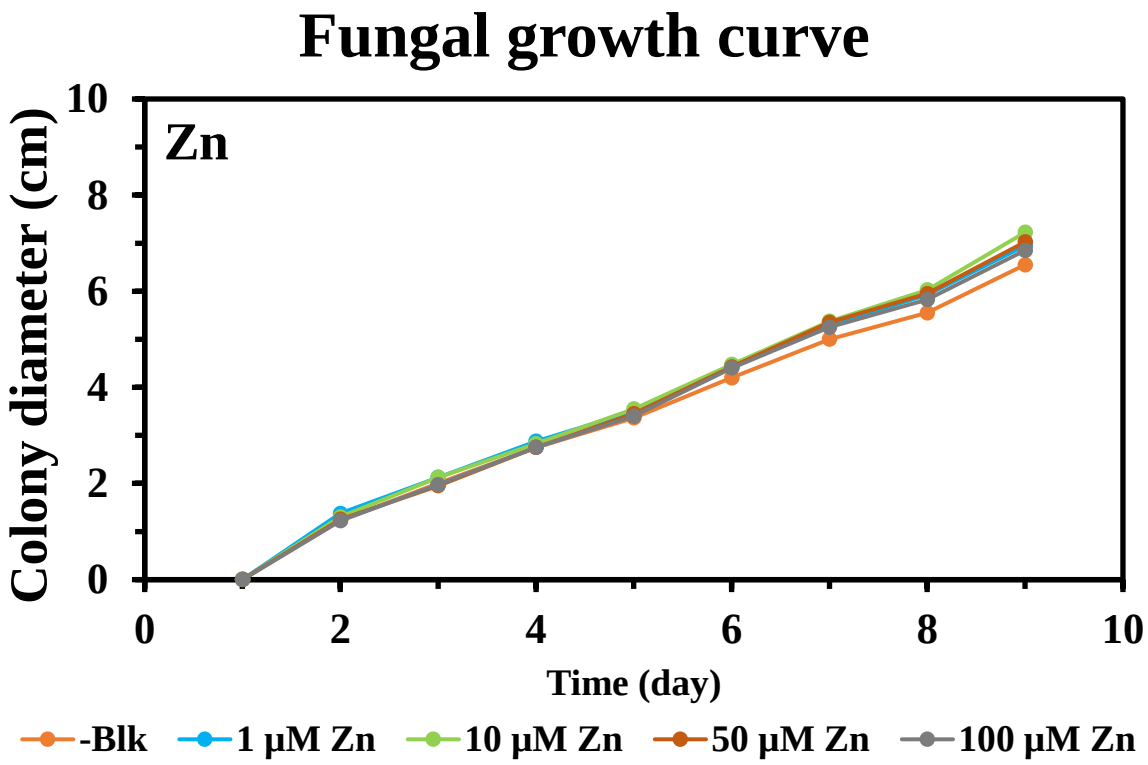
Zn and Pb show distinct impacts on fungal growth and Mn(II) oxidation



- Pb inhibited the formation of Mn oxides and the growth of fungi.
- Zn inhibited the formation of Mn oxides but didn't inhibit the growth of fungi.

Zn and Pb show distinct impacts on fungal growth and Mn(II) oxidation





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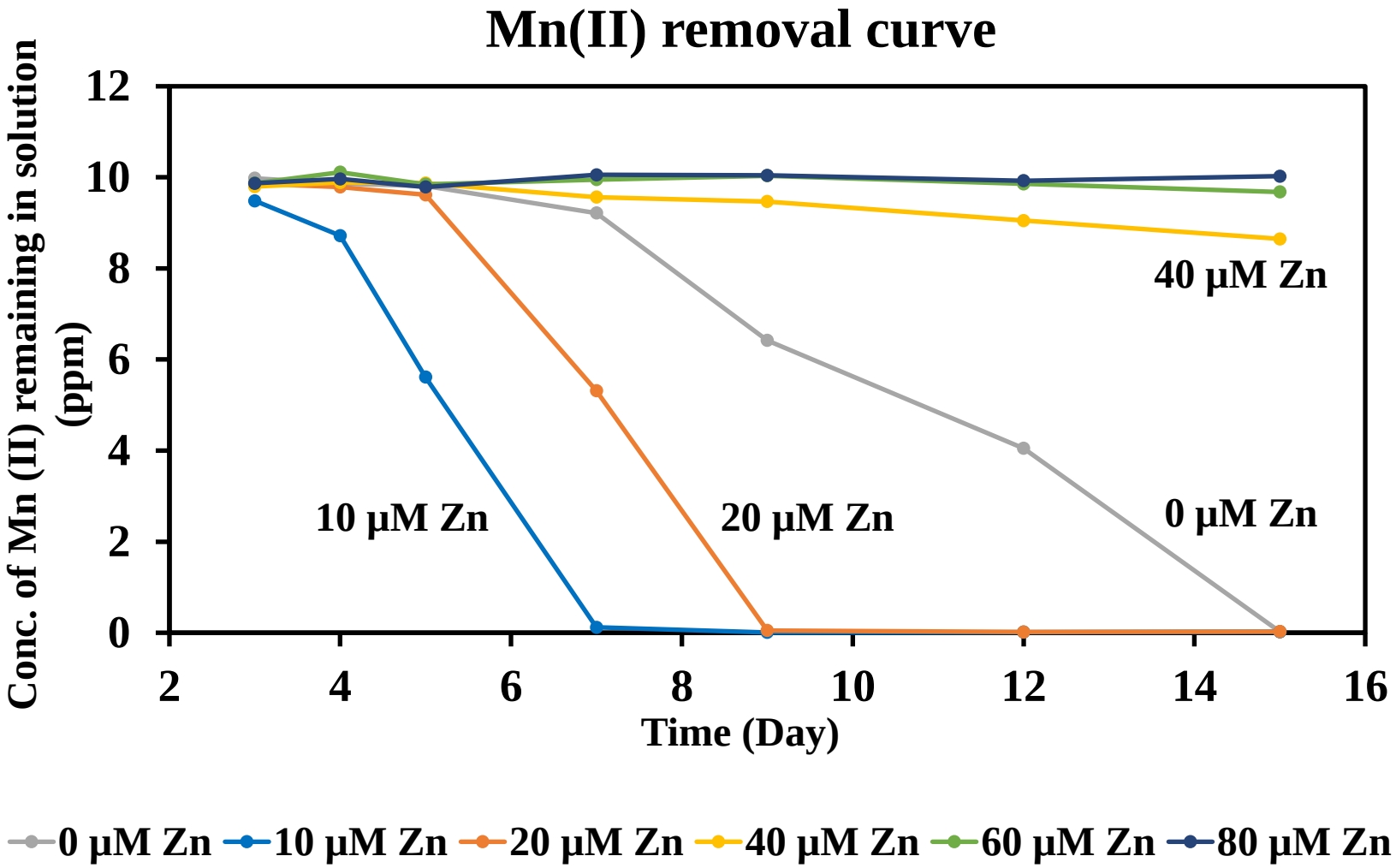
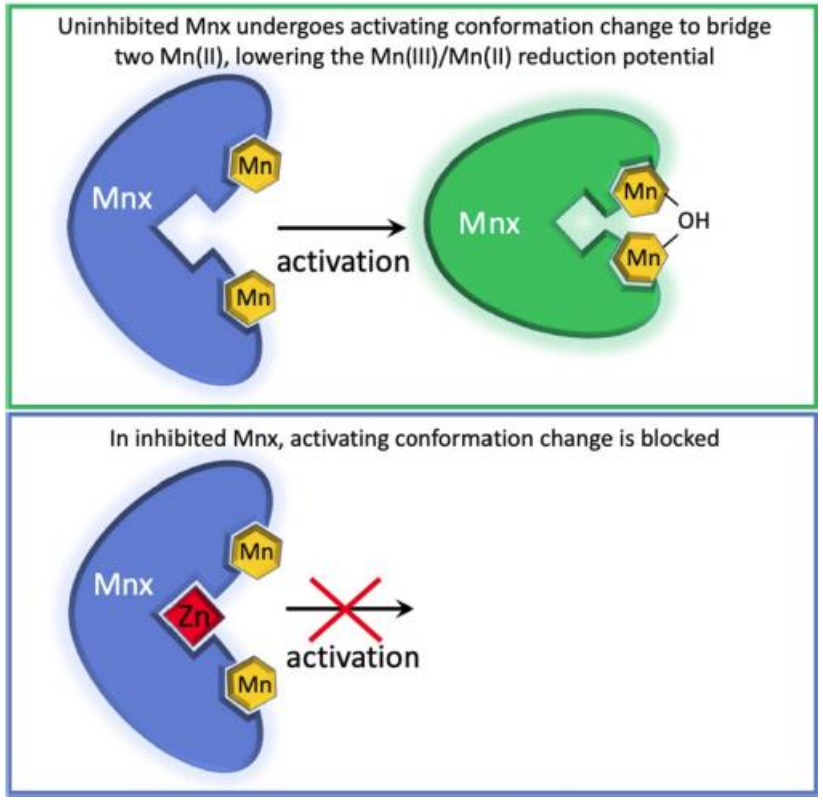
Zn inhibited the formation of Mn oxides



- The formation of Mn oxides was inhibited by Zn, especially when the concentration of Zn was above **40 µM**.

Possible mechanism of Zn inhibiting Mn(II) oxidation



- **Hypothesis**
- If Mn(II) oxidation of our fungus is regulated by multicopper oxidases (MCOs), additional Cu should alleviate the inhibitory effect of Zn.

Zn(II) changed structure of multicopper oxidases in *Bacillus* sp. to inhibit the oxidation.
Soldatova (2021) J. Inorg. Biochem.

The effect of Cu on the formation of Mn oxides

Setup

- AY liquid media with 200 µM Mn(II)
- Spike in

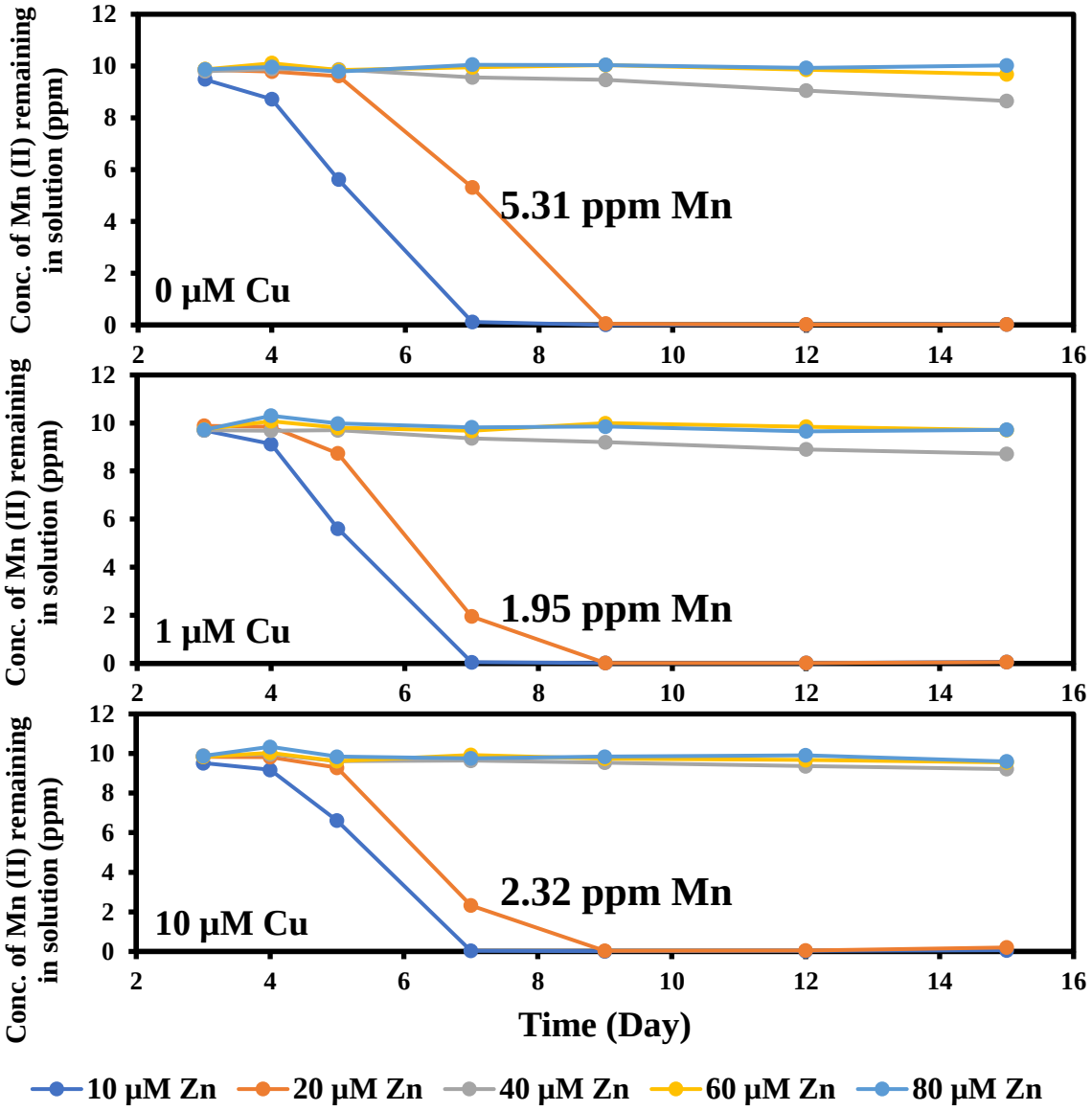
  Zn(II) 10, 20, 40, 60, 80 µM

  Cu(II) 0, 1, 10 µM

Results

- Cu increased Mn removal rate.
- Cu didn't alleviate the inhibitory effect of Zn.

Mn(II) removal curve



TC1: *Curvularia lunata*

(GenBank accession number: OP531858)

Results

- Fungus TC1 can **effectively remove Mn(II)**.
- High concentrations of Pb and Zn have different inhibition mechanisms on the oxidation of Mn(II) and the growth of fungi.
- The oxidation mechanisms of our fungus should be led by superoxide or another enzyme.

Future work

- **Study the detailed mechanisms of Mn(II) oxidation by fungus TC1**
  - Ongoing experiments are focused on elucidating potential pathways of myco-genic Mn(II) oxidization process.
  - We are trying to figure out whether our fungus oxidizes Mn(II) by enzymes, superoxide, or a mixture of them.

Acknowledgements

Collaborators

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Thank you! Question?