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Linking Mn(II)-oxidizing bacteria to natural attenuation at a former U mining site

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Uranium mining near Ronneburg, Germany resulted in widespread environmental contamination with acid mine drainage (AMD) and high concentrations of heavy metals and radionuclides. Despite physical remediation of the area, groundwater is still a source of heavy metal contaminants, e.g., Cd, Ni, Co, Cu and Zn, to nearby ecosystems. However, natural attenuation of heavy metals is occurring in Mn oxide rich soils and sediments ranging in pH from 5 to 7. While microorganisms readily oxidize Mn(II) and precipitate Mn oxides at pH $\sim$7 under oxic conditions, few studies describe Mn(II)-oxidizing bacteria (MOB) at pH $\sim$5 and/or in the presence of heavy metals. In this study we (1) isolated MOB from the contaminated Ronneburg area at pH 5.5 and 7 and (2) evaluated the biological formation of Mn oxides. We isolated nine MOB strains at pH 7 (members of the *Proteobacteria*, *Actinobacteria, Bacteroidetes*, and *Firmicutes* phyla) and a single isolate at pH 5.5 (Oxalobacteraceae isolate AB_14, within the β-*Proteobacteria*). LA-ICP-MS showed that all isolates accumulated Mn and Fe in their biomass. However, the Oxalobacteraceae isolate AB_14 oxidizes more Mn without additional Fe in the medium. Preliminary FTIR analysis indicated that all isolates formed precipitates, which showed absorption bands that were characteristic for birnessite. High resolution TEM showed variable morphology of precipitates and EDS confirmed the presence of Mn oxides. Isolate AB_14 was not surrounded with precipitates whereas our *Actinobacteria* isolate AB_18 was encrusted with Mn oxides. Electron diffraction is currently being used to confirm the presence of birnessite and other Mn oxide phases. This, the first known report of any organism capable of Mn oxidation at low pH, demonstrated that MOB can be involved in the natural attenuation of both moderately acidic and neutral pH soils and sediments via the formation of biogenic Mn oxides. Future work will fully evaluate the minerals formed in this process as well as their interactions with contaminating heavy metals and radionuclides.