

Microbial reduction and biomineralization of Cu by model metal-reducing bacteria and enrichment cultures

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Cu distribution in soil is affected by climatic, geological, and pedological factors. Aside from geological sources, exogenous inputs from industrial, mining, and agricultural processes can also contribute to soil Cu concentrations. Although a micronutrient, Cu can also be highly toxic in excess. The bioavailability and mobility of Cu is influenced by soil pH, organic content, and mineralogy as well as by its redox chemistry. For example, mobilization of Cu and co-contaminants in flooded soils has been linked to microbial reduction of Cu [1]. Despite the importance of Cu redox chemistry, relatively little is known about microbial interactions that could facilitate Cu redox reactions in the environment.

To explore the potential role of microorganisms in Cu biogeochemical cycling, we investigated the potential for direct microbial reduction of Cu(II) by two model metal-reducing bacteria, *Shewanella oneidensis* and *Geobacter sulfurreducens*. Both organisms were able to reduce Cu(II) in solution, with up to 50 μM and 5 μM Cu(II) reduced by *S. oneidensis* and *G. sulfurreducens*, respectively. Potential electron transfer pathways involved in Cu(II) reduction were probed using cytochrome deletion mutants of *S. oneidensis* that suggested the Mtr pathway, commonly used for metal reduction in this bacterium, was not important here. Bioreduction of Cu(II) by both organisms resulted in the biomineralization of Cu nanoparticles (CuNPs). Extensive characterisation of these CuNPs revealed different biomineralization products were formed by the different bacteria, with metallic CuNPs produced by *S. oneidensis* and Cu₂S-like CuNPs produced by *G. sulfurreducens*.

To investigate further the potential diversity of Cu(II)-reducing microbes in soils, we established enrichment cultures from a Cu-rich soil from a former mining site in Cheshire, UK. When challenged with high concentrations of Cu(II) (200 μM), the enrichment cultures became dominated by two species of bacteria, as revealed by 16S rRNA sequencing. Cu reduction was observed in the enrichments and the resulting CuNPs were characterised as a mixture of metallic Cu and Cu_xS. These data highlight the potential for microbes to influence Cu biogeochemistry through direct Cu bioreduction with the speciation of the resulting biominerals, metallic and/or sulphide, depending on the microbial community present.

[1] Weber et al (2009) *Nat Geosci* 2:267–271.