

## Seeing is believing: Making heavy metal ions visible under a confocal laser scanning microscope

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Biofilms and microbial aggregate are common at interfaces, which bind toxic heavy metal ions and control their fate and bioavailability in the environment due to the high sorption capacity. The spatial relationship of metal ions to biomacromolecules such as extracellular polymeric substances in biofilms with microbial cells and biogenic minerals is complex and occurs at the micro- and submicrometer scale, it also remains unclear to which of these component(s) contribute to the metals binding in complex microbial aggregates and biofilm.

To clarify this question, our present study focuses on 3D mapping of heavy metals sorbed to cells, glycoconjugates that comprise the majority of EPS constituents, and Fe(III) mineral aggregates formed by the phototrophic Fe(II)-oxidizing bacteria *Rhodobacter ferrooxidans* SW2 using confocal laser scanning microscopy in combination with metal- and glycoconjugates-specific fluorophores. To evaluate the influence of glycoconjugates, microbial cell surfaces, and (biogenic) Fe(III) minerals, and the availability of ferrous and ferric iron on heavy metal sorption in the microbial aggregates at the sub- $\mu\text{m}$  scale.

The results showed that the mineral surfaces, bacterial cell and the glycoconjugates provided most of sorption sites for heavy metals. Statistical analysis revealed that all heavy metals tested showed relatively similar sorption behaviour. Simultaneously, ferrous and ferric iron ions competed with the heavy metals for sorption sites on the organic compounds. The benefit of this method is that the samples can be analyzed in their natural hydrated state, avoiding artefact. It will further our understanding of the behavior of metals in environmental systems, also is essential to understand the underlying mechanisms of microbe–mineral–metal interactions.