Microorganisms and Biofilms

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Tracking mineral particles and metal(oid)s from armor stones in freshwater biofilms

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Industrially produced copper slag is widely used as armor stone in inland waterways. Copper slag still contains significant amounts of sulfidic minerals and leaching of metal(oid)s poses a potential risk for the aquatic environment [1]. Biofilms may function as a sink due to metal(oid)s binding to functional groups of extracellular polymeric substances and cell surfaces or through intracellular storage. Additionally, the biofilm matrix may trap small, abrasive particles. These bound metal(oid)s or mineral particles may be transferred to organisms of higher trophic levels, particularly to biofilm grazing organisms. Furthermore, biofilms may harbor microorganisms, which enhance the dissolution of the trapped mineral particles, e.g. the oxidative dissolution of sulfidic minerals. The fate of metal(oid)s and small mineral particles released from armor stones remains so far unclear. Little is known about how these particles integrate into a biofilm matrix and how they interact with the biota.

In cooperation with the German Federal Institute of Hydrology, an environmental impact assessment of copper slag (CUS) was performed with basanite (BAS) as reference material of natural origin. Six indoor stream mesocosms were set up containing water and sediment from the river Rhine. CUS and BAS were applied as crushed sand (grain size < 2 mm). The experiments were run for 6 months, after which biofilm was sampled from acrylic glass walls. In order to unveil the pathways of dissolved metal(oid)s or released mineral particles within biofilms, ultrathin sectioned embedded biofilm samples were investigated by transmission electron microscopy. EDX was used to determine particle elemental composition in order to discriminate prime armor stone minerals from secondary minerals formed by microbial activity. Scanning electron microscopy was employed to demonstrate the diverse nature and complex structure of investigated biofilms.

SEM of biofilms showed a dominance of a variety of diatom species accompanied by a sheathed, filamentous cyanobacterium affiliated to the genus *Leptolyngbya* (Fig. 1a). In addition, various smaller, morphologically diverse bacterial cells were observed, either attached on diatom shells, cyanobacterium sheaths or mineral surfaces (Fig. 1b). TEM of ultrathin sections showed both the cells of the different biofilm microorganisms as well as many mineral particles, which were very small in size (<500 nm in diameter). These particles were apparently loosely attached to cell surfaces or extracellular structures such as sheaths or capsules and did not reveal specific adhesion or incorporation. Their elemental composition reflected the main elements present also in the original armor stone minerals (spectra in Fig. 1c, 1d). However, a high spatial heterogeneity on a very small scale was detected revealing iron rich spots in BAS biofilms and iron and sulfur rich spots in CUS biofilms.

The incorporation of small mineral particles from copper slag can initiate enhanced interaction with biota due to their high surface to volume ratio. Oxidative dissolution of sulfidic minerals by microorganisms present in the biofilm may result in a stronger release of dissolved metal(oid)s and decrease in pH. The small particle size and the heterogeneous mineral composition may contribute to the overall heterogeneity of the biofilm and hence its metabolic and physiological diversity. A direct evidence for the removal of dissolved metal(oid)s from the water phase by precipitation (secondary mineral formation), i.e. the selective enrichment of specific elements on cell surfaces or extracellular structures, could not be found.

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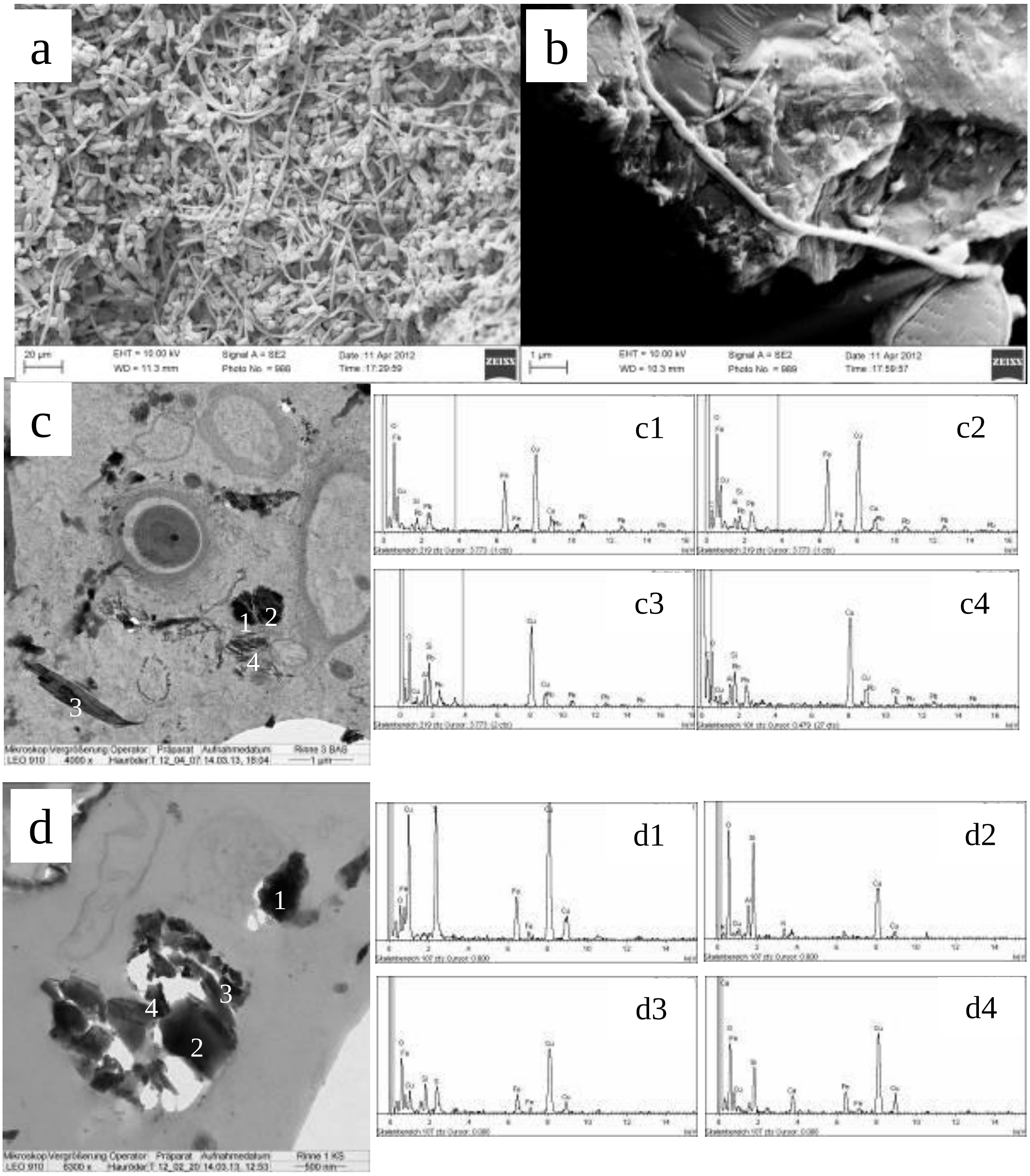

Figure 1. SEM images of biofilm from mesocosm 1 (CUS) (a,b), TEM images and EDX spectra of biofilm from mesocosm 3 (BAS) (c) and from mesocosm 1 (CUS).