Biofilms are of crucial importance for the remediation of contaminated waters. Cyanobacteria of the genus *Phormidium* form dense mats in alpine creeks. The bacterial filaments are covered by a gelatinous sheath that adsorbs a variety of elements. In a copper contaminated creek in the Austrian Alps, an extensive, *Phormidium* dominated biofilm accumulates 3.9 ± 1.8% copper, thereby completely remediating the water of the creek by immobilising the copper. This study investigates the structure and ultrastructure of the biofilm with special regard to the localisation and speciation of the copper. The biofilm was chemically fixed or plunge frozen; further preparation included cryo-substitution with ultrathin sectioning as well as freeze drying, conventional sectioning and analysis of whole mounts. Microscopic techniques comprised confocal microscopy, polarised and phase contrast light microscopy, X-ray microanalysis as well as scanning and transmission electron microscopy. The biofilm exhibits an extraordinary thickness of up to 22 cm with only the top layer containing living cells. It consists almost exclusively of filamentous *Phormidium* growing in clearly distinct layers (Fig. 1). Other bacteria like *Bacillus* are restricted to the surface of the biofilm. The copper is not evenly distributed in the biofilm but occurs as distinct crystals, probably consisting of the secondary copper mineral Sampleite \([\text{NaCaCu}_5(\text{PO}_4)_4 \cdot \text{H}_2\text{O}]\) with a diameter of about 10 µm between the bacterial filaments (Fig. 2). Furthermore, copper is also found in the sheaths of the bacteria. Here, transmission electron microscopy suggests that this copper is not simply adsorbed but occurs in abundant submicroscopic electron-dense particles at the surface of the sheaths. These results show that the process of copper immobilisation by *Phormidium* biofilms is far more complex than simple passive adsorption and includes processes of biomineralisation. The speciation of the copper as mineral particles within the biofilm indicates that the immobilisation of metals is permanent which is confirmed by analysis of old, subfossil layers of the biofilm. These findings do not only enlighten the mechanism of metal immobilisation by *Phormidium* but also encourage its use for bioremediation of mine waste waters.

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Fig. 1: Cross section of the biofilm after Dapi staining. The filamentous Cyanobacteria (yellow-white) form several clearly distinct layers. Non-photosynthetic microorganisms (blue) are restricted to the surface (CLSM).

Fig. 2: Between the Cyanobacteria, globular, copper rich mineral particles are found (SEM).