Bryophytes inhabit extremely different habitats, ranging from dry fallen river banks (e.g. *Physcomitrella patens*) to metal contaminated sites (e.g. *Pohlia drummondii* and *Mielichhoferia elongata*). Therefore, some bryophyte species are considered stress tolerant, and even the supposedly metal sensitive moss *P. patens* showed increased tolerance to Cu-EDTA in earlier studies.

For the present experiments, the bryophytes were cultivated on sterile agar plates and tested for zinc (as Zn-EDTA, ZnCl$_2$ and ZnSO$_4$) and copper (as Cu-EDTA, CuCl$_2$ and CuSO$_4$) over a period of five weeks (Fig. 1).

Despite of the high tolerance towards Cu-EDTA of *P. patens*, we measured changes in growth and metal uptake analyzed by X-ray microanalysis in a scanning electron microscope (Fig. 2) if the metal is offered with different anions. Here, especially the uptake of EDTA chelated metals was significantly lower compared to metal offered as ionic salt. Modelling of ion availability explained most of the differences in toxicity.

Changes in the cellular content of reactive oxygen species (ROS) after staining with 2,7-dichlorofluorescein diacetate (H$_2$DCFDA) were analyzed in a confocal scanning microscope (Fig. 3) and the three different bryophyte species compared. *P. patens* showed only low H$_2$DCFDA fluorescence in control cells, in contrast to metal treated cells were increased ROS could be detected for chloroplast associated mitochondria, the nuclear region and the cell wall region.

Further investigation of cellular localization of metal deposition was performed using FluoZin-3 and is ongoing in transmission electron microscopy studies.

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Fig. 1: P. patens plants grown on control and zinc spiked media over a period of 5 weeks.

Fig. 2: Scanning electron microscope micrograph of P. patens leafy gametophyte (scale bar = 1 mm).

Fig. 3: P. patens leaf cells stained with H$_2$DCFDA for H$_2$O$_2$ detection. a, Control cells; b, Plants grown on 1 mM ZnCl$_2$ and c, plants grown on 0.1 mM CuSO$_4$ show increased fluorescence in close vicinity of the chloroplast, in the nuclear region (*), mitochondria (arrowhead) and the cell wall (scale bar = 10 µm).