The heavy metal distribution in two copper tolerant bryophytes *Pohlia drummondii* and *Mielichhoferia elongata*

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Pohlia drummondii and *Mielichhoferia elongata* are known to occur on copper rich substrate [1,2]. We used plant material from old copper-mines in the Grossarl valley near Hüttschlag in Salzburg (Hohe Tauern, Austria) and *in vitro* cultures were established for further investigations. These relate to the possible uptake of copper from the substrate and to stress reactions influencing the protonemal growth.

After testing different media for cultivation it was decided to use modified Benecke-medium [2] for resistance tests. Both mosses were cultivated on agar plates enriched with Cu-EDTA from 1 µM up to 0.1 M. *P. drummondii* survived up to a concentration of 10 mM and this limit was streched further on plates with 13 mM, 16 mM and even up to 24 mM. By contrast *M. elongata* has an extraordinary high tolerance limit of 0.1 M.

The heavy metal distribution in the shoots was measured in three groups: basis, middle and tip, by X-ray microanalysis (EDX) in combination with SEM. The mosses were air dried and carbon coated. The EDX data were evaluated using SPSS. For both mosses the results show a similar pattern: A significantly higher uptake on plates with 1mM Cu-EDTA and above could be detected as compared to the control. *P. drummondii* indicated a tendency of accumulation in the basis from 10 mM up to 16 mM but shows a decline in 24 mM (Fig. 1c). In *M. elongata* a higher content of copper could be measured in the basis and in the middle of the shoots as compared to the tip when grown at 10mM (Fig. 1d). At all other concentrations no significant differences in the three zones could be detected.

Effects of copper on the protonemal growth were examined by measuring the cell-lengths of chloronemata (Fig. 2a) and caulonemata (Fig. 2b) in the light microscope. The cell-lengths of *M. elongata* from 10 mM copper medium show an increase for chloronemata and caulonemata compared with the cells from control medium. In *P. drummondii* only the chloronema cells showed an elongation and the caulonema did not (Fig. 2c).

In conclusion the EDX data suggest an copper enrichment in both *P. drummondii* and *M. elongata*. The higher tolerance of *M. elongata* may be due to the lower uptake compared with *P. drummondii*. How and why the changes in the protonemal cell-lengths occur has yet to be discovered and will be part of further investigations. These will also include the exact cellular localization of copper with electron energy loss spectroscopy (EELS).

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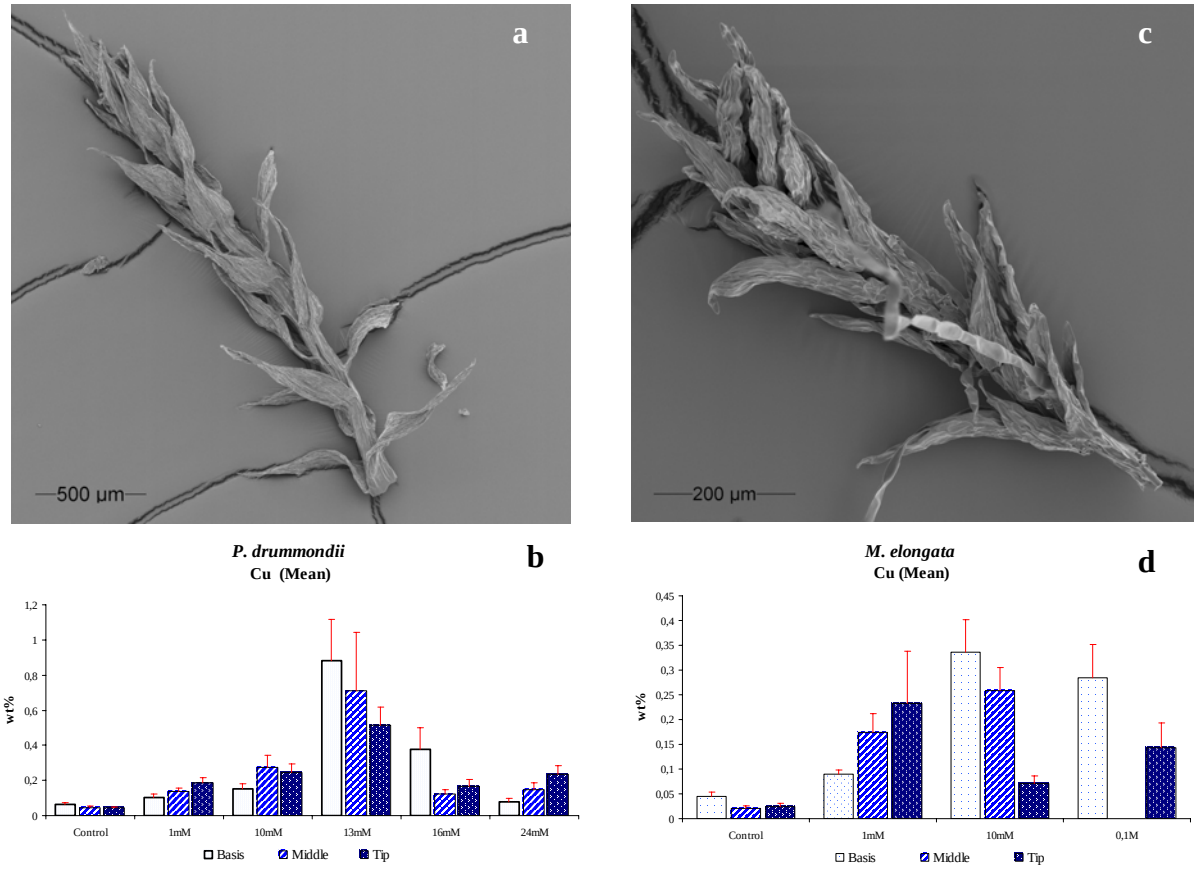

Figure 1. a SEM image of *P. drummondii*, **b** histogram of copper distribution in *P. drummondii* grown on different copper concentrations, **c** SEM image of *M. elongata*, **d** histogram of copper distribution in *M. elongata* grown in different copper concentrations

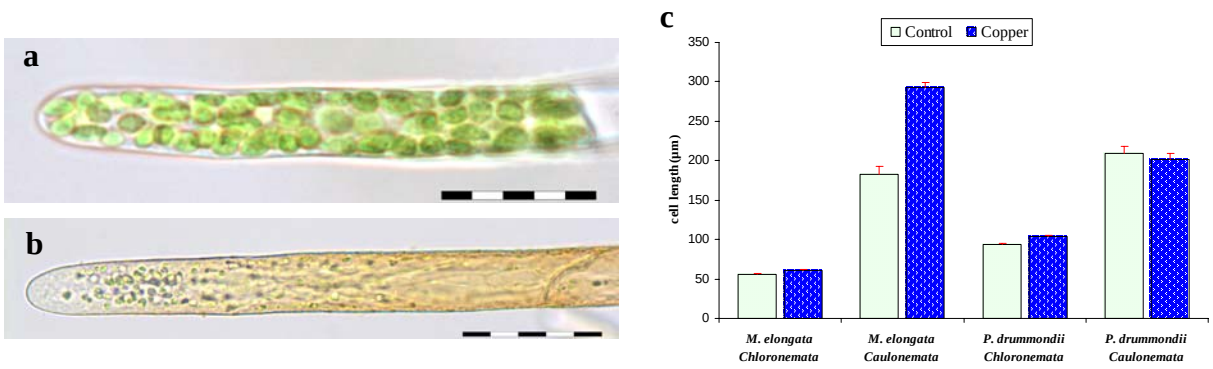

Figure 2. light microscope images of **a** chloronema cell (bar = 20µm) and **b** chaulonema cell (bar = 50µm), **c** histogram showing the effects of copper on the protonemal growth of chloronemata and caulonemata in *P. drummondii* and *M. elongata*.

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