

S. Sassmann, W. Adlassnig, M. Puschenreiter, E. J. P. Cadenas, M. Leyva, I. K. Lichtscheidl, I. Lang:

Free metal ion availability is a major factor for tolerance and growth in *Physcomitrella patens*

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Abstract:

Metal rich sites are populated by only a few specialized vascular plant species allowing less competitive bryophytes to inhabit these ecological niches. On such sites, many different stress factors may interact with the plants and foreclose statements on the individual effects of a single factor, therefore the means for this tolerance are difficult to investigate. This study uses the cultivation of the moss *Physcomitrella patens* on solid media under controlled environmental conditions in order to study the growth inhibiting effects of copper, zinc and cadmium and the contribution of corresponding anions as chlorides, sulfates and ethylenediaminetetraacetic acid, respectively. Availability of the metals to plants was estimated both by water extraction with subsequent measurement by inductively coupled plasma mass spectroscopy and by modeling metal speciation using Visual MINTEQ a free chemical equilibrium model software. A decreased ratio of gametophyte to protonema growth (G:P) was observed as a first reaction to even very low metal levels; G:P measurements can therefore be used as sensitive stress indicators in *P. patens*. Though all metals caused inhibiting effects with all anions, ethylenediaminetetraacetic acid chelates showed up to three orders of magnitude less toxic. Using Visual MINTEQ, we modeled the water solubility of zinc almost perfectly but achieved less accurate results for copper. For both metals, water solubility was rather under- than overestimated, indicating that adsorption to the agar played only a minor role in controlling metal solubility. Free metal cations were useful to explain growth inhibition which could not be fully explained by total metal concentration, calculated or experimentally determined water solubility. Especially the low toxicity of ethylenediaminetetraacetic acid chelates could be explained satisfactory by shielding of the metal ions.