Title	**Evaluation of cations and chelating agents as extracellular extractants for Cu, Pb, V and Zn in the sequential elution technique applied to the terrestrial moss *Pseudoscleropodium purum***
Author	Pérez-Llamazares A, Galbán- Malagón CJ, Aboal JR, Angel Fernández J, Carballeira A.
Journal	Ecotoxicology and Environmental Safety
Abstract	Three experiments were carried out to select the best extractant for use in the sequential elution technique, to enable extraction of Cu, Pb, V and Zn from the extracellular fraction of the terrestrial moss *Pseudoscleropodium purum*. The optimal concentrations of the extractants tested (CoCl2, NiCl2, Pb (NO3)2, SrCl2, dimercaprol, EDTA, penicillamine) were determined on the basis of the maximum extraction of Zn achieved without any alteration of the plasma membrane. The capacity of these agents (at the optimal concentrations established) to extract the extracellular fractions of Cu, Pb, V and Zn was then evaluated. Extraction with 10 mM EDTA is recommended for all 4 elements considered. As a second option, the use of 50 mM penicillamine is recommended to extract Cu, 30 mM dimercaprol to extract Pb and V and 20 mM NiCl2 to extract Zn. It was also concluded that these results cannot be extrapolated to other cryptogams, and that separate assays are required.
Year	2010
Pages	507-14
keywords	*Pseudoscleropodium purum*, Cu, Pb, V, Zn

Title	**Modelling exchange kinetics of copper at the water-aquatic moss (*Fontinalis antipyretica*) interface: Influence of water cationic composition (Ca, Mg, Na and pH)**
Author	Daniel Ferreira, Philippe Ciffroy, Marie-Hélène Tusseau-Vuillemin, Cédric Garnier, Jean-Marie Garnier
Journal	Chemosphere, Volume 74(8)
Abstract	The present study investigated the effect of water cationic composition (Ca, Mg, Na, pH) on the bioaccumulation and elimination rates of copper by an aquatic moss (*Fontinalis antipyretica*), under laboratory conditions. For this purpose, mosses were exposed to copper at an environmentally relevant and usually non-toxic concentration (5 μg L^{-1}) in natural waters where cationic composition and concentrations were varied. To describe

	copper bioaccumulation by aquatic mosses, a two-compartment model was the first-order kinetics, was developed and calibrated under a wide range of water cationic composition. Bioaccumulation rates of Cu in mosses were significantly reduced as the concentrations of competitive cations in solution increased. Hence, in hard-water, Ca and Mg cations play a protective role as they compete with Cu^{2+} ions for the absorption on transport sites at the organism–water interface. Based on the relationships between each major cation concentration and the exchange kinetics on mosses, the binding constants (K_{CiBL}) of each competing cations to the biological surfaces were derived. Using the present cationic-dependant kinetic model, it is now feasible to incorporate water cationic composition in the (re)interpretation of bryophytes contamination levels and in the (re)definition of Water Quality Criteria (WQC) as illustrated through two selected examples of biomonitoring programmes. In the framework of future national water quality guidelines revisions, a such flexible and mechanistic biomonitoring tool (integrating the protective effects of competing cations) may greatly improve the ability of regulators to derive site-specific Cu (metal) guidelines for protecting aquatic biota, while limiting the use of conservative assumptions.
Year	2009
Pages	1117- 1124
keywords	water cationic composition, bioaccumulation, water quality criteria

Title	**Pectinous cell wall thickenings formation—A response of moss protonemata cells to lead**
Author	Magdalena Krzeslowska, Marta Lenartowska, Ewa J. Mellerowicz, Slawomir Samardakiewicz, Adam Wozny
Journal	Environmental and Experimental Botany, Volume 6(1)

Abstract	Lead poisoning constitutes one of most detrimental environmental hazards to all living organisms. Plants developed a variety of avoidance and tolerance mechanisms that are activated in response to lead exposure. Plant cell walls were suggested to play important role in these reactions by creating an efficient barrier to lead entry to the protoplasts, but the molecular mechanisms involved in such shielding reaction have not been elucidated. Tip growing protomemata of *Funaria hygrometrica* (Hedw.) were used as model for studying effects of lead exposure on plant cell walls (CWs). Forty-eight hour-treatment 4 μM $PbCl_2$ resulted in the appearance of cell wall thickenings (CWT_s) at the tip of the apical cell, which is the lead entry site to the cell protoplast [Krzeslowska, M., Wozny, A., 1996. Lead uptake localization and changes in cell ultra structure of *Funaria hygrometrica* protonemata. Biol. Plant. 38, 253–259]. The nature of these thickenings differed from the one of cell wall in unexposed plants as revealed by immuno labelling with monoclonal antibodies and histochemical analyses. The most striking difference was the appearance high amount of low-esterified (JIM5 epitope) and unesterified (PAM1 epitope) homogalacturonan, which were absent from the tip cell wall of control protonemata and are known as the compounds able to bind and immobilise Pb^{2+}. Furthermore, the cell wall thickenings commonly contained callose and at least two kinds of lipid compounds known as the substances preventing metal ions entry to the protoplast. Observations in transmission electron microscope (TEM) showed that CWTs contained a few distinct, varied structurally regions. The dominant one was the region of a granular structure—never found in the control CW. This region contained both the highest amount of JIM5 pectins—and the most numerous lead deposits. In many cases gold particles, identifying JIM5 pectins, appeared to be bound to lead deposits. It indicated that JIM5 pectins which accumulated in CWTs were involved in immobilisation of high amounts of Pb^{2+}. Because the region of lead accumulation occupied the largest volume of the CWTs, we concluded that CWTs appear to be a very important repository for Pb^{2+} in protonemata cells. Thus, we postulate that, CWTs localized at the tip of the apical cell—the main region of lead uptake [Krzeslowska, M., Wozny, A., 1996. Lead uptake localization and changes in cell ultrastructure of Funaria hygrometrica protonemata. Biol. Plant. 38, 253–259] rich in JIM5 pectins, callose and lipids function as the effective barrier against lead ions penetration into the protonema protoplast. The findings substantiate previous hypotheses that lead ions can be sequestered in cell walls and point to the possibility that capacity for lead binding might increase in cell response to lead.

Year	2009
Pages	119-131
keywords	*Funaria hygrometrica*, Lead poisoning, protonemata cells

Title	**Responses to copper by the moss Plagiomnium cuspidatum: Hydrogen peroxide accumulation and the antioxidant defense system**
Author	Yanfang Wu, Yahua Chen, Yanjun Yi, Zhenguo Shen
Journal	Chemosphere, Volume 7(9)
Abstract	Using both histochemical and cytochemical methods, we investigated the effects of copper (Cu) on the production of hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\cdot-}$) in the leaves of the moss Plagiomnium cuspidatum. Cu treatment significantly increased the contents of total thiobarbituric acid-reactive substances and H_2O_2, as well as the activity of guaiacol peroxidase and superoxide dismutase (SOD). Native PAGE detected all three forms of SOD (Mn-SOD, Fe-SOD and CuZn-SOD) in P. cuspidatem, and the increase in the total SOD activity appeared to be mainly caused by an increase in CuZn-SOD activity. According to cytochemical results, H_2O_2- dependent CeCl3 precipitates were primarily localized in the plasma membranes and cell walls, and O2.- was chiefly localized on the inner side of the plasma membrane and in the cytoplasm surrounding the chloroplasts. Experiments using imidazole as an inhibitor of NADPH oxidase, N-N-diethyldithiocarbamate as an inhibitor of Cu Zn-SOD, and 1,2-dihydroxybenzene-3,5-disulphonic acid as an O2.- scavenger indicated that a partial source of H2O2 in the cell walls may be NADPH oxidase. The results also showed that peroxidase (POD) are involved in the detoxification of H2O2. Increased POD activity induced by Cu may remove excess H_2O_2 caused by Cu.
Year	2009
Pages	1260- 1265
keywords	*Plagiomnium cuspidatum*, Cu, H_2O_2, $O_2^{\cdot-}$

Title	**Spatial structure of trace elements in extensive biomonitoring surveys with terrestrial mosses**
Author	M.T. Boquete, J.A. Fernández, J.R. Aboal, C. Real, A. Carballeira
Journal	Science of The Total Environment, Volume 408(1)

Abstract	The size of the area affected by contamination processes mainly depends on the type of contaminant and the way it is emitted. In regular sampling, the size of the sampling grid will therefore interact with the scale at which the contamination processes are produced, for each contaminant, so that the grid will only enable characterization of those processes that occur at scales larger than the grid, i.e. large scale processes. The present study analysed the data corresponding to tissue concentrations of Cd, Cu, Fe, Hg, Mn, Pb, Se, V and Zn in the terrestrial moss *Pseudoscleropodium purum* obtained in regular sampling surveys with grids of different sizes (15 × 15, 7.5 × 7.5 km) plus a series of accessory points located at 1 km from the 15 × 15 km sampling grid; all sampling sites were located in Galicia and were sampled in March 2004. The objective of the study was to characterize the interaction between the scale of the sampling grid and the scale at which the contamination processes are produced, for each contaminant, to enable adjustment of the size of the grids to the scale at which the contamination processes are mainly produced. For this purpose, the spatial structure of the concentration of each element was analysed by use of semivariograms created with a robust estimator of the semi variance. The results of the study verified the existence of a real interaction between the scale of the sampling grid and the scale of the contamination processes. The results also demonstrated how in the study area, the contamination processes associated with Cd, Pb and V are generally small scale processes, whereas those associated with Cu, Hg and Se are generally large scale processes.
Year	2009
Pages	153-162
keywords	*Pseudoscleropodium purum*, heavy metals, contamination processes