BIOLOGIA PLANTARUM 43 (1): 93-98, 2000

Wall thickenings - moss protonema apical cell reaction to lead

M. KRZESŁOWSKA and A. WOŹNY

Institute of Experimental Biology, Faculty of Biology, Adam Mickiewicz University, Al. Niepodległości 14, 61-713 Poznań, Poland

Abstract

Two-days-old protonemata of *Funaria hygrometrica* Hedw. growing in *in vitro* conditions were treated with 4 μM $PbCl_2$ for 48 h. After this time grey or brown colour thickenings of the cell wall appeared in protonemata cells. They were then localized in tip parts of apical cells. Electron microscopical (TEM) observations showed that the thickenings were structurally connected with the wall and sometimes they included lead deposits. Cytochemically it was found that they contained pectic polysaccharides, callose, lipid substances (probably suberin or sporopollenin) and only few cellulose. Other compounds of cell wall, *e.g.* lignin and cutin were not detected.

Additional key words: heavy metal, *Funaria hygrometrica*, pectic polysaccharides, callose, cellulose, suberin, sporopollenin.

Introduction

Mosses are able to accumulate heavy metal ions in high quantity (Tyler 1990). It was found for example that in *Sphagnum* cells the ability to bind heavy metals in cell wall depends on the level of pectic substances, especially polygalacturonic acids (Clymo 1963). Among many heavy metals present in the environment, lead has the highest affinity to these compounds (Ernst *et al.* 1992). Cell wall in mosses contained a considerable quantity of polygalacturonic acids (Tyler 1990). It was found that among many moss species lead was immobilized also in walls of *Funaria hygrometrica* cells (Basile *et al.* 1994, Krzesłowska and Woźny 1996). Numerous disturbances caused by this element were described, *e.g.*, inhibition of spores germination (Krupińska 1976, Basile *et al.* 1995), protonemata growth (Krzesłowska *et al.* 1994, Basile *et al.* 1995). Moreover apical cells of protonemata treated with lead were characterized, *e.g.*, by swollen tips (Basile *et al.* 1995), lower microtubule number and their disturbed array - not parallel to longer axis of the cell (Basile *et al.* 1995, Krzesłowska 1995), absence of exclusion zone in the top part of this cell and some disturbances at the ultrastructural level, especially well visible in chloroplasts (Krupińska 1976, Basile *et al.* 1995, Krzesłowska and Woźny 1996).

The results of this study showed that in apical cells of *Funaria hygrometrica* protonema treated with lead commonly appeared some structures which were morphologically similar to cell wall thickenings. Moreover from our earlier studies it was known that apical cell is also one of the main place of lead uptake (Krzesłowska and Woźny 1996). Therefore the aim of the study was to show the morphology, ultrastructure and composition of the thickenings. The structure and especially chemical composition of the thickenings also may indirectly suggest their role in cells treated with toxic metal.

Materials and methods

Two-days-old protonemata of *Funaria hygrometrica* Hedw. growing on the Kofler (1959) medium supplemented with microelements according to Heller (1953) were treated with 4 μM $PbCl_2$ for 48 h inhibiting the growth of protonemata in about 50 %.

Received 9 November 1998, *accepted* 22 March 1999.
Fax: (+48) 61 8523615, e-mail: adaw@main.amu.edu.pl

Identification of the compounds building cell wall thickenings was carried out using specific cytochemical methods and stainings for light and fluorescence microscopy: calcofluor white for identification of cellulose, aniline blue - callose, ruthenium red - pectic polysaccharides (Fry 1994), auramine - cutin (Wędzony 1996), Sudan IV - lipid substances (cutin, suberin, sporopollenin), floroglucine with HCl - lignin (Młodzianowski and Woźny 1982). Lignin, cutin and phenolic compounds were also identified by their autofluorescence (Młodzianowski and Woźny 1982, Eckey-Kaltenbach *et al.* 1994). The procedure of preparing the material to observation in TEM was described earlier (Krzesłowska and Woźny 1996).

Results

In apical cells of *Funaria hygrometrica* protonemata treated with lead commonly appeared (in about 60 %) wall thickenings (Fig. 2) not present in the control material (Fig. 1). The thickenings were localized mostly in the tips of apical cells (called tip thickenings) which were usually swollen. Sometimes they occurred in lateral walls along the thread (lateral thickenings). They differed in size, shape and colour (Fig. 2). The thickenings present in swollen tips often occupied almost whole area of such a tip (Figs. 4, 5, 6). In some cells they were strongly developed and formed the structure similar to a helmet above the protoplast. Lateral thickenings have usually rounded shape and occupied the area less than half of the cross section of the cell (Fig. 7). In some cases, however, they were larger and formed an additional cross wall in the cell. The colour of thickenings was grey or brown (Fig. 2). In some cases brown colour did not cover the whole area of the thickenings and was limited to the region near the protoplast. Then the top was grey. The differences of the thickenings colour suggested its various chemical composition, what was confirmed by cytochemical investigations (see later).

The study in TEM showed that both tip thickenings and lateral ones were always structurally connected with cell wall surrounding protoplast (Fig. 7). Their structure was not identical with typical cell wall (Fig. 10) but rather similar to it. In many cases tip thickenings were of homogenic nature on the whole area (Fig. 7). However, these ones which were brown, contained a few different homogenic and granular areas (Fig. 8). Lateral thickenings were homogenic or homogenic with some number of granules (Fig. 7). All thickenings contained a low electron density (similar to microscopical image of callose) layer next to the plasma membrane. In some cases this layer was wide and well visible (Fig. 9) but its width was not the same in all observed thickenings. Especially interesting is that the thickenings contained lead deposits - sometimes in large quantity (Fig. 7). The size of deposits and their number were different.

The cytochemical studies of the thickenings composition showed that they contained compounds typical for plant cell wall. The most common were pectic polysaccharides (Fig. 5). These substances were present almost in all thickenings. In many cases they occurred on the whole area of the structure (Fig. 5). On the other hand cellulose, one of the most characteristic substance building plant cell wall, was present only in few tip thickenings and occupied relatively small area (Fig. 3). The other compound often present in the structures was callose (Fig. 4). Some of wall thickenings were covered by callose layer on the whole area. There were, however, also such ones which did not contain any callose. Brown colour of some thickenings suggested a possible presence of phenolic compounds inside of them. The negative result of lignin staining and no specific autofluorescence both for this substance and other phenolic compounds showed us that these materials were not present in the thickenings. Apart from phenolic compounds brown colour may be caused by some lipid substances, *e.g.* suberin. Sudan IV staining gave the positive result (Fig. 6) within brown areas of the thickenings. It might be caused by the presence of three substances: cutin, suberin or sporopollenin. The presence of cutin we excluded - there was no characteristic autofluorescence for this compound and the result of the reaction with auramine was also negative. It showed indirectly that the lipid substance present in the thickening was probably suberin and/or sporopollenin.

The studies showed that four compounds characteristic for plant cell wall built the thickenings in apical cells of *Funaria hygrometrica* protonemata: pectic polysaccharides, callose, lipid substances (suberin or sporopollenin) and cellulose. The most common were pectic polysaccharides present almost in all structures. Sometimes the composition of two or more thickenings was the same. They often differed, however, one from another in areas contained several compounds. Moreover not all described structures were built from all detected substances. Lateral thickenings, present sometimes along the protonema threads, were built from pectic polysaccharides, callose and lipid substances (suberin or sporopollenin). However, cellulose was not found there.

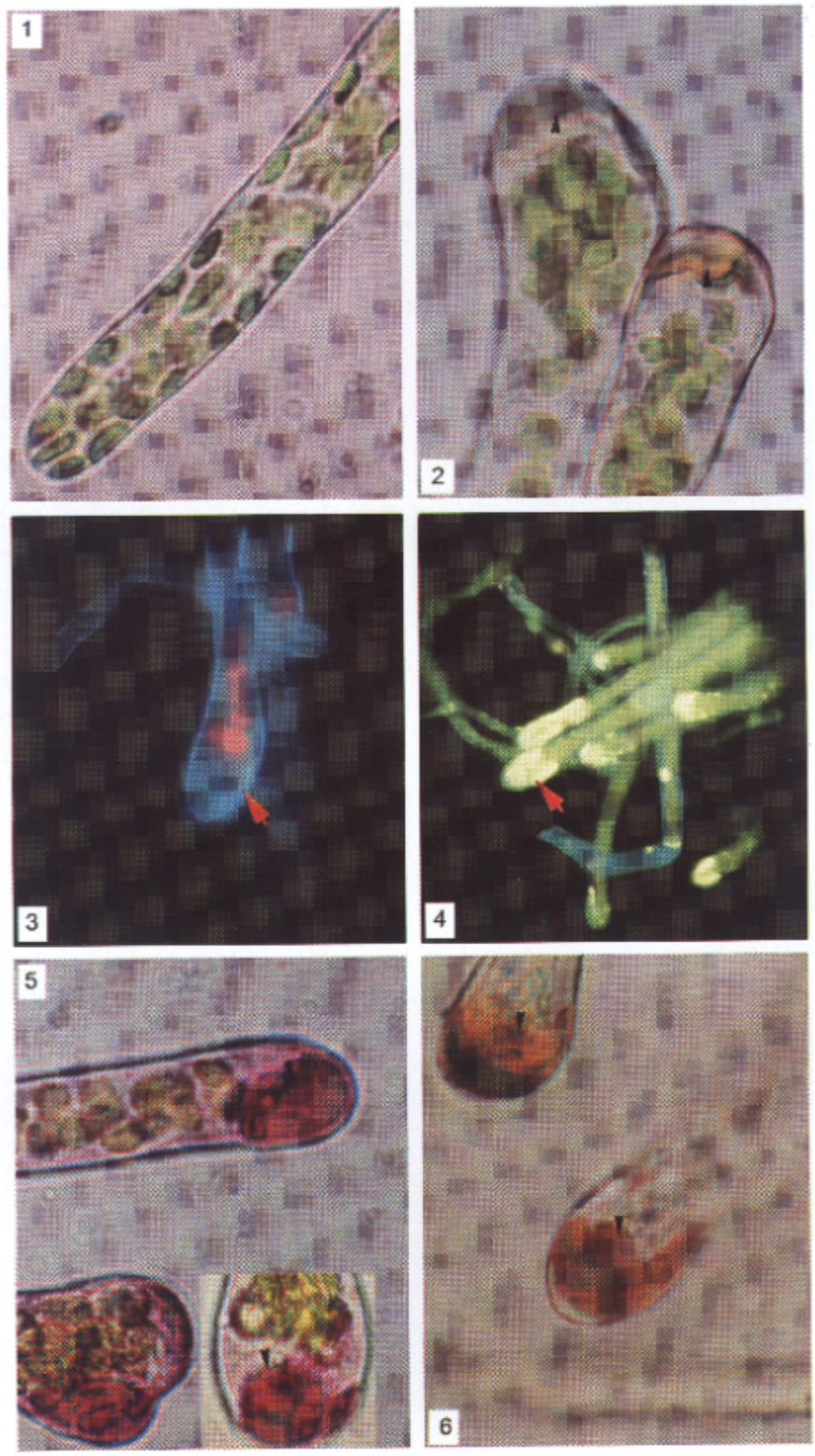
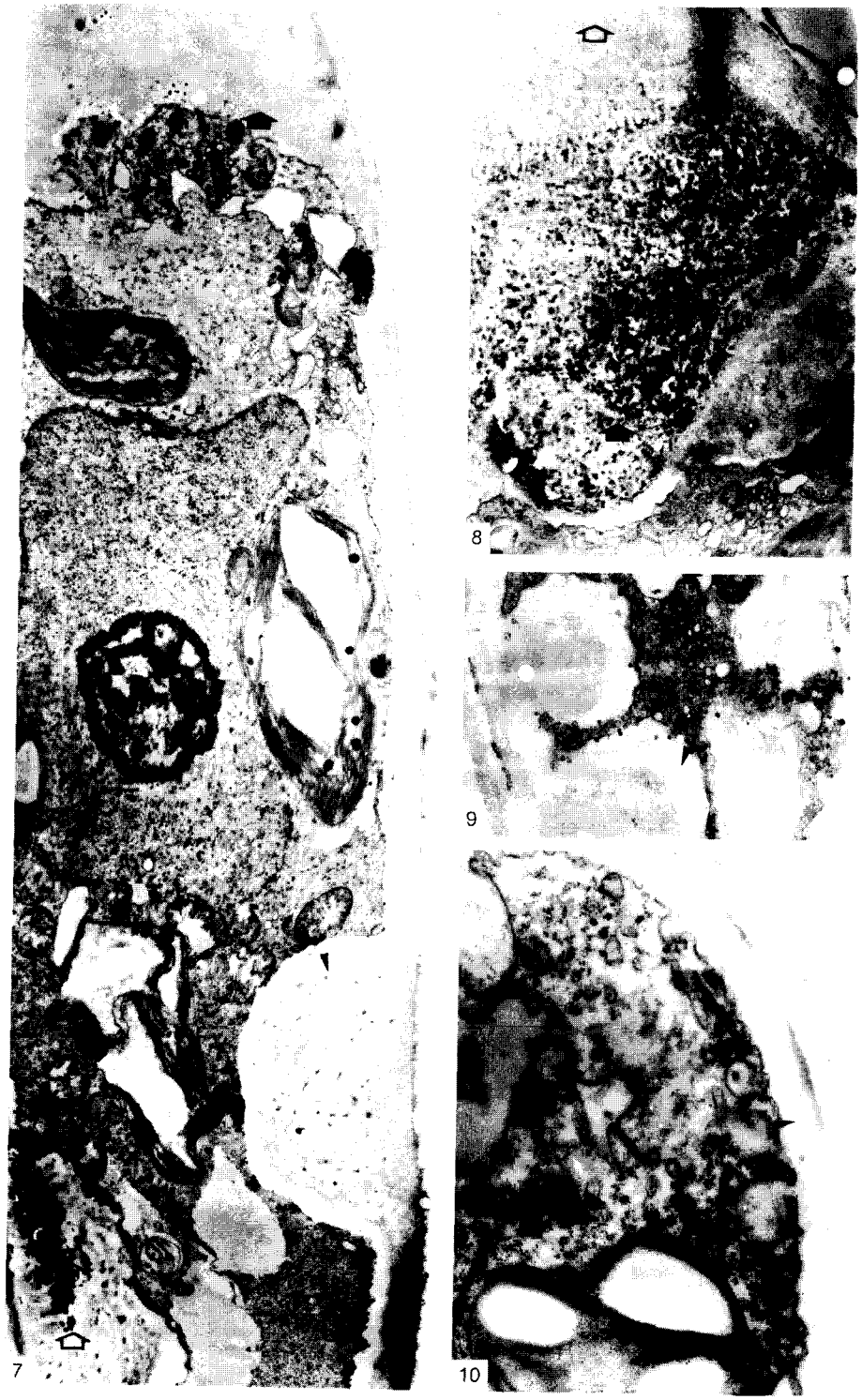


Fig. 1. Morphology of cell wall in the apical cell in control *Funaria hygrometrica* protonema (no staining, ×1 440).
Fig. 2. Cell wall thickenings in protonemata treated with lead: tip thickenings (*arrowheads*) of wall in apical cells (no staining; ×1 440).
Figs. 3 - 6. Substances building cell wall thickenings which appeared in protonemata treated with lead: cellulose (*arrow*), (Fig. 3; ×400); callose (*arrow*), (Fig.4; ×1 440), pectic polysaccharides (*arrowheads*), (Fig. 5; ×1 440); lipid substances - suberin or sporopollenin (*arrowheads*), (Fig. 6; ×1 440).



Figs. 7 - 10. Ultrastructure of wall thickenings in apical cell of *Funaria hygrometrica* treated with lead and cell wall in control: longitudinal section of apical cell treated with lead - tip thickening (*arrow*), lateral thickening (*arrowhead*), lead deposits (*open arrows*), (Fig. 7; ×15 000), tip thickening of cell wall containing granular (*arrow*) and homogenic areas (*open arrow*), (Fig. 8; ×30 000), thickenings of the wall on the tip of apical cell - the low electron density layer similar to callose (*arrowhead*) is well visible, (Fig. 9; ×20 000), typical wall of control apical cell (*arrowhead*), (Fig. 10; ×25 000).

Discussion

Typical protonemata cell wall of *Funaria hygrometrica* contains cellulose, pectic polysaccharides and in cross walls callose (Bopp *et al.* 1991). Lead caused, however, a considerable increase of pectic polysaccharides, of callose and sometimes of cellulose. Moreover suberin or sporopollenin appeared which are not present, as a rule, in protonemata cell wall. Cell wall thickenings in *Funaria hygrometrica* protonemata were observed also as the result of other stress conditions, *e.g.*, hypertonic solution of glycine (Schnepf *et al.* 1986), D_2O (Schmiedel and Schnepf 1980), colchicine (Schnepf *et al.* 1982). In general, both the structure and composition of the thickenings were different from these ones which appeared in *Funaria* protonemata as the result of treating them with lead. However, they have a few features common: localization - in tips of apical cells, different from typical cell wall structure and in some cases (Schmiedel and Schnepf 1980) the presence of callose.

The appearance of cell wall thickenings rich in pectic polysaccharides induced by lead have not been shown yet. In available literature, we found only a few examples of the pectic polysaccharides synthesis caused by other stress factors, *e.g.*, the appearance of cell wall thickenings, built mainly from calcium pectate, in cells of tobacco, poplar, birch and alder as the result of ozone treatment (Günthardt-Goerg 1996, Günthardt-Goerg *et al.* 1997). The thickenings were faced to intercellular spaces and had character of droplets. Moreover, the synthesis of pectic polysaccharides was observed on the surface of mechanical injuried callus in *Picea sitchensis* (Miller and Barnet 1993) and in cells of tobacco as the result of high concentration of NaCl (Iraki *et al.* 1989).

Lead caused the appearance of cell wall thickenings, *e.g.*, in *Chara* (Heumann 1987), but they were completely different in structure and composition from these in *Funaria.* Moreover, in *Lilium longiflorum* pollen tubes, characterized like moss protonemata by tip growth, some aberrations in cell wall structure occurred (Röderer and Reiss 1988) as the result of lead treatment. The composition of these aberrations was not studied but the authors supposed that they could contain pectic polysaccha-rides. In *Lilium longiflorum* some thickenings of cell wall also occurred. That was, however, the result of treating the plant with other heavy metals, *e.g.*, cadmium and mercury (Sawidis and Reiss 1995). The thickenings were localized in or near the top region of apical cells but their structure was different from the thickenings found in *Funaria* treated with lead. It was found that lead also caused the synthesis of some cell wall compounds which probably did not form any thickenings, *e.g.*, callose (Lummerzheim *et al.* 1995, Samardakiewicz *et al.* 1996) and suberin (Breckle and Kahle 1992). Presence of suberin found in roots of lead treated *Fagus silvatica* seedlings caused that the roots become brown (Breckle and Kahle 1992). This fact suggest that suberin was also the component of wall thickenings in *Funaria hygrometrica* protonemata treated with lead because many of them were brown and Sudan IV staining showed the presence of a lipid substance there. Another lipid compound present in thickenings could be the sporo-pollenin because it was observed indentical colour of fluorescence both in spores walls and in thickenings (Konieczna-Koperska - personal communication).

The chemical composition strongly suggest that formation of wall thickenings in apical cells of *Funaria hygrometrica* protonemata treated with lead would be a kind of a resistance reaction to this metal. Because lead ions have especially high affinity to pectic poly-saccharides (Ernst *et al.* 1992) rich in this compound wall thickenings could effectively immobilize the ions (lead deposits were often observed in these structures). Such function was also supposed for thickenings in *Lilium* pollen tube treated with heavy metals (Röderer and Reiss 1988, Sawidis and Reiss 1995). Additionally, the presence of callose and suberin might limit the contact of lead ions with plasma membrane and also their entering into the protoplast. Also sporopollenin is generally known as the substance protecting spores, pollen grains, *etc.*, from many of environmental stress factors (Zetzche 1932). Wall thickenings appeared mostly in tip zone of apical cell which is exclusion and uptake region therefore their role as the structure protecting protoplast from lead ions entering and its toxicity could be especially effective.

However, wall thickenings in *Funaria hygrometrica* could be, simply, the result of tip growth disturbances in apical cells caused by toxic metal. The explanation of the problems will be the subject of further studies.

References

Basile, A., Giordano, S., Cafiero, G., Spagnuolo, V., Castaldo-Cobianchi, R.: Tissue and cell localization of experimentally supplied lead in *Funaria hygrometrica* (Hedw.) using X-ray SEM and TEM microanalysis. - J. Bryol. **18**: 69-81, 1994.

Basile, A., Giordano, S., Spagnuolo, V., Alfano, F., Castaldo-Cobianchi, R.: Effect of lead and colchicine on morphogenesis in protonemata of moss *Funaria hygrometrica.* - Ann. Bot. **76**: 597-606, 1995.

Bopp, M., Ouader, H., Thoni, C., Sawidis, T., Schnepf, E.: Filament disruption in *Funaria* protonemata: Formation and disintegration of tmema cells. - J. Plant Physiol. **137**: 273-284, 1991.

Breckle, S.W., Kahle, H.: Effects of toxic heavy metals (Cd, Pb) on growth and mineral nutrition of *Fagus sylvatica.* - Vegetatio **101**: 43-53, 1992.

Clymo, R.S.: Ion exchange in *Sphagnum* and its relation to bog ecology. - Ann. Bot. **27**: 309-324, 1963.

Eckey-Kaltenbach, H., Ernst, D., Heller, W., Sandermann, H., Jr.: Biochemical plant responses to ozone. - Plant Physiol. **104**: 67-74, 1994.

Ernst, W.H.O., Verkleij, J.A.C., Schat, H.: Metal tolerance in plants. - Acta bot. neerl. **41**: 229-248, 1992.

Fry, S.C.: Chemical analysis of components of the primary cell wall. - In: Harris, N., Oparka, K.J. (ed.): Plant Cell Biology. A Practical Approach. Pp.199-219. IRL Press, Oxford 1994.

Günthardt-Goerg, M.S.: Different responses to ozone of tobacco, poplar, birch, and alder. - J. Plant Physiol. **148**: 207-214, 1996.

Günthardt-Goerg, M.S., McQuattie, C.J., Scheidegger, C., Rhiner, C., Matyssek, R.: Ozone-induced cytochemical and ultrastructural changes in leaf mesophyll cell walls. - Can. J. Forest Res. **27**: 453-463. 1997.

Heller, R.: Recherches sur la nutrition minerale des tissus végétaux cultivés *in vitro.* - Ann. Sci. Nat. Bot. Biol. Vég. **14**: 1-223, 1953.

Heumann, H.G.: Effects of heavy metal on growth and ultrastructure of *Chara vulgaris.* - Protoplasma **136**: 37-48, 1987.

Iraki, N.W., Bressan, R.A., Carpita, N.C.: Cell walls of tobacco cells and changes in composition associated with reduced growth upon adaptation to water and saline stress. - Plant Physiol. **91**: 48-53, 1989.

Kofler, L.: Contribution à l'étude biologique des mousses cultivées *in vitro.* Germination des spores, croissance et dévelopment du protonéma chez *Funaria hygrometrica.* - Rev. Bryol. Lichenol. **24**: 1-199, 1959.

Krupińska, I.: Influence of lead tetraethyl on the growth of *Funaria hygrometrica* L. and *Marchantia polymorpha* L. - Acta Soc. Bot. Pol. **45**: 421-432, 1976.

Krzesłowska, M., Pustelnik, L., Woźny, A.: Differentiated lead-induced inhibition of growth rate of *Funaria hygrometrica* Hedw. protonemata. - Bull. Soc. Amis Sci. Lett. (Poznań) **30**: 33-36, 1994.

Krzesłowska, M.: The visualization of microtubule system in cells of *Funaria hygrometrica* protonemata by the immunofluorescence method and some disturbances caused by lead present in the medium. - Bull. Soc. Amis Sci. Lett. (Poznań) **32**: 39, 1995.

Krzesłowska, M., Woźny, A.: Lead uptake, localization and changes in cell ultrastructure of *Funaria hygrometrica* protonemata. - Biol. Plant. **38**: 253-259, 1996.

Lummerzheim, M., Sandroni, M., Castresana, C., De Oliveira, Van Montagu, M., Roby, D., Timmerman, B.: Comparative microscopic and enzymatic characterization of the leaf necrosis induced in *Arabidopsis thaliana* by lead nitrate and by *Xanthomonas campestris* pv. *campestris* after foliar spray. - Plant Cell Environ. **18**: 499-509, 1995.

Miller, H., Barnett, J.R.: The structure and composition of bead-like projections on Sitka spruce callus cells formed during grafting and in culture. - Ann. Bot. **72**: 441-448, 1993.

Młodzianowski, F., Woźny, A.: [Cell wall.] - In: Lectures and Practices from Plant Cell Biology. Pp. 331-339. Wyd. Nauk. Uniw. Im. Adama Mickiewicza, Poznań 1982. [In Polish.]

Röderer, G., Reiss, H.D.: Different effects of inorganic and triethyl lead on growth and ultrastructure of lily pollen tubes. - Protoplasma **144**: 101-109, 1988.

Samardakiewicz, S., Strawiński, P., Woźny, A.: The influence of lead on callose formation in roots of *Lemna minor* L. - Biol. Plant. **38**: 463-467, 1996.

Sawidis, T., Reiss, H.D.: Effects of heavy metals on pollen tube growth and ultrastructure. - Protoplasma **163**: 156-161, 1995.

Schmiedel, G., Schnepf, E.: Polarity and growth of caulonema tip cells of the moss *Funaria hygrometrica.* - Planta **147**: 405-413, 1980.

Schnepf, E., Deichgräber, G., Bopp, M.: Growth, cell wall formation and differentiation in the protonema of the moss, *Funaria hygrometrica*: effects of plasmolysis on the developmental program and its expression. - Protoplasma **133**: 50-65, 1986.

Schnepf, E., Hrdina, B., Lehne, A.: Spore germination, development of the microtubule system and protonema cell morphogenesis in the moss, *Funaria hygrometrica*: effects of inhibitors and growth substances. - Biochem. Physiol. Pflanz. **177**: 461-482, 1982.

Tyler, G.: Bryophytes and heavy metals: a literature review. - Bot. J. linn. Soc. **104**: 231-253. 1990.

Wędzony, M.: [Fluorochromes stainings.] - In: Mikroskopia Fluorescencyjna dla Botaników. [Fluorescence Microscopy for Botanists.] Pp. 47-70. Drucpol, Kraków 1996. [In Polish.]

Zetzsche F.: Sporopollenine. - In: Klein, G. (ed.): Handbuch der Pflanzenanalyse. Vol. 3. Springer, Wien 1932.