doi:10.4149/gpb_2011_01_52

Effect of cadmium and lead on the membrane potential and photoelectric reaction of *Nitellopsis obtusa* cells

Renata Kurtyka, Zbigniew Burdach and Waldemar Karcz

Department of Plant Physiology, Faculty of Biology and Environmental Protection, University of Silesia, Katowice, Poland

Abstract. The effects of Cd and Pb on membrane potential (E_m) and photoelectric reaction of *Nitellopsis obtusa* cells were investigated. It was found that Cd and Pb at 1.0 mM caused a depolarization of the E_m, whereas both metals at lower concentrations changed the E_m in a different way. Pb at 0.1 mM and 0.01 mM hyperpolarized the E_m, whereas Cd at the same concentrations depolarized and did not change the E_m, respectively. In the presence of 0.01 mM Pb, the light-induced hyperpolarization of the E_m was by 18% higher as compared to the control, whereas at 1.0 mM Pb it was by 40% lower. Pb at 0.1 mM and Cd at 0.01 mM or 5 × 0.01 mM did not change the light-induced membrane hyperpolarization. However, in the presence of Cd at 0.1 mM and 1.0 mM this hyperpolarization was 2-fold lower or was completely abolished, respectively. These results suggest that at high Cd and Pb concentrations both depolarization of the E_m and decrease of light-induced membrane hyperpolarization in *Nitellopsis obtusa* cells are probably due to inhibition of the plasma membrane H^+-ATPase activity, whereas both metals at lower concentrations differ in mechanism of membrane potential changes.

Key words: Cadmium — Lead — Membrane potential — Photoelectric reaction — *Nitellopsis obtusa*

Introduction

Metals such as cadmium (Cd) and lead (Pb) are very harmful environmental pollutants which are widely known inhibitors of plant metabolism. These metals by binding into specific sites of membranes such as amino, carboxyl, hydroxyl, phosphoryl and sulfhydryl groups have a destructive influence on structure and function of plant membranes (De Filippis 1979). The modification of membrane properties by heavy metals may be caused by changes of their permeability, fluidity, protein and lipid composition (Jones et al. 1987; Stefanov et al. 1993, 1995a,b; Fodor et al. 1995; Ouariti et al. 1997; Llamas et al. 2000; Rucińska and Gwoździ 2005; Karcz et al. 2009; Miśkiewicz et al. 2010). It is also postulated that plasma membrane may have a role in metal tolerance by preventing or reducing the entry of metals to the cells and it is the first target for ions toxicity, causing changes, among others, in electrical potential (Verkleij and Schat 1990; Sanita` di Toppi and Gabbrielli 1999; Karcz and Kurtyka 2007). Across the plasma membrane there is a membrane potential difference, value of which depends on plant species and type of the cells (Stolarek and Karcz 1987; Elzenga et al. 1995; Lehtonen and Saari 2000). The plasma membrane potential (*E*m) consists of two components; the first, the active component, which is generated by an plasma membrane H^+-ATPase (PM H^+-ATPase) and the second, the passive one, which depends on membrane permeability. The activity of the PM H^+-ATPase is affected among other by temperature (Karcz and Burdach 2007), ion concentrations (Felle 1991) and illumination (Stolarek et al. 1988; Elzenga et al. 1995; Michelet and Boutry 1995).

The dark/light signals cause in plant cells an electrical reaction which depends on plant species and cell type (Karcz 1991; Karcz et al. 1991; Shabala and Newman 1999; Lehtonen and Saari 2000). In *Characeae* cells a typical reaction on dark/light transition consists of two phases – a rapid transient depolarization of the E_m followed by a slow membrane hyperpolarization (Fujii et al. 1979; Stolarek and Karcz 1987; Stolarek et al. 1988). In turn, light/dark transition causes similar responses but in

Correspondence to: Renata Kurtyka, Department of Plant Physiology, Faculty of Biology and Environmental Protection, University of Silesia, ul. Jagiellońska 28, 40 032 Katowice, Poland
E-mail: renata.kurtyka@us.edu.pl

the opposite direction (Shimmen et al. 1994). It was previously shown in experiments performed with *Elodea densa* cells and *Nicotiana tabacum* mesophyll protoplasts that light-induced electrogenic H^+ extrusion (membrane hyperpolarization) is associated with increased of K^+ uptake and alkalinization of cell sap, which are the symptoms of an increase of PM H^+-ATPase activity (Marrè et al. 1989; Blom-Zandstra et al. 1997). Among the different ions which are responsible for electrical reaction to light the most likely candidate for membrane depolarization is Ca^{2+}, whereas K^+ seems to function as the equilibrium ion, moving passively across the plasma membrane to compensate for light-induced charge movement of Cl^- or H^+ (Shabala and Newman 1999). It has also been shown that the effects of light on membrane potential changes are suppressed by treatment the cells with the photosynthetic 3-(3'-4'-dichlorophenyl)-1,1-dimethylurea (DCMU), and PM H^+-ATPase inhibitors (Marrè et al. 1989; Karcz 1991; Okazaki et al. 1994; Harada et al. 2002). It was also proposed that in photosynthetically active cells the light signal from chloroplasts to the PM H^+-ATPase is carried by electrons (Elzenga and Prins 1989), and PSII system (Köhler et al. 1985).

The main goal of the present study was to compare the effects of Cd and Pb ions on the membrane potential and photoelectric reaction in internodal cells of *Nitellopsis obtusa*. The internodal cells have proved to be the most suitable material for analyzing the electrical characteristics of membranes of plant cells because of their large cell size and diameter (Shimmen et al. 1994; Tazawa and Shimmen 2001).

Materials and Methods

Plant material and growth conditions

The experiments were carried out with internodal cells of giant algae *Nitellopsis obtusa* (*Characeae*), which were grown in an aquarium in the natural pond water at 25°C, under fixed light cycle of 16 h light and 8 h dark. Before the experiments the cells were incubated within 2 h in basic solution (APW – artificial pond water) containing: 1.0 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$; pH of the medium was 5.8–6.0. After this period one of them was transferred into a perfusion plexiglass chamber which was mounted on a vertically placed microscope stage.

Measurement of membrane potential

The standard electrophysiological technique was used for membrane potential measurements, as previously described by Stolarek and Karcz (1987) and Karcz and Burdach (2002). The E_m differences were measured by recording the voltage between a 3 M KCl-filled glass micropipette inserted into the vacuole and a reference electrode in the external medium. The microelectrodes were inserted into *Nitellopsis obtusa* cells under microscope by using of micromanipulator (Hugo Sachs Electronik, March-Hugstteten, Germany). Micropipettes were pulled on a vertical pipette puller (model L/M-3P-A, List-Medical, Germany) from borosilicate glass capillaries (type 1B150F-3, World Precision Instruments, USA). Tip diameters were less than 1 μm. After stabilization of E_m (<20 min) the external medium was changed for a new one, at the same salt composition, containing additionally $CdCl_2$ or $PbCl_2$ at the final concentration 1.0, 0.1 and 0.01 mM. Medium changes were done by means of peristaltic pump (type Peri-Star PRO, World Precision Instruments, USA). The membrane potential in the presence of metals was measured continuously within 45 min.

The next part of our experiments concerned with the effects of Cd (1.0, 0.1, 5 × 0.01 and 0.01 mM) and Pb (1.0, 0.1 and 0.01 mM) on light-induced electrical reaction (photoelectric reaction) in *Nitellopsis obtusa* cells. In this case, the visible light (70 $W{\cdot}m^{-2}$) from a 100 W halogen bulb (model ZH 100, PZO Warsaw) was switched on twice; first, for 30 min after stabilization of E_m in the dark in control medium and second, after 30 min the treatment *Nitellopsis obtusa* cells with cadmium or lead.

Statistical analysis

Data were analyzed by using the computer software Statistica by StatSoft Inc. (data analysis software system), version 8.0. (www.statsoft.com). Differences between individual treatments and control were analyzed using one-way ANOVA and LSD test. Statistical significance was $p < 0.05$.

Results

The changes in electric potential differences between the vacuole and the medium in cells of *Nitellopsis obtusa* incubated in the presence of Cd and Pb were determined. The E_m of these cells, before being changed in response to Cd and Pb was –151.4 ± 6.9 mV (means ± SE, $n = 8$) and –156.5 ± 3.8 mV (means ± SE, $n = 8$), respectively. The electric potential measurements in the cells of *Nitellopsis obtusa* clearly showed a dependence of their E_m on Cd and Pb concentrations (Figs. 1, 2). Both metals, at 1.0 mM caused an immediate depolarization of E_m, value of which was significantly higher for Cd (depolarization by 81.9 ± 3.9 mV, $n = 8$) than for Pb (depolarization by 40.6 ± 1.9 mV, $n = 8$) (Figs. 1, 2). Treatment of internodal cells with Cd at 0.1 mM caused a depolarization of E_m by 57.9 ± 2.4 mV (means ± SE, $n = 8$) whereas Cd at 0.01 mM did not change the E_m (Fig. 1). Pb, in contrast to Cd, applied at 0.01 mM and 0.1 mM brought about membrane hyperpolarization by 19.8 ± 0.9 mV (means ± SE, $n = 8$) and 15.6 ± 0.8 mV (means ± SE, $n = 8$), respectively (Fig. 2).

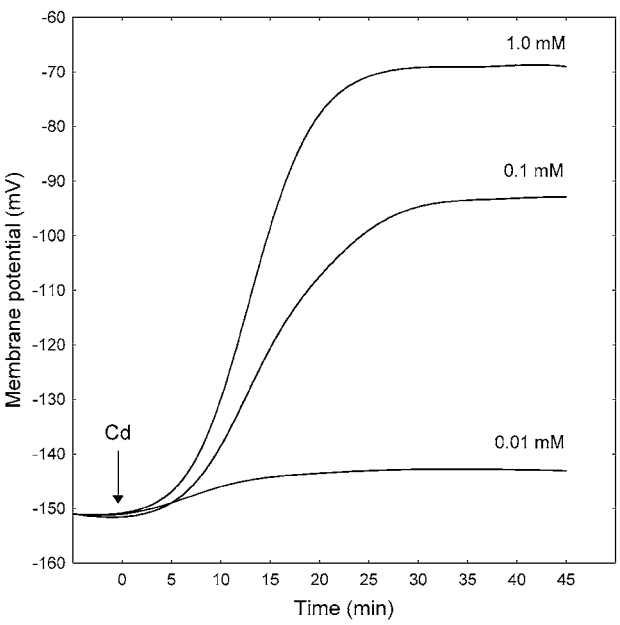

Figure 1. Effect of Cd (1.0, 0.1, 0.01 mM) on the membrane potential (E_m) of *Nitellopsis obtusa* cells. (At time 0, the control medium was changed for a new one, at the same salt composition, containing in addition Cd). Standard error (SE) did not exceed 5%, $n = 8$.

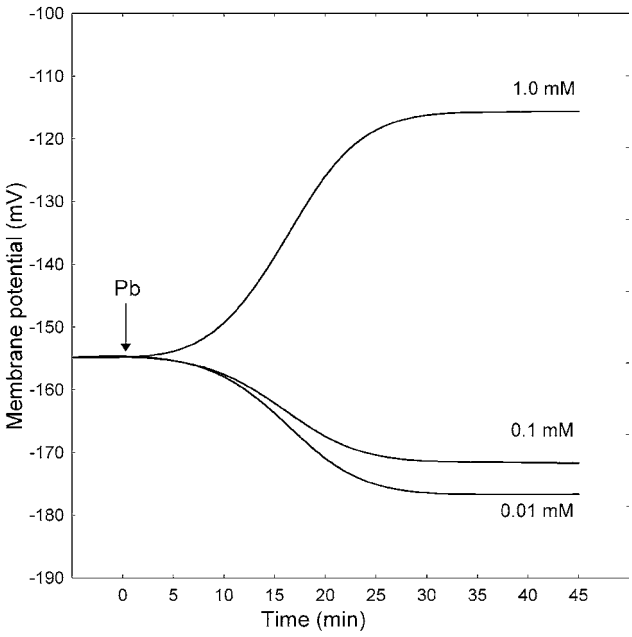

Figure 2. Effect of Pb (1.0, 0.1, 0.01 mM) on the membrane potential (E_m) of *Nitellopsis obtusa* cells. (At time 0, the control medium was changed for a new one, at the same salt composition, containing in addition Pb). Standard error (SE) did not exceed 5%, $n = 8$.

The kinetics of light-induced membrane potential changes in *Nitellopsis obtusa* cells incubated in the presence of Cd or Pb was also investigated. We have showed that the exposure of the cells (medium without metal) to visible light caused a transient depolarization of E_m (which was usually finished within 2 min) after which a delayed hyperpolarization of *E*m by 65.7 ± 2.9 mV (means ± SE, $n = 8$) was observed (Fig. 3A). The same cells treated again with light (after period of 60 min in the dark) showed practically identical kinetics of the membrane potential changes as in the first case (Fig. 3A, Tab. 1). The data presented here show that the photoelectric reaction (light-induced membrane hyperpolarization) of *Nitellopsis obtusa* cells was changed in the presence of Cd or Pb (Fig. 3 and Tab. 1). It was found that at 0.01 mM Pb the

Table 1. Effect of Cd and Pb ions on the photoelectrical reaction of *Nitellopsis obtusa* cells. The visible light was switch on twice; first, for 30 min after stabilization of E_m in the dark in control medium and second, after 30 min the treatment *Nitellopsis obtusa* cells with cadmium or lead

Treatment		Membrane potential, E_m (mV)				
		after stabilization in the control medium	30 min after light-on	60 min after light-off	30 min after treatment with metal or with control solution	30 min after light-on
Control		-153.7 ± 7.8^{fghi}	-219.4 ± 5.5^{b}	-166.0 ± 9.0^{def}	-162.2 ± 7.5^{deg}	-217.0 ± 8.4^{b}
Cd (mM)	0.01	-150.2 ± 3.3^{fghi}	-221.1 ± 4.8^{b}	-160.9 ± 4.3^{eh}	-155.0 ± 6.4^{ehi}	-222.3 ± 6.2^{b}
	0.05				-139.6 ± 8.9^{ij}	-188.3 ± 6.9^{c}
	0.1				-93.9 ± 7.8^{kl}	-125.9 ± 6.1^{j}
	1.0				-72.1 ± 6.2^{m}	-75.7 ± 5.8^{lm}
Pb (mM)	0.01	-155.1 ± 10.4^{ehi}	-221.3 ± 3.4^{b}	-58.2 ± 9.9^{ehi}	-178.1 ± 6.2^{cd}	-256.7 ± 8.2^{a}
	0.1				-174.9 ± 8.4^{ce}	-235.9 ± 5.6^{b}
	1.0				-104.9 ± 5.2^{k}	-132.3 ± 8.5^{j}

The data are means of at least eight independent experiments. Error indicate ± SE. Means followed by the same letter are not significantly different from each other using the LSD-test ($p < 0.05$).

light-induced hyperpolarization of the E_m was by 12.4 ± 0.9 mV (means ± SE, n = 8) higher as compared to the control, whereas Cd at the same concentration as well as Pb at 0.1 mM did not change ligt-induced membrane hyperpolarization. However, Cd at 5 × 0.01 mM decreased this hyperpolarization by 22 mV. In the presence of 0.1 mM Cd and 1.0 mM Pb the light-induced hyperpolarization of E_m was 2-fold smaller as compared to the control. Interestingly, Cd at the highest concentration (1.0 mM) totally abolished the photoelectric reaction of *Nitellopsis obtusa* cells.

Discussion

The data presented in this paper show that Cd and Pb at 1.0 mM caused a depolarization of the E_m, whereas both metals at lower concentrations changed the E_m in a different way. Pb at 0.1 mM and 0.01 mM hyperpolarized the E_m, whereas Cd at the same concentrations depolarized and did not change the E_m, respectively (Figs. 1, 2). Similar results were also obtained in our previous study (Karcz and Kurtyka 2007) in experiments performed with parenchymal cells of maize coleoptile segments which, incubated at 0.1 mM Cd, were depolarized by approx. 60 mV. We have suggested that the effect of Cd on the membrane potential of parenchymal cells might be, at least in part, caused *via* reduced PM H^+-ATPase activity. Effect of Cd on both membrane-bound ATPase activity and K^+ uptake in *Beta vulgaris* L. roots has been studied by Lindberg and Wingstrand (1985). They showed that *in vitro* application of Cd inhibited the PM H^+-ATPase activity and K^+ uptake. In turn, Ros et al. (1992) reported that Cd modified the PM H^+-ATPase activity from rice (*Oryza sativa* L.), but this effect depended on the performed assay (*in vivo* or *in vitro*). The results reported by Perfus-Barbeoch et al. (2002) should also be mentioned. These authors obtained that Cd^{2+} mimics Ca^{2+} and enteres *Vicia faba* guard cells through voltage-dependent Ca^{2+} channels, whereas K^+ channels were insensitive to external Cd^{2+} application. In turn, Lindberg et al. (2004) showed that cadmium uptake into the cytosol of wheat protoplasts partly takes place by channels permeable to calcium and potassium, and is dependent on membrane potential. Taking into account that cadmium is more easily taken up into the cells than Pb (Pahlsson 1989) it could be suggested that Pb only at high concentration (1.0 mM) caused a depolarization, while Cd ions do this at lower concentrations (Cd at 0.1 mM and to some extent 0.01 mM Cd). The hyperpolarization of the E_m in the presence of lead acetate was also found by Morse and Spanswick (1984) in cells of *Nitella translucens* and was ascribed by the authors to block K^+ channels. Morse and Spanswick (1984), in contrast to our data, observed hyperpolarization of the E_m in the presence of a very high Pb concentration (5 mM). This discrepancy between electrical responses of *Nitellopsis obtusa* and *Nitella translucens* to Pb probably results from different lead salts used (Sharma and Chopra 1987; Małkowski et al. 1996). The effect of Cd and Pb on the E_m of *Nitellopsis obtusa* cells observed here

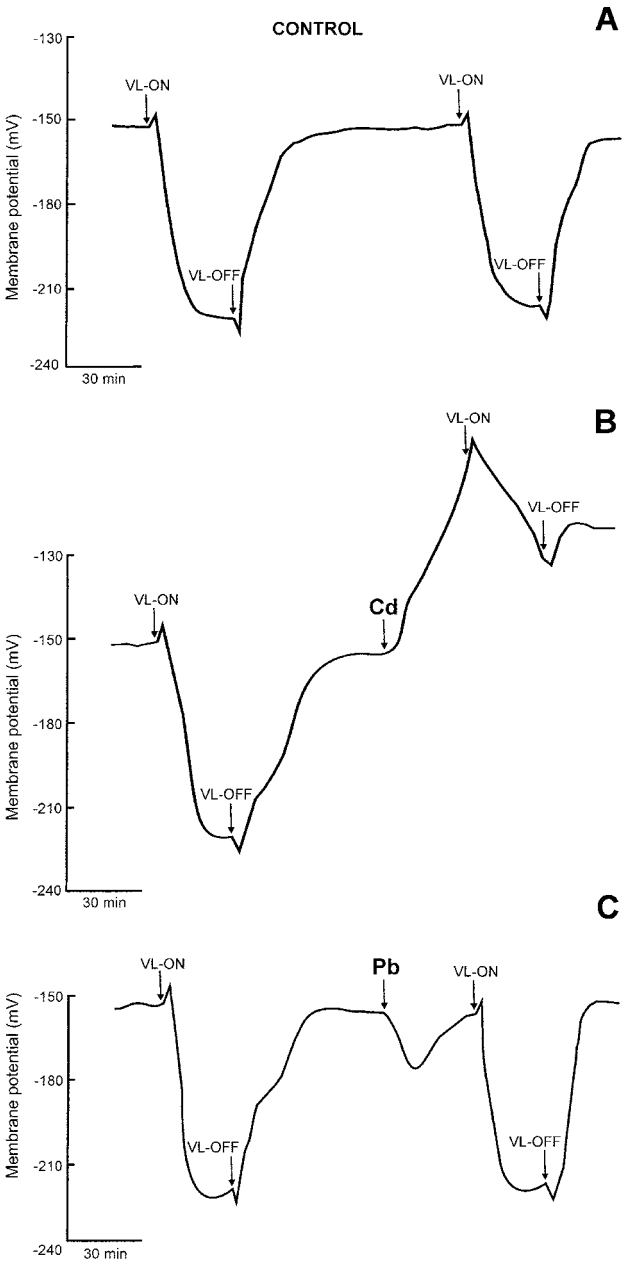

Figure 3. Responses of *Nitellopsis obtusa* cells (representative traces) to illumination (VL-ON) and darkening (VL-OFF) **A.** Control (exposure of the cells to visible light caused a transient, small depolarization of the membrane potential after which a delayed hyperpolarization of E_m was observed). **B.** Treatment with 0.1 mM Cd ions. **C.** Treatment with 0.1 mM Pb ions. Adequate mean values are indicated in Table 1.

may be caused by both the decrease of the PM H^+-ATPase activity and the changes in plasma membrane permeability, especially for Ca^{2+} and K^+ ions (Morse and Spanswick 1984; Małkowski et al. 2005; Karcz and Kurtyka 2007). This suggestion is supported by our previous study (Małkowski et al. 2005), in which we showed that the Cd ions at high concentration (1.0 mM) decreased the content of Ca^{2+} in root cells of *Zea mays* L., and also by other authors who showed that Cd, but not Pb, induced a depolarization of the membrane potential (Llamas et al. 2000; Pavlovkin et al. 2006; Karcz and Kurtyka 2007) which resulted from decreased PM H^+-ATPase activity (Kennedy and Gonsalvez 1987, 1989; Burzyński and Buczek 1994; Fodor et al. 1995; Astolfi et al. 2003).

It has already been demonstrated that the membrane potential of *Characean* cells is very sensitive to light/dark and dark/light transitions (for review see Shimmen et al. 1994). In photosynthetic plant cells, the light can rapidly modulate the PM H^+-ATPase activity and voltage-dependent ion channels. The typical light-induced reaction consists of a quick initial depolarization of the E_m (Spalding and Cosgrove 1992; Elzenga et al. 1995; Shabala and Newman 1999; Stahlberg and van Volkenburgh 1999), followed by a slow membrane repolarization which very often resulted in plasma membrane hyperpolarization (Karcz 1991; Spalding and Cosgrove 1992; Blom-Zandstra et al. 1997; Stahlberg and van Volkenburgh 1999). Similar to the above cited authors we have showed that the exposure of the *Nitellopsis obtusa* cells to visible light caused a transient depolarization of E_m (which was usually finished within 2 min) after which a delayed hyperpolarization of E_m by 65.7 ± 2.9 mV (means ± SE, n = 8) was observed (Fig. 3A, Tab. 1). Several authors (Ermolayeva et al. 1996; Johannes et al. 1997; Shabala and Newman 1999) have suggested that Ca^{2+} could be a potent depolarizing ion which enters cytosol can stimulate the PM H^+-ATPase activity, resulting in an increase of H^+ efflux (Shabala and Newman 1999). It has also been previously shown (Okazaki et al. 1994) that H^+ ions apart from being extruded out of the cells are also taken up into the organelles such as vacuoles and chloroplasts.

The data presented in this paper clearly show that the Cd^{2+} ions are much more effective, than Pb^{2+} ions, in altering the light-induced membrane hyperpolarization (Fig. 3 and Tab. 1). This effect is probably connected with the fact that Cd^{2+} ions, which are more easily taken up by cells than Pb, alter the membrane permeability, especially for Ca^{2+} and K^+ ions. Furthermore, it is also possible that the inhibitory effect of Cd on photoelectric reaction is related to inhibition of photosynthesis by this metal (Siedlecka and Krupa 1996; Tukaj et al. 2007).

Taken together, these results suggest that at high Cd and Pb concentrations both depolarization of the E_m and decrease of light-induced membrane hyperpolarization in *Nitellopsis obtusa* cells are probably due to inhibition of the PM H^+-ATPase activity, whereas both metals at lower concentrations differ in mechanism of membrane potential changes.

References

Astolfi S., Zuchi S., Chiani A., Passera C. (2003): In vivo and in vitro effects of cadmium on H^+ATPase activity of plasma membrane vesicles from oat (Avena sativa L.) roots. J. Plant Physiol. **160,** 387–393 doi:10.1078/0176-1617-00832

Blom-Zandstra M., Koot H., van Hattum J., Vogelzang S.A. (1997): Transient light-induced changes in ion channel and proton pump activities in the plasma membrane of tobacco mesophyll protoplasts. J. Exp. Bot. **48,** 1623–1630

Burzyński M., Buczek J. (1994): The influence of Cd, Cu, Pb and Ni on NO_3– uptake by cucumber seedlings. II. In vitro and in vivo effect of Cd, Cu, Pb and Ni on the plasmalemma ATPase and oxidoreductase from cucumber seedling roots. Acta Physiol. Plant. **16,** 297–302

De Filippis L. F. (1979): The effect of heavy metal compounds on the permeability of Chlorella cells. Z. Pflanzenphysiol. **92,** 39–49

Elzenga J. T. M., Prins H. B. A. (1989): Light-induced polar pH changes in leaves of Elodea canadensis. II. Effects of ferricyanide: Evidence for modulation by the redox state of the cytoplasm. Plant Physiol. **91,** 68–72 doi:10.1104/pp.91.1.68

Elzenga J. T. M., Prins H. B. A., van Volkenburgh E. (1995): Light-induced membrane potential changes of epidermal and mesophyll cells in growing leaves of Pisum sativum. Planta **197,** 127–134 doi:10.1007/BF00239948

Ermolayeva E., Hohmeyer H., Johannes E., Sanders D. (1996): Calcium-dependent membrane depolarization activated by phytochrome in the moss Physcomitrella patens. Planta **199,** 352–358 doi:10.1007/BF00195726

Felle H. H. (1991): The role of the plasma membrane proton pump in short-term pH regulation in the aquatic liverwort Riccia fluitans L. J. Exp. Bot. **42,** 645–652 doi:10.1093/jxb/42.5.645

Fodor E., Szabo-Nagy A., Erdei L. (1995): The effect of cadmium on the fluidity and H^+-ATPase activity of plasma membrane from sunflower and wheat roots. J. Plant Physiol. **147,** 87–92

Fujii S., Shimmen T., Tazawa M. (1979): Effect of intracellular pH on the light-induced potential change and electrogenic activity in tonoplast-free cells of Chara australis. Plant Cell Physiol. **20,** 1315–1328

Harada Y., Okazaki S., Takagi S. (2002): Photosynthetic control of the plasma membrane H^+-ATPase in Vallisneria leaves. I. Regulation of activity during light-induced membrane hyperpolarization. Planta **214,** 863–869 doi:10.1007/s00425-001-0689-x

Johannes E., Ermolayeva E., Sanders D. (1997): Red light-induced membrane potential transients in the moss Physcomitrella

patens: ion channel interaction in phytochrome signalling. J. Exp. Bot. **48,** 599–608
doi:10.1093/jxb/48.Special_Issue.599

Jones G. J., Nichols P. B., Johns R. S., Smith J. B. (1987): The effect of mercury and cadmium on the fatty acid and sterol composition of the marine diatom Asterionella glacialis. Phytochem. **26,** 1343–1348
doi:10.1016/S0031-9422(00)81809-9

Karcz W. (1991): Effects of visible light and fusicoccin on membrane potential and pH of the incubation medium of Sagittaria leaf cells. Acta Biol. Sil. **17,** 16–22

Karcz W., Stolarek J., Kurtyka R. (1991): Photoelectrical reactions in Cucurbita pepo L. modified by action potentials induced by electrical and thermal stimuli. Acta Biol. Sil. **17,** 24–33

Karcz W., Burdach Z. (2002): A comparison of the effects of IAA and 4-Cl-IAA on growth, proton secretion and membrane potential in maize coleoptile segments. J. Exp. Bot. **53,** 1089–1098
doi:10.1093/jexbot/53.371.1089

Karcz W., Burdach Z. (2007): Effects of temperature on growth, proton extrusion and membrane potential in maize (Zea mays L) coleoptile segments. Plant Growth Regul. **52,** 141–150
doi:10.1007/s10725-007-9184-0

Karcz W., Kurtyka R. (2007): Effect of cadmium on growth, proton extrusion and membrane potential in maize coleoptile segments. Biol. Plant. **51,** 713–719
doi:10.1007/s10535-007-0147-0

Karcz W., Trela Z., Burdach Z., Przestalski S. (2009): Effect of trimethyllead chloride (Met_3PbCl) on SV channels and volume changes in the red beet vacuoles. Acta Biol. Crac. Ser. Bot. **51** (Suppl. 2), 105–106

Kennedy C. D., Gonsalves F. A. N. (1987): The action of divalent zinc, cadmium, mercury, copper and lead on the trans-root potential and H^+ efflux of excised roots. J. Exp. Bot. **38,** 800–817
doi:10.1093/jxb/38.5.800

Kennedy C. D., Gonsalves F. A. N. (1989): The action of divalent Zn, Cd, Hg, Cu and Pb ions on the ATPase activity of a plasma membrane fraction isolated from roots of Zea mays. Plant Soil **117,** 167–175
doi:10.1007/BF02220709

Köhler K., Steigner W., Simonis W., Urbach W. (1985): Potassium channels in Eremosphaera viridis. I. Influence of cations and pH on resting membrane potential and on an action-potential-like response. Planta **166,** 490–499

Lehtonen J., Saari J. (2000): Illumination does not affect the membrane potential or the plasma membrane H^+-ATPase activity in Micrasterias. Physiol. Plant. **110,** 436–442
doi:10.1111/j.1399-3054.2000.1100402.x

Lindberg S., Wingstrand G. (1985): Mechanism of Cd^{+2} inhibition of (K^+ + Mg^{+2}) ATPase activity and K^+ ($^{86}Rb^+$) uptake in young roots of sugar beet (Beta vulgaris). Physiol. Plant. **63,** 181–186
doi:10.1111/j.1399-3054.1985.tb01900.x

Lindberg S., Landberg T., Greger M. (2004): A new method to detect cadmium uptake in protoplasts. Planta **219,** 526–532
doi:10.1007/s00425-004-1256-z

Llamas A., Ullrich C. I., Sanz A. (2000): Cd^{2+} effects on transmembrane electrical potential difference, respiration and membrane permeability of rice (Oryza sativa L.) roots. Plant Soil **219,** 21–28
doi:10.1023/A:1004753521646

Małkowski E., Stolarek J., Karcz W. (1996): Toxic effect of Pb^{2+} ions on extension growth of cereal plants. Pol. J. Environ. Stud. **5,** 41–45

Małkowski E., Kurtyka R., Kita A., Karcz W. (2005): Accumulation of Pb and Cd and its effect on Ca distribution in maize seedlings (Zea mays L.). Pol. J. Environ. Stud. **14,** 203–207

Marrè M. T., Albergoni F. G., Moroni A., Marrè E. (1989): Light induced activation of electrogenic H^+ extrusion and K^+ uptake in Elodea densa depends on photosynthesis and is mediated by the plasma membrane H^+-ATPase. J. Exp. Bot. **40,** 343–352
doi:10.1093/jxb/40.3.343

Michelet B., Boutry M. (1995): Update on H^+-ATPase: The plasma membrane H^+-ATPase: A highly regulated enzyme with multiple physiological functions. Plant Physiol. **108,** 1–6

Miśkiewicz J., Trela Z., Przestalski S., Karcz W. (2010): Superstatistics analysis of the ion current distribution function: Met_3PbCl influence study. Eur. Biophys. J. **39,** 1397–1406
doi:10.1007/s00249-010-0594-y

Morse M. J., Spanswick R. M. (1984): Effect of lead acetate on the K^+ influx in Nitella translucens. In: Membrane Transport in Plants. (Eds. W. J. Cram, K. Janáček, R. Rybová and K. Sigler), pp. 336–337, Academia, Prague

Okazaki Y., Tazawa M., Iwasaki N. (1994): Light-induced changes in cytosolic pH in leaf cells of Egeria densa: measurements with pH-sensitive microelectrodes. Plant Cell Physiol. **35,** 943–950

Ouariti O., Boussama N., Zarrouk M., Cherif A., Ghorbal M. H. (1997): Cadmium- and copper-induced changes in tomato membrane lipids. Phytochem. **45,** 1343–1350
doi:10.1016/S0031-9422(97)00159-3

Pahlsson A. M. B. (1989): Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants: literature review. Water Air Soil Pollut. **47,** 287–319
doi:10.1007/BF00279329

Pavlovkin J., Luxová M., Mistríková I., Mistrík I. (2006): Short- and long-term effects of cadmium on transmembrane electric potential (E_m) in maize roots. Biol. (Bratisl.) **61,** 109–114
doi:10.2478/s11756-006-0016-x

Perfus-Barbeoch L., Leonhardt N., Vavasseur A., Forestier C. (2002): Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. Plant J. **32,** 539–548
doi:10.1046/j.1365-313X.2002.01442.x

Ros R., Morales A., Segura J., Picazo I. (1992) In vivo and in vitro effects of nickel and cadmium on the plasmalemma ATPase from rice (Oryza sativa L.) shoots and roots. Plant Sci. **83,** 1–6
doi:10.1016/0168-9452(92)90055-Q

Rucińska R., Gwóźdź E. A. (2005): Influence of lead on membrane permeability and lipoxygenase activity in lupine roots. Biol. Plant. **49,** 617–619
doi:10.1007/s10535-005-0059-9

Sanita` di Toppi L., Gabbrielli R. (1999): Response to cadmium in higher plants. Environ. Exp. Bot. **41,** 105–130
doi:10.1016/S0098-8472(98)00058-6

Shabala S., Newman I. (1999): Light-induced changes in hydrogen, calcium, potassium, and chloride ion fluxes and concentrations from the mesophyll and epidermal tissues of bean leaves. Understanding the ionic basis of light-induced bioelectrogenesis. Plant Physiol. **119,** 1115–1124 doi:10.1104/pp.119.3.1115

Sharma S. D., Chopra R. N. (1987): Effect of lead nitrate and lead acetate on growth of the moss Semibarbula onentalis (Web.) Wijk. et Marg. grown in vitro. J. Plant Physiol. **28,** 243–249

Shimmen T., Mimura T., Kikuyama M., Tazawa M. (1994): Characean cells as a tool for studying electrophysiological characteristics of plant cells. Cell Struct. Funct. **19,** 263–278 doi:10.1247/csf.19.263

Siedlecka A., Krupa Z. (1996): Interaction between cadmium and iron and its effects on photosynthetic capacity of primary leaves of Phaseolus vulgaris. Plant Physiol. Biochem. **34,** 833–841

Spalding E. P., Cosgrove D. J. (1992): Mechanism of blue-light-induced membrane depolarization in etiolated cucumber hypocotyls. Planta **188,** 199–205 doi:10.1007/BF00216814

Stahlberg R., van Volkenburgh E. (1999): The effect of light on membrane potential, apoplastic pH and cell expansion in leaves of Pisum sativum L. var. Argenteum. Planta **208,** 188–195 doi:10.1007/s004250050549

Stefanov K., Popova I., Kamburova E., Pancheva T., Kimenov G., Kuleva L., Popov S. (1993): Lipid and sterol changes in Zea mays caused by lead ions. Phytochem. **33,** 47–51 doi:10.1016/0031-9422(93)85394-7

Stefanov K., Seizova K., Popova I., Petkov V., Kimenov G., Popov S. (1995a): Effect of lead ions on the phospholipid composition in leaves of Zea mays and Phaseolus vulgaris. J. Plant Physiol. **137,** 243–246

Stefanov K. L., Pandev S. D., Seizova K. A., Tyankova L. A., Popov S. S. (1995b): Effect of lead on the lipid metabolism in spinach leaves and thylakoid membranes. Biol. Plant. **37,** 251–256 doi:10.1007/BF02913222

Stolarek J., Karcz W. (1987): Effects of UV-C radiation on membrane potential and electric conductance in internodal cells of Nitellopsis obtusa. Physiol. Plant. **70,** 473–478 doi:10.1111/j.1399-3054.1987.tb02845.x

Stolarek J., Karcz W., Pietruszka M. (1988): Mathematical modelling of light-induced electric reaction of Cucurbita pepo L. leaves. Acta Soc. Bot. Pol. **57,** 271–278

Tazawa M., Shimmen T. (2001): How characean cells have contributed to the progress of plant membrane biophysics. Aust. J. Plant Physiol. **28,** 523–539

Tukaj Z., Baścik-Remisiewicz A., Skowroński T., Tukaj C. (2007): Cadmium effect on the growth, photosynthesis, ultrastructure and phytochelatin content of green microalga Scenedesmus armatus: A study at low and elevated CO_2 concentration. Environ. Exp. Bot. **60,** 291–299 doi:10.1016/j.envexpbot.2006.12.002

Verkleij J. A. C., Schat H. (1990): Mechanisms of metal tolerance in plants. In: Heavy Metal Tolerance in Plants: Evolutionary Aspects. (Ed. A. J. Shaw), pp. 179–193, CRC Press, Boca Raton

Received: June 24, 2010
Final version accepted: October 7, 2010