Changes of photosynthetic parameters in cucumber leaves under Cu, Cd, and Pb stress

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Abstract

The effects of Cu, Cd, and Pb toxicity on photosynthesis in cucumber leaves (*Cucumis sativus* L.) were studied by the measurements of gas exchange characteristics, chlorophyll (Chl) fluorescence parameters, and Chl content. Concentrations of metals in sequence of 20 µM Cu, 20 and 50 µM Cd, and 1 000 µM Pb decreased the plant dry mass to 50–60 % after 10 d of treatment whereas 50 µM of Cu decreased it to 30 %. The content of Cd in leaves of plants treated with 50 µM Cd was three times higher than the contents of Cu and Pb after plant treatment with 50 µM Cu or 1 000 µM Pb. Hence Cd was transported to leaves much better than Cu and Pb. Nevertheless, the net photosynthetic rate and stomatal conductance in leaves treated with 50 µM Cu or Cd were similarly reduced. Thus, Cu was more toxic than Cd and Pb for photosynthesis in cucumber leaves. None of the investigated metals decreased internal CO_2 concentrations. Also the effect of metals on potential efficiency of photosystem 2, PS2 (F_v/F_m) was negligible. The metal dependent reduction of PS2 quantum efficiency (Φ_{PS2}) after plant adaptation in actinic irradiation was more noticeable. This could imply that reduced demand for ATP and NADPH in a dark phase of photosynthesis caused a down-regulation of PS2 photochemistry. Furthermore, in leaves of metal-treated plants the decrease in water percentage as well as lower contents of Chl and Fe were observed. Thus photosynthesis is not the main limiting factor for cucumber growth under Cu, Cd, or Pb stress.

Additional key words: chlorophyll content and fluorescence; Fe; growth; heavy metals; internal CO_2 concentration; net photosynthetic rate; stomatal conductance; water relations.

Introduction

Plant exposition to heavy metals has always resulted in a strong reduction of plant growth as a consequence of significant alterations in many metabolic pathways. Among the processes limiting plant growth and productivity, the most significant is photosynthesis. Several studies have shown that photosynthetic efficiency of many plants is affected by heavy metals (Krupa and Baszyński 1995). Metals have multidirectional effect on photosynthesis (for reviews see Krupa and Baszyński 1995 and Prasad and Strzałka 1999). The excess of Cu, Cd, or Pb inhibits directly the photosynthetic electron transport (Krupa and Baszyński 1995, Myśliwa-Kurdziel 2002) as well as the activities of Calvin-Benson cycle enzymes or net assimilation of CO_2 (Prasad and Strzałka 1999 and references therein). In addition, metals can also alter the photosynthetic activity indirectly, decreasing the content of photosynthetic pigments or damaging the photosynthetic apparatus on every level of its organisation, structure of chloroplasts (Molas 2002), or lipid and protein composition of thylakoids (Skórzyńska-Polit and Baszyński 1997).

Heavy metals affect two distinct parameters which are crucial for both photosynthetic activity and plant growth, *i.e.* water relations and ionic relations. During the exposure to metals the water status of leaves (water potential, transpiration rate, and relative water content) is distorted, resulting in reduction of leaf expansion (Poschenrieder and Barcelo 1999). Furthermore, disturbances in the uptake of ions and in their tissue distribution can lead to premature senescence of adult leaves, and in consequence to the reduction of the total photosynthetic area (Krupa *et al.* 2002). Additionally, changes of these parameters depend on the severity and duration of metal stress and on plant species.

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Abbreviations: C_i = internal CO_2 concentration; DM = dry mass; F_{0o} = minimal level of chlorophyll fluorescence; F_m, F_m' = maximum fluorescence of dark- and light-adapted leaves, respectively; F_v, F_v' = variable fluorescence of dark- and light-adapted state, respectively; F_v/F_m = potential efficiency of PS2; g_s = stomatal conductance; P_N = net photosynthetic rate; PS = photosystem; Q_A – plastoquinone; Φ_{PS2} = light-adapted PS2 quantum efficiency.

Although the effects of heavy metals on the separate elements, such as the light or dark phase of photosynthesis, chlorophyll (Chl) content, or water relations, were intensively examined in the past, there is still lack of complex and parallel analysis of those parameters. Therefore, we simultaneously examined Chl content, efficiency of photosystem 2 (PS2) photochemistry, photosynthetic electron transport chain, and net photosynthetic rate (P_N), as well as water content under heavy metal stresses. Additionally, the yield (dry mass) of plants after metal treatment was measured to estimate whether the negative action of heavy metals on photosynthetic apparatus was the main reason for plant growth reduction.

Materials and methods

Cucumber (*Cucumis sativus* L.) seeds germinated in darkness at 25 °C were transferred to the modified Hoagland solution 3 times diluted (Burzyński 2001). After 7 d (at the second leaf stage) Pb, Cd, or Cu in the form of chlorides were added to the nutrient solutions. Plants were grown hydroponically under a 16 h photoperiod (180 μmol m^{-2} s^{-1}) at 25 °C during the day and 22 °C during the night. After the next 10 d of growth, plants were harvested and the second, fully expanded leaf was used for the analyses. At this time the control plants as well as the plants treated with 20 μM Cu or Cd or 1 000 μM Pb have developed three leaves, whereas the plants treated with 50 μM Cu or Cd have formed only two leaves.

Chl fluorescence was measured with a pulse amplitude modulated system (model *FMS2*, *Hansatech Instruments*, UK). Prior to the measurements, the leaves were dark adapted for 20 min in order to obtain the potential fluorescence (F_v/F_m). After the dark period, all centres of PS2 were "open", *i.e.* their electron acceptors Q_A were oxidised. The minimum Chl fluorescence (F_0) was measured using a 594 nm amber modulating beam whereas the maximum fluorescence (F_m) corresponding to all PS2 centres in the "close state" was induced by a 1-s pulse of saturating "white light" (10 800 μmol m^{-2} s^{-1}). The variable fluorescence (F_v) was calculated from the difference between F_m and F_0. The actual quantum efficiency of PS2 [$\Phi_{PS2} = (F_m' - F_s)/F_m'$] was determined after adaptation of leaves to an actinic "white light" (600 μmol m^{-2} s^{-1}), when "steady state" of the fluorescence yield (F_s) was reached. In order to achieve the F_m', the saturating pulse was given to "close" all the PS2 reaction centres.

P_N, stomata conductance (g_s), and internal CO_2 concentration (C_i) were measured with portable photosynthesis measuring system *CIRAS-1* (*PPSystems*, Hitchin, U.K.) fitted with broad leaf cuvette, at irradiance of 300 μmol m^{-2} s^{-1}, 350 cm^3 m^{-3} CO_2 in air, and leaf temperature of 25 °C.

Leaves used for photosynthesis and Chl fluorescence measurements were then removed from plants and dried at 70 °C for 2 d. Then each leaf was digested with $HClO4/HNO_3$ mixture in the microwave system and contents of Cu, Cd, and Pb were determined by an atomic absorption spectrophotometer (*Perkin-Elmer 3300*).

Chl amount was determined in 80 % acetone solution, according to the procedure of Arnon (1949). The values of dry mass (DM), Chl *a* and *b* contents, and contents of metals were analysed and the standard deviations (SE) were calculated for twelve replications from four independent experiments. Gas exchange and fluorescence parameter measurements were performed on ten to twenty individual plants per treatment.

Results

Experiments were performed with leaves of cucumbers growing at 20 and 50 μM of Cu or Cd and 1 000 μM of Pb. Although these metal concentrations significantly reduced the fresh mass of plants, they were not lethal. The DM of cucumber treated for 10 d with 20 μM Cu and 20 and 50 μM Cd similarly as with 1 000 μM Pb was reduced to 50–60 % (Fig. 1). When plants were grown in nutrient solution with 50 μM Cu, their DM was decreased up to 30 %. Among the steams, leaves, and roots, the highest reduction of biomass was found in roots.

The highest metal accumulation in plant leaves treated with Cd, Cu, or Pb was observed for cadmium (Table 1). At 20 μM, Cd tissue accumulation was 1.12 mmol compared with 0.75 mmol of Cu per kg of dry matter. Pb achieved comparable leaf content at 1 000 μM concentration in nutrient solution. Interestingly, elevation of external Cd from 20 to 50 μM almost tripled its content in leaves, whereas similar raise of external Cu increased only slightly (about 20 %) the metal content in leaves. Our results clearly indicate that the relative toxicity profile of investigated metals for plant growth was Cu>Cd>Pb.

The ratio of variable fluorescence to maximum fluorescence (F_v/F_m), independently of Cu and Cd concentrations, was reduced only slightly (a few percent) as compared to the control (Table 1). Over the same range of Cu and Cd concentrations, the actual PS2 quantum efficiency (Φ_{PS2}; Chl fluorescence measured without dark adaptation) was more markedly altered (greater than 10 %). However, its reduction was still relatively small. Pb effect on the ratio of F_v/F_m and Φ_{PS2} was negligible.

P_N for control plants was about 8.5 μmol(CO_2) m^{-2} s^{-1}. Inclusion of Cu and Cd at concentration of 50 μM to the nutrient solution drastically decreased P_N (up to 10 and 20 %, respectively, Fig. 2*A*). Lower concentrations of these two metals (20 μM) similarly as 1 000 μM Pb were

Table 1. The photosynthetic fluorescence parameters in the second leaf of plants treated with 20 or 50 μM Cu or Cd or 1 000 μM Pb. Means ±SE.

Metal content in nutrient solution [μM]	plant leaves [mmol kg^{-1}(DM)]	F_v/F_m	% of control	Φ_{PS2}	% of control
Control		0.839 ± 0.015		0.740 ± 0.022	
Cu 20	0.75 ± 0.19	0.829 ± 0.009	98	0.666 ± 0.019	90
50	0.90 ± 0.21	0.811 ± 0.012	96	0.629 ± 0.011	85
Cd 20	1.12 ± 0.25	0.825 ± 0.012	98	0.651 ± 0.012	87
50	3.51 ± 0.37	0.815 ± 0.010	97	0.658 ± 0.014	89
Pb 1 000	1.22 ± 0.21	0.832 ± 0.013	99	0.725 ± 0.019	98

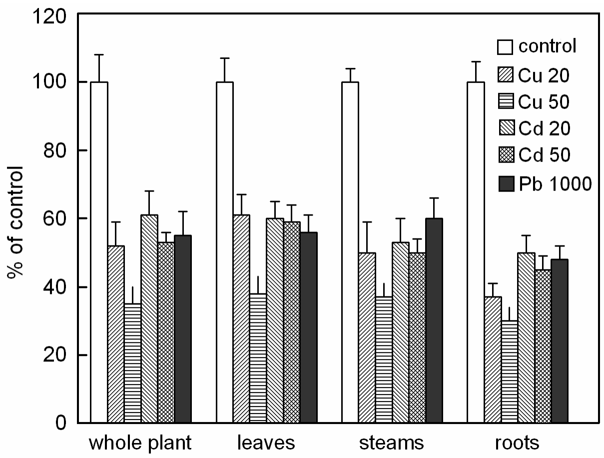

Fig. 1. Dry mass of whole plants, leaves, stems, and roots after plant treatment with Cu, Cd (20 and 50 μM), or Pb (1 000 μM). Mean % of control plants ± SE. Control values: whole plant 6.18 g, leaves 3.03 g, stem 1.78 g, roots 1.37 g.

less effective and P_N values in these combinations were decreased only to 80 % of control trials.

g_s was also strongly decreased by 50 μM Cu or Cd. In leaves of plants treated with 1 000 μM Pb and 20 μM Cu or Cd, g_s was similar as in the control (Fig. 2*B*) leaves.

1 000 μM Pb and 20 μM Cu or Cd did not change C_i (Fig. 2*C*), whereas in plants treated with 50 μM Cu or Cd higher C_i was found in comparison to the control plants.

Cd, Cu, and Pb only slightly disturbed the water relations in leaves (Table 2). The most visible effect was observed in leaves of cucumber treated with 50 μM Cu (about 10 % lower water content comparing to the control). The effects of metals on the contents of Chl *a* and *b* were much more obvious. In leaves of plants growing with 20 μM Cu or Cd as well as with 1 000 μM Pb the Chl content decreased up to 80 or 90 %. In combinations with 50 μM Cu the contents of pigments were reduced even up to 68 % of control. All studied metals also lowered the content of Fe in leaves (Table 2). As

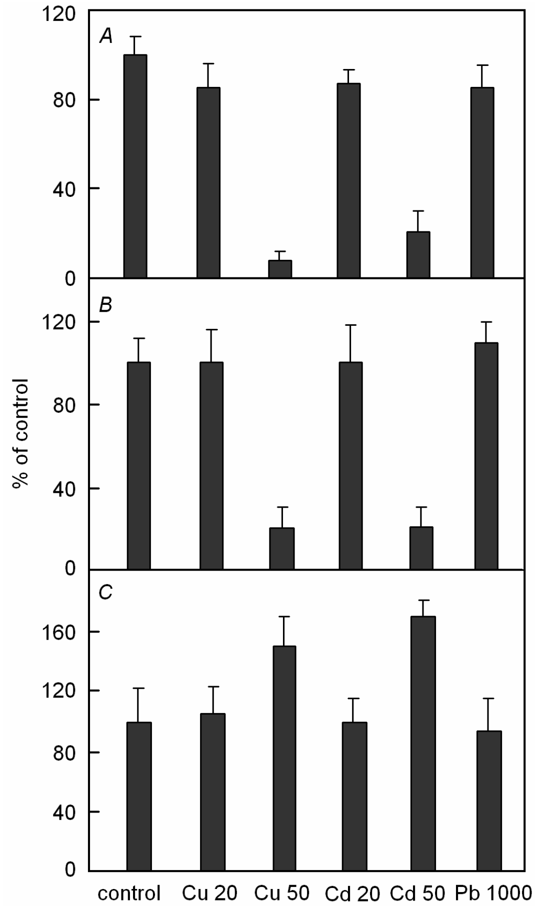

Fig. 2. Net photosynthetic rate, P_N (*A*), stomatal conductance, g_s (*B*), and internal CO_2 concentration, C_i (*C*) in the second leaves of cucumber treated with Cu, Cd (20 and 50 μM), or Pb (1 000 μM). Mean % of control plants ± SE. Control values: P_N 8.5 μmol(CO_2) m^{-2} s^{-1}, g_s 120 mmol(CO_2) m^{-2} s^{-1}, C_i 130 cm^3 m^{-3}.

external concentration of Cu and Cd increased, Fe contents in leaves decreased.

Discussion

Translocation of Cu, Cd, or Pb from root to stem of different plants is very weak (Burzyński and Buczek 1989, Ernst *et al*. 1992, Greger 1999). Especially lead is a metal largely accumulated in the roots, while Cd, in general, is more mobile than Pb and Cu (Greger 1999). Our results confirmed those findings. In almost every concentration, with the exception of 50 µM Cd, the amounts of metals in leaves detected after 10 d of treatment were as low as 1 mmol kg^{-1}(DM). Translocation of Cd from roots to leaves was three times higher than the translocation of Cu, when metal concentration in nutrient solution was 50 µM. Similarly, the movement of Cd from roots to leaves was three times higher, in comparison to Pb, even if concentration of lead in nutrient solution was as high as 1 000 µM.

Table 2. Relative contents of chlorophyll (Chl) *a*+*b*, water, and Fe in second leaves of cucumber treated with Cu, Cd, or Pb. Control values: Chl *a*+*b* 4.2 g kg^{-1}(DM), water 890 g kg^{-1}(fresh mass), Fe 175 mg kg^{-1}(DM).

Metal [µM]	Water [%]	Chl *a*+*b*	Fe
Control	100 ± 3	100 ± 2	100 ± 5
Cu 20	95 ± 2	80 ± 2	80 ± 5
50	90 ± 3	68 ± 4	65 ± 7
Cd 20	96 ± 2	88 ± 3	71 ± 4
50	95 ± 2	80 ± 5	63 ± 5
Pb 1 000	95 ± 4	88 ± 6	88 ± 8

Treatment of plants with Cd, Cu, and Pb decreased markedly the plant growth (Fig. 1). On the contrary, only a mild effect of metals was observed on the potential PS2 efficiency (0.811–0.829 for metals compared to 0.839 per control, Table 1). According to Björkman and Demmig (1987) if F_v/F_m is higher than 0.8, the potential efficiency of PS2 is not affected. Slight change of PS2 efficiency in excess of Cu was also observed by Ciscato *et al.* (1997) in *Triticum durum.* The concentration of Cu, which, similarly as in our experiments, drastically reduced the growth of *Phaseolus*, only slightly decreased the efficiency of PS2 photochemistry (Cook *et al*. 1997). Scardelis *et al*. (1994) also did not find changes of PS2 efficiency in *Sonchus* spp. or *Taraxacum* spp. growing on soil contaminated with Pb, Zn, and Cu. On the other hand, numerous studies indicate (Maksymiec *et al*. 1994, Poskuta and Wacławczyk-Lach 1995, Maksymiec and Baszyński 1996, Krupa and Moniak 1998, Larsson *et al*. 1998) that heavy metals alter this photosynthetic parameter more markedly. Thus, the disturbances in potential efficiency of PS2 under heavy metals' stress depend on the plant genera and age, time of exposure to metals, and their contents in leaf tissues.

Φ_{PS2} was more noticeably diminished than F_v/F_m in plants treated with Cu and Cd. Nevertheless, we did not detect such a drop of Φ_{PS2} in plant leaves treated with Pb. Similar relation between F_v/F_m and Φ_{PS2} was obtained for plants treated with an excess of Mn (Kitao *et al*. 1997, Subrahmanyam and Rathore 2000). Reduction of Φ_{PS2} could be explained by the decreased capacity of the carbon metabolism and/or by a low utilisation of ATP and NADPH in a dark phase of photosynthesis (Kitao *et al*. 1997, Subrahmanyam and Rathore 2000).

In this study, among determined parameters of photosynthesis, P_N was most evidently affected by Cu, Cd, and Pb. This fact confirms the hypothesis that carbon reduction and its subsequent dark metabolism is more sensitive to metals than the light phase of photosynthesis. We found that P_N in plants treated with 50 µM Cu and Cd was drastically reduced (up to 10 and 20 % of control, respectively). Simultaneously, in these plants very low g_s (20 % of control) was observed. Nevertheless, significant reduction of P_N in plants after 50 µM Cu or Cd treatment was not caused by a low g_s and low CO_2 concentration in chloroplasts, because C_i values were even higher in leaves of those plants than in the control leaves (Fig. 2*C*). High C_i could be explained by a low P_N and/or by an increase in the dark respiration rate, which was often detected in plants treated with heavy metals (Lee *et al.* 1976, Burzyński and Buczek 1994, Romanowska *et al*. 2002). The elevated rate of dark respiration after metal treatment of plants was usually connected with the senescence of leaf tissues. To sum up, high C_i observed in cucumber treated with 50 µM Cu or Cd indicated that drastic reduction in P_N was not due to the direct effect of metals on the stomata closure. Similar results indicating that an opening state of stomata had only a minor effect on CO_2 assimilation were obtained for leaves of plants treated with Mn (Sage and Reid 1994, Subrahmanyam and Rathore 2000). It cannot be excluded that the reduction of P_N is also due to the effect of Cd and Cu on the photosynthetic CO_2 reduction cycle. There are many reports concerning the effect of Cu, Cd, or Pb on the dark reactions of photosynthesis (for review see Prasad and Strzałka 1999). A drop in CO_2 reduction during photosynthesis could influence the photochemical efficiency (lower Φ_{PS2} observed in cucumber treated with Cu or Cd, Table 1) *via* an imbalance between the absorbed photon energy and the energy utilised by the carbon metabolism. Finally, our results have shown that the photosynthetic electron transport is more tolerant to Cu, Cd, and Pb stress than CO_2 reduction.

We also suggest that the influence of metals on the photosynthetic reactions is rather indirect. The excess of heavy metals usually decreased the content of Chl, often by lowering the transport rate of Fe to the leaves (Table 2). Furthermore, the low Fe content could influence not only the Chl synthesis but also the structure of chloroplasts and indirectly the light and dark phases of photosynthesis (Siedlecka and Krupa 1999). Heavy metals usually disturb water relations in plants, which is widely recognized as the first and very important reason of metal toxicity (Poschenrieder and Barcelo 1999). It is consistent with our observations that the Cu and Cd treatment of cucumber reduced the water content in plant leaves.

Considering the relation of Cu, Cd, and Pb contents in leaves and in the nutrient solution with their effect on the photosynthetic parameters, toxicity profile of investigated metals would follow as Cu>Cd>Pb. As mentioned earlier, Pb, Cu, and Cd accumulate mainly in roots. In the aboveground plant parts, metals were predominantly found in the apoplast and, to a lesser extent, in the vacuoles. Only a small amount of metals, which is taken up by plants, have reached chloroplasts in leaves. Therefore, it might be possible that a direct effect of heavy metals on the photosynthetic apparatus is largely restricted.

The drastic reduction of cucumber DM was observed for all used heavy metal concentrations (Fig. 1) independent on the metal level. On the contrary, only the highest concentrations of Cu and Cd affected photosynthetic parameters. These findings suggest that photosynthesis is not the main process which limits the growth of cucumber under Cu, Cd or Pb stress.

References

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. – Plant Physiol. **24**: 1-15, 1949.

Björkman, O., Demmig, B.: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. – Planta **170**: 489-504, 1987.

Burzyński, M.: Influence of pH on Cd and Cu uptake, distribution and their effect on nitrate reductase activity in cucumber (*Cucumis sativus* L.) seedling roots. – Acta Physiol. Plant. **23**: 201-206, 2001.

Burzyński, M., Buczek, J.: Interaction between cadmium and molybdenum affecting the chlorophyll content and accumulation of some heavy metals in the second leaf of *Cucumis sativus* L. – Acta Physiol. Plant. **11**: 137-145, 1989.

Burzyński, M., Buczek, J.: The influence of Cd, Pb, Cu and Ni on NO_3^- uptake by cucumber seedlings I. Nitrate uptake and respiration of cucumber seedlings roots treated with Cd, Pb, Cu and Ni. – Acta Physiol. Plant. **16**: 291-296, 1994.

Ciscato, M., Valcke, R., Van Loven, K., Clijsters, H., Navari-Izzo, F.: Effect of in vivo copper treatment on the photosynthetic apparatus of two *Triticum durum* cultivars with different stress sensitivity. – Physiol. Plant. **100**: 901-908, 1997.

Cook, C.M., Kostidou, A., Vardaka, F., Lanaras, T.: Effects of copper on the growth, photosynthesis and nutrient concentrations of *Phaseolus* plants. – Photosynthetica **34**: 179-193, 1997.

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

Greger, M.: Metal availability and bioconcentration in plants. – In: Prasad, M.N.V., Hagemayer, J. (ed.): Heavy Metal Stress in Plants. From Molecules to Ecosystems. Pp. 1-29. Springer-Verlag, Berlin – Heidelberg – New York 1999.

Kitao, M., Lei, T.T., Koike, T.: Effects of manganese toxicity on photosynthesis of white birch (*Betula platyphylla* var. *japonica*) seedlings. – Physiol. Plant. **101**: 249-256, 1997.

Krupa, Z., Baszyński, T.: Some aspects of heavy metals toxicity towards photosynthetic apparatus – direct and indirect effects on light and dark reactions. – Acta Physiol. Plant. **17**: 177-190, 1995.

Krupa, Z., Moniak, M.: The stage of leaf maturity implicates the response of the photosynthetic apparatus to cadmium toxicity. – Plant Sci. **138**: 149-156, 1998.

Krupa, Z., Siedlecka, A., Skórzynska-Polit, E., Maksymiec, W.: Heavy metal influence on the light phase of photosynthesis. – In: Prasad, M.N.V., Strzałka, K. (ed.): Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Pp. 287-303. Kluwer Academic Publishers, Dordrecht 2002.

Larsson, E.H., Bornman, J.F., Asp, H.: Influence of UV-B radiation and Cd^{2+} on chlorophyll fluorescence, growth and nutrient content in *Brassica napus*. – J. exp. Bot. **49**: 1031-1039, 1998.

Lee, J., Cunningham, B.A., Paulson, G.M., Liang, G.H., Moore, R.B.: Effects of cadmium on respiration rate and activities of several enzymes in soybean seedlings. – Physiol. Plant. **36**: 4-6, 1976.

Maksymiec, W., Baszyński, T.: Different susceptibility of runner bean plants to excess copper as a function of the growth stages of primary leaves. – J. Plant Physiol. **149**: 217-221, 1996.

Maksymiec, W., Russa, R., Urbanik-Sypniewska, T., Baszyński, T.: Changes in acyl lipid and fatty acid composition in thylakoids of copper non-tolerant spinach exposed to excess copper. – J. Plant Physiol. **140**: 52-55, 1992.

Maksymiec, W., Russa, R., Urbanik-Sypniewska, T., Baszyński, T.: Effect of excess Cu on the photosynthetic apparatus of runner bean leaves treated at two different growth stages. – Physiol. Plant. **91**: 715-721, 1994.

Molas, J.: Changes of chloroplast ultrastructure and total chlorophyll concentration in cabbage leaves caused by excess of organic Ni(II) complex. – Environ. exp. Bot. **47**: 115-126, 2002.

Myśliwa-Kurdziel, B., Prasad, M.N.V., Strzałka, K.: Heavy metal influence on the light phase of photosynthesis. – In: Prasad, M.N.V., Strzałka, K. (ed.): Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Pp. 229-257. Kluwer Academic Publ., Dordrecht 2002.

Poschenrieder, C., Barcelo, J.: Water relation in heavy metals stressed plants. – In: Prasad, M.N.V., Hagemayer, J. (ed.): Heavy Metal Stress in Plants. From Molecules to Ecosystems. Pp. 207-231. Springer-Verlag, Berlin – Heidelberg – New York 1999.

Poskuta, J.W., Wacławczyk-Lach, E.: *In vivo* responses of primary photochemistry of photosystem II and CO_2 exchange in light and in darkness of tall fescue genotypes to lead toxicity. – Acta Physiol. Plant. **17**: 233-240, 1995.

Prasad, M.N.V., Strzałka, K.: Impact of heavy metals on photosynthesis. – In: Prasad, M.N.V., Hagemayer, J. (ed.): Heavy Metal Stress in Plants. From Molecules to Ecosystems. Pp. 117-139. Springer-Verlag, Berlin – Heidelberg – New York 1999.

Romanowska, E., Igamberdiev, A., Parys, E., Gardestron, P.:

Stimulation of respiration by Pb^{2+} ions in detached leaves and mitochondria of C_3 and C_4 plants. – Physiol. Plant. **116**: 148-154. 2002.

Sage, R.F., Reid, C.D.: Photosynthetic response mechanisms to environmental change in C3 plants. – In: Wilkinson, R.E. (ed.): Plant-Environment Interactions. Pp. 413-499. Marcel Dekker, New York – Basel – Hong Kong 1994.

Scardelis, S., Cook, C.M., Pantis, J.D., Lanaras, T.: Comparison of chlorophyll fluorescence and some heavy metal concentrations in *Sonchus* spp. and *Taraxacum* spp. along an urban pollution gradient. – Sci. total. Environ. **158**: 157-164. 1994.

Siedlecka, A., Krupa, Z.: Cd/Fe interaction in higher plants – its consequences for the photosynthetic apparatus. – Photosynthetica **36**: 321-331, 1999.

Skórzyńska-Polit, E., Baszyński, T.: Diferences in sensitivity of the photosynthetic apparatus in Cd-stressed runner bean plants in relation to their age. – Plant Sci. **128**: 11-21, 1997.

Subrahmanyam, D., Rathore, V.S.: Influence of manganase toxicity on photosynthesis in ricebean (*Vigna umbellata*) seedlings. – Photosynthetica **38**: 449-453, 2000.