ACTA BOT. CROAT. VOL. 39, 33—40, 1980.

UDC 58
CODEN: ABCRA2
YU ISSN 0365—0588

UDC 581.174.582.542.1:546.815 = 20

THE EFFECT OF LEAD ON THE STRUCTURE AND FUNCTION OF WHEAT PLASTIDS

MERCEDES WRISCHER and DARINKA MEGLAJ

(Laboratory of Electron Microscopy, Ruđer Bošković Institute, Zagreb)

Received January 10, 1980

Introduction

In living organisms lead affects a series of important metabolic activities (Vallee and Ulmer 1972, Huang et al. 1974, Foy et al. 1978). It has been reported that in plants lead inhibits cell division (Hammett 1928, 1929) and elongation (Zegers et al. 1976), different enzyme activities (Vallee and Ulmer 1972, Maier 1978), the electron transport in mitochondria (Koeppe and Miller 1970, Bittell et al. 1974), and photosynthesis (Miles et al. 1972. Bazzaz et al. 1975, Wong and Govindjee 1976).

Plants tolerate rather high concentrations of lead, as it has been shown on plants growing in zones of high automobile traffic and in the vicinity of lead mines (Foy et al. 1978). Lead is absorbed by plants and is accumulated preferentially in the walls of roots and stems, where it can be detected with the electron microscope due to its high electron density (Hammett 1928, Gullvåg et al. 1974, Malone et al. 1974, Sharpe and Denny 1976, Zegers et al. 1976). Dark inclusions containing lead have been found also inside the cells, predominantly in the nuclei (Hammett 1929, Skaar et al. 1973), in dictyosome vesicles (Malone et al. 1974, Zegers et al. 1976), and occasionally also in chloroplasts, mitochondria and peroxysomes (Bittell et al. 1974, Opus and Gullvåg 1974, Sharpe and Denny 1976). It has been suggested that lead reacts preferentially with phosphates by forming insoluble lead compounds (Ophus and Gullvåg 1974, Zegers et al. 1976).

It has been further reported that plants, growing in areas of high automobile traffic, show signs of chlorosis as a consequence of lead poisoning, and that lead inhibits normal greening and chlorophyll synthesis

in leaves of plant seedlings (Hampp and Lendzian 1974). This inhibition is probably due to an inactivation of some enzymes of the chlorophyll biosynthesis (Hampp and Ziegler 1974). The purpose of this research was to study the fine structure, the pigment content, and the photosynthetic activity of plastids of etiolated and green leaves held for several days on water containing lead salts.

Material and Methods

Wheat seedlings (*Triticum aestivum* cv. »super zlatna«) were grown at 25 °C either in darkness or in light for 8—9 days. For experiments the second and third leaf were detached from the seedlings and put into petri dishes on filter paper wetted either with an 0.5, 1, 5, or 10 mM solution of $PbCl_2$ or $Pb(NO_3)_2$ in distilled water, or with distilled water alone. In some experiments leaves held on adequate solutions of NaCl or $NaNO_3$ were also used as control. Green leaves — of seedlings grown in light — were exposed directly to light (2 fluorescent tubes 20 W, 4500 °K, illumination intensity 4000 lx) for 4, 24, 48, 72, or 96 hours. Etiolated leaves were at first incubated for 3 hours in darkness and then exposed to light for 4, 24, 48, or 72 hours.

For electron microscopic examinations small pieces of leaves were fixed in 1% glutaraldehyde in cacodylate buffer at 1 °C, postfixed in 1% OsO_4 and after dehydration embedded in Araldite. Thin sections were examined in a Siemens Elmiskop I, either without any further staining, or after staining with uranyl acetate and lead citrate.

The pigments were extracted from whole leaves or from isolated plastids in 85% acetone. The quantitative determination of chlorophyll a, chlorophyll b and total carotenoids was performed at wavelengths of 663, 644, and 452.5 nm (after Röbbelen 1957, cited by Urbach et al. 1976).

Plastids were isolated by grinding the leaves with pestle and mortar in cold medium containing 0.05 M tricine (N-Tris-(hydroxymethyl)-methylglycine; pH 7.8), 0.4 M sorbitol or sucrose, 0.01 M NaCl, and 0.5% bovine serum albumine. The hogomenate was squeezed through one layer of nylon gauze and two layers of cotton flannel. The tissue debris was removed by a 3 min. centrifugation at 120×g. The plastids were then sedimented by a 10 min. centrifugation at 1000×g, washed and centrifuged in the same medium and resuspended in it again.

The photosynthetic activity (O_2 exchange) was measured with an O_2 electrode (Hansatech Ltd., Norfolk, England) at 20 °C. The suspensions were illuminated with a halogen lamp giving an intensity of 10^4 lx. The Hill reaction (evolution of O_2) was measured in the reaction mixture containing 50 mM tricine (pH 7.8), 0.1 M sorbitol, 5 mM $MgCl_2$, 2.5 mM $K_3Fe(CN)_6$, 3 mM NH_4Cl and chloroplasts of 50 — 100 μg chlorophyll (Evans 1975). The activity of photosystem 1 was measured as O_2 uptake in the reaction mixture containing 50 mM tricine (pH 7.8), 0.1 M sorbitol, 0.1 mM methyl viologen, 0.5 mM NaN_3, 2.5 μM 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea, 2 mM sodium ascorbate, 5 μM 2,6-dichlorophenol indophenol, and chloroplasts of 50 — 100 μg chlorophyll (Evans 1975). The effect of lead ions on the photosynthetic activity of isolated chloroplasts was measured after a 30 min. preincubation in darkness in the isolation medium containing $PbCl_2$.

Results

There were no differences between $PbCl_2$ and $Pb(NO_3)_2$ in their effect on the cells and plastids of both etiolated and green leaves. The detached leaves survived on a 1 mM or 5 mM concentration of lead salts for several days, while still higher concentrations (10 mM) were lethal already after about 2 days.

Etiolated leaves

Concentrations of lead salts of 0.5 mM or lower had only little effect on the speed of the greening of etiolated leaves exposed to light. After 3 or more days in light the leaves were only slightly less green than the control ones. Higher concentrations of lead, i. e. 1 mM and 5 mM, inhibited the greening to a much higher degree. After 2 or 3 days in light the leaves still remained yellow, although they were often unevenly coloured, with yellow and light green portions.

Electron microscopic examinations showed that in leaves, held in light on 1 mM or 5 mM lead solutions, the transformation of etioplasts into chloroplasts was strongly inhibited. The first stage of this transformation, i. e. the disintegration of prolamellar bodies, was not affected at all. Both in the control leaves and in the leaves treated with lead, the tubular structures of the prolamellar bodies disintegrated and were transformed into single thylakoids already after 3 — 4 hours in light. The following developmental stage — the formation of grana — was, on the contrary, strongly inhibited by lead. While in the control leaves, after 48 hours in light, there were already chloroplasts with well developed grana (Fig. 1), in the yellow portions of leaves held on lead solutions (1 mM or 5 mM) there still were roundish plastids containing only few single thylakoids (Fig. 2); in light green leaf portions the plastids contained somewhat more thylakoids aggregated into rudimentary grana. If maintained constantly on lead salts plastids did not differentiate further and remained in the stage of etiochloroplasts till the death of the leaf. If, however, after some hours the lead treatment was interrupted and the leaves transferred for 2 or more days to water, they eventually succeeded in developing chloroplasts and regreening.

Lead inhibited the synthesis of both chlorophyll and carotenoids. The degree of chlorophyll inhibition increased with the increasing concentration of lead. After 48 hours in light on a 5 mM lead solution the concen-

Table 1. Total chlorophyll, total carotenoids and photosynthetic activity of *etiolated leaves* maintained 48 h in light on water and $PbCl_2$.

	Total chlorophyll (mg/g fr. wt.)	Total carotenoids (mg/g fr. wt.)	Hill reaction (μM O_2/mg chlorophyll/h)	Photosystem 1 activity (μM O_2/mg chlorophyll/h)
Water	0.90	0.14	109.53	78.52
$PbCl_2$ 5 mM	0.46	0.11	98.86	115.90

tration of total chlorophyll reached only about half of that of control leaves (Table 1). The ratio of chlorophyll a/b was also different: in the control leaves it was 3.3, and in the leaves held on lead 3.9. The concentration of total carotenoids was also lower than in the control (Table 1). The photosynthetic activity of these plastids was low, but when calculated to the very low concentration of chlorophyll present in them, the Hill reaction reached almost the value found in untreated plastids, while the activity of photosystem 1 was even higher than in the control (Table 1).

The values for photosynthetic activity for both the control and lead treated plastids were rather high, especially those of the Hill reaction. This may be partly the consequence of the addition of uncoupler, but may reflect also the characteristics of young chloroplasts, which normally possess rather high photosynthetic activities (L e e c h 1977). Besides that, the preservation of these plastids during isolation, as shown by light and electron microscopy, was better than that of grown up chloroplasts.

Electron microscopic examinations of unstained sections showed that there were no dark granular inclusions in the plastids, which would indicate a deposit of lead compounds. Dark deposits were, however, found sometimes in nuclei, and very often in dictyosome vesicles (Fig. 2). In addition to that, some portions of the cell walls contained large dark deposits, and the regions between them and the plasmalemma were dilated and filled with dark particles, some less dense material and small vesicles. Several dictyosomes always lay in the vicinity of these inclusions (Fig. 2).

Green leaves

The examination of chloroplasts of lead treated green leaves showed that, in spite of the fact that their ultrastructure was similar to that of untreated chloroplasts (Figs. 3, 5), the concentration of the total chlorophyll was somewhat lower than in the control, at least in the leaves held for 2 or more days on fairly high lead concentrations (Table 2). The photosynthetic activity was inhibited in relation to the increasing concentration of lead and the duration of the treatment. After 48 hours on a 5 mM solution of lead both the Hill reaction and the activity of photosystem 1 were considerably lower than in the chloroplasts of the control leaves (Table 2).

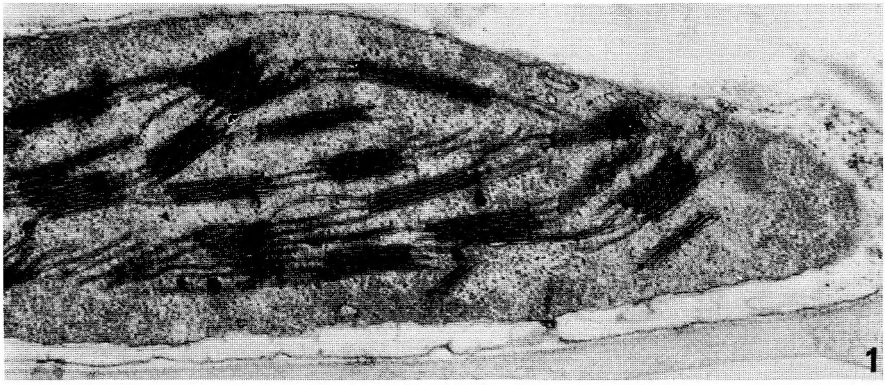
Fig. 1. Etiolated wheat leaf maintained on water for 48 h in light. Young chloroplast with grana. 34,000 : 1.

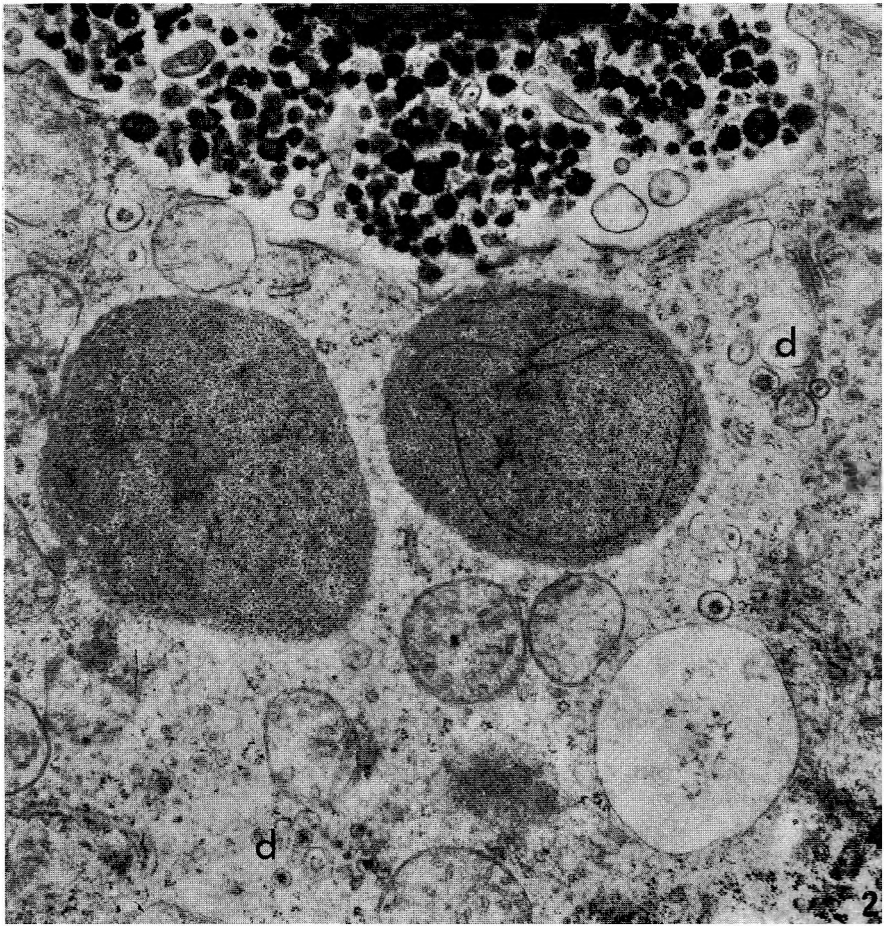
Fig. 2. Etiolated wheat leaf treated with 1 mM $PbCl_2$ for 48 h in light. There are only single thylakoids in the etiochloroplasts. Vesicles of the dictyosomes (d) are filled with dark material. In the cell wall dark deposits (upper part of the picture). 20,000 : 1.

Fig. 3. Green wheat leaf — control. Part of a chloroplast with large grana. 48,000 : 1.

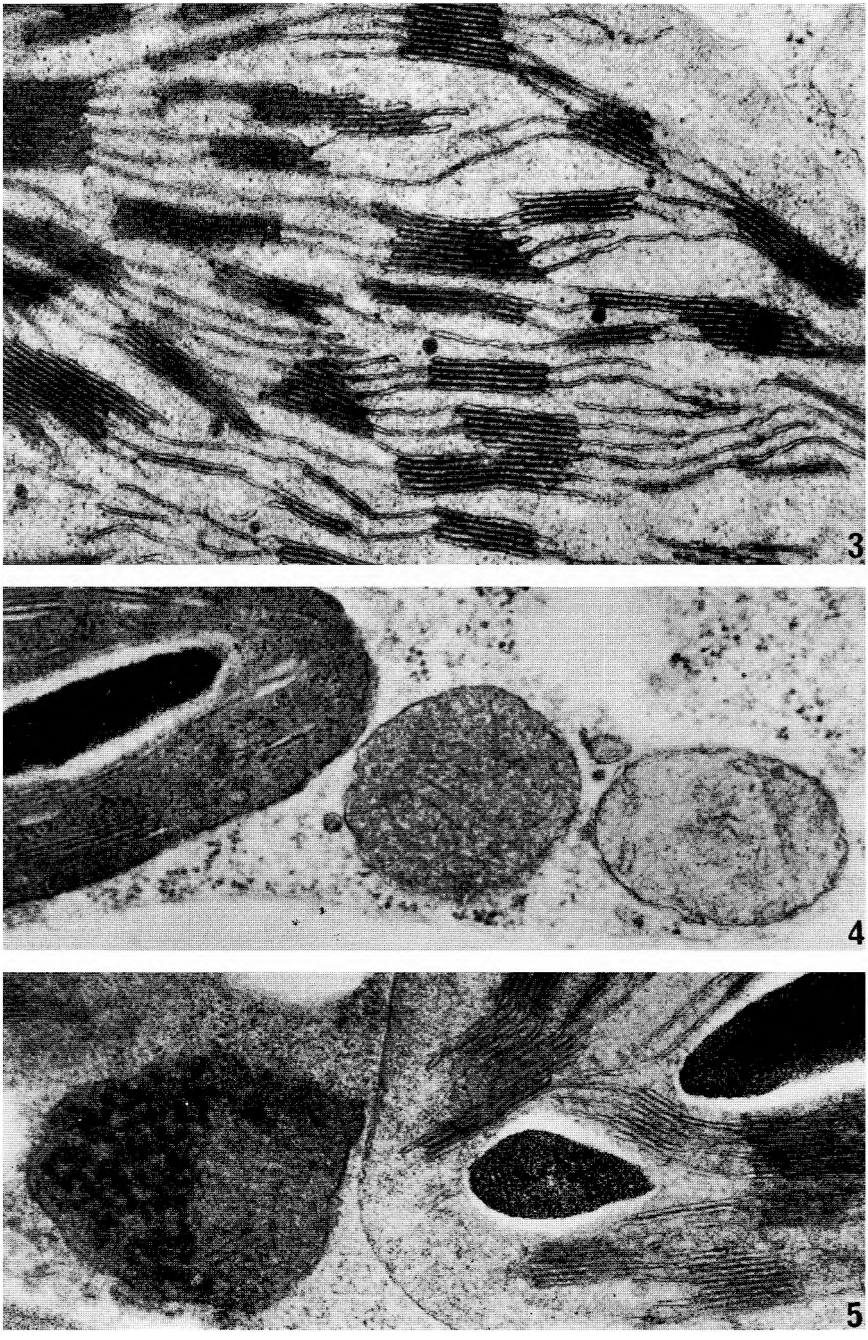
Fig 4. Green wheat leaf — control. Part of a mesophyll cell with a mitochondrion, part of a chloroplast and a peroxysome (in the middle of the picture), containing fibrillar inclusions in the matrix. 48,000:1.

Fig. 5. Green wheat leaf held 4 days on 1 mM $PbCl_2$. Part of a chloroplast and a peroxysome (on the left) with a large amorphous inclusion. 48,000 : 1.





Figs. 1—2.



Figs. 3—5.

Table 2. Total chlorophyll, total carotenoids and photosynthetic activity of *green leaves* maintained 48 h in light on water and $PbCl_2$.

	Total chlorophyll (mg/g fr. wt.)	Total carotenoids (mg/g fr. wt.)	Hill reaction (μM O_2/mg chlorophyll/h)	Photosystem 1 activity (μM O_2/mg chlorophyll/h)
Water	1.29	0.14	41.55	61.76
$PbCl_2$ 5 mM	0.82	0.14	37.94	33.08

As shown by electron microscopic examination of unstained sections, dark deposits were found only in some nuclei and in dictysome vesicles. Some portions of cell walls contained large dark deposits, similar to those found in etiolated leaves.

In all green leaves treated with lead solutions there were marked differences in the fine structure of peroxysomes. In the control leaves these organelles usually contained only some fibrillar inclusions embedded in a granular matrix (Fig. 4). Peroxysomes of leaves held on lead solutions — independently of the concentration and length of the treatment — were filled with large amorphous inclusions (Fig. 5). According to the low contrast, as observed in unstained sections, these inclusions did not contain lead.

Isolated chloroplasts

In order to establish how far lead affects directly photosynthetic activity, isolated chloroplasts were held in media containing lead salts for 30 minutes. These experiments showed that the inhibition of the photosynthetic activity of isolated chloroplasts depended of the concentration of lead salts present in the medium, while a prolongation of the treatment with lead for more than 30 minutes was without remarkable effect. These experiments also showed, that photosystem 1 seemed to be less sensitive to lead ions than photosystem 2 (i. e. the Hill reaction). At a 10 mM concentration of lead the Hill reaction dropped already to about half (23.16 μM O_2/mg chlorophyll/h) of that in untreated chloroplasts (41.55 μM 0_2/mg chlorophyll/h), while at the same time the activity of photosystem 1 was about 85% (52.94 μM O_2/mg chlorophyll/h) of that of the control (61.76 μM O_2/mg chlorophyll/h).

Discussion

When used at rather high concentrations lead delays and inhibits normal transformation of etioplasts into chloroplasts. Plastids remain in the stage of etiochloroplasts and contain only a very poorly developed thylakoid system and much less chlorophyll than those in the control leaves. Both the high ratio of chlorophyll a/b and the rather high photosynthetic activity confirm that lead blocks their differentiation already at an early stage (Plesničar and Bendall 1973, Leech 1977, Šestak et al. 1977). The present experiments indicate that both photosystems 1 and 2 are inhibited by lead, although in isolated chloroplasts

photosystem 1 seems to be somewhat less sensitive to lead. Miles et. al. (1972) have observed that in isolated chloroplasts only photosystem 2 has been inhibited by lead, the inhibition lying probably between the primary electron donor of photosystem 2 and the site of water oxidation. These authors used somewhat lower lead concentrations, than the ones used in the present experiments in which they inhibited the activity of photosystem 1. Our results are, however, in accordance with those of Wong and Govindjee (1976) who — using high concentrations of lead ions — have observed an inactivation of the reaction centre P 700 of photosystem 1.

When treated with lead the peroxysomes of green leaves develop large amorphous inclusions. The meaning of these structures formed after lead treatment is for the moment unknown, as the function of the s. c. "nucleoids" in peroxysomes is still unknown (Frederick et al. 1975). In any case, the formation of large "nucleoids" after lead treatment could indicate a change in the activity of these organelles.

It is striking that leaves and isolated chloroplasts tolerate rather high concentrations of lead. It has been reported that isolated plant mitochondria can absorbe ten times more lead than other heavy metal ions, and that precipitations of lead with some compounds, above all with phosphates, are the cause of this high lead tolerance (Bittell et al. 1974, Zegers et al. 1976). According to our ultrastructural investigations, dark inclusions probably containing lead, are occasionally found in nuclei, although the cell wall seems to be the prime site of lead deposition. As dictyosome vesicles also contain dark material, it seems that dictyosomes may play an important role in the formation of cell wall deposits (Malone et al. 1974, Zegers et. al. 1976) and in the detoxification of lead in plant cells.

Summary

The effect of lead salts on the structure and function of plastids in etiolated and green wheat leaves exposed to light has been studied by ultrastructural investigations, pigment analysis and determination of photosynthetic activity (O_2 exchange).

The investigations have shown that in plastids of etiolated leaves, after they were exposed to light, the development of new thylakoids and the synthesis of pigments, especially of chlorophyll are strongly affected by lead salts ($PbCl_2$ or $Pb(NO_3)_2$ at concentration of 1 mM or 5 mM). In relation to their low chlorophyll content, the photosynthetic activity (both the Hill reaction, and the activity of photosystem 1) in these plastids is rather high. This indicates that lead inhibits the normal differentiation of plastids so that they remain in their early etiochloroplast developmental stage.

There are no prominent ultrastructural changes in the chloroplasts of green leaves held on lead solutions, although their chlorophyll content and photosynthetic activity are lower than in chloroplasts of untreated leaves. It has been shown that, when isolated chloroplasts are treated with lead salts, photosystem 1 is less affected than the Hill reaction.

Some other ultrastructural changes found in leaves held on lead solutions are also described.

*

The authors wish to thank Professor Z. Devidé and Dr. N. Ljubešić for helpful discussions and valuable comments.

References

Bazzaz, F. A., R. W. Carlson, G. L. Rolfe, 1975: Inhibition of corn and sunflower photosynthesis by lead. Physiol. Plant. *34,* 326—329.

Bittell, J. E., D. E. Koeppe, R. J. Miller, 1974: Sorption of heavy metal cations by corn mitochondria and the effects on electron and energy transfer reactions. Physiol. Plant. *30,* 226—230.

Evans, M. C. W., 1975: Photosynthetic oxygen evolution and the relationship between electron transport and ATP synthesis in spinach chloroplasts. In: Laboratory manual of cell biology *(D. O. Hall, S. E. Hawkins,* eds.), pp. 151—153. Unibooks Univ. Press Ltd.

Foy, C. D., R. L. Chaney, M. C. White, 1978. The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol. *29,* 511—566.

Frederick, S. E., P. J. Gruber, E. H. Newcomb, 1975: Plant microbodies (Review article). Protoplasma, *84,* 1—29.

Gullvåg, B. M., H. Skaar, E. M. Ophus, 1974: An ultrastructural study of lead accumulation within leaves of *Rhytidiadelphus squarrosus* (Hedw.) Warnst. A comparison between experimental and environmental poisoning. J. Bryol. *8,* 117—122.

Hammett, F. S., 1928: Studies in the biology of metals. I. The localization of lead by growing roots. Protoplasma *4,* 183—186.

Hammett, F. S., 1929: Studies in the biology of metals. III. The localization of lead within the cells of growing roots. Protoplasma 5, 135—141.

Hampp, R., K. Lendzian, 1974: Effect of lead ions on chlorophyll synthesis. Naturwiss. *61,* 218—219.

Hampp, R., H. Ziegler, 1974: Der Einfluss von Bleiionen auf Enzyme der Chlorophyllbiosynthese. Z. Naturforsch. *29c,* 552—558.

Huang, C.-Y., F. A. Bazzaz, L. N. Vanderhoef, 1974: The inhibition of soybean metabolism by cadmium and lead. Plant Physiol. *54,* 122—124.

Koeppe, D. E., R. J. Miller, 1970: Lead effects on corn mitochondrial respiration. Science *167,* 1376—1378.

Leech, R. M., 1977: Etioplast, structure and its relevance to chloroplast development. In: Etioplast, a colloquium organized on behalf of the bioenergetic organelle group *(J. W. Bradbeer,* ed.). Biochem. Soc. Trans. *5,* 81—84.

Maier, R., 1978: Untersuchungen zur Wirkung von Blei auf die saure Phosphatase in *Zea mays* L. Z. Pflanzenphysiol. *87,* 347—354.

Malone, C., D. E. Koeppe, R. J. Miller, 1974: Localization of lead accumulated by corn plants. Plant Physiol. *53,* 388—394.

Miles, C. D., J. R. Brandle, D. J. Daniel, O. Chu-Der, P. D. Schnare and *D. J. Uhlik,* 1972: Inhibition of photosystem II in isolated chloroplasts by lead. Plant Physiol. *49,* 820—825.

Ophus, E. M., B. M. Gullvåg, 1974: Localization of lead within leaf cells of *Rhytidiadelphus squarrosus* (Hedw.) Warnst. by means of transmission electron microscopy and x-ray microanalysis. Cytobios *10,* 45—58.

Plesničar, M., B. S. Bendall, 1973: The photosynthetic activities and electron carriers of developing barley leaves. Biochem. J. *136,* 803—812.

Sharpe, V., P. Denny, 1976: Electron microscope studies on the absorption and localization of lead in the leaf tissue of *Potamogeton pectinatus* L. J. exper. Bot. *27,* 1155—1162.

Skaar, H., E. Ophus, B. M. Gullvåg, 1973: Lead accumulation within nuclei of moss leaf cells. Nature *241,* 215—216.

Šestak, Z., J. Zima, H. Strnadova, 1977: Ontogenetic changes in the internal limitations to bean-leaf photosynthesis. 2. Activities of photosystems 1 and 2 and non-cyclic photophosphorylation and their dependence on photon flux density. Photosynthetica *11,* 282—290.

Urbach, W., W. Rupp, H. Sturm, 1976: Experimente zur Stoffwechselphysiologie der Pflanzen. G. Thieme Verl., Stuttgart.

Vallee, B. L., D. D. Ulmer, 1972: Biochemical effects of mercury, cadmium and lead. Ann. Rev. Biochem. *41,* 91—128.

Wong, D., Govindjee, 1976: Effect of lead ions on photosystem I in isolated chloroplasts: Studies on the reaction center *P 700.* Photosynthetica *10,* 241—254.

Zegers, P. V., K. H. Harmet, L. Hanzely, 1976: Inhibition of IAA-induced elongation in *Avena* coleoptile segments by lead: a physiological and electron microscopic study. *Cytobios 15,* 23—35.

SAŽETAK

UTJECAJ OLOVA NA STRUKTURU I FUNKCIJU PLASTIDA PŠENICE

Mercedes Wrischer i Darinka Meglaj

(Laboratorij za elektronsku mikroskopiju, Institut »Ruđer Bošković«, Zagreb)

Istražen je utjecaj olovnih soli na strukturu i funkciju plastida u osvijetljenim etioliranim i zelenim listovima pšenice. Istraženi su ultrastruktura, sadržaj pigmenata i fotosintetska aktivnost plastida tretiranih i kontrolnih listova.

Istraživanja su pokazala da u plastidima etioliranih osvijetljenih listova olovne soli [$PbCl_2$ ili $Pb(NO_3)_2$ koncentracije 1 mM ili 5 mM] snažno inhibiraju razvoj tilakoida i sintezu pigmenata, napose klorofila. S obzirom na niski sadržaj klorofila, fotosintetska aktivnost (Hillova reakcija i aktivnost fotosistema 1) u tim je plastidima razmjerno visoka. Ovi nalazi upozoravaju na to da olovo inhibira normalnu diferencijaciju plastida, tako da oni zaostanu u ranom razvojnom stadiju etiokloroplasta.

Kloroplasti zelenih listova, koji su držani na otopini olovnih soli, ne pokazuju nikakvih većih ultrastrukturnih promjena, iako su i sadržaj klorofila i fotosintetska aktivnost niže od one netretiranih kloroplasta. U kloroplastima koji su nakon izolacije držani 30 minuta u mediju s dodatkom olovnih soli, aktivnost fotosistema 1 manje je zakočena od Hillove reakcije.

U radu su opisane i neke druge ultrastrukturne promjene u stanicama listova pšenice izazvane prisutnošću olovnih soli.

Dr. Mercedes Wrischer
Institut »Ruđer Bošković«
Bijenička 54
YU-41000 Zagreb (Jugoslavija)