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Influence of lead on the development of lupin seedlings and ultrastructural localization of this metal in the roots

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Abstract

The effect of lead on the early phases of development of yellow lupin seedlings was investigated. In the presence of this metal the number of germinating seeds was found to diminish distinctly, the hypocotyls and roots were shorter and the fresh weight and anthocyanin content in the cotyledones were markedly decreased. In the root cap cells lead was present in the vacuoles, ER, dictyosomes, the nuclear envelope and cell walls.

INTRODUCTION

One of the many factors polluting the natural environment are lead compounds. In contrast to the relatively well known effect of lead on animal organisms, little is still known of its influence on plants. The changes it causes in plants depend among other things on the substrate on which the plant is cultivated (Foy et al. 1978). Accumulation of this element in plant cells depends also on its chemical form and concentration (Ahlf et al. 1980). The attenuating influence of some ions as for instance calcium on the toxic action of lead is also known (Garland and Wilkins 1981). Under the same culture conditions the presence of lead may produce different effects according to the plant species.

It results from the available literature that the toxic action of lead was most pronounced in root cells (Griffith 1919, Bonnet 1922, Wilkins 1957, Świeboda 1976, Lane and Martin 1980, Krupińska 1981). Hammett (1929, quoted after Simola 1977) attributed the decreased rate of root growth of *Zea* to the reduction, under the influence of this element, of mitotic activity in the growth apexes. Röderer (1979) published data concerning cytokinesis disturbances by lead compounds.

The observed morphological changes found their reflection in cytological investigations. Malone et al. (1974) when examining cells of the root epidermis of *Zea mays* found lead in the dictyosomes and ER. They advanced the hypothesis that the lead taken up by the plant is at first accumulated in vesicles of dictyosomal origin. The latter then move to the plasmalemma and lead is removed to the cell wall where it is accumulated in the form of crystals of various size. The authors did not observe deposits of this metal either in the mitochondria, plastids or within the cell nuclei. Observation of the cells of *Rhytidiadelphus squarrosus* by Gullvåg et al. (1974) revealed the presence of lead mainly in vacuoles, the cell nucleus and plasmalemma.

The present study was undertaken to establish the influence of various $PbCl_2$ concentrations on seed germination and hypocotyl and root morphology in early stages of development of yellow lupin seedlings. Localization of the metal in cells of the root cap was also done by means of a transmission electron microscope.

MATERIAL AND METHODS

Seeds of yellow lupin (*Lupinus luteus* L. cv. Jantar) from the 1978 harvest were soaked in tap water for 12 h. After swelling they were placed on Petri dishes of 20 cm diametre lined with lignin moistened with 20 millilitres of distilled water or with an aqueous solution of $PbCl_2$ in concentrations of 10, 100, 200, 1000 and 5000 ppm. The dishes with the seeds were placed in darkness (20°C, air moisture 80%). After 24 and 48 h the per cent of germinating seeds was counted under green light and after 72 h they were transferred to a germinating box with distilled water (control) or with water $PbCl_2$ solution and left in the dark for 72 h. The lenght of the hypocotyls and roots (each time in 10 seedlings) was measured (significant differences were only found at 500, 1000 and 5000 ppm concentrations) and the fresh weight of the cotyledones and anthocyanin level were determined by Mohr's method (1981). The ultrastructure of the root cap cells was also examined. Material for study was fixed with glutaraldehyde and OsO_4, dehydrated in an acetone graded series and with propylene oxide and embedded in Epon 812. Ultrathin sections were cut on an LKB "Ultratome III" and were left unstained for observation on a transmission electron microscope JEM 7 A at 50 or 80 kV.

RESULTS

After 24 h the control seeds (treated with distilled water) germinated in 35 per cent, and after further 24 h in 83 per cent. Seeds treated with aqueous $PbCl_2$ solutions of 500, 1000 and 5000 ppm concentrations germinated much worse, the differences being more pronounced after 24 than

after 48 h. The lowest germination per cent (7%) was noted after 24 h in seeds treated with the 5000 ppm solution of plumbous salt (Fig. 1). A characteristic feature of seeds germinating on $PbCl_2$ solution was the production of abnormally long root hairs.

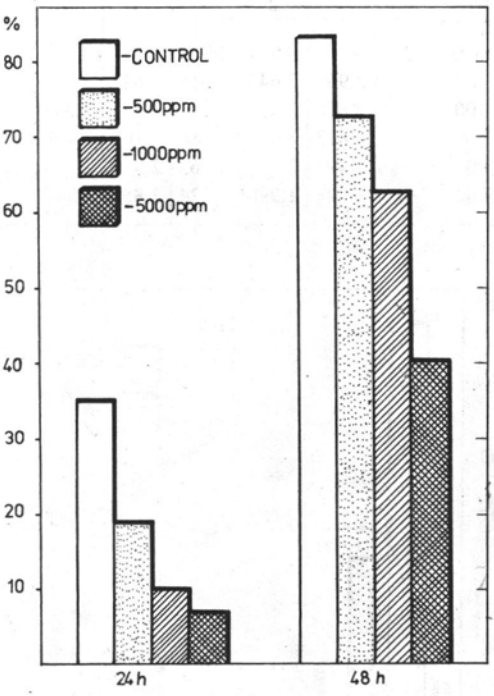


Fig. 1. Effect of $PbCl_2$ on the percentage of germinating yellow lupin seeds, mean values from five replications

Control seedlings showed after 6 days a normal morphological structure. The cotyledones had an intensive brownish red colour. The mean hypocotyl length was 24.8 mm and that of the root 39.1 mm. Seedlings grown in various plumbous salt concentrations exhibited a decrease in cotyledone fresh weight (Table 1) and a considerable shortening of the hypocotyls and roots (Figs. 2 and 3), the growth inhibition being more pronounced in the roots than in the hypocotyls.

The lupin cotyledones belong to organs which synthesise in darkness large quantities of anthocyanins (Majorek 1976). This process was inhibited by lead compounds. The brownish-red colour of the cotyledones changed to orange at 10-500 ppm concentrations, to yellow at 1000 ppm and pale yellow at 5000 ppm (Table 2). A marked thickening of the root neck and increased browning of the roots were observed in proportion to the $PbCl_2$ solution concentration. Frequently the root tips died back, particularly at higher lead concentrations.

Table 1

Fresh weight of 23 cotyledones (in grams) serving for analysis of anthocyanin level. Incubation—6 days in the dark

$PbCl_2$ concentrations, ppm	Cultures					Mean from 5 replications
	1	2	3	4	5	
Control	3.681	3.871	2.964	2.871	3.892	3.456
10	2.958	3.643	2.615	2.641	3.673	3.106
100	2.858	3.598	2.201	2.253	3.341	2.850
500	2.813	3.017	1.988	2.108	3.098	2.607
1000	2.493	2.876	1.763	2.979	2.094	2.441
5000	1.923	1.998	1.124	2.851	1.997	1.979

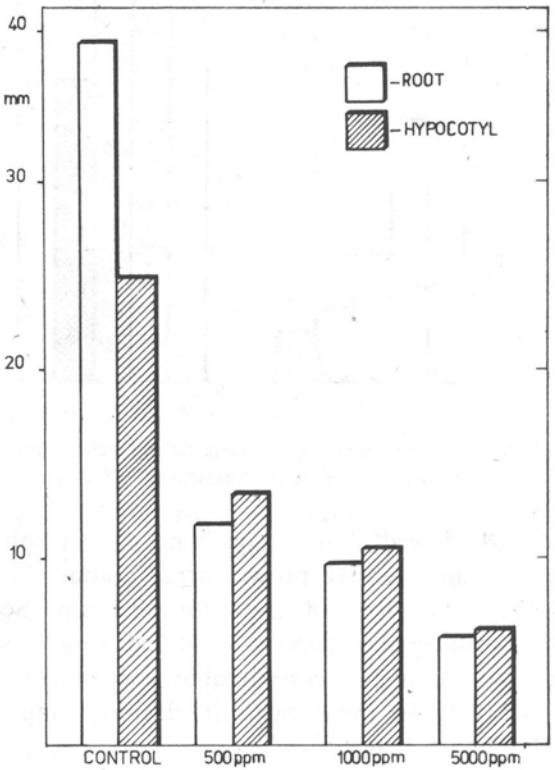


Fig. 2. Hypocotyl and root length of lupin seedlings growing in water and in $PbCl_2$ water solutions, means of five replications

For ultrastructural analysis only root caps were used because of their direct contact with the plumbous salt, their high sensitivity to it and the relatively homogeneous anatomical structure. Lead content was highest in the vaculoes and in the endoplasmic reticulum (Figs. 5, 6 and 7).

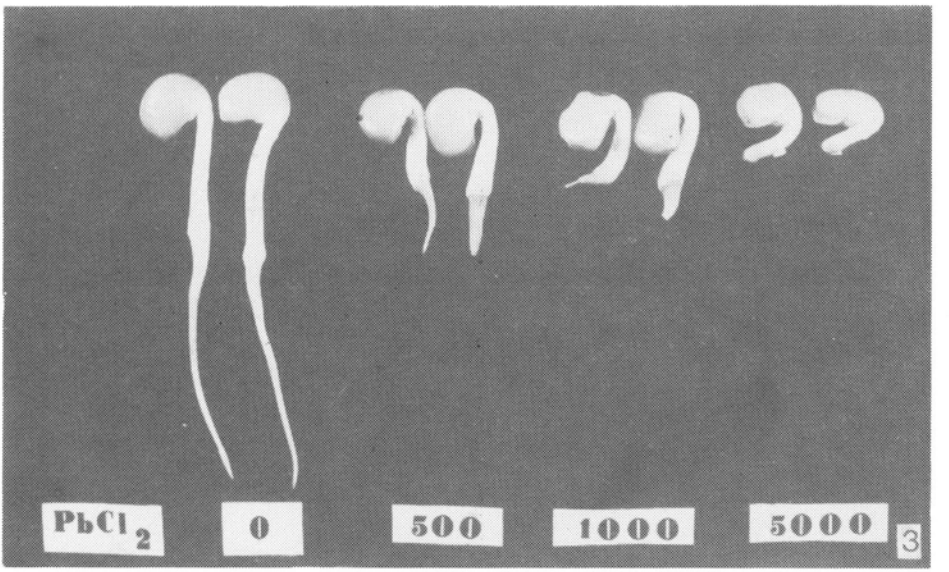
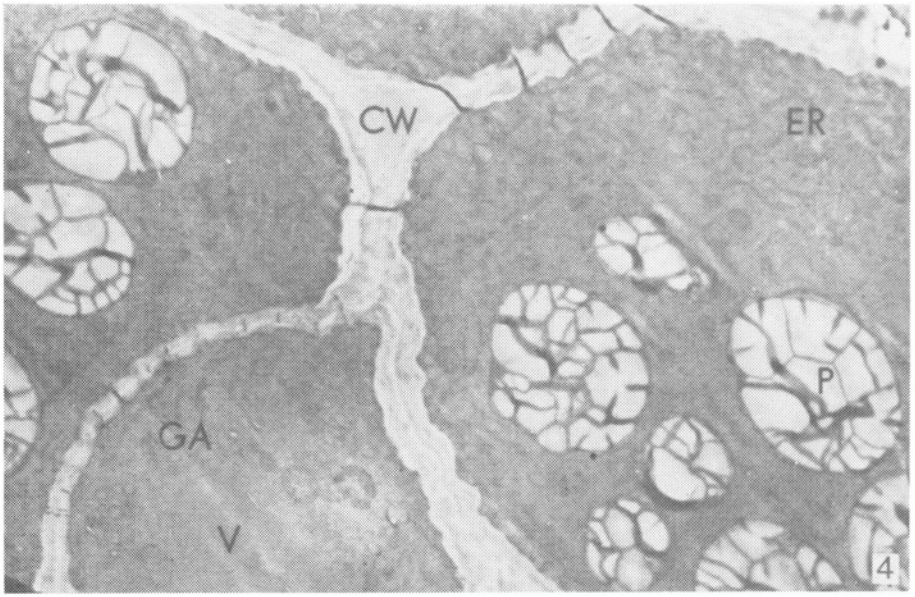
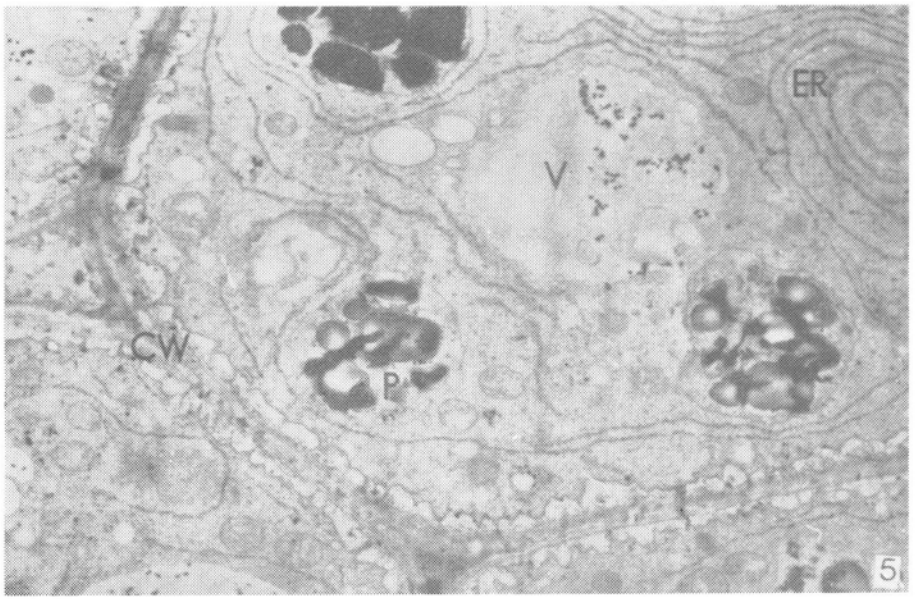


Fig. 3. Yellow lupin seedlings cultured in darkness and treated for 72 h with water or various $PbCl_2$ concentrations

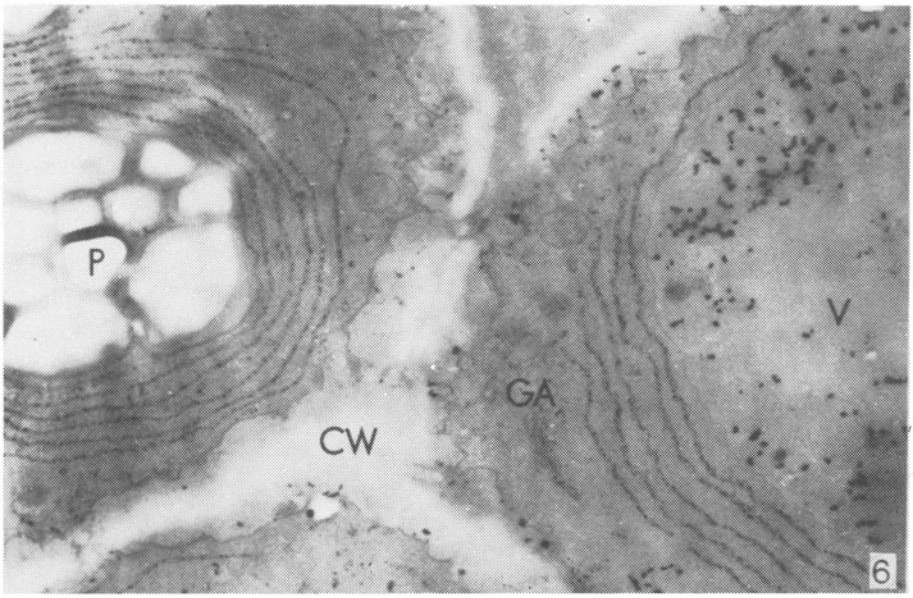


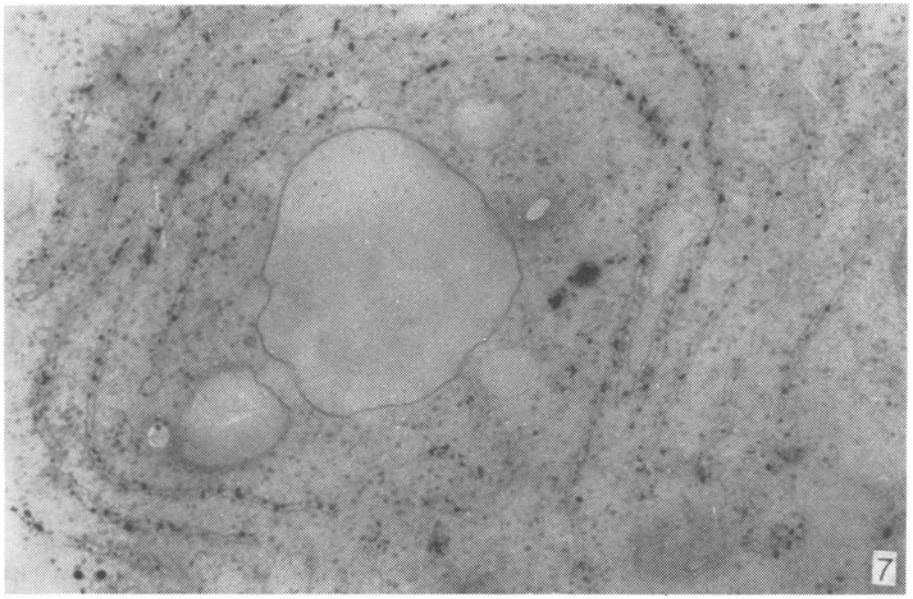


Figs. 4 and 5. Root cap cells of yellow lupin seedlings after 6 days of culture in darkness. Gluteraldehyde/OsO_4, Epon 812, CW—cell wall, ER—endoplasmic reticulum, GA—Golgi apparatus, N—nucleus, NE—nuclear envelope, P—plastid, PL—plasmalemma, V—vacuole

Fig. 4. Control cells (seedlings cultured in water). x 9 000

Fig. 5. Seedling cells treated with water $PbCl_2$ solution 5 000 ppm. Lead deposits in vacuole, ER, cell wall. x 10 000

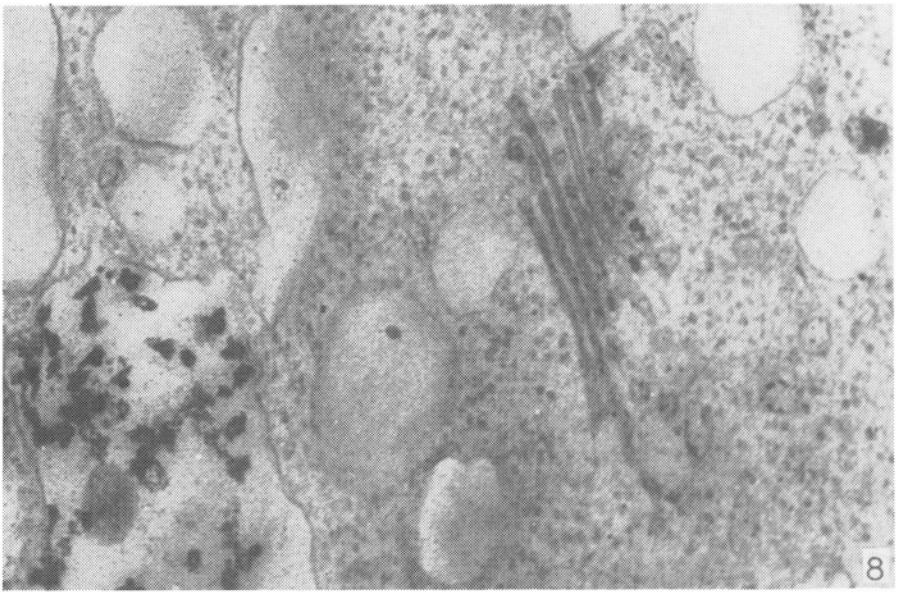


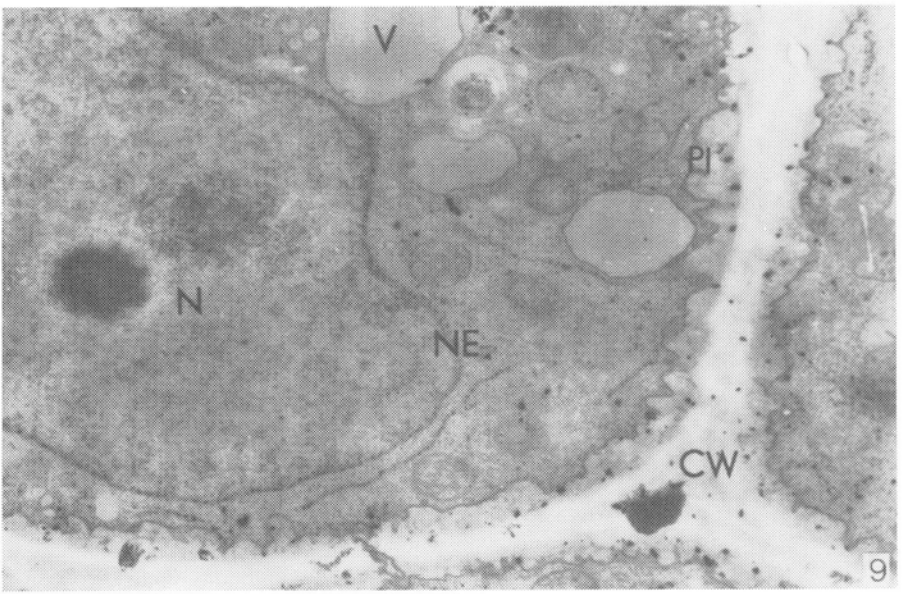


Figs. 6 and 7. Root cap cells of yellow lupin seedlings after 6 days of culture in darkness. Glutaraldehyde/OsO_4, Epon 812

Fig. 6. Fragments of cells with lead doposits in vacuole, ER, the cisterns of which surround both the vacuole and amyloplast in the dictyosome and cell wall ($PbCl_2$—5 000 ppm). x 13 000. Abbreviations as in Figs. 4-5

Fig. 7. Endoplasmic reticulum in higher magnification. ($PbCl_2$—5 000 ppm). x 36 000





Figs. 8 and 9. Root cap cells of yellow lupin seedlings after 6 days of culture in darkness. Glutaraldehyde/OsO_4, Epon 812

Fig. 8. Lead deposits present in dictyosome and one of the vacuoles ($PbCl_2$—5 000 ppm) x 50 000

Fig. 9. Minute lead grains in perinuclear space of nuclear envelope, ER and in space between cell wall and plasmalemma as well as in the wall itself. ($PbCl_2$—5 000 ppm). x 10 000. Abbreviations as in Figs. 4-5

Table 2

Anthocyanin level expressed in corrected absorption et $\lambda = 535$ nm after Mohr (1981). In each experiment 23 cotyldons were analysed

$PbCl_2$ concentrations, ppm	Cultures					Mean from 5 replications
	1	2	3	4	5	
Control	0.177	0.282	0.288	0.183	0.282	0.242
10	0.104	0.160	0.190	0.134	0.160	0.150
100	0.092	0.144	0.155	0.097	0.139	0.125
500	0.085	0.108	0.105	0.085	0.085	0.094
1000	0.020	0.029	0.041	0.031	0.020	0.028
5000	0.016	0.022	0.021	0.021	0.016	0.019

Large amounts were also found in the dictyosomes and vesicles, probably of dictyosomal origin (Figs. 6 and 8). Minute lead grains were, moreover, observed in the perinuclear spaces of the nuclear envelope and the spaces between the plasmalemma and the cell wall (Fig. 9). The walls contained only small amounts of lead deposits (Figs. 5 and 9). No traces of lead could be revealed in the mitochondria and amyloplasts, although around the latter frequently ER coils could be seen concentrically arranged and filled with lead deposits (Fig. 6).

The fact is interesting that, in spite of the considerable lead amounts found in the root cap cells, no major deviations were noted in their structure (the ER perhaps excepted) as compared with the pictures of the control cells (Fig. 4).

DISCUSSION

Investigations on lupin seed germination in the presence of $PbCl_2$ demonstrated the marked inhibitory action of lead on this process. Dilling (1926) when using 0.2 per cent lead observed even a complete loss of germination ability of mustard seeds.

Lead causes in lupin seeds a marked decrease in the cotyledon fresh weight. This effect was the more pronounced the higher was the plumbous salt concentration. With the decrease of fresh weight a shortening of the hypocotyls and roots and thickening of the latter were observed. Similar results concerning the effect of lead on the fresh weight of *Avena sativa* seedlings were obtained by Fiusello and Molinari (1973). Changes in the lenght and thickeness of roots were noted by many authors such as for instance Wilkins (1957) or Lane and Martin (1980).

Lupin cotyledones are known to intensively synthesize anthocyanin pigments (Majorek 1976). The presence of lead distinctly inhibited this synthesis. Fritsch and Grisebach (1975) suggest that at least part of

the enzymes engaged in anthocyanin synthesis are connected with the tonoplast. Weissenböck and Effertz (1974) and McClure (1975) consider plastids as responsible for this synthesis and believe the products are transported from here to the vacuoles. Steinitz and Bergfeld (1977) found that a necessary condition for anthocyaninin accumulation is the formation of a central vacuole. Unfortunately it is known at which of these stages lead exerts its inhibitory influence.

Ultrastructural location of lead demonstrated in lupin roots its presence in nearly all the cellular structures. Lead deposits were observed between the plasmalemma and cell wall and in the wall itself. Similar results are reported by Malone et al. (1974) for the cells of root tips of *Zea mays* and by Lorch and Schafer (1981) for *Phymatodocis nordstedtiana* cells. Much lead was found in lupin within the endoplasmic reticulum, dictyosomes and vesicles of dictyosomal origin. The same localisation of lead is also reported by Malone et al. (1974) in *Zea*.

Quite free of lead were in the lupin root cap cells the mitochondria and amyloplasts, this being in agreement with the results of Gullvåg et al. (1974) for instance. Walton (1973) has demonstrated that isolated animal mitochondria will accumulate lead. Similar results under *in vitro* conditions were obtained by Malone et al. (1974) for *Zea mays* root cells. These authors did not, however, successed in demonstrating lead uptake by mitochondria in *Zea* under *in vivo* conditions.

To recapitulate, it may be affirmed that the most pronounced effect of $PbCl_2$ on lupin consisted in changes within the roots and hypocotyls. Moreover, in seedlings cultured in the presence of lead general debilitation, disturbances in pigment synthesis and frequent necrotic changes were observed.

Accumulation of lead in the ER and in the secretory system connected with the latter—the Golgi apparatus—indicate no doubt the primary stage of binding of this metal in the protoplast. Considerable deposits in the vacuole and intercellular spaces may be the result of the process of lead excretion beyond the living protoplast. Only further detailed investigations with treatment at various dates with lead and various times of postincubation of material freee of lead may give a continuous sequence of ultrastructural pictures illustrating the process of penetration, excretion and accumulation of Pb in the cell.

REFERENCES

Ahlf W., Irmer U., Weber A., 1980. Über die Anreicherung von Blei durch Süsswassergrünalgen unter Berücksichtigung verschiedener Aussenfaktoren. Z. Pflanzenphysiol. 100: 197-207.

Bonnet E., 1922. Action des sels solubles de plomb sur les plantes. Compt. Reut. 174: 488-491.

Dilling T. W., 1926. Influence of lead and the metalic ions of copper, zinc, thorium, beryllium and thallium on the germination of seeds. Ann. Appl. Biol. 13: 160-167.

Fiusello N., Molinari M. T., 1973. Effects of lead on plant growth. Allonia 19: 89-96.
Foy C. W., Chaney R. L., White M. C., 1978. The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol. 28: 511-566.
Fritsch H., Grisebach H., 1975. Biosynthesis of cyanidin in cell cultures of *Haplopappus gracilis*. Phytochem. 14: 2437-2442.
Garland C. J., Wilkins D. A., 1981. Effect of calcium on the uptake and toxicity of lead in *Hordeum vulgare* L. and *Festuca ovina* L. New Phytol. 87: 581-593.
Griffith J. J., 1919. Influence of mines upon land and livestock in Cardiganshire. J. Agric. Sci. 9: 366.
Gullvåg B. M., Skaar H., Ophus E. M., 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.
Krupińska I., 1981. Działanie związków ołowiu na rozwój mchów w wraunkach hodowli *in vitro*. Ph. D. Thesis, Warsaw University.
Lane S. D., Martin E. S., 1980. An evaluation of the effect of lead on the gross morphology of *Raphanus sativus*. Z. Pflanzenphysiol. 98: 437-452.
Lorch D. W., Schäfer H., 1981. Laser microprobe analysis of the intracellular distribution of lead in artificially exposed cultures of *Phymatodocis* (*Chlorophyta*). Z. Pflanzenphysiol. 101: 183-188.
Majorek L., 1976. Wpływ kinetyny i antybiotyków na syntezę chlorofilu i antocyjanów w izolowanych liścieniach łubinu. Mickiewicz University, Poznań.
Malone C., Koeppe D. E., Miller R. J., 1974. Localization of lead accumulated by corn plant. Plant Physiol. 53: 388-394.
McClure J. W., 1975. Physiology and function of flavonoids. In: The flavonoids. Harbone J. B., Marby T. J., Marby H. (eds.). Chapman and Hall. London. pp. 970-1055.
Mohr L. S., 1981. Analysis of phytochromediated anthocyanin. Plant Physiol. 47: 649-655.
Röderer G., 1979. Hemmung der Cytokinese und Bildung von Riesenzellen bei *Poterioochromonas malhamensis* durch organische Bleiverbindungen und andere Agenzien. Protoplasma 99: 39-51.
Simola L. K., 1977. The tolerance of *Sphagnum fimbriatum* towards lead and cadmium. Ann. Bot. Fennici. 14: 1-5.
Steinitz B., Bergfeld R., 1977. Pattern formation underlying phytochrommediated anthocyanin synthesis in the cotyledons of *Sinapis alba* L. Planta 133: 229-235.
Świeboda M., 1976. The use of biological tests for establishing the influence of the dust from lead and zink works on plant development. Acta Soc. Bot. Pol. 45: 17-31.
Walton J. R., 1973. Granules containing lead in isolated mitochondria. Nature 243: 100-101.
Weissenböck G., Effertz B., 1974. Entwicklungs und Lichtabhängige Akkumulation von C-Glycosylflovonone im Haferkeimling (*Avena sativa* L.). Z. Pflanzenphysiol. 74: 298-326.
Wilkins D. A., 1957. A technique for the measurement of lead tolerance in plants. Nature 180: 37-38.

Rozwój siewki łubinu w obecności ołowiu oraz jego ultrastrukturalna lokalizacja w korzeniach

Streszczenie

Badano wpływ ołowiu na wczesne etapy siewek rozwoju siewek łubinu żółtego. W obecności tego metalu stwierdzono wyraźne zmniejszenie liczby kiełkujących nasion, skrócenie hypokotyli i korzeni, a także wyraźnie mniejszą świeżą masę i zawartość antocyjanów w liścieniach. W komórkach czapeczki korzeniowej ołów jest obecny w wakuolach, ER, diktiosomach, otocze jądrowej i ścianach komórkowych.