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The Effect of Lead on Early Stages of *Phaseolus vulgaris* L. Growth *in vitro* Conditions

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Abstract. Lead chloride (10^{-5} M) inhibited the growth of the main root, the duration of development, the number and growth of lateral roots, primary and trifoliate leaves, and also the mitotic index in root apical meristems. Lead strongly inhibited root growth rate, mainly by reducing the number of dividing cells. Other mechanisms of this inhibition are discussed.

Lead is one of the most important metals that pollute the natural environment due to man's impact. It is widespread especially in the urban environment. Its permanent presence in the atmosphere, water and food may be poisonous to people (Mahaffey 1977). Lead is absorbed by plants and causes many destructive changes in them, if present in sufficiently large quantities in the tissue (*e. g.* Baumhardt and Welch 1972, Mukherji and Maitra 1976, Woolhouse 1983, Burzyński 1987, Stiborová *et al.* 1987, Woźny 1987), which changes are reflected in plant morphology.

This morphological study has been undertaken since relatively little is known about the effect of lead on the morphology of organs, at early stages of growth. It also attempts to explain the cause of the inhibitory effect of lead on root growth.

MATERIAL AND METHODS

Beans (*Phaseolus vulgaris* L. cv. Saxa Aurea) were used for experiments. Seeds displaying identical morphologic characteristics were surface sterilized with 1 % calcium hypochlorite solution for 10 min, rinsed with distilled water and placed in lignin-lined Petr dishes. The lignin was soaked in:

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(1) Hogland's fluid medium containing microconstituents after Arnon and ferric citrate (Hewitt 1966): the control medium,
(2) a medium like under (1) with added lead chloride $PbCl_2$, at an adequate concentration.

Values of pH of the media were adjusted to 5.0. After 96 h of germination in the dark at the temperature of 19 ± 2 °C, only germinated seeds that had 12 mm long roots were further tested. They were placed in Erlenmeyer flasks 100 ml in volume, filled with 110 ml of Hogland's medium containing microconstituents after Arnon (Hoagland and Arnon 1950) and ferric citrate – the control medium (Hewitt 1966) or 110 ml of the same medium with the addition of $PbCl_2$. Cardboard funnels were placed in the flask necks in order to allow the right immersion of plants in the medium. Only the roots were immersed. The culture was grown in a growth room, under continuous illumination of *ca.* 4 300 lux at the temperature of 25 ±3 °C and the relative humidity of 50 per cent.

Initial experiments were conducted with several lead concentrations, from 5.10^{-7} to $1.5.10^{-5}$ M, in order to establish the concentration at which the increase in root length was about 50 per cent slower after 48 h growth in culture than in the control medium (RG_{50}). The index of tolerance was used to find out the desirable concentration (Wilkins 1957). It is given by the following formula:

$$IT = \frac{\text{average root length increase in Pb}^{2}\text{-containing medium}}{\text{average root length increase in control medium}} \times 100$$

The Effect of Lead on Morphology

Germinated seeds with 12 mm long roots were placed in Hogland's medium (the control medium) and the medium containing extra lead at the concentration of 10^{-5} M (RG_{50}). The culture was maintained for three weeks. Every week the medium was replaced by a fresh one. After 1, 3, 5, 12, 16 and 21 d of culture, the length of the main root was measured. Information was supplied on their number and shape, the time at which lateral roots appeared, the colour of the main root and lateral ones, root thickness, and the time of appearance of primary and trifoliate leaves. After 5, 7 and 12 d of culture (primary leaves) and 12 and 21 d (trifoliate leaves), measurements were made of the surface area of leaf blades, with the accuracy of 1 mm^2.

The Effect of Lead on Cell Division

The culture of germinated seeds was grown on Hogland's medium (the control medium) and the culture with the addition of $PbCl_2$ at the Pb concentration of 10^{-5} M in the growth room for 48 h. After 24 and 48 h 1–2 cm long root tips were taken and fixed in Carnoy's fixative. For making slides the root tips were macerated

in 1 M HCl (10 min, 60 °C) and then rinsed in distilled water. The macerated root tips were directly squashed in a cetocarmine. The mitotic index (MI = the percentage of dividing nuclei) was checked in the region of 0 to 2 mm from the apex..The MI was determined in 5 fields of view, *i. e.* about 1000 cells. Moreover, the diameter of nuclei and the surface area of cells were measured in an OK 15 KM (PZO) micrometric eyepiece and the Reichert light microscope. All the experiments were repeated three times.

RESULTS

The aim of initial experiments was to define the effect that various concentrations of lead had on the root growth and to determine the lead concentration that reduced the root growth to half that of the control (RG_{50}) after 48 h of culture growth (Table 1). The presence of lead at 10^{-5} M in the medium caused a reduction of the main (Table 2) and lateral (Table 3) roots, as well as the thickening of the main root. After

TABLE 1

The effect of different concentrations of lead on the root growth in cm (the mean of 3 assays on 30 plants ± standard deviation)

Lead concentr. [M]	Root length after 96 h of seed germination on the control medium (initial material)	Root length increase after 48 h of growth on the lead-containing medium	Index of tolerance [%]
0	1.2 ± 0.1	2.8 ± 0.2	100
5×10^{-7}	1.2 ± 0.2	2.2 ± 0.5	85
10^{-6}	1.2 ± 0.2	1.9 ± 0.6	77.5
5×10^{-6}	1.2 ± 0.3	1.3 ± 0.4	62.5
10^{-5}	1.2 ± 0.1	1.0 ± 0.1	55
1.5×10^{-5}	1.2 ± 0.3	0.9 ± 0.3	42.5

TABLE 2

The effect of lead on the main root length in cm (the mean of 3 assays on 30 plants ± standard deviation)

Pb concentr. [M] / Days	0	1	3	5	12	16	21
0	1.2 ± 0.1	2.6 ± 0.5	5.4 ± 0.8	11.0 ± 1.3	12.5 ± 1.0	13.7 ± 0.8	14.6 ± 0.5
10^{-5}	1.2 ± 0.1	1.8 ± 0.2	2.6 ± 0.4	6.5 ± 1.2	7.0 ± 0.9	7.2 ± 0.3	7.4 ± 0.2

TABLE 3

The effect of lead on the lateral root length in cm (the mean of 3 assays on 30 plants ± standard deviation)

Pb concentr. [M] \ Days	3	5	16	21
0	7.0 ± 1.1	8.0 ± 0.9	10.0 ± 0.8	11.0 ± 1.0
10^{-5}	0.6 ± 0.2	2.0 ± 0.5	4.0 ± 0.7	4.0 ± 0.7

as early as 24 h of culture the circumference of the root in the transition region was larger than in the control medium. Thus symptom prevailed throughout the experiment (Table 4). On the second day of culture the root tips of plants grown on the lead containing medium became brown. After the next two days, the whole root systems turned brown.

Lateral roots developed on the second day of culture. The number of lateral roots of plants grown on the lead-containing medium was smaller, compared with the control. After three days of culture the main root had 7 ± 2 lateral roots (the control material 11 ± 3), whereas it had 19 ± 4 and 50 ± 8 lateral roots, respectively, after five days. Besides, the lateral roots of seedlings grown on the lead-containing medium were shorter than the control roots. No changes were observed in the appearance of cotyledons of plants grown on the lead-containing medium. Cotyledons of the control and lead-treated plants became dry on the 5th or 6th day of culture and fell off between the 6th and 9th days of plant growth.

Primary leaves developed on the third day of culture. Out of 30 specimens, 9 control and 4 lead-treated plants had primary leaves. After four days primary leaves formed in all the specimens.

TABLE 4

The effect of lead on the main root circumference in cm (measurement in the transition region; the mean of 3 assays on 30 plants ± standard deviation)

Pb concentr. [M] \ Days	0	1	3	5	12	16	21
0	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.7 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
10^{-5}	0.2 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.9 ± 0.2	1.0 ± 0.1	1.2 ± 0.1	1.2 ± 0.1

Trifoliate leaves appeared on the 7th day of culture. Out of 30 plants grown on the lead-containing medium, nine had trifoliate leaves, whereas fourteen out of the same number of the control plants possessed them. On the 9th day of culture trifoliate leaves were formed in all plants. The surface area of the blades of primary and trifoliate leaves was smaller in the lead-treated plants than in the control ones. Lead inhibited an increase in the blade area more intensely in the primary leaves than the trifoliate ones. The greatest difference was recorded on the 7th day. The surface area of the primary lead blades in the lead-treated plants was then about 11 ± 3 cm^2 (50 per cent) smaller than that of the control leaf blades (Figs. 1A, 1, 2). As far as similar trifoliate leaves are concerned, differences in the blade surface area were considerably less serious (Figs. 1B, 4, 5.) No changes occurred in the colour of leaves and stems of plants grown on the lead-containing medium.

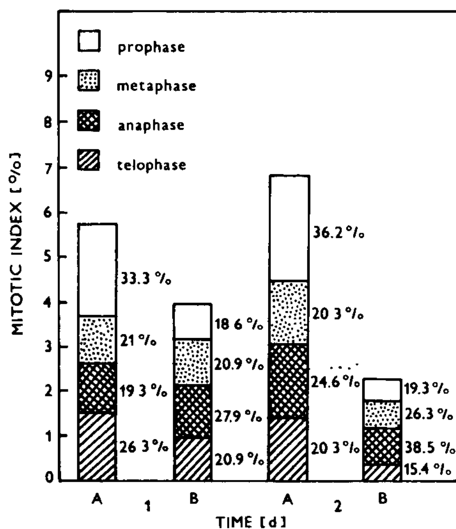

Fig. 1. The effect of lead on leaf blade surface area in cm^2 (the mean for 30 blades ± standard deviation). – A: Primary leaves after 5, 7 and 12 d of culture, B: trifoliate leaves after 12 and 21 d of culture.

After 24 and 48 h of culture growth, the mitotic index (MI) was estimated for root apical meristems of the plants grown on the lead-containing medium and the control ones by the use of the squashed material method. Values of MI decreased under the influence of lead. After 48 h they were 62.3 per cent lower than those of the control meristems (Fig. 6). The percentage of cells at particular stages of mitosis was also calculated. Under the influence of lead the number of prophases and telophases became reduced, whereas that of metaphases increased (Fig. 6). At the lead concentration employed, *i. e.* 10^{-5} M, neither disturbance in the morphology of chromosomes nor their abnormal divisions were observable. Despite the earlier data on lupin (PRZYMUSIŃSKI and WOŹNY 1985), it has been established in this study that

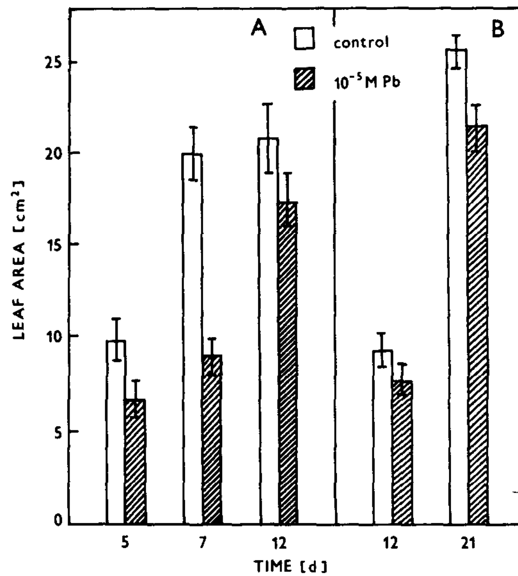

Fig. 6. The effect of lead on the mitotic index (MI) for apical meristem cells (about 1000 on the average) in control (A) and 10^{-5} M lead-treated (B) roots after 24 (1) and 48 (2) hours of culture. Numbers on the right of histograms indicate the percentage of particular phases of mitosis.

the size of meristem cells and nuclei of the lead-treated material is nearly identical with that of the control ones.

DISCUSSION

The results indicate that lead inhibited largely growth at the concentration of 10^{-5} M. This effect was most rapidly and severely demonstrated by the inhibition of root growth. Less severe influence of lead on the stem was most likely due to its weak mobility and hence, lower lead contents of the above-ground organs. For instance, there was a nearly linear rise in the lead contents of pea (*Pisum sativum* L.) stems as the metal concentration in the medium increased. Simultaneously, the lead contents of roots reached their maximum at the lowest concentration of lead in the medium and several times exceeded those of stems (Poskuta *et al.* 1987). As the relationship between lead contents of the stem and those of the root was a linear function of lead concentration in the medium, the root should be considered to be the main lead storage portion (Poskuta *et al.* 1987).

The mechanism of growth inhibition by lead is not known in detail. However, the avaiable evidence suggests that one of the ways of growth inhibition involves a decrease in the number of dividing cells under the influence of lead. After 48 h of bean culture growth on the lead-containing medium, the mitotic index (MI) was over 60 per cent lower than that for the control meristems. Mitosis is directed by

microtubule. It has been postulated that lead compounds impair microtubule organization causing effects qualitatively identical with those of colchicine (Röderer 1979, 1986). Röderer (1986) in the unicellular alga *Poterioochromonas malhamensis* stated that the selective interaction of triethyl lead (organolead) with mitotic and cytoplasmic microtubules is the primary cause of the selective inhibition of mitosis and cytokinesis. The lead-induced inhibition of mitotic divisions has been described for other plants as well (Hammett 1928, Ahlberg *et al.* 1972, Świeboda 1976, Wierzbicka 1984, 1988, Przymusiński and Woźny 1985, 1987, Romaniuk *et al.* 1986). A reduction in growth is likely to be not only the result of a decrease in the number of mitosis processes. Large amounts of lead are accumulated in the cell wall components, especially pectic substances and hemicelluloses (Lane *et al.* 1978, Lorch 1978 quoted after Lorch and Schäfer 1981, Książek *et al.* 1984). As a consequence, as well as because of a lead-induced decrease in IAA quantities (Mukherji and Maitra 1977), the walls become less extensible (Lane and Martin 1980, Burzyński and Jakób 1983). A lead-induced decrease in water intake (Burzyński 1987a) results in severe disturbance in cell elongation. Of some importance for growth is also a lead-induced reduction in the absorption of some elements by roots, including phosphorus, calcium, potassium and iron (Misra and Pandy 1974, Maene *et al.* 1977, Burzyński 1987b). However, the study of this reduction has yet to be continued.

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Figs. 2–5 at the end of the issue.