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Cell division in *Lemna minor* roots treated with lead

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Abstract

Treatment of *Lemna minor* L. roots with 15 μM Pb^{2+} supplied as $Pb(NO_3)_2$ in 50-fold diluted Wang medium caused a progressive reduction in mitotic activity in the root tip. The percentage of dividing nuclei after 1, 6, 12 and 12 h of lead treatment was 6.25, 4.4, 3.4 and 0.3, respectively as compared to 7.1–7.7% in the control. After 6 h of lead treatment the number of cells in metaphase and anaphase was reduced by four- and nine-fold, respectively and after 12 h these phases were not detected. There were 3- and 10-fold fewer cells in telophase after 6 and 24 h, while those in prophase were reduced only in the 24 h treatment (a 30-fold reduction). These effects were associated with an increase in the number of cells exhibiting disturbances including lagging chromosomes, chromosome bridges, micronuclei, and nuclei with more condensed chromatin. The formation of micronuclei in root cells of *L. minor* cells at a very low dose of lead indicates that roots of this aquatic plant may be more sensitive to lead than those of terrestrial plants.

Keywords: Cell division; Lead; Root; Duckweed; *Lemna minor* L.

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1. Introduction

Lead is one of the most important heavy metals responsible for anthropogenic pollution of the environment. Under normal conditions the concentration of lead in water ranges from 0.003 to 0.6 μM (Demayo et al., 1982), though concentrations as high as 10 μM (in the river sediments even 48 M) have been reported (Ciszewski, 1998). Uptake of this metal can cause destructive changes in plants, in particular, inhibition of root growth, which is considered to be one of the earliest morphological effects of metal toxicity (Breckle, 1991). Disturbances in cell division, such as C-mitosis, formation of chromosome bridges, micronuclei and chromosome stickiness, are among reasons cited for inhibited root growth (Przymusiński and Woźny, 1985; Wierzbicka, 1988, 1995; Woźny and Jerczyńska, 1991; Ivanov et al., 1998; Jiang and Liu, 1999, 2000; Liu et al., 2000; El-Ghamery et al., 2003). These problems have been relatively well studied in terrestrial plants, but little is known about the influence of lead on cell division in aquatic vascular plants. The aim of this study was to trace the effect of lead on successive stages of cell division in root tips of the aquatic plant *Lemna minor* L.

2. Materials and methods

2.1. Plant material and treatments

L. minor L. was cultured on a liquid medium according to Wang (1990), at pH 5.7, 23 ± 1 °C, and constant irradiance (66 μmol m^{-2} s^{-1}). For the experiments, only young individuals with roots about 2.5 mm long were transferred to 60 mL of 50-fold diluted Wang medium containing 15 μM Pb^{2+} as lead nitrate. The floating plants were incubated in this liquid medium for 1, 6, 12 or 24 h. Control plants were kept for the same period in 60 mL of 50-fold diluted Wang medium.

The doses used were based on the results of our earlier experiments (Samardakiewicz and Woźny, 2000). We demonstrated that 15 μM was the concentration of lead at which root length was reduced by 50% relative to controls after 12 h of incubation.

2.2. Mitotic index

The mitotic index, which represents the percentage of dividing nuclei, was estimated for the apical root meristem of control and lead-treated plants. Root tips (1.5 mm long) were excised, fixed in Carnoy's fixative, macerated in 1N HCl (15 min, 60 °C), and rinsed in distilled water. Macerated root tips were stained with acetocarmine and squashed directly on glass slides. Specimens were observed with a light microscope and photographs taken with Fujicolor 100 film.

2.3. Data analysis

The experiment was conducted in three series. The mitotic index was determined in six fields of view with about 1200 cells. The significant differences were assessed by the Student's *t*-test (for $p < 0.05$).

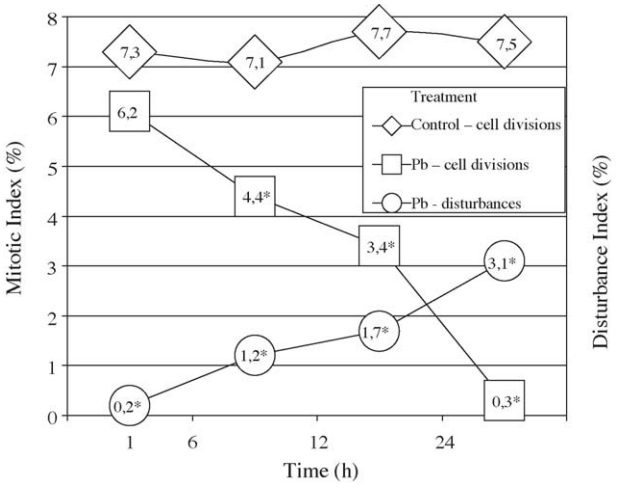

Fig. 1. The percentage of dividing nuclei (mitotic index) and cells with disturbances (disturbance index) in apical meristem of *L. minor* roots: control (control-cell division) and lead-treated plants (Pb-cell divisions, Pb-disturbances), $^{*}p \leq 0.005$.

3. Results

3.1. Control plants

The mitotic index values of root tip meristem cells in plants transferred from complete Wang medium to 50-fold diluted medium and incubated for 1, 6, 12 and 24 h, remained at a similar level and the differences were not statistically significant (Fig. 1). Irrespective of

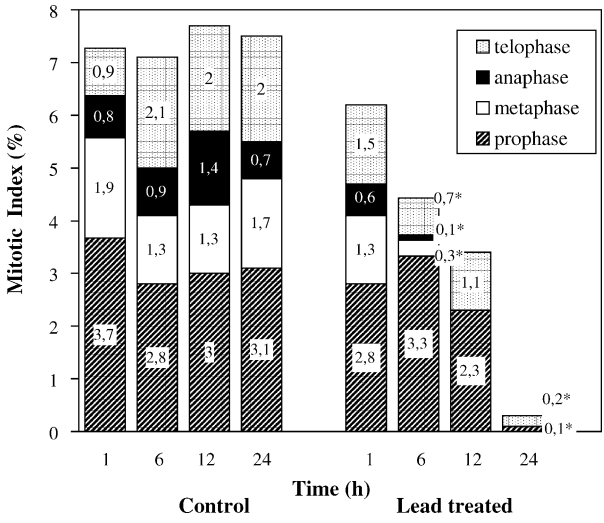

Fig. 2. The percentage of various phases of mitosis in the apical meristem of *L. minor* roots with and without treatment with lead, $^{*}p \leq 0.005$.

the duration of the incubation period, prophase dominated root tip meristem cells (Fig. 2). Metaphase ranked second after 1 h, while telophase ranked second after 6 h and later. Anaphase was usually the least frequent (except after 12 h, when metaphase ranked last).

3.2. Lead-treated plants

The number of mitotic divisions in roots of lead-treated plants at all times was lower than those of the control, except after 1 h when the difference was not significant, and decreased as the time of lead treatment was prolonged. After 24 h cell divisions were nearly completely inhibited.

After 1 h of lead treatment numbers of cells in the different phases, except telophase, were slightly lower in lead-treated plants than in the control (Fig. 2). After 6 h of incubation with lead, the number of cells in all phases, except prophase, were significantly lower than in the control. After 12 h a characteristic difference between lead-treated and control plants was the lack of metaphase and anaphase. Numbers of cells in the other phases appeared lower than in the control, but the differences were not statistically significant, and prophase was more frequent than telophases, as in the control. After 24 h differences between lead-treated and control plants were the most distinct. Prophase and telophase were respectively 30 and 10 times less frequent than in the control, while the other phases were not detected at all. In contrast to control and lead-treated plants after 1, 6 and 12 h, cells in telophase were more frequent than those in prophase only after 24 h of lead treatment (Fig. 2).

As the mitotic index values declined in lead-treated plants, the number of cells with disturbances grew with increasing duration of the treatment (Fig. 1). After 1 h their number

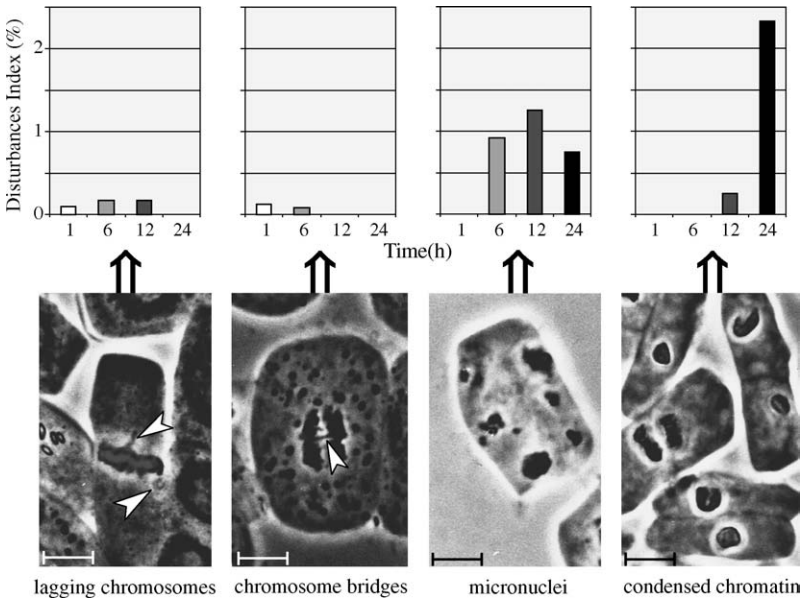

Fig. 3. The percentage of cells with disturbances in apical meristem of *L. minor* roots after lead treatment. Bar represents 3 μm.

was low and the disturbances included nearly exclusively lagging chromosomes and chromosome bridges (Fig. 3). After 6 h the number of cells with disturbances was several times higher (Fig. 1). Beside lagging chromosomes and chromosome bridges (still infrequent), micronuclei were characteristic of that time (Fig. 3). After 12 h of lead exposure a further increase in the number of cells with disturbances was observed; they were nearly 1.5 times more frequent than after 6 h (Fig. 3). Apart from micronuclei and lagging chromosomes, interphase nuclei with more condensed chromatin were also noticed. After 24 h such nuclei were the dominant type of disturbance (Fig. 3). The total number of cells with disturbances was about twice as high at 24 h as compared to 12 h (Fig. 1).

4. Discussion

The inhibitory effect of lead on root growth has often been reported in terrestrial plants. It has been attributed to several factors, including a lowering of mitotic activity (Hamett, 1928; Przymusiński and Woźny, 1985; Wierzbicka, 1988, 1995; Woźny and Jerczyńska, 1991; Ivanov et al., 1998; Jiang and Liu, 1999, 2000; Liu et al., 2000). Our results indicate that lead causes inhibition of cell division in *L. minor* roots, as well. The data suggest that in the first hours of lead treatment, mitosis in *L. minor* is inhibited probably after prophase, since there was a significant reduction in the number of cells in metaphase and anaphase. Many events essential for mitosis take place during prophase. One of these is the organization of microtubules of the mitotic spindle. It has been postulated that lead compounds impair microtubule organization, causing effects qualitatively similar to those of colchicine (Röderer, 1986; Wierzbicka, 1988, 1995; Eun et al., 2000). In *L. minor* the disturbances observed may also have been caused by lead-induced changes of these microtubules, since some of the disturbances are similar to the effects of colchicine treatment, for example, condensed chromatin. In addition, results of research on the influence of lead on the microtubule cytoskeleton in *L. minor* root cells confirm this hypothesis (Samardakiewicz, 2000). Disturbances observed in the latter study include diffusive fluorescence of tubulin, which may be a symptom of microtubule depolymerization, and changes in microtubule configuration in the preprophase band and the phragmoplast. These disturbances may indicate that lead affects cell divisions not only through changes in microtubule configuration but also through loss of their stability. However, disturbances in microtubules detected in *Zea mays* suggest that lead exerts a stronger influence on malfunction of microtubule organization rather than on polymerization (Eun et al., 2000).

Apparently, all disturbances in cell divisions may be explained by dysfunction of the cytoskeleton. An example may be the lead-induced formation of micronuclei. The presence of the latter has been explained either as the final result of C-mitoses, chromosome bridges (as a consequence of disturbances of the spindle apparatus) or the chromosome breakage and extraction into the cytoplasm (Wierzbicka, 1988; Fenech, 2000; Krishna and Hayashi, 2000; Uhl et al., 2003). However, in *L. minor* only chromosome bridges, but not all stages of C-mitoses were observed. Thus, the formation of micronuclei in some other way cannot be excluded. It is also possible that the micronuclei in *L. minor*

may represent nuclear material extruded from the nucleus into the cytoplasm, similar to the nuclear material in the cytoplasm of *Z. mays* cells treated by lead (Eun et al., 2000). Verification of this, in the case of *L. minor*, requires further detailed studies.

Formation of micronuclei in plant cells has recently been used as a bio-indicator of lead contamination in plants (Knasmüller et al., 1998; Steinkellner et al., 1998; Majer et al., 2002). In *L. minor* cells micronuclei were observed at a very low concentration of lead (15 μM) and after a short incubation (6 h). This indicates that roots of this aquatic plant are more sensitive to lead compared to the roots of terrestrial species, such as *Vicia*, *Tradescantia* and *Zea*, in which micronuclei were induced at lead concentrations of 4 mM, 1 mM and 200 μM, respectively (Steinkellner et al., 1998; Eun et al., 2000). In conclusion, the aquatic plant *L. minor* seems to be more suitable than the above-mentioned terrestrial plants for bio-monitoring lead since it is more sensitive to lead and requires very short treatment periods. In addition, the micronuclear bioassay with *Lemna* is relatively easy and inexpensive.

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