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EFFECTS OF LEAD ON ROOT GROWTH, CELL DIVISION, AND NUCLEOLUS OF *ALLIUM CEPA*

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Abstract

The effects of different concentrations of lead nitrate on root growth, cell division, chromosome morphology and nucleolus in root tip cells of Allium cepa *were studied. The concentrations of lead nitrate ($Pb(NO_3)_2$) were 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} M. It was observed that lead reduces root growth and causes mitotic irregularities, including c-mitosis, anaphase bridges, and chromosome stickiness. Also, interphase cells with micronuclei, irregularly shaped nuclei and nuclei with decomposed nuclear material were noticed. The c-mitotic effect reached its maximum in the meristem at above 10^{-4} M Pb, when practically almost all the anomalous dividing cells are of this type. After treatment with Pb, there were many similar silver-stained particulate materials scattered in the nucleus in short, rounded meristem cells and in long, oblong root cap cells.*

INTRODUCTION

Lead (Pb) exists in many forms in natural sources throughout the world. The content of Pb in soil is 15–17 ppm (Dou, 1988). Generally speaking, when organic matter and other mineral nutrients are in abundant supply then Pb toxicity does not appear (Baumhardt & Welch, 1972). Lead toxicity occurs most commonly on waste heaps from mining operations where the organic matter and nutrient content of the soil are low. If a large amount of Pb is accumulated in plants and crops, it will probably poison them seriously. There are reports on Pb poisoning of humans (Wan *et al.*, 1991), soil (Yang *et al.*, 1982; Zhang *et al.*, 1982; Xie *et al.*, 1987), and crops (Dou & Hu, 1987; Radecki *et al.*, 1989). Many studies on the effects of lead on the cytological effects of *Allium cepa* L. have been published (e.g. Levan, 1945; Ahlberg *et al.*, 1972; Ramel, 1973; Lerda, 1992).

This study is aimed at increasing our understanding of the effects of different concentrations of Pb^{2+} on the root growth, cell division, and nucleolus of *Allium cepa*.

MATERIALS AND METHODS

Healthy and equal-sized bulbs were chosen from a population of the common onion *Allium cepa* L. ($2n = 16$). The bulbs had not started formation of green leaves or root growth. Before starting the experiment, the dry scales of the bulbs and the brownish bottom plate were removed and the ring of the root primordia was left intact. Twelve onions were the starting material in each series, and the best 10 onions were selected for testing (Fiskesjö, 1988).

The concentrations of lead nitrate ($Pb(NO_3)_2$) were 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} M, which were prepared in tap water. The bulbs were placed directly in the test liquids which were changed every 24 h. The bulbs were allowed to germinate roots in beakers at a room temperature of about 20°C for 24, 48, 72 and 96 h. The roots were protected from direct sunlight. Macroscopic observations were made at the end of each time interval. For the observation of chromosomal morphology, some roots were fixed in Carnoy's reagent, followed by squashing in Carbol Fuchsin solution (Li, 1982), and for the observation of nucleolus change, some were fixed in 3 parts 95% ethanol : 2 parts acetic acid for 4–5 h and hydrolysed in 5 parts 1 M hydrochloric acid : 3 parts 95% ethanol : 2 parts acetic acid for 5 min at 60°C, followed by squashing in 45% acetic acid, drying, and on day 2 staining with silver nitrate (Li *et al.*, 1990). Some of the slides were also stained with Methylene Blue after the silver staining.

RESULTS

Macroscopic effects of Pb^{2+} on root growth

The effect of Pb^{2+} on root growth of *Allium cepa* varied with the different concentrations of lead nitrate solutions used (Figs 1 and 2(a)). The rate of root growth per day decreased with increasing duration of the treatment and increasing Pb^{2+} concentration up to 10^{-5} M Pb. At higher concentrations (10^{-4}–10^{-2} M Pb), the inhibitory effect was strong, and slight or no root growth occurred after 24 h treatment.

Also, the effects of Pb^{2+} on the morphology of the roots varied depending on concentration. After exposure to 10^{-7}–10^{-6} M Pb, the morphology of the roots was nearly normal during the whole treatment (4 days). At 10^{-5} M, the roots showed a twisted appearance after 48 h of treatment, and the roots continued to be twisted during the longer treatments. At 10^{-4}–10^{-3} M, the roots were slightly brownish or blackish, stunted

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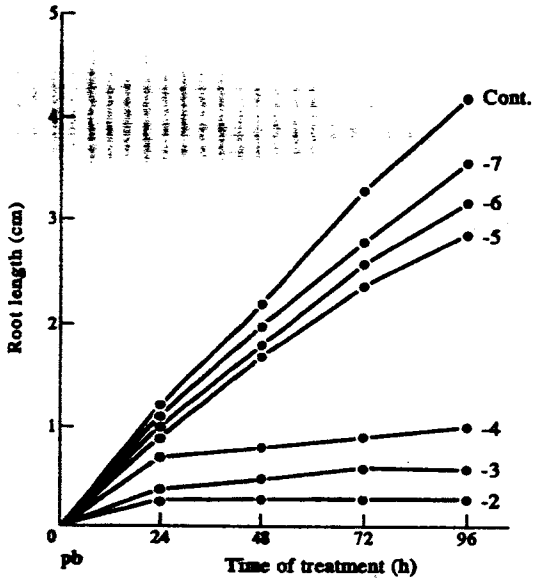

Fig. 1. Effects of different concentrations (10^{-7}–10^{-2} M) of lead nitrate on root growth of *Allium cepa*.

and bent in various directions (Fig. 2(b)) after 48 h of treatment, and at 10^{-2} M, the roots were brownish and blackish, rod-like in appearance and rotten after 48 h treatment (Fig. 2(c)).

Microscopic effects of Pb^{2+} on cell division

Mitotic index

The mitotic index reflects the frequency of cell division and is regarded as an important parameter in evaluating the rate of root growth. This investigation showed that the mitotic index decreased with increased Pb concentration and treatment duration (Table 1, Fig. 1). This fits in well with the above mentioned effects of lead nitrate on root growth.

Effects on chromosome morphology

According to the standard types of aberrant chromosome behaviour referred to in the modified *Allium cepa*, introduced by Fiskesjö (1985). Pb induced certain general toxic effects on the chromosomes.

c-mitosis. Colchicine mitosis was first described by Levan (1938) in the root tip mitosis of *Allium cepa* as an inactivation of the spindle followed by random scattering of the condensed chromosomes. c-mitoses were noted in all treated roots at Pb concentrations from 10^{-7} to 10^{-2} M. As can be seen from Table 1, the frequency of cells with c-mitoses is variable. At 10^{-7}–10^{-5} M Pb, the frequency of cells with c-mitoses increases progressly, but at above 10^{-4} M, this frequency increase sharply with a decrease in the normal dividing cells (metaphases and anaphases) after 48 h of treatment (Fig. 3(a)).

Chromosome bridges. Anaphase bridges involving one or more chromosomes (Fig. 3(b)) were observed after the treatments with Pb^{2+}, and sparse fragments were found. The frequency of cells with chromosome bridges was variable (Table 1).

Chromosome stickiness. Sticky chromosomes were observed in the treated roots at Pb concentrations from 10^{-6} to 10^{-2}. Almost all of the sticky chromosomes exhibit c-mitosis (Fig. 3(c)). The frequency of cells with chromosome stickiness progressively increases with increasing lead nitrate concentration and duration of treatment.

Effects on nuclei in interphase. In addition to the aberrations mentioned above, interphase cells with micronuclei were observed after 48 h of treatment with 10^{-6}–10^{-2} M lead nitrate (Fig. 3(d)). Also, irregularly shaped nuclei were found after 48 h of treatment with 10^{-4}–10^{-2} M Pb (Fig. 3(e)). Furthermore, with membranes unbroken, nuclei in which nuclear material was decomposed into particulates were noticed after 96 h treatment with 10^{-2} M Pb (Fig. 3(f)).

Effects of nucleoli in root tip cells

Normally, the diploid nucleus of *Allium cepa* contains one or two nucleoli (Fig. 3(g)). After 48 h of treatment with 10^{-3}–10^{-2} M Pb and 96 h of treatment with 10^{-4} M, some similar silver-stained particulate material was scattered in the nucleoli in short, rounded meristem

Table 1. Effects of lead nitrate ($Pb(NO_3)_2$) on roots of *Allium cepa*[a]

Time (h)	Treatment (M)	Root length per 24 h (cm, mean)	Mitotic index	Number of cells	Normal dividing cells (%)			Anomalous dividing cells (%)			Anomalous mitoses (%)
					Prophases	Metaphases	Anaphases	c-mitosis	Chromosome bridges	Chromosome stickiness	
48	10^{-2}		5	91	17·6			60·4		22·0	82·4
	10^{-3}	0·2	13	509	30·8			68·4		0·8	69·2
	10^{-4}	0·2	20	1 000	35·1	7·7	5·7	50·6	0·6	0·3	51·5
	10^{-5}	0·8	31	1 000	44·4	31·0	21·6	1·0	1·8	0·2	3·0
	10^{-6}	0·8	32	1 000	38·2	35·8	24·0	0·5	1·4	0·1	2·0
	10^{-7}	0·9	33	1 000	45·2	31·1	23·0	0·2	0·5		0·7
	Control	1·0	39	1 000	38·5	38·0	23·3	0·1	0·1		0·2
96	10^{-2}		0	0							
	10^{-3}	0·1	3	102	11·8			28·4		59·8	88·2
	10^{-4}	0·1	9	673	38·8	2·5	4·2	44·3	0·1	10·1	54·5
	10^{-5}	0·5	27	1 000	45·4	30·4	18·4	2·7	1·4	1·7	5·8
	10^{-6}	0·6	30	1 000	41·1	38·5	17·1	0·7	1·7	0·9	3·3
	10^{-7}	0·8	32	1 000	42·4	40·3	16·0	0·2	0·7	0·4	1·3
	Control	0·9	39	1 000	41·3	41·0	17·3	0·1	0·3		0·4

[a] Micronuclei and fragments are not statistical.

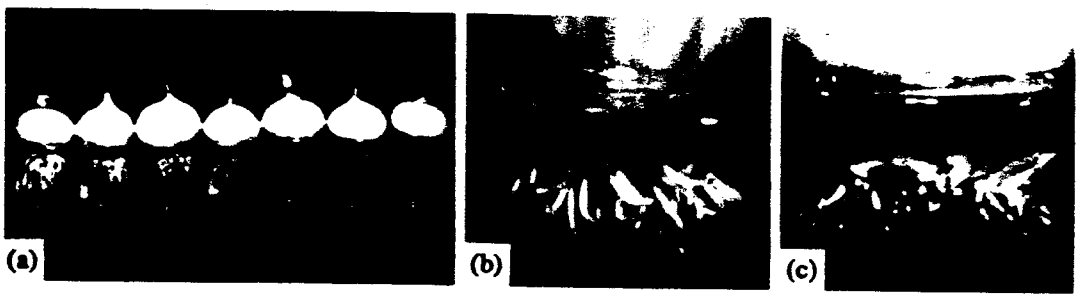

Fig. 2. Macroscopic effects of Pb on *Allium cepa* roots: (a) roots treated with different concentrations of Pb^{2+}; (b) Pb-treated roots stunting and bending; (c) Pb-treated roots, appearing rod-like (time of treatment: 96 h; test concentration: (a) control (tap water), 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} M Pb from left to right; (b) 10^{-3} M Pb; (c) 10^{-2} M Pb).

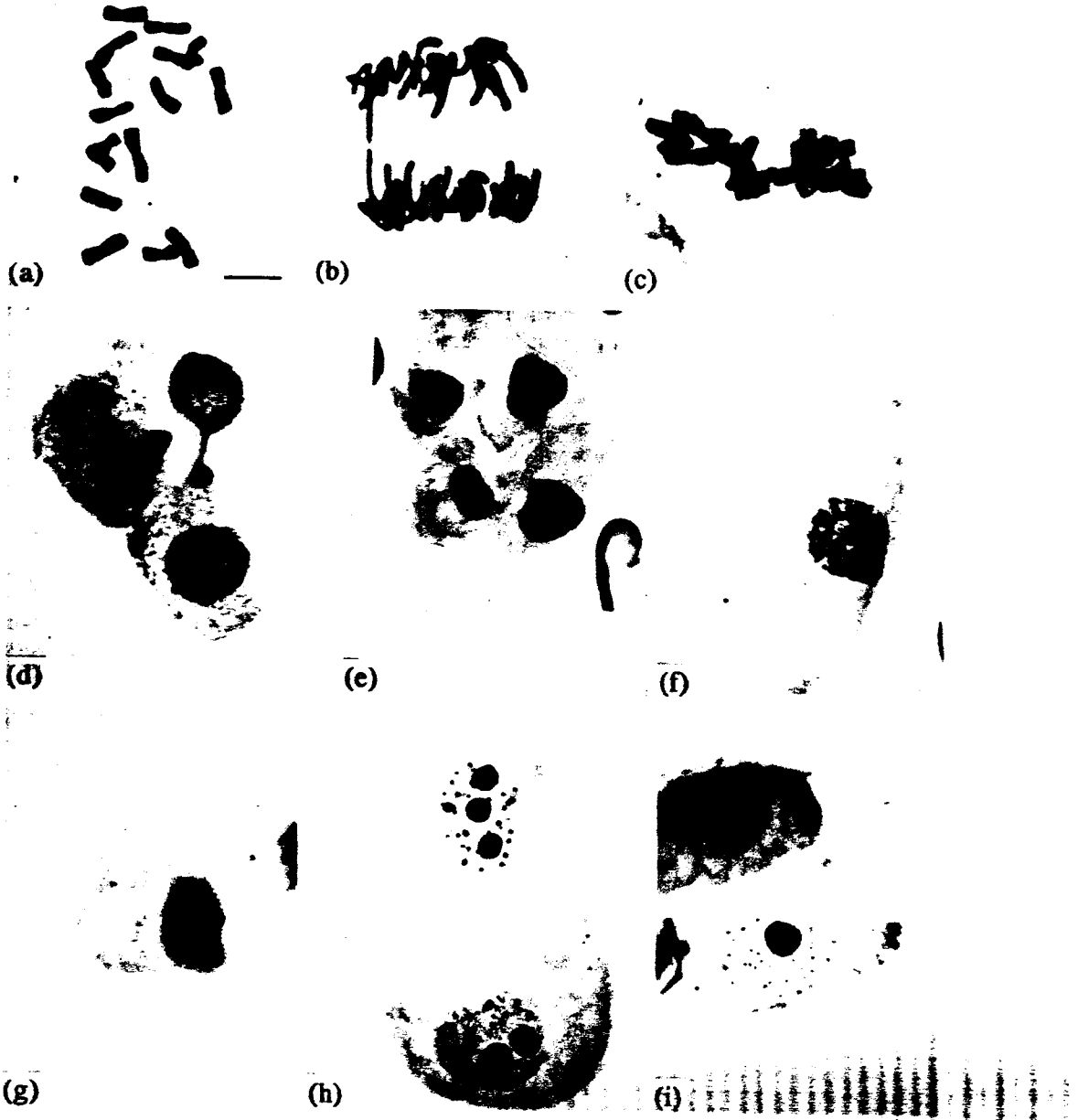

Fig. 3. The effects of Pb^{2+} on root tip cell division of *Allium cepa*: (a–c) effects of Pb^{2+} on chromosomal morphology: (a) c-metaphase (10^{-5} M Pb^{2+}, 48 h); (b) chromosome bridge (10^{-4} M Pb^{2+}, 48 h); (c) chromosome stickiness (10^{-2} M Pb^{2+}, 96 h). (d–f) Effects of Pb^{2+} on nuclei: (d) Micronuclei (10^{-6} M Pb^{2+}, 48 h); (e) irregularly shaped nuclei (10^{-3} M Pb^{2+}, 96 h); (f) nuclei with decomposed nuclear material (10^{-2} M Pb^{2+}, 96 h). (g–i) Effects of Pb^{2+} on nucleoli: (g) control cells (tap water). (h–i) Many silver-stained particulate material scattered in the nuclei: (h) 10^{-2} M Pb^{2+} (in short, rounded meristem cells); (i) 10^{-2} M Pb^{2+} (in long oblong root cap cells) (bars = 10 μm: (a–f) stained with Carbol Fuchsin solution; (g–i) stained with silver nitrate).

cells (Fig. 3(h)) and in long, oblong root cap cells (Fig. 3(i)). The nucleolar material was not extruded from the nucleus into the cytoplasm.

DISCUSSION

The results indicate that lead nitrate inhibits the root growth of *Allum cepa* at all of the concentrations used; the Pb effects on chromosomal morphology include c-mitosis, anaphase bridges and chromosome stickiness; at above 10^{-4} M Pb, almost all of anomalous dividing cells are c-mitoses: irregularly shaped nuclei and nuclei with decomposed nuclear material were only found in the treatment with 10^{-4} and 10^{-2} M Pb respectively; Pb in higher concentrations (10^{-4}–10^{-2} M Pb) induces toxic effects on nucleoli, causing some similar silver-stained particulate material scattered in the nuclei and once the nucleolus was poisoned the root growth nearly stopped or completely stopped. The Pb effects on the chromosomal morphology is in agreement with the early findings of Levan (1945).

Lead is not generally considered as an essential element for the growth of plants, but appears to stimulate plant growth in some plants in small amounts (Dou, 1988). Lane *et al.* (1978) indicated that the inhibiting effects of Pb on growth may arise from the interference of Pb with auxin-regulated cell elongation. Mukherji & Maitra (1977) stated that a primary cause of inhibition of cell growth arises from a Pb-induced stimulation of IAA oxidation. Wallace *et al.* (1966) thought that Ca^{2+} is readily replaced by other cations from its binding sites at the exterior surface of the plasma membrane. It has been claimed that Ca^{2+} can be replaced by Pb^{2+}, because they have similar radii (Ca^{2+} 0·99 Å; Pb^{2+} 1·25 Å) (Chai & Zhu, 1983). Means & Dedman (1980) found that calmodulin (CaM) (Cheung 1980–1983) was located specifically in the mitotic spindle and involved in the processes of chromosome movement through regulation and control of depolymerization and polymerization of the microtubules (Li & Sun, 1991). With what is indicated, we infer that after Pb treatment, the expression of c-mitosis may result from calcium deficiency in the cells and CaM failing to activate Ca-ATPase (Xu, 1985) and regulate calcium concentration in the cells, which, in turn, disturbs and destroys the physiological activity and regulation of CaM. In this way, CaM does not regulate and control microtubules, or tubulin synthesis may be inhibited.

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