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Investigations on the cytotoxic influence of zinc on *Allium cepa* L. roots

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Abstract

The influence of various (0.1, 0.08, 0.04, 0.02, 0.01, 0.005, 0.0025 and 0.00125 M) zinc chloride and zinc acetate concentrations on the mitotic activity of the apical meristem of onion adventitious roots was investigated after 24 h of incubation. It was found that the tested compounds have a mitodepressive action and distinctly inhibit root elongation. The results of topochemical analysis are reported.

INTRODUCTION

Zinc is one of the microelements playing an important role in cell metabolism. It is present in many enzymatic systems and activates a number of enzymes. It also influences hydrocarbon metabolism and protein synthesis. When zinc is dificient, the metabolic rhythm is disturbed and sometimes inhibited. According to Price and Valle (1962) and Altmann et al. (1968) this element acts directly on ribonucleic acids, when, namely zinc is lacking in plants, the RNA content greatly decreases. This, according to Kessler (1961), is due to the enhanced activity of ribonuclease. Under normal growth and development conditions of plants zinc is mainly accumulated in young physiologically active tissues. Accumulation of this element in the meristems seems to point to its influence on growth processes (Gerebtzoff and Ramaut 1970).

An excessive zinc content in the atmosphere and substrate is the cause of accumulation of this element in various tissues and organs (Kaźmierczakowa and Rams 1974, Karweta 1976) and sometimes leads to inhibition of growth of the plants, leaf chlorosis and even to dying out of species (Antonovics et al. 1971).

Among the abundant data concerning the toxicity of zinc but few deal with its influence on the mitotic cycle (Świeboda 1976). It was therefore, considered useful to investigate the effects of some chosen zinc compounds on the mitotic activity of root meristem and the induction by it of mitotic disturbances. It was also attempted to detect the relation between its mitodepressive action and the accumulation of this element in the meristem and the root elongation zone.

MATERIAL AND METHODS

Rooted *Allium cepa* L. bulbs (grown in tap water at room temperature) with roots 1-2 cm long were divided into halves and incubated for 24 h in a thermostat at 24° ± 1°C — one half in a water solution of the zinc compound, the other in water. In the first combination bulbs for cytological examination were incubated in aqueous 0.1, 0.08, 0.04, 0.02, 0.01, 0.005, 0.0025 and 0.00125 M solutions of $ZnCl_2$ and $Zn(CH_3COO)_2$. The roots were measured with millimetre paper. The cut off roots were fixed for 24 h in Carnoy's fluid and stained with acetoorcein (Zeilinga 1956) at room temperature for a dozen hours or so. Then squashes were prepared from the root apexes of about 3 mm length. For each concentration 5 preparations were made. In each 1000 cells were counted and the mitotic indexes were calculated.

In the second experimental combination with samples prepared for topochemical analyses one half of the rooted bulb was incubated for 24 h at 24° ± 1°C in aqueous solutions of zinc acetate of 0.1, 0.04, 0.005, 0.00125 M concentration, the corresponding halves of the onions were incubated in water. After incubation the roots were fixed in 95 per cent ethanol. Zinc in the tissues was revealed by the modified method of McNary (Gerebtzoff and Ramaut 1970) in sections prepared on the freezing microtome. The sections were shortly washed in bidistilled water, then placed in a mixture of 0.01 per cent dithizone solution with absolute acetone and bidistilled water, and stained for 5 min with 0.1 per cent azure II solution. After washing with bidistilled water the sections were embedded in glycerogel (Zawistowski 1970).

RESULTS

Even macroscopic analysis of the control and tested material allows the evaluation of the toxicity of both the zinc salts applied. The inhibitory action on root growth proved to be positively correlated with the concentration of zinc chloride and acetate and the potency of both compounds was more or less similar (Table 1). They were toxic in higher concentrations (0.04-0.1 M) and blocked completely root elongation. In lower concentrations they exerted a strong inhibitory effect.

This was not accompanied, however, either by changes in firmness, thickening or else turning brown of the roots.

Table 1

Inhibition of elongation growth of *Allium cepa* L. roots by zinc salts

Concentration of zinc solution, M	$ZnCl_2$			$Zn(CH_3COO)_2 \times 2H_2O$		
	mean length increment of roots, cm		growth inhibition, %	mean length increment of roots, cm		growth inhibition, %
	control	incubated		control	incubated	
0.1	0.7	0	100.0	0.8	0	100.0
0.08	0.9	0	100.0	0.9	0	100.0
0.04	1.2	0	100.0	0.8	0	100.0
0.02	1.4	0.1	92.9	0.5	0.1	80.0
0.01	0.8	0.1	87.5	0.4	0.1	75.0
0.005	0.7	0.1	85.7	0.8	0.2	75.0
0.0025	0.6	0.1	83.5	0.3	0.1	66.7
0.00125	0.9	0.3	66.7	0.5	0.2	60.0

Both compounds had a considerably mitodepressive effect on the tested meristem, which was also positively correlated with the concentration of the solutions (Table 2).

Table 2

Mitosis inhibition in root meristems of *Allium cepa* L. by various zinc salt concentrations

Concentration of zinc solution, M	$ZnCl_2$			$Zn(CH_3COO)_2 \times 2H_2O$		
	mitotic index of control meristems, %	mitotic index of tested meristems, %	mitosis inhibition, %	mitotic index of control meristems, %	mitotic index of tested meristems, %	mitosis inhibition, %
0.1	9.53±0.21	3.68±0.13	61.4	8.37±0.13	3.12±0.06	62.7
0.08	9.04±0.28	4.38±0.08	51.5	8.01±0.08	3.60±0.03	55.1
0.04	9.37±0.20	4.37±0.05	49.5	8.28±0.18	4.14±0.07	50.0
0.02	9.20±0.11	5.38±0.04	41.5	8.30±0.10	4.60±0.04	44.6
0.01	9.41±0.06	6.03±0.11	35.9	8.11±0.09	5.48±0.03	32.4
0.005	9.46±0.14	6.69±0.09	29.3	8.63±0.06	6.34±0.06	26.5
0.0025	9.46±0.11	7.34±0.07	22.4	9.11±0.29	7.18±0.10	21.2
0.00125	9.64±0.19	7.99±0.17	17.1	8.82±0.15	7.37±0.04	16.4

Inhibition of mitotic activity was associated with changes in the percentual proportion of the particular phases of mitosis (Figs. 1 and 2). Under the influence of higher $ZnCl_2$ concentrations a large number of prophases appeared. Whereas in the control root meristems the number of prophases amounted to 66.6 per cent, under the action the 0.1 M solution they reached 91.8 per cent. As weaker dilutions were applied, the high percentage of prophase cells gradually decreased, and at the

lowest concentration (0.00125 M) it was close to the control values. With the rise of the percentual proportion of prophases the per cent of the remaining phases of mitosis decreased, this appearing quicker for anaphase (complete disappearance at 0.1 M), somewhat slower for telophase and latest for metaphase (Fig. 1). Similar results were obtained for meristems treated with zinc acetate solution (Fig. 2).

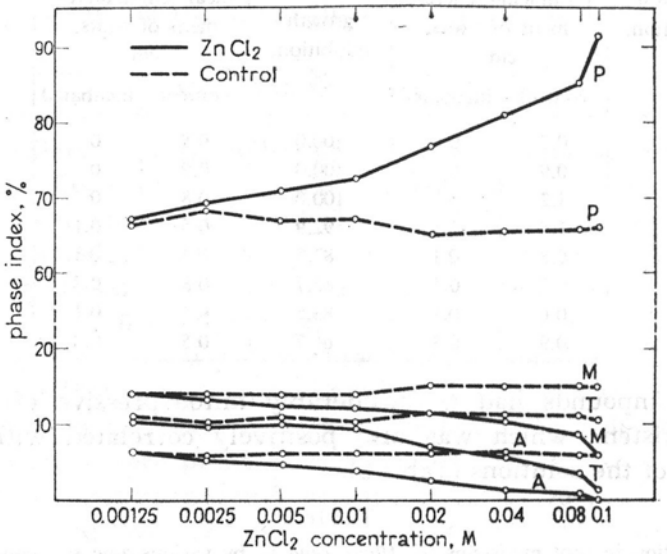

Fig. 1. Mean phase indexes for *Allium cepa* L. root meristems incubated in zinc chloride solutions. P — prophase, M — metaphase, A — anaphase, T — telophase

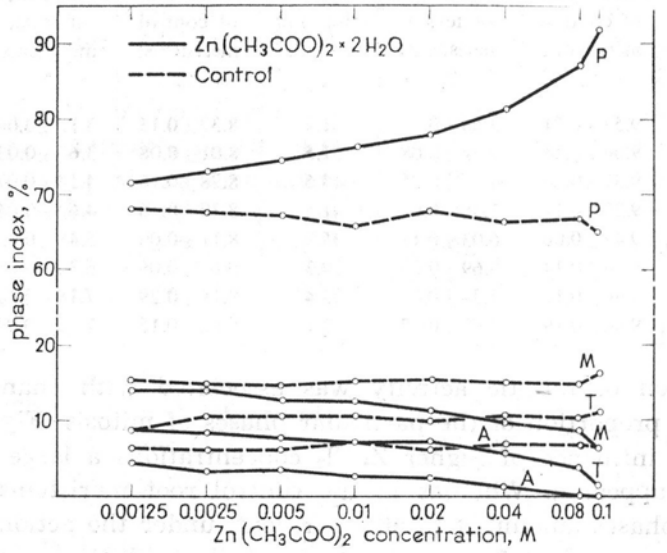

Fig. 2. Mean phase indexes for *Allium cepa* L. root meristems incubated in zinc acetate solutions. P — prophase, M — metaphase, A — anaphase, T — telophase

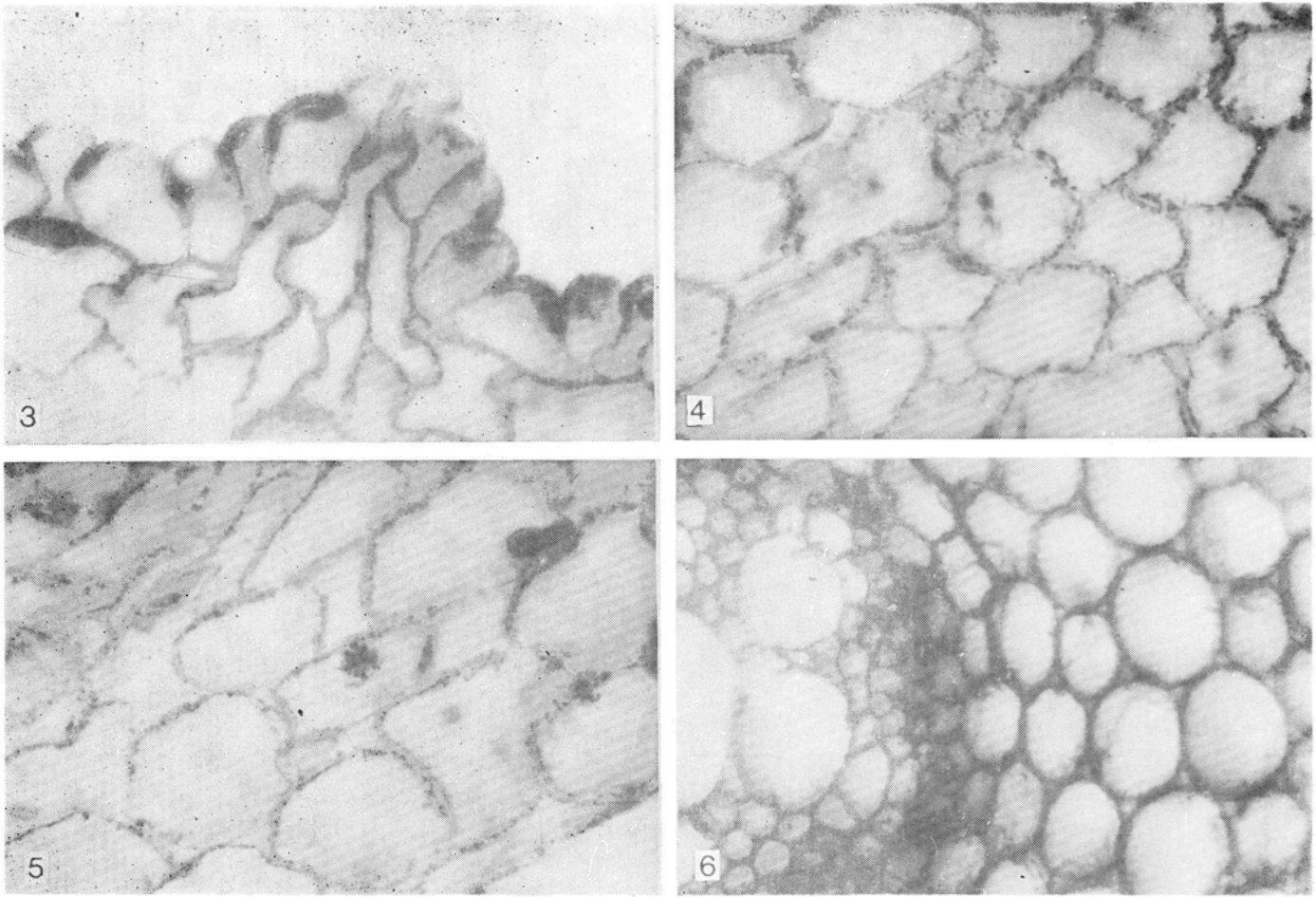

Fig. 3. Zinc inclusions in the rhizoderm of *Allium cepa* L. roots. Incubation in 0.00125 M $Zn(CH_3COO)_2 \times 2\,H_2O$. × 500
Figs. 4 and 5. Zinc deposits in primary cortex of *Allium cepa* L. roots. Incubation in 0.1 M $Zn(CH_3COO)_2 \times 2\,H_2O$. × 500
Fig. 6. Cross section through *Allium cepa* L. root incubated in water (control). × 500

At the lowest zinc acetate concentration (0.00125 M) nonuniform deposition of metal precipitate was observed on the surface and in the rhizodermis. Minute granules were deposited at the cell wall and inside the cell. The subdermal cells (Fig. 3), accumulated the minute grains in the zone close to the wall. The amount of precipitate distinctly decreased in the primary cortex layers (Figs. 4 and 5). Zinc was also detected in the cell wall and the lumen of pericycle cells. The phloem cells contained both rather numerous granular forms and sporadic large amorphous inclusions. In the xylem elements zinc precipitates were but seldom seen.

It is generally believed that meristematic tissue is the site of particular zinc concentration. Histochemical analysis of this root zone, incubated in the lowest concentration of zinc acetate applied, confirmed this opinion. The distinctly purple colour of the cytoplasm of meristematic cells, particularly in the perinuclear zone, is evidence of the presence of zinc. At higher concentrations (0.1, 0.04 and 0.005 M) the zonality of accumulation of this element in the root tissues continued to prevail, whereas the number of deposits increased markedly and their colour became deeper.

DISCUSSION

Heavy metals are mitotic poisons acting probably on enzymes by blocking the sulphydryl groups (B i e s e l 1958). They cause karyokinesis inhibition, sometimes also karyorhexis or karyolysis. The author does not specify, however, which elements induce the above mentioned effects. Zn^{2+} ions are known to increase the frequency of aberrations caused by mono- and polyfunctional alkylating agents (R i e g e r and M i c h a e l i s 1967).

To the toxic action of zinc is ascribed the mitodepressive influence on the root meristem, the shift in the duration of the particular phases of the mitotic cycle and inhibition of root elongation growth.

It has been demonstrated that the antimitotic effect is accompanied by accumulation of zinc in the root tissues, particularly in the meristematic zone. According to G e r e b t z o f f and R a m a u t (1970) the interference of zinc in the process of mitosis is possible, at least indirectly, the more so, since in the meristem in which mitotic activity is intensive this element tends to accumulate in larger quantities than in the root cap.

The intensity of the colour reaction indicated that the degree of accumulation of zinc inclusions seems to be proportional to the zinc acetate concentration. The site of highest concentration of zinc are the protoplasts of meristematic cells, whereas in the suprameristematic area

accumulation of this metal seems to be of zonal character. Numerous zinc deposits in the form of granules accumulated in the rhizoderm and pericycle cells, and smaller quantities in the primary cortex. Distinct inclusions were also found in the phloem cells, whereas in the xylem they occurred but seldom. Similar zones of accumulation of this metal were described by Gerebtzoff and Ramaut (1970) in root meristems of *Hordeum vulgare*. They also demonstrated the presence of zinc in the assimilative parenchyma cells of leaves. According to the mentioned authors, zinc in ion form penetrates to the root through root hairs, part of it spreads in the meristematic zone and root cap, and the rest is translocated to the leaves, where it acts as one of the organogenetic factors.

The cytotoxic effects of zinc detected by us proved to be correlated with its concentration in the solution, notwithstanding the kind of salt applied. The tested compounds induce but few chromosome aberrations, but they produce changes in the percentual proportion of the particular mitotic phases, with an increase in the prophase index and depression of the indexes of the remaining phases. This becomes particularly pronounced when high concentrations of zinc chloride and acetate are used. The differences in cytotoxicity of both salts are not significant.

REFERENCES

Altmann H., Fetter F., Kaindl K. 1968. Untersuchungen über den EinfluB von Zn-Ionen auf die m-RNS-Synthese in *Chlorella* zellen. Z. Naturforschung 23b: 395-396.

Antonovics J., Bradshaw A. D., Turner R. G. 1971. Heavy Metal Tolerance in Plants. Adv. Ecolog. Res. 7: 1-80.

Biesel J. J. 1958. Mitotic poissons and cancer problem. Amsterdam-Houston-London-New York.

Gerebtzoff A., Ramaut J. L. 1970. Essai de localisation histochimique du zinc chez *Hordeum* gr. *vulgare* et toxicité. Physiol. Plant. 23: 574-582.

Karweta S. 1976. Zmiany zawartości Zn i Pb w roślinach jako skutek zanieczyszczenia powietrza przez zakład hutniczy metali nieżelaznych. Zesz. Probl. Nauk Roln. 179: 583-587.

Kaźmierczakowa R., Rams B. 1974. Wpływ przemysłowych zanieczyszczeń powietrza na zawartość ołowiu i cynku w wybranych roślinach leczniczych w rejonie olkuskim. Herba Polonica 20: 373-378.

Kessler B. 1961. Plant analysis and fertilizer problems. W. Reuther, (ed.) American Inst. Biological Sciences, Washington, pp. 314-322.

Price C. A., Valle B. L. 1962. *Euglena gracilis*, A Test Organism for Study of Zinc. Plant Physiol. 37: 428-433.

Rieger R., Michaelis A. 1967. Die Chromosomenmutationen. Verlag Jena.

Swieboda M. 1976. The use of biological tests for establishing the influence of flue dust from lead and zinc works on plant development. Acta Soc. Bot. Pol. 45: 17-32.

Zawistowski S. 1970. Technika histologiczna, histologia oraz podstawy histopatologii. PZWL, Warszawa.
Zeilinga A. E. 1956. An improved acetic orcein squash method for serial cytological preparations. Euphytica 5: 171-174.

Badanie cytotoksycznego wpływu cynku na korzenie Allium cepa L.

Streszczenie

Określono wpływ różnych stężeń wodnych roztworów chlorku i octanu cynku na aktywność mitotyczną merystemu korzeniowego *Allium cepa* L. po 24 godzinnej inkubacji. Stwierdzono mitodepresyjne działanie, przesunięcie czasu trwania poszczególnych faz mitozy oraz inhibicję wydłużania korzeni. Histochemicznie, zmodyfikowaną metodą Mc Nary zlokalizowano cynk w strefie merystematycznej oraz w komórkach ryzodermy, perycyklu i we floemie w strefie elongacyjnej.