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Environmental Contamination and Toxicology

Effects of Mg^{2+}, Co^{2+}, and Hg^{2+} on the Nucleus and Nucleolus in Root Tip Cells of *Allium cepa*

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Metal toxicity in plants has been known for a long time (Foy et al. 1978; Roy et al. 1988; Ganrot 1986; Mukhopadhyay and Sharma 1991; Mishra and Kar 1974; Fernandes and Henriques 1991). Much importance has increasingly been attached to the problems of metal pollution with the development of modern industry and agriculture. If metals in plants are accumulated to a large extent, it might seriously affect them. The cytological efftcts of cobalt and mercury have been studied in *Allium cepa* by documentation of c-mitosis (Levan 1945). Also, the quantification of chromosome aberration in *Vicia faba* root-tip cells treated by magnesium sulphate (Abraham and Nair 1989) and in *Allium cepa* by metyl mercury chloride and mercuric chloride (Fiskesjö 1988) has been reported. Cytological research on the poisoning effects of Mg, Co and Hg on the nuclei and nucleoli in root-tip cells of plants has hardly been reported.

The aim of this study was to determine the effects of different concentrations of magnesium, cobalt and mercury ions on root growth, and on the nuclei and nucleoli of root tip cells of *Allium cepa.*

MATERIALS AND METHODS

Healthy and equal-sized bulbs were selected from a population of the common onion *Allium cepa* L. The onions were neither sprouting green leaves or had any root growth. The outer

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scales of the bulbs and the brownish bottom plate were removed and the ring of the root primordia was left intact before the start of the experiments. Because onions may grow poorly, even the controls, it was first necessary to use twelve onions at each concentration of the test chemicals and in the control. After 1 or 2 days, the ten best onions in each series were used for the experiment (Fiskesjö 1988).

The test concentrations of Mg^{2+}, Co^{2+} and Hg^{2+} ions were made up from magnesium sulphate ($MgSO_4 \cdot 7H_2O$), cohaltous nitrate ($Co(NO_3)_2 \cdot 6H_2O$) and mercuric chloride ($HgCl_2$), respectively, ranging from 10^{-7} to 10^{-1} M. The solutions were prepared in tap water (pH=6.5). Tap water was used for the control experiment. The onions were placed directly in the test media in beakers and the beakers were changed daily. The bulbs were allowed to germinate producing roots and were observed at 24, 48 and 72 hr. The experiment wes performed at a room temperature of 21-23℃ and the roots were protected from direct sunlight. Macroscopie observations were made at the end of each time interval. Some roots were cut and fixed in Carnoy's reagent, followed by squashing in Carbol Fuchsin solution (Li 1982) to enable observation of nucleus morphlogy. For the observation of nucleolus change, excised roots were fixed in 3 parts 95% ethanol : 2 parts acetic acid for 4 to 5 hr. They were then hydrolyzed in 5 parts 1 M hydrochloric acid : 3 parts 95% ethanol : 2 parts acetic acid for 4-5 min at 60℃, followed by squashing in 45% acetic acid, drying, and on day 2 staining with silver nitrate (Li et al. 1990).

RESULTS AND DISCUSSION

The effects of Mg^{2+}, Co^{2+} and Hg^{2+} on root growth of *Allium cepa* varied with different concentrations of magnesium, cobalt and mercury ions in solution (Fig. 1). The root growth decreased progressively with increasing Mg^{2+}, Co^{2+} and Hg^{2+} concentrations, and was seriously inhibited in concentrations above 10^{-2} M Mg^{2+}, 10^{-3} M Co^{2+} and 10^{-4} M Hg^{2+}. There was slight or on growth after 24 hr treatment. Hg^{2+} exerted more growth inhibition than the other metal ions.

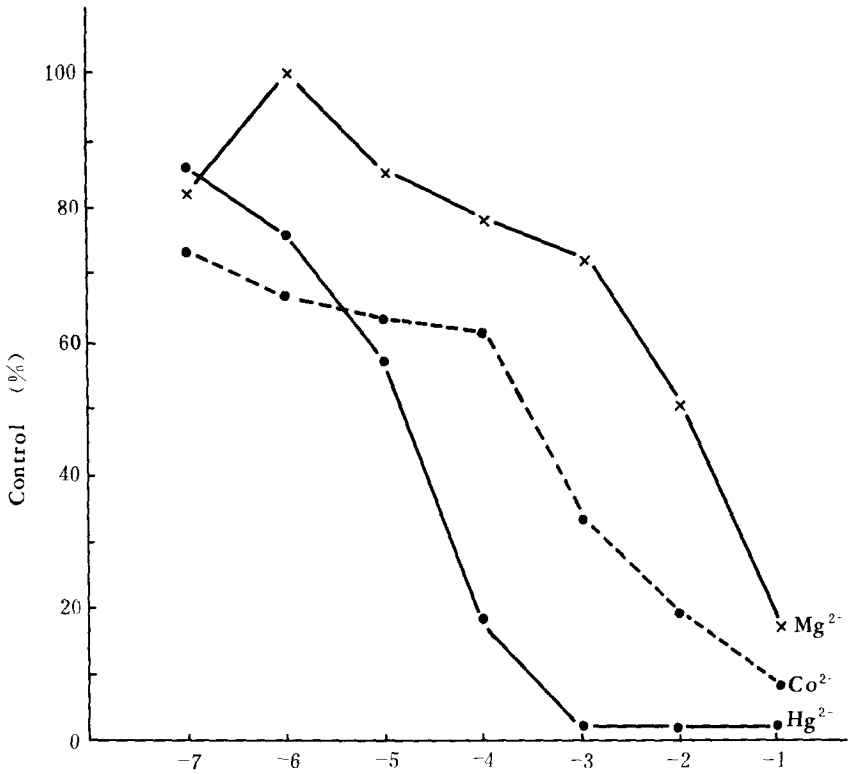

Figure 1. Effects of different concentration (10^{-7} to 10^{-1} M) of Mg^{2+}, Co^{2+} and Hg^{2+} on root growth of *Allium cepa* after 72 hr of treatment.

The effects of Mg^{2+}, Co^{2+} and Hg^{2+} on the morphology of the roots also varied depending on concentration. The roots were more or less normal during the treatments at 10^{-7} to 10^{-3} M Mg^{2+}, 10^{-7} to 10^{-5} M Co^{2+} and 10^{-7} to 10^{-6} M Hg^{2+}, but became twisted at 10^{-2} M Mg^{2+}, 10^{-4} to 10^{-3} M Co^{2+} and 10^{-5} M Hg^{2+}, and stunted and bent in different directions at 10^{-1} M Mg^{2+}, 10^{-2} to 10^{-1} M Co^{2+} and 10^{-4} to 10^{-2} M Hg^{2+}.

The effects of Hg^{2+} and Mg^{2+} on nuclei in the root-tip cells varied with the different concentrations tested. Interphase cells with small amounts of micronuclei were observed after 24 hr of

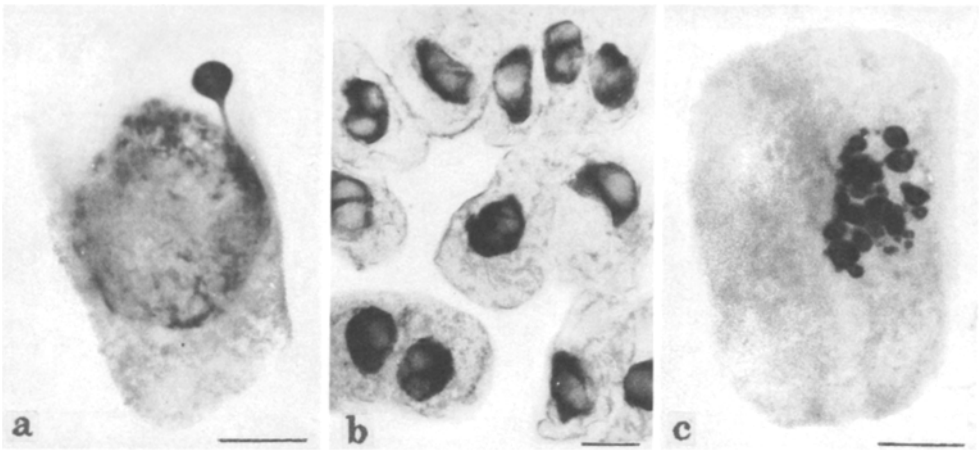

Figure 2a-c. The effects of Mg^{2+}, Co^{2+} and Hg^{2+} on root tip cells of *Allium cepa*. a. Micronucleus, 10^{-5} M Mg^{2+} 24 hr. b. Irregularly shaped nuclei, 10^{-2} M Co^{2+}, 24 hr. c. The nucleus disintegrated into irregularly shapd bodies, 10^{-3} M Hg^{2+}, 24 hr. Scale = 10 μm.

treatment with 10^{-7} to 10^{-4} M Mg^{2+} or 10^{-7} to 10^{-5} M Hg^{2+} solutions (Fig. 2a), but not found in Co-treated roots. Irregularly shaped nuclei were noticed more often after 24 hr teratment with 10^{-3} to 10^{-1} M Mg^{2+}, 10^{-5} to 10^{-1} M or 10^{-6} M (48 hr) Co^{2+} and 10^{-4} to 10^{-1} M or 10^{-5} M (48 hr) Hg^{2+} (Fig. 2b). More pronounced changes were observed. The nucleus was disintegrated into irregularly shaped bodies after 24 hr Mg^{2+} (10^{-2} to 10^{-1} M), Co^{2+} (10^{-2} to 10^{-1} M) and Hg^{2+} (10^{-4} to 10^{-1} M; 48 hr, 10^{-5} M) treatment (Fig. 2c).

Normally, the diploid nucleus of *Allium cepa* contains 1-2 nucleoli (Fig, 3a). The effects of Mg^{2+}, Co^{2+} and Hg^{2+} also varied depending on concentration. In Fig. 3b, a few particulates of silver-stained material were observed together with the main nucleolus/nucleoli in the nucleus of some root-tip cells after 24 hr at 10^{-7} to 10^{-5} M Hg-treatment. This phenomenon was observed also after 24 hr of teratment with

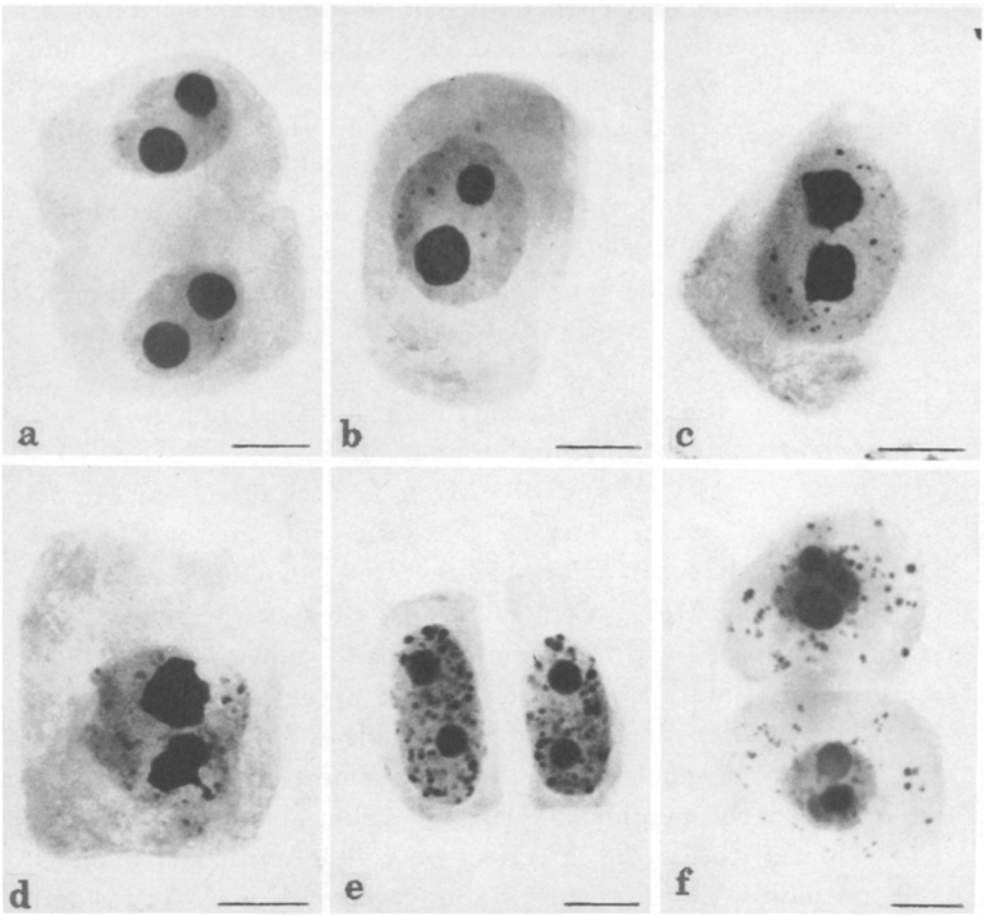

Figure 3a-f. The effects of Mg^{2+}, Co^{2+} and Hg^{2+} on nucleoli in root tip cells of *Allium cepa.* a. Control cells (tap water). b. A few particulate silver-stained materials scattered in the nucleus, 10^{-6} M Mg^{2+}, 24 hr. c-d. Some particulate silver-stained materials scattered around the irregularly shaped nucleoli in the nuclei. c. 10^{-4} M Co^{2+}, 48 hr. d. 10^{-3} M Hg^{2+}, 48 hr. e. Many silver-stained particulates scattered in the nucleus, 10^{-2} M Hg^{2+}, 48 hr. f. Nucleolar material located in the cytoplasm, 10^{-3} M Hg^{2+}, 72 hr. Scale= 10 μm.

10^{-6} M Co^{2+} and 10^{-4} to 10^{-6} M Mg^{2+}. Figs. 3c-d showed that some more particulate silver-stained material was scattered

around the irregularly shaped nucleoli in the nucleus after 24 hr of teratment with 10^{-3} to 10^{-1} M Mg^{2+}, 10^{-5} to 10^{-4} M Co^{2+} and above 10^{-4} M Hg^{2+}. As seen in Fig. 3e, many silver-staind particles were distributed in the nucleus (above 10^{-3} M Hg^{2+} and Co^{2+}) after 48 hr of treatment. At concentrations above 10^{-3} M Hg^{2+}, nucleolar material was extruded from the nucleus into the cytoplasm (Fig. 3f) in some roots. This phenomenon was not found in the roots treated with Co^{2+} and Mg^{2+}.

The results in the present study indicated that root growth of *Allium cepa* was strongly inhibited at concentrations above 10^{-2} M Mg^{2+}, 10^{-3} M Co^{2+} and 10^{-4} M Hg^{2+}. The effects of Mg^{2+}, Co^{2+} and Hg^{2+} on the nucleus resulted in micronuclei and irregularly shaped and disintegrated nuclei. At low concentrations, Mg^{2+}, Co^{2+} and Hg^{2+} affected the nucleoli of some root-tip cells so that similar silver-stained particulate materials appeared in the nucleus. At higher concentrations, many silver-stained particulate materials did in it (Co^{2+} and Hg^{2+}), and the nucleolar material became extruded from the nucleus into the cytoplasm (Hg^{2+}). From the results above, Hg^{2+} showed the strongest effect on root growth and nucleus and nucleolus development when compared with Co^{2+} and Mg^{2+}, whereas Co^{2+} showed a stronger effect than Mg^{2+}. The effects of Mg, Co and Hg on nucleoli are, to some degree, similar to those observed after aluminium treatments of *A. cepa*. *A. sativum* and *Vicia faba* (Fiskesjö 1983, 1990; Liu and Jiang 1991; Liu et al. 1993a and b), and copper treatment of *A. cepa* (Liu et al. 1994), but with a few differences. Firstly, in the present study some silver-stained particulate material was observed at 10^{-6} to 10^{-1} M Mg^{2+} and Co^{2+}, and 10^{-7} to 10^{-1} M Hg^{2+} after 24 hr of treatment, while the phenomenon noticed by Liu et al. (1993a and b; 1994) was recorded after 24 hr of treatment with 10^{-2} to 10^{-1} M Al^{3+} (*A. sativum*) and 10^{-1} M Al^{3+} (*Vicia faba*, 48 hr), 10^{-7} to 10^{-1} M Cu^{2+} (*A. cepa*), and 10^{-2} to 10^{-1} M Cr Ⅵ (*A. cepa*) (Liu et al., unpublished). Secondly, the nucleolar material from the

nucleus into the cytoplasm observed in the present investgation was not as large as those found by Liu and Jiang (1991) and Liu et al, (1993a and b; 1994).

Kihlman (1971) indicated that the standard method for the detection of chromosome aberrations in plants involves treatment and analysis of root-tip meristematic cells. In later years, studies have shown that this classical assay for induced chromosome aberrations can be used widely and effectively to monitor environmental chemicals (Grant 1978; Klekowski 1978; Fiskesjö 1988; Liu et al. 1992). The evidence in the present investigation also indicated that the toxic effects on nucleoli in root-tip cells of plants caused by metal ions can be observed and studied by using the silver staining techique and the cytological toxic characters may be considered an important cytological parameter for monitoring purposes.

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REFERENCES

Abraham S, Nair RB (1989) Production of mitotic abnormalities by magnesium sulphate in *Vicia faba* L. Cytologia 54: 559-563

Fernandes JC, Henriques FS (1991) Biochemical, physiological, and structural effects of excess copper in plants. Bot Rev 57: 246-273

Fiskesjö G (1983) Nucleolar dissolution induced by aluminium in root cells of *Allium.* Physiol Plant 59: 508-511

Fiskesjö G (1988) The Allium test - an alternative in environmental studies: the relative toxicity of metal ions. Mut Res 197: 243—260

Fiskesjö G (1990) Occurrence and degeneration of 'Al-structures' in root cap cells of *Allium cepa* L. after Al-treatment. Hereditas 112: 193-202

Foy CD, Chaney RL, White MC (1978) The physiology of metal toxicity in plants. Annu Rev Plant Physiol 29: 511-566

Ganrot PO (1986) Metabolism and possible health effects of aluminum. Environ Health Perspect 65: 363-411

Grant WF (1978) Chromosome aberrations in plants as a monitoring system. Environ Health Perspect 27: 37-43

Kihlman BA (1971) Root tips for studying the effects of chemicals on chromosomes. In: Hollaender A (ed) Chemical Mutagens: Principles and Methods for Their Detection, Plenum Press, New York, p 489-514

Klekowski EJ, JR (1978) Screening aquatic ecosystems for mutagens with fern bioassays. Environ Health Perspect 27: 99-102

Levan A (1945) Cytological reactions induced by inorganic salt solutions. Nature 156-751

Li MX (1982) Introducing a good stain used for nuclei and chromosome. Bull Biol 5:53

Li MX, Zhang ZP, Yuan JZ, Jin XL (1990) An improved method for Ag-NOR staining and its application to plant chromosomes. Chin Bull Bot 7: 56—59

Liu DH, Jiang WS(1991) Effects of Al^{3+} on the nucleolus in root tip cells of *Allium cepa* . Hereditas 115: 213-219

Liu DH, Jiang WS, Li MX (1992) Effects of trivalent and hexavalent chromium on root growth and cell division of *Allium cepa.* Hereditas 117: 23-29

Liu DH, Jiang WS, Li DS (1993a) Effects of aluminiym ion on root growth, cell division, and nucleoli of garlic (*Allium sativum* L.). Environ Pollut 82: 295-299

Liu DH, Jiang WS. Wang HL (1993b) Effects of Al^{3+} on root growth, cell division and nucleolus of *Vicia faba* L. Chin J Bot 5: 138-144

Liu DH, Jiang WS, Lu C, Zhao FM, Hao YQ, Guo L (1994) Effects of copper sulfate on the nucleolus of *Allium cepa* root-tip cells. Hereditas 120: 87-90

Mukhopadhyay MJ, Sharma A (1991) Manganese in cell metabolism of higher plants. Bot Rev 57:117-149

Roy AK, sharma A, Talukder G (1988) Some aspects of aluminum toxicity in plants. Bot Rev 54: 145—178