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Cytogenetic toxicity effects of inorganic nickel and organic Ni(II) complexes on *Brassica oleracea* L. root meristem

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Abstract. Experiments were carried out on the effect of nickel as an inorganic compound ($NiSO_4 \cdot 7H_2O$) and organic Ni(II) complexes (i.e. Ni(II)-Glu and Ni(II)-EDTA) in concentrations of 20, 40 and 85 μM dm^{-3} on meristematic cells of root tips of *Brassica oleracea* L. cv. Sława from Enkhouizen. All three tested chemical forms of nickel had a mitodepressive effect and inhibited root elongation. With respect to the degree of root elongation inhibition and mitodepressive effect, the tested forms of nickel can be put in the following order: Ni(II)-Glu $NiSO_4 \cdot 7H_2O$ Ni(II)-EDTA. In all three tested forms, nickel caused disturbances in mitotic divisions, resulting in anaphase bridges and binuclear cells, whose nuclei were joined by a bridge of condensed chromatin or separated. Inorganic nickel and Ni(II)-Glu in higher concentrations damaged nuclei (the amount of condensed chromatin increased), nucleoli (their structure became more condensed and vacuolisation was observed), endoplasmic reticulum (fragmentation, swelling of cisternae) and mitochondria (structure condensation).

Key words: cabbage, cytotoxicity, genotoxicity, inorganic nickel, mitosis, Ni(II) complexes, stress tolerance, root meristem.

Introduction

Phytotoxic concentrations of heavy metal ions, like cadmium, lead, copper, zinc and nickel, originate most frequently from industrial and agricultural activities (KABATA-PENDIAS, PENDIAS 1999). Excessive accumulation of these heavy metal in plants is a stress factor causing structural, physiological and genetical alterations (MISHRA, KAR 1974, van ASSCHE, CLIJSTERS 1990, CHAKRAVARTY,

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SRIVASTOVA 1992, PUNZ, SIEGHARDT 1993, MOLAS1997a, b, SRESTY, MADHAVA RAO 1999). Since root is the first target tissue confronted with the excessive concentrations of heavy metals, the toxic symptoms seem to appear more in roots than in shoots (BAKER, WALKER 1989). The apical meristem of the root is particularly exposed to the activity of heavy metals, including nickel.

The response of plants to nickel stress depends on plant genotype (MACNAIR 1993) and on the concentration and chemical form of nickel both *ex vivo* and *in vivo* (KRÄMER et al. 1996, MOLAS 1997b,c, KABATA-PENDIAS, PENDIAS 1999). Nickel in soil solution occurs as free ions (Ni^{2+}) and complex ions combined with organic and inorganic ligands (ADRIANO 1986, KABATA-PENDIAS, PENDIAS 1999); in these forms nickel is absorbed by plants. In plants nickel also occurs as free ions and complex compounds, usually combined with organic compounds, such as amino acids and organic acids (PELOSI et al. 1976, LEE et al. 1978, KRÄMER et al. 1996); nickel affects cells in these forms. It should be emphasised that the few tests concerning the cytotoxic and genotoxic influence of nickel on plants that have been carried out so far concentrated mainly on the effect of inorganic forms of this element, whereas very little is known about the effect of its organic forms in which this metal is absorbed by plants and occurs *in vivo*.

The main purpose of the conducted experiments was to examine the differences, if any, in the effect of inorganic and organic complexes of nickel, differing with respect to mobility, on the structural organisation of the genetic apparatus and mitotic activity of the root meristem in cabbage seedlings.

Material and methods

Plant culture

Cabbage seeds (*Brassica oleracea* L., cv. Sława from Enkhouzien) were treated with 1% NaOCl for 7 min, rinsed in distilled water, and then germinated at 22°C on cotton moistened with distilled water. After 72 h, seedlings with radicles measuring 25 ± 2 mm were transferred to 2.5-L beakers (10 seedlings per beaker) containing half-strength modified Hoagland's solution at pH 5.3 (HOAGLAND, ARNON 1950) supplemented with nickel, added as $NiSO_4 \cdot 7H_2O$ and organic Ni(II) complexes (metal : ligand 1 : 1): Ni(II)-ethylene-diamine-tetraacetate (Ni(II)-EDTA) and glutamatonickelate (Ni(II)-Glu). The complex with glutamate acid is mobile, and the complex with EDTA is inert (HAY 1987). Nickel was added in all the three chemical forms to half-strength Hoagland's nutrient solution in the concentration of 0 (control), 20, 40 and 85 μMdm^{-3}. Every series was conducted in four replications and was repeated two times. The following growth conditions were maintained constant: 16 h and 8 h day and night, respectively, under illumination of ca. 105 W m^{-2}, at the temperature of 21 ± 1°C, and relative humidity of 75 ± 5%. The nutrient solution was aerated every 2 days. After 8 days of

growth the plants were harvested, and then the length of the main root of seedlings from every series was measured in order to determine the tolerance index; samples for microscopic examination were also taken.

Tolerance index (TI) to each nickel compound was calculated according to the following formula (WILKINS 1978):

$$TI(\%) = \frac{\text{mean length in metal solution}}{\text{mean length in the control}} \times 100\% .$$

Mitotic activity of root meristem

Root fragments were fixed for 2 h in Carnoy's solution and stained with acetoorcein (ZEILINGA 1956) at room temperature. Then squashes were prepared from the root apices of about 3-4 mm in length. For each series 10 preparations were made. Mitotic activity of the meristem was determined taking into account all cell divisions in 1000 cells from 7 fields of light microscope (mitotic index), with attention paid to the numbers of particular mitotic phases (phase indices). The squashed preparations were also used for determination of changes in chromosomal structure.

Microscopic study

For transmission electron microscopy (TEM), the terminal 2.5-mm-long radicles were fixed overnight at 4°C in 2.5% glutaraldehyde solution buffered in 0.1 M sodium phosphate buffer (pH 7.2). The root tips were post-fixed in a buffered 1% solution osmium tetroxide for 2 h at 4°C in the dark and then washed thoroughly with buffer. The root tips were then dehydrated in graded acetone; finally, the root tips were embedded in Spur's low-viscosity epoxy resins. Ultra-thin sections were cut on a Reichert-Jung Ultracut E ultramictrotome and then stained with uranyl acetate and Reynolds lead citrate for 15 minutes. Finally, ultra-thin sections of root apex were examined and photographed with a Tesla BS 500 transmission electron microscope. A minimum of seven samples were examined from every Ni treatment.

Results

As shown in Table 1, the tolerance to nickel in a cabbage genotype depended on the chemical form in which this element was absorbed by plants. As the tolerance index shows, tolerance to nickel was much higher when this metal occurred in the form of inert complexes than when it occurred in inorganic form (i.e. as nickel sulphate) and in organic form as mobile complexes Ni(II)-Glu. The tolerance to inorganic nickel and Ni(II)-Glu was similar; however, nickel as Ni(II)-Glu in a higher concentration was slightly more toxic than its inorganic form. Nickel as

Table 1.Tolerance index (in % of the control) of inorganic nickel and organic Ni(II) complexes in cabbage

Ni concentration ($\mu M\ dm^{-3}$)	$NiSO_4 \cdot 7H_2O$	Ni(II)-Glu	Ni(II)-EDTA
20	96.6	97.8	112.3*
40	64.5*	57.3*	98.2
85	32.6*	31.7*	80.7 *

*significantly different from the control at P = 0.05.

Ni(II)-EDTA in a low concentration (20 μM) stimulated root elongation; consequently the tolerance index exceeded 100%. At the highest concentration of nickel in this form, i.e. at 85 μM, reduction of root elongation was observed; however, the degree of reduction was relatively small.

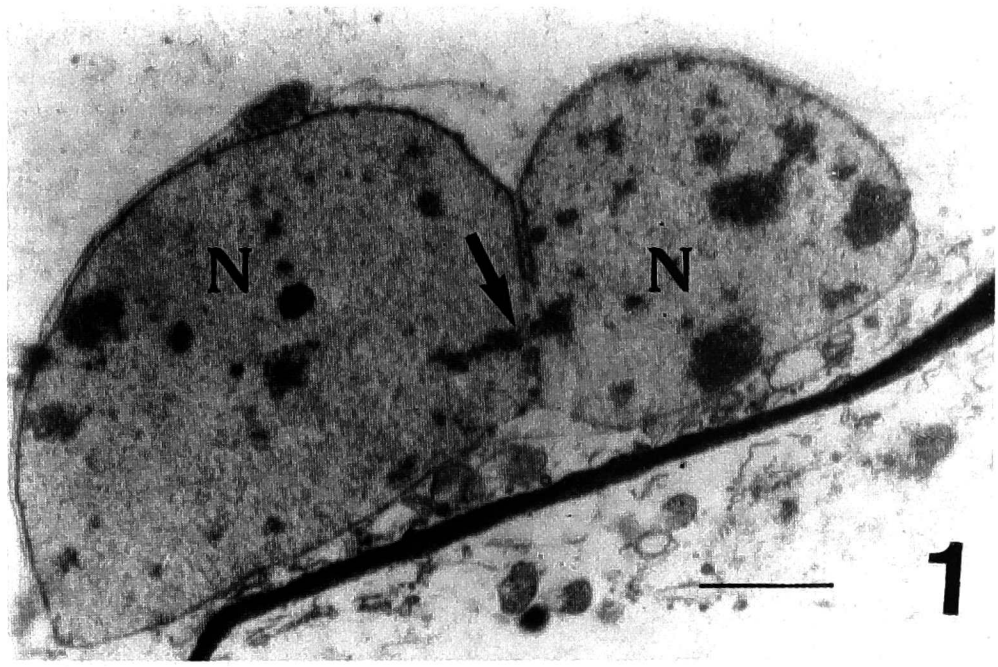
When nickel occurred in inorganic (i.e. sulphate) form and in the form of organic complexes Ni(II)-Glu in concentrations of 40 μM and 85 μM, it had a mitodepressive effect on the cells of cabbage root meristem (Table 2). After 8-day exposure of roots to nickel as $NiSO_4 \cdot 7\ H_2O$ and Ni(II)-Glu at a concentration of 40 μM, the mitotic activity of root meristem was reduced by about 40%, while at a concentration of 85 μM Ni mitosis was completely inhibited. Nickel as

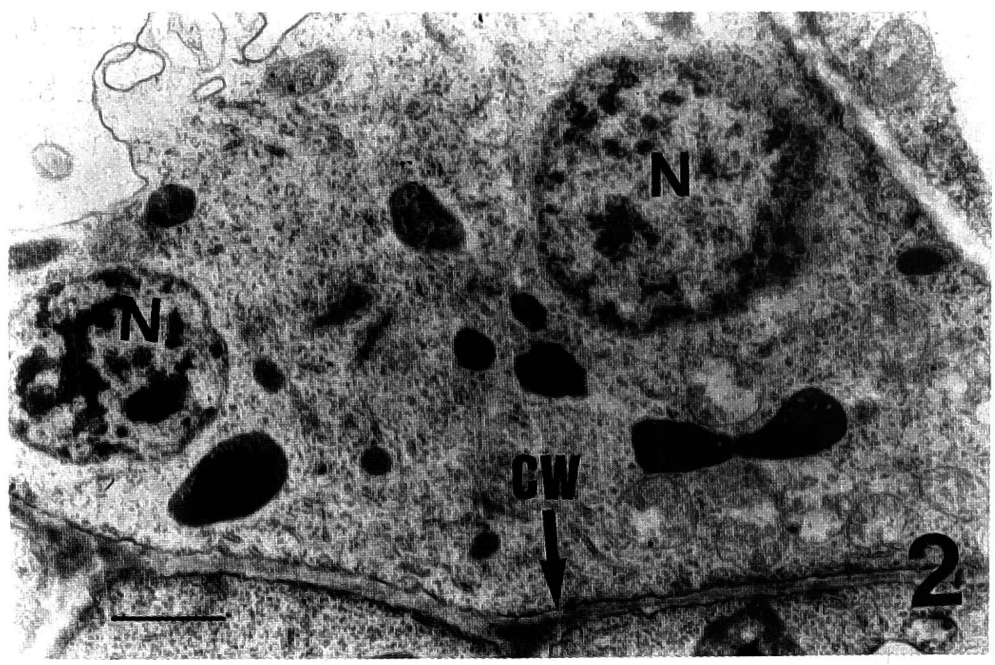
Table 2. Mitotic and phase indices in the tip meristem of cabbage roots in plants treated with inorganic nickel and organic Ni(II) complexes

Index (%)	Control	$NiSO_4 \cdot 7H_2O$ ($\mu M\ Ni\ dm^{-3}$)			Ni(II)-Glu ($\mu M\ Ni\ dm^{-3}$)			Ni(II)-EDTA ($\mu M\ Ni\ dm^{-3}$)		
		20	40	85	20	40	85	20	40	85
Mitotic	5.7	5.4	3.6*	0.0	5.3	3.1*	0.0	6.5*	5.5	4.7*
Prophase	36.7	38.8	48.3*	0.0	38.6	51.2*	0.0	34.9	35.7	41.8*
Metaphase	24.5	24.8	20.7*	0.0	23.7	20.1*	0.0	24.8	24.8	22.6*
Anaphase	20.7	19.1	15.8*	0.0	19.0	13.9*	0.0	21.1	21.0	18.1*
Telophase	18.3	17.3	15.1*	0.0	18.5	12.7*	0.0	19.0	18.6	17.1*

*significantly different from the control at P = 0.05.

inert complexes Ni(II)-EDTA at a low concentration of 20 μM stimulated mitotic divisions of the cells of the tip root meristem of cabbage seedlings. At a concentration of 40 μM, nickel did not affect the divisions of meristem cells, and the meristem's mitotic activity was almost the same as in control plants. Nickel as Ni(II)-EDTA in a high concentration, i.e. at 80 μM, reduced the number of cells at mitosis but the reduction was small, i.e. about 17.5% as compared to control plants. In all three forms and in mitosis-inhibiting concentrations, nickel increased





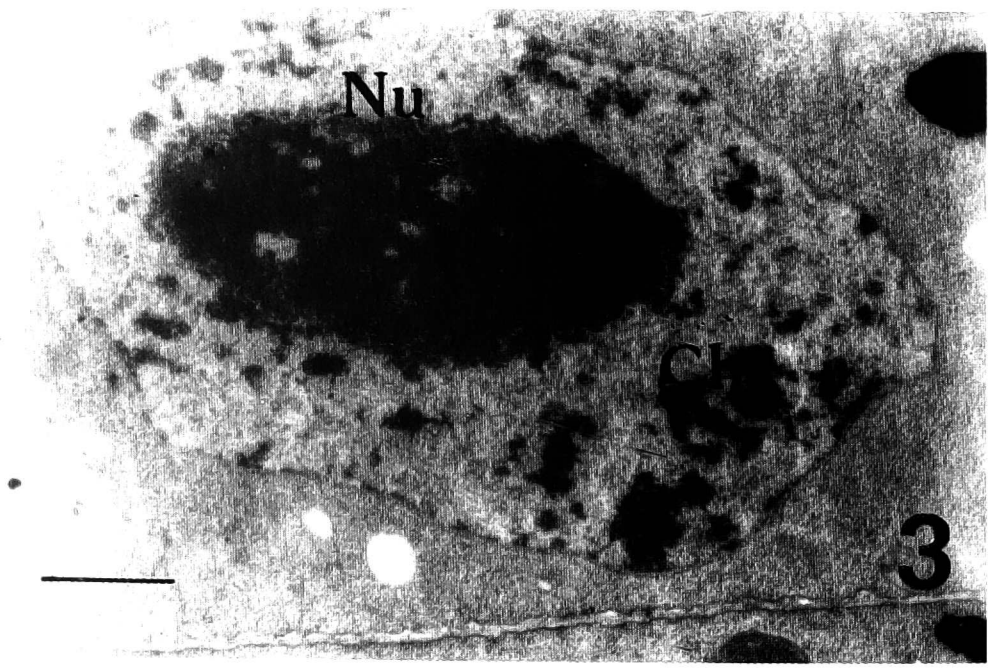
Figures 1-2. Binuclear cells identified in cabbage, in apical root meristem. N = nucleus (arrow indicates chromatin bridge), CW = cell wall. Scale bars = 2.5 M.

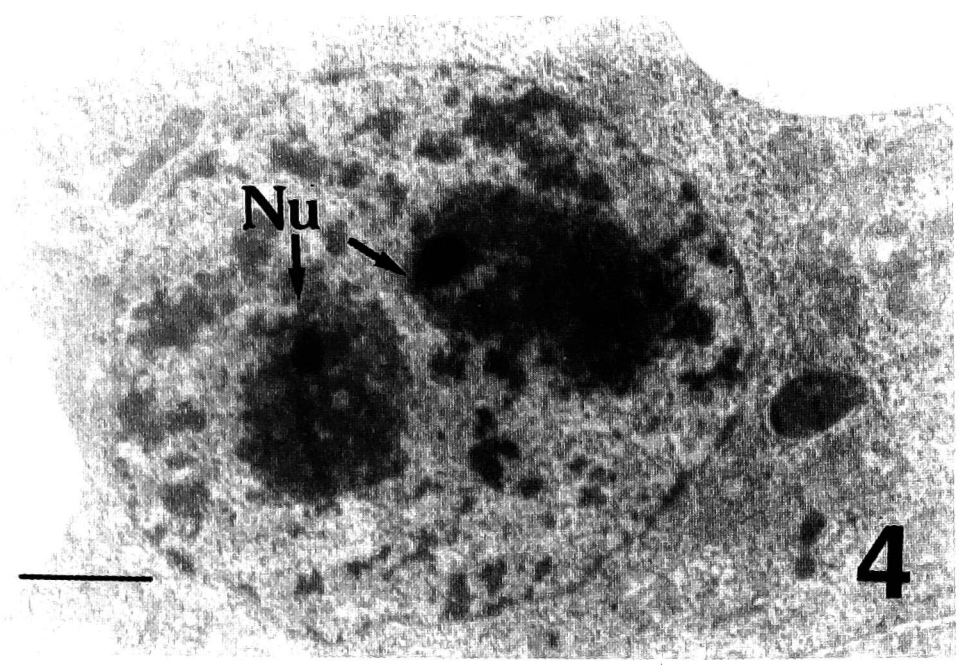
the number of prophases and reduced the numbers of metaphases, anaphases and telophases. The reduction in the numbers of the last two mitotic stages, i.e. anaphase and telophase, were found to be the highest (Table 2).

Nickel in low concentration of 20 and 40 μM, added as $NiSO_4 \cdot 7\ H_2O$ and Ni(II)-Glu, disturbed cell divisions; however, mitotic abnormalities were observed more often in the series with 40 μM Ni (Table 3). In the apical root meristem, mainly in its older part, i.e. in the differentiation zone, binuclear cells were observed (Figures 1 and 2). In many of the binuclear cells, nuclei were joined with a chromatin bridge (Figure 1). On squashed preparations chromatid bridges in anaphase were observed. They were more numerous in root meristems of seedlings treated with Ni(II)-Glu than in those treated with $NiSO_4 \cdot 7H_2O$ (Table 3).

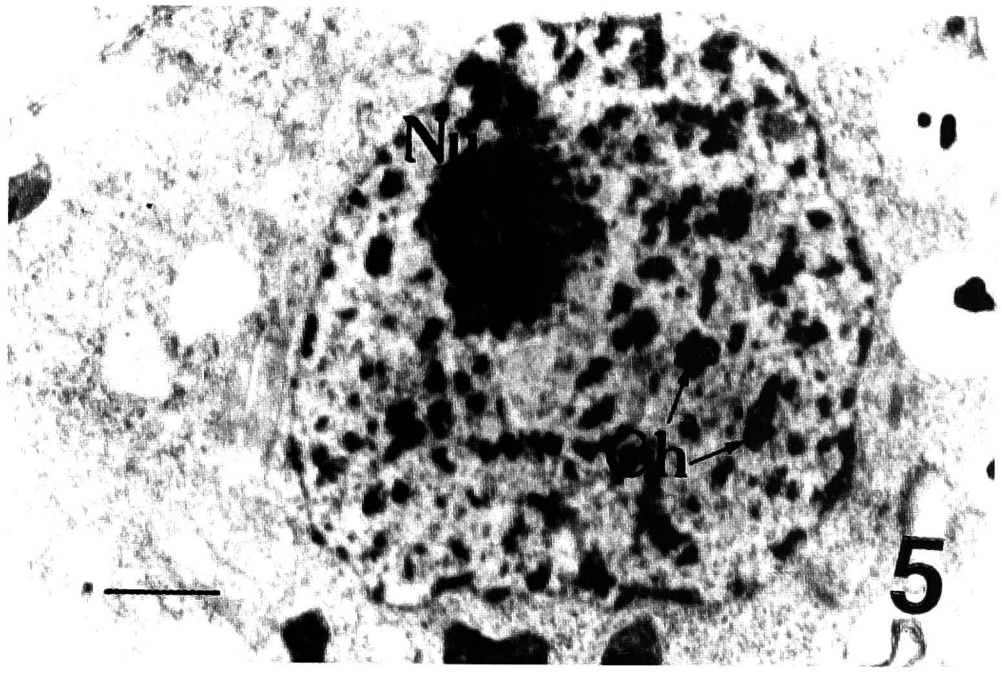
Table 3. Mitotic abnormalities in the tip meristem of cabbage roots treated with inorganic nickel and organic Ni(II) complexes

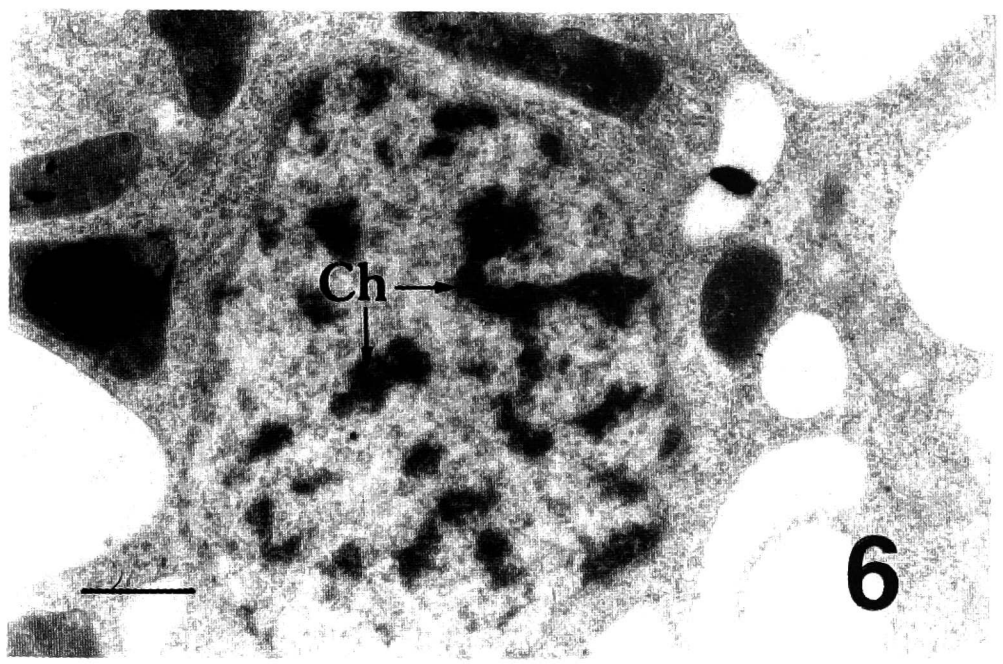
Mitotic abnormalities	Control	$NiSO_4 \cdot 7H_2O$ (μM Ni dm^{-3})			Ni(II)-Glu (μM Ni dm^{-3})			Ni(II)-EDTA (μM Ni dm^{-3})		
		20	40	85	20	40	85	20	40	85
Anaphase bridges (% of dividing cells)	0.0	2.1	3.6	0.0	2.8	4.2	0.0	0.0	single	single
Binuclear cells (%)	0.0	0.8	2.7	0.0	1.3	3.2	0.0	0.0	single	single

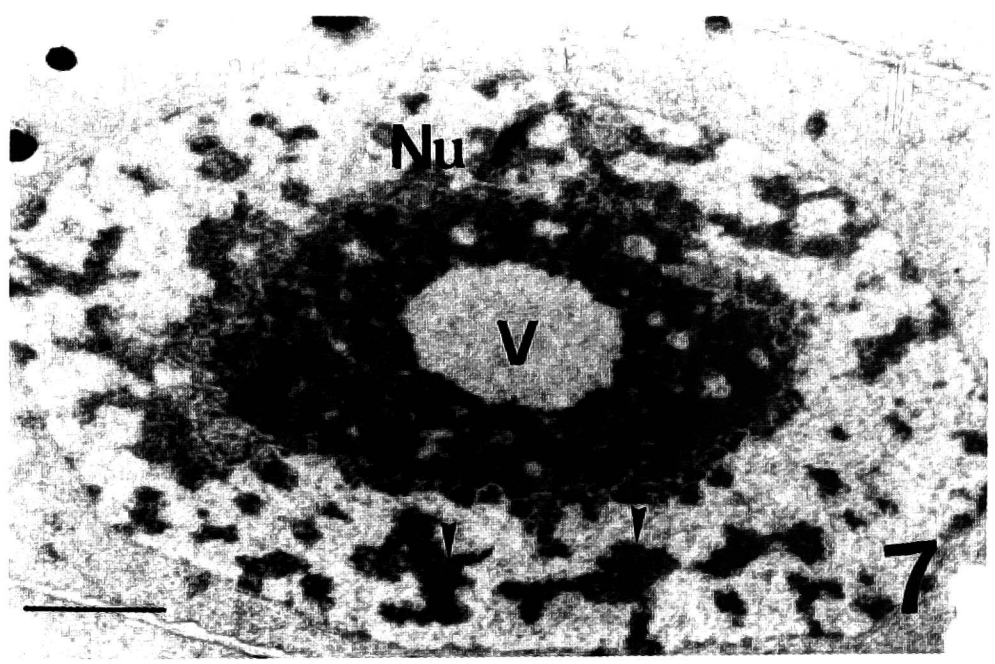




Figures 3-4. Interphase nuclei of root meristem cells. Nu = nucleolus, Ch = condensed chromatin. Scale bars = 2.5 M.







Figures 5-7. Nuclei of root meristem cells of cabbage seedlings treated with high concentrations of inorganic nickel and Ni(II)-Glu. N= nucleus, Nu = nucleolus, V = vacuole, Ch = condensed chromatin. Scale bars = 2.5 M.

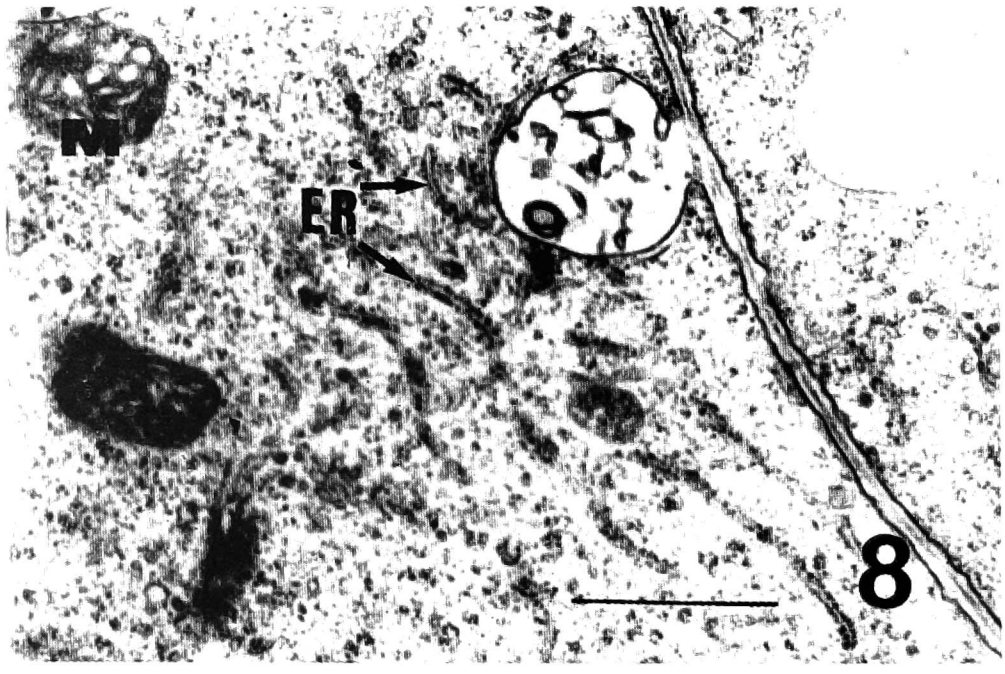


Figure 8. Fragment of root meristem cell of cabbage seedling treated with high concentrations of inorganic nickel and Ni(II)-Glu.ER = endoplasmic reticulum, M = mitochondrion. Scale bars = 2.5M.

No other abnormalities in cell divisions were observed. Interphase nuclei in the apical meristem of roots treated for 8 days with Ni as $NiSO_4 \cdot 7H_2O$ and Ni(II)-Glu in the concentration of 40 μM were considerably larger and contained more condensed chromatin (Figures 3 and 4) than the interphase nuclei of control plants. Nucleoli were also larger, and they were usually condensed and had a high electron density (Figure 3). In some cells, nucleoli were joined with one or two nucleolar organiser regions (NOR); some nuclei contained two nucleoli (Figure 4). Cell divisions were usually quite normal in the root meristem of seedlings grown on the medium with Ni as Ni(II)-EDTA in all concentrations used in the experiments. Sporadically, in the series with 40 and 85 μM Ni, chromatid bridges in anaphase and binuclear cells were identified (Table 3). In binucleate cells, nuclei were joined with a condensed chromatin bridge, just like in the series with Ni(II)-Glu and $NiSO_4 \cdot 7H_2O$. Interphase nuclei and nucleoli in tip meristems of roots treated only with 85 μM as Ni(II)-EDTA were only slightly larger, as compared to the control. However, no symptoms of damage were observed.

The genetic apparatus of root meristem in plants grown on the medium treated with high concentrations of nickel, i.e. 85 μM as $NiSO_4 \cdot 7H_2O$ and Ni(II)-Glu, was clearly changed and symptoms of damage were observed. Some nuclei were morphologically deformed. Some of them had no nucleoli at all (Figure 5), while the other nuclei had nucleoli of a condensed structure (Figure 6); sometimes they were vacuolised (Figure 7) and morphologically deformed. The so-called condensed chromatin was identified in nuclei; it was located either in the perimeter of the nucleus, or all around it (Figures 5-7). More free ribosomes and fewer endoplasmic reticulum (ER) cisternae were identified in the cytoplasm, as compared to control plants or plants treated with nickel in lower concentrations. ER

cisternae underwent fragmentation, forming short swollen fragments (Figure 8). The root cells of plants treated with Ni as Ni(II)-Glu had very few ribosomes and ER cisternae. Mitochondria were in the so-called condensed form (Figure 8).

Discussion

The results show that nickel applied in both the inorganic form, i.e. as $NiSO_4 \cdot 7H_2O$, and in the form of organic complexes used in the experiments, i.e. Ni(II)-Glu and Ni(II)-EDTA, had a mitodepressive effect on the root meristem of cabbage plants and disturbed mitotic divisions. The typical symptoms of the genotoxic effect of the examined chemical forms of nickel in a cabbage genotype were the characteristic chromatid bridges in anaphases and binuclear cells. These abnormalities show that all three examined forms of nickel disturb karyokinesis (anaphase bridges, nuclei joined by chromatin bridge); nickel can also cause disturbances in cytokinesis (binuclear cells, in which nuclei were separated and cell wall was not formed). No other chromosomal abnormalities during cell divisions in the cabbage genotype were observed. However, other authors have observed them in other plant species. After treatment with inorganic nickel numerous chromosomal abnormalities (anaphase bridges, stickiness, chromosome breaks, micronucleus, polyploids, diplo-chromatids) were observed by CHAKRAVARTY and SRIVASTOVA (1992) in root cells of *Helianthus annuus*, while binuclear cells were observed by SRESTY and MADHAVA RAO (1999) in root cells of *Cajanus cajan*. No changes pointing to the mutagenic effect of inorganic nickel (as $NiSO_4 \cdot 6H_2O$) on genetic apparatus were observed by L'HUILLIER et al. (1996) in two cultivars of *Zea mays* that differed with respect to tolerance of plants to nickel. It should be emphasised that in our study nickel applied as $NiSO_4 \cdot 7H_2O$ and Ni(II)-Glu caused abnormalities in mitosis when applied in lower concentrations (i.e. 20 and 40 μM); in a high concentration, i.e. 85 M, nickel in this form inhibited mitosis completely and caused structural damage of the genetic apparatus, i.e. damage of the nucleus, nucleolus, and of other cell structures, including ER and mitochondria.

In the form of inert complexes, i.e. Ni(II)-EDTA, and in concentrations of 40 and 85 μM, nickel caused disturbances of karyokinesis; however, even in the highest concentration it did not damage the structure of the genetic apparatus. It can be assumed that nickel as $NiSO_4 \cdot 7H_2O$ and Ni(II)-Glu in a high concentration, i.e. 85 μM, may inhibit the process of transcription and translation of DNA in cabbage. A visual proof of that was the condensation of chromatin in nucleus and an increase in nucleolus structure condensation. According to ČIAMPOROVÁ and MISTRÍK (1993), such a condensation of nuclear chromatin and the compact nucleolus structure may reflect limitations in DNA replication and transcription. This relationship was observed in many plant species as a response to abiotic stress conditions (ČIAMPOROVÁ, MISTRÍK 1993). According to investigations

conducted by LYNN et al. (1997), nickel treatment increases cellular reactive oxygen species (ROS). According to the hypothesis of these authors, in the presence of the ROS (especially H_2O_2) nickel may exhibit a synergistic inhibition of both DNA polymerization and ligation; it also caused protein degradation *in situ*. Free oxygen radicals generated under nickel stress may also initiate lipid peroxidation in plants (PANDOLFINI et al. 1992). On the basis of these hypotheses it may be suggested that the damages of the nucleus and membrane structures, especially ER, which we observed in root meristem cells, may be a result of oxygen stress caused by high nickel concentrations, during which the reactive oxygen species are generated. It should be emphasised that damages of ER contributed to the reduction of protein synthesis, whereas the condensed form of mitochondrion contributed to disturbances in cell energy management (KAWIAK et al. 1995).

Conclusions

The results of the conducted experiments show that nickel in all three tested chemical forms (i.e. $NiSO_4 \cdot 7H_2O$, Ni(II)-Glu and Ni(II)-EDTA) had a mitodepressive and genotoxic effect on cabbage root meristem. The most frequent symptoms of the genotoxic effect of the examined chemical forms of nickel were chromatid bridges in anaphase, binuclear cells, condensation of nuclear chromatin and compact structure of nucleoli. The mitodepressive and genotoxic effect of nickel depends on the chemical form and concentration of a given form of this metal. With respect to the cytotoxicity degree, the tested forms of nickel can be put in the following order: Ni(II)-Glu $NiSO_4 \cdot 7H_2O$ Ni(II)-EDTA. These differences may result from chemical reactivity of these Ni forms as well as from differences in their bio-assimilation. From the sulphate (inorganic) form and from Ni(II)-Glu, nickel is absorbed by cabbage plants faster and is accumulated in larger amounts than from Ni(II)-EDTA (MOLAS 1997c). Experiments conducted by other authors also show that in the form of complexes with EDTA or with amino acids, such as histidine, nickel is absorbed by plants in smaller amounts than from the inorganic form (ALBASEL, COTTENIE 1985, KRÄMER et al. 1996). This may explain different effects of the tested forms of nickel on the structure of the genetic apparatus and on the mitotic activity of root meristems of the examined cabbage cultivar.

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