ULTRASTRUCTURAL RESPONSE OF CABBAGE OUTER LEAF MESOPHYLL CELLS (*BRASSICA OLERACEA* L.) TO EXCESS OF NICKEL

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ABSTRACT

Changes in the structure and in the ultrastructure of cabbage outer leaf mesophyll cells [*Brassica oleracea* L.] cv. Sława from Enkhouizen were examined by means of light and electron microscopy. The examined plants were grown on the basic Murashige and Skoog medium with addition of excessive concentrations of nickel (added as $\text{NiSO}_4 \times 7\text{H}_2\text{O}$), i.e. Ni 5, Ni 10 and Ni 20 mg/dm^3 . In Ni 5 mg/dm^3 samples mainly adaptation changes to the conditions of stress were observed. These changes were manifested by the increase of cytoplasm content and by cytoplasm vacuolization, by the increase of nucleus and nucleolus volume, nucleolus vacuolization, the increase of plasmalemma invaginations and of the amount of rough ER, by the central arrangement of smooth ER and of the thylakoids of chloroplasts; it was also shown by the growth of the number of mitochondria and of peroxisomes in the cell. In Ni 10 mg/dm^3 samples, apart from adaptation changes, such as the increase of the nucleus volume, increase of plasmalemma invaginations, cytoplasm and nucleolus vacuolization, degeneration changes were also observed. They concerned mainly the nucleus (the increasing amount of condensed chromatin), ER (swelling and fragmentation of rER and sER), mitochondrion (swelling and reduction of cristae), Golgi apparatus (disintegration and decay) and chloroplasts (changes of shape, swelling and reduction of thylakoids, disappearance of starch and presence of big plastoglobuli). In Ni 20 mg/dm^3 samples cell protoplasts were in different stages of degeneration and the cell organelles that were identifiable, were usually damaged.

KEY WORDS: *Brassica oleracea* L., cell ultrastructure, leaf mesophyll, nickel.

INTRODUCTION

The excess of nickel in soil environment, especially in serpentine soil, is caused by natural processes, and also by man's activity: agricultural chemicals, industrial and urban wastes, etc. (Crooke 1956, Kabata-Pendias and Pendias 1993). Nickel in mobility forms is very easily absorbed by plants, although it has some influence on their growth and on physiological and metabolic processes (Mishra and Kar 1974, Eskew et al. 1984, Van Asche and Clijsters 1990, Bishnoi et al. 1993, Moya and Ros 1993). Excess of nickel induced visible symptoms of phytotoxicity, i.e. chlorosis and necrosis of leaves, morphological deformation of plant organs, especially of root; it also depressed the growth and the dry matter of plants (Hunter and Vergnano 1952, Anderson et al. 1973, Hara and Sonoda 1979, Spiak 1993, Szymańska and Molas 1995).

The numerous studies that have been done so far concerning the toxicity of nickel, focus mainly on the ability of plants to accumulate this metal in their natural environment and on the disturbance of metabolic and physiological processes, especially the disturbance of photosynthesis. However, very few studies are concerning the ultrastructural response of plant cells to excess of nickel [L'Huillier et al. 1996]; this response is, on the hand, a result of metabolic and physiological processes but, on the other hand, it determines them.

This paper presents the results of examination conducted by means of light and transmission electron microscope. The examination concerned the changes in the ultrastructure of outer leaf mesophyll cells of cabbage plants grown on the medium with the addition of rising excess of nickel.

MATERIAL AND METHODS

Plant culture

Seeds of cabbage [*Brassica oleracea* L.] cv. Sława from Enkhouizen, were surface-sterilized in NaOCl (ca. 1% active chlorine) for 5 minutes, rinsed in sterilized, distilled water and placed on agar medium in glass pots. In control series the medium used contained macroelements and microelements in the quantity according to Murashige and Skoog (1962) and 0.8% agar. In experimental series the basic MS medium was supplemented with nickel, added as $\text{NiSO}_4 \times 7\text{H}_2\text{O}$, in concentrations of 5, 10 and 20 mg/dm^3 . The value of the media pH was adjusted to 5.2. Seedlings growth was conducted in *in vitro* conditions, day and night, 16 and 8 h respectively, with $240 \pm 10 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ photon flux density of leaf level, in temperature 24°C.

After 16 days from seeds germination primary outer cabbage leaves were prepared from control series and from the ex-

Fig. 1-4. LM micrographs of cross sections of outer leaves of cabbage plants grown without (Fig. 1) or with nickel in concentrations of 5 mg (Fig. 2), 10 mg (Fig. 3) and 20 mg (Fig. 4) in the medium. $\times 125$.

Abbreviations for TEM micrographs shown in Figs 5-32:

V – cytoplasmic vacuole, W – cell wall, Vs – vesicle, PM – plasmalemma, PS – periplasmic space, T – tonoplast, rER – rough endoplasmic reticulum, sER – smooth endoplasmic reticulum, R – ribosomes, L – lysosomes, N – nucleus, Nu – nucleolus, P – peroxisomes, IM – chloroplast intra membranes (thylacoids), S – starch, F – myeline figure, CH – chloroplast, MV – multivesicular body.

perimental series with 5 and 10 mg Ni/dm³, and the samples for microscope examination were collected. From the experimental series with 20 mg Ni/dm³ the samples of outer leaves were collected after 21 days from seeds germination, because their development was delayed.

Microscopic study

Samples of leaf tissues were cut out from the middle parts of leaf areas of the control and of Ni-treated plants. The samples were fixed in 3.5% glutaraldehyde solution buffered in 0.05 M sodium cacodylate (pH 6.9) for 12 h at room temperature. Then the samples were postfixed in 2% OsO₄ (similarly buffered) for 1 h, dehydrated in graded ethanol and embedded in Araldit resin. For light microscopy semithin sections were obtained with the LKB ultramicrotome and stained with azure B and toluidyne solutions. They were examined and photographed by means of light microscopy (Leica DM RBE). For electron microscopy purposes ultrathin sections were obtained with the Reichert-Jung Ultracut E ultramicrotome and contrasted with 8% uranyl acetate as well as Reynolds lead citrate solutions for 15 minutes. Finally, they were examined and photographed with Tesla BS 613 transmission electron microscope.

RESULTS

Light microscopy

Light micrograph cross sections of cabbage leaves revealed differences among the samples examined [Figs 1-4]. As shown by Figs 1-3, the size of Ni-treated leaf mesophyll cells and intercellular spaces (Ni 10 mg and Ni 20 mg samples) were smaller than those of the control leaves. Those parameters in Ni-treated plants (Ni 5 mg) were almost the same as in the control [Figs 1, 2]. Examination of cross sections of those plant leaves treated with 5 and 10 mg of Ni (observed by means of LM) showed that their mesophyll cells had more cytoplasm than the cells in the control; the cells of Ni 20 mg sample had very little cytoplasm and when they were examined by means of light microscope, they gave the impression of being optically empty [Figs 1-4]. The examined samples differed also with respect to the number of chloroplasts. When the concentrations of nickel in cabbage plants medium was increasing, the number of chloroplasts was decreasing [Figs 2-4].

Fine structure

The ultrastructural examination of cabbage leaf mesophyll cells was carried out in the presence of increasing concentration of nickel; the studies focused mainly on the comparison of ultrastructural responses of the individual cell components to various degrees of the concentration of this metal.

Cytoplasm

The mesophyll cells of the examined plant samples had a different amount of cytoplasm; the electron density of cytoplasm and the degree of vacuolization was also different in particular samples [Figs 5-7]. Ni 5 mg sample had the biggest amount of cytoplasm and its electron density was also greatest [Fig. 5]. Ni 10 mg sample had less cytoplasm and its electron density was smaller [Fig. 6]. Ni 20 mg sample had the least amount of cytoplasm; the electron density of this cytoplasm was also the smallest [Fig. 7]. Ni 5 mg sample and Ni 10 mg sample contained more cytoplasm than those in the control series, whereas the cells of Ni 20 mg sample contained

less cytoplasm than those in the control. Cytoplasm was vacuolized only in Ni 5 mg and Ni 10 mg samples; it was vacuolized to the biggest degree in Ni 10 mg sample [Figs 5, 6]. Electron-dense deposits were identified in some cytoplasmic vacuoles [Fig. 8]; in some cases they were surrounded by membranes.

Plasma membranes

In all the examined samples (Ni 5 mg, Ni 10 mg, Ni 20 mg) plasmalemma formed invaginations [Figs 9-12] and, as a result, periplasmic space was identified in many mesophyll cells between the cell wall and plasmalemma [Fig. 10]. In numerous cells, especially in Ni 10 mg and Ni 20 mg samples, plasmalemma was associated with electron-dense granules or occupied with electron-dense precipitates [Figs 9-12]. Electron-dense granules and vesicles (which membranes were associated with electron-dense precipitates) were identified on the exoplasmic and endoplasmic side of plasmalemma [Figs 10, 11]. In some cells the invaginations of plasmalemma terminated with vesicles which originated in the process of endocytosis [Fig. 12]. The tonoplast that surrounded the vacuole was shrunk to a smaller extent in Ni 5 mg sample and to a greater extent in Ni 10 mg sample. In many cells of both samples the tonoplast was occupied by electron-dense material [Figs 13, 14]. The electron-dense deposits were identified in the vacuole of many cells. The tonoplast in the cells of Ni 20 mg sample was not identified in most cases, and if it was, it was disintegrating.

Endoplasmic reticulum

Rough endoplasmic reticulum (rER) was identified only in the cells of Ni 5 mg and Ni 10 mg samples [Figs 15, 16]. In Ni 5 mg sample it was more numerous than in the control cells. In Ni 10 mg sample rER membranes were swollen [Fig. 16] and they were often identified as short fragments in the cytoplasm. RER was not identified in the cells of Ni 20 mg samples. In the cytoplasm of cells of Ni 5 mg and Ni 10 mg samples free ribosomes were identified [Figs 15, 16]; they were more numerous in Ni 5 mg sample. Smooth endoplasmic reticulum (sER) was identified in the cells of all examined samples; in Ni 20 mg sample it was very rare and had the shape of short, swollen fragments. In Ni 5 mg sample sER membranes were not swollen in most cases. Their arrangement was usually parallel. In Ni 10 mg sample sER membranes were swollen and fragmented. Their arrangement was usually parallel [Fig. 18] or central (and their structure was often similar to myelin figures) [Fig. 17]; the cup-shape arrangement was often observed [Fig. 18].

Nuclei and nucleoli

Generally, in mesophyll cells of Ni 5 mg and Ni 10 mg samples examined by TEM the nuclei were bigger than in the control. In both samples the nucleus was located close to the cell wall or in the cell center. In Ni 5mg sample the nucleus was usually round, only seldom was it layered, and in Ni 10 mg sample it was prolate or layered and only seldom round [Figs 19-21]. In both samples the nuclei contained dispersed chromatin and there were few clusters of condensed chromatin in circumference [Figs 19-21]. The nucleoli were more compact and electron-dense in Ni samples than in the control. In both samples numerous nucleoli were associated with 1 or 2 nuclear organizer regions (NOR) [Figs 20-21]. In Ni 5mg sample few nucleoli usually contained only one vacuole [Fig. 19]; in Ni 10 mg sample numerous nucleoli were vacuolized

Fig. 5-7. TEM micrographs showing content, density and vacuolization degree of cytoplasm of plants treated with Ni 5 mg (Fig. 5), Ni 10 mg (Fig. 6) and Ni 20 mg (Fig. 7). x 6 000.

Fig. 8. TEM micrograph showing cytoplasmic vacuole with electron-dense deposits (arrowheads). x 14 000.

(contained 2-3 vacuole). In Ni 20 mg sample cells, the nuclei were prolate and often shrunk [Fig. 22]. They contained some dispersed chromatin but also quite a lot of condensed chromatin [Fig. 22]. The nucleolus in Ni 20 mg sample was either in the process disintegrating or it had the biggest electron density of all examined samples.

Mitochondria

The examined samples showed a different number of mitochondria in one mesophyll cell of a leaf; their structure also varied. In Ni 5 mg and 10 mg samples the mitochondria were more numerous than in the control; Ni 5 mg sample contained the largest amount of mitochondria. Ni 20 mg sample contained few mitochondria. In all examined samples the mitochondria were bigger than in the control. They were most swollen in Ni 10 mg and Ni 20 mg samples, their shape was round, or prolate and sometimes they were deformed [Figs 23, 24]. In

Ni 5 mg and 10 mg samples the electron density of mitochondrion matrix was bigger; to a greater extent in Ni 10 mg sample and to a smaller extent in Ni 20 mg sample [Figs 15, 23, 24]. Only in Ni 10 mg and Ni 20 mg samples the reduction of the number of interior cristae was observed; the reduction was biggest in Ni 20 mg sample, where their arrangement was often accidental [Fig. 23].

Chloroplasts

Chloroplasts of cabbage plants treated with 5 mg of Ni were generally similar to those in the control, with respect to the size, shape and internal structure. These plastids usually contained starch, and their thylakoids had the typical arrangement of grana and stroma lamellae [Figs 25, 26]. In few chloroplasts the reduction of the number and size of grana (stocked thylakoids) and the growth in number of not combined lamellae which often, had a central arrangement was observed

Fig. 9-12. TEM micrographs showing invaginations of plasmalemma and presents of the electron-dense granules and percipitates (arrows), vesicles (arrowheads), which membranes were associated with electron-dense granules and percipitates (Fig. 11), and vesicles which ended plasmalemma invaginations (Fig. 12). x 24 000 (Fig. 9-11); x 32 000 (Fig. 12).

Fig. 13-14. Tonoplast occupied by electron-dense material in leaf mesophyll cells of plants treated with Ni 5 mg (Fig. 13) and Ni 10 mg (Fig. 14). x 18 000; x 10 000.

Fig. 15-18. TEM micrographs showing arrangement of ER in mesophyll cells of plants treated with Ni 5 mg (Fig. 15), Ni 10 mg (Fig. 16-18). Fig. 15 – parallel arrangement of rER, Fig. 16 – swelling and fragmented rER cisternae, Fig. 17 – concentric arrangement of sER (which formed structure similar to myeline figure), Fig. 18 – parallel (sER) and cup-shape (thick arrows) arrangement of smooth ER, L – probably late lysosomes. x 18 000 (Fig. 15, 16); x 24 000 (Fig. 17, 18).

Fig. 19-22. TEM micrographs showing shape and intra structure of nuclei and nucleoli in mesophyll cells of plants treated with Ni 5 mg and Ni 10 mg (Fig. 19-20), and Ni 20 mg (Fig. 22). Thick arrows indicate condensed chromatin. x 10 000 (Fig. 19-21); x 8 000 (Fig. 22).
Fig. 23-24. TEM micrographs showing shape and intra structure of mitochondria with Ni 20 mg sample (Fig. 23) and with 10 mg sample (Fig. 24). Thick arrow indicates reduced cristae. x 24 000; x 18 000.

[Fig. 26]. The chloroplasts of Ni 10 mg sample were usually rounded, sometimes even spherical, and their intra membranes were swollen and reduced [Figs 27, 28]. The plastids contained big plastoglobuli and some starch [Fig. 27]. The chloroplasts of Ni 20 mg sample were small, spherical or prolate, shrunk and often deformed [Figs 29-31]. They contained few swollen not combined lamellae, plastoglobuli and big osmophilic substance whose shape similar to that of starch grain [Fig. 30], but whose electron density was definitely different [Figs 27, 30].

Other structures

In Ni 10 mg sample cells lysosomes were very seldom identified. In Ni 20 mg sample cells there were more lysosomes. In Ni 10 mg sample these organelles had the shape of the so-called electron-dense body or multivesicular body, and in Ni 20 mg sample late lysosomes were identified only as the so-called multivesicular body [Fig. 32]. Undamaged Golgi apparatus was observed only in 5 mg sample cells. In Ni 10 mg and Ni 20 mg samples Golgi apparatus was very seldom identified and if it was, it was severely damaged, and a gradual disintegration of its dictyosomes was observed [Figs 16, 31]. The organelles were characterized by compactly stocked, short cisternae, and small or no vesicles; sometimes these organelles were cup-shapes [Figs 16, 31]. Microbodies such as peroxisomes were more numerous than in the control and they exist side by side with chloroplasts in Ni 5 mg sample [Fig. 25]. In Ni 10 mg sample they were observed very seldom and there were none at all in Ni 20 mg sample. In Ni 10 mg and Ni 20 mg samples of cabbage leaf mesophyll cells numerous structures similar to those of the so-called myelin figures were identified [Figs 17, 31].

DISCUSSION

The changes in the ultrastructure of the components of leaf mesophyll cells of cabbage plants exposed to excess of nickel depended on the concentration of this metal and they were usually adaptative changes to the conditions of stress and/or degeneration changes. Some of these changes were similar to those that take place in the process of physiological ageing of leaf cells. Cytoplasm vacuolization, central arrangement of intracellular membranes, the increase of plasmalemma invaginations, the increase of nucleus volume and nucleolus vacuolization, the increase of the amount of ER rough in a cell – such changed were observed in the cells of plants that were exposed to different kinds of environmental stress (Čiamporová and Mistrík 1993, Woźny 1995); these changes were sometimes considered to be adaptative ones. These changes were also observed in leaf mesophyll cells of cabbage plants exposed to nickel in concentration of 5 mg/dm³; some of these changes were observed in cells of cabbage plants exposed to nickel in concentration of 10 mg/dm³. Cytoplasm vacuolization is one of the ways a cell uses to adapt to changing osmotic conditions (Hajibagheri et al. 1985, 1987). The increase of plasma membrane invaginations is a good way to increase the surface of membranes, which, in turn, ensures the proper progress of processes connected with them (Yeo et al. 1977, Landsberg 1986, Čiamporová and Mistrík 1993). The increase of nucleus volume under stress conditions is interpreted in different ways, however in most cases it is closely connected with increase of cell metabolic activity (transcription and translation processes, and protein synthesis) (Kłyszczko-Stefanowicz 1995, Woźny 1995). The more so, that it is accompanied by the increase of the amount of rER (it was ob-

served in Ni 5 mg sample) the presence and quantity of which is an indicator of the intensity of protein synthesis in a cell. Naturally, as a reaction to stress many proteins are synthesized in plant cells, including the so-called stress proteins (i.e. HSP), or the peptides which take active part in the transport of metal, i.e. phytochelatins (Cooper and Ho 1987, Vartanian et al. 1987, Porankiewicz and Gwóźdź 1993, Tukiendorf 1993). Phytochelatins take part in the process of transporting heavy metals, including nickel, from cell cytoplasm to vacuole (Tukiendorf 1993, Salt and Rauser 1995). It needs to be emphasized that nickel with phytochelatins, with free amino acids (such as histidine) and with organic acids (such as citric acids) forms intracellular chelate complexes thus appearing in chemical forms that are less toxic than ionic form of nickel [Tukiendorf 1993, Krämer et al. 1996, Lee et al. 1977, 1978]. The ability to chelate metal ions in a cells is one of the main mechanisms of its intracellular detoxification [Lewitt 1972, Woźny 1993]. Nucleus vacuolization ensures a balance between rRNA synthesis and its transport to cytoplasm under stress conditions (Fransolet et al. 1979, Čiamporová and Mistrík 1993).

The increase of cytoplasm amount in cells (which was observed in Ni 5 mg and Ni 10 mg samples) may point to the increase of their metabolic activity and also to the change of plasma membrane permeability and to the disturbance of electrolyte-water balance (Kawiak et al. 1995). It seems that in Ni 5 mg sample cells the increase of cytoplasm amount may have resulted from the increased metabolic activity of the cells, which is further indirectly confirmed by the number and the ultrastructure of their organelles. In Ni 10 mg sample cells the increase of cytoplasm amount may have been a results of damage and plasmalemma permeability change, although it is hard to eliminate the increase of cells metabolic activity. In this sample (Ni 10 mg), apart from adaptative changes in the cells such as cytoplasm vacuolization, the increase of plasmalemma invaginations, the increase of nucleus volume and nucleolus vacuolization, many changes degeneration type were also observed. These changes affected ER (swelling and fragmentation rER and sER), Golgi apparatus (damage and disintegration), mitochondrion (swelling, increase of matrix density, reduction of the number of cristae), chloroplasts (change of shape, intra membranes swelling and reduction, presence of big plastoglobuli) and the nucleus and nucleolus (increase of condensed chromatin contain, increase of nucleolus density). The presence of many structures similar to myelin figures which originate as a result of cell organelles disintegration (Kawiak et al. 1995) confirms the presence of disintegration processes in the cells of this sample and also the presence of quite numerous lysosomes. It also needs to be said that Ni 10 mg sample cells were deprived of repair mechanisms that was revealed by the damage of rER and sER, by the damage and disintegration of Golgi apparatus, by the increase of the amount of condensed chromatin in nucleus. Mesophyll cells protoplasts of plants treated with 20 mg Ni were degenerated to a different degree, they contained a lot of late lysosomes and those organelles that were identifiable, were seriously damaged. Degeneration changes in plant cell ultrastructure (leaf, root) of different plant species, similar to the degeneration changes that were observed in Ni-treated cabbage plants, have generally been observed under different environmental stress conditions, including caused by the presence of such metals as: Pb, Cd, Cu, Hg (Heumann 1987, Ouzounidou et al. 1992, Čiamporová and Mistrík 1993, Woźny 1995). The characteristic presence of electron-dense precipitates or electron-dense substance on cell plasma membranes in all

Fig. 25-30. TEM micrographs showing shape and intra structure of chloroplasts of plants treated with Ni 5 mg (Fig. 25, 26), Ni 10 mg (Fig. 27, 28) and Ni 20 mg (Fig. 29, 30). Thick arrows indicate plastoglobuli, thin arrow indicates electron-dense deposit (shape like starch grain). x 18 000 (Fig. 25-29); x 24 000 (Fig. 30).

Fig. 31. Leaf mesophyll cell of plant treated with Ni 20 mg, containing damaged chloroplasts, mitochondria, Golgi apparatus and myeline figures. x 14 000.

Fig. 32. Multivesicular bodies in degenerative mesophyll cells of plants treated with Ni 20 mg. x 18 000.

examined samples points to the transport of this metal from cell apoplast to cell cytoplasm, i.e. probably by means of endocytosis (the presence of vesicles that originated in the process of endocytosis confirm these suggestion). It is known, that nickel is bound to plasmalemma and it can be transported actively or/and passively to cytoplasm across plasmalemma (Morgutti et al. 1981, Körner et al. 1986); however, it is transported from cytoplasm to vacuole by means of phytochelatines (Tukiendorf 1993). In Ni 5 mg and Ni 10 mg samples electron-dense deposits were identified mainly in vacuole and in cytoplasmic vacuoles, i.e. in the structures that are non-active metabolically. It needs to be said that the deposit of toxic substances in non-active metabolically cell structures is one of the mechanisms of plant tolerance to chemical stress factor (Lewitt 1972, Baker 1981).

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ULTRASTRUKTURALNA ODPOWIEDŹ KOMÓREK MEZOFILU
ZEWNĘTRZNEGO LIŚCIA ROŚLIN KAPUSTY (*BRASSICA OLERACEA* L.) NA NADMIAR NIKLU

STRESZCZENIE

Metodą mikroskopii świetlnej i elektronowej badano zmiany w strukturze i ultrastrukturze komórek mezofilu pierwszego, zewnętrznego liścia roślin kapusty [*Brassica oleracea* L. cv. Sława z Enkhoiuzen] rosnących na podstawowej pożywce MS z dodatkiem nadmiarowych koncentracji niklu ($\text{NiSO}_4 \times 7\text{H}_2\text{O}$), tzn. 5, 10 i 20 mg/dm^3 . W obecności 5 mg Ni obserwowane zmiany wykazywały charakter zmian adaptacyjnych do warunków stresu. Przejawiały się one we wzroście zawartości cytoplazmy w komórce i jej wakuolizacji, wzroście objętości jądra i jąderka, wakuolizacji jąderka, wzroście inwaginacji plazmalemmy i ilości błon szorstkiej siateczki śródplazmatycznej (rER), centrycznym układzie błon gładkiej siateczki śródplazmatycznej (sER) oraz błon tylakoidów chloroplastów, jak też we wzroście liczby mitochondriów i peroksysomów w komórce. W obecności 10 mg Ni obok zmian adaptacyjnych (takich jak: wzrost objętości jądra, wakuolizacja cytoplazmy i jąderka, wzrost inwaginacji plazmalemmy) obserwowano zmiany o charakterze degeneracyjnym. Były one obserwowane na terenie jądra (wzrost ilości chromatyny zwartej), rER i sER (spęczniecie i fragmentacja błon), mitochondrium (spęczniecie matriks i redukcja wewnętrznych błon), aparatu Golgiego (dezintegracja i zanik) oraz chloroplastów (zmiana kształtu, spęczniecie i redukcja błon tylakoidów, redukcja skrobi, obecność dużych plastoglobul). W obecności 20 mg Ni protoplasty komórek były w mniej lub bardziej zaawansowanym stadium degeneracji, a identyfikowane nieliczne organella komórkowe były uszkodzone.

SŁOWA KLUCZOWE: *Brassica oleracea* L., mezofil liścia, nikiel, ultrastruktura komórki.