THE EFFECT OF RADIONUCLIDES AND HEAVY METALS ON THE ULTRASTRUCTURAL FEATURES OF LEAVES IN *HELIANTHUS ANNUUS*

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Key words: heavy metals; radionuclides; Helianthus annuus; leaf ultrastructure.

ABSTRACT

The ultrastructural features of the leaf in mature plants of Helianthus annuus (during flowering), cultivated in three different areas: in normal culture (Floresti village, Cluj district) and in cultures on sites polluted with heavy metals and radionuclides: (a) near the power station Turceni and (b) on sterile waste dump in the Pedological Research Station of Rovinari (Gorj district), were analyzed. The soil analysis in the three sites showed a higher content in radionuclides and heavy metals, especially on the soil from Turceni, as compared to Control. The ultrastructural analysis pointed out the effect of the presence of high amounts of radionuclides and heavy metals, as well as the plant reaction: the parietal disposition of the heterochromatin in the nucleus in the shape of ***bodyguard****, synthesis and accumulation of some substances (probably with antioxidant or chelating role) in vacuoles, which interacted with exogenous particles, a/o.*

INTRODUCTION

The *Helianthus annuus* (*Asteraceae*) is an important oilseed crop all over the world. This species has also been traditionally used as an antiinflammatory, antimalarial, antiasthmatic, antioxidant, antitumoral and antimicrobial agent, for thousands of years (Saini and Sharma, 2011), as well as a cosmetics plant. Research studies have shown that it can prevent cancer and many more harmful diseases (Saini and Sharma, 2011). Medicinal plants contain various phytochemicals that are very important to human life for the treatment of various diseases. Antioxidants present in the plants play an essential role in protecting the cells and tissues against damage caused by reactive oxygen species (ROS). Plants containing flavonoids have been reported to possess strong antioxidant properties (Ara and Nur, 2009). Previous studies have shown that *Helianthus annuus* has an antimicrobial activity and an antioxidant capacity (Singh et al., 2010), playing an important role in the protection of cells and tissues against damages caused by reactive oxygen species. A common consequence of heavy metal toxicity is the excessive accumulation of reactive oxygen species and methylglyoxal (MG), both of which can cause peroxidation of lipids, oxidation of proteins, inactivation of enzymes, DNA damage and/or interaction with other vital constituents of plant cells. The heavy metals that enter the cells may be captured by amino acids, organic acids, glutathione (GSH), or by specific metal-binding ligands (Hossain et al., 2012). Some adulterations in the organelle ultrastructure and their amount in the cell can offer information about of the metabolic state of the cell. Angaji et al. (2012) synthesized the actual knowledge about antioxidants. Subashini and

Rakashitha (2012) analyzed the phytochemical screening, the antimicrobial and antioxidant activities of crude extracts from seeds of *Helianthus annuus* in order to explore new bioactive compounds belonging to this species. Kumar and Gowda (2010) have recently determined that there are polyphenols, flavonoids, proteins, α-tocopherol, carotenoid pigments, such as lutein, a/o, in petals of *Helianthus annuus*. Lutein is used in preventive medicine against diuresis, diarrhea and several inflammatory diseases.

In the investigations carried out in the POLMEDJIU grant, the capacity of different plant species to have phytoremediatory properties, as well as the interaction of heavy metals or radionuclides with cellular organelles (Corneanu, 2011), were analyzed. This paper presents the investigations carried out in *Helianthus annuus* plants developed on Control site, unpolluted, or in two polluted areas with a high amount of heavy metals and radionuclides. The interaction of heavy metal particles with cellular organelles was analyzed, as well as the plant reaction of defense against these stress factors (great amount of heavy metals and radionuclides).

MATERIAL AND METHOD

Biological material. The investigations were performed on mature plants of *Helianthus annuus* (during flowering) developed in normal culture on unpolluted soil (Control, Floresti village, Cluj district) and in two areas polluted with heavy metals and radionuclides in the Gorj district on the middle Jiu river valley. The two polluted area were: (a) near the power plant Turceni and (b) on a sterile waste dump in the Rovinari Research Station. The radionuclide and heavy metal content of the soil was analyzed, as well as the ultrastructural features of the leaves.

Ultrastructural features of the leaves were established in mature plants (during flowering). Leaf fragments of about 1 mm^3 were prefixed in a 25% glutaraldehyde solution (2 ½ h), postfixed in a 1% Millonig solution (1 ½ h) and then imbibed and infíltrated in EPON 812. Seriated sections about 80-90 nm thick were contrasted with uranyl acetate and lead citrate. The examination was made by a TEM JEOL-JEM 1010 apparatus, in the Electron Microscopy Center, *Babes-Bolyai* University of Cluj-Napoca.

Radionuclide activity (Bq/kg) was determined by the Duggan method, with the help of a gamma spectrometer with HPGe detector, plan crystal and Be fenster; mixed testing and ISOCS Canberra program; matrix sources from IAEA-Vienna (Duggan, 1999).

Heavy metal amount (mg/kg) from the soil samples was determined by the spectrophotometric method. The spectrophotometry with atomic absorption, with solution atomization in the air-acetylene flame was used.

RESULTS AND DISCUSIONS

The soil pollution degree. In the middle Jiu river basin, the coal mining and energy industries determined the soil remodeling, by bringing to the soil surface a great amount of rock and setting up sterile and ash waste dumps, as a result of the activity of the two power plants, Turceni and Rovinari.

As compared to the values recorded in Control, the radionuclide activity has higher values on the site from Turceni power plant and on the sterile waste dump from Rovinari, except Ra-226 (Table 1).

Table 1.

The activity of some radionuclides in soil samples (Bq/kg soil)

Site / Radionuclide	Floresti-Cluj	Rovinari-Station	TEPP-Turceni
Th-234 / U-238	12.7 ± 5.2	55.4 ± 6.0	43.75 ± 4.92
Ra-226	38.2 ± 9.12	25.3 ± 1.4	28.3 ± 0.8
Pb-210	18.8 ± 1.67	42.1 ± 14.1	40.84 ± 6.07
Bi-214	13.3 ± 0.61	24.9 ± 1.5	26.3 ± 0.85

Pb-214	13.7 ± 0.35	26.2 ± 1.4	29.5 ± 0.77
U-235	2.31 ± 0.56	4.95 ± 0.6	4.82 ± 0.86
Ac-228 / Th-232	23.5 ± 3.12	35.5 ± 2.0	39.6 ± 4.49
Pb-212	23.7 ± 0.65	39.5 ± 2.8	45.5 ± 1.22
K-40	378.1 ± 1.22	487.9 ± 18.1	533.27 ± 15.4
Be-7	< 8.59	< 14.1	< 10.1
Cs-137	3.57 ± 0.68	45.7 ± 1.47	12.6 ± 0.43

Relating to the heavy metal amount in the soil, except zinc, for all the other elements the values recorded near TEPP Turceni and Rovinari Pedological Station were much higher than the values recorded in Control (Table 2).

Table 2.

The heavy metal amount in soil (mg/kg soil)

Site	Heavy metal (mg/kg soil)								
	Zn	Cu	Fe	Mn	Pb	Ni	Cr	Co	Cd
Floresti	41.4	1.86	458	29.5	10.5	2.27	Udl.	3.44	0.035
Rovinari	58.8	19.0	22,995	422	15.6	29.6	26.1	10.3	Udl
Turceni	30.6	46.2	22,214	363	42.6	35.1	40.9	8.8	0.9

Udl = under detection limit

Ultrastructural features of the mature leaf

***Helianthus annuus* L., Control, Floresti**

The plants from Control batch have a normal ultrastructure of the leaf, with some features due to their character of phytoremediatory species.

Epidermial cells are covered with a thick cuticle. The pelicular cytoplasm has very few cytoplasmic organelles.

Parenchyma consists of polygonal cells with spaces between them (Fig. 1). The parietal cytoplasm presents numerous cellular organelles. Some chloroplast are transformed in mielinic corpuscles (Fig. 1). In the central vacuole there is a diverse matter. Between the two epidermises, there is the aerial intercellular conducting system. In this variant, the chloroplasts of lenticular shape contain numerous grana, disposed on their length. Every grana consists of numerous thylakoids (up to 25-30). Plastoglobuli are rarely present and starch grains are practically absent (Fig. 2). In the nucleus, the heterochromatine is disposed in the form of thick cords (Fig. 2). Mitochondria present a normal matrix and cristas (Fig. 2). The cells from lacuna parenchyma usually present a pellicular cytoplasm. In the central vacuole, there are matter particles (probably of exogenous origin) usually of granular and acicular shape (Fig. 3).

Central cylinder consists of fundamental parenchyma, liberian and ligneous vessels. The cells of the fundamental parenchyma are metabolically active, with a normal ultrastructure of the cellular organelles: nuclei, mitochondria, small size fusiform chloroplasts, with a few stroma thylakoids oriented on the chloroplast length.

In the parenchyma cells, there are drops of a synthesized substance, which are transferred in the conducting vessels (Fig. 4). This substance can represent a chelating or an antioxidant substance.

Helianthus annuus is a phytoaccumulator species, having the capacity of absorbing some heavy metals from the environment (Zn, Cd, Cr, Ni, As, Fe, Pb, Cu, a/o; Walliwalagedra et al., 2000; Chen and Cutright, 2001; Jadia and Fulkar, 2008, a/o). The capacity of synthesizing some substances with chelating role was exposed by some authors (Jadia and Fulekar, 2008; Hossain et al. 2012, a/o).

Dat et al. (1998) stated that the primary role of antioxidants was to prevent degradation induced by free radical reactions, as well as by other stress factors, such as heavy metals and radionuclides. Al-Qubaie (2012) analyzed the reaction of *Helianthus annuus* cv. Giza 102 to the three antioxidants, which are traps for the free radicals of ROS produced in the natural processes or as a result of mutagen factor action. Jadia and Fulekar (2009) synthesized data on the phytoremediation process of the heavy metals and established that different extractive and transport substances are involved in this process. The drops of substances noticed in the *Helianthus annuus* cell probably represent these substances.

Helianthus annuus **L., sterile waste dump, Rovinari Pedological Station**

The leaf epidermal cells are covered with a thick cuticle. In the pellicular cytoplasm there are very few cellular organelles. In the epidermal cells there is exogenous matter having an acicular shape, parietally disposed. As a reaction to the presence of exogenous matter, the metabolism of the parenchyma cells is stimulated in *Helianthus annuus* and in the parenchyma cells the number of peroxisomes is high as compared to Control (Fig. 5). Research carried out by Sandalio et al. (2001) regarding the cadmium accumulation in the pea leaves pointed out that, in the cadmium presence, the level of H_2O_2 produced in peroxisomes was double. In the presence of a moderate amount of radionuclides and heavy metals, the metabolic activity of the cell is more intense in this hyperaccumulator species. The stimulation of the metabolic activity is underlined by the presence of starch grains in chloroplasts (Fig. 7). As chloroplasts have a well represented structure, they have numerous grana groups oriented on their length. Every grana consists of 20-25 normal thylakoids. A few small size plastoglobuli and 1-2 starch grains are present (Fig. 7). The mitochondria have a normal matrix and some crystas. The nucleus has heterochromatin in its mass, as well as exogenous acicular matter (Fig. 6). The presence of a small amount of exogenous matter among the chromatin fibers probably does not affect the nucleus structure and function. The exogenous acicular matter is adherent to the inner side of the cell wall (Fig. 8). The acicular particles can penetrate the cell wall and in some cases they can break it.

Helianthus annuus **L., TEPP – Turceni**

Near the power plant Turceni, the amount of radionuclides and heavy metals is higher than in Control and on the sterile waste dump from Rovinari Pedological Station. As a consequence, the cells present some ultrastructural alterations and/or adaptations. Sandalio et al (2001) have previously reported some senescence processes of the leaves, as a result of the toxicity of the high amount of cadmium. The ultrastructural features reported in the senescent leaves were the disintegration of organelle structures, the increase of lipid peroxidation and membrane leakiness (Buchanan-Wollaston, 1997, cited by Sandalio et al., 2001), the increase of the number and size of plastoglobuli from the chloroplast (Smart, 1994, cited by Sandalio et al., 2001), a reduction of chloroplast size, the invagination of the tonoplast into the vacuole, the chromatin condensation in the nucleus, the loss of cytoplasmic components, a/o (Inada et al., 1998, cited by Sandalio et al., 2001). In this experiment the leaf cell ultrastructure of the plants from TEPP-Turceni presents some adulterated features.

As in other cases, the epidermal cells are covered with a thick cuticle. The side walls present some prolongations with the role of a strong adherence between cells. The parenchyma cells present numerous cellular organelles in the parietal cytoplasm, while the central vacuole is in the middle. There is a high number of chloroplasts with 1-2 starch grains (Fig. 9). The ultrastructural features of the cellular organelles from the parenchyma cells are affected by the presence of the high amount of heavy metals and radionuclides.

The lower epidermis is covered with a thinner cuticle (Fig. 16).

The nucleus has large blocks of parietal heterochromatin (Fig. 10), which has been previously reported by Smart (1994), Sandalio et al. (2001), a/o. A structure similar to the metabolic structure of ***bodygaurd*** type results. It has been previously described by Hsu (1975) and also noticed in numerous plant or animal species (Craciun and Corneanu, 1985). This structure represents the last barrier protecting the cell from the action of a strong stress factor.

In the vacuole of the parenchyma cell, there is a fine granular matter, spreading in the plant through conducting vessels (Fig.11). Exogenous particles are also present in the intercellular spaces.

The investigations carried out by Daud et al. (2009) in two transgenic cotton genotypes exposed to different cadmium concentrations pointed out the presence of some adulterations of the cytoplasm and nucleus in the root cells, as well as the presence of cadmium particles in vacuoles.

The chloroplasts present one or more starch grains (Figs. 9, 11), in some cases the chloroplasts being transformed into amyloplasts (Fig. 11). The presence of acicular matter near amyloplasts can suggest the presence of a metabolic relation between them (Fig. 15).

In some conducting vessels, there are numerous exogenous acicular particles (Fig. 12). Their presence induces the synthesis of a viscous substance (chelator and/or antioxidant), which immobilizes the exogenous particles and thus limits their dispersion in the folliar tissue (Fig. 13). The synthesis of this substance by the cells was also described in other researches in different plant species (Yeh et al., 2000; Chen and Cutright, 2001; Jadia and Fulekar, 2008, 2009; Al-Qubaie, 2012; Hossain et al., 2012, a/o). The high amount of acicular exogenous particles in the cell can break the tonoplast, the cell wall, as well as the cell (Figs. 14, 15). The acicular matter can be accumulated in vacuoles. This matter can penetrate in cell, through stomata (Fig. 16).

The severe effects at the leaf cell ultrastructure in *Helianthus annuus* plants developed near TEPP-Turceni (in comparison with the values recorded in the other two sites), can be due to bigger amount content in soil in some radionuclide's (Pb-212, Pb-214, Ac-228; Table 1), and heavy metal (Cu, Pb, Ni, Cr, Cd; Table 2).

CONCLUSIONS

The *Helianthus annuus* plants were cultivated in different environmental conditions: (a) Control with a normal amount of heavy metals and radionuclides, (b) sterile waste dump of the Rovinari Pedological Station, and (c) near TEPP-Turcerni, both with a great amount of heavy metals and radionuclides. The ultrastructural features of the leaf cells belonging to *Helianthus annuus* plants developed on the soil with different amounts of heavy metals and radionuclides reveal different features.

1. The synthesis of a viscous substance (with chelating or antioxidant role) takes place in some cells. It agglutinates and/or neutralizes the exogenous particles present in conducting vessels and in other cells of the leaf tissue.
2. The presence of a moderate amount of exogenous matter (heavy metals and radionuclides) intensifies the metabolic activity of the chloroplast and synthesizes starch grains (absent in the Control from Floresti, Cluj district), and later transforms chloroplasts into amyloplasts.
3. Through the parietal heterochromatinization of a high amount of nuclear chromatine, a structure of ***bodyguard*** type resulted, with the role of protecting the genetic matter from the presence of a great amount of heavy metals and radionuclides in the environment.
4. Depending on the amount of heavy metals and radionuclides in the environment, their effects on the leaf ultrastructure in *Helianthus annuus* were different: in moderate amounts they determined a stimulation of metabolism and defence systems of the cell (sterile waste

dump from Rovinari Pedological Station); in high amounts they induced adulterations of the cell organelle ultrastructure (TEPP Turceni).

Acknowledgements. This research was sponsored from the research grant POLMEDJIU, PN-II nr. 32,150/2008, by CNMP-Bucharest, Romania.

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Plate 1 *Helianthus annuus* L., Control.

Fig. 1. Parenchyma cell.

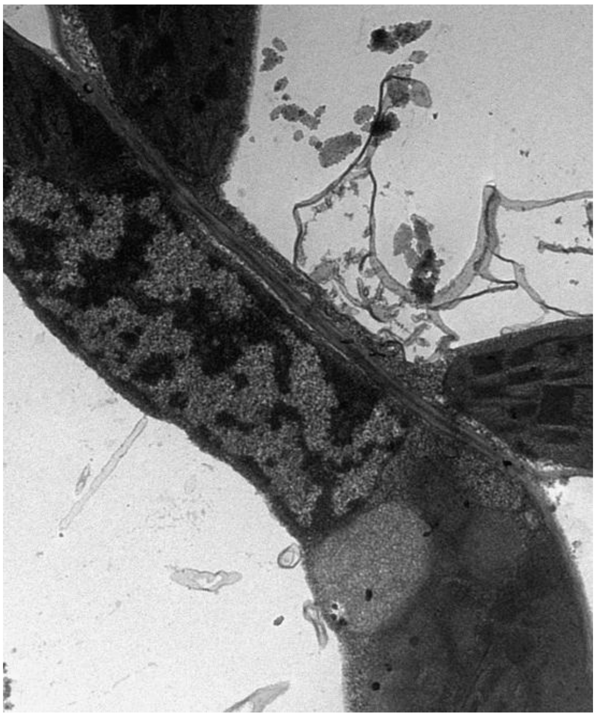

Fig. 2. Nucleus, mitochondria, chloroplast in Control.

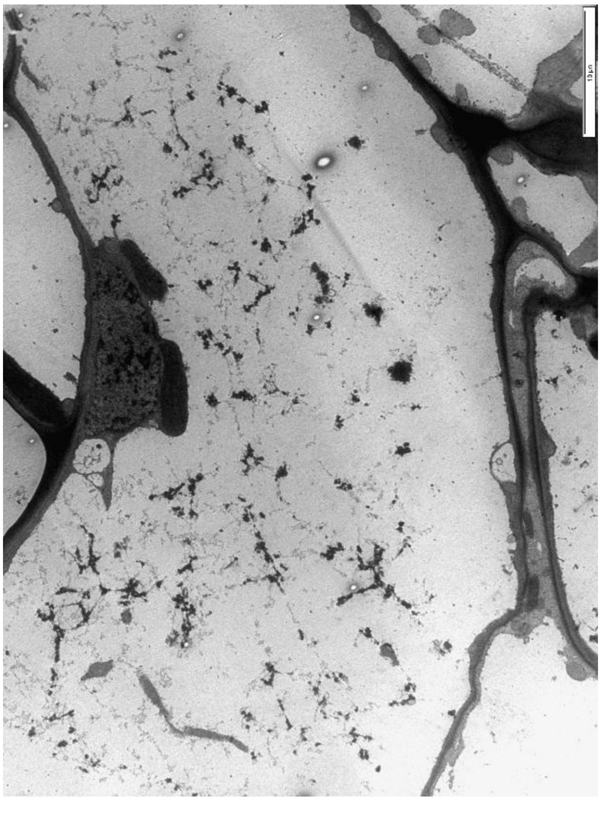

Fig. 3. Parenchyma cell with exogenous matter.

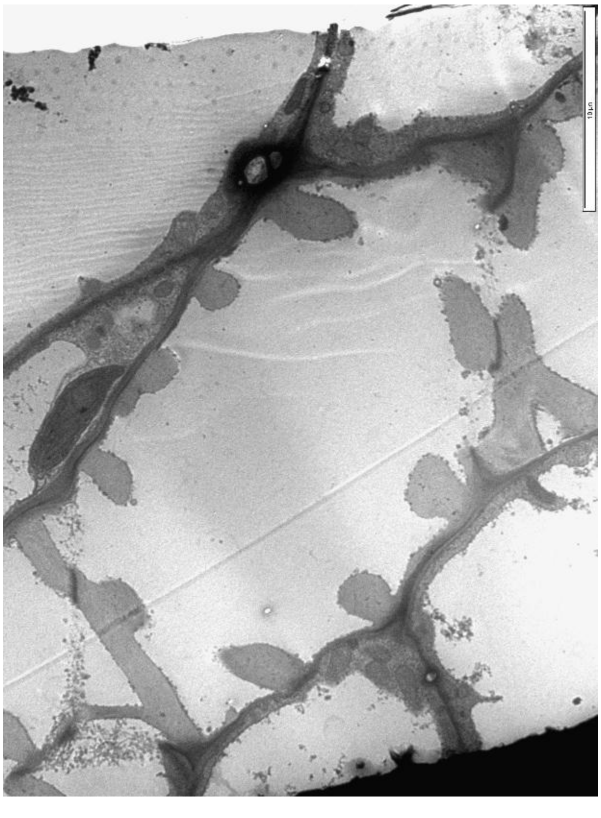

Fig. 4. Conducting vessel with drops of chelator.

Plate 2. *Helianthus annuus*, sterile waste dump in Rovinari Pedological Station.

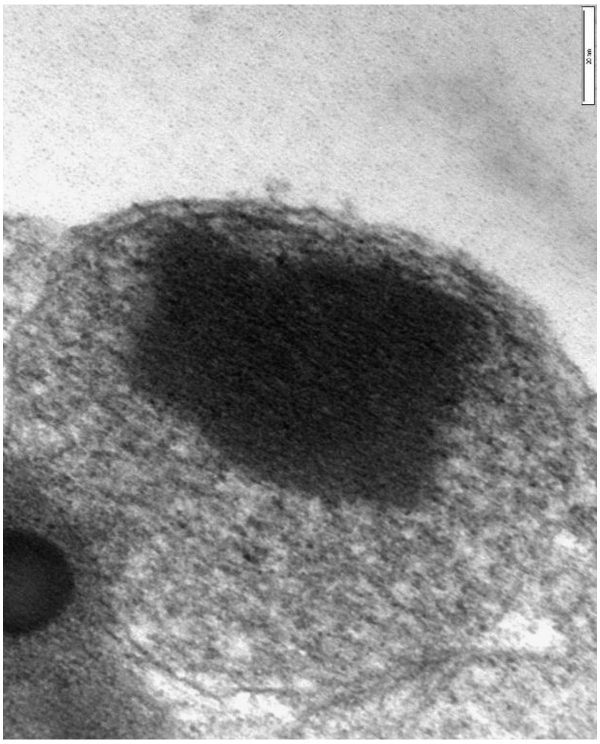

Fig. 5. Peroxisome in parenchyma cell.

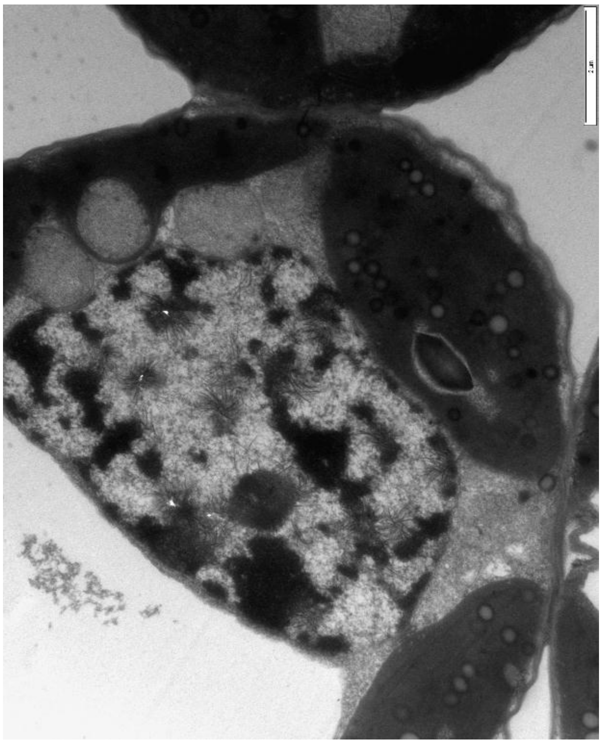

Fig. 6. Nucleus with heterochromatine and exogenous matter.

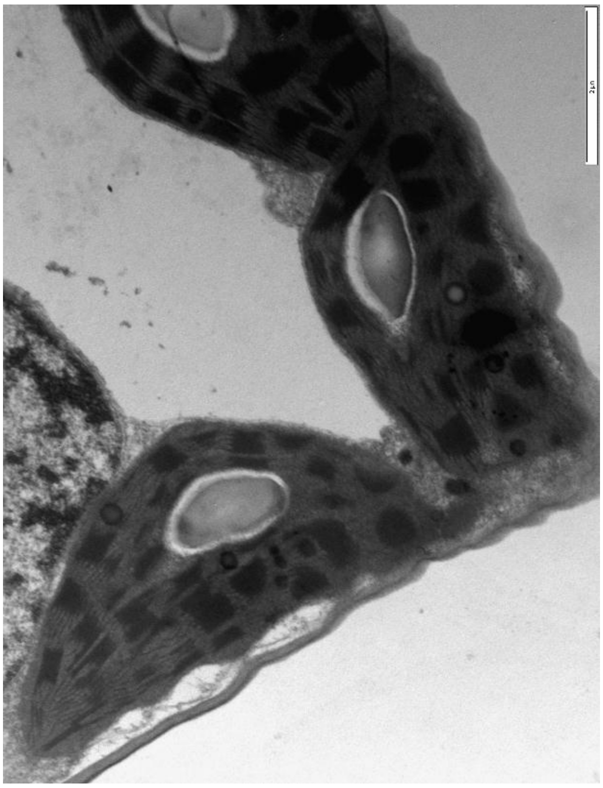

Fig. 7. Chloroplast with normal structure.

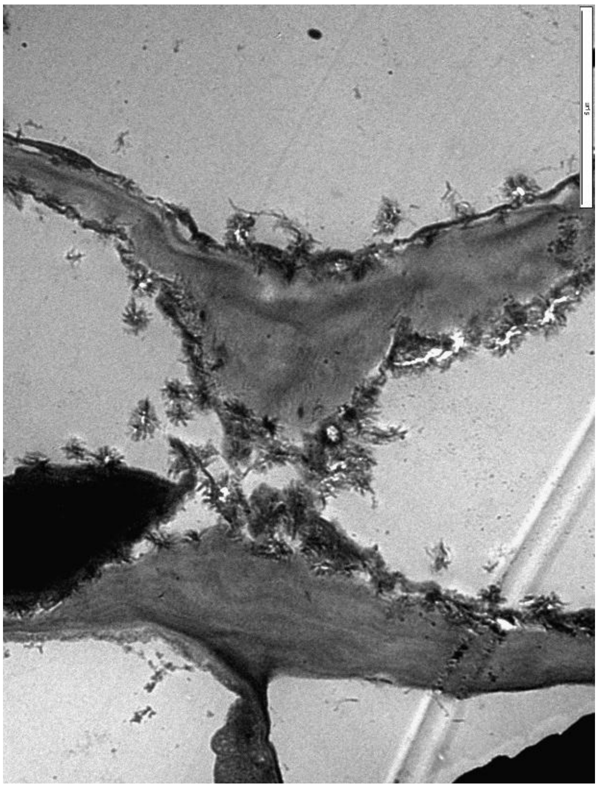

Fig. 8. Acicular exogenous matter on the cell wall.

Plate 3. *Helianthus annuus*, near TEPP – Turceni.

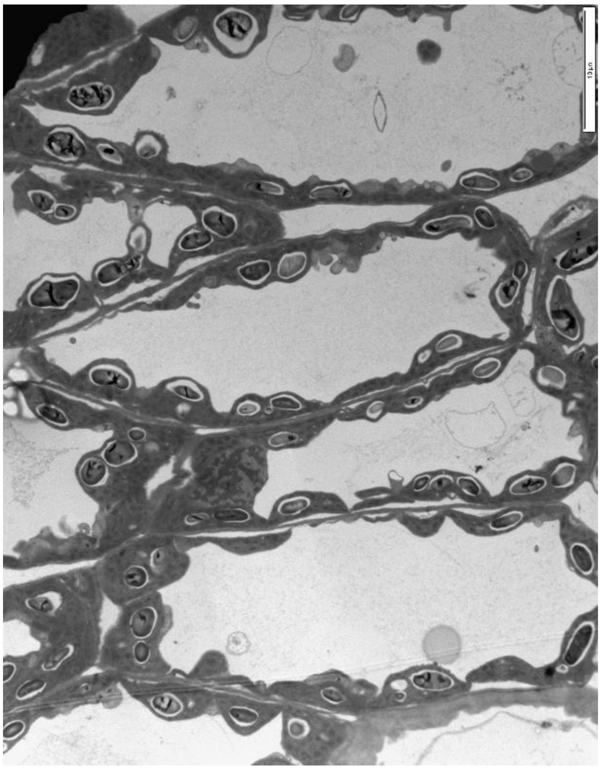

Fig. 9. Palisade parenchyma cells.

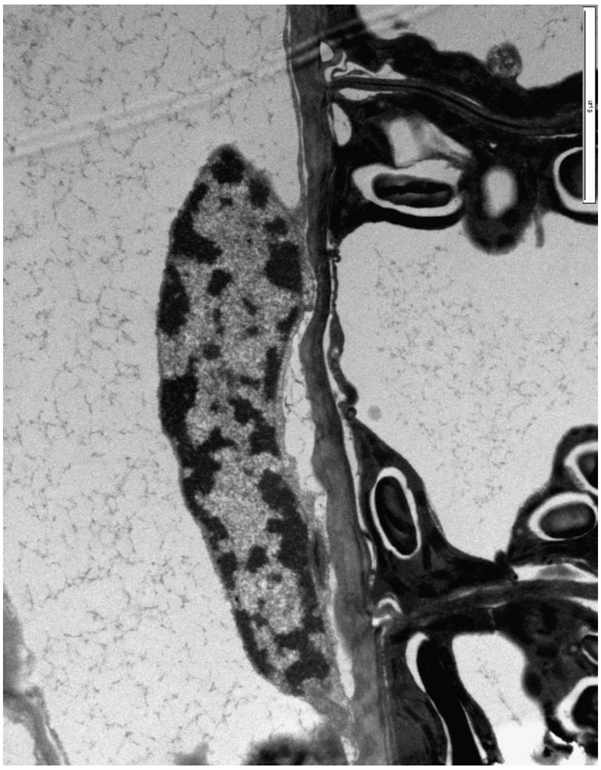

Fig. 10. Nucleus with parietal heterochromatine.

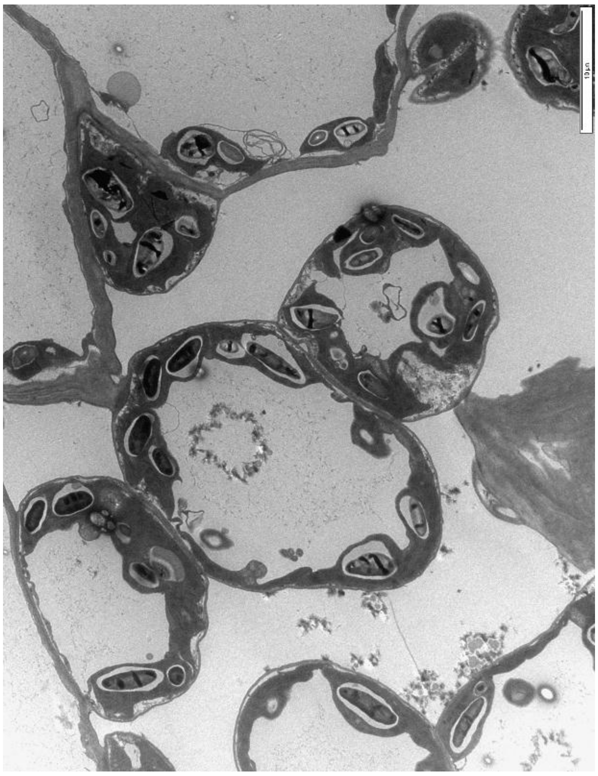

Fig. 11. Lacuna parenchyma; chloroplasts, amyloplasts.

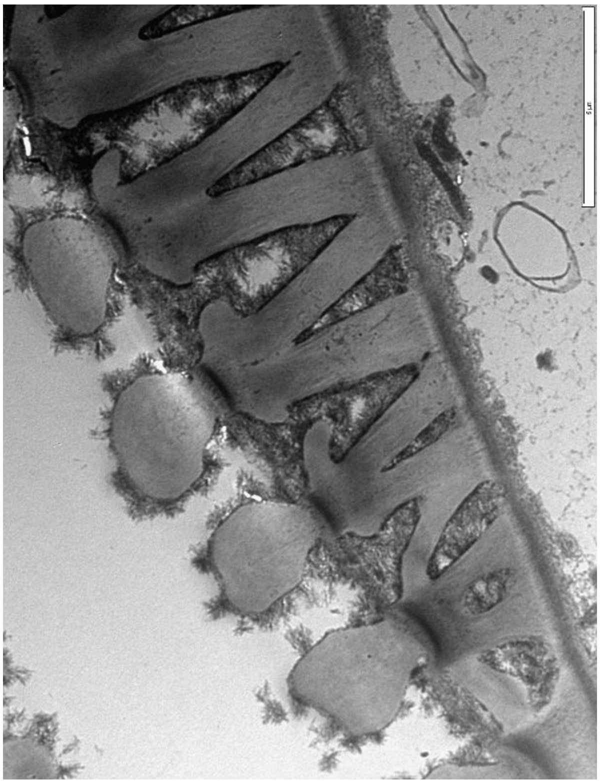

Fig. 12. Conducting vessel, chelating subst., exogenous part.

Plate 4. *Helianthus annuus*, near TEPP – Turceni.

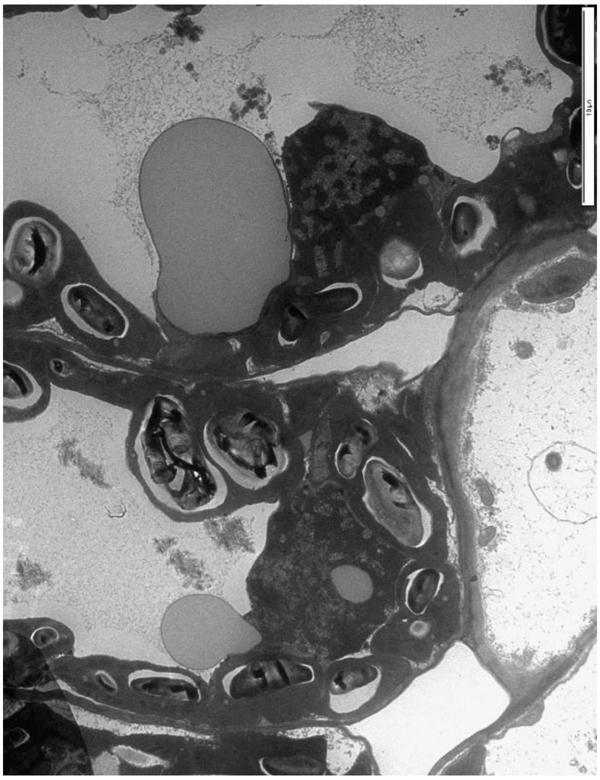

Fig. 13. Parenchyma cell; chelating substance, exogenous part.

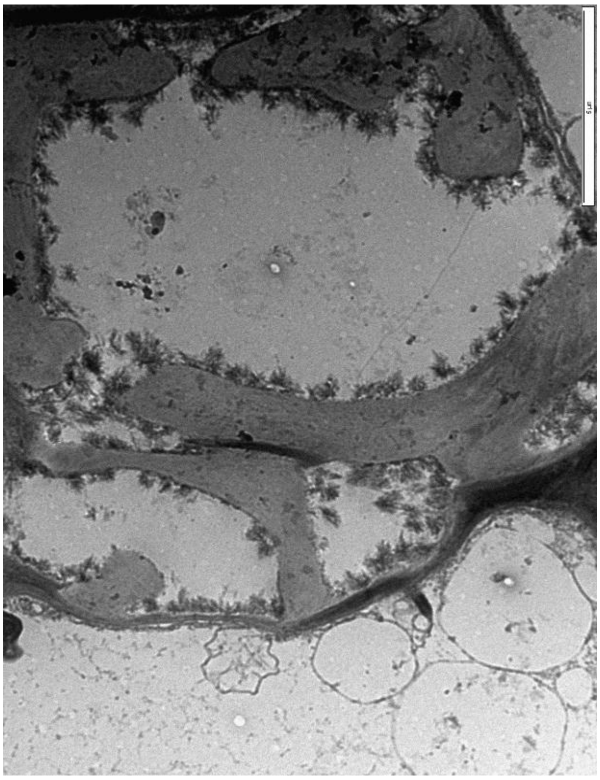

Fig. 14. Acicular particles in an affected cell.

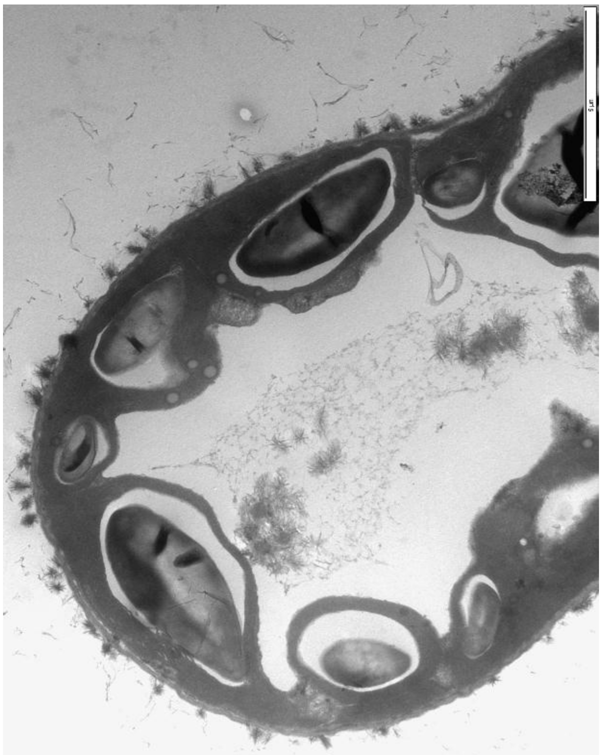

Fig. 15. Exogenous matter near amyloplast and tonoplast.

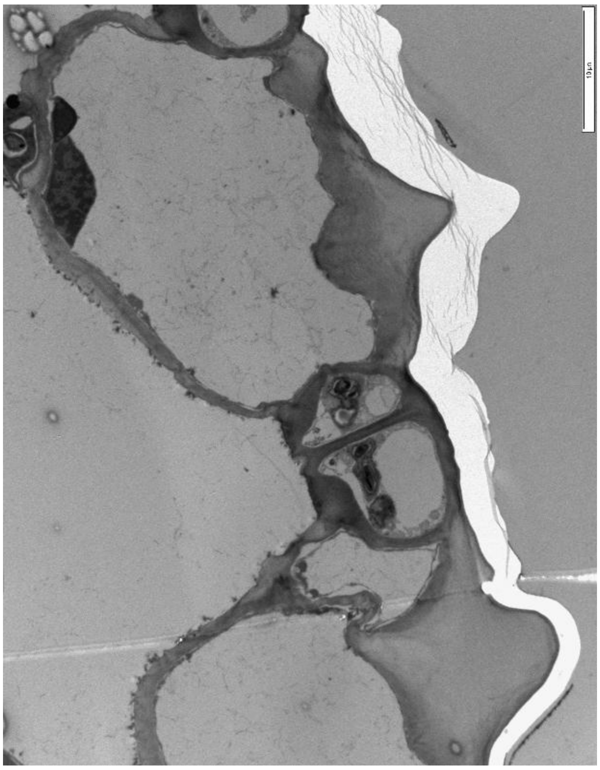

Fig. 16. Lower epidermis, stomata and exogenous matter.