ULTRASTRUCTURAL ASPECTS OF CELLULAR RESISTANCE TO POLLUTION. CASE STUDY - *Phragmites australis* (CAV.) TRIN. EX STEUD.

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Abstract. Hiperaccumulator plants possess specific pathways for adaptation and growth in hostile environments, in the presence of large amounts of exogenous material, with harmful action. Reed (*Phragmites australis* (Cav.) Trin. ex Steud), a hyperaccumulator plant for cadmium, is also resistant to other heavy metals and radionuclides. It is a species widespread on Earth's surface with a great capacity to adapt to different environment conditions. After the analysis of the ultrastructural characteristics of three reed populations, developed in natural conditions, in different locations in the Middle Jiu Valley (Gorj, Romania), containing slightly different amounts of heavy metals and radionuclides, was established a possible resistance mechanism to heavy metals. Exogenous particles of different shapes, enter in the leaf through the stomata or penetrate the cuticle and the cell wall. In leaf tissue, the exogenous material is distributed through plasmodesmata to the neighbouring cells. Reed manifests a natural resistance to some stress factors achieved by: ferritin granules synthesis, as well as of chelating substances, with role in binding metal and in the transport of exogenous materials and active metabolic pathways, that allow a cell intense activity, even in the presence of exogenous material in the nucleus or mitochondria. In addition to this, all exogenous materials can be accumulated and stored in large vacuoles, located in some deposit cells, from the lacunar parenchyma, near the vascular system and stomata annex cells.

Keywords: *Phragmites australis*, coal exploitation, cell ultrastructure, cellular adaptations for resistance to pollution.

Rezumat. Aspecte ultrastructurale ale rezistenței celulare la poluare. Studiu de caz - *Phragmites australis* (Cav.) Trin. ex Steud. Plantele de tip hiperacumulator posedă căi specifice de adaptare și creștere în medii ostile, în prezența unor cantități mari de material exogen, cu acțiune dăunătoare. Stuful (*Phragmites australis* (Cav.) Trin. ex Steud.), o plantă hiperacumulatoare pentru cadmiu, este, de asemenea, rezistentă la alte metale grele și la radionuclizi. Este o specie larg răspândită pe suprafața Pământului, cu o mare capacitate de a se adapta la diferite condiții de mediu. După analiza caracteristicilor ultrastructurale ale celor trei populații de stuf, dezvoltate în condiții naturale, în diferite locații din Bazinul Mijlociu al Jiului (Gorj, România), conținând cantități ușor diferite de metale grele și radionuclizi, s-a stabilit un posibil mecanism de rezistență la metalele grele. Particulele exogene de diferite forme, pătrund în frunză prin stomate sau penetrează cuticula și peretele celular. În țesutul frunzelor, materialul exogen este distribuit prin plasmodesmata celulelor vecine. Stuful manifestă o rezistență naturală față de anumiți factori de stres realizată prin: sinteza granulelor de feritină, precum și a substanțelor chelatoare, cu rol în legarea metalului și în transportul materialelor exogene și căilor metabolice active, care permit o activitate intensă a celulelor, chiar și în prezența materialului exogen în nucleu sau mitocondrii. În plus, toate materialele exogene pot fi acumulate și stocate în vacuole mari, localizate în unele celule de depozitare, din parenchimul lacunar, în apropierea celulelor vasculare și celulelor anexe ale stomatelor.

Cuvinte cheie: *Phragmites australis*, exploatări carbonifere, ultrastructura celulei, adaptare celulară pentru rezistența la poluare.

INTRODUCTION

The existing life forms on planet Earth are adapted to different environmental conditions (ecological niches) and in different geochemical conditions, that allow differential development. In time, some species have acquired resistance and can grow on media that are unsuitable for other species (high activity of radionuclides, toxic amounts of heavy metals, etc.). As a result of experimental investigations, many species of plants involved in the process of phytoremediation have been established, able to reduce the soil pollutants level, and thus modify their geochemical composition, so as to be favourable for human health and for the health of other species (AIT ALI et al., 2004; ALKORTA et al., 2004; WEIS & WEIS, 2004; BRAGATO et al., 2006; RAJAKARUNA et al., 2006; WANG & JIA, 2009; KUMARI & TRIPATHI, 2015; PARDO et al., 2016). These techniques were used to "clean" the environment after some natural or anthropogenic disasters (explosions at Chernobyl or Fukushima nuclear plants, earthquakes, and tsunamis, etc.). After a series of experiments conducted by IANNELLI et al. (2002), PIETRINI et al. (2003) was established that the species *Phragmites australis* (Cav.) Trin. ex Steud is a hyperaccumulator species for cadmium (Cd). In a comparative experiment on the decontamination capacity of the wastewater by *P. australis* and *Thyfa latifolia* L.*,* it was concluded that reed is more efficient for adsorbtion of copper (Cu), cadmium (Cd), chromium (Cr), nickel (Ni), iron (Fe), lead (Pb) and zinc (Zn); superior results can be obtained by combining the two species (KUMARI & TRIPATHI, 2015). PARDO et al. (2016) demonstrated the enhanced capacity of rhizofiltration for arsenic (As) of the reed, after external iron addition and iron plaque formation at the root level, in a hydroponic experiment. Researches in molecular biology have led to the discovery of genes involved in phytoremediation processes, functioning of specific metabolic pathways, synthesis of stress-protective substances. Thus, DAVIES et al. (2006) using transcriptomics technology, have identified the genes responsible for phytoremediation activity in *P. australis*. Through gradual adaptation of the parental cell line (HJ16) at a high concentration of H2O2, AL-QENAEI et al. (2013) have obtained a new resistant cell line H_2O_2 Jurkat.

Adaptation of the organisms to environmental conditions, with different geochemical composition and through different metabolic pathways, is possible through the synthesis of stress-protective substances (ferritin, etc.), genes amplification or gene mutations. Cell biology studies have shown exogenous polluting particles interaction with organelles, modification of metabolic pathways (CORNEANU, 2011).

Reed occupies disturbed, primary places and can also thrive in damp places with high acidity. It is particularly common along roads, ditches and roadside debris piles deployed, wherever there is a small depression with water. PENKO (1993) observed stunted reed plants on acidic wastes from abandoned copper mines in Vermont. Also were found near waste dumps on middle Jiu valley, in Romania (CORNEANU, 2011), and near abandoned mines in Upper Silesia, Poland (CISZEWSKI et al., 2013). Different types of changes and / or man-made damages may contribute to the spread of reed. For example, a flooding due to tidal restrictions may lead to a decrease in the mass of water that may favour reed (ROMAN et al., 1984). Also, sedimentation may promote the spread of reed, by raising the water level and effective reduction in the frequency of floods.

P. australis is a clonal herbaceous species with a concave woody stem that can grow up to 6 m tall. The leaves are lanceolate, frequently 20-40 cm long and 1-4 cm width. The flowers that form in midsummer, are arranged in reddish spikelets. Ripening seeds is highly variable and occurs in autumn and winter, being important for colonization of new areas. In nature, there are cytotypes with different somatic chromosomes numbers (4x - 16x), with a good adaptation to various environmental conditions, tetraploid (4x) and octoploid forms (8x) being the most common. Different degree of polyploidy leads to differences in ontogeny, stem morphology, leaf anatomy and morphological differences between clones. These differences are sustained when the plants are grown in a common environment (HANSEN et al., 2007; LAMBERTINI et al., 2008). WEIS & WEIS (2004) consider that the reed is an invasive species, accumulating a greater amount of heavy metal compared to some native species and makes it suitable for use in wet locations, for phytoremediation and restoration of wetlands. Researches of LAMBERTINI et al. (2008) on numerous reed populations of various origin and ploidy level underlined that "populations of *Phragmites australis* in Europe can be considered as part of a single meta-population."

This experiment was conducted on *P. australis*, phytoremediator species that naturally vegetated in three different sites regarding the content of heavy metals and radionuclides. The three locations are part of the same areas situated in the Middle Jiu Valley, fragmented by surface coal exploitations, at a distance of 30 km between them (Oltenia region, Gorj county, Romania). There were performed investigations regarding: soil contents in heavy metals and radionuclides activity, analysis of ultrastructural characteristics of mature plant leaf cells, determination of ultrastructural characteristics that support the adaptation of the plants to a substrate with a high content of pollutants.

MATERIALS AND METHODS

Site description. The middle basin of the river Jiu is located in the Getic Piedmont. In the mining basin of North Oltenia is the main reserve of coal, valued at about 3 million tons, providing one-third of the total electricity produced annually in Romania. An important role in this regard of the mines and surface coal (lignite) exploitation and the two existing thermoelectric power plants (TEPP) (Turceni and Rovinari) in the Middle Jiu Valley (Fig. 1). Mining and energy production leads to environmental degradation through pollution and habitat fragmentation. The area is heavily populated and the villages in the area are affected by coal dust and ash from coal power plants, as well as from ash pits.

Biological material. Investigations were performed on three populations of reed harvested from three wetlands located in the Middle Jiu Valley (Gorj county, Romania), currently separated (but originating from a single initial population spread over a length of 30 km):

- the population nearby the village Ţânţăreni (air pollution from ash pit and fly ash from TEPP-Turceni, as well as from the European road E66);
- the population from a wetland nearby TEPP-Turceni (200m) (air pollution from ash pit and fly ash from TEPP-Turceni);
- the population located at the base of a closed sterile waste dump, 30 years old, near the village Cocoreni. The site is about 15 km North of TEPP Turceni and at the same distance from TEPP Rovinari. The location is in the vicinity of a coal deposit (500 m) (air pollution from ash pit and fly ash from TEPP-Turceni, as well as coal dust from coal deposit).

In Fig. 1 are presented the sample locations, as well as the potential pollution sources.

Biological material samples were collected from mature leaves, from plants located in the middle of the three areas.

Amounts of heavy metals and radionuclides activity from the soil. The soil samples were harvested from the rhisosphere horizon (the 5-20 cm).

The heavy metals analysis were done in National Research and Development Institute for Soil Science, Agrochemistry and Environment Protection Bucharest, Romania. The amount of heavy metals in soil (expressed in mg / kg soil) was determined using flame atomic mass spectrometry method (LĂCĂTUŞU et al., 2011).

Radionuclides activity was done in Radioactivity Monitoring Station, Environment Protection Agency Craiova, Dolj, Romania Radionuclides activity was determined by the Duggan method (1988), (IAEA TECDOC 1092 directives), with a gamma spectrometry system, analyzer SPECTRUM-MASTER-ADCAM, model 92X. For the energy and efficiency of calibration standard gamma punctiform and volume sources with energies of the gamma radiation in the range of interest (5 – 20.000 keV) were used; Am241, Cs137, Co60, Eu152, Ba133. The collecting time of the natural background amounted to 2000.000 s. Radionuclides activity was expressed in Bq/kg, confidence level 95%.

Ultrastructural investigations. Leaf samples were harvested from mature plants. Pieces of about 1 mm^3 taken from the middle of the leaf were prefixed in a solution of 2.7% Glutaraldehyde solution (2 ½ hours), postfixed in a 1% solution of osmium tetroxide (1 ½ hours), infiltrated and soaked in EPON 812 resin. Serial sections of about 80-90 nm thick were contrasted with uranyl acetate and lead citrate. Analysis of leaf ultrastructural characteristics were determined with a TEM JEOL JEM 1010 electron microscope (Electron Microscopy Center, Babeş-Bolyai University, Cluj-Napoca, Romania.

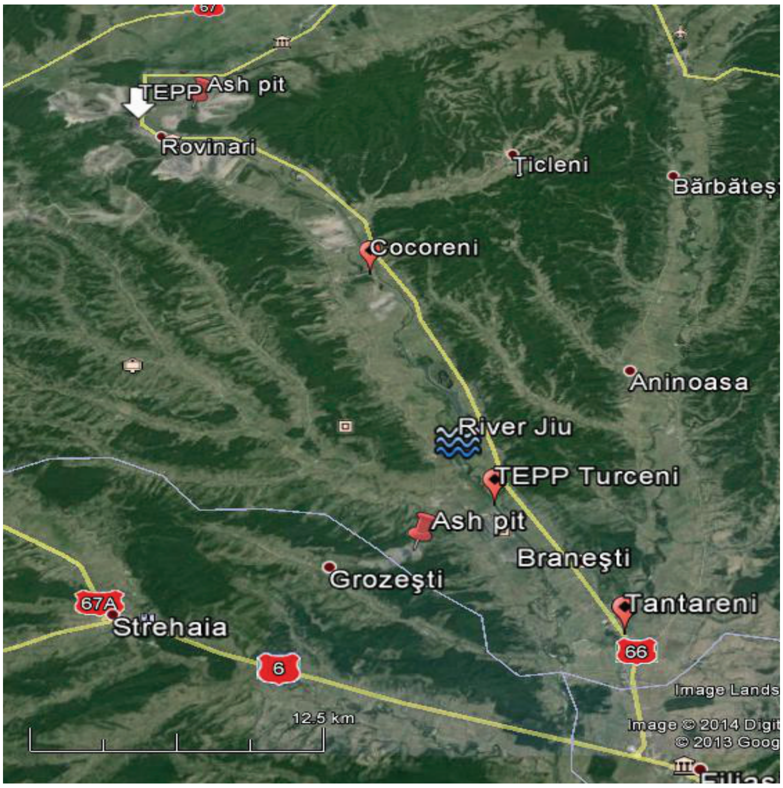

Figure 1. Sample location and the potential pollution sources (original).

RESULTS AND DISCUSSION

The levels of pollutants in soil (radionuclides activity and the amount of heavy metals in topsoil). Coal, like any ore, contains a discrete quantity of radionuclides, existing primary naturally such as: K-40, U-238, U-235, Th-232, Ra-226. Natural radionuclides are released in the environment from by-products or wastes, resulted from energy production. They have physico-chemical properties similar to some constituent chemical elements of living organisms, are metabolized and arrive finally, through different natural trophic chains, into the human organism (CHIOSILĂ, 2004). During coal combustion, most uranium, thorium and their decay products are released from the coal matrix and distributed in gas phase as well as in solid combustion products (fly ash and bottom ash).

Soil analysis revealed the presence of natural radionuclides, belonging from ash and coal dust, as well as of Cs-137 (artificial radionuclide), of Chernobyl provenance. Radionuclides activity was dependent on the considered site and the distance from the source of pollution. Values over the accepted limits for Romania were recorded for U-238, U-235 and Cs-137 (artificial radionuclide) in Ţânţăreni site, and U-238, U-235, Pb-210, Cs-137 in Cocoreni site. The soil samples harvested near TEPP-Turceni were recorded the highest values for U- 238, Pb-210, Pb-214, U-235 and Cs-137 (Table 1). The radionuclides activity in samples from the ash pit (bottom ash) was 10-20 times higher than in soil and the ash participate to air pollution, being spread by the wind, over the entire considered area.

The amount of heavy metals recorded values slightly over the normal content in soil, but under the alert limits for: Pb, Ni Cr, Cu in the three sites. Thus, the highest values for Pb, Ni and Cr were recorded in the Ţânţăreni site, while values slightly over normal for Cu, Pb and Ni were recorded at the basis of sterile waste dump from Cocoreni. Nearby TEPP-Turceni the highest values were recorded for Zn, Cu, Pb, Ni and Cr (Table 2).

Table 1. Radionuclides activity in topsoil, ash and coal dust (Bq · kg^{-1} soil)
(bold letters mark the values over the limit for Romania).

Radionuclide	**Ţânţăreni village Mean ± SD**	**TEPP-Turceni Mean ± SD**	**Cocoreni Mean ± SD**	**Limits for Romania**	**Bottom ash (ash pit) Mean ± SD**	**Lignite dust Mean ± SD**
U-238/Th-234	**43.20 ± 5.55**	**51.30 ± 6.47**	**34.10 ± 4.51**	25.0	**656.60± 76.90**	<10.5
Ra-226	25.50 ± 2.00	38.90 ± 2.50	22.50 ± 1.40	10.0-90.0	**509.0± 186.60**	19.1±1.75
Pb-210	33.40 ± 1.47	**79.15 ± 6.46**	**44.78 ± 1.54**	20.0-40.0	**568.60± 42.70**	0
Bi-214	26.70 ± 2.37	36.96 ± 3.22	22.0 ± 1.40	20.0-40.0	**267.40± 13.10**	23.50±1.91
Pb-214	24.10 ± 1.18	**41.50 ± 2.15**	23.30 ± 1.10	20.0-40.0	**385.70± 19.40**	14.80±1.28
U-235	**4.49 ± 0.41**	**5.26 ± 0.54**	**3.33 ± 1.23**	2.0	**32.90± 8.26**	2.18±1.83
Ac-228/Th-232	35.80 ± 7.72	34.80± 1.90	35.60 ± 1.80	13.0-65.0	**207.31 ± 24.0**	19.10±1.72
Pb-212	4.15 ± 1.39	49.3± 1.82	30.0 ± 1.65	20.0-50.0	**324.90± 20.70**	0
K-40	485.40 ± 22.0	464.0 ± 25.9	388.90± 21.60	330.0-800.0	929.30± 76.0	<43.0
Cs-137	**15.90 ± 0.83**	**6.54 ± 1.96**	**5.77 ± 0.55**	0	**114.70± 10.10**	<1.9

Table 2. The amount of heavy metal in topsoil and bottom ash (mg · kg^{-1} soil)
(bold letters mark the values over normal content in soil).

Heavy metal	**Ţânţăreni village**	**TEPP-Turceni**	**Cocoreni**	**Normal content in soil**	**Allert limits**	**Bottom ash (Ash pit)**
Zn	49.1	**306.0**	58.7	100	300-700	63.0
Cu	13.8	**45.2**	**22.4**	20	100-250	19.0
Fe	20,899	22,214	21,205	*	*	25,004
Mn	593.0	363.0	310	900	1500-2000	252.0
Pb	**22.5**	**42.6**	**22.1**	20	50-250	2.53
Ni	**68.6**	**35.1**	**25.7**	20	75-200	**50.0**
Cr	**47.8**	**40.9**	21.2	30	100-300	20.0
Co	9.8	8.8	8.28	*	*	7.38
Cd	Traces	0.9	Traces	1.0	3.0-5.0	0.154

*There are no available data in Romanian standards

Ultrastructural characteristics of leaf. The ultrastructural leaf analysis of the mature plants reveals some minor differences between the three genotypes of *P. australis*. Ultrastructural investigations showed that species adapts to the presence in the environment of the stressors (heavy metals and radionuclides).

Penetration and accumulation of exogenous material in leaf parenchyma cells. In the three sites were recorded similar issues regarding the penetrating paths of the exogenous pollutant particles, their dissemination and accumulation. Exogenous particles, acicular shape, granular or squamous, enter in the leaf tissue through stomata or through penetration of cuticle and epidermal cell wall (Fig. 2).

Similar to other species found in the same site (*T. latypholia*), they can be extracted from the environment by the plant root system and spread through the vascular circulation (unpublished data). In the parenchymal tissue adjacent cells, exogenous particles are scattered by plasmodesmata (Fig. 3). In the cell vacuole (Fig. 4) exogenous particles are usually aggregate, acicular particles are usually found in the form of needle-shaped aggregates of particles. Squamous particles are also aggregate, especially in the circulatory system into the inner wall of the leading vessel. Granular particles form different sized granules, by aggregation. Also, in the cells in this area, near plasmodesmata, multivesicular bodies (MVB) can be present (Fig. 4).

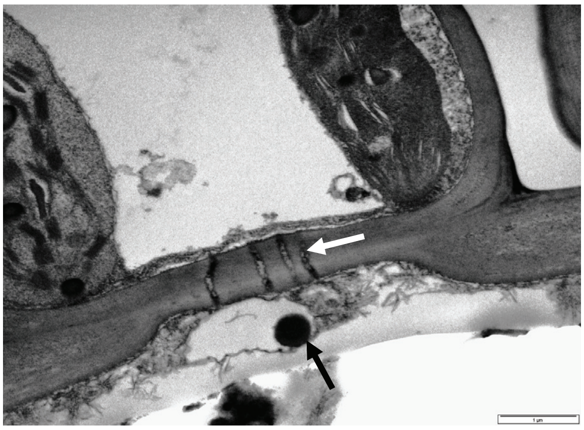

Figure 2. Longitudinal section through a plasmodesma, on its tract being visible exogenous material (white arrow). In the vesicle situated under the cuticle, there are exogenous material and anthocyanin granules (black arrow) (scale bar 1µm) (original).

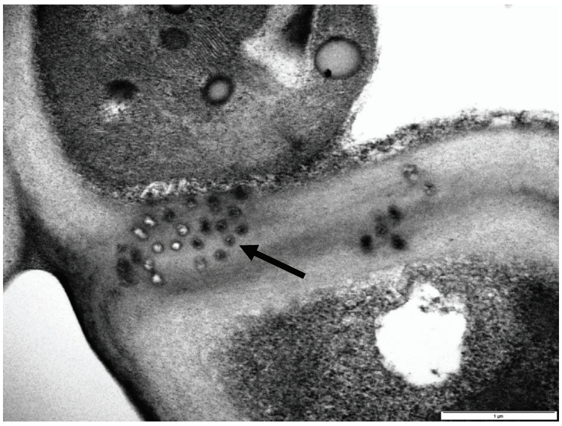

Figure 3. Transversal section through a plasmodesma. In some canalicles there is exogenous matter, which migrate from one cell to another (arrow) (scale bar 1µm) (original).

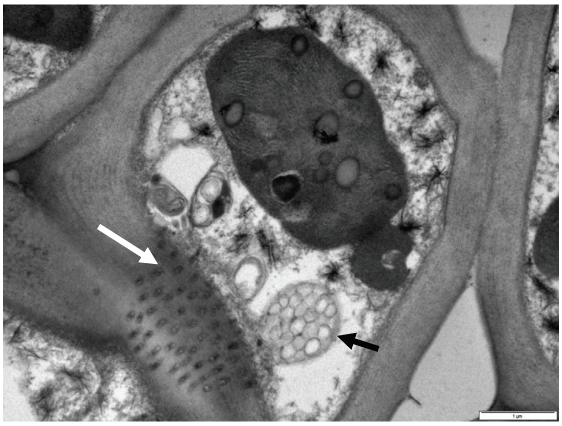

Figure 4. Transversal section through a plasmodesma (white arrow). In the cell cytoplasm, there is a MVB (black arrow) and accumulation of exogenous matter (scale bar 1µm) (original).

Exogenous particle interaction with organelles. Acicular particles are usually grouped near plasmalemma, on the tonoplast surface (Fig. 5) or within it, in the vacuole (Fig. 6), on the surface or inside the cell wall (Fig. 7), and in intercellular space, which forms aeriferous circulatory system, where they reach by crossing stomata ostiole (Fig. 8). They can also be found on the external surface of the endoplasmic reticulum channels, inside the nucleus and of some organelles. The plasmodesmata presence, as well as of the exogenous particles in the cell wall, confirm that exogenous particles end up in the leaf tissue by penetrating the cuticle and the epidermal cell wall. From the leaf tissue cells, they are spread mainly through the vascular circulation, exogenous particles being present in libber and ligneous cells, as well as in fundamental parenchyma cells (Figs. 9, 10).

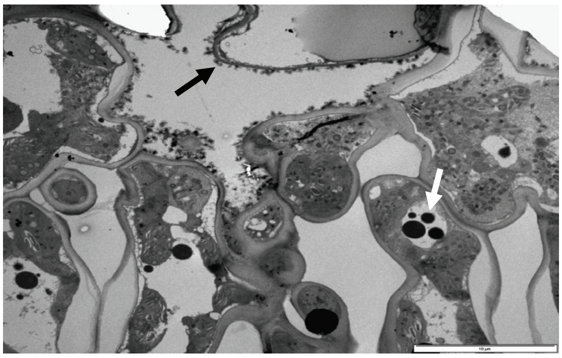

Figure 5. Acicular exogenous matter accumulated and disposed on the vacuole (black arrow) tonoplast. In some vacuole there are anthocyanin granules (white arrow) (scale bar 10µm) (original).

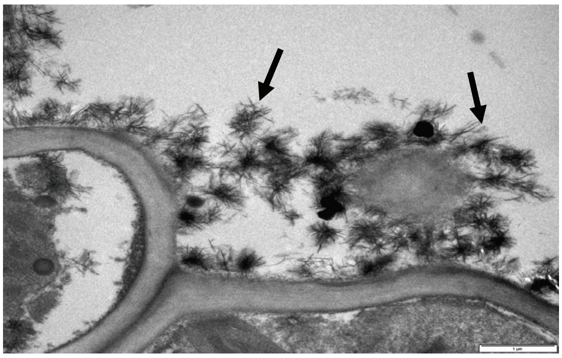

Figure 6. Exogenous acicular matter, disposed in vacuole and on tonoplast (detail), (scale bar 1µm) (original).

Another pathway for their presence in leaf tissue is their penetration through the stomata. In Fig. 8 in the stomata annex cells (which are in "open" position) there are vesicles with a chelating substance having an active defense action of the plant, in the presence of exogenous material. Exogenous particles were also found in some cell organelles, especially in the nucleus and mitochondria. In the nucleus, in the presence of small quantities of exogenous acicular particles, which are accumulated in karyolymph, the ultrastructure is relatively normal (Fig. 11).

Figure 7. Acicular exogenous particles arranged on the exterior walls of the cell, in the intercellular space (black arrow) and in the cell walls (white arrow), (scale bar 1µm) (original).

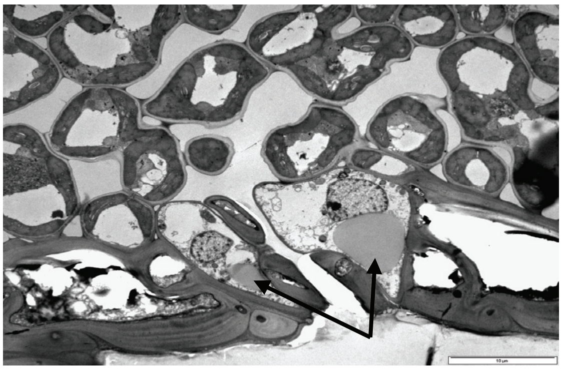

Figure 8. Stomata in "open" position, with chelating substance (arrow) in the annexes cells (scale bar 10µm) (original).

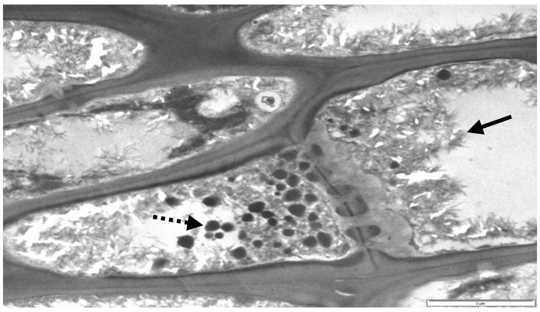

Figure 9. Massive accumulation of acicular exogenous particles (simple arrow) in libberian cells, together forming an accumulation of antyhocyanin granules (dots arrow) (scale bar 2µm) (original).

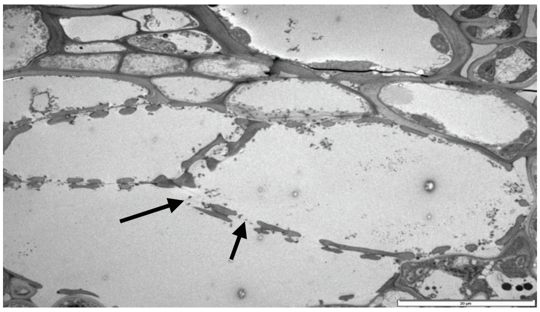

Figure 10. The cells from the ligneous vessels with a very lax constitution, which permit an easy penetration of the exogenous material (arrow) (scale bar 20µm) (original).

If the amount of acicular exogenous material is great, karyolymph shows varying degrees of alteration. In addition, the exogenous material is present in the cell vacuole (Fig. 12). The defensive cell reaction is the presence in the vacuole of the anthocyanin vesicles, as well as with chelating substance (Fig. 12).

In mitochondria, exogenous particles were found on the surface of mitochondrial crysta and in the matrix (Fig. 13). As a result of the accumulation of a big quantity of exogenous particles in chloroplast, its ultrastructure is altered, the main aspects of the alterations being the presence of a small amount of plastoglobuli and the grana fragmentation (Fig. 14).

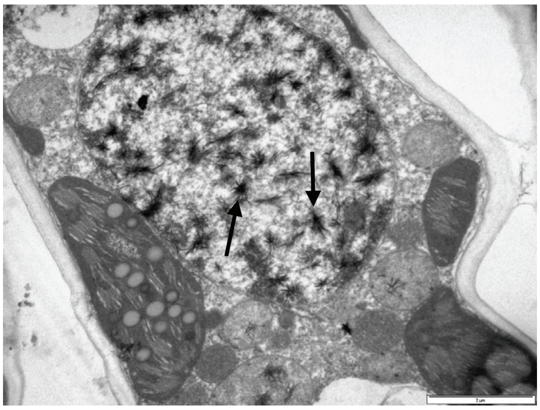

Figure 11. Nucleus with a moderate amount of acicular particles (arrow), accumulated in karyolymphe (scale bar 2µm) (original).

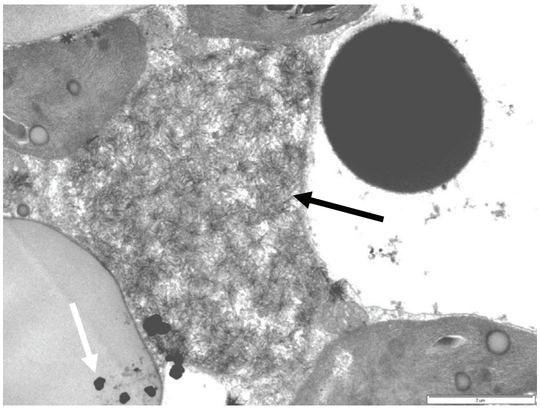

Figure 12. Nucleus with an altered structure (black arrow). In a vacuole (right) there is a giant anthocyanin granule. In left there are vesicles with chelating substance in which there are exogenous particles (white arrow) (scale bar 2µm) (original).

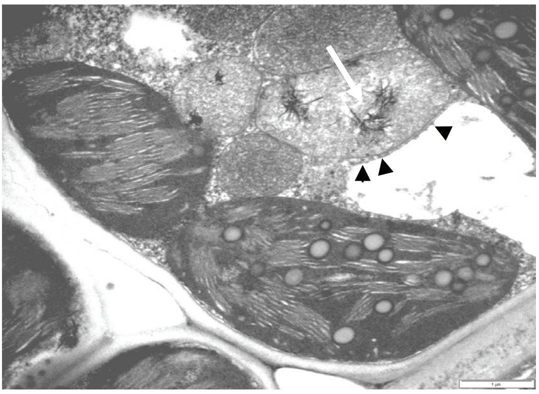

Figure 13. Mitochondria with an accumulation of exogenous acicular particles (white arrow). The chloroplast contains numerous plastoglobules. In mitochondria take place the ferritin synthesis, disposed as granules in cytoplasm (black arrows), (scale bar 1µm) (original).

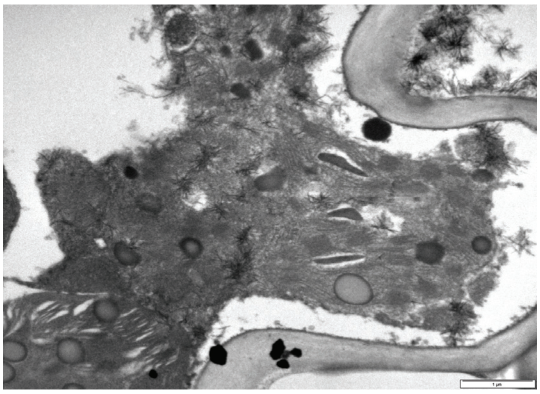

Figure 14. Chloroplast with altered structure as a result of the exogenous particles accumulation in the cell (scale bar 1µm) (original).

Defense reaction of the plant cell. In the presence of exogenous particles (of heavy metals and radionuclides), the reaction of the plants from the three sites was similar, with some particularities.

Synthesis of chelating substances (probably phytochelatins) accumulated in vesicles of different sizes usually accompanied by granules with exogenous substances, or by electron-dense (osmiophilic) granules. These vesicles are present in the plants from all three sites. These vesicles can be merged (Fig. 15, several small vesicles located in the vacuole arranged around the exogenous acicular particles), resulting electron-light vesicles, relatively uniform in size (5.0 to 7.6 µm), present mainly in aerial circulatory system (Fig. 8) or in circulatory system, in addition to electron-dense granules (Turceni site), or in addition of exogenous aggregate particles (sites Ţânţăreni and Cocoreni). The exogenous particles presence on the surface or inside vesicles with chelating substance, especially in the both circulatory systems (liberrian and ligneous), highlights their involvement in the chelating and the transport of this exogenous material (Fig. 16).

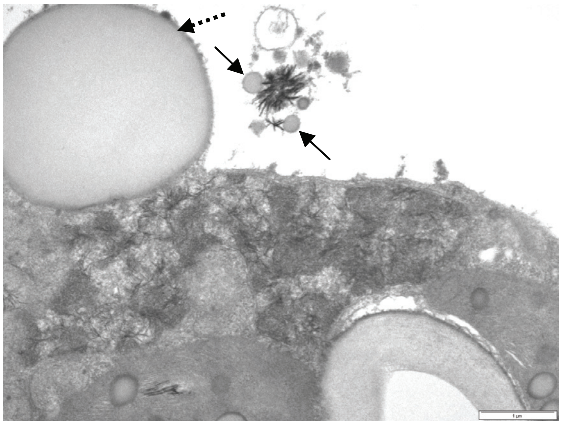

Figure 15. Many small vesicles with chelating substance present in a vacuole, around of an acicular exogenous particle (simple arrow). As result of their merging, result big vesicles full with chelating substance (dot arrow), (scale bar 1µm) (original).

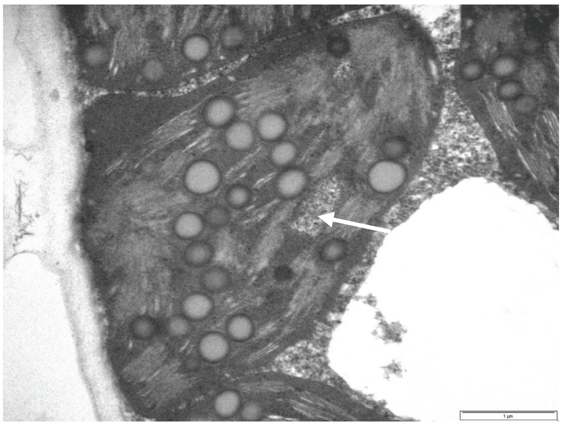

Figure 16. Chloroplast with many plastoglobuls. In stroma there is the "crystal-like bodies" area (arrow) in which take place the ferritin synthesis (scale bar 1µm) (original).

Ferritin synthesis has a role in defense and adaptogenic properties. Iron is an essential element for all life forms and limits oxidation to the ferric form, having a profound impact on the productivity of the organisms. As a consequence of an inappropriate diet, about 30% of the global population suffers from different forms of iron deficiency (GOTO et al., 2001). In all organisms are ubiquitous but Ferritin, a metalloprotein of 450 kDa, rich in iron, composed of 24 subunits (THEIL, 1987). Ferritin in plants shows an important role in redox reactions. The function of ferritin in plants consists of keeping the iron for a short or long time, to protect the cell from the toxic effects of free iron, thus serving as a primary antioxidant. PRASAD & NIRUPAM (2007) considers that ferritin acts against biotic and abiotic stress factors, accumulating heavy metals, as well as a protector of the genome. Ferritin genes have been found at different species of plants, especially vegetables, being reported ferritin synthesis in the chloroplast and mitochondria. HARRISON & AROSIO (1996) reported the presence in the chloroplast stroma of the regions without thylakoid (named "crystal-like bodies"), formed by the accumulation of ferritin (Fig. 16). DUY et al. (2007) reported the synthesis of ferritin in the chloroplast of the species *Phragmites australis*. In plants from Ţânţăreni site, where it was recorded a large amount of iron in the substrate, in the chloroplast was revealed the existence of ferritin granules, spherical or small rod-shaped. Histochemical methods were not used in this research to identify ferritin features. However, it is possible that in Fig. 13, to be evidenced ferritin synthesis, in mitochondria. In mitochondria matrix and cytoplasm around mitocondria are located some electron-dense particles of similar size. They are synthesized in the mitochondria and subsequently migrate into the cytoplasm.

Granules of anthocyanin. Anthocyanins are water-soluble pigments, substances belonging to the group of flavonoids, found in all tissues of higher plants (leaves, stems, roots, flowers and fruits). In leaves and sometimes in stem, they serve to protect the cells from the damage "high-light" by absorbing blue-green and ultraviolet light (high-light stress). Anthocyanin synthesis occurs in chloroplast, anthocyanin molecules being arranged on the thylakoid surface (Fig. 17). They migrate to the periphery of the chloroplast and then into the cytoplasm. After their aggregation, resulted small granules virions shape like, disposed on internal surface of the tonoplast (Fig. 18). They arrived in the in vacuole, merges resulting initially small granules, and then large anthocyanins granules in the cell vacuole (Fig. 12).

In the vacuoles there is a different amount of exogenous material, free or stored in multivesicular bodies (together or not with the remnants of the destroyed cells) vesicles with chelating substance, a.o. The presence of the anthocyanins granules in these cells blocks the action (toxic or not) of exogenous material and so the plant can accumulate a large amount of toxic substances in the cells (Fig. 19).

Multivesicular Bodies (MVB) are endosomal organelles containing small vesicles (exosome), formed following the inward budding of the outer endosomal membrane. Studies on the biogenesis, structure and function of multivesicular bodies were carried out by different authors (AN et al., 2007; PIPER & KATZMANN, 2007; OTEGUI & REYES, 2010). Their presence in the cell is associated with exchanges between neighbouring cells by plasmodesmata, as it shows in Fig. 4 (Ţânţăreni site). DUY et al. (2007) found that plant cells can secrete endosomes derived from multivesicular bodies. After OTEGUI (2014): "MVBs are endosomes that consist of a limiting membrane and internal vesicles". "The internal vesicles arise from invaginations of the limiting membrane and carry membrane proteins targeted for degradation in the lysosome/vacuole. MVBs play a crucial role in both the endocytic and the secretory pathways of all eukaryotic cells, sorting proteins for degradation or recycling, down -regulating receptors, and mediating the transport of proteins to the vacuole/lysosome. Thus, MVB functions are tightly related to cell signalling, differentiation, and transport of vacuole cargoes" (Figs. 20, 21).

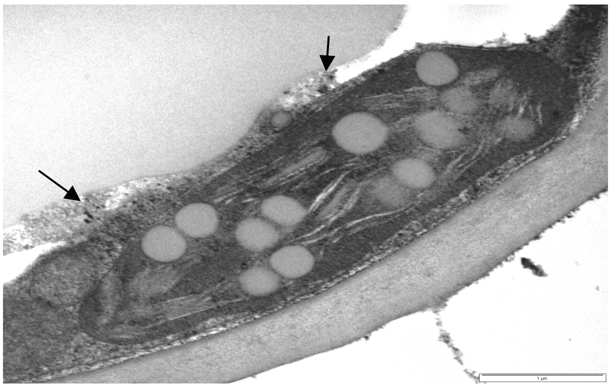

Figure 17. Young chloroplast with plastoglobules and electron-dense granules (anthocyanin granules), synthesized at the thylakoids level (scale bar 1μm) (original).

Figure 18. The synthesis and formation of the anthocyanin granules. After synthesis in the chloroplast, the small anthocyanin granules will be aggregated, resulting big granules which accumulated in the vacuole (arrow), (scale bar 2μm) (original).

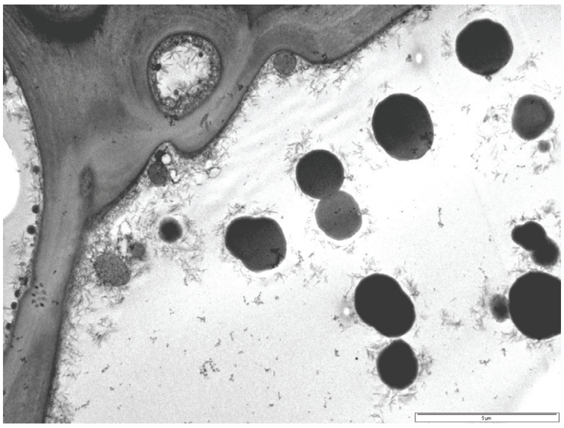

Figure 19. Acicular exogenous particles and anthocyanin granules, accumulated in the vacuole of a lacuna parenchyma cell (scale bar 5μm) (original).

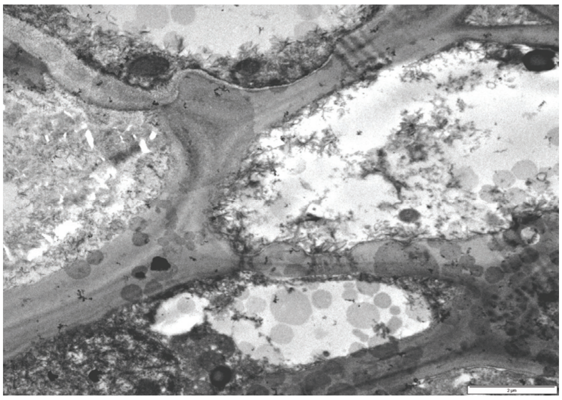

Figure 20. Accumulation of acicular exogenous material and alogen granules in the cells of libberian vessels, which will become deposit cells. The nucleus and cytoplasm present alterations (scale bar 5μm) (original).

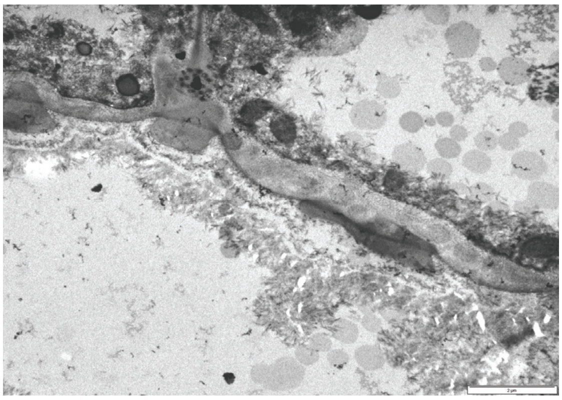

Figure 21. Accumulation of acicular exogenous particles and destroyed cell vestigial, in the cells situated near central cylinder. These cells will become also deposit cells (scale bar 2μm) (original).

Deposit cells. Exogenous material, along with the remnants of the destroyed cells is accumulated in deposit cells, located on the underside of the leaf, near the lower epidermis, circulatory system (libberian cells or ligneous cells) and aeriferrous circulatory system (formed from intercellular spaces) or in the fundamental parenchyma from the central cylinder, represent deposit cells of exogenous material, and/or remnants of the organelles (Figs. 20, 21). In these cells, multivesicular bodies are also, involved in exocytosis processes. Between deposit cells, there are plasmodesmata, that facilitate the transport of this material. In the core of plasmodesmata canalicles or in transit, through the cell wall, there is present this exogenous material. Also in the stomata annex cells, located in the vicinity, there are vesicles with chelating substances (Fig. 8). The presence of all these factors in the deposit cells (exogenous material, multivesicular bodies, vesicles with chelating substances, anthocyanins granules and other structures and substances such as Ferritin, a.o.) situated near the lower epidermis and the circulatory system, supporting their involvement in the inactivation and / or exocytosis of the toxic waste materials from the plant. These findings are in accord with the ones described in a review, regarding the complexation of the metals with different ligands and their mechanisms by LEITENMAIER & KÜPPER (2013).

CONCLUSIONS

After the distribution of the various pollutant elements in the soil in the area under consideration, the major sources of pollution are TEPP Turceni, TEPP Rovinari (fly ash), ash pits (bottom ash) and the coal deposit Cocoreni (coal dust). In the analyzed soil samples was registered an activity of the natural and artificial radionuclides over the normal limits for Romania (U- 238, Pb-210, Pb-214, U-235 and respectively Cs-137), but the content in heavy metals, even is over the normal soil content (Pb, Ni, Cr, Cu), is still under the alert limits. The most polluted site is placed nearby TEEP Turceni.

P. australis cosmopolitan, hyperaccumulator species is very suitable for the study of the penetration of exogenous particles and various cellular strategies of containment, neutralization and their storage at the tissue level. Exogenous particles entering in the plant, on the one hand by absorption, at the root level, and arrive in leaf tissue through the leading vessels and on the other hand, arrive directly through the cuticle and penetration of the cell wall or through stomata. They are spread in leaf tissue through plasmodesmata, located between adjacent cells. In the cell, the exogenous particles are visible on the tonoplast or in the vacuoles, near endoplasmic reticulum tubules and in some organelles like the nucleus, mitochondria, and chloroplasts.

The active cell reaction is demonstrated by the presence of specific structure and synthesis of stress-protective substances (multivesicular bodies, vesicles with a chelating substance, anthocyanin granules, ferritin particles, a/o). Chelators synthesized in the chloroplast thylakoids, gather and incorporate exogenous particles, transporting them through the intercellular spaces and the circulatory system, in cells located near the lower epidermis.

The exogenous particles and remnants of cell organelles are clustered in cell vacuoles, located in the lower epidermis, and nearby leader system vessels, which become deposit cells. Anthocyanin granules accumulated in cell vacuoles block the action of exogenous toxic particles. The ferritin molecules, synthesized in mitochondria and chloroplasts, have an important role in many important redox reactions. They keep the iron in the cell and act as primary antioxidants. The presence of multivesicular bodies (MVB), in cells located near the lower epidermis, suggests the existence of an exocytosis activity, through which are probably removed the accumulations of deposit cells.

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