PHYTOREMEDIATION OF SOME HEAVY METALS AND RADIONUCLIDES FROM A POLLUTED AREA LOCATED ON THE MIDDLE JIU RIVER. CASE STUDY: *TYPHA LATIFOLIA* L.

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Abstract. *Typha latifolia* (cattail) presents phytoremediatory properties, being capable to accumulate pesticide residues, lead and other heavy metals and radionuclides from the environment in its rhizomes. Cattail, a hyperaccumulator plant for arsenic, lead, plutonium and selenium, is also resistant at other heavy metals and radionuclides. *Typha* presents different uses for its medicinal properties: diuretic or for sores, boils, wounds, burns, and smallpox pustules, wounds, carbuncles, inflammations, scalds as jelly-like paste against dysentery and diarrhoea, for abdominal cramps or for its aphrodisiac properties, etc. Moreover, *Typha* is used as a source of starch in ethanol production, being a bioenergy crop. In the present paper, there were analysed the ultra-structural features of *Typha latifolia* leaves from plants developed in two sites on the middle Jiu river valley (Gorj County, Romania): (a) Control site near Ţânţăreni village, and (b) the plants developed on a 35 year-old waste dump, near Cocoreni. It was evidenced the presence of different exogenous particles in the epidermal and parenchyma cells, the synthesis and presence of some anti-stress compounds (ferritin, anthocyanin and others bioactive substances), as well as the presence of some structural adaptations at the plants developed on an environment with a big amount of heavy metals and radionuclides (the plasmodesmata presence or the absence of cell wall in small areas, for facilitating of the material exchange between cells, through the median membrane; the absence of the cuticle on the epidermal cells; the presence of some deposit-cells in which the exogenous matter or destroyed cell organelles accumulate, etc). At the same time, in the plants developed on the waste dump from Cocoreni, it was evidenced the presence of a metabolic structure of NAB type (nucleolus associated body) in the nucleus, near the nucleon, associated with an intense normal or pathological metabolic activity (presence of a stress factor, etc).

Keywords: *Typha latifolia*, coal exploitation, heavy metals, radionuclides, cell ultrastructure adaptations; resistance to stress factors.

Rezumat. Fitoremedierea unor metale grele şi radionuclizi într-o zonă poluată pe valea mijlocie a râului Jiu. Studiu de caz: *Typha latifolia* L. *Typha latifolia* (papura lată) prezintă proprietăţi fitoremediatoare, fiind capabilă să acumuleze în rizomi, reziduuri de pesticide, plumb şi alte metale grele sau radionuclizi din mediu. *Typha* este o plantă hiperacumulator pentru arseniu, plumb, poloniu şi seleniu, fiind de asemenea rezistentă şi la alte metale grele şi radionuclizi. *Typha* prezintă diferite utilizări pentru proprietăţile sale medicinale: diuretic sau la prepararea unei paste utilizată în cazul rănilor, opăririlor, arsurilor, cicatrice sau pustule rămase după variolă, râie, antrax sau diferite forme de furuncule şi abcese, inflamaţii, contra disenteriei şi diareei, în cazul crampelor abdominale, afrodisiac ş.a. De asemenea, *Typha* este folosită ca o sursă de amidon pentru obţinerea de etanol, fiind o cultură bioenergetică. În prezentul studiu, au fost analizate caracteristicile ultrastructurale ale frunzelor la plantele de *Typha latifolia* dezvoltate în două situsuri pe valea mijlocie a râului Jiu (judeţul Gorj, România): (a) situsul Control, situat lângă localitatea Ţânţăreni şi (b) plante dezvoltate pe o haldă de steril, veche de 35 ani, situată lângă localitatea Cocoreni. A fost evidenţiată prezenţa diferitelor particule exogene în celulele epidermale şi parenchimatice, sinteza şi prezenţa unor compuşi antistres (ferritin, antocianin şi o altă substanţă bioactivă), precum şi prezenţa unor adaptări structurale la plantele dezvoltate pe un mediu cu mari cantităţi din unele metale grele şi radionuclizi (prezenţa plasmodesmelor sau absenţa peretelui celular în mici zone, pentru a facilita schimbul de material exogen între celule, prin membrana mediană; absenţa cuticulei de pe celulele epidermale; prezenţa unor celule-depozit unde se acumulează material exogen sau organite celulare distruse, ş.a.). De asemenea, la plantele dezvoltate pe halda de steril de la Cocoreni, a fost evidenţiată prezenţa în nucleu, lângă nucleol, a unei structuri metabolice tip NAB's (nucleolus associated body's), însoţită de o intensă activitate metabolică, normală sau patologică (prezenţa unui factor de stres, ş.a.).

Cuvinte cheie: *Typha latifolia*, exploatări de cărbune, metale grele, radionuclizi, adaptări celulare ultrastructurale, rezistenţă la factori de stres.

INTRODUCTION

Botanical features and uses

Typha is a plant species, which is presently re-discovered, even if it was used by human populations 10,000 years ago. *Typha latifolia* L. (cattail) is a perennial herbaceous plant, native in Europe, Asia, Africa, North and South America. In Australia and Hawaii, it is considered a noxious weed. Its presence was reported also in Indonesia, Malaysia, New Zealand, Papua - New Guinea and Philippines. *T. latifolia* is a wetland species, met in different climates (tropical, subtropical, southern and northern temperate, humid coastal and dry continental climates), up to an altitude of 2,300 m. Cattail develops in flooded areas in which water depth does not exceed 0.8 meters, being met in fresh water and also in slightly brackish marshes. The plant height is between 1.5 and 3 metres, the alternate leaves being 2-4 cm broad. The plant is monoecious, with unisexual flowers developed in dense racemes. *T. latifolia* can hybridize with other related species (*Typha angustifolia*), forming hybrids as *Typha* × *glauca* (*Typha angustifolia* × *T. latifolia*), white cattail.

Typha presents numerous uses, the plants being frequently eaten by wetland mammals (muskrats) and its seeds by birds. Some parts of the plant are edible for humans. Thus, the starchy rhizomes present a protein content comparable to that of maize and rice (MORTON, 1975), the obtained flour having 266 kcal per 100 grams (after other authors, only 106 kcal/100 g). Because they are fibrous, the starch must be scraped or sucked from the tough fibbers. The seeds have a high content of linoleic acid, being used to feed cattle and chickens in some African countries (Ghana).

Different parts of the cattail have been eaten and used in a variety of ways. The written references for this species are disposables dating back to the 1600's; others were found in some caves from Ohio dating from 800-1400 A. D. The rootstock is mostly starchy and edible and early colonists used it as food. The young shoots can be eaten like asparagus, the immature flower spikes can be boiled and eaten like corn on the cob, and the sprouts at the tip of the rootstock can be used in salads or boiled and served as greens. Cattail pollen can also be used as a flour substitute in bread making and it was additionally used in religious ceremonies by Native Americans.

In addition, *Typha* was used by local human populations for its medicinal properties. Thus, the boiled rootstocks are used as a diuretic, or mashed to make a jelly-like paste for sores, boils, wounds, burns, scabs, and smallpox pustules (MAIDEN, 1889). Recent experiments point out that *Typha* plants are able to remove arsenic from drinking water. The Amerindian population poultice jelly-like pounded roots on wounds, sores, boils, carbuncles, inflammations, scalds, burns. Fuzz from mature female flower heads is applied to scalds, burns and prevents chafing in babies. Young flower heads were eaten for diarrhoea. The infused roots were used against dysentery and diarrhoea (Foster and Duke). It can be also used as a dressing to pack burns. The Omaha tribe pulverized the root to form a paste used to heal burns, then covered the paste with the cattail flowers, while the Cheyenne took the powdered root for abdominal cramps. The recent investigations point out that in 100 g rhizome there are numerous bioactive substances, such as: carbohydrates (5.14 g), sugars (0.22 g), dietary fibber (4.5 g), protein (1.18 g), fats (0.00 g), numerous vitamin (A-vitamin and beta-carotene, thiamine, riboflavin, niacin, pantothenic acid, B6-vitamin, folate, choline, C-vitamin, K-vitamin) and other chemical elements (calcium, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc). After MORTON (1975), the delivered energy reaches 266 kcal/100 g (USDA Nutrient Database).

Typha latifolia presented also a magical reputation as an aphrodisiac. In China, cattail pollen (Pu Huang) from the species *Typha latifolia* L. or *Typha angustata* Bory et Chaub. was consumed as an aphrodisiac (RÄTSCH & MÜLLER-EBELING, 1986). *Typha capensis* is also used as aphrodisiac for men and women (Botanical Source, 2014). In traditional Chinese medicine, however, it is primarily used to treat nosebleeds (BENSKY & GAMBLE, 1986). The pollen of *Typha angustata* contains isorhamnetin, pentacosane, sitosterol, palmitic acid and a-typhasterol (BENSKY & GAMBLE, 1986).

Some human populations (from Peru and Bolivia, around Titicaca Lake) use *Typha* to build different boats. The United States Navy used it during of the World War II, as substitute for kapok in life vests and aviation jackets, which resisted even after 100 hours of submersion. *Typha* was used as thermal insulation in buildings, as an organic alternative to conventional insulating materials such as glass wool or stone wool.

Native Americans used the feathery seeds for baby beds. When mixed with ash and lime, the seeds form cement that is reported to be harder than marble. For more than 10,000 years, cattail leaves have provided Native Americans with a source for thatched roofs, woven floor mats and sandals. Leaves were twisted into rings and used under a collar to keep a horse neck from being injured. The stems produce a substance used as an adhesive. The Menomini and Meskwaki peoples used the root as a caulk to seal leaks in their boats. From stem, there were obtained fibres up to 4 meters long, used for raw textiles, an alternative to cotton or linen. The mean production is 7 - 10 tons per hectare annually, the yield of leaf fibre in *Typha glauca* being of 30-40%. *Typha* is also used as a source of starch for ethanol production. As it presents a good productivity at northern latitudes, *Typha* is considered a bioenergy crop (DUBBE et al., 1988). *Typha* is also used as an ornamental plant or to make paper. Because cattail blooms from March to May, this species represents the unique flower perfect for dried arrangements.

Phytoremediatory properties

In different experiments performed by different authors, *Typha latifolia* species emphasized its feature of phytoremediatory species, resistant to the presence of a big amount of heavy metals and/or radionuclides or other stress factors in the environment. In a synthesis performed by HAZRA et al. (2011), it is pointed out that the common aquatic plant species used in wastewater treatment are *Eichhornia cressipes*, *Azolla* spp., *Ceratophyllum demersum*, *Chara* spp., *Hygrophila polysperma*, *Ipomoea aquatica*, *Pistia stratiotes*, *Typha latifolia*, *Brassica juncea*, *Helianthus annuus* and *Medicago sativa*. They are basically responsible for the removal of nutrients such as COD, heavy metals like Cd, Cr, Cu, Fe, Ni, Pb, Zn, nitrate, phosphorus, as well as hydrocarbons and suspended solids. BRANKOVI et al. (2011) analysed the concentration of different heavy metals in different organs belonging to different plant species, developed in water and in other environments. They established the heavy metal content (Fe, Mn, Cu and Pb) in stem and leaves (*Bidens tripartitus*, *Polygonum amphibium*, *Lycopus europaeus*), and in root, stem and leaves (*Typha angustifolia* and *Typha latifolia*). In the aquatic plants, the highest concentration in Fe and Pb was recorded in the root of *Typha latifolia*, while the highest concentration in Mn and Cu was recorded in the stem and leaves of the investigated species.

Typha latifolia presents phytoremediatory properties, as it accumulates pesticide residues, lead and other heavy metals from the environment in their rhizomes. For this reason, the plants growing in polluted water are not used in alimentation (GORE, 2007). In accordance with the results obtained by different researchers, *Typha latifolia* manifests a hyperaccumulatory activity for As, Pb and Pl, as well as for Se (PILON-SMITS et al., 1999); phytoextraction for As and an phytostabilization activity for As and Pb.

MANIOS et al. (2002) analysed the development of *Typha latifolia* in a mixture of sewage sludge compost, commercial compost and perlite, and in the presence of different concentrations of Cd, Cu, Ni, Pb and Zn, for a period of 10 weeks. The experimental results did not emphasize the presence of an inhibition in the plants development in the presence of heavy metals in three of the four groups. In another experiment, MANIOS et al. (2003) point out an increase in the chlorophyll hydrolysis due to the accumulation of some heavy metals (Cd, Cu, Ni, Pb and Zn) in *Typha latifolia* leaves.

CARDWELL et al. (2002) analysed 15 species of aquatic and marsh plants overgrowing watercourses in Brisbane (Australia). They recorded much higher concentrations of heavy metals in the rhizomes of plants in comparison with the concentrations found in the leaves mainly in case of *Typha domingensis* Pers. and *Schoenoplectus validus* (Vahl) A. & D. Löve. The concentration in rhizomes was so high that it exceeded the values recorded in the adjacent bottom deposits. These findings are determined by the migration of these compounds, from leaves and accumulation in rhizomes, in every autumn, similar with *Phragmites* sp.

SCHRÖDER & LYUBENOVA (2005) established that *Typha latifolia* was very effective for phytoremediation of wastewater contaminated with low concentrations of anthropogenic substances (pharmaceuticals, pesticides and other xenobiotics). The researchers point out that *Typha latifolia* has the potential to remove and detoxify xenobiotics from wastewater and bed-load sediments contaminated with heavy metals (Cu, Pb, Cd, As in concentration of 20 to 250 μM) and organic pollutants. These changes are induced by changes in the activity of the detoxification enzymes.

SUKUMARAN (2013) point out that the emergent plant *Typha latifolia* (together with the floating plant *Eichornia* sp.) has proved as a promising technology for removing pollutants from latex industry effluents. Its rooted nature has favoured increased rhizosphere activity, thereby enhancing nutrient and pollutant removal.

MATERIAL AND METHODS

Site description

The middle basin of the river Jiu is located in the Getic Piedmont. 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 is played by the mines and surface lignite exploitations and the two thermoelectric power plants (TEPP-Turceni and TEPP-Rovinari) located in the Middle Jiu Valley. The mining exploitation and energy production leads to environmental degradation through pollution and habitat fragmentation. The area is densely populated and the villages in the area are affected by coal dust and ash from coal power plants, as well as from ash pits (CORNEANU et al., 2012; 2014).

Biological material

The investigations were performed on two populations of cattail harvested from two wetlands located in the Middle Jiu Valley (Gorj district, Romania), currently separated (but originating from a single initial population spread over a length of 30 km):

(a) the population nearby Ţânţăreni village (air pollution from the ash pit and fly ash from TEPP-Turceni, as well as from the European road E66);

(b) the population located at the base of a 35 year-old sterile waste dump, near Cocoreni village. 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 the coal deposit).

The biological material samples were collected from mature leaves, from plants located in the middle of the two areas.

Amounts of heavy metals and radionuclides activity from the soil

The soil samples were harvested from rhizosphere horizon (5-20 cm).

The heavy metals analyses were done at the 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 at the Radioactivity Monitoring Station, Environment Protection Agency Craiova, Dolj, Romania. Radionuclides activity was determined by Duggan method (DUGGAN, 1989); IAEA TECDOC 1092 directives), with a gamma spectrometry system, analyser SPECTRUM-MASTER-ADCAM, model 92X. For the energy and efficiency of calibration, there were used standard gamma punctiform and volume sources with energies of the gamma radiation in the range of interest (5 – 20.000 keV); Am-241, Cs-137, Co-60, Eu-152, Ba-133. The collecting time of the natural background amounted to 2000.000 s. Radionuclides activity was expressed in Bq/kg soil, 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. The analyses of leaf ultrastructural characteristics were performed with a TEM JEOL JEM 1010 electron microscope (Electron Microscopy Center, Babeş-Bolyai University, Cluj-Napoca, Romania).

RESULTS AND DISCUSSIONS

Soil content in radionuclides and heavy metals

Soil analysis revealed the presence of natural radionuclides, originating in 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 in Ţânţăreni site, and U-238, U-235, Pb-210, Cs-137 in Cocoreni site (Table 1). The radionuclides activity in the samples from the ash pit (bottom ash) was 10-20 times higher than in the soil and the ash participates in air pollution, being spread by the wind, over the entire considered area.

Table 1. Radionuclide activity in topsoil (5-20 cm level), bottom ash and lignite dust (Bq/kg soil). With bold, the values over the limit for Romania.

Radionuclide	Ţânţăreni village	Cocoreni waste dump	Limits for Romania	Bottom ash	Lignite dust
U-238/Th-234	**43.20 ± 5.55**	**34.10 ± 4.51**	25.0	**656.6 ± 76.9**	< 10.5
Ra-226	25.50 ± 2.00	22.50 ± 1.40	10 – 90	**509.0 ± 186.6**	19.1 ± 1.75
Pb-210	33.40 ± 1.47	**44.78 ± 1.54**	20 – 40	**568.6 ± 42.7**	0
Bi-214	26.70 ± 2.37	22.00 ± 1.40	20 – 40	**267.4 ± 13.10**	23.5 ± 1.91
Pb-214	24.10 ±1.18	23.30 ± 1.10	20 – 40	**385.7 ± 19.4**	14.8 ± 1.28
U-235	**4.49 ± 0.41**	**3.33 ± 1.23**	2.0	**32.9 ± 8.26**	2.18 ± 1.83
Ac-228/Th232	35.80 ± 7.72	35.60 ± 1.80	13 – 65	**207.31 ± 24.0**	19.10 ± 1.72
Pb-212	4.15 ± 1.39	30.00 ± 1.65	20 – 50	**324.9 ± 20.7**	0
K-40	485.4 ± 22.0	388.9 ± 21.6	330 – 800	929.8 ± 76.0	< 43.0
Cr-137	**15.90 ± 0.83**	**5.77 ± 0.55**	0	**114.7 ± 10.10**	< 1.9

Table 2. The amount of heavy metals in topsoil (5-20 cm level) and bottom ash (mg/kg soil). With bold, the values over the normal content in soil in Romania.

Heavy metal	Ţânţăreni village	Cocoreni waste dump	Normal soil content	Alert limits	Bottom ash
Zn	49.1	58.7	100	300 – 700	63.0
Cu	13.8	**22.4**	20	100 – 250	19.0
Fe	20,899	21,205	*	*	25,004
Mn	593.0	310	900	1500 – 2000	252
Pb	**22.5**	**22.1**	20	50 – 250	2.53
Ni	**68.6**	**25.7**	20	75 – 200	**50.0**
Cr	**47.8**	**21.2**	30	100 – 300	20.0
Co	9.8	8.28	*	*	7.38
Cd	Traces	Traces	1.0	3.0 – 5.0	0.154

- Not available data in Romania

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 two sites. Thus, in Ţânţăreni site, there were recorded the highest values for Pb, Ni and Cr, while at the base of the waste dump from Cocoreni there were recorded only values slightly over the normal threshold for Cu, Pb and Ni (Table 2). In case of Cocoreni waste dump, it was recorded a diminished amount of some radionuclides (Ra-226, U-238, Bi-214, Pb-212, Cs-137) and some heavy metals (Mn, Ni, Cr), as a result of the phytoremediatory action of plants and of half time values (Tables 1, 2).

Compounds and structures associated with the resistance at the stress factors

Similar with the case recorded in other species (CORNEANU et al., 2014) in *Typha latifolia*, it was evidenced the presence of some compounds, which confer resistance at some stress factors such as heavy metals, radionuclides and other pollutant factors.

Ferritin is a metal protein of 450 kDal, present in all organisms, rich in iron (GOTO et al., 2001). The ferritin is associated with redox reactions, protecting the cell against the toxic effects of free iron, thus serving as a primary antioxidant (CORNEANU et al., 2014). HARRISON & AROSIO (1996) reported the presence of a ferritin accumulation in chloroplast in an area without thylakoids, named “crystal-like bodies”. DUY et al. (2007) and CORNEANU (2011) reported the ferritin synthesis in the chloroplast of the species *Phragmites australis*. In the leaves of *Phragmites australis*, in mitochondria matrix, as well as in the cytoplasm situated around mitochondria, there also appear some electron-dense particles of similar size with ferritin molecules (CORNEANU et al., 2014). Presently, the investigations performed on *Typha latifolia* leaves point out the ferritin synthesis in mitochondria and in chloroplast.

Similarly, ZHAO et al. (2014) reported the ferritin synthesis in *Typha latifolia* leaves. The investigations performed in *Triticum* grains (SINGH et al., 2013) enabled the estimation of the proportion of iron species in mature grains: 14–23 ± 3% is ferrous and the rest is ferric. During grain development, metal ions are remobilized from leaves to developing grains via phloem tissues, due to xylem discontinuity, seeds being non-transpiring organs (HELL &

STEPHAN, 2003; TANAKA & TANAKANA, 2007). In this process, ferritin is involved, which regulates the percent amount between ferrous and ferric ions.

Anthocyanins (flavonoids) are water-soluble pigments, present 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; HERNEA et al., 2014). Their synthesis takes place in chloroplast, the synthesized anthocyanin molecules being arranged on the thylakoid surface. From chloroplast, they migrate in cytoplasm, being accumulated in cell vacuole, together with many other corpuscles and structures: exogenous matter free or stored in multivesicular bodies, exogenous matter, etc. (CORNEANU et al., 2014). The presence of anthocyanin particles in these cells stops the toxic action of the toxic substances accumulated in these cells.

ARCHETTI (2009) analysed the leaves colour in autumn at 2,368 species belonging to almost 400 genera from the temperate region of the earth, establishing the phylogenetic relation between them. After OUGHAM et al. (2008), the reason for the anthocyanin production in autumn is not clear; for many species, the anthocyanin production is beneficial. Thus, in autumn, the anthocyanin from leaves migrates and accumulates in rhizomes, assures the plant resistance in the next year, when the stem and leaves of the next year develop from rhizomes (CORNEANU et al., 2014). The anthocyanin synthesis in the *Typha latifolia* leaves was also signalled in other papers GOLOVINA et al., 1998).

Other bioactive substances are synthesized in cytoplasm and accumulate as some amorphous masses, in cytoplasm or in vacuole.

Multivesicular Bodies (MVB) are endosomal organelles containing small vesicles (exosome), formed following the inward budding of the outer endosomal membrane, met in parenchyma cells or in the cells from the proximity of the conducting vessels. Their presence was recorded in different species by different authors (AN et al., 2007; PIPER & KATZMANN, 2007; OTEGUI & REYES, 2010; CORNEANU et al., 2014). Their presence in the cell is associated with exchanges between neighbouring cells by plasmodesmata, or at the level of the areas only with membrane media in the cell wall. The plant cells can secrete endosomes derived from multivesicular bodies. After OTEGUI (2014) "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".

Deposit cells. In the studies performed in *Phragmites australis*, CORNEANU et al. (2014) point out that the exogenous material together or not with the remnants of the destroyed cells is accumulated in some deposit cells, located on the underside of the leaf, near the lower epidermis, vascular circulatory system and aeriferous circulatory system or in the fundamental parenchyma from the central cylinder. They represent **deposit cells** of exogenous matter and/or remnants of the organelles. In these cells, multivesicular bodies are also involved in exocytosis processes. Between deposit cells there are plasmodesmata that facilitate the transport of this material, or some cells present small areas with a cut up in the cell wall. The presence in the deposit cells with exogenous matter and cell remainders, together with substances and some structures (multivesicular bodies) implied in their expulsion from the cell supports the deposit cells implication in the inactivation and / or exocytosis of the toxic waste materials from the plant. These findings are in accordance with the described mechanism of foreign expulsion in *Phragmites australis*, as well as with the ones described in a review, regarding the complexation of metals with different ligands and their mechanisms by LEITENMAIER & KÜPPER (2013).

Plasmodesma and presence of some **small areas in the cell wall, performed only the median membrane**, facilitate the matter exchange between neighbouring cells.

Nucleolus associated body (NAB) is a metabolic structure of the nucleus, of spherical-oval-shape, of 0.5 µm in diameter situated near the nucleolus or in close relation with it. Their presence indicates an intense normal or pathological metabolic activity (CRĂCIUN et al., 1996).

Absence of the cuticle from the epidermis cells favours the migration of the exogenous particles in the cattail leaf.

Ultra-structural features of *Typha latifolia* leaves

A. Control, Ţânţăreni village.

Upper epidermis composed of a single cell layer is covered with a thick cuticle. The pellicular cytoplasm contains a small number of cellular organelles disposed especially to the basal pole of the cell. In some epidermis cells, there is an exogenous matter, of squamous and granular shape (Fig. 4).

Palisade parenchyma is composed of long cells, with small spaces between them. The cytoplasm with cell organelles is parietally disposed. The cell wall, in small areas on its length, presents only the **median membrane** (Fig. 1), thus facilitating the exchanges of matter between the neighbouring cells, because the plasmodesmata number is reduced. In some ducts of the plasmodesmata, the presence of an electron-dense matter is visible. The exogenous matter is frequently met in the vacuole of the parenchymatous cells, as well as in the spaces between them (Fig. 4). In both parenchyma types, the ultra-structural features of the cells are similar.

The nucleus presents big heterochromatin areas disposed at its periphery or in its inner mass (Figs. 2, 3).

The chloroplast contains thylakoids structured in grana (Figs. 2, 3), numerous plastoglobules and starch grains. In some chloroplasts, especially in those situated in the lacuna parenchyma, the thylakoids number in grana is very high. The grana groups can be oriented or not on the chloroplast length. In the cells from the lacuna parenchyma, between the thylakoids from grana, **ferritin particles** appear (Fig. 2), which are synthesized in the "**like crystalloid body**" areas (Fig. 2).

Mitochondria usually presents dilated crysta (Fig. 2). The leaf cells are involved in an intense metabolic activity, some mitochondria being in division, and in others, there are the synthesized ferritin granules (Fig. 2).

In cytoplasm, neighbouring chloroplast, it occurs the synthesis of a bioactive substance. In an initial phase, it is visible the presence of brilliant small areas disposed in the cytoplasm near the chloroplast (Fig. 2). These particles joint and form amorphous masses, which are accumulated in cytoplasm, and finally in the cell vacuole (Fig. 3).

In the parenchyma cells, it also occurs the **anthocyanin synthesis**. The anthocyanin particles of different size are present in the vacuole from the parenchyma cells (Fig. 4), in epidermis cells, or in the cells situated near the conducting system.

In some parenchyma cells **the central vacuole compartmentation** is visible (Fig. 1), process also met in other species (CORNEANU et al., 2012). The vacuole compartmentation is important to preceed the metabolic cell activity in optimal conditions.

Multivesicular bodies (MVB) are met in vacuoles from the parenchyma cells or in the proximity of the conducting vessels (Fig. 3). After OTEGUI (2014) “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”.

The presence of the exogenous toxic factors in a big amount leads to the tonoplast breaking. In case of the Control variant, they were not met in the vacuole structures of myelin type, present because of some ageing processes or adulteration processes of the cell (CORNEANU et al., 2014).

The **central cylinder** is composed of conducting vessels with very thick wall (Fig. 1) and fundamental parenchyma. In the cells from the conducting vessels, the vacuole tonoplast can be broken. Both on tonoplast and in vacuole, there is an electron-dense matter, performed especially through anthocyanin granules (Fig. 4).

Lacuna parenchyma is composed of cells with rounded corners, with rich cytoplasm and numerous cell organelles. Between cells, there are bigger intercellular spaces.

Lower epidermis is composed of cells slightly flattened, with pellicular cytoplasm and a few cell organelles, covered with a thin cuticle. Both in the parenchyma cells and in epidermal cells, there are particles of exogenous matter (Fig. 4).

B. 35 year-old sterile waste dumps, Cocoreni.

The leaf tissue presents some particular features, as an adaptation to the environment condition (a bigger amount of some heavy metals and radionuclides).

Upper epidermis consists of cells with thin walls, covered with a very thin cuticle. The epidermis cells present prolongers for their joining (Fig. 5). Thus, it results a structure resistant at the action of environmental factors (a bigger amount of heavy metals and radionuclides), although the component cells possess a pellicular, rarefied cytoplasm, with a few cellular organelles (Fig. 5). In some epidermal cells, there are exogenous particles deposits especially on the tonoplast and on cell wall (Fig. 6).

Palisade parenchyma is composed of two parallelepiped cell layers, perpendicularly disposed on the epidermis surface, with small free spaces between them. Sometimes, the cells from the first layer of the palisade parenchyma present a prolonger with role in the solidarity process between tissue cells (Fig. 7). The cells present a rich amount of cytoplasm with numerous cellular organelles. The chloroplasts usually of lenticular shape present **granal structure** having numerous plastoglobules. The nucleus of spherical shape, slightly flattened, consists of discrete blocks of heterochromatin, disposed in its mass (Fig. 5). Between the neighbouring cells, there is a small number of **plasmodesmata**, for the exchange of matter between them. In the vacuole, there are **anthocyanin granules** and amorphous structures from a bioactive substance. In the vacuole, it is also present the compartmentation process (Fig. 5).

Lacuna parenchyma is composed of big cells with rarefied cytoplasm parietally disposed, with a few cellular organelles. The cell walls (usually the laterally walls) also present numerous prolongers for solidifying cells in leaf tissue. In some small areas, the wall cell is represented only through the median membrane, being thus facilitated the exchange between neighbouring cells.

Central cylinder presents the ligneous vessel cells, poorly lignified.

Lower epidermis is made up of big cells, pellicles cytoplasm and a few cellular organelles. The cuticle is practically absent.

In both parenchyma type, the cellular ultra-structure is relatively similar.

The chloroplasts from the lacuna parenchyma cells present a different shape (spherical-elongate, ovoid or lenticular shape), being usually composed of 3-5 groups of 2-3 stroma thylakoids and a few plastoglobules (Fig. 6). In some chloroplasts, it was pointed out the presence of some electron-dense particles, similar with ferritin particles (Fig. 6). In both chloroplasts from this population, starch grains were not present.

Mitochondria are sometimes long, with slightly dilated crysta (Fig. 6), in some mitochondria being present ferritin particles (Fig. 6). In addition, some mitochondria can be in division (Fig. 6).

In some cells, the cytoplasm is poor, rarefied (Fig. 8), with a few cellular organelles. The vacuole is delimited by tonoplast (Fig. 5). Other cells present a rich cytoplasm with many cell organelles. Between the neighbouring cells, there are plasmodesmata (Fig. 9). In some cells, near the nucleolus, a metabolic structure of NAB type is present (Fig. 10). Its presence indicates the presence of a stress factor (represented in this case by a big amount of exogenous matter).

In the vacuole, it can be present exogenous matter, cellular rests, tonoplast; sometimes the tonoplast (Figs. 8, 9) and the cell wall can break. The exogenous matter is free in the vacuole (Fig. 5), or parietals disposed on tonoplast and on the cell wall. In the vacuole, amorphous masses composed of different bioactive substances accumulate (Fig. 5).

In the cell vacuole and in the intercellular spaces, there are numerous **anthocyanin particles** (Figs. 5, 7), with role in stopping the toxicity processes, produced as a result of presence of the exogenous matter and the cellular rests (Fig. 8).

CONCLUSIONS

The content in heavy metals and radionuclides from the two sites from the middle Jiu river valley (Gorj district, Romania) and the ultra-structural features of the mature leaf from *Typha latifolia* L were analysed. It was also analysed the plant structures involved in plant resistance developed in an environment polluted with some heavy metals and radionuclides.

Typha latifolia is a hyperaccumulator for arsenic, lead, plutonium, selenium and other compounds, being also an energetic plant (in northern countries), having some medicinal and pharmaceutics properties, etc.

The substrate from the waste dump from Cocoreni (level 5-20 cm depth) presents a lower content in some heavy metals (Mn, Ni, Cr) and radionuclides (U-238, Ra-226, Bi-214, Pb-212, Cs-137), in comparison with the same level substrata, from the Control area (village Ţânţăreni), as a result of the phytoremediatory plants action and half-time value.

The cell wall in some areas from the cell leaf length is reduced at the median membrane, being thus facilitated the exchanges of matter between cells, the plasmodesmata number being reduced in *Typha latifolia* leaf (especially in the Control site).

In the plants developed on the sterile waste dump from Cocoreni, the epidermis and parenchyma cells present numerous prolongers, which assure a compact structure of the leaf, performed from cells with a rarefied cytoplasm, usually with a few cellular organelles.

The *Typha latifolia* plants from the Control site (near Ţânţăreni village) present a thick cuticle over the epidermal cells. The exogenous matter and rests of proper cells destroyed from the leaf tissue are accumulated in some **deposit cells**. These are represented through epidermal cells and some cells situated near the conducting vessels.

In the leaf cells, it occurs the synthesis of some bioactive substances with an antioxidant effect (ferritin, which is synthesized in chloroplast and mitochondria), anthocyanin and a bioactive substance, which stopped the infectious processes, a/o. These substances are accumulated in the leaf cells.

In the presence of a greater amount of exogenous matter (heavy metals and radionuclides), the plant reacts through the intensification of the cell metabolism, in some cells the mitochondria being in division.

The multivesicular bodies are in a small number, the elimination of the toxic accumulated mater and proper cellular rests, take place on other pathways.

The plants developed on the sterile waste dump from Cocoreni present, in nucleus the ***NAB*** corpuscle (Nucleolus Associated Body), a metabolic structure, which increases the plant resistance at the presence of a stress factor in a big amount.

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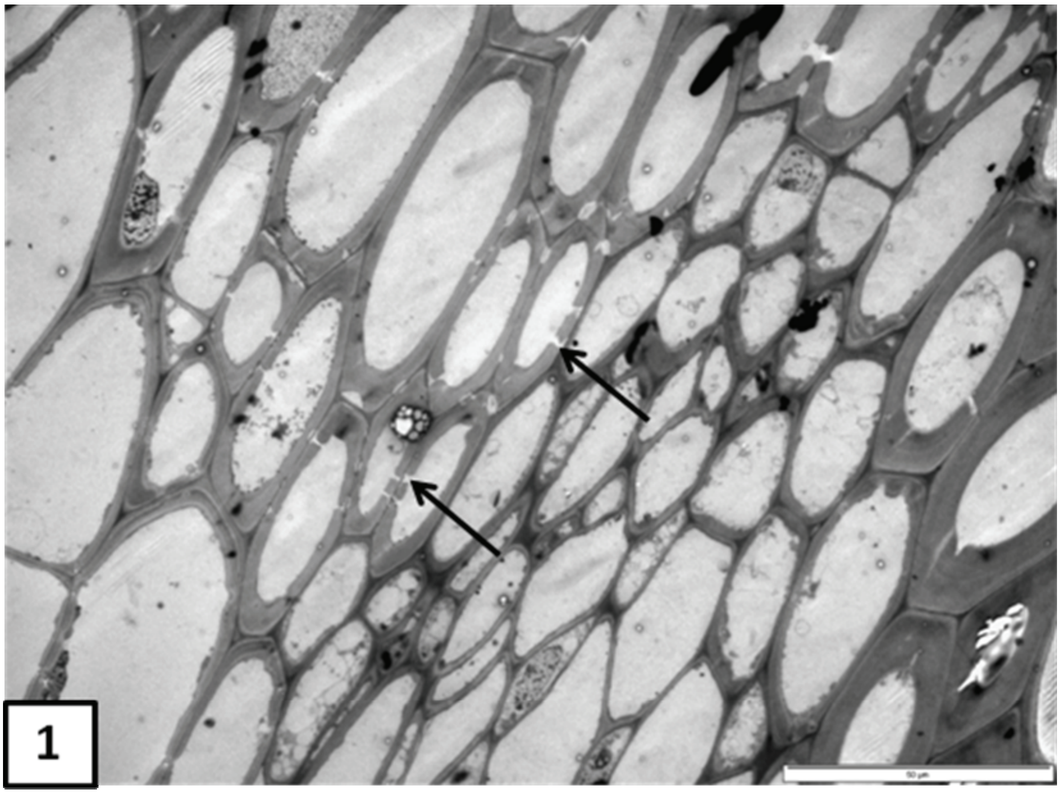
PLATE 1. *TYPHA LATIFOLIA*, CONTROL SITE, ŢÂNŢĂRENI VILLAGE.



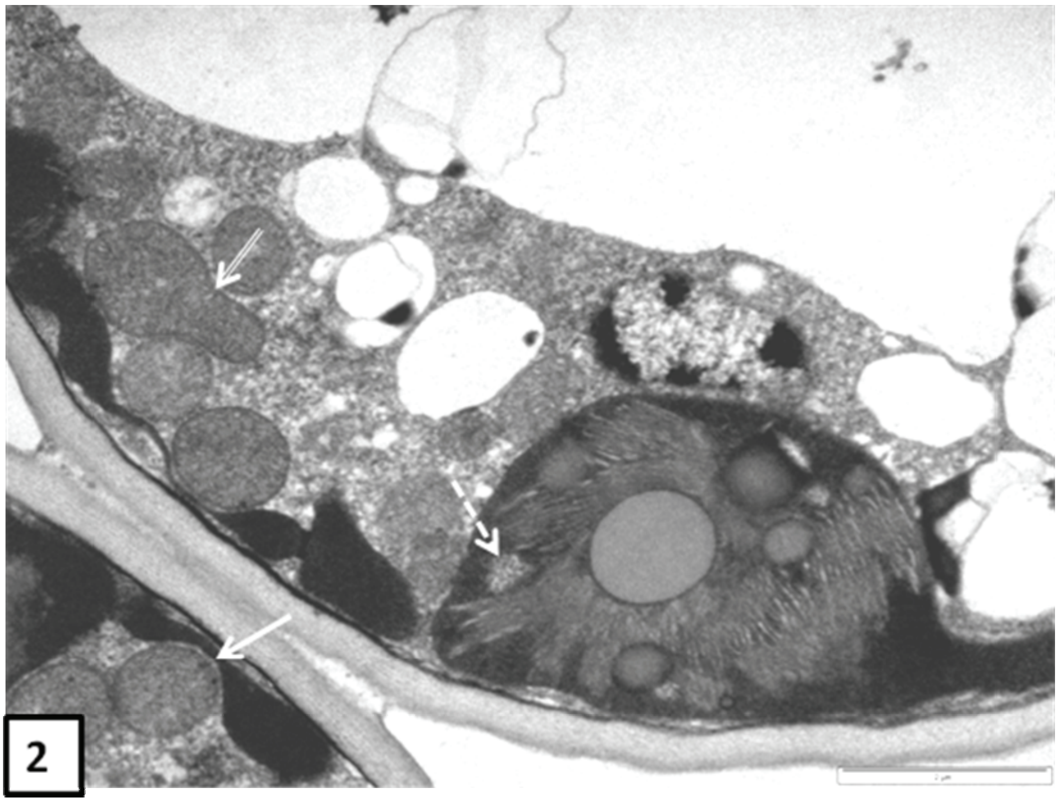
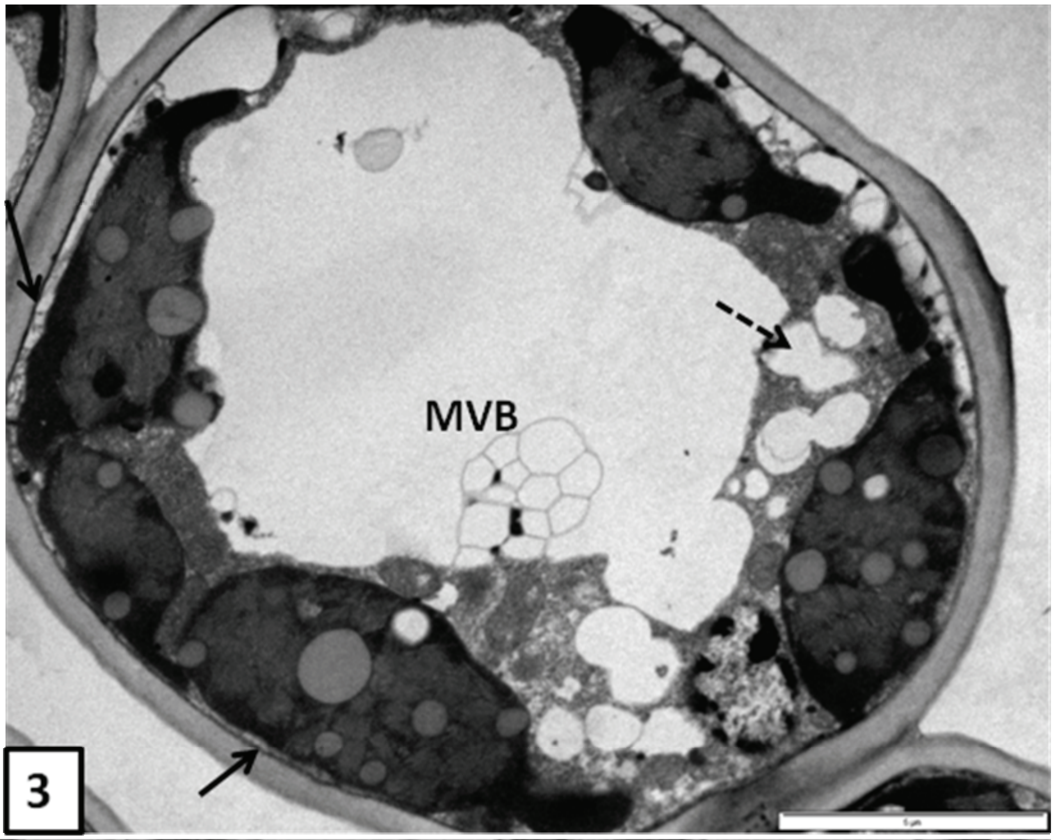


Figure 1. Leaf parenchyma and central cylinder. Membrane media from cell wall (**arrow** →). Vacuole compartmentation.
Figure 2. Chloroplast with a rich grana and a “like crystalloid body” area (**dots arrow**). Mitochondria in division (**double arrow**), with dilated crysta, or with ferritin granules in matrix (**arrow** →).
Nucleus with heterochromatin. Synthesis of a bioactive substance in leaf cell (also in figure 3).

PLATE 2. *TYPHA LATIFOLIA*, CONTROL SITE, ŢÂNŢĂRENI VILLAGE.



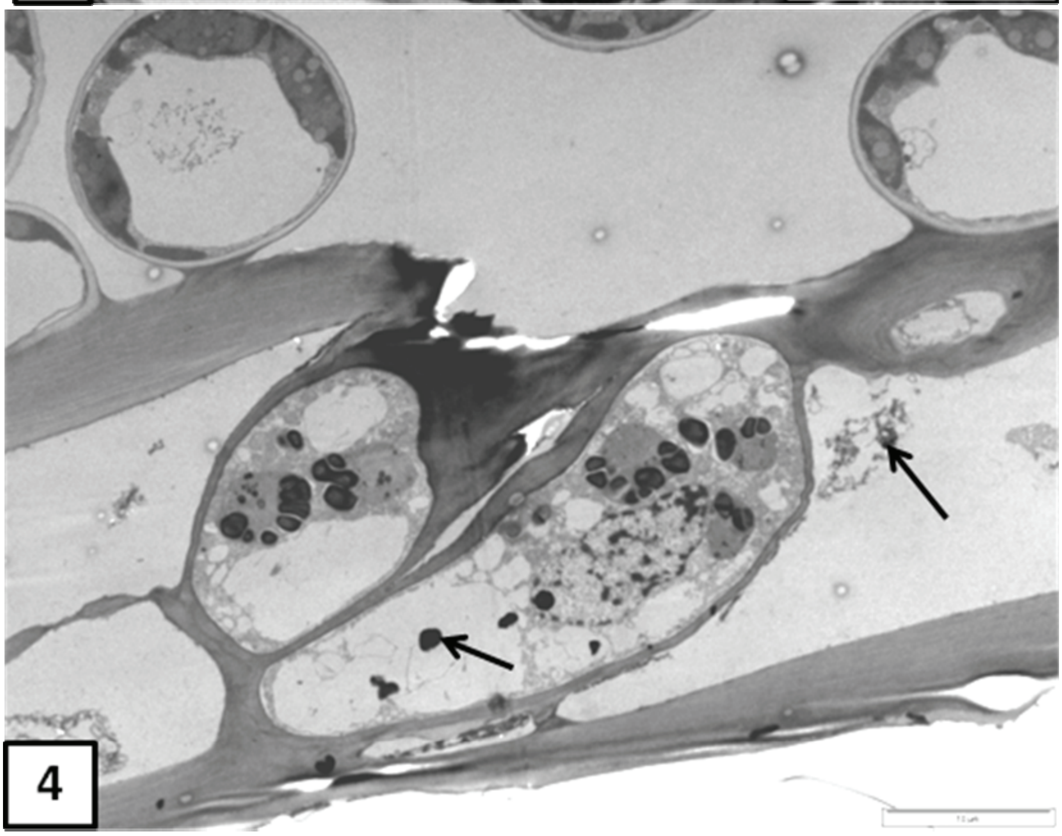


Figure 3. Synthesis of BAS near chloroplast (**arrows** →) and electron-clear BAS blocks in cytoplasm (**double arrow** ⇒). Microvesicular bodies (**MVB**) in cell vacuole. Nucleus with heterochromatin at periphery.
Figure 4. Anthocyanin granules in cell (**arrow** →). Exogenous matter in vacuole from lacuna parenchyma and lower epidermis.

PLATE 3. *TYPHA LATIFOLIA,* STERILE WASTE DUMP FROM COCORENI.

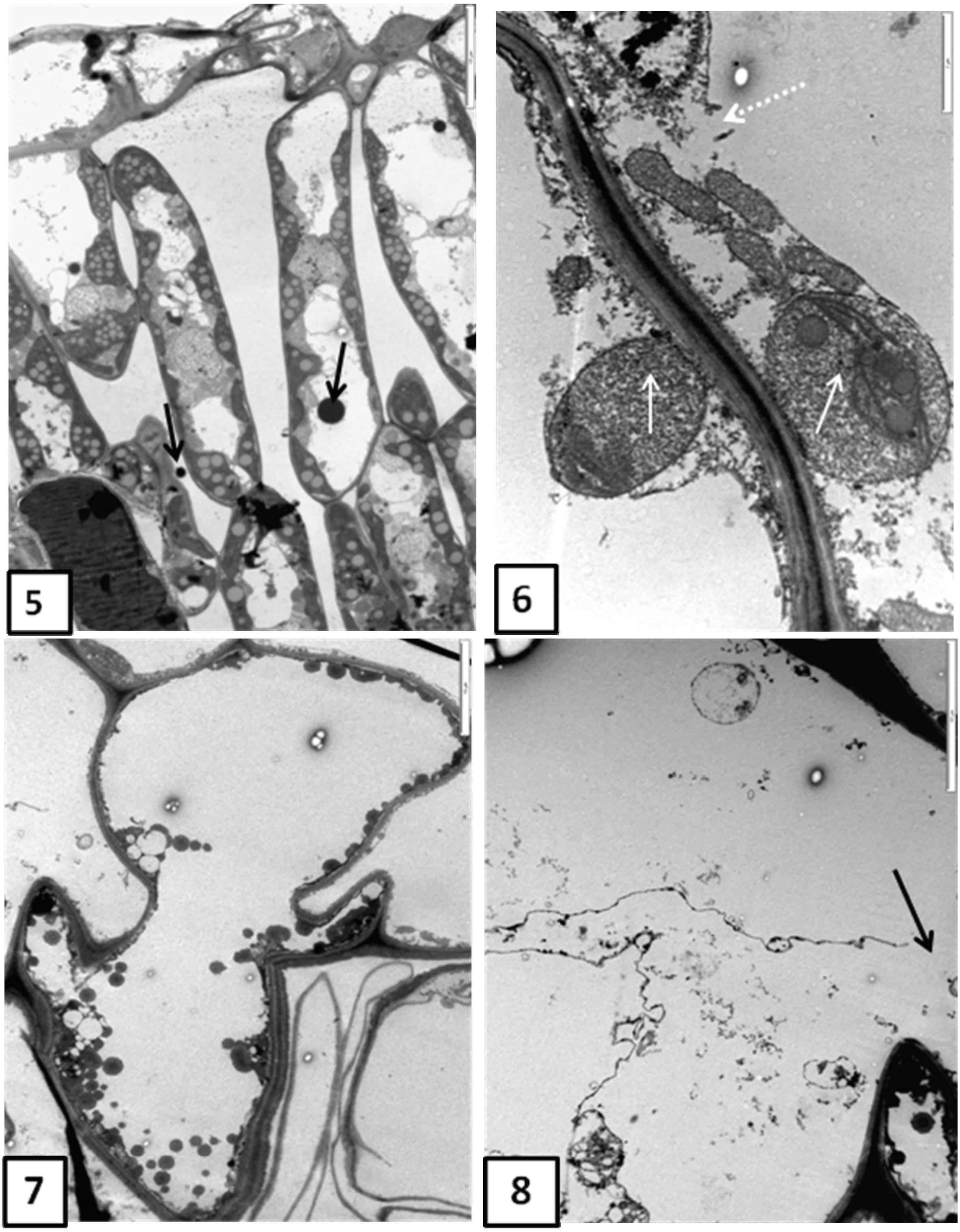
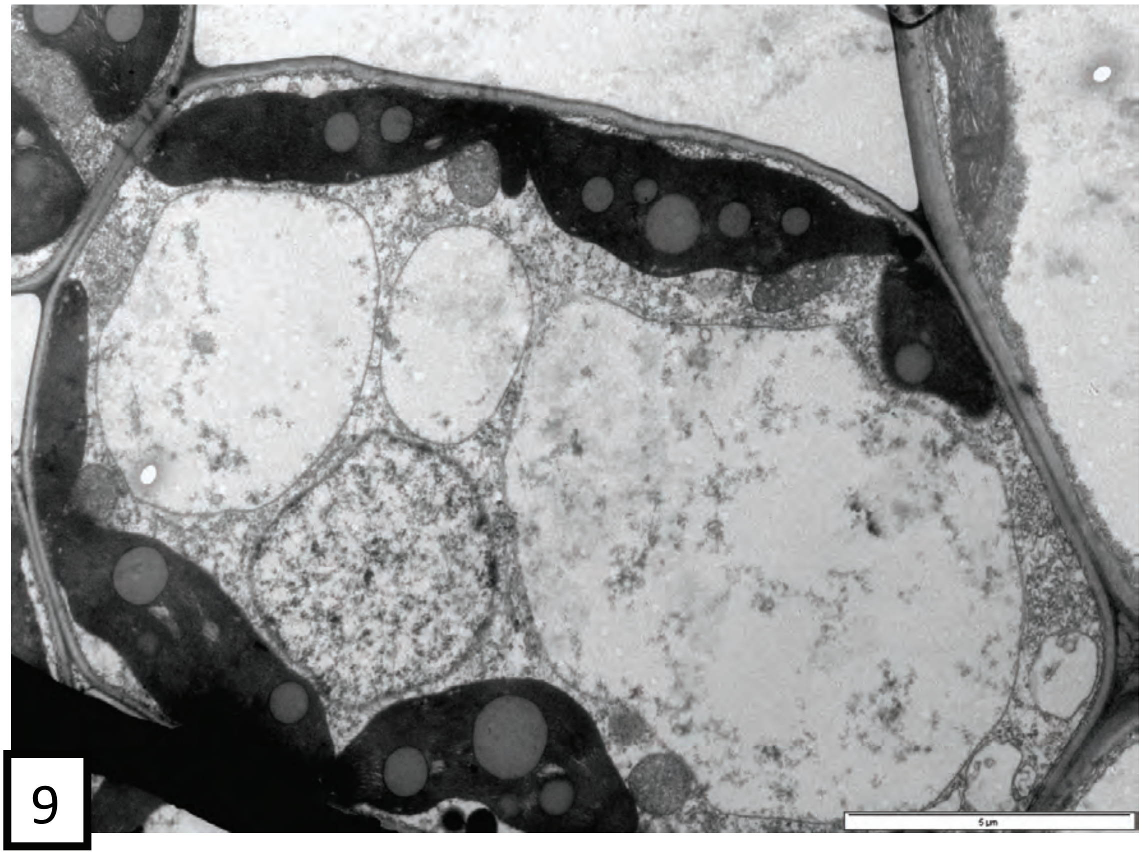


Figure 5. Epidermal cell with prolongers: pellicle cytoplasm with few cell organelles. Palisade parenchyma cells: Nucleus and chloroplast; Anthocyanin granules in vacuole and intercellular spaces (**arrow →**); compartmentation vacuole.
Figure 6. Chloroplast with stroma thylakoids, plastoglobules and ferritin (**arrow →**).
Elongated mitochondria in division. Brocken tonoplast (**dots arrow**).
Figure 7. Parenchyma cells with prolongers and anthocyanin. Brocken tonoplast and areas with membrane media.
Figure 8. Brocken tonoplast (**arrow→**). Exogenous matter and cell rests in cytoplasm.

PLATE 4. *TYPHA LATIFOLIA*, STERILE WASTE DUMP FROM COCORENI.



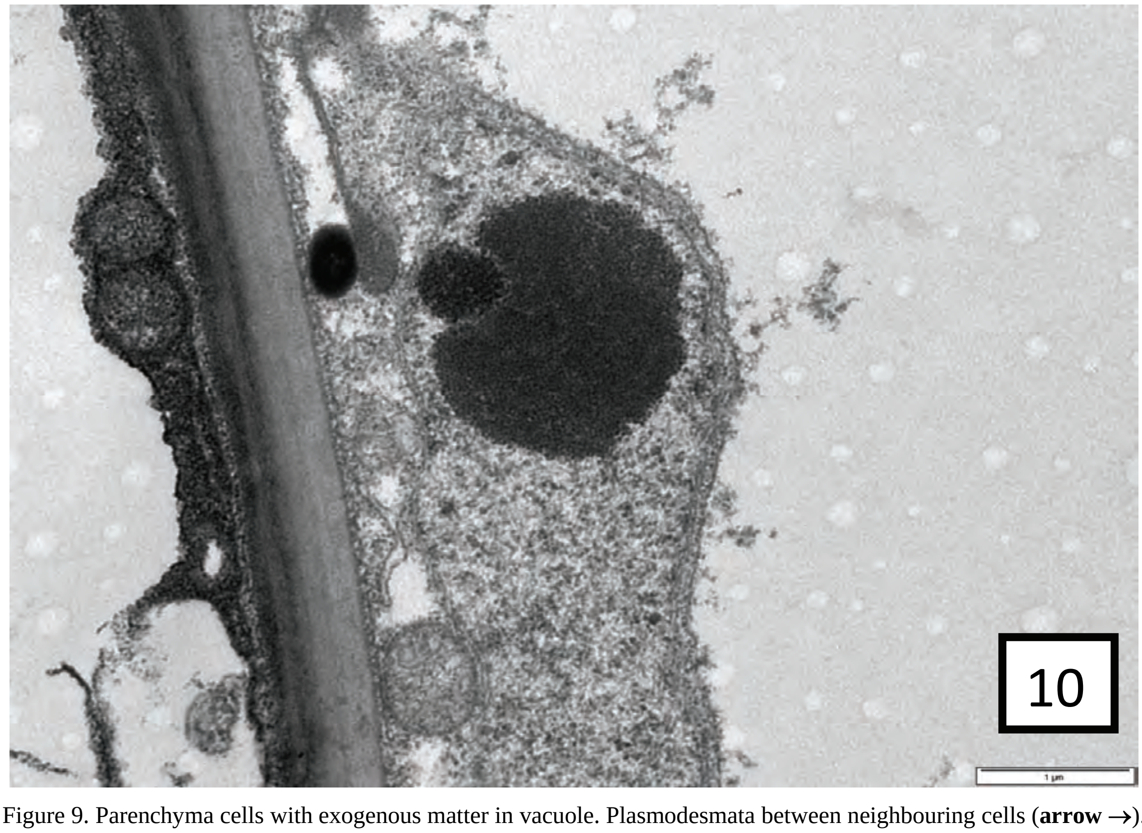


Figure 9. Parenchyma cells with exogenous matter in vacuole. Plasmodesmata between neighbouring cells (**arrow →**).
Figure 10. Parenchyma cell: nucleus with NAB (**arrow →**), mitochondria with dilated crysta, exogenous matter in vacuole.