Morphological studies of *Typha Australis* under stress environmental factor

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Abstract. Bioindication studies based on morphological parameters of *Typha australis* showed that the influence of stress ecological factor is seen not only in peculiarities of accumulation and distribution of heavy metals in plant tissues, but also, at morphological-anatomical and ultrastructural level.

1. Introduction

Coastal-aquatic vegetation plays a multifunctional role in littoral ecosystems, being an important factor determining the quality of soils, bottom sediments and water. The degree of its activity is specific to biological characteristics of plant species, vegetation periods, adaptation capabilities, intensity of anthropogenic action. Of the toxicants entering water bodies, heavy metals (HM) represent a serious environmental problem. The indicators of the state of macrophyte plants under conditions of habitat change include anatomical-physical-production processes, the specificity of which is determined by the peculiarities of plant growth. Marker changes of plants growing in areas with increased anthropogenic load include: abnormal changes in morpho-physiological signs and various deformations of organs [1, 2]. *Typha* L., like other macrophytes, are able to absorb biogenic elements and physiologically active substances – phenols, pesticides [3, 4] from water, they are characterized by high biological ability to accumulate metals. The systems of plant macrophytes are very labile, allowing to quickly change the direction of metabolic processes in conditions of habitat change, in particular under the anthropogenic load. This ability can be used in determining the environmental plasticity of coastal-aquatic plants under stress on toxicants with different mechanisms of action. However, the autecological mechanisms for regulating the adaptability of aquatic plants to adverse habitat conditions remain insufficiently studied. In this regard, the study of their response-reactions to loading in terms of toxicants, including HM, seems quite relevant.

The analysis of the characteristics of ultrastructural cell organization of plants under the action of heavy metals is an important factor to understand physiological changes caused by heavy metals.

The purpose of this work is to study anatomical and ultrastructural features of indicator helophyte – *Typha australis* Schum. & Thonn under HM loads.

2. Methods and materials

The primary focus was given to the territories with the highest level of anthropogenic pollution on the example of the sea edge of the Don Delta, the mouth zones of small rivers entering the basin of the
Azov Sea, the northern coast of the Gulf of Taganrog in the vicinity of Taganrog city with its industrial enterprises, roads and a harbor.

Alluvial-meadow, alluvial-layered, meadow, meadow-bog and boggy soils of different extent of salinization are widespread in the floodplains of small rivers, especially near the delta of the Don River.

Route-field study was carried out on the Russian territory of northern and southern coasts of the Gulf of Taganrog of the Azov Sea and the mouth of the Don River in 2017–2018. Soil and plant samples were taken at the monitoring sites.

To define the morphobiometrical measurements of Typha *australis* (Schum. & Thonn), standard cuts were taken in September 2017–2018 in natural habitats due to mass distribution of this species in coastal ecotopes, when vegetative and generative parts of Typha *australis* plants reach their maximum. An average sample of plants belonging to the same species was formed from each monitoring site. The combined plant sample consisted of 8–10 incremental samples. The study of Typha *australis* population in these territories revealed elementary morphological anomalies (teratormorphs) localized in generative organs – hypogenesis (a kind of deformity characterized by underdevelopment of organs or parts) and polymerization (increase in the number of organs). The Typha *australis* species with anomalies have two pistillate ears on one plant of various shape, length and width. Morphometric indicators were measured in normal and abnormal instances. Abnormal plants were determined according to A.A. Fedorov [5]. The following morphological signs were measured: height of a sprout, length and width of ears, length and width of the second ear on peduncle, as well as the distance between the ears were measured in the Typha *australis* with two ears. After selection, the plants were dried to air-dry state and ground. The root was pre-cleaned of soil particles before grinding.

Mineralization of Typha *australis* samples (Typha australis (Schum. & Thonn)) was carried out by dry combustion method according to GOST 26657-85. Low-pH extraction of HM from ash was carried out by dissolution in 20 % HCl solution followed by AAS [6]. The HM content in the model vegetation under study was compared with the maximum permissible levels (MPL) of chemical elements in farm animal feed and feed additives.

Plant samples from the most contaminated and least contaminated monitoring sites were taken for microscopic studies. The samples for microscopy were prepared according to standard procedure of double fixation of plant tissue modified to study hydrophytes [7]. Fragments of plant roots and leaves about 1 mm in size were taken for the study. The samples were fixed with 2.5 % glutaraldehyde solution on phosphate buffer (PBS) for one hour at 20–22 °C. Then the postfixation of 1 % solution of osmic acid was carried out in PBS within 120 min. For convenience of observation the samples were placed in flat capsules and filled in in epon. Ultrathin sections were made on the Leika EM UC6 microtome (Leica, Germany), studied and photographed on the translucent electronic microscope Tecnai Spirit G2 (Philips, Holland). For light-optical observations, half-thin sections (about 1 μm thick) were studied in light transmission microscope MICMED-6 (LOMO, Russia).

### 3. Results

#### 3.1. Heavy metal content in plants

Most of the plants of the studied area are contaminated with Cr, Zn, Ni, Cd, least with Pb (Table 1). All plant samples show an excess of Cr MDU. There was an excess of MPL in the analysis of Typha *australis* (Schum. & Thonn) in terms of Cr – by 26 times, Zn – up to 4 times, Ni – up to 4 times and Cd – up to 2 times. Metal content in plants is a reliable indicator of element mobility in soils. This may indicate that plants absorb a significant amount of these metals not only from the soil, but also directly from the waters of the nearby water body, which flood the territory during the seiche storm fluctuations of the water level of the sea edge of the Don River Delta and the zone of the coast of the Gulf of Taganrog [8]. The Zn content in the roots of Typha *australis* reaches 174.1±13.6 mg/kg and is many times higher than that in the above-ground parts of vegetative and reproductive organs of Typha *australis* (Schum. & Thonn), and accordingly higher than MPL (Table 1). The amount of Mn and Cu
in soils under *Typha australis* populations does not exceed the lower threshold concentration of these metals in plant feed.

The physiological significance of a number of analyzed elements acting as “essential” macro- and microelements of the plant organism is undeniable. At the same time, it is known that in case of significant anthropogenic contamination “microelements” can accumulate in the body at concentrations exceeding physiologically necessary levels [9]. Therefore, it is possible that high concentrations of Zn in the roots of the macrophyte plant – *Typha australis* (Schum. & Thonn) in this case are related to the impact of natural-anthropogenic factors, since the mouth region of large and small rivers is considered as part of a marginal filter or a cascade of barrier zones with avalanche sedimentation, interpenetration, mixing and transformation of river and sea waters containing large amounts of biogenic elements and organic and inorganic pollutants [10, 11].

3.2. Morphological observations

1) Root

Light-optical observations showed that parenchyma cells are located in the primary cortex between exoderm (EX) and endoderm (EN). In mesoderm, air-bearing cavities were observed – channels bounded from each other by several layers of parenchymal cells. Behind the endoderm there is an axial cylinder (AC) with conducting elements – xylem and phloem (Fig. 1a). Xylem occupies the center of the stela. Water absorbed from the soil moves through the epiderm, cortex, pericycle, and then enters the xylem. In plants that grew on contaminated soil, the root diameter was significantly smaller than the control samples. The breakdown of orderly arrangement of exoderm cells was also observed (Fig. 1b). The optical density of central cylinder structures decreased.

The analysis of electronograms showed that in the control root cells had ultrastructural characteristics similar to those of other authors [12]. They had intact cell walls, cytoplasmic membranes and a large central vacuole (Fig. 2c). Mitochondria measuring about 1 to 2 microns contained rare, arbitrarily oriented, extended cristae (2e) in their matrix. Significantly swollen endoplasmic reticulum vacuoles and few ribosomes were observed in the cytoplasm (Fig. 2e). Nuclear chromatin of low concentration is uniformly dispersed throughout the karyoplasm area.

The matrix of mitochondria plants that grew up on contaminated soil looked enlightened, cristae mitochondriales in most of the organelles were destroyed (Fig 3d, f). In many cells the cell membrane integrity was impaired and cytoplasm contents penetrated the central vacuole. In some cells, the cytoplasm looked condensed (Fig. 3 d).

### Table 1. Content of Mn, Cr, Cu, Zn, Pb, Ni and Cd in *Typha australis* (Schum. & Thonn) at monitoring sites in Fluvisols on the coast of the Gulf of Taganrog of the Azov Sea, the sea edge of the Don River Delta and small rivers, mg/kg

| sample          | Plant components | Mn    | Zn     | Cu    | Pb    | Cr     | Ni    | Cd    |
|-----------------|------------------|-------|--------|-------|-------|--------|-------|-------|
| Unpolluted site | leaves           | 101±7 | 17.5±1 | 3.9±0.2| 0.6±0.1| 2.4±0.2| 1.1±0.1| 0.04±0.003 |
|                 | roots            | 144±9 | 78.9±3.5| 9.9±0.7| 0.7±0.1| 4.5±0.3| 2.6±0.2| 0.06±0.007 |
|                 | flower heads     | 202±15| 62.6±4.3| 7.9±0.5| 1.0±0.2| 5.6±0.8| 1.4±0.1| 0.01±0.001 |
| Polluted site   | leaves           | 118±9 | 118.0±8.5| 4.4±0.1| 1.1±0.1| 7.6±0.5| 0.8±0.5| 0.51±0.01 |
|                 | roots            | 178±13| 174.1±13.6| 13.5±0.3| 6.5±0.3| 9.7±0.6| 9.3±0.5| 0.03±0.02 |
|                 | flower heads     | 357±26| 98.0±7.6| 8.8±0.2| 1.7±0.1| 3.2±0.2| 1.6±0.1| 0.02±0.001 |

Note: 1) in bold – excess over MPL (1987).
Figure 1. Cross section of the cattail root: a, c, e – control; b, d, f – pollution; Ep – epidermal cells; Ct – cortex; S – central cylinder (stele); TEM micrographs of cross ultrathin sections the cattail root. M – mitochondria; CW – cell wall; V – vacuole; ER – endoplasmic reticulum. The scale bar is (μk): a – 100, b – 100, c – 0.5, d – 1, e – 0.5, f – 1

2) Leaf
The surface of the leaf is covered with an epidermis consisting of tight-fitting, epidermal cells (Ep) (Fig. 2a). The space between the adaxial and abaxial epidermis is occupied by mesophyll cells (Me). Voids divided by transverse partitions into squares form a complex system of air-carrying channels (Vc). Voids of increased optical density – druzes (Dr) can occasionally be observed near conducting beams.

The effects of HM toxicity on plants grown on contaminated soil caused an increase in the thickness of the leaf plate (Fig. 2b), as well as a decrease in the number of mesophyll cells.
Figure 2. TEM micrographs of cross ultrathin sections the cattail leaf in control – a, c, e and with pollution – b, d, f. CW – cell wall; Cl – chloroplast; P – plastoglobule; Thy – thylakoids gran; M – mitochondria; P – pyroxsome, V – vacuole. The scale bar is (μk): a – 100, b – 100, c – 2, d – 1, e – 1, f – 1
Most of the cell section area in samples grown on pure soil is occupied by a central vacuole, in which individual clusters of fine particles can often be observed (Fig. 2c). A cytoplasm containing nucleus, plastids, mitochondria, ribosomes, and other organelles fits tightly to the cell wall. Chloroplasts of mainly ellipsoid shape contain a dense matrix, on the background of which grans with the number of thylakoids up to 20 units are clearly observed. Plastoglobules, which can number up to 4 units and the maximum diameter making 0.4 μm, are located mainly in the center of the organelle. The oval-shaped mitochondria contain a moderately dense matrix in which numerous and slightly swollen cristae are evenly distributed (Fig. 2e). The degree of ribosome concentration in the cytoplasm is quite high. Peroxisomes contain a fine grain uniform matrix, and their diameter is about 0.8 μm. The core is elongated and separated from the cytoplasm by a double-circuit shell.

In the plastids of plant cells grown on contaminated soil, there was a decrease in electron density and a vacuolization of the matrix at certain sites. Large (more than 0.5 μm in diameter) and dense plastoglobules are located throughout the area. Their number in the cell can reach 10 units or more (Fig. 2d, f). The concentration of ribosomes in the cytoplasm is relatively low. The shape of the most mitochondria is rounded, the matrix is enlightened, and the organelles themselves look swollen (Fig. 2f). The few peroxisomes contain a fine-grained matrix of reduced electron density. Nuclear chromatin is evenly distributed throughout the organelle area.

4. Conclusion
Light-optical and TEM observations showed that the HM toxicity caused a significant reduction in root size and air voids as well as the disruption of cell membrane integrity in some cells. The decrease in aeration degree is also observed in water roots of another macrophyte – Typha angustifolia L. under conditions of load on acetic acid lead acetate [13]. The TEM microscopy method showed partial degradation of the cell wall of the root parenchyma of Typha angustifolia under toxic effect of Pb(NO₃)₂ [14]. The hydroponic study on rice carried out by [15] also showed that Cu can be taken, transported, bioprocessed and transferred to the root epidermis, exoderm, cortex and endodermis. Obviously, such structural changes in the root directly contacting dissolved compounds allow macrophyte plants adapting to adverse habitat conditions. Disorders of chloroplast ultrastructure in the presence of HM are one of the key reasons to decrease the pigment content in plants and, in general, the decrease in photosynthesis intensity. In the presence of high concentrations of Cd, the structure of the outer membrane of chloroplasts is changed [16], as well as the membranes of thylakoids, the number of grains is reduced, their shape and structure are disturbed [17]. Chloroplasts, peroxisomes and mitochondria are the main organelles of photosynthesis, so the destructive changes in these organelles are obviously associated with a decrease in the level of metabolic processes contributing to plant growth.

The increase in the number of plastoglobules is probably caused by changes in the membrane structure of plastids. At the same time, it is known that it is in plastoglobules that the components of photosynthetic membranes accumulate – lipids, proteins and pigments released during reconstruction of the grand under extreme factors [18]. Thus, it can be assumed that an increase in the number of plastoglobules is a protective mechanism against damage to the photosynthetic apparatus under HM contamination.

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