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Intracellular localization, accumulation and distribution of heavy metals in plants

Abstract: Heavy metals uptake, distribution and accumulation processes in plants are very important for their impact on physiological and biochemical processes and, consequently, on plant growth and development. The distribution of heavy metals in plant parts and cell organelles are discussed. There are detoxification mechanisms in cell compartments, like binding with cell wall, with organic acids in vacuoles, complexes with phytochelatins and etc. What proportion of given metal ions would be the in free form, and what – bound with organic molecules; it depends from pH of the environment and chemical properties of element. The stability of metals complexes decreases in the case of deviation pH of environment from neutral: at low pH there is a competition of protons with metal ions for binding sites in molecules, at high pH – by the reason of the competition of hydroxyl groups with ligand.

Key words: intracellular localization, heavy metals, distribution, accumulation.

Main body

Plant roots contain the greatest amount of heavy metals [1, 2]. Less heavy metal accumulate in stems and leaves, and even less – in the grain. Concentration of heavy metals in the grain and aboveground organs is mainly due to «the effect of detention» in their roots, which are more tolerant as compared to sensitive plant species. An effective mechanism for retarding the heavy metals exists in the roots. Comparison of heavy metals contents in soils and plant parts showed that the dependence of content in plants by soil concentration increases in the following order: Cd > Zn > Cu > Pb > Cr for monocots and depends on the mobility of metal in the soil. For dicots, this pattern is less pronounced [3].

The character distribution of heavy metals in cell organelles plays an important role in protective mechanism in plants [4-8]. The absorption of metal ions by the root system from the soil and nutrient solution is carried out in various ways, on which the likelihood of intake of ions directly into the cytoplasm of cells and the rate of movement of the tissues and organs of plants depends. The character of metal accumulation in organs of plants depends on the plant species and a metal nature (Table 1).

Zinc at high concentration (25 mg/kg) was accumulated in significant amounts in the aerial parts of wheat and beans. The protective function of the roots towards Cd was more expressed than for Zn, which is not accumulated in the roots, and moved in the stems and leaves. This is probably due to unequal role of these elements in the plant metabolism. Translocation rate of cadmium and lead to the aboveground part as compared to Zn was much lower. Most of the Pb is retained in the root system. Localization of the metal in parts of the plant is dependent on its mobility. According to the researchers, Pb in plants of lupine was contained mostly in the tips of the roots, less – in the basal part, and hypocotyls. Most Pb detected in cortical parenchyma in comparison with central cylinder. This fact was explained by Pb lower mobility as compared to other metals.

Patterns of distribution of Pb, Cd and Zn in the tissues of the root are not well understood. The apical root sections on metal content may vary from basal. Many authors have noted that at high concentrations of metals in the environment of the basal part of the roots accumulate significantly more Pb, Cd and Zn, than apical one, especially in resistant populations. Other authors suggest that most of the metal accumulates in meristematic parts of the roots [3].

Different plant species have unequal protective opportunities, as evidenced by their tolerance to different heavy metals. According to one hypothesis, the main role in the development of resistance belongs to the binding of metal cell walls in the roots. Study of the intracellular localization of heavy metals found that they bind to the cell membrane and accumulate in the vacuoles. The relatively high concentration in the cell walls of Pb and Cd detected in a number of species. According to another hypothesis, their role

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is a compartmentalization and metal accumulation in vacuoles of root cells. Cell walls of monocotyledonous and dicotyledonous plants differed in content of pectin and hemicelluloses, thereby manifest differences in their ability to bind cations. The bond strength of certain metal ions with the components of the cell wall varies. It correlates with different values of stability constants (Log K) metal complexes with functional groups of carbohydrates. For Pb, it is equal to 6.4, for Cd – 4.9. Therefore, Pb binds more strongly with cellular membranes than Cd, and slowly moves along the apoplast. The affinity of other metals to polygalacturonic acid decreases in the following order: Pb > Cr > Cu > Ca > Zn. It was not studied the binding of Pb and Cd apoplast proteins. In barley leaf apoplast Cd increased the content of these proteins. However, the role of these proteins is uncertain. Under the influence of heavy metals can be enhanced suberin and callose deposition, reducing the absorption of metals [3].

Table 1 – Intercellular localization of Cd and Pb in plants (I.V. Seregin, V.B. Ivanov, 2001)

Plant species, Metal, tissue	Cell wall	Vacuole	Golgi apparatus	Endoplasmic reticulum	Nucleus	Research method
Zea mays (Cd)						
Differentiated cells (Cd – $2x10^{-1}$ mMol; $3x10^{-3}$ mMol)		+			+	The X-ray microanalysis
Mature cortex and stele cells	+	+				Electronic microscopy Histochemistry
Rhizodermis	+					
Endodermis	+					
Pericycle	+/-					
Xylem parenchyma	+					
Rhytidiadelhus squarrosus (Pb – 4.8 mMol)		+	+		+	Electronic microscopy
Lemna minor (Pb – $3x10^{-3}$ mMol)					+	
The outer layer of root cap	+					Electronic microscopy Histochemistry
The other cell layers of root caps	+		+	+		
Epidermis			+	+		
Cortex	+		+	+		
The basal part of the root	+	+				
Allium cepa (Pb-7.5x 10^{-3} mMol)	+	+	+			Autoradiography
Zea mays (Pb – $8x10^{-1}$ mMol; $7.5x10^{-3}$ mMol)	+	+	+			Electronic microscopy X-ray microanalysis
Glycine soya (Pb)	+	+	+			
Lupinus luteum (Pb – $4.3x10^{-2}$ mMol)						Electronic microscopy
The cortex parenchyma	+		+	+		Histochemistry
Stele	+/-		+/-	+/-		Electronic microscopy
Pisum sativum (Pb)	+	+	+			Electronic microscopy
Raphanus sativus (Pb – 5 mMol)	+		+			Electronic microscopy

Heavy metals were also detected in the intercellular space, dictyosome, endoplasmic reticulum, nuclear membrane. In the cytoplasm ions can bind to biomolecules. In this case, the chelate is derived from a cell or accumulated therein (mostly in the vacuoles). Accumulation of toxic ions into vacuoles in the form of inactive compounds is more typical for plant tolerance to heavy metals. Fraction remaining in the cytosol as free ions or soluble complexes is moving simplastically from the root to the stem and then – to the leaves of plants by charged sites of xylem or carried away by the transpiration stream of water.

Cd accumulated in the shell in less than inside the cell. It can bind to intracellular lysosome like granules largest amount of the metal is in the cytoplasm. It is known that the usual response to the Cd is the induction of synthesis of low molecular weight, cysteine-rich proteins – metallothioneins, phytochelatins [5, 9-15].

Bond Cd with organic ligands is much stronger than other metals. At high concentrations of Cd-phytochelatins complexes are localized in the vacuoles. At low concentrations 86-100% of Cd found in the cytoplasm in *Datura inoxia* (Rauser, 1987). Under these conditions, there is no need to isolate the cells of Cd in the vacuoles. At high concentrations Cd binds with organic acids, and low – with glutathione in the cytosol [11-13, 16].

Cadmium is found in the cytoplasm and nucleus vacuoles bentgrass and roots of corn, and also indicates the presence of Cd in the cell wall of roots of maize. Other studies have detected a high concentration of Cd, associated with phosphorus and calcium in the sea fern *Azolla filiculoides* L. [17]. Most of them have been identified in the cell wall of the xylem vascular bundles. It is shown that the Cd concentration decreases with increasing content of Se, Al, K, Ca, P. The aerial parts and roots of wheat subjected to the effect of Cu, the metal was found in the cell wall and vacuoles. In the presence of Cu in the growth medium, the ratio of the metal content in the cell walls and vacuoles increased in favor of the latter [17, 18].

Copper was found in the matrix of the cell walls of *Enteromorpha compressa* [19]. The study of Pb effect on *Lemna minor* L. it has been found that Pb is present in the vacuoles, vesicles and the cell wall. At high concentrations of Pb found in symplast. Changing the ratios of content of Pb in vacuoles and cell walls after a 6- and 12-hour of exposure in favor of the latter indicates a redistribution of the metal cell walls [20]. It was found that in *Thlasi caerulescens* L., subjected to the effect of Zn, the metal is concentrated in the epidermal cells and vacuoles [21]. At low concentrations the greatest amounts of Zn was found in the vacuole. Perhaps tonoplast of epidermal cells of leaves of *T. caerulescens* has a higher ability to transport Zn in the vacuole than mesophilic cells. The ability to isolate in *T. caerulescens* Zn in epidermal vacuole is an important aspect in this type of hypertolerance to heavy metals. The preferential localization of Zn in the epidermis, apparently contributes to the protection of mesophyll cells from damage and maintains the functions of mesophyll cells at a high concentration of Zn in the leaves. *Arabidopsis halleri* L. is pseudometallophyte, i.e. it is growing in contaminated and uncontaminated soils. It is hyperaccumulators of Zn, as well as Cd. Recent studies using electron microscopy demonstrated the cellular distribution of Zn in the tissues *A. halleri*, grown in a hydroponic environment. Zinc in the plant leaves mainly found in the base of trichomes – hairs, present on the surface of plant leaves, and mesophyll cells [22, 23].

Thus, the character distribution of heavy metals in cell organelles plays an important role in protective mechanism in plants. The above data show that not all ions uptake by plants actively influence at its metabolism, so as a definite part of metals can bind with organic acids and low-molecular proteins and concentrate in an metabolically low-active compartments. Part of toxic ions turns out of firmly bound with reactive-capable portions on the surface cell wall and in the apoplast, and are penetrating across the plasmalemma – with intracellular biomolecules. It is also important to take into account, that the multiply charged ions form more stable complexes, than the singly charged, possessing lesser charge density.

Thus, the heavy metals are mainly concentrated in the roots of plants, which limits their movement in the generative organs. A common feature of interstitial and intracellular distribution is the concentration of large amounts of metals in the surface structures and protecting cells from the toxic effect of metals. Concentration of heavy metals occurs by binding them into soluble compounds having a different nature. Despite the significant accumulation of heavy metals in a metabolically inactive cell compartments (vacuoles and cell walls), some of the metals enters the cytoplasm and exerts multiple toxic effects, and this may be due to both direct effect of metals and reducing the activity of some of the processes as a result of violation of others.

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