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Potential of Higher Plants as Environmental Remediators

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ABSTRACT. Elimination of contaminants from the environment by microorganisms of different taxonomic groups is a genetically determined property, which has already been widely discussed. Until recently, plants still occupying above 40% of the world land, were considered as organisms having only a limited potential for contaminants conjugation and accumulation within cell organelles. Analysis of experimental data of last two decades revealed the high ecological potential of plants. It has been exposed deep degradation processes proceeding in higher plants and in the majority leading to complete detoxification of anthropogenic contaminants: the enzymes carrying out oxidation and conjugation processes have been revealed and characterised; formation of anthropogenic contaminants conjugates with endogenous compounds and enzymes participating in this process has been shown. However, still there are uncertain questions closely related to the contaminants multistage degradation process in plants, the authors are making an attempt to evaluate different aspects of plants, ecological potential from the modern understanding, revealing the criterion for the evaluation of deviations under the action of contaminants in the ultrastructural architectonics of plant cells. *© 2007 Bull. Georg. Natl. Acad. Sci.*

Key words: *functionalization, conjugation, compartmentation, detoxification enzymes, cell ultrastructure.*

Natural contaminations such as the emission of poisonous gases during a volcanic eruption and earthquakes, swamps poisoned evaporations, synthesis of toxic compounds by lower (microorganisms) and higher plants, etc., in comparison with the human contribution in the environmental contamination is much less impressive. In spite of difficulties in quantitative, as well as in qualitative estimation, and having a tendency to increase, the amount of spread out contaminants exceeds annually one billion tons. Most dangerous among these contaminants are considered as emergent because of their persistence, bioaccumulation, and toxicity along with our awareness of their prominent occurrence in the environment. In different ways, huge amounts of these hazardous substances or toxic intermediates of their incomplete transformations are accumulated in the different niches of biosphere, significantly affecting the ecological balance. Lately, many ecological technologies have been elaborated, targeted to minimize the flow of toxic compounds to the biosphere and to control their level or state [1, 2]. The international character of this problem being determined by global migration of contaminants (migration between soil, air and water, geographical, biotic, etc.) leads to distribution of toxic compounds of different structure and overall increasing the level of toxicity.

Nevertheless, the plants kingdom members assimilate toxic compounds, removing them from the environment, naturally providing long-term protection and monitoring against their environmental dispersal. Plants being recently recognized as important ecological tools and in order to properly evaluate their detoxification potential should be emphasized according to the following features:

- Higher plants simultaneously contact three main ecological niches: soil, water and air.
- Well-developed root system of higher plants determines soil-plant-microbial interaction, representing a

unique process, significantly affecting the overall plant metabolism.

- Large assimilating surface area of plant leaves (adaxial and abaxial), significantly exceeding in size the above ground surface under the plant, permits the absorption of contaminants in a big quantity from air via the cuticle and stomata.

- Unique internal transportation system in both directions, distributing all penetrated compounds throughout the entire plant.

- Autonomous synthesis of vitally important organics and requirement of extra energy during prolonged remediation process.

- Existence of enzymes catalysing oxidation, reduction, hydrolysis, conjugation and other reactions of multistage detoxification process.

- Large intracellular space to deposit heavy metals and conjugates of organic contaminants.

- Functionalization and further transformation of organic contaminants in plant cells (conjugation, deep oxidation, etc.).

The intensity of the contaminants absorption process, characterized by various regulations, depends on contaminants solubility, molecular mass, concentration, polarity, pH, temperature, soil humidity, etc. [1, 3].

Nowadays there are experimental data obviously demonstrating that plants activate a definite set of biochemical and physiological processes to resist the toxic action of contaminants by the following mechanisms: excretion, conjugation of contaminants with intracellular compounds and further compartmentalization of conjugates into cellular structures, decomposition of environmental contaminants to standard cell metabolites or their mineralization.

Commonly, plants gradually degrade entering cells organic contaminants to avoid their toxic action. According to contaminants assimilating potential plants sometimes differ up to four orders of magnitude, allowing to classify plants as strong, average and weak absorbers of different structure contaminants. For instance the most active assimilators uptake up to 10 mg of benzene per 1kg of fresh biomass per day, the assimilation potential of the weak absorbers is measured in hundredths of mg [4].

The simplest pathway of organic contaminants entering the plant cell is excretion. The essence of excretion is that the toxicant molecule does not undergo chemical transformation, and being translocated through the apoplast, is excreted from the plant. This pathway of xenobiotic (contaminant) elimination is rather rare and takes place only at high concentrations of highly mobile (phloem-mobile or ambi-mobile) xenobiotics.

In the great majority, contaminants being absorbed and penetrated into plant cell undergo enzymatic transformations leading to the increase of their hydrophilisity-process simultaneously accompanied by decreasing of toxicity. Below there are presented successive phases of contaminants initial transformations in accordance to Sanderman's green liver concept [5] (Fig. 1):

Functionalization is a process whereby a molecule of a hydrophobic organic xenobiotic acquires hydrophilic functional group (hydroxyl, carboxyl, amino, etc.) as a result of enzymatic oxidation, reduction, hydrolysis, etc. Due to the introduction of functional group the polarity and correspondingly reactivity of the toxicant molecule is enhanced. That promotes an increase of intermediates affinity to enzymes, catalyzing further transformation.

Conjugation takes place as a basic process of phytoremediation and is determined by formation of a chemically coupled contaminant to endogenous cell compounds (proteins, peptides, amino acids, organic acids, mono-, oligo-, polysaccharides, lignin, etc.) forming of peptide, ether, ester, thioether or other type covalent bonds. Intermediates of contaminants initial transformations or contaminants themselves possessing functional groups capable of reacting with intracellular endogenous compounds are susceptible to conjugation.

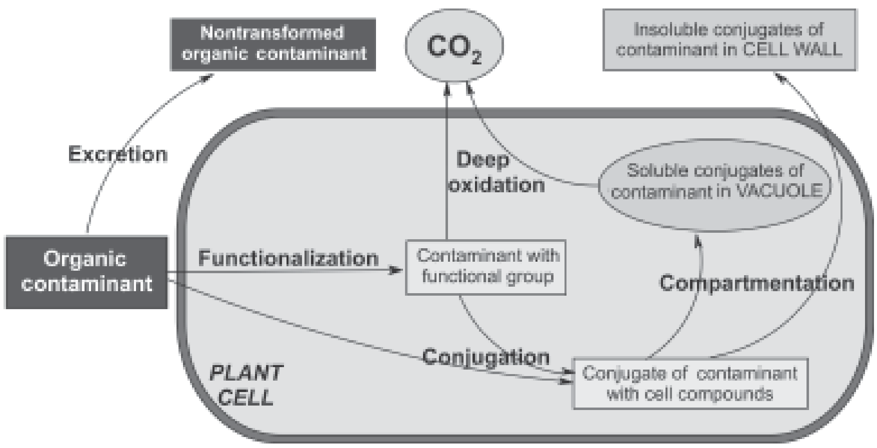

Fig. 1. The main pathways of organic contaminant transformation in plant cells

Commonly, the main part of the toxicant molecules undergoes conjugation and only a small amount is deeply degraded (0.1-2% depending on contaminants structure). Conjugation is a wide spread defence mechanism in higher plants especially in cases when penetrated into plant cell concentration of the contaminants exceeds the plant's transformation (decomposition) potential. Increased amount of deep degradation to regular plant sell metabolites, or CO_2 and water is achieved in case of linear, low molecular structures of contaminants [6, 2]. Relatively quickly, after the termination of plant incubation with the contaminant, conjugates are no longer found in plant cells.

Although conjugation is one of the most widely distributed pathways of plant self-defence, it cannot be assumed as energetically and physiologically advantageous for the plant metabolism process. Firstly formation of conjugates leads to the depletion of vitally important cellular compounds, and secondly unlike deep degradation, formation of conjugates is maintaining contaminants basic molecular structure, and hence results only in partial and provisional decreasing of its toxicity.

Compartmentation. In most cases the final step of conjugates processing temporary (short or long) storage of conjugates in defined compartments of the plant cell takes place. Soluble conjugates of toxic compounds (coupled with peptides, sugars, amino acids, etc.) are accumulated in cell structures (primarily in vacuoles), while insoluble conjugates (coupled with lignin, starch, pectin, cellulose, xylan) are moved out of the cell via exocytose in the apoplast being accumulated in the cell wall [5]. The compartmentation process is analogous to mammalian excretion, essentially removing the toxic part from metabolic tissues. The major difference between detoxification in mammals and plants is that plants do not have a special excretion system for the removal of contaminants conjugates from the organism. Hence they use a mechanism of active transport for the removal of the toxic residues away from the vitally important sites of the cell (nuclei, mitochondria, plastids, etc.). This active transport is facilitated and controlled by the ATP-dependent glutathione pump [7], known as “storage excretion” [8].

The above described pathway of toxic compound processing i.e., functionalization $\rightarrow$ conjugation $\rightarrow$ compartmentalization, is well illustrated by the processing of contaminants of different structures. One of such examples demonstrating the transformation of organochlorine pesticides is the hydroxylation of 2,4-D followed by conjugation with glucose and malonyl residues and deposition in vacuoles [9].

The Enzymes. Contaminants decomposition process is closely related to many aspects of higher plants cellular metabolism. In prolonged and multifunctional detoxification processes quite a few enzymes are actively involved. According to catalyzed reactions they directly or indirectly participate in the detoxification process.

Transformations of contaminants during functionalization, conjugation and compartmentation are of enzymatic nature. It is remarkable that due to their unusual flexibility in the absence of xenobiotics, in plant cell these enzymes catalyze reactions typical for regular plant cell metabolism. Based on multiple literature data the following enzymes directly participate in the transformation process of anthropogenic contaminants:

- **Oxidases, catalyzing hydroxylation, dehydrogenation, demethylation and other oxidative reactions (cytochrome P450-containing monooxygenases, peroxidases, phenoloxidases, ascorbatoxidase, catalase, etc.).**
- **Reductases, catalyzing the reduction of nitro groups (nitroreductase).**
- **Dehalogenases, splitting atoms of halogens from halogenated and polyhalogenated xenobiotics.**
- **Esterases, hydrolyzing ester bonds in pesticides and other organic contaminants.**

Conjugation reactions of contaminants in plant cell are catalyzed by transferases: glutathione S-transferase (GST), glucuronozyl-O-transferase, malonyl-O-transferase, glucosyl-O-transferase, etc. Compartmentation of intermediates of contaminants transformation-conjugates takes place under the action of ATP-binding cassette (ABC) transporters [10]. Depending on the structure of the contaminant some other enzymes may also be involved in their degradation process.

Cellular decomposition of contaminants prolonged in time involves participation of enzymes providing plant cell with extra energy needed for the defence processes, induction of the enzymes, and provision of cells by vitally important secondary metabolites. Enzymes involved in these and similar processes obviously indirectly participate in the detoxification of contaminants processes. The correlation between the penetration of organic contaminants (alkenes, aromatic hydrocarbons, polycyclic aromatic hydrocarbons) in plant cells and the corresponding changes in the activities of enzymes participating in energy supply (malate dehydrogenase) and nitrogen metabolism (glutamate dehydrogenase, glutamine synthetase) has been revealed. As has been shown, the activities of the enzymes are highly affected by xenobiotics concentration, exposure time and mode of illumination [3].

Ultrastructure. To evaluate the ecological potential of plants, the data proving responses at the level of cell ultrastructure under the action of contaminants, as the most precise indications of plants exploitation, should also be emphasized. Undoubtedly, penetration even of a small concentration of contaminants into plant cells leads to invisible, but most often measurable deviations in cell metabolic processes such as: induction of enzymes, inhibition of some intracellular metabolic processes, change the level in regular secondary metabolites, etc. The existence in plant cell contaminants in increased concentrations provokes clearly noticeable deviations in cells ultrastructural organization. It has been shown that the complex of changes and alterations in the main metabolic processes of plant cell elicited by organic pollutants (pesticides, hydrocarbons, phenols, aromatic amines, etc.), are connected with the deviations of cell ultrastructural architecture. The sequence and deepness of destruction in plant cell organelles are determined by the variety of plant, chemical nature, concentration and duration of the contaminant action, etc. [11, 12]. This course of events has been experimentally demonstrated by authors in a number of various higher plants exposed to different ^{14}C-labelled toxic compounds. In these experiments due to the penetration, movement and localization of contaminants in plant cells changes in ultrastructural organization has been shown. Apparently, the negative effects of toxic compounds on cell ultrastructure, depending on its concentration, could be divided in two types, being different for each contaminant and plant:

- metabolic, which is digested by the plant in spite of some negative effect by the mobilization of plants internal potential.

- lethal, leading to indigestible deviations and to the plant death.

In Figure 2 maize root apex cells are shown, exposed to ^{14}C-nitrobenzene action, its penetration across the plasmalemma and localization in subcellular organelles. Studies of the penetration of ^{14}C–labelled xenobiotics into the plant cell indicate that labelled compounds at the early stages of exposure (5–10 min) are detected in the cell membrane, in the nuclei and nucleolus (in small amounts), and, seldom, in the cytoplasm and mitochondria. As a result of prolonged exposure the amount of a label significantly increases in the nucleus, at the membranes of organelles, in tonoplasts, and further in vacuoles [13], i.e. xenobiotic becomes distributed in most of subcellular organelles, but ultimately there is a tendency of contaminants primary accumulation in vacuoles.

Obviously plants, as remediators, for a long time most effectively act at low and shallow contamination of soil and air, when no significant changes in cell ultrastructure may be detected. Nevertheless, it should be underlined that plants subjected to high concentrations for relatively short periods in most cases are able to recover from slight deviations in cell ultrastructure and thus maintain their ecological activities.

Transgenic plants have also been studied in connection with degradation of several (some) particular contaminants. For this purpose the widely distributed explosive TNT has generally been chosen. In order to increase the degradability of TNT and similar compounds, the transgenic plants (several) contained the gene of bacterial enzyme (pentaeritrole tetranitrate reductase, EC 1.6.99.7) were received [14]. Transgenic tobacco has been analysed for its ability to assimilate the residues of TNT and trinitroglycerine. Seedlings of transgenic plants extracted explosives from the liquid area much faster, accomplishing denitration of nitro groups, than the seedlings of common forms of the same plants, in which growth was inhibited by the contaminants [15]. Transgenic tobacco thus differed substantially from the common plant by its tolerance, fast uptake and assimilation of significant amounts of TNT. Analogous experimental results have been obtained with other plant species [16].

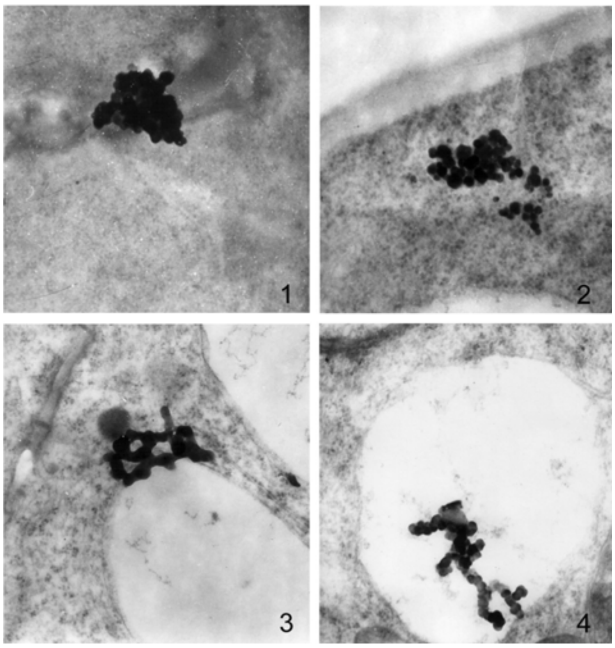

Fig.2. Electron micrographs showing the penetration and movement of ^{14}C-labelled nitrobenzene (0.15 mM) in a maize root apex cell [13].
The xenobiotic penetrated through the plasmalemma (1), moved to the cytoplasm (2), and thereafter translocated into vacuoles (3,4).
1 – x 48 000; 2 – x 36 000; 3 – x 50 000; 4 – x 30 000

There are dozens of publications concerning successful improvement of plant detoxification abilities by cloning the genes of transferases and oxidases, which intensively participate in contaminants transformation processes [17, 18].

Finally owing to the still wide terrestrial and aquatic distribution of plants we should consider these organisms as a very important biological instrument having tremendous ecological potential.

ბიოტექნოლოგია

უმაღლესი მცენარეების, როგორც ეკოლოგიური რემედიატორების პოტენციალი

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უკანასკნელი ორი ათეული წლის მონაცემებმა მცენარეების საკმაოდ ძლიერი ეკოლოგიური პოტენციალი გამოავლინა. დადგენილ იქნა მცენარეებში მიმდინარე ორგანული ტოქსიკანტების ღრმა დეგრადაციული პროცესები, რომელთა შედეგადაც ტოქსიკანტების სრული დაშლა მიიღწევა.

სადღეისოდ ერთმნიშვნელოვნადაა დამტკიცებული, რომ უჯრედში ორგანული ტოქსიკანტის შეღწევა იწვევს გარკვეული ბიოქიმიური და ფიზიოლოგიური პროცესების გააქტივებას, იმისათვის რომ მცენარემ თავიდან აიცილოს ან მნიშვნელოვნად შეამციროს ტოქსიკანტის მავნე ზემოქმედება. ასევე გარკვეულია მცენარეში მოხვედრილი ტოქსიკური ნაერთის ბედი. როგორც დადგინდა, უჯრედში შეღწეული ტოქსიკანტის ბედი შემდეგ მექანიზმებს ემორჩილება: ექსკრეცია, უჯრედის ენდოგენურ ნაერთებთან კონიუგაცია და შემდგომი კომპარტმენტაცია (განაწილება) უჯრედულ სტრუქტურებში, ორგანული ტოქსიკური ნაერთების დეგრადაცია (უპირატესად ჟანგვითი) სტანდარტულ უჯრედულ მეტაბოლიტებამდე ან სრული მინერალიზაცია.

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