

ECOLOGICAL POTENTIAL OF PLANTS

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To date increasing military activities, production of chemicals, unpredictable growth of industry and transport, urbanization, the permanent increase of contamination of all biological sources by chemical compounds of toxic nature are observed. Naturally formed emission of poisonous gases, the washing of toxic elements out of ore during floods or earthquakes, formation by microorganisms toxic compounds etc. are a very little as compared with human anthropogenic contribution in environments contamination. 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 using following physiological/biochemical mechanisms. Ecotechnologies based on combination of microorganisms and plants allow widening the most modern understanding of remediation potential on a scale significantly exceeding any local or even national level. Elaboration of a new ecological concept, unifying experience accumulated for last 3-4 decades and based on effective use phytoremediation/remediation (plants/microbial) joint potential should be highly beneficial for the whole world, by increasing its ecological potential.

Key words: ecology, plants, plant growing, chemical compounds, phytiremediation.

Introduction. The most important global problem of modern world (the end of XX – beginning XXI century) is still ecological. In general, the contradiction between natural processes and human's activity has become extremely urgent. "Natural restoration of nature" is impossible. Science and industry, having risen the welfare of the most part of the mankind to the incredible height, damage the natural environment and threaten the existence of people. Science creates new ecologically dangerous technologies resulting in pseudonatural processes by means of changing regimes of natural processes. This way gives temporal results necessary for the mankind, but its consequences destroys nature and society. It is growing the scale of resistance of natural, organismic and artificially natural, technological, created processes, becoming a conflict which cannot be ignored. One of the most dangerous technological processes is the chemical pollution of biosphere.

As a result of increased military activities, production of chemicals, unpredictable growth of industry and transport, urbanization, the permanent increase of contamination of all biological sources by chemical compounds of toxic nature are observed. Naturally formed emission of poisonous gases, the washing of toxic elements out of ore during floods or earthquakes, formation by microorganisms toxic compounds etc. are a very little as compared with human anthropogenic contribution in environments contamination. According to some data around 600 millions of tons of chemicals of different nature are annually produced in the world. By different ways the great amount of this different nature hazardous compound or their incomplete metabolic transformations, still having high toxicity, are accumulated in biosphere, significantly affecting the ecological balance. Chemically synthesized stable compounds which do not undergo or participate in a very small extent in enzymatic transformations, such as plant protection and pest control agents, solvents and emulsifiers, etc. are especially dangerous for all kind of organisms. Disposal of municipal sewage and wastes accumulated by industry should also be considered as the priority for human settlements, as serious contamination source. Uncontrolled discharge of all kinds of wastes always creates functioning biological source of contamination. The elimination of contaminants from the environment by microorganisms of different taxonomic groups is a well established, genetically determined property, which has already been widely discussed. For instance efficiency of microbial processes of decontamination of fat/oil containing effluents is connected with a serious technological benefit by applying specialized strains decomposing oil and fat [1].

Until recently, plants, which still occupy about 40% of the world's land area, were considered as organisms just accumulating contaminants but having no potential to transform them into harmful compounds. According to existing ideas plant could only slightly transform toxic compounds, than conjugate them and deposit in vacuoles [2].

Analysis of experimental data in the last two-three decades has revealed the visible ecological

potential of plants. It has been exposed the deep degradation processes proceeding in higher plants, depending on the structure of contaminants quite often leading to mineralization or deep degradation of contaminants. As a result, the enzymes carrying out partial/deep oxidation, conjugation, and compartmentation processes have been revealed and characterised; the formation of anthropogenic contaminants conjugates with endogenous compounds has been shown. Although, there are still some unlearned steps in detoxification process carried out in plants closely related to the contaminants multistage degradation process, the authors are making attempts for the evaluation of different aspects of plants ecological potential from the modern understanding, revealing the criterion for the evaluation of deviations under the action of contaminants in ultra structural architectonics of plant cells. In table 1 it is presented contaminants on the ability of plants to absorb and metabolize examined by authors.

Table 1

List of tested toxicants

Hydrocarbons	Methane, Ethane, Propane, Butane	Pentane, Hexane, Cyclohexane	Benzene, Toluene, Naphthalene	1,2-Benz(a)anthracene, 3,4-Benzopyrene, Dibenz(a,h)anthracene, 3-Methylcholantrene
Organochlorine solvents	Chloroform			
Alcohols	Methanol, Ethanol	Isopropanol, Butanol	Pentanol, Hexanol	Octanol, Benzyl alcohol
Phenols	Phenol, o-Cresol, m-Cresol, p-Cresol	Catechol, Hydroquinone, Methylhydroquinone, Resorcin	Pyrogallol, a-Naphthol, Fluoroglucine, Thymol, Guaiacol	Oxyhydroquinone, Toluhydroquinone, Thymohydroquinone, Durohydroquinone
Quinones	0-Benzoquinone, p-Benzoquinone	Toluquinone, Timoquinone	Duroquinone, Anthraquinone	2-Methyl-1,4-naphthoquinone, 2-Hydroxy-1,4-naphthoquinone
Aldehydes and Ketones	Formaldehyde	Acetaldehyde	Acetone	
Organic acids	Formic acid, Acetic acid	Acetahydrate, Propionic acid	Butyric acid, Valeric acid	Caproic acid, Benzoic acid
Nitroderivates	o-Nitrophenol, p-Nitrophenol	2,4-Dinitrophenol, p-Nitroanisole	Nitrobenzene, Dinitrobenzenes	2,4,6-Trinitrotoluene (TNT), Hexahydro-1,3,5-trinitro-1,3,5-triazin (RDX)
Amines	Aniline	N,N-Dimethylaniline	Benzidine	
Pesticides (Herbicides)	Phenoxyacetic acid, 2,4-Dichloroacetic acid (2,4-D)	Atrazine, Simazine, Lindane	Carbaryl (Sevan)	2,4-Dinitro-o-Cresol (DNOC), Dichlorodiphenyltrichloroethane (DDT)
Drugs	Aminopyrine	Ethylmorphine		

In spite of difficulties in quantitative, as well as qualitative estimation, and having a tendency to be increased, level of spread out contaminants significantly exceeds permissible standards. 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 different niches of biosphere, significantly affecting ecological balance. Lately, many ecological technologies targeted to minimize the flow of toxic compounds into the biosphere and monitoring of their level or state have been developed [3, 4]. Despite some positive effect from the realization of these technologies (physical, chemical, mechanical etc), the intensive flow of toxic compounds to the biosphere is still increasing [2, 5].

The international character of this problem being determined by global migration of contaminants (migration between soil, air and water, geographical, biotic, etc.) leads to the distribution of toxic compounds of different structure and overall increase of the toxicity level.

Nevertheless, the members of the plant kingdom assimilate toxic compounds, removing them from the environment, naturally providing long-term protection and monitoring against their environmental dispersal. Obviously, microorganisms and plants represent the main power of nature permanently defending the ecological balance. Plants being recently recognized as an important ecological tool and in order to properly evaluate their detoxification potential should be emphasized according to following features:

- Higher plants simultaneously contact three main ecological niches: soil, water and air.
- A well-developed root system of higher plants determines the soil-plant-microbial interaction, representing a unique process by producing exudates, significantly affecting the overall plant metabolism.
- The large assimilating surface area of plant leaves (adaxial and abaxial) significantly exceeds in size the corresponding aboveground surface located under the plant, and permits the absorption of contaminants in large quantities from the air via the cuticle and stomata.
- The unique internal transportation system in both directions, distributing all the penetrated compounds throughout the entire plant.
- The autonomous synthesis of vitally important organics and extra energy by using intracellular potential during the prolonged remediation process.
- The existence of enzymes catalysing oxidation, reduction, hydrolysis, conjugation, compartmentation and other reactions of the multistage detoxification process.
- The large intracellular space to deposit heavy metals and conjugates of organic contaminants.
- Functionalization and further transformation of organic contaminants by following conjugation, deep oxidation, etc. in plant cells.

In order to penetrate into a leaf, xenobiotics (contaminants) should pass through the stomata, or traverse the epidermis which is covered by film-like wax cuticle. Generally, stomata are located on the lower (abaxial) side of a leaf, and the cuticular layer is thicker on the upper (adaxial) side. Gases and liquids penetrate through the stoma into the leaves. The permeability for gases depends on the degree of opening of stomata apertures (4-10 nm) and for liquids, on moistening of the leaf surface, the surface tension of the liquid and morphology of the stomata. The majority of toxic compounds of low and average molecular weight quite easily penetrate into a leaf as solutions (pesticides, liquid aerosols, etc.).

The contaminant penetration into the roots essentially differs from the leaves. Substances pass into roots only through cuticle-free unsubsided cell walls. Therefore, roots absorb substances much less selectively than leaves. Roots absorb environmental contaminants in two phases: in the first fast phase, substances diffuse from the surrounding medium into the root; in the second they gradually distribute and accumulate in the tissues. The intensity of the contaminants absorption process, characterized by various regulations, depends on the contaminant solubility, molecular mass, concentration, polarity, pH, temperature, soil humidity, etc. [3, 4].

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 using following physiological/biochemical mechanisms:

- Excretion.
- Conjugation of contaminants with intracellular compounds following by compartmentalization of the conjugates in cellular structures.
- Decomposition of environmental contaminants (or their significant part) to standard cell metabolites or their mineralization.

Commonly, plants gradually degrade penetrated through cell wall organic contaminants to avoid their toxic action. According to contaminants assimilating potential plants sometimes are differing up to four orders of magnitude that allowed classifying 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, whereas the assimilation potential of the weak absorbers is measured in hundredths of mg [5] (Table 2).

Plants according to leaves potential to assimilate benzene and toluene

Plants	Amount of absorbed aromatic hydrocarbon by 1 kg of fresh leaves, for 24 hours, in nig.	Plants	
Strong absorbers	1.0-10.0	Maple (<i>Acer campestre</i>) Oleaster (<i>Elaeagnus angustifolia</i>) Locust (<i>Robinia pseudoacacia</i>) Wild pear (<i>Pyrus caucasica</i>) Walnut (<i>Juglans regia</i>) Almond-tree (<i>Amigdalus communis</i>) Cherry-tree (<i>Cerasus avium</i>) Amorphia (<i>Amorpha fruticosa</i>) Cherry-tree (<i>Cerasus vulgaris</i>) Chestnut (<i>Castanea sativa</i>)	Apple-tree (<i>Malus domestica</i>) Zelkova (<i>Zelcova caprinifolia</i>) Poplar (<i>Populus canadensis</i>) Ryegrass (<i>Lolium perene</i>) Lilac (<i>Siringa vulgaris</i>) Weeping willow (<i>Salix</i>) Catalpa (<i>Catalpa bignonioides</i>) Platan-tree (<i>Platanus orientalis</i>) Sophora (<i>Sophora japonica</i>)
Average absorbers	0.1-1.0	Alder (<i>Alnus barbata</i>) Asp (<i>Populus tremula</i>) Elm (<i>Ulmus fdiacea</i>) Ash (<i>Fraximus excelsior</i>) Tea (<i>Camellia sinensis L.</i>) Persimmon (<i>Diospyros lotus</i>) Bay laurel (<i>Laurus nobilis</i>)	Gleditdchia (<i>Gleditschia triacanthos</i>) Kidney (<i>Phaseolus vulgaris</i>) Pine (<i>Pinus</i>) Pine (<i>Pinus eldarica</i>) Thuja (<i>Tuja</i>) Apricot (<i>Prunus armenicana</i>) Vine (<i>Vitis vinifera</i>)
Weak absorbers	0.001-0.1	Fir (<i>Picea abies</i>) Mulberry (<i>Morus alba</i>) Lime-tree (<i>Tilia cauxasica</i>) Reed (<i>Phragmites communis</i>) Maize (<i>Zea mays</i>) Wild plum (<i>Prunus divaricata</i>) Kiwi (<i>Apteryx australis</i>) Aloe (<i>Aloe</i>) Medlar (<i>Mespilus germanica</i>) Rose (<i>Rosa</i>) Platan-tree (<i>Platanus</i>)	Cypress (<i>Cupressus sempervirens var. Pyram idalis</i>) Geranium (<i>Pelargonium roseum</i>) Privet (<i>Ligustrum vulgare</i>) Fig (<i>Ficus carica</i>) Pomegranate (<i>Punica granatum</i>) Rhododendron (<i>Rhododendron ponticum</i>) Peach-tree (<i>Persica vulgaris</i>) Potato (<i>Solanum tuberosum</i>) Tomato (<i>Lycoperssicum esculentum</i>) Pussy-willow (<i>Salix alba</i>) Cherry-plum (<i>Prunus vachushtii</i>)

The fate of the entered plant cell contaminants depends on their chemical nature, external temperature, variety of plants and phase of vegetation, etc. The simplest pathway of entered the plant cell organic contaminants is excretion. The essence of excretion is that the toxic molecule does not undergo chemical transformation, and being translocated through the apoplast, is excreted from the plant. This pathway of xenobiotics (contaminants) elimination is rather rare and takes place at high concentrations of highly mobile (phloem-mobile or ambi-mobile) xenobiotics.

In the majority of cases, contaminants being absorbed and penetrated into plant cell undergo enzymatic transformations leading to an increase in their hydrophilicity - process simultaneously accompanied by contaminants toxicity decreasing. Below are presented successive phases of contaminant initial transformations in accordance with Sandermann's "green liver" concept [6] (Fig. 1):

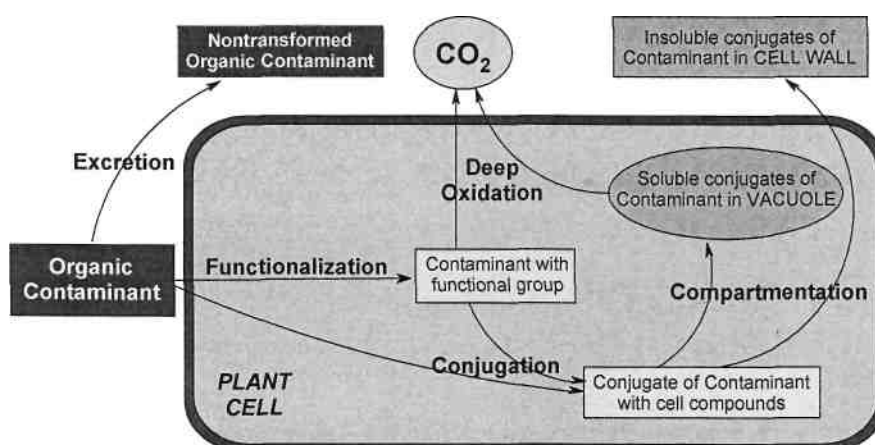


Fig. 1. The main pathways of organic contaminant transformation in plant cells

Functionalization is a process whereby a molecule of a hydrophobic organic xenobiotic acquires a 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 corresponding reactivity of the toxicant molecule is enhanced. This promotes an increase of intermediate's affinity to enzymes, catalyzing further transformation.

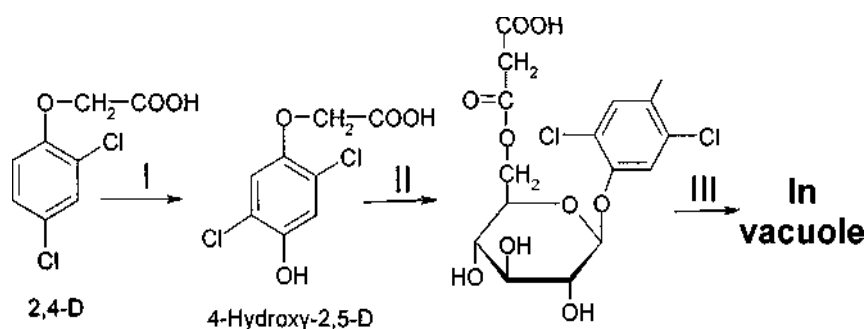
Conjugation takes place as a basic process of phytoremediation and is determined by the formation of chemical coupling of the contaminant to the endogenous cell compounds (proteins, peptides, amino acids, organic acids, mono-, oligo-, polysaccharides, lignin, etc.), so forming peptide, ether, ester,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

Commonly, the main part of the toxicant molecules undergoes conjugation and only a small amount is deeply degraded (0.1-2%, depending on structure of contaminants). Conjugation is a widespread defence mechanism in higher plants, especially in cases when the penetrated contaminant concentration is exceeding the plant transformation (decomposition) potential. Increased amounts of deep degradation to regular plant sell metabolites, or CO₂ and water, most often is achieved in the case of linear, low molecular weight structures of contaminants [7, 3]. The toxicity of the conjugates compared to parent compounds is significantly decreased due to creating the new compound containing large non-toxic part. Conjugates are kept in the cell for a certain period of time without causing visible pathological deviations in from cell homeostasis. The conjugate formation also gives the plant cell extra time for the internal mobilization, and the induction of enzymes responsible for contaminants further transformation. Relatively quickly, after the termination of plant incubation with the contaminant, conjugates are no longer found in plant cells.

Some attempts have been made by authors (unpublished data) to estimate different plant (soybean, ryegrass) cells' potential to accumulate conjugated benzene in their cells in the case of toxicant saturation. In spite of incomplete information, it was supposed that for genetically non-modified plants, it could be, as a minimum, several molecules of contaminant conjugates per one of plant sell. Although conjugation is the most widely distributed pathways of plant self-defence, it cannot be assumed as energetically and physiologically advantageous for metabolic processes in plants. Firstly, the formation of conjugates leads to the depletion of vitally important cellular compounds, and secondly, unlike deep degradation, the formation of conjugates maintains the contaminant basic molecular structure, and hence results only in partial and provisional decreasing of its toxicity.

Compartmentation in most cases is the final step of conjugates processing. Soluble conjugates of toxic compounds (coupled with peptides, sugars, amino acids, etc.) are accumulated in the cell structures (primarily in vacuoles), while insoluble conjugates (coupled with lignin, starch, pectin, cellulose, xylan) are moved out of the cell via exocytosis into the apoplast and accumulated in the cell wall [6]. The compartmentalization 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 contaminant 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 [8, 9] and is known as "storage excretion".

The above described pathway of toxic compound processing, i.e., functionalization → conjugation → compartmentalization, is well illustrated by the processing of anthropogenic 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 [10].



2,4-D transformation for deposition in vacuole

Enzymes. Anthropogenic organic toxicants decomposition processes are 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 are directly or indirectly participating in 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 contaminants, 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.

The first step of contaminants transformation in majority of cases is carried out by oxidative enzymes, the most often contaminants oxidation is performed by the following metabolically active enzymes having the various metabolic functions:

- **Cytochrome P450-containing monooxygenases (EC 1.14.14.1)** are mixed-function enzymes located in the membranes of the endoplasmic reticulum (microsomes).

Monooxygenase system contains redox-chain for electron free transport, the initial stage of electron transfer is a NADPH-cytochrome P450 reductase (EC 1.6.2.4); the intermediate carrier—cytochrome, and the terminal acceptor of electrons—cytochrome P450. When NADPH is used as the only source of reductive equivalents, the existence of an additional carrier, a NADH-dependent flavoprotein is required. NADH may also be oxidized by the NADPH-dependent redox system. In the latter case cytochrome b₅ is not required. The cytochrome P450-containing monooxygenases use NADPH and/or NADH reductive equivalents for the activation of molecular oxygen and incorporation of one of its atom into lipophilic organic compounds (XH) that results in formation of hydroxylated products (XOH) [11]. The second atom of oxygen is used for the formation of a water molecule (Fig. 2).

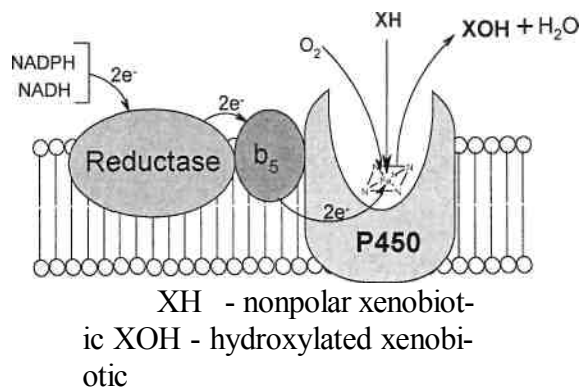
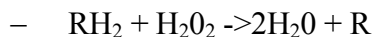


Fig. 2. Microsomal monooxygenase system

- Plant cytochrome P450-containing monooxygenases play an important role in the hydroxylation of organic contaminants [6]. The enzymes participate in the reactions of C- and N-hydroxylation of aliphatic and aromatic compounds, N-, O-, and S-dealkylation, sulpho-oxidation, deamination, N-oxidation, oxidative and reductive dehalogenation, etc. [11, 12]. The resistance of plants against herbicides is mediated by their rapid intracellular transformation into hydroxylated products and subsequently conjugated to carbohydrate moieties in the plant cell wall. For examples, N-demethylation and ring-methyl hydroxylation of the phenylurea herbicide chlorotoluron in wheat and maize is cytochrome P450-dependent processes. For some phenyl urea herbicides in the Jerusalem artichoke cytochrome P450-mediated N-demethylation is sufficient to cause significant or complete loss of phytotoxicity [13].

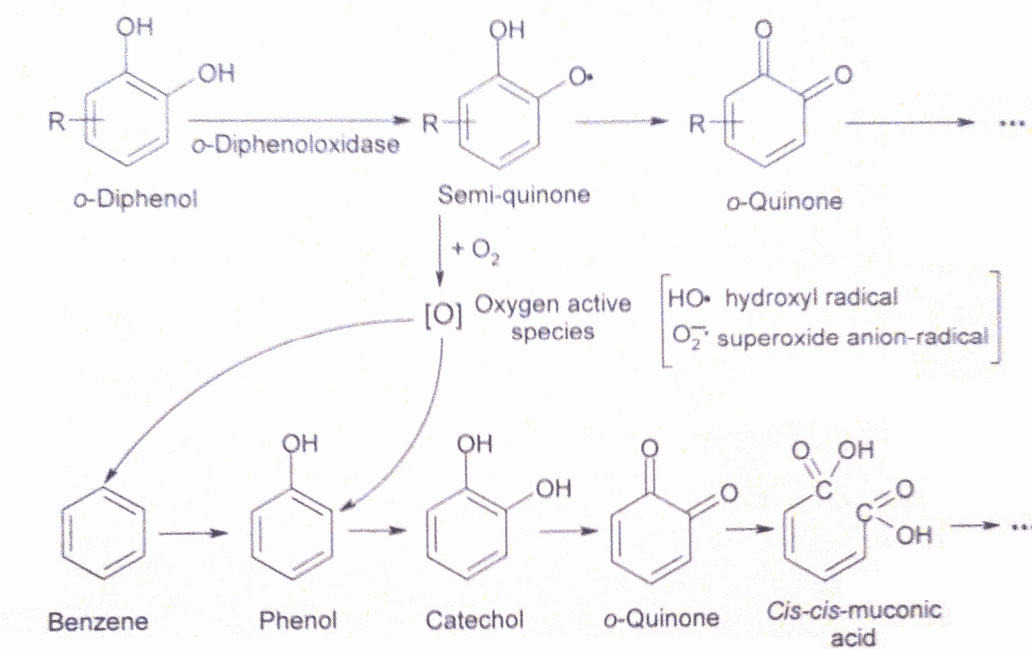
- **Peroxidase.** In higher plants, peroxidase activity increases in response to stress. Among multiple functions of this enzyme one of major is the protection of cells from oxidative reactions imposed of all photosynthetic plants. The great catalytic versatility of the peroxidase is its predominant characteristic, and, therefore, no single role exists for this multifunctional enzyme.

- The peroxidase is defined by the following reaction:



– The peroxidases catalyze a number of free radical reactions. Alternatively, the compound that is directly oxidized by the enzyme further oxidizes other organic compounds, including xenobiotics. This notion is based on the wide ubiquitous distribution of this enzyme in plants (the isozymes of peroxidase in green plants occur in the cell walls, plasmalemma, tonoplasts, intracellular membranes of endoplasmic reticulum, plastids and cytoplasm), and the high affinity and wide substrate specificity of plants peroxidases to organic xenobiotics of different chemical structures. The participation of plant peroxidases in hydroxylation reactions of xenobiotics has been widely discussed. For example, peroxidases from different plants are capable of oxidizing N,N-dimethylaniline, 3,4-benzopyrene, 4-nitro-o-phenylen-diamine, 4-chloroaniline, phenol, aminofluorene, acetaminophen, diethylstilbestrol, butylated hydroxytoluene, hydroxyanisoles, benzidine, etc. [6]; horseradish (*Armoracia rusticana*) peroxidase oxidizes tritium-labelled [C^3H_3] TNT (14).

– **Phenoloxidases**, group of the copper-containing enzymes (other names-tyrosinase, monophenol monooxygenase, phenolase, monophenol oxidase, etc.) are spread within the plant cell organelles catalyzing both monooxygenase and oxygenase reactions: the *o*-hydroxylation of monophenols (monophenolase reaction) and the oxidation of *o*-diphenols to *o*-quinones (diphenolase reaction). Currently accepted enzyme nomenclature classifies hydroxylating phenol oxidase as monophenol monooxygenase (EC 1.14.18.1) and *o*-diphenols oxidizing phenol oxidase as catechol oxidase (EC 1.10.3.1). Plant phenol oxidases appear to be a group of specific enzymes, oxidizing wide range of *o*-diphenols, such as DOPA (dihydroxyphenylalanine), catechol, etc, but unable to convert *m*- or *p*- diphenols to the corresponding quinons, Substrate specificity of catechol oxidase from *Lu-copus europaeus* and characterization of the bioproducts of enzymatic caffeic acid oxidation. The active center of phenol oxidases contains two copper atoms and exists in three states: "met", "deoxy" and "oxy". Phenoloxidases actively participate in the oxidation of xenobiotics of aromatic structure. As it has been demonstrated phenoloxidase from spinach, analogously to many other plants, oxidizes aromatic xenobiotics (benzene, toluene), by their hydroxylation and further oxidation to quinone [5]. In a number of the cases, if the xenobiotic is not a substrate for the phenoloxidase, it may undergo co-oxidation in the following manner: the enzyme oxidizes the corresponding endogenous phenol by forming quinones or semi-quinones or both, i.e. compounds with a high redox potential. These compounds activate molecular oxygen by forming oxygen radicals, such as superoxide anion radical ($O_2^{\cdot-}$) and hydroxyl radical ($\cdot OH$) [15], that gives compounds the capacity for the further oxidation of xenobiotic. The formation of these radicals enables phenoloxidase to participate in contaminants degradation processes also by co-oxidation mechanism presented below.



Enzymatic oxidation of *o*-diphenols (upper) by phenoloxidase and non-enzymatic co-oxidation of benzene (lower)

Analogously, nitrobenzene is oxidized to *m*-nitrophenol, and the methyl group of [C^3H_3] TNT [14] is oxidized by phenoloxidase from tea plant. The information confirming participation of this

enzyme in the oxidative degradation of xenobiotics in higher plants is sparse [5], despite the fact that participation of phenoloxidase should definitely be expected while xenobiotics degradation. Laccase of basidial fungi, analogous to higher plant phenoloxidase, have been better explored. Laccase degrades different aliphatic and aromatic hydrocarbons, and actively participates in the enzymatic oxidation of alkenes [16]. Crude preparations of laccase isolated from the white rot fungus *Trametes versicolor* oxidizes 3,4-benzopyrene, anthracene, chrysene, phenanthrene, acenaphthene and some other PAHs. The intensity of oxidation of these antropogenic contaminants is increased in the presence of such mediators as: phenol, aniline, 4-hydroxybenzoic acid, 4-hydroxybenzyl alcohol, methionine, cysteine, reduced glutathione, and others compounds-substrates of laccase. These data indicate that in the cases of fungal laccase and plant o-diphenoloxidase, the oxidation of hydrocarbons is carried out by a co-oxidation mechanism [6].

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 [17]. Depending on the structure of the contaminant some other enzymes may also be involved in their degradation process.

Prolonged in time cellular decomposition of contaminants 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. 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 it has been shown the activities of the enzymes are highly affected by xenobiotics concentration, exposure time and mode of illumination [4].

Ecologically the most advantageous pathway of organic contaminants transformation in plants is their deep oxidative degradation. In higher plants mainly the following enzymes are responsible for this process: cytochrome P450-containing monooxygenase, peroxidase and phenoloxidase. To correctly evaluate the universality of the action of these enzymes, responsible for the degradation of different structure organic contaminants, some of their specificities should be emphasized (Table 3).

Table 3

Plants oxidative metalloenzymes

Enzyme	Physiological function	Existence in cell	Localization	Specificity to toxicants	Limiting factors	Stability
Cytochrome P450 containing monooxygenase	Participation in a number of intracellular synthesizing reactions	Small amount, inductive nature	Endoplasmatic reticulum, cytosole	Very high affinity to nonpolar toxicants	NADPH, NADH	Labile, inactivating during substrate oxidation
Peroxidase	Hormonal regulation, lignification, response on stress, removing of peroxides	Large amount, inductive nature	Cell wall, vacoules, cytosole, tonoplasts, plastids, plasmalemma	Affinity to aliphatic compounds	Hydrogen peroxide or organic hydroperoxides	Stable
Phenoloxidase	Oxidative transformation of phenols, lignification, cell defence reactions	Large amount, presents in latent form too, inductive nature	Chloroplasts, cell wall, cytosole, tonoplasts	Affinity to aromatic compounds	Endogenous phenols	Stable

Ultrastructure. To evaluate the ecological potential of plants, the data proving the responses at the level of cellular ultrastructure under the action of contaminants, as the most precise indications of plants exploitation, should also be emphasized. Undoubtedly, penetration even a small concentrations 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 of secondary metabolites, etc. The existence in plant cell contaminants in increased concentrations provokes clearly noticeable deviations in cellular ultrastructure under the action of contaminants, as the most precise indications of plants 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.) is connected with the deviations of cell ultrastructural architecture. The sequence and deepness of the destruction in plant cell organelles are determined by the variety of plant, chemical nature, concentration and duration of the contaminant action, etc. [18, 19]. 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 affects 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 insignificant negative effect
- lethal, leading to indigestible deviations and to the plant death.

Figure 3 illustrates maize root apex cells exposed to ^{14}C -nitrobenzene action, its penetration across the plasmalemma and localization in subcellular organelles. Studies of penetration of 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 [20], i.e. xenobiotic becomes distributed in most of subcellular organelles, but ultimately there is a tendency of contaminants primary accumulation in vacuoles.

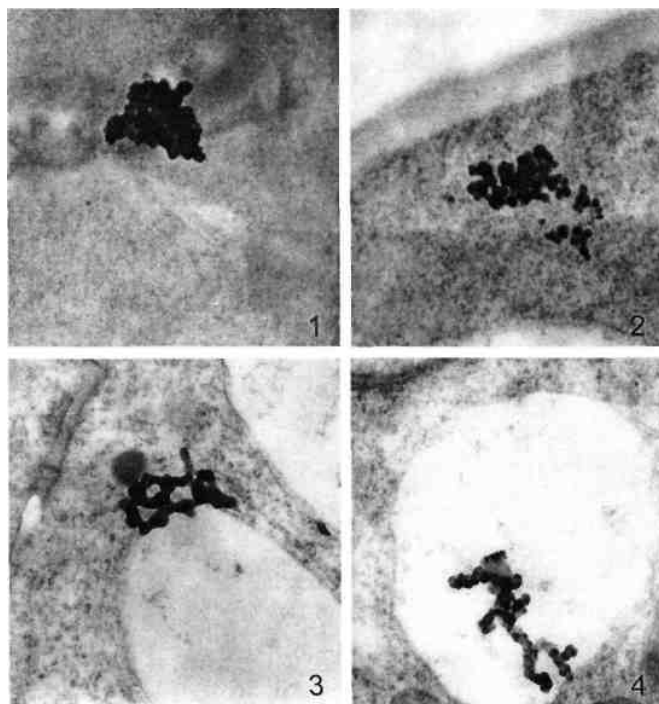


Fig. 3. Electron micrographs showing the penetration and movement of C-labelled nitrobenzene (0.15 mM) in a maize root apex cell [20].

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.

Obviously plants, as remediators, for a long time the most effectively act at low and shallow contamination of soil and air, when no significant changes in cell ultrastructure take place. Planting of almost any kind of vegetation, including agricultural vegetation, is beneficial for environment. However, in order to make the exploitation of most ecological potential of each particular plant, the

selection should be carried out according to the plants potential to assimilate/accumulate toxic compounds of different structure.

Phytoremediation is a unique cleanup strategy. The realization of phytoremediation technologies implies the planting on a contaminated area with one or more specific, previously selected plant species with the potential to extract contaminants from soil. A precise survey of the vegetation *on site* should be undertaken to determine what species of plants would have the best growth at the contaminated site. Based on the number of experimental results including the use of labeled xenobiotics and electron microscopic observations, the deep degradation of anthropogenic contaminants in plants could be considered as narrow but permanently acting pathway having much less potential than conjugates formation process (especially in case of contaminants saturation).

Transgenic plants have also been studied in connection with degradation of some particular contaminants. For this purpose the widely distributed explosive TNT (trinitrotoulene) has generally been chosen. In order to increase the degradability of TNT and similar compounds, the transgenic plants contained the gene of bacterial enzyme (pentaeritrole tetranitrate reductase, EC 1.6.99.7) were received [21]. Transgenic tobacco has been analysed for its ability to assimilate the residues of TNT and trinitroglycerine. Seedlings of the 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 [22]. 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 [23].

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 [24, 12].

Obviously, the attempts to improve artificially ecological potential of higher plants will be continued, and the results will be the more substantial from the viewpoint of their eventual practical realization. The positive effect of these investigations could be much more impressive if all aspects of the quite complicated and multistage detoxification process would be better elucidated with regard to plant physiology and biochemistry. Such information would allow the creation of more rational and effective strategy for the gene engineering potential application.

The cost of phytoremediation technologies. Bioremediation is a completely natural process based on the joint detoxification action of plants and microorganisms. Phytoremediation technologies are economically competitive, compared with existing conventional ones. Dozens of scaled up examples have demonstrated the superiority of plant-based remediation technologies, mainly due to the following reasons [25]: phytoremediation, being a natural, solar energy-driven process, does not require any additional energetic or significant material or other input; phytoremediation takes place *in situ* and requires no digging or hauling; little mechanical equipment is needed to operate the phytoremediation process. The cost components for the implementation of phytoremediation technologies include:

- Detailed characterization of the polluted site (soil type, water content, type of contaminant(s), concentration of contaminant(s) in the soil, etc.).
- Selection of appropriate plants and consortia of microorganisms.
- An corresponding irrigation system.
- Capital cost, materials, monitoring, including required instrumentation, indirect costs, etc.
- Operation and maintenance (labour, materials, chemicals, laboratory analyses, etc.).

Phytoremediation offers cost advantages, but it should be underlined that the time needed for full remediation is typically lengthy. Table 4 gives estimates of the costs of phytoremediation as compared with existing conventional technologies.

Table 4

Estimated cost savings through the use of phytoremediation rather than conventional treatment, according to EPA data (25)

Contaminant	Phytoremediation		Conventional Treatment		Projected
	Application	Estimated cost	Application	Estimated	
Lead in soil (1 acre)	Extraction, harvest, and disposal	\$ 150,000-250,000	Excavate and landfill	\$ 500,000	50-65%
Solvents in groundwater (2.5 acres)	Degradation and hydraulic control	\$ 200,000 for installation and initial maintenance	Pump and treat	\$ 700,000 annual operating cost	50% cost saving by 3 rd year
Total petroleum hydrocarbons in soil (1 acre)	<i>In situ</i> degradation	\$ 50,000-100,000	Excavate and landfill or incinerate	\$ 500,000	80%

Plants, solely or preferable in combination with specially selected microorganisms (or their consortia), are very promising detoxifiers allowing to create ecologically friendly technologies around or along hotbeds of contamination. It should be clearly realized that ecotechnologies based on combination of microorganisms and plants allow widening the most modern understanding of remediation potential on a scale significantly exceeding any local or even national level. Elaboration of a new ecological concept, unifying experience accumulated for last 3-4 decades and based on effective use phytoremediation/remediation (plants/microbial) joint potential should be highly beneficial for the whole world, by increasing its ecological potential. Realization of this conception supposes the creation of new forms of organization activity on the scale of interstate collaboration, different from today's practicing ones. The main demand for these forms is not to create but to eliminate contradiction of new technologies leading to accumulation of toxic compounds of various structure and strategy of phytoremediation. Thus the touched problem is behind the concept of the present research.

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