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Mechanisms of Organic Contaminants Uptake and Degradation in Plants

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Abstract**—**As a result of urbanization, the unpredictable growth of industry and transport, production of chemicals, military activities, etc. the concentration of anthropogenic toxicants spread in nature exceeds all the permissible standards. Most dangerous among these contaminants are organic compounds having great persistence, bio-accumulation, and toxicity along with our awareness of their prominent occurrence in the environment and food chain. Among natural ecological tools, plants still occupying above 40% of the world land, until recently, were considered as organisms having only a limited ecological potential, accumulating in plant biomass and partially volatilizing contaminants of different structure. However, analysis of experimental data of the last two decades revealed the essential role of plants in environment remediation due to ability to carry out intracellular degradation processes leading to partial or complete decomposition of carbon skeleton of different structure contaminants. Though, phytoremediation technologies still are in research and development, their various applications have been successfully used.

The paper aims to analyze mechanisms of organic contaminants uptake and detoxification in plants, being the less studied issue in evaluation and exploration of plants potential for environment remediation.

Keywords**—**organic contaminants, Detoxification, metalloenzymes, plant ultrastructure.

I. Introduction

As a result of urbanization, the unpredictable growth of industry and transport, production of chemicals for agriculture, military activities, etc. the concentration of anthropogenic toxicants spread in nature, especially in some regions exceeds all the permissible standards [1]. In spite of difficulties in quantitative, and qualitative estimation, and having a tendency to be increased, the amount of spread out contaminants exceeds billions of tons annually. Most dangerous among these contaminants are those having great persistence, bio-accumulation, and toxicity along with our awareness of their prominent occurrence in the environment and food chain. 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 ecological balance [2, 3]. The international character of this problem being determined by such factors as global migration of contaminants (migration between soil, air and water, geographical, biotic, etc) consists in overall distribution of contaminants of different structure and level of toxicity.

Among natural ecological tools, plants still occupying above 40% of the world land, until recently, were considered as organisms having only a limited ecological potential, accumulating in plant biomass and partially volatilizing contaminants of different structure. However, analysis of experimental data of the last two decades revealed the essential role of plants in ecological processes. It has been exposed that plants in addition to accumulation of heavy metals, carry out intracellular degradation processes leading to partial or complete decomposition of carbon skeleton of different structure contaminants [3]. Nevertheless, representatives of plant kingdom assimilate toxic compounds with different velocity, removing them from the environment, naturally providing long term protection and monitoring against their environmental dispersal. Although heavy metals are a problem at many hazardous waste sites, a large number of sites, contaminated with petrochemicals, oil hydrocarbons, pesticides, etc., are met. In a number of such cases phytoremediation seems to be the real alternative and cheap way of traditional clean up technologies. Though, phytoremediation technologies still are in research and development, their various applications have been successfully used.

The paper aims to analyze plants potential for organic contaminants uptake and detoxification, determining their role in environment remediation and protection.

II. Plants and Plant Cell: Uptake and Transformation of Organic Contaminants

Nevertheless, the plants kingdom members 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 straggling for the maintaining of ecological balance.

Plants becoming recognized as important ecological tool and in order to properly evaluate their detoxification potential, the following ecobiological specificities of these organisms should be emphasized:

– 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

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unique process, significantly affecting the overall plant metabolism.

– The large assimilating surface area of plant leaves (adaxial and abaxial), significantly exceed the above ground surface beneath the plant, permit the absorption of contaminants in a big quantity from air via the cuticle and stomata.

– The unique internal transportation system in both directions, distributing all penetrated compounds throughout the entire plant.

– The autonomous synthesis of vitally important organics and extra energy during prolonged remediation process.

– The universal for all kinds of plants 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, compartmentalization, etc.).

In order to penetrate into a leaf, the organic contaminant 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.

Through stoma into leaves penetrate gases and liquids. The permeability for gases depends on the degree of opening of stomata apertures (4–10 nm), and for liquids the permeability depends on moistening of the leaf surface, surface tension of liquid and morphology of stomata. The majority of toxic compounds penetrate into a leaf as solutions (pesticides, liquid aerosols, etc.). It was established for the leaves of zebrine (*Zebrina purpusii*) that the crossover surface tension of their lower surface is 25–30 dyne/cm (for comparison: the surface pressure of water equals 72.5 dyne/cm, for ethanol 22 dyne/cm) [4]. Liquids with a surface tension less than 30 dyne/cm have a constant angle of contact with the surface of a leaf and instantly penetrate into the stomata. Liquids with surface tension above than 30 dyne/cm penetrate into the stoma without moistening the leaf surface.

Still in the middle of seventeenth possible pathways of lipophilic organic contaminant penetration in leaves were shown by absorption of gaseous hydrocarbons by hypostomatic leaves [5]. The leaves of the field maple (*Acer campestre*), wild Caucasian pear (*Pyrus caucasica*), vine (*Vitis vinifera*) and narrow-leaved oleaster (*Elaeagnus angustifolia*) were placed in an atmosphere containing ^{14}C-methane or [1–6-^{14}C] benzene. Contact with labelled hydrocarbon occurred only from one side of the leaf. The total radioactivity of the non-volatile metabolites formed show the absorption of gaseous alkanes and vapours of aromatic hydrocarbons is implemented by leaves not only through stomata, but also through cuticle; the way through the stomata looks preferable.

Similar results have been reported for a number of herbicides (α-naphthylacetic acid, 2,4-D, picloram and derivatives of urea), applied in soluble form to leaves [6, 7]. The abaxial side of a leaf, rich in stomata, absorbs the organic substances more intensively than by the adaxial side. These results forcefully imply the active participation of stomata during the absorption of toxic compounds (Fig. 1).

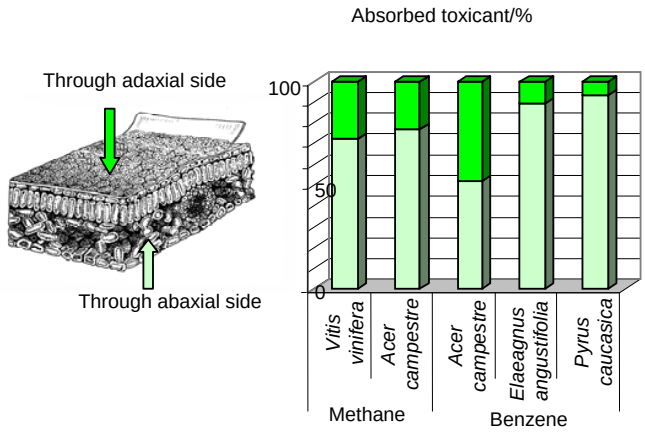


Fig. 1. Absorption of ^{14}C-methane (specific radioactivity 1 μCi/ml) and [1–6-^{14}C] benzene (specific radioactivity 4.9 μCi/ml) by the hypostomatic leaves of plants. Concentration of methane in the air is equal to 1.5% by volume; 8 h exposure under illumination. Concentration of benzene in the air is equal to 2 mg/l, 4 h exposure in darkness [8].

The contaminants penetration into the roots essentially differs from the leaves. Substances pass into roots only through cuticle-free unsuberized 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 contaminants solubility, molecular mass, concentration, polarity, pH, temperature, soil humidity, etc. [3, 9].

Nowadays there are experimental data obviously demonstrating that plants are able to 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 are differing up to four orders of magnitude that allowed to 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, the

assimilation potential of the weak absorbers is measured in hundredths of mg [10].

The fate of 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 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 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 are presented successive phases of contaminants initial transformations in accordance to Sandermann's green liver concept [6] (Fig. 2):

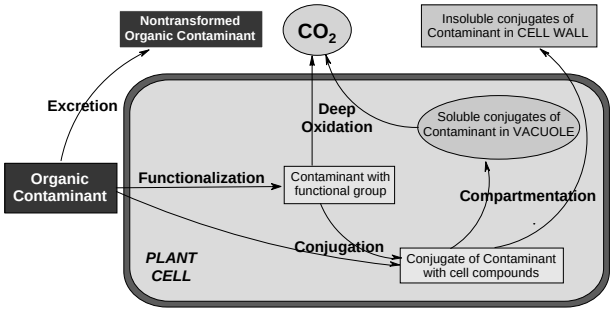


Fig. 2. The main pathways of organic contaminant transformation in plant cells

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. This promotes an increase of intermediates affinity to enzymes, catalysing further transformation.

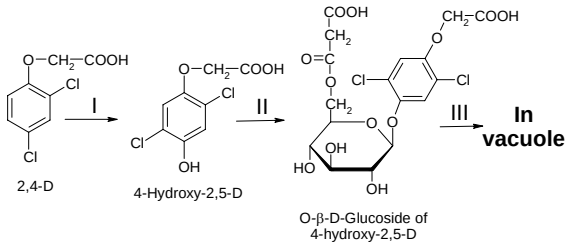
Conjugation takes place a basic process in phytoremediation and consists in formation of chemically coupled contaminant to endogenous cell compounds (proteins, peptides, amino acids, organic acids, mono-, oligo-, polysaccharides, lignin, etc.) forming of peptide, ether, ester, thioether or other type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

Commonly, immediately after penetration, the main part of the toxicant molecules undergoes conjugation and only a small amount is deeply degraded (0.1-5% 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 [3, 8]. The toxicity of conjugates compared to parent compounds is decreased due to binding with non-toxic cellular compounds. Conjugates are kept in a cell for a certain period of time without causing visible pathological deviations in cell homeostasis. Conjugates formation also gives the plant cell extra time for the internal mobilization, 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 case of toxicant saturation. In spite of incomplete information it was suggested that for genetically non modified plants it could be, as a minimum, several molecules of contaminant conjugates per each plant sell. 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 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 cell wall [6]. The compartmentalization process is analogous to mammalian excretion, essentially removing 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 [11] and is known as "storage excretion" [12].

The described above pathway of toxic compound processing i.e., functionalization $\rightarrow$ conjugation $\rightarrow$ 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 [13] .



2,4-D transformation for deposition in vacuoles

III. The Enzymes participating in detoxification processes

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.

Ttransformations 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 catalyse 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 [14]. 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 other enzymes and first of all of those of energy exchange. Energy is in demand in cell under contamination stress. In addition to catalyses of immediate transformation reactions, extra energy is needed for translocation of xenobiotics, induction of enzymes, both oxidation and conjugation, for synthesis of compounds forming conjugates with xenobiotics, etc. The correlation between the penetrations of organic contaminants (alkanes, aromatic hydrocarbons, polycyclic aromatic hydrocarbons) in plant cells and the corresponding changes in the activities of malate dehydrogenase and glutamate dehydrogenase has been revealed [15, 16]. As it has been shown the activities of these enzymes are highly affected by xenobiotics concentration, exposure time and mode of illumination. Activation of glutamate dehydrogenase, catalyzing oxidative deamination of L-glutamic acid and thus providing Tricarboxylic acid cycle with carbon skeleton of amino acids for their further oxidation indicates energy demand in cell. Activation of observed in some cases of glutamine synthetase, enzyme involved in ammonia primary assimilation could be explained as compensation of the amino acid expense. Thus, enzymes involved in energy generation, ammonia assimilation, and probably some others obviously indirectly participate in the detoxification of contaminants.

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 monooxygenese, 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 1).

TABLE
Plants oxidative metalloenzymes

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

Cytochrome P450-containing monooxygenases (EC 1.14.14.1) are mixed-function enzymes located in the membranes of the endoplasmic reticulum (microsomes) [17]. 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 b_5, 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 b_5 is not required [18]. 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) [19]. The second atom of oxygen is used for the formation of a water molecule (Fig.3).

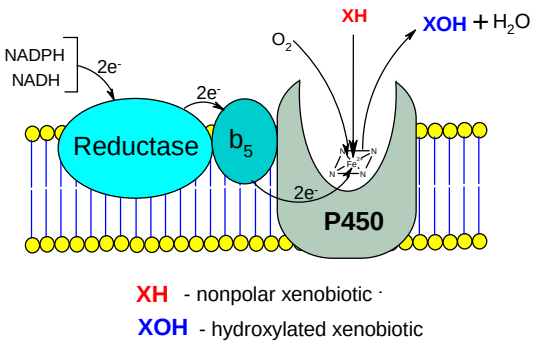


Fig. 3. Microsomal monooxygenase system

Plant cytochrome P450-containing monooxygenases play an important role in the hydroxylation of organic contaminants [7]. 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. [19, 20]. 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 [21, 22]. For some phenylurea herbicides in the Jerusalem artichoke cytochrome P450-mediated N-demethylation is sufficient to cause significant or complete loss of phytotoxicity [23].

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:

$$RH_2 + H_2O_2 \rightarrow 2H_2O + R$$

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. According to the current hypothesis the great majority of organic contaminants in plants are oxidized by peroxidases [24]. 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. In literature 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 [25], 3,4-benzpyrene, 4-nitro-*o*-phenylendiamine [26], 4-chloroaniline [27], phenol, aminoflourene, acetaminophen, diethylstilbestrol, butylated hydroxytoluene, hydroxyanisoles, benzidine, etc. [7]; horseradish (*Armoracia rusticana*) peroxidase oxidizes tritium-labelled [C^3H_3] TNT [28].

Phenoloxidases, group of the copper-containing enzymes (other names-tyrosinase, monophenol monooxygenase, phenolase, monophenol oxidase, etc.) are wide 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) [29]. Currently accepted enzyme nomenclature classifies hydroxylating phenol oxidase as monophenol monoxygenase (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 *Lucopus europaeus* and characterization of the bioproducts of enzymatic caffeic acid oxidation, FEBS Letters, 445, 103-110). The active center of phenol oxidases contains two cooper atoms and exists in three states: “met’, “deoxy” and “oxy”. A catalytic cycle for the phenoloxidase also may involve non-enzymatic reaction, with participation of *o*-quinone intermediate [30].

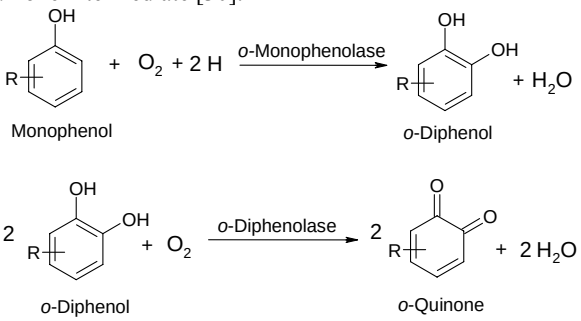


Fig.4. Action of phenoloxidases on monophenol and *o*-diphenol

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 [11]. 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 super oxide anion radical ($O^{2-\cdot}$) and hydroxyl radical ($^{\cdot}OH$) [31, 32], 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 (Fig. 5):

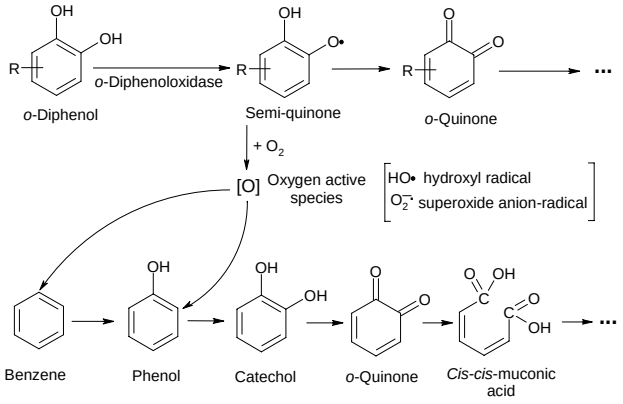


Fig. 5. Enzymatic oxidation of *o*-diphenols (upper) by phenoloxidase and non-enzymatic co-oxidation of benzene (lower)

Analogously, nitrobenzene is oxidized to *m*-nitrophenol, and the methyl group of [C^3H_3] TNT [1] 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 [33], 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 [34], and actively participates in the enzymatic oxidation of alkenes [35]. Crude preparations of laccase isolated from the white rot fungus *Trametes versicolor* oxidizes 3,4-benzopyrene, anthracene, chrysene, phenanthrene, acenaphthene and some other PAHs [36]. 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 [37]. 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 [11].

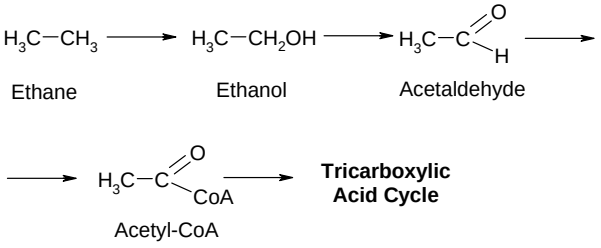
Apparently metalo-enzymes differing in their localization in plant cell organelles, structural organization, mechanisms of action, substrate specificity allow plants firstly to oxidise wide spectrum of organic contaminants including aromatic structures containing contaminants and secondly regulate inter-replacement of these enzymes during contaminants (xenobiotics) oxidative degradation caused due to inability or decreased potential of any of these enzymes to carry out further oxidation of structurally unsuitable intermediates.

Deep degradation of organic xenobiotics (contaminants) is multistage, mainly oxidative enzymatic process and only insignificant amount of toxic molecules undergo direct degradation, the majority of the conjugated with endogenous secondary metabolites contaminants (above 80%) are accumulated in vacuoles and apoplasts and their further transformation takes place with some delay. The emission of $^{14}CO_2$ (up to 5% in case of labelled linear contaminants) indicates that in plant cells the formation of conjugates and their compartmentalization is followed by deep oxidation of the toxic parts of their [9, 17].

Based on the number of experimental data we suppose that the most rate-limiting stage of the whole process of xenobiotics transformation seems to be the initial hydroxylation of nonpolar contaminants. As a result of functional group introduction molecule of transformed contaminants becomes easily accessible for further enzymatic transfomation.

IV. Metabolism of Organic Toxicants in Plant Cells

The transformation of the small molecular weight aliphatic xenobiotics as methane in tea plant (*Thea sinensis*) proceeds by the formation of fumaric acid. Transformation of ethane, propane and pentane leads to the formation of low molecular mass compounds largely composed by di- and tricarbon organic acids. Labelled fumaric, succinic, malonic, citric and lactic acids are identified in plant leaves exposed to these low molecular mass alkanes, with most of the radioactivity incorporated into succinic and fumaric acids. The absence of oxalic acid directly indicates that ethane in plants is oxidized monoterminally. The oxidation of ethane at one terminal carbon atom leads to the formation of acetyl-CoA, which in turn participates in the Krebs cycle [38, 39].



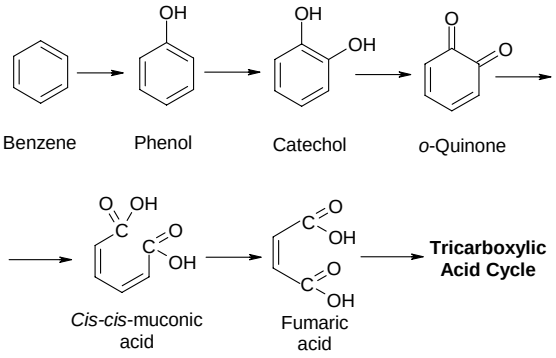
Transformation of ethane in higher plants

At one terminal carbon atom propane oxidation forms propionic acid, successively undergoing further β-oxidation and resulting in the formation of malonyl-CoA, and decarboxylation by formation acetyl-CoA. Formation of low molecular mass compounds such as monocarbonic acids allows suggesting that propane and pentane could be oxidized

monoterminally, by intermediates incorporation into the Krebs cycle or forming valeric acid.

Long chain alkanes are subjected to similar transformations. For instance, after 40 min of incubation of leek leaves with an emulsion of exogenous [^{14}C] octadecane in water, 9.6% of the total label is detected in esters, 6.4% in alcohols, and 4% in organic acids [40].

The most significant input in understanding in plants detoxification process has been revealed nearly 40 years ago by discovery of plants ability to transform (oxidatively decompose) benzene and phenol via aromatic ring cleavage. As a result of such degradation carbon atoms of contaminants are incorporated into organic acids and amino acids. Similar data were reported for nitrobenzene, aniline, toluene, α-naphthol, and benzidine transformation in plants [41, 42], [3, 43-45]. Oxidation of benzene and phenol by crude enzyme extracts of plants forms muconic acid as a result of ring cleavage, with catechol formation, as intermediate [46, 47]:



Oxidative degradation of benzene in plant cells

Further oxidation of muconic acid results in formation of fumaric acid. Labelled muconic and fumaric acids are found in plants exposed to labelled benzene or phenol. Cleavage of the aromatic ring in endogenous substrates proceeds by the transformation of 3,4-dihydroxybenzoic acid into 3-carboxymuconic acid [48]. Phenoxyalkyl-carboxyl acids containing four and more carbon atoms in their side chain often undergo β-oxidation in plants. For instance, 2,4-dichlorophenoxybutyric acid is oxidized resulting by formation of 2,4-D [48-50].

Finally contaminants degradation proceeds to standard cell metabolites or mineralization. Degrading xenobitic the plant cell not only avoids its toxic action but also utilizes its carbon, nitrogen, and other atoms for intracellular biosynthetic and energetic needs. The totality of such transformations is the essence of the plants detoxification process. Direct complete xenobiotic degradation in a plant cell is however accomplished only at low, metabolic, concentrations of environmental contaminants and respective time (it may last days or weeks).

V. Plant Cells Ultrastructure

To evaluate the ecological potential of plants, the data proving the 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 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 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 the destruction in plant cell organelles are determined by the variety of plant, chemical nature, concentration and duration of the contaminant action, etc. [50-52]. This course of events we have experimentally demonstrated 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 on two types, being different for each contaminant and plant:

- metabolic, which is digested by the plant in spite of insignificant negative effect by the mobilization of plants internal potential
- lethal, leading to indigestible deviations and to the plant death.

On the Figure 5 is shown maize root apex cells exposed to ^{14}C-nitrobenzene action, its penetration across the plasmalemma and localization in subcellular organelles. Studies of 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 [8], i.e. xenobiotic becomes distributed in most of subcellular organelles, but ultimately there is a tendency of contaminants primary accumulation in vacuoles.

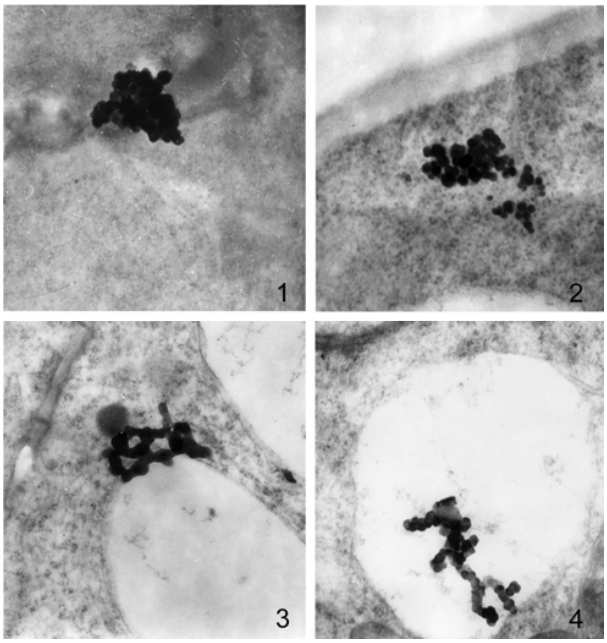


Fig. 6. Electron micrographs showing the penetration and movement of ^{14}C-labelled nitrobenzene (0.15 mM) in a maize root apex cell.

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

The general picture of the evolving action of organic contaminants on plant cells with duration of exposure is the following:

– Initially, changes in the configuration of the nucleus become noticeable. Simultaneously inhibition of DNA synthesis takes place. The barrier function of the plasmalemma and its ability to accumulate calcium are damaged. Ca^{2+} concentration in the cytoplasm is increased; Ca^{2+}–ATPase activity is inhibited. Mitochondria with swollen cristae and packed matrix becomes noticable, the plastids are electron-dense and enlarged.

– Prolonged action of contaminants leads to a widening of the cisternae of the endoplasmic reticulum and Golgi apparatus, vacuolization of the cytoplasm. The size of cytoplasm is thereby decreased and the periplasmic space concomitantly enlarged. In some cortical cells of the root apices, the number of ribosomes in the hyaloplasm is increased, and the formation of polysomes is observed. Lysis of mitochondria and depletion of ribosomes from the endoplasmic reticulum of membranes take place. Multiple contacts between the endoplasmic reticulum and the plasmalemma, vacuoles, nucleus, and membranes of the mitochondria are detectable. The enhancement of the size of the nucleus and chromatin coagulation, indicating a disturbance of the DNA synthesis process, is observed. Nuclei acquire deviant shapes because of the development of many protuberances of the nuclear membrane. In leaf cells, chloroplast shape and composition become ill defined, the external membrane is not visible, the orientation of the system is disturbed, and matrix is brightened with large osmiophilic inclusions. In the cytoplasm accumulation of the differentiated cells of the root caps that secrete mucus, is visible. Some of these hypertrophied vesicles are fused forming a large deposit of mucus. Inhibition of the process of maturing secretory vesicles translocation towards the cell periphery is often correlated not only with the swelling of vesicles, but also with the disappearance of the normal dictyosomes.

Prolonged exposure to environmental contaminants causes extensive destruction of the cell and plant death.

VI. PLANTS AS REMEDIATORS

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 might 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 vital activities.

Planting of almost any kind of vegetation, including agricultural flora are beneficial for the environment. However, in order to make the exploitation of most ecological potential of each particular plant, the selection should be carried out according to 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 higher plants could be considered as narrow but permanently working pathway having much less potential than conjugates formation process (especially in case of contaminants saturation). Finally plants, greatly depending on the variety, completely eliminate toxicants by metabolic degradation.

During the last decade phytoremediation from a conceptual methodology has become into ecologically important commercial technology for the cleaning of environment. The successful realization of phytoremediation technologies greatly depends on the synergetic action of microorganisms and plants. In order to increase the ecological potential of plants, definite progress has already been achieved by the cloning of genes of the enzymes participating in contaminants transformation/accumulation. A number of modified plants having especially high accumulation ability and a corresponding large intracellular volume to deposit metabolite – xenobiotic conjugates have been created. Some of recent publications [52-55] are devoted to the discussion of these and other problems concerning the uptake of inorganic contaminants. In these publications where transgenic plants,

characterized by enhanced tolerance to cadmium and lead (70-75 mM), which inevitably points to their hyperaccumulation potential, are described. Data indicate the doubling of the lead content in transgenic plants has also been published [56].

Among the large diversity of plants with perspectives for phytoremediation the poplar family attracts special interest. Owing to its strong root system it is characterized by the increased absorption ability. Multiple gene-engineering modifications of this plant have presented convincing evidence for the expediency in practical usage of some plants-transformants generated. Cloning of Glutathione S-transferase was successful in creation of several perspective transgenic clones. The transfer of cytochrome P450 genes to different plants has been a wide spread activity for last decade [57]. Some of the created transgenic plants generally are characterized by high resistance to herbicides of different structure and have clearly observable high detoxification potentials [20].

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 [36]. 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 [58]. 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 [59].

There are douzens of publications concerning successful improvement of plant detoxification abilities by cloning the genes of transferases and oxidases, participating in contaminant transformation processes [20, 57].

Obviously, 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 plants physiology and biochemistry. Such information would allow the creation of more rational and effective strategy for the gene engineering potential application.

Until recently plants were considered as organisms having a naturally limited potential for contaminants conjugation and accumulation. Last decade have clearly revealed the potential of plants to absorb and decompose organic contaminants and accumulate inorganic contaminants from soil, water and air. Depending on the nature of the organic xenobiotic and the type of plant, typically 1 kg of green biomass takes up from the air daily amounts ranging from microgram's to tents of milligrams of pollutants [4, 11, 60]. Plants possessing the universal cleaning up (i.e. applicable to soil, groundwater and air) capabilities are the only agents carrying out the process of remediation by transporting metals to above ground parts of plants. Some plants are indeed known as hyper accumulators of metals. For the superterranean instance transgenic plants of Indian mustard, poplar, tobacco, *Thlaspi*, *Arabidopsis*, etc. possess especially high potentials for metal accumulation and transportation [56, 61].

Elimination of contaminants located deeper than two metres in the soil is connected with limitations in time, since mass transfer processes at that depth and deeper proceeds much more slowly than in upper parts. Hence extraction by roots and the subsequent transport may become the rate-limiting factor of the whole process. Therefore, plant-microbial action-based technologies would need excessive time to achieve a satisfactory clean standard of soil. In case of contaminants high concentration, phytoremediation as a final "polishing step" must follow other technologies such as excavation, treatment or disposal, etc. Other case when phytoremediation is not successfully applied is the high concentrations of soil contaminants such as polychlorinated biphenyls and dioxins. At high concentrations of these compounds no plants can grow up in contaminated soil. In such extraordinary cases phytoremediation technology alone in any realistic time cannot clean up the soil.

On the other hand plants are very promising detoxifiers *qua* ecologically safe technologies around hotbeds of contamination (Green filter [4], Vegetation cap, Phytoremediation cover, Hydrologic control, Evapotranspiration cover or any other plant based technology) — ecologically friendly and of significant ecological importance. Elaboration of a new ecological concept, unifying worldwide experience accumulated for last 30 years and realizing of new plant-based approaches in the world scale should lead to the increase of the ecological potential of the whole planet. The universality of phytoremediation consists in the uptake nearly of all types of organic contaminants and heavy metals and their accumulation in intracellular structures or oxidative degradation to carbon dioxide.

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.

REFERENCES

[1]. Graham C, Ramsden JJ (2008) *Introduction to global warming. Complexity and Security*. IOS press, 147-184.
[2]. Tkhelidze P (1969) Oxidative transformation of benzene and toluene in vine grapes (in Russian). *Bull Georg Acad Sci* 56: 697–700.
[3]. Kvesitadze G, Khatisashvili G, Sadunishvili T, Ramsden JJ (2006) *Mechanisms of detoxification: the basis of phytoremediation*. Berlin Heidelberg, Springer, 262p.
[4]. Schönherr J, Bukovac MJ (1972) Penetration of stomata by liquids. Dependence on surface tension, wettability, and stomatal morphology. *Plant Physiol* 49: 813–823.
[5]. Ugrekhelidze D, Durmishidze S (1980) The biosphere chemical pollution and plant (in Georgian). Metsniereba, Tbilisi.
[6]. Sandermann H (1994) Higher plant metabolism of xenobiotics: the "green liver" concept. *Pharmacogenetics* 4: 225–241
[7]. Sharma MP, Vanden Born WH (1970) Foliar penetration of picloram and 2,4-D in aspen and balsam poplar. *Weed Sci* 18: 57–65.

[8]. Ugrekhelidze D (1976) *Metabolism of exogenous alkanes and aromatic hydrocarbons in plants* (in Russian). Metsnieraba, Tbilisi
[9]. Korte F, Kvesitadze G, Ugrekhelidze D, Gordeziani M, Khatisashvili G, Buadze O, Zaalishvili G, Coulston F (2000) Review: Organic toxicants and plants. *Ecotoxicol Environ Saf* 47: 1–26.
[10]. Ugrekhelidze D, Korte F, Kvesitadze G (1997) Uptake and transformation of benzene and toluene by plant leaves. *Ecotoxicol Environ Saf* 37: 24–28.
[11]. Martinova E (1993) An ATP-dependent glutathione-S-conjugate "export" pump in the vacuolar membrane of plants. *Nature* 364: 247–249.
[12]. Coleman JOD, Mechteld MA, Kalff B, Davies TGE (1997) Detoxification of xenobiotics in plants: chemical modification and vacuolar compartmentalization. *Trends Plant Sci* 2: 144–151
[13]. Sandermann H (1987) Pestizid-Rückstände in Nahrungspflanzen. Die Rolle des pflanzlichen Metabolismus. *Naturwissenschaften* 74: 573–578.
[14]. Eckardt NA (2001) Move it on out with MATEs. *Plant Cell* 13: 1477–1480.
[15]. Betsiashvili M., Sadunishvili T., Amashukeli N., Tsulukidze N., Shapovalova N., Dzamukashvili N., Nutsubidze N. Effect of aromatic hydrocarbons on main metabolic and energetic enzymes in maize, ryegrass and kidney bean seedlings. *Bull. Georgian Acad. Sci*, 2004, vol. 170, No 1, 172-174.
[16]. Chrikishvili D, Sadunishvili T, Zaalishvili G (2006) Benzoic acid transformation via conjugation with peptides and final fate of conjugates in higher plants. *Ecotoxicol Environ Saf* 64, 3, 390-399.
[17]. Robineau T, Batard Y, Nedelkina S, Cabello-Hurtado F, LeRet M, Sorokine O, Didierjean L, Werck-Reichhart D (1998) The chemically inducible plant cytochrome P450 CYP76B1 actively metabolizes phenylureas and other xenobiotics. *Plant Physiol* 118: 1049–1056
[18]. Hansikova H, Frei E, Anzenbacher P, Stiborova M (1994) Isolation of plant cytochrome P450 and NADPH:cytochrome P450-reductase from tulip bulbs (*Tulipa fosteriana*). Gen Physiol Biophys, 13: 149–169
[19]. Schuler MA (1996) Plant cytochrome P450 monooxygenases. *Crit Rev Plant Sci* 15: 235–284.
[20]. Morant M, Bak S, Moller BL, Werck-Reichhart D (2003) Plant cytochromes P450: tools for pharmacology, plant protection and phytoremediation. *Curr Opin Biotechnol* 2: 151–162.
[21]. Fonné-Pfister R, Kreuz K (1990) Ring-methyl hydroxylation of chlortoluron by an inducible cytochrome P450-dependent enzyme from maize. *Phytochemistry* 9: 2793–2804.
[22]. Mougin C, Cabanne F, Canivenc M-C, Scalla R (1990) Hydroxylation and N-demethylation of chlortoluron by wheat microsomal enzymes. *Plant Sci* 66: 195–203.
[23]. Didierjean L, Gondet L, Perkins R, Lau S-MC, Schaller H, O'Keefe DP, Werck-Reichhart D (2002) Engineering herbicide metabolism in tobacco and *Arabidopsis* with CYP76B1, a cytochrome P450 enzyme from Jerusalem artichoke. *Plant Physiol* 130: 179–189.
[24]. Stiborova M, Anzenbacher P (1991) What are the principal enzymes oxidizing the xenobiotics in plants: cytochrome P-450 or peroxidase? *Gen Physiol* 10: 209–216.
[25]. Shinohara A, Kamataki T, Ichimura Y, Opochi H, Okuda K, Kato R (1984) Drug oxidation activities of horse-redish peroxidase, myoglobin and cytochrome P-450$_{cam}$ reconstituted with synthetic hemes. *Jap J Pharmacol* 45: 107–114.
[26]. Wilson L. Williamson T. Gronowski J, Gentile GI, Gentile JM (1994) Characterization of 4-nitro-*o*-phenylendiamine activities by plant systems. *Mutation Res* 307: 185-193.
[27]. Laurent FMG (1994) Chloroaniline peroxidation by soybean peroxidases. *Pestic Sci* 40: 25– 30.
[28]. Adamia G, Ghoghoberidze M, Graves D, Khatisashvili G, Kvesitadze G, Lomidze E, Ugrekhelidze D, Zaalishvili G (2006) Absorption, distribution and transformation of TNT in higher plants. *Ecotoxicol Environ Saf*, 64, 136-145.
[29]. Sánches-Ferrer A, Rodrígez-López JN, García-Cánovas F, García-Carmona F (1994) Tyrosinase: a comprehensive review of its mechanism. *Biochim Biophys Acta* 1247: 1–11.
[30]. Rodrígez-López JN, Tudela J, Varón R, Fenoll LG, García-Carmona F, García-Cánovas F (1992) Analysis of a kinetic model for melanin biosynthesis pathway. *J Biol Chem* 267: 3801–3810.
[31]. Guillén F, Martýnez MJ, Muñoz C, Martýnez AT (1997) Quinone redox cycling in the ligninolytic fungus *Pleurotus eryngii* leading to extracellular production of superoxide anion radical. *Arch Biochem Biophys* 339: 190–199.
[32]. Guillén F, Gómez-Toribio V, Martýnez MJ, Martýnez AT (2000) Production of hydroxyl radical by the synergistic action of fungal laccase and aryl alcohol oxidase. *Arch Biochem Biophys* 382: 142–147.
[33]. Ugrekhelidze D, Phiriashvili V, Mithaishvili T (1986) Uptake of salicylic acid and aniline by pea roots (in Russian). *Fiziol Rast* (Moscow) 33: 165–170.
[34]. Colombo JC, Cabello MN, Arambarri AM (1996) Biodegradation of aliphatic and aromatic hydrocarbons by natural soil microflora and pure culture of imperfect and ligninolytic fungi. *Environ Pollut* 94: 355–362.
[35]. Niku-Paavola ML, Viikari L (2000) Enzymatic oxidation of alkenes. *J Mol Cat* 10: 435–444.
[36]. Collins PJ, Dobson ADW (1997) Regulation of laccase gene transcription in *Trametes versicolor*. *Appl Environ Microbiol* 63: 3444–3450.
[37]. Johannes C, Majcherczyk A (2000) Natural mediators in the oxidation of polycyclic aromatic hydrocarbons by laccase mediator systems. *Appl Environ Microbiol* 66: 524–528.
[38]. Durmishidze S, Ugrekhelidze D (1968) Absorption and conversion of butane by higher plants (in Russian). *Dokladi Akademii Nauk SSSR* 182: 214–216
[39]. Durmishidze S, Ugrekhelidze D (1968). Oxidation of ethane, propane and pentane by higher plants (in Russian). *Bull Georg Acad Sci* 50: 661–666
[40]. Cassagne C, Lessire R (1975) Studies on alkane biosynthesis in epidermis of *Allium porrum* L. leaves. 4. Wax movement into and out of the epidermal cells. *Plant Sci Lett* S5: 261–266
[41]. Durmishidze S, Ugrekhelidze D, Djikiya A (1974) Absorption and transformation of benzene by higher plants (in Russian). *Fiziologiya i Biochimiya Kulturnikh Rastenii* 6: 217–221.
[42]. Durmishidze S, Ugrekhelidze D, Djikiya A (1974) Absorption and transformation of toluene by higher plants (in Russian). *Appl Biochem Microbiol* 10: 673–676.
[43]. Jansen EF, Olson AC (1969) Metabolism of carbon-14-labelled benzene and toluene in avocado fruit. *Plant Physiol* 44: 786–791.
[44]. Mithaishvili T, Scalla R, Ugrekhelidze D, Tsereteli B, Sadunishvili T, Kvesitadze G (2005) Transformation of aromatic compounds in plants grown in aseptic conditions. *Z Naturforsch*, 60, 97-102.
[45]. Ugrekhelidze D, Kavtaradze L (1970) The question of metabolism of α-naphthol in higher plants (in Russian). *Bull Georg Acad Sci* 57: 465–469.
[46]. Durmishidze S, Ugrekhelidze D, Djikiya A, Tsevelidze D (1969) The intermediate products of enzymatic oxidation of benzene and phenol (in Russian). *Dokladi Akademii Nauk SSSR* 184: 466–469
[47]. Durmishidze S, Djikiya A, Lomidze E (1979) Uptake and transformation of benzidine by plants in sterile conditions (in Russian). *Dokladi Akademii Nauk SSSR* 247: 244–247.
[48]. Tateoka TN (1970) Studies on the catabolic pathway of protocatechuic acid in mung bean seedlings. *Bot Mag* (Tokyo) 83: 49–54.
[49]. Hawf LR, Behrens R (1974) Selectivity factors in the response of plants to 2,4-D. *Weed Sci* 22: 245–249.
[50]. Buadze O, Sadunishvili T, Kvesitadze G (1998) The effect of 1,2-benzanthracene and 3,4-benzpyrene on the ultrastructure on maize cells. *Int Biodeterior Biodergad* 41: 119–125.
[51]. Zaalishvili G, Lomidze E, Buadze O, Sadunishvili T, Tkhelidze P, Kvesitadze G (2000) Electron microscopic investigation of benzidine effect on maize root tip cell ultrastructure, DNA synthesis and calcium homeostasis. *Int Biodeterior Biodergad* 46: 133–140.
[52]. Sargent JA, Blackman GE (1972) Studies on foliar penetration. 9. Patterns of penetration of 2,4-dichlorophenoxyacetic acid into the leaves of different species. *J Exp Bot* 23: 830–839.

[53]. Li ZS, Szczypka M, Lu YP, Thiele DJ, Rea PA (1996) The yeast cadmium factor protein (YCF1) is a vacuolar glutathione S-conjugate pump. *J Biol Chem* 271: 6509–6517.
[54]. Lu YP, Li ZS, Rea PA (1997) AtMPR1 gene of *Arabidopsis* encodes a glutathione S-conjugate pump: isolation and functional definition of a plant ATP-binding cassette transporter gene. *Proc Natl Acad Sci* USA 94: 8243–8248.
[55]. Song WY, Sohn EJ, Martinoia E, Lee YJ, Yang Y-Y, Jasinski M, Forestier C, Hwang I, Lee Y (2003) Engineering tolerance and accumulation of lead and cadmium in transgenic plants. *Nat Biotechnol* 21: 914–919.
[56]. Peuke AD, Kopriva S, Rennenberg H (2004) Phytoremediation with the help of transgenic trees. In: *Phytoremediation: environmental and molecular biological aspects.* OECD workshop, Hungary, Abstr, p 33Phytoremediation: environmental and molecular biological aspects. OECD workshop, Hungary, Abstr, p 27.
[57]. Ohkawa H, Tsujii H, Ohkawa Y (1999) The use of cytochrome P450 genes to introduce herbicide tolerance in crops: a review. *Pestic Sci* 55: 867– 874.
[58]. Hannink N, Rosser SJ, French CE, Basran A, Murray JA, Nicklin S, Bruce NC (2001) Phytodetoxification of TNT by transgenic plants expressing a bacterial nitroreductase. *Nat Biotechnol* 19: 1168–1172.
[59]. Hannink N, Rosser SJ, Bruce NC (2002) Phytoremediaition of explosives. *Crit Rev Plant Sci* 21: 511–538
[60]. Tsao DT (2003) *Phytoremediation. Advances in biochemical engineering and biotechnology*. Springer, Berlin Heidelberg New York.
[61]. Macek T, Macková M, Pavlíková D, Száková J, Truska M, Singh-Cundy A, Kotraba P, Yancey N, Scouten WH (2002) Accumulation of cadmium by transgenic tobacco. Acta *Biotechnologica* 22: 101–106 .