UDK 581.526.56+642.125

DEGRADATION OF ANTHROPOGENIC CONTAMINANTS BY HIGHER PLANTS

G. KVESITADZE

Georgian National Academy of Sciences, Georgia

E-mail: giorgi@kvesitadze@science.org.ge

Elimination of contaminants from the environment by microorganisms of different taxonomic groups is an evolutionarily determined property, which have already been widely discussed. Until recently, plants still occupying above 40% of the world land, were considered as organisms having only a limited potential for contaminants conjugation and accumulation within cell organelles. Based on 40 years experience in this area author is making an attempts for the evaluation of different aspects of plants ecological potential from the modern understanding; to assume mechanism of inter replacement of enzymes participating in oxidative degradation of organic contaminants in higher plants; to stress the importance of phenoloxidase, enzyme hitherto unknown to participate actively in remediation processes (contaminants oxidative decomposition); to reveal the criterion for the evaluation under the action of contaminants of such precise indicator of plant detoxification potential as deviations in ultrastructural level of plant cells.

Natural contaminations such as the emission of poisonous gases during a volcanic eruption and earthquakes, swamps poisoned evaporations, synthesis of toxic compounds by lower (microorganisms) and higher plants, etc., in comparison with the human contribution in the environmental contamination is much less impressive. 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. In spite of difficulties in quantitative, as well as in qualitative estimation, and having a tendency to be increased, the amount of spread out contaminants exceeds annually one billion of tons. Most dangerous among these contaminants are considered as emergent because of their persistence, bioaccumulation, and toxicity along with our awareness of their prominent occurrence in the environment. In different ways, huge amounts of these hazardous substances or toxic intermediates of their incomplete transformations are accumulated in the different niches of biosphere, significantly affecting ecological balance. Lately, many ecological technologies have been elaborated, targeted to minimize the flow of toxic compounds to the biosphere and to control their level or state [1, 2]. Despite the definite positive effect from the realization of these technologies (physical, chemical, mechanical etc), the intensive flow of toxic compounds to the biosphere is still increasing.

Nevertheless, the plants kingdom assimilates 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 being recently 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 unique process, significantly affecting the overall plant metabolism.

– Large assimilating surface area of plant leaves (adaxial and abaxial), significantly exceeding in size the above ground surface

under the plant, permits the absorption of contaminants in a big quantity from air via the cuticle and stomata.

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

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

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

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

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

The 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. [2, 3].

Nowadays there are experimental data obviously demonstrating plants potential 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 [4].

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 [5] (Fig. 1).
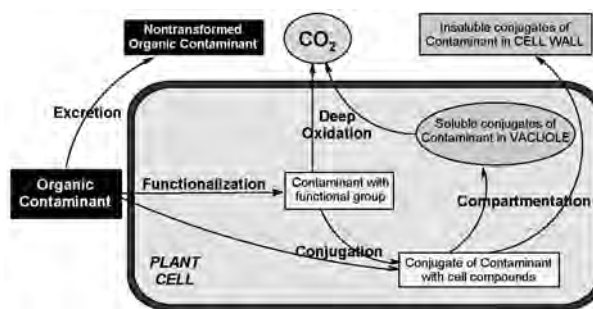

Fig. 1. **The main pathways of organic contaminant transformation in plant cells**

Functionalization is a process whereby a molecule of a hydrophobic organic xenobiotic acquires 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 [2]. 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 [5]. 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 [6] and is known as «storage excretion» [7].

The described above 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 [8].
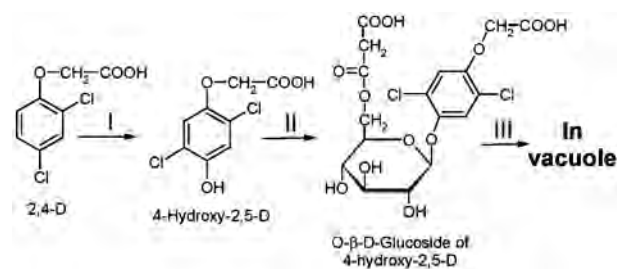
2,4-D transformation for deposition in vacuoles

The 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.

Ttransformations of contaminants during functionalization, conjugation and compartmentation are of enzymes function. 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. Below are presented enzymes directly participating 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 [9]. 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 [10].

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.

Cytochrome P450-containing monooxygenases (EC 1.14.14.1) are mixed-function enzymes located in the membranes of the endoplasmic reticulum (microsomes) [11]. 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

Plants oxidative metalloenzymes

Enzyme	Physiological function	Existence in cell	Localization	Specificity to toxicants	Limiting factors	Stability
Cytochrome P_{450} 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

by the NADPH-dependent redox system. In the latter case cytochrome b_5 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) [12]. The second atom of oxygen is used for the formation of a water molecule (Fig. 2).
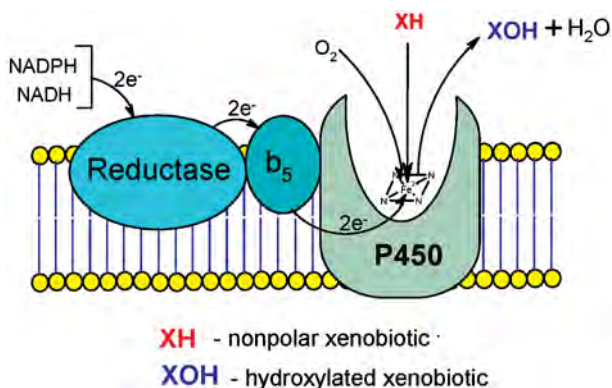

Fig. 2. **Microsomal monooxygenase system**

Plant cytochrome P450-containing monooxygenases play an important role in the hydroxylation of organic contaminants [5]. 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. [12, 13]. 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 [14, 15]. For some phenylurea herbicides in the Jerusalem artichoke cytochrome P450-mediated N-demethylation is sufficient to cause significant or complete loss of phytotoxicity [16].

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:

$$RH_2 + H_2O_2 \rightarrow 2H_2O + R.$$
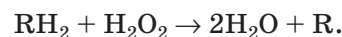
The peroxidases catalyze a number of free radical reactions. Alternatively, the compound that is directly oxidized by the enzyme further oxidizes other organic compounds, including xenobiotics. This notion is based on the wide ubiquitous distribution of this enzyme in plants (the isozymes of peroxidase 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 [17], 3,4-benzpyrene, 4-nitro-*o*-phenylendiamine [18], 4-chloroaniline [19], phenol, aminoflourene, acetaminophen, diethylstilbestrol, butylated hydroxytoluene, hydroxyanisoles, benzidine, etc. [5]; horseradish (*Armoracia rusticana*) peroxidase oxidizes tritium-labelled $[C^3H_3]$ TNT [20].

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 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».

Phenoloxidases actively participate in the oxidation of xenobiotics of aromatic struc-

ture. 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 [4]. 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-}) and hydroxyl radical ($\cdot$OH) [21], 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. 3).
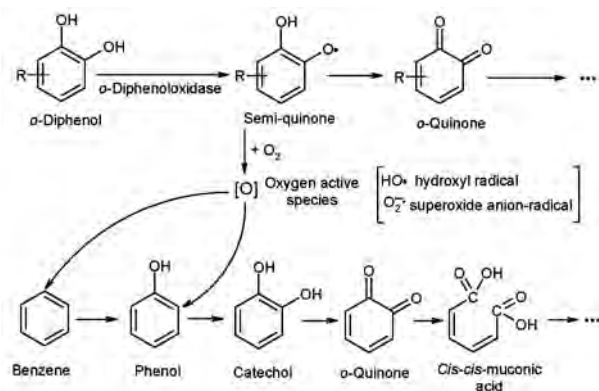
Enzymatic oxidation of *o*-diphenols (upper) by phenoloxidase and non-enzymatic co-oxidation of benzene (lower)

Analogously, nitrobenzene is oxidized to *m*-nitrophenol, and the methyl group of [C_3H_3] TNT [20] 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 [4], 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 [22], and actively participates in the enzymatic oxidation of alkenes [23]. Crude preparations of laccase isolated from the white rot fungus *Trametes versicolor* oxidizes 3,4-benzopyrene, anthracene, chrysene, phenanthrene, acenaphthene and some other PAHs [24]. The intensity of oxidation of these antro-

pogenic 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 [25]. 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 [4].

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 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 molecules [4, 26].

Based on the number of experimental data it is supposed 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.

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 [27].
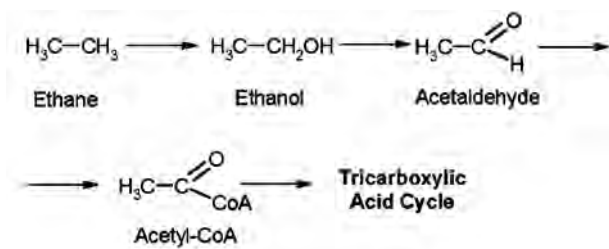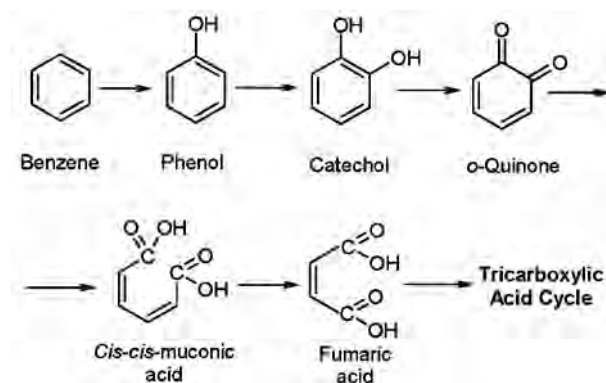

Transformation of ethane in higher plants

Oxidative degradation of benzene in plant cells

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 [28].

The most significant input in understanding in plants detoxification process has been revealed 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 [29, 30]. 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.

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 [31]. Phenoxyalkyl-carboxyl acids containing four and more carbon atoms in their side chain often undergo β-oxidation in plants. For instance, 2,4-dichlorophenoxy-butyric acid is oxidized resulting by formation of 2,4-D [32–34].

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).

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. [35, 36]. This course of events has been 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 Fig. 3 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, i.e. xenobiotic becomes distributed in most of subcellular organelles, but ultimately there is a tendency of contaminants primary accumulation in vacuoles.

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
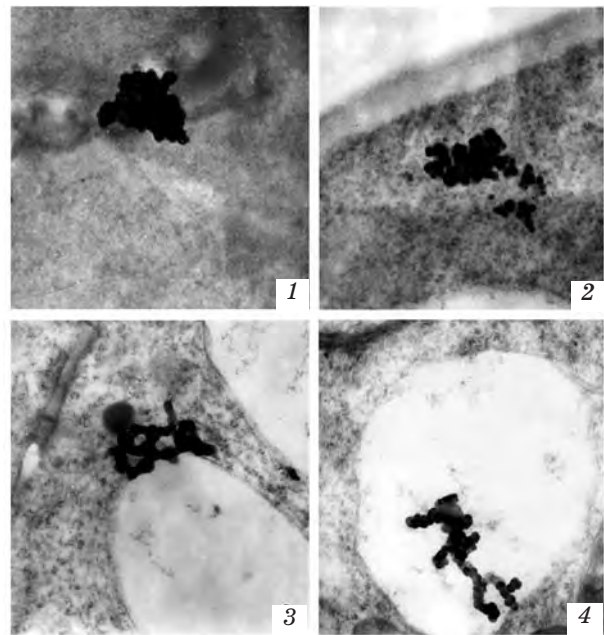

Fig. 3. **Electron micrographs showing the penetration and movement of ^{14}C-labelled nitrobenzene (0.15 mM) in a maize root apex cell**
The xenobiotic penetrated through the plasmalemma (*1*), moved to the cytoplasm (*2*), and thereafter translocated into vacuoles (*3, 4*).
1 — ×48 000; *2* — ×36 000;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

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.

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.

Phytoremediation is a unique cleanup strategy. The realization of phytoremediation

139

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).

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 genetically modified plants having especially high accumulation potential and correspondingly large intracellular volume to deposit metabolite — xenobiotic conjugates have been created. Some publications [37, 38] 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 detected [39].

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 [40]. Some of the created transgenic plants generally are characterized by high resistance to herbicides of different structure

and have clearly observable high detoxification potentials [13].

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 [41]. 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 [42]. 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 [43].

There are douzens of publications concerning successful improvement of plant detoxification abilities by cloning the genes of transferases and oxidases, which intensively participate in contaminant transformation processes [13, 40].

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 technique 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 [2, 4, 44]. 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 [39, 45, 46].

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 [2], 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 mineralization.

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. *Tsao D. T.* Phytoremediation. Advances in biochemical engineering and biotechnology 78. — Springer, Berlin Heidelberg, New York, 2003. — 206 p.
2. *Kvesitadze G., Khatisashvili G., Sadunishvili T., Ramsden J. J.* Mechanisms of detoxification: the basis of phytoremediation. — Berlin Heidelberg, Springer, 2006. — 262 p.
3. *Korte F., Kvesitadze G., Ugrekhelidze D. et al.* Review: Organic toxicants and plants // Ecotoxicol. Environ Saf. — 2000 — V. 47. — P. 1–26.
4. *Ugrekhelidze D., Korte F., Kvesitadze G.* Uptake and transformation of benzene and toluene by plant leaves // Ibid. — 1997. — V. 37. — P. 24–28.
5. *Sandermann H.* Higher plant metabolism of xenobiotics: the «green liver» concept // Pharmacogenetics. — 1994. — V. 4. — P. 225–241.
6. *Martinova E.* An ATP-dependent glutathione-S-conjugate «export» pump in the vacuolar membrane of plants // Nature. — 1993. — V. 364. — P. 247–249.
7. *Coleman J. O. D., Mechteld M. A., Kalff B., Davies T. G. E.* Detoxification of xenobiotics in plants: chemical modification and vacuolar compartmentalization // Trends Plant Sci. — 1997. — V. 2. — P. 144–151.
8. *Sandermann H.* Pestizid-Rückstände in Nahrungspflanzen. Die Rolle des pflanzlichen Metabolismus // Naturwissenschaften. — 1987. — V. 74. — P. 573–578.
9. *Eckardt N. A.* Move it on out with MATEs // Plant Cell. — 2001. — V. 13. — P. 1477–1480.
10. *Kvesitadze G., Gordeziani M., Khatisashvili G. et al.* Review: Some aspects of the enzymatic basis of phytoremediation // J. Biol. Physics Chem. — 2001. — V. 1, 2. — P. 49–57.
11. *Robineau T., Batard Y., Nedelkina S. et al.* The chemically inducible plant cytochrome P450 CYP76B1 actively metabolizes phenylureas and other xenobiotics // Plant Physiol. — 1998. — V. 118. — P. 1049–1056.
12. *Schuler M. A.* Plant cytochrome P450 monooxygenases // Crit. Rev. Plant Sci. — 1996. — V. 15. — P. 235–284.
13. *Morant M., Bak S., Moller B. L., Werck-Reichhart D.* Plant cytochromes P450: tools for pharmacology, plant protection and phytoremediation // Curr. Opin. Biotechnol. — 2003. — V. 2. — P. 151–162.
14. *Fonné-Pfister R., Kreuz K.* Ring-methyl hydroxylation of chlortoluron by an inducible cytochrome P450-dependent enzyme from maize // Phytochemistry. — 1990. — V. 9. — P. 2793–2804.

15. *Mougin C., Cabanne F., Canivenc M.-C., Scalla R.* Hydroxylation and N-demethylation of chlortoluron by wheat microsomal enzymes // Plant Sci. — 1990. — V. 66. — P. 195–203.

16. *Didierjean L., Gondet L., Perkins R. et al.* Engineering herbicide metabolism in tobacco and *Arabidopsis* with CYP76B1, a cytochrome P450 enzyme from Jerusalem artichoke // Plant Physiol. — 2002. — V. 130. — P. 179–189.

17. *Shinohara A., Kamataki T., Ichimura Y.* Drug oxidation activities of horse-redish peroxidase, myoglobin and cytochrome P-450_{cam} reconstituted with synthetic hemes // Jap. J. Pharmacol. — 1984. — V. 45. — P. 107–114.

18. *Wilson L. Williamson T. Gronowski J. et al.* Characterization of 4-nitro-*o*-phenylendiamine activities by plant systems // Mutation Res. — 1994. — V. 307. — P. 185–193.

19. *Laurent F. M. G.* Chloroaniline peroxidation by soybean peroxidases // Pestic Sci. — 1994. — V. 40. — P. 25–30.

20. *Adamia G., Ghoghoberidze M., Graves D. et al.* Absorption, distribution and transformation of TNT in higher plants // Ecotoxicol. Environ Saf. — 2006. — V. 64. — P. 136–145.

21. *Guillén F., Gymez-Toribio V., Martýnez M. J., Martýnez A. T.* Production of hydroxyl radical by the synergistic action of fungal laccase and aryl alcohol oxidase // Arch. Biochem. Biophys. — 2000. — V. 382. — P. 142–147.

22. *Colombo J. C., Cabello M. N., Arambarri A. M.* Biodegradation of aliphatic and aromatic hydrocarbons by natural soil microflora and pure culture of imperfect and ligninolytic fungi // Environ Pollut. — 1996. — V. 94. — P. 355–362.

23. *Niku-Paavola M. L., Viikari L.* Enzymatic oxidation of alkenes // J. Mol. Cat. — 2000. — V. 10. — P. 435–444.

24. *Collins P. J., Dobson A. D. W.* Regulation of laccase gene transcription in *Trametes versicolor* // Appl. Environ Microbiol. — 1997. — V. 63. — P. 3444–3450.

25. *Johannes C., Majcherczyk A.* Natural mediators in the oxidation of polycyclic aromatic hydrocarbons by laccase mediator systems // Appl. Environ Microbiol. — 2000. — V. 66. — P. 524–528.

26. *Chrikishvili D., Sadunishvili T., Zaalishvili G.* Benzoic acid transformation via conjugation with peptides and final fate of conjugates in higher plants // Ecotoxicol. Environ Saf. — 2005. — (In press YEES 3185).

27. *Durmishidze S., Djikiya A., Lomidze E.* Uptake and transformation of benzidine by plants in sterile conditions (in Russian) // Dokl. Akad. Nauk SSSR. — 1979. — V. 247. — P. 244–247.

28. *Cassagne C., Lessire R.* Studies on alkane biosynthesis in epidermis of *Allium porrum* L. leaves. 4. Wax movement into and out of the epidermal cells // Plant Sci. Lett. — 1975. — V. S5. — P. 261–266.

29. *Durmishidze S., Ugrekhelidze D., Djikiya B.* Absorption and transformation of toluene by higher plants (in Russian) // Appl. Biochem. Microbiol. — 1974. — V. 10. — P. 673–676.

30. *Mithaishvili T., Scalla R., Ugrekhelidze D. et al.* Transformation of aromatic compounds in plants grown in aseptic conditions // Z. Naturforsch. — 2005. — V. 60. — P. 97-102.

31. *Tateoka T. N.* Studies on the catabolic pathway of protocatechuic acid in mung bean seedlings // Bot. Mag. (Tokyo). — 1970. — V. 83. — P. 49–54.

32. *Hawf L. R., Behrens R.* Selectivity factors in the response of plants to 2,4-D // Weed. Sci. — 1974. — V. 22. — P. 245–249.

33. *McComb A. J., McComb J. A.* Differences between plant species in their ability to utilize substituted phenoxybutyric acids as a source of auxin for tissue culture growth // Plant Sci. Lett. — 1978. — V. 11. — P. 227.

34. *Taylor H., Wain R.* Studies of plant growth-regulating substances. 52. Growth retardation by 3,5-dichlorophenoxyethylamine and 3,5-dichlorophenoxybutyric acid arising from their conversion to 3,5- dichlorophenoxyacetic acid in tomato plants // Ann. Appl. Biol. — 1978. — V. 89. — P. 271–277.

35. *Buadze O., Sadunishvili T., Kvesitadze G.* The effect of 1,2-benzanthracene and 3,4-benzpyrene on the ultrastructure on maize cells // Int. Biodeterior Biodergad. — 1998. — V. 41. — P. 119–125.

36. *Zaalishvili G., Lomidze E., Buadze O. et al.* Electron microscopic investigation of benzidine effect on maize root tip cell ultrastructure, DNA synthesis and calcium homeostasis // Ibid. — 2000. — V. 46. — P. 133–140.

37. *Lu Y. P., Li Z. S., Rea P. A.* 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. — 1997. — V. 94. — P. 8243–8248.

38. *Song W. Y., Sohn E. J., Martinoia E. et al.* Engineering tolerance and accumulation of lead and cadmium in transgenic plants // Nat. Biotechnol. — 2003. — V. 21. — P. 914–919.

39. *Peuke A. D., Kopriva S., Rennenberg H.* Phytoremediation with the help of transgenic trees. In: Phytoremediation: environmental and molecular biological aspects. — OECD workshop, Hungary, Abstr, 2004. — P. 33.

40. *Ohkawa H., Tsujii H., Ohkawa Y.* The use of cytochrome P450 genes to introduce herbicide tolerance in crops: a review // Pestic Sci. — 1999. — V. 55. — P. 867–874.
41. *French C. E., Hosser S. J., DaviesG. J. et al.* Biodegradation of explosives by transgenic plants expressing pentaerythritol tetranitrate reductase // Nat. Biotechnol. — 1999. — V. 17. — P. 491–494.
42. *Hannink N., Rosser S. J., French C. E. et al.* Phytodetoxification of TNT by transgenic plants expressing a bacterial nitroreductase // Ibid. — 2001. — V. 19. — P. 1168–1172.
43. *Hannink N., Rosser S. J., Bruce N. C.* Phytoremediaition of explosives // Crit.

Rev. Plant Sci. — 2002. — V. 21. — P. 511–538.
44. *Ugrekhelidze D., Durmishidze S.* The biosphere chemical pollution and plant (in Georgian). — Tbilisi: Metsniereba, 1980.
45. *Macek T., Macková M., Pavlнková D. et al.* Accumulation of cadmium by transgenic tobacco // Acta Biotechnol. — 2002. — V. 22. — P. 101–106.
46. *Macek T., Sura M., Francova K. et al.* Approaches using GM plants for the removal of xenobitics (Cd, Ni, PCB) including experiments in real contaminated soils. In: Phytoremediation: environmental and molecular biological aspects. — OECD workshop, Hungary, Abstr, 2004 — P. 27.

ДЕГРАДУВАННЯ АНТРОПОГЕННИХ КОНТАМІНАНТІВ ВИЩИМИ РОСЛИНАМИ

Г. Квеситадзе

Національна академія наук Грузії

E-mail: giorgi@kvesitadze@science.org.ge

Еволюційно зумовлена здатність мікроорганізмів різних таксономічних груп до елімінації забруднювальних речовин з навколишнього середовища загальновідома й широко обговорюється в літературі. Водночас донедавна вважали, що рослини, які займають близько 40% суші, мають обмежений потенціал зв'язування забруднювальних речовин і накопичення їх усередині клітинних органел. Автор статті, ґрунтуючись на 40-річному досвіді роботи в цій галузі, розглядає з погляду сучасних уявлень різні аспекти екологічного потенціалу рослин; механізм заміни ензимів, що беруть участь в оксидативній деградації органічних забруднювальних речовин; роль у цьому процесі фенолоксидази; критерії оцінки потенціалу рослин до детоксифікації за таким точним індикатором, як зміна рослинних клітин на ультраструктурному рівні під дією забруднювальних речовин.

Ключові слова: вищі рослини, антропогенні забруднювальні речовини, оксидативна деградація, фенолоксидаза.

ДЕГРАДАЦИЯ АНТРОПОГЕННЫХ КОНТАМИНАНТОВ ВЫСШИМИ РАСТЕНИЯМИ

Г. Квеситадзе

Национальная академия наук Грузии

E-mail: giorgi@kvesitadze@science.org.ge

Эволюционно обусловленная способность микроорганизмов различных таксономических групп к элиминации загрязняющих веществ из окружающей среды общеизвестна и широко обсуждается в литературе. В то же время до недавнего времени считали, что растения, занимающие около 40% суши, обладают ограниченным потенциалом для связывания загрязняющих веществ и накопления их внутри клеточных органелл. В представленной статье автор, основываясь на 40-летнем опыте работы в данной области, рассматривает с точки зрения современных представлений различные аспекты экологического потенциала растений; механизм замены энзимов, участвующих в оксидативной деградации органических загрязняющих веществ; роль в этом процессе фенолоксидазы; критерии оценки потенциала растений к детоксификации по такому точному индикатору как изменение растительных клеток на ультраструктурном уровне под действием загрязняющих веществ.

Ключевые слова: высшие растения, антропогенные загрязняющие вещества, оксидативная деградация, фенолоксидаза.