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PLANT AS A MAIN TOOL IN CREATION OF ECOLOGICAL BALANCE

Abstract: 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. As it has been exposed contaminants deep degradation processes proceeding in higher plants requires: the enzymes carrying out oxidation/reduction and conjugation processes; the ability of some plant varieties to accumulate huge amount of heavy metals and deposit them in cellular structures; formation of anthropogenic contaminants conjugates with endogenous compounds and enzymes participating in this process. Although, still there are uncertain questions closely related to the contaminants multistage degradation process in plants. Based on 30 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);

Key words: *plant, enzyme, contaminants, ecological potential, detoxification*

INTRODUCTION

More than 500 millions of tons of chemicals are produced annually in the world. In different ways, huge amounts of these hazardous substances or toxic intermediate products of their incomplete transformations are accumulated in the biosphere, significantly affecting ecological balance. Nevertheless, members of the plant kingdom (lower microorganisms and higher-plants) can assimilate environmental contaminants, and be successfully directed to remove toxic compounds from the environment, providing long-term protection against their environmental dispersal in ever increasing doses [1]. Undoubtedly, the great majority of chemically synthesized

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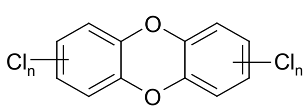
compounds such as plant protection and pest control agents (pesticides), paints and varnishes, solvents and emulsifiers, petroleum products, products of applied chemistry, many chemicals (monomers, dyes, plasticizers, stabilizers, etc.) widely used in the polymer industry, products of the pharmaceutical industry, surfactants, refrigerants, aerosols, explosives, heat-generating elements of nuclear power stations, conservation agents and packing materials, according to their nature are toxic. Some of these products are characterized by a much higher toxicity than otherwise comparable natural compounds. Examples of exceptions (highly toxic compounds of natural origin) are cyanogenic glycosides, glucosinolates, glycoalkaloids, lectins, phenols, coumarins and some other secondary metabolites of plants. Toxins of microorganisms are specific poisons elaborated by both prokaryotic and eukaryotic microorganisms. These toxins are often polypeptides varying in molecular mass and contain up to one hundred thousand of amino acids [2]. Low molecular mass organic compounds are also encountered as microbial toxins. In spite of their high toxicity, these compounds exist in nature at such low concentrations in comparison with toxic compounds of anthropogenic nature that they even cannot be considered as contaminants.

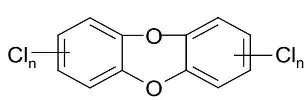
Pesticides. Pesticides, compounds for plant protection and pest control, were the most widely distributed chemical contaminants of the environment in the twentieth century. According to data gathered by the United States Environmental Protection Agency (EPA) and the World Health Organization (WHO), over 1000 compounds are used as pesticides, representing compounds of many different chemical classes: carbamates, thiocarbamates, dipyridyls, triazines, phenoxyacetates, coumarins, nitrophenols, pyrazoles, pyrethroids, and organic compounds containing chlorine, phosphorus, tin, mercury, arsenic, copper, etc. Millions of tons of pesticides are produced and used annually in tight combination with agriculture. Many articles, reviews and books are devoted to pesticides [3, 4]; Due to wide spread and long term application of pesticides in agriculture, soils, ground waters and reservoirs in many areas are now heavily contaminated. The toxicity of pesticides makes them hazardous when incorporated into the food chain.

Dioxins. The group of polychlorinated dibenzodioxins and dibenzofurans called dioxins are distinguished by especially high toxicity [5].

These compounds are always found as a complex mixture.



Polychlorinated dibenzo-*p*-dioxin

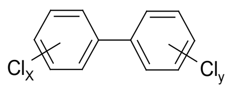


Polychlorinated dibenzo-*p*-furan

n is the number of chlorine atoms and varies from 4 to 8 for the entire molecule

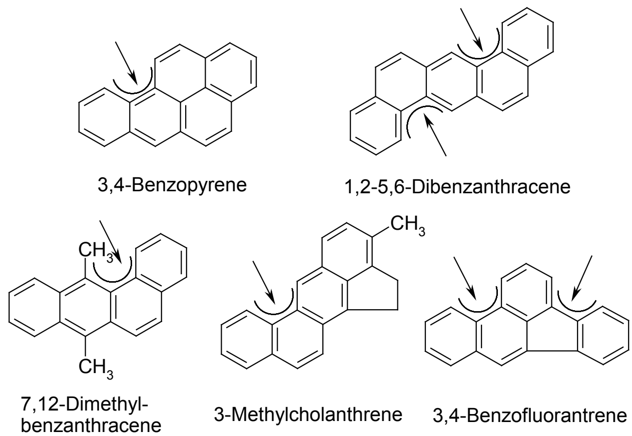
In spite of their high resistance, dioxins do appear to undergo very slow biodegradation. In the literature there are however no data reporting the ability of plants to transform dioxins, but some microorganisms are able to mineralize these harmful toxic compounds. The deep degradation of dioxin molecules is conducted by the joint action of aerobic and anaerobic microorganisms.

Polychlorinated biphenyls (PCBs). PCBs, a family of over two hundred compounds, are characterized by extremely high toxicity among polychlorinated aromatic compounds. Due to their high flame resistance, PCBs are used as anti-flash additives in electronics, printing equipment, transformer capacitors, packing materials, and as plasticizers in plastics, and as liquid thermofors components of many technical oils.



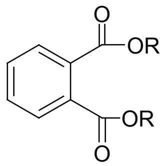
PCBs. Aroclor–1254 has x + y=5, for example

Polycyclic aromatic hydrocarbons (PAHs). As with PCBs, the aromatic hydrocarbons contain condensed rings. PAHs are almost insoluble in water, have high boiling points and are difficult to decompose [6].



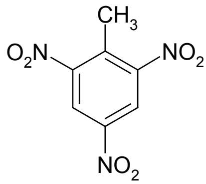
All these compounds have at least one re-entrant cavity (marked by arrows) in their molecular structure. This feature is a characteristic for many carcinogenic compounds. No reliable information on PAHs release on the industrial scale is available. Compounds of this class are formed during combustion, and, many natural products contain them. PAHs can be found in pitches, bitumen, soot, and humus components of soil.

Phthalates. Phthalates, esters of phthalic acid, are another group of aromatic ring– containing toxic compounds. These esters are used as water softeners in the production of polyvinyl chloride and other polymeric materials. Esters of phthalic acid are widely used in the production of solvents, lubricants, pesticides, lacquers and dyes, paper, perfumery, etc. [162].



Ester of phthalic acid

2, 4, 6-Trinitrotoluene (TNT). A very toxic contaminant, TNT is used as an explosive compound and intermediate in the production of dyes and photographic materials.

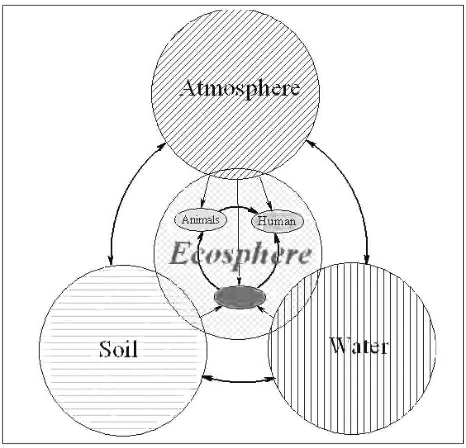


2, 4, 6-Trinitrotoluene

The production and use of TNT for military purposes has led to its wide distribution. TNT is one of the most toxic explosives in the military arsenal, and has contaminated thousands hectares of ground. TNT mobility in soil is limited due to its strong adsorption on soil particles.

Other contaminants: Chlorinated alkanes and alkenes; Benzene and its homologues; Heavy metals; Arsenic; Lead; Mercury; Cadmium; Gaseous contaminants: Carbon monoxide (CO); Carbon dioxide (CO_2); Sulphur dioxide (SO_2); Nitrogen oxides (NO_x); etc.

The circulation of toxicants in the ecosphere can be presented by the following informative scheme (Picture 1.).



Picture 1. The circulation of contaminants in the ecosphere [14]

Migration of contaminants is first of all the tendency of compounds of different structures and molecular masses to be dispersed in the environment. It is a very complicated multistage process controlled by many physical, chemical and biological mechanisms and factors, in particular:

- Basic physical-chemical characteristics of substances such as molecular mass, water solubility, "hydrophobicity" (calculated as the coefficient of substrate partitioning between nonpolar and polar solvents – typically *n*-octanol and water – designated as K_{ow}); vapour pressure (the determinant of substrate volatility); the presence of reactive functional groups, etc.
- Physical processes of mass transfer of substances, such as adsorption, desorption, diffusion, impedance, convection, dispersion, dry and wet precipitation, etc.
- Such chemical processes as oxidation, hydrolysis, photolysis, conjugation of toxic compounds or their derivatives with natural raw materials, etc.
- Geographical processes of substance circulation such as wind, precipitation, ocean flow, river transportation, etc.
- Biological activities of organisms participating in the global processes of substance circulation in nature – bioconcentration, biomultiplication, bioaccumulation, biotransformation, biodegradation, biotic transportation of substances, etc.

The total substance transport velocity characterizing substance volatility is equal to [7]:

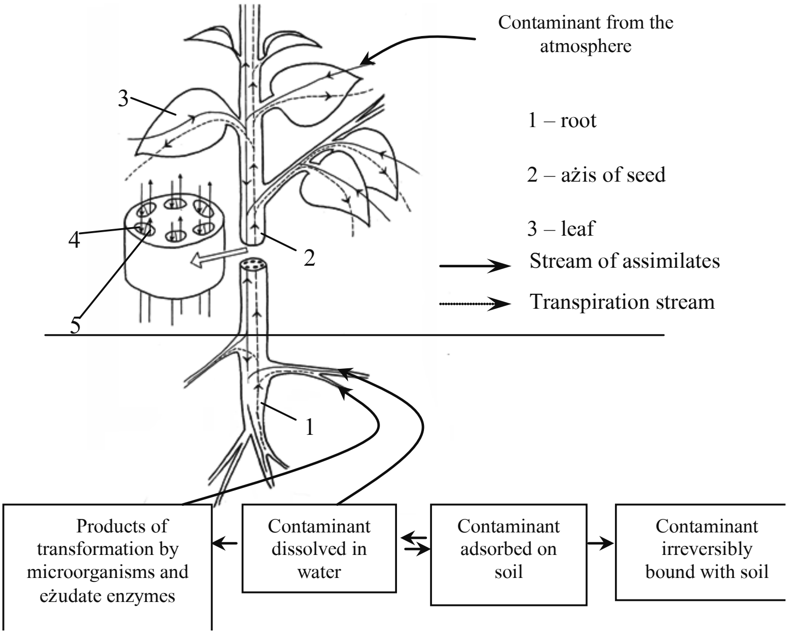
$$K = \left[\frac{1}{k_1} + \frac{RT}{k_g H} \right]^{-1}$$

K is the total velocity of substance flow, *R* is the universal gas constant, *T* the absolute temperature, k_1 the substance transport velocity in liquid phase, k_g the substance transport velocity in the gas phase and *H* is Henry's constant. The accumulation of contaminants in any part of biosphere is resulted in formation of: Smog; Oil contamination; Soil contamination; Chemical accidents.

The constantly increasing level of toxic compounds has a rather negative effect on nature, especially on such vitally important biological processes as respiration, photosynthesis, fixation of molecular nitrogen, reproduction, etc. High concentrations of environmental contaminants may, via mutagenesis lead to the destruction of some particular living species and the creation of new ones, which often degenerate and weaken.

Environmental contaminants enter plant cells from air, soil and water. Plants absorb contaminants primarily through their roots and leaves. Contaminants get into leaves as a result of the direct spraying of plants with agrochemicals and by absorption of gaseous contaminants existing in the air. Below ground, foreign compounds penetrate in plants together with water and nutrients by the roots. Chemical compounds are absorbed by roots less selectively than by leaves. The physiology of the processes of toxic compound absorption by leaves and roots differ essentially from each other. Compounds penetrated into the cell became substrates for enzymes able to transform them. The ability of the plants to permit contaminants of definite structure, lipophilicity, molecular mass and charge to enter in their intercellular space are those initial characteristics that also determine the detoxification potential of plants.

Summarizes the pathways of penetration and translocation of toxic compounds in plants.



Picture 2. Ways of environmental contaminants penetration and translocation in plants

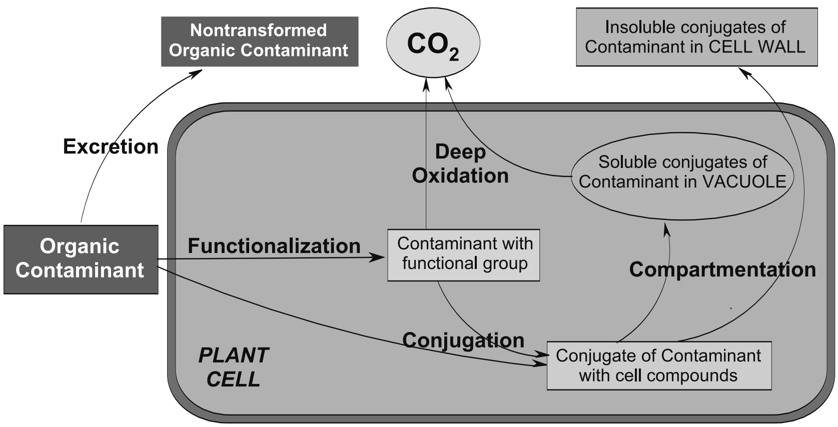
Initially absorbed by roots and leaves, contaminants are distributed into the cells of all organs of the plants by the transpiration stream and assimilate flows and deeply degraded (to CO_2) or accumulated in plant cell vacuoles and intercellular space. Due to this ability plants are already used in different phytoremediation technologies. However for the full realization of ecological potential, the enzymes of plant origin causing deep degradation of organic contaminants to carbon dioxide and by this way returning carbon atoms to natural circulation processes should be carefully analyzed. The mechanisms of the induction of these enzymes and the intracellular regulation of their activities seem to be especially promising avenues of further investigation and wide application. Another important characteristic for phytoremediation aims is resistance of plant cells to environmental contaminants of different structure.

The plant's abilities to absorb, deposit (conjugate), and deeply degrade pollutants, and to mineralize organic and to accumulate inorganic pollutants, within its cells determines the ecological potential of the plant. These abilities are the main technological parameters determining the application of plants in novel phytoremediation technologies. To investigate the ecological potential of plants, in addition to ascertaining their physiological and biochemical characteristics, studies at the level of cell ultrastructure are also very important. Transmission and scanning electron microscopy, in combination with autoradiographic methods, with well-developed techniques of fixation of plant tissues and getting ultrathin sections, allows the deleterious effects of toxic contaminants to be revealed at the ultrastructural level, and the fate of toxicants in the plant cell to be followed. Organic contaminants are absorbed by plants from the air and the soil after being dissolved in water and taken up together with nutritional compounds [1, 8]. Thus, it is evident that after contaminants penetration into the plant cell, structure-function changes take place as a result of contaminant toxicity and of cell reorganization directed to pollutant detoxification.

Nowadays there are many experimental data obviously demonstrating that plants are able to activate a definite set of biochemical and physiological processes to resist the toxic action of environmental contaminants, namely:

- Excretion.
- Conjugation of environmental contaminants with intracellular compounds and further compartmentation of conjugates.
- Degradation of environmental contaminants to standard cell metabolites, and finally to carbon dioxide.

Plants totally or partially detoxify environmental organic contaminants entering their cells. As has been already noted, these features allow plants to be used in several well adapted field technologies for the removal of, or at least a significant decrease the amount of contaminants [9].



Picture 3. The main pathways of organic contaminant transformation in plant cells

In the majority of cases, environmental pollutants absorbed by plants penetrate into the cells where they are subjected to enzymatic transformations leading to the decrease of their toxicity.

Phase I: functionalization is a process whereby a molecule of a hydrophobic organic contaminant acquires hydrophilic functional group (hydroxyl, amino, carboxyl, etc.) as a result of enzymatic transformations (oxidation, reduction, hydrolysis, etc.). Due to the functional group the polarity and reactivity of the toxicant molecule is enhanced. **Phase II: conjugation** is a process when a contaminant is chemically coupled to cell endogenous compounds (proteins, peptides, amino acids, organic acids, mono-, oligo- and polysaccharides, lignin, etc.) by formation of peptide, ether, ester, thioether or other bonds of a covalent nature. **Phase III: compartmentation** is in most cases the final step of conjugate processing. In this phase temporary (short or long term) 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 vacuoles, while insoluble conjugates (coupled with protein, lignin, starch, pectin, cellulose, xylan and other polysaccharides) are moved out of the cell via exocytosis and are accumulated in the apoplast or cell wall [10].

Excretion. The term "excretion" in plants implies the partial release of environmental contaminants absorbed by the plant in unchanged form through the leaves or the root system [1, 11].

Conjugation with endogenous compounds. Detoxification via conjugation is one of the defense mechanisms of higher plants. The toxicity of conjugates if compared with the toxicity of initial forms of toxic compounds is significantly decreased because of binding with non-toxic cellular compounds. Obviously, in such form, conjugates are kept in a cell for a definite interval without causing any pathological deviation in cell homeostasis. Conjugation of toxic compounds penetrated into the plant cell takes place with: carbohydrates; amino acids; peptides, proteins, lignin and hemicellulose.

Degradation pathways. One the most desirable ecological feature of plants is their potential to carry out deep degradation (oxidation) of environmental organic contaminants. Such transformations lead to the partial decomposition of the carbon skeletons of toxic molecules, to regular cell metabolites, or to mineralization to CO_2 and further participation of carbon atoms in characteristic natural cycles (plant cell processes). Albeit depending on the variety of the plant, the nature of the contaminant and its concentration, nevertheless a relatively small proportion of the environmental contaminant penetrating into the plant cell undergoes deep oxidation. At high contaminant concentrations most of the contaminant, that finds its way into the cell, is conjugated and deposited inside the cell. Ultimately it is released from the sites of deposition and degraded. Conjugation thus allows the plant to defer the possible energetically costly degradation process until it is in a stress free condition and can afford to expend the necessary resources on the task.

The decomposition of organic contaminants in plant cells mainly is carried out by oxidative enzymes. Basically, this process consists of several consecutive stages and terminates by the release of CO_2 (sometimes only in trace amounts however). Since carbon dioxide is considered to be an inorganic compound, the conversion of the organic pollutant into CO_2 is known as the process of mineralization. The verification of the concept of phytotransformation has been confirmed at different scales: in laboratory, greenhouse and field experiments.

1. THE ECOLOGICAL IMPORTANCE OF PLANTS

In order to properly understand the ecological power of plants and evaluate their detoxification potentials, the anatomical-morphological and physiological-biochemical particularities of plants responsible for establishing the basis for their action as environmental protectors and remediators should be emphasized:

- Higher plants contact with soil and water through roots and with the air by leaves, so they interact simultaneously with two different environments.
- Soil-plant-microbial interactions engender unique processes influencing the overall plant metabolism as well as transformations of contaminants.
- Highly developed root systems allow plants to control large areas of soil at different depths and create micro conditions convenient for the multiplication of microorganisms in the rhizosphere with the help of exudates.
- The large surface of plant leaves permits absorption of pollutants from the air via the cuticle (lipophilic compounds) and stomata (gases).
- A well developed internal transportation system for nutrients works in both directions, allowing environmental contaminants to be distributed throughout the entire plant.
- Plant-microbial interaction creates a microenvironment resulting in the concentration and penetration of contaminants at and into the roots.
- The autonomous synthesis of vitally important organic compounds by means of photosynthesis requires primary ammonia (via uptake of nitrate or ammonia from the soil, or in the case of leguminous plants as a result of symbiotic nitrogen fixation). This process is important since remediation of polluted sites requires additional metabolic force especially in the case of prolonged contacts with contaminants.
- Plants contain the apparatus required for the full set of biochemical and physiological processes of detoxification, and are sufficient no need for additional non-plant-microorganism-based technological help.
- Plant ecological potential is directed to remove contaminants from the environment. The purposeful application of plants can be of long-term or of short-term advantage, depending on the goal of the operation. For phytoremediation, long-term application of the plantation system to exploit and amortize their potential and maintain their continued effectiveness is recommended. Monitoring should follow short-term cleanup technology. Essentials of monitoring whose results are used for plant selection include the following: elements, constituents or other parameters to be monitored; frequency and duration of monitoring; monitoring and sampling methods; monitoring of locations; and

quality control requirements [13]. Sometimes, depending on the time available for remediation processes, could be too prolonged in time and hence not be acceptable. All phytoremediation technologies depend on the rate of growth of the plants and when these are characterized by seasonal activity this usually limits their application efficiency. Probably several growing seasons are needed for plants to attain the effective age of maturity for optimal phytoremediation.

Plants are very promising detoxifiers allowing the creation of safe technologies around or along hotbeds of contamination (Green filter, Vegetation cap, Phytoremediation cover, Hydrologic control, Evapotranspiration cover or any other plant–based technology) – ecologically friendly, and definitely positive, and of significant local importance [15]. Since there is no universal ecotechnology to clean up all kinds of contamination, phytoremediation solely or in combination with other convenient types of technologies should be considered as the best solution of the problem. Elaboration of a new ecological concept, unifying experience accumulated for the last three decades in particular and the realization of new plant-based approaches on a world scale should lead to a beneficial increase of the ecological potential of the whole planet.

CONCLUSION

Creation of a good foundation for the realization of a new, ecological concept, namely "Plants for a healthy planet", requires additional activities necessary for the world-scale realization of the concept. To realize in practice such a project, among the most important aims that should be considered are the following:

- Investigations needed for the detailed biochemical and physiological analysis of the whole process of phytoremediation – a group of innovative technological approaches (detailed characterization of all types of oxidases catalyzing degradation of organic contaminants; transferases and other enzymes participating in detoxification process etc. should be continued; creation of new, modified, genetically stable, environmentally safe, highly effective vegetation (growing under different climatic conditions: trees, grasses, legumes, terrestrial or aquatic plants) and selection of microorganisms (bacteria, fungi, actinomycetes), with a special attention to symbiotic action; scaling up of phytoremediation processes.
- International recognition and worldwide support for and realization of a new ecological concept (international cooperation, including financing; involvement of such international organizations as: NATO, UNESCO, U. S. Environmental Protection Agency (EPA), European Environmental Agency (EEA), United Nations Environment Program (UNEP), and National and International Green Movement, Greenpeace, Global Greens, etc.
- Organization of scientific branches, responsible for the establishment of a world pollution and remediation map and the creation of corresponding mathematical models of existing hotbeds, contaminated sites, distribution of contaminants and creation of approaches for their remediation, etc.

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