Metabolism of ^{14}C-Containing Contaminants in Plants and Microorganisms

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Abstract The most important researches of organic contaminants metabolism in plants and microorganisms using ^{14}C-labeled compounds are reviewed. The data that indicate on biodegradation and full detoxification (mineralization) of organic contaminants, such as aliphatic and monoaromatic hydrocarbons and their derivatives, polycyclic aromatic hydrocarbons, organochlorine pollutants, and, 2,4,6-trinitrotoluene, in microorganisms and to lesser extent in plants are presented.

Keywords Phytoremediation · Mineralization · Aliphatic hydrocarbons · Monoaromatic hydrocarbons · Polycyclic aromatic hydrocarbons · Organochlorine contaminants

Contents

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1 Introduction

Radiocarbon, ^{14}C is the natural radioactive isotope of carbon (half-life of 5,730 years), which is formed in the upper layers of the atmosphere as a result of cosmic rays action. After production, the ^{14}C atoms react to form ^{14}CO, which subsequently oxidizes to $^{14}CO_2$. The gas mixes rapidly and becomes evenly distributed throughout the atmosphere. Radioactive carbon dioxide also dissolves in water and thus permeates the oceans, but at a slower rate (Ramsey 2008).

Radiocarbon is beta-emitter but ^{14}C itself does not represent a pollutant, as its natural content in compounds is very low (natural abundance is 1 part per trillion). Labeled with ^{14}C organic pollutants are widely used in phytoremediation researches, as ^{14}C presence allows to follow pollutants metabolism in plants, by means of autoradiography monitor their translocation in plant organs and distribution among tissues and separate ultracellular organelles. Application of ^{14}C-labeled compounds gives the most trustworthy and convincing data on whether full detoxification (mineralization) of organic pollutant takes place or not during the process of phytoremediation.

The chapter describes the most important data obtained as a result of study of ^{14}C-labeled organic pollutants metabolism in plants and microorganisms.

2 Metabolism of ^{14}C-Labeled Aliphatic and Monoaromatic Hydrocarbons and their Derivatives

As a result of incubation of numerous plants (55 representatives of annual and perennial plants) with ^{14}C-labeled carbohydrates, it was demonstrated that all of them uptake and metabolize alkanes and arenes with different intensities (Durmishidze and Ugrekhelidze 1975; Durmishidze et al. 1974a, b). Benzo[a]-pyrene and benz[a]anthracene are actively uptaken, transported, and metabolized by plants (Devdariani 1988; Devdariani and Durmishidze 1983; Devdariani and Kavtaradze 1979a, b; Devdariani et al. 1979a, b; Müller 1976). The products of transformation of hydrocarbons uptaken by leaves flow along the stem to the roots and from the roots the intermediates of absorbed and metabolized hydrocarbons migrate to leaves (Korte et al. 2000).

By using labeled with ^{14}C compounds, it has been shown that ryegrass (*Lolium perenne* L.) has the capacity of taking up radioactive C_1–C_6 and C_8 monatomic alcohols, benzyl alcohol, C_1–C_2 aldehydes, acetone, acetaldehyde, C_1–C_6 monocarbonic acids, acetoacetic acid, acetic anhydride, cyclohexane, benzene, toluene, phenol, α-naphthol, naphthalene, and chloroform from the air by leaves (Durmishidze and Beriashvili 1979). It has been established that these compounds undergo oxidative degradation in ryegrass leaves, resulting with destruction of carbon skeleton, cleaving cyclic and bicyclic rings, and labeled carbon atoms of tested xenobiotics are incorporated into the common metabolites of the cell such

as: sugars (glucose, fructose, sucrose, raffinose), organic acids (malic, succinic, fumaric, citric, glycolic, glyoxylic, malonic, muconic, and other), amino acids (threonine, serine, glutamic and aspartic acids, lysine, alanine, phenylalanine valine, tryptophan, methionine, leucine, proline, glycine, etc.), and biopolymers. The authors suggest that as a result of hydroxylation, decarboxylation, and other transformation of xenobiotics intracellular concentration of $^{14}CO_2$ increased that induce photosynthetic formation of sugars and other intracellular compounds.

The experiments with ^{14}C-labeled hydrocarbons proved that sterile plant seedlings, placed in an atmosphere containing low-molecular mass alkanes (C_1–C_5) or cyclohexane, uptake these compounds, exposing their molecules to deep oxidative transformations to $^{14}CO_2$ (Durmishidze and Ugrekhelidze 1967, 1968a, 1975). In a plant cell, these hydrocarbons are oxidized and form the corresponding carbonic acids. On the basis of identified intermediate products, it was concluded that these alkanes undergo monoterminal oxidation, with formation of the corresponding primary alcohols, followed by oxidation to carboxyl acids, while cyclohexane oxidizes via ring cleavage (Korte et al. 2000; Ugrekhelidze 1976). The emission of $^{14}CO_2$ in the dark during this process serves as evidence for the occurrence of mineralization and can be easily measured (depending on the time of exposure, the percent of mineralization was found to be as high as 30 %). Consequently, organic and amino acids are among the end products of this transformation, and they can be used for further cell metabolism (Penner and Early 1973).

The transformation of [^{14}C] methane in higher plant cells is carried out by primary hydroxylation and successive forming of methanol, formaldehyde, and formic acid (Kvesitadze et al. 2006; Ugrekhelidze 1976). The oxidation of [1,2-^{14}C] ethane at one-terminal carbon atom leads to the formation of acetyl-CoA, which in turn is able to participate in the Krebs cycle. Concerning hypothesis of monoterminal oxidation of ethane: if ethane was oxidized at both terminal carbon atoms, instead of one, the carbon atoms originating from ethane would be incorporated into glycolic, glyoxalic or oxalic acids, but the ^{14}C carbon atoms originating from ethane are incorporated basically into succinic and fumaric acids (Ugrekhelidze 1976).

Based on the identified low-molecular mass degradation ^{14}C-products (succinic, fumaric, malonic, malic, and citric acids), which are formed during mineralization of [1–3-^{14}C] propane to $^{14}CO_2$ by plants, it is suggested that propane is also oxidized monoterminally. The primary oxidation of propane at one-terminal carbon atom leads to the formation of [1–3-^{14}C] propionic acid. This intermediate consequently transforms to malonyl-CoA as a result of β-oxidation. Last product undergoes decarboxylation resulting in the formation of acetyl-CoA (Kvesitadze et al. 2006; Ugrekhelidze 1976). Acetyl-CoA is transferred to carboxyl groups of succinic acid that can be incorporated into the Krebs cycle.

The presence of [^{14}C] fumaric, glycolic, and malic acids and the absence of labeled γ-isobutyric and succinic acids among radioactive intermediates forming as a result transformation of [1–4-^{14}C] butane indicate on monoterminal mechanism of butane mineralization in plants (Ugrekhelidze 1976). The incorporation of butane carbon skeleton in Krebs cycle is possible via β-oxidation of formed butyric

acid to C_2-acids, e.g., to glycolic acid, identified as one of radioactive metabolite of butane.

The tea (*Thea sinensis*) seedlings, grown in an atmosphere containing [1–5-^{14}C] pentane, release $^{14}CO_2$ (approximately 30 % from total radioactivity) after being transferred to a pentane-free atmosphere. The radioactive label of absorbed pentane is observed in the low-molecular mass compounds. Among them, nonvolatile organic acids (fumaric and succinic acids) and amino acids (alanine and glutamic acid) were identified, but the sugar fraction remained nonradioactive. The radioactive label was almost equally inserted in organic acids (fumaric and succinic acids) and amino acids (the most radioactivity was found in alanine). Absence of the labeled carbon atoms in the sugars can be explained by the fact that being in the dark, photosynthesis and, correspondingly, the biosynthesis of sugar does not take place. The presence of the carbon label of pentane in components of the Krebs cycle indicates that [1–5-^{14}C] pentane metabolism in the plant cells proceeds via monoterminal oxidation as well as in case of C_1–C_4 alkanes, but oxidation of butane leads to forming of valeric acid and its successive conversion to acetyl-CoA, which can be inserted into the Krebs cycle (Durmishidze and Ugrekhelidze 1968b; Ugrekhelidze 1976; Ugrekhelidze and Durmishidze 1984; Varazashvili and Pruidze 2005). The above described transformation of pentane can be sketched in Fig. 1.

Obviously, the longchain alkanes also are subjected to oxidative transformations in plants and the steps of their degradation are similar to conversion of short chain alkanes. For instance, after 40 min of incubation of leek (*Allium porum* L.), 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 (Cassagne and Lessire 1975).

The evolution of $^{14}CO_2$ during incubation of plants with [1-^{14}C] cyclohexane indicates that the ring of this hydrocarbon is cleaved with the formation of aliphatic products. The major component among them is succinic acid (Ugrekhelidze 1976). The incorporation of radioactive carbon atoms in tyrosine and phenylalanine is also observed. It was supposed that in plants the first step of cyclohexane transformation is its hydroxylation into cyclohexanol. The inferred scheme of cyclohexane metabolism in plants is in Fig. 2.

As is seen from the scheme (Fig. 2), in the initial stage, cyclohexane undergoes oxidation to unsaturated cyclic intermediates with oxo- or hydroxyl-groups. In the next stage of metabolism, these intermediates form cyclohexene-3-diol-1,2, which is oxidized with cleavage of carbonic cycle and is transformed into adipinic acid. This metabolite can form fumaric acid and thus, incorporates into general metabolism of organic acids in plant cell.

The experiments with using ring-labeled arenes (^{14}C-benzene and ^{14}C-toluene) and avocado (*Persea americana*) fruit demonstrate that hydrocarbons are transformed to a series of compounds, toluene to a greater extent than benzene. After 4 h of exposure time, both arenes formed a little amount of $^{14}CO_2$ (Jansen and Olson 1969). It have been established that vapors of [1–6-^{14}C] benzene and [1-^{14}C] toluene penetrated in plants (maple (*Acer campestre*), apple (*Malus domestica*) and

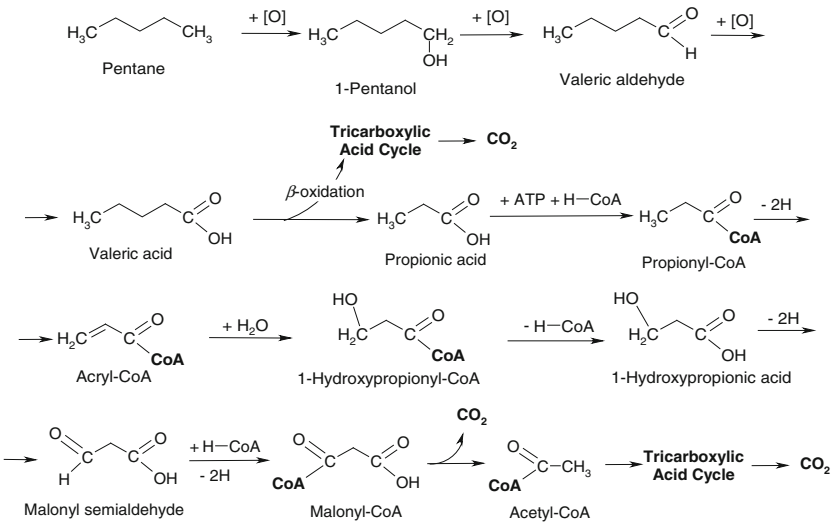

Fig. 1 Hypothetic pathway of pentane metabolism in the higher plant cells

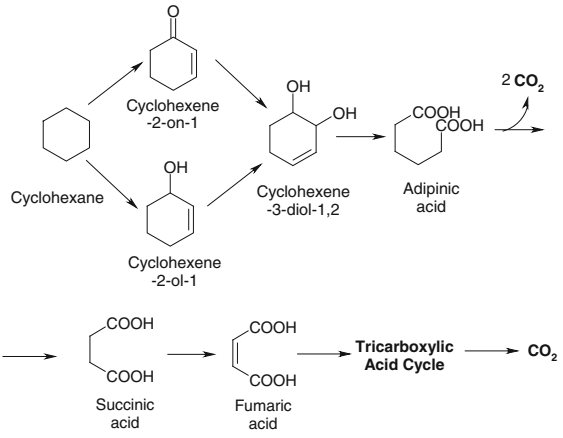

Fig. 2 Oxidative degradation of cyclohexane in plants

vine (*Vitis vinifera*)) through hypostomatous leaves from both sides, whereas hydrocarbons are more intensively up taken by the stomatiferous side and more actively taken up by young leaves (Kvesitadze et al. 2006; Ugrekhelidze et al. 1997). Transformation of these radioactive arenes in leaves includes the aromatic ring cleavage and labeled carbon atoms are mainly incorporated into nonvolatile organic acids (basically into muconic and fumaric acids in case of benzene, and into muconic and malic acids in case of toluene), while their incorporation into amino acids (particularly, into tyrosine and phenylalanine in case of benzene, and into tyrosine and aspartic acid in case of toluene) is less intensive. It has been shown that intact spinach (*Spinacia oleracea*) leaves mineralize the absorbed [1–6-^{14}C] benzene and

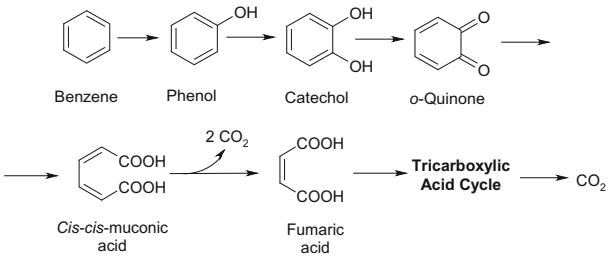

Fig. 3 Oxidative degradation of benzene in plant cells according to Kvesitadze et al. (2006)

[1-^{14}C] toluene to $^{14}CO_2$, and this process is strongly stimulated in light. Oxidation of [1–6-^{14}C] benzene by spinach chloroplasts or by enzyme preparation from spinach leaves is almost completely inhibited by chelating of copper ions and slightly by chelating of iron ions. Benzene oxidation by enzyme preparation is significantly stimulated by NADH and NADPH; in their presence, [1–6-^{14}C] phenol, as hydroxylation product of the benzene, is formed. It is worth noting that the labeled phenol was isolated from sterile seedlings of maize, pea, and pumpkin incubated on the solution of [1–6-^{14}C] benzene.The phenol was present in tissues in negligible amounts, though the degree of benzene label incorporation into aliphatic products was much higher (Korte et al. 2000; Ugrekhelidze et al. 1977). It is supposed that the first step of oxidative transformation of benzene in plant leaves is hydroxylation via enzyme containing copper as the prosthetic group (Ugrekhelidze et al. 1997). Further, oxidation of phenol in catechol leads to the aromatic ring cleavage and formation of muconic acid, which subsequently transforms into fumaric acid. The last easily incorporates in tricarboxylic acid cycle, and as a result, full mineralization of aromatic hydrocarbons to carbon dioxide takes place (see scheme in Fig. 3) (Durmishidze et al. 1969, 1974a, b, c). These dicarbonic acids are often found in plants exposed to benzene or phenol. Such cleavage of the aromatic ring is typical for endogenous substrates (Tateoka 1970). If toluene degradation performs via similar pathway, labeled α-methylmuconic acid will be identified among the intermediates of [1-^{14}C] toluene transformation in plants. However, such compound, as well as the benzoic acid were not identified (Ugrekhelidze 1976).

It has been demonstrated that [1–6-^{14}C] phenol (hydroxybenzene) penetrated through the roots of mung bean (*Vigna radiata*) and wheat seedlings is coupled to low-molecular-weight peptides, forming phenol–peptide conjugates (Chrikishvilli et al. 2005; Ugrekhelidze et al. 1999). The part of [1–6-^{14}C] phenol absorbed by higher plants is detoxified by oxidative cleavage of the aromatic ring. Thus, the sterile plants absorbing $^{14}C_6H_5OH$ are able to release $^{14}CO_2$ after exposure to darkness; in addition, in tissues of these plants labeled muconic acid and fumaric acid were found (Ugrekhelidze 1976; Ugrekhelidze et al. 1999). Rather specific products of conjugation are produced from pentachlorophenol in soybean (*Glycine max*) and wheat (*Triticum aestivum*) (Sandermann et al. 1991; Schmitt et al. 1985). In these plants, pentachlorophenol is transformed into *β*-D-glycosyl and *O*-malonyl*β*-D-glucosyl conjugates.

The investigations of the fate of different exogenous monatomic phenols in plants show that the main pathway of their transformations is conjugation with the low-molecular mass peptides. For instance, [1-^{14}C] *o*-nitrophenol, [1-^{14}C] 2,4-dinitrophenol, and [1-^{14}C] α-naphthol, after penetration into sterile pea (*Pisum sativum*) seedlings, bind with such peptides (Arziani et al. 1983, 2002). The part of these monatomic phenols is irreversibly bound to proteins via quinone–protein interaction and only a small part are transformed via aromatic ring cleavage. Monatomic phenol with high dissociation constant (dinitrophenol) causes a corresponding stimulation of peptide accumulation in plant tissues. It is also worth noting that in contrast to polyatomic phenols, which are metabolized in higher plants via glycosylation (Glass and Bohm 1971; Pridham 1964), the above mentioned monatomic phenols do not form corresponding glycosides in tissues of investigated plant.

Transformation of labeled benzene derivatives, [1–6-^{14}C] nitrobenzene, ([1–6-^{14}C] aniline, [1-^{14}C] and [7-^{14}C] benzoic acid, in axenic seedlings of maize (*Zea mays* L.), kidney bean (*Phaseolus vulgaris* L.), pea, and pumpkin (*Cucurbita pepo* L.) have been studied (Mithaishvili et al. 2005). After penetration in plants, these pollutants undergo oxidative or reductive transformations, which lead to the cleavage of aromatic ring or to the conjugation with peptides and biopolymers. Ring cleavage is accompanied by formation of labeled organic acids of the Krebs cycle and subsequent emission of $^{14}CO_2$. The analysis of conjugates has been shown that as a result of reduction or hydroxylation of [1–6-^{14}C] nitrobenzene, correspondingly, labeled aniline or *o*-nitrophenol is produced (*p*-nitrophenol was identified in trace amount). Labeled metabolites of [1–6-^{14}C] aniline are involved in the formation of peptide conjugates. Concerning [^{14}C] benzoic acid, this xenobiotic is directly linked to the amino groups of peptides at the expense of own carboxylic group. In other experiments with [1-^{14}C] and [7-^{14}C] benzoic acids, it has been established that after removal of the plants (sterile seedlings of maize and pea) from the labeled benzoic-acid-containing hydroponic medium, the amount of conjugation products gradually decreases and the process is accompanied by the emission of labeled carbon dioxide (Chrikishvili et al. 2006). This indicates that conjugates eventually release their toxic part unchanged, which further undergo mineralization by plant cell enzymes.

Although plants have the inherent ability to detoxify xenobiotics, they generally lack the catabolic pathway for the complete degradation of these compounds as compared to microorganisms (Abhilash et al. 2009). Due to their fast growing ability, much more easily regulated adaptation, fast inductive processes, and the wide spectrum of the enzymes participating in the degradation of organic xenobiotics, microorganisms are much more active detoxifiers when expressing their activity per unit of dry biomass (or in any other way) (Kvesitadze et al. 2006). Such advantages of microorganisms also are revealed in degradation of hydrocarbons. As it will be shown below, researches using ^{14}C-labeled compounds confirmed that microorganisms from different taxonomic groups have capabilities

to mineralize wide spectrum of aliphatic alkanes, cycloalkanes, arenes, and their derivatives in both aerobic and anaerobic conditions.

For instance, it has been demonstrated that strain *Rhodococcus* sp. EH831 isolated from the enriched hexane degrading bacterial consortium was able to mineralize approximately half part of the [1–6-^{14}C] hexane into $^{14}CO_2$ (Lee et al. 2010). Among intermediates, the oxidative degradation products of hexane such as alcohols, ketones, and aldehydes are identified.

Entomopathogenous fungi are able to transform [1-^{14}C] *n*-hexadecane into different lipid products, part of which is subsequently utilized for energy formation, and the complete catabolism of hexadecane ended by a significant release of $^{14}CO_2$ (Napolitano and Juárez 1997). The study of the ability of filamentous fungi to degrade crude oil components shows that [1-^{14}C] *n*-hexadecane is being mineralized, not simply transformed to intermediate metabolites (April et al. 1999). [1-^{14}C] Hexadecane was also anaerobically oxidized to $^{14}CO_2$. Molybdate, a specific inhibitor of sulfate reduction, inhibited the hexadecane oxidation (Coates et al. 1996). These results demonstrate that a wide variety of hydrocarbon contaminants can be degraded under sulfate-reducing conditions in hydrocarbon-contaminated sediments.

In some cases, ^{14}C-labeled components of oil hydrocarbons were used for determination of remediation potential of microorganisms. For instance, the rate of utilization of [1-^{14}C] hexadecane was used for estimating the hydrocarbon-degrading potential of bacteria (Walker and Colwell 1976). In experiments, conducted for estimation of aerobic biodegradation potential of the microbial community, contaminated sediments were incubated with ^{14}C-labeled organic compounds (among them [1–6-^{14}C] benzene and [1–6-^{14}C] toluene), and the evolution of $^{14}CO_2$ was measured over time (Aelion and Bradley 1991).

It has been established that the enrichment of halophilic and halotolerant bacteria completely degraded labeled benzene, toluene, ethylbenzene, and xylenes within 1–2 weeks under aerobic conditions. Community structure analysis revealed that *Marinobacter* spp. were the dominant members of the enrichment (Nicholson and Fathpure 2004). Bacterial community from hypersaline soil samples has the capability to use of [1–6-^{14}C] benzene as the sole carbon and energy source, and herewith, 1/3 of the assimilated [1–6-^{14}C] benzene was converted to $^{14}CO_2$ (Nicholson and Fathpure 2005).

Studies with labeled [1–6-^{14}C] benzene and [1–6-^{14}C] toluene showed substantial mineralization of these compounds to $^{14}CO_2$ by the lignin-degrading basidiomycete *Phanerochaete chrysosporium* (Yadav and Reddy 1993a). *P. chrysosporium* was also shown to degrade monatomic phenols (simple phenol and *p*-cresol) (Kennes and Lema 1994). [^{14}C] Benzene can be mineralized in both aerobic and anaerobic conditions (Vogt et al. 2011). Aerobic microbial degradation of aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylenes occurs by means of preliminary hydroxylation and consequent cleavage of aromatic ring, similar to plants (Gibson and Parales 2000; Jindrová et al. 2002; Tao et al. 2004).

Under anaerobic conditions in methanogenic enrichment cultures, amended with ^{14}C-benzene degradation of this compound by formation of $^{14}CO_2$ and $^{14}CH_4$

was demonstrated (Grbic-Galic and Vogel 1987; Vogel and Grbic-Galic 1986). The mass balance showed that less than 6 % of ^{14}C-labeled benzene added was converted to $^{14}CO_2$. In other experiments, more than 90 % of ^{14}C-labeled benzene was mineralized to $^{14}CO_2$ by aquifer-derived microorganisms under strictly anaerobic conditions, by using sulfide-reduced mineral medium (Edwards and Grbic-Galic 1992). It has been demonstrated that the addition of sulfate stimulates anaerobic benzene degradation in methanogenic sediments (Weiner et al. 1998). In experiments with ^{14}C-labeled benzene, more than 90 % labeled carbon of the benzene was released as CO_2 (Burland and Edwards 1999). Complete mineralization of benzene to carbon dioxide and methane were significantly higher under methanogenic conditions (Kazumi et al. 1997; Ulrich and Edwards 2003).

Diverse strains of anaerobic bacteria have been isolated that degrade alkylbenzenes anaerobically, using nitrate, iron(III), or sulfate as electron acceptors (Spormann and Widdel 2000). It has been shown that the denitrifier strain *Azoarcustolulyticus* Tol-4 is capable to anaerobic mineralization of [1–6-^{14}C] toluene: 68 % of ^{14}C was found in carbon dioxide and 30 % in biomass (Chee-Sanford et al. 1996).

Anaerobically, in presence of nitrates, *Dechloromonas* strain RCB completely degrades [1–6-^{14}C] benzene (concentration 160 μM) to $^{14}CO_2$ within 5 days (Coates et al. 2001). This process is nitrate-dependent and involves an initial hydroxylation, subsequent carboxylation, and loss of the hydroxyl group to form benzoate (Chakraborty and Coates 2005). According to suggestions of the authors, all anaerobic benzene degrading microorganisms, regardless of their terminal electron acceptor carry out benzene degradation by this pathway. In addition to nitrate, strain RCB could alternatively degrade benzene both aerobically and anaerobically with perchlorate or chlorate as a suitable electron acceptor (Chakraborty et al. 2005). Also, this strain was capable to anaerobically degrades other monoaromatic hydrocarbons, and toluene and ethylbenzene were completely mineralized to CO_2.

3 Metabolism of ^{14}C-Labeled PAHs

Because of wide spread occurrence and carcinogenic properties, PAH represent one of the most dangerous groups of environmental pollutants.

PAH represent one of the groups of environmental pollutants of great concern. The low solubility in water and the high chemical stability of polycondensed aromatic structure predetermine the recalcitrance of PAHs to biodegradation. Microbial degradation is being the primary route of mineralization of PAHs in soils (Rojo-Nieto and Perales-Vargas-Machuca 2012). However, it is not the only way of biotransformation of PAHs in the environment, since the great number of investigations with using ^{14}C-labeled PAHs demonstrate that higher plants have capacity to uptake and degrade these compounds.

Persistence of the PAHs increases as the number of condensed rings and also depends on the location of the rings in a molecule (Bezalel et al. 1996a, b).

Respectively, the simplest PAH naphthalene is more easily exposed to mineralization. The possibility of naphthalene degradation by bacterial strains under nitrate-reducing anaerobic condition has been confirmed by measuring mineralization of [^{14}C] naphthalene to $^{14}CO_2$ (Bregnard et al. 1996). Similar results are under sulfate-reducing conditions for [^{14}C] naphthalene and [^{14}C] phenanthrene (Coates et al. 1997). It has been demonstrated that microbial mineralization of [^{14}C] naphthalene to labeled carbon dioxide may be coupled to sulfate reduction in aquifer-derived sediments (Bedessem et al. 1997). As much as 66 % of labeled naphthalene was mineralized to $^{14}CO_2$ over 13 days, and molybdate inhibited the intensity of this process by 44 %. By using [^{14}C] naphthalene, it has been established that the abundance of the naphthalene dioxygenase gene *nahAc* was correlated with the aerobic naphthalene mineralization potential in oxic soil layer of petroleum hydrocarbon-contaminated sites (Tuomi et al. 2004).

Some plant species are distinguished by high ability to uptake PAHs from environment (Slaski et al. 2000). In early studies, it has been shown that the absorbed by roots of maize and kidney bean [1,2-^{14}C] benzo(a)pyrene was transported to leaves and some its part undergo biotransformation that was accompanied by the release of labeled carbon dioxide (Ugrekhelidze 1976). Radioactive carbon dioxide as the final product of the oxidation of [9-^{14}C] benzo(a)anthracene was also detected in herbaceous plants ryegrass and alfalfa (*Medicago glutinosa*) (Devdariani and Kavtaradze 1979a, b). Among the metabolites of [7,10-^{14}C] benzo(a)pyrene enzymatic oxidation-labeled 1,6-benzo(a)pyrene-quinone, 6,12-benzo(a)pyrene-quinone, 3,6-benzo(a)pyrene-quinone, 9,10-dihydrodiol-BP, 7,8-dihvdrodiol-benzo(a)pyrene, 4,5-dihydrodiol-benzo(a)pyrene and 3-hydroxo-benzo(a)pyrene are identified (Durmishidze et al. 1979b). In most cases, further oxidation of formed metabolites followed by ring cleavage. It has been also shown that separate organelles isolated from pea seedlings oxidize [7,10-^{14}C] benzo(a)pyrene to carbon dioxide (Devdariani et al. 1979b). Maize, kidney bean, and pumpkin revealed ability to cleavage of [3,4-^{14}C] benzo(a)pyrene B and C rings under sterile conditions by the formation of radioactive organic acids and amino acids and by emission of $^{14}CO_2$ (Durmishidze et al. 1979a). For such PAHs as naphthalene, pyrene, anthracene, and dibenzanthracene, the same kind of transformation is observed (Devdariani 1988). It is supposed that hydroxylation is the primary reaction in the transformation of polycyclic hydrocarbons in plants. The analogous transformation of PAHs was determined in cell suspension cultures (Harms 1975; Harms et al. 1977; Trenk and Sandermann 1978).

The extent of PAHs destruction in the environment is of high importance. It has been shown that partial degradation of PAHs in soil can become a reason of the groundwater pollution with toxic metabolites (Schmidt et al. 2010). Mineralization of labeled metabolites, produced by fungus *Cunninghamella elegans* from [^{14}C] phenanthrene, [^{14}C] fluoranthene, and [^{14}C] pyrene, was compared to mineralization of the parent [^{14}C] PAHs in soil slurries. It was supposed that reduction of lipophlicity and raising bioavailability of the metabolites compared to the parent PAHs would enhance the degree of their mineralization in soil slurries (Schmidt

et al. 2010). Unexpectedly, the mineralization of the labeled metabolites was in all cases extremely slow as compared to the mineralization of the parent [^{14}C] PAHs.

Comparing the PAHs-degradation abilities of the microorganisms from different taxonomic groups might be suggested that fungi are distinguished by greatest ability. It has been demonstrated that the white rot fungus *Pleurotus ostreatus* mineralized to labeled carbon dioxide 7.0 % of [^{14}C] catechol, 3.0 % of [^{14}C] phenanthrene, 0.4 % of [^{14}C] pyrene, and 0.19 % of [^{14}C] benzo(a)pyrene by day 11 of incubation. It also mineralized [^{14}C] anthracene (0.6 %) much more slowly (35 days) and [^{14}C] fluorene (0.19 %) within 15 days (Bezalel et al. 1996a, b). In other experiments, this fungal strain degraded [^{14}C] benzo(a)pyrene and 40 % of the compound was removed after one month of incubation. The mineralization degree (estimated by measuring of released $^{14}CO_2$) as compared to unsterile control soil without tested fungal strain increased from 0.1 to 1 % (Eggen and Majcherczyk 1998). Fungal strain *Stropharia coronilla* mineralized approximately 12 % of the added [^{14}C] benzo(a)pyrene in Mn^{2+} supplemented cultures within 6 weeks, whereas only 1 % was evolved as $^{14}CO_2$ in non-supplemented cultures (Steffen et al. 2003).

The increase of bioavailability promotes the biotransformation of PAHs. So, the effects of pig manure compost (PMC) and a nonionic surfactant Tween 80 on the transformation of [^{14}C] pyrene in a soil–plant system (*Agropyron elongatum*) have been studied (Cheng and Wong 2008). The results showed that the mineralization of [^{14}C] pyrene depends on the dissipation degree of PAH in vegetated soil, and the co-addition of Tween 80 and PMC could improve the intensity of both dissipation and mineralization processes.

Plant-microbial interaction is important factor for improving rhizodegradation of PAHs. Thus, plant root extracts of osage orange (*Maclura pomifera*), hybrid willow (*Salix alba* × *matsudana*), or kou (*Cordia subcordata*), or plant root exudates of white mulberry (*Morus alba*) supported 15–20 % benzo[a]pyrene removal over 24 h. Mineralization of [7-^{14}C] benzo(a)pyrene by *Sphingomonas yanoikuyae* JAR02 yielded in 0.2 to 0.3 % $^{14}CO_2$ when grown with plant root exudates (Rentz et al. 2005). Experiments with ^{14}C-labeled pyrene in a sand amended *Mycobacterium* strain KMS and barley plants showed that greater release of $^{14}CO_2$ was observed in the system with barley colonized by KMS than in microcosms containing just the bacterium inoculum or sterile barley plants (Child et al. 2007).

4 Metabolism of ^{14}C-Labeled Organochlorine Pollutants

The organochlorine pollutants are widely distributed and include industrial chemicals, solvents, pesticides, drugs, polychlorinated biphenyls, dioxins, etc. The content of chlorine atoms on the one hand determines the toxicity of these pollutants, and on the other hand defines their resistance to the complete detoxification via oxidative degradation. Therefore, the investigations by using labeled

organochlorine pollutants that assess the degree of mineralization of such compounds are very important to evaluate the effectiveness of various tools for remediation technologies.

The main pathway of [^{14}C] 2,4-dichlorophenoxyacetic acid (2,4-D) metabolism in plants is the formation of esters with glucose and malonyl residues (Feung et al. 1976; Sandermann 1987; Viana and Mantell 1998). For example, after penetration of [^{14}C] 2,4-D into the root cells of barley (*Hordeum vulgare*) seedlings the labeled conjugates were detected in the vacuoles and among these conjugates, 80 % was *O*-*β*-D-glycosides of the 2,4-D metabolites (Chkanikov 1985). Other literature data indicates on possibility of formation of some conjugates of 2,4-D metabolites with peptides (Durmishidze et al. 1982; Kakhniashvili 1988; Kakhniashvili et al. 1979). Furthermore, the authors reported that in maize, pea, and kidney bean, along with transformation of the side chain of 2,4-D, aromatic ring degradation takes place. The labeled acyclic organic acids, amino acids, sugars, and CO_2 as products formed from the [1-^{14}C] 2,4-D have been isolated and identified. However, the mineralization degree is low and equals to 1 %.

2,4-D is biodegradable in soils, while adsorption/desorption is influenced by both soil organic matter content and soil pH (Boivin et al. 2005). For example, the greatest mineralization (up to 30 %) of [1–6-^{14}C] 2,4-D occurred in sandy soils containing the least amount of organic carbon (Cycon et al. 2010). Microbial degradation of 2,4-D in soil involves hydroxylation, cleavage of the acid side-chain, decarboxylation, and ring opening (Tomlin 2006). The forming labeled 2,4-dichloroanisole and 2,4-dichlorophenol from ring-labeled [^{14}C] 2,4-D in the soil indicates that hydrolytic cleavage and decarboxylation are potential starting reactions in the transformation of 2,4-D by rhizospheric microorganisms (Smith 1985).

Fungi, especially basidiomycetes, are distinguished by high ability of complete destructin of 2,4-D. Extensive mineralization of ^{14}C-labeled 2,4-D by white rot basidiomycetes *P. chrysosporium* and *Dichomitus squalens* has been demonstrated in liquid media (Reddy et al. 1997; Yadav and Reddy 1992, 1993b). Fungal biotransformation of 2,4-D involve an initial ether cleavage resulting in the formation of 2,4-dichlorophenol and acetate. Further, degradation of chlorophenol intermediate is carried out by ligninolytic peroxidases that catalyze subsequent oxidative dechlorination to a benzoquinone metabolite followed by aromatic ring cleavage and finished by mineralization to $^{14}CO_2$. These microorganisms can degrade polyhalogenated hydrocarbons, which hardly undertake degradation by plants. For instance, as a result of biotransformation of the insecticide lindane (1,2,3,4,5,6-hexachlorocyclohexane) by the white rot fungi *P. chrysosporium*, the polar metabolites such as tetrachlorocyclohexane, tetrachlorocyclohexane epoxide, and tetrachlorocyclohexenol, as well as carbon dioxide are identified (Mougin et al. 1996). Similar metabolites are found among intermediates when lindane undergoes degradation by other fungal strains, e.g., *Trametes hirsutus, Cyathus buller,* and *Phanerochaete sordida* (Singh and Kuhad 1999, 2000). According to these results, hypothetic scheme of lindane mineralization has been proposed (see Fig. 4).

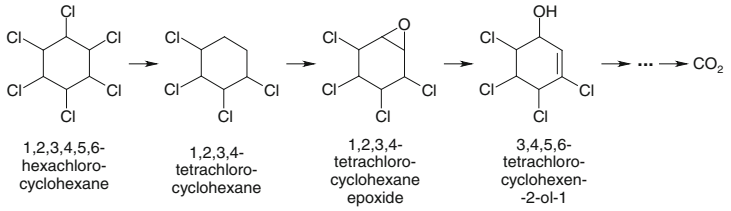

Fig. 4 Hypothetical pathway of lindane degradation by white rot fungi according to Marco-Urrea and Reddy (2012)

The widely used degreasing solvent such as trichloroethylene (TCE) is problematic environmental pollutant due to its chemical stability and toxicity for living organisms. Studies that used [^{14}C] TCE have revealed biological tools for effective removing this pollutant from environment. It has been demonstrated that TCE is mineralized by lignin peroxidase of *P. chrysosporium* cultures grown aerobically (Khindaria et al. 1995). Later research showed that most of using [^{14}C] TCE as the substrate for *P. chrysosporium* undergoes total degradation to $^{14}CO_2$ (Yadav et al. 2000). The plants can significantly enhance TCE mineralization by indigenous microorganisms in soil. It has been shown that plant species bahiagrass (*Paspalum notatum*), Chinese bushclover (*Lespedeza cuneata*), loblolly pine (*Pinus teada*), and goldenrods (*Solidago* sp.) significantly rise degradation level of TCE to carbon dioxide in rhizosphere (Walton and Anderson 1990). The presence of the broad-leaved cattail (*Typha latifolia*) resulted in increased production of labeled CO_2 from 3.2 to 5.3 % in wetland microcosms (Bankston et al. 2002).

Despite the fact that their production is currently prohibited, polichlorinated biphenyls (PCBs) remain to be one of the most problematic pollutants because they hardly undergo degradation. In general, the degree of mineralization of PCBs decreased with an increase in chlorine content. Thus, mineralization of [^{14}C] Aroclor-1242 (42 % chlorine by weight) by *P. chrysosporium* was about 20 %, while that of [^{14}C] Aroclor-1254 (54 % chlorine by weight) ranged from 10 to 14 % (Bumpus and Aust 1987; Eaton 1985; Yadav et al. 1995). Besides strains of *P. chrysosporium,* other fungal cultures are also characterized with capacity to degrade congeners of PCBs containing three and more chlorine atoms in molecule. For instance, *Trametes versicolor*, *Pleurotus ostreatus,* and *Bjerkanderaadusta* mineralize [^{14}C] 2,4,5-trichlorobiphenyl (Beaudette et al. 1998, 2000); *Phlebia brevispora*, as well as *P. chrysosporium* degrades labeled 3,3′,4,4′-tetrchloro-, 2,3′,4,4′,5-pentachloro- and 3,3′,4,4′,5,5′-hexachloro-biphenyls (Kamei et al. 2006).

The metabolism of PCB congeners in plants significantly depends on the plant species, degree of chlorination, and molecular configuration of PCBs (Wilken et al. 2009). In studies with [^{14}C] 2-chlorobiphenyl) in soybean cultures, one dihydroxylated and six different monohydroxylated compounds were detected among conjugates. Hydrolysis of metabolites of [^{14}C] 2,2′,5,5′-tetrachlorobiphenyl in wheat cell cultures yielded four monohydroxylated and three dihydroxylated metabolites.

Polychlorinated dibenzodioxins (PCDDs) are highly toxic and most difficultly degradable environmental pollutants. It has been established that PCDD, similar to PCBs, undergo degradation by several species of white rot fungi (Marco-Urrea and Reddy 2012). For example, *Phlebia lindtneri, Phlebia* sp. MG-60, and an unidentified white rot fungus degraded [^{14}C] 2,7-diCDD to a maximum extent of 6.5 % (Mori and Kondo 2002). *Phlebia* species are able to mineralize tri- and tetra- substituted dioxins, such as 2,3,7-triCDD (18.4–27 %), 1,2,8,9-tetraCDD (11.9–21.1 %), and 1,2,6,7-tetraCDD (14.2–21.5 %) (Kamei et al. 2005).

5 Metabolism of 2,4,6-trinitrotoluene Labeled with ^{14}C in Plants and Microorganisms

Labeled with ^{14}C pollutants were broadly applied for the study of TNT metabolism in plants and microorganisms. For example, the penetration and localization of [1-^{14}C] TNT in soybean plant cells (and maize) was studied via electron microscopic autoradiography. In soybean root cells, [1-^{14}C] TNT was detected as electron-dense label in cell walls, endoplasmic reticulum, mitochondria, plastids, nuclei, nucleolus, and vacuoles (Fig. 5) (Adamia et al. 2006; Kvesitadze et al. 2006); in leaves the label appeared primarily in cell walls, chloroplasts, and vacuoles (Fig. 6) (Adamia et al. 2006).

Attention should be paid to the localization of [1-^{14}C] TNT on membrane structures participating in the transport of reducing equivalents (membranes of the endoplasmic reticulum, mitochondria, and plastids). Supposedly, TNT transformation proceeds in these subcellular organelles.

The results of experiments using [1-^{14}C] TNT for studying the fate of absorbed TNT in soybean seedlings indicate on universal distribution of TNT-labeled carbon atom in low- and high-molecular mass compounds in roots and aboveground parts of plants. The data of these experiments prove ones again the high mobility of TNT and its metabolites in plants (Adamia et al. 2006). Content of labeled TNT and its metabolites among low-molecular compounds in plant roots are much higher than in above ground parts. On the contrary, labeled high-molecular compounds are more intensively in aboveground parts of soybean. It should be proposed that most part (up to 70 %) of metabolites of TNT is conjugated with biopolymers. The physical–chemical analysis of insoluble in 80 % ethanol high-molecular ^{14}C compounds fraction indicates to the existence of two types of TNT metabolites (with amino and carboxyl groups, correlation 4:1), which bind with high-molecular compounds of plants. Apparently, formation of amino and carboxyl groups as a result of TNT transformation by plant enzymes promotes their conjugation with endogenous compounds (Adamia et al. 2006; Khatisashvili et al. 2009). Based on these results and literature data, the hypothetic scheme of TNT metabolism in plants could be presented in Fig. 7.

As is seen from the Fig. 7, the metabolism of TNT in plants proceeds in following way: initially either nitro groups of TNT are reduced to amino groups,

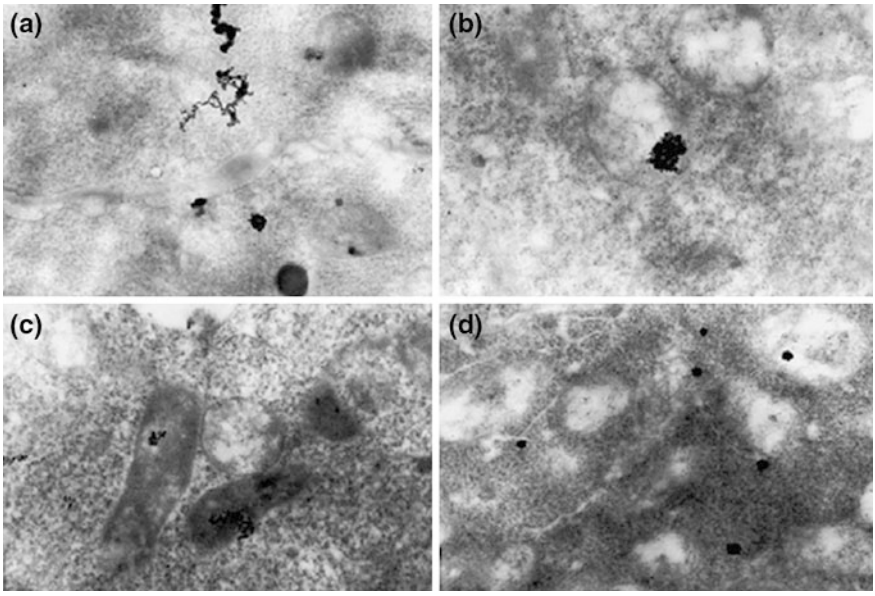

Fig. 5 Cortical cells of roots of soybean seedlings grown in 0.5-mM [1-^{14}C] TNT. **a** Label in mitochondria and on the plasmalemma in the endoplasmic reticulum ×28,000. **b** Label in mitochondria ×48,000. **c** Label in plastids ×36,000. **d** Label in mitochondria and endoplasmic reticulum ×20,000 (Adamia et al. 2006; Kvesitadze et al. 2006)

catalyzed by nitroreductase, or methyl group of the molecule is transformed to carboxyl group, catalyzed by oxidation enzymes (phenoloxidase, preferably). Set of different transferases forming soluble low-molecular (∼30 %) and insoluble high-molecular mass conjugates (∼70 %) ends the transformation process of TNT. However, the relation between the high molecular conjugates formed in both cases indicates that main part of TNT (80–85 %) is transformed via reduction pathway. Activation of some enzymes of cell basic metabolism, providing the nitroreductase with reduced equivalents of NAD(P)H, suggests their indirect participation in the xenobiotic detoxification (Khatisashvili et al. 2009). Similar works conducted by other authors could be a base for such supposition (Best et al. 1999a, b; Hughes et al. 1997; Schoenmuth and Pestemer 2004; Sens et al. 1998, 1999). After uptake, [1–6-^{14}C] TNT by roots of kidney bean, labeled with ^{14}C conjugates with lignin (20 %), hemicellulose (14 %), and pectin (5 %) are identified (Sens et al. 1998, 1999). Such biopolimers, being widely presented in plant tissues and possessing many free functional groups, actively participate in conjugation with amino groups of intermediates of TNT metabolism. It has been shown that aminodinitrotoluenes (ADNTs), primary products of TNT reduction, conjugate with hemicellulose in the roots of hybrid willow (*Salix* sp.) and Norway spruce (*Picea abies*) trees used in dendroremediation of soils polluted by TNT (Schoenmuth and Pestemer 2004).

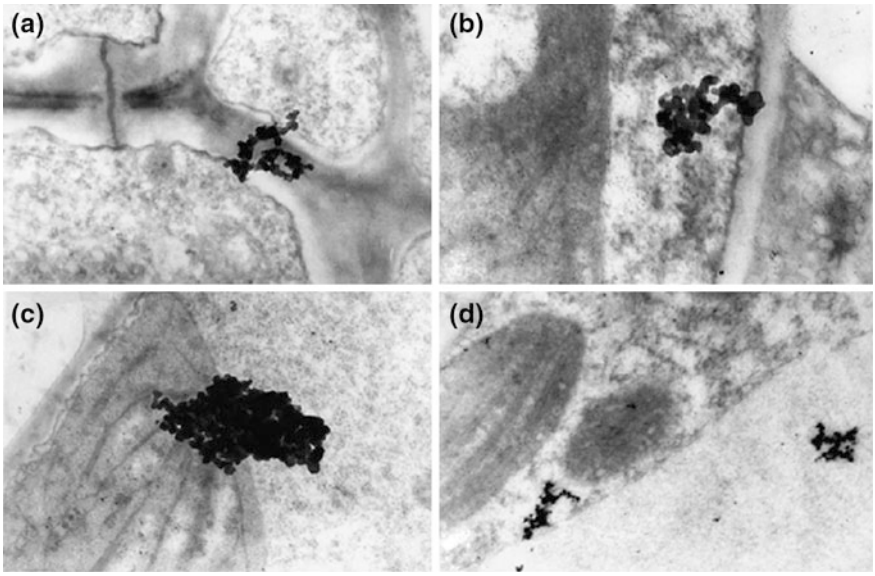

Fig. 6 Cells of leaves of soybean seedlings, grown in 0.5-mM [1-^{14}C] TNT. **a** Label in cell wall and on plasmalemma of the two cells ×64,000. **b** Label on plasmalemma and in periphery cytoplasm ×88,000. **c** Label in a chloroplast ×48,000. **d** Label in vacuole and cytoplasm ×48,000 (Adamia et al. 2006)

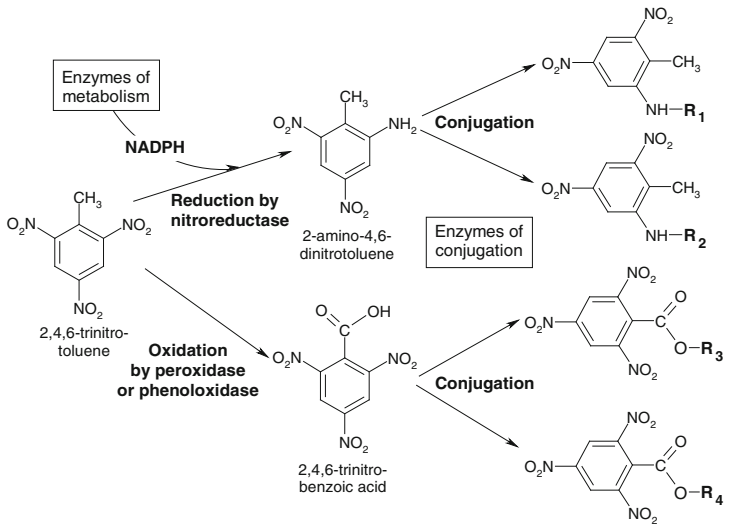

Fig. 7 Supposed pathways of TNT transformation in plants. R_1 and R_3 in scheme are low-molecular mass soluble compounds; R_2 and R_4—high-molecular mass insoluble compounds (Khatisashvili et al. 2009)

Plants ability to uptake and metabolize TNT was confirmed by Hughes et al. (1997). Three plant systems, viz. Madagaskar periwinkle (*Catharanthus roseus*) hairy root cultures, axenic, and native watermilfoil plants (*Myriophyllum* sp.) were exposed to demonstrate reduction of uniformly labeled [^{14}C] TNT, and to evaluate the fates of the labeled carbon atoms. TNT is completely transformed in all plant systems containing viable plant tissue. Aminonitrotoluenes, some unidentified ^{14}C-labeled compounds, extractable plant-associated [^{14}C] fractions that could not be identified as reduction products and bound-residues (plant-associated material that could be quantified after combustion of the plant tissue) are the metabolites that have been found.

Release of $^{14}CO_2$ was not observed in none of the cases of study of [^{14}C]-labeled TNT metabolism in plants. This fact indicates that plants are not able to complete mineralization of this pollutant. Main part of TNT carbon skeleton remains unchanged in plants, however, it is less dangerous to plant cell as it is conjugated with intracellular compounds and deposited in such compartments (cell wall, vacuoles) that are distanced from living important organelles (nucleus, mitochondria, plastids, etc.). Obviously, in such form, conjugates are kept in a cell for a definite period without causing any pathological deviation in cell homeostasis.

First step of transformation of TNT in microorganisms, as well as in plants, is reduction of nitro groups to amino groups. Despite of this similarity, majority of microorganisms more intensively assimilate TNT than plants. It have been shown that microorganisms of different taxonomic groups (bacteria, fungi, yeast) have abilities to assimilate [1-^{14}C] TNT and in all cases the carbon skeleton of TNT undergoes deep biotransformation that is testified by radioactivity of the fractions of organic acids and amino acids (Khatisashvili et al. 2004). Carbon atoms of assimilated and transformed [1–6-^{14}C] TNT are basically used by microorganisms for the biosynthesis of amino acids. In cultivation medium of microscopic fungi, the presence of labeled amino acids is not observed. Among the amino acids, the compounds with aromatic ring (basically phenylalanine and tyrosine) were prevalent, while for organic acids the radioactive label of TNT was mostly detected in fumaric and succinic acids. Fumaric acid is one of the products of biodegradation of the aromatic ring and is easily metabolized into succinic acid. It can be concluded that after reduction of the main part of the assimilated [1–6-^{14}C] TNT molecules, their oxidation follows which leads to removal of the amino groups and cleavage of the aromatic ring, and as a result organic acids are formed as standard cell metabolites. Thus, successive reduction and oxidation reactions complete detoxification of TNT and the atoms of this toxicant become involved in the vital processes of the organism.

Using [^{14}C] TNT, it was shown that microorganisms differently from plants possess the ability of TNT complete mineralization that is proved by the facts of $^{14}CO_2$ release after incubation of certain strains with [1–6-^{14}C] TNT. For example, *Pseudomonas* sp. JLR11 is able to assimilate [1–6-^{14}C] TNT and about 85 % of total TNT was incorporated as cell biomass, and about 1 % of was recovered as $^{14}CO_2$ (Esteve-Núnez and Ramos 1998). The study of biotransformation of labeled TNT by *P. chrysosporium* has shown that in less than 2 weeks, TNT disappeared

completely, 11 different labeled metabolites were identified, but mineralization (liberated $^{14}CO_2$) did not exceed 1 %. After 30 days, all of these metabolites disappeared, but mineralization did not exceed 10 % even after the incubation period was increased to 120 days. The biotransformation of TNT was accompanied by the appearance of manganese peroxidase and lignin-dependent peroxidase activities (Hawari et al. 1999).

Application of ^{14}C-labeled TNT contributed to researchers to establish optimum conditions for TNT mineralization. Thus, the cometabolic transformation of 2,4,6-trinitrotoluene (TNT) by an immobilized *P. chrysosporium* culture was investigated under different TNT and/or glycerol feeding conditions. As a result, full mineralization of [1–6-^{14}C] TNT was achieved to a level of 15.3 % following a 41-day incubation period (Rho et al. 2001). Also, it has been shown that the using of surfactant Tween 80 significantly enhanced [1–6-^{14}C] TNT mineralization by *P. chrysosporium,* in particular, 39.0 % of the TNT was respired on day 68 (Hodgson et al. 2001). Radiolabeled [^{14}C] TNT studies revealed 4.14 % mineralization after an incubation period of 163 days by a mixed culture acclimated and maintained on crude oil-containing media (Jason et al. 2004). In other experiments, by using modified Fenton's reagents ($Fe^{2+} + H_2O_2$) and aerobic microorganisms was achieved significant increase in TNT mineralization (Schrader and Hess 2004). These results show promise in the use of combined abiotic-biotic treatment processes for soils contaminated with high concentrations of TNT. From abiotic factors promoting TNT mineralization by *Pseudomonas* strains, pretreatment with alkali should also be mentioned (Herrmann et al. 2007).

6 Conclusion

The reviewed data on studies with application of ^{14}C-labeled xenobiotic allow concluding that plants and microorganisms are capable to degrade wide spectrum of environmental contaminants up to their mineralization, and microorganisms are more potent in this ability.

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