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Transformation of TNT by Aquatic Plants and Plant Tissue Cultures

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The ability of plants to uptake and transform 2,4,6-trinitrotoluene (TNT) was investigated using the aquatic plant *Myriophyllum spicatum,* axenic *Myriophyllum aquaticum,* and *Catharanthus roseus* hairy root cultures. Studies demonstrate that *Myriophyllum*, with or without its periphyton, and *C. roseus* transform TNT. Low concentrations of aminated nitrotoluenes (2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene) were observed in the extracellular medium and tissue extracts. Primary products of transformation were not identified, and mineralization was not observed. Mass balances demonstrate that a large percentage of the unknown TNT transformation products were associated with the plant. This fraction could be at least partially recovered from the plant tissue with methanol extraction. A soluble fraction was also present in the medium. The absence of periphyton had little impact on the results observed. Medium concentrations of 4-amino-2,6-dinitrotoluene were greater in systems in which the periphyton was not removed. For the first time, the intrinsic ability of plants to transform TNT has been confirmed. The formation of soluble, uncharacterized transformation products is a concern for potential phytoremediation applications.

Introduction

Munitions production and handling over the past 90 years has led to widespread and persistent 2,4,6-trinitrotoluene (TNT) contamination at a number of government facilities (*1*). Since TNT is toxic to many aquatic (*2*−*4*) and terrestrial species (*5*), the remediation of contaminated soils is necessary. Excavation and thermal processes are currently the most common remedial actions (*1*), but are costly. Recent efforts have sought to reduce the cost of remediation with alternative technologies such as bioremediation (*6*) and phytoremediation (*7, 8*).

Phytoremediation processes rely on the ability of plants to uptake and, in some cases, to metabolize pollutants. Information regarding the potential for TNT uptake or transformation in plants is cited in only a few studies. Palazzo and Leggett (*9*) reported TNT uptake from soil by bush beans and identified traces of several reduction products (e.g., aminonitrotoluenes) in leaf extracts. Wolfe et al. (*7*) reported rapid TNT disappearance from an aqueous medium in the presence of Stonewort (*Nitella*), also identifying low levels of aminonitrotoluenes in the aqueous medium. In both these studies, however, the experimental systems contained plant-associated microflora, so the ability of plants to transform TNT without the participation of associated microbes remained arguable. Moreover, because mass balances were not performed, it was impossible to evaluate the final distribution of TNT and/or transformation products.

In this paper, we report studies on the fate of [U-^{14}C]TNT in two plant systems: (1) native *Myriophyllum spicatum* with its associated microflora and (2) aseptic plant systems using axenic *Myriophyllum aquaticum* and *Catharanthus roseus* hairy root tissue cultures. *M. spicatum* was selected because of its availability at a munitions-contaminated facility (Alabama Army Ammunition Plant, Childersburg, AL) and the commercial availability of axenic *Myriophyllum* plant cultures. *C. roseus* hairy root cultures were employed for their rapid growth rates and as a microbe-free surrogate for plant-root activity. All systems were sediment-free to avoid complicating results with sorption or irreversible binding processes. Following incubations, mass balances were performed. These studies demonstrate the ability of plants to transform TNT rapidly and, interestingly, without the stoichiometric accumulation of aminonitrotoluenes or azoxy dimers.

Experimental Section

Chemicals. 2,4,6-Trinitrotoluene, trinitrobenzene, 2,6-dinitrotoluene, 2,4-dinitrotoluene, nitrobenzene, *o*-nitrotoluene, *p*-nitrotoluene, and toluene (ChemService, Westchester, PA); [ring-U-^{14}C]-2,4,6-trinitrotoluene (NEN Research Products, Boston, MA), specific activity of 1.3 mCi/mmol and purity of 98%; 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene (AccuStandard Inc., New Haven, CT); 2-hydroxylamino-4,6-dinitrotoluene, 4-hydroxylamino-2,6-dinitrotoluene, and 2,4-diamino-6-nitrotoluene (Dr. Ron Spanggord, SRI International, Menlo Park, CA); 2,6-diamino-4-nitrotoluene, 2,4,6-triaminotoluene, 2,2′,6,6′-tetranitro-4,4′-azoxytoluene, and 4,4′,6,6′-tetranitro-2,2′-azoxytoluene (Prof. Deborah Roberts, University of Houston, Houston, TX); $NaH^{14}CO_3$ (New England Nuclear, Boston MA) specific activity of 3.0 mCi/mmol; 2-propanol (EM Science, Gibbstown, NJ) were used.

Plants and Tissue Cultures. Native samples of *M. spicatum* were collected from ponds located at the Alabama Army Ammunition Plant, Childersburg, AL. Plants were cultured outdoors in 20-gal containers recharged naturally with rainwater. The water depth in these systems was typically 40 cm. Sediments collected from ponds located at the Alabama Army Ammunition Plant were added to these containers with limestone gravel as a source of trace nutrients and carbonates and to provide for a rooting matrix.

Axenic *M. aquaticum* plants were purchased from American Type Culture Collection (ATCC, Rockville, MD) and propagated vegetatively on NH_4^+-free Murashige Skoog (MS) medium (Sigma Plant Culture, St. Louis, MO) supplemented with agar. Axenic plants were maintained in magenta boxes at room temperature under light from cool white and broad spectrum bulbs for a 14-h photoperiod at a light intensity of 100 μEinstein/m^2. Axenic plants were grown for 3 months to obtain sufficient biomass for harvesting.

Hairy root cultures of *C. roseus* LBE-6-1 were grown in the dark at 26 °C on an orbital shaker (110 rpm) in 250-mL Erlenmeyer flasks containing 50 mL half-strength Gamborg's B5 salts (Sigma Plant Culture, St. Louis, MO) and 30 g/L sucrose. Medium pH was adjusted to 5.7 with HCl prior to filter sterilization (0.22 μm). Roots were subcultured every 3−4 weeks by transferring five tips, approximately 35−40 mm in length and 0.04 g total, into a flask with fresh medium. Fresh weight of the root biomass was determined by medium displacement as in Bhadra et al. (*10*).

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Analytical Methods. TNT and related nitroaromatics were quantified by micellar electrokinetic capillary electrophoresis (MECE) and high-pressure liquid chromatography (HPLC). MECE analysis was carried out using a Hewlett Packard 3D capillary electrophoresis system equipped with a diode array UV/vis detector. HP Chemstation software was used for data acquisition and analysis on a HP Vectra 486 personal computer. TNT and reduction products were separated with a 75-μm capillary using an SDS/boric acid/borate running buffer (pH 8.57). The system was calibrated daily with known standards. Peak identification was based on comparison of retention times and UV spectra with authentic standards. Spectra were aquired from 200−400 nm and chromatograms extracted for quantification at 230 nm. The limit of detection with this method was 1−4 ppm, depending on the compound of interest.

The HPLC analysis, described by Waters Millennium Application Brief (MB15, Method B 8/02/93), employed a Waters (600E System Controler) HPLC pump, a Waters (717 plus) autosampler, a Waters C_8 Nova-Pak Cartridge (3.9 mm × 15 cm), a guard column, and a Waters 996 photodiode array detector (Waters Associates, Milford, MA). Data acquisition and analysis were performed on a NEC 486es personal computer with Waters Millennium 2010 software. Nitroaromatics were separated on the column under isocratic conditions using a 82:18 mixture of water:2-propanol at a flow rate of 1.0 mL/min. Spectra were aquired from 200 to 400 nm and chromatograms were extracted for quantification at 230 nm. Peak identification was based on comparison of retention times and UV spectra with authentic standards. The detection limit for TNT with this method was determined to be nominally 0.20 ppm.

^{14}C was quantified with a Beckman LS 3801 scintillation counter. Samples (1 mL) were added to disposable scintillation vials containing 10 mL of Ready Gel (Beckman). Counting times were 10 min. The fraction of ^{14}C associated with plant matter was determined by combusting plant samples with an OX 600 biooxidizer (R. J. Harvey) and collecting $^{14}CO_2$. A predetermined weight of plant sample (typically 0.5 g) was combusted at 900 °C for 4 min under a stream of oxygen. The $^{14}CO_2$ that evolved was trapped in the scintillation cocktail (R. J. Harvey ^{14}C Cocktail) and quantified with the scintillation counter. Standards of mannitol and dried plant materials, spiked with known [^{14}C]TNT levels, indicated an average efficiency of 95%. CO_2 production was determined by LS counting of KOH solutions from traps included in incubation flasks. Trapping efficiency, tested with a solution of [^{14}C]$NaHCO_3$, was 93%.

Transformation Studies. Experiments were carried out in batch studies regardless of the plant used at temperatures ranging from 20 to 26 °C in the absence of light. Reaction vessels were 250-mL Erlenmeyer flasks containing a known mass of plants (wet weight) and a sufficient volume of medium to ensure that the plants were completely submerged. TNT medium was prepared by adding ring-labeled [^{14}C]TNT that would yield 15 000−30 000 dpm per flask (depending on the particular experiment) as well as unlabeled TNT to the two media described previously for plant growth. After TNT addition, media were filter sterilized (0.22 μm) and the concentrations of TNT and ^{14}C confirmed.

For *Myriophyllum* transformation studies, plants were added to flasks containing TNT medium to an approximate concentration of 50 g/L (wet weight basis). Autoclaved plants were used as heat-inactivated controls. Native *Myriophyllum* was washed repeatedly with tap water to remove sediment, and axenic *Myriophyllum* plants were rinsed with sterile deionized water to remove agar; plants were then suspended in Smart solution (*11*) (pH = 7.5). In hairy root studies, TNT and [^{14}C]TNT were added aseptically to flasks containing 28−32-day-old roots at an approximate concentration of 100 g/L. Again, autoclaved cultures were used as controls. All experiments and heat-inactivated systems were performed in triplicate. A single bottle served as a medium control.

In all cases, medium samples were taken for transient analysis of extracellular nitroaromatic concentrations and ^{14}C analysis. After 7 days of incubation, systems were dismantled and mass balances on ^{14}C were performed. Samples were taken of the growth medium and KOH trap and counted directly. Next, plants or hairy roots were rinsed with 1% acetic acid to remove surface-associated media and ^{14}C, and this fraction was counted directly. Total plant-associated ^{14}C was determined by oxidation of plant samples. The amount of extractable ^{14}C associated with plant tissue was then evaluated using a modification of the method of Bhadra et al. (*10*). Tissue was frozen and then lyophilized until no further weight loss occurred. Wipe tests of the lyophylizer and ^{14}C loss in controls confirmed that no volatilization losses occurred during freeze drying. Samples (100−300 mg of freeze-dried tissue) were extracted with 20−30 mL of methanol for 8−10 h in a water-bath sonicator. The temperature was controlled below the flash point of methanol (56 °C) with an ice bath. The extract was filtered (0.22 μm) and concentrated to a volume of 2 mL, and the ^{14}C level was determined. Nitroaromatic concentrations remaining at the time of mass balances were determined by HPLC analysis of medium samples and tissue extracts. To ensure that microbial contamination of aspetic cultures had not occurred, medium samples (1.0 mL) were spread on agar plates prepared with half-strength nutrient broth and incubated for 3 days at 25 °C. Colony formation was not observed in any of the systems reported.

TNT Recovery Experiments. Experiments were conducted over a 7-day period to assess potential losses of TNT or ^{14}C in heat-inactivated controls and medium controls. For these studies, heat-inactivated native *Myriophyllum* and *C. roseus* hairy roots were used. These systems were treated in a manner identical to that discussed in transformation studies except that they were sampled only at the completion of 7 days of incubation. Thus, no changes in liquid to plant ratios were incurred that could influence TNT partitioning. Experiments were conducted in triplicate.

Results

Medium Sampling. Results from the temporal sampling of medium for native *Myriophyllum* are presented in Figure 1. Figure 1A shows the concentration of TNT in viable plant systems as compared to heat-inactivated controls and medium controls. In systems containing plant tissue (viable and heat-inactivated), an initial decrease in TNT concentration was observed, presumably due to partitioning. In viable systems, however, medium TNT concentrations continued to decrease and by day 7 had dropped below detection limits. Medium TNT concentration in heat-inactivated controls stabilized after day 1, and medium controls were constant throughout. The medium concentrations of identifiable TNT transformation products are shown in Figure 1B. The only compounds observed at detectable concentrations were 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene, and they represent approximately 16% conversion at 168 h. In Figure 1C, medium ^{14}C concentrations are presented. No losses were observed in medium controls. An initial decrease of ^{14}C was observed in heat-inactivated controls. In viable systems, ^{14}C levels decreased rapidly over the first 3−4 days and then remained essentially constant throughout the remainder of the experiment with approximately 50% of the initial ^{14}C remaining in the medium.

Figures 2 and 3 show results from axenic *Myriophyllum* and *C. roseus* studies. Similar trends were observed in these systems as compared to the native *Myriophyllum*, although variability in results was greater. The increase in variability was apparently the result of less uniform biomass concentration within replicates. *C. roseus* systems were sampled at

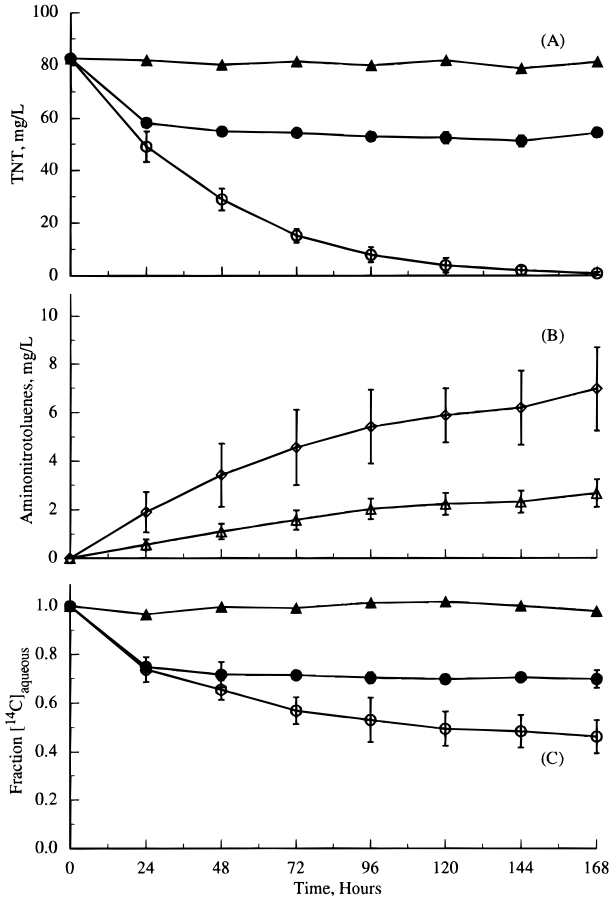

FIGURE 1. Results of temporal medium sampling from experiments with native *Myriophyllum*: (A) average TNT concentrations in medium control (▲), heat-inactivated controls (●), and viable plant systems (○); (B) average concentrations of 2-amino-4,6-dinitrotoluene (△) and 4-amino-2,6-dinitrotoluene (◇) in viable plant systems; and (C) the average fraction of ^{14}C remaining in medium control (▲), heat-inactivated controls (●), and viable plant systems (○). Error bars represent one standard deviation (in some cases they are not visible due to the presence of the symbol).

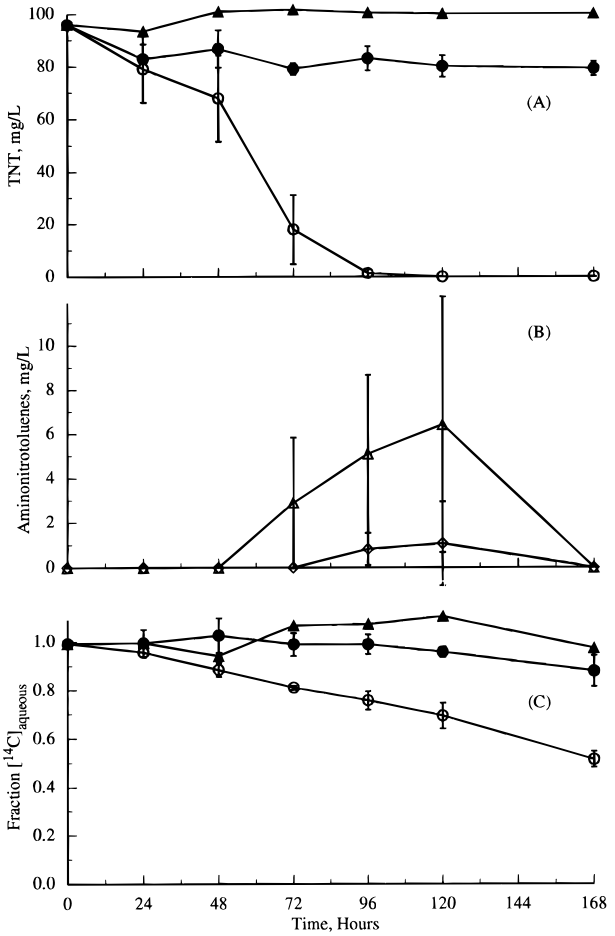

FIGURE 2. Results of temporal medium sampling from experiments with axenic *Myriophyllum*: (A) average TNT concentrations in medium control (▲), heat-inactivated controls (●), and viable plant systems (○); (B) average concentrations of 2-amino-4,6-dinitrotoluene (△) and 4-amino-2,6-dinitrotoluene (◇) in viable plant systems; and (C) the average fraction of ^{14}C remaining in medium control (▲), heat-inactivated controls (●), and viable plant systems (○). Error bars represent one standard deviation (in some cases they are not visible due to the presence of the symbol).

regular intervals for only the first 2 days, as compared to a 7-day sampling of *Myriophyllum* experiments. The rationale for more frequent and rapid sampling was the higher *C. roseus* biomass concentrations, which generally result in more rapid disappearance of TNT. At the end of 2 days, TNT was still present in *C. roseus* cultures, but samples taken after 5 days had no detectable levels of TNT (data not shown).

^{14}C Mass Balances. After 7 days of incubation, the systems were dismantled, and a mass balance was performed on added ^{14}C. Results from the three cultures are presented in Table 1. The average recoveries of ^{14}C ranged from 93% (± 4.8%) to 99.4% (± 9.5%), and significant levels of mineralization were never observed (i.e., no measured value exceeded 0.1% of the ^{14}C added). In the *Myriophyllum* systems (native and axenic), ^{14}C levels were distributed almost equally between the medium and the plants (as measured by tissue oxidation). In the *C. roseus* cultures, a larger fraction was present in the roots, which may be a result of higher biomass levels. An additional difference between the hairy roots and the intact plants was that extraction of the roots yielded complete recovery of ^{14}C (when compared to tissue oxidation). Efficiencies of recovery are comparable to those reported in similar studies investigating the uptake of xenobiotic chemicals in terrestrial plants (*12*). Moreover, combustion of the plant samples resulted in a residual ash. Any ^{14}C associated with this residue is not accounted for in these results.

Also shown in Table 1 are the percent recoveries of TNT, 4-amino- 2,6-dinitrotoluene, and 2-amino-4,6-dinitrotoluene. These values represent the molar percent that could be accounted for by combining levels found in the medium and in tissue extracts. Despite the fact that mass balances were nearly 100%, only traces of aminated nitrotoluenes could be detected in experimental systems. Elution profiles were compared against retention times of other nitroaromatics listed previously, but no matches were found. Products either had limited UV/vis absorption, eluted in the polar solvent front, or both.

TNT Recovery Experiments. Results of TNT recovery control studies are shown in Table 2. The recoveries of ^{14}C and TNT in all systems ranged from 94.8% (± 3.5%) to 108.3% (± 6.2%), and TNT recovered was never significantly different from ^{14}C recoveries. These results demonstrate that TNT does partition to the plant matrix, but in heat-inactivated plant tissue the TNT can be extracted. Also, these studies confirm that neither abiotic losses (e.g., reactions with the medium, volatilization, irreversible binding, or others) or losses in sampling can explain the disappearance of TNT observed in viable systems.

Discussion

The purpose of these studies was to quantitatively evaluate the uptake and transformation of TNT by plants, with and without the contribution of plant-associated periphyton. Results of experiments with native *Myriophyllum*, axenic

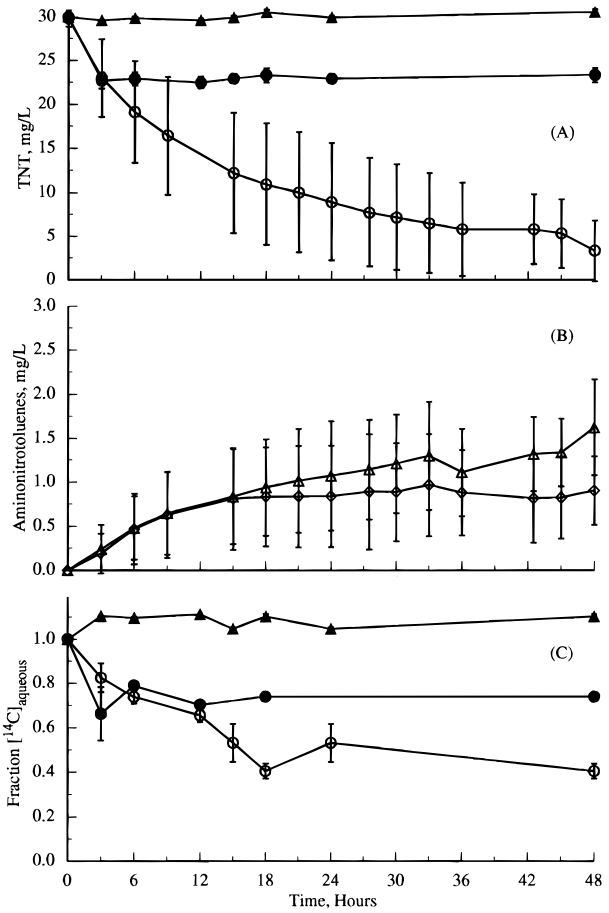

FIGURE 3. Results of temporal medium sampling from experiments with *C. roseus* hairy root cultures: (A) Average TNT concentrations in medium control (▲), heat-inactivated controls (●), and viable plant systems (○); (B) average concentrations of 2-amino-4,6-dinitrotoluene (△) and 4-amino-2,6-dinitrotoluene (◇) in viable plant systems; and (C) the average fraction of ^{14}C remaining in medium control (▲), heat-inactivated controls (●), and viable plant systems (○). Error bars represent one standard deviation (in some cases they are not visible due to the presence of the symbol).

Myriophyllum, and hairy root cultures of *C. roseus* indicate that plants are capable of TNT transformation and that products of transformation are partially taken up by the plants. The only identifiable transformation products observed in any system were two aminated nitrotoluenes: 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene. These were observed in the plant growth medium and in plant extracts. No other metabolites could be identified, and no mineralization was observed.

It is difficult to determine from these studies whether uptake precedes transformation. In only one case (native *Myriophyllum*) was TNT detected in plant extracts, and this was at low levels. It is possible, therefore, that the TNT is transformed prior to or during transport into the plant tissue. Reduction products (4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene) were observed outside the plants, indicating that transformation occurs at the plant surface, or that these transformation products can be exchanged between the extracellular and intracellular compartments.

The results of these studies generate interesting questions regarding the pathways of TNT transformation in plants. No oxidative pathways yielding even traces of CO_2 were observed. Samples from the growth medium and from the plant tissue extracts were analyzed for a range of compounds that could result from the reduction (2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2-hydroxylamino-4,6-dinitrotoluene, 4-hydroxylamino-2,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene, and 2,6-diamino-4-nitrotoluene) and/or elimination of nitro groups (2,6-dinitrotoluene, 2,4-dinitrotoluene, *o*-nitrotoluene, *p*-nitrotoluene, toluene); from demethylation of the aromatic ring (trinitrobenzene, nitrobenzene); or from condensing reactive intermediates (2,2′,6,6′-tetranitro-4,4′-azoxytoluene and 4,4′,6,6′-tetranitro-2,2′-azoxytoluene). Reduction of TNT is a common observation in microbial (*13*), mammalian (*14*, *15*), and abiotic reactions (*16*). Elimination of nitro groups from TNT forming toluene has been observed following reduction processes (*17*) or during light-catalyzed abiotic reactions forming nitrotoluenes (*18*). Oxidation of the methyl group of TNT has been observed as a result of abiotic and light-catalyzed reactions (*18*). TNT reduction was observed, but the products of these reactions were always at concentrations below what would be expected if stoichiometric conversion had occurred. Further reduction may have occurred, but neither isomer of diaminonitrotoluene or triaminotoluene was observed. It is important to note that triaminotoluene may not have been observed even if it were produced, since triaminotoluene reacts spontaneously with oxygen and these systems were aerobic. In aerobic microbial systems, reduction beyond the monoaminodinitrotoluene level is uncommon (*13*), but no information exists regarding the potential reduction of TNT in plants.

Based upon the compounds observed in these studies, it appears that plant systems can reduce TNT to the level of monoaminonitrotoluenes, with or without associated periphyton. Following this reduction, no further identification of metabolites was possible. Some metabolites remain within the plant, while a fraction remains in the medium. Of the fraction within the plant, the degree to which it can be extracted varies. These observations are consistent with studies of the metabolism of pesticide/herbicide in terrestrial plants and plant cell cultures. A recent summary of xenobiotic transformation by plants and a conceptual model for plant "detoxification" processes can be found in Trapp and McFarlane (*12*). In this model, the xenobiotic is taken up by the plant through transpiration processes; transformed by oxidation, reduction, or hydrolysis reactions; conjugated with compounds such as D-glucose, glutathione, amino acids, or malonic acid; and finally compartmentalized in a vacuole, cell wall material, or lignin. Trapp and MacFarlane illustrate this process with the compounds 2,4-dichlorophenoxyacetic acid (2,4-D) and pentachlorophenol. 2,4-D transformation is initiated by hydroxylation reactions, followed by conjugation with D-glucosyl or malonyl residues and subsequently transported into cell vacuoles, where it remains in a soluble form. Similarly, PCP is initially hydrolyzed and then conjugated with lignin, forming an insoluble "residue".

Our results are consistent with the Trapp and McFarlane model. TNT levels in the medium decrease as TNT contacts the plant; the TNT is transformed/conjugated and is either transported back to the medium in a soluble form or remains associated with the plant. The nature of the associated form may vary somewhat depending on the time of incubation and, presumably, the plant species used. Whether TNT is conjugated directly or undergoes an initial transformation process is difficult to ascertain. The presence of aminated nitrotoluenes in all systems may represent the first step of a conjugation process or a means of reducing TNT toxicity. Conjugation of 3,4-dichloroaniline with malate by wheat and soybean roots was reported by Bockers et al. (*19*). No reports of conjugation to an aryl nitro group were found in the literature. Further studies will be required to determine the role of reductive processes and whether plants do conjugate TNT.

Experiments with axenic cultures and hairy roots demonstrated similar trends to those observed with native plants. For example, the rates of TNT disappearance were similar in the native and axenic *Myriophyllum*, and the same reduction products were observed. An interesting difference between

TABLE 1. Mass Balance Data from All Systems after 7 Days of Incubation[a]

plant	sample	^{14}C (% added)	TNT (% mol added)	4A26DNT (% mol added)	2A46DNT (% mol added)
Myriophyllum, native	medium	46.1 ± 6.8	ND[b]	9.6 ± 2.3	3.7 ± 0.8
	plant, oxidation	46.9 ± 6.2	NA[c]	NA	NA
	plant, extraction	22.4 ± 4.9	0.4 ± 0.19	1.5 ± 0.46	1.4 ± 0.27
	total[d]	93.0 ± 4.8	0.4 ± 0.19	11.1 ± 2.8	5.1 ± 1.04
Myriophyllum, axenic	medium	51.5 ± 3.3	ND	ND	ND
	plant, oxidation	44.1 ± 9.1	NA	NA	NA
	plant, extraction	32.6 ± 1.75	ND	ND	0.42 ± 0.14
	total[d]	95.6 ± 6.5	ND	ND	0.42 ± 0.14
C. roseus, hairy roots	medium	29.4 ± 4.6	ND	1.2 ± 1.7	1.1 ± 1.0
	root, extraction	70.0 ± 4.9	ND	1.3 ± 0.3	1.3 ± 0.9
	total	99.4 ± 9.5	ND	2.5 ± 2.0	2.4 ± 1.9

[a] Results presented as a percent of TNT added. [b] ND, not detected. [c] NA, not applicable. [d] Total is the sum of medium and *plant oxidation.*

TABLE 2. Recovery of TNT after 7 Days of Incubation in Heat-Inactivated and Media Controls

		heat-inactivated controls		media controls	
plant	sample	^{14}C (%)	TNT (%)	^{14}C (%)	TNT (%)
myriophyllum	medium	61.2 ± 8.5	63.7 ± 8.5	108.3 ± 6.2	101.8 ± 3.0
	plant	33.6 ± 3.17	31.7 ± 6.7	NA	NA
	total	94.8 ± 3.5	95.4 ±15.2	108.3 ± 6.2	101.8 ± 3.0
C. roseus, hairy roots	medium	71.3 ± 5.8	82.8 ± 7.0	101.2 ± 0.9	98.9 ± 0.9
	roots	23.1 ± 2.8	17.1 ± 3.1	NA	NA
	total	94.4 ± 8.6	99.9 ± 10.1	101.2 ± 0.9	98.9 ± 0.9

[a] NA, not applicable.

the native *Myriophyllum* and both axenic *Myriophyllum* and *C. roseus* is in the concentrations of observed aminated nitrotoluenes. In the native cultures, the 4-amino-2,6-dinitrotoluene isomer was present at a higher concentration than 2-amino-4,6-dinitrotoluene, while this trend was reversed in the aseptic systems. Furthermore, there were differences in the extent of plant-associated ^{14}C and its extractability. The largest fraction of ^{14}C associated with plant tissue was observed in *C. roseus* cultures. This may have been the result of higher biomass concentrations (due to partitioning), greater metabolic activity, or intrinsic differences between species. The *C. roseus* cultures were exposed to lower initial TNT concentrations. This also may have affected the final distribution. If TNT were toxic to or diminished accumulation processes, lower levels of accumulation would occur at higher initial TNT concentrations. Certainly, the products of TNT metabolism by *C. roseus* were more readily extractable. The plant-associated ^{14}C observed in axenic and native *Myriophyllum* were similar, but slightly more was extractable from the axenic cultures.

While the final products of TNT transformation by *Myriophyllum* and *C. roseus* remain uncertain, these studies present the first quantitative demonstration of TNT transformation by aquatic plants. Mass balances and plant extractions show that TNT disappearance is not the result of accumulation processes, and studies with axenic plants and tissue culture confirm the intrinsic ability of plants to facilitate the transformation process. In these experiments, mineralization did not occur, and aminated nitrotoluenes were observed in the medium and in plant extracts. After 7 days of incubation, levels of TNT and its reduction products were highest in systems containing native *Myriophyllum* (16.6% ± 4.0% of the moles added). If phytoremediation of TNT-contaminated materials is to be pursued, further studies need to be conducted to evaluate transformation pathways and the potential toxicity of products. From these experiments, it is clear that plants are capable of TNT transformation, but as the products of TNT metabolism are not contained solely within the plant, extracellular products need to be characterized.

Acknowledgments

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