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Uptake and Phytotoxicity of TNT in Onion Plant

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<요 약 문>

The uptake of ^{14}C-2,4,6-trinitrotoluene (TNT) in hydroponics was studied using onion plants. Of the total TNT mass (5 μM concentration), 75% was in the roots, 4.4% in the leaves, and 21% in the external solution at 2 days. The percent distribution in roots was lower with higher concentration in the external solution, but in leaves it was comparable at all concentrations (5-500 μM). Root concentration factor (RCF) in hydroponics was more than 85 in constant hydroponic experiment (CHE) at 5 μM and 150 in non-constant hydroponic experiment (NHE) at 5 μM. The maximum RCF values in the hydroponic system were greater with lower solution concentration. Transpiration stream concentration factor (TSCF) values in the present study (NHE only: 0.31-0.56) were relatively similar to the values with predicted values (0.43-0.78), increasing with higher external TNT concentration. For phytotoxicity tested in hydroponics and wet paper method, 500 μM was toxic to onion plant, 50 μM was non-toxic for plant growth but limited the transpiration rate, and 5 μM was non-toxic as control.

Key Word : Hydroponic; Phytotoxicity; ^{14}C-2,4,6-trinitrotoluene (TNT); Root Concentration Factor (RCF); Transpiration Stream Concentration Factor (TSCF)

1. Introduction

The uptake of a chemical through the roots depends on the physiological properties of the root, transpiration rate (providing mass flow of solutes), and physico-chemical properties of the contaminant. Among the physical chemical properties of the organic contaminant, water solubility, vapor pressure, molecular weight, and octanol/water partition coefficient are the most important factors in this connection.

Uptake of organic molecules by plants is a highly efficient removal mechanism for moderately hydrophobic organic chemicals, with octanol-water partition coefficients (log K_{ow}) in the range of 1-3.5 (Schnoor, 1990). A corresponding log K_{ow} for a maximum TSCF value is 2.5 (Burken, 1998). Moderately lipophilic compounds include most BTEX chemicals, chlorinated solvents, nitrogenous explosive compounds (including TNT), and short-chain aliphatic chemicals. Hydrophobic chemicals (higher log K_{ow}) by contrast are easily crossed in the mucigel associated with the root surface and in

the lipid membranes, but their concentration in the cytosol of the root cells (aqueous phase) is very low. Because of their low aqueous concentration, they cannot easily be translocated within the plant by mass flow either in the aqueous phase during symplastic transport to the xylem or in transport within the xylem. By contrast, chemicals that are quite water-soluble (lower log K_{ow}) are not sufficiently transported through the lipid membranes during entry into the symplasm.

The resistance of plant species to a potentially phytotoxic chemical is one of the vital factors in choosing a plant species for phytoremediation. Earlier studies on TNT uptake from soil or from hydroponics have been done with duckweed, yellow nutsedge, tall fescue, switchgrass and smooth bromegrass, *Myriophyllum spicatum* (aquatic plant), Canada blue grass and swamp meadow grass, oat, wheat, cress and turnip and mixtures of plants. TNT phytotoxicity has been tested by its effects on seed germination, growth rate, chlorosis and transpiration.

The specific objectives of the present study were: (1) to determine values for RCF and TSCF for onion grown in hydroponics, and compare between two different hydroponic systems (NHE and CHE) and (2) to investigate the phytotoxicity of TNT to onion plants, and compare it with the sensitivity of other species used in phytoremediation.

2. Materials and Methods

At about 60 days, onions were removed from the pots and the roots were cut off at the base of the stem (shoot). The onion stem bases were washed in water and transferred into a plastic container, which were filled with nutrient solution and installed with an air bubbling system to oxygenate the roots. Those onions were allowed to grow new roots at the stem base for two weeks. Then, the onions with newly grown roots were transferred into 100-ml test tubes that containing 60 ml TNT-nutrient solution and equipped with the air-bubbler.

TNT solutions were prepared to give 5 μM, 50 μM, and 500 μM TNT in the nutrient solution. Volumes of TNT in nutrient solution (60 ml) were measured into each test tube and 0.05 μCi ^{14}C-TNT was added into each tube, giving three different specific activities. In experiments where the TNT concentration was maintained constant at constant hydroponic experiments (CHE), solutions were replaced with new solutions every 6 hours. In non-constant hydroponic experiments (NHE) designed to allow TNT to be depleted from the solution by root uptake, the initial solution was not replaced.

^{14}C-TNT in the hydroponic solutions was measured by liquid scintillation spectrometry using a Hewlett-Packard Tricarb Counter. ^{14}C-TNT accumulated by the plant root was directly counted by placing roots in the liquid scintillation cocktail, in which they became translucent. For the leaf samples, because of interference (color quenching) from chlorophyll, a wet combustion method was employed. Corrections were made for chemical quenching using an external standard.

Four methods were used to test for phytotoxicity in this study. For direct observation of root cell structure, transverse sections were cut, stained with 1.5% Evans blue for 3 minutes, mounted in water on a slide and examined with a microscope equipped with a projection screen. For growth rate measurement, 3-day old onion seedlings were put on filter papers in Petri dishes, which were wetted with each TNT solution (5 μM, 50 μM, 500 μM, and control, no TNT), and weighed daily for 5 days. For root color change tests, plants were grown essentially as described for the CHE work. Additionally, changes in transpiration provided evidence of phytotoxicity.

3. Results

The non-constant hydroponic experiments determined the distribution of TNT in each compartment (root, leaf and solution) at different initial TNT concentrations in the solutions (5 μM, 50 μM, and 500 μM) (Fig. 1). TNT partitions between root and solution (known as RCF) at three initial concentrations (5 μM, 50 μM, and 500 μM TNT) at 48 hours were examined in the comparison of RCF with studies published earlier in the literature (Table 1). The average TNT concentration in the transpiration stream was calculated using total TNT accumulated in the leaf part and total water transpired through the plant. TSCF values for the present study were similar to those previously calculated based on K_{ow} (Table 1).

The Leaf Concentration Factor (LCF) is a new factor introduced in the present study due to the weakness of TSCF, and it is a convenient factor for describing the translocation of an organic chemical into leaves:

$$LCF = C_L/C_S \qquad (1)$$

where C_L is the concentration in leaves and CS is the concentration in external solution.

The absolute concentrations of TNT in the roots were measured with time at the three concentrations (5 μM, 50 μM, and 500 μM) for the constant hydroponic experiments (Fig. 2). The concentration of TNT translocated to the leaves was very small and similar to that in the experiments (NHE). RCF values at 48 hours were examined and compared with the precious values (Table 1).

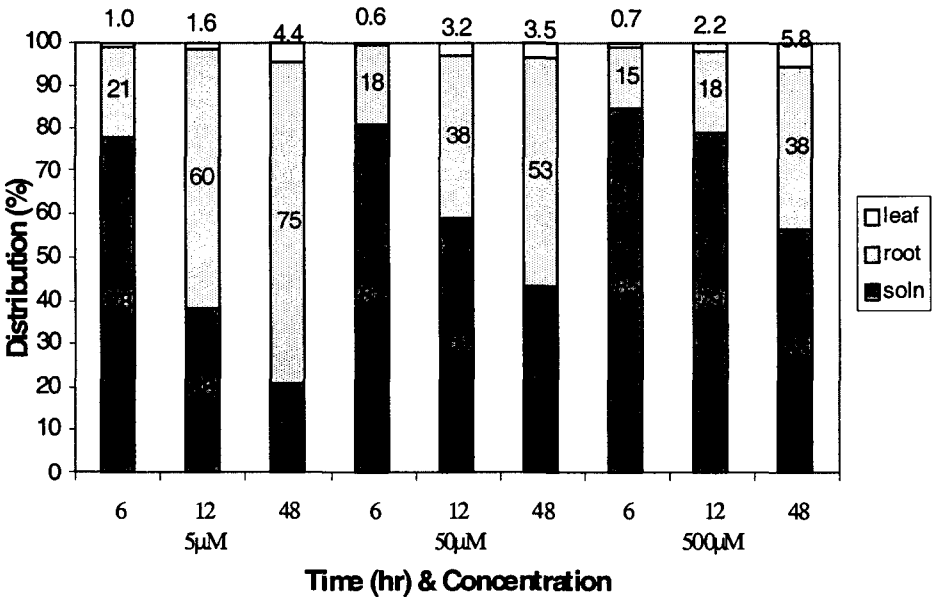

Figure 1. Distribution of TNT among the root, leaf and solution for an onion plant in non-constant hydroponic treatment. The values were calculated based on nmol per plant part and nmol remaining in the solution.

Table 1. Previous and present studies of calculated and experimental RCF (Root Concentration Factors) and TSCF (Transpiration Stream Concentration Factors) for TNT.

Log K_{ow}	RCF(ml/g)	TSCF	Plant Species	Type of Values	Sources
1.9	1.70	0.78	Barley	Calculated[a]	Briggs, 1982
1.9	1.73	-	Barley	Calculated	Topp, 1986
1.9	-	0.43	Soybean	Calculated	Hsu, 1990
1.9	3.50	0.66	Hybrid Poplar Tree	Calculated	Burken, 1998
-	49.0	0.46	Hybrid Poplar Tree	Experimental	Thompson, 1998
1.9	150.2	0.25	Onion	5 μM NHE	This Study
1.9	90.9	0.24	Onion	50 μM NHE	This Study
1.9	41.3	0.56	Onion	500 μM NHE	This Study
1.9	84.8	-	Onion	5 μM CHE	This Study
1.9	20.9	-	Onion	50 μM CHE	This Study
1.9	11.2	-	Onion	500 μM CHE	This Study

[a] RCF and TSCF values were calculated using the equations developed in the literature.

The toxicity of TNT was tested at three different concentrations using the wet paper method with 3-day old seedlings (Fig. 3), and also detected by color change of the roots, from white to yellow (Not shown). Additionally, using two-month-old onions grown hydroponically, toxicity of 500 μM TNT was again indicated by color change of the roots (Not shown). Observation of transverse sections of root indicated 500 μM TNT showed damage to a few isolated cells in the cortex (Not shown).

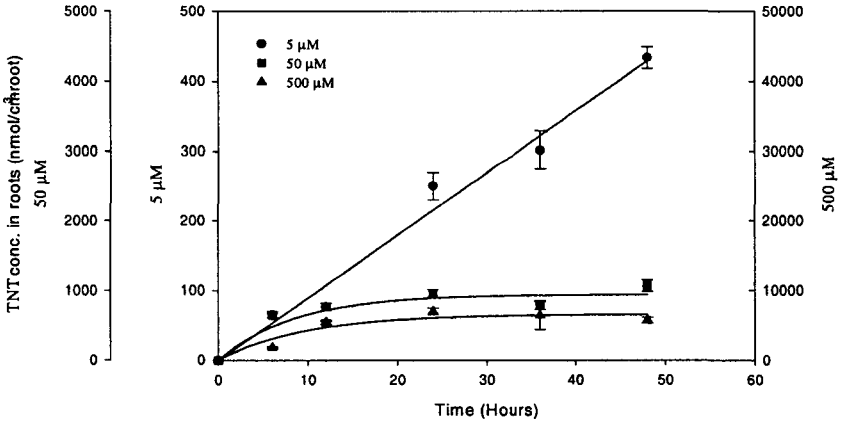

Figure 2. TNT concentration in roots (nmol/cm^3-root) in constant hydroponic treatment. at 5 μM, 50 μM and 500 μM (n = 6, S. E.).

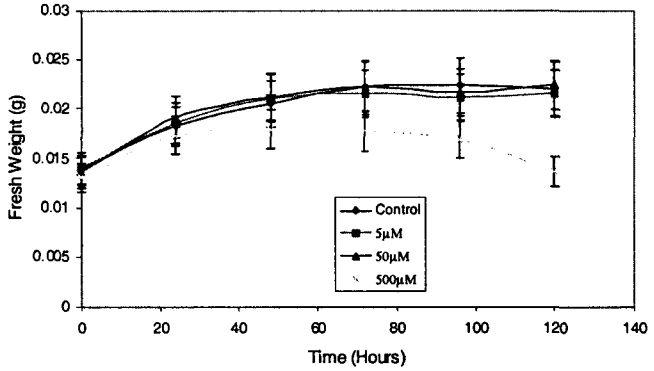

Figure 3. Toxicity of TNT at four different concentrations (Control - no TNT, 5 μM, 50 μM and 500 M) using the wet paper method with 3-day old onion seedlings. Values are fresh weight per seedling (n = 9, S. E.).

4. Conclusions

The apparent steady state concentration of TNT in roots at 5 μM in NHE (not in CHE) was caused by depletion of TNT from the hydroponic solution. At 50 and 500 μM, the steady state concentrations may represent a true equilibrium between root cells and the external solution, or may result from TNT toxicity. The concentration of TNT in the leaves was very low compared to TNT concentration in the root, and reflects relatively low translocation rate to leaves from roots.

RCF values were different at the different TNT concentrations in both NHE and CHE. Values of TSCF in NHE appear to be independent of RCF because the time-dependent patterns of TSCF were different from those of RCF. TSCF values might be overestimated due to lowering TNT concentration with time in the external solution. Furthermore, the high value at 500 μM appeared to result from low transpiration, causing a high TNT concentration. This can be supported by LCF (leaf concentration factor) values.

The results from all tests indicated that 500 μM TNT was toxic to the onion plants, whereas plants were tolerant to 50 μM TNT. However, transpiration was slowed at 50 μM TNT. 5 μM TNT seems to be non-toxic for the plant growth and function for transpiration.

After all, it is strongly recommended to use a specific RCF or TSCF value according to different TNT concentration once applying for the rate of chemical uptake by plant in a contaminated site where the concentration is below the threshold value for phytotoxicity.

5. References

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