FOURIER-TRANSFORM INFRARED SPECTROSCOPY AS A TOOL TO MONITOR CHANGES IN PLANT STRUCTURE IN RESPONSE TO SOIL CONTAMINANTS

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ABSTRACT

Fourier-transform infrared spectroscopy (FTIR) can be used as a tool to monitor changes in plant cell structures caused by organic soil contaminants. This study specifically focuses on the use of FTIR to determine the effect of benzotriazoles (BTs) on the structure of sunflower plants. BTs are corrosion inhibitors commonly used in aircraft deicing fluids, and they are found in chemical formulations used to prevent freezing of cooling systems. These chemicals have been detected in ground and surface waters, and soils near facilities that utilize and/or produce them. Our group currently studies possible methods to remediate BTs by way of phytoremediation using sunflowers. However, the fate of BTs within the plant is not well known. FTIR can be used to determine the fate of these contaminants within the plant and how they change the plant cell structure. Previous studies have provided evidence that BTs can be taken up by sunflower plants and can possibly be incorporated into the plant structure by lignification, since they cannot be extracted from dry plant matter. The structure of sunflower plants grown in Hoagland's solution with 30, 60, and 90 mg/L of BT were analyzed and compared to untreated plants to observe changes in plant structure. Based on our observations, the FTIR technique shows potential for detecting structural changes induced by the presence of these contaminants and may be used to prove the capability of plants to phytotransform organic contaminants.

Key words: FTIR, benzotriazole, phytoremediation, phytotransformation, plants

INTRODUCTION

Fourier-transform infrared spectroscopy (FTIR) has been extensively applied in plant cell wall analysis (McCann et al., 2001; Kacuráková et al., 2000; Sene et al., 1994). However, very few studies use FTIR to examine changes in plant cell wall structure due to the absorption or uptake of organic contaminants. Interest in phytoremediation over the past decade has led to an increased need to find methods to study fate and transport of organic contaminants in plants. FTIR has been proven to be useful in studying compositional changes in plant cell walls during development (McCann et al., 1997). Therefore, it can possibly be used to determine changes in cell wall architecture upon exposure to organic contaminants.

This study specifically uses FTIR to determine changes in the plant cell wall of sunflowers exposed to benzotriazole (BT). BT is a corrosion inhibitor commonly found in airplane deicing fluid, antifreeze, and gasoline. The 4 or 5 methyl derivatives of BT are also widely used. BTs have been detected in soil and surface and ground waters near facilities that utilize or produce them (Bausmith and Nuefeld, 1999; USEPA, 1977). BTs

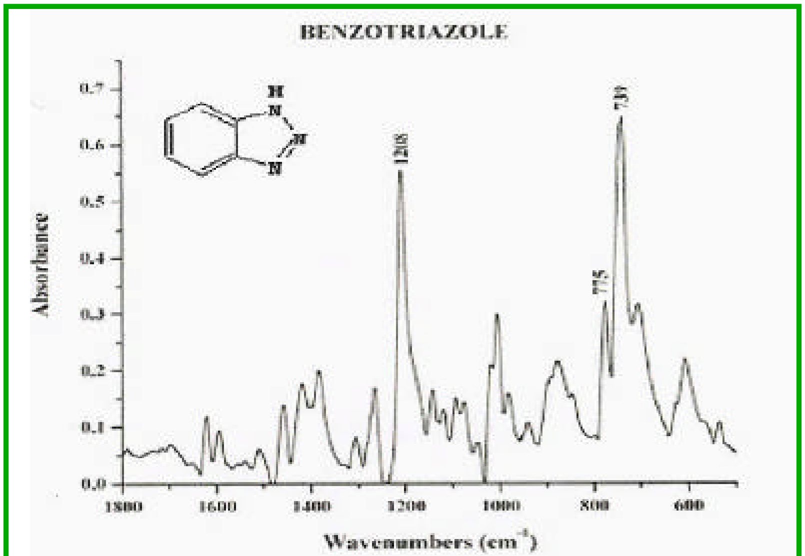

Figure 1. FTIR spectrum of benzotriazole.

are toxic to many aquatic organisms, and bacteria and is mildly carcinogenic (N.C.I., 1978; USEPA, 1977). It is resistant to ultraviolet radiation and very soluble in water, rendering it highly stable and mobile in the environment. At present, there are no known bacterial degradation pathways for BT (Rollinson and Callely, 1986).

Sunflowers have shown potential for remediating BT from contaminated waters and soil (Castro et al., 2001; 2000). However, the fate of BTs within the sunflower is unknown. Acid and organic solvent extraction of BTs from treated sunflower plants have been unsuccessful. However, previous studies provided evidence that BTs may be incorporated into the plant structure by lignification (Castro et al., 2001). Due their aromatic structure, BTs could possibly be biotransformed by the sunflower and mimic the phenylpropanoid subunits that constitute lignin. Lignin has been widely studied by FTIR due to its negative impact on paper pulping and forage digestibility (Akin et al., 1993; Jung and Deetz, 1993; Thomas, 1970). This provides the ability to determine spectral differences in the lignin portion of the plant cell wall sunflowers that are treated with BT. In this paper, we discuss the ability of FTIR to determine changes in the structure of the sunflower plants due to exposure to BT.

PROCEDURE

Sunflower seeds were germinated in vermiculite moistened with Hoagland's solution for one week under continuous lighting. After the germination period, the seedlings were transferred into amber stock bottles containing Hoagland's (nutrient) solution. The plants were separated into four stratified groups. Each group was given Hoagland's solution containing 0, 30, 60, and 90 mg/L benzotriazole, respectively. Hoagland's solution with and without benzotriazole was prepared as previously reported by Castro et al. (2001). The solution level was maintained by adding fresh solution daily. The plants were kept under continuous lighting for 15 days. After this growth period, the fresh weights of the plants were recorded. A sample was taken 10 minutes. after the addition of solution to the plants and after the growth period. The samples were analyzed by high-performance liquid chromatography (HPLC) for BT concentration. The plants were harvested and the roots, stems, and leaves were separated. The roots, stems, and leaves were dried, ground with a Wiley mill, and sieved to 100 mesh. Dried root samples (about 1g), untreated and treated with benzotriazole, were spiked by mixing with 0.1 mL of a 5% benzotriazole solution in methanol. The spiked samples were allowed to dry for 24 hours. All samples were analyzed in triplicate on a Nicolet Nexus 670 Fourier-transform infrared (FTIR) spectrometer. Potassium bromide pellets were made consisting of 5% plant matter by weight or 1% for pure benzotriazole. Sixty-Four scans at 2 wavenumber resolution were conducted for each sample. Spectral analysis was performed using OMNIC E.S.P. 5.2 software.

RESULTS

The FTIR spectrum of benzotriazole seen in Figure 1 resembles previously reported spectra by Mohan and Settu (1993). The absorbance bands at 1208, 775, 745, and 739 cm^{-1} are of particular importance due to their high intensity. These bands represent C-H in-plane and out-of-plane bending for the benzene ring of benzotriazole as seen in Table 1. Since these bands are highly intense, they should be easily seen in sunflower plants treated with benzotriazole. Absence of these peaks may suggest that the sunflower plant could have

Table 1. Assignment of important bands for FTIR spectra of pure benzotriazole and sunflower root samples (Mohan and Setu, 1993; Colthup et al., 1990).

Frequency	Assignment	Comments
1208 cm^{-1}	C — H in-plane bending	benzene ring in benzotriazole
870 cm^{-1}	Aromatic C — H wag isolated H of *meta*, unsymmetrical tri, symmetrical tri substituted benzene	aromatic rings in lignin
775 cm^{-1}	C — H out-of-plane bending	benzene ring in benzotriazole
745, 739 cm^{-1}	C — H out-of-plane bending	benzene ring in benzotriazole

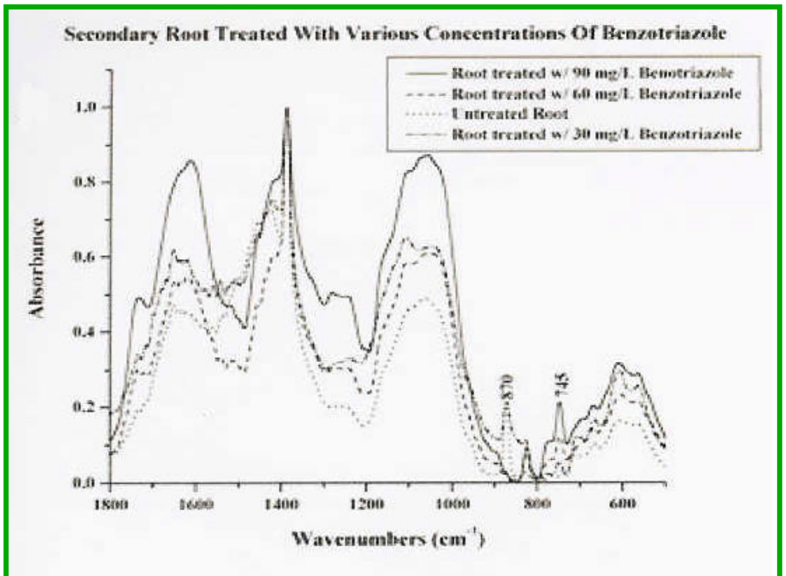

Figure 2a. FTIR spectra of sunflower roots samle untreated and treated with 30, 60, and 90 mg/L benzotriazole. FTIR spectrum from 1800-600 cm^{-1}.

transformed benzotriazole in some manner. This is a definite possibility because the benzotriazole could not be extracted from treated dry plant matter as mentioned previously.

Obvious spectral differences between untreated and treated root samples with various concentrations of benzotriazole can be observed in Figure 2a. The disappearance of the intense peak at 1208 cm^{-1} is quite noticeable. Spectral subtraction of untreated root sample from the treated root samples did not yield a peak at 1208 cm^{-1}, further suggesting a change in the benzotriazole structure upon absorption by the sunflower plant. However, the appearance of peaks at around 745 cm^{-1} in the treated samples does represent a part of benzotriazole that may have not been modified by the plant. The other interesting point to notice is the concentration dependence in absorption intensity at 745 cm^{-1} (Figure 2a). The absorption intensity increases as the concentration of benzotriazole increases. The disappearance of the peak at 870 cm^{-1}, representing an aromatic C-H wag of lignin (Table 1), may imply that the plant structure is changed when treated with higher concentrations (60 and 90 mg/L) of benzotriazole.

A simple experiment can be conducted in order to determine if the sunflower plant actually biotransforms benzotriazole. Dried untreated root samples can be spiked with benzotriazole and subsequently be analyzed by FTIR. Results of this procedure can be observed in Figures 2a and 2b. The 1208cm^{-1} and 775cm^{-1} bands represent C-H in-plane and out-of-plane bending for the aromatic ring in benzotriazole. Both appear in the spiked sample. Also, the lignin peak at 870 cm^{-1} still can be observed (Figure 2b) within spiked sample as well. Upon spectral subtraction of the untreated root sample and spiked sample, a spectrum that is similar to

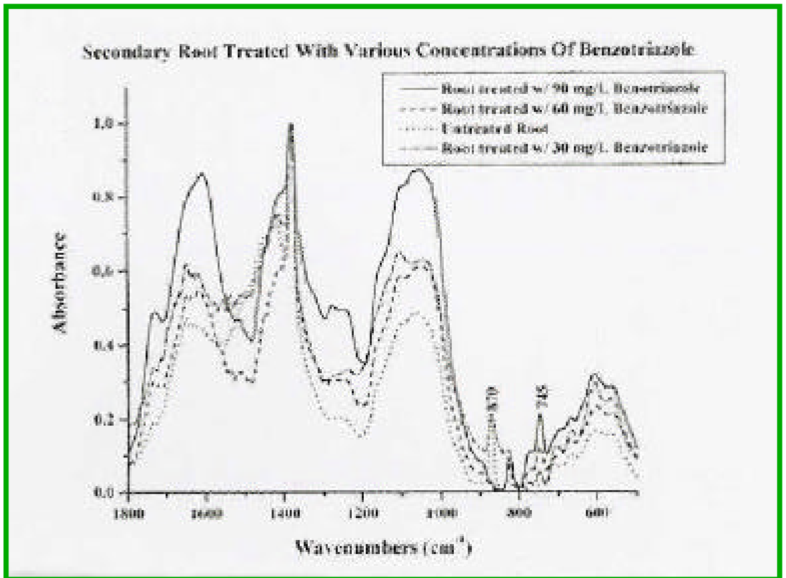

Figure 2b. FTIR spectra of sunflower roots sample untreated and treated with 30, 60, and 90 mg/L benzotriazole. FTIR spectrum from 900-7000 cm^{-1}.

benzotriazole is produced. This provides further evidence that the benzotriazole is being transformed by the sunflower plant.

CONCLUSIONS

Sunflower plants treated with benzotriazole display spectral changes in regions characteristic of the benzotriazole structure, particularly at 745 cm^{-1}. However, the intense peaks at 1208cm^{-1} and 775 cm^{-1}, characteristic to benzotriazole, were not observed in the treated samples. The sunflower plants treated at higher concentrations (60 and 90 mg/L) of benzotriazole display a change in the plant structure due to the reduction or disappearance of the lignin peak at 870 cm^{-1}. This can be attributed to a modification in the production of this structural component within the plant due to the uptake of benzotriazole. The spiked samples display every major intense peak due to benzotriazole and the major lignin peak at 870cm^{-1}. This means that the transformation of benzotriazole requires more than just contact with plant material. A living plant system is needed for the process to occur. This demonstrates that FTIR can be applied to studying changes in plant structure due to adsorption of organic contaminants. These results will help lead to the construction of a cost-effective, environmentally friendly phytoremediation system capable of phytotransforming benzotriazole and other similar organic contaminants using sunflower plants.

ACKNOWLEDGEMENTS

Although this article has been funded by the U.S. Environmental Protection Agency under assistance agreements R-819653, R-825549, and R-825550 through the Great Plains/Rocky Mountain Hazardous

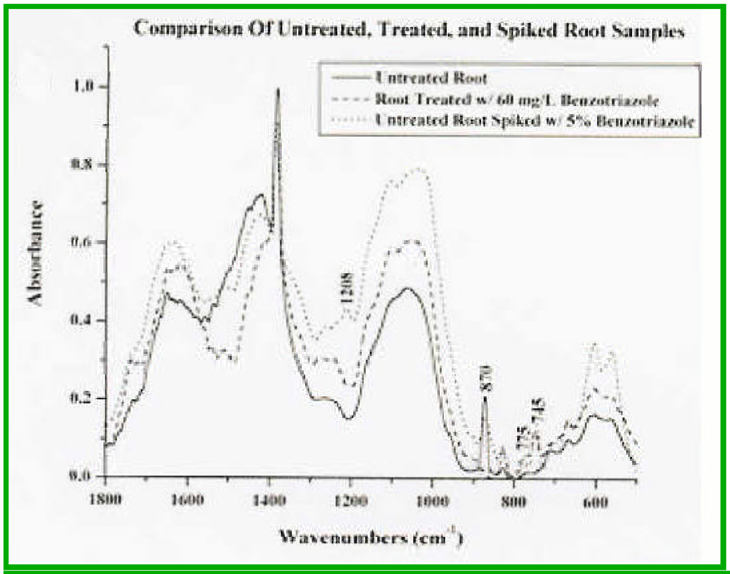

Figure 3a. FTIR spectra of sunflower roots sample untreated, untreated spiked with 5% benzotriazole, and treated with 60 mg/L benzotriazole. FTIR spectrum from 1800-600 cm^{-1}.

Substance Research Center headquartered at Kansas State University, it has not been subjected to the agency's peer and administrative review and therefore may not reflect the views of the agency, and no official endorsement should be inferred.

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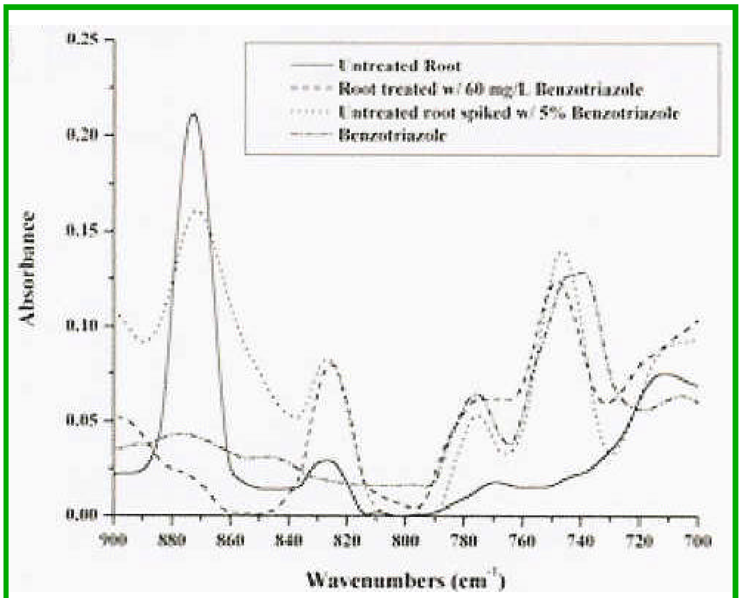

Figure 3b. FTIR spectra of sunflower roots sample untreated, untreated spiked with 5% benzotriazole, and treated with 60 mg/L benzotriazole. FTIR spectrum from 900-700 cm-1 (spectrum of pure benzotriazole included.

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