NITRIFICATION INHIBITION USING BENZOTRIAZOLES

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ABSTRACT

The common corrosion inhibitor, 1H-benzotriazole (Bz), found as a component of glycol-based aircraft deicers, and its derivatives such as 5-chloro-1H-benzotriazole (CBz), 1-hydroxy-benzotriazole (HBz), and 5-methyl-1H-benzotriazole (MBz), are all potential nitrification inhibitors. The inhibitory effect on the nitrification of urea-N fertilizer in agricultural soils was examined over a four-week period. All the compounds tested, except HBz, have the ability to inhibit the nitrification of urea-N fertilizer effectively. Their inhibitory capabilities depend on the structural components of the soil, particularly organic matter (O.M.). In the soil with the largest percent of O.M. (2.3%), inhibition by all the benzotriazole derivatives was considerably decreased. In the soil with least O.M. (0.3%), even HBz, the poorest of the inhibitors, showed signs of inhibition. For Bz, MBz, and CBz, in the range of 90% inhibition was observed in the soil with the lowest O.M. content during a four-week treatment period. On a mass basis, Bz had the greatest inhibitory effect followed by MBz and CBz, whereas HBz showed little inhibition of the nitrification of urea-N in soils with >0.5% O.M. If the inhibitory effects were looked at on a molar concentration basis, there would be little variation in inhibitory potency for MBz, CBz, and Bz. It was concluded that all three are effective nitrification inhibitors with urea-N fertilizer. The incorporation of benzotriazoles as inhibitors could help economize N fertilizers by helping prevent leaching and denitrification. However, the environmental fate of these compounds has not been determined, and they may produce toxicity to plants and other organisms.

Key words: nitrification, benzotriazoles, deicing fluids, corrosion inhibitors, methylbenzotriazole, aircraft deicing fluids (ADFs)

INTRODUCTION

Benzotriazoles have high stabilities both at high temperatures and in presence of UV light (US EPA, 1977), and have a wide range of industrial uses. Since they complex strongly with some metals (Cornell et al., 2000), they are commonly used as a corrosion inhibitor in glycol-based aircraft deicing fluids (ADFs). The majority of simple benzotriazoles currently produced go into such anticorrosion applications. Typical applications include the protection of copper-containing parts (for which benzotriazole excels) by inclusion of benzotriazoles in automobile antifreeze solutions, in recirculating water systems such as power plant and commercial air-conditioning cooling systems, and in coatings for protection of copper alloys in architectural and decorative applications. More complex nonpolar derivatives are also used widely to stabilize plastics and similar materials against the decomposition that would otherwise take place upon exposure of these materials to ultraviolet radiation. Metal chelation ability (to silver) accounts for their use in photography, mainly as an antifogging constituent of films to improve their photographic characteristics (US EPA, 1977).

Recently, together with laccase, benzotriazoles have been proposed as mediators in pulp bleaching to decrease the amount of bleaching chemicals in the papermaking process (Ander et al., 1997a; Bourbonnais and Paice, 1996; Call and Mücke, 1996, 1997; Paice et al., 1996).

Human safety is a concern with all these various industries involved with the consumption of benzotriazoles. These compounds present an environmental problem due to their appreciable water solubility, persistence under environmental conditions, and toxicity to microorganisms and plants (Pillard, 1995). Until now, there have not been reports revealing ways for treating waste streams containing benzotriazoles by conventional methods, due to the lack of microorganisms that can degrade most benzotriazoles (Rollinson and Callely, 1986). A non-conventional system for treating triazole-contaminated water could have significant merit. In modern agro-ecosystems, where large quantities of N fertilizers are used continuously, the efficiency of their use may be low and may result in environmental pollution (Puttanna, Nanje Gowda, and Prakasa Rao, 1999, 2001). Much fertilizer N applied to soils is in the form of ammonium or ammonium-producing compounds such as urea, and is usually oxidized quite rapidly to nitrate by nitrifying microorganisms in soil. The nitrate thus produced is susceptible to loss by leaching and denitrification, and there is international concern about pollution of ground and surface waters by fertilizer-derived nitrate. This concern has stimulated research to find compounds that will effectively inhibit nitrification of fertilizer N when applied to soils in conjunction with N fertilizers, and numerous compounds have been patented or proposed for this purpose (McCarty and Bremner, 1989). Efficient N management techniques, such as the use of nitrification inhibitors, may help in economizing N fertilizers in situations where leaching or denitrification is a problem. McCarty and Bremner (1989) first reported that benzotriazole was a nitrification inhibitor, while Puttana et al (1999) have demonstrated its efficacy in a field study.

By studying the possible use of benzotriazole derivatives as nitrification inhibitors in agricultural soils, we may obtain a better understanding of two environmental concerns, nitrate loss and benzotriazole fate in the environment.

MATERIALS AND METHODS

Soils used for all of the experiments reported here were surface soils obtained from agricultural sites near Manhattan, Kans. Properties are summarized in Table 1. The "river bank" soil was from a field along the Kansas River, while the "Manhattan pile" was topsoil that had been stored. The samples designated

"north" and "south" were from arable fields north and south of the river. Before use, each sample was air dried and crushed to pass to through a U.S. Standard Sieve (No. 8, 2380 microns, 0.0937 inches). Samples of the soils were then sent to the Kansas State Soil Testing lab to be tested for pH, sand %, silt %, clay %, and organic matter % (O.M.) (Table 1).

The benzotriazole derivatives used in the inhibition test were 1H-benzotriazole, 1-hydroxy-benzotriazole, 5-methyl-1H-benzotriazole, and 5-chloro-1H-benzotriazole. All were purchased from Sigma-Aldrich and used as purchased by preparing stock water solutions of 0.5 to 2 g/L, which were diluted as needed for soil treatment. Urea and potassium nitrate were from Fisher Scientific.

The procedure used to determine the effects of the test compounds on nitrification in soil was as follows: 100 grams of selected soil sample were placed in an amber glass jar (~0.5-0.8 L) and treated with enough solution to bring the soil to ≈60% field capacity. (The appropriate amount of water for each soil type was determined in a separate preliminary experiment.) The test solutions used consisted of deionized water, 10μg/g of soil of a specific test compound, and 100 μg/g of N as NH_2CONH_2 (urea-N). Controls were also made for all the soils consisting of a H_2O control (soils treated to ≈ 60% with a water solution only), a urea-N control (soils treated with 100 μg/g of soil of urea-N but omitting nitrification inhibitor), and a KNO_3 control (soils treated with 100 μg/g of soil of N as KNO_3 to represent 100% nitrification of N). The jars were covered but unsealed to incubate at room temperature (~22 degrees C).

Table 1. Soil analysis.					
Sample ID	pH	Sand %	Silt %	Clay %	Organic Matter %
River Bank	7.6	54	32	14	0.3
Manhattan Pile	6.2	22	45	33	2.1
North	7.4	44	42	14	2.3
South	7.5	26	58	16	1.6

The soils were analyzed for NO_3–N every week for a maximum of four weeks. Samples were analyzed using a Hach DR/2000 (direct reading spectrophotometer) following the method outlined by the manufacturer. Precisely weighed samples of soil (~6 g) containing a known proportion of water were treated with 42 mL of a solution of 0.3 % calcium sulfate to flocculate soil to provide clear samples for NO_3-N testing. After centrifugation, 25 mL of the clear supernatant were used for analysis. The Hach reagents were added, mixed for a controlled time of 1 min, and read after a fixed time. From the absorbance values obtained with a standard solution of nitrate, concentrations of the unknown solutions were determined. They were multiplied by 7 to convert them to mg/kg of fresh soil. The input amount of urea or nitrate was calculated to give 100 mg/kg N, in addition to the background amount present in the soil. The percent nitrification for each sample was calculated from (C-T)/Cx100, where T is the actual colorimeter absorbance level of the nitrification of urea-N in soil samples treated with the test compounds, and C is the actual absorbance level from the nitrification of urea-N controls. No correction was made for the initial amount of nitrate present in the soil. Thus the percent of inhibition was underestimated. An alternative calculation could use a correction for the initial amount of nitrate present in the soil, giving ΔC and ΔT values. As shown below, the nitrate level in untreated (water control) soils changed over time so that assumptions about inhibition of the endogenous ammonification and nitrification would be needed to get a more precise estimate.

DISCUSSION AND ANALYSIS OF RESULTS

Figures 1 through 4 show results for the indicated soil types as a function of time of incubation. In all cases, the measured absorbance level is corrected to an equivalent mass of soil, based on the actual sample mass taken. It is readily apparent from the water controls that the level of endogenous nitrate varies widely between soils. The good agreement over time in the measured nitrate from the potassium nitrate-treated soils indicates the reproducibility of the analytical method. For some soils, there is apparently an increase in endogenous nitrate, presumably due to nitrification of organic matter. Different soils show markedly different rates of nitrification of added urea. The ability of benzotriazoles to inhibit nitrification also differs for the different soils, mainly as a function of the organic matter content of the soil but also due to other conditions such as pH- when comparing the Manhattan pile soil to the north or south, which have similar levels of O.M.
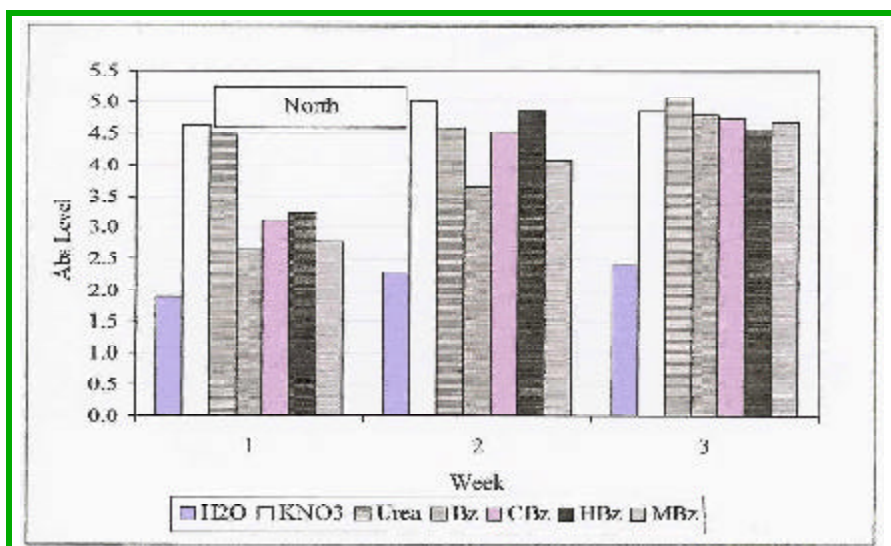
Figure 1. Soil from the north side of the Kansas River. Treatments and times are indicated on the figure. See Materials and Methods for details of treatment.

Table 2 provides a summary of the results expressed as relative inhibition of nitrification for various soil types and times of incubation. For each time interval, the extent of nitrate formation by urea alone was determined and set equal to zero percent inhibition. The different inhibitor treatments were then compared to that value. The extent of nitrification of urea differed considerably between soil types. It was found that the north soil was the first to completely nitrify the urea. By the first week of analysis, the concentration of nitrate-N in the urea-N only control was almost the same as the potassium-nitrate control, which represents the full nitrification of urea-N. The nitrate control had a concentration of 176 mg/L, whereas the urea-N control gave a concentration of 170 mg/L, only a 3.5% difference (see Figure 1). In treatments with the benzotriazole derivatives (Bz, HBz, MBz, and CBz), there was 28-41% less nitrate after the first week in the north soil. Even the 1-hydroxy-benzotriazole (HBz), found to have the least inhibiting ability of all the compounds throughout the experiment, inhibited the nitrification of urea-N in the north soil by 30 % during the first week. It is not clear if the observed rates represent the real inhibition capabilities of Bz derivatives because of the full nitrification of the urea-N control. At two weeks the urea-N control was at a level of 174 mg/L, while the concentration of the nitrate control was 190 mg/L. During the second week, inhibition by the test compounds was considerably less than the week before. The trends are consistent in showing that Bz and MBz have the ability to inhibit the nitrification of urea-N more effectively than CBz and HBz. The third week of analysis of the North soil, where differences between treatments are relatively small, most likely indicates just the variation in analysis. In the north soil, there is a large population of bacteria in the soil

equipped for the task of converting ammonia to nitrate rather well. In all cases, even with the presence of the test compounds, the urea-N fertilizer was completely transformed into nitrate-N by the third week. This could also be related to the large amount of nitrate-N already present in the soil. This level of nitrate-N present without the addition of urea-N fertilizer or potassium nitrate was observed for the three-week period in the H_2O control. Values were 72 mg/L in the first week, 87 mg/L in the second week, and 92 mg/L in the third week (see Figure 1). The slight increase in nitrate concentration over the three-week period in the water control could possibly indicate pre-existing ammonia or biomass N not yet converted into nitrate, which was converted upon moistening the air-dry soil. This high and changing background of nitrate complicates interpretation of the nitrification inhibition data, resulting in underestimation of the efficacy of the inhibitors.

Table 2. Nitrification inhibition by benzotriazole derivatives (%).

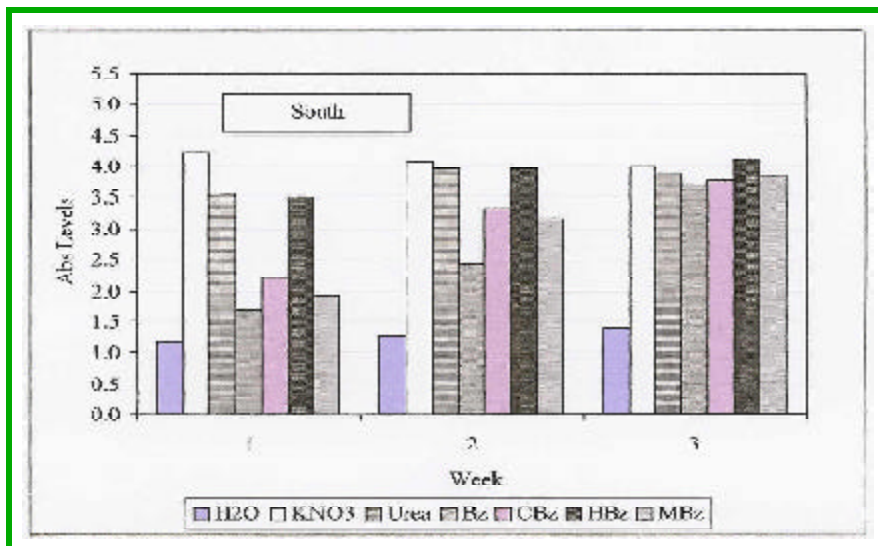| Soil Type	Treatment	Week 1	Week 2	Week 3	Week 4
North	Urea	0	0	0	---
North	Bz	40.8	20.4	5.2	---
North	CBz	30.2	1.5	6.8	---
North	HBz	27.7	-5.8	9.7	---
North	MBz	38.0	11.3	7.3	---
South	Urea	0.0	0.0	0.0	---
South	Bz	52.4	38.8	4.7	---
South	CBz	38.0	15.6	2.3	---
South	HBz	1.6	-0.4	-5.9	---
South	MBz	46.1	20.4	1.1	---
Manhattan Pile	Urea	0.0	0.0	0.0	0.0
Manhattan Pile	Bz	29.2	34.8	24.6	26.6
Manhattan Pile	CBz	15.3	22.2	41.5	47.4
Manhattan Pile	HBz	9.0	16.7	17.3	25.1

Figure 2. Soil from the south side of the Kansas River. Treatments and times are indicated on the figure. See Materials and Methods for details of treatments.

The south soil (Figure 2) had many properties similar to the north soil. Like the north soil, the urea-N control for the south soil reached near complete nitrification of urea-N by the first week. The nitrate concentration of the urea-N control at the end of the first week was 135 mg/L, while the nitrate control was 160 mg/L, indicating about 85% of full nitrification. As in the north soil, the HBz had little effect on the nitrification inhibition. Bz again had the largest inhibition (52 %), followed by MBz and CBz. The H_2O control of the south soil behaved similarly to the H_2O control of the north soil. Each week it slightly increased in its nitrate-N, from 44 mg/L after one week to 53 mg/L after three weeks. During the second week, the relative efficacy of inhibition remained in the same order as for week one. There was little inhibition evident in the third week.

The Manhattan pile soil behaved rather differently from the north and south soils in terms of the rate of nitrification (see Figure 3). By the end of the first week, the urea-N control was only at a concentration of 38 mg/L, while the nitrate control was at 166 mg/L, and the water control was 35 mg/L. During the second week, the Manhattan pile soil went from 22 % to 33 % nitrification in comparison to the nitrate control, which was at 158 mg/L. By the third week, the level reached 43 %, and by the fourth week it was 54 % nitrified in comparison to the nitrate control. The H_2O control had similar nitrate-N levels as the south soil, starting with a concentration of 35 mg/L and going up slightly to 44 mg/L. Overall, the Manhattan pile soil had a low nitrification rate. It would have been interesting to examine the samples for the Manhattan pile until the urea-N control reached full nitrification of the urea fertilizer, but the relative inhibition pattern generally was constant over the four-week testing period.
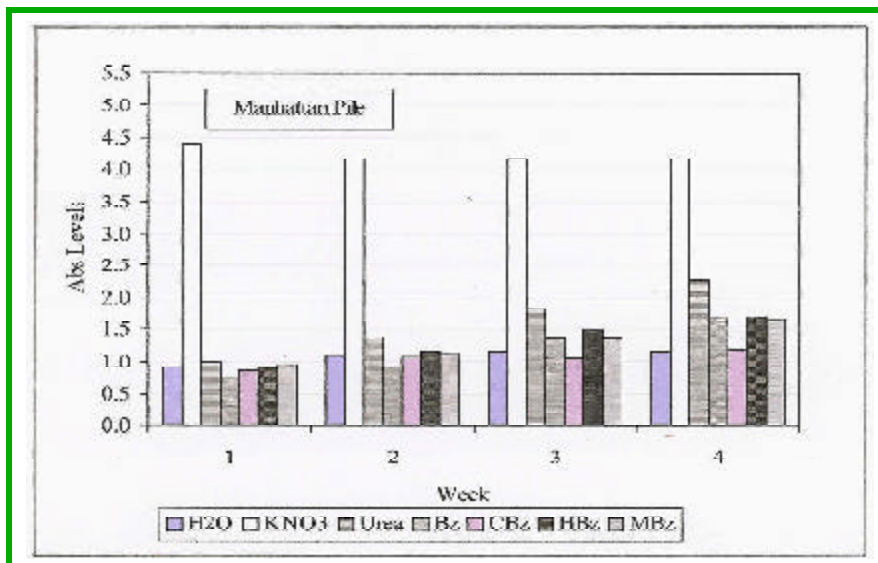
Figure 3. Soil from the Manhattan storage pile. Treatments and times are indicated on the figure itself. See Materials and Methods for details of treatments.

Like the Manhattan pile soil, the river bank samples were also tested for four weeks because of the slow nitrification rates of the test samples. The test compounds inhibited the nitrification of urea-N best in the river bank soil samples (see Figure 4). After the first week, the urea-N control was only 33 mg/L, 23 % relative to the nitrate control at 143 mg/L. All of the test compounds inhibited the rate of nitrification by at least 50%. The levels of nitrate found with the test compound samples were even less than the concentration of the H_2O control (18 mg/L). By the second week, the urea-N control had jumped to 95 % of the nitrate control. At three weeks, there was essentially 100% nitrification with a concentration of 147 mg/L. Concentrations of nitrate-N were still lower than the H_2O control (22 mg/L) in the cases of Bz, CBz, and MBz. Even at four weeks, these compounds kept concentrations of nitrate-N lower than the H_2O control (22 mg/L).

It is unclear what an average nitrification inhibition percent would be for an average soil, given the wide variation between soils. The makeup of the soils (specifically % O.M.) greatly affects the inhibitory strength of the compounds. The North soil had the highest organic matter (2.3%), and the compounds had the least effect in inhibiting the nitrification of the urea fertilizer. The effect of the test compounds in the river bank soil was the greatest, while organic matter level in the river bank soil was the lowest (0.3%). It was mostly sand (54%). It appears that the benzotriazoles bind to the organic matter, decreasing the amount available to inhibit nitrification. Even the Manhattan soil shows this trend, where although the initial rate of nitrification is relatively slow compared to the other soils, the inhibition percents are low. The Manhattan pile also has the second largest percent of organic matter (2.1%). There is also another aspect of the Manhattan
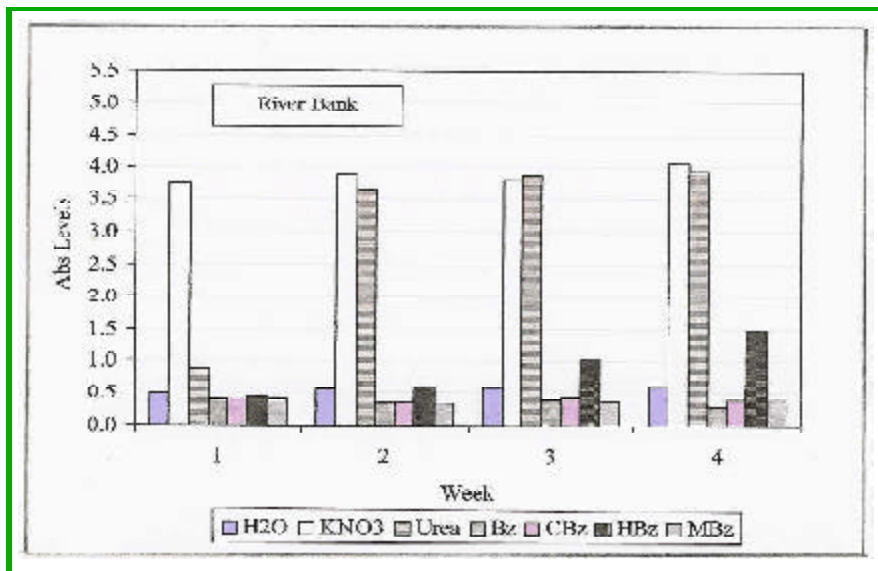
Figure 4. Soil from the bank of the Kansas River. Treatments and times are indicated on the figure itself. See Materials and Methods for details of treatments.

soil that makes it unique and possibly accounts for the slow nitrification rate of the soil. The pH of the Manhattan pile soil (6) is the lowest by a considerable amount. This low pH level could indicate the inactivity of bacteria in the soil responsible for the nitrification of ammonia. Another very important point is the consistent trends in potency of the inhibition. It is clear that HBz has little effect on the nitrification of urea-N. Consistently Bz has the greatest effect, where MBz has the second greatest effect and CBz has the least effect of the three. On a molar concentration basis, there is more Bz per mass of soil than MBz or CBz, because all treatments used the same weight ratio of inhibitor to supplied N (1:10). The formula weight of CBz is 153.5 g/mol, while Bz is 119 g/mol, a 32.5% difference. Thus the greater inhibition effect of Bz may simply indicate that it was present at a higher concentration, not that it is a more effective inhibitor.

CONCLUSION

The purpose of this research was to determine if benzotriazole (Bz) and its derivatives, such as 5-chlorobenzotriazole (CBz), 1-hydroxy-benzotriazole (HBz), and 5-methyl-1H-benzotriazole (MBz), could be used as nitrification inhibitors for fertilizers to increase their effectiveness and reduce the leaching of nitrate-N. All compounds have some inhibitory capabilities. Even HBz inhibited the nitrification of urea-N in some soils. The compounds Bz, MBz, and CBz were found to have significant nitrification inhibition in all soil types. Even in the extremely active north soil, all three effectively inhibited nitrification in the first week. In the instance of the inactive river bank soil, the three inhibited the nitrification up to 90 %.

It is unclear how the observed nitrification inhibition relates to environmental releases of aircraft deicing fluids (ADFs). Soil concentrations attained by land application of the ADFs may reach the levels studied here even when fairly small quantities of ADF are applied to the soil. The dosage used in these studies was 10 mg/kg soil, which would correspond to about 10 mL ADF as applied to aircraft with 20 % glycol in the ADF solution containing 0.5% by weight of Bz per weight of glycol, which is a typical composition (Castro et al., 2001). We have applied diluted glycol solution at levels of 5 g/L to soil for months without toxicity to grass. Just 400 such applications (400 d) would result in a cumulative dose of 10 mg/kg Bz, if it were present at the indicated ratio of 0.5% by weight relative to the glycol.

In the absence of proven pathways for degradation of the benzotriazoles, their application to soil is problematic. The experiments cited where it has been tested under field conditions are in another country, not under the same regulations that apply in the U.S. It is evident from the present work and that of Puttana et al. (1999, 2001) that inhibition of nitrification can occur if the benzotriazoles reach a critical concentration dependent on the soil organic matter content. It is also clearly established that benzotriazoles are toxic to plants and other organisms at higher concentrations, in the range of 10-50 mg/L solution (Cornell et al., 2000; Castro et al 2001). If the average soil water content is 20% by weight, a dosage of 10 mg/kg corresponds to 50 mg/L in the aqueous phase for a soil with zero O.M. Thus with <one application of the nitrification inhibitor, significant growth inhibition may be observed in plants growing in sand, unless they are able to detoxify it. Fortunately, it appears that higher plants are able to do so (Castro et al., 2001). Soils with high O.M. will show lesser growth inhibition. Hart et al. (in preparation) have determined the sorption properties of the soils studied here for each of the benzotriazoles. Knowing the sorption coefficient, it is possible to predict the available benzotriazole in the aqueous phase, and hence, the likely extent of toxicity to plants. Data is lacking on the capabilities of aquatic organisms to degrade or detoxify the benzotriazoles. It appears that during ADF treatments currently being used, dilution is the only attempted solution of the pollution problem. Clearly more needs to be done to manage these common contaminants.

ACKNOWLEDGMENTS

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