PHYTOREMEDIATION OF AIRCRAFT DE-ICER AND ANTIFREEZE FORMULATIONS

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ABSTRACT

Aircraft de-icer fluids (ADFs) and antifreeze solutions are mainly formulations of either propylene or ethylene glycol and additives such as benzotriazole methyl substitution isomers (tolyltriazoles), and surfactants. Although the formulations are proprietary, in general the glycols are present in concentrations that vary from 20 to 95 % and the triazoles at levels up to 1 g/L. The ADFs are used and discharged to the environment in large quantities, constituting a terrestrial and aquatic environmental risk. Although the *in situ* biodegradation of glycols has been achieved under controlled conditions, the (bio)degradation potential and mode of toxicity of the triazoles remain uncertain. Triazoles are identified as possible carcinogens and inducers of toxic responses in aquatic flora and fauna. Lignin peroxidase, which is the catalyst in the lignification process in higher plants, is an enzyme that is able to degrade triazole. We found a threshold for toxicity of triazoles to common grass and to sunflowers, of about 0.1 g/L. It was not possible to extract triazoles from the treated plants. The presence of glycols and surfactants in the ADF mixture impacts plant growth and health. These compounds can reduce the ability for the plant to immobilize the triazoles.

Key words: *aircraft de-icing fluid (ADF), phytoremediation, tolyltriazole, propylene glycol, ethylene glycol*

INTRODUCTION

Human safety is an overriding concern at airports, and the Federal Aviation Administration (FFA) requires the use of de-icing chemicals to ensure passenger safety during winter operations (Mericas and Wagoner, 1994). Aircraft de-icing fluid (ADF) is an aqueous glycol-based mixture that is routinely used to control ice formation on aircraft before takeoff. De-icing fluids are applied by spraying on the aircraft before departure. A significant portion of ADF runs off the aircraft, where it can enter storm drains and nearby surface or ground water, causing potential damage (Cancilla et al., 1998).

The discharge of untreated ADF wastes to surface water and storm water collection systems is of both regulatory and practical concern. Airports are searching for alternative methods for managing ADF wastes, but municipalities are often reluctant to accept untreated ADF waste as influent to their treatment systems. On-site active treatment and/or recycling are costly options, applicable to larger airports. On-site dryland or wetland treatment is a favorable approach because airports necessarily cover a significant area of land (Revitt et al., 1997; Roseth et al., 1998). Land treatment of propylene glycol-based ADF under controlled conditions is potentially an effective means of remediation for ADF solutions in soils for concentrations up to 20% by weight of propylene glycol (Bausmith et al., 1999). However, benzotriazoles and surfactants may inhibit bacterial decomposition of glycols. Although the solutions can be diluted to prevent this inhibition, the benzotriazoles may seep into groundwater systems prior to undergoing biodegradation. Surfactants in ADF may

increase the toxicity of triazoles to microbes or plants. While actual compositions of ADFs are proprietary, when diluted for use they are approximately as follows: propylene glycol, 20-30%; tolyltriazole, 0.05-0.2%; surfactants and viscosity enhancers 1-2%; other additives 1-2%; water 65-80%. Some properties of the main components in the ADF are shown in Table 1 (Sax and Lewis, 1989; USEPA, 1977; Howard and Meylan, 1998).

As a way to address the ADF disposal problem, we are investigating alternatives to handle components present in the formulation individually as well as the complete formulation, focusing on the corrosion inhibitors (tolyltriazoles). The chemical structure of 1-H-benzotriazole is shown in Figure 1.

Methyl groups on the benzene ring yield the 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole (USEPA, 1977), collectively referred to as "tolyltriazole," which contains

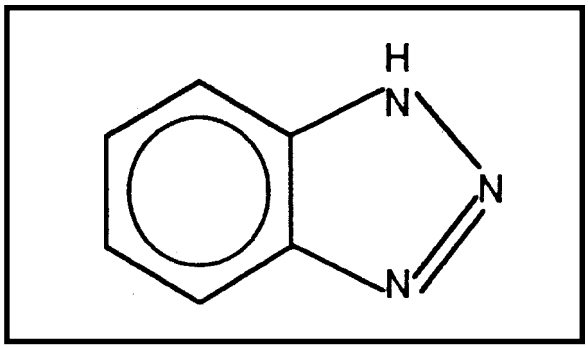

Figure 1. Chemical structure of 1-H-benzotriazole.

approximately equal amounts of the two isomers. Benzotriazole and its derivatives are stable to high temperature and dissipate absorbed ultraviolet (λ_{max} around 275 nm) as heat. The low vapor pressure of triazoles (about 4 x 10^{-5} to 5 x 10^{-5} atm) ensures little of these contaminants in the atmosphere; however, because of their appreciable water solubility, they can potentially migrate to groundwater. A retardation factor of about 2 in soil with an organic matter content of 1% indicates that

Table 1. Relevant properties of ADF components of our concern.

Characteristic	Propylene Glycol (PG)	Methyl Benzotriazole (MBz)
Boiling point (°C at 1 atm)	188.2	> 300
Freezing point (°C at 1 atm)	- 59	76 - 87
Vapor pressure (mm Hg at 20°C)	0.08	0.03
Solubility in water (at 20°C)	Hygroscopic	20 g / L
Specific gravity	1.05	1.24 (solid)
Theoretical oxygen demand (mg O_2 / mg)	1.68	1.56
Log octanol-water partition coefficient (Log K_{OW})	-0.92	1.44 [a]
Henry's Law constant (atm m^3 / mol) at 25°C	1.29 x 10^{-8}	3.17 x 10^{-7}

[a] The value reported for 1 H-Benzotriazole is 1.44, while for methyl benzotriazole, the log Kow estimated using heptanol is 1.81 (USEPA, 1978).

triazoles are weakly sorbed to soil (our unpublished observations).

Stability is a key concept in benzotriazole chemistry, giving it utility in many beneficial applications but leading to undesirable persistence under environmental conditions. Benzotriazoles do not readily oxidize or hydrolyze; they are not reactive under sunlight irradiation; and there is no evidence of biodegradation of them by bacteria (Rollinson and Callely, 1986). Their structural similarity to naturally occurring substances (e.g., adenine, guanine, indole) suggests that they could inhibit the production of proteins, enzymes, and RNA in mammalian systems and affect the central nervous and endocrine systems as well (USEPA, 1977). Although the evidence is not strong, they have been identified as possible carcinogenic substances (NCI, 1978). They have been shown to induce toxic responses in model ecosystem receptors including fish, invertebrates, and marine and soil bacteria at relatively low concentrations (Cornell et al., 2000; Cancilla et al., 1997; Hartwell et al., 1995).

Little is known about the mode of toxicity of benzotriazoles in plants. They have some similarity to natural plant growth regulators such as auxin and cytokinin (Klingensmith, 1961; USEPA, 1977). They are also effective metal chelators, which is the basis of their anticorrosion properties. Graham (1986) proposed that copper chelation could result in male sterility in wheat treated with benzotriazole. Damage to the root system seems to be the main effect that we observed. We have noted growth inhibition with pumpkin, horseradish, sunflower, and fescue at solution concentrations below 100 mg/L (Wu et al., 1998).

It is believed the majority of the production of benzotriazoles goes into anticorrosion applications. This includes the protection of copper-containing parts by inclusion of benzotriazoles in automobile antifreeze solutions, in the formulation of aircraft de-icer solutions, in recirculating water systems such as power plant and commercial air-conditioning systems, and in coatings for the protection of copper alloys in architectural and decorative applications. Their use may have markedly increased since the 1977 estimate of 28,000 tons per year (USEPA, 1977), because aircraft de-icers have increased in use and more vehicles are on the road. Recently the 1-hydroxy derivative is being considered as an alternative laccase mediator in biopulping for paper production, a process from which enormous amounts of a by-product, benzotriazole, might be discharged to the environment (Call and Mucke, 1997). There is no openly published environmental impact report on this application.

We have investigated the fate of benzotriazoles in plant-based remediation for use at airports. We have reported that triazole degradation may be achieved by the metabolic action of the enzyme lignin peroxidase (Wu et al., 1998). However, the fungus culture conditions under which lignin peroxidase is produced are fairly specific (Aust et al., 1997). These characteristics make it difficult to develop a feasible on-site treatment with the fungus. Higher plants strengthen their cell walls by lignification, which is the free-radical catalyzed

polymerization of methoxylated aromatic alcohols with laccase and lignin peroxidases as the catalysts (Ruhland et al., 1958). This suggests that benzotriazole may be reactive in plants where lignin is being synthesized.

MATERIALS AND METHODS

Pure benzotriazole, 1-hydroxybenzotriazole, and methyl benzotriazole were purchased from Aldrich / Sigma Chemical Co. Tolyltriazole was a gift of Mark Hernandez, University of Colorado. The compounds were kept as aqueous stock solutions to treat plants and as calibration standards for chromatography. Stored solutions appeared to be stable for at least two years. De-icing fluid was obtained from the local airport in the diluted-as-applied form. It contained 22% (v/v) propylene glycol (PG) and about 1 g/L tolyltriazole.

For separation and quantification of methyl benzotriazole (MBz), detection at 275 nm was used with liquid chromatography on a Hamilton PRP-1 Column, with methanol (50-70%) + water as eluent at a flow rate of 1.0 mL/min. Phenolic exudates from plants elute at a shorter time than the tolyltriazole, so the methanol level was adjusted to optimize resolution of compounds of interest in each experiment. Appropriate standards were run each day of analysis.

The monitoring of PG was done indirectly, measuring the depletion of sodium periodate (absorbance decrease at 260 nm) by the oxidation reaction. The adaptation for the flow injection analysis work included the reaction at 65°C and acid conditions (pH between 4 and 5) to improve the sensitivity. The mobile phase for the assay was a solution of sodium p-periodate 0.1 mM, in sodium hydroxide (0.05 M), boric acid (0.1 M), and acetic acid (10 ml/L). The samples were injected and mixed with the mobile phase using a HPLC pump and loop injector. Reaction was on high-density polyethylene tubing for four minutes at 65°C. The system allowed us to detect solutions below 1 mM (~0.1 g/L) very rapidly, even for a large number of samples.

Plants selected for study were fescue grass and sunflowers. The grass, (*Festuca arundinacea*) K-31 cultivar, is a very common grass used at airports and represents a perennial monocot with an extensive fibrous root system. The sunflowers (*Helianthus annuus*) are rapidly growing dicots that produce woody stems in a short season, thus guaranteeing the production of peroxidase from the lignification process. The plants were cultured in different media: aqueous solution (for the wild sunflowers only), pure fine vermiculite, or in a mixture of silty sand topsoil / vermiculite (volumetric ratio 1:2). The soil was obtained from a site at a closed landfill on a river floodplain that has been extensively used for our previous studies. While grass was grown in vermiculite, sunflower seeds were planted in moist vermiculite for 10 days and then the seedlings were transplanted to different containers (with either vermiculite or soil / vermiculite mixture) to apply some specific treatment. Pure vermiculite has a higher cation exchange capacity and therefore the availability for ionic compounds (nutrients, contaminants) is higher. For the sunflowers, when the first two

main leaves were 4 cm long, the plants began to be watered with the corresponding solution. The lighting was done with regular fluorescent light bulbs, either 24 or 12 hours a day.

Some nutrients (based on Hoagland's solution [a]) were added to the medium; if not specified, the concentration was ¼ of that recommended by Hoagland. The solutions of triazole, PG, and ADF were prepared in the nutrient solution as well. Three kinds of containers were used: 600 mL (~10 cm diameter, 12 cm high), 800 mL (~7 cm diameter, 22 cm high), and 1500 mL (~10.5 cm diameter, 22 cm high). Details for particular experiments are given below.

RESULTS

Sorption of Triazoles to Culture Media

Given their organic nature, triazoles are sorbed to the culture media in which the plants are grown. For the concentration ranges tested (50 to 200 mg/L), sorption factors were measured as the ratio of the concentration of an aqueous sample from the media to the input concentration. For vermiculite, this ratio was approximately 0.71, which means that the sorbed fraction was 0.29 (29% of MBz was sorbed to vermiculite). The mixture of vermiculite and top soil gave a ratio of approximately 0.44 (56% of MBz was sorbed) and for silty sand topsoil rich in roots, the ratio was approximately 0.26 (74% of MBz was sorbed). These results show clearly that the effective concentration of MBz in the aqueous phase of the culture media depends greatly on the organic matter content of the media. While tolyltriazole gave similar results to the sorption values of MBz, benzotriazole showed a lower sorption (in the soil / vermiculite mixture. Its ratio was 0.66 with 34% sorbed), possibly because the absence of the methyl group makes the benzotriazole less hydrophobic.

Tolerance of Grass to Methyl-Benzotriazole

Fescue grass seedlings were transplanted to vermiculite (10 per 600 mL container) and treated with either methyl-benzotriazole or benzotriazole solution. At a concentration 50 mg/L, they grew nearly normally while those treated at 100 mg/L were stunted. For more mature fescue grass, watered with solutions of triazoles for about 50 days, the biomass production (as percentage in weight of leaves produced with respect to a healthy untreated control) and the disappearance of triazole from the medium (measured as the loss of the compound with respect to the material introduced) were recorded. The results are shown in Table 2. The leaves were trimmed after the first 20 doses and then again after 20 more doses. The aqueous phase was analyzed after the total of 40 doses.

A concentration greater than 50 mg/L of either methyl-benzotriazole or benzotriazole was toxic, and it was lethal at a concentration of 150 mg/L. For concentrations below 50 mg/L, the

[a] Standard Hoagland's solution contains: KNO_3 (404 mg/L), KH_2PO_4 (109 mg/L), $MgSO_4.7H_2O$ (394 mg/L), $Ca(NO_3)_2.4H_2O$ (1476 mg/L), $FeSO_4.7H_2O$ (6 mg/L), EDTA Na_2 (8 mg/L), H_3BO_3 (3 mg/L), $MnCl_2.4H_2O$ (2 mg/L), $ZnSO_4.7H_2O$ (0.2 mg/L), $CuSO_4.5H_2O$ (0.2 mg/L), and $H_2MoO_4.H_2O$ (0.2 mg/L).

plants grew somewhat less vigorously than untreated controls, but could be maintained for long periods with no cumulative toxicity. Benzotriazole was somewhat less toxic than methyl-benzotriazole.

Several extraction methods were investigated for the recovery of the triazole from the stems and leaves of the plants. Solvents including water, methanol, ethanol, and acetone in different sequences, extraction times, and temperatures (as high as 96°C) were tried unsuccessfully. Even with 1 M NaOH or 1 M HCl solutions at low and high temperatures, there was no recovery. This leads us to think that the triazole is being bonded and/or transformed within the plants resulting in a non-extractable form of the compound, maybe as part of the plant structure.

Tolerance of Sunflower to Methyl Benzotriazole

After two months of growth outside, wild sunflowers were transferred to the laboratory and maintained in aqueous solutions containing different concentrations of triazoles. Fresh solution was added based on their consumption such that solution volume remained constant. In general, the rate of water consumption per gram of initial fresh plant material decreased with time of triazole treatment. This effect was larger when the triazole concentration was greater than 100 mg/L. It was possible to observe the gradual damage of the roots, which first turned brown, followed by little new root growth. The plants apparently accumulated triazole and transformation products within their structure (up to about 3.0 to 5.0 mg of triazole incorporated per gram of fresh initial plant material over a period of about 35 days). The accounting of triazole was done by measuring the concentration of the solution in the container at different times and comparing this with the concentration expected in this solution based on the amount added. Similar triazole extraction methods as those done for the fescue grass, including

Table 2. Biomass production and triazole disappearance for fescue grass treated with methyl-benzotriazole and benzotriazole solutions.

Compound	Concentration Fed (mg/L)	% Biomass produced relative to control		Triazole that disappeared	
		After 20 doses	After 20 more doses	(mg)	%
Methyl-Benzotriazole	50	35.8	17.3	23.7	49.8
	100	44.3	5.1	17.6	18.9
	150	43.8	1.45	3.21	2.4
Benzotriazole	50	46.2	26.4	39.2	78.3
	100	31.3	3.6	33.9	36.5
	150	27.7	0.6	33.5	24.8

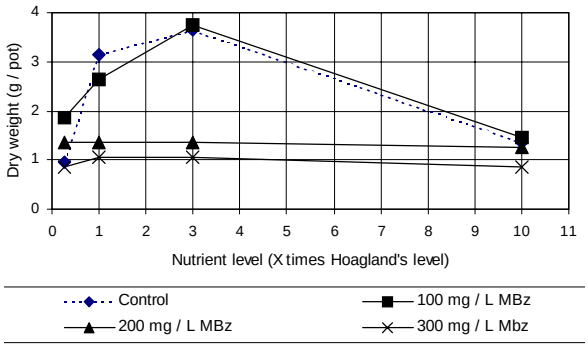

Figure 2. Biomass produced in soil (dry weight of plant material) by sunflowers treated with different MBz levels: effect of nutrient amendments.

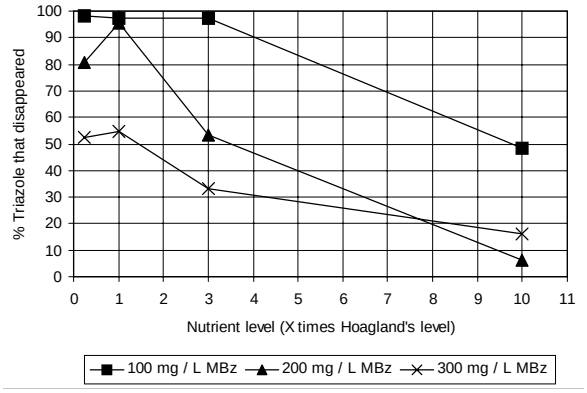

Figure 3. Percentage of MBz disappearing from soil solution: effect of nutrient amendments.

stems and leaves, were also unsuccessfully attempted, even at these high levels of accumulation. Either the triazoles were degraded in solution or they were incorporated and transformed to plant biomass.

In a one-month treatment, sunflower seedlings (three plants per 800 mL container) grew in the soil / vermiculite mixture almost normally (as high as the controls) when watered with methyl benzotriazole solution at 25 mg/L. The leaves, however, turned yellow and brown and were less vigorous than the controls, with the effect more pronounced in the bottom leaves. The top leaves were nearly normal and the plants flowered about five days before the controls did. These effects were more evident at 50 mg/L. At a concentration of 100 mg/L the plants were 20% shorter than the controls and produced small precocious flowers. The leaves and roots, however, showed much more damage.

In order to improve plant growth conditions while being treated with the triazole, we studied the effect of adding an extra supply of nutrients to the soil/vermiculite mixture. Sunflower 10-day old seedlings (2 per 800 mL container) were watered with solutions containing the triazole at concentrations of 100, 200, and 300 mg/L prepared in the nutrient solution at four levels of strength: ¼ (¼X), 1 (1X), 3 (3X), and 10 (10X) times the concentration recommended by Hoagland; and then compared with control solutions of the same concentration of nutrients without any triazole. The results are shown in Figures 2 and 3. Figure 2 shows the biomass produced (dry weight of the plant material) for the different triazole treatments with the different nutrient levels during the 30-day period. As mentioned above, it should be noted that the effective concentration of triazole is reduced as much as one half by the sorption of it to the organic matter in the soil. It is clear that the plants can tolerate a low level of triazole (100 mg/L) for all different levels of nutrients; however, increasing the nutrient level has a negative effect possibly because of the high level of nitrogen (A separate study showed that an N level greater than 700 ppm i.e., 3X Hoagland's, is toxic to the plants.).

For concentrations of MBz equal or greater than 200 mg/L, the nutrient effect in the tolerance is not appreciable. With respect to

the triazole disappearance (Figure 3), increasing the nutrient level has a positive effect up to about 1 X (the level recommended by Hoagland) for all triazole treatments.

Josten and Kutschera (1999) reported that borate is an essential element for formation of new (adventitious) roots on sunflower seedlings. Because root formation appeared to be the first inhibited process in triazole-treated plants, we tested the effect of increased borate on the triazole inhibition of plant growth. Plants (4 per 600 mL container) were grown for 20 days in vermiculite and watered with a fixed amount of ¼-strength Hoagland's solution per day, containing supplementary borate at two different levels (5 x and 10 x the control rate (¼ Hoagland's)) and varied levels of triazoles (100 and 200 mg/L). Plants treated with the higher levels of triazole and borate died from excess water because the triazole markedly decreased water use and plant growth compared to the controls. Plants treated with the lower extra level of borate (5/4 Hoagland's) and 100 mg/L triazole concentration grew as much as 75% of the controls.

A second experiment was done with a soil/vermiculite mixture in 1500 mL containers with four plants. Plants were treated with either tolyltriazole or MBz at 100 mg/L or 200 mg/L. The solution input was more closely controlled to match water use than in the first experiment. This makes description of the dosage regime more difficult, but plants were treated until those exposed to the highest input level of triazoles were killed. Supplemental borate at three and nine times the control rate (the later is more than twice that of standard Hoagland's solution) showed no significant difference from the lowest level (¼ Hoagland's), indicating that borate did not have a marked effect on the triazole toxicity. Indeed, after 20 days of treatment, the plants exposed to the highest level of triazole and no extra level of borate looked better than those with the highest level of triazole and extra borate, but still not as good as the controls. There was no observable difference between the plants treated with MBz and tolyltriazole at similar concentrations.

A different way to look at the problem is by considering the metal- binding capacity of the triazole. Although required by the plants in very small amounts, metals such as Zn, Mo, Cu, and Mn (also called trace elements or micronutrients) are essential for the plant development in different biochemical pathways. We thought that the triazole toxicity could be the result of a chelating process that prevents metal uptake by the plant. Therefore, we tried to enrich the soil by using Hoagland's nutrient solution and adding an extra supply of the micronutrients at three different levels: 1X, 3X, and 10X, where X is the concentration of the micronutrients in the original Hoagland's formulae. We did not add an extra supplement of borate since it already showed no improvement.

Two sunflower seedlings were transplanted to pure vermiculite in the 600 mL containers and were treated with MBz solutions (100 and 200 mg/L) prepared in the modified Hoagland's. The plants were watered every day until the desired concentration was achieved. We observed that the plants with higher micronutrients were able

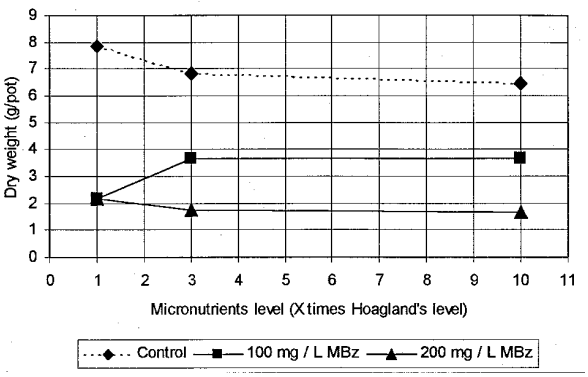

Figure 4. Biomass produced in vermiculite (dry weight of plant material) by sunflowers treated with different MBz levels: effect of micronutrient amendments.

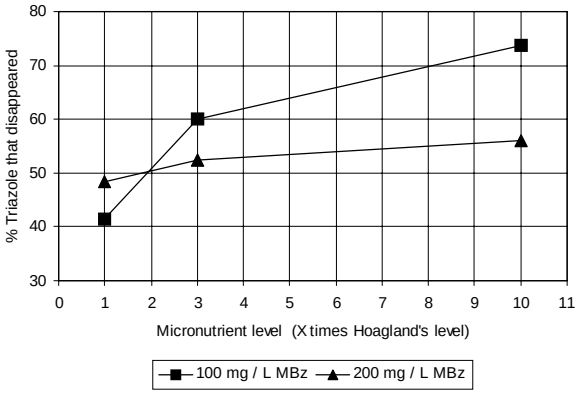

Figure 5. Percentage of MBz disappearing from solution: effect of micronutrient amendments in vermiculite.

to tolerate more MBz, so we held the plants at that level (maintaining volume with water) for seven days and then we continued watering with the solution until the predicted soil solution level was doubled. The results are shown in Figures 4 and 5. There is not a dramatic effect in the plant growth (biomass production) when no triazole is present. The level of micronutrients can be increased even up to 10 times that recommended by Hoagland's with no toxic effect. The plant tolerance to MBz was increased somehow, but their final weights were very comparable. However, more MBz disappeared at the higher levels of micronutrients, leading us to think that indeed the triazole toxicity might have to do with metal uptake inhibition.

Tolerance of Sunflower to Propylene Glycol

Sunflowers at about 10 days age, grown from seeds in soil/vermiculite mixture, were treated in 800 mL containers with ADF solutions at concentrations of 1.0, 2.0, and 4.0 % of ADF (2.3, 4.6, and 9.2 g/L of glycol), watering from the top with a daily dose of about 50 mL depending on the excess of runoff observed. After a period of 24 days, the plants treated with 4% of ADF had died, while the ones treated with 1% survived, although with limited growth and some leaf-damage. We think that PG absorption by the plants leads to its accumulation in the leaves, which subsequently causes an osmotic effect that damages the plant.

Therefore, the conditions of the experiment were changed to try to achieve aerobic degradation of the glycol prior to its contact to the plant roots. We utilized part of the soil previously used to degrade glycol and combined it with fresh soil. This time the watering was done from the bottom, allowing enough time for the soil/plant system to take up the solution (with ADF doses reduced to 0.5, 1, and 2%) and for oxygen to diffuse in. From this experiment we saw rather than PG accumulation, some kind of nutrient deficiency in the plants, maybe because of intense bacterial activity that consumed nutrients required by the plant.

Under similar conditions in another experiment, plants (3 per 800 mL container) were

watered from the bottom with solutions of PG, ethylene glycol, antifreeze, and ADF solutions at a concentration of 2 g/L of glycol in standard 1 X Hoagland's solution or Hoagland's solution + 10 x potassium phosphate. With the higher level of phosphate, the plants can grow in PG, ethylene glycol, or antifreeze with no particular damage. The ADF-treated plants (equivalent to 2 g/L of glycol and 8 mg/L of triazole) show some leaf burn with or without additional phosphate, while the standard phosphate treated ones also consumed less water than the others.

Plants were treated in 800 mL containers (three per container) with ADF solutions at different levels. Those treated with 5.0 g/L of glycol (~20 mg/L of triazole) showed poor growth but survived while plants treated with 10 g/L of glycol and 40 mg/L of triazole showed acute toxicity with just three days of treatment, perhaps because the triazole level at this concentration inhibits the glycol degradation and affects the bacterial populations and root development. The surfactants in the de-icer formulation may be an additional contributing factor in the toxicity of ADF to plants.

CONCLUSIONS

We propose that triazoles might be transformed by lignin peroxidases and laccase of plants to incorporate and immobilize them into the lignin fraction. When added at a level beyond the ability of the plants to cope, MBz causes root death and eventually plant death. The dose-response curve for triazole is peculiar, with a doubling of input concentration changing from a "no-effect" to a toxic response. The toxic threshold of MBz in an aqueous solution (about 100 mg / L) seems to be about the same for several plant species (Wu et al., 1998 and our unpublished observations), and is the same whether plants are grown hydroponically, with vermiculite, or in a low-organic soil. The tolerance of the plants and their triazole-degradative capability is enhanced by the addition of nutrients to the soil media. The improvement is greater when an extra supplement of trace elements (excluding borate) up to 10 times that recommended by Hoagland's is added to the soil. A screening process that might reveal the compound (or compound mixture) that promotes this plant-resistance is under investigation in the search for the mechanism of toxicity of MBz to plants. Oxalate oxidase, an enzyme that may generate peroxide for the lignin peroxidase, is a manganese enzyme (Requena and Bornemann, 1999). Laccase requires copper. The triazoles are known to be effective copper chelators at high concentrations (USEPA, 1977) and may also bind other metals. The toxicity could be such a direct effect or it could be through mimicking a plant growth regulator (Klingensmith, 1961).

In situ soil biodegradation of PG from ADF is possible under controlled conditions and is promoted by maintaining aerobic conditions and supplying nutrients, including an extra addition of phosphate. However, the toxic effect of the PG on plants (mainly the alteration of the leaves osmotic equilibrium) causes plant weakness and inhibits the triazole immobilization in plants. At the same time, bacteria degrading the glycol might be inhibited by the presence of

high levels of triazoles. It is still necessary to find more appropriate conditions, i.e., concentration of contaminants, supplementation of nutrients, soil pre-adaptation, and location of the input for which the degradation of both contaminants can be achieved in a field scale. Soil tilling and drip irrigation techniques can be used in field applications to assure oxygen diffusion and aerobic conditions.

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