PHYTODEGRADATION KINETICS OF METHYL BENZOTRIAZOLE

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ABSTRACT

Plant roots may promote the disappearance of organic pollutants from soil solution. We have studied the activity of plants that react with benzotriazole (Bz) and some of its derivatives, such as tolyltriazole (Tz), a commonly used corrosion inhibitor in aircraft deicing formulations, 5-methyl benzotriazole (5-MBz); and 1-hydroxy benzotriazole (HBz). At levels below the toxic threshold (about 100 mg/L was found for several plant species), all the triazoles appear to be degraded by plant enzymatic action, since their concentration in the aqueous phase of the culture decreases with time and they cannot be recovered from the plant tissue by solvent extraction. Hydroponic studies with sunflowers (*Helianthus annuus*) were designed to investigate the behavior of the solution concentration vs. time and to determine kinetic parameters (reaction order and rate constant) for phytodegradation of triazoles. Analyses were performed to determine whether the disappearance behaved as commonly found for enzyme-catalyzed reactions, fitting the Michaelis-Menten kinetic model of rate vs. concentration. The data for some of the plant systems followed the Michaelis-Menten model better than a first-order model. Experiments with other plant species are in progress.

***Key words**: corrosion inhibitor, methyl benzotriazole, kinetics, sunflowers, phytodegradation*

INTRODUCTION

Because benzotriazoles have high stability (USEPA, 1977) and complex strongly with some metals (Cornell et al., 2000), tolyltriazole, a mixture of the 4- and 5- isomers of methyl benzotriazole (MBz), is commonly used as a corrosion inhibitor in glycol-based aircraft deicing fluids (ADFs). Nonetheless, 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 triazoles by conventional methods due to the lack of microorganisms that can degrade both isomers of the tolyltriazole mixture (Rollinson and Callely, 1986).

A non-conventional system for treating triazole-contaminated water has been proposed by Castro et al. (2000). This consists of a plant-based *in situ* land-remediation technique, under the rationale that vegetation is capable of immobilizing triazole compounds, either by irreversible sorption or phytodegradation, when the effective bioavailable concentration is not greater than 100 mg/L of MBz in soil solution. Above that concentration, the triazoles are visibly toxic to plants and inhibit root growth and normal plant development. This paper presents an experimental investigation of the disappearance achieved in hydroponic culture of sunflowers (*Helianthus annuus*) for four different members of the triazole family (see Figure 1): 1-H-benzotriazole (Bz), 1-hydroxy-

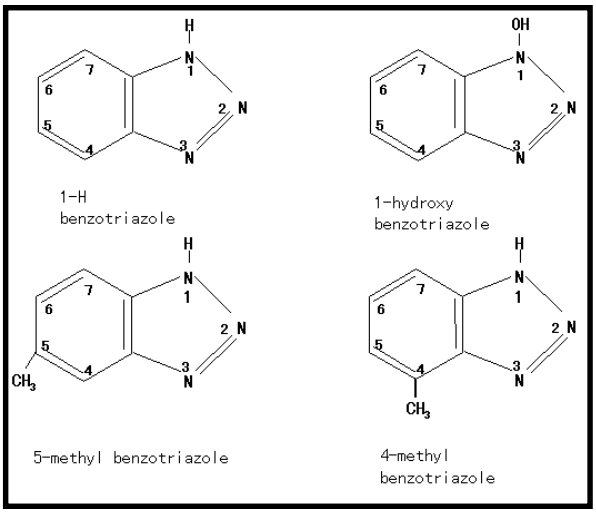

Figure 1. Chemical structure of some triazole compounds. Tolyltriazole is a mixture of 4-methly benzotriazole and 5-methyl benzotriazole.

benzotriazole (HBz), 5-methyl-benzotriazole (MBz), and tolyltriazole (Tz). The experiments were designed to obtain more information about the interactions of plants with these compounds, measuring concentration decrease and its relation to water uptake, initial concentration, and photoperiod.

MATERIALS AND METHODS

A batch of 30 sunflower seeds was placed in moist vermiculite under continuous lighting with 40-watt, cool white fluorescent tubes. The light energy available for photosynthetic activity fell in the range of 150 – 200 μmol/m^2, measured with a quantum meter (Apogee instruments). After 10 days, the most healthy and similar looking seedlings were selected (19 in total) and placed individually in brown glass bottles containing 500 mL of Hoagland's solution [a]. The plants were held by open-cell polyurethane foam plugs, assuring that only the roots were in contact with the water phase. The water level was maintained nearly constant by adding fresh water every other day. The plants were kept under continuous lighting for a period of 15 days, after which their fresh weight was obtained. The weights varied from 7.5 to 14 g/plant (average 10.5 g/plant).

The plants were distributed into five stratified groups, four with four plants and one with three plants. Each group had about the same mean weight. For the first phase of the experiment, the solution in the bottles was then replaced with a solution of 30 mg/L of the triazole prepared in Hoagland's solution. Each of the four-plant groups had a different triazole and was maintained in continuous light, while the three-plant group (with 5-MBz) was used for the 12:12 h dark:light cycle and designated as MBz 12 h. After about 10 min from addition of the stock solution, samples were taken from each bottle, and then every 12 h, distilled water was added to bring the volume back to 500 mL and more samples were taken. Right after sampling, the samples were analyzed in a liquid chromatograph on a Hamilton PRP-1 column (150 x 4.1 mm), with 60% methanol in water as eluent at a flow rate of 1.0 mL/min. This system allowed a minimum detection level of 3±1 mg/L. After five days of treatment, the final fresh weights of the plants were recorded and the solution in the bottles was replaced by uncontaminated Hoagland's solution, where they were kept for about five days and then treated for

[a]Standard Hoagland's solution contains KNO_3 (404 mg/L), KH_2PO_4 (109 mg/L), $MgSO_4.7H_2O$ (394 mg/L), $Ca(NO_3)_2$ (1476 mg/L), $FeSO_4.7H_2O$ (6 mg/L), EDTA Na_2 (8 mg/L), H_3BO_3 (3 mg/L), $MnCl_2.4H_20$ (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).

four and one-half more days with a higher concentration of the same triazole at 60 mg/L in Hoagland's solution, with the same lighting scheme as previously. The fresh weights at the beginning and end of the second phase were also recorded. Three plants appeared to be infected with some kind of fungus in the first phase and therefore, their triazole compound assignment was interchanged with healthy plants to see if the illness was related to the contaminant. One of the sick plants recovered and the other two showed a partial recovery, while two more got the infection in the second phase. However, these responses did not appear to be related to the triazole treatment. Because the data for fungal-infected plants differed significantly from the others, no data from sick plants or recovering plants were used in the analysis.

After the second phase, the more healthy plants (two from each triazole group for a total of 8) were selected and the contaminated solution was replaced with fresh Hoagland's solution, where they were kept for about seven days under continuous lighting. Since the plants were larger and there were fewer of them, the containers were moved to enable all plants to receive light without being covered by other plants. The third phase of the experiment began by replacing the clean solution with triazole-contaminated Hoagland's solution at a level of 60 mg/L. The daily water uptake was replenished with more of this triazole solution instead of distilled water, maintaining all plants under continuous light. Samples to monitor the concentration change were taken every 24 hrs after bringing the volume back to 500 mL. After seven days of treatment, the plants were divided into roots and shoots and the fresh weights of each section were recorded. The roots were placed into 50-mL-assay tubes and a volume of methanol equal to approximately two times the fresh weight was added to extract any triazole reversibly bound. The tubes were closed and kept in the dark and after two days, a sample from the supernatant was analyzed for triazole content.

RESULTS

During the first and second phases, the triazole concentration decreased similarly with time for all the triazole treatments except for the hydroxy-benzotriazole (HBz), which showed a slower decrease and in some cases the plants did not appear to have the necessary enzymes to transform it. During the first phase, the concentrations of the four bottles in each group of plants at each sampling time were close enough to consider them as replicates. For the second phase, the plants that were infected with the fungus suffering from stress, which was evident by observing the decrease in their water uptake, biomass production, and triazole-transforming capacity such that the concentration of triazole did not decrease at the same rate as the others for the same group. Consequently these infected plants were not included to obtain averages. Figure 2 shows the average concentration at every sampling time for the plants in the first phase (notice the lower and smaller disappearance for HBz); error bars (± one standard deviation) are indicated for the 5-MBz-24h treatment and are similar for the other

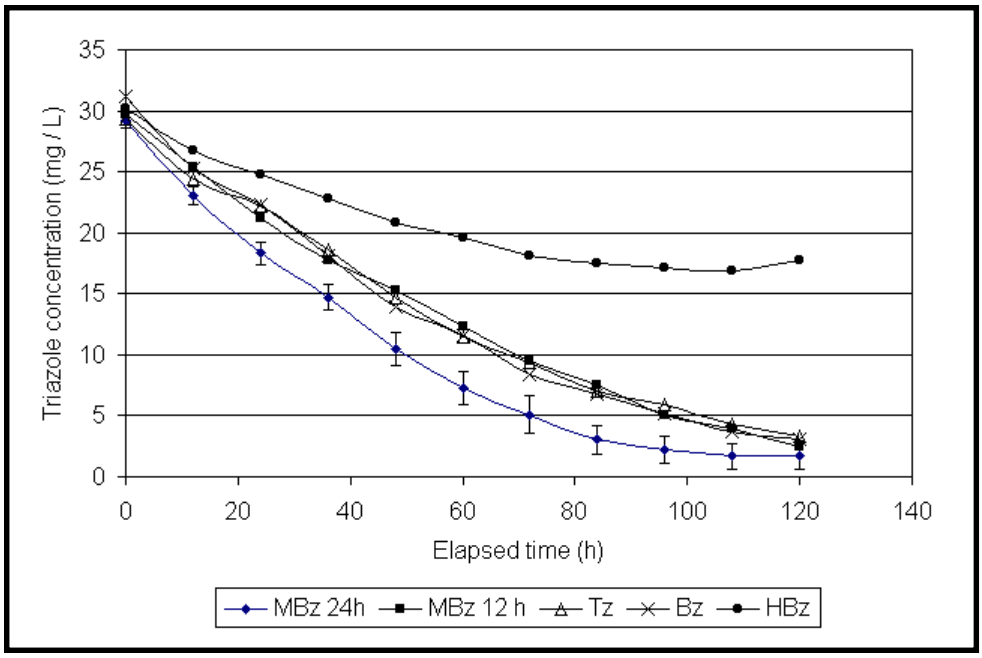

Figure 2. Concentration curves for sunflowers treated with low initial triazole concentration (30 mg/L, phase 1).

compounds. Figure 3 shows the same plot just for the benzotriazole-treated plants in the second phase, with the average obtained without including the infected plant. During the third phase, the concentration of all triazoles but the HBz decreased with time, indicating again the incorporation of benzotriazole and its methyl isomers into the plant matrix and the different uptake and transformation pattern of the hydroxy benzotriazole. However, in this phase, the concentration did not decrease as fast as during the other phases since the respective triazoles were being added daily; nevertheless, the trend of the concentration curve was to level off to an approximately constant value around 100 mg / L for the HBz and 40 mg / L for the other triazoles (See Figure 4).

The plant tolerance to the different triazoles was determined by observing their water uptake (Trapp et al., 2000) and/or comparing their biomass production. As shown in Figure 5, during the first and second phases, the average water uptake was similar for all triazole-treated plants within the phase and it increased as the plant was growing. For constant illumination, measured values ranged from 38 to 70 mL / 12 h for phase 1 and 62 to 93 ml / 12h for phase 2. It should be noted that the plants under the 12:12 h dark:light cycle presented the expected variability for the water uptake. At night the overall water uptake was reduced to as low as 15 mL, while during the day it was similar to the others. At the beginning of phase 3, the plants were taking up more than 200 mL of solution per 12 h; but during this phase, all plants showed a decrease from about 230 ml / 12h to 123 mL / 12h except for the HBz-treated plants, which consumed no less than 180 mL / 12h. This indicates that triazoles influence water uptake, but the mechanism is unknown.

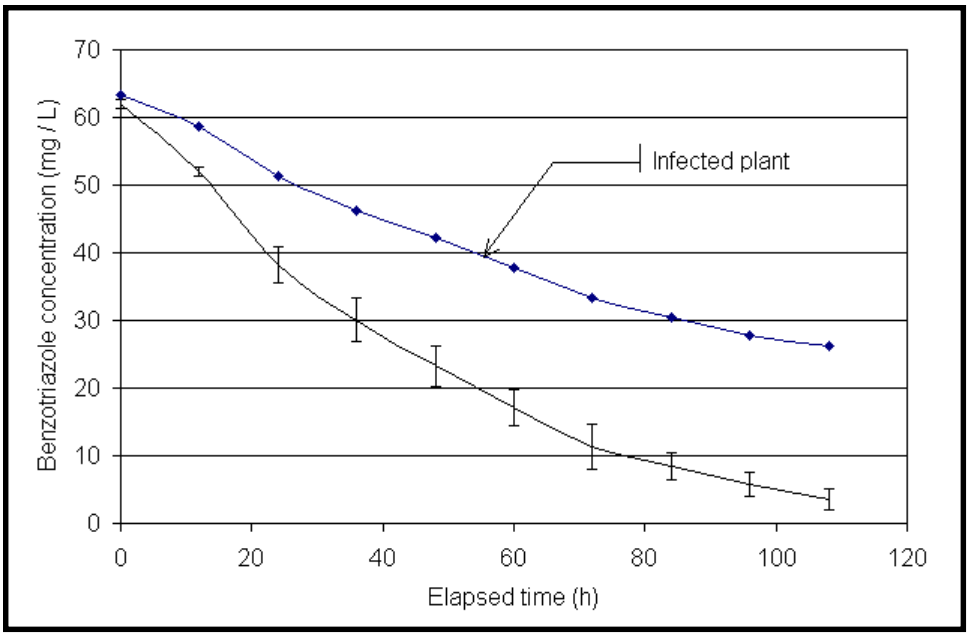

Figure 3. Triazole concentration for sunflowers treated with high initial Bz concentration (60 mg/L) under 24 h lighting.

The biomass production, evaluated as the percent change in the total fresh weight of the plants, showed the expected trend with respect to the stage of plant growth and lighting scheme. As presented in Figure 6, during phase 1 the percent increase in fresh weight was similar for all triazole treatments at about 67% for the 24h light scheme but lower for the 12h light scheme at about 47.5 %. In phase 2 it was about 33% for all triazole-treated plants under 24h lighting and 27% for the 12h light scheme. Thus it was concluded that during the first two phases, the biomass production did not show any particular trend regarding the triazole to which the plants were exposed. As discussed before, the fungal-infected plants presented a reduced plant growth (change between 0 and 10 %), and they were not included in the averaging. However, during phase 3 (with a constant supply of triazole solution), the HBz-treated plants showed a much higher biomass production (22%) compared to the other triazole-treated plants (from 4 to 8.6 % change in fresh weight). These results demonstrated that all the triazoles but the HBz were interacting with the sunflowers and affecting their natural development, but the effect was more evident when the plants received a daily supply of the triazole to maintain it at a high level. Although the capability of plants to phytotransform triazole derivatives was not strongly affected by the stage of growth, initial concentration, or lighting scheme, since in all phases the concentration decreased, the water uptake and biomass production seemed to be somewhat affected. The results in Figures 4, 5, and 6 show that the plants were able to transform HBz (1-hydroxy benzotriazole), but the process of transport and transformation was different in some way from that observed for the other triazoles. In phase 3, the growth of the sunflowers was better for plants with HBz, and the water uptake was largest for plants with

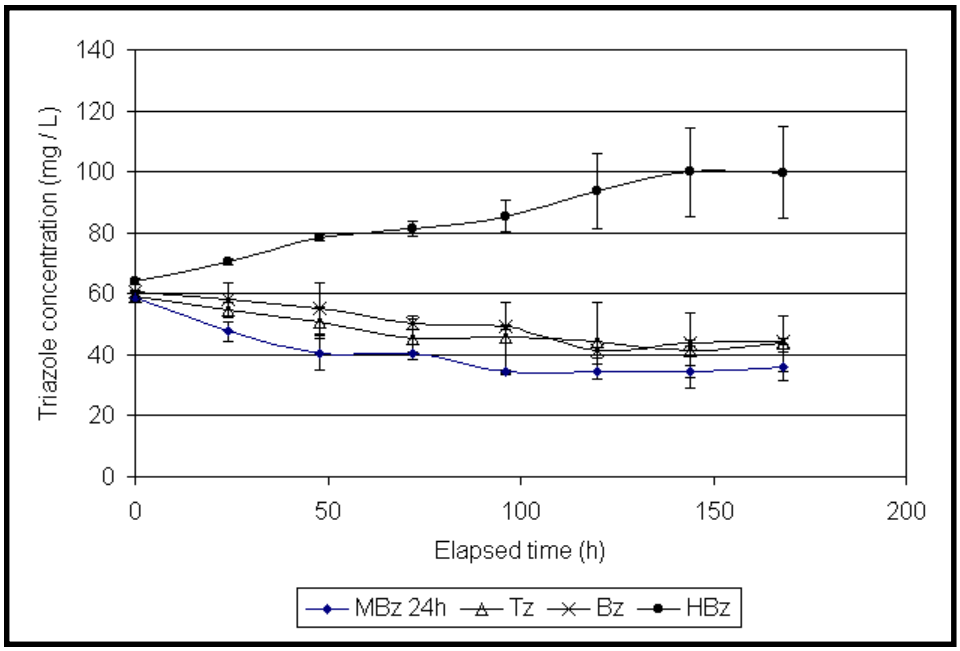

Figure 4. Concentration curves for sunflowers treated with constant supply of high triazole concentration (60 mg/L, phase 3).

HBz. The steady state condition observed in Figure 4 includes a feed concentration of 60 mg/L of HBz and a solution concentration of about 100 mg/L. If all the HBz enters the plant with water uptake, this would give a transpiration stream concentration factor of 0.6.

For each time interval, the initial concentration and water uptake were measured and the final concentration was calculated using the amount of triazole present after replenishing the uptake (for which the concentration was measured), minus the amount added with the solution (which was zero for the first two phases), divided by the final volume for the interval (taken as the total volume minus the volume of solution added). The ratio of the total triazole disappearance (calculated as the initial mass of triazole minus the final mass of triazole for the interval) to the disappearance due to water uptake (calculated as the volume taken up by the plant times the average concentration for the interval) was obtained for each subgroup. Figure 7 shows this ratio for the plants treated at 60 mg/L initial triazole concentration, excluding the hydroxy-benzotriazole-treated plants. It is clear that the total amount that disappeared was always greater than the amount taken up with the evapotranspiration stream, since the ratio was about two and tended to be higher as the treatment time passed. It is also evident that during the night, the process by which the triazoles were being incorporated into the plants did not stop, making the ratio as high as eight in Figure 7. Similar results were seen during phase 1, and for phase 3 this ratio oscillated around 1.5 to 2.5 without showing a particular trend. In the case of hydroxy-benzotriazole, this ratio was never greater than one. The observations that the loss of triazole did not depend directly on water uptake, was not greatly affected by triazole concentrations less than about 100 mg/L, and continued happening at night indicate that

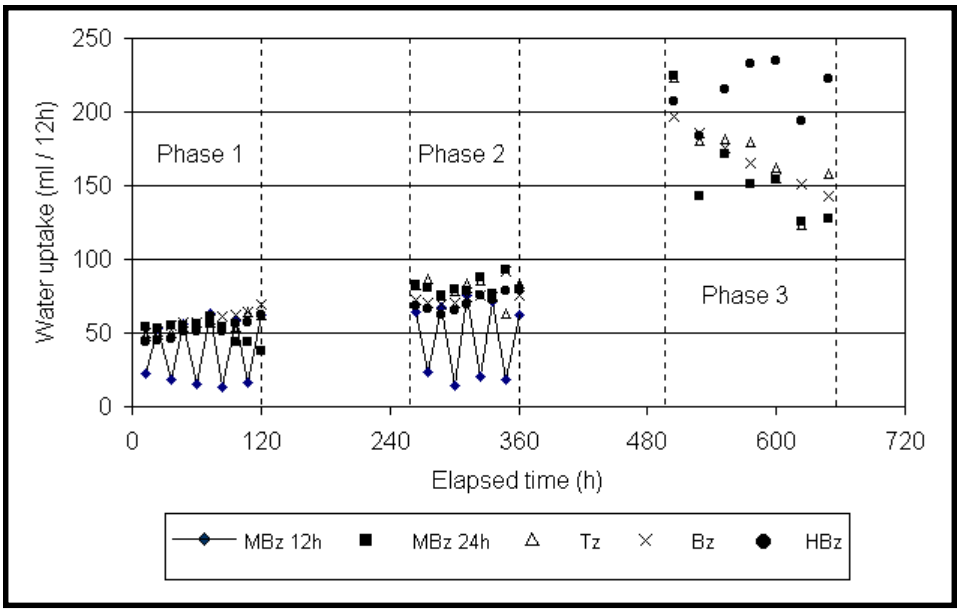

Figure 5. Water uptake for sunflowers treated with different triazoles for the three phases.

the process is happening in the roots. Additionally, the benzotriazole, 5-methyl benzotriazole, and tolyltriazole could not be recovered from the plant material, either in roots or shoots after 24-h extraction with methanol. By the end of the third phase, the plants had taken up considerable amounts of triazoles. These results point toward a nonreversible binding or chemical incorporation in the roots, possibly by lignification. On the other hand, the presence of plant phenolic compounds of strong absorbance at the UV wavelength of the system (275 nm) did not allow an appropriate resolution to separate and distinguish the hydroxy benzotriazole in the methanol extract, even when the percent methanol eluent was reduced to 50%. This did not allow us to establish the degree of non-reversible binding of HBz.

ANALYSIS OF KINETICS

The kinetics of the process were approached by performing nonlinear-least-squares analysis in a spreadsheet such as Excel (Orvis, 1996). Because these kinds of processes have been described with models such as first-order rate with exponential decay (Susarla et al., 2000), or Michaelis-Menten enzymatic kinetics (Castro et al., 2000), these two models were explored. The concentration-time data was used as an integrated reaction progress curve (Yun and Suelter, 1977) to obtain the parameters of the models. The nomenclature used for the mathematical expressions is defined in Table 1.

The concentration was converted to the amount present at each moment of sampling by multiplying concentration by the total volume. The amount-time data were converted to rate or velocity using the numerical method of finite differences. This method has the advantage of taking into account more than two points for estimating the rate, therefore better assessing the trend. It was necessary to use this method since during some time intervals the concentration varied more than 10%, which means an assumption of constant concentration and velocity

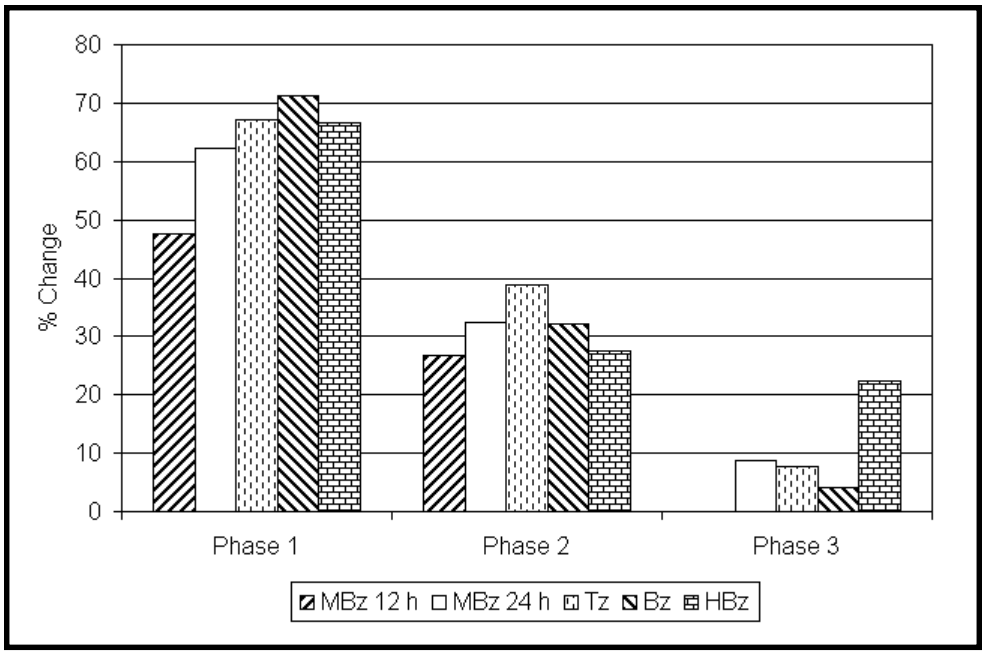

Figure 6. Percent change in biomass for the sunflowers treated with different triazoles for duration of the three phases. Phase 1 was 5 days; phase 2 was 4.5 days, and phase 3 lasted 7 days.

does not apply. The numerical method requires that the data points of the independent variable, time, be equally spaced:

$$\Delta t = t_{i+1} - t_i \qquad i = 1,2, ..., N-1 \quad (1)$$

This was achieved during the experimentation because samples were taken every 12±0.2 h. The rate at each average concentration value for each interval can be calculated using the following formulas (Fogler, H.S., 1999):

•Initial point:

$$V_1 = \left(\frac{dA}{dt}\right)_1 = \left(\frac{\Delta A}{\Delta t}\right)_1 = -\frac{-3A_1 + 4A_2 - A_3}{2\Delta t} \quad (2)$$

•Interior points:

$$i = 2, 3, \ldots, N\text{-}1$$

$$V_1 = \left(\frac{dA}{dt}\right)_i = \left(\frac{\Delta A}{\Delta t}\right)_i = -\frac{A_{i+1} - A_{i-1}}{2\Delta t} \quad (3)$$

•Last point:

$$V_N = \left(\frac{dA}{dt}\right)_N = \left(\frac{\Delta A}{\Delta t}\right)_N = -\frac{A_{N-2} - 4A_{N-1} + 3A_N}{2\Delta t} \quad (4)$$

The velocity was correlated to the average of the initial and final concentrations for each interval for each of the models. The first-order rate constant, k_f (L/h), was obtained by using the following expression:

$$\left(\frac{dA}{dt}\right)_i = -k_f C_{favi} \qquad i = 1, 2, \ldots, \text{N-1} \quad (5)$$

Here the subscript *f* refers to first-order estimate and the average concentration for the interval was taken as:

$$C_{favi} = \sqrt{(C_i * C_{i=1})} \qquad i = 1, 2, \ldots, \text{N-1} \quad (6)$$

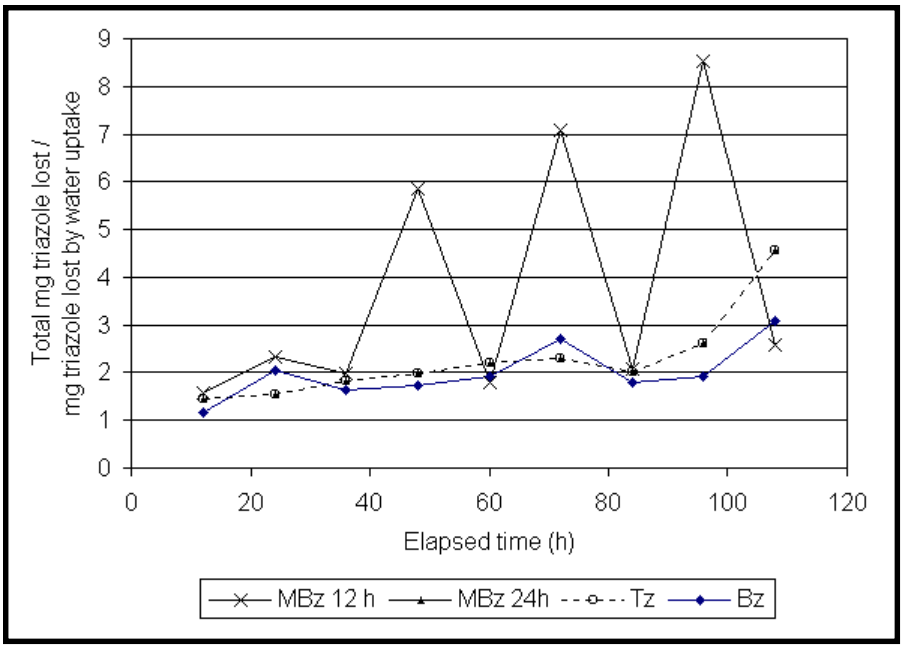

Figure 7. Ratio of total triazole loss to estimated loss by water uptake for sunflowers treated with high initial triazole concentration (60 mg/L).

because for each interval the concentration decrease should follow the same first-order rate model.

The parameters of the Michaelis-Menten model (subscript *M*):

$$V_i = \left(\frac{\Delta A}{\Delta t}\right)_i = \left(\frac{dA}{dt}\right)_i = \frac{V_{\max} C_{Mavi}}{K_M + C_{Mavi}}$$

$$i = 1, 2, \ldots, \text{N-1} \qquad (7)$$

were obtained for each interval using the equations:

$$K_{Mavi} = \frac{V_{i+1} C_i C_{i+1} - V_i C_i C_{i+1}}{V_i C_{i+1} - V_{i+1} C_i} \qquad (8)$$

$$V_{\max avi} = \frac{V_i \left(\frac{V_{i+1} C_i C_{i+1} - V_i C_i C_{i+1}}{V_i C_{i+1} - V_{i+1} C_i} \right) + V_i C_i}{C_i}$$

$$i = 1, 2, \ldots, \text{N-1} \qquad (9)$$

These parameter values and value of the average velocity:

$$V_{avi} = \frac{V_i + V_{1+1}}{2} \qquad i = 1, 2, \ldots, \text{N-1} \qquad (10)$$

were then used to find an average value of concentration for the same time interval such that the Michaelis-Menten model was satisfied in the interval; that is:

$$C_{Mavi} = \frac{V_{avi} K_{Mavi}}{V_{\max avi} - V_{avi}} \qquad i = 1, 2, \ldots, \text{N-1} \qquad (11)$$

Using the values of $V_{av\,i}$ and $C_{M\,av\,i}$, the values of the parameters K_M and V_{max} for the total treatment period were estimated using a nonlinear least-squares routine. Parameter values for the different plants were used to obtain the mean values presented in Table 2. In all cases, the value for the regression coefficient

(r^2) for the Michaelis-Menten model was better than for the first-order model; the r^2 for the first-order model was as low as 0.389 with the average being 0.744, while for the saturation model the values of r^2 fell between 0.798 and 0.989 with an average of 0.931. To evaluate the goodness of fit, a Chi-square test was performed comparing the estimated values for the rates (E) with the experimental observations (O):

$$x^2 = \sum_{i=1}^{N-1} \frac{(O_i - E_i)^2}{E_i} \qquad (12)$$

for each of the models. Larger values of the sample statistic, x^2, indicate greater differences between the proposed model and the experimental data. The critical value will depend on the level of significance, α, and the number of degrees of freedom, d.f. (Brase and Brase, 1995). For the plants treated with an initial triazole concentration of 30 mg/L, a total of 11 samples were taken (d.f. = 10) and for the treatment with initial triazole concentration of 60 mg/L, the number of samples was 10 (d.f. = 9). Thus, with $\alpha = 0.995$, the critical x^2 values are 2.16 for phase 1 and 1.73 for phase 2 while with $\alpha = 0.95$, the critical x^2 values are 3.94 for phase 1 and 3.33 for phase 2. These results are

Table 1. Nomenclature for data analysis.

Symbol	Definition	Units
$i = 1,2, \ldots, N$	Number of samples during treatment time	
C_i	Measured triazole concentration	mg/L
C_0	Initial triazole concentration	mg/L
$C_{f\,i}$	Triazole concentration estimated from the first-order rate model	mg/L
V_{sln}	Volume of solution around the roots	L
A_i	Amount of triazole (concentration times volume)	mg
k_f	First-order rate constant	L/h
V_i	Rate (velocity) of triazole loss	mg/h
$V_{\max}$	Maximum rate of triazole concentration decrease for a single plant	mg/h
K_M	Michaelis or half-saturation constant	mg/L
V_{avi}	Arithmetic average of the rate between two consecutive samples	mg/h
$C_{M\,avi}$	Average triazole concentration between two consecutive samples based on Michaelis-Menten model	mg/L
$K_{M\,avi}$	Michaelis constant between two consecutive samples	mg/L
$V_{\max\,avi}$	Maximum rate of triazole concentration decrease between two consecutive samples	mg/h
$C_{M\,i}$	Triazole concentration estimated from the Michaelis-Menten model	mg/L
$C_{f\,avi}$	Average triazole concentration between two consecutive samples based on first-order rate model	mg/L
fw_0, fw_N, fw_i	Initial, final, and estimated fresh weights of the plant	kg
$V\max$	Maximum rate of triazole dissappearance per unit plant fresh weight at sampling	mg/kg h

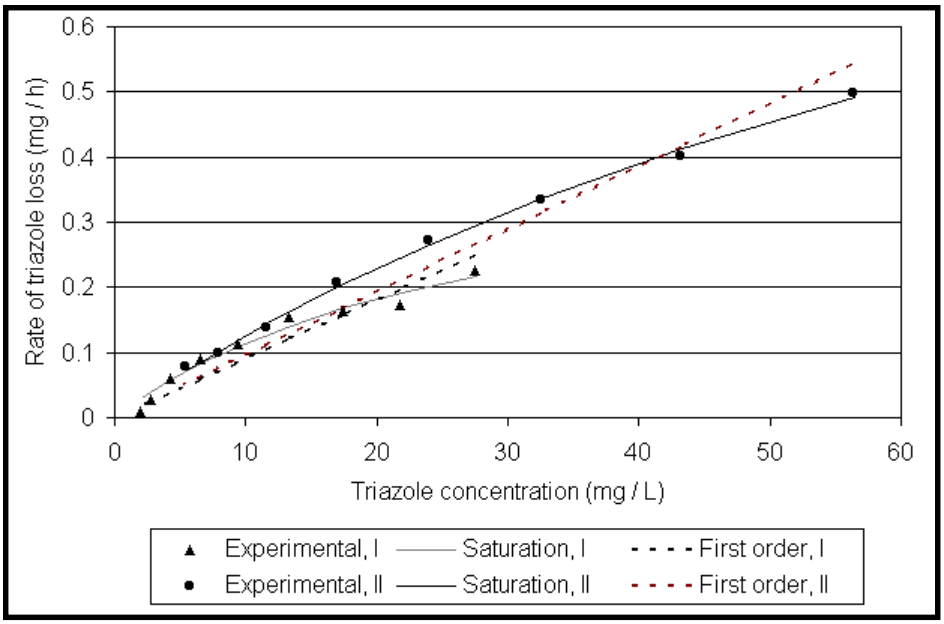

Figure 8. Experimental and modeled rates of triazole concentration decrease calculated as finite difference vs. the average concentration for sunflowers treated with low (I) and high (II) initial 5-MBz level and 24h lighting.

also shown in Table 2. Even though in most cases both models fitted the data quite reasonably, it is clear that the saturation model always gave better values for the sample statistic. Thus, it was concluded that a Michaelis-Menten model can represent the triazole disappearance by sunflowers better than the first-order model. However, when comparing the first and second phases, the model parameters are always lower for the first phase (small plants and low initial triazole concentration) than the ones for the second phase (larger plants and high initial triazole concentration). The average value of K_m was 27 mg/L in the first phase and 156.4 mg/L in the second phase, while Vmax was 0.37 mg/h in the first phase and 1.84 mg/h in the second phase. This effect can be seen by plotting the data for the two phases together, as shown in Figure 8 for the 5-MBz-treated plants under continuous lighting. The other triazole treatments

Table 2. Summary of results of model fitting for tiazole-treated sunflowers.

			First Order				Saturation					
Triazole	Phase	No. of Plants	k_f (x10²) (L/h)	Std. Dev.	r^2	Chi² x 10^3	Km (mg/L)	Std. Dev.	Vmax (mg/h)	Std. Dev.	r^2	Chi² x 10^3
5-MBz 12h	1	3	0.62	1.5E-04	0.389	25.4[a]	13.8	0.16	0.25	0.063	0.975	0.6
	2	3	0.70	9.8E-04	0.841	30.4	114.9	3.48	1.02	0.171	0.919	27.1
5-MBz 24h	1	4	0.91	6.6E-04	0.839	98.8	19.9	4.58	0.37	0.064	0.937	38.0
	2	3	0.98	2.3E-03	0.879	148.5	112.8	97.0	1.61	1.258	0.963	63.3
Tz 24h	1	4	0.66	1.1E-03	0.422	77.0	17.9	5.07	0.26	0.003	0.798	21.8
	2	2	1.01	4.2E-04	0.978	35.1	243.8	2.73	2.91	0.082	0.989	21.6
Bz 24h	1	4	0.72	9.8E-04	0.693	50.8	31.0	11.1	0.39	0.119	0.896	13.7
	2	3	0.90	8.1E-04	0.908	73.5	153.9	129.7	1.82	1.276	0.971	16.8

[a]Chi² = 0.0254; all values of Chi square are smaller than the table values for $\alpha = 0.995$.

(except the hydroxy benzotriazole), at the different initial concentrations and lighting schemes, showed similar trends. During each phase, the mass of each plant increased during the kinetic experiment. Thus, we have a possibly increasing mass of plant enzymes and a decreasing concentration of triazole, which exhibits a response that fits the Michaelis-Menten kinetic model better than the first-order model. For phase 1, the percent increase in plant mass is larger compared to phase 2 because the plants are smaller. This may contribute to the smaller values of the model parameters that are found in phase 1.

Therefore, for making a more fair comparison between the phases, it was necessary to include some kind of measure of the amount of available sites for the phytotransformation of the triazole, since the plants had changed during each treatment and intermediate period. As an approximation, it was assumed that the concentration of binding sites (or reactive enzyme) was proportional to the fresh weight of the plant. Since only the initial and final fresh weights for each phase were known, it was also assumed that the plants were following an exponential growth scheme. Thus, the rates in $mg_{triazole}$ / h were normalized to $mg_{triazole}$ / h $kg_{fresh\ plant}$ by dividing by the estimated fresh weight (kg) at the moment of sampling. Hence, equation (7) now is written as:

$$V'_i = \left(\frac{dA}{dt}\right)_i * \left(\frac{1}{fw_i}\right) = -\frac{V'_{\max} C_{Mavi}}{K'_M C_{Mavi}}$$

$$i = 1, 2, \ldots, \text{N-1} \qquad (13)$$

where the primed letters are for the normalized case. The first-order model was also normalized by including the plant fresh weight into the first-order rate constant:

$$iC_{fi} = C_0 e^{-k_f \left(\frac{1}{fw_i}\right) t_i} \qquad = 1, 2, \ldots, \text{N-1} \qquad (14)$$

Figure 9 shows the results for the 5-MBz-treated plants under continuous lighting for both phases after normalizing. From observing the plot, it was clear that normalizing to the fresh weight did not improve the closeness of the data between first and second phases. Table 3 shows the regression coefficient and x^2 values for the two models. For the saturation model, some parameter estimates were not meaningful ($>1x10^6$ = N/A in Table 3). The values for the first-order rate constant, however, remained within a wide but reasonable range (16 to 62 L kg/h), although the trend was opposite to what was seen before (high k_f for the lower initial concentration). The fit (r^2) with the first-order model was better compared to the first-order results in Table 2. Some other attempts for normalizing the data were performed, such as normalizing to fresh weight but assuming linear growth for the plants or plant growth based on water uptake, and normalizing to the rate of fresh-weight change, but none of them showed a consistent improvement in the comparison of the first and second phases. It is clear that the data need to be normalized since bigger plants presented higher values for the parameters, but a measure of the concentration of binding sites (or enzyme) within the plant more appropriate than the fresh weight appears to be required to obtain values varying within a smaller range.

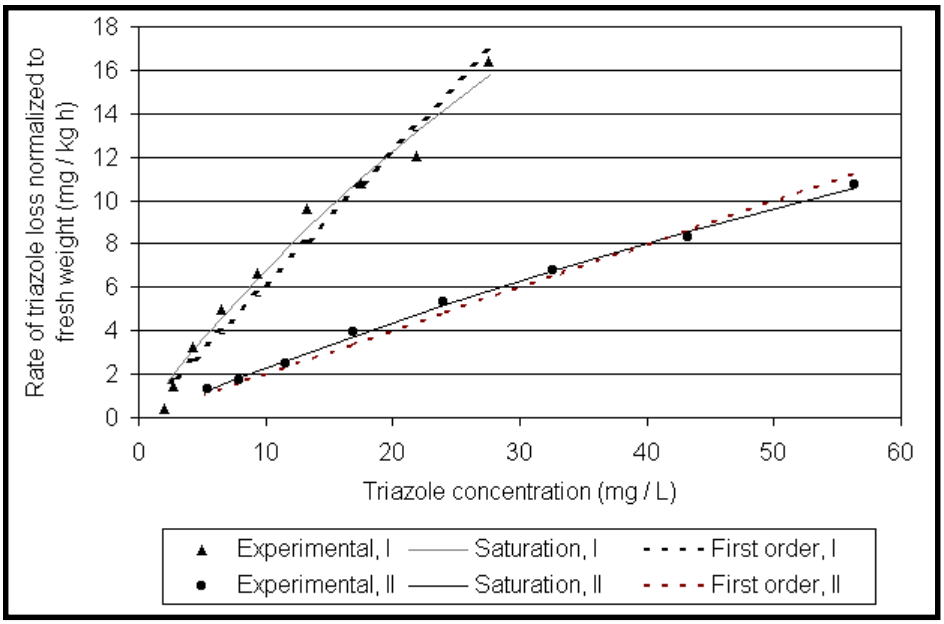

Figure 9. Experimental and modeled rates of triazole concentration decrease normalized to plant fresh weight vs. the average concentration for sunflowers treated with low (I) and high (II) initial 5-MBz level and 24h lighting.

One possibility could be the root-surface area, but it presents the difficulty of measuring it experimentally at different sampling times. The results in Table 3 and Figure 9 show that the first-order model gives a reasonable fit for most of the cases and that the values of the parameters in the Michaelis-Menten model are unusually large and not appropriate in some cases.

CONCLUSIONS

Benzotriazole and its 4- and 5-methyl derivatives disappeared from fertilized aqueous solution in the presence of roots of living sunflowers. The plants were able to tolerate the triazole and remain healthy with similar average water uptake even at a concentration as high as 60 mg/L. The disappearance of the triazole

Table 3. Summary of results of model fitting after normalizing to fresh weight.[a]

			First Order				Saturation					
Triazole	Phase	No. of Plants	k_f (x10^2) (L/h)	Std. Dev.	r^2	Chi^2 x 10^3	Km (mg/L)	Std. Dev.	Vmax (mg/h)	Std. Dev.	r^2	Chi^2 x 10^3
5-MBz 12h	1	3	39.9	3.0E-02	0.890	0.51	51.3	22.6	28.7	0.063	0.987	0.04
	2	3	16.2	2.0E-02	0.905	0.53	203.9	38.1	38.4	6.1	0937	0.56
5-MBz 24h	1	4	61.6	6.3E-02	0.934	3.14	N/A	23.3	N/A	N/A	0.866	5.75
	2	3	20.4	1.6E-02	0.942	1.90	N/A	N/A	N/A	N/A	0.909	2.89
Tz 24h	1	4	40.0	3.1E-02	0.837	1.68	64.4	35.7	34.6	15.7	0.886	0.00
	2	2	21.1	2.5E-02	0.992	0.28	N/A	N/A	N/A	N/A	0.827	3.24
Bz 24h	1	4	44.9	2.2E-02	0.921	1.15	186.6	140.7	96.6	68.2	0.940	0.90
	2	3	24.8	1.2E-01	0.975	0.65	N/A	N/A	N/A	N/A	0.960	1.08

[a]N/A These values are very large and are not meaningful.

appears be happening in the roots, since the rate was greater than the loss with the water uptake. The disappearance also happened at night, and no triazole was extractable with methanol from the plant material.

When the increase in plant mass with plant growth was not included in the model, the rate of triazole loss was better represented by a Michaelis-Menten kinetic model than a simple first-order or exponential decay model, as shown by the goodness-of-fit test. The V_{max} values, ranging from 0.25 to 2.91 mg / h, reflected a significant metabolic activity with triazole as substrate. The fit for the first-order model was improved when normalizing to the plant fresh weight. When the data was normalized, the first-order model gave a better fit than the Michaelis-Menten model.

Even though hydroxy-benzotriazole is structurally similar to the other benzotriazole derivatives, its disappearance did not follow the same pattern and in some cases, the plants did not appear to have the necessary enzymes to transform it at low concentration. It appears that there exist specific mechanisms for triazole incorporation into and/or transformation in the plants, and they differ for hydroxy benzotriazole.

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