Atrazine Degradation in Subsurface Soil by Indigenous and Introduced Microorganisms†

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Significant atrazine degradation (50%) but only 1% mineralization was detected in samples taken from the upper soil layer (0−25 cm), but not in samples taken from deeper horizons. Thin layer chromatography analysis of noninoculated soil indicated dealkylation to be a major degradation pathway with deethylatrazine favoring deisopropylatrazine. Inoculation with *Pseudomonas* sp. strain ADP (*P*.ADP) resulted in 90−100% mineralization of [^{14}C]atrazine in all samples after 15 days. Atrazine was degraded in the soil via dechlorination as the first mineralization step. C-source competition was not responsible for differences in initial mineralization rates. Higher organic matter content in the upper soil level did not result in a sorption-related decrease in degradation rates. It is concluded that the limiting factor for atrazine mineralization in the tested soil profiles was the absence of atrazine-mineralizing microorganisms. Therefore, bioaugmentation may be preferable to enhancement of intrinsic atrazine-degrading activity when complete atrazine mineralization is the goal of bioremediation activity.

Keywords: *Atrazine; subsurface; biodegradation; microbial activity; soil organic matter*

INTRODUCTION

The fate of commonly used herbicides such as atrazine in natural environments has been thoroughly investigated in the past 40 years (Cheng, 1990). However, less attention has been given to the subsoil environments (Johnson and Fuhrmann, 1993) that form the last barrier before pesticides leach into the water table. Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] is one of the herbicides that frequently compromises groundwater quality (Belluck et al., 1991; Barker and Richards, 1992; Thurman et al., 1992). Atrazine has been used worldwide since 1952 to control annual weeds in corn and sugarcane (Ma and Selim, 1996), and the U.S. Environmental Protection Agency (U.S. EPA) estimates that between 32 000 and 34 000 metric tons of atrazine were used in U.S. agricultural crop production in 1993, making it the most heavily used of all pesticides (Aspelin, 1994).

To date, most of the studies on atrazine degradation have focused on the root zone and on dissipation and leaching into the subsurface, and only a few have considered the degradation in the unsaturated zone (Ritter,1990; Linn et al., 1993; Bottoni et al., 1996; Ma and Selim, 1996). However, since even small amounts of pesticides can seriously contaminate groundwater (Kordel et al., 1992), information on microbial degradation in the unsaturated zone is important for the understanding of the overall fate of atrazine. It is an important input into pesticide leaching models such as PELMO (Klein et al., 1993).

Chemical and biological degradation of atrazine plays an important role in determining the fate and transport of the herbicide in soils. These aspects have been reviewed (Cook, 1987; Erickson and Lee, 1989; Ma and Selim, 1996). Since atrazine ring carbons are in the oxidation state of CO_2, they do not serve for catabolic or biosynthetic roles in atrazine degradative pathways, and only oxidation of the side chain carbons can yield energy. Indeed, many of the reported bacteria could utilize side chains exclusively and failed to cleave the ring moiety. Therefore, in a number of studies, additional substrates have been used to provide carbon and energy sources for microbial growth, while atrazine was utilized as a sole nitrogen source (Cook, 1987). Simultaneous catabolism both of side chain carbons and of all molecular nitrogens has been reported in several studies of bacterial pure cultures (Yanze-Kontchou and Gschwind, 1994; Mandelbaum et al., 1995; Radosevich et al., 1995).

It has repeatedly been reported that microbial counts and carbon content decrease with increasing soil depth. Therefore, it is to be expected that atrazine molecules that are leached below the root zone would be less susceptible to microbial degradation (Dobbins et al., 1987; Moorman and Harper, 1989; Radosevich et al., 1996; Veeh et al., 1996). It is widely accepted that atrazine degradation in the subsoil is slower than in the root zone (Adams and Thurman, 1991; Fomsgaard, 1994), but the reasons for the increased persistence have rarely been addressed. In a recent study by Radosevich et al. (1996), an atrazine-degrading bacterium was used to probe subsurface soils for atrazine degradation potential. The enhancement of mineralization by both tested soils in the inoculated sediments suggests that atrazine mineralization in the subsurface is limited by the absence of atrazine degraders rather than by sorption, nutrient availability, or other inherent sediment properties. Interestingly, Sparling and Aislabie (1996) detected greater atrazine degradation at lower depths than at the surface soil level and attributed it

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to the presence of a larger population of atrazine-degrading bacteria (G. Sparling, personal communication).

Pseudomonas sp. strain ADP (*P*.ADP) was recently isolated, and its ability to mineralize atrazine via a defined metabolic pathway has been fully characterized (Mandelbaum et al., 1995; De-Souza et al., 1995, 1996; Boundy-Mills et al., 1997). Its ability to mineralize atrazine with dechlorination as the first degradative step (Mandelbaum et al., 1995) could differentiate its activity from that of the indigenous microflora that degrade atrazine mainly via dealkylation pathways.

The aim of the present study is to assess atrazine degradation in situ along a soil profile by indigenous and introduced microorganisms. The influence of soil organic matter, nutrient availability, and presence of atrazine-degrading bacteria is discussed.

In spite of previous reports that atrazine dechlorination in soils occurs mainly due to hydrolytic chemical dechlorination, this work indicated that bacterial dechlorination of atrazine as a first degradation step occurs in situ in soil (as predicted from laboratory experiments) in the presence of *Pseudomonas* sp. strain ADP.

The degradation rate and pathway depended on the composition of the atrazine-degrading bacterial population more than on the availability of nutrients or the interaction of atrazine with the soil organic matter.

MATERIALS AND METHODS

Chemicals. Atrazine 98% and [U-*ring*-^{14}C]atrazine (chemical purity = 97.3%, specific activity = 14.6 mCi/mg, radiochemical purity = 98.6%) were a gift from the Ciba-Geigy Corp., Greensboro, NC. [^{14}C]Citrate was purchased from Sigma Chemical Co. (St. Louis, MO); the specific activity was 50 mCi/mmol.

Soil. Fine-loamy thermic mixed Mollic Haploxeralt soil samples were collected from a site of the Agricultural Research Organization, Bet Dagan, Israel. Soil samples were taken to a depth of 4 m using a Geoprobe apparatus (Geoprobe Systems, Salina, KS). Two adjacent profiles were sampled (10 m apart). The sampled soil was air-dried and sieved (2 mm mesh size). The profile was analyzed for its microbial activity, and subsamples from five different segments where chosen for further study according to the microbial activity along the profile. Particle size was analyzed by hydrometer method according to the procedure of Day (1965) and total organic carbon according to the procedure of Snyder and Trofymow (1984).

Microbial Cultures. An atrazine-degrading bacterium, *Pseudomonas* sp. strain ADP used in this study, has been described previously (Mandelbaum et al., 1995). The culture was grown in 250 mL of liquid atrazine medium at 35 °C on an orbital shaker (125 rpm) according to the method of Mandelbaum et al. (1993). The cells were harvested after 24 h of incubation by centrifugation (6000*g*, 10 min), washed twice with sterile saline solution (0.8% NaCl), and resuspended in the same solution to a final concentration of 1×10^7 cells/mL.

Atrazine Degradation. *Application of Atrazine to the Soil.* Fifty gram samples of air-dried soil from selected depths were each mixed with 50 mL of aqueous atrazine solution (33 ppm) to form a homogeneous soil slurry. The slurry was dried at 40 °C and ground with a mortar and pestle. The resulting dry soil was then thoroughly mixed to ensure even distribution of atrazine.

Atrazine Mineralization by P.ADP and by Indigenous Soil Bacteria. Subsamples from the atrazine-amended soil (5 g), in two replicates, were placed in 50 mL tubes and wetted with 350 μL of distilled water containing [U-*ring*-^{14}C]atrazine, to yield a final radiolabel concentration of 5000 cpm/g of soil. The tubes were preincubated in an incubator at 30 °C. After 24 h of preincubation, 1150 μL of distilled water containing *P*.ADP bacteria and sodium citrate were added, to a final concentration of 2.3×10^6 cells and 2 mg of citrate/g of soil (final water content = 30%). The inoculated soil samples were thoroughly mixed until the soil was homogeneously wet. In each tube, an uncapped Eppendorf tube containing 750 μL of 1 N NaOH was used for trapping the $^{14}CO_2$ released by atrazine mineralization. The samples were further incubated at 30 °C during the rest of the experiment. At predetermined times, the radioactivity in the NaOH solution was determined in a liquid scintillation counter (1217 Rackbeta, LKB, Sweden).

Control subsamples that had not been amended with *P*.ADP were similarly tested. Three months after the initiation of the experiments, the residual atrazine and the metabolites formed were extracted with methanol similarly to the procedure of Bottoni et al. (1996). Five gram soil samples (30% water content w/w) were extracted with 10 mL of methanol. The soil suspension was extracted for 24 h in a reciprocal shaker operated at 200 cycles/min. The methanol was evaporated to dryness, and the extract was redissolved in 50 μL of methanol and applied to a TLC plate (Uniplate, 20 × 20 cm, 250 μm, Analtech), which was developed in chloroform/methanol/formic acid/water 75:20:4:2 (v/v). The radioactivity on the plate was analyzed with a Bio-Imaging analyzer (FUJIX BAS 1000, Japan).

Atrazine Degradation by Native Soil Bacteria. The degradation of atrazine by native microorganisms was determined in laboratory incubation experiments. Atrazine was added to the soils of different soil depths. The samples were aged at 40 °C for several days. Sixteen soil samples (2.5 g) from each atrazine-amended soil were incubated in 10 mL capped test tubes. Nonradiolabeled citrate was added to a final concentration of 0.2% (w/w) to half of the samples. The final water content of all samples was adjusted to 30% (v/w). At each sampling time, a pair of samples was extracted (scarified), and residual atrazine was determined by gas chromatography. The samples were incubated for 0, 7, 14, and 30 days at 30 °C. Each sample was extracted by a mixture of 4 mL of distilled water and 2 mL of ethyl acetate/hexane (1:1 v/v). The samples were extracted for 24 h under constant shaking (200 rpm), and the two phases were separated by centrifugation (1200*g*). The organic phase was collected and analyzed with a Varian Star 3400 cx gas chromatograph (Varian, Harbor City, CA) equipped with an autosampler (Varian 8200 cx). The GC was fitted with a temperature programmable injector (Varian 1077), a thermionic specific detector, and a DB-1 megabore column having the dimensions 30 m × 0.53 mm i.d., 1.5 μm film (J&W Scientific, Folsom, CA). The GC operating conditions were as follows: injector temperature, 240 °C; detector temperature, 300 °C; column oven temperature, 180 °C. The He (carrier gas) flow rate was 10 mL/min and the injection volume was 1 μL.

Soil Microorganism Activity Measurements. Microbial biomass was determined using the chloroform fumigation N extraction method (Brookes et al., 1985). Two methods for the determination of the microbial activity were used: (i) dehydrogenase activity, according to the method of Tabatabai (1982); and (ii) fluorescein diacetate (FDA) hydrolysis, according to the method of Schnurer and Rosswall (1982).

Adsorption Studies. Soil samples (4 g) were mixed with 6 mL of aqueous solution of atrazine in a glass screw-capped centrifuge tube with a Teflon septum. The solutions were prepared in the following concentrations: 5, 10, 15, 20, and 25 mg of atrazine/L of a 0.01 mol/L $CaCl_2$ solution. The tubes were shaken in an end-over-end agitator for 24 h at 25 ± 1 °C. The suspensions were centrifuged for 5 min at 1200*g*, and the supernatant was filtered through a 0.7 μm glass-fiber filter (Whatman). The amount of atrazine in the supernatant was determined by HPLC using an HPLC controller (SSI, PA) with autosampler (Merck Hitachi L7200, Tokyo, Japan). Detection was made at 220 nm with a UV detector (Varian 9050). Separation was obtained on a C_8 5 μm (250 × 4 mm) reversed-phase column (Merck, LiChrocart RP-8, Darmstadt, Germany) with a flow rate of 1 mL/min. The mobile phase was 70:30 (v/v) methanol/water with 50 mM ammonium acetate.

Herbicide adsorption at equilibrium was calculated as the difference in herbicide concentration between the initial solu-

Table 1. Composition and Adsorbability of Soil Samples from Profiles A and B

profile	depth (cm)	OM[a] (%)	clay (%)	silt (%)	sand (%)	K_d (L kg^{-1})	n
A	0−25	1.6	12.5	7.5	80	0.41	1.07
	50−75	1.0	31.9	13.8	54.4	0.36	1.03
	140−175	0.6	32.5	13.8	53.8	0.27	1.02
	225−255	0.4	36.9	9.4	53.8	0.20	0.98
	370−400	0.2	21.3	2.5	76.3	0.04	0.82
B	0−25	1.6	6.3	6.3	87.5	0.37	0.86
	50−75	0.6	22.5	8.8	68.8	0.18	0.84
	140−175	0.8	32.5	12.5	55	0.18	0.81
	225−255	0.5	30.0	9.4	60.6	0.14	0.91
	370−400	0.2	8.8	5.0	86.3	0.07	0.94

[a] OM, organic matter.

tion and the solution in equilibrium with the soil. The distribution coefficient, K_d (L/kg) was calculated from the Freundlich equation

$$x/m = K_d C_e^{1/n}$$

where x = mass of adsorbed atrazine (mg), m = soil mass (kg), C_e = atrazine concentration (mg/L) of the supernatant solution at equilibrium, and n = a constant. Since n was determined to be near unity in the present study, the constant K_d was calculated by dividing the concentration of adsorbed atrazine in the soil by the atrazine concentration in the solution.

RESULTS AND DISCUSSION

Two soil profiles (10 m apart) were sampled to a depth of 4 m; soil analysis data (Table 1) indicated spatial heterogeneity. In both soil profiles clayey soil was sandwiched between two sandy layers. The surface sandy layer (0−25 cm) was underlayed by more clayey soil (50−255 cm), which is followed by another sandy layer. In the surface layer, higher organic matter content and K_d values were measured. These values declined with increasing soil depth, and soil microbial biomass (biomass N) and activity (hydrolase and dehydrogenase) had already fallen below the experimental detection limits at a soil depth of 2.5 m (Table 2). Generally, the decrease in microbial activity along the soil profiles was not linear, and there were steady levels of microbial activity and biomass in the 75−175 cm horizon. The highest microbial activity and biomass were associated with the upper soil layer, which contained >1% organic matter. The zone between 75 and 175 cm, where the activity was relatively constant, had an organic matter content of 0.6−1%, and in the zone with no activity, soil organic matter content was below 0.4%. Atrazine degradation was measured at various soil depths along the soil profile. Significant atrazine degradation was detected in samples taken from the upper soil level. More than 50% of the applied atrazine (33 ppm) was degraded in 30 days of incubation (Figure 1). However, in soil samples taken from lower parts of the profile, significantly less atrazine degradation occurred. Less than 33% degradation was recorded by the end of 30 days in all soil samples from >50 cm. The results of our experiments are in line with previous results that indicated lower degradation rates in subsurface soils because of their lower microbial populations (Fomsgaard, 1995; Kordel et al., 1995; Veeh et al., 1996). Atrazine mineralization in the top layer with most activity occurred to a much lesser extent, thus indicating that only partial degradation of atrazine in the soils took place. After 30 days, about 1% of the initial atrazine amount in the upper soil layer had been mineralized (Figure 1), while in soil samples from deeper parts of the profile no atrazine mineralization had taken place (data not shown). After 3 months of incubation, atrazine metabolites in the soil samples were analyzed by thin layer chromatography (TLC). Deethylatrazine and deisopropylatrazine were the main degradation products in the upper soil level (Figure 2), while mineralization totaled <5% (data not shown). Deethylatrazine was the main degradation product, as indicated by the intensity of its spot on the TLC plate (Figure 2). Indeed, the most common biological metabolism of atrazine in soils proceeds via dealkylation of the ethylamine side chain to produce deethylatrazine (Adams and Thurman, 1991; Durand and Barcelo, 1992; McMahon et al., 1992; Nair and Schnoor, 1992; Wienhold et al., 1993). However, it is known that dealkylation products maintain their herbicidal activity and may even pose an increased risk of contamination of groundwater, because of their increased mobility (Thurman et al., 1994).

Since it was clear that atrazine mineralization in the soils was very slow, we inoculated the soil with *P*.ADP, which harbors genes for the rapid and complete mineralization of atrazine. This bacterium has previously been reported to be capable of atrazine mineralization in soils (Mandelbaum et al., 1995). Inoculation with *P*.ADP and the amendment with citrate were aimed at maximizing atrazine degradation. Under these conditions, we could identify possible inhibition of atrazine degradation in different soil strata.

When *P*.ADP was inoculated in soils amended with [U-*ring*-^{14}C]atrazine, 90−100% of the label was recovered as $^{14}CO_2$ in 15 days after inoculation (Figure 3). However, marked differences in the initial mineralization rates of atrazine were recorded. Samples from depths of 0−75 cm and 370−400 cm showed enhanced rates of mineralization, whereas mineralization in the strata between these levels lagged. In these experiments citrate was added as an external carbon source to support the activity of *P*.ADP.

Increased herbicide degradation activity in subsurfaces amended with organic nutrients has been observed previously under aerobic conditions (Pothuluri et al., 1990), indicating that nutrient availability may limit biodegradation. It was suspected that reduced atrazine mineralization rates could be caused by nutrient competition between the soil indigenous microflora and *P*.ADP. It was hypothesized that the high level of organic matter in the upper soil layer could support both the activity of *P*.ADP and that of the indigenous microflora and, therefore, that mineralization was less susceptible to competition for the carbon source (citrate) in this layer than in the 140−175 and 225−255 cm layers, where the initial biomass was low. It was suggested that in the absence of alternative available C sources, competition for citrate would be more significant in terms of its effect on atrazine mineralization. To test this hypothesis, [^{14}C]citrate was added to the soil and its degradation kinetics were monitored (Figure 4). Surprisingly, similar citrate mineralization rates were recorded in all soil layers. Moreover, in experiments in which citrate but not *P*.ADP was added to the soil samples, only minimal stimulation of atrazine degradation was observed (Figure 1). From these results, we concluded that competition for citrate was not an important factor in attenuating the atrazine mineralization rates along the soil profile. The microbial transformation of atrazine in the natural environ-

Table 2. Biomass and Dehydrogenase and FDA Activities in Soil Profiles A and B

	profile A			profile B		
depth (cm)	dehydrogenase (mg kg^{-1} day^{-1})	FDA (mg kg^{-1} 30 min^{-1})	biomass (mg kg^{-1})	dehydrogenase (mg kg^{-1} day^{-1})	FDA (mg kg^{-1} 30 min^{-1})	biomass (mg kg^{-1})
0−25	3.0	1.9	114.8	3.7	3.3	82.3
25−50	2.3	1.0	118.3	1.9	2.3	63.7
50−75	3.8	1.3	83.0	0.6	1.2	43.8
75−100	1.9	1.1	68.5	0.5	0.8	43.4
100−125	2.7	0.9	56.0	0.3	1.2	42.7
125−140	2.0	0.9	45.5	0.4	1.4	36.8
140−175	2.0	0.7	50.8	0.3	0.5	29.8
175−200	2.3	0.3	38.5	0.5	0	32.6
200−225	0.3	0.1	35.0	0.5	0	21.0
225−255	0.1	0	11.4	0.3	0	8.1
255−275	0	0	4.0	0	0	0
>275	0	0	0	0	0	0

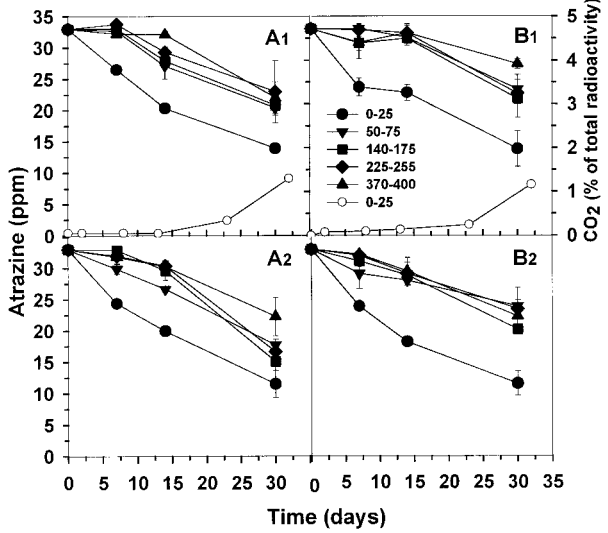

Figure 1. Atrazine degradation (solid symbols) and mineralization (open symbols) in nonaugmented soil samples: Al, soil from profile A nonamended with citrate; A2, soil from profile A amended with 0.2% (w/w) of sodium citrate; B1 and B2 represent similar treatments in soil profile B. Mineralization was not measured in the soils amended with sodium citrate. Numbers in B1 refer to soil depths in cm.

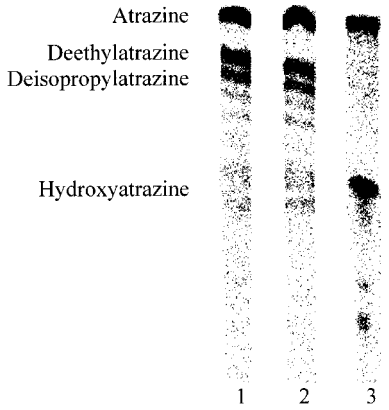

Figure 2. Thin layer chromatogram (TLC) of ^{14}C-labeled atrazine degradation products: 1, sample from profile A (0−25 cm); 2, sample from profile B (0−25 cm); 3, standards of hydroxyatrazine and atrazine. Deethylatrazine and deisopropylatrazine were determined according to their comigration with nonlabeled standards (data not shown).

ment is greatly influenced by the total carbon and organic content of the soil (Cook, 1987), and in the tested soil profiles, lower organic contents and lower atrazine distribution coefficients (K_d) were recorded with increasing soil depth. Similar results had previously been obtained with other herbicides (Koskinen et al., 1996).

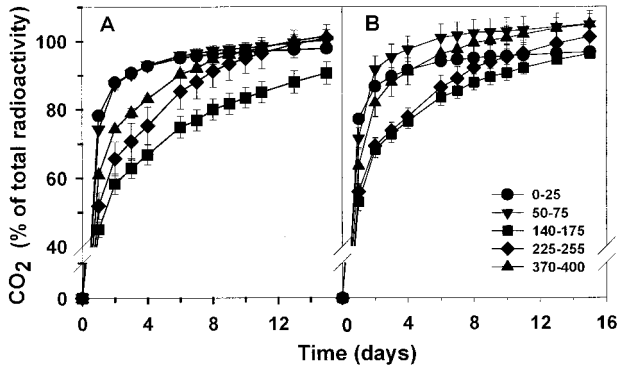

Figure 3. Atrazine mineralization in soil samples taken from soil profiles A and B and inoculated with *Pseudomonas* sp. strain ADP. Number refer to soil depths in cm.

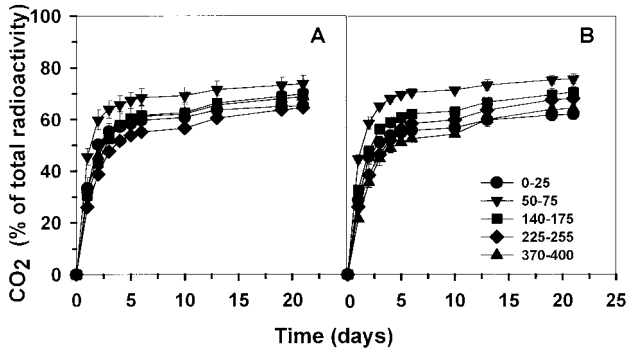

Figure 4. Sodium [^{14}C]citrate mineralization in soil samples taken from soil profiles A and B. Numbers refer to soil depths in cm.

In contrast to observations in which atrazine mineralization was negatively correlated with organic matter content (Radosevich et al., 1996), in the present study indigenous atrazine degradation was highest in the upper soil level; that is, higher persistence of atrazine was associated with deeper strata.

From the present study, we conclude that the potential for atrazine degradation exists in all soil strata. The most important factor that governs atrazine mineralization in the soils is the presence of an atrazine-mineralizing population. From previous pesticide leaching data collected in the same location (data not shown) and in accordance with previously published data (Walker et al., 1996; Azevedo et al., 1997), pesticide distribution 1 year after application was mostly in the 10−100 cm layer. It may be concluded that bioremediation of soils in the upper level or in deeper strata may be feasible by amendment with the bacterium *P.*ADP. However, in the present study, the soil was first excavated and only then inoculated; therefore, further study is needed

to assess the ability of the bacterium to mineralize atrazine in situ if *P.*ADP inoculation is to be considered for in situ bioremediation.

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LITERATURE CITED

Adams, C. D.; Thurman, E. M. Formation and transport of deethylatrazine in the soil and vadose zone. *J. Environ. Qual.* **1991**, *20*, 540−547.

Aislabie, J.; Lloyd-Jones, G. A review of bacterial degradation of pesticides. *Aust. J. Soil Res.* **1995**, *33*, 925−942.

Aspelin, A. L. *Pesticides Industry Sales and Usage—1992 and 1993 Market Estimates*; OPP, Office of Prevention Pesticides and Toxic Substances, U.S. Environmental Protection Agency: Washington, DC, 1994; p 13.

Azevedo, A. S.; Kanwer, R. S.; Singh, P.; Ahuja, L.; Pereira, L. S. Simulation of atrazine transport using root zone water quality model for Iowa soil profiles. *J. Environ. Qual.* **1997**, *26*, 153−164.

Barker, B. D.; Richards, P. R. Herbicides concentrations in Ohio's drinking water supplies: A quantitative exposure assessment. In *Rational Readings on Environmental Concerns*; Lehr, J. H., Ed.; Van-Nostrand Reinhold: New York, 1992; pp 84−97.

Belluck, D. A.; Benjamin, S. L.; Dawson, T. Groundwater contamination by atrazine and its metabolites. In *Pesticide Transformation Products. Fate and Significance in the Environment*; Somasandarum, L., Coats, J. R., Eds.; ACS Symposium Series 459; American Chemical Society: Washington, DC, 1991; pp 254−273.

Bottoni, P.; Keizer, J.; Funari, E. Leaching indices of some major triazine metabolites. *Chemosphere* **1996**, *32*, 1401−1411.

Boundy-Mills, K. L.; De-Souza, M. L.; Mandelbaum, R. T.; Wackett, L. P.; Sadowsky, M. J. The *atz*B gene of *Pseudomonas* sp. strain ADP encodes the second enzyme of a novel atrazine degradation pathway. *Appl. Environ. Microbiol.* **1997**, *63*, 916−923.

Brookes, P. C.; Landman, A.; Pruden, G.; Jenkinson, D. S. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* **1985**, *17*, 837−842.

Cheng, H. H. *Pesticides in the Soil Environment. Processes Impacts and Modeling*; SSSA Book Series 2; Soil Science Society of America: Madison, WI, 1990.

Cook, A. M. Biodegradation of s-triazine xenobiotics. *FEMS Microbiol. Rev.* **1987**, *46*, 93−116.

Day, P. R. Particle fractionation and particle-size analysis. In *Methods of Soil Analysis—Part 1*; Black, C. A., Evants, D. D., White, J. L., Ensminger, L. E., Clark, F. E., Eds.; Agronomy Monograph 9; ASA: Madison, WI, 1965; pp 545−565.

De-Souza, M. L.; Wackett, L. P.; Boundy-Mills, K. L.; Mandelbaum, R. T.; Sadowsky, M. J. Cloning, characterization, and expression of a gene region from *Pseudomonas* sp. strain ADP involved in the dechlorination of atrazine. *Appl. Environ. Microbiol.* **1995**, 3373−3378.

De-Souza, M. L.; Sadowsky, M. J.; Wackett, L. P. Atrazine chlorohydrolase from *Pseudomonas* sp. strain ADP: gene sequence, enzyme purlfication, and protein characterization. *J. Bacteriol.* **1996**, *178*, 4894−4900.

Dobbins, D. C.; Thornton-Manning, J. R.; Jones, D. D.; Federle, T. W. Mineralization potential for phenol in subsurface soils. *J. Environ. Qual.* **1987**, *16*, 54−58.

Durand, G.; Barcelo, D. Environmental degradation of atrazine, linuron, and fenitrothion in soil samples. *Toxicol. Environ. Chem.* **1992**, *36*, 225−234.

Erickson, E. L.; Lee, H. K. Degradation of atrazine and related s-triazines. *Crit. Rev. Environ. Cont.* **1989**, *19*, 1−13.

Finklea, H. C.; Fontenot, M. F. Accelerated bioremediation of triazine contaminated soils: a practical case study. In *Bioremediation Science and Applications*; Skipper, H. D., Turco, R. F., Eds.; Soil Science Society of America: Madison, WI, 1995; pp 221−234.

Fomsgaard, I. S. Degradation of pesticides in subsurface soils, unsaturated zone—a review of methods and results. *Int. J. Environ. Anal. Chem.* **1995**, *58*, 231−245.

Johnson, R. M.; Fuhrmann, J. J. Degradation of atrazine and metolachlor in subsoils from an Atlantic coastal plain watershed. In *Sorption and Degradation of Pesticides and Organic Chemicals in Soil*; Linn, D. M., Carski, T. H., Brusseau, M. L., Chang, H-F., Eds.; Soil Science Society of America: Madison, WI, 1993; pp 27−31.

Klein, M. *User Manual, PELMO Pesticides Leaching Model*; Fraunehofer-Institut für Umweltchemie und Ökotoxikologie, 1993.

Kordel, W.; Herrchen, M.; Klein, M.; Traub-Eberhard, U.; Kloppel, H.; Klein, W. Determination of the fate of pesticides in outdoor lysimeter experiments. *Sci. Total Environ.* **1992**, *123/124*, 421−434.

Kordel, W.; Wahle, U.; Knoche, H.; Hund, K. Degradation capacities of chlorotoluron and simazine in subsoil horizons. *Sci. Total Environ.* **1995**, *171*, 43−50.

Koskinen, W. C.; Stone, D. M.; Harris, A. R. Sorption of hexazinone, sulfometuron methyl, and tebuthiuron on acid, low base saturated sand. *Chemosphere* **1996**, *32*, 1681−1689.

Linn, D. M.; Carski, T. H.; Brusseau, M. L.; Chang, H.-F. *Sorption and Degradation of Pesticides and Organic Chemicals in Soil*; SSSA Special Publication 32; Soil Science Society of America: Madison, WI, 1993.

Ma, L.; Selim, H. M. Atrazine retention and transport in soils. In *Reviews of Environmental Contamination and Toxicity*; Ware, G. W., Ed.; Springer-Verlag: New York, 1996; pp 129−173.

Mandelbaum, R. T.; Wackett, L. P.; Allen, D. L. Mineralization of the s-triazine ring of atrazine by stable bacterial mixed cultures. *Appl. Environ. Microbiol.* **1993**, *59*, 1695−1701.

Mandelbaum, R. T.; Wackett, L. P.; Allen, D. L. Isolation and characterization of a *Pseudomonas* sp. that mineralizes the s-triazine herbicide atrazine. *Appl. Environ. Microbiol.* **1995**, *61*, 1451−1457.

McMahon, P. B.; Chapelle, F. H.; Jagucki, M. L. Atrazine mineralization potential of alluvial-aquifer sediments under aerobic conditions. *Environ. Sci. Technol.* **1992**, *26*, 1556−1559.

Moorman, T. B.; Harper, S. S. Transformation and mineralization of metribuzin in surface and subsurface horizons of a Mississippi Delta soil. *J. Environ. Qual.* **1989**, *18*, 302−306.

Nair, D. R.; Schnoor, J. L. Effect of two electron acceptors and atrazine mineralization rates in soils. *Environ. Sci. Technol.* **1992**, *26*, 2298−2300.

Pothuluri, J. V.; Moorman, T. M.; Obenhuber, D. C.; Wauchope, R. D. Aerobic and anaerobic degradation of alachlor in samples from a surface-to-groundwater profile. *J. Environ. Qual.* **1990**, *19*, 525−530.

Radosevich, M.; Traina, S. J.; Hao, Y. L.; Tuovinen, O. H. Degradation and mineralization of atrazine by soil bacterial isolate. *Appl. Environ. Microbiol.* **1995**, *61*, 297−302.

Radosevich, M.; Traina, S. J.; Tuovinen, O. H. Biodegradation of atrazine in surface soils and subsurface sediments collected from an agricultural research farm. *Biodegradation* **1996**, *7*, 137−149.

Ritter, W. F. Pesticide contamination of groundwater in the United States—a review. *J. Environ. Sci. Health* **1990**, *B25*, 1−29.

Schnurer, J.; Rosswall, T. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Appl. Environ. Microbiol.* **1982**, *43*, 1256−1261.

Snyder, J. D.; Trofymow, J. A. A rapid accurate wet oxidation diffusion procedure for determining organic and inorganic carbon in plant and soil samples. *Commun. Soil Sci. Plant Anal.* **1984**, *15*, 587−597.

Sparling, G.; Aislabie, J. Soil type affects the rate of atrazine decomposition and potential for aquifer contamination. Presented at the Conference on the Contribution of Plant and Animal Poduction to the Nation's Environment, Rukura, NZ, Aug 1996 (abstract).

Tabatabai, M. A. Soil enzyme. In *Methods of Soil Analysis—Part 2*; Page, A. L., Miller, R. H., Keeney, D. R., Eds.; Agronomy 9; American Society of Agronomy: Madison, WI, 1982; pp 937−940.

Thurman, E. M.; Goolsby, D. A.; Meyer, M. T.; Mills, M. S.; Pomes, M. L.; Kolpin, D. W. A reconnaissance study of herbicides and their metabolites in surface water of the Midwestern United States using immunoassay and gas chromatography/mass spectrometry. *Environ. Sci. Technol.* **1992**, *26*, 2440−2447.

Thurman, E. M.; Meyer, M. T.; Mills, M. S.; Zimmerman, L. R.; Perry, C. A. Formation and transport of deethylatrazine and deisopropylatrazine in surface water. *Environ. Sci. Technol.* **1994**, *28*, 2267−2277.

Veeh, R. H.; Inskeep, W. P.; Camper, A. K. Soil depth and temperature effect on microbial degradation of 2,4-D. *J. Environ. Qual.* **1996**, *25*, 5−12.

Walker, A.; Welch, S. J.; Melacini, A.; Moon, Y. H. Evaluation of three pesticides leaching models with experimental data for alachlor, atrazine and metribuzine. *Weed Res.* **1996**, *36*, 37−47.

Wienhold, B. J.; Sadeghi, A. M.; Gish, T. J. Effect of strach encapsulation and temperature on volatilization of atrazine and alachlor. *J. Environ. Qual.* **1993**, *22*, 162−166.

Yanze-Kontchou, C.; Gschwind, N. Mineralization of the herbicide atrazine as a carbon source by a *Pseudomonas* strain. *Appl. Environ. Microbiol.* **1994**, *60*, 4297−4302.

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