THE EFFECTS OF PH AND FERRIC IRON ON PHOTOCHEMICAL AND MICROBIAL DEGRADATION OF ATRAZINE IN A FRESHWATER ENVIRONMENT

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INTRODUCTION

The herbicide atrazine is a photosynthetic inhibitor used to selectively control broadleaf weeds in agricultural crops. The half-life of atrazine in aquatic environments has been found to range from 3 days to over 8 months. Numerous environmental factors (including pH, light intensity, and inorganic salts) affect the degradation of atrazine. Photolysis and microbial degradation both have been shown to be important degradative processes affecting many organic pollutants in aquatic environments (Hwang et al. 1996). Photosensitizers have been used to induce or amplify the rate of photodegradation for several compounds including atrazine (Rejto et al.1993). However, photoinduced toxicity has been reported for some organic contaminants (Landrum et al. 1987; McConkey et al. 1997).

Iron is a common component of the earth and a trace nutrient. Ferric iron acts as a natural catalyst in aquatic photochemical reactions (Faust 1994). The use of ferric iron in the photodegradation of atrazine has been recorded in several studies. Most studies range between pH 3 and pH 8, and rely on formation of active oxidants (Balmer and Sulzberger, 1999; Arnold, Hickey, and Harris 1995). Ferric chloride is a strong oxidant used in water treatment plants and the printing industries. It has been used to form counter ions as well in organic degradation studies. Hydrolysis of atrazine occurs at both alkaline and acidic pHs and may be important for atrazine transformation (Trotter et al. 1990). Studies have also shown that isolated pure cultures and stable mixed communities may aid in biodegradation of s-triazines and their photoproducts, but there is very little information on the presence of ferric salts and their effects on atrazine degradation (McMahon and Chapelle 1992; Shapir, Mandelbaum, and Jacobsen 1998; Kontchou and Gschwind 1999).

The objective of this study was to attempt to assess the quantitative effects of ferric iron salts and initial pH adjustments on microbial communities during atrazine degradation.

MATERIALS AND METHODS

Sampling and Chemicals

Surface water samples was collected from the Ross Barnett reservoir near Jackson, MS. Once the water was removed, the temperature and pH of the water samples were measured (Fisher 995 pH meter). Temperatures ranged between 26-29 C, and pH ranged from 6.5-7.5. Assays were initiated within one hour of collection.

UL-^{14}C-Atrazine (s.a. 20.1 mCi/mmol; purity: 98.4%) was obtained from Sigma Chemical Company. Unlabeled atrazine (98% purity) was obtained from ChemService (West Chester, PA). All atrazine was dissolved in HPLC grade acetonitrile (Fisher). UL-^{14}C-glucose (s.a. 265 mCi/mmol; purity 99%) was obtained from Moravek Biochemicals (Brea, CA). All solutions were prepared with filtered sterile water.

Incubation and Degradation

Atrazine Mineralization. ^{14}C-labeled atrazine (70 -101 µg/l) was added to 50 ml of live or killed (autoclaved or addition of formaldehyde at 1% final conc.) water samples in 150-ml quartz flasks (GM Associates Inc., Oakland, CA) for study of photochemical and microbial degradation. The quartz flasks allowed 100% transmission of light of wavelengths ≥300 nm. For simulated dark studies, 160-ml Pyrex milk bottles (Corning Co., USA) were covered with aluminum foil.

To determine the effect of pH on photolysis and microbial degradation, 0.1 N NaOH and 10% acetic acid were added to water samples to adjust the initial pH to 8.5 and 3.5 respectively before degradation experiments were conducted. To assess the effect of ferric salts on photolysis and microbial degradation of atrazine, sterile stock solutions of ferric compounds were prepared and

aliquots were added to make the final concentration of the exposure 1, 10, and 100 μM before the incubation started. The bottles were then incubated for up to three days in an outdoor tub containing continuous running water to maintain sample water at an ambient temperature (Hwang et al. 1998).

For mineralization measurements, the samples were acidified after incubation and $^{14}CO_2$ was collected using the two-trap method with 1N NaOH and β-Phenylethylamine (Hwang and Maloney, 1996). These bottles were allowed to sit at least 12 hours before completion. When samples were ready to be run, 8.0mL of UltimaGold scintillation fluid was added to disposable vials, the filter papers were placed in the vials with the fluid, and the radioactivity was measured with liquid scintillation spectroscopy (Packard Model 1600 TR).

^{14}C-Glucose Mineralization. 25 ml of autoclaved sample water and atrazine (20 mg/l) was exposed to direct sunlight (light exposure) or covered in foil (dark exposure) for up to three days. The selected treatment groups were: control (no atrazine), acidic pH, basic pH, and ferric salts (20 & 200 μM final concentration).

After exposure, 25 ml of fresh sample water was added to each bottle and incubated for 12 hours in darkness. ^{14}C-glucose (2 μg/l) was then added to the bottles and allowed one additional hour of incubation. Following incubation, the procedure discussed in the previous section was employed to trap $^{14}CO_2$.

Microbial Viability. 50 ml of fresh sample water was added to quartz flasks (light exposure) or Pyrex bottles (dark exposures) along with unlabeled atrazine (10 mg/l final conc.). The treatment groups included: control (no atrazine) acidic pH, basic pH, and ferric salts (10 &100 μM). After up to three days of incubation in a water bath, agar plates were spread with samples from the bottles to assess the effects of the exposures on the microbial populations of the samples (Hwang et al. 1998).

RESULTS AND DISCUSSION

^{14}C- UL- Atrazine Mineralization. After incubating for up to three days, ^{14}C-UL-atrazine was slowly mineralized. As seen in Figure 1, the greatest mineralization percentage was recovered from the dark live group and was 0.4, 0.9, and 2.7% respectively for day 1, 2, and 3. Percentages remained low in killed groups throughout incubation (<0.02%). The mineralization percentages increased from 0.05% after day 1 to 0.09% after day 3 for light live group and increased from 0.06% after day 1 to 2.7% after day 3 for the dark live group. From this we concluded that photolysis does not enhance total microbial degradation for atrazine mineralization in the short-term study of microbial assemblages in freshwater samples.

The results from the three-day pH exposure experiments are listed in Table 1. The results indicate that except for the dark killed treatment mineralization percentage in the control (unamended) groups was higher than the acidic group (pH 3.5). The mineralization activity was highest in the dark control group indicating that the indigenous microbes may perform better under neutral conditions. However, the mineralization rates were much lower than the previous experiments. Acidity appears to inhibit atrazine mineralization in light exposure groups. Microbial mineralization of atrazine at pH 8.5 was not different from the control neutral pH in light exposures. The dark pH 8.5 group (0.052%) was slightly larger than dark control (0.043%), but the difference was not significant. Overall, the mineralization percentage in this experiment was lower than 1.6% (i.e. the impurity of the compound).

The effect of ferric compounds on atrazine mineralization is shown in Table 3. At the concentrations examined (1, 10, & 100 μM), $FeCl_3$ failed to affect microbial mineralization of atrazine. Microbial mineralization of atrazine was still higher in dark exposures than that of light exposure groups.

^{14}C- Glucose Mineralization. When measuring glucose mineralization (Table 5), the dark exposures were generally larger than light exposures among all groups. The largest percentages were found in dark exposures at pH 8.5 (6.5 %). This was larger than the dark control (4 %); all the other dark exposures were less than the control.

The largest mineralization from the light exposures was the light with atrazine exposure (5.1 %). This was about twice the light control count (2.5 %). This stimulation may be due to microbial utilization of atrazine photoproducts as growth substrates.

The greatest inhibition of glucose mineralization was observed in the light initial pH 3.5 exposure group (0.9 %). Since the purity of the glucose was 99 %, our assumption is that the lower pHs severely inhibits the microbial metabolism.

Ferric salts exhibited no significant effects on mineralization at the 10 μM concentration in light (3.3 %) and dark (3.8 %) groups; however, at 100 μM concentrations, the salts severely inhibited the mineralization of atrazine in both the light (1.3 %) and dark (1.67 %) groups. This adverse observation may be because the final pH at concentration 10 μM remained near natural waters, while 100 μM concentrations dropped the pH to ~4.5.

Microbial Viability. Measurements of bacterial viability show that atrazine reduced the viability of microbes after three days of light exposure when compared to the light control (no atrazine) group in the pH experiments (Table 2). The most viable organisms were found in the dark exposures. At both pH 3.5 and 8.5, the dark exposures were greater than the control group, but the dark unamended samples were lower. This may be a by-product of hydrolysis reactions which occur more in acidic and alkaline pHs than neutral (Trotter et al. 1990).

As seen in Table 4, the addition of atrazine increased the bacterial growth in the $FeCl_3$ experiment under both light and dark exposures, but the effect of the ferric compound at concentrations (1, 10, & 100 μM) was not clear because the numbers fluctuated in a common range. Since the $FeCl_3$ crystals transmit red light and reflect green light. We speculate that light attenuation by the crystals reflected some of the harmful light radiation allowing more cells to remain viable (Heiserman 1992).

Transformation. Our most recent HPLC data collected indicate that atrazine is transformed after one day rather slowly. Light live exposures show a loss of 9.45% of the parent compound in one day, but light killed results indicate no degradation (0.66% of parent compound). Dark live exposures show 5.77% degradation over the same period. These preliminary results may indicate a synergistic relationship between photolysis and microbial degradation of atrazine.

Conclusion. During this series of experiments, we determined that even though pH changes and the presence of ferric salts can affect the transformation of atrazine in aquatic environments, they have no significant effect on the mineralization of the compound. The presence of ferric iron at 100 μM concentration caused the pH of the solution to drop to pH 4.5 and greatly reduced metabolic activity. We believe that the lowering of the solution pH in both studies had adverse effects on the microbial community and thus inhibited mineralization.

The results of our preliminary transformation experiments appear to support further investigation of the relationship between photolysis and microbial degradation. Even though complete mineralization was not observed, the transformation of the parent compound was observed. Further investigation may reveal a better understanding of this relationship.

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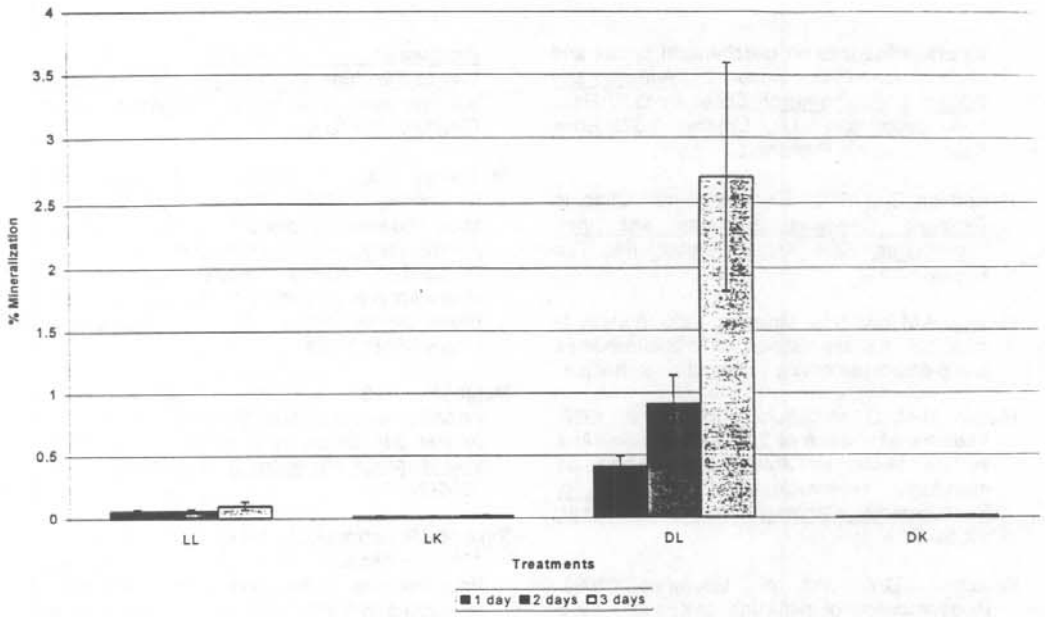

Figure 1. Atrazine Mineralization Percentage after 3 days incubation.

Table 1. The Effects of initial pH change on Atrazine Mineralization

Treatment	Amount Mineralized (ug/L)	Percent Mineralization
Dark Control	0.05 ± 0.02	0.055 ± 0.003
Dark Initial pH 3.5	0.023 ± 0.008	0.024 ± 0.008
Light Control	0.04 ± 0.03	0.05 ± 0.03
Light Initial pH 3.5	0.024 ± 0.009	0.026 ± 0.009
Dark Control	0.031 ± 0.002	0.043 ± 0.003
Dark Initial pH 8.5	0.04 ± 0.01	0.05 ± 0.02
Light Control	0.03 ± 0.04	0.04 ± 0.02
Light Initial pH 8.5	0.020 ± 0.028	0.028 ± 0.002

Table 2. The Effects of initial pH change & Atrazine on microbial viability

Treatment	C.F.U./ ml
Light Control (No atr)	18,300 ± 3,300
Light with Atr	9,900 ± 1,730
Light initial pH 3.5	3,200 ± 2,340
Light initial pH 8.5	9,000 ± 3,660
Dark Control	22,000 ± 5,000
Dark with Atr	15,100 ± 4,270
Dark initial pH 3.5	32,700 ± 4,350
Dark Initial pH 8.5	36,600 ± 7,640

Table 3. The Effects of Ferric Salts on atrazine mineralization

Treatment:(live samples)	Amount Mineralized (µg/L)	Percent Mineralization
Light Control (No Fe^{3+})	0.03 ± 0.01	0.04 ± 0.02
Light 1 µM $FeCl_3$	0.021 ± 0.001	0.029 ± 0.001
Light 10 µM $FeCl_3$	0.020 ± 0.001	0.027 ± 0.001
Light 100 µM $FeCl_3$	0.021 ± 0.005	0.029 ± 0.001
Dark Control	0.031 ± 0.002	0.043 ± 0.003
Dark 1 µM $FeCl_3$	0.04 ± 0.01	0.05 ± 0.02
Dark 10 µM $FeCl_3$	0.030 ± 0.008	0.04 ± 0.01
Dark 100 µM $FeCl_3$	0.05 ± 0.02	0.07 ± 0.03

Table 4. The Effects of Ferric Salts & Atrazine on Microbial Viability

Treatment	C.F.U./ml
Light Control (No Atr)	44,417 ± 8,480
Light with Atr	35,100 ± 9,960
Light 1 µM $FeCl_3$	45,767 ± 4,179
Light 10 µM $FeCl_3$	38,633 ± 6,716
Light 100 µM $FeCl_3$	38,500 ± 4,636
Dark Control (No Atr)	12,233 ± 4,030
Dark with Atr	12,867 ± 3,612
Dark 1 µM $FeCl_3$	20,200 ± 15,627
Dark 10 µM $FeCl_3$	41,433 ± 1,457
Dark 100 µM $FeCl_3$	32,733 ± 6,045

Table 5. Glucose Mineralization After 3 days of Exposure

Treatment	Percent Mineralization
Dark Control (No Atr)	4.0 ± 1.3
Dark with atr	3.3 ± 0.8
Dark pH 3.5	1.8 ± 1.4
Dark pH 8.5	6.5 ± 3.4
Dark $FeCl_3$10 µM	3.8 ± 1.0
Dark $FeCl_3$ 100 µM	1.7 ± 0.8
Light Control	2.5 ± 0.3
Light with atr	5.1 ± 1.7
Light pH 3.5	0.9 ± 0.2
Light pH 8.5	3.2 ± 0.8
Light $FeCl_3$ 10 µM	3.3 ± 0.6
Light $FeCl_3$ 100 µM	1.3 ± 0.5