Effect of Swine Manure Application on Atrazine Mineralization, Biomass Carbon and Atrazine Degradation

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Abstract

The effect of swine manure application on atrazine degradation and fate was investigated in two soils (Kennebec and Floyd) sites near Treynor and Nashua, IA. The Kennebec soil has a history of continuous corn and continuous atrazine application while the Floyd soil has a history of corn and soybean rotation. The half life of atrazine in Floyd was longer (55 days) than in Kennebec soil (7 days). The biomass carbon in both soils increase up to day 7 then decreased with time. Mineralization of UL-ring-^{14}C atrazine ($^{14}CO_2$ production) was faster in the Kennebec soil than in Floyd soil. Although swine manure increased microbial carbon over time in untreated soils, manure inhibited atrazine mineralization in the Floyd soil. In Kennebec soil, application of manure equivalent to 448 kg N/ha inhibited the atrazine mineralization during the first 35 days of incubation. Nitrogen from the manure may have inhibited cleavage of the atrazine ring. The differences between soils in atrazine degradation and mineralization probably resulted from the continuous application history at Treynor site, which may have increased the population of atrazine degrader.

Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is one of the most commonly used herbicides in the Midwest USA. Because of its wide use and persistence in the environment, atrazine is causing increased problems with groundwater contamination (USEPA, 1990a). The frequency of atrazine detection in groundwater is related to extensive usage, moderate persistence, and mobility through the soil profile (Spalding et al., 1989).

Biodegradation refers to the biological transformation of an organic chemical to another form, without regard to extent.

Transformation may be involve minor modifications such as an oxidation of reduction or the loss of a functional group from a molecule or may involve many sequential changes to the compound. Complete biodegradation to inorganic molecules is termed mineralization. Mineralization under aerobic conditions results in the formation of additional microbial biomass, CO_2, water and inorganic products. Mineralization under anaerobic condition results in the formation of additional microbial biomass, water, inorganic products and any one or combination of partially oxidized compounds (i.e., methane, organic acids, or others) (Skaladamy et al., 1992). Rates of atrazine degradation are relatively slow and may be influenced by several factors, including application history, soil nutrient conditions, and the presence of degrader microorganisms (Alvey et al., 1995). Microbial degradation products include hydroxyatrazine, N-dealkyl metabolites (Behki et al., 1986, 1993; siron et al., 1973; Donnelly et al., 1993; and Winkelmann et al., 1991) and readily metavolized, ring-cleavage products that lead to complete mineralization (Grigg et al., 1993; Mandelbaum et al., 1993; and Radosevich et al., 1993).

The mineralization of atrazine is affected by the form of C and N in the system. Alvey et al. (1995) found that inorganic N at high levels inhibited atrazine mineralization. The effects of N levels on the rate of atrazine mineralization were more evident when N was supplied as inorganic N added as plant debris or present in the soil organic matter. Assaf et al. (1994) observed that although the addition of N and C at the same time as the herbicide application did not affect atrazine degradation, the mineralization of atrazine metabolites increased following the later addition of a high level of soluble carbon, indication that degradation of atrazine metabolites was controlled by carbon level. Manure contains significant quantities of organic C and N. Mineralization resulted mainly from the further degradation of hydroxyatrazine and partially from deethylatrazine and deisopropylatrazine. Although the processes and pathways of atrazine degradation in soils have been widely studied, there is little information available on the effects of organic C and organic N in manure on degradation and mineralization of ^{14}C atrazine.

Microbial biomass carbon accounts for 1-3% of soil organic C (Jenkisor, 1997). It represents a labile pool of C and nutrient elements (Martens, 1995). Microbial biomass carbon may indicate potential microbial activity because it stores energy for microbial processes (Rice et al., 1996).

Kirchner et al. (1993) reported that the fertilization affects the soil microbial biomass carbon by increasing root biomass, root exudates, and crop residues, thus providing increased substrate for microbial growth. Microbial biomass levels tended to be greater in the fertilized soil than in unfertilized soil but difference between the two treatments were not significant. Martyniuk and Wagner (1978) also reported that microbial populations were greater in fertilized soil than in unfertilized soil. Saratchandra et al. (1998) found that nitrogen fertilization increased levels of organic C in soil. Fauci and Dick (1994) reported that a long-term history of organic amendments, such as animal manure, generally maintain higher levels of microbial carbon, even in the presence of recent organic amendments.

Although some literature is available on biomass carbon in soil, little information is available on the effects of manure on biomass carbon. The objectives of this study were to determine the effect of swine manure application on atrazine mineralization, biomass carbon, and atrazine degradation.

Materials and Methods

Soils

Soils were collected from Iowa State University's Northeast Research Center near Nashua, Iowa (Floyd) and from the site near Treynor, Iowa (Kennebec). The Kennebec soil has a history of continuous corn and continuous atrazine application while the Floyd soil has a history of corn and soybean rotation. The Floyd soil received a nitrogen application rate equivalent to 168 kg/ha of *N* from 1977 to 1992 (Kanwar et al., 1993). The total atrazine usage from 1972 to 1992 in Kennebec soil is 16.15 kg/ha. However, there was no atrazine usage on Floyd soil in 1978 to 1984, 1986 to 1987, 1991 to 1992 and 1996. Selected properties of both soils are given in Table 1.

Table 1
Selected physical and chemical characteristics for the Kennebec soil and the Floyd soil at 15 cm depth.

Soil	Parameters				
	Sand, %	Silt, %	Clay, %	pH	Organic C, %
Kennebec	ND	ND	ND	ND	1.67
Floyd	44	34	22	6.74	2.56

Swine Manure

Swine manure were collected from Swine Nutrition Center, Ames, Iowa. The total *N* and *C* contents were 4.56% and 39.42%, respectively. The application rates used in this study were 224 and 448 kg N/ha. Included in the total *N* analysis were NH_4 concentrations of 3095 mg/l and 1 mg/l of NO_3.

Mineralization Studies

Mineralization studies were performed by using biometer flasks containing 50 g (oven dry basis) soil from the top 15 cm of Floyd and Kennebec soils. Swine manure was added at the rates equivalent to 0, 224, 448, kg N/ha. Treatment solutions containing (UL-ring-^{14}C) atrazine (99% purity, Chem service, West Chester, PA) were prepared in methanol with a specific activity of 388.7 μ Ci/mmol. Atrazine concentration following addition to soil was 1 mg/kg soil. Triplicate flasks were prepared for each rate and flasks were incubated in the dark at 25°C. The $^{14}CO_2$ trapping solution (0.5 M *NaOH*) was changed at 3, 7, 14, 28, 35, 49, 63, 91, 105,130, 160, and 171 days and the radioactivity determined by liquid Scintillation Spectrophotometer (Model 1900 TR, Packard, Meriden, CT). Samples were prepared by adding 4 ml of Ultima Gold Cocktail XR (Packard, Meriden, CT) and 3 ml of *NaOH* taken from the CO_2 trap.

Microbial Biomass Carbon Studies

Floyd and Kennebec soils were incubated with swine manure at the rates equivalent to 0, 224, 448 kg N/ha and atrazine was added to soils at the rates of 1 mg/kg soil. At day 0, 7, 14, 28, 70 and 120, 8-mm-sieved soil samples were measured for biomass carbon by fumigation and direct extraction with 0.5 M K_2SO_4 (Tate et al., 1988). Organic *C* in the fumigated and non-fumigated extracts was measured using Dohrman DC-180 carbon analyzer (Rosemount analytical

Services, Santa Clara, CA) calibrated with potassium phthalate standards. The correction factor for calculating biomass carbon is k = 0.33 (Sparling and West, 1988).

Atrazine Degradation Studies

Kennebec soils and Floyd soils were mixed with manure at the rates equivalent to 0, 224, 448, kg N/ha and atrazine at 1 mg/kg soil. Triplicate samples were taken at 0, 7, 14, 28, 70 and 120 days. Ten grams of soils were extracted by 20 ml of the methanol/water (4:1, v/v) extraction solution. The samples were vortexed and left to equilibrate for 12 hours at room temperature. Then the automated procedures were done by Zymark Robotic System in which the samples were vortexed and centrifuged at 2,700 rpm for 10 minutes. Repeat three times with an additional 14 ml of extraction solution. The combined extracts were evaporated under air at 50°C for 65 minutes. The remaining of the two ml of methanol followed by two ml of water. Elution of the atrazine from the *C*18 were done by the addition of 2 ml of ethyl acetate which contain terbuthyazine (0.5 ng/μL). These extracts were analyzed by Gas Chromatography with HP-5 (Cross-linked 5% phenyl, 95% methyl silicone) column, 80 kPa of head pressure, and *He* as a carrier gas with the flow rate of 1 ml/min.

Results and Discussion

Effect of Swine Manure on Mineralization of Atrazine

Atrazube mineralization in the non-manured Floyd soil were obviously higher than the Floyd soils mixed with manure at equivalent rate of 224, 448 kg N/ha (Figure 1). The average mineralization of ^{14}C atrazine at 171 days after addition was equal to 50.7%, 18.8% and 27.4% of applied ^{14}C atrazine for the soil mixed with manure equivalent to 0, 224 and 448 kg N/ha, respectively. These demonstrated that atrazine mineralization in Floyd soil were inhibited by manure in which the nitrogen from the manure inhibited ring cleavage of atrazine in the soil mixture resulting in lower mineralization rates in manure samples. This was consistent with the work of Alvey et al. (1995), who reported that inorganic *N* at high levels inhibited atrazine mineralization and the effects of N levels on the rate of atrazine mineralization were more evident when *N* was supplied as inorganic *N* at high levels than as organic *N*.

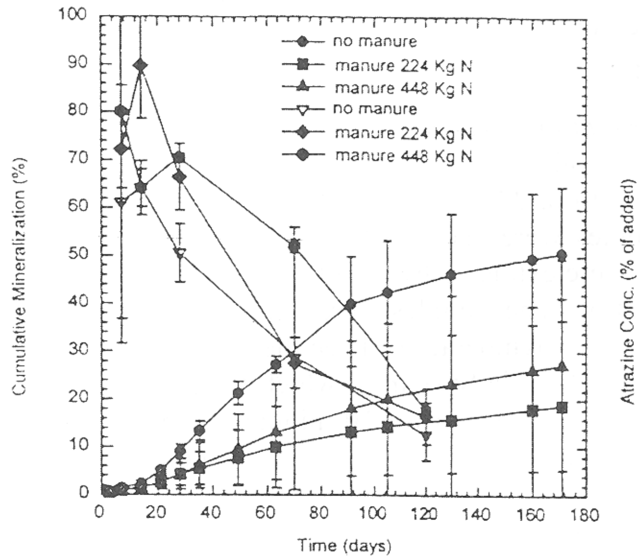

Figure 1. Cumulative mineralization of (^{14}C-UL-ring) atrazine and swine manure N application rates in Floyd soil as a function of time.

In the Kennebec soil, application of manure equivalent to 448 kg N/ha inhibited atrazine mineralization during the first 35 day incubation (Figure 2). The rate of mineralization in the Kennebec soil was faster than in the Floyd soil. This may result from the more frequent atrazine application history at Treynor site, which may had increased the population of atrazine degraders. The results (Figure 2) also showed that there was not much difference in the total mineralization between the soil without manure and the soil samples mixed with manure at rates of 224 and 448 kg N/ha (5.7% and 2.9%, respectively). However, the mineralization rate in soil treated with 224 kg N/ha was a little bit higher than in the control. This could be explained that the degraders-degraders utilized the triazine ring as a *N* source. Possibility the nitrogen from the manure inhibited ring cleavage of atrazine in the soil mixture resulting In lower mineralization rates in manured samples.

After day 171 the ^{14}C bound residues were measured after methanol-water extractions and the results showed that the remaining ^{14}C activity was bound to the soils (Table 2). Possibility that *C* added in manure increased in sorption which, in turn, increased bound residues.

Table 2
Distribution of 14C 171 days after additional of (14C-UL-ring)

Soil	Manure	Bound	Mineralized	Extracted
	kg N/ha equiv	%	%	%
Kennebec	0	9.1	70.0	5.0
Kennebec	224	10.6	75.6	3.0
Kennebec	448	9.9	67.0	3.0
Floyd	0	30.0	50.7	25.2
Floyd	224	51.7	18.8	25.5
Floyd	448	44.7	27.4	23.7

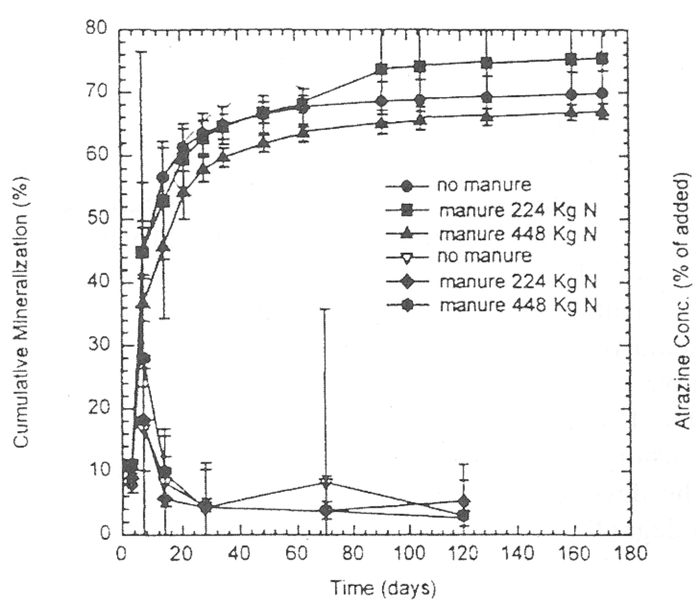

Figure 2. Cumulative mineralization of (^{14}C-UL-ring) atrazine and swine manure N application rates in Kennebec soil as a function of time.

Effect of Swine Manure on Biomass Carbon

In the Kennebec soil, the biomass carbon at day 0 for non-manured, manure at equivalent rate of 224, 448 kg N/ha samples were 302, 504 and 597 mg/kg dry weight of soil, respectively. In the Floyd soil, the biomass carbon at day 0 for the non-manured, manure at equivalent rate of 224, 448 kg N/ha samples were 218, 232 and 297 mg/kg dry weight of soil, respectively. Biomass carbon in non-manured Floyd and Kennebec soil, respectively. Biomass carbon in non-manured Floyd and Kennebed soils and these soils mixed with manure at equivalent rate of 224, 448 kg N/ha increased up to day 7 then decreased with time. The rapid increase of biomass carbon at day 7 may by attribute to the large population of the microorganisms and the large quantity of organic *C* and *N* in manure. It was possible that the biomass carbon after day 7 decreased because the nutrients for microorganisms became depleted.

Effect of Swine manure on Atrazine Degradation

The results showed that there was no effect of swine manure application to the degradation of atrazine in both soils (Figure 3 and 4). The half lives were determined by using the regression analysis. The half life of atrazine in Floyd was longer (55 days) than in Kennebec soil (7 days). This may due to the amounts of organic matter in the soils. Kennebec soil had less organic matter (1.67%) than Floyd soil (2.56%) so there was less sorption of atrazine to Kennebec soil which resulted to more atrazine available for being degraded. This was consistent with the work of Hamaker et al. (1972) who reported that the sorption was the primary mechanism for atrazine retention in soil and organic matter was the most important constituent for sorption. Greater bound residues in manure treated Floyd soils may be due to accumulation of hydroxyatrazine, which was more strongly sorbed than atrazine. It was also possible that the Kennebec soil from Treynor site had a history of the more frequent atrazine application which may result in the development of an adapted, acclimated microorganisms capable of degrading atrazine as a *N* sourece.

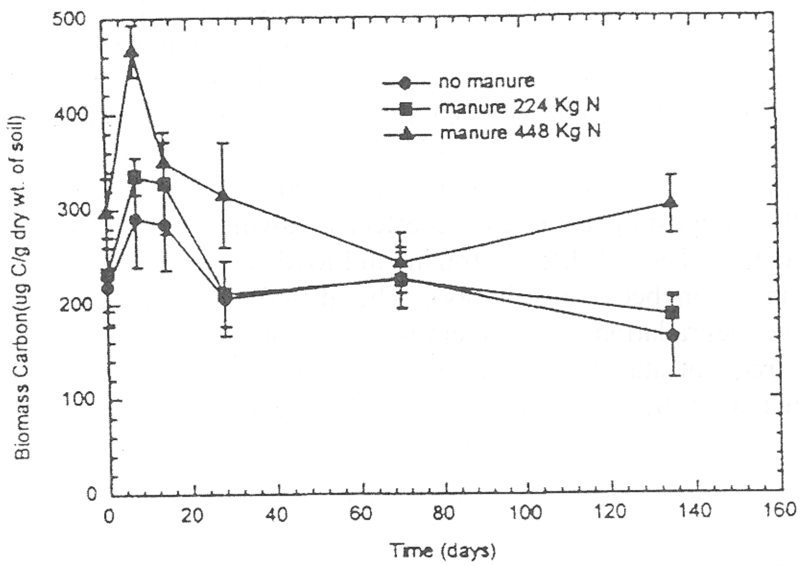

Figure 3. Biomass carbon of Floyd soil as a function of time.

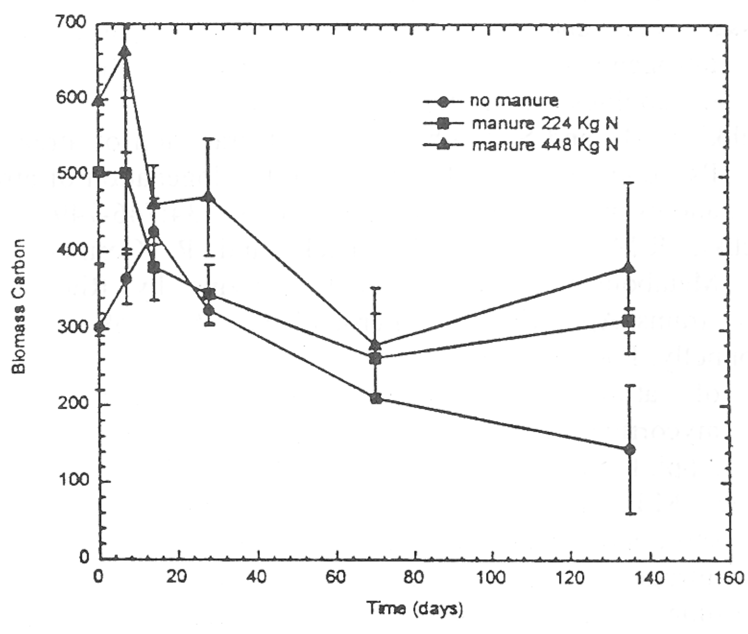

Figure 4. Biomass carbon of Kennebec soil as a function of time.

Conclusion

Studies were conducted to investigate the effects of swine manure application on atrazine mineralization, biomass carbon and atrazine degradation. Samples were collected from Treynor (Kennebec) and Nashua (Floyd) sites in Iowa and were mixed with manure at three rates (0, 224, 448 kg N/ha). The results of this study indicate that the swine manure inhibited atrazine mineralization slightly, suggesting a repressive effect of swine manure on atrazine degradation. The half life of atrazine in Floyd soil was longer (55 days) than in Kennebec soil (7 day). The differences between soils in atrazine degradation and mineralization probably resulted from the more frequent atazine application history at Treynor site, which may had increased the population of atrazine degraders.

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