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Biodegradation of atrazine in soil using poultry litter

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Abstract

Microorganisms, including *Pseudomonas* and *Actinomycetes* species, are known to degrade atrazine and other pesticides in soil. Poultry litter has a large number of microorganisms along with many nutrients. Atrazine is applied to soil at times soon after using poultry litter as manure. The objective of this research was to study the degradation of atrazine (2 or 3 ppm) in soil using poultry litter. The soil + atrazine mixture was treated with either poultry litter, gamma irradiated poultry litter or water extract of the irradiated litter in order to differentiate between the effects of microorganisms, nutrients and organic matter. Atrazine in the soil was extracted with water and methanol and analyzed by pesticide immunoassay (ELISA) 1, 5, 10, 30 or 60 d after poultry litter treatment. The small loss of atrazine from soil treated with the irradiated litter was almost the same as from the sterile soil with no poultry litter. Atrazine was significantly (86%) degraded in soil with untreated poultry litter within 30 d. Degradation was virtually completed within 60 d. The rate of atrazine biodegradation with poultry litter was almost 2 times faster than without the litter. The toxicity (EC_{50}) of the samples after treatments, to *Photobacterium phosphoreum* ("Microtox"), was also measured. The toxicity of the soil + atrazine mixture treated with poultry litter (both the untreated and the gamma irradiated) was the same as that of the soil + litter mixture; no significant concentrations of toxic by-products were produced from the biodegradation of atrazine.

Keywords: Biodegradation; Atrazine; Poultry litter; Microrganism; Toxicity

1. Introduction

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine], a derivative of the s-triazines is a selective herbicide, controlling broadleaf and grassy weeds in corn, sorghum, rangeland, sugar cane, grass sod, and other crops [1]. Annual use of atrazine is of the order of 75–90 million pounds in the United States [2]. Depending on the type of soil and an application rate of 2–4 lbs per acre the half-life of atrazine has

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been reported as 40–190 d [3, 4]. Half-life of atrazine in soil at pH 4 (25 °C) has been reported to be 244 d [5]; in the presence of humic acid the half-life at pH 2.9, 4.5, 6.0 and 7.0 was found to be 34.8, 174, 398 and 742 d, respectively [5]. Atrazine remains highly mobile in soils, does not strongly adsorb to sediments and is not expected to bioconcentrate or volatilize [5]. Atrazine has been shown to be acutely and chronically toxic to freshwater and estuarine fauna [6]. Aerobic degradation of atrazine in the soil environment is a combination of biological and chemical processes. The main degradation pathways of atrazine are dehalogenation and n-dealkylation. Abiotic dehalogenation and subsequent hydrolysis result in the formation of hydroxyatrazine; direct dechlorination of atrazine by microorganisms has not been readily observed. Mandelbaum et al. [7] have isolated the bacterium *Pseudomonas sp.* that mineralizes atrazine from a herbicide spill site. Dechlorination of deethylatrazine and deisopropylatrazine was facilitated by *Pseudomonas putida*, *Pseudomonas fluorescens* and *Pseudomonas stutzeri* [8, 9]. The only bacterium that rapidly hydrolyzed atrazine was *Fusarium roseum* [10]. *Nocardia* can utilize atrazine as its sole source of carbon [8]. The n-dealky-lation pathway is the mechanism that microorganisms use to breakdown atrazine. The *Pseudomonas* preferentially remove the isopropyl side chain more than the ethyl side chain [8]. Anaerobic biodegradation is not known to play a significant role in the breakdown of atrazine in field soils; anaerobic biodegradation produces more toxic end products [11].

Poultry litter has a large number of microorganisms that can potentially act as degradative agents. The microbial population of litter is acidophilic bacteria, fungi, algae and aerobic heterotrophs; this includes *Pseudomonas*, *Actinomycetes* and *Nocardia* [12]. Tao [13] found that poultry litter could degrade gasoline in the soil. Poultry litter has a large amount of nutrients (nitrates and phosphates) that can speed up the bioremediation of soil contaminated by organic compounds [14]. Each ton of poultry manure contains 80 lbs of nitrogen, 50 lbs of phosphate and 40 lbs of potash [15]. Poultry litter is commonly applied to the soil as manure before application of atrazine.

The objective of this study was to determine the effect of poultry litter on the biodegradation of atrazine in soil and to monitor the toxicity of the end products.

2. Materials and methods

2.1. Soil and poultry litter samples

Soil was collected randomly (top 10 cm) from a 30 m square plot (divided in to 30 sub-plots), following standard procedures [16], on the university farm and sieved (<1 mm). The soil was a sandy loam with a hydraulic conductivity of 0.85 cm s^{-1} (moderate drainage). The permeability of the soil was 5.9 cm h. Nitrogen levels in the soil were 45 ppm nitrates and Kjeldahl nitrogen each. The poultry litter was collected from the university poultry house in sterilized bags, air dried and sieved under sterile conditions. The litter was five flock old (about 300).

2.2. Gamma irradiation treatment

The soil and one-half of the poultry litter were gamma irradiated separately with ^{60}Co using a dose rate of 316.28 KRAD/h (3.1628 KGRAY/h) for 9.5 h [17]. No microorganisms were found in the gamma-irradiated soil or poultry litter indicating complete sterilization [18].

2.3. Water extract of poultry litter

Thirty milliliter of sterile water (with 1% peptone) was added to each of the 10 g portions of the radiated litter in sterilized plastic centrifuge bottles. The water + poultry litter mixture was hand shaken for 5 min, then centrifuged at 3500 rpm for 15 min. The leachate was then decanted into a sterile Erlenmeyer flask and stored at −10 °C.

2.4. Soil treatments

Poultry litter was added to each flask (0 or 10 gm; to simulate actual field conditions with or without the litter) from the following treatments:

(1) Poultry litter. This treatment indicates the role of water soluble nutrients, organic matter and microorganisms in the biodegradation process.

(2) Gamma irradiated (sterile) litter. This treatment indicates the role of nutrients and organic matter without any microorganisms in the biodegradation process.

(3) Water extract of sterile litter. This treatment indicates the role of water soluble nutrients alone in the biodegradation process.

Five time periods (1, 5, 10, 30 or 60 d; based on preliminary testing that showed complete degradation before 60 d) were used to determine the biodegradation. The moisture content of the soil was kept at 50% by weight during the course of the experiment. The flasks were incubated at 22 ± 1 °C. The flasks were periodically shaken to maintain aerobic conditions.

2.5. Atrazine solutions and extraction procedures

Analytical grade atrazine (a gift from the Ciba-Geigy Co.) solutions were prepared in aqueous methanol (15 ml/1) to ensure complete dissolution of the atrazine and added to the soil. Atrazine solution was added to the soil to get a final concentration of 0, 2 or 3 ppm atrazine; 3 ppm is more than the amount normally used in agricultural fields. At the designated time period, the soil mixture was extracted for atrazine. One-hundred-fifty milliliter of deionized water was added to each flask, the mixture was transferred to a centrifuge bottle, shaken at 180 cycles per min for 8 h and then centrifuged at 30 000 rpm for 15 min. The water extract was decanted and stored at −10 °C until analyzed. The soil was further extracted twice with methanol:water (80:20, v/v) first for 12 h and then for 8 h. The methanol extracts were kept at −10 °C.

2.6. Atrazine analyses

Atrazine was measured in the soil using pesticide immunoassay (Quantix Systems, Moorestown, NJ) along with an ELISA (enzyme linked immunosorbant assay) plate reader. This method is highly sensitive to atrazine in soil or water. The degradation products of atrazine do not significantly interfere with the results of the test. Atrazine can be detected at levels as low as 0.05 ppb in soil or water. This method correlates well with traditional methods of gas chromatography and high-performance liquid chromatography with the benefit of less cleanup and shorter analysis time for each sample [19].

Sterile soil, with 2 or 3 ppm atrazine, was used as control. No microorganisms were detected in these samples up to 60 d.

2.7. Toxicity

Each of the methanol extracts was combined with the water extract for toxicity analyses. Toxicity was determined with the use of "Microtox Bioassay" [20]. This bioassay uses a strain of luminescent microorganisms, *Photobacterium phosphoreum*, to measure toxicity of a chemical. These organisms emit light as a byproduct of their respiration. If respiration is inhibited in some way, light output is reduced or growth is stopped [21]. The reduction in light output is proportional to the degree of toxicity of a sample and the drop in light output can be converted into an effective concentration where 20% (EC_{20}) or 50% (EC_{50}) of the organisms are affected by the toxic substance. This bioassay is sensitive to 1–2 ppb atrazine.

2.8. Statistical design and analyses

The design of the experiment was a randomized complete design, with 4 poultry litter and 3 atrazine treatments, 5 time periods and 3 replications. Means of these 3 replicates with standard errors are given in Tables 1 and 2. Analysis of variance and Duncans Multiple Range test were used to determine significant differences ($p \leqslant 0.05$) among each treatment and within groups of treatments.

3. Results and discussion

3.1. Atrazine degradation

Background levels of atrazine found in the soil or poultry litter were less than 90 ppb. Sterilized soil mixed with 2 ppm atrazine alone degraded the herbicide very slowly with time (Table 1, Fig. 1). After 30 d, the amount of atrazine left in soil mixed with atrazine (2 ppm) and poultry litter was only 0.27 ppm showing an 86% loss of atrazine. Almost complete degradation (96%) occurred in 60 d. The rate of atrazine degradation with poultry litter was almost 2 times faster than without litter. The small loss of atrazine from the sterile soil treated with the irradiated

Table 1
Atrazine concentration (ppm) in soil samples

Amendment	Days after treatment				
	1	5	10	30	60
	2 ppm Atrazine				
Control	2.19^{aw}	1.81^{ax}	1.58^{ax}	0.506^{ay}	0.313^{ay}
	(0.31)	(0.54)	(0.33)	(0.071)	(0.023)
Poultry litter	2.01^{bw}	1.08^{bx}	0.85^{bx}	0.274^{by}	0.077^{cy}
	(0.30)	(0.091)	(0.031)	(0.026)	(0.009)
Poultry litter (sterile)	1.96^{bw}	1.47^{abx}	1.56^{awx}	0.488^{ay}	0.119^{bcy}
	(0.075)	(0.051)	(0.059)	(0.013)	(0.005)
Sterile litter's water extract	1.93^{bw}	1.22^{bx}	1.52^{aw}	0.587^{ay}	0.189^{bz}
	(0.04)	(0.05)	(0.05)	(0.02)	(0.01)
	3 ppm Atrazine				
Control	3.27^{aw}	2.21^{ax}	2.21^{ax}	1.63^{ay}	0.379^{az}
	(0.12)	(0.13)	(0.17)	(0.99)	(0.01)
Poultry litter	2.60^{bw}	2.05^{aw}	2.05^{bw}	0.55^{dx}	0.232^{ax}
	(0.23)	(0.27)	(0.31)	(0.01)	(0.01)
Poultry litter (sterile)	2.97^{aw}	2.08^{ax}	2.03^{bx}	0.99^{cy}	0.243^{az}
	(0.19)	(0.11)	(0.12)	(0.10)	(0.01)
Sterile litter's water extract	2.74^{abw}	2.05^{ax}	2.14^{abx}	1.15^{by}	0.371^{az}
	(0.13)	(0.11)	(0.14)	(0.91)	(0.03)

Standard error of means of 3 replicates is given in parentheses.
a, b, c and d are column-wise comparisons.
w, x, y, and z are row-wise comparisons.
Means with the same letters are not significantly different ($p \leqslant 0.05$).

poultry litter was almost the same as from the sterile soil without the addition of litter. No significant change in these results was noticed on using soil without irradiation. The atrazine (1–2 ppm) loss in soils amended with bovine manure has been reported [21, 22] in the range of 70–95 d. Wastewater sludge can lower atrazine levels in the soil; some of the organisms in the sludge are found in poultry litter as well [23]. Singh et al. [24] showed that atrazine loss was greater in soil that contained microorganisms than in soil with no microorganisms. The results of this experiment show that the microorganisms in the litter played a significant role in the biodegradation of atrazine. The pH of the soil changed from 7.0 to 7.6 on the addition of the poultry litter. An increase in pH from 6.0 to 7.0 (in the presence of humic acid and other organic materials) increased the half life of atrazine from 398 to 742 days [5]; in the experiment reported here the half life of atrazine is reduced, in the presence of poultry litter (which is mostly organic matter), pointing towards biodegradation by the microorganisms in the litter.

The (small) loss of atrazine in soil with sterile litter or its water extract was the same as in the soil with no litter. This shows that the sterile litter's organic matter or its water extract's nutrients did not play any role in the degradation of atrazine. Sterilized soil that was mixed with 3 ppm atrazine and poultry litter also showed

Table 2
EC_{50} (ppm) of soil samples

Amendment	Days after treatment				
	1	5	10	30	60
	2 ppm Atrazine				
Control	31.53^{bx}	23.91^{axy}	18.31^{ay}	42.38^{aw}	24.91^{axy}
	(2.57)	(2.01)	(1.91)	(3.75)	(2.44)
Poultry litter	10.05^{cwx}	12.15^{bw}	6.38^{bxy}	5.78^{by}	7.02^{bxy}
	(1.07)	(1.93)	(1.13)	(1.02)	(1.05)
Poultry litter (sterile)	7.81^{cx}	8.44^{cwx}	7.30^{bx}	9.23^{bwx}	9.80^{bw}
	(1.77)	(1.98)	(1.85)	(1.23)	(1.65)
Sterile litter's water extract	49.56^{aw}	21.60^{ax}	17.89^{ax}	34.53^{awx}	23.02^{ax}
	(3.69)	(3.01)	(1.97)	(3.02)	(2.87)
	3 ppm Atrazine				
Control	33.49^{bxy}	18.32^{ay}	16.79^{ay}	53.98^{ax}	73.36^{aw}
	(3.05)	(1.77)	(1.53)	(4.15)	(4.79)
Poultry litter	9.17^{cwxy}	6.12^{bxy}	5.84^{by}	10.58^{bwx}	11.46^{bw}
	(1.02)	(1.33)	(1.61)	(1.98)	(2.71)
Poultry litter (sterile)	9.60^{cw}	6.78^{bw}	6.95^{bw}	6.81^{bw}	8.09^{bw}
	(1.72)	(0.79)	(1.37)	(1.62)	(1.79)
Sterile litter's water extract	75.72^{aw}	19.47^{ay}	23.88^{ay}	43.84^{ax}	62.49^{aw}
	(5.41)	(2.73)	(2.31)	(3.78)	(5.43)

Standard error of means of 3 replicates is given in parentheses.
a, b, c and d are column-wise comparisons.
w, x, y, and z are row-wise comparisons.
Means with the same letters are not significantly different ($p \leqslant 0.05$).

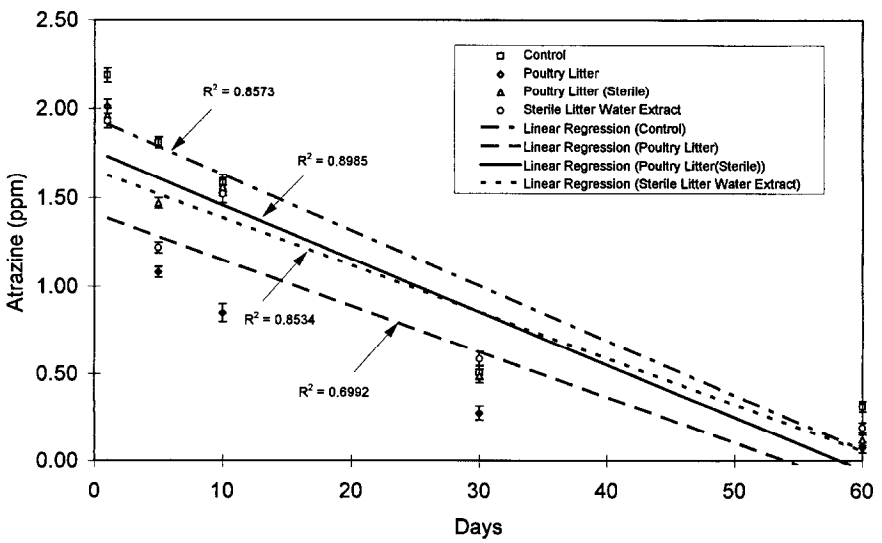

Fig. 1. Changes in 2 ppm atrazine concentration.

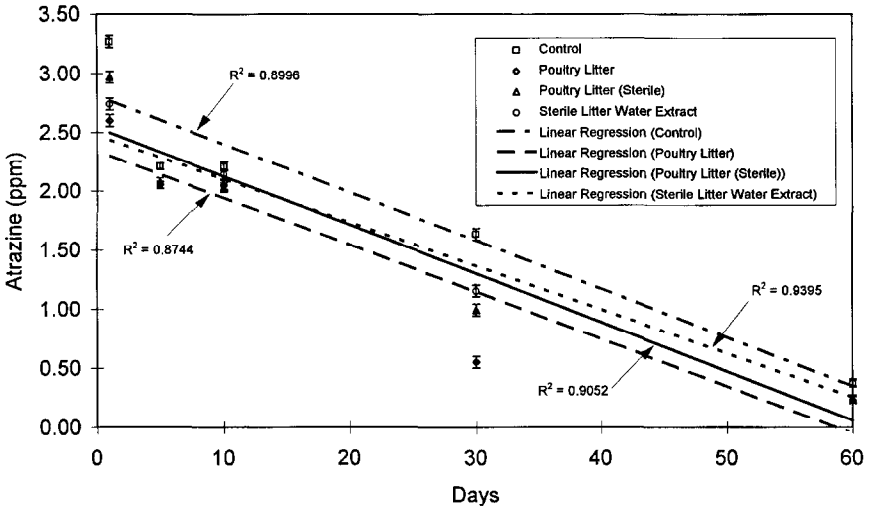

Fig. 2. Changes in 3 ppm atrazine concentration.

similar degradative effects (Table 1, Fig. 2). A statistically significant difference between the results from the sterile and non-sterile litter treatments was noticed only at the 30 d time point with 3 ppm atrazine in soil. Thirty days after mixing atrazine (3 ppm) with soil alone its concentration changed to 1.63 ppm but with the addition of poultry litter the atrazine concentration was reduced to 0.55 ppm.

3.2. Toxicity analyses

The lower the EC_{50} value the more toxic the substance is. No significant differences were seen in the toxicity of soil, soil + atrazine or soil + sterile litter's water extract. The toxicity of the soil mixed with poultry litter (untreated or sterile) was significantly higher than the toxicity of the soil alone. The poultry litter has a significant amount of toxicants including ammoniacal compounds, metals and antibiotics [15] which were not found in the soil. The toxicity in poultry litter is not from the bacteria that make up the litter, but from the toxicants in the litter as demonstrated by the same levels of toxicity both with the sterile and untreated litter [15].

The toxicity of the soil + atrazine (2 or 3 ppm; Table 2) mixed with the poultry litter (both untreated and sterile) was significantly greater than the toxicity of soil + atrazine alone but was not different from the toxicity of the soil + litter mixture. This toxicity did not change significantly with time indicating that the degradation products were not toxic as the experiment was conducted under aerobic conditions. Kross et al. [25] reported that degradation products of atrazine were less toxic than the parent compound to *Photobacterium phosphoreum*.

From these results it can be concluded that poultry litter's addition to sterile soil resulted in the biodegradation of atrazine twice as fast as the degradation in soil with no litter and that on degradation no toxic byproducts were produced.

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