Chapter 7. [U-ring-^{14}C] atrazine mineralization and incorporation in a bioremediated soil

7.1. Introduction

In Chapter 5, declines in atrazine leachability in several soil treatments amended with organic sorbents and nutrients was examined. It was found that a significant amount of atrazine was lost during 120 days of incubation. The purposes of this study were: 1) to determine more precisely the mass balance of atrazine in contaminated soil during bioremediation which includes the amount of volatilization, mineralization, transformation, sorption and incorporation, and relative importance of these different pathways in terms of their overall contribution to atrazine dissipation; 2) to explore the mechanisms of sorption and incorporation of atrazine and its metabolites into soil humic substances and minerals. I tested hypothesis that a major portion of the atrazine residue associated with the soil humic acid fraction was covalently bound to humic materials.

Radiolabeled atrazine was used as a tracer for determining the overall metabolism/fate of atrazine incubated in soil matrices. This experiment was conducted in a closed system with air circulated through radiolabeled incubation chambers. Soil containing [U-ring-^{14}C] atrazine was amended with corn meal, fertilizers (NPK) and peat moss, etc., and was incubated in the chambers for 16 weeks. Corn meal was added to the amended samples and the matrix was mixed at 8 weeks. Samples were taken at 0, 8, and 16 weeks intervals for analysis (Materials & Methods 3.6.). Volatile compounds released from contained system in the form of $^{14}CO_2$ and other ^{14}C-volatiles were trapped by 5 N KOH solution and polyurethane foam plugs (PUFs), respectively. The amount of activity released in the form of $^{14}CO_2$ was used to measure the degree of atrazine mineralization by soil microbes. Volatilization was based on the radioactivity extracted from the polyurethane foam plugs (PUFs).

7.2. Fractionation of radioactivity in atrazine contaminated soil

In addition to mineralization, atrazine may also be transformed into various polar and nonpolar metabolites, and incorporated into portions of soil organic matter (SOM),

and sorbed to soil minerals. At the end of incubation intervals, atrazine contaminated soil was removed from the incubation chambers and extracted exhaustively with ethylacetate and separated into two fractions (ethylacetate extract I and organic solvent insoluble) (Figure 3-1). Atrazine in the organic solvent insoluble fraction was further extracted with 0.1 NaOH, producing two subsequent fractions (alkali soluble and alkali insoluble). During alkali extraction, part of the atrazine and its metabolites bound into the SOM were also presumed to be released into the alkaline solutions. The types of SOM bound atrazine and its metabolites can be complex. Binding may be due to a range of interactions such as van der Waals' forces, weak or strong chemical forces, etc. It is also possible that the residue could be physically trapped in soil micropores. In order to determine whether physical entrapment played a major role in sequestering atrazine, alkali soluble and insoluble fractions were further extracted by methylene chloride and ethylacetate. It was assumed that a large portion of the physically trapped atrazine and its metabolites would be partitioned into non-polar organic solvent phases (methylene chloride as Methylene chloride extract, and ethylacetate as Ethylacetate extract II) once a portion of the SOM macromolecular structures were partially relaxed under alkaline conditions. Methylene chloride was selected as the solvent for extracting matrices from alkali soluble fraction because of its well-defined separation from alkaline solutions (aqueous) when it was used as an extracting agent. Alkali soluble fractions easily form emulsions with most other organic solvents when they are mixed. The methylene chloride and ethylacetate extractions were used to remove portions of those partitioned residue in alkali soluble and insoluble fractions, respectively. However, in addition to physically trapped atrazine, other weakly bound atrazine and its metabolites could also enter into methylene chloride and ethylacetate phase. After ethylacetate extraction, the alkali insoluble solids were combusted forming $^{14}CO_2$. The radioactivity associated with the $^{14}CO_2$ represented the radioactivity associated with alkali insoluble. The radioactivity in ethylacetate extract I, ethylacetate extract II and methylene chloride was separated into different metabolites and quantified by high performance thin layer chromatography (HPTLC) and liquid scintillation counting (LSC) (Figure 3-1).

7.3. Recovery of radioactivity from bioremediated soil study

Radiolabeled [U-ring-^{14}C] atrazine used for bioremediated soil study was recovered from various fractions after 8 and 16 weeks of incubation (Table 7-1). The results indicate that the overall radiolabeled recoveries from 8 and 16 week amended and unamended treatments ranged from 92% (3702 pCi) to 95% (3793 pCi) based on the total radiolabeled recovered from the 16 week frozen controls. The amended treatment recovered 94% (8 weeks, 3749 pCi) and 92% (16 weeks, 3702 pCi) of the initial radioactivities; while the corresponding unamended treatments recovered 94.7% (8 weeks, 3793 pCi) and 95% (16 weeks, 3822 pCi) of the initial sample radioactivity.

7.4. Volatilized ^{14}C-organic compounds

Volatile materials were released from the incubation media, this might include not only metabolites of ^{14}C-atrazine, but also a small amount of volatilized ^{14}C-atrazine. Volatile radioactivity was collected by two consecutive polyurethane foam plugs described as volatile I and volatile II (Materials and Methods 3.6.4.). The amount of radioactivity in volatile I and volatile II is tabulated in Table 7-1. Overall, only a small percentage of radioactivity was associated with these fractions. The amount of radioactivity in volatile I ranged from 0.2% (7.8 pCi) to 0.9% (35.3 pCi) of total radioactivity applied. The corresponding radioactivity in volatile II ranged from 0.01% (0.5 pCi) to 0.07% (2.6 pCi) of total radioactivity. For the volatile II, only approximately 21% radioactivity was detected after the first 8 week incubation interval, most activity (79%) was released from the second 8 week incubation interval. The cumulative volatile II in unamended treatments was evenly distributed between the first 8 week and second 8 week intervals, with each consisting of 50% of total volatile II activity (Figure 7-1). However, the unamended treatments had 4.5 and 3.5 times more radioactivity in volatile I than that of the corresponding amended treatments after 8 and 16 weeks of incubation, respectively (Table 7-1). Based on total activity in volatile I and II (Sum of volatile I and II), the unamended treatments had 4.3 and 2.8 times more radioactivity than the corresponding amended treatments after 8 and 16 weeks of incubation. Except for the 16 week amended treatments (77%), more than 93% of the total radioactivity was associated

Table 7-1. Summary of radioactivity associated with various fractions of remediated atrazine soil residues after 8 and 16 weeks of incubation[1,2]

	Radioactivity in pCi (% of total radioactivity)				
		Incubation interval			
Fractions	0	8 Weeks		16 Weeks	
	Frozen Control	Unamended	Amended	Unamended	Amended
Carbon dioxide	0(0)C	10.1 ± 3.0(0.3)B	24.6 ± 20.2(0.6)B	20.5 ± 15(0.5)B	190.6 ± 44(5)A
Volatile I	0(0)C	35.3 ± 2.7(0.9)A	7.8 ± 0.6(0.2)B	30.2 ± 9(0.8)A	8.6 ± 4.6(0.2)B
Volatile II	0(0)C	0.7 ± 0.1(<0.1)B	0.5 ± 0.4(<0.1)B	1.3 ± 0.8(<0.1)AB	2.6 ± 1(<0.1)A
Total volatiles	0(0)C	36 ± 2.6 (0.9)A	8.3 ± 0.5(0.2)B	31.5 ± 9(0.8)A	11.2 ± 4.4(0.3)B
Ethylacetate I	3955 ± 128(98.8)A	2949 ± 109(74)B	1450.4 ± 63.8(36)D	2478 ± 121(62)C	1132.9 ± 88(28)E
Ethylacetate II	1.6 ± 0.3(<0.1)B	8.4 ± 2.5(0.2)A	9.4 ± 2(0.2)A	9.9 ± 0.5(0.3)A	7.1 ± 1.2(0.2)A
Methylene chloride	0.9 ± 0.2(<0.1)C	16.9 ± 15(0.4)B	13.9 ± 2.1(0.4)AB	59.3 ± 29(1.5)A	10.9 ± 4.1(0.3)B
Fulvic acid	27.9 ± 2.3(0.7)C	211.6 ± 52(5)B	229 ± 19.9(5.7)B	371.1 ± 30(9)A	304.4 ± 42(8)AB
Humic acid	3.6 ± 0.3(<0.1)D	107.2 ± 41(3)C	149.6 ± 28.7(4)BC	187 ± 17(5)AB	232.6 ± 14(6)A
Alkali insoluble	18.4 ± 1.3(0.5)C	453.6 ± 58(11)B	1863 ± 57.7(47)A	664.1 ± 189(17)B	1812 ± 63(45)A
Total	4007 ± 4	3793 ± 60	3749 ± 13	3822 ± 70	3702 ± 70
Recovery(%)	100	95	94	95	92

[1]Values represent the mean ± SE based on 3 replicate samples.

[2]Upper case letters indicate comparison within columns between treatments. Concentrations with the same letter are not significantly different. Analysis is completed through analysis of variance (MANOVA) with Tukeys multiple range test on the means (Anonymous, 1985; Hair et al., 1992; $P > 0.05$).

with the radioactivity in volatile I. No effort was made to determine the identify of the
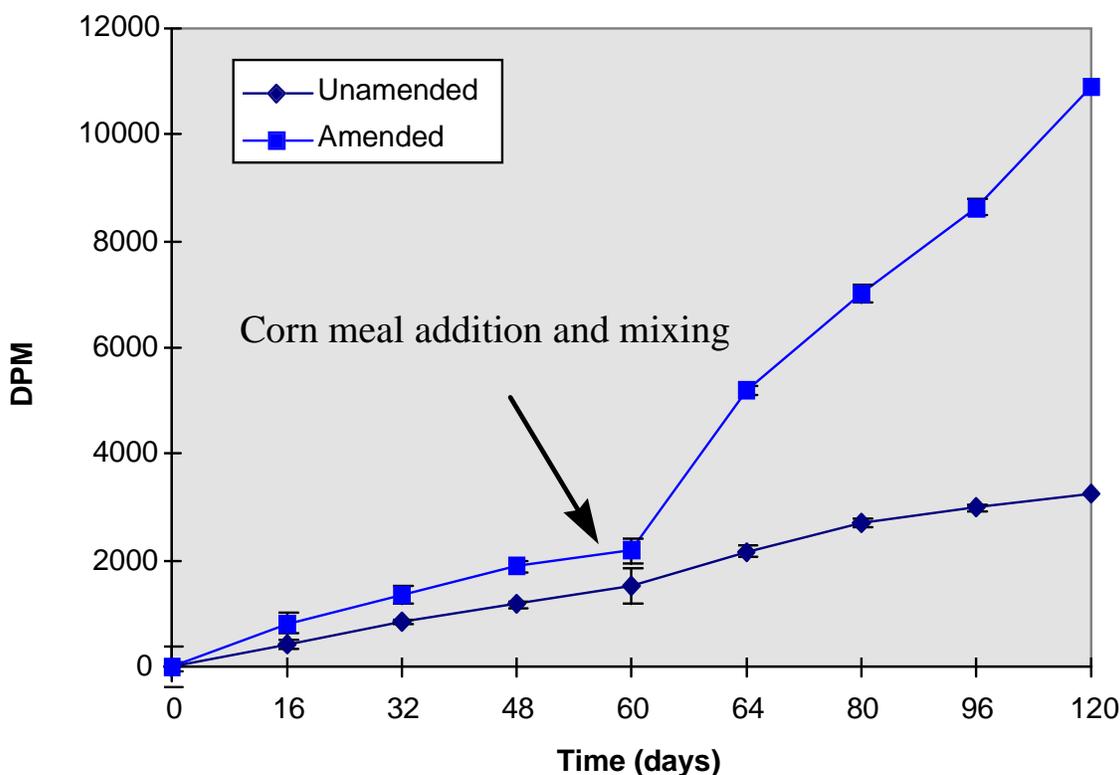volatiles trapped in the polyurethane foams (PUFs).

Figure 7-1. Cumulative ^{14}C-volatiles in the volatile II evolved from atrazine bioreactors

7.5. Atrazine mineralization as indicated by evolved $^{14}CO_2$

Based on the amount of $^{14}CO_2$ released, the amount of atrazine mineralized was
minimal. The amount $^{14}CO_2$ released was less than 5% in both amended and unamended
treatments (Table 7-1). Although only a small amount of total radiolabeled atrazine was
recovered as $^{14}CO_2$, the amended treatment produced 2.5 (8 weeks) and 9.3 (16 weeks)
times more $^{14}CO_2$ than the corresponding unamended treatments. The cumulative $^{14}CO_2$
released over 16 weeks was significantly higher in amended treatments (190.6 pCi) than
in unamended treatments (20.5 pCi) (P < 0.5, Figure 7-2; Table 7-1). It should be noted
that approximately 87% of $^{14}CO_2$ was produced during the second 8 week incubation
period while only about 13% of $^{14}CO_2$ was released from the first 8 week incubation
period in the amended treatments. However, comparison of the first 8 and second 8
week intervals of the unamended soil produced about same level of $^{14}CO_2$ (ca. 20.5 pCi).

The difference in $^{14}CO_2$ released from 8 week (30.6 pCi) versus 16 week (215 pCi) incubation period was statistically significant (P< 0.5) for all amended and unamended treatments.
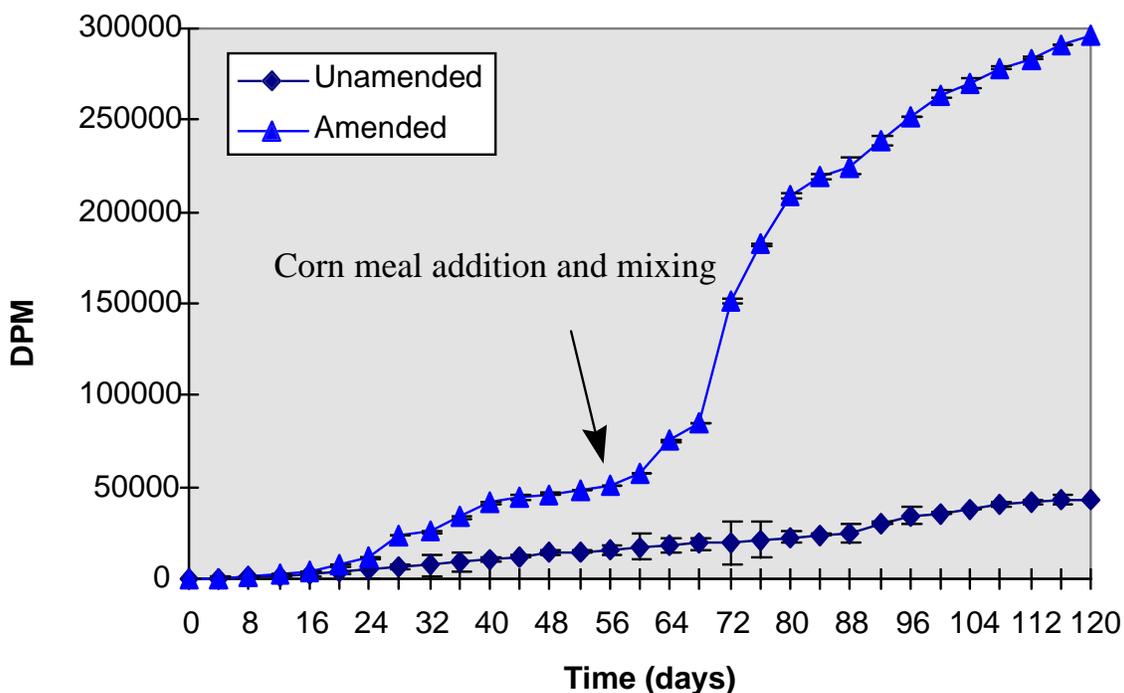

Figure 7-2. Cumulative $^{14}CO_2$ evolution from atrazine bioreactors

7.6. Solvent extractable fraction of ^{14}C-atrazine and its associations

Most of the total applied radioactivity was found in the ethylacetate extract I fraction. The percentage of the ethylacetate radioactivity in the amended treatments was 36 and 28% for 8 weeks (1450 pCi) and 16 weeks of incubation (1133 pCi), respectively; and 74 (8 weeks, 2949 pCi) and 62% (16 weeks, 2478 pCi) were found in the corresponding unamended treatments for the same time interval. The ethylacetate radioactivity was 3955 pCi or 98.7% of the total applied radioactivity in controls. Statistical analysis showed that the controls had significantly higher radioactivity in the ethylacetate I fraction than any of the treatments (P < 0.05). Approximately twice as much ethylacetate extractable atrazine and its metabolites were found in the unamended treatments than in the amended treatments. Overall, the unamended and 8 week

treatments had higher radioactivity in ethylacetate I than the corresponding 16 week amended treatments.

7.7. Radioactivity associated with soil organic matter

A major portion of radioactivity associated with the soil organic matter was indicated by the amount of radioactivity found in the soil humic acids and fulvic acids. The combined percentages of radioactivity associated with humic acids and fulvic acids ranged from 8 (8 weeks, unamended) to 14% (16 weeks, amended and unamended) of total radioactivity (Table 7-1). The amount of radioactivity in the fulvic acids was slightly higher than that of humic acids for each treatment after the 16 week incubation interval. The overall radioactivity in the fulvic acid and humic acid fractions from the amended treatments increased from 379 pCi (8 weeks) to 537 pCi (16 weeks). Similar results were obtained in the unamended treatments, which were from 319 pCi (8 weeks) to 558 pCi (16 weeks). No significant differences were found between unamended or amended treatments for the radioactivity associated with fulvic acids or humic acids in the either the 8 week samples or the 16 week samples. The amended treatments tended to contain more ^{14}C-radioactivity in the humic acids than the unamended treatments.

Less than 1% of the total radioactivity (< 10 pCi) was present in the ethylacetate extract II (Figure 3.1; Materials and Method 3.6.5.). The radioactivity found in these extracts was similar ranging from 7 to 10 pCi per treatment (P > 0.05). The radioactivity recovered from methylene chloride extracts was also less than 1% of total applied radioactivity (< 17 pCi) except for the 16 week unamended incubation, which was 1.5% (59.3 pCi).

7.8. Recovery of radioactivity in the alkali insoluble fraction

Approximately 47% (1863 pCi) and 45% (1812 pCi) of the total radioactivity was associated with the alkali insoluble fractions in the amended treatments for 8 and 16 week incubation interval, respectively. Only 11% (453.6 pCi) and 17% (664.1 pCi) of the total radioactivity were found in the corresponding 8 and 16 week unamended

treatments. Amended treatments had significantly higher radioactivity in the alkali insoluble fraction than the unamended treatments for either the 8 week or 16 week incubation interval.

7.9. Thin-layer chromatography (TLC) of ethylacetate extract I

In order to characterize the potential metabolites formed during incubation in the ethylacetate extract I, thin-layer chromatography (TLC) was used to determine the activity associated with different atrazine metabolites. TLC results of amended and unamended treatments in the ethylacetate extract I after 16 weeks are shown in Table 7-2. The radioactivity was reported as dpm in 10 μl of total 5 mL in the final ethylacetate extract I volume. The means and their standard errors (SE) were based on three replicates. The percentage of each compound was calculated based on total radioactivity in the ethylacetate extract I. (Materials and Methods 3.6.7. to 3.6.9.).

It was unexpected to find that the frozen ethylacetate I controls contained all six atrazine metabolites as well as atrazine. Although the ^{14}C-atrazine standard provided by the manufacturer was labeled as high as 99% purity, it is possible that it contained a small percentage of impurities (less than 1%). Because of the extremely high sensitivity of radioactivity detection technique and the relatively high radioactivity that was used in the experiment, the low radioactivity in minor impurities could be within the detection limits of thin layer chromatography combined with liquid scintillation counting (TLC-LSC). Thin-layer chromatography revealed that the [U-ring-^{14}C] atrazine stock solution prepared from the standard contained the following materials: atrazine (91%), deethylatrazine (2.8%), deisopropylatrazine (1.7%), diaminoatrazine (2.1%), OH-atrazine (1.4%), OH-deethylatrazine (0.5%), OH-deisopropylatrazine (0.4%). The results were presented in the Table 7-2.

Table 7-2. Atrazine and its metabolites in the ethylacetate extract I after 8 and 16 weeks of incubation[1,2,3]

	Radioactivity in dpm (% of total radioactivity)				
	Incubation interval				
Compounds	0	8 Weeks		16 Weeks	
	Control	Unamended	Amended	Unamended	Amended
Atrazine	16064 ± 1640(89)A	9575 ± 421(86)B	6192 ± 382(89)C	8766 ± 296(74)B	1319 ± 625(36) D
Deethylatrazine	569 ± 144(3)B	691 ± 111(6)AB	322 ± 95(5)B	1037 ± 231(9)A	415 ± 155(11)B
Deisopropyl-atrazine	580 ± 127(3)B	377 ± 29(3)BC	196 ± 66(3)C	1076 ± 237(9)A	345 ± 128(9)BC
Diaminoatrazine	388 ± 144(2)AB	261 ± 63(2)BC	92 ± 26(1)C	338 ± 39(3)BC	608 ± 150(17)A
OH-atrazine	168 ± 81(1)BC	103 ± 37(1)BC	46 ± 1(0.7)C	215 + 21(2)B	434 ± 72(12)A
OH-deethylatrazine	117 ± 37(0.7)A	85 ± 22(0.8)A	45 ± 9(0.6)A	116 ± 26(1)A	353 ± 315(10)A
OH-deisopropyl-atrazine	106 ± 28(0.6)BC	63 ± 9(0.6)BC	42 ± 5(0.6)C	319 ± 90(3)A	168 ± 23(5)B
Total	17992 ± 1555	11154 ± 524	6934 ± 687	11867 ± 796	3643 ± 1033
Measured	17401	12977	6382	10905	4985
Recovery (%)	103	86	109	109	73

[1]Values represent the mean ± SE based on 3 replicate samples; dpm = disintegrations per minute.

[2]Upper case letters indicate comparison within a row between treatments. Concentrations with the same letter are not significantly different. Analysis through analysis of variance (MANOVA) with Tukeys multiple range test on the means (Anonymous, 1985; Hair et al., 1992; $P > 0.05$).

[3]Thin-layer chromatography revealed that the [U-ring-14C] atrazine stock solution prepared from the standard contained the following materials: atrazine (91%), deethylatrazine (2.8%), deisopropylatrazine (1.7%), diaminoatrazine (2.1%), OH-atrazine (1.4%), OH-deethylatrazine (0.5%), OH-deisopropylatrazine (0.4%).

Overall recovery was calculated based on the sum of atrazine radioactivity and its metabolites as compared to the total radioactivity measured in the ethylacetate extract I. The recoveries ranged from 73% (16 weeks, amended) to 109% (8 weeks, amended and 16 weeks, unamended), which indicates good correlation with the results of direct measurement using LSC. The percentage distribution of radiolabel activity in atrazine and its metabolites were quite similar among the 8 week amended, unamended treatments. Atrazine was the major compound contributing to the overall radioactivity and about 10% of radioactivity was derived from the other six atrazine metabolites. The higher concentrations of less polar metabolites such as deethylatrazine and deisopropylatrazine were present than the other relatively more polar metabolites such as OH-atrazine, OH-deethylatrazine, OH-deisopropylatrazine and diaminoatrazine in the 8 week incubation treatment.

A change in the pattern of the percentage of radioactivity distribution was observed for the 16 week amended and unamended treatments. The total activity of the 16 week unamended treatment samples was almost the same as that of 8 week samples. However, the percentage of atrazine decreased from 86% to 74%, while the percentage of deethylatrazine, deisopropylatrazine and OH-deisopropylatrazine increased approximately 2% (OH-deisopropylatrazine) to 6% (deisopropylatrazine). This suggests that atrazine was converted to these metabolites, but the further transformation of those metabolites was relatively slow because of the lack of microbial activity or other pathways that may have been involved but not resolved by the TLC system. On the other hand, atrazine in the amended treatments experienced rapid degradation during the second 8 week of soil incubation. The total radioactivity in the ethylacetate extract I in the 16 week amended treatment was only 29% of that of controls (4985 dpm vs. 17,401 dpm in 10 ul ethylacetate extract I). The amount of ^{14}C-atrazine remaining after 16 weeks was low (36%, 1319 dpm) compared with the 8 week amended treatments (89%, 6192 dpm) or controls (96%, 16064 dpm). The six metabolites were present in higher concentrations at the 16 week incubation interval than they were at the 8 week

incubation interval. In fact, OH-atrazine, diaminoatrazine and deethylatrazine each represented more than 10% of the total radioactivity (Table 7-2).

7.10. Atrazine and its metabolites in ethylacetate extract II

The levels of atrazine and its metabolites in the ethylacetate extract II are reported in Table 7-3. As expected, controls had a relatively low level of activity in the ethylacetate extract II. Unamended treatments had the highest level of radioactivity in the ethylacetate extract II, and the amended treatments had lower recoveries, compared with the unamended and controls. Total radioactivity recovery obtained from the TLC separated spots ranged from approximately 62 (8 weeks, amended treatment) to 101% (8 weeks, unamended treatment) based on the total radioactivity.

In terms of composition, OH-atrazine was present in higher amounts in the amended treatments than the corresponding unamended treatments. Approximately 17% (651 dpm) and 31% (1081 dpm) of the radioactivity in the OH-atrazine fraction was found in the 8 week and 16 week interval amended treatments, while only 10% (565 dpm) and 9% (562 dpm) was found in the corresponding spots recovered from the 8 week and 16 week unamended treatments. A higher activity of atrazine, deethylatrazine and deisopropylatrazine was present in the unamended than in the amended treatments. After the 16 week incubation interval, only 24% (821 dpm) of the radioactivity was recovered as atrazine in the amended treatments while 50% (3037 dpm) of radioactivity was recovered as atrazine in the unamended treatment. It should also be noted that the percentage of atrazine in the amended treatment decreased from 42% (8 weeks) to 24% (16 weeks) after 16 weeks of incubation. The deisopropylatrazine concentration apparently remained constant since approximately 15% of overall radioactivity in the both 8 week (822 dpm) and 16 week (909 dpm) was found in the unamended treatments. But its concentration was lower in the 8 week (9%; 358 dpm) and 16 week (7%; 249 dpm) amended treatments respectively ($P > 0.05$). Similar results

Table 7-3. Atrazine and its metabolites in the ethylacetate extract II after 8 and 16 weeks of incubation[1,2,3]

	Radioactivity in dpm (% of total radioactivity)				
	Incubation interval				
Compounds	0	8 Weeks		16 Weeks	
	Control	Unamended	Amended	Unamended	Amended
Atrazine	283 ± 88(28)C	2429 ± 141(44)A	1627 ± 200(42)B	3037 ± 578(50)A	821 ± 94(24)C
Deethylatrazine	125 ± 27(12)C	643 ± 34(12)AB	362 ± 186(9)BC	820 ± 176(13)A	316 ± 51(9)C
Deisopropyl-atrazine	150 ± 18(15)B	822 ± 108(15)A	358 ± 182(9)B	909 ± 285(15)A	249 ± 43(7)B
Diaminoatrazine	112 ± 18(11)B	577 ± 256(10)A	461 ± 120(12)AB	489 ± 80(8)AB	643 ± 175(18)A
OH-atrazine	164 ± 40(16)B	565 ± 99(10)AB	651 ± 143(17)AB	562 ± 142(9)AB	1081 ± 265(31)A
OH-deethylatrazine	118 ± 24(11)B	302 ± 65(5)A	224 ± 90(6)AB	187 ± 61(3)AB	279 ± 28(8)A
OH-deisopropyl-atrazine	73 ± 16(7)A	205 ± 114(4)A	171 ± 96(4)A	92 ± 89(2)A	94 ± 11(3)A
Total	1025 ± 137	5544 ± 384	3855 ± 820	6098 ± 1238	3484 ± 594
Measured	1070	5511	6230	6508	4660
Recovery (%)	96	101	62	94	75

[1]Values represent the mean ± SE based on 3 replicate samples; dpm = disintegrations per minute.

[2]Upper case letters indicate comparison within a row between treatments. Concentrations with the same letter are not significantly different. Analysis through analysis of variance (MANOVA) with Tukeys multiple range test on the means (Anonymous, 1985; Hair et al., 1992; $P > 0.05$).

[3]Thin-layer chromatography revealed that the [U-ring-14C] atrazine stock solution prepared from the standard contained the following materials: atrazine (91%), deethylatrazine (2.8%), deisopropylatrazine (1.7%), diaminoatrazine (2.1%), OH-atrazine (1.4%), OH-deethylatrazine (0.5%), OH-deisopropylatrazine (0.4%).

were found for the deethylatrazine, with higher activity present in the unamended treatment. There was only a small amount of radioactivity associated with OH-deethylatrazine and OH-deisopropylatrazine in either unamended or amended treatments over 16 week incubation period. However, there was a small increase in the amount of diaminoatrazine in the 16 week amended treatments. Approximately 18% (643 dpm) of the extract radioactivity was identified as diaminoatrazine in the 16 week amended treatments (Table 7-3).

7.11. Atrazine and its metabolites in the methylene chloride extract

Most of the radioactivity in the methylene chloride extract (methylene chloride extract I) was associated with atrazine in the 8 week and 16 week unamended treatments (Table 7-4). Approximately 63% (6576 dpm) and 68% (15,980 dpm) of radioactivity was present in the fractions associated with atrazine in the 8 week and 16 week unamended treatments, respectively. All the six metabolites had relatively low (< 10%) radioactivity in the unamended treatments. Compared with unamended treatments, the amended treatments had a relatively low proportion of radioactivity associated with the atrazine fraction. Only 39% (2585 dpm) and 31% (1040 dpm) of the radioactivity was found in the 8 week and 16 week amended treatments, respectively. Hydroxyatrazine, deisopropylatrazine and diaminoatrazine had a relatively high activity in the amended treatments representing from 8% (555 dpm) to 26% (1744 dpm) of total radioactivity in the methylene chloride extract. Compared with the 16 week treatments, a significantly higher amount of radioactivity was detected in the fractions of atrazine (15,980 dpm vs. 1040 dpm), deethylatrazine (1315 dpm vs. 309 dpm), deisopropylatrazine (2140 dpm vs. 437 dpm) and diaminoatrazine (1135 dpm vs. 339 dpm) in the amended treatments than in the unamended treatments measured for the same time interval (P < 0.05). The amount of atrazine, deethylatrazine and deisopropylatrazine had also increased over time in the unamended treatments (Table 7-4).

Table 7-4. Atrazine and its metabolites in the methylene chloride extract after 8 and 16 weeks of incubation[1,2,3]

Compounds	Radioactivity in dpm (% of total radioactivity)				
	Incubation interval				
	0	8 Weeks		16 Weeks	
	Control	Unamended	Amended	Unamended	Amended
Atrazine	230 ± 129(15)C	6576 ± 3004(63)B	2585 ± 1003(39)BC	15980 ± 1776(68)A	1040 ± 87(31)C
Deethylatrazine	97 ± 23(6)C	638 ± 142(6)B	383 ± 125(6)BC	1315 ± 357(6)A	309 ± 58(9)BC
Deisopropyl-atrazine	95 ± 34(6)B	719 ± 345(7)B	555 ± 143(8)B	2140 ± 597(9)A	437 ± 100(13)B
Diaminoatrazine	288 ± 207(18)B	635 ± 290(6)AB	565 ± 104(9)AB	1135 ± 495(5)A	339 ± 87(10)B
OH-atrazine	459 ± 128(29)A	1082 ± 369(10)A	1744 ± 809(26)A	1998 ± 902(9)A	641 ± 634(19)A
OH-deethylatrazine	263 ± 164(17)A	419 ± 215(4)A	472 ± 90(7)A	552 ± 378(2)A	315 ± 171(9)A
OH-deisopropyl-atrazine	140 ± 66(9)A	323 ± 246(3)A	285 ± 63(4)A	435 ± 193(2)A	245 ± 59(7)A
Total	1572 ± 493	10392 ± 2474	6589 ± 1153	23555 ± 1398	3327 ± 1087
Measured	2085	11187	9226	39164	4800
Recovery (%)	76	93	72	60	69

[1]Values represent the mean ± SE based on 3 replicate samples; dpm = disintegrations per minute.
[2]Upper case letters indicate comparison within a row between treatments. Concentrations with the same letter are not significantly different. Analysis through analysis of variance (MANOVA) with Tukeys multiple range test on the means (Anonymous, 1985; Hair et al., 1992; $P > 0.05$).
[3]Thin-layer chromatography revealed that the [U-ring-14C] atrazine stock solution prepared from the standard contained the following materials: atrazine (91%), deethylatrazine (2.8%), deisopropylatrazine (1.7%), diaminoatrazine (2.1%), OH-atrazine (1.4%), OH-deethylatrazine (0.5%), OH-deisopropylatrazine (0.4%).

7.12. Discussion

Atrazine dissipation from contaminated soil can result from volatilization, mineralization, transformation, sorption and incorporation. Volatilization and mineralization were not significant, but transformation, sorption and incorporation were the main dissipation routes for atrazine in the experimentally contaminated soils. This discussion is organized in the order of results reported in the previous sections.

The overall volatilization of atrazine and its metabolites from both unamended and amended soils was not significant. Less than 1% of ^{14}C-atrazine applied was collected in the fractions of volatile fractions I and II. This is consistent with its relatively low vapor pressure. In addition to this, volatilization was lower in organically-based material amended soils. There was more than three times the ^{14}C-activity captured from the unamended treatments than those from the corresponding amended treatments. The amended treatments containing more organic matter may reduce volatilization of atrazine and its metabolites from bioreactors due to increased sorption. These volatiles probably consisted of both atrazine and its metabolites. Due to the placement order of the PUFs in the incubation system, volatile I may have contained the ^{14}C-activity associated with atrazine and less volatile metabolites, while volatile II may have contained the ^{14}C-activity associated with more volatile atrazine metabolites. It might be that more atrazine was lost due to volatilization in the unamended treatment. However, more ^{14}C-activity was detected associated with volatile II in the amended treatments, especially in the second 8 weeks incubation interval (Figure 7-1). This suggests that more metabolic activity was occurring in the amended treatment and hence more ^{14}C-activity was lost in the form of more volatile atrazine metabolites. Addition of corn meal at the 8 weeks interval may provide additional carbon resource for microbial activity. It is also possible that microbial acitivities in amended treatments was stimulated as a result of mixing process

The atrazine rates mineralization in (represented as the ^{14}CO$_2$ data) both the unamended and amended treatments were low. Less than 1% of [U-ring-^{14}C] atrazine

was transformed to $^{14}CO_2$ in the unamended treatment over 16 weeks, while approximately 5% of atrazine was mineralized in the amended treatments during the same time period. After 16 weeks of incubation, the $^{14}CO_2$ released from the amended contaminated soil samples was ten times higher than that released from the unamended treatments. These results support the hypothesis that microbes were involved in atrazine mineralization process. They are also in agreement with the results reported by other workers (Wagner and Chahal, 1966; Goswami and Green, 1971; Dao et al., 1979; Assaf and Turco,1994).

Soil microorganisms have shown the potential for cometabolizing atrazine. As discussed in Chapter 5, energy and nutrient resources such as corn meal, vegetable oil and fertilizers may increase soil bacterial and fungal populations, elevating their metabolic activities. Enhanced microbial activity can increase the potential for cometabolism of atrazine. It is likely that soil microbes have the capability of cometabolizing atrazine since many microbial enzymes have a broad substrate spectrum (McGilvery, 1970). These enzymes synthesized for one purpose could have the influence of catalyzing the detoxification of atrazine and its metabolites through various reactions such as deamination, dechlorination and/or dealkylation. However, in terms of metabolic efficiency, cometabolism of atrazine might not be quite as efficient, since these transformations depend on other unrelated activities and could not directly benefit soil microorganisms as a nutrient and energy source. In this study, only a small percentage of atrazine was mineralized, therefore, cometabolism most likely was a main contributor of overall atrazine transformation. In addition to this, nutrient amendments had a significant influence in mineralizing rate of either the 8 week or the 16 week treatments. Similar to volatile II, addition of corn meal increased $^{14}CO_2$ release from amended treatments (Figure 7-2). These provided additional evidence that atrazine cometabolism might be one major degradation pathway of atrazine mineralization since cometabolism usually increases with enhanced microbial activity.

It is known that complete mineralization can be achieved by a specific group of microorganisms. Species from the genera *Pseudomonas* and *Rhodococcus* that possesses the capability of using atrazine as sole carbon and nitrogen sources have been isolated and identified from soil with extended periods of atrazine enrichment (Mandelbaum et al., 1995; Radosevich et al., 1995). In this study, microbial cultures obtained from contaminated bioreactor with 2-3 years atrazine usage was added to both unamended and amended atrazine contaminated soils. However, since the overall mineralization rates were relatively low for both the unamended and the amended treatments, the existence of a large soil microbial population that was capable of efficiently mineralizing atrazine was unlikely. If these kinds of organisms were present, certain environmental factors such as substrate availability, soil pH, and temperature, etc., may have limited their activities and hence hindered their efficiency for atrazine utilization.

As indicated in Chapter 5, not all microbial activities lead to the complete degradation of soil xenobiotics, especially for those recalcitrant compounds such as atrazine. Certain aspects regarding atrazine transformation into various intermediate metabolites through various microbial activities has been already considered, details on these transformations will now be discussed. It was found that a significant decrease in solvent extractable atrazine was observed on the organic amended treatments after the 8 and 16 weeks of incubation (Table 7-1). Within these solvent extractable fractions, the presence of atrazine and its six major metabolites including deethylatrazine, deisopropylatrazine, OH-atrazine, OH-deethylatrazine and diaminoatrazine have been determined. Almost an equal percentage of radioactivity came from these atrazine metabolites for the 8 week unamended and amended treatments (14 vs. 11%), while 26% and 64% of radioactivity resulted from atrazine metabolites for the 16 week unamended and amended treatments, respectively (Table 7-2). Over time, more radioactivity was detected in these six metabolites. Comparing the amended treatments with the unamended treatments during the 16 week incubation, there was a relatively larger percentage of radioactivity found in the polar atrazine metabolites which include OH-

atrazine, OH-deethylatrazine, OH-deisopropylatrazine and diaminoatrazine. While only a few bacteria have been identified as having the capability of mineralizing atrazine, a number of bacteria and fungi can partially degrade atrazine to its dealkylated or deaminated metabolites (Radosevich et al., 1995). These kinds of bacteria and fungi most likely were present in the soil mixture in this study, and nutrient amendments increased their abundance and activities.

In addition to mineralization and transformation, atrazine could be physically trapped, sorbed and bound to soil humic substances and minerals. Humic substances are considered to be the most important soil components which affect the fate of herbicides in soil (Senesi and Chen, 1989; Senesi, 1993). In this study, humic substances were extracted by 0.1 N NaOH and then further separated into humic and fulvic acids based on their solubilities at different pH. Soil humic and fulvic acids interact with atrazine through various types of mechanisms including physical trapping (Xing et al., 1996), proton exchange (Hayes, 1970), hydrogen bonding (Sposito et al., 1996), electron transfer (Senesi et al., 1995; Sposito et al., 1996), hydrophobic interaction (Lerch et al., 1997) and possibly covalent binding (Bollag, 1990; Dankwardt et al., 1996). Soil organic matter is a dynamic macromolecule which has a highly solvated, expandable and flexible segmental structure (Hayes and Himes, 1986) and is often times described as having holes within its molecular structure (Schulten, 1996). Sorption of atrazine was physically modeled as a bimodal or dual-phase process which included both a fast and a slow sorption phase (Pignatello and Xing, 1996; Xing et al., 1996). The model was based on the facts that competitive sorption occurred between atrazine and other organic compounds such as prometon and cyanazine in mineral and peat soils, and humic acids. These workers proposed that both partition and hole filling contributed to the sorption of atrazine by soil organic matter and estimated as much as one-third to one-half of atrazine sorption could occur in the hole domain. The formation of hydrogen bonding between atrazine and NH_2, OH, COOH at soil organic matter was considered to primary interaction in immobilization of atrazine at hole sites.

Chemical interactions involving in the sorption of atrazine by humic substances were investigated by a number of researchers with spectroscopic techniques including UV-visible, infrared, and electron spin resonance spectroscopy (Martin-Neto, et al., 1994; Senesi et al., 1995; Sposito et al., 1996). The study by Sposito et al. (1996) revealed that s-triazines interact with humic acid functional groups with the weak interactions including proton transfer, hydrogen bonding, and electron transfer. The actual interactions depend on the basicity of s-triazine molecule, functional groups of humic substances and the pH of media. They suggested that proton transfer is more likely occurring where humic substances have high acidic functional group contents and the s-triazines have low basicity, and electron-transfer mechanisms are more likely occurring where the humic substance have low acidity functional groups and the s-triazines have a high basicity. In this study, atrazine and its associations were extensively extracted with ethylacetate. Due to the relatively weak forces of the proton transfer and hydrogen bonding, most untrapped atrazine and its associations with these weak interactions would most likely be recovered by ethylacetate extraction (Ethylacetate extract I). The radioactivity associated with these weak interactions decreased over time, and the amendments with lignocellulosic material and microbial nutrients decreased the portions of radioactivity associated with these weak interactions. As indicated by Xing et al. (1996), significant amount of atrazine might be trapped within the holes of humic substance macromolecules. These holes or hydrophobic sites which contribute to the binding of nonionic pesticides such as atrazine exist at low pH in fulvic and humic acids, but are destroyed by conformational changes associated with carboxyl deprotonation at higher pH levels (Li et al., 1992; Martin-Neto, et al., 1994). Therefore, most physically entrapped atrazine and its association by humic substances would be recovered into the methylene chloride extract. The results from this study indicate that both atrazine and its metabolites were physically or weakly absorbed by soil organic matrices, however, only a small percentage (2%) of those physically absorbed atrazine and its metabolites were present in either the amended or unamended treatments. Similar to the radioactivity distribution in the ethylacetate extract I, the more polar atrazine metabolites were found in those physically trapped residues in the 16 week amended treatments. It appears that

the amount of physically entrapped atrazine by humic substances were not substantial or most of these entrapped residues were recoverable by solvent extraction (ethylacetate extract I). However, since the experiment was only conducted within a relatively short period time (16 weeks), the fate of these physically trapped atrazine and its associations in a long time frame are still need to be investigated.

Chemical binding or covalent bonding was considered to be the most important factor for *s*-triazine dissipation in soil (Calderbank, 1989) and composting environment (Berry et al., 1993a, b; Judge, 1996). Hydrophobic interactions may assist in the incorporation of atrazine to soil organic matter at the initial stage but are considered to be reversible. However, incorporation is viewed as being a long term stabilization process resulting in atrazine or its metabolic byproducts becoming chemically or covalently bound to soil organic matter (Calderbank, 1989; Berry et al., 1993a, b). Due to the intrinsic complex physiochemical heterogeneity of soil organic matter, direct evidence of atrazine and its degradative products covalently bound to humic substance has not yet been confirmed. Nevertheless, the chemical bond formation between atrazine and humic substance most likely occurs due to microbial enzymatic activities or/and the catalysis of some minerals such as manganese dioxides. Dealkylation is one of the most important mechanism in the microbial degradation of atrazine (Behki and Khan, 1986). Atrazine dealkylated metabolites may covalently bound to humic substance at the amino moieties of the triazine ring. Berry and Boyd (1985) found that aromatic amines (e.g., anilines) covalently bound to quinone with the catalysis of horseradish peroxidase while dealkylated atrazine and anilines are not aromatic, atrazine contains three annular nitrogens which might reduce the reactivity of the substituted amine.

Atrazine may form covalent bonds with humic substances at the chloride position of the triazine ring. Haider et al. (1993) demonstrated the fungicide anilizine, 4,6-dichloro-N-(2-chloro-phenyl)-1,3,5-triazine-2-amine, was able to covalently bound into soil organic matter. Anilizine metabolites was bound to various functional OH-groups of humic substances by dechlorization of triazine ring of aninline metabolites in the forms

of ethers or/and esters. Based the specific positions of immunoconjugate formation, Dankwardt et al. (1996) also proposed that atrazine was probably bound to humic and fulvic acids via the substitution of the chloride atom in the triazine ring. These workers detected up to 50 ng of bound residues in one gram of soil. More bound residue was associated with humic acids than the corresponding fulvic acids. The amount of non-extractable atrazine recovered was different from soils with different maize grown practices (yearly vs. every 2nd year). The results suggest that bound atrazine was associated with microbial activities, but the authors did not discuss this aspect. In this study, the total amount of activity associated with fulvic and humic acids was about 14% for both unamended and amended treatments after 16 weeks of incubation (Table 7-1). As discussed in the previous sections, the amount activity associated with humic and fulvic acids were unlikely to be residues resulting from weak interactions such as proton transfer, hydrogen binding and physical trapping. Most of this radioactivity probably represented the amount of atrazine and its metabolites covalently bound to humic substances. It is possible that atrazine may concurrently bind to soil humic substance by two mechanisms mentioned here or other mechanisms that we have not discussed, due to the complexity of soil humic substances, the heterogeneity of soil environment and enormous potential microbial enzymatic systems.

Since about 14% of overall applied radioactivity residues were associated with humic and fulvic acid fractions at unamended and amended treatments, incorporation to humic substances was clearly one of the most important interactions that contributes to atrazine dissipation in the contaminated soil samples. However, as much as 45% of the applied radioactivity was found in the alkali insoluble fraction for the 16 weeks amended treatments. Soil organic alkaline extraction efficiency was considered to be only about 80% according to the estimation of Stevenson (1982). Therefore, the alkaline insoluble fractions might include unrecoverable humic and fulvic acids, unextractable humins and partially degradative cellulose substances, minerals, etc. It is unlikely that atrazine and its degradative products interact with these soil insoluble fractions in a single dominant interaction. As discussed in the interactions of atrazine with humic and fulvic acids, the

humic substances remaining in the alkaline insoluble fractions most likely interact with atrazine through the formation of covalently bonding or physically trapped in the hydrophobic holes within their macromolecules. The interactions with soil clays could be complex depending on the types of clay, soil structure, soil pH and moisture level, etc.

Sorption by clay minerals may play an important role in the dissipation of atrazine and its metabolites in amended contaminated soil. A closely-related *s*-triazine tricyclazole were reported strongly sorbed to Ca-montmorillonite, Ca-kaoline and soil organic matter (humic and fulvic acids) (Xiao, et al., 1991b). They found the pH of soil-water system had a strong influence of tricyclazole sorption. Most *s*-triazines and their degradation products are weak bases with dissociation constants (pK_a) ranging from 1.7 to 5.3 (Lerch et al., 1995), cation exchange might play an important role for sorption of *s*-triazine and their metabolites either to clay or soil organic matter if soil-water pH is within 2 pH units of the pK_a. Since the pH near colloid surfaces has been reported to be approximately 0.5-2 units lower than that of the bulk soil solution (Weber, 1970), atrazine and its metabolites would be protonated at these conditions (i.e. pH is within 2 units of pK_a). Weber et al. (1969) reported that the maximum adsorption of several *s*-triazines by organic matter occurred near the pK_a of each compound.

A number of hydroxylated atrazine including hydroxyatrazine, deethylhydroxyatrazine, deisopropylhydroxyatrazine interacted predominately with soil through cation exchange and hydrophobic interaction (Lerch et al., 1997). These workers claim that cation exchange was more important than hydrophobic interaction or covalent bonding for *s*-triazines bound residue in soil supported by their experiment with several different so-called cation exchange, hydrophobic, and mixed-mode extractants. However, the strong ionic extractant such as KCl solution may induce or enhance the formation of ionic hydroxylated atrazine degradative products and hence increase the partition of hydroxylated atrazine degradative products into extractants. Laird (1996) proposed that interactions of atrazine with smectite (2:1 phyllosilicate) is a combination of water bridging between electronegative moieties on the atrazine molecule and

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adsorbed metal cations and hydrophobic interaction between the alkyl-side chains on the atrazine molecule and hydrophobic microsites on the smectite surface. The protonation and hydrolysis of absorbed atrazine was catalyzed by surface acidity. The hydroxylated atrazine may exist either as a hydroxy or a keto forms. Tautomerism and resonance of atrazine hydroxylated degradative products allow its strong binding to smectite surface and result in the persistence of atrazine hydroxylated products. The amount of sorbed atrazine within the hole domain was substantial for either soil mineral and organic materials. About one-third to one-half of atrazine sorption was associated with the hole domain in the mineral (3% organic matter) and peat soil (93% organic matter) (Xing et al., 1996).

Most of applied radioactivity was associated with alkali insoluble fraction in the amended treatments (Table 7-1). Attempts to recover the physically trapped atrazine only provided less than 2% of total applied radioactivity (ethylacetate extract II). However, due to the limit penetrating power of ethylacetate in soil solid matrix, it is unlikely ethylacetate could extract any significant amount of these physical trapped residues in soil micropores. The TLC-LSC experiments indicated significant increases of hydroxylated atrazine in the 16 weeks treatment compared to that obtained in the 8 weeks treatments in the ethylacetate extracts I, ethylacetate extracts II and methylene chloride extracts. This was especially true for the amended treatments (Table 7-2, 7-3 and 7-4). When atrazine is partially degraded to more polar hydroxylated atrazine degradative products which are relatively stable in soil, these atrazine degradation products could be covalently bound to organic matter or trapped/sorbed into soil clay or/and soil organic matter through various kinds of mechanisms. Ionic bonding is more likely formed between hydroxylated atrazine and soil organic matter or clay, while covalent binding is more likely formed between atrazine and its dealkylated metabolites (Lerch et al., 1997). Hydroxylated atrazine as well as atrazine may be physically trapped in soil micropores or/and hydrophobic holes within soil organic matter. The tautomerism and resonance of atrazine hydroxylated degradative products enhance its stability with hydrophobic micropores through the weak to medium strong molecular interactions. The

main dissipation pathway appears to be that covalently bound to soil humic substances and partial atrazine degradation to its various metabolites through dechlorination and dealkylation.

Atrazine and its metabolites may dynamically interact with soil matrix in a multiple forms of weak and strong chemical interactions as well as entrapment. Different forms of interactions may dominate overall interaction at specific stages when atrazine is applied to soil. Weak interactions such as hydrogen bonding, proton transfer, hydrophobic interaction, etc. may contribute to major portion of interaction between atrazine/metabolites and soil matrix at the initial incubation stage. After 8 and 16 weeks amendments, those weak and physically interactions may be replaced by strong chemical interactions such as covalent bonding and electron transfer as well as entrapment into hydrophobic sites in soil organic matter or micropores in soil minerals as a major interaction (Figure 7-3). On the other hand, only a small percentage of atrazine was lost due to volatilization and mineralization.
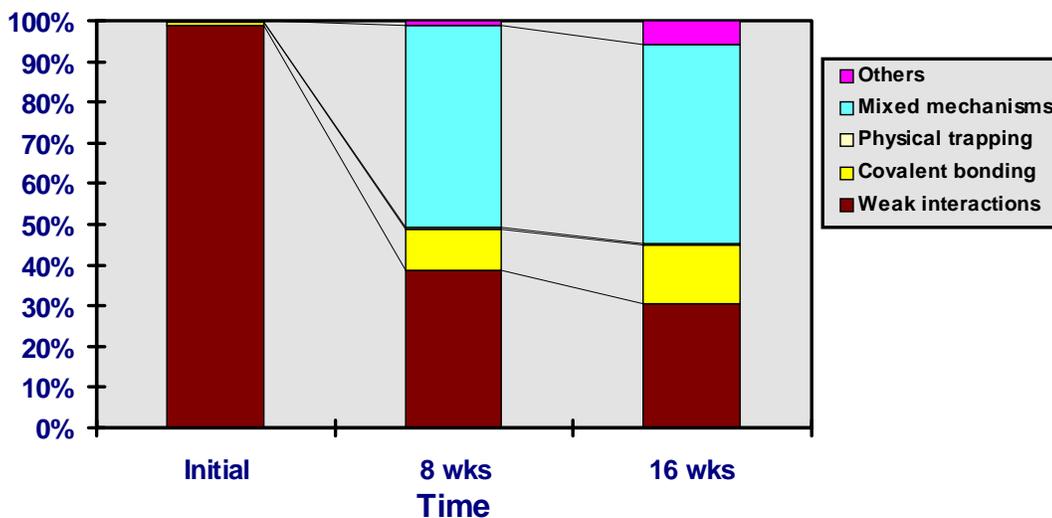

Figure 7-3. Dynamic interactions of atrazine and its metabolites with soil matrix[1,2]
[1]The results are based on the amended treatments.
[2]Weak interactions are hydrogen bonding, proton transfer, hydrogen bonding, etc.

Mixed mechanisms include physically entrapment, covalent bonding, electron transfer, etc. Others are the portion of radioactivity associated with volatilization and mineralization.

The goal of bioremediation is detoxification of hazardous compounds. Mineralization represents one of most complete process of detoxification. However, for some recalcitrant chemicals, it is not always feasible for complete transformation into inorganic compounds. A number of dissipation routes including physical trapping, sorption, partial transformation, and covalent binding to soil organic matter may occur simultaneously for many pesticides. Physical entrapment may reduce pesticide bioavailability. Partially degraded products may undergo repetitive cycles of an trappment and sorption, and covalent binding and transformation, etc. However, atrazine and its associations covalently bound to soil organic matter result in the loss of their identities and thus their toxicity (Kearney, 1976; Berry and Boyd, 1985; Bollag, 1991). Therefore, the formation of covalent binding to soil organic matter should be considered as a process of detoxification. However, some reports indicated that covalently bound toxic compounds can be slowly released in its original forms from the complex due to the microbial activities. Nevertheless, the process is slow and the amount released is most likely negligible (Khan and Ivarson, 1981; Bollag, 1991).

In summary, atrazine in bioremediation soils dissipated through volatilization, mineralization, transformation, sorption and incorporation. Volatilization resulted only in less than 2% of overall loss at 30 °C during 16 weeks of incubation. Lignocellulosic amendments reduced atrazine volatility compared with unamended soils. Mineralization occurred to a small extent in both amended and unamended treatments. Less than 5% of atrazine was recovered from treatments over 16 weeks of incubation. Addition of lignocellulosic sorbents and nutrients stimulated mineralization, especially in the second 8 week incubation interval. Most radioactivity (62%) was found in the ethylacetate extract I for unamended treatments whereas 45% of applied radioactivity was associated with alkaline insoluble fraction. The total incorporated or covalently bound radioactivity

in soil organic matter (humic and fulvic acids) was 14% of initial applied radioactivity in both unamended and amended treatments based on the sum of radioactivity in humic and fulvic acids. Physisorbed residues from soil organic matter or alkali insoluble fractions contributed less than 2% of overall radioactivity as measured radioactivity in methylene chloride extract and ethylacetate extract II. A number of atrazine metabolites including OH-atrazine, deethylatrazine, deisopropylatrazine, OH-deethylatrazine, OH-deisopropylatrazine and diaminoatrazine were found in these fractions. A higher percentage of the more polar atrazine metabolites were recovered from the 16 week amended treatments. Atrazine appears to be primarily incorporated to soil organic matter and transformed into hydroxylated atrazine which may be sorbed or/and trapped in soil minerals in the amended treatments. Physically trapping in soil organic matter and clays may reduce the bioavailability of applied atrazine while covalently bound to soil organic matter yield a detoxification process. The enhanced covalently bound to soil organic matter could be considered to be an effective process in bioremediation.

Chapter 8. Conclusions

The primary objective of this research was to investigate various factors affecting the efficacy of the bioremediation of soil containing high concentrations of chlorpyrifos and atrazine. Nutrient addition is one of the bioremediation strategies considered for treatment of pesticide-contaminated soils in a cost-effective and environmentally sound manner. Various biotic and abiotic factors and their interactions determine whether microbial nutrient enhancement can be successfully used to dissipate pesticides and related compounds to acceptable levels. Therefore, it is critical that we have a complete understanding of factors affecting rates of bioremediation of these toxic chemicals as well as how to maximize their degradation and incorporation in the laboratory. Two basic criteria used for the evaluation of this type of bioremediation technology are extractability and leachability. Extractability was used as a measure of dissipation based on solvent-extractable residue from soil and indicated how fast pesticides were degraded or incorporated; Leachability measured the amount of chlorpyrifos or atrazine leached from soil using standardized procedures and suggested whether chlorpyrifos or atrazine from contaminated sites could possibly contaminate water resources.

As discussed in the introduction, chlorpyrifos and atrazine are two important pesticides used in the USA, based on their relative persistence in the environment and wide use in agriculture. They represent two different formulations: emulsion (Dursban[®] 4E) and liquid formulation (AAtrex[®] 4L). In addition, chlorpyrifos and atrazine, as well as other pesticides at high concentrations, were found to be more persistent and mobile in soils than at low concentrations (Dzantor and Felsot, 1991; Racke et al., 1994). Therefore, this dissertation provides information on the fate of formulated chlorpyrifos and atrazine in contaminated soil at high concentrations in term of various routes of dissipation and leachability.

The results obtained indicated that bioremediation procedures using biostimulation and organically-based amendment might be used for the detoxification and containment of chlorpyrifos contaminated soils. After 90 days of incubation, solvent

extractable chlorpyrifos had declined by approximately 70% in contaminated soils amended with vegetable oil and peat moss, compared with 49% in the corresponding unamended soils. A number of dissipation routes including volatilization, chemical hydrolysis, soil microbial mineralization and transformation, adsorption and covalent binding to soil matrices were possible for chlorpyrifos. Volatilization of chlorpyrifos might contribute to some losses from soil, depending on temperature, soil moisture levels and soil amendments. For unamended soil, the losses from volatilization could reach a higher level at a low moisture level. Hydrolysis of chlorpyrifos to TCP was considered to be the major dissipation pathway of chlorpyrifos in soil. Both abiotic and biotic hydrolysis contribute to chlorpyrifos dissipation. It is likely that nutrient amendments increased microbially-mediated hydrolysis of chlorpyrifos to TCP. Lignocellulosic materials may act as sorbents for chlorpyrifos and its metabolites, providing additional sites for microbial development and some low levels of nutrients supporting microbial activities. As a result, the use of lignocellulosic amendments might reduce toxic effects of high concentrations of chlorpyrifos and TCP in contaminated soil. Vegetable oil is an excellent energy and carbon source for microbial metabolism. Amendments with lignocellulosic sorbents and vegetable oil may enhance microbial activity, which facilitate microbially-mediated transformation of chlorpyrifos. Most mineralization of chlorpyrifos is considered to be the result of cometabolism from soil microorganisms. However, one fungal species (*Phanerochaete chryosporium*) has been reported to be capable of completely mineralizing chlorpyrifos. Microbes did appear to enhance chlorpyrifos degradation and possible mineralization to some extent in this study. Soil microbial activity was directly involved on the degradation of TCP in other studies (Racke and Robbins, 1991; Feng et al., 1997a, b). The overall incorporation of soil organic matter may be limited due to the relatively inert chemical structure of chlorpyrifos and TCP. In addition to enhanced chlorpyrifos dissipation, organically-based amendments decreased the leaching hazard by reducing its leaching potential from contaminated soils in a short time period. An 82% reduction in chlorpyrifos leachability from contaminated soils amended with vegetable oil and peat moss was observed while only a 28% reduction in the corresponding controls was found after 30 days of

incubation. Similarly, there was a 96-98% reduction in chlorpyrifos leachability in the treatments amended with lignocellulosic sorbents and microbial nutrients and an 86% reduction in chlorpyrifos leachability in the unamended control based on the 4 h shaking method after 90 days of incubation. The reduction was considered to be associated with the surfactants and other adjuvants used in formulation (Dursban® 4E). Treatments with formulated chlorpyrifos had a 43% reduction in leachability compared with only negligible decreases in the treatments with technical grade chlorpyrifos after the first 3 days of application.

Atrazine dissipation was similar to that of chlorpyrifos in amended soils. Approximately 85% of atrazine was dissipated in two treatments amended with vegetable oil, peat moss, and fertilizers versus approximately 40% dissipation in the corresponding unamended treatments. Atrazine can be dissipated from contaminated soils through volatilization, chemical hydrolysis, soil microbial mineralization and transformation, adsorption and covalent binding to soil matrices. The results from [U-ring-^{14}C] atrazine incubation study indicated that only a small portion of atrazine was lost through volatilization directly from soil. Overall, less than 1% of atrazine was lost through volatilization for either amended or unamended treatments after 16 weeks of incubation (Table 7-1). Atrazine might be abiotically hydrolyzed to hydroxyatrazine; this process could be catalyzed by Fe and Al in soil which increases the electron deficiency of C-Cl bond and subsequently facilitates the nucleophilic attack of Cl in atrazine molecules (Armstrong et al., 1967). Complete microbially mediated mineralization for atrazine only occurred to a small degree. Less than 5% of [U-ring-^{14}C] atrazine was mineralized to $^{14}CO_2$ in treatments amended with organic-based material for a period of 16 weeks. Microbially mediated transformation of atrazine was more important for its dissipation. Both dealkylated and dechlorinated atrazine metabolites were detected at the 8 week and 16 week incubation samples. Relatively more dechlorinated metabolites in amended treatments might provide evidence of the microbially mediated hydrolysis/dechlorination of atrazine and its metabolites. This is consistent with the results reported by Ciardi et al. (1985). These workers found that hydrolysis rates can also be enhanced by microbial

catalysis. The unamended treatment had a higher percentage of dealkylated atrazine metabolites. It indicated that the further dissipation of these atrazine metabolites might depend on the microbial mediated processes. Atrazine may be sorbed by humic substances by proton exchange (Hayes, 1970), hydrogen bonding (Sposito et al., 1996), electron transfer (Senesi et al., 1995; Sposito et al., 1996), and physical trapping (Xing et al., 1996). The atrazine absorption by electron transfer is unlikely due to its relatively low basicity. On the other hand, there is an increased possibility of electron transfer for hydroxylated atrazine degradative products sorbed to humic acids due to their relatively high pKa. The mechanisms of atrazine and its metabolites being sorbed by humic substances depend on the basicity of compounds and acidity of functional groups on humic acids. A general rule is that more triazines are sorbed by proton exchange where the s-triazines have a low basicity and humic substances have a high acidity functional group, while more triazines are sorbed by electron transfer where the s-triazines have a high basicity and humic substances have a low acidity functional group (Sposito et al., 1996). Most atrazine sorbed to humic substances by weak interactions such as proton transfer and hydrogen binding should be recovered by extensive ethylacetate extraction. A significant decrease of ethylacetate extractable radioactivity was observed in the amended treatments over 16 weeks of incubation. This suggests that there is a decline in the importance of weak physical and chemical interactions between atrazine and soil humic substances. In addition to weak interactions such as proton exchange and hydrogen bonding, atrazine and its metabolites might be physically trapped into hydrophobic microsites within humic macromolecules (Xing et al., 1996). These hydrophobic holes are presumed to be eliminated during the alkaline extraction due to configuration changes resulting from pronation of functional groups of humic substances (Li et al., 1992). Therefore, most of the physically trapped atrazine and its metabolites will be partitioned into methylene chloride phases during extractions. Contrary to the explanation proposed by Pignatello and Xing (1996), only less than 2% of the overall applied radioactivity was recovered in the methylene chloride extract. However, a portion of the trapped atrazine residues might be extracted by ethylacetate (ethylacetate extract I) since most of the trapped atrazine residues probably interact with humic

substances through weak chemical and physical interactions. After extraction with ethylacetate and methylene chloride, which most likely removed most of physically trapped and weakly sorbed atrazine and its metabolites, a major part of atrazine and its metabolites was present in soil organic matrices (humic and fulvic acids). The radioactivity associated with humic and fulvic acids were probably covalently bound to functional groups such as COOH, OH, NH_2 in the humic substances. The binding sites may be chloride and amino positions at the triazine ring for atrazine and its dealkylated metabolites. However, actual binding mechanisms might be more complex than discussed here and merits further study.

As much as 45% of ^{14}C-radioactivity was recovered from the alkali insoluble fraction of amended soil matrices. This was similar to the results obtained by Willems et al. (1996) with carbofuran in lignocellulosic compost. During the 8 week solid state fermentation with peat moss as the organic matrix, Willems et al. (1996) determined approximately 60% of radioactive carbofuran was associated with the alkali insoluble fraction after intensive ethylacetate and 0.1 M NaOH extraction. However, in a soil environment, the radioactivity associated with the alkaline insoluble fraction might be more complex than in the lignocellulosic matrix due to the heterogeneity of soil. The components in soil might include unrecoverable soil humic and fulvic substances, unextractable humins, and lignin materials. Therefore, it is most likely that atrazine interacted with the alkaline insoluble fraction involving several different mechanisms. The humic substances may have interacted with atrazine and its metabolites in covalent binding. Hydrophobic microsites in humic substances may entrap some molecular atrazine or pronated atrazine. Meanwhile, sorption and physical entrapment may have played an important role with regard to interactions of atrazine and its metabolites with soil minerals. Hydroxylated atrazine degradative products were considered to primarily interact through cation exchange, and to a lesser degree, hydrophobic interaction with soil (Lerch et al., 1997). Atrazine metabolites, especially polar metabolites, could be simply absorbed by soil minerals or soil organic matter-mineral complexes and therefore difficult to extract by organic solvents. Microbial activity was responsible for the

transformation of atrazine to polar metabolites through dealkylation and dechlorination whereas further degradation of these metabolites requires ring cleavage which is slow, therefore, these metabolites may accumulate in soil.

A number of dissipation routes resulting from microbial activity of xenobiotics are possible. These include mineralization, transformation, sorption/physical trapping and covalent binding to soil organic matter. Mineralization, representing an ultimate goal of bioremediation, is a process where toxic chemicals are degraded into nontoxic inorganic compounds. Physical trapping will not alter the toxicity of these contaminants. These trapped compounds could be potentially released into the environment when environmental conditions change. Unlike physically entrapped residue, covalently bound atrazine and its metabolites lose their identities and toxicity. There is evidence that these bound residues could be released into the environment in their original forms or modified toxic forms based upon the influence of microbial enzymatic activities. The rate of release is very low and usually rapidly degraded into nontoxic compounds by microbial activities (Khan and Ivarson, 1981). Specifically, the non-extractable radioactivity associated with humic substances was most likely covalently bound to functional groups of humic substances. Furthermore, the formation of these non-extractable residues can be considered as a functionally equivalent alternative of detoxification for some recalcitrant chemicals such as atrazine.

Based on the results of the TCLP method, atrazine leachability was approximately 70% of the initial level (30% decrease in leachability) in the treatments with organic amendment versus 80% of the initial level (20% decrease in leachability) in unamended treatments. The differences between amended and unamended treatments were more prominent with the TLCP method than with the 4 h shaking method. Organically-based amendments might be able to withstand more severe leaching conditions than the corresponding unamended soils. Atrazine leachability may depend more on its form in AAtrex® 4L which is similar to a flowable formulation. Atrazine existed in a particulate form in its formulation, and was weakly sorbed by soil matrices

once it was applied to soil. Therefore, it was relatively easily leachable compared with that of chlorpyrifos. However, organically-based material amendments substantially reduced atrazine dissipation in contaminated soils as discussed above, which in turn, decreases the leachability by reducing leachable atrazine in a long run.

From the results presented here, bioremediation of these recalcitrant chemicals is possible by enhanced degradation and containment in contaminated sites even at high concentrations (5 g kg^{-1}). However, there are no general strategies suitable for every chemical. Studies are needed to evaluate different strategies suitable for different contaminants. The approach used in this study was diagrammed in Figure 8-1. For chlorpyrifos, lignocellulosic sorbents such as peat moss combined with vegetable oil result in declines in extractability as well as prevent its leaching from contaminated soils. Addition of other nutrient sources such as fertilizers and corn meal might further assist in its dissipation and can be used if it is determined to be economically feasible. Atrazine requires microbial nutrients such as vegetable oil, fertilizers and lignocellulosic materials for relatively fast dissipation rates. However, atrazine leachability appears to be dependent on its formulation. Microbial enhancement may pay off in the long term since it decreases leachability by reducing its leachable concentration.
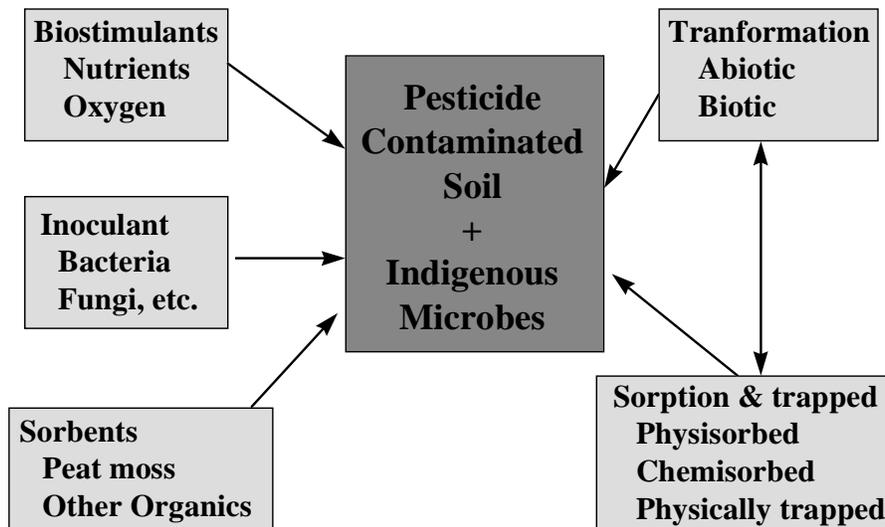

Figure 8-1. Microbial remediation approach for enhancing pesticide dissipation

It is important to point out that all of the factors involved in remediation techniques and strategies interacted and may be interdependent with each other. There are tradeoffs between the techniques that might be used to dissipate these contaminants. For example, the lignocellulosic amendments enhance chlorpyrifos and atrazine degradability and other dissipation routes possibly through reducing pesticide toxicity on soil microbial populations, increasing sorption/physical trapping and microbial degradation, and decreasing their leachability. However, the amendments may decrease pesticide availability for the soil microbial population because of strong sorption and possibly increased physical sorption in micosites of humic substances. Nutrient amendments have similar tradeoffs in term of efficiency of degradation and economy. The more corn meal used, the more microbial activity. However, soil microbes may switch to these easily accessible and degradable nutrient resources under excess nutrient conditions, hence the degradability of toxic compounds will be reduced. Therefore, a good bioremediation protocol can only be obtained based on fully understanding all of these interactions, cause affects relationships and tradeoffs.

As a promising technology, bioremediation through biostimulation may provide an efficient, economical and environmentally sound method to recover contaminated sites. The dissipation of chlorpyrifos and atrazine was significantly enhanced by organic-based material amendments. Leachability of chlorpyrifos was reduced by lignocellulosic materials and vegetable oil amendments within a short time period. However, there was only a small decline of atrazine leachability in amended contaminated soils. Organically-based amendments not only enhanced atrazine mineralization but also increased unextractable atrazine in soil humic substances and minerals. Most of that was considered to be unavailable and nontoxic to its environment. Further studies are needed to provide the method of containment of relatively mobile contaminants such as atrazine, and to optimize the amendments of organically-based materials for contaminant dissipation.

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VITA

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In 1992, he traveled from Hangzhou to Blacksburg, Virginia to pursue his graduate study in the Entomology department of Virginia Tech. Currently, he is a Ph.D. candidate in Entomology. He was married to Wenkai Zhu in 1992. They had a daughter (Katharine Julia Xiao) on August 23, 1994.