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Monomethylhydrazine Degradation and Its Effect on Carbon Dioxide Evolution and Microbial Populations in Soil

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Monomethylhydrazine (MMH), along with hydrazine and 1,1-dimethylhydrazine are the main components of hydrazine fuels (Schmidt, 1984). Information on the fate of MMH in soil and its overall effect on soil microbial activity is not known, though MMH is known to be toxic to a number of soil bacteria, including Enterobacter cloacae (London and Mantel, 1983; London et al., 1983; Mantel and London, 1980), autotrophic nitrifiers Nitrosomonas and Nitrobacter, and denitrifiers (Kane and Williamson, 1983). Kane and Williamson (1983) reported that among the three hydrazines MMH was the most toxic to bacteria.

Despite the fact that axenic bacterial cultures are inhibited by the three hydrazines, Ou and Street (1987) reported that soil respiration, and total bacterial and fungal populations in soil, were not inhibited by hydrazine at concentrations of 100 µg/g and lower. Even at 500 µg/g, only total bacterial populations in soil were inhibited by the presence of hydrazine. They also reported that hydrazine rapidly disappeared in soil. We initiated this study to investigate the effect of MMH on soil microbial activity and on degradation of the chemical in soil.

MATERIALS AND METHODS

Arredondo fine sand (Grossarenic Paleudult) used for this study had never been exposed to MMH or hydrazine. The soil sample (10 kg) was collected from the 5-20 cm soil depth, air-dried and sieved to pass a 2-mm sieve. Key properties of the soil have been reported previously (Ou and Rao, 1986; Ou and Street, 1987).

Two hundred g of soil (oven-dry weight basis) were placed in 500-mL wide-mouth glass bottles with screw caps, and 16 mL of deionized water were added. MMH was applied to the soil samples to give concentrations of 0, 10, 50, 100, 200, and 500 µg/g. After mixing, glass beakers (50 mL) containing 20 mL of 0.2-0.5N KOH were placed in the bottles. The KOH solutions were used for trapping CO_2 evolved from the samples. Bottles without beakers were used for assessment of the effect of MMH on total aerobic

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bacterial and fungal populations. Similar procedures for determination of CO_2 evolution, and of bacterial and fungal populations, had been used formerly for hydrazine-treated soils (Ou and Street, 1987). The values of least-significant difference at the 5% level ($LSD_{0.05}$) for total cumulative CO_2 production from the MMH treated and untreated samples were determined by a modified Student's t-test using a pooled error variance (Steel and Torrie, 1960).

MMH was determined by the colorimetric method of Reynolds and Thomas (1965). Briefly, 10 g of soil were placed in plastic centrifuge tubes and extracted three times with 20 mL of cold and deoxgenated 0.1M HCl. One hundredth to 1.0 mL of the extracts were transferred to 4 mL of 10% trichloroacetic acid and adequate amounts of deionized water were added to give a final volume of 5 mL. Four mL of each mixture were mixed with 5 mL of the color-reagent, p-dimethylaminobenzaldehyde. After 30 minutes, optical densities of the resultant mixtures were read at 485 nm.

In addition, uniformly-labeled [14C]MMH, along with unlabeled MMH, were also used for determination of disappearance and mineralization of MMH in soil. [14C]MMH was purchased from Amersham Corporation (Arlington Heights, IL) and had a specific activity of 6 µCi/mmol and 98% radio-purity. $[^{14}C]CO_2$ trapped in KOH and radioactivity in the HCl extracts were determined by scintillation counting. Radioactivity remaining in the extracted soil was determined by combusting to $[^{14}C]CO_2$ in a Packard Tri-Carb sample oxidizer as described previously by Ou et al. (1982). The evolved $[^{14}C]CO_2$ was trapped in a commercial scintillation solution containing an organic amine (Packard Instrument Co., Downers Grove, IL), and counted by scintillation counting. All soil samples had been incubated at 25°C.

RESULTS AND DISCUSSION

MMH at concentrations ranging from 10 to 500 µg/g did not inhibit soil respiration in Arredondo soil. Unlike hydrazine, which initially inhibited total CO_2 production by soil (Ou and Street, 1987), total CO_2 evolution was actually enhanced initially by the treatment with MMH (Tables 1 and 2). In fact, initial total CO_2 production became progressively larger as MMH concentration was increased. Total cumulative CO_2 production during 21 days for all of the MMH-treated samples was significantly higher than for the untreated samples.

Total aerobic bacterial populations and total fungal populations were also not inhibited by 10 µg/g of MMH (Table 3) and, at 100 µg/g and larger, total aerobic bacterial populations (Tables 3 and 4) were actually significantly larger than for the control treatments throughout the entire 21 days of incubation, with total bacterial populations becoming progressively larger as MMH concentration was increased. At 100 µg/g, total fungal populations were either not affected or were increased as well, though total fungal populations in soil treated with 200 and 500 µg/g of MMH were

Table 1. Total CO_2 production from Arredondo soil treated with MMH at 0, 10, 50 and 100 µg/g.

Days	Rates of CO_2 Production (mg CO_2 - C/100g soil/day)[a]			
	MMH (µg/g)			
	0	10	50	100
0-1	4.61 ± 0.12	5.00 ± 0.15	5.24 ± 0.18	5.56 ± 0.15
1-2	3.66 ± 0.14	3.82 ± 0.14	4.09 ± 0.18	4.17 ± 0.13
2-5	2.00 ± 0.06	2.13 ± 0.03	2.41 ± 0.34	2.40 ± 0.29
5-7	1.47 ± 0.09	1.57 ± 0.06	1.64 ± 0.11	1.65 ± 0.09
7-11	1.16 ± 0.05	1.25 ± 0.00	1.33 ± 0.09	1.34 ± 0.05
11-14	1.05 ± 0.01	1.21 ± 0.15	1.14 ± 0.06	1.20 ± 0.04
14-18	0.88 ± 0.04	1.09 ± 0.13	1.01 ± 0.05	1.05 ± 0.05
18-21	0.78 ± 0.04	0.92 ± 0.09	0.90 ± 0.03	0.93 ± 0.04
Total	32.80	36.29	37.19	38.16
		$LSD^{b}_{0.05}$ = 2.32		

[a] N=3.
[b] $LSD_{0.05}$ least-significant difference at the 5% level.

Table 2. Total CO_2 production from Arredondo soil treated with MMH at 0, 200 and 500 µg/g.

Days	Rates of CO_2 Production (mg CO_2 - C/100g soil/day)[a]		
	MMH (µg/g)		
	0	200	500
0-1	4.39 ± 0.28	4.81 ± 0.19	5.26 ± 0.18
1-2	3.85 ± 0.27	4.42 ± 0.30	4.78 ± 0.28
2-4	2.44 ± 0.16	2.78 ± 0.20	3.17 ± 0.13
4-7	1.70 ± 0.08	1.84 ± 0.08	1.96 ± 0.05
7-10	1.41 ± 0.12	1.56 ± 0.12	1.71 ± 0.03
10-14	1.17 ± 0.08	1.28 ± 0.08	1.37 ± 0.02
14-17	1.00 ± 0.07	1.14 ± 0.15	1.33 ± 0.09
17-21	0.89 ± 0.06	0.96 ± 0.11	1.14 ± 0.05
Total	33.71	37.37	41.44
		$LSD^{b}_{0.05}$ = 3.12	

[a] N=3.
[b] LSD least-significant difference at the 5% level.

Table 3. Total aerobic bacterial and fungal populations in Arredondo soil treated with MMH at 0, 10 and 100 µg/g.

MMH (µg/g)	Days		
	7	14	21
		Bacteria (cfu/g[a] x 10^{-5})	
0	201a ± 22	151a ± 6	126a ± 11
10	192a ± 14	172ab± 23	185b ± 7
100	271b ± 14	175b ± 14	166b ± 20
		Fungi (cfu/g[a] x 10^{-3})	
0	76a ± 9	87a ± 5	79a ± 3
10	81a ± 4	86a ± 6	77a ± 5
100	83b ± 13	104a ± 14	100b ± 7

[a]cfu/g colony-forming units per gram of soil.

Table 4. Total aerobic bacterial and fungal populations in Arredondo soil treated with MMH at 0, 200 and 500 µg/g.

MMH (µg/g)	Days		
	7	14	21
		Bacteria (cfu/g[a] x 10^{-5})	
0	224a ± 15	128a ± 11	150a ± 41
200	284b ± 34	216b ± 45	273b ± 39
500	446c ± 67	296c ± 21	296c ± 49
		Fungi (cfu/g[a] x 10^{-3})	
0	90a ± 5	78a ± 10	80a ± 4
200	43b ± 8	54b ± 4	66b ± 6
500	42b ± 3	61b ± 6	58b ± 5

[a]cfu/g colony-forming units per gram of soil.

significantly but not severely reduced. The effect of 500 µg/g of MMH was in contrast to that of hydrazine, which severely reduced bacterial populations but enhanced fungal populations (Ou and Street, 1987).

MMH disappeared rapidly from both nonsterile and sterile soils. MMH at 10 µg/g completely disappeared from nonsterile and sterile soils in 30 minutes. Even at 100 and 500 µg/g, only 41.8 and 67.4% of the applied MMH were detected in nonsterile soils (Table 5), respectively, 30 minutes after application. After 48 hours, only small amounts of MMH remained in either nonsterile or sterile samples. The percentage of MMH remaining in sterile soils was

Table 5. MMH in nonsterile and sterile Arredondo soil.

Hours	MMH (%)			
	100 µg/g		500 µg/g	
	Nonsterile	Sterile	Nonsterile	Sterile
0.5	41.8	49.8	67.4	70.6
4	8.7	ND[a]	23.4	ND
24	1.6	5.3	1.3	4.3
48	0.7	2.3	0.6	1.2

[a]Not determined.

consistently slightly higher than in nonsterile soils. This suggested that chemical degradation was the most important factor contributing to the disappearance of MMH from soil. Biological degradation also contributed to the disappearance of MMH, though much less significantly.

Despite the fact that biological degradation played only a minor role in the disappearance of MMH from soil, it was found that substantial amounts of [14C]MMH in nonsterile soil were mineralized to CO_2. The evolved and trapped radioactivity in the KOH traps was principally [14C]CO_2, since little radioactivity remained in the supernatants after precipitation with $BaCl_2$ (Table 6). Degradation of MMH to CO_2 is a microbial process. After 9 days of incubation, 46.5 and 42.6% of the applied [14C] were trapped in KOH for Arredondo soil treated with 100- and 500-µg/g of [14C]MMH, respectively (Table 7). More than 95% of the trapped [14C] was found to be associated with [14C]CO_2. Furthermore, 6.9 and 4.7% of the applied [14C] in the 100- and 500-µg/g treatments could be extracted with 0.1N HCl, respectively, and 26.9 and 28.8% of applied [14C] remained in the extracted 100 and 500 µg/g

Table 6. Radioactivity evolved and trapped in KOH from Arredondo soil treated with [14C]MMH.

Days	% of Applied [14C]	
	100 µg/g	500 µg/g
1	12.3[a] (0.6)[b]	6.8[a] (0.4)[b]
2	32.3 (1.7)	14.5 (1.2)
3	38.7 (1.9)	27.1 (1.4)
6	44.1 (2.1)	39.7 (1.6)
9	46.5 (2.2)	42.6 (1.9)

[a]Radioactivity in KOH before addition of $BaCl_2$.
[b]Radioactivity in KOH after addition of $BaCl_2$. $BaCl_2$ and [14C]carbonate forms insoluble [14C]$BaCO_3$.

Table 7. [14C] distribution in nonsterile and sterile Arredondo soil treated with [14C]MMH after 9 days of incubation.

MMH (µg/g)	% of Applied [14C]				
	KOH		HCl Extract	Extracted Soil	Recovery
	Before addition of $BaCl_2$	After addition of $BaCl_2$			
			Nonsterile		
100	46.5	2.2	6.9	26.9	80.3
500	42.6	1.9	4.7	28.8	76.1
			Sterile		
100	4.1	4.0	ND[a]	ND	ND
500	3.9	4.1	ND	ND	ND

[a]Not determined.

treated soils, respectively. Total [14C] recoveries for the 100 and 500 µg/g treatments were 80.3 and 76.1%, respectively. MMH at 25°C has a vapor pressure of 49 mm Hg (Schmidt, 1984), which is somewhat higher than the vapor pressure of water. Hence, at least a part of the unaccounted for [14C] have been lost due to volatilization. No $[^{14}C]CO_2$ was evolved from sterile soil treated with [14C]MMH (Table 7).

Although MMH disappeared rapidly from both nonsterile and sterile soils, our findings suggest that the nature of MMH degradation in nonsterile soil may be different from that in sterile soil. Alternatively, it is possible that MMH in both nonsterile and sterile soils was initially oxidized chemically to an oxidation product, while the product was subsequently degraded microbiologically to CO_2 in nonsterile soil. The product in sterile soil was not degraded further.

The enhancement of CO_2 production in MMH-treated soils was in part due to the increase in aerobic bacterial populations and in part due to the mineralization of MMH to CO_2. Since degradation of MMH to CO_2 is principally microbial, it is possible that MMH-degrading microorganisms can be isolated from soil and used for detoxification of contaminated soils, water, and wastes.

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