Bull. Environ. Contam. Toxicol. (1987) 38:179–183

Environmental Contamination and Toxicology

Hydrazine Degradation and Its Effect on Microbial Activity in Soil

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Considerable information has been accumulated on the toxicity of hydrazine to soil bacterial cultures and on the degradation of hydrazine by soil bacterial cultures. The activities of the autotrophic nitrifiers Nitrosomonas and Nitrobacter and of denitrifying bacteria (Kane and Williamson, 1983), and the growth of Enterobacter cloacae (London and Mantel, 1983; London et al., 1983; Mantel and London, 1980), were all inhibited by hydrazine. Kane and Williamson (1983) reported that hydrazine was degraded cometabolically to N_2 gas in the presence of $(NH_4)_2SO_4$ as a second nitrogen source. Stiefel et al. (1977) found an enzyme system in heterotrophic N_2-fixing bacteria capable of degrading hydrazine.

Information concerning the effect of hydrazine on microbial activity in soils is not available, however. Accidental spills to soil can occur during transportation and storage. Therefore, this study was initiated to determine degradation rates of hydrazine in soils and its effect on soil microbial activity.

MATERIALS AND METHODS

Arredondo fine sand (Grossarenic Paleudult) was used for this study. The soil, which had never been exposed to hydrazine, was air-dried and sieved to pass a 2-mm sieve. The sample had a pH of 5.7, and 1.7% of organic carbon.

Two hundred g of soil (oven-dry weight basis) were placed in 500 mL glass bottles or flasks, and 16 mL of distilled water were added. Appropriate amounts of hydrazine sulfate were added to give hydrazine concentrations of 0, 2.5, 10, 25, 100, 125, 250, and 500 µg/g. After mixing, the bottles were weighed and incubated at 25°C. For determination of hydrazine residue, as well as bacterial and fungal populations in the soil, 10 g of soil samples were removed. For determination of nitrate and ammonia, 15 g of samples were removed. Once a week the weights of the soil

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samples were checked, and distilled water was added to compensate any water loss.

Hydrazine was determined by the colorimetric method of Watt and Chrisp (1952). In essence, plastic centrifuge tubes containing 10 g soil samples were deoxygenated by flushing with a stream of N_2, and the samples were extracted three times with 20 mL of deoxygenated 0.1M NaCl. 0.1 to 1 mL aliquots were transferred to 10 mL of the color-developing agent, p-dimethylaminobenzaldehyde, and the resulting mixtures were diluted to 25 mL by adding 1 M HCl. Specific-ion electrodes were employed to determine NH_4^+ and NO_3^- in the soil. After mixing with 0.1 g of calcium sulfate, 15 g soil samples were extracted with 45 mL distilled water. 20 mL and 10 mL aliquots were used for determination of NO_3^- and NH_4^+, respectively.

Dilution-plate count methods as described by Ou et al. (1978a) were used to determine bacterial and fungal populations in the soil samples. Carbon dioxide evolved from the samples in closed glass bottles was trapped in KOH and determined by titration (Ou et al., 1978b). All experiments were duplicated with the exception of the soil respiration experiment, which was done in triplicate.

Table 1. Hydrazine in sterile and nonsterile Arredondo soil.

Days	Hydrazine (%)			
	100 µg/g		500 µg/g	
	Sterile	Nonsterile	Sterile	Nonsterile
0.05	83±11	62±3	97±3	93±2
1	8±0	0	71±1	62±2
2	0	0	52±0	39±3
3	0	0	39±2	25±4
6	0	0	13±1	3±1
8	0	0	8±1	0

RESULTS AND DISCUSSION

At low concentrations, hydrazine disappeared rapidly from Arredondo soil. For example, at 10 µg/g, hydrazine disappeared completely in 1.5 hours. Autooxidation appeared to be the principal factor contributing to the disappearance of the chemical from soil, as less than 3% of the applied hydrazine was recovered from sterile soil. Even at 100 µg/g hydrazine disappeared completely in 1 day and, at 500 µg/g, the chemical disappeared completely in 8 days (Table 1). Biological degradation was a relatively minor factor, although hydrazine consistently disappeared from sterile soil at somewhat slower rates. By comparing the hydrazine loss from sterile and nonsterile soils, it appeared that biological degradation was responsible for about 20% of the disappearance.

Since hydrazine is partly degraded biologically in this soil, it is of interest to determine if hydrazine is metabolized to ammonia, which can serve as a nitrogen source for growth. We found no evidence of hydrazine being converted to ammonia. The levels of NH_4^+ in Arredondo soil, both with and without treatment with hydrazine at 100 µg/g, were essentially the same following 7 days of incubation.

Table 2. Effect of hydrazine on soil respiration in Arredondo soil treated with hydrazine at 0, 2.5, 25 and 125 µg/g.

Days	Rate of CO_2 Production (mg CO_2-C/100g soil/day)			
	Hydrazine (µg/g)			
	0	2.5	25	125
1	2.79±0.11	2.41±0.07	1.99±0.10	1.64±0.26
3	1.55±0.02	1.66±0.05	1.76±0.04	1.68±0.04
7	0.70±0.04	0.82±0.08	0.80±0.03	0.86±0.09
11	0.72±0.11	0.75±0.11	0.65±0.06	0.73±0.10
14	0.54±0.04	0.50±0.02	0.49±0.05	0.69±0.09
18	0.51±0.10	0.41±0.02	0.41±0.06	0.61±0.10
21	0.50±0.10	0.41±0.01	0.44±0.06	0.52±0.03
Total*	16.73	16.38	15.74	17.43
		$LSD_{0.05}^{**} = 1.93$		

* Total cumulative CO_2 production in 21 days.
** $LSD_{0.05}$ least-significant difference at the 5% level.

Table 3. Effect of hydrazine on soil respiration in Arredondo soil treated with hydrazine at 0, 250 and 500 µg/g.

Days	Rate of CO_2 Production (mg CO_2-C/100g soil/day)		
	Hydrazine(µg/g)		
	0	250	500
1	2.52±0.04	1.43±0.15	1.27±0.08
2	1.77±0.03	1.97±0.13	1.57±0.06
3	1.31±0.02	1.47±0.13	2.14±0.06
6	0.85±0.06	0.89±0.05	1.09±0.02
10	0.58±0.05	0.62±0.06	0.61±0.02
14	0.50±0.01	0.56±0.04	0.52±0.01
17	0.53±0.05	0.57±0.04	0.55±0.06
21	0.43±0.02	0.52±0.10	0.47±0.06
Total*	15.78	16.05	16.30
		$LSD_{0.05}^{**} = 1.64$	

* Total cumulative CO_2 production in 21 days.
** $LSD_{0.05}$ least-significant difference at the 0.05% level.

Soil respiration (total CO_2 evolution) in hydrazine-treated soils was initially inhibited, with the degree of inhibition progressively increasing as hydrazine concentration increased (Tables 2 and 3). However, the inhibition was temporary. In fact, not only had all samples recovered from the inhibition within 2 days, but CO_2 production was actually enhanced. CO_2 production then levelled off after 6 or 7 days. Total cumulative CO_2 production in all treatments was not significantly different ($\bar{p} = 0.05$) after 21 days.

Table 4. Effect of hydrazine on bacterial and fungal populations in Arredondo soil.

Concentration of hydrazine (µg/g)	Days 1	7	14	21	28
		Bacteria (cfu/g* x 10^{-6})			
0	13.92	15.45	9.83	7.63	7.21
100	1.35	24.90	42.60	25.80	16.40
500	0.82	0.68	0.91	0.44	0.56
		Fungi (cfu/g* x 10^{-4})			
0	1.05	2.26	2.70	2.22	2.61
100	0.87	6.71	9.36	9.59	9.70
500	1.00	3.85	11.44	9.49	7.84

* cfu/g colony forming units per gram of soil.

Similar to CO_2 production, bacterial populations in hydrazine-treated soils were also reduced initially (Table 4), although fungal populations were not affected. The reduction of bacterial populations appeared to be the principal cause of the inhibition in CO_2 evolution. For the 100 µg/g treatment, bacterial populations quickly recovered. This reflected the fact that, at this concentration, hydrazine was completely degraded within 1 day (Table 1). In fact, bacterial populations were enhanced in 7 days and remained larger than the control treatment thereafter. In contrast, bacterial populations for the 500 µg/g treatment were at least 10 times smaller than for the control treatment throughout the 28 days of incubation. After 7 days fungal populations for the 100 and 500 µg/g treatments were significantly larger than for the control treatment. Because of the magnitude of the reduction in bacterial populations for the 500 µg/g treatment, not only nitrifying bacteria, denitrifying bacteria, anaerobic bacteria (Kane and Williamson, 1983), and Enterobacter cloacae (London et al., 1983) would be killed, but many other bacteria could be killed as well.

Hydrazine at concentrations of 10 and 100 µg/g did not exert any adverse effect on nitrification after 49 days for the Arredondo soil (Table 5). However, nitrification did not take place to a

significant extent in the 500 µg/g treatment. As mentioned above, bacterial populations in this treatment were profoundly reduced, and nitrifying bacteria most likely would be killed at this concentration. Our results suggest that, at concentrations of 100 µg/g and lower, hydrazine exerts no adverse effect or only a short, temporary effect on soil microbial activity.

Table 5. Effect of hydrazine on nitrification in Arredondo soil.

Hydrazine concentration (µg/g)	NH_4^+-N* (µg/g)	NO_3^--N* (µg/g)
0	79.6±0.9	60.6±0.8
10	69.3±11.1	60.9±2.0
100	82.7±0.5	53.8±0.5
500	123.1±3.0	12.6±0.2

* Results at 49 days

Acknowledgements. We thank Bill Harb and Kevin Carter for technical assistance. This study was supported by Contract No. F08635-83-C-0136 from the US Air Force. Florida Agricultural Experiment Station Journal Series No. 7256.

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Received May 5, 1986; accepted August 9, 1986.