APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1976, p. 399-403
Vol. 31, No. 3

Printed in U.S.A.

Microbial Formation and Degradation of Dimethylamine

ROBERT L. TATE III[1] AND MARTIN ALEXANDER*

Laboratory of Soil Microbiology, Department of Agronomy, Cornell University, Ithaca, New York 14853

Received for publication 8 October 1975

Dimethylamine was formed from trimethylamine in soils of different pH values. The rate of disappearance of the secondary amine from soil was affected by pH and was markedly reduced under anaerobiosis. The accumulation of dimethylamine in cultures of *Micrococcus* sp. provided with trimethylamine depended on the nitrogen sources available to the bacterium but was not greatly influenced by the C-N ratio of the medium. Dimethylamine and nitrite accumulated in large amounts at pH 6.0 to 8.0 in cultures containing the tertiary amine and nitrate, but dimethylnitrosamine was apparently not produced.

The *N*-nitrosamines represent a class of chemicals that can be carcinogenic as well as teratogenic in low concentrations. Ayanaba and Alexander (1) and Ayanaba et al. (2, 3) have shown that these toxicants are generated in samples of soil, lake water, and sewage so that a potential exists for the formation of such hazardous compounds in natural ecosystems. Of considerable environmental importance also is the finding that these carcinogens, once formed, are quite stable (15). Thus, if the nitrosamines were produced, they could persist sufficiently long to reach drinking or recreational waters, to be retained on the surfaces of root crops, or to be assimilated by higher plants. Indeed, it has recently been postulated that the increased rate of occurrence of gastric cancer in at least one community is linked with high nitrate levels in its public water supply, the nitrosamines generated from the nitrate, via nitrate reduction, presumably being the responsible carcinogen (7). Because nitrate synthesis and accumulation are widespread in aerated soils, it is likely that a major limiting factor for the formation of nitrosamines is the production of the secondary amine rather than the inorganic nitrogen precursor. These amines could arise in the decay of plant material or through the use of certain pesticides (5, 14), which are or can be transformed to yield secondary amines.

The present study was conducted to establish some of the factors affecting the accumulation of one secondary amine, dimethylamine (DMA), that might occur in nature during the degradation of a simple tertiary amine, trimethylamine (TMA), found naturally in some higher plants and fungi (11) as well as in fish.

[1] Present address: Agricultural Research and Education Center, P. O. Drawer A, Belle Glade, Fla. 33430.

MATERIALS AND METHODS

The four soils tested were Hudson Collamer silt loam (pH 4.5), Langford channery silt loam (pH 5.2), Williamson silt loam (pH 6.8), and Lima loam (pH 7.0). Their organic matter levels were 1.8, 4.1, 1.6, and 3.6%, respectively. For some studies, the pH of the Williamson silt loam was adjusted by the addition of 0.1 N sulfuric acid; the soil was then air dried, the moisture level was adjusted to 40% (wt/wt), the sample was incubated for 3 weeks to allow the microbial community to adjust to the new pH, and then the soil was dried in air.

To investigate TMA or DMA metabolism in soil, air-dried soil was passed through a 1.0-mm sieve and placed in test tubes (15 by 1.5 cm). The various amendments were introduced, the moisture content of the soil was adjusted to approximately 40% by weight, and the samples were incubated at 23 C. In studies involving anaerobic conditions, 1.0 mg of glucose was added per g of the samples, and anaerobiosis was maintained by means of GasPak anaerobic systems (BBL). At intervals, the soil was extracted with 1 N $CaCl_2$, and the particulate matter was removed by centrifugation at 10,000 × *g* for 10 min. This procedure gave quantitative recovery of the amines tested. Secondary amines were obtained from this extract by steam distillation (6). A minimum of three samples was prepared at each interval.

The DMA concentration in the steam distillates was determined by the colorimetric method of Pribyl and Nedbalkova (9). The identity of the amine was verified by gas-liquid chromatography (14) or by thin-layer chromatography. For thin-layer chromatography, the amines were separated in a solvent of butanol-acetic acid-water (80:20:20), and the secondary amines were detected with nitroprusside(sodium)-acetaldehyde spray (13). Nitrite was detected colorimetrically by the method of Montgomery and Dymock (8), and nitrosamines were measured by the procedure of Daiber and Preussmann (4).

The inorganic salts solution previously described (15) was amended with appropriate levels of filter-

sterilized carbon sources in order to grow the test organisms. The pH of the solution was varied by use of 0.05 M potassium phosphate buffer of the appropriate pH in place of the original buffer.

Micrococcus sp., a soil isolate obtained from Williamson soil by enrichment culture with TMA as the sole carbon source, was provided by Suzanne H. Atkin. The organism was grown at 30 C with shaking in 500-ml Erlenmeyer flasks containing 150 ml of medium. Growth was measured by determining the optical density at 540 nm. For DMA analysis of the culture, the cells were removed from the liquid by centrifugation, and the supernatant fluid was then steam distilled. The culture was identified by standard methods (10, 12).

RESULTS

To study the formation of DMA from TMA in soil, Williamson silt loam at two pH levels and Hudson Collamer silt loam were amended with 1.0 mg of TMA per g of soil. At intervals during the incubation period, samples of the soil were extracted and analyzed for DMA. As shown in Fig. 1, DMA accumulated in each of the soils. The maximum concentration was nearly 70 μg/g in the Hudson Collamer silt loam. Surprisingly, the yield was appreciably greater in Williamson silt loam at the acid than at the near-neutral pH value. The secondary amine was then further degraded, and none was detectable at 21 days in any of the soils.

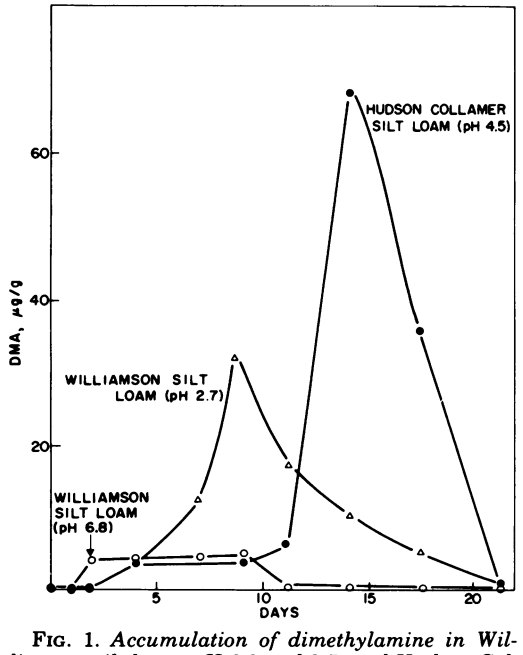

FIG. 1. *Accumulation of dimethylamine in Williamson silt loam, pH 6.8 and 2.7, and Hudson Collamer silt loam, pH 4.5, amended with 1.0 mg of trimethylamine per g of soil.*

The accumulation of secondary amines in nature is governed not only by their rate of synthesis but also by the rates of their degradation. The rate of DMA decomposition was assessed in soils with different pH values. DMA was metabolized in samples of all the soils tested. In the Williamson silt loam adjusted to different pH values, the rate and extent of DMA disappearance decreased as the acidity increased (Fig. 2). In the three other soils, the disappearance was slowest in Hudson Collamer silt loam (pH 4.5) and was more rapid in Lima loam (pH 7.0) and Langford channery silt loam (pH 5.2). Thus, pH appears to have an effect on the rate of loss, the disappearance being slowest in acid soils, but soil constituents probably are impor-

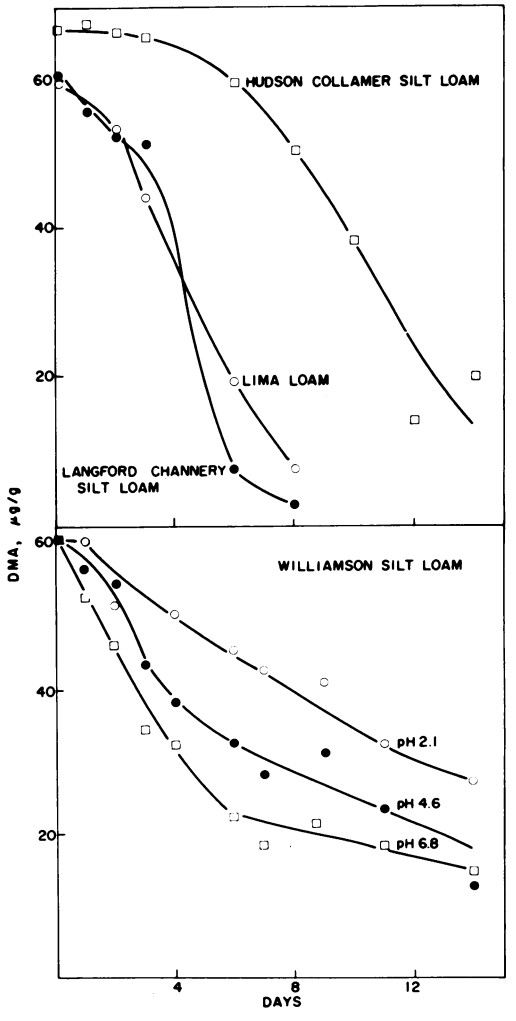

FIG. 2. *Disappearance of dimethylamine from soils of varying pH. The Williamson soil was adjusted to the pH values shown.*

tant in governing the rate of loss.

Anaerobiosis also had a significant effect on the stability of the secondary amine in soil (Fig. 3). Although DMA was metabolized in Williamson silt loam in the presence and absence of oxygen, the process was markedly retarded under anaerobiosis.

To study the factors affecting the microbial formation of DMA, *Micrococcus* sp. was grown in a medium containing 0.20% TMA or glucose as carbon source and 200 μg of N/ml as ammonium, nitrite, or nitrate. The organism could use the three inorganic ions as nitrogen sources. The amount of DMA accumulating in a medium containing TMA varied from 67 to 355 μg/ml, depending upon the nitrogen source (Table 1). The concentration of DMA was measured at 24 h, which was in the late exponential phase of growth. No DMA accumulation occurred when glucose was substituted for the TMA in the growth medium so that the secondary amine seems to be derived from the tertiary amine.

The kinetics of DMA production by *Micrococcus* sp. was observed in the salts solution supplemented with 0.4% (wt/vol) TMA, 0.025% yeast extract, and 0.1% $(NH_4)_2SO_4$. Little accumulation of the secondary amine occurred during the phase of active growth (Fig. 4). DMA appeared in substantial amounts during the late exponential phase and reached a maximum of approximately 2.0 mg/ml in the early stationary phase. The decrease in DMA concentration after 24 h of growth probably resulted from the ability of the organism to use DMA as a carbon source.

TABLE 1. *Effect of carbon and nitrogen source on dimethylamine formation by Micrococcus sp.*

Carbon source	Inorganic nitrogen source	Dimethylamine (μg/ml)
Trimethylamine	Nitrite	355
	Nitrate	174
	Ammonium	67
Glucose	Nitrate	0

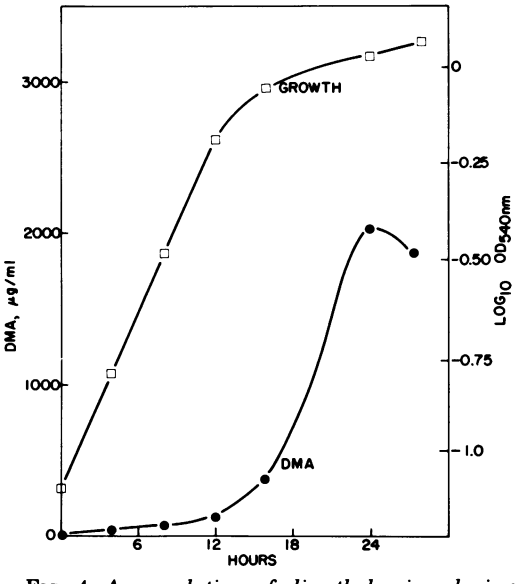

FIG. 4. *Accumulation of dimethylamine during growth of Micrococcus sp.*

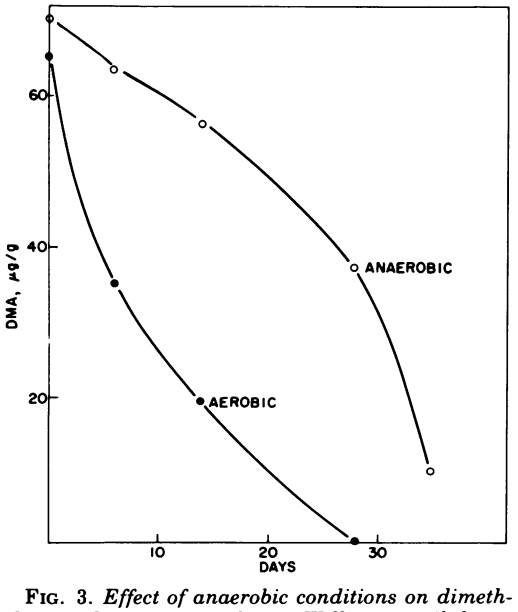

FIG. 3. *Effect of anaerobic conditions on dimethylamine disappearance from a Williamson silt loam.*

Because pH is important in nitrosamine formation, spontaneous nitrosation being enhanced under acidic conditions, the effect of this parameter on DMA accumulation was measured. *Micrococcus* sp. is also capable of reducing nitrate to nitrite so the effect of pH on this reaction was also observed. The growth medium consisted of the salts solution supplemented with 200 μg of nitrate-nitrogen/ml and 4.0 mg of TMA/ml and adjusted to various pH values. Growth, DMA and nitrite levels, and the pH of the medium were measured at 24 h. No significant change in the pH of the growth medium occurred during the experiment. The results showed that the maximum yields of DMA and nitrite were at pH 6.5 (Fig. 5). No change in the cell yield was evident at higher pH levels, but the amount of DMA and nitrite formed decreased at neutral or slightly alkaline pH values. Nitrosamine was not detected in any of the samples, showing that this bacte-

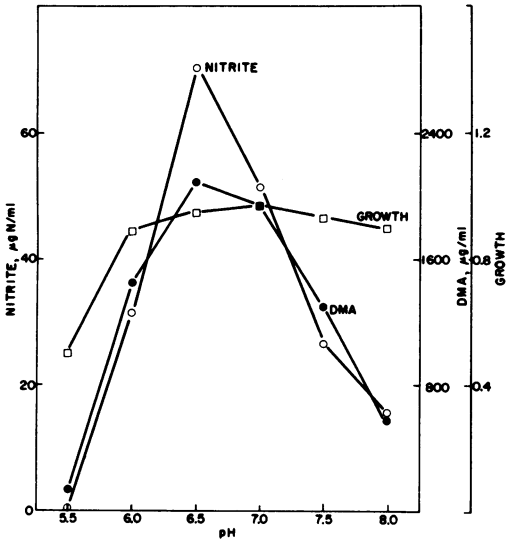

FIG. 5. *Effect of pH on dimethylamine and nitrite accumulation in cultures of Micrococcus sp. growing on trimethylamine and nitrate.*

rium does not form the toxicant under these growth conditions. A lower pH would be necessary for spontaneous nitrosamine synthesis to be significant.

The carbon-nitrogen ratio of culture media is known to affect the yield of various nitrogenous products. To determine the influence of variation of this ratio on the accumulation of DMA, the micrococcus was grown in the salts solution containing 0.4% TMA plus $(NH_4)_2SO_4$ in concentrations to give carbon-nitrogen ratios from 60:1 to 1:1. Although some differences were noted in the quantity of DMA that accumulated in 24 h (Table 2), the differences were probably not significant since the standard deviation associated with the sampling, distillation, and analytical procedure employed was 12%.

DISCUSSION

Secondary amines are known to be formed in soils in the degradation of certain pesticides (14), and data showing that nitrosamines sometimes accumulate indicate that the secondary amine precursor is synthesized in sufficient quantities and is of sufficient persistence to allow for these potential carcinogens to be produced. The synthesis of nitrosamines in the soils here used was not examined because formation of these carcinogens in soil has already been demonstrated.

An increase in the duration of the secondary amine accumulation in nature could increase the probability of nitrosamine formation. The present data show that DMA loss is slow in some soils and rapid in others, but acidity is not the sole factor determining the rate of degradation. The studies of the micrococcus, although not necessarily directly applicable to natural ecosystems, suggest that the prevailing C-N ratio may not affect the accumulation of the secondary amine in the presence of TMA, although the relative amounts of carbon and nitrogen frequently alter greatly the extent of accumulation of nitrogenous metabolites.

TABLE 2. *Effect of the carbon-nitrogen ratio on dimethylamine accumulation by Micrococcus sp.*

C-N ratio	Dimethylamine (μg/ml)
60:1	831
24:1	882
12:1	726
6:1	814
3:1	955
1:1	838

Nitrosamines have yet to be detected in natural habitats, but conversely there has been no significant effort to assess their occurrence in soils and waters. It is likely that the detection and identification of the trace quantities of these compounds that may occur in such environments will be quite difficult, but information on the environmental conditions which contribute to the build-up of their secondary amine precursors will at least allow attention to be focused on the more likely sites for toxicant synthesis.

ACKNOWLEDGMENT

This work was supported by Public Health Training grant ES0098 from the Division of Environmental Health Sciences.

LITERATURE CITED

1. **Ayanaba, A., and M. Alexander.** 1974. Transformations of methylamines and formation of a hazardous product, dimethylnitrosamine, in samples of treated sewage and lake water. J. Environ. Qual. 3:83-89.
2. **Ayanaba, A., W. Verstraete, and M. Alexander.** 1973. Formation of dimethylnitrosamine, a carcinogen and mutagen, in soils treated with nitrogen compounds. Soil Sci. Soc. Am. Proc. 37:565-568.
3. **Ayanaba, A., W. Verstraete, and M. Alexander.** 1973. Possible microbial contribution to nitrosamine formation in sewage and soil. J. Natl. Cancer Inst. 50:811-813.
4. **Daiber, D., and R. Preussmann.** 1964. Quantitative colorimetrische Bestimmung organische N-Nitroso-Verbindungen durch photochemische Spaltung der Nitrosaminbindung. Z. Anal. Chem. 206:344-352.
5. **Elespuru, R. K., and W. Lijinsky.** 1973. The formation of carcinogenic nitroso compounds from nitrite and some types of agricultural chemicals. Food Cosmet. Toxicol. 11:807-817.
6. **Heath, D. F., and J. A. E. Jarvis.** 1955. The polarographic determination of dimethylnitrosamine in animal tissue. Analyst 80:613-616.
7. **Hill, M. J., G. Hawksworth, and G. Tattersall.** 1973.

Bacteria, nitrosamines and cancer of the stomach. Br. J. Cancer 28:562-567.
8. **Montgomery, H. A. C., and J. F. Dymock.** 1962. The determination of nitrite in water. Analyst 86:414-416.
9. **Pribyl, M., and J. Nedbalkova.** 1967. Spektrophotometrische Bestimmung von Dimethylamin in Dimethylformamid. Z. Anal. Chem. 232:261-267.
10. **Skerman, V. B. D.** 1967. A guide to the identification of the genera of bacteria. The Williams and Wilkins Co., Baltimore.
11. **Smith, T. A.** 1971. The occurrence, metabolism and functions of amines in plants. Biol. Rev. Cambridge Philos. Soc. 46:201-241.
12. **Society of American Bacteriologists.** 1957. Manual of microbiological methods. McGraw-Hill Book Co., New York.
13. **Stahl, E.** 1969. Thin-layer chromatography. Springer-Verlag, New York.
14. **Tate, R. L., and M. Alexander.** 1974. Formation of dimethylamine and diethylamine in soil treated with pesticides. Soil Sci. 118:317-321.
15. **Tate, R. L., and M. Alexander.** 1975. Stability of nitrosamines in samples of lake water, soil, and sewage. J. Natl. Cancer Inst. 54:327-330.