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Accelerated Degradation of Methyl Bromide in Methane-,2,4-D-, and Phenol-Treated Soils

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The use of the soil fumigant methyl bromide (bromomethane, MeBr) in the United States is scheduled to be phased out after January 1, 2001 (Noling and Becker, 1994). The ban of MeBr is due to its' potent ozone depletion in the stratosphere (Watson *et al.*, 1992). The search for alternative chemicals and strategies is being actively, pursued (Stephens, 1996). If no effective chemicals or strategies can be identified before the ban takes place, economic impacts to agricultural communities could be substantial.

Although at ≥ 4°C MeBr is a gas (Yates *et al.*, 1996), up to 70% of injected MeBr can be degraded in soil (Yagi *et al.*, 1995), not volatilized into the atmosphere. Three degradation pathways have been identified as responsible for the disappearance of MeBr in soil (Oremland *et al.*, 1994: Ou *et al.*, 1997; Shorter *et al.*, 1995: Yagi *et al.*, 1995): a) chemical hydrolysis to form methanol and bromide ion, b) methylation to soil organic matter and release of bromide ion, and c) microbial oxidation to form formaldehyde and bromide ion. Biological hydrolysis and other microbial processes also likely contribute to the disappearance of MeBr in soil. A strain of methane oxidation bacterium *Methylcoccus capsulatus* (Oremland *et al.*, 1994) and several species of ammonia oxidation bacteria (Rasche *et al.*, 1990) had the capacity to oxidize MeBr through the action of methane- and ammonia-monoxygenase, respectively. Oremland *et al.* (1994) showed that MeBr in methanotrophic soils under aerobic conditions disappeared more rapidly than in autoclaved soils, indicating biological degradation by methanothrophic bacteria and other microorganisms. Stimulation of MeBr degradation was observed in soil treated with ammonium sulfate (Ou *et al.*, 1997). Application of the ammonia fertilizer apparently stimulated nitrification activity in soil, and MeBr, in turn, was degraded during the bacterial oxidation of ammonia.

In this study, attempts were made to stimulate MeBr degradation in soil collected from an undeveloped forest site pretreated with methane, the herbicide 2,4-D (2,4-dichlorophenoxyacetic acid), or phenol. Similar to the involvement of the methane monooxygenase on the initial step of methane degradation, 2,4-dichlorophenol hydroxylase and phenol hydroxylase are responsible for the initial step of degradation of 2,4-D and phenol, respectively (Harker and Kim, 1990). 2,4-

dichlorophenol hydroxylase and phenol hydroxylase are monooxygenases, and these monooxygenases may also have the capacity to oxidize MeBr concurrently during the oxidation of the respective substrates.

MATERIALS AND METHODS

Surface (0 - 15 cm depth) and subsurface (15 - 30 cm depth) soil samples were collected from an undeveloped forest site at the University of Florida campus Gainesville, Florida. This site and sampling technique have been described previously (O u *et al.*, 1997). Soil was classified to be Arredondo fine sand. Soil-water contents, organic matter contents and pH values of the surface and subsurface samples were 65 and 5 g kg $^{-1}$. 11.8 and 7.7 g kg^{-1}, and 5.50 and 5.70, respectively. Both samples consisted of more than 90% sand.

Methyl bromide (99.5% purity) and methan gas (99% purity) in metal cylinders were purchased from Matheson Gases (Morrow, GA) and used as supplied. Analytical grade phenol and 2,4-D were obtained from Fisher Scientif,c (Orlando. FL) and Eastman Kodak (Rochester, NY), respectively.

Surface and subsurface soil samples (500 g. oven dry weight basis) were incubated in 1 L glass Erlenmeyer flasks, or in 1 L glass bottles with screw caps. The soil samples in Erlenmeyer flasks were treated with either phenol (4 µg g^{-1}) or 2,4-D (10 µg g^{-1}). Soil samples in glass bottles were supplied with 1 part (150 ml) of methane and 3 parts (450 ml) of air, and closed immediately with caps. To prevent leakage, the threads of the bottles were wrapped with Teflon tape. These samples were incubated in the dark at 25°C. After 3 weeks of incubation, phenol and 2,4-D treated samples were retreated with the respective chemical. Immediately after the third treatment, soil samples were used for methyl bromide degradation experiments. The methane treated soil samples were retreated with methane and air once a week 5 times. One week after the fifth treatment, these samples were used for methyl bromide degradation experiments without retreatment of methane.

Treated and untreated soil samples (10 g oven dry weight basis) were placed in 22 mL headspace-gas chromatograph (GC) glass vials. A known amount of MeBr (200, 500, or 1000 µg) was introduced onto the soil surface using a air tight glass microsyringe as described previously by O *et al.* (1997). The vials were then immediately closed with Teflon-lined, butyl rubber septa caps. These vials were sonicated for 15 minutes, and then incubated in the dark at 25°C. At selected time intervals, two vials from each treatment were sonicated for 15 minutes, and then subjected to headspace-GC determination of MeBr residues.

A Perkin Elmer Autosystem GC (Norwalk. CT) equipped with a headspace autosampler, a flame ionization detector (FID), split-splitless injector, Turbochrom 4 software, and a 486 computer was employed for determination of MeBr residues the headspace-GC vials. Gas chromatographic parameters and operational conditions have been described previously (O *et al.*, 1997). The retention time

for MeBr was 3.8 minutes.

RESULTS AND DISCUSSION

At the application of 500 µg of MeBr (50 g g $^{-1}$). MeBr in untreated soil samples disappeared slowly but steadily (Figure 1). After 35 days of incubation, 23 and 32% of the applied MeBr remained in the surface and subsurface samples, respectively. Disappearance of MeBr in treated samples was much more rapid than in the untreated samples, and the disappearance was initially more rapid in the surface samples than in the corresponding subsurface samples. Methyl bromide in the treated surface and subsurface samples disappeared completely in 3 and 1 day, respectively. At 1000 µg (100 µg g $^{-1}$). MeBr in the methane treated surface and subsurface samples initially disappeared more rapidly than in the corresponding untreated samples, especially in the treated surface sample. Disappearance of MeBr in the treated surface sample was initially more rapid than in the treated subsurface sample; however, the trend was reversed after 7 days. As a result, at the end of 35 days of incubation, the amounts of MeBr in the two samples were nearly similar, 10 and 15%, respectively. For the untreated soil samples, after slightly higher initial disappearance of MeBr in the surface sample, disappearance of MeBr in the two samples was generally similar throughout the rest of the incubation period. At 200 µg (20 µg g^{-1}), no McBr residues in methane treated surface and subsurface soil samples were detected after two hours of incubation (data not shown). Whereas, MeBr in untreated surface and subsurface soil samples disappeared completely in 14 and 21 days, respectively (see Figure 2).

Ou *et al.,* (1997) found that chemical degradation was the principal factor responsible for the MeBr disappearance in untreated soil, and biological degradation was minor. Therefore, the differentials in the disappearance rates of MeRr in methane treated and untreated soils were likely due to the action of methanotrophic bacteria stimulated by repeated applications of methane. Oremland *et al.,* (1994) attributed similar results to the action of methane monooxygenase, produced by bacteria for the oxidation of methane to methanol. Methanotrophic bacteria were apparently responsible for the majority of the degradation in the methane treated soil samples that received 200 and 500 µg of MeBr. At the application of 1000 µg, contribution frem mcthanotrophic bacteria in the methane treated soil was less significant. Twenty-two to 40% of the degradation in the treated surface soil during the first 7 days was attributed to methane oxidation bacteria. After 7 days, contribution from these bacteria was much less, about 10% of the degradation. In the treated subsurface soil, contribution from the methanotrophic bacteria in the first 3 days was about 10%, and less than 5% thereafter. At this concentration, the activity of methane monooxygenase could be inhibited by MeBr.

At applications of 200 µg of MeBr, stimulation of MeBr degradation in 2,4-D treated surface and subsurface soil samples was much less than in methane treated soil samples (Figure 2): but MeBr degradation in the 2,4-D treated soil samples

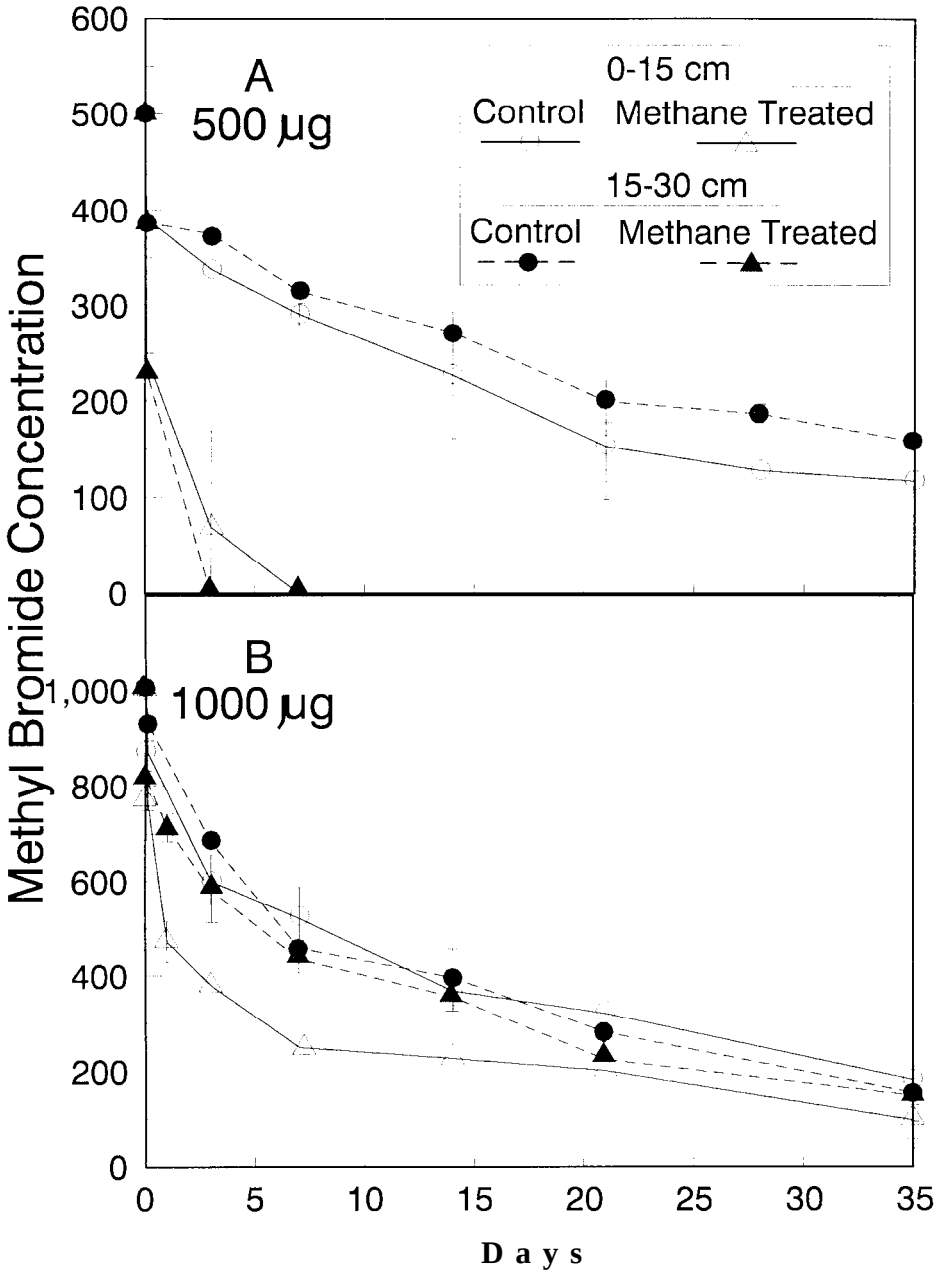

Figure 1. Disappearance of MeBr in methane-treated and untreated surface (0 - 15 cm depth) and subsurface (15 - 30 cm depth) Arredondo soil. Soil samples (10 g) received 500 or 1000 µg of MeBr.

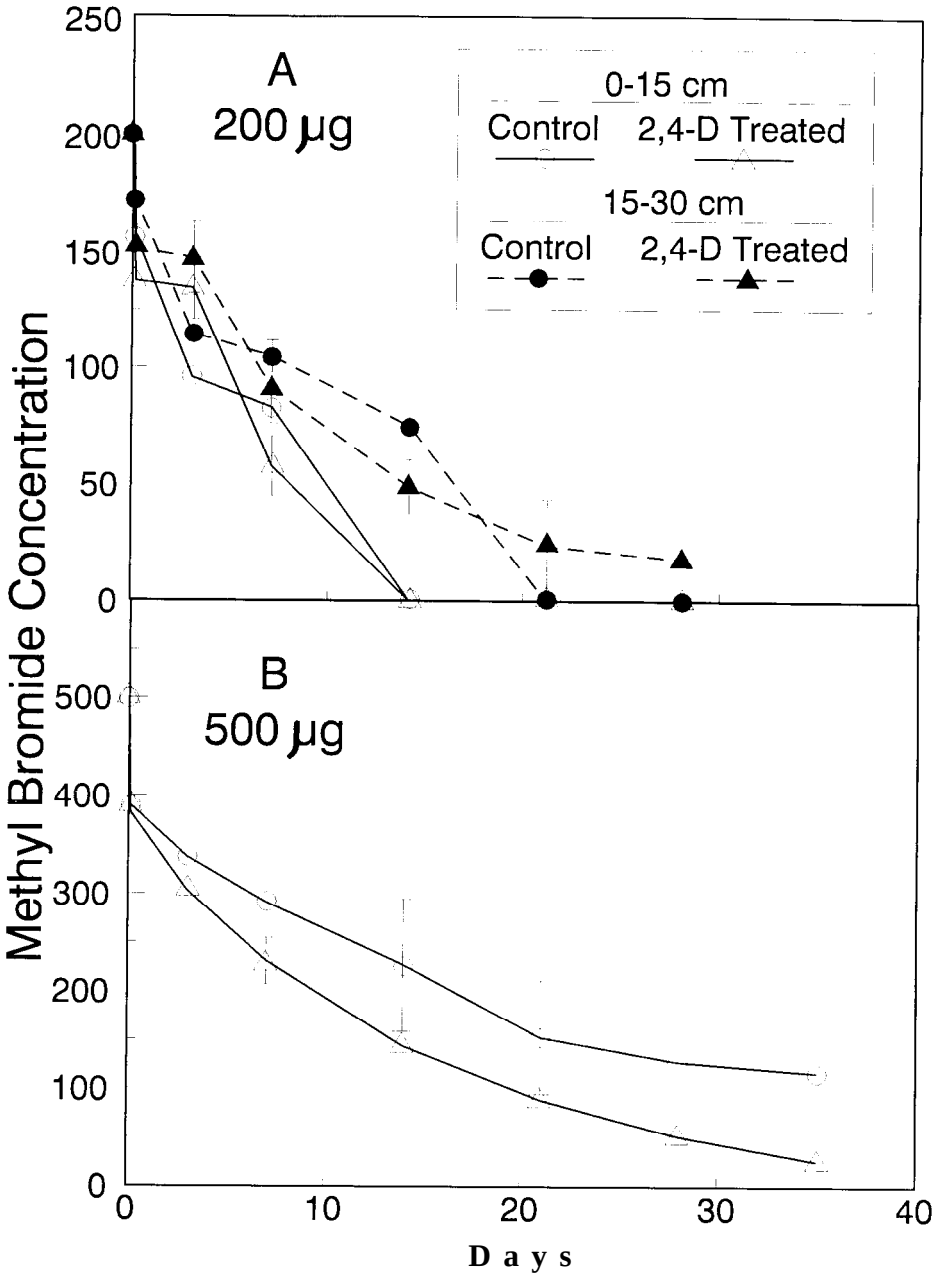

Figure 2. Disappearance of MeBr in 2,4-D-treated and untreated surface (0 - 15 cm depth) and subsurface (15 - 30 cm depth) Arredondo soil. Soil samples (10 g) received 200 or 500 µg of MeBr.

was initially more rapid than in untreated samples. After 7 days of incubation, a smaller amount (29%) of MeBr remained in the 2,4-D treated surface sample, whereas, 42% of MeBr remained in the untreated surface sample. Similar to the untreated surface sample, MeBr in the treated surface sample also completely disappeared in 14 days. Although the disappearance of MeBr in the treated subsurface sample was generally more rapid during the first 14 days of incubation than in the untreated subsurface sample, small amounts of MeBr still remained in the sample after 28 days of incubation. On the other hand, MeBr in the untreated sample completely disappeared in 21 days. At 500 µg, MeBr in treated and untreated surface samples disappeared slowly, and the disappearance was more rapid in the treated sample than in the untreated sample. After 35 days of incubation, 5% of the applied MeBr remained in the treated soil, whereas, 23% of the applied MeBr remained in the untreated soil.

In a manner similar to the 2,4-D treatment, effects of phenol treatment were not as great as the methane treatment. At the application of 200 µg of MeBr (20 µg g^{-1}), MeBr in treated and untreated surface and subsurface samples disappeared completely in 14 and 21 days, respectively. (Figure 3). Stimulation of MeBr degradation in the phenol treated samples was not evident until after 3 to 14 days of incubation. Stimulation or MeBr was greater in soil samples treated with 500 µg of MeBr than in soil samples treated with 200 µg of MeBr, and stimulation was greater in the subsurface soil than in the surface soil. Although phenol degrading population densities in these soil samples are not known, phenol degrading populations likely increased resulting in increased MeBr degradation. after a lag period. Bacteria that utilized phenol as a sole source of carbon and energy were readily isolated from the phenol treated soil samples (unpublished observation).

Harker and Kim (1990) reported that the 2,4-D degrade *Alcaligenes eutrophus* JMP134 produced two monooxygenases (3,4-dichloropheliol hydroxylase and phenol hydroxylase) during the degradation of 2,3-D and phenol, respectively, and that these enzymes also had the capacit to cometabolically degrade trichloecthylene (TCE) a short-chained chlorinated aliphatic hydrocarbon. Apparently, enzymatic activities of 2,4-dichlorophenol hydroxylase and phenol hydroxylase toward the oxidation of MeBr produced by 2,4-D degraders and phenol degraders in the 2,4-D-treated soil and the phenol-treated soil, respectively, were much lower than the activity of methane monooxygenase produced by methane oxidation bacteria in the methane-treated soil. Methane monooxygenase has a much greater specific enzymatic activity toward oxidation of TCE than 2,4-dichlorophenol hydroxylase and phenol hydroxylase (Wackett, 1995).

From agricultural standpoint, it is not practical to use methane to eliminate MeBr residues in soil after fumigation is completed. For soil fumigation, MeBr is generally injected into nonflooded soil at 15 to 30 cm depth (Noling and Becker, 1994). Under such conditions, there is little chance for soil to create methanogenic conditions to generate a significant amount of methane from organic matter.

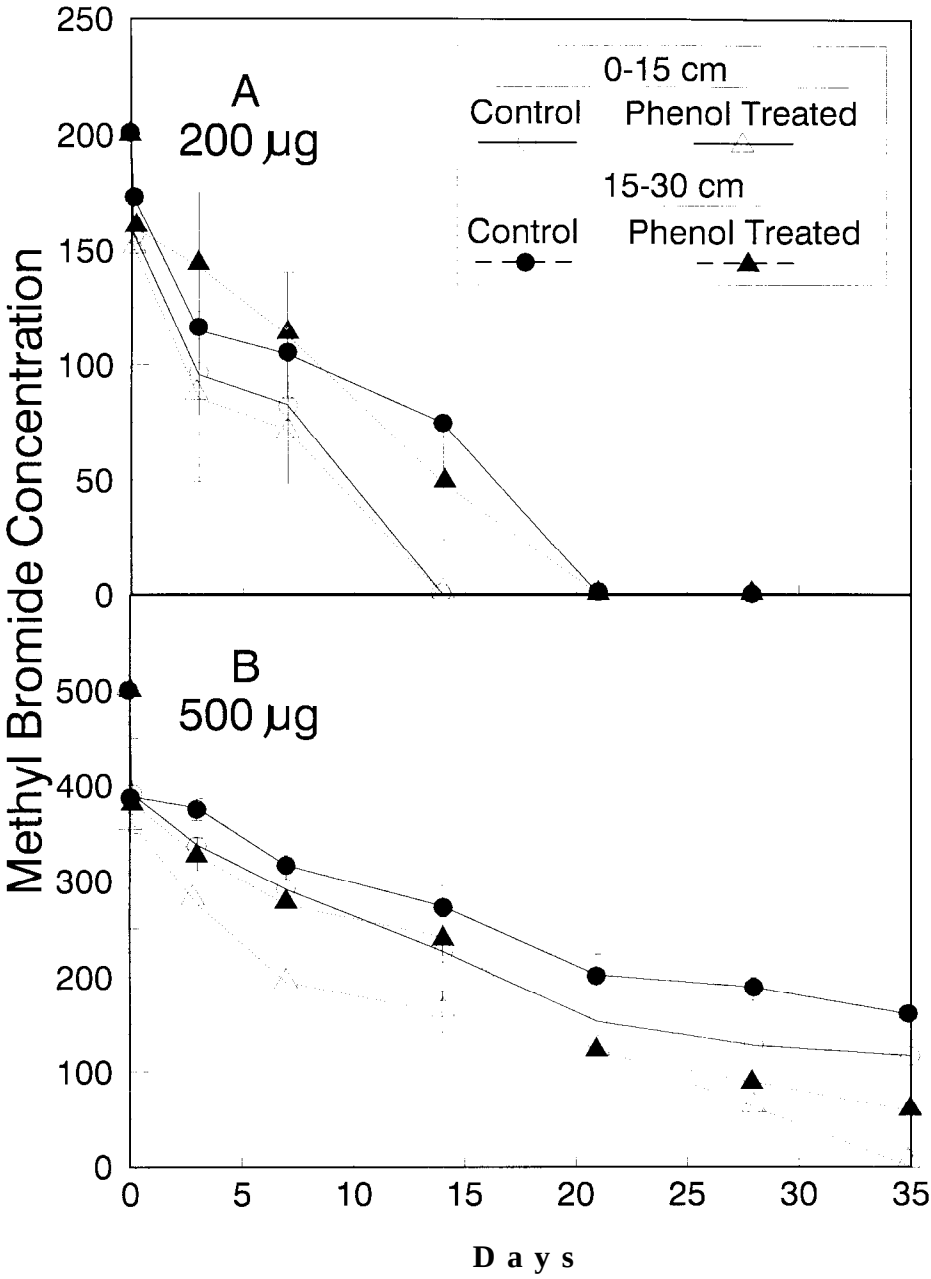

Figure 3. Disappearance of MeBr in phenol-treated and untreated surface (0 - 15 cm depth) and subsurface (15 - 30 cm depth) Arredondo soil. Soil samples (10 g) received 200 or 500 µg of MeBr.

Application of ammonium sulfate may serve dual purposes, as an N-fertilizer and as a stimulator of MeBr degradation. 2,4-D may also serve dual purposes as well, as a herbicide and stimulation of MeBr degradation.

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