# Vadose Zone Processes and Chemical Transport

## Volatilization and Degradation of Soil-Applied Dimethylselenide

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### ABSTRACT

**Dimethylselenide (DMSe) is a highly volatile gas that is produced by indigenous microorganisms in seleniferous soils and sediments; however, little is known about the soil conditions that affect the persistence of DMSe and its transport to the atmosphere. In this study we investigated the effect of moisture content, temperature, and organic amendments on the degradation of soil-applied DMSe. The degradation of DMSe was entirely a result of biological mechanisms, but changes in temperature (20–40°C) and soil moisture content (30–70% of the maximum water holding capacity) had little influence on the degradation rate. In contrast, amending soil with either 1% casein or gluten (by weight) had an inhibitory effect on the degradation of DMSe. After 18 d, 2.1 times more DMSe was present in the casein-amended soil and 2.6 times more DMSe was present in the gluten-amended soil. The transport of DMSe in packed soil columns was also investigated. Increasing the depth to soil surface was found to significantly decrease the amount of DMSe transported to the air. After 6 d, 57% of DMSe injected 10 cm below the soil surface was volatilized. At an injection depth of 20 cm the cumulative emissions were reduced by 38% and at 30 cm the cumulative emissions were reduced by 51%. In columns containing 1% casein or gluten in the top 5 cm of soil the cumulative loss of DMSe was about 9% higher than in unamended soil. Increasing our understanding of the soil conditions that influence the gaseous diffusion of DMSe should help in determining the feasibility of using Se volatilization as a remediation technique.**


High concentrations of selenium (Se) in the environment pose a hazardous threat to wildlife and humans (Ohlendorf et al., 1986). In seleniferous soils, sediments, and waters, Se oxyanions and organo–Se compounds are microbiologically transformed into volatile Se compounds, which are subsequently released to the atmosphere (Francis et al., 1974; Doran and Alexander, 1977a). In California's San Joaquin Valley, where Se-contaminated soils are derived from Se-bearing geologic strata, Se volatilization has been proposed as a cost-effective bioremedial approach (Karlson and Frankenberger, 1988). Dimethylselenide (DMSe, $[CH_3]_2Se$), the major volatile Se gas detected in the environment, plays a significant role in the biogeochemical cycling of Se and is less toxic than either selenate ($SeO_4^{2-}$, $Se^{6+}$) or selenite ($SeO_3^{2-}$, $Se^{4+}$) (Franke and Moxon, 1936; McConnell and Portman, 1952), which are the predominant Se species found in seleniferous soils and waters.

For DMSe the vapor pressure is 32 kPa, the Henry's constant (dimensionless) is 0.058, and the solubility is 0.024 g $g^{-1}$ water (Karlson et al., 1994). As a result of DMSe's high solubility in water, soil moisture content may heavily influence Se volatilization, since soil moisture decreases the rate of gaseous diffusion in soil (Zhang et al., 1999). Other soil factors known to affect the rate of DMSe volatilization are temperature, pH, and redox status (Frankenberger and Karlson, 1994a). These soil factors, and also moisture content, directly control microbial activity and, hence, the Se biomethylation process. The addition of organic amendments to Se-contaminated soil and sediment is known to increase the Se volatilization rate by stimulating indigenous microorganisms to methylate Se (Doran and Alexander, 1977a; Karlson and Frankenberger, 1989, 1990). Apparently, organic C is a limiting factor in the microbial methylation of Se. Microorganisms have also been isolated that are capable of demethylating volatile Se compounds (Doran and Alexander, 1977b; Oremland and Zehr, 1986). Thus, demethylation of DMSe may be an additional significant rate-controlling factor of Se volatilization.

The majority of Se volatilization studies have looked at the formation of DMSe in soils and sediments that were either naturally contaminated or amended with Se (Ganje and Whitehead, 1958; Hamdy and Gissel-Nielsen, 1976; Abu-Erreish et al., 1968; Francis et al., 1974; Doran and Alexander, 1977a; Zieve and Peterson, 1981; Frankenberger and Karlson, 1995). In these studies, Se volatilization was only studied from the perspective of Se biomethylation, while an understanding of the fate and transport of DMSe was largely unknown. Therefore, if Se volatilization is to be effectively used as a remediation technique, the biomethylation of Se and fate and transport of DMSe should be studied as separate phenomena. Only recently have several researchers have tried to address the latter of these two (Zhang and Frankenberger, 1999; Zhang et al., 1999; Martens and Suarez, 1999; Guo et al., 1999).

In this laboratory study we characterized the degradation and emission of DMSe added to soil under a variety of environmental conditions. The specific objectives were to: (i) quantify the contribution of microbial and chemical processes to the total degradation of DMSe as a function of temperature, (ii) determine the effect of moisture content and organic amendments on the degradation of DMSe in soil, and (iii) determine the volatilization potential of DMSe from packed soil columns as affected by soil depth, moisture content, and organic amendments. It is hoped that through this study

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**Abbreviations:** DMSe, dimethylselenide; $WHC_{max}$, maximum water holding capacity.

we will have acquired a better understanding of the conditions that control DMSe transport and emission from soil. Ultimately, it may be possible to enhance DMSe emissions by targeting the optimum soil conditions required for both the biomethylation and volatilization of Se. This study is unique in that we have shown that organic amendments known to stimulate the biomethylation of Se also increase the persistence of DMSe in soil and allow for increased DMSe emissions.

## MATERIALS AND METHODS

### Chemicals and Soil

Casein and gluten were purchased from Sigma Chemical Co. (St. Louis, MO). Dimethylselenide (99% purity) was purchased from Strem Chemical Co. (Newburyport, MA). The soil used in this study was Arlington sandy loam (coarse-loamy, mixed, thermic Haplic Durixeralf), obtained from the University of California, Riverside, Agricultural Experiment Station. The soil was removed from the Ap horizon, passed through a 2-mm sieve, and briefly stored at 5°C until used. The soil has a pH of 7.2, organic matter content of 0.92%, maximum water holding capacity ($WHC_{max}$) of 0.2 kg $kg^{-1}$, and Se concentration of $<0.1$ mg $kg^{-1}$.

### Degradation Experiments

To better understand diffusive transport in soil, experiments were conducted to assess the effect of organic amendments (i.e., casein [milk protein] and gluten [wheat protein]) and soil moisture content on the degradability of DMSe. Each of the organic amendments was applied at 1% (w/w, dry wt. basis). The amended soils were prepared by thoroughly mixing the organic amendment and soil in plastic bags. The moisture content of the soil mixtures was then adjusted to 30% of the $WHC_{max}$ with deionized water. After which, 10 g (dry wt.) of soil was added to 21-mL glass headspace vials.

To determine the effect of moisture content on DMSe degradation, the soil moisture content was adjusted to 30, 50, and 70% of the $WHC_{max}$. To adjust the soil moisture content, soil was first added to the headspace vials (10 g dry wt. per vial), followed by the addition of an appropriate volume of deionized water. The samples were then covered with Parafilm (American National Can, Chicago, IL) and allowed to stand overnight at room temperature before being spiked with DMSe.

To differentiate between chemical and microbial transformations at different temperatures (i.e., 20, 30, and 40°C), DMSe degradation was measured with nonsterile and autoclaved soil. Vials containing 10 g of soil (dry wt.) were autoclaved twice (1 h at 121°C), with a 24-h period between the first and second autoclaving. After the second autoclaving, the soil moisture content was determined and readjusted to 30% of the $WHC_{max}$ with sterile deionized water.

To initiate each of the experiments, 4 μL of 99% pure DMSe standard was added to the treated vials with a gas-tight microsyringe, and then the vials were immediately capped with Teflon-faced septa and incubated in the dark at the specified temperatures. Triplicate samples were removed from each treatment at different times and stored at −20°C until analyzed. A Hewlett-Packard (Wilmington, DE) 5890 gas chromatograph (GC) equipped with a micro-electron capture detector (μECD) was used to analyze DMSe in the headspace of each vial. Column injections were made from a Tekmar (Cincinnati, OH) 7000 headspace autosampler. Prior to the column injection, each of the vials were heated to 90°C for 15 min. The GC conditions were as follows: injector temperature, 230°C; detector temperature, 280°C; carrier gas, He, 0.76 mL $min^{-1}$; oven temperature, isothermal at 100°C for 11.9 min; capillary column, AT-624 60 m × 0.25 mm × 1.4 μm (Alltech, Deerfield, IL). The average extraction efficiency of DMSe residues from the time-zero matrix samples was >91%. All statistical analyses were performed at the 0.05 significance level.

### Column Experiments

The effect of soil depth, moisture content, and organic amendments on DMSe emission losses were studied with packed stainless steel soil columns. The system consisted of the soil column (12.5-cm i.d. × 70-cm length) and a sampling chamber (5-cm i.d. × 5-cm length) sealed to the top of the column (Gan et al., 2000). The columns were packed with Arlington sandy loam in 5-cm increments with an appropriate mass of soil to result in a uniform bulk density ($\rho_b$) of 1.65 g $cm^{-3}$ throughout the soil column. At this bulk density, a total of 14.2 kg of dry soil was used to pack each column. To test the effect of soil depth on DMSe emissions, DMSe standard (0.5 mL) was injected into the column at 10, 20, or 30 cm below the soil surface through sampling ports on the side of the column. To test the effect of moisture content on DMSe emissions, the soil moisture content was adjusted to 30, 50, and 70% of the soil's $WHC_{max}$. To test the effect of organic amendments on DMSe emissions (soil moisture adjusted to 30% $WHC_{max}$), casein and gluten were applied at 1% (w/w, dry wt. basis) in the top 5 cm of soil. For the moisture and organic amendment experiments, DMSe standard (0.5 mL) was injected 10 cm below the soil surface. A continuous air flow (150 mL $min^{-1}$) in the sampling chamber was used to sweep DMSe emitted from above the soil surface; these samples from each column were collected through the use of an automated gas sampling system (Wang et al., 1999) and analyzed for DMSe with a Hewlett-Packard 5890 GC equipped with a μECD. The GC conditions were set as follows: injector temperature, 120°C; detector temperature, 270°C; carrier gas, He, 14 mL $min^{-1}$; oven temperature, isothermal at 100°C for 5 min; capillary column, AT-624 60 m × 0.53 mm × 3 μm (Alltech).

## RESULTS AND DISCUSSION

### Dimethylselenide Degradation Experiments

In Arlington sandy loam, the degradation of DMSe was a result of biological mechanisms only, since a significant ($P < 0.05$) loss of DMSe only occurred in nonsterile soil (Fig. 1). With respect to chemical degradation of DMSe, it is not a significant process in Arlington soil; however, chemical degradation of DMSe may be significant in other soil types. Over the 18-d experimental period, 68% of the applied DMSe was degraded at 20°C, while about 81% of the DMSe was degraded at 30 and 40°C. Due to the presence of large numbers of diverse microorganisms in soils, the biodegradation of DMSe may be affected by factors that influence microbial activity. Surprisingly, an increase in temperature from 20 to 30 and 40°C did not substantially increase nor decrease the biological degradation rate (Fig. 1), although there was a significant difference ($P < 0.05$) between the 20 and 30°C treatments. It appears that the microbes responsible for DMSe degradation are active over a wide temperature range. The biological degradation of DMSe occurs when a methyl group is removed

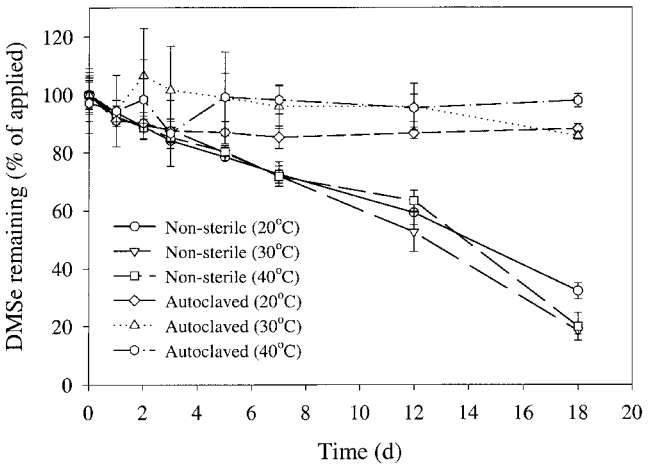


**Fig. 1. Degradation of dimethylselenide in nonsterile and autoclaved Arlington sandy loam as affected by temperature. The soil was autoclaved twice at 120°C for 1 h. Soil moisture content = 30% maximum water holding capacity ($WHC_{max}$). Error bars represent the standard deviation of triplicate samples.**

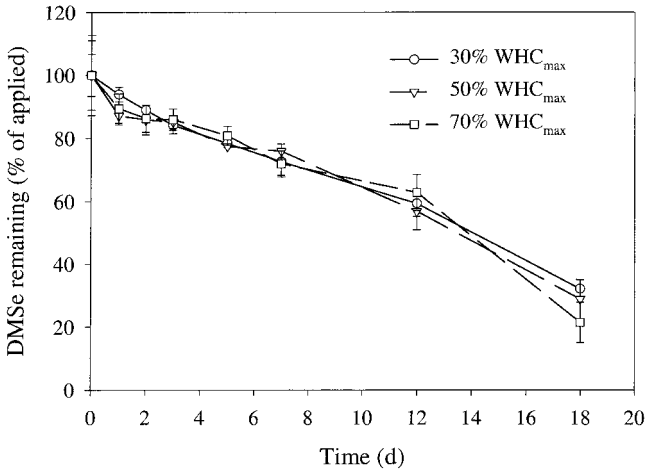


**Fig. 2. Effect of various soil moisture levels on the degradation of dimethylselenide in Arlington sandy loam. The samples were incubated at 20°C. Error bars represent the standard deviation of triplicate samples.**

by cleavage of the Se–C bond (Wang and Burau, 1995), which is also commonly known as a demethylation reaction. Dimethylsulfide ($[CH_3]_2S$), the analog compound of DMSe, is degraded by both aerobic (Kanagawa and Kelly, 1986; Suylen et al., 1986) and anaerobic (Kiene et al., 1986; Visscher and van Gemerden, 1991; Oremland et al., 1991) bacteria. Although there is abundant information on Se methylating microorganisms in the literature, very little information is available with respect to DMSe demethylating organisms. Doran and Alexander (1977b) isolated two demethylating pseudomonads able to utilize DMSe as a sole carbon source. In anoxic sediments, the demethylation of DMSe was reported to occur by an obligate methyltroph (Oremland and Zehr, 1986). It cannot also be ruled out that the demethylation of DMSe occurs as a result of fortuitous metabolism (Dagley, 1984), not catabolism. The study of DMSe demethylating organisms is clearly an area of vast research opportunities.

Soil moisture not only presents a physicochemical impediment to Se volatilization, but like that of temperature, it also influences microbial activity. In soil, the degradation of fumigant pesticides, which have similar vapor pressures and Henry's constants to that of DMSe (Yates and Gan, 1998), has been found to increase with increasing soil moisture content (Gan et al., 1999). It is commonly believed that pesticides in the dissolved phase are readily accessible for soil microorganisms (Shelton and Parkin, 1991; Walker et al., 1992; Garciá-Valcárcel and Tadeo, 1999). It could therefore be expected that DMSe degradation should also increase with increasing soil moisture content. However, in this study, the rate of DMSe degradation was unaffected ($P > 0.05$) by changes in soil moisture content when adjusted to 30, 50, and 70% of the soil's $WHC_{max}$ (or 6, 10, and 14% moisture by weight) (Fig. 2). Guo et al. (1999) found that changes in soil moisture content ≥10% by weight to as high as 43.9% (oversaturation) also had no effect on DMSe degradation.

Figure 3 demonstrates that DMSe degradation is more rapid in unamended soil than in organically amended soil. After 18 d, 2.1 and 2.6 times more DMSe (a statistically significant amount, $P < 0.05$) was present in the casein- and gluten-amended soil, respectively. Both casein and gluten were chosen because they have been shown to increase the biomethylation of Se in seleniferous soil and water, resulting in higher Se volatilization rates (Frankenberger and Karlson, 1989; Thompson-Eagle and Frankenberger, 1990). It has been proposed that the microbial volatilization of Se can be used as a bioremediation technique to remove Se from soils and sediments (Frankenberger and Karlson, 1994b). Apparently, the DMSe degrading microorganisms are readily utilizing the casein and gluten as a C source over that of DMSe, which results in less DMSe being degraded. In another study, the degradation of DMSe was also found to be inhibited in gluten-amended soil (Guo et al., 1999). The addition of steer manure to the same soil

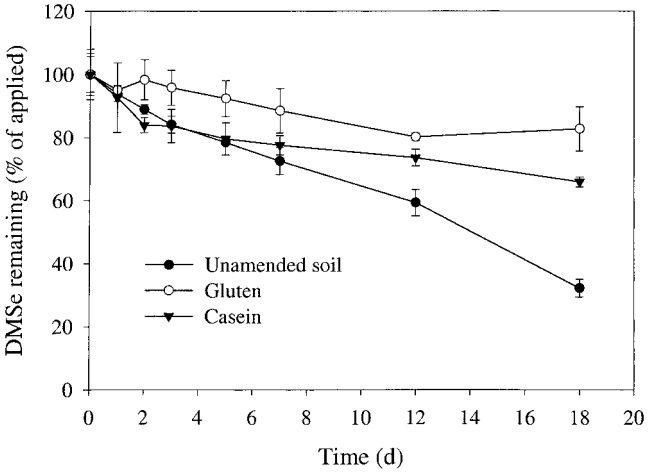


**Fig. 3. Degradation of dimethylselenide in gluten- and casein-amended Arlington sandy loam. The amendments were applied at 1% (dry wt. basis), and the incubation temperature was 20°C. Soil moisture content = 30% maximum water holding capacity ($WHC_{max}$). Error bars represent the standard deviation of triplicate samples.**

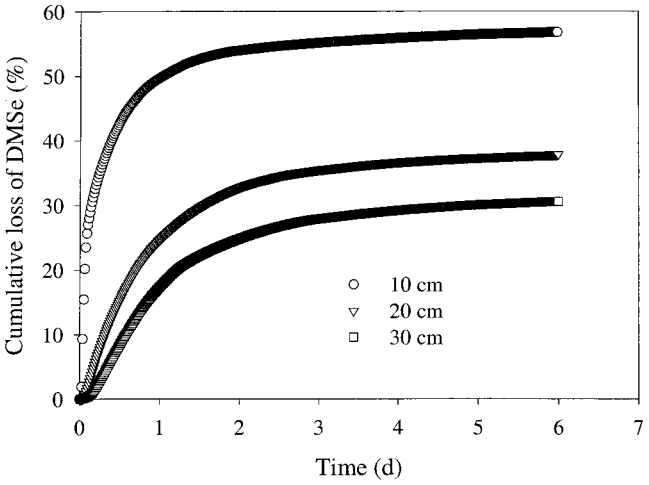


**Fig. 4. Relationship between dimethylselenide injection depth and emissions from packed soil columns. The columns were packed with Arlington sandy loam to a bulk density of 1.65 g cm$^{-3}$ and incubated at room temperature. Soil moisture content = 30% maximum water holding capacity ($WHC_{max}$).**

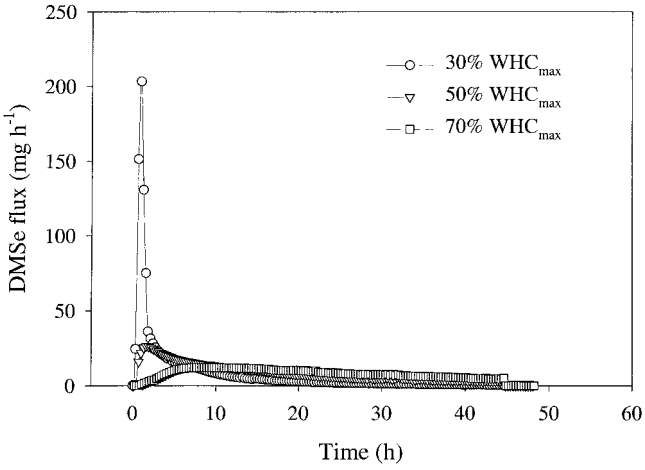


**Fig. 5. Comparison of various soil moisture levels on dimethylselenide flux from packed soil columns. The columns were packed with Arlington sandy loam to a bulk density of 1.65 g cm$^{-3}$ and incubated at room temperature. To initiate the experiment, 0.5 mL of dimethylselenide standard was injected 10 cm below the soil surface.**

produced a similar result. These results demonstrate that the persistence of DMSe may be increased by the addition of a number of organic amendments as long as they are readily biodegradable by the soil microflora. Theoretically, increased persistence of DMSe should then allow for more DMSe to be released from soil through volatilization.

## Dimethylselenide Emission Experiments

The volatilization of DMSe was related to soil depth, moisture content, and organic amendments (Fig. 4, 5, and 6). In soil at a moisture content of 30% the $WHC_{max}$ and an injection depth of 10 cm, 57% of the applied DMSe was volatilized after 6 d (Fig. 4). Increasing the injection depth to 20 and 30 cm reduced DMSe emissions by 38 and 51%, respectively. Since DMSe is predominantly degraded through biological mechanisms in Arlington soil, the deeper injection depth may allow DMSe longer time to react with the soil microorganisms, thus, increasing the amount of DMSe degraded and reducing the amount released through volatilization. Therefore, under natural conditions, the majority of volatile Se produced near the soil surface will be released to the atmosphere, while DMSe produced deeper in the soil profile will be trapped. Experiments by Martens and Suarez (1999) found that DMSe is predominantly found in the phosphate-soluble Se pool, suggesting microbial oxidation to $Se^{6+}$ and $Se^{4+}$. In field experiments, increased Se emission rates occurred after tillage of seleniferous sediments (Frankenberger and Karlson, 1992). Tillage increases porosity, which enhances the diffusion of gaseous Se and may also increase contact between the Se and methylating microorganisms. Although varying bulk densities were not tested in the soil columns, it could be expected that soil bulk density less than 1.65 g cm$^{-3}$ would have brought about higher DMSe emissions at all injection depths.

As previously mentioned, DMSe is highly soluble in water and, as a result, soil moisture can be an important factor affecting DMSe volatilization to the atmosphere. When the soil moisture level was increased, a decrease in the maximum DMSe emission rate occurred (Fig. 5). At a soil moisture content of 30% the $WHC_{max}$ (6% by weight) the maximum DMSe emission rate was 203 mg h$^{-1}$, while at 50 and 70% of the $WHC_{max}$ (10 and 14% moisture by weight, respectively) the maximum emission rate was only 26.4 and 12.1 mg h$^{-1}$, respectively. In addition, the time to reach the maximum emission rate was delayed by increases in soil moisture; at 30, 50, and 70% of the $WHC_{max}$ the maximum flux of DMSe occurred at 0.9, 1.6, and 9.5 h, respectively. Despite DMSe's high vapor pressure, the ability of water to dissolve large amounts of DMSe may account for these differences. Zieve and Peterson (1985) found that a loam soil from London (UK) (at about 14% moisture by weight) sorbed 2.1 times more DMSe than the same soil when air-dried. The importance of soil moisture content on Se volatilization has been reported by other workers. In soil column (4-cm i.d. × 4.5-cm length; bulk density = 1.33) experiments conducted by Zhang and Frankenberger (1999), 96% of the total injected DMSe from an air-dried soil was emitted to the air, whereas only 14% was emitted from a water-saturated soil. Although increases in the soil moisture content delayed and reduced the maximum flux rate of DMSe in this study, the cumulative loss of DMSe was similar between the moisture treatments (data not shown). At 30 and 50% of the $WHC_{max}$, 56% of the applied DMSe, and at 70% of the $WHC_{max}$, 51% of the applied DMSe was emitted after 45 h. The average DMSe emission rates were 8.8, 8.7, and 8.1 mg h$^{-1}$, respectively. Since moisture content within the $WHC_{max}$ range of 30 to 70% had no effect upon the rate of DMSe degradation, the slight difference in cumulative emissions between the 70% and 30 and 50% treatments can probably be explained by increased DMSe absorption. The soil moisture levels used in this study were chosen because they fall within

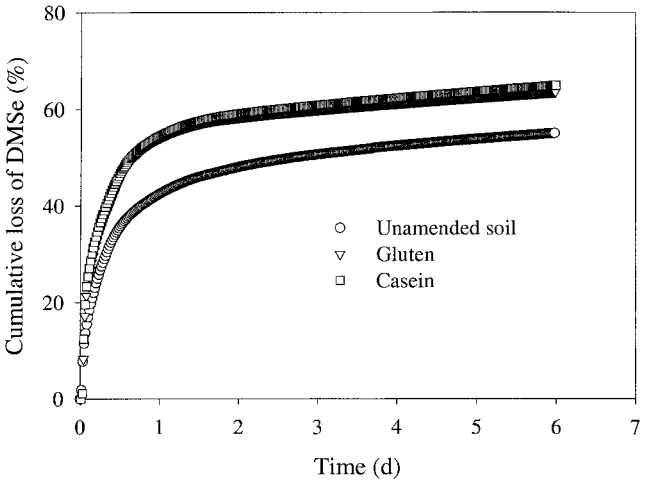


**Fig. 6. Effect of organic amendments on dimethylselenide emissions from packed soil columns. The top 5 cm of soil in column was amended with 1% (dry wt. basis) of either gluten or casein. The soil columns were packed to a final bulk density of 1.65 g cm$^{-3}$ with Arlington sandy loam and incubated at room temperature. Soil moisture content = 30% maximum water holding capacity ($WHC_{max}$).**

moisture optimum ranges (i.e., 18 to 70% of the $WHC_{max}$) that permitted maximal Se biomethylation and volatilization (Abu-Erreish et al., 1968; Hamdy and Gissel-Nielsen, 1976; Zieve and Peterson, 1981; Frankenberger and Karlson, 1989). Based on our results and those obtained by other workers, it appears that soil moisture levels below saturation would be most effective in a remediation strategy since they are known to promote Se biomethylation and allow for increased DMSe transport and volatilization.

As shown in Fig. 3, the addition of either casein or gluten to soil increased the persistence of DMSe. When casein or gluten was mixed into the top 5 cm of soil in the columns, 9.9 and 8.5% more DMSe was released through volatilization, respectively (Fig. 6). This evidence further supports the premise that the DMSe degrading microorganisms preferentially degrade the added casein and gluten, which then allows more DMSe to be released through volatilization. Therefore, the addition of casein or gluten to Se-contaminated soils or sediments serves a two-fold purpose: (i) stimulating the biomethylation of Se and (ii) enhancing Se volatilization by virtue of reduced DMSe degradation. Although it is clear that organic amendments do enhance the transport of DMSe in soil, the rate-limiting step governing Se volatilization is the biomethylation of Se. The biomethylation of Se is not only controlled by factors such as available organic C and soil moisture, but most importantly by the form of Se present. Selenate and selenite are the forms most readily available for microbial uptake and subsequent volatilization. Therefore, conditions that maintain the soluble pool of Se while allowing for maximum Se biomethylation and DMSe transport will increase the usefulness of Se volatilization as a remediation technique.

## CONCLUSIONS

The results from this study demonstrate that DMSe transport and emissions are affected by soil depth, organic amendments, and degradation by microorganisms. The effect of soil depth on volatilization is suspected to be a result of the increased contact time with soil, which then allows more time for biological degradation to occur. Changes in soil moisture content below saturation had no substantial effect on the degradation and cumulative loss of DMSe. Organic amendments, such as casein and gluten, which are known to stimulate the biomethylation of Se, were found to inhibit the degradation of DMSe. Microorganisms appear to utilize the added C sources more readily than DMSe, which increases the persistence of DMSe in soil. The increased persistence then permits more DMSe to be released through volatilization. Our results further support the usefulness of increasing soil porosity through tillage and application of organic amendments, not only as a means to stimulate Se biomethylation, but also to increase the transport of DMSe in soil and its subsequent volatilization to the atmosphere.

### ACKNOWLEDGMENT

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