which increases in concentration progressively is supported by the bromine profile of the Zechstein II. The bromine:chlorine ratio of halite increases smoothly upward in the section, in marked contrast to its erratic behavior reported from other deposits (*11*).

The new predicted evaporation path for seawater has removed mineralogical discrepancies with observed sequences including glauberite, polyhalite, and kainite. We offer no insight into the presence of sylvite, which often is considered to be secondary after carnallite (*12, 13*). The new hydrologic model can account for the observed anhydrite-halite mass relationships without the need to assume reflux or sulfate enrichment. By comparing the mineral sequences observed in a particular evaporite deposit with the predictions obtained in the more complete system, it may be possible to evaluate the contributions of stable equilibrium, metastable equilibrium, and fractional crystallization processes.

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Selenium Biomethylation Products from Soil and Sewage Sludge

Abstract. *Inorganic selenium compounds are converted to volatile methylated species (dimethyl selenide, dimethyl diselenide, and dimethyl selenone or methyl methylselenite) by microorganisms in sewage sludge and soil. In the absence of added selenium, no volatile selenium compounds were detected. All samples were evaluated without the addition of nutrients and in the presence of air or nitrogen. The methylation process may be an important step in the detoxification process for microorganisms exposed to high concentrations of selenium.*

In trace amounts selenium is essential for human health, but at higher concentrations it can be harmful (*1*). Concentrations of atmospheric selenium in remote areas of the earth are far in excess of predictions from anthropogenic or known natural sources (*2*). Up to 50 percent of the atmospheric selenium passes through filters capable of collecting 99 percent of the particles with diameters in excess of 0.1 μm (*3*). This vapor-phase, or very fine particle selenium, could be inorganic forms such as Se or SeO_2, which have relatively high vapor pressures, or organic selenium.

A potentially important source of atmospheric selenium is natural biomethylation. Selenium is known to be biomethylated, producing organic metabolites that are more volatile than the original inorganic forms (*4*). Other metals such as lead (*5*), arsenic (*6*), tin (*7*), and mercury (*8*) can also be biomethylated, forming organic compounds that are usually more toxic than the inorganic forms. Selenium is of interest as a potential environmental toxicant because of the small margin between the necessary nutritional levels and human toxicity (*9*).

Rats, plants, fungi, bacteria, molds, and microorganisms can produce methylated forms of selenium when exposed to inorganic or certain organic forms. Rats fed selenate or selenite exhale dimethyl selenide, $(CH_3)_2Se$ (*10*). Nonaccumulator plants, such as cabbage, give off $(CH_3)_2Se$, and accumulator species, such as *Astragalus racemosus*, produce dimethyl diselenide, $(CH_3)_2Se_2$, when exposed to selenite (*11*). Eleven microorganisms have been isolated from the soil that are capable of producing $(CH_3)_2Se$ (*12*). A strain of *Penicillium* that produced $(CH_3)_2Se$ from inorganic selenium compounds was isolated from raw sewage (*13*), and in the presence of air soils containing added inorganic selenium and glucose evolve $(CH_3)_2Se$ (*14*). Recently, $(CH_3)_2Se$ and an unknown volatile selenium compound were detected in an aqueous extract of biologically active lake sediment with and without added inorganic or organic selenium compounds (*6*). Since selenium is highly susceptible to biomethylation, this reaction may be an important step in its transformation and ultimate transport in the environment.

Our investigation was carried out to ascertain the quantity and the volatile forms of selenium that could be released to the atmosphere from soil and sewage sludge by methylation when selenium in different oxidation states is added under aerobic and anaerobic conditions. Soil and sewage sludge media were chosen because their high biological activities render them a likely ecosystem for biomethylation.

The selenium reactor system consisted of a 500-ml flask fitted with a ground-glass stopper containing an inlet glass tube that extended to near the bottom of the flask and an outlet tube. The inlet tube was connected to a gas cylinder and the outlet to a Teflon tube (6 cm by 1 cm in outside diameter) containing 0.25 g of Spherocarb (60 to 80 mesh) used for the room temperature collection of the gaseous selenium metabolites. A stream of either high-purity nitrogen or air was passed through the sampling flask, which contained 100 g of sewage sludge or soil, to sweep the volatile metabolites present in the headspace into the Spherocarb trap. The Spherocarb quantitatively retains organic selenium forms so that they can be extracted for subsequent chemical analysis. The flow rate was 50 to 100 ml/min, sufficient to flush the system in 10 minutes. The sewage sludge or soil was first passed through a Nuclepore filter with 0.2-μm pores to ensure the removal of any particles released from the media by physical processes. In preliminary experiments with radioactive tracer, we found that less than 5 percent of the $(CH_3)_2Se$ was retained by the Nuclepore filters and the other parts of the sampling system. The Spherocarb was treated with 3 ml of methanol, which extracts the volatile selenium metabolites quantitatively. These metabolites were analyzed with a gas chromatograph–microwave plasma detector system which is specific for the identification of selenium compounds and is capable of detecting as little as 20 pg of $(CH_3)_2Se$ (*15*).

We confirmed $(CH_3)_2Se$ and $(CH_3)_2Se_2$ by gas chromatography–mass spectrom-

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etry, using known compounds for comparison. The identity of the third metabolite was not originally known but was suspected of being $(CH_3)_2SeO_2$. We confirmed the structure of dimethyl selenone or methyl methylselenite $[(CH_3)_2SeO_2]$ by comparing the mass spectra with the spectrum reported by Rebane (*16*). From the mass spectrum, it is impossible to determine which of the two possible molecular forms of $(CH_3)_2SeO_2$ is actually present.

We observed $(CH_3)_2Se$, $(CH_3)_2SeO_2$, and $(CH_3)_2Se_2$ in soil and sewage sludge samples inoculated with sodium selenite (Na_2SeO_3) or elemental selenium (Se^0) (Table 1). The sewage sludge and soil samples were incubated at 21°C and contained 6 and 1 percent water (by weight), respectively. In the absence of added selenium, no volatile selenium metabolites were observed in sewage sludge, which had natural selenium concentrations of 3.2 μg per gram of sewage (dry weight).

The quantities of selenium metabolites evolved from the two media during 30-day incubation periods are shown in Table 1. The initial rate of evolution was more rapid in the sewage sludge than in the soil, probably because a greater quantity of microorganisms and nutrients was present. In soil, the production of volatile forms of selenium occurred at a relatively uniform rate. All species showed a slight increase during the fourth day in the presence of air or nitrogen, but the fluctuations were not statistically significant. When elemental selenium was used, there was a marked reduction in the quantity of all selenium species produced.

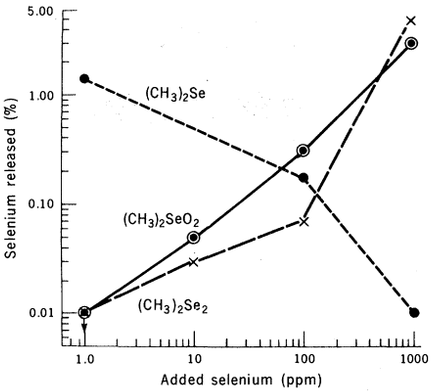

Fig. 1. Amounts of three selenium metabolites emitted from sewage sludge with respect to the amount of selenite added.

To verify that the methylation process was of a biological origin, we autoclaved sewage sludge samples spiked with sodium selenite and incubated them for 2 weeks. No volatile selenium species were detected ($\leq$ 1 ng).

Soil and sewage sludge were exposed to either air or nitrogen to ascertain which environment was more conducive to selenium biomethylation. In all cases, samples exposed to air produced larger quantities of each selenium species than corresponding samples exposed to nitrogen. Sewage sludge exposed to nitrogen methylated selenium at a slower rate (by a factor of approximately 80) than a similar sample exposed to air. Soil exposed to nitrogen also exhibited a decrease in the production of volatile selenium, but only by a factor of < 10.

One purpose of this study was to determine the effect of the oxidation state of the added selenium on the production rate of metabolites. Selenite and elemental selenium were chosen because of their differences in solubility (selenium is insoluble in water and selenite is soluble). Soil spiked with selenite produced eight times more selenium metabolites than similar soil samples spiked with elemental selenium. The total quantity of selenium species recovered ranged from 0.034 to 7.9 percent of that added as sodium selenite and from 0.0005 to 0.005 percent of that added as elemental selenium. Similarly, sewage sludge spiked with selenite produced about 100 times the quantity of organic selenium compounds as sewage spiked with elemental selenium. In all cases, by the end of 30 days, there were reddish deposits present in the media, an indication that some selenite had been reduced to elemental selenium. In both media, the reduction of selenite to elemental selenium by biological methods was confirmed by the absence of reddish deposits in the autoclaved media. At high selenite concentrations (1000 μg/g), this reddish color was evident within the first few days.

To determine the interrelationships of the metabolites, we studied the production of each selenium species with respect to added selenite. Sewage sludge

Table 1. Organic vapor-phase selenium evolved (in micrograms) in 30-day incubations.

Media conditions* and form of Se	Added Se	$(CH_3)_2Se$		$(CH_3)_2SeO_2$		$(CH_3)_2Se_2$		Total evolved (%)
	(μg/g)	(μg)†	(%)‡	(μg)†	(%)‡	(μg)†	(%)‡	
Soil-air (Na_2SeO_3)	1000	10.8	0.01	2.0	0.002	21.1	0.02	0.034
Soil-N_2 (Na_2SeO_3)	1000	5.8	0.006	5.4	0.006	12.9	0.0013	0.024
Sewage-air (Na_2SeO_3)	1000	10.6	0.01	3000	3.0	4000	4.9	7.9
Sewage-N_2 (Na_2SeO_3)	1000	20.0	0.02	58.4	0.06	8.2	0.008	0.086
Sewage-air (Na_2SeO_3)	100	16.5	0.17	31.2	0.31	7.1	0.07	0.55
Sewage-air (Na_2SeO_3)	10	5.4	0.54	0.52	0.05	0.29	0.03	0.62
Sewage-air (Na_2SeO_3)	1	1.3	1.3	< 0.001		< 0.001		1.3
Sewage-air (Se^0)	500	2.6	5.2×10^{-3}	< 0.001		< 0.001		5.2×10^{-3}
Sewage-N_2 (Se^0)	500	0.25	5×10^{-4}	< 0.001		< 0.001		5×10^{-4}
Soil-air (Se^0)	200	0.58	2.9×10^{-3}	0.25	1.3×10^{-3}	0.007	4×10^{-5}	4.2×10^{-3}
Soil-N_2 (Se^0)	200	0.39	2×10^{-3}	0.20	1×10^{-3}	0.004	2×10^{-6}	3×10^{-3}

*Wet sludge or soil. †Micrograms of methylated species released. ‡Percentage of selenium added to the sample that was released as methylated species.

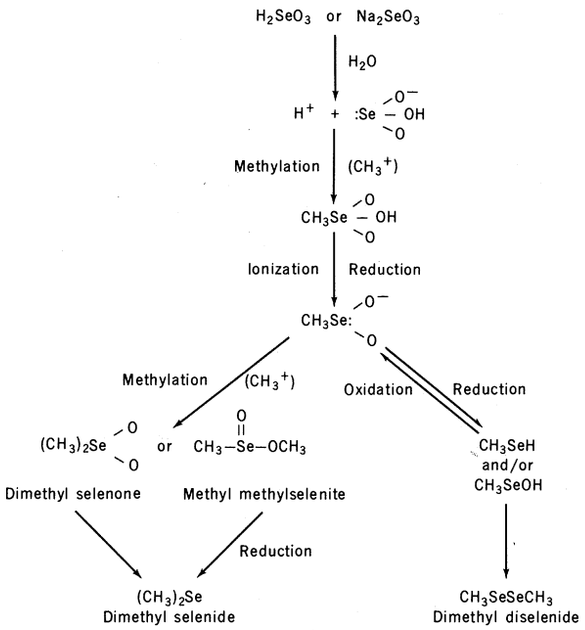

Fig. 2. Proposed mechanism for the methylation of selenium.

was chosen as the medium because of its high rate of production (Table 1 and Fig. 1). With an initial selenite concentration of 1000 μg/g, the dominant species were $(CH_3)_2SeO_2$ and $(CH_3)_2Se_2$, which accounted for the release of 3.0 and 4.9 percent, respectively, of the added selenium. The microorganisms may in some cases release these organoselenium species to detoxify themselves. At lower selenite concentrations, the relative quantity of $(CH_3)_2Se$ increased whereas the quantities of $(CH_3)_2Se_2$ and $(CH_3)_2SeO_2$ decreased markedly. The relative abundances of $(CH_3)_2SeO_2$ and $(CH_3)_2Se_2$ produced depend directly and that of $(CH_3)_2Se$ produced depends indirectly upon the concentration of added selenite. It appears that the higher concentration of selenium inhibited certain organisms and therefore forced the decrease in the production of $(CH_3)_2Se$, whereas organisms that formed $(CH_3)_2SeO_2$ and $(CH_3)_2Se_2$ were unaffected.

The results show that the biological methylation of selenium occurs rapidly in soil and sewage sludge and has the potential to occur in the environment if selenium is present in a soluble state. In the absence of added selenium, the sewage sludge did not evolve volatile selenium species, presumably because of the low solubility of the selenium or the lack of available selenium. Thus, the evolution of organic selenium must require either that the selenium be in chemical form which can be utilized by the microorganisms responsible for biomethylation or that the selenium be at a high enough concentration that the organisms may use biomethylation as a detoxification step. Our inability to detect natural selenium metabolites from the sewage sludge may have been due to a methylation rate that was too slow to produce detectable amounts of selenium metabolites.

The identification of $(CH_3)_2SeO_2$ as a volatile metabolite gives some credence to the methylation mechanism proposed by Challenger (*17*). He proposed the formation of $(CH_3)_2SeO_2$ as the final intermediate prior to its reduction to $(CH_3)_2Se$. The proposed mechanism is shown in Fig. 2, where Challenger's pathway to $(CH_3)_2Se$ is given as the left branch. The unknown species referred to by Chau *et al.* (*4*) in their work with lake sediments is probably $(CH_3)_2SeO_2$. For the gas chromatographic separation they used the same type of column as used in this work, and their unknown peak corresponds to that of $(CH_3)_2SeO_2$. These and other studies have shown that microbial methylation of selenium is a widespread occurrence in various media and under different conditions. Thus, biomethylation could be a pathway for the mobilization of selenium to the atmosphere.

With the data presented here, we cannot verify or dispute Challenger's methylation mechanism, although his mechanism includes no steps for the formation of $(CH_3)_2Se_2$, which was observed. The mechanism could be modified to include a concentration-dependent branch to produce $(CH_3)_2Se_2$. This could occur at the CH_3SeO_2 intermediate, where reduction could form either CH_3SeOH or CH_3SeH, which would rapidly produce $(CH_3)_2Se_2$. This type of mechanism is similar to that of the corresponding sulfur species. From our data it appears that the right-hand pathway in Fig. 2 may be active as a detoxification mechanism for microorganisms present in the sludge. When the selenium concentration is sufficiently high, this mechanism proceeds rapidly to reduce the $(CH_3)_2Se_2$ and the left-hand mechanism is abbreviated at the $(CH_3)_2SeO_2$ step, where this compound is expelled. It is probably energetically easier for the organism to expel the $(CH_3)_2SeO_2$ than to reduce it to $(CH_3)_2Se$. A second and probably more plausible possibility is that different microorganisms are responsible for the formation of each species and that the microorganisms responsible for the production of $(CH_3)_2Se$ are hindered at high selenium concentrations, whereas the microorganisms producing $(CH_3)_2Se_2$ are more selenium-tolerant.

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