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Mineralization of Selenium-Containing Amino Acids in Two California Soils

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ABSTRACT

Organic forms of Se as the selenoamino acids, selenomethionine (SeMet) and selenocystine (SeCys), are detected in plants grown on seleniferous soil, yet little is known about the speciation and distribution of Se upon decomposition of SeMet and SeCys in soil. To determine the mineralization rate of selenoamino acids in soil, three concentrations of SeMet, SeCys, methionine (Met), or cystine (Cys) were added to samples of a Panoche (fine-loamy, mixed [calcareous], thermic Typic Torriorthent) or a Panhill (fine-silty, mixed, thermic Typic Haplargid) soil and aerobically incubated at 22°C for up to 168 h. The amino acid concentrations were analyzed by high performance anion chromatography. Methionine additions closely followed first-order reaction kinetics and SeMet additions followed pseudo-first-order kinetics, with order dependent on SeMet concentration. The time required for 50% mineralization of SeMet additions in the Panhill and Panoche soils was 23.5 and 3.2 h (5 mg Se kg^{-1}), 41.6 and 15.5 h (25 mg Se kg^{-1}), and 47.1 and 36.0 h (50 mg Se kg^{-1}), respectively. The majority of the SeMet and Met additions were recovered as volatile species (50–80%). In contrast to SeMet, SeCys was rapidly nonextractable (<6 h) from both soils, with little to no volatile Se detected and was initially recovered as phosphate-soluble selenite and selenide after 6 h of incubation. These results suggest that Se present in seleniferous plant tissue as SeMet will not accumulate in soil due to extensive volatilization. In contrast, additions of seleniferous plant residues rich in SeCys will result in organic Se mineralization to inorganic Se forms in soil.

CARBON-BONDED S, a major S component of photosynthetic leaves, may represent an important source of SO_4 for terrestrial systems (Likens and Bormann, 1974). The sulfur-containing amino acids, Met and Cys, constitute a large portion of the plant C-bonded S and may account for 5 to 35% of the S present in soil (Freney et al., 1970). Fitzgerald et al. (1988) reported that both Met and Cys were mineralized to SO_4 when added to forest soils with greater Met incorporation into biomass and soil organic matter than Cys additions. Methionine metabolism can lead to formation of volatile S compounds such as dimethyl sulfide (DMS). This volatilization may account for >90% of the natural S emissions from marine regions and about 50% of the global biogenic S entering the atmosphere (Andreae, 1990). One intermediate in the formation of DMS from Met that has received much interest is dimethylsulfoniopropionate (DMSP) (Challenger and Simpson, 1948; White, 1982; Talyor and Gilchrist, 1991). The formation of DMSP from Met has been documented in marine environments (Talyor and Gilchrist, 1991), but no reports have been published on the occurrence of DMSP in terrestrial soils. In contrast to our knowledge of the transformations of S amino acids, the mobility and mineralization of SeMet and SeCys in soil has been a neglected research topic (Haygarth, 1994)

The importance of C-bonded Se in the environment is not well known. Plants have been reported to assimilate soluble inorganic Se into analogs of the S amino acids from seleniferous soils (Gupta et al., 1993). Abrams and Burau (1989) and Rael and Frankenberger (1995) have presented unequivocal mass spectrum data proving that SeMet is present in seleniferous soils and sediments. The origin of this selenoamino acid in the soil materials was not identified as either plant or microbial in nature nor were concentrations provided. Doran (1982) suggested that microbial metabolism of SeMet paralleled the metabolism of the S analog. Doran and Alexander (1977), however, reported that four isolated Met-utilizing soil bacteria were unable to utilize SeMet as a sole C source, and SeMet (10^{-4} *M*) also inhibited growth of organisms in a Met-supplemented growth medium. Frankenberger and Karlson (1989) reported that addition of different SeMet concentrations to soil resulted in rapid and extensive volatilization of Se as dimethylselenide (DMSe) with very limited DMSe production from SeCys additions to soils. They suggested that stimulation of Se volatilization from seleniferous soils upon SeMet addition results from the donation of a methyl group from SeMet during the methylation of inorganic Se. Information was not presented concerning the fate of the nonvolatilized Se from soil SeMet or SeCys additions and no additional literature has been located pertaining to the mineralization rates or fate of selenide (Se^{-II}) present as Se amino acids in soils.

In contrast to our knowledge of the importance of S volatilization in natural systems, little is known about the pathway of Se volatilization from soils (Gao and Tanji, 1995). Selenium is known to be highly enriched (1000- to 6000-fold) in atmospheric aerosols relative to its crustal abundance (Duce et al., 1975), suggesting that Se volatilization pathways may be important in

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Abbreviations: Cys, cystine; DMDS, dimethyl disulfide; DMDSe, dimethyl diselenide; DMS, dimethyl sulfide; DMSe, dimethyl selenide; $DMSe^+$-R, dimethylselenonium ion; DMSeP, dimethylselenopropionate; DMSP, dimethylsulfoniopropionate; HGAAS, hydride generation atomic absorption spectrophotometry; HPIC, high performance ion-exchange chromatography; *k*, reaction-rate constant; Met, methionine; PED, pulsed electrochemical detector; SeMet, selenomethionine; SeCys, selenocystine; Se-methyl-SeMet, Se-methyl selenomethionine; S-methyl-Met, S-methyl methionine.

Se cycling. Cooke and Bruland (1987) found dissolved DMSe, dimethyl diselenide (DMDSe), and a nonvolatile dimethylselenonium ion ($DMSe^+$–R) in surface waters of seleniferous sites in California. The $DMSe^+$–R compound was suggested to be Se–methyl selenomethionine (Se–methyl-SeMet). Cooke and Bruland (1987) postulated that Se was volatilized in a pathway similar to the pathway proposed for S compounds by Andreae and Barnard (1984).

This work was conducted to determine the mineralization rate of Se amino acids introduced into a soil environment and speciation of the derived products under aerobic conditions. These results are compared with the mineralization rates and fate of the S amino acid analogs.

MATERIALS AND METHODS

Materials

Soil samples (0–15-cm depth) taken from the Panoche and the Panhill soils used in this study had the following chemical characteristics: pH, 8.06 and 7.98; 5.6 and 5.8 g organic C kg^{-1} soil; 1.19 and 1.12 g total N kg^{-1} soil; and 0.1 and 0.3 mg total Se kg^{-1} soil, respectively. Particle-size range was determined in the Panoche and Panhill soils as 320 and 460 g sand kg^{-1} soil and 360 and 320 g clay kg^{-1} soil, respectively. Soil pH was determined on a 2.5:1 water to soil ratio, total C content was determined by dry combustion with a Coulometric C analyzer (UIC, Inc., Joliet, IL)[1], total N content by the method described by Bremner and Mulvaney (1982), and texture by the hydrometer method of Gee and Bauder (1986). The soils were collected from the Panoche Fan in the west-central San Joaquin Valley, California. The elevated Se levels, compared with nearby nonfan soils, suggest that soil microbial populations may have been previously exposed to seleniferous plant residues. The soils were screened to pass a 2-mm mesh and stored in collected air-dried state.

The amino acids standards were purchased from Sigma Chemical Co. (St. Louis, MO). The remaining chemicals (ACS reagent grade) used were purchased from Baker (J.T. Baker, Inc., Buffalo Grove, IL).

Instruments

Hydride generation atomic absorption measurements were made with a Perkin Elmer 3030B spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) equipped with a Varian Model VGA-76 (Varian Associates, Mulgrave, Victoria, Australia) vapor generation apparatus for Se analysis. A Se electrodeless discharge lamp (Perkin-Elmer) operated at 6 W was used as the radiation source. The operational conditions were as follows: acetylene flow, 2.4 L min^{-1}; air flow, 6.0 L min^{-1}; purge gas flow, Ar, 90 mL min^{-1}; sample flow, 1.0 mL min^{-1}; 6 *M* HCl flow, 1.0 mL min^{-1}; reagent flow, 0.6% $NaBH_4$–0.5% NaOH, 0.33 mL min^{-1}; wavelength, 196 nm; and slit width, 2.0 nm. Quality assurance procedures for Se analysis by HGAAS were employed as follows. Duplicate samples were analyzed with calibration, reagent blanks, and NIST Se standard reference material 3149 (National Institute of Standards and Technology, Gaithersburg, MD) spikes (20 ng Se mL^{-1}) to check for interferences at the beginning and end of each HGAAS run. Acceptable data quality objectives were as follows: spikes, 90–103% recovery; precision, 10%; detection limit, 0.2 mg kg^{-1}.

The soil amino acid concentrations were determined by modification of the procedure described by Martens and Frankenberger (1992). Briefly, the extracted amino acids, Met and SeMet, were isocratically eluted with a Dionex (Dionex Corp., Sunnyvale, CA) gradient pump (150-μL injection loop) by high performance ion-exchange chromatography (HPIC) on an AminoPac PA1 column (50 m*M* NaOH with 15 m*M* $NaBO_4$) and detected with a Dionex pulsed electrochemical detector (PED) equipped with a gold electrode using the following times and potentials in the integrated voltametry mode (1-μC output range). At time (t_s) = 0.00, the potential (E_v) = 0.25, at t_s = 0.20, E_v = 0.25; at t_s = 0.30, E_v = 0.65; at t_s = 0.40, E_v = 0.65; at t_s = 0.50, E_v = 0.25; at t_s = 0.70, E_v = 0.25; at t_s = 0.71, E_v = 1.20; at t_s = 0.90, E_v = 1.20; at t_s = 0.91, E_v = −0.60; at t_s 1.0, E_v = −0.60, with the integration period from 0.2 to 0.70 s. Peak areas and retention times were determined with a Hewlett-Packard 3396 recording integrator (Hewlett-Packard Co., Fullerton, CA). The PED did not require an alkaline postcolumn addition when operated in the integrated voltametry mode. The amino acids Cys and SeCys were isocratically eluted (50 m*M* NaOH, 15 m*M* $NaBO_4$ and 250 m*M* NaOAc) on the AminoPac PA1 column with detection by PED. Our preliminary experiments have shown that coelution of Met with SeMet, SeCys with Cys, or coelution with other amino acids was not a problem with this methodology.

Soil headspace gas composition was analyzed on a Varian 3700 gas chromatograph equipped with a DB-5 capillary column (J.W. Scientific, Folsom, CA) with a 0.25-mm inner diameter, a 0.25-μm film thickness, and a 30-m length. The operational conditions were as follows: flame ionization detector, 115°C; column temperature, 60°C; injector, 80°C; carrier gas (He), 1.0 mL min^{-1}; make-up gas 30 mL min^{-1}; H_2, 30 mL min^{-1}; air, 300 mL min^{-1}. Peak areas and retention times were determined with a Hewlett–Packard 3396 recording integrator. A standard solution of DMSe was obtained from Strem Chemical Co. (Newburyport, MA), DMDSe and DMS solutions were obtained from Aldrich Chemical Co. (St. Louis, MO), and dimethyldisulfide (DMDS) solution was obtained from Sigma Chemical Co.

To investigate the influence of biological decomposition of the S- and Se-containing amino acids in soil, we compared mineralization rates on sterilized and nonsterilized soils. Sterilized soils were obtained by autoclaving samples of the two soils for 2 h at 121°C and 103 kPa on consecutive days. Sterile soil was then placed in previously sterilized centrifuge tubes and treated with filter-sterilized solutions of amino acids and incubated as outlined below.

Procedures

Amino Acid Mineralization

The mineralization rates of S and Se amino acids were determined by addition of 5 g (air-dry basis) of Panoche or Panhill series soil to 40-mL Teflon centrifuge tubes with additions of water (−0.034 MPa moisture potential) with or without 5, 25, or 50 mg S or Se kg^{-1} soil as Met, SeMet, Cys; or 5, 10, or 25 mg Se kg^{-1} soil as SeCys. The lower SeCys application rates were due to the limited water solubility of SeCys. The tubes containing the treated soils were connected to an apparatus train for collection of CO_2 and volatilized S and Se gases (Fig. 1) and aerobically incubated at ambient temperatures (22 ± 2°C) for various times up to 168 h. Compressed

[1] Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product listed by the USDA.

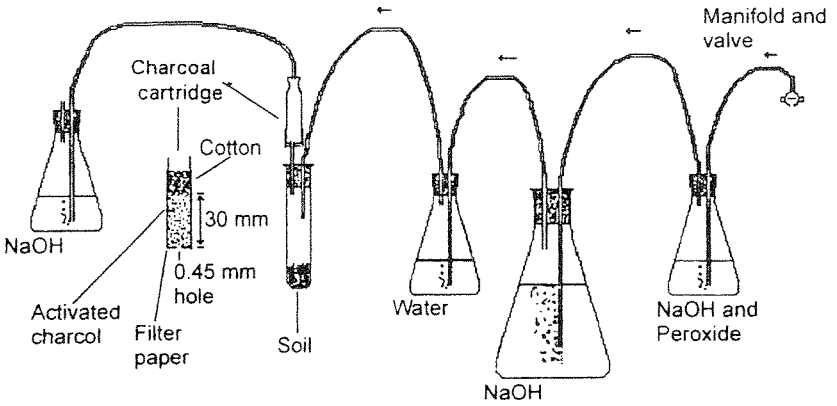

Fig. 1. Apparatus used to capture gases evolved during aerobic incubation of amino acids in soils. The arrows indicate airflow direction.

air was first passed through a 6% H_2O_2 (pH 9.0) solution, then a 0.5 *M* NaOH solution, and finally deionized water to humidify the air (Fig. 1). A sample flow rate of 10 mL min^{-1} was maintained at each 40-mL tube in the experiment via individual needle valves. The 10 mL min^{-1} flow rate was chosen to effectively aerate the 40-mL tubes for collection of CO_2 and volatile Se and S gases. The CO_2 evolution was determined by back titration (0.25 *M* HCl) of a 10 mL 0.5 *M* NaOH trap treated with excess $BaCl_2$ (Hassink, 1994). Exposed rubber surfaces were treated with Fluoroglide Teflon spray (Aldrich Chemical Co.) to limit surface exposure of evolved gases. The evolved Se gases were trapped on a charcoal cartridge. The charcoal cartridge was then emptied into a 40-mL centrifuge tube and the Se present extracted with 10 mL of a H_2O_2–HNO_3 solution (80 mL 30% H_2O_2 L^{-1}; 58.8 mL 17 *M* HNO_3 L^{-1}) incubated at room temperature for 1 h. The samples were then centrifuged (10 000 *g*, 20 min) and the supernatant removed for analysis by 6 *M* HCl reduction and HGAAS. The S gases were eluted from the cartridge with methanol, digested with 5 mL of H_2O_2 (30%) for 6 h (80°C) and analyzed for SO_4 by ion chromatography with suppressed conductivity detection (Dionex Corp.). The tubes were arranged in a completely randomized design with two replications and the mineralization experiments were conducted a minimum of two times and a maximum of four times.

At the specified times, duplicate soil samples were removed from the apparatus, treated with 10 μL of threonine (2.5 g L^{-1}) as an internal standard, extracted with 25 mL (pH 7.0) of 0.1 *M* KH_2PO_4–K_2HPO_4 (P-buffer) and shaken for 1 h on a horizontal shaker (120 oscillations min^{-1}). The sample was then centrifuged for 20 min at 10 000 *g* and the supernatant decanted. A sample aliquot was then filtered (0.1 μm) and diluted for amino acid analysis. To confirm the Se content of the detected amino acids, elution of the suspected Se amino acid (confirmed by co-chromatography with pure standards) was captured with a fraction collector (ISCO, Inc., Lincoln, NE), and the fractions were digested with 2 mL of 0.1 *M* $K_2S_2O_8$, reduced by 6 *M* HCl, and analyzed for Se by HGAAS.

Selenium Extraction and Speciation

To determine the Se speciation after mineralization of the Se amino acids, the seleniferous soils were further extracted as described by the Se speciation protocol of Martens and Suarez (1997). Briefly, the soils were treated with 0.1 *M* $K_2S_2O_8$ (90°C) for 2 h after the P-buffer extraction, centrifuged for 20 min at 10 000 *g*, and the supernatant collected for analysis. In addition, the P-buffer and persulfate extracted samples were treated with 2.5 mL of 17 *M* HNO_3 for 0.5 h (90°C) then diluted with 20 mL of water and heated for an additional 1.5 h, centrifuged (20 min; 10 000 *g*) and the supernatant collected for analysis. The P-buffer, $K_2S_2O_8$, and HNO_3 extractions released soluble Se^{+VI}, Se^{-II}, and ligand-exchangeable Se^{+IV}, tightly held Se^{+IV} and Se^{-II} and insoluble Se^0 fractions, respectively (Martens and Suarez, 1997). The Se concentrations and speciation present in the three extractions were determined by a HGAAS methodology described by Martens and Suarez (1997).

Caution: Timing of the HCl reduction step for Se^{+VI} analysis is critical. The reduction of Se^{+VI} with 6 *M* HCl will oxidize Se^{-II} present in the sample to Se^{+IV}, resulting in elevated Se^{+VI} results. Our research determined that a 6 *M* HCl reduction (90°C) for 20 to 30 min limited Se^{-II} oxidation to Se^{+IV} yet still allowed for quantitative reduction of Se^{+VI}. A 1-h reduction time under these conditions converted 98 to 100% of the added Se^{-II} to Se^{+IV}.

Sulfur and Selenium Volatilization

The detection of volatile S and Se gases during soil incubation of the S and Se amino acids was performed as follows. Samples of the Panoche or Panhill soil (5 g) were placed in a 125-mL Erlenmeyer flask treated with the specified level of the S or Se amino acid (−0.034 MPa moisture potential), equipped with a Mininert gas sampling valve (Aldrich Chemical Co.), and incubated statically at ambient temperatures. At the specified time, air samples were removed from the soil headspace with a 1-mL gas-tight Series 2 Pressure-Lok gas syringe (Alltech Associates, Deerfield, IL) and injected onto the gas chromatograph.

The detection method described by White (1982) was modified as follows to determine if the reported S volatilization intermediates, DMSP or S-methyl methionine (S–methyl-Met), were produced in soil during Met metabolism. A 1-mL aliquot of the P-buffer extract from the Met-treated soils was added to a 10-mL screw-top vial, treated with 1 mL of 2 *M* NaOH, immediately equipped with a Mininert gas sampling valve, incubated for 2 h at ambient temperatures, and the headspace sampled for the appearance of DMS. The same sample, after ambient temperature sampling, was then heated for 2 h at 100°C and the headspace again sampled for S gas production. The ambient DMS (DMSP) formed was subtracted from the heated DMS (S–methyl-Met plus DMSP) concentration to determine the concentrations of S–methyl-Met and DMSP, respectively. White (1982) reported that DMSP and S–methyl-Met are the only S compounds known to react with NaOH under the conditions outlined to yield DMS. The alkaline hydrolysis reactions must be conducted immediately upon extraction from the soil. The release of DMS from the Panoche extracts upon NaOH treatment de-

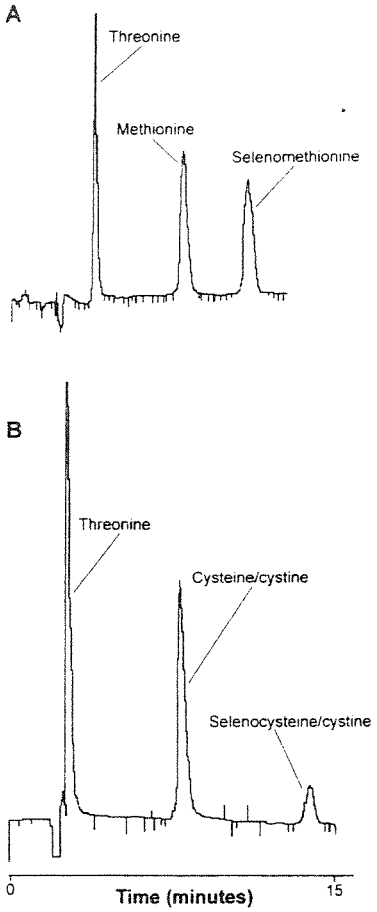

Fig. 2. Chromatographic traces of (A) 2.5 mg threonine L^{-1} and 5.0 mg Met and SeMet L^{-1} (eluant, 50 m*M* NaOH; 15 m*M* $NaBO_4$) and (B) 2.5 mg threonine L^{-1} and 5.0 mg Cys and SeCys L^{-1} (eluant, 50 m*M* NaOH; 15 m*M* $NaBO_4$; 250 m*M* NaOAc).

creased to zero after storage of the extracts at −20°C (or 4°C) for more than 2 d.

RESULTS AND DISCUSSION

Investigations of the fate of Se amino acids in soil has been hindered by a lack of suitable methodology (Banuelos et al., 1993). Reverse-phase chromatography of derivatized or nonderivatized amino acids has led to coelution problems, since the retention properties of S- and Se-containing amino acids are similar to other common amino acids (Tschursin and Wolf, 1993; Tschursin et al., 1994). Martens and Frankenberger (1992) reported that anion exchange separation with pulsed amperometric detection was a rapid and sensitive technique for quantification of amino acids from complex matrices. Figure 2 shows HPIC chromatographic traces of standards for Met and SeMet (Fig. 2a) and for Cys and SeCys (Fig. 2b). The alkaline eluant employed with this methodology converted the thiol groups in cysteine to the disulfide bond (cystine). The same reaction occurred for the Se amino acid analogs, resulting in one peak for each amino acid pair.

Methionine Mineralization

Sulfur recovery of the 50 mg Met–S kg^{-1} soil addition to the Panhill and Panoche soils incubated at 22°C for

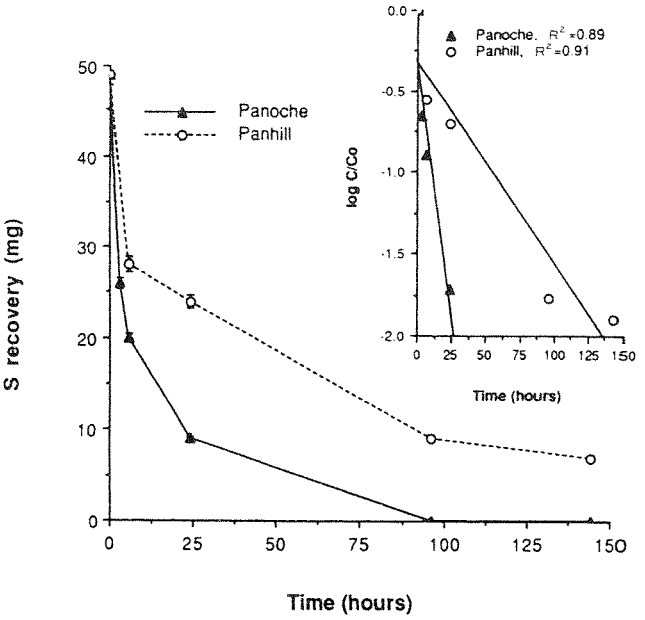

Fig. 3. Sulfur recovery (mean ± standard deviation) from the Panhill and Panoche soils amended with 50 mg Met–S kg^{-1} soil.

up to 168 h is shown in Fig. 3. Plots of the logarithm of the remaining amino acid as a function of time resulted in an approximation of a linear relationship ($R^2 > 0.99$) for the different Met additions and similar reaction-rate constant (k) values suggested that the disappearance of Met from the two soils followed first-order kinetics. The rapid decrease in the Met concentrations determined during the initial sampling times suggested that rapid Met adsorption and/or microbial assimilation was/were the dominant process(es) occurring in these soils (Fig. 3). The 5 and 25 mg Met–S kg^{-1} soil addition recovery rates and k values were similar to the reaction of the 50-mg addition rate (data not presented). Analysis of the data showed that SO_4 did not accumulate in the soils with mineralization of added Met nor were CO_2 evolution rates significantly different in the Met-treated soils compared with the control soils (no Met addition) until after free Met was not detected in the treated soil. Hadas et al. (1992) reported that ^{14}C-alanine additions to soil resulted in rapid assimilation, with $^{14}CO_2$ evolution proceeding at a rapid rate only after alanine was removed from solution. Ion chromatographic analysis of the charcoal traps indicated that up to 80% of the Met–S added to these soils was volatilized as DMS and/or DMDS. The results suggested that the majority of the added Met–S was volatilized from these soils under aerobic conditions, with the remaining S being incorporated into microbial biomass.

Selenomethionine Mineralization

Selenium recovery from additions of SeMet to the treated Panhill and Panoche soils and the distribution of the Se oxidation states resulting from mineralization of the selenoamino acid is shown in Fig. 4.

The SeMet additions to the Panoche soil were mineralized faster than SeMet additions to the Panhill soil. The SeMet additions did not show rapid sorption or direct microbial assimilation in the two soils as was

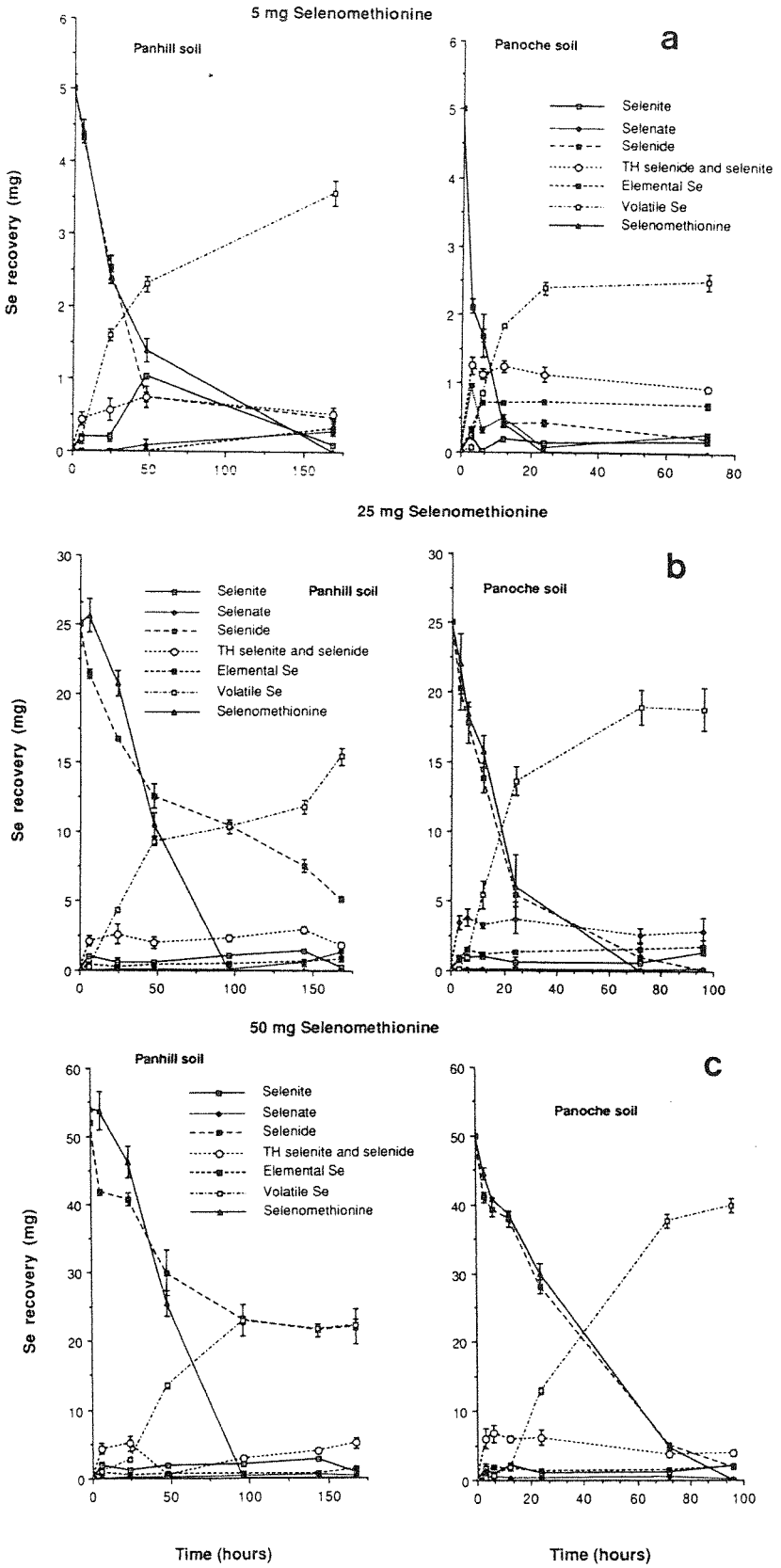

Fig. 4. Selenium recovery (mean ± standard deviation) from the Panhill soil and the Panoche soil amended with (a) 5 mg SeMet–Se kg^{-1} soil, (b) 25 mg SeMet–Se kg^{-1} soil, (c) 50 mg SeMet–Se kg^{-1} soil.

noted for the Met additions. The time required for 50% mineralization of the SeMet additions was calculated as 3.2 and 24.5 h (5 mg), 15.5 and 41.6 h (25 mg), and 36.0 and 47.1 h (50 mg) for the Panoche and Panhill soils, respectively. The SeMet additions followed pseudo-first-order decomposition kinetics, with order depen-

dent on SeMet concentrations. Both the Panoche and the Panhill showed apparent first-order kinetics at the 5-mg SeMet addition rate, and apparent zero-order kinetics at the higher SeMet additions of 25 and 50 mg. Figure 5 shows a time series of HPIC chromatographic traces detailing the disappearance of the 50 mg SeMet–Se kg^{-1} soil application rate in the Panoche soil at the 6-, 24-, and 72-h sampling periods. The Panhill traces were nearly identical to the Panoche traces except for the remaining SeMet concentrations (data not shown). The faster SeMet mineralization rates of the Panoche soil, compared with the Panhill soil, may be partially explained by the cultivation history of the two soils. The Panoche soil when sampled was under intensive vegetable cultivation in the Central San Joaquin Valley of California, but the nearby Panhill soil had not been subjected to recent cultivation. Monreal and McGill (1989b) found that Cys mineralization ($^{14}CO_2$ production) was greater in heavily cultivated soils than the respective noncultivated soils, and the differences were attributed to different C allocation patterns among microbially mediated processes.

The amounts of Se volatilized were different for the two soils tested. The Panhill soil evolved 70, 60, and 40% of the added SeMet at the 5, 25, and 50 mg SeMet–Se kg^{-1} soil addition rates, respectively. In contrast, the Panoche soil evolved 50, 76, and 80% of the added Se at the same rates of addition. The decrease in Se volatilized from the Panhill soil with increasing SeMet levels may be due to the accumulation of a P-buffer-soluble organic Se compound as determined by HGAAS analysis (Fig. 4b and 4c). This unidentified compound reached a maximum concentration of 21.5 mg kg^{-1} (43%) of the 50 mg SeMet kg^{-1} applied to the Panhill (Fig. 4c). Extensive chromatographic analysis determined that this accumulated organic Se compound was not SeMet or a Se derivative of homoserine. Cooke and Bruland (1987) proposed that homoserine would be an intermediate in the Se volatilization process from SeMet. Although not probable, we cannot dismiss the possibility that the failure to detect homoserine was due to the rapid mineralization of homoserine in these soils.

Figure 6 shows chromatographic traces of the different Se compounds volatilized from the Panhill (Fig. 6a) and the Panoche soil (Fig. 6b) when treated with 25 mg SeMet–Se kg^{-1} soil and incubated statically for up to 48 h. The traces clearly show that two mechanisms for Se volatilization are present in the different soils. The Panhill soil evolved predominately DMDSe during the

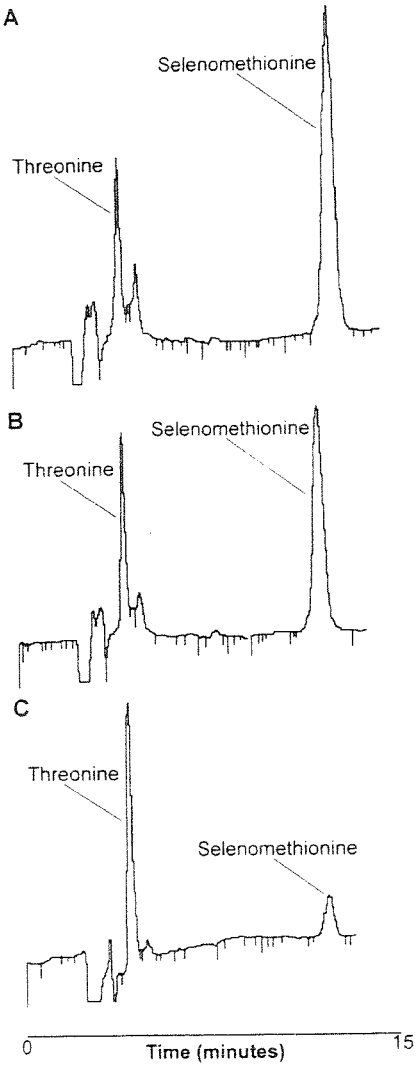

Fig. 5. Chromatographic traces of SeMet concentrations (50 mg SeMet–Se kg^{-1} soil) remaining in the Panoche soil after incubation for (A) 6 h, (B) 24 h, and (C) 72 h.

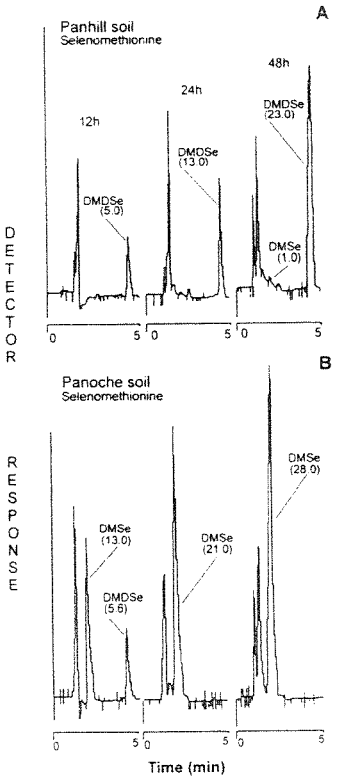

Fig. 6. Chromatographic traces of DMSe and DMDSe produced from 25 mg SeMet–Se kg^{-1} addition to the (A) Panhill soil and (B) Panoche soil statically incubated at ambient temperatures and sampled at the times specified. The value in parentheses indicates the concentration (in micrograms) of Se gas present in the headspace.

Table 1. Sodium hydroxide catalyzed formation of DMS or DMSe from methylation pathway intermediates of S and Se amino acid-treated soil extracts incubated at ambient (23°C) or 100°C temperatures for 2 h.†

Soil	Amino acid	0 h		24 h		48 h		72 h	
		23°C	100°C	23°C	100°C	23°C	100°C	23°C	100°C
		mg intermediate kg^{-1}							
Panoche	Met	ND	ND	1.80	1.22	1.10	2.45	ND	ND
	SeMet	ND	ND	2.61	1.62	3.31	1.97	1.62	1.14
Panhill	Met	ND	ND	ND	ND	ND	ND	ND	ND
	SeMet	ND	ND	ND	ND	ND	ND	ND	ND

† Phosphate buffer extracts (25 mg S or Se kg^{-1} soil) were treated with 1 mL 2 *M* NaOH in a 10 mL vial equipped with a Mininert gas sampling valve and incubated at ambient or 100°C for 2 h. The headspace was analyzed for DMS or DMSe. The data are presented as mg DMSP or DMSeP kg^{-1} soil determined at 23°C and mg S-methyl-Met and Se-methyl-SeMet kg^{-1} soil determined at 100°C. The values for mg S-methyl-Met and Se-methyl-SeMet were determined by subtraction of mg DMS or DMSe present in ambient temperature headspace (DMSP and DMSeP) from the total mg DMS and DMSe present in the heated samples. ND, not detected.

study. This trend was evident up to the 168-h sampling (data not shown), although the measured DMDSe concentrations began to decline in the headspace after the 48-h sampling. In contrast, the Panoche soil exhibited both DMSe and DMDSe evolution after 12 h, but subsequent headspace sampling showed only DMSe present. A reduction in the headspace concentration of DMSe was also noted after the 48 h sampling, suggesting that sorption of volatilized DMDSe and DMSe was occurring in the soils tested. Since S- and Se-containing amino acid additions to sterilized soils remained unchanged with no detectable volatilization with incubation for up to 5 d, the results of this study indicate that Se volatilization from SeMet addition to nonseleniferous soils results from the biological conversion of SeMet to DMSe or DMDSe.

Headspace analysis of the Met-treated Panhill and Panoche soils showed both DMS and DMDS evolution (data not shown), suggesting that several pathways are involved in the terrestrial S volatilization process, as previously reported for marine organisms (Talyor and Gilchrist, 1991). Their study reported evidence for two mechanisms of S volatilization from aerobic coastal sediments. The first pathway involved a methylation pathway and the intermediate DMSP and resulted in DMS volatilization. The second pathway involved demethylation reactions and resulted in only methane thiol (CH_3SH) evolution. Methane thiol was also reported to be oxidized to DMDS by sediment organisms (Talyor and Gilchrist, 1991). White (1982) established that DMSP could be detected in algae due to the evolution of DMS upon ambient treatment with 1 *M* NaOH, while the DMSP precursor, S–methyl-Met, released DMS only upon incubation with 1 *M* NaOH at 100°C for 2 h. Phosphate buffer extractions of the Met-treated Panoche soil incubated at ambient temperature and at 100°C released DMS, suggesting that both DMSP and S–methyl-Met are present in Met-treated Panoche soil (Table 1). This is the first evidence that terrestrial soils evolve DMS similar to the pathways established for S loss by both marine bacteria and plants. The lack of DMS release from Met-treated Panhill soil extracts suggests that a methylation pathway was not prevalent in the Panhill soil.

Application of the alkaline incubation procedure also released DMSe from the Panoche soil treated with SeMet, but DMSe was not released from the SeMet-treated Panhill soil. Evolution of DMSe was measured with ambient NaOH incubation, suggesting the presence of dimethylselenopropionate (DMSeP), and evolution of DMSe with the 100°C NaOH incubation suggests the presence of Se–methyl-selenomethionine (Se–Methyl-SeMet). The detection of the possible intermediates of Se volatilization from SeMet additions to soils has not been reported, but Cooke and Bruland (1987) reported evidence of the presence of Se–methyl-SeMet in seleniferous surface waters. This evidence suggests that several pathways for Se volatilization are active in the soils tested, similar to the pathways found for S volatilization. The failure to detect DMSeP or Se–methyl-SeMet evolution from the alkaline-treated Panhill soil extracts suggests that the organic Se compound that accumulated in the SeMet-treated Panhill soil was 3-selenol-propionate ($HSeCH_2CH_2CO_2H$). This Se compound is analogous to a 3-mercaptopropionate ($HSCH_2CH_2CO_2H$) compound found to accumulate with aerobic marine bacteria that metabolize the demethylation pathway intermediate, 3-methiolpropionate ($CH_3SCH_2CH_2CO_2H$) (Talyor and Gilchrist, 1991).

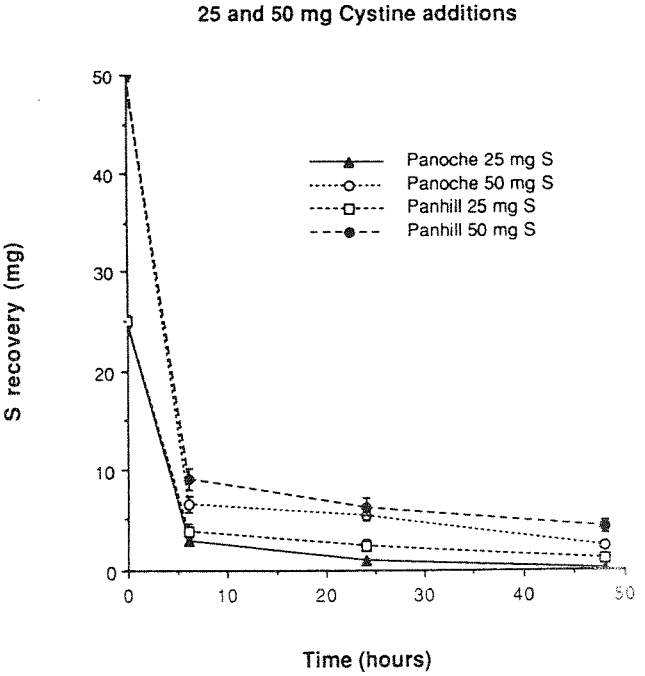

Fig. 7. Sulfur recovery (mean ± standard deviation) from the Panhill and Panoche soils amended with 25 and 50 mg Cys–S kg^{-1} soil.

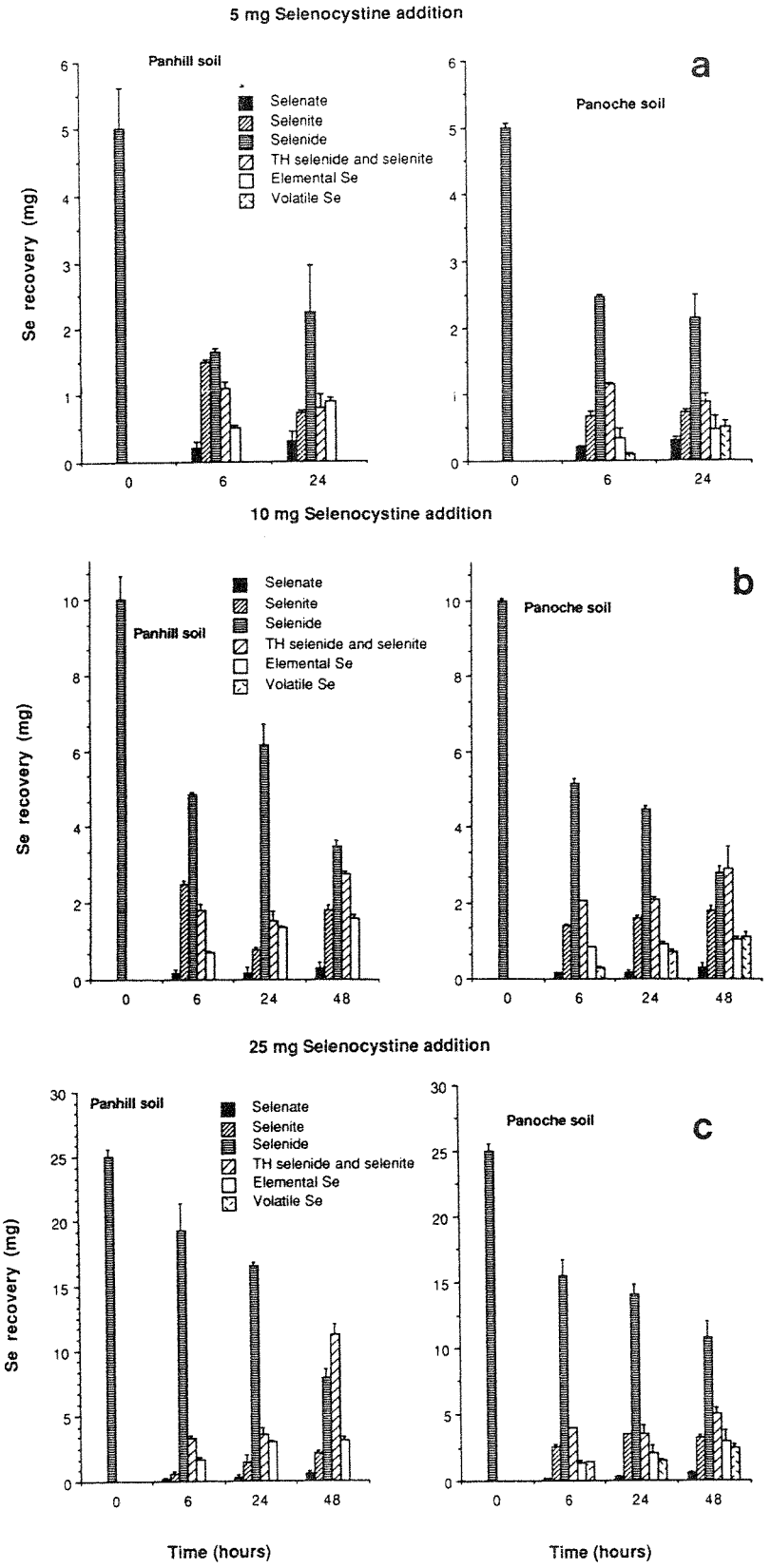

Fig. 8. Selenium recovery (mean ± standard deviation) from the Panhill and Panoche soils amended with the (a) 5 mg SeCys–Se kg^{-1} soil, (b) 10 mg SeCys–Se kg^{-1} soil, (c) 25 mg SeCys–Se kg^{-1} soil.

Addition of Se^{-II} as SeMet to soil results in the majority of the Se^{-II} lost as volatile Se, but sequential extractions with HGAAS analysis determined that low levels of inorganic Se forms were found in the treated Panhill and Panoche soils (Fig. 4). The Se^{+VI}, Se^{+IV}, and Se^{-II} concentrations presented in Fig. 4 were determined by HGAAS analysis of the P-buffer extraction. The tightly held Se^{+IV} and Se^{-II} values were determined by HGAAS analysis of the persulfate extracts and the elemental Se^0 was determined by HGAAS analysis of the HNO_3 extractions (Martens and Suarez, 1997). The concentration of Se remaining in the SeMet-treated soils increased from 2.5 and 1.4 mg Se kg^{-1} with the 5-mg SeMet addition to 10 and 28.5 mg Se kg^{-1} with the 50-mg SeMet addition in the Panoche and Panhill soils, respectively. It has been previously noted that DMSe gas adsorbed by soils resulted in increased levels of soil Se (Zieve and Peterson, 1985). At present we cannot determine if the increased levels of Se determined in the SeMet-treated soils resulted from the sorption of the released Se gas, as noted by Zieve and Peterson (1985), or from the oxidation of the nonvolatilized Se^{-II}. Additional research is needed to determine the extent of adsorption of Se gases and Se speciation resulting from the adsorbed Se by soils. In general, our results suggest that the majority of SeMet–Se released in natural systems will be lost due to microbial volatilization, and that mineralization of SeMet through seleniferous plant residue inputs to natural systems will contribute little to soil or water Se inventories.

Cystine Mineralization

Addition of Cys to the Panhill and Panoche soils resulted in a rapid decrease in Cys concentrations within 6 h (Fig. 7). The CO_2 evolution rate from the treated soils was not different from the controls (no Cys added) during the 48 h incubation and free SO_4 levels did not increase during the incubation time period in the Cys-treated soils. This evidence suggests that Cys additions were rapidly bound by the soil components or incorporated by soil microorganisms. Previous research (Fitzgerald et al., 1988) established that forest organic soil horizons initially incorporate greater amounts of ^{35}S-Cys and ^{35}S-Met than are mineralized. Monreal and McGill (1989a) also reported that ^{14}C-Cys additions were rapidly removed from solution, with <2% remaining after 1 min and >60% of the DPMs added recovered from the microbial biomass after incubation times as short as 1 h. Their results showed that low levels of ^{14}C-Cys additions will also persist due to stabilization in soil by humification, mineral complexation, or physical entrapment processes.

Selenocystine Mineralization

In contrast to the extensive Se volatilization noted with application of SeMet to the two soils, no measurable volatile Se was observed for SeCys applications to the Panhill soil and much lower levels (maximum of 18% of SeCys–Se applied) of volatile Se were measured with SeCys applications to the Panoche soil (Fig. 8). Challenger (1951) suggested that DMDSe formation in natural systems was the result of SeCys metabolism with concomitant release of the amino acid serine. Headspace analysis of the Panoche soil treated with SeCys determined that only DMSe was present and extensive amino acid analysis failed to detect the release of serine. After incubation for 6 h, SeCys was not detected in the P-buffer extracts of either soil, yet the majority of the Se remaining in both soils was determined to be Se^{-II} by HGAAS. The rapid oxidation of the SeCys (Se^{-II}) to Se^0 and Se^{+IV} is in contrast to published reports on the very slow conversion of soil-amended Se^0 to Se^{+IV} (Geering et al., 1968). Solutions of SeCys used in this study for amino acid calibration were only quantitative for a maximum of 48 h (4°C) due to formation of a red precipitate in deionized water (pH 9.0). These results indicate that SeCys is very unstable in natural systems and that SeCys present in biological materials will rapidly oxidize and the majority of the released Se will be transformed to inorganic Se. The quantity and distribution of the two selenoamino acids SeMet and SeCys in seleniferous plant material have not been completely described. Wheat (*Triticum aestivum* L.), soybean [*Glycine max* (L.) Merr.], and other grains have been reported to contain SeMet (Beilstein and Whanger, 1986; Yasumoto et al., 1988), while *Allium* spp. and *Brassica* spp. have been reported to be rich sources of the amino acids SeCys and methylselenocysteine (Cai et al., 1995).

The results presented here indicate that the majority of Se added as SeMet to semiarid soils will be volatilized, resulting in low levels of added Se remaining in the treated soil. The metabolism of SeMet and Met additions to terrestrial soils was similar and resulted in the same products as reported for marine environments. In contrast, the majority of the Se added as SeCys will enrich the Se inventory in the treated soil. Research is needed to understand the distribution of the Se-containing amino acids in seleniferous plant residues to predict the impact of the added Se on the environment. The volatile Se compounds detected in this study also displayed an affinity for the soil components, but little is known about the distribution of Se oxidation states in soil resulting from sorption of Se gases.

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