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Changes in the Distribution of Selenium Oxidation States with Sample Storage

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Abstract

Significant changes in the distribution of selenium (Se) oxidation states of fresh soil were noted with oven-drying (90°C) or with outdoor storage (>5 yr) in compromised plastic containers compared with small changes in Se oxidation state distribution in air-dried samples. Compromised outdoor storage resulted in a 25% increase in the selenate (Se^{6+}) concentrations with a concomitant 18.4 and 5.7% decrease in elemental Se and selenite (Se^{4+}) concentrations, respectively, on a total Se basis. Substantial increases in phosphate-soluble selenide from 15.7% of total Se for fresh seleniferous alfalfa (*Medicago sativa* L.) to 32.6% phosphate-soluble Se were noted with air-dried alfalfa. The results indicate that air-drying will not substantially alter the distribution of soil Se oxidation states, but will result in a significant increase in the concentration of phosphate-soluble Se in seleniferous alfalfa residue.

Selenium has become one of the major elemental contaminant problems in irrigation drainage waters in the western portion of the USA due to the toxicological effects of Se on fish and wildlife. An overview of the Se problem in California evaporation ponds including the Kesterson evaporation ponds is presented by Ohlendorf and Santolo (1994). In environmental and biological samples, Se can exist in inorganic forms as elemental Se (Se^0), metal selenides (Se^{2-}), and selenite (Se^{4+}) and selenate (Se^{6+}), and as organic species with direct Se–C bonds such as methylated compounds, seleno-amino acids, and selenoproteins. Research has identified that the toxic dose of Se is very much dependent on its chemical form, with different toxicity for organic and inorganic Se compounds (Heinz et al., 1987, 1988, 1989). Thus, identification of each Se oxidation state in soil, plant, and water samples is more important than the determination of a total Se concentration (Martens and Suarez, 1997).

Accurate determination of the distribution of Se oxidation states, especially in water samples, has been shown to be dependent on sample preparation and storage (Wang, 1994; May and Kane, 1984; Robberecht and Van Greiken, 1982; Cheam and Agemian, 1980). Possible loss of Se from fresh plant tissue with high concentrations of Se has been noted upon drying for sample preparation (Grant, 1981), but currently little information is available to document the changes in the distribution of Se oxidation states in organic material with different sample preparation or storage methods.

Soil Sampling and Storage

The first sampling of soil from the research plots of W.T. Frankenberger in Kesterson evaporation pond 4 was conducted in 1987. At the time of sampling, a subsample was immediately air-dried and stored in the laboratory in a closed wide-mouth polyethylene bottle. The remaining soil was stored outside between two glasshouse enclosures in plastic trash containers. This soil was soon exposed to the elements due to the loss of the original lids and the development of cracks in the sides of the trash containers. A second sampling of the Kesterson evaporation pond 4 soil near the site of the initial sample collection was collected May 1996, 2 d after a 3-cm rain event. The field-moist soil was transported via an ice chest back to the laboratory and immediately sieved to pass a 2-mm opening in the field-moist state to remove large pieces of organic material. The fresh soil sample was immediately analyzed for Se oxidation state distribution, then stored in a field-moist state at 4°C. A subsample was then air-dried (ambient temperatures) for 48 h in the laboratory and stored in a wide-mouth polyethylene plastic bottle. The Kesterson soil (Turlock fine loamy, mixed, thermic Albic Natraqualf) had the following chemical and physical properties: pH, 7.63; organic C, 26.7 g C kg^{-1} soil; inorganic C, 3.3 g C kg^{-1} soil; total N, 4.96 g N kg^{-1} soil; sand content, 580 g kg^{-1} soil; clay content, 150 g kg^{-1} soil; and total Se, 47.2 mg Se kg^{-1} soil (1987 sampling; Frankenberger and Karlson, 1994) and 31.4 mg Se kg^{-1} soil (1996 sampling). The soil chemical and physical parameters were determined as described by Martens and Suarez (1997).

Seleniferous alfalfa was produced in sand culture tanks irrigated every 5 h with a 0.5 *M* Hoagland's solu-

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Abbreviations: HGAAS, hydride generation atomic absorption spectrophotometry; MANOVA, one-way multivariate analysis of variance; LSD, least significant difference.

tion (Hoagland and Arnon, 1950) or with a 0.5 *M* Hoagland's solution containing 2 mg Se L^{-1} as sodium selenate. The alfalfa was harvested at the 0.1 bloom stage for Se analyses.

Determination of Selenium Oxidation States by Sequential Extraction

Hydride generation atomic absorption measurements (HGAAS) were made with a Perkin Elmer 3030B spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) equipped with a Varian Model VGA-76 (Mulgrave, Victoria, Australia) vapor generation apparatus for Se analysis. Operating conditions and quality assurance program for HGAAS were followed as detailed by Martens and Suarez (1997). Alfalfa was digested for total Se content by the HNO_3 digestion method described by Martens and Suarez (1997). To determine the distribution of Se oxidation states present in the seleniferous alfalfa and soils before and after drying, 5 g soil (oven-dry basis) or 200 mg alfalfa was placed into a 40-mL PTFE (Teflon) centrifuge tube and exposed to a duplicated sequential extraction scheme composed of a water extraction, a buffered phosphate (P-buffer) extraction, a sodium hydroxide (NaOH) or sodium persulfate extraction, and a nitric acid extraction (HNO_3) (Martens and Suarez, 1997). Distribution of Se oxidation states Se^{4+}, Se^{6+}, and Se^{2-} solubilized by the water and P-buffer extractions was determined by selective HGAAS determination of the Se^{4+} oxidation state (Martens and Suarez, 1997). The Se oxidation states Se^{4+} and Se^{2-} were determined in the NaOH extraction and the elemental Se (SeO) concentration was measured as Se^{6+} in the HNO_3 oxidation by HGAAS procedures. All allotropes of SeO, insoluble S–Se associations, and possible S–amino acid–Se complexes are grouped together in the SeO fraction (Weres et al., 1989). Storage conditions evaluated for the first set of collected Kesterson 4 soil were (i) air-dried for 9 yr and (ii) stored outside for 9 yr. Storage conditions evaluated for the second set of Kesterson 4 soil were (i) field-fresh soil, (ii) air-dried soil (1 d), (iii) air-dried soil (4 mo), and (iv) oven-dried (18 h; 90°C).

A second plant extraction methodology, a methanol/chloroform/water (M/Ch/W) extraction was performed to fractionate the soluble, polar Se compounds present in the alfalfa tissue from the nonpolar, insoluble components. Before and after air-drying, alfalfa tissue (200 mg) was added to 40 mL centrifuge tubes and extracted with 15 mL M/Ch/W (15:5:3, v/v/v) overnight with gentle shaking (60 oscillations min^{-1}). Alfalfa remaining was separated by centrifugation, dried, and digested for total Se. To isolate the polar compounds (M/W phase), from the pigments and lipids (Ch plase), 6-mL DI water was added and the isolated lipid phase digested for total Se. Distribution of Se oxidation states in M/W phase was as described for the water and P-buffer extractions. The phase separation and Se analysis of the aqueous, Ch and insoluble phases are described by Martens and Suarez (1997, unpublished data).

Treatment differences were evaluated by a one-way multivariate analysis of variance (MANOVA) model (SAS, SAS Inst., Cary, NC) and mean differences were analyzed by a Tukey's studentized range and least-significant difference (LSD) test at the 1% level. The same significant treatment differences were obtained by the two tests.

Table 1. Distribution of Se oxidation states in Kesterson pond 4 (1987 sampling) upon storage in an air-dry state or with compromised outside storage for 9 yr as determined by sequential extractions.

Treatment	Selenium oxidation state				Total
	Se^{6+}	Se^{4+}	Se^{0}	Se^{2-}	
	mg Se kg^{-1}				
Air-dry	2.48a** (0.30)† [5.2]‡	4.36a (0.56) [9.1]	27.73a (1.5) [58.0]	13.23a (0.35) [27.7]	47.80a
Outside	13.21b (0.56) [30.0]	1.49b (0.16) [3.4]	17.43b (0.08) [39.6]	11.93a (0.28) [27.1]	44.06a

** Means with the same letter are not significantly different at the 1.0% level.
† Value in parentheses indicates standard deviation of the mean values.
‡ Value in brackets indicates percentage of total Se contained in each oxidation state.

Results

The distribution of the four Se oxidation states in the Kesterson pond 4 soil sampled in 1987 and stored in the laboratory or outside is presented in Table 1. Prolonged outside storage compared to in-laboratory preservation resulted in an increased Se^{6+} concentration of 24.8% and in a decreased Se^{4+} and SeO concentrations of 5.7 and 18.4%, respectively. The increase in Se^{6+} was probably due to the oxidation of SeO and Se^{4+} to Se^{6+}. A loss of 3.74 mg Se kg^{-1} soil was most likely due to volatilization by soil microorganisms and not by leaching of the soluble Se^{6+}, because the bottoms of the plastic trash containers were intact. Very little change was noted in the Se^{2-} concentration with storage. The noted changes in the distribution of Se oxidation states were probably due to the additions of moisture from the winter rains followed by the very warm conditions between the glasshouse structures during the spring and summer in southern California. Since this methodology was not available for use with the field fresh samples in 1987, we can only speculate that the Se oxidation state distribution did not change significantly since the total Se concentration in the soil (43.3–47.2 mg total Se) determined by Frankenberger and Karlson (1994) was similar to the laboratory-stored air-dried soil (47.8 mg total Se).

To evaluate short-term storage conditions on the distribution Se oxidation states in soil, fresh samples were obtained from the Kesterson pond 4 adjacent to the former plots of W.T. Frankenberger (source of first samples) and stored as fresh, air-dried (48 h), air-dried (4 mo), and oven-dried samples. Analysis of the results from the extraction procedure coupled with HGAAS speciation determined that two significant oxidation state changes were taking place in the dried soils. First, a significant decrease in Se^{6+} concentrations and an increase in the Se^{4+} concentrations were noted with an increase in the length of the dry storage and intensity

Table 2. Distribution of Se oxidation states in Kesterson pond 4 (1996 sampling) upon storage in a field fresh state, an air-dry state (immediately analyzed), an air-dry state for 4 mo and an oven-dry state as determined by sequential extractions.

	Extract							
	Water		P-buffer		NaOH		HNO_3	
Treatment	Se^{4+}	Se^{6+}	Se^{4+}	Se^{2-}	Se^{4+}	Se^{2-}	Se^{0}	Total
	mg Se kg^{-1}							
Fresh soil	0.10a**	1.77a	0.77a	0.90a	0.32a	10.06a	17.80a	31.72
	(0.01)†	(0.1)	(0.01)	(0.01)	(0.2)	(0.15)	(0.46)	
Air-dry‡	0.12a	1.79a	0.81a	0.91a	0.24a	9.78a	17.60a	31.25
	(0.02)	(0.01)	(0.02)	(0.03)	(0.04)	(0.04)	(0.52)	
Air-dry§	0.14a	1.75a	1.02b	0.65b	0.23a	10.25a	16.62b	30.66
	(0.02)	(0.04)	(0.04)	(0.01)	(0.06)	(0.62)	(0.47)	
Oven-dry	0.32b	1.11b	1.67c	1.67c	0.25a	11.40b	14.00c	30.42
	(0.01)	(0.01)	(0.02)	(0.07)	(0.01)	(0.43)	(1.21)	

** Means with the same letter are not significantly different at the 1.0% level.
† Value in parentheses indicates standard deviation of the mean values.
‡ Air-dry and immediately analyzed.
§ Air-dry with 4 mo storage.

of drying, especially oven-drying (Table 2). A second change in oxidation state was noted with the decrease in refractory SeO concentrations as measured by the HNO_3 oxidation and an increase in the Se^{2-} concentrations measured in the P-buffer and NaOH extractions. These reductions, related to the intensity of the drying conditions are in contrast to oxidative changes measured in the compromised outside storage, where the major influence may have been biological activity. Zhang and Moore (1996), employing a less precise sequential extraction procedure, reported that oven-drying (75°C) Montana pond sediments containing Se, increased soluble Se concentration 44 to 225% and absorbed Se concentration 0 to 260% and decreased the organic matter Se fractions 0 to 30%. Due to these oven-induced changes, Zhang and Moore (1996) conducted the reported research on samples dried at <40°C, but no information on changes in the Se solubility was reported on the <40°C samples.

Unpublished results (Johnson et al., 1966) reported by Grant (1981) showed that volatile Se compounds that are normally present in plants exposed to Se, can be liberated during the drying process. For this study, seleniferous alfalfa was grown to evaluate the standard practice of air-drying plant tissue on the distribution of Se oxidation states and total Se concentration. Sequential extractions revealed that upon air-drying the phosphate-extractable selenide concentrations from the fresh alfalfa significantly increased from 15.7 to 32.6% of the total Se inventory (Table 3) with a concomitant decrease in the less-soluble forms of plant Se. The M/Ch/W extraction had similar increases in polar Se concentrations in the aqueous methanol phase from 14.0% with fresh alfalfa to 37.7% of the total Se inventory with the air-dried treatment (Table 3). The total extractable Se from air-dried alfalfa was not significantly different from the fresh alfalfa (Table 3). Grant (1981) reported that loss of Se from the drying of fresh biological materials with normal Se concentrations was not a problem and that total Se content of dried vegetable matter was not affected by storage at room temperature for up to 10 yr. It is unknown at the present time what effects air-drying will induce on the mineralization of organic Se compounds present in plant tissue compared to incorporation of fresh plant materials in the environment.

The results show that improper storage can significantly change the distribution of Se oxidation states in soil. Air-drying soil appears acceptable for long-term storage with minor losses of elemental Se and some redistribution of NaOH-extractable Se^{4+} to P-buffer-extractable Se^{4+}. Fresh samples should be utilized where possible. Air-drying plant materials is not acceptable for characterization of Se oxidation states in alfalfa, and fresh samples must be analyzed immediately for Se distribution or possibly frozen for future use.

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Table 3. Changes in distribution of Se oxidation states in fresh and air-dried alfalfa determined by sequential extractions or methanol/chloroform/water (M/Ch/W) extraction.

	Sequential extraction				
Treatment	P-buffer Se^{6+}	P-buffer Se^{2-}	Persulfate	Nitric	Total
	mg Se kg^{-1}				
Air-dry	2.50 (0.08)a**	14.00 (0.11)a	12.18 (0.14)a	14.30 (0.03)a	42.98
Fresh	1.05 (0.15)a	6.90 (0.15)b	17.30 (1.6)b	18.76 (2.8)b	44.01
	M/Ch/W extraction				
	Polar Se	Lipid Se	Nonpolar Se	Total Se	
Air-dry	18.99 (1.37)a	4.24 (0.18)a	21.18 (1.02)a	44.41	
Fresh	7.40 (0.86)b	1.86 (0.03)b	34.72 (1.25)b	43.98	

** Means with the same letter are not significantly different at the 1.0% level.
† Value in parentheses indicates standard deviation of the mean values.

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