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Understanding the speciation of the multioxidation states of selenium is vital to predicting the mineralization, mobilization, and toxicity of the trace element in natural systems. Asequential extraction scheme (SES) was developed for identification of Se oxidation states that first employed 0.1 M (pH 7.0) K_2HPO_4—KH_2PO_4 (P-buffer) to release soluble selenate (Se^{+VI}) and selenide (Se^{-II}) and ligand-exchangeable selenite (Se^{+IV}). The second step involved oxidation of organic materials with 0.1 M $K_2S_2O_8$ (90 °C) to release Se^{-II} and Se^{+IV} associated or occluded with organic matter. The final step used HNO_3 (90 °C) to solubilize insoluble Se remaining in the sample. The solubilized Se compounds were speciated by a selective hydride generation atomic absorption spectrophotometry technique. Accuracyofthe developed SES method (96-103% recovery) was verified by use of prepared Se compounds of known speciation, NISTstandard reference materials, and existing seleniferous soils. The average precision (relative standard deviation) for the P-buffer extraction ranged from 5.5 to 7.7% (n= 12);the precision of the persulfate extraction ranged from 2.6 to 8.4% (n = 12); and the precision of the nitric acid extraction ranged from 2.8 to 7.4% (n = 12) for three soils extracted at four different time periods. The method was applied to analyze Se species in seleniferous plant, soil, and sediment samples.

Introduction

Selenium has become one of the major contaminants of concern in irrigation drainage water in arid and semi-arid portions of the western United States (I). Accurate determination of Se speciation is vital to understanding the potential for mobilization and toxicity of the element. In environmental samples, any or all of the four Se oxidation states, selenate (Se^{+VI}), selenite (Se^{+IV}), elemental (Se^0), and selenide (Se^{-II}), maybe present. The mobility, bioavailability, and toxicity of Se are controlled by the chemical speciation (2), indicating that it is desirable to determine the concentration of individual Se species in a sample rather than a total Se content. Synchrotron-based X-ray absorption spectroscopy has shown promise for definitive determination of the distribution of Se oxidation states in natural samples under ambient conditions (3). Two factors limiting this nondestructive method are the relatively high detection limit for Se [0.01 g (kg of soil)$^{-1}$] and the need for a radiation source.

Chemical extraction techniques are a more traditional approach for Se oxidation state identification. Tessier et al. (4) first proposed the use of sequential extractions for investigations of trace element speciation in biological samples. Though sequential extractions provide much information on element distribution, sequential extraction techniques have often been criticized for failure to address element redistribution during extraction and for lack of selectivity, based on results obtained with model substances to imitate natural soils or sediments (5- 7) The use of certain extractants such as NaOCl or mixed acid digestions can alter the oxidation state of the element and can accurately provide only a "total" element concentration in the extract. In addition, there has been limited research into the problem of extraction efficiency. Despite these drawbacks, partial extractions have been used with considerable success to obtain information on the bioavailability (8, 9) and the geochemistry of trace elements (I0, 11).

Since Se chemistry bears little resemblance to transition metal chemistry and geochemical behavior, it is not surprising that the sequential extraction schemes devised for transition metals are not applicable to Se extraction (12). The principles of sequential extraction techniques, specifically the use of progressively stronger extractants to solubilize the element from different sources, are applicable to Se extraction and can provide meaningful results when carefully interpreted.

Analysis of Se present in evaporation drainage pond soils from the San Joaquin Valley of California contaminated with Se-laden drainage water revealed that the vast majority of the Se present in the irrigation drainage water (Se^{+VI} and Se^{+IV}) became concentrated in the surface 15 cm of the containment ponds, in forms whose mineralization and mobilization potential are not well understood, and did not percolate into the groundwater (13, 14). The inability of presently employed extraction techniques to identify the forms of Se in Se-contaminated materials has emphasized the need to develop an efficient Se extraction methodology for natural systems. Detailed Se speciation is required in order to predict Se mobility and leaching from seleniferous lands and evaporation ponds. Treatment and management of these lands to minimize Se leaching and toxicity also requires knowledge of Se redox status and speciation.

Selective Se removal from sediment or soil samples is complicated by the fact that Se exists in more than one oxidation state, each with its unique mechanism of retention (15, I6). In addition, the solubility of Se-containing minerals ranges from the very soluble Se^{+IV} and Se^{+VI} minerals to the extremely insoluble Se^{-II} and Se^0 minerals. The insoluble Se forms may represent major environmental Se sinks (12).

Most of the proposed Se extraction schemes (17-20) have not employed the use of standard reference compounds or the addition of materials with known Se speciation and content to calculate the proposed method extraction efficiency. The objective of this work was to develop an effective sequential extraction scheme (SES) that is compatible with hydride generation atomic absorption spectrophotometry (HGAAS) for determination of Se species in natural samples. Hydride generation AAS methodology was utilized because it is the most studied method for Se determination and the sequence for determination of the different Se oxidations states is well-known (21). This procedure was verified with seleniferous reference materials and then used to analyze plant, soil, and sediment samples of unknown Se content and speciation.

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Materials and Methods

Apparatus. Atomic absorptionmeasurements (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. 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, argon, 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 mn; and slit width, 2.0 mn. A quality assurance procedure for Se analysis by HGAAS was employed as follows: Duplicate samples were analyzed with calibration, reagent blanks, NIST samples, and spikes (20 ng of Se mL^{-1}) to check for interferences at the beginning and end of each HGAAS run. Acceptable data quality objectives were as follows: spike recovery, 90-103%; precision, 10%; detection limit, 0.01 mg kg^{-1}. All glassware was cleaned with detergent, rinsed, soaked overnight in 4 M HNO_3, rinsed six times with deionized (DI) water ($>5 \times 10^{-5}$ dS m^{-1}), and air-dried prior to use. Many methods have been employed for Se analysis of environmental samples, and the pros and cons of each, including HGAAS, are discussed in a review by Robberecht and Van Grieken (22). The use of proper blanks, spikes, and reference materials with a certified Se content and a VGA-76 hydride generator for precise signal integration results in a very reliable and sensitive method for Se analysis.

Reagents and Standards. Stock solutions (1000 mg of Se L^{-1}) of Se (+IV and +VI) were prepared by dissolution of Na_2SeO_3 and Na_2SeO_4 (Aldrich Chemical Co., St Louis, MO) in DI water. Working standards of lower concentrations were prepared by serial dilution of stocks using DI water. Baker (J. T. Baker, Inc., Buffalo Grove, IL) instra-analyzed grade HCl, HF, and HNO_3 were used for all specified analyses. $NaBH_4$ and $K_2S_2O_8$ were used as received from Aldrich, and K_2HPO_4 and KH_2PO_4 were obtained from Baker. The Se reference standards, wheat flour 1567a (1.1 mg of Se kg^{-1}), bituminous coal fly ash 1633b (10.26 mg of Se kg^{-1}), and standard Se reference material 3149 (10 g of Se L^{-1}) were obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD). Goethite was prepared by reaction of dissolved $Fe(NO_3)_3$ with NaOH (23). The Se^0 compounds 1 and 2 were prepared by reacting 0.12 M SeO_3^{2-} (as Na_2SeO_3) with 0.10 M ascorbic acid and Se^0 no. 3 was prepared by reacting 0.12 M SeO_3^{2-} (as Na_2SeO_3) and 0.13 M cysteine (24). The precipitated material was repetitively washed with DI water and centrifuged to remove unreacted substrates. The precipitated material was then air-dried (Se^0 1) or oven-dried at 50 °C (Se^0 nos. 2 and 3). The Se content, determined by HNO_3 dissolution and HGAAS, was 99, 100, and 26% for Se^0 nos. 1-3, respectively. The poorly crystalline structure in Se^0 nos. 1 and 2 was confirmed by X-ray diffraction measurements (Phillips Electronics Instruments, Mt. Vernon, NY). Se^0 no. 3 was found by X-ray diffraction to be contaminated by SeS_2 and/or selenotrisulfldes, which accounted for the lower Se content as reported by Kice (24).

Sample Preparation. Excess water in the collected soil (0- 15 cm depth) and sediment samples was allowed to drain and then the samples were air-dried. Plant material that appeared partially decomposed was removed from the Kesterson pond no. 4 soil sample before air-drying. The soil, Se reference, and plant material were ground with an agate mortar and pestle to pass through a sieve (150-μm openings), dried at 40 °C to constant weight, and stored in polyethylene, wide-mouth bottles. Soil pH was determined on a 2.5: 1 water to soil ratio, total C content was determined by dry combustion (935 °C) with a Coulometric C analyzer (UIC, Inc., Joliet, IL), and inorganic C content was determined by CO_2 evolved by acid treatment. Organic C content was determined by subtracting inorganic C content from total C content. Total N was determined by the method of Bremner (25) and texture by a hydrometer method (26).

Total Se Analysis. Prepared soil and reference material (0.20-0.50 g) were placed in a 100-mL PTFE (Teflon) beaker with 5 mL of aqua regia (3:1; $HCl:HNO_3$) and 2 mL of HF, covered with a watch glass, and heated at 110 °C in a sand bath for 2 h (27). Boric acid (1 g) was then added to complex the excess HF, and the sample was diluted to 100 mL with DI water and transferred to a 100-mL polyethylene bottle. All samples were then stored at 4 °C and analyzed within 7 day of digestion.

Plant material (alfalfa and partially decomposed Kesterson pond no. 4 plant residue) was digested for total Se content by modification of the HNO_3 method described by Banuelos and Pflaum (28) Briefly, 0.5- 1.0 mL of 17 M HNO_3 was added totheplantmaterial (0.01-0.20g) in a 25-mL graduated glass test tube, covered, and allowed to react overnight, and then the mixture was slowly heated on a sandbath to 130 °C until brown fumes no longer were evolved (3 h). The samples were then treated with 1 mL of 30% H_2O_2, heated at 130 °C, and 1 h later treated with 1 mL of 0.1 M $K_2S_2O_8$ for 30 min (130 °C) and cooled, and 0.5 mL of saturated NH_2OH HCl was added to reduce excess HNO_3. The sample was then reduced with 6 M HCl until bubbling ceased. Samples were analyzed for Se by HGAAS.

Proposed Method for Selenium Extraction and Speciation. The following SES was developed for analysis of soluble, adsorbed, organic, and insoluble Se present in seleniferous samples.

Phosphate Extraction. Prepared material (0.1-5.0 g) was placed into a 40-mL PTFE centrifuge tube and fractionated as follows: A 25-mL aliquot 0.1 M (pH 7.0) K_2HPO_4–KH_2PO_4 buffer (P-buffer) was added to the prepared material, capped, and shaken (130 oscillations min^{-1} at ambient temperature) on a horizontal shaker for 1 h. When <5.0 g of seleniferous material was fractionated, Panoche soil was added to equal 5.0 g of material. The sample was then centrifuged (10000g; 20 min), and the supematant was decanted to a 30-mL polyethylene bottle. The sample was then shaken for 2 min with 5 mL of DI water and centrifuged, and the supematants were combined. A water extraction (25 mL; shaken for 1 h) can be inserted before the P-buffer extraction to determine water-soluble Se.

The concentration of each Se oxidation state was determined by HGAAS on three treatments of the P-buffer or water extract. This procedure enables speciation of Se^{+IV}, Se^{+VI}, and Se^{-II} solubilized by the water or the P-buffer by selective determination of the Se^{+IV} oxidation state. We added from 0.1 to 2.0 mL of the water or the P-buffer to 25-mL graduated glass test tubes and added the following: (1) 6 M HCl for Se^{+IV} concentration (no heating; 25 mL total); (2) 6 M HCl heated at 90 °C for 30 min for Se^{+IV} and Se^{+VI} concentrations (25 mL total); (3) 1 mL of 0.1 M $K_2S_2O_8$ (90 °C) for 30 mln to oxidize Se^{-II} to Se^{+VI} and then addition of 6 M HCl (25 mL total; 90 °C) for 30 min to determine Se^{+IV}, Se^{+VI}, and organic Se^{-II} concentrations. Persulfate treatment (analysis 3) results in complete oxidation of all water or P-buffer-soluble Se species to the Se^{+VI} oxidation state. The Se^{-II} concentration was calculated by subtracting the Se^{+IV} and Se^{+VI} determination (analysis 2) from the persulfate oxidation values (analysis 3). The Se^{+VI} concentration was calculated by subtracting the Se^{+IV} concentrations (analysis 1) from the Se^{+IV} and Se^{+VI} (analysis 2) determinations. The difference between HGAAS readings determined with and without persulfate oxidation cannot be equated with Se^{-II} unless good recoveries of Se^{+VI} spikes occur without persulfate additions (12, 29, 30). Recovery tests with Se^{+IV}, Se^{+VI}, or selenomethionine (Se^{-II}) spiked to a nonseleniferous soil and extracted with the P-buffer resulted in >95% spike recovery rates by the listed HGAAS speciation procedure. CAUTION: 6 M HCl can reduce the Se^{+VI} present in a sample aliquot to Se^{+IV}. The

TABLE 1. Pmpetties of Soils Used

soil	pH[a]	organic C[b] [g (kg of soil)$^{-1}$]	inorganic C[c] [g (kg of soil)$^{-1}$]	total N[d] [g (kg of soil)$^{-1}$]	sand [g (kg of soil)$^{-1}$]	clay [g (kg of soil)$^{-1}$]	total Se[e] [mg (kg of soil)$^{-1}$]
Peck	7.95	4.6	3.0	1.29	230	290	11.7
Kesterson pond no. 4	7.63	26.7	3.3	4.96	580	150	47.2
Kesterson pond no. 7	7.28	19.3	3.0	4.80	420	180	6.7
Kesterson pond no. 11	7.70	15.1	2.8	3.61	380	200	5.2
Lost Hills pond A	7.60	28.5	3.2	6.43	320	230	43.9
Panhill	7.98	5.8	2.5	1.12	460	320	0.3
Panoche	8.06	5.6	4.2	1.19	320	360	0.1
San Luis Drain sediment	7.91	33.3	56.5	9.51	10	750	83.8

• The pH values were determined on a 1:2.5 soil to water ratio. [b] Organic C content was determined by difference between total C content and inorganic C content by Coulometric C analyses. [c] inorganic C content was determined by Coulometric C analysis. [d] Total N was determined by block digestion and colorimetric NH, determination. 'Total Se content was determined by mixed acid digestion.

TABLE 2. Extraction Efficiency of 0.1 M KH_2PO_4—K_2HPO_4 (ptl 7.0) for Selenite Added te Samples of Goethite and Soils'

materiel	selenite addition (mg kg^{-1})	selenite recovered (mg kg^{-1})	recovery (C/C_o)
Goethite	1.0	0.72	0.72
	5.0	4.03	0.81
	10.0	9.12	0.91
Panoche soil	1.0	0.96	0.96
	5.0	4.98	0.99
	10.0	10.05	1.00
Panhill soil	1.0	0.97	0.97
	5.0	4.97	0.99
	10.0	9.98	0.99

• 15-mL aliquots of 0.1 g of goethite $L^{-1}10^{-3}$ M NaCl were placed in 40-mLTeflon centrifuge tubes and incubated at ambient temperatures for 2 h after addition of 0.1 mL of water containing the specified level of selenite. Selenite desorption was determined with the addition of 5mL of 0.4M P-buffer and measured with HGAAS. Panhill and Panoche soils (1 g) were placed in 40-mLTeflon tubes and incubated for 2 h after theaddition of 0.1 mL of water containing the specified levelofselenite. Selenite desorption was determined with addition of 5 mL of 0.1 M P-buffer and measured with HGAAS.

TABLE 3. Comparison of Potassium Persulfate ($K_2S_2O_8$) levels for Se Extraction fmm Soils or Metallic Se^0 .

material	$K_2S_2O_8$ concn (M)	extraction: first (μg of Se recovered)	extraction: second (μg of Se recovered)	Σ1 and 2	total Se[b]
Lost Hills A	0.04	5.4	3.7	9.1	43.9
(4.57)	0.10	10.4	8.7	19.1	
	0.13	10.9	5.5	16.4	
	0.20	13.8	5.6	19.5	
Kesterson	0.04	5.7	3.0	6.7	47.2
Pond no. 4	0.10	8.8	5.3	14.1	
(4.36)	0.13	11.6	5.4	17.0	
	0.20	14.5	5.2	19.8	
metallic	0.04	41.0	50.2	91.2	100000
Se (11.56)	0.10	141.0	193.0	334.0	
	0.13	150.0	337.0	487.0	
	0.20	518.0	927.0	1440.0	

• Material (1 g of soil or 0.1 g of metallic Se) was boiled for 1 h with 10 mL of specified persulfate concentration in a 40-mL PTFE centrifuge tube and centrifuged, the supernatant was carefully removed, then an additional 10 mL of specified persulfate concentration wss added, and the process was repeated. The supernatantswere analyzed by HGAAS. The values in parentheses indicate the Se values (μg) determined for the P-buffer extractions of the soil or Se material. *Total Se content [mg (kg-' of soil)$^{-1}$] determined by mixed acid hydrolysis.

HGAAS analysis for Se^{+IV} should be completed within 20 min after addition of 6 M HCl (analysis 1). High levels of extractable C can interfere with Se^{+VI} reduction to Se^{+IV}. The use of aXAD-8 resin (Supelco, Inc., Bellefonte, PA) will remove organic interferences from extracts before Se^{+VI} reduction.

Persulfate ***Oxidation.*** The material remaining after the P-buffer extraction was then treated with 25 mL of 0.1 M $K_2S_2O_8$ (90 °C) for 2 h. The persulfate-soil suspension was centrifuged (10000g; 20 min), the supematant was decanted, the sample was mixed with 5 mL of DI water and centrifuged, and the supematants were combined in a 30-mL polyethylene bottle. Due to the oxidation of the reactive Se species to Se^{+VI} by the persulfate treatment, HGAAS analysis was performed on aliquots (0.5-2.0 mL) treated with 6 M HCI (total 25 mL) and heated at 90 °C for 30 min. A NaOH extraction (0.1 M; 90 °C; 2 h) can be substituted for the $K_2S_2O_8$ extraction, and Se^{+IV} and Se^{-II} can be identiiied in the NaOH extracts as outlined in the P-buffer section, HGAAS step 1 (Se^{+IV}) and step 3 (Se^{-II} and Se^{+IV}).

Nitric ***Acid Oxidation.*** The material remaining after P-buffer and persulfate extractions was treated with 2.5 mL of 17 M HNO_3, warmed to 90 °C for 30 min, and thoroughly cooled, then 20 mL of DI water was added, and the sample was heated at 90 °C for 1.5 h. The resulting 2 M HNO_3 solution was cooled to ambient temperature and centrifuged (10000g; 20 min) and decanted, and Se analysis (Se^{+VI}) was performed on sample aliquots (0.1-1.0 mL) treated with 6 M HCl (total 25 mL) heated at 90 °C for 30 min. The refractory Se solubllized by the nitric acid treatment is referred to as Se^0.

CAUTION: If the sample contains carbonates, possible sample loss may occur upon rapid addition of 17 M HNO_3 due to extreme effervescence in the sample. Nitric acid should be added very slowly to samples to minimize this reaction. In addition, the CO_2 generated by heating the sample with concentrated nitric acid must be released slowly after cooling to limit the possible loss of sample and to limit analyst exposure to the acid.

Results and Discussion

Seleniferous Soils. The chemical and physical properties of the eight soils used in this study are presented in Table 1. Soil and sediment samples were obtained from the San Luis Drain (delivered seleniferous drainage water, Merced County, CA); from the Kesterson Reservoir evaporation ponds 4,7, and 11 (Merced County, CA); from the Lost Hills District evaporation pond A (Kern County, CA); and from the Sumner Peck Ranch (Fresno County, CA).

Selenium Speciation. ***Water Extraction. Low*** levels of Se^{+IV} species (<2% of Se inventory) in selenlferous soils are present in a water-extractable form (31). Results from a water-extraction (5 g of soil, 2 g of fly ash material, 25 mL of water) step before the use of the P-buffer indicated that 3.3,1.0,1.4, and 7.8% of the Se inventory was water-soluble Se^{+IV} in the Peck, Kesterson ponds 4 and 7 soils, and NIST fly ash 1633b, respectively. The water extraction quantatively removed the Se^{+VI} species present in the materials tested, and only trace levels of Se^{+IV} were detected.

Phosphate Buffer Extraction. Phosphate-based solutions are commonly used for extraction of adsorbed forms of Se

TABLE 4. Potassium Persulfate Extraction Efficiency of Selenifemus Soils and Standard Materials@

material	$K_2S_2O_8$ extraction	mixed acid extraction	recovery (C/C_0)
Peck (4.23)	9.83	11.7	0.84
Kesterson pond no. 4 (4.36)	9.88	47.2	0.21
Kesterson pond no. 11 (1.47)	3.00	5.2	0.58
Lost Hills A (4.57)	10.6	43.9	0.24
Kesterson pond no. 4 plant residue (7.34)	16.4	84.5	0.19
San Luis Drain (5.95)	10.3	83.8	0.12
NIST fly ash 1633b (2.65)	9.32	10.2	0.91

@A 1 g sample was placed in a 40-mL PTFE centrifuge tube and heated for at 90 °C for 2 h after treatment with 0.1 M $K_2S_2O_8$. The tube contents were centrifuged (10000g) for 20 min. and the aupernatant was recovered for HGAAS analysis. Thevalues in parentheses indicate the Se content of the P-buffer for the seleniferous material specified.

from soil/sediments **(17, 32, 33).** Laboratory screening of potential extractants showed that an equal molar ratio of KH_2PO_4–K_2HPO_4 (0.05 M; pH 7.0) resulted in quantitative recovery of Se^{+IV} and Se^{+VI} spikes by HGAAS. Table 2 shows the extraction efficiency of the P-buffer for selenite spiked into goethite and samples of the Panhill and Panoche series soils. Lipton (34) noted that incomplete extraction of selenite with phosphate from goethlte was due to the presence of binuclear Fe sites selective for selenlte. The 0.1 M (pH 7.0) K_2HPO_4–KH_2PO_4 buffer was developed for extraction of soluble and llgand-exchangeable Se to avoid the low pH value (4.8) of the KH_2PO_4, which has been shown to introduce Se redistribution errors in soil extractions (34). The quantitative recovery of selenlte spikes from the Panhill and Panoche series soils suggests that the P-buffer limited possible redistribution effects and the noted specific Se^{+IV} binding processes are not significant in these semi-arid soils. In addition, recent studies (35) have suggested that the acidity of unbuffered KH_2PO_4 (pH 4.8) extractions may overestimate exchangeable Se concentrations due to solubilization of Se associated with amorphous and carbonaceous soil constituents.

Research has also found that 0.1 M potassium phosphate is an effective extractant for plant protein (36) and that it extracts seleniferous proteins if present in nondecomposed seleniferous plant material in soil. We achieved > 95% spike recovery rates by the HGAAS speciation procedure when we spiked [1 μg of each Se species (g of soil)$^{-1}$] a nonselenlferous soil with selenate, selenlte, and selenomethionine and extracted with P-buffer. Application of the P-buffer to seleniferous soils resulted in a low recovery of Se^{+IV}, Se^{+VI}, and Se^{-II} (< 10%) when compared to the mixed, acid total Se analysis of the respective soils (Table 1). These results are in agreement with previously results, which also determined < 10% of the total Se inventory in these soils as soluble and (or) exchangeable Se (32, 33). Mixed acid digestion of the soils after the P-buffer extraction confirmed that the majority of the Se inventory was not extracted by the P-buffer.

Persulfate Extraction. Chao and Sanzolone (19) reported that the majority of Se present in seleniferous California soils required additional strong oxidation steps for extraction after removal of soluble Se. Cutter (I 7) reported that a 2% $K_2S_2O_8$ addition was an effective oxidant to eliminate the organic interferences present in sediment-NaOH extractions. The use of $K_2S_2O_8$ to solubilize and release Se that may remain after the P-buffer extraction was investigated. The data presented in Table 3 show that $K_2S_2O_8$ concentrations from 0.84 to 0.2 M released greater amounts of Se than did P-buffer extraction from the soils tested, with a limited Se release from the spiked metallic Se (<0.34% of spiked metallic Se) at a 0.1 M $K_2S_2O_8$ concentration. We established that prolonged oxidizing conditions (>0.1 M $K_2S_2O_8$; >2h heating) released additional amounts of Se from insoluble forms that may be present in the sample. Based on these extractions, we conclude that optimum extraction of Se from soil and sediments with limited oxidation of insoluble Se forms is obtained with 0.1 M $K_2S_2O_8$ [5.0 mL (g of soil)$^{-1}$] reacted at 90 °C for 2 h.

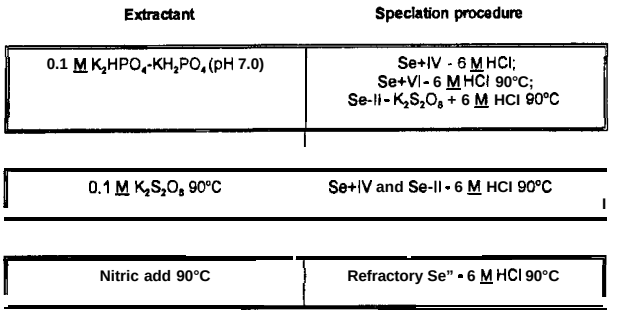

FIGURE 1. Flow diagram of the sequential extraction scheme and the HGAAS speciation procedure.

Table 4 shows that a 0.1 M $K_2S_2O_8$ extraction for 2 h at 90 °C recovered from 91% (NIST fly ash) and 84% (Peck soil) of the mixed acid total Se inventory to <22% of the Se inventory from the Kesterson pond no. 4 soil, pond no. 4 plant residue, and the San Luis Drain sediments. Niss et al. (18) found by ion chromatographlc analysis that the Se present in the NIST fly ash 1633a and other tested fly ash materials leached with NaOH was predominantly selenite. Our analyses of NIST flyash 1633b determlned that only 26% of the total Se inventory was present as P-buffer soluble or exchangeable Se, suggesting that the majority of the Se ('65%) in the fly ash sample was in a strongly-bound phase. The use of $K_2S_2O_8$ oxidation results in solubihzed Se extracted as Se^{+VI} species. This oxidation effectively limits the noted re-distribution effects that would bias results but would also eliminate the potential for further Se speciation.

To determine which Se species were present in the $K_2S_2O_8$ extractions, a 0.1 M NaOH extraction (25 mL) was substituted for the $K_2S_2O_8$ extraction after a P-buffer extraction in the Peck, Kesterson pond no. 4, and Lost Hills A soils. Speciation of extracted inorganic Se has been reported to remain unchanged in 0.5 M NaOH concentrations for as long as 4 h (13, 16). In addition, our research has shown that amorphorous forms of Se are not extracted by use of NaOH (data not shown) or the 0.1 M $K_2S_2O_8$ extractions. Sodium hydroxide extracts were found to contain 85, 42, and 27% of the extractable Se as nonwater-soluble or ligand-exchangeable Se^{+IV} species in the Peck, Lost Hills A, and Kesterson pond no. 4 soils, respectively. The remaining Se in the NaOH extracts was recovered after a $K_2S_2O_8$ oxidation, indicating that Se^{-II} was present as selenlferous plant material. These results show that with the exception of the Peck and Kesterson pond no. 11 soils and the NIST flyash 1633b, the majority of Se (>75%) determined by mixed acid digestion for the Kesterson pond no. 4 and Lost Hill pond A soils and San Luis Drain sediments was not accounted for by the water, the P-buffer, or the 0.1 M $K_2S_2O_8$ extractions.

Nitric Acid Extractions. Discussions with NIST technical staff (J. S. Kane, personal communication) determined that NIST Se reference standard 3149 was prepared by solubilization of metallic Se in concentrated nitric acid followed by dilution. This technique suggested that refractory Se remaining in these soils after P-buffer and $K_2S_2O_8$ extractions could also be extracted by a HNO_3 treatment. Preliminary experiments showed that 1 or 2 M HNO_3 (l-48 h shaking; 25 °C) was ineffective for the removal of additional amounts of Se from the soils in question. Addition of 2.5 mL of 17 M HNO_3 (90 °C) for 30 min to samples of the Kesterson pond no. 4 soil that had been previously extracted with P-buffer and 0.1 M $K_2S_2O_8$ removed 70% of the remaining Se inventory. Dilution to 2 M H^+ with water and additional heating time

TABLE 5. Recovery of Selenium Species from a Panoche Soil Spiked with Reference, Amorphous Se and Plant Material[a]

material	rep	0.1 M KH_2PO_4–K_2HPO_4 (pH 7.0) Se^{+IV} (μg of Se recovered)	0.1 M KH_2PO_4–K_2HPO_4 (pH 7.0) Se^{+VI} (μg of Se recovered)	0.1 M KH_2PO_4–K_2HPO_4 (pH 7.0) Se^{-II} (μg of Se recovered)	$\sum$P-buffer (μg of Se recovered)	$K_2S_2O_8$ (0.1 M) (μg of Se recovered)	HNO, (μg of Se recovered)	$\sum$Se (μg of Se recovered)	total Se (μg)[b]
metallic Se	1	0.21	0.78	11.6	12.6	1009.0	63750.0	64770.0	67700.0
	2	0.01	0.45	1.25	1.71	792.0	35425.0	36220.0	38200.0
amorphous Se 1	1	0.08	1.87	13.11	15.1	592.0	20901 .o	21510.0	22500.0
	2	0.09	1.67	8.58	10.3	402.0	22951 .o	23360.0	25300.0
amorphous Se 2	1	0.41	0.57	7.43	8.41	272.0	18459.0	18740.0	20000.0
	2	0.32	0.42	11.8	12.5	225.0	17964.0	18200.0	17400.0
amorphous Se 3	1	0.64	1.72	12.9	15.2	60.5	19102.0	19180.0	19900.0
	2	0.64	1.79	12.9	14.0	39.5	18765.0	18820.0	19500.0
alfalfa	1	0.33	0.11	87.1	87.5	23.0	26.75	137.0	140.3
	2	0.28	0.2	87.2	87.6	25.4	22.25	135.0	137.2
Kesterson pond no. 4 plant residue	1	0.43	1.33	5.58	7.34	5.08	14.70	27.1	26.73
	2	0.64	1.58	3.99	6.21	6.06	13.74	26.0	25.37
NIST fly ash 1633b	1	2.39	0.41	ND[c]	2.8	4.82	6.70	14.3	13.91
	2	3.11	0.5	ND	3.61	5.67	7.41	16.7	17.29
NIST flour 1567a	1	ND	ND	0.80	0.80	0.75	ND	1.55	1.48
	2	ND	ND	0.64	0.64	0.55	ND	1.19	1.29

[a] A determined sample weight of seleniferous material was added to a Panoche soil to equal 5 g in a 40-mL PTFE centrifuge tube. The sample was treated sequentially with the phosphate buffer, $K_2S_2O_8$ (90 °C) and HNO_3 (90 °C). The sample was centrifuged (10000g) after each extraction, and the supernatant was removed. The sample was then shaken with 5 mL of water, the sample was centrifuged, and the supernatants were combined and treated with the next extractant. [b] Total Se content determined by mixed acid hydrolysis. [c] ND, not detected.

TABLE 6. Extraction of Selenium Species from Seleniferous Soil with the SES[a]

soil	0.1 M $K_2H_2PO_4$–KH_2PO_4 (pH 7.0) Se^{+IV} [mg of Se (kg of soil)$^{-1}$]	0.1 M $K_2H_2PO_4$–KH_2PO_4 (pH 7.0) Se^{+VI} [mg of Se (kg of soil)$^{-1}$]	0.1 M $K_2H_2PO_4$–KH_2PO_4 (pH 7.0) Se^{-II} [mg of Se (kg of soil)$^{-1}$]	$\sum$P-buffer [mg of Se (kg of soil)$^{-1}$]	0.1 M $K_2S_2O_8$ [mg of Se (kg of soil)$^{-1}$]	HNO_3 [mg of Se (kg of soil)?	$\sum$Se [mg of Se (kg of soil)$^{-1}$]	total Se[b]
Peck	3.39	0.81	0.03	4.23	5.71	2.24	12.2	11.7
Kesterson pond no. 4	1.94	2.42	ND[c]	4.36	6.31	37.3	47.9	47.2
Kesterson pond no. 7	0.24	0.30	0.06	0.60	0.31	5.60	6.51	6.7
Kesterson pond no. 11	0.17	1.20	0.10	1.47	1.63	2.19	5.29	5.2
Lost Hills pond A	1.60	2.80	0.17	4.57	4.99	34.9	44.5	43.9
San Luis Drain Sediment	0.17	1.59	4.19	5.95	1.72	68.1	75.7	83.8

[a] Five-gram sample of soil was placed in a 40-mL PTFE centrifuge tube and sequentially treated with phosphate buffer, $K_2S_2O_8$ (90 °C), and HNO_3 (90 °C). The sample was centrifuged and the supernatant removed. The sample was then shaken with 5 mL of water, the sample was centrifuged, and the supernatants were combined and treated with the next extractant. [b] Total Se content determined by mixed acid hydrolysis. [c] ND, not detected.

(1.5 h) quantitatively removed the remaining Se from this soil. These results suggest that a strong nitric acid oxidation is suitable to recover insoluble Se forms remaining in soils after treatment with P-buffer and $K_2S_2O_8$ extractions. The proposed three-extraction procedure provides a means for accounting for Se species from environmental samples. A flow chart detailing this extraction procedure and the Se species extracted is diagramed in Figure 1.

SES Efficiency. The effectiveness of the developed SES protocol for selective Se recovery was tested by the addition of a range of seleniferous materials with known and unknown Se speciation into samples of the Panoche series soil. The results show that the three extraction steps in this sequential extraction scheme are very effective for the recovery of added Se (Table 5). The recovery rates of added Se ranged from 95 to 103%. The results show that <1.5% of the metallic or amorphous Se forms were solubilized by the P-buffer or $K_2S_2O_8$ solutions while extracting the more soluble Se compounds. Data from this extraction method indicate that >60% of the Se present in the nondecomposed seleniferous alfalfa is in the P-buffer extraction with <18% in the HNO_3 extraction. This Se distribution is in contrast to the partially decomposed Kesterson pond no. 4 plant residues of which <27% of the Se found in the P-buffer extraction with 48% of the Se was recovered in the HNO_3 extraction. The depletion of the P-buffer-soluble Se from the partially decomposed plant material as compared to the nondecomposed plant material suggests that the P-buffer-extractable Se is available to soil microorganisms. At present, Se speciation resulting from the soil mineralization of organic Se present in seleniferous plant residues is not known.

Application of this method to seleniferous soils with unknown Se speciation found that the San Luis Drain sediment, the Lost Hills pond A, and the Kesterson ponds 4 and 7 soils, which had been exposed to ponded water for extended periods of time, had *80-84%* of the mixed acid digestible Se inventory present as HNO_3-soluble Se forms (Table 6). These results are not surprising since water ponding conditions is expected to produce anaerobic conditions conducive to Se reduction into the "refractory pool" of Se. The Kesterson pond no. 11, which experienced infrequent water ponding, had 42% of the Se inventory in these Se forms with the majority of the Se present in the P-buffer and persulfate extractions. These extraction steps represent the adsorbed and organic Se pools of Se present in the soil. The mixed redox status of Se in this material is consistent with intermittent ponding, producing periods of highly reduced and aerobic conditions. In contrast, the Peck soil, which experienced unknown water ponding, had only 18% of the Se inventoryin the HNO_3-soluble Se forms. The small quanity of reduced Se is consistent with the predominantly aerobic conditions in the Peck pond. Tokunaga et al. (37) reported > 70% of the total Se inventory in the Kesterson pond no. 11 soil was as organic-associated Se extractable with NaOCl.

TABLE 7. Extraction of Selenium Species from Seleniferous Material with Water, P-Buffer, NaOH, and HNO_3 Sequential Extraction[a]

material	Se^{+VI} (mg of Se kg^{-1})	Se^{+IV} (mg of So kg^{-1})	Se^{0} (mg of Se kg^{-1})	Se^{-II} (mg of Se kg^{-1})	total Se[b] (mg of Se kg^{-1})
Peck	0.81 (100)	8.24 (3.3)	2.24	0.89	12.18
Keeterson pond no. 4	2.48 (100)	4.36 (1 .0)	27.73	13.23	47.80
Keeterson pond no. 7	0.50 (100)	1.11 (1.4)	2.29	2.80	6.70
NIST fly ash 1633b	0.15 (100)	7.94 (7.0)	1.87	0.15	10.11

[a] Material (5 g of soil or 2 g of NIST fly ash 1633b) was sequentially extracted with water, 0.1 M P-buffer, 0.1 M NaOH (90 °C), and HNO_3 in a 40-mL PTFE centrifuge tube. The aupernatants were analyzed by HGAAS apeciation techniques. [b] Total Se content [mg (kg of soil)$^{-1}$] is the sum of the Se^{+VI} (water extraction), Se^{+IV} (water, phosphate, and NaOH extractions), Se^{0} (nitric acid extraction) and Se^{-II} (phosphate end NaOH extractions) analysis of the four extractions. Values in parentheses indicate the percentage Se present as water-soluble forma.

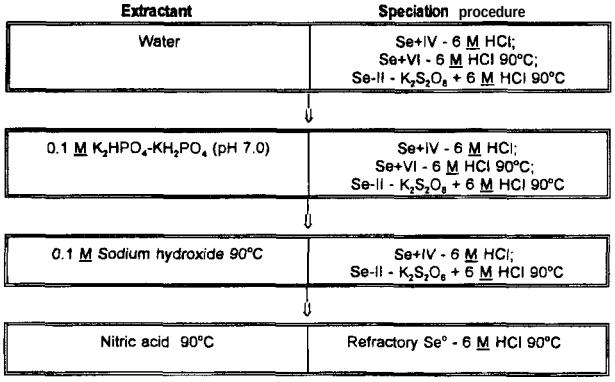

FIGURE 2 Flow diagram of the expanded sequential extraction scheme and the HGAAS speciation procedure.

This NaOCl extraction, however, will also extract elemental Se forms from this soil, which is not desirable if we wish to distinguish organic bound Se from elemental forms. In our study, we determined that 31% of the total Se inventory of the pond no. 11 soil was present in the $K_2S_2O_8$ extraction and 41% in the HNO_3 extraction for a total of 72% Se^{+IV} and Se^{-II} plus Se^{0}.

Recovery and Precision. The recovery rate of the extraction procedure, as shown in Table 5, ranged from 96 to 103% Se recovery with the NIST flyash 1633b (n = 4). We analyzed four different triplicate extractions of the Peck, Kesterson pond no. 4, and Lost Hills A soil samples over a period of 12 months to determine the precision of the developed SES. The mean values (+ the relative standard deviation) for the P-buffer were 4.32 (0.24), 4.30 (0.33), and 4.65 (0.31) mg of Se (kg of soil)$^{-1}$ for the Peck, Kesterson pond no. 4, and Lost Hills A soils, respectively. The mean values for the persulfate extractionsrangedfrom5.70 (0.15), 6.38 (0.25), and4.99 (0.42) mg of Se (kg of soil)$^{-1}$ for the Peck, Kesterson pond no. 4 and Lost Hills A soils, respectively; and the mean values for the nitric acid extractions ranged from 2.04 (0.15), 37.05 (1.05), and 34.90 (0.98) mg of Se (kg of soil)$^{-1}$ for the Peck, Kesterson pond no. 4, and Lost Hills A soils, respectively.

A more intensive approach to the above listed extraction scheme to account for Se speciation includes addition of a water extraction [25 mL (5 g of soil)$^{-1}$] to account for water-soluble Se and substitution of NaOH [25 mL (5 g of soil)$^{-1}$; 90 °C) for the persulfate oxidation to determine Se^{+IV} and Se^{-II} concentrations. Table 7 shows the speciation of Se inventories in the Peck soil, the Kesterson ponds 4 and 7 soils, and the NIST fly ash 1633b. The results show that excellent recovery of the Se inventories is possible with this extraction scheme and that the method allows a total accounting of the Se^{+VI}, Se^{+IV}, Se^{-II}, and Se^{0}. For example, the Peck soil Se inventory was determined to be composed of 6.6% Se^{+VI}, 67.6% Se^{+IV}, 7.3% Se-“, and 18.3% insoluble Se^{0}. A flow chart detailing the expanded Se extraction scheme and the Se species each step extracts is shown in Figure 2.

The combination of a sequential extraction protocol with a subsequent HGAAS speciation scheme fully accounted for the soluble, ligand-exchangeable, organic, and insoluble Se forms in the samples analyzed. The lack of interferences combined with limited redistribution effects and the noted accuracy and precision provide a valuable research tool for investigations into the very complex reactions involved with Se in natural systems.

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