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ARTICLE *in* ANALYTICAL CHEMISTRY · DECEMBER 1985

Impact Factor: 5.64 · DOI: 10.1021/ac00291a045

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RECEIVED for review April 17, 1984. Resubmitted May 24, 1985. Accepted July 1, 1985. L. Van Vaeck thanks the National Fund for Scientific Research (N.F.W.O.), Belgium, for a "Research Associate" grant. J. De Waele is indebted to the "Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw" (I.W.O.N.L.), Belgium, for financial support. The work was funded by the Interministrial Commission for Science Policy, Belgium (Research Grant, 80-85/10). The text has been elaborated within the framework of the Belgian Programme for the reinforcement within the potential in the new technologies—PREST (Prime Minister's Office for Science Policy). The scientific responsibility for the text is assumed by its authors.

Determination of Selenium Speciation in Biogenic Particles and Sediments

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Selenium can exist in a variety of chemical forms in the suspended particles and bottom sediments of natural waters. A procedure for sediments and planktonic material has been developed that uses a multistep nitric/perchloric acids digestion to solubilize total selenium and a weak sodium hydroxide treatment to release selenite and selenate. The solubilized selenium species are determined by a selective hydride generation/atomic absorption technique. Accuracy was verified by using a combination of standard reference materials, radiotracers, and existing sediment leach methods. For field and reference samples the average precision (relative standard deviation) for total selenium determinations is 8.8% (n = 8 samples) and 19.3% for selenite + selenate determinations (n = 6 samples). The detection limit for total particulate selenium is 10 ng/g using a sample size of 0.2 g. The method has been used on a variety of plankton, planktonic detritus, and sediment samples.

The accurate determination of particulate-bound trace-element concentrations in natural water systems is of extreme importance to geochemical studies. This "elemental reservoir" represents the major sink of trace elements removed from the dissolved state and a potentially large source that can be remobilized into the surrounding water. With respect to removal and remobilization, not only the total concentration but also the manner in which an element is associated with the particulate matter must be determined. By use of a sequential extraction procedure such as that described by Tessier et al. (*1*), the partitioning of trace elements between exchangeable, carbonate, iron and manganese oxides, organic and resistant mineral fractions is revealed; these data can be termed "phase speciation". Information on phase speciation is vital to understanding the processes of removal from the dissolved to the particulate state and the potential for remobilization to the water and biota (often referred to as bio-availability).

For multiple-oxidation-state elements such as selenium, the existence of different chemical forms necessitates further analysis of particulate associations. Selenium has four formal oxidation states: -II, 0, IV, and VI. In natural waters the principal dissolved selenium species are Se(IV) and Se(VI), which exist as selenite and selenate, respectively (*2*, *3*). Within particulate material, any of selenium's four oxidation states may be found. Since the biological uptake and toxicity of selenium are controlled by its chemical form (*4*, *5*), an evaluation of this chemical speciation in particulate matter is needed. Furthermore, many processes that affect the selenium cycle in natural waters can be elucidated with particulate chemical speciation data.

The problem with all sequential extraction procedures (i.e., for phase speciation) is that they cannot preserve the chemical form of selenium due to the reagents and conditions employed. The purpose of this paper is to describe a method that can quantitatively reveal the chemical speciation of selenium in particulate materials such as plankton, planktonic detritus, and sediments. This method interfaces with a selective hydride generation/atomic absorption technique which is used for dissolved selenium speciation determinations (*6*, *7*). However, the method should be amenable for use with any procedure capable of determining the speciation of dissolved selenium.

EXPERIMENTAL SECTION

Apparatus. The hydride generation/trapping/detection apparatus is thoroughly described elsewhere (*6*). The system includes a helium-purged glass stripping vessel, glass U-tube immersed in dry ice/2-propanol (water vapor trap), glass U-tube packed with DMCS-treated glass wool immersed in liquid nitrogen (hydride trap), and an atomic absorption spectrometer (Perkin-Elmer 4000) fitted with a quartz tube and air/hydrogen flame atomizer. For particulate selenium determinations, solution volumes are small

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(e.g., 10 mL) and a smaller stripper than that described by Cutter (6) can be utilized. The small stripper is equivalent in design to the larger unit except that a 29/42 ground-glass joint is used and the stripper can hold 70 mL of solution. The following parameters are also adjusted (refer to ref 6 and 7): 40 mL total sample volume, 22 mL of concentrated HCl, 0.5 mL of 2% sulfanilamide, 3 mL of 4% borohydride solution, and 7-min strip/trap time. All tubing and fittings are Teflon, and all glass surfaces are treated with dimethyldichlorosilane (DMCS) in order to reduce hydride loss by adsorption. Spectrometer signals are processed, and peak areas determined, by a Hewlett-Packard 3390A digital integrator.

Glassware used in sample preparation is cleaned with detergent and acetone, rinsed, heated overnight in a 7 M nitric acid bath, rinsed with distilled water, and air-dried prior to use. Plasticware is similarly treated, but soaked overnight in a 4 M HCl bath (cold) in place of nitric acid.

Reagents and Standards. Reagents for the hydride determination are exactly as described by Cutter (6–8). Reagents for the phase speciation procedure are those of Tessier et al. (1), with the omission of the peroxide–nitric acid solution used for organic matter dissolution (discussed below). All reagents and acids are reagent grade (Baker) with the exception of Baker "Instra-Analyzed" nitric acid.

Amberlite XAD-8 resin (Supelco), 16–50 mesh, is rinsed at least four times with distilled water, and the resin fines are removed by decanting. This washed resin is stored in a refrigerator to prevent bacterial growth which destroys the resin. The XAD-8 column consists of 5 cm of washed resin in a 0.8-cm-i.d. glass column (with Teflon metering stopcock). The column is preconditioned with the following reagents at 2 mL/min: 20 mL of pH 12 solution (KOH-adjusted water), then 15 mL of pH 1.6 solution (HCl-adjusted water).

^{75}Se selenite and selenate (New England Nuclear) are stored as pH 7 and 11 solutions, respectively. The degree of selenite–selenate cross contamination in the two solutions is determined by selectively removing the selenite from solution using activated alumina (9) and then determining changes in activity. No detectable contamination was found in the radiotracers used for this work.

The following standard reference materials were employed: National Bureau of Standards (NBS) Bovine Liver (SRM 1577a), Oyster Tissue (SRM 1566), River Sediment (SRM 1645), Estuarine Sediment (SRM 1646), and International Atomic Energy Agency (IAEA) copepod homogenate (MA-A-1).

Procedures. *Sample Preparations.* Biogenic material and sediments are stored frozen between sampling and analysis. A thawed sample is placed in a beaker, covered with a watch glass, and heated at 40 °C until dry. The material is then ground with an agate mortar and pestle, and the powder is sieved with a plastic mesh (150-μm openings) to remove larger particles (which are reground and sieved). This prepared material is kept in a polyethylene bottle. Standard reference materials are dried at 40 °C to constant weight prior to use.

Total Selenium. Prepared material (0.02–0.20 g) is placed in a 50-mL beaker with 5 mL of concentrated nitric acid, covered with a watch glass, and heated at a gentle reflux for 3 h. Perchloric acid (0.2 mL) is added, and the sample is refluxed for an additional 3 h. The beaker is then uncovered and the acids are allowed to evaporate until only a slight amount of moisture remains. The nitric/perchloric reflux process is repeated once again. At no time is the sample allowed to go dry or char since selenium losses can occur. A third 3-h nitric acid (only) reflux is performed, and the sample is again carefully evaporated until most of the acid is removed (moist residue). Ten milliliters of 4 M HCl is added to the beaker contents to facilitate residue dissolution (heated if necessary), and this solution is transferred, after filtration through 0.45-μm membrane filters, to polyethylene bottles for storage.

Aliquots of the HCl solution (0.5–1.0 mL) are diluted to 40 mL with distilled water and analyzed by using the dissolved selenite + selenate procedure described by Cutter (7), with the small stripper modifications noted above. Calibration for all samples is performed via the standard additions technique (using selenite).

Chemical Speciation. The prepared material (0.20–0.50 g) is placed in a 50-mL Tefzel centrifuge tube and 2 mL of distilled water is added. This slurry is sonically disrupted (20 kHz) for 3 min; 2 mL of 2 M NaOH is added and the tube is capped and placed in a sonic bath for 4 h. The leach solution is then adjusted to pH 1.6–2.0 with concentrated HCl (ca. 0.4 mL) and centrifuged at 10 000 rpm for 10 min. The supernatant is decanted into a Teflon beaker, the pellet is rinsed with 1 mL of pH 1.6 solution, and the tube is respun. This supernatant is added to the first, and the rinse procedure is repeated two additional times. The pH of the combined supernatant is adjusted to 1.6–1.8 using HCl or NaOH and passed through a prepared XAD-8 column at a maximum flow rate of 2 mL/min; the column flow-through is collected in a 30-mL polyethylene bottle. The Teflon beaker is rinsed 3 times with 3 mL of pH 1.6 solution; the rinses are passed through the XAD column and collected with the first supernatant. The treated leachate should be stored in a refrigerator and analyzed within 3 weeks.

Selenite is determined on aliquots (ca. 0.5–1.0 mL) of the leachate diluted to 40 mL using the procedures described for water samples (7) and the small stripper modifications noted above. Determination of selenite + selenate entails boiling a similarly diluted aliquot for 15 min with 0.5 mL of persulfate solution (2% w/v) after acidification to 4 M HCl; the resulting solution is treated as a selenite sample. Since the determination of total selenium includes all possible oxidation states (–II, 0, IV, VI), the amount of particulate selenide + elemental selenium is estimated to be the difference between total selenium and selenite + selenate determinations. Similarly, the concentration of particulate selenate is calculated as the difference between particulate selenite + selenate and selenite determinations.

Phase Speciation. Selenium in exchangeable, carbonate, and iron and manganese oxides phases is released by using the sequential leach procedure described by Tessier et al. (1). Due to incomplete recoveries, Tessier's peroxide/nitric acid (organic) leach is replaced by the concentrated nitric/weak perchloric acids digest used for total selenium. No selenium has been detected in the resistant mineral (residual) phases of the materials examined thus far. Therefore the mineral (residual) phase leach/digestion (ref 1) is not routinely performed.

Total selenium in each phase is is determined by using 1.0-mL aliquots from the respective leachate diluted to 40 mL with distilled water. This solution is then subjected to the total dissolved selenium procedure (i.e., total sample volume of 40 mL adjusted to 4 M HCl, 0.5 mL of 2% potassium persulfate added, and 0.5-h boiling reflux—ref 8).

RESULTS AND DISCUSSION

Sample Handling and Preparation. The handling of samples must not alter the concentration or chemical speciation of particulate selenium. For biogenic materials and sediments, speciation changes can occur via bacterial degradation during storage. In order to prevent this, samples are placed in polyethylene bottles and frozen immediately. Laboratory processing, sample drying in particular, can lead to selenium loss through volatilization (10); therefore samples are dried slowly at 40 °C. Thorough grinding of the dried material with an agate mortar and pestle and sieving are included to assure sample homogeneity.

Total Particulate Selenium Determination. Most procedures for the determination of total particulate selenium employ "wet" oxidations to solubilize selenium (see ref 11 for a compilation). Digestions which include hydrofluoric acid (12) must be conducted in sealed containers since SeF_4 and SeF_6 are volatile. Procedures utilizing oxidizing reagents (e.g., nitric, perchloric, and sulfuric acids, hydrogen peroxide, and potassium persulfate or permanganate) limit such volatility problems without requiring specialized apparatus. However, selenium contamination in sulfuric acid can be too large for trace determinations, while permanganate is a severe interferent in the hydride determination (13).

With these considerations, the use of nitric acid, hydrogen peroxide, and dilute perchloric acid for particle digestions was investigated. So that accuracy could be assessed, standard reference materials (NBS Bovine Liver, Oyster Tissue, River Sediment, and Estuarine Sediment, and IAEA Copepods) were employed in this work. The hydride generation method re-

Table I. Recoveries of Total Selenium for Standard Reference Materials[a]

type	certified value	recovered value
Bovine Liver (NBS SRM 1577a)	1.1 ± 0.1	1.1 ± 0.1 (n = 4)[b]
Oyster Tissue (NBS SRM 1566)	2.1 ± 0.5	2.6 ± 0.3 (n = 3)
Copepod Homogenate (IAEA MA-A-1)	3.1 ± 0.2	3.0 ± 0.2 (n = 6)
River Sediment (NBS SRM 1645)	1.5[c]	1.7 ± 0.3 (n = 4)
Estuarine Sediment (NBS SRM 1646)	0.6[c]	0.43 ± 0.02 (n = 3)

[a] Concentrations in micrograms of selenium per gram. [b] "n" is the number of separate samples processed; each sample is determined in triplicate (as a minimum). [c] Only reported values (not certified) are obtainable.

quires selenium be in the form of selenite for direct determination (*6*). Since an oxidative digestion might lead to the production of selenate, which may be found in particulate material as well, aliquots of the digestion solution are treated as dissolved selenite + selenate samples (i.e., 15-min boil of samples acidified to 4 M HCl) in order to produce selenite exclusively (*7*).

Initial experiments using nitric acid (only) refluxes and a nitric acid/hydrogen peroxide digestion (*14*) demonstrated that full recoveries of the reported selenium concentrations are not achieved (i.e., <75%). For the types of particulate matter tested, quantitative recoveries are found when a small amount of perchloric acid (0.2 mL) is added at the end of nitric refluxing, with additional refluxing thereafter. The nitric–perchloric acids reflux is repeated twice, with an evaporation step intervening. The combination of acid reflux and evaporation is very effective at oxidizing organic material, especially when the solution volume is reduced and copious acid fumes are generated. However, care must be taken to avoid sample drying or charring at this stage (i.e., the sample must remain moist). With reference materials, selenium losses greater than 50% were found when charring occurred; similar behavior has been observed previously (*11*). To ensure removal of perchloric acid that might interfere with the hydride determination, a third nitric acid (only) reflux and evaporation step is employed. The results for standard reference materials using this multiple reflux procedure can be seen in Table I. While the potential hazards of perchloric acid make its use undesirable, only small quantities are added, and complete selenium recoveries for a variety of sample types are obtained. For the standard reference materials used, an average procedural precision (as relative standard deviation) of 9.9% is obtained. The detection limit for total particulate selenium can be calculated by using a maximum sample size of 0.2 g and an analytical detection limit of 0.2 ng; a value of 10 ng of Se/g of sample is obtained.

Selenium Speciation. Although total selenium concentrations are reported for standard reference materials, virtually no information on the actual chemical form (e.g., oxidation state) of selenium in these materials is available. Thus the accuracy of any particulate speciation method is difficult to assess. To overcome this deficiency, a review of the possible chemical speciation of selenium in particles found in natural waters is first necessary. For plankton, the works of Wrench (*15*), Wrench and Campbell (*16*), and Foda et al. (*17*) demonstrate that selenium is predominantly found as selenoamino acids in proteins (i.e., organic selenide species). Anionic selenite and selenate might also be bound to biogenic material via adsorption; in comparison to sulfur, biological selenate esters are uncommon (*5*). Elemental selenium has only been reported in cultures of certain microorganisms where the dissolved selenium concentrations were far above natural values (*18*).

The chemical and phase speciation of sedimentary selenium is more complex than that in biological materials. Inorganic selenide may exist as insoluble metal selenides (e.g., iron selenide) or may be found in ferroselite ($Fe^{II}Se_2$), a pyrite analogue (*19*). Since biological detritus can form a portion of surficial sediments, organic selenide species (noted above) may also be associated with sediments. As potentially adsorptive species, selenite and selenate may be found in phases such as carbonate, ferric oxides, and manganese oxides. Further, a portion of sedimentary selenium may exist in the elemental state, depending on the ambient redox conditions (*19*). Overall, the type of sedimentary association falls into two categories, adsorbed selenite and selenate and covalently bound selenide (inorganic and organic forms); elemental selenium might also be classified as covalently bound.

Planktonic Selenium. ^{75}Se selenite and selenate and a mixed plankton sample from the Northeast Pacific Ocean were used to determine recoveries for the leaching of selenite and selenate from planktonic material. Efforts were focused on these two selenium species since they are the predominant dissolved forms of the element in most natural waters (*2*, *3*). In order to label the sample with adsorbed selenium, the homogenized planktonic material was placed in pH 8, ^{75}Se selenite and selenate labeled seawater for 48 h. After this time, the material was rinsed with unlabeled seawater to remove any excess (nonadsorbed) radiolabels; portions of this ^{75}Se labeled plankton were then used in desorption experiments. A 1 M NaOH, 4-h leach in a sonic bath removed 94 ± 2% (n = 4) of the labels (material counted before and after treatment); 1 M HCl removed less than 70% of the adsorbed selenite and selenate. In order to ensure that no speciation change occurs during the sodium hydroxide treatment, in particular selenite oxidation, nonradiogenic selenite standards were subjected to the same leach conditions. Over the duration of the leach (4 h) selenite displayed no detectable speciation change (recovery, 107 ± 4.3%, n = 4).

The reference materials, NBS Oyster Tissue and IAEA Copepods, were subjected to this 4-h, 1 M NaOH leach procedure. In order to maximize the surface area exposed to the alkaline solution, the material is first suspended in distilled water and sonically disrupted (20 kHz); the alkaline treatment follows. The leachate is collected after acidification to pH 1.5–2.0 via centrifugation and rinsing of the remaining particles. Planktonic matter is visibly degraded after alkaline leaching, presumably by protein hydrolysis. While aiding desorption, this type of degradation adds dissolved organic compounds (and organic selenide) to the leach solution, which may interfere with the subsequent hydride determination (*20*). By use of the procedure of Roden and Tallmann (*20*), the leachate is passed through an Amberlite XAD-8 column in order to remove dissolved organic material; selenite and selenate are not retained by XAD-8. The column effluent and column rinses are collected for analysis. In addition to interference, the presence of dissolved proteins in the alkaline leachate might lead to selenite contamination through organic selenide oxidation. This possibility was examined by subjecting a dissolved selenocysteine standard to the alkaline leach procedure. No conversion to selenite was detected with this labile organic selenide species.

The determinations of selenite and selenate are made with aliquots of the XAD effluent and the dissolved speciation procedures already in use (*6–8*). However, addition of selenate standards to XAD-treated leachates showed that the recovery of this species is reduced by nearly 50% compared to that for water samples (*7*); the reason is unclear. Addition of 2% persulfate solution prior to boiling removes this effect.

Table II. Chemical Speciation of Selenium in Reference Materials and One Freshwater Sediment[a]

type	total Se	Se^{IV}	Se^{VI}	predicted[b] Se^{IV+VI}
		A. Biological Materials		
Copepod (IAEA MA-A-1)	3.00 ± 0.20	0.02 ± 0.01 (n = 3)[c]	0.02 ± 0.01 (n = 3)	–
Oyster Tissue (NBS 1566)	2.60 ± 0.30	<0.01 (n = 3)	<0.01 (n = 3)	–
		B. Sediments		
River Sediment (NBS 1645)	1.70 ± 0.30	0.02 ± 0.01 (n = 8)	0.08 ± 0.03 (n = 8)	0.11 ± 0.02 (n = 4)
Estuarine Sediment (NBS 1646)	0.43 ± 0.02	0.001 ± 0.0006 (n = 8)	0.04 ± 0.02 (n = 8)	0.05 ± 0.01 (n = 4)
Hyco Reservoir sediment	6.45 ± 0.66 (n = 4)	0.21 ± 0.03 (n = 4)	0.51 ± 0.09 (n = 4)	0.63 ± 0.04 (n = 3)

[a] Concentrations in micrograms of selenium per gram. [b] Predicted Se^{IV+VI} equal to total selenium in the exchangeable + carbonate + iron/manganese oxide phases. [c] n = The number of separate samples processed; each sample is determined in triplicate.

Table III. *Total Selenium and Selenium Speciation in Some Biogenic Materials and Sediments from Natural Waters*[a]

type/location	total Se	Se^{IV}	Se^{VI}	Se^{-II+0}[b]
euphausiids/North Pacific Ocean[c]	3.60	0.11	<0.01	3.49
105-m sediment trap material/North Pacific[c]	3.24	0.04	0.21	2.99
232-m sediment trap material/North Pacific[c]	2.48	<0.01	0.16	2.32
sediment/Catfish Lake, Wilmington NC[d]	9.59 ± 0.52 (n = 3)	0.10 ± 0.03 (n = 3)	0.54 ± 0.03 (n = 3)	8.95 ± 0.52
sediment/Great Marsh, DE[e]	0.41 ± 0.02 (n = 3)	0.02 ± 0.01 (n = 3)	0.01 ± 0.005 (n = 3)	0.38 ± 0.02

[a] Concentrations in micrograms of selenium per gram. [b] Determined as the difference between total Se and Se^{IV+VI}. [c] Due to small sample size, replicate samples not analyzed. [d] From phase leaches on this sediment, Se^{IV+VI} predicted to be 0.61 ± 0.15 μg of Se/g (n = 3); 0.64 ± 0.04 μg of Se/g recovered. "n" is the number of replicate samples. [e] From phase leaches on this sediment, Se^{IV+VI} predicted to be 0.03 ± 0.01 μg of Se/g (n = 3); 0.03 ± 0.01 μg of Se/g recovered. "n" is the number of replicate samples.

Moreover, no contamination to the selenite + selenate fraction from organic selenides occurs due to the removal of organic species by XAD-8. The results for the speciation leach of biogenic reference materials can be found in Table IIA. If the work of Wrench and co-workers (*15–17*) is considered (i.e., selenium is primarily organic selenide species in biogenic material), then the amount of organic selenide can be estimated from the difference between total selenium and selenite + selenate.

Sedimentary Selenium. The radiotracer studies demonstrated that selenite and selenate can be desorbed by using alkaline conditions. Due to the possible variety of selenium species in sediments, selenite and selenate leach accuracy was examined in an additional manner. As discussed above, adsorbed selenite and selenate would be concentrated in the inorganic phases of a sediment particle. These phases can be selectively solubilized by using procedures described by Tessier et al. (*1*), and the concentration of total selenium within each phase can be determined (these leaches destroy chemical speciation). The concentration of selenite and selenate in a sediment would then be equal to the sum of total selenium in the exchangeable, carbonate, and iron and manganese oxides phases; this represents a working assumption *only*. It should also be pointed out that these phase definitions are operational; for example, the organic fraction represents any selenium solubilized by oxidation (e.g., elemental selenium, organic selenide, or inorganic selenide such as ferroselite).

The Tessier procedure (*1*) was used on two reference sediments (Estuarine and River) and a sediment from Hyco Reservoir, the cooling water source for Carolina Power and Light's Roxboro Steam Electric Plant (Roxboro, NC). The results for these selenium phase leaches (expressed as percentages of the total selenium) can be seen in Figure 1. With the above working assumption and phase leach data, the amount of adsorbed selenite and selenate in the sediments should be 0.11 ± 0.02 μg of Se/g (7.3 ± 1.3% of reported total Se) for River Sediment, 0.05 ± 0.01 μg of Se/g (8.3 ± 1.6% of reported total Se) for Estuarine Sediment, and 0.63 ± 0.04 μg of Se/g (9.8 ± 0.6% of total Se) for the Hyco sediment.

The same 1 M NaOH leach method used for plankton samples was applied to the reference and Hyco Reservoir sediments. An alkaline sediment leach has several advantages in that biogenic selenite and selenate can be released (this work) and selenite in hydrous ferric oxides (a common mineral phase in sediments) is solubilized (*19*). Furthermore, the

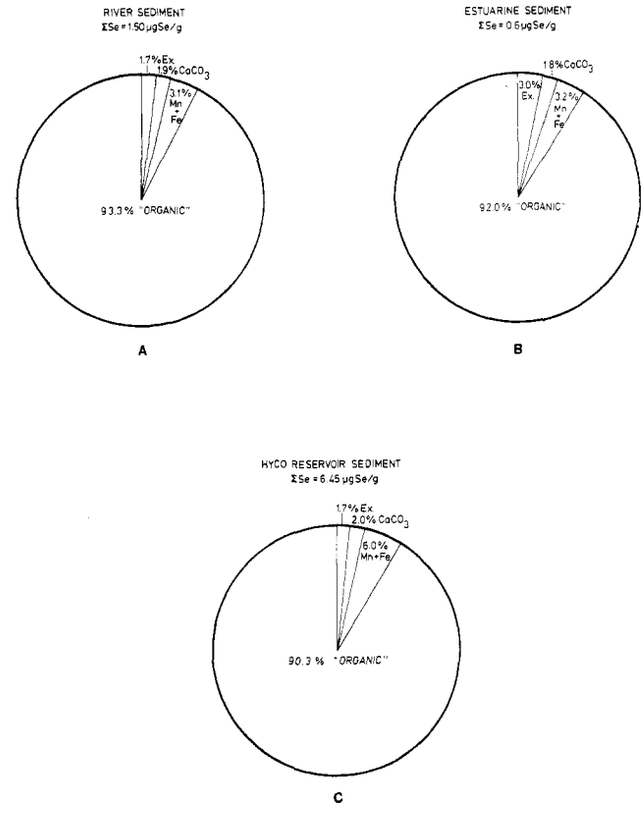

Figure 1. Association of sediment-bound selenium in exchangeable (Ex.), carbonate ($CaCO_3$), iron and manganese oxides (Fe + Mn), and organic ("Organic") phases as defined by Tessier et al. (ref 1) for: (A) NBS River Sediment (percentages of reported selenium concentration based on n = 4 samples); (B) NBS Estuarine Sediment (percentages of reported selenium concentration based on n = 4 samples); and (C) Hyco Reservoir (North Carolina) sediment (percentages of total selenium concentration based on n = 3 samples).

selenite stability experiment above was repeated in the presence of sediments, and no detectable speciation change is observed. Since organic compounds (notably humic substances) are released by sodium hydroxide treatment (*21*), the leachate (after pH adjustment and centrifugation) is passed through XAD-8 columns as previously discussed.

Results for the alkaline sediment leaches for River, Estuarine, and Hyco sediments can be found in Table IIB. Close agreement between recovered (speciation leach) and predicted (phase leach) selenite + selenate concentrations is shown. The alkaline leach appears to quantitatively solubilize selenium in the sediments' exchangeable, carbonate, and iron and manganese oxides phases. The stability of selenium species under alkaline conditions further suggests that selenite and selenate comprise the selenium content of these three inorganic phases, in support of the original working assumption. Until a suitable chemical speciation standard for environmental materials is available, more rigorous evaluations of speciation accuracy will be difficult to obtain.

The concentrations of adsorbed selenite and selenate in the sediments (Table IIB) are very low (compared to the total selenium concentration), leading to larger analytical variability (e.g., the average relative standard deviation for selenite + selenate is 27%). Some of this variability may also be due to sample inhomogeneity. However, the results in Table IIB show the alkaline leach to be accurate and relatively precise for sediments in natural waters. By use of the total oxidative selenium digest (nitric/perchloric) and the sodium hydroxide leach, the concentration of selenide + elemental selenium can be estimated from the difference between total sedimentary selenium and adsorbed selenite + selenate.

Sample Storage for Speciation Leaches. The stability of selenite and selenate during storage of chemical speciation leachates was examined by using artifically spiked samples (30 mL linear polyethylene bottles; 10 mL pH 1.6 (HCl) water, 1.2 ng of Se/mL as selenite or selenate, refrigerated storage). Refrigeration is used since some of the leachates (e.g., from plankton) might support the growth of microorganisms and result in speciation changes. After 25 days of storage, 95 ± 16% of the selenite (n = 6) and 100 ± 10% of the selenate (n = 6) spikes were recovered. While much of the observed variability may be due to spiking (pipetting) errors, the data are similar to other reports (*22*) and show that storage over a 3-week time period is acceptable.

Application to Field Samples. In addition to the Hyco Reservoir sediment used in methods development, many different types of field samples have been subjected to these speciation procedures; some of the results are presented in Table III. If the data in Tables I–III are considered together, the procedural precision (expressed as relative standard deviation) is 8.8% (n = 8) for total particulate selenium, and 19.3% (n = 6) for particulate selenite + selenate. These data indicate that selenite and selenate are a small percentage of sediment-bound and planktonic selenium.

ACKNOWLEDGMENT

The author thanks the Center for Marine Studies (University of California, Santa Cruz) for the use of their analytical facilities during the radionuclide portion of this work and Carolina Power and Light (New Hill, NC) for the sample of Hyco Reservoir sediment.

Registry No. Selenium, 7782-49-2; selenite, 14124-67-5; selenate, 14124-68-6; water, 7732-18-5.

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RECEIVED for review June 7, 1985. Accepted July 24, 1985. The sediment work was supported by Grant RP 2020-1 from the Electric Power Research Institute, Palo Alto, CA.