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Measurement of organic-^{35}S and organic-S in lake sediments: Methodological considerations

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Abstract. Three protocols for the determination of inorganic and organic sulfur fractions were tested for their suitability to estimate total indigenous organic sulfur (S_{org}) and $^{35}S_{org}$ formed from added $^{35}SO_4^{2-}$ in sediments of chemically dilute lakes in the ELA. The protocols tested have all been reported in the literature. It was found that two protocols involving sequential analyses for S fractions following acid treatment gave estimates of both S_{org} and $^{35}S_{org}$ up to 87% lower than a non-sequential protocol. The low estimates were largely due to hydrolysis and solubilization of solid phase S which was then removed in a rinsing step. The non-sequential protocol, in which total reduced inorganic sulfur and total sulfur were determined on separate aliquots, is recommended as the most reliable of the three. Individual analyses in this protocol were verified for these lake sediments using a variety of S standards.

Introduction

Organic-S (S_{org}) constitutes the majority of sedimentary S (often $>80\%$) in lakes and consists of C-bonded and sulfate ester forms (Mitchell et al. 1984; David & Mitchell 1985; Nriagu & Soon 1985). Although organic matter imported from the watershed and produced by water column biota can be major sources of sedimentary S_{org}, stable and radioisotope studies have shown that it is also formed *in situ* from sulfate reduction, through reactions of H_2S with organic compounds (Landers et al. 1983; Nriagu & Soon 1985; Rudd et al. 1986a; Landers & Mitchell 1988; Baker et al. 1989). These, and similar results in freshwater peat (Behr 1985; Brown 1986), have stimulated interest in the nature of sedimentary S_{org} that forms as a result of sulfate reduction, as opposed to S_{org} that was previously thought to be derived solely from plant and algal remains. Formation of S_{org} in this way is of particular importance in freshwater systems because it provides a pathway for long term alkalinity production from sulfate reduction (Rudd et al. 1986a) other than the formation of insoluble iron sulfide minerals by the reaction of H_2S and Fe (e.g. Kelly et al. 1982; Cook & Schindler 1983; Berner 1984).

Our understanding of the importance of S_{org} in the lacustrine S cycle depends greatly on the ability to quantify it accurately. S_{org} in sediments is estimated indirectly either as the difference between inorganic sulfur (S_{inorg}) and total S (S_{total}), or as the fraction remaining after all S_{inorg} has been removed (Fig. 1). Thus, accurate determination of both S_{total} and S_{inorg} is necessary to obtain accurate values of S_{org}. Previous investigations, however, have shown that in some instances S_{org} is underestimated by current methods for S_{total} (Amaral et al. 1989) and S_{inorg} (Brown 1986). Difficulties are not surprising given the complex variety of S compounds found in sediments, and the fact that analytical methods often involve multiple sequential determinations (Table 1). To date, there is no reliable direct method for determining total S_{org} in sediments.

The same methods (Table 1) are also used in sulfate reduction studies to track incorporation of radiolabelled sulfate-S into organic and inorganic pools. Newly formed ^{35}S fractions have a different distribution from pre-existing cold S fractions (Rudd et al. 1986a; Amaral 1991) and may have different chemical characteristics. Thus, it is important to know the accuracy of these methods for both ^{35}S and cold S in order to further our understanding of the short term, as well as long term, fate of sulfur in lake sediments.

In this study we examine three of the common methods currently in use as to their suitability for measuring S_{org} and $^{35}S_{org}$ in lake sediments. Towards this end, two important questions are considered. First, what are the specificities and efficiencies of the different analytical steps involved? Second, what is the effect of the order in which the analytical steps are done?

Materials and methods

Site description

Epilimnetic sediment was obtained from several lakes at the Experimental Lakes Area (ELA). The ELA (northwestern Ontario 93°30′–94°00′W and 49°30′–49°45′N) is situated on the Precambrian Shield (Brunskill & Schindler 1971). Sediment was also obtained from Lake 5-0, a small acid

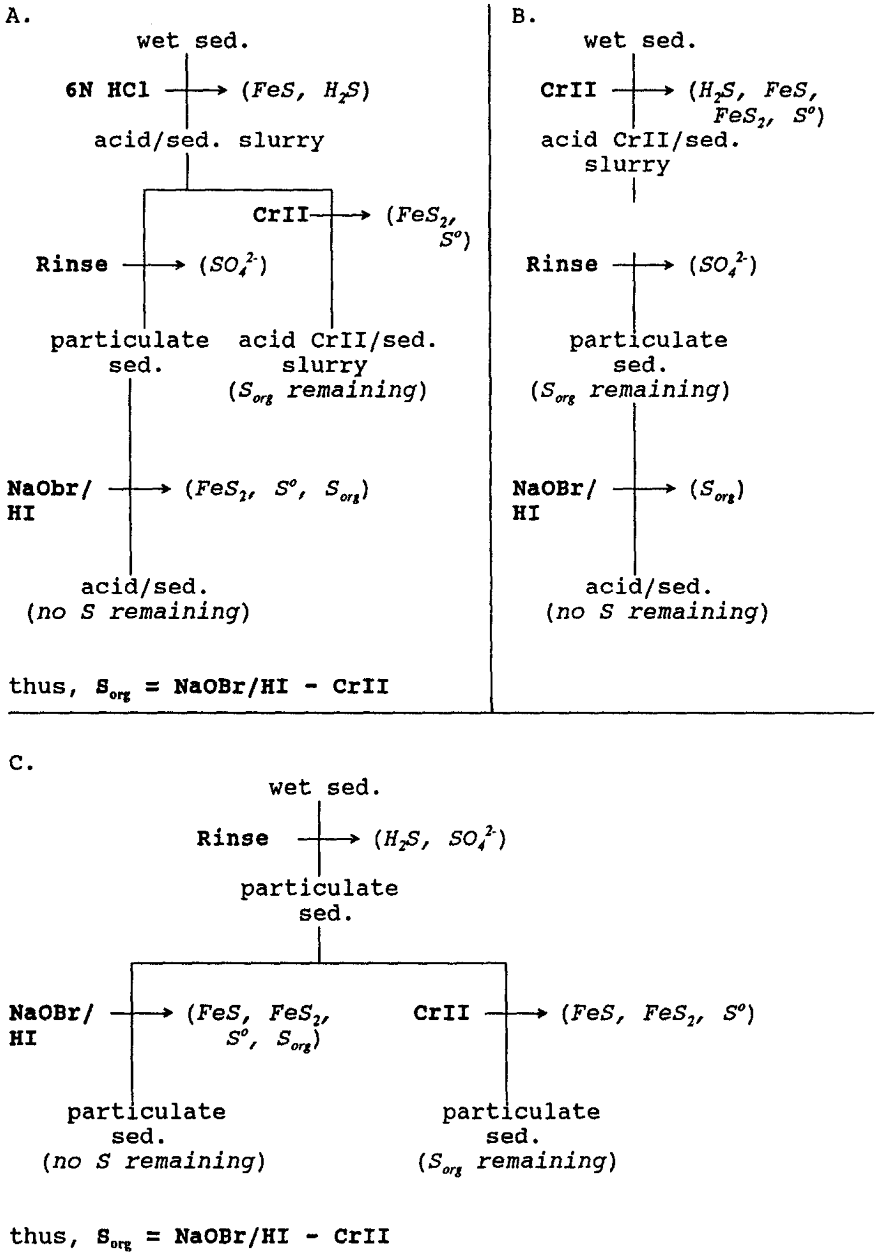

Fig. 1. Three protocols used in the analysis of S and ^{35}S fractions in sediments and peats. All three protocols have been described in the literature (A, Rudd et al. 1986a; B and C Wieder & Lang 1988), and the major S compounds assumed to be measured at each step are given in brackets. More detailed descriptions of the compounds obtained by different analyses are given in Table 1.

Table 1. Sulfur species contained in some S fractions described in the literature. Details of the treatments used to obtain each S fraction are given in the text.

Sulfur fraction	Main sulfur species	Example references
HCl volatile (AVS)	Dissolved H_2S and HS^-, FeS	1
HCl soluble (pre-water rinse)	Pore-water SO_4^{2-}, $S_2O_3^{2-}$, polythionates, polysulfides, soluble organic-S, HCl-hydrolyzable organic S (e.g. sulfated polysaccharides and amino acids)	2
CrII reducible	FeS, FeS_2, S^0, $S_2O_3^{2-}$, poly-sulfides (organic/inorganic), polythionates	3, 4 5, 6
S_{org} (S_{total} — CrII reducible)	C-O-S (ester sulfates), C-N-S (sulfamic acid), C-S (e.g. amino acids, petides, protein, sulfolipids, sulfonic acids, heterocyclics.	2,6 7
water soluble	Pore water SO_4^{2-}	8

Modified from Krairapanond et al. (1991).

[1] Jorgensen & Fenchel (1974)

[2] Nriagu & Soon (1985)

[3] Zhabina & Volkov (1978)

[4] Wieder et al. (1985)

[5] Canfield et al. (1986)

[6] Amaral (1991)

[7] Fitzgerald (1976)

[8] for ELA sediment; this work

lake in the Florida panhandle, for use in S recovery experiments (see below).

The ELA lakes (114, 223, 224, 239, 240 and 302 South) are small (A_0 = 10.9—56.1 × 10^4 m^2), shallow ($\bar{Z}$ ■ 1.7—10.5 m), and among the most chemically dilute in the world with overlying water sulfate concentrations in the order of 31 μmol L^{-1} (Brunskill & Schindler 1971; Armstrong & Schindler 1971). Lake 302 South has been experimentally acidified with sulfuric acid (Rudd et al. 1990) and at the time of sampling had an epilimnetic sulfate concentration of 104—125 μmol L^{-1}. Pore water SO_4^{2-} concentrations in ELA lakes are low with values of 3—37 μmol L^{-1} reported for Lakes 114 and 302 South (Rudd et al. 1986a).

Sediment description and handling

Lake 114 sediment was of high porosity (>0.95; where porosity =

volume of water/volume of water + solid sediment) and high organic content (loss on ignition (L.O.I.) = 58.5% by weight; Brunskill et al. 1971). Sediment from Lake 302 South was of low porosity (0.5—0.64) and visually described as sandy. This type of sediment contains little organic matter, with values of organic C and N $< 2.0\%$ on a dry weight basis (Sweerts et al. 1986). More details of sedimentary chemical characteristics are given in Rudd et al. (1986b). The sediment obtained from Lakes 223, 224, 239, and 240 was visually similar to that from Lake 302 South, and was of comparable porosity (0.55—0.63). Sand from lake 5-0 was very fine and compact (porosity < 0.5) and had very low S content (< 1 μmol S g^{-1} dry weight), making it ideal for experiments testing the effect of sediment on recovery of S from different compounds.

Cores were retrieved by diver from depths of 1.5 to 2 m. Radiolabelled ^{35}S-sulfate (carrier-free) was added to the overlying water of undisturbed cores from Lake 302 South within 30 hours of collection in order to generate $^{35}S_{org}$. The cores were incubated at 21 °C for 24—48 hours and then sliced at 1 cm intervals. The slices were immediately frozen in whirlpack bags under a N_2 atmosphere until analysis. Indigenous S_{org} and recently formed $^{35}S_{org}$ were measured in cores from Lake 302 South by three analytical protocols (see below). The presence of sulfate in the overlying water, as well as significant pool sizes of reduced S in this sediment (Rudd et al. 1986a), should minimize incomplete recovery of ^{35}S due to adsorption during analysis, thus addition of carrier S was unnecessary. Other sediments were homogenized (top 2 cm) before use in S recovery experiments.

Chemical analyses

Acid volatile sulfur (AVS = mostly H_2S and amorphous FeS) was determined by acidification of sediment with deoxygenated 6N HCl (25 °C) under N_2 gas (Rudd et al. 1986a). The resulting acid slurry may contain several non-volatile, soluble S components (Table 1). The low temperature used should minimize underestimation of AVS by potential oxidation of S^{2-} to S^0 by FeIII (Chanton & Martens 1985; Cornwell & Morse 1987).

Total reduced S_{inorg} (primarily FeS, FeS_2 and S^0) was determined by conversion to H_2S by boiling with acidic 1M $CrCl_2$ for 1.5 h (Zhabina & Volkov 1978). This S fraction is commonly referred to as chromium (CrII) reducible S (CRS), although for some compounds (e.g. FeS) conversion of S to H_2S is via reductive dissolution by acid, the S being already in its most reduced state (S^{2-}).

Total sulfur was determined by preliminary oxidation with NaOBr (250 °C) followed by hydriodic acid (HI) reduction of the resulting sulfate to H_2S as described by Tabatabai & Bremner (1970) and Amaral et al. (1989). The analysis was done on wet sediment to avoid S losses due to drying (Amaral et al. 1989). This reducing solution of HI, hypophosphorous and formic acids of Johnson & Ulrich (1959) was prepared as described in Amaral et al. (1989).

The H_2S and $H_2^{35}S$ produced by acid volatilization, and CrII and HI reduction was sparged from the sediment by N_2, trapped in zinc acetate-NaOH traps (Howarth & Teal 1979), and quantified iodometrically. Radiolabel was measured by counting trap aliquots in a Rackbeta (LKB) liquid scintillation counter.

Specificities and efficiencies of S recovery by CrII reduction and alkaline oxidation-reduction (NaOBr-HI) were determined using several inorganic and organic standards (10—20 μmoles) in the presence of sediment from two of Lakes 114, 302 South and 5-0 (1.5—5 g). The recovery efficiency of the NaOBr-HI method for S_{total} was also determined using standards alone. The efficiency of the AVS method is 95% with a standard of CdS (Rudd et al. 1986a).

Unreacted $^{35}SO_4^{2-}$ was determined by rinsing the sediment once with deoxygenated 2.5 M $MgSO_4.7H_2O$ and distilled water to displace any adsorbed $^{35}SO_4^{2-}$ (Rudd et al. 1986a) followed by 3 more rinses with deoxygenated, distilled water alone. Rinse water and sediment were separated by centrifugation (20 min. at 3000 rpm). ^{35}S isotope in aliquots of the supernatants was measured by scintillation counting. The efficiency of the rinsing step was tested by mixing $^{35}SO_4^{2-}$ with sediment for 30 min., at 4 °C, and under room atmosphere to minimize sulfate reduction, then rinsing and quantitating in the above manner.

Unlabelled sulfate and H_2S are negligible in ELA pore waters ($< 1\%$ of total S; Amaral unpub. data) and concentrations of dissolved volatile S_{org} (dimethyl sulfide, methanethiol, carbonyl sulfide and dimethyl disulfide) are also very low (Richards et al. 1991). Therefore, these fractions were not analyzed. This may not be the case in other systems, such as in salt marsh sediments, where the concentration of soluble reduced S compounds is high (Luther et al. 1986) and where cold and radiolabelled H_2S may accumulate.

Analysis protocols

In addition to testing the specificity and efficiency of the chemical analyses with freshwater lake sediments, the effect of order of analysis was also examined. Three different protocols (i.e. the order in which chemical analyses are done; Fig. 1), all of which have been described in the literature, were examined for the ability to accurately estimate S_{org} and $^{35}S_{org}$ in these low S sediments (0.03—0.5% S by weight; Amaral unpub. data).

In the first protocol (A), AVS was determined first on wet sediment. One half of the resulting sediment-acid slurry was then analyzed by CrII reduction, while the other half was first rinsed, to remove unreacted $^{35}SO_4^{2-}$, and then analyzed for total remaining S (Rudd et al. 1986a). This protocol has been used to measure the distribution of $^{35}SO_4^{2-}$ due to sulfate reduction, and employs an aqua regia oxidation to determine total ^{35}S (but not unlabelled S). For this study, the aqua regia step was replaced with the alkaline oxidation method (NaOBr-HI) in order to quantify both labelled and unlabelled S pools. Estimates of $^{35}S_{org}$ and S_{org} were obtained as the difference between the CrII and NaOBr-HI measurements (Fig. 1).

In the second protocol (B), CRS (all non-sulfate S_{inorg}) was determined first on wet sediment. The sediment was then rinsed to remove unreacted $^{35}SO_4^{2-}$, and analyzed (by NaOBr-HI) for total remaining S which, at this stage, should be only S_{org} (Wieder & Lang 1988).

In the third protocol (C), S_{total} (i.e. NaOBr-HI analysis) and CRS were determined on separate sediment aliquots after the sediment was rinsed to remove soluble sulfur (i.e. H_2S and SO_4^{2-}). S_{org} was estimated as the difference between CRS and S_{total} measurements (e.g. Carignan & Tessier 1988, Wieder & Lang 1988). One important difference between previous and the current work is the fact that we did not dry the sediments prior to determination of S_{total}, to avoid large losses of S which lead to low S_{org} estimates (Amaral et al. 1989).

Results

All three analytical methods tested in this study (CrII reduction, alkaline oxidation-reduction, rinsing) showed, in the presence of sediment, high S(^{35}S) recoveries from compounds for which they were specific (Tables 2—4).

Recovery efficiencies by CrII reduction of S from Fe-S compounds and S^0 were $>90\%$, and interference from recovery of S_{org} and sulfate was very low ($<1\%$) (Table 2). The presence of sediment did not appear to inhibit the action of the CrII reagent, as recoveries of non-sulfate S_{inorg} were high and comparable to previously reported efficiencies for standards alone (Howarth & Merkel 1984; Wieder et al. 1985; Rudd et al. 1986a; Canfield et al. 1986; Hseih & Yang 1989).

The efficiency of the NaOBr-HI method for total S, although high, was affected differently by different sediments (Table 3). Recoveries of 85—

Table 2. Specificity and efficiency of the chromium reduction method for the measurement of S in different chemical compounds. Analyses were carried out in the presence of 5 g of sediment from Lakes 114 and 302 South (see text for sediment description).

Chemical species	% of S recovered ($n = 2$–5)
$FeS_{1.8}$[1]	100 ± 10
$Na_2S_2P_3$	68 ± 3
Na_2SO_4	<0.1
Pyrite ore	95 ± 3
Pyrite ore[2]	92 ± 7
S^0	91 ± 9
Methionine	0.0
Phenylthiourea	5.9 ± 0.5
SDS[3]	1.2 ± 0.3

[1] Prepared as described in Wada (1977)
[2] Analyzed with sand from Lake 302 South
[3] Sodium dodecyl sulfate

Table 3. Efficiency of the total sulfur method for several S compounds in the absence and presence of sediment.

System analyzed	Mean S content μmol/g	μmol S added	% Recovery Range	Mean	SD	n
S Standards (alone)[1]	—	9.2—19.7	90.2—98.4	94.5	3.0	6
Sediment (L114, ELA)	7.77	9.2—19.7	89.6—94.0	91.3	1.9	4
Sediment (L50, Florida)	0.26	9.2—19.7	80.7—90.5	84.5	3.2	6

[1] The sulfur standards used were; phenylthiourea, 2-methyl-2-thiopseudourea sulfate, L-methionine and K_2SO_4. Approximately 1.5 g and 5 g of floccy and sandy sediments were tested (wet weight). See text for description of sediments.

95% were obtained for several organic and inorganic S standards, depending on the presence or absence of sediment (Table 3). The same efficiencies of recovery were obtained with standards analyzed alone and in the presence of sediment from Lake 114. However, sand from Lake 5-0

Table 4. The removal of unreacted $^{35}SO_4^{2-}$ from two sediments by rinsing with $MgSO_4$ and distilled water. Duplicate determinations were carried out on ≈ 30 g of sediment from each of Lake 302 South and Lake 114.

Rinse number[1]	Cumulative recovery of added $^{35}SO_4^{2-}$ (%)	
	L. 302 South	L. 114
1	95.6 ± 9.5	80.0 ± 1.0
2	101.0 ± 9.6	91.5 ± 1.1
3	102.2 ± 9.3	99.0 ± 1.0
4	102.5 ± 9.2	101.1 ± 1.5

[1] Rinse 1 consisted of shaking sediment for 5 min. with 25 mL of 2.5 M $MgSO_4 \cdot 7H_2O$, before adding 150 mL of distilled water and centrifuging. Rinses 2—4 were done using 180—200 mL of distilled water. Further details are given in the text.

interfered with the method and S recovery was 10% lower than for S standards alone (Table 3).

The removal of sulfate by rinsing (shown by $^{35}SO_4^{2-}$) was found to be 80—95% complete in the first rinse for two different sediments (Table 4). The remainder of the $^{35}SO_4^{2-}$ was recovered by subsequent rinses with distilled H_2O, confirming that the rinsing procedure presented here removes all of the unreacted $^{35}SO_4^{2-}$ from lake sediments. Free $H_2{}^{35}S$, as well as unlabelled SO_4^{2-} and H_2S, if present, would also be removed at this step. However, 95% of soluble radiolabel recovered in the above manner from incubated ELA sediments had the same mobility as a $^{35}SO_4^{2-}$ standard under paper electrophoresis and was precipitated by $BaCl_2$ (data not shown), indicating that $^{35}SO_4^{2-}$ was the only important radiolabelled sulfur component in these water rinses.

Estimates of total S_{org} and $^{35}S_{org}$ in lake sediments were severely affected by the order in which individual analyses were done. When protocol A was used on sediment from two Lake 302 South cores, 7 out of 12 core slices analyzed gave negative estimates of $^{35}S_{org}$ (i.e. after $AV^{35}S$) was removed, the $CR^{35}S$ fraction was greater than that assumed to contain both $CR^{35}S$ and $^{35}S_{org}$) (Table 5). These are clearly impossible values indicating that there are problems associated with this protocol. Interestingly, unlabelled S_{org} measured by this same protocol in the same sediment samples were never negative and were generally greater than 50% of total S (Table 5), suggesting indigenous S_{org} is chemically different from S_{org} produced from recent sulfate reduction (Rudd et al. 1986a; Amaral 1991).

Table 5. Estimates of S_{org} and $^{35}S_{org}$ in two sediment cores from Lake 302 South (1.5 m) as determined by protocol A.

Core	Sediment depth (cm)	unlabelled S_{org}	$^{35}S_{org}$
		% of total reduced S(^{35}S)	
1	0–1	70.2	−2.2
	1–2	51.8	−5.8
	2–3	57.0	4.1
	3–4	54.1	−2.4
	4–5	49.4	17.0
	5–6	16.9	72.6
2	0–1	82.9	−11.5
	1–2	77.2	3.2
	2–3	71.0	4.1
	3–4	83.4	−0.7
	4–5	53.0	−1.8
	5–6	37.7	−9.6

Estimates of $^{35}S_{org}$ using protocol B, which unlike protocol A is a sequential approach which does not include a calculation by difference (Fig. 1), were positive for the samples tested (Table 6). However, $^{35}S_{org}$ measured by protocol B was much lower than when analyzed by the third alternative, protocol C, which gave 75–85% higher estimates of $^{35}S_{org}$ (Table 6). The difference in the two protocols was due to the greater amount of soluble ^{35}S with protocol B (>30%) than with protocol C

Table 6. Comparison of ^{35}S-species estimates using two different analysis schemes on each of three slices from a core from Lake 302 South. Refer to Fig. 1 for details of protocols used.

Protocol	Core slice (cm)	Reduced $^{35}S_{inorg}$	Soluble $^{35}S^{1}$	$^{35}S_{org}$
		(% of total ^{35}S recovered)		
B	0–1	64.7	32.3	3.0
	1–2	55.4	37.4	13.0
	2–3	44.9	46.6	8.5
C	0–1	75.0	1.9	23.0
	1–2	47.4	0.5	52.0
	2–3	64.6	5.0	35.4

[1] Soluble ^{35}S refers to the rinse step (see Fig. 1).

(0.5—5%) (Table 6). The water rinse for $^{35}SO_4^{2-}$ recovery in protocol C was shown to be specific for $^{35}SO_4^{2-}$, but the rinse in protocol B (which follows an acidification step) apparently solubilizes more than just unreacted $^{35}SO_4^{2-}$.

Similar results were obtained for estimates of cold S_{org} in sediments from different lakes (Table 7). Protocol B resulted in significantly lower (48—66%) S_{org} estimates than protocol C in 4 out of 5 sediments analyzed (Table 7), indicating much of the indigenous S_{org} was solubilized or hydrolyzed by hot acid during the CrII reduction step (Fig. 1).

Table 7. Estimates of S_{org} in sediment as determined by two different protocols (B and C). Single determinations by each protocol were done on subsamples from core slices of the top 2 cm of sediment from several lakes.

Sediment source	S_{org} (μmol S g^{-1} wet weight)		
	Protocol B	Protocol C	B/C
L 223	0.9	1.7	0.52
L 224	1.7	1.7	1.00
L 239	0.9	6.2	0.15
L 240	1.1	2.5	0.44
L 302S	1.4	2.7	0.52

Discussion

The results showed that current methodologies may severely underestimate the content of S_{org} and production of $^{35}S_{org}$ from $^{35}SO_4^{2-}$ reduction in lake sediments. Potential problems can arise in three areas, these being the (i) efficiency, (ii) specificity, and (iii) sequence of individual chemical analyses used to determine different S fractions. Each of these points are in turn discussed below with a view to identifying the procedure expected to give the most accurate results.

In previous studies, the efficiencies of the CrII reduction and NaOBr-HI methods have been determined with several S-containing standards (e.g. Tabatabai & Bremner 1970; Howarth & Merkle 1984; Wieder et al. 1985; Canfield et al. 1986; Rudd et al. 1986a; Amaral et al. 1989), but the possible variable effects by the matrices in which they are to be measured in nature have not always been examined. We found that the efficiency of the NaOBr-HI method for S_{total} varied depending on the type of sediment being analyzed. The method was equally effective in the absence as in the presence of highly organic, floccy sediment (Table 3; Amaral et al. 1989), as has been observed for soil (Tabatabai & Bremner 1970) and peat (Wieder & Lang 1988). However, sandy sediment interfered with the method, decreasing recoveries by about 10% as compared to the standards alone (Table 3). The reason for this decrease in recovery was not examined but it is interesting to note that Freney et al. (1970) found that removal of silicates from soil, by hydrofluoric acid extraction, often resulted in slightly higher recoveries of HI-reducible S. In contrast, chromium reduction was not affected by the presence of sediment, since our observed recoveries (Table 2) were high and comparable to those obtained with standards alone (Wieder et al. 1985; Canfield et al. 1986; Rudd et al. 1986a). Sediment from a *Juncus* marsh has also been shown not to interfere with the method (Hseih & Yang 1989), while others have reported slight interference (Howarth & Merkel 1984).

Failure to correct the NaOBr-HI method for lower efficiency in sandy sediment would lead to underestimates of both S_{total} and S_{org} (often calculated as S_{total} − CRS). For example, in radiotracer experiments with some ELA sediments, where about 25% of reduced $^{35}SO_4^{2-}$ distributes into the $^{35}S_{org}$ fraction (Amaral 1991), a 10% underestimate of $^{35}S_{total}$ results in a 40% underestimate of the $^{35}S_{org}$ pool.

Because of the sequential nature of the methods used (Fig. 1), accurate estimates of S_{org} depend on the specificity of each of the individual analytical steps for different S fractions. When tested with a variety of S compounds, CrII reduction was specific for non-sulfate S_{inorg} (Table 2), as has been previously found for inorganic and organic S standards including Fe-S minerals, protein and thiophene (e.g. Wieder et al. 1985; Canfield et al. 1986). However, because the nature of sedimentary S_{org} is largely unknown there is no sure way to test whether some S_{org} species are chromium reducible. Indeed, recent detailed work on the extraction and characterization of sedimentary S_{org} (Amaral et al., in prep.) has shown that a portion of the S in some organic compounds is released by chromium reduction (Table 8), and the conclusion that only S_{inorg} is measured by this technique is inaccurate. A similar observation was also made by Brown (1986) who measured chromium reducible S in peat from which all S_{inorg} had been removed and obtained greater than 14% of S_{org}. Similarly, we have found that 10—20% of $^{35}S_{org}$ was obtained by this analysis in an ELA sediment (Amaral et al., in prep). Therefore, chromium reduction may overestimate the amount of S_{inorg} in sediments and so underestimate S_{org}.

The most important factor in the determination of $(^{35}S)S_{org}$ in sediments was the sequence in which individual analyses were performed. The most

Table 8. Chemical characterization of $^{35}S_{org}$ components isolated from 2.5 N HCl extracts of $^{35}SO_4^{2-}$-incubated sediment from Lake 303 at the ELA. The components were separated by Sephadex G-10 and LH-20 chromatography.

Component name[1]	% of total $^{35}S_{org}$ produced	% of $^{35}S_{org}$ that is CrII reducible
HCl i	10.5	40
HCl ii	5.1	25
HCl iii	4.0	15
HCl iv	4.4	6
HCl v	nd[2]	nd
HCl vi	3.4	33

Modified from Amaral et al. (in prep).

[1] The absence of $^{35}S_{inorg}$ in components HCl i to vi was verified by electrophoretic and chromatographic techniques (Amaral et al., in prep), so that all $CR^{35}S$ is considered to be derived from $^{35}S_{org}$. Soluble S_{inorg} was removed by extraction with pyrophosphate buffer prior to HCl extraction.

[2] nd = not determined

problematic protocols, A and B, were those which involved a preliminary treatment with strong acid (Fig. 1). These protocols gave low (and even negative) estimates of S_{org} and $^{35}S_{org}$ (Tables 4, 6 & 7), due in part to solubilization and hydrolysis of solid phase S_{org} (Table 1). Proof of this was obtained by chromatography of ^{35}S extracted from sediment with 2.5 N HCl, which showed that the radiolabel was associated with $^{35}S_{org}$ compounds (Table 8; Amaral et al., in prep.). Similarly, many ester sulfates, a labile group of S_{org} compounds, release free sulfate by acid hydrolysis (King & Klug 1980).

If this solubilization and rinsing away of $^{35}S_{org}$ were the only problem, protocol A would not lead to negative $^{35}S_{org}$ estimates. For example, in the worst case, if all $^{35}S_{org}$ was solubilized and removed in the rinse, only $^{35}S_{inorg}$ would remain, and calculating $^{35}S_{org} = {}^{35}S_{total} - CR^{35}S$, would result in a value of zero. However, sulfur in some of these acid soluble S_{org} compounds, contrary to current assumptions, is recovered by CrII reduction (Table 8). If these compounds, as well as inorganics such as $S_2O_3^{2-}$, polythionates and polysulfides (Table 1), are present as soluble components in the acid supernatant, they will be measured by CrII reduction but not by NaOBr-HI (i.e. S_{total}) analysis, since protocol A involves a rinse before this step (Fig. 1). This situation can lead arithmetically to negative estimates of $^{35}S_{org}$. Therefore, CrII reducible $^{35}S_{org}$ in the acid supernatant causes a decrease in the estimate of total $^{35}S_{org}$ in two ways: i) by increasing the apparent size of the S_{inorg} fraction, and ii) by being rinsed away before NaOBr-HI analysis.

Thus, protocol A gives minimum estimates of $^{35}S_{org}$ in sediments. The degree of underestimation will depend on how much $^{35}S_{org}$ is acid-solubilized and rinsed away and the amount of soluble ^{35}S (organic and inorganic) that is CrII reducible. The potential problems associated with this protocol are summarized in Fig. 2.

Protocol B has been used to measure the endproducts of $^{35}SO_4^{2-}$ reduction in peat, where it has some verification (Wieder & Lang 1988). However, as previously discussed, because the procedure involves sequential determinations and an initial contact of the sediment with hot, concentrated HCl (during the CrII reduction step) it too underestimates $^{35}S_{org}$ in these sediments. This was apparent from a comparison analysis with protocol C in which the latter gave much higher estimates of $^{35}S_{org}$ (75—87%; Table 7) and S_{org} (48—66%; Table 7). The large percentages of soluble ^{35}S obtained with protocol B as compared to protocol C (Table 6)

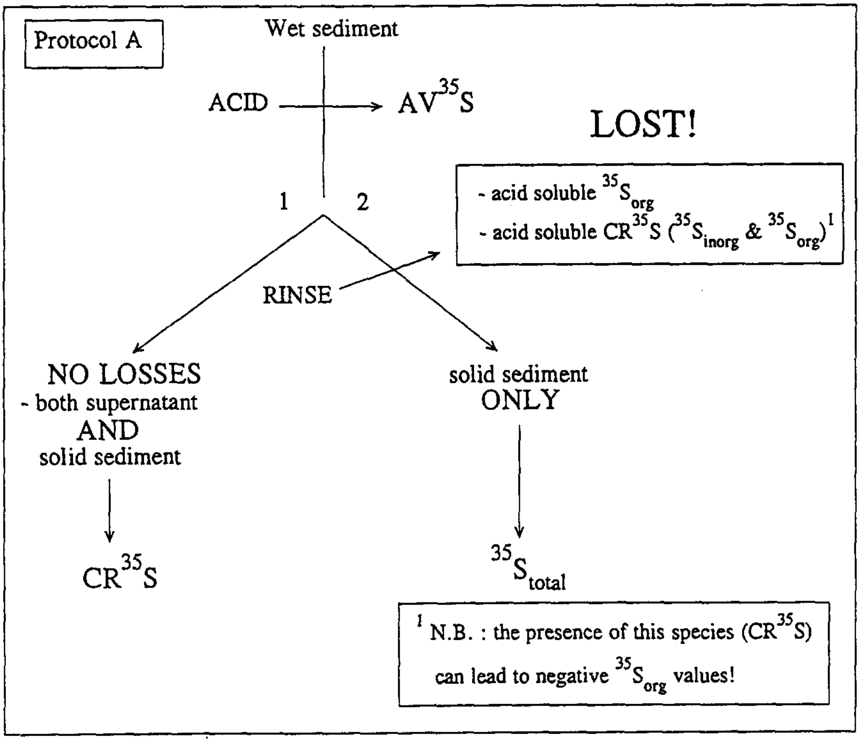

Fig. 2. Summary outline of the possible problems leading to underestimates and negative estimates by protocol A (see Fig. 1 and text).

substantiate the hypothesis that acid addition solubilizes more than just unreacted $^{35}SO_4^{2-}$.

Interestingly, ester sulfate compounds in peat from Big Run Bog, where protocol B has been used (Wieder & Lang 1988; Yavitt & Lang 1990), are resistant to acid hydrolysis (Jarvis et al. 1987), and in this respect differ from their lacustrine counterparts (King & Klug 1980). This fact illustrates how a method may be suitable for use in some but not all environments.

Protocol C, which has been used to determine unlabelled S fractions in peat (Wieder & Lang 1988) and, with only minor modifications, in freshwater sediments (e.g. Carignan & Tessier 1988; Landers et al. 1983), is non-sequential and does not involve acid treatment preceding either CrII or NaOBr-HI analyses (Fig. 1). Therefore, underestimates of $^{35}S_{org}$ due to acid hydrolysis do not occur. Although protocol C gave higher estimates of S_{org} and $^{35}S_{org}$, the question still remains as to whether or not it is accurate. This accuracy depends on the efficacy of the three analytical steps: rinse, NaOBr-HI analysis and CrII reduction (Fig. 1).

As previously discussed, the NaOBr-HI method is reliable provided the efficiency is tested with each sediment used (Table 3) and the analysis is done on wet sediment (Amaral et al. 1989). Similarly, the rinse method was found to be specific for unreacted $^{35}SO_4^{2-}$ (and expectedly SO_4^{2-}) in the sediments studied (Table 4; see Results). However, evidence that some S_{org} is CrII reducible (Table 8) means that protocol C can underestimate S_{org} if this type of compound is present in significant quantities. Such underestimates may be higher, for example, in salt marsh sediments where greater formation of CrII reducible organic polysulfides (Table 1) is believed to occur due to reaction of high concentrations of polysulfides with organic matter (Boulegue et al. 1982). Nevertheless, this protocol is the preferred of the three since it minimizes underestimation of S_{org}. This is evident from the results (Tables 4, 6 & 7).

Protocols B and C affected labelled and unlabelled S fractions the same way, whereas unlabelled S_{org} was more resistant than $^{35}S_{org}$ to loss during analysis by protocol A (Table 5). The high S_{org} estimates obtained with protocol A suggest that strong acid alone (at 25 °C) is not sufficient to solubilize a large percentage of this material without heat (Table 4). This difference may be because S_{org} already present in the sediment originated from algal and terrestrial plant and animal sources and not only from sulfate reduction. Furthermore, long-term diagenetic changes in S_{org} produced from sulfate reduction could lead to very acid resistant types of S compounds, such as sulfonic acids (Ferdelman et al. 1991).

In conclusion, it was found that sequential analysis of S fractions in several ELA sediments lead to underestimates of S_{org} and $^{35}S_{org}$ if preliminary analyses involved strong acid. A scheme which determines total reduced S_{inorg} and S_{total} on separate aliquots of a sediment gave the most reliable estimates of both S_{org} and $^{35}S_{org}$. The use of protocols A and B can lead to an underestimation of the importance of S_{org} in the sulfur cycle as a mechanism of sulfur storage. As a consequence, the amount of alkalinity generated by sulfate reduction in lake sediments may also be severely underestimated if the S_{org} produced by this process is not quantitatively measured.

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