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Improved, high-throughput approach for phosphorus speciation in natural sediments via the SEDEX sequential extraction method

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Appendix 1: Detailed SPExMan-SEDEX protocol

I. Preparation and general procedural information
  A. Equipment
    1. Freeze dryer (lyophilizer)
    2. Shaker table
    3. Repipette or other volumetric reagent dispenser, capacity 15 to 30 mL (precision ±0.1 mL) (acid-clean in 10% HCl)
    4. Agate mortar and pestle (acid-clean in 10% HCl).
    5. Adjustable-volume pipettes (10–100 µL, 100–1000 µL, 1000–5000 µL)
    6. Analytical balance (minimum precision 0.0001 g)
    7. No. 120 U.S.A. Standard Testing Sieve (Tyler equivalent 115 mesh) with 125-µm openings
    8. 0.40- or 0.45-µm polycarbonate filters
      a. acid clean in 10% HCl 24–48 h and rinse copiously with distilled H_2O immediately before use
      b. filters can be stored in 10% HCl for prolonged periods
    9. SPExMan manifold and reaction vessels as described in the text
  B. Glassware/plasticware (clean in phosphate-free detergent, soak 24–48 h in 10% HCl, and oven- or air-dry completely before use)
    1. SPExMan sediment extraction vessels, filter holders, stopcocks, caps
    2. 8- and 20-mL polyethylene scintillation vials with polyethylene linerless caps (Wheaton #986704) for collection of filtered supernatants
    3. 50- or 100-mL waste beaker
    4. Glass ashing jars
      a. must withstand 550°C muffle furnace
      b. Corning Pyrex culture jars with orange polypropylene caps and clear polypropylene gaskets [Corning cat. no. 1395-100] are ideal
      c. in addition to acid cleaning, premuffle jars at 550°C before use; plastic gaskets must be removed before muffling and replaced before extraction
    5. Spatulas for weighing samples (metal), dithionite (ceramic), and for scraping ashing jars to resuspend sediment (metal), if necessary after ashing in muffle furnace during step V
  C. Preparation of samples
    1. Freeze-dry sediment samples.
    2. Disaggregate dry sediments in an agate mortar and pestle; homogenize completely.
    3. Take a split from the homogenized sample for sieving, archiving the remainder.
    4. Pass entire sample split through a sieve with 125-µm openings. Use the mortar and pestle to grind sparingly when necessary, so that the entire split passes through the sieve. Minimal grinding of sediments can be achieved by repeated transfer between sieve and mortar to remove material <125 µm in size at numerous times during the grinding of a single sample. Excessive grinding will alter the surface area of the sample and change its inherent solubility characteristics. It is important that the entire split be passed through the sieve to have a sample split representative of the homogenized parent sample.
    5. Weigh out sample for extraction on glassine weighing paper, on an analytical balance, and transfer into acid-cleaned, assembled SPExMan reaction vessels with acid-cleaned filter installed. For sequential extraction of sediments, 0.10 gdw is recommended.
    6. If filtered concentrates of suspended sediments are to be extracted, place entire filter within reaction vessel assembled as described in 5 above.
  D. Important procedural details of extraction: Extraction conditions used in the standardization experiments detailed in Ruttenberg (1992) must be replicated identically when the SEDEX method is applied to sediments so that the operationally defined separations of different forms of phosphorus apply. These conditions are summarized below.
    1. Sediment grain size ≤ 125 µm, with minimum grinding necessary to achieve this size
    2. Temperature of extraction 25°C
    3. Extractant composition (Appendix 3 below; see also Ruttenberg 1992, Table 1).
    4. Duration of extraction (Fig. 1)
    5. Solid:solution ratios 1:100
      a. This is an especially critical parameter because use

of excess solids can result in incomplete dissolution of target phases due to saturation of the solution with respect to that target phase. For most sediments, we have found that a solid:solution ratio of 1:100 is optimal. Using SPExMan reaction vessels, and 8- or 20-mL supernatant collection vials, we have found it convenient to use 0.10 g sediment:10 mL solution for all steps except steps IIA and IIB, for which we use 15 mL solution.

b. This ratio may need modification for a specific sediment sample. For example, during SEDEX standardization experiments, we observed incomplete dissolution of ferric oxyhydroxides at solid:solution ratios below 1:2000 and incomplete dissolution CFA at solid:solution ratios below 1:2000. Incomplete dissolution was attributed to supersaturation of the extractant solution. In sediments with high concentrations of CFA or ferric oxyhydroxides, the SEDEX solid:solution ratios for steps IIA and IIIA may need to be increased. Alternatively, steps IIA and IIIA may be repeated before moving on to the $MgCl_2$ wash steps.

6. Effective resuspension: Constant suspension of solids must be maintained throughout an extraction step, otherwise complete dissolution of target phases will not occur. Constant suspension throughout a given extraction step is achieved by agitating the samples on a shaker table with reaction vessels oriented at 45 degrees to the plane of the shaker table motion (Fig. 4A).
7. Timing: The efficiency and specificity of each step of the SEDEX scheme is based on standardization with analog phases. In many instances, separation of different phases is imperfect (see Ruttenberg 1992), and some fraction of nontarget phases can dissolve in a given extraction step. In some cases, the separation is based on choosing extraction conditions that maximize differences in kinetic rates of dissolution of target versus nontarget phases. For this reason, it is important not to let sediment residue remain in any of the primary extractants for extended periods after completion of the primary extraction step. If the SEDEX scheme cannot be performed in its entirety without breaks, it is always preferable to break after the last $MgCl_2$ wash in a given step. This is particularly critical for steps I–III. Sample tubes should be stored refrigerated until initiation of the next primary extraction step.
8. Supernatant oxidation for total dissolved phosphorus (TDP) determination
   a. Although not explicitly included in the SEDEX protocol as published (Ruttenberg 1992), we have found that there often is a quantity of phosphorus solubilized during steps I–IV that is unreactive to the molybdate-blue colorimetric reaction. To account for this unreactive phosphorus pool (which is likely either organic phosphorus or polyphosphate), we use the oxidation method of Monaghan and Ruttenberg (1999) on splits of the combined supernatants for each SEDEX step.
   b. We have developed two record sheets for use in preparation and execution of the SEDEX protocol. We have found these to be helpful in expediting planning, preparation, and execution of the protocol and in keeping track of experimental details. These are included as Tables A1-2 and A1-3 in Appendix 1 (this appendix).

II. Chemical analysis of supernatants

A. Phosphate: For all steps except steps IIA and IIB, the single-solution molybdate-blue spectrophotometric method is recommended (Koroleff 1976). Separate standards and reagent blanks must be made up for each step such that the solution matrix of the standards and reagent blanks match, as closely as possible, the solution matrix of the sample. If there is significant residual volume from a preceding step, this must be taken into account when making up standards and reagent blanks. Acetate buffer and $MgCl_2$ supernatants, when not greatly diluted, require acidification to pH 1 before analysis. Precision of measurements is usually better than 2% to 3%. For additional information, see Appendix 4: Helpful Tips for Orthophosphate Analysis in SEDEX Supernatant Solutions.

B. Phosphate/CDB: For supernatants from steps IIA and IIB, the citrate and dithionite interfere with reduction of phosphomolybdate to form the blue complex. It is therefore necessary to use the solvent extraction method of Watanabe and Olsen (1964). In this method, the molybdate complex is extracted into isobutanol before reduction with dilute $SnCl_2$ solution. The interferants are left behind in the aqueous phase, having been complexed with $FeCl_3$ (Lucotte and d'Anglejan 1985). The procedure is as follows; for more detailed protocol, see Appendix 5: Butanol Extraction/Molybdate Blue Colorimetric Method for Phosphate in CDB.

1. Transfer 2.5-mL splits of step IIA supernatant to 8-mL scintillation vials. The remainder of the supernatant is archived for permanent storage and for analysis of dissolved iron.
2. Spike sample splits with 1 M $FeCl_3$ at 100:1 (vol/vol): 0.025 mL for 2.5 mL supernatant.
3. Simultaneously, spike CDB standards and reagent blanks with 1 M $FeCl_3$ at 100:1 (vol/vol).
4. Cap vials, shake to homogenize, and leave uncovered until the initially yellow reagent blank, turns to a stable clear, and samples turn from dark to pale yellow (several days), indicating that the reaction is complete.
5. Remove aliquots of 0.65 mL sample to volumetrically marked glass culture tubes, dilute them to 3.0 mL with appropriate reagent blank solution, and extract into 2 mL isobutanol, discarding the aqueous phase.

6. React samples with molybdate reagent (Watanabe and Olsen 1962), wash with 1N H_2SO_4, and reduce with $SnCl_2$.
7. After making up to 3 mL total volume with ethanol, measure absorbances spectrophotometrically at 725 nm.
8. It is critical that the volume ratio of CDB to final volume be the same for reagent blanks, standards and samples. This can be accomplished by diluting aliquots of a standard phosphate stock solution made up in CDB with aliquots of a reagent blank made up in CDB, where both the stock solution and the reagent blank have the same solution composition as the sample. Precision is variable but is usually better than 5%.

C. Dissolved Fe: Dissolved Fe in step IIA–C is measured by flame atomic absorption spectroscopy. It is critical that standards and reagent blanks be made such that their solution composition matches that of the sample as closely as possible. Precision of measurements is better than 2%–3%.

III. SPExMan-SEDEX sequential extraction protocol

The procedure below is written for 0.10-g sediment samples and a solid:solution ratio of 1:100 (except steps IIA and IIB, which have solid:solution ratios of 1:150).

Install filters into reaction vessel filter holders, assemble reaction vessels, and plug into stopcocks that have been screwed (finger tight) into manifold plate; close all stopcocks.

Weigh freeze-dried, sieved sample into vessel; record weights.

Fill calibrated repipette with extractant for step I.

A. Step I: 1 M $MgCl_2$ (pH 8)
   1. Step IA
      a. Add 10 mL $MgCl_2$ to sample in reaction vessel via repipette. Note time on SEDEX record sheet.
      b. Cap reaction vessel with solid cap and place on shaker table for 2 h. Note shaker table *Start* and *Stop* times on SEDEX record sheet.
      c. Vacuum filter and collect supernatant.
         i) Place open collection vials on rack inside vacuum chamber.
         ii) Remove manifold plate from shaker table and seat onto vacuum chamber.
         iii) Align stopcock needles with open collection vials; needles should be positioned such that tips extend a few millimeters below vial rims.
         iv) Attach vacuum pump to vacuum chamber and set vacuum to pull at ≤5 psi.
         v) Remove caps from reaction vessels.
         vi) Turn on vacuum, open stopcocks, and pull vacuum until reaction vessels are evacuated, collecting filtered supernatant in vials.
         vi) Close stopcocks and turn off vacuum.
      g. Add next extractant according to procedure.
      h. Replace caps.
      i. Remove manifold plate from vacuum chamber and place on shaker table for next step.
      j. Cap and remove collection vials from chamber and acidify if necessary.
   2. Step I-B
      a. Add 10 mL $MgCl_2$ to sample residue in reaction vessel via repipette.
      b. Repeat steps b–j outlined for step IA.

B. Step II: CDB (pH 7.6)
   1. Step II-A
      a. Weigh out aliquots of $Na_2S_2O_3$ (Na-dithionite), one aliquot per sample, into small weigh boats (ca. 0.37 g/15 mL citrate-bicarbonate solution).
      b. Transfer dithionite to reaction vessel containing sample residue.
      c. Add pH 7.6 citrate-bicarbonate solution to reaction vessel via repipette, *slowly* to prevent excessive effervescence.
      d. Close with caps that have integrated check valves or bacterial air vents.
      e. Place on shaker table for 8 h. Note *Start* and *Stop* times on SEDEX record sheet.
      f. Collect and filter supernatant following step IA-c.
      g. After initiating step IIB, remove splits of filtered supernatants just collected and spike with 1.0 M $FeCl_3$. (See separate Appendix 5, Butanol Extraction/Molybdate Blue Colorimetric Method for Phosphate in CDB, for detailed protocol.) Save unspiked splits for analysis of dissolved Fe.
   2. Step IIB: identical to step IB.

C. Step III: acetate buffer (pH 4)
   1. Step IIIA
      a. Add 10 mL buffer to sample residue in reaction vessel. Note time on SEDEX record sheet.
      b. Repeat steps b–j outlined for step IA.
   2. Step IIIB: identical to step IB
   3. Step IIIC: identical to step IB

D. Step IV: 1 M HCl
   1. Add 10 mL of 1 M HCl to sample residue in reaction vessel.
   2. Repeat steps b–j outlined for step IA.

E. Step V: 550°C ash/1 M HCl
   1. Transfer filter with sediment residue from step IV quantitatively to 100-mL Corning Pyrex ashing jars. Remove plastic gaskets before transferring sample to jars. If necessary, use small quantities of distilled H_2O to wash any sediment grains adhering to walls of reaction vessel or to filter holder into ashing jar.
   2. Add 0.10 mL 100% (wt/vol) $Mg(NO_3)_2$ to each jar, making sure that sediment residue is entirely wetted.
   3. Place in oven at ≈110°C until dry, covering each jar with a ribbed watch glass to prevent contamination while permitting water vapor to escape.
   4. Cover samples with glass Petri dish and ash for 2 h at 550°C in a muffle furnace.
      a. Note: It is important to ensure that Petri dish cover is flush with rim of jar to prevent contamination.
      b. Note: Label jars w/ no. 2 pencil; this will remain

after ashing. Also note position of jars in furnace in case labels burn off.
5. After jars have cooled, replace gaskets.
6. Add 10 mL of 1 M HCl via repipette to sample residue in ashing jar.
7. Resuspend sediment, using spatula to scrape ashed sediment from jar walls and bottom, if necessary to achieve complete resuspension. Sometimes resuspension is difficult and requires vigorous scraping. Rinse spatula with additional 1 mL of 1 M HCl when resuspension is complete. Note: It is critical that the exact volume of rinsate be noted for each jar for supernatant phosphate concentration to be correctly calculated.
8. Cap jars securely and place jars on shaker table for 16 h. Note *Start* and *Stop* times on SEDEX record sheet.
9. After 16-h extraction, remove jars from shaker table and transfer supernatants to new or acid-cleaned polypropylene syringes with 0.45- or 0.40-µm polycarbonate syringe filters attached. Do NOT rinse sediment residue into tubes at this point, or supernatant phosphate will be diluted.
10. Discard first few drops passed through filter and collect remainder of filtrate in collection vials.

Table A1-1. Expendable supplies for SPExMan-SEDEX.

Item	Supplier	Catalog no.	Cost/unit	Notes
Whatman 25-mm diameter polycarbonate filters, 0.4 µm	VWR, Fisher Scientific	Whatman #09-300-62	$63.57/pk 25	
B-D 20-cc disposable syringes (nonsterile)	VWR, Fisher Scientific	BD309661	$57/case of 4 pks of 40	For use with acrodisc syringe filters (next line item), for filtering step V supernatant.
0.45-µm Acrodisc GHP 25-mm diameter syringe filters	VWR, Fisher Scientific	VWR #28143-888	$1424/case 1000	Can also use 0.40-µm polycarbonate syringe filters.
8-mL Nalgene LDPE narrow-mouth bottles	VWR, Fisher Scientific	NN #2003-9025	$44/case 72	Supernatant filtrate collection vials; 10 mL capacity
Wheaton, 20-mL HDPE scintillation vials	VWR, Fisher Scientific	Wheaton #986700	$123/case 500	Supernatant filtrate collection vials when using 15 mL extractant (e.g., steps IIA and IIB)
Repipette with bottle (Barnstead)	VWR, Fisher Scientific	3020AL (Barnstead#)	$406.00	For supernatant delivery to reaction vessels: any device that can accurately deliver reproducible volumes is acceptable.
Pipette to dispense 100–1000 µL	VWR, Fisher Scientific	022472101 (Eppendorf)	$289.00	Needed to acidify supernatant (100 µL) and add $Mg(NO_3)_2$ to residue of step IV before muffling (1000 µL)
Analytical balance	VWR, Fisher Scientific	various	variable	Able to measure 0.1 ± 0.001 g sediment
Glass ashing jars (100 mL Pyrex)	Fisher Scientific	06-414-1A	$79.61/case 10	For muffling residue of step IV, any glass jar resistant to 550°C is acceptable
Pyrex ribbed watch glasses	Fisher Scientific	02-613A	$69.52/case 12	To cover ashing jars when drying and ashing, any cover with ribbing to allow evaporation without loss of sample due to accidental splattering
Media/storage bottles, 1-L and 2-L Kimax	VWR	89000-932 (1-L); 89000-934 (2-L)	$124.71/case 10 (1-L); $131.41/case 4 (2-L)	Reagent storage
Corning hotplate/ stirrer, 5 by 7 inches	Fisher Scientific	S50448HP	$399.00	Any combination stir/hot plate will do
Accumet pH meter 50; accompanying solutions	VWR or Fisher Scientific	various	variable	Any pH meter will do
Other: forceps, spatulas, glassine weigh paper, stir bars				

Contact information: VWR, 800-932-5000 or www.vwrsp.com; Fisher Scientific, 800-766-7000 or www.fishersci.com.

Table A1-2. SEDEX time table.

Current step	Date	Day	Required extraction time, h	Actual extraction time	Time start	Time stop
I-A			2			
I-B			2			
II-A			8			
II-B			2			
III-A			6			
III-B			2			
III-C			2			
IV			16			
V			16			
Transfer to jars:						
Ash @ 550°C:			2			
Out of muffle furnace:						

Table A1-3. SEDEX record sheet.

SEDEX#:
Notes:

• Place acid-clean 0.2-µm* polycarbonate or Pall GHP (hydrophilic polypropylene) filters on manifold filter holders.
• Weigh out and transfer 0.1 g of ≤125 µm sample to each reaction vessel.
• Actual weights:

#	Sample	Sieved <125 µm	Sample mass, g	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				

*Note: Can also opt for the more traditional 0.40- or 0.45 µm pore size filters.

SEDEX#

Step IA 2-h (10 mL 1 M $MgCl_2$)

Name of person running step:_____

$MgCl_2$—date made/made by: _____

Adjust pH to 8.0—date/analyst: _____

Pipette calibration—vol/date/analyst): _____

1) Add 10 mL pH-adjusted $MgCl_2$ (pH 8.0)—date/time:_____ pH:_____

2) Cap reaction vessels with solid caps

3) Start shaking for 2 h—date/time:_____

4) Set up collection vials

5) Stop shaker table—date/time:_____

6) Filter and collect $MgCl_2$ supernatants—date/time:_____

7) Initiate step IB ($MgCl_2$), then come back and acidify supernatants from this step

Volume 12 M HCl added (should be 100 µL for 10-mL sample):_____

Notes (Step IA):_____

Step IB 2-h (10 mL 1 M $MgCl_2$)

Name of person running step:_____

$MgCl_2$—date made/made by: _____

Adjust pH to 8.0—date/analyst: _____

Pipette calibration—vol/date/analyst): _____

1) Add 10 mL pH-adjusted $MgCl_2$ (pH 8.0)—date/time:_____ pH:_____

2) Cap reaction vessels with solid caps

3) Start shaking for 2 h—date/time:_____

4) Set up collection vials

5) Stop shaker table—date/time:_____

6) Filter and collect $MgCl_2$ supernatants—date/time:_____

7) Initiate step IIA (CDB), then come back and acidify supernatants from this step

Volume 12 M HCl added (should be 100 µL for 10-mL sample):_____

Notes (Step IB):_____

SEDEX#

Step IIA 8-h (15 mL CDB)

Name of person running step:_____

CB—date made/made by: _____

Adjust pH to 7.6—date/analyst: _____

Pipette calibration—vol/date/analyst:_____

1) Add approximately 0.38 g Na-dithionite to each reaction vessel
2) Slowly add 15 mL pH-adjusted CB (pH 7.6)—date/time:_____ pH:_____
3) Cap reaction vessels with perforated caps fitted with bacterial air vents
4) Start shaking for 8 h—date/time:_____
5) Make up standards for Watanabe and Olson butanol extraction for analysis of P in CDB (see Appendix 1, section B, and Appendix 5)
6) Set up collection vials
7) Stop shaker table—date/time:_____
8) Filter and collect CDB supernatants—date/time:_____
9) Initiate step IIB ($MgCl_2$), then come back and prepare samples from this step for Watanabe and Olson butanol extraction CDB (see Appendix 1, section B, and Appendix 5)

Notes (Step IIA):

Step IIB 2-h (15 mL 1 M $MgCl_2$)

Name of person running step:_____

$MgCl_2$—date made/made by: _____

Adjust pH to 8.0—date/analyst: _____

Pipette calibration—vol/date/analyst:_____

1) Add 10 mL pH-adjusted $MgCl_2$ (pH 8.0)—date/time:_____ pH:_____
2) Cap reaction vessels with solid caps
3) Start shaking for 2 h—date/time:_____
4) Set up collection vials
5) Stop shaker table—date/time:_____
6) Filter and collect $MgCl_2$ supernatants—date/time:_____
7) Initiate step IIIA (NaAc-HAc), then come back and acidify supernatants from this step
   Volume 12 M HCl added (should be 124 µL for 15-mL sample):_____

Notes (Step IIB):

SEDEX#

Step IIIA 6-h (10 mL acetate buffer)

Name of person running step:_____

Na-Acetate—date made/made by: _____

Adjust pH to 4.0—date/analyst: _____

Pipette calibration—vol/date/analyst:_____

1) Add 10 mL pH-adjusted acetate buffer (pH 4.0)—date/time:_____ pH:_____

2) Cap reaction vessels with perforated caps fitted with bacterial air vents

3) Start shaking for 6 h—date/time:_____

4) Set up collection vials

5) Stop shaker table—date/time:_____

6) Filter and collect NaAc-HAc supernatants—date/time:_____

7) Initiate step IIIB ($MgCl_2$), then come back and acidify supernatants from this step

Volume 12 M HCl added variable (predetermine on 10-mL aliquot of extractant):_____

Notes (Step IIIA):_____

Step IIIB 2-h (10 mL 1 M $MgCl_2$)

Name of person running step:_____

$MgCl_2$—date made/made by: _____

Adjust pH to 8.0—date/analyst: _____

Pipette calibration—vol/date/analyst:_____

1) Add 10 mL pH-adjusted $MgCl_2$ (pH 8.0)—date/time:_____ pH:_____

2) Cap reaction vessels with solid caps

3) Start shaking for 2 h—date/time:_____

4) Set up collection vials

5) Stop shaker table—date/time:_____

6) Filter and collect $MgCl_2$ supernatants—date/time:_____

7) Initiate step IIIC ($MgCl_2$), then come back and acidify supernatants from this step

Volume 12 M HCl added (should be 100 µL for 10-mL sample):_____

Notes (Step IIIB):_____

SEDEX#

Step IIIC 2-h (10 mL 1 M $MgCl_2$)

Name of person running step:_____

$MgCl_2$—date made/made by: _____

Adjust pH to 8.0—date/analyst: _____

Pipette calibration—vol/date/analyst:_____

1) Add 10 mL pH-adjusted $MgCl_2$ (pH 8.0)—date/time:_____ pH:_____

2) Cap reaction vessels with solid caps

3) Start shaking for 2 h—date/time:_____

4) Set up collection vials

5) Stop shaker table—date/time:_____

6) Filter and collect $MgCl_2$ supernatants—date/time:_____

7) Initiate step IV (HCl), then come back and acidify supernatants from this step

Volume 12 M HCl added (should be 100 µL for 10-mL sample):_____

Notes (Step IIIC):_____

_____

_____

_____

Step IV 16-h (10 mL 1 M HCl)

Name of person running step:_____

HCl—date made/made by:_____

Pipette calibration—vol/date/analyst:_____

1) Add 10 mL HCl—date/time:_____

2) Cap reaction vessels with solid caps

3) Start shaking for 16 h—date/time:_____

4) Set up collection vials

5) Stop shaker table—date/time:_____

6) Filter and collect HCl supernatants—date/time:_____

Notes (Step IV):_____

_____

_____

_____

SEDEX#

Step V Muffle 550°C/extract 16-h (10 mL 1 M HCl)

Name of person running step:_____

100% (wt/vol) $Mg(NO_3)_2$—date made/made by: _____

HCl—date made/made by:_____

Pipette calibration—vol/date/analyst:_____

1) Transfer sample residue and filter from step IV to 100-mL Corning jars
   Use minimal MQ-H_2O to rinse residue from reaction vessel and filter holder into jar
   date/time:_____
2) Add 0.4 mL of 100% (wt/vol) $Mg(NO_3)_2$:_____
3) Place sample jars covered with speedy-vaps in oven (110°C) until just dry—date/time:_____
4) Place sample jars covered with Pyrex Petri dish or watch glass in muffle furnace (550°C) for 2 h—date/time:_____
5) Rewrite sample numbers on jars with Sharpie pen when jars are removed from furnace
6) Add 10 mL HCl—date/time:_____
7) Scrape residue with spatula to resuspend in jars and rinse with additional 1 M HCl as necessary
   Volume added to rinse spatula (≈1 mL; *must record exact volume used*):_____
8) Replace gaskets on jars and cap tightly
9) Start shaking for 16 h—date/time:_____
10) Set up collection vials
11) Stop shaker table—date/time:_____
12) Filter through 0.4-µm GHP acrodisc filters using 20-mL syringe
13) Collect HCl supernatants—date/time:_____

Notes (Step V):_____

Record position in furnace here: