Standard Operating Procedures for Determining Total Phosphorus, Available Phosphorus, and Biogenic Silica Concentrations of Lake Michigan Sediments and Sediment Trap Material

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1.0 Available Phosphorus

1.1 Available phosphorus is determined by extracting sediments with 0.1N NaOH, following the analytical procedures described by Williams et al. (1967; 1980). This chemical extraction procedure has compared favorably with algal assay estimates of available phosphorus (Sagher et al., 1975; Williams et al., 1980; Sonzogni et al., 1982; Dorich et al., 1985).

Prior to Analysis:

1.1.1 Weigh 25-35 mg (for box cores) or 75-100 mg (for ponars or sandy samples) of freeze-dried, homogenized, sediment on the Mettler AT250 balance. Calibrate balance at beginning and end of session with standard weights. Weigh sediment onto pre-tared glassine weigh paper. Transfer to pre-labeled, 50 mL polypropylene tubes. Record sample weight and vial number in log book.

1.1.2 Include one procedural blank (no sediment), and two reference sediment samples with each batch.

1.1.3 Add 30 mL of 0.1N NaOH using the Brinkman repeator pipet. Cap tightly and mix well.

1.1.4 Place tubes into a 25°C shaking water bath and shake for 17 hours.

On day of Analysis:

1.1.5 Add 3 mL of 1.0 N HCl to neutralize sample using Eppendorf pipet.

1.1.6 Cap and mix well. Record the sample extract volume. (i.e. 33 mL)

1.1.7 Allow samples to settle for one-half to one hour.

1.1.8 Pipet 10 mL of DDW into pre-labeled Falcon polypropylene sampling tubes using a Brinkman repeator pipet. Next, pipet 1.0 mL of sample into each tube of DDW, rinsing the pipet tube once with each new sample before dispensing. These volumes will give a dilution factor of 11 (11/1) for the extract concentration. Record dilution volumes used on the trace and spreadsheet.

1.1.9 Analyze P concentration using standard procedures outlined in Davis & Simmons (19) lab manual.

1.1.10 Standards are made up in sample matrix and should account for any P-contamination introduced by the reagents. Matrix and procedural blanks are included with each run to

test for contamination or interferences. Matrix and procedural blanks should ideally be the same and should approximate the intercept value of the standard regression.

1.1.11 Determine P concentration of sample extract (applying dilution factor) against standard regression, then calculate P mass for the extract volume.

1.1.12 Calculate Available P-content of sediment by dividing mass by sample weight. Report units as µg P/mg DW.

1.2 Standards

1.2.1 Standards are made in the same matrix as samples. The 30 mL NaOH/3 mL HCl sample is diluted 1/11 before analysis, so for standards in 100 mL flask add 8.2 mL 0.1N NaOH and 0.82 mL 1.0 N HCl and bring up to volume.

1.2.2 Create standards from two stock of 1000 µg P/L. Make up in designated 100 mL flasks.

1.2.3 Expected Range for samples using the extract sample dilution of 11 is 2-20 µg/L. Suggested Stds: 2, 4, 8, 12, 16, 20.

1.3 Glassware

Standards are created in dedicated 100 mL volumetric flasks. Flasks are acid washed with 10% HCL and rinsed six times with DDW between each use. Sediment samples are extracted in new, used once only, polypropylene centrifuge tubes which have been shown to be free of contaminants. Extracts are neutralized and diluted in Falcon polypropylene test tubes which have been shown to be free of contaminants and then analyzed from these tubes

1.4 Waste

All sample wastes are collected and neutralized prior to disposal down the drain. There are no known toxic wastes generated by these procedures which would require special handling and disposal.

2.0 Total Phosphorus

2.1 Total phosphorus content is determined using a modification of the combustion and hot HCl extraction procedure of Andersen (1976).

Prior to Analysis:

2.2.1 Weigh 25-35 mg (for box cores) or 75-100 mg (for ponars or sandy samples) of freeze-dried, homogenized, sediment on the Mettler AT250 balance. Pre-calibrate balance with standard weights. Weigh material onto pre-tared glassine weigh paper. Transfer to pre-numbered (etched), acid-washed Pyrex test tube. Record sample weight and vial number in log book.

2.2.2 Include one procedural blank (no sediment), and two reference sediment samples with each batch.

2.2.3 Remove caps and cover tubes with Al-foil. Combust Pyrex tubes with sediment at 500°C for two hours.

On Day of Analysis:

2.2.4 Add 25 mL of 1.0 N HCl using the Eppendorf pipet. Cap tightly and mix well.

2.2.5 Place tubes into a boiling water bath (99°C) for 30 minutes.

2.2.6 After cooling, add 25 mL of DDW to bring sample volume to 50 mL. Mix well and let samples sit for one hour to cool and settle. Record the sample extract volume.

2.2.7 Pipet 10 mL of DDW into pre-labeled Falcon polypropylene sampling tubes using a Brinkman repeator pipet. Next, pipet 0.4 mL of sample into each tube of DDW, rinsing the pipet tube once with each new sample before dispensing. These volumes give a sample dilution factor of 26 (10.4/0.4) for the extract concentration. Record dilution volumes on the trace and in the spreadsheet.

2.2.8 Analyze P concentration on the Auto Analyzer II using the standard molybdate/ascorbic acid procedures described by Davis and Simmons (1979). There is no need to neutralize the sample prior to analysis. Blanks are included to test for contamination or interferences.

2.2.9 Determine P concentration of sample extract (applying dilution factor) against standard regression, then calculate P mass for the extract volume.

2.2.10 Calculate P-content of sediment by dividing mass by sample weight. Report units as µgP/mg DW.

2.3 Standards

2.3.1 Standards are made in the same matrix as samples. The 50 mL sample extract is in 0.5N HCl and is diluted by a factor of 26 to make a final sample matrix of 0.019N HCl. For standards, add 1.9 mL 1.0N HCl to the 100 mL flask and bring up to volume.

2.3.2 Create standards from 2° stock of 1000 µg P/L. Make up in designated 100 mL flasks.

2.3.3 Expected Range for samples using the extract sample dilution of 26 is 10-100 µg/L.

2.4 Glassware

Standards are created in dedicated 100 mL volumetric flasks. Flasks are acid washed with 10% HCl and rinsed six times with DDW between each use. Sediment samples are extracted in acid-washed Pyrex test tubes. Between analyses the Pyrex tubes are rinsed with DDW three times to remove any sediment or acid residue and then soaked in a 25% HCl batch for at least 24 hrs.

Tubes are rinsed six times with DDW and inverted on clean paper towels to dry. Extracts are diluted in Falcon polypropylene test tubes which have been shown to be free of contaminants and then analyzed from these tubes.

2.5 Waste

All sample wastes are collected and neutralized prior to disposal down the drain. There are no known toxic wastes generated by these procedures which would require special handling and disposal.

3.0 Biogenic Silica

3.1 Biogenic silica refers to silica which has been assimilated by diatoms and incorporated into their frustules as an amorphous polymorph of silica (Krausse et al., 1983). Biogenic silica is determined using a wet alkaline digestion with 1% Na_2CO_3 at 85°C. Mineral contributions to the silica pool are corrected for by using a timed extraction procedure and the differential rates of extraction for biogenic bersus mineral forms (DeMaster 1981).

Prior to Analysis:

3.1.1 Weigh 25-35 mg for clay/silty sediment or 50-70 mg for sandy sediment of freeze-dried, homogenized, sediment on the Mettler AT250 balance. Calibrate balance at beginning and end of session with standard weights for 10, 30, 50 mg. Weigh material onto pre-tared glassine weigh paper. Transfer to pre-labeled, 50 mL polypropylene tubes. Record sample weight and vial number in log book.

3.1.2 Include one procedural blank (no sediment), and two reference sediment samples with each batch.

On day of Analysis:

3.1.3 Pre-label Falcon sample tubes (five tubes for each sample: T1, T2, T3, T4, T5).

3.1.4 Fill each sample tube with 0.19 mL 1N HCl and then add 10 mL DDW.

3.1.5 Add 40 mL of 1% Na_2CO_3 to each sediment sample using the Brinkman repeator pipet. Cap tightly and mix well.

3.1.6 Place centrifuge tubes into a 85°C shaking water bath (100 rpm) and shake for six hours taking 1.0 mL sub-samples at intervals of 2, 3, 4, 5, and 6 hours. Set up samples in batches of 10-12 for subsampling. Stagger a 2^{nd} batch 15 minutes apart from 1^{st}.

3.1.7 Sub-sample each sample as follows: Stop shaker. Remove samples. Mix well and then let settle for five minutes. Remove 1 mL of extract from sample and add it to sample tube with 10 mL mL DDW and 0.19 mL of 1N HCL. NB: Be careful to get consistent volumes when pipetting the hot samples. Keep tip at constant temp. Need to calibrate actual volume withdrawn using 1.0 mL pipet. Return sediment samples to bath ASAP and re-start shaker.

3.1.8 Analyze SiO_2 concentration using standard procedures. Blanks are included to test for contamination or interferences.

3.1.9 Determine SiO_2 concentration of sample extract against regression for 10-50 ppm standards. Don't multiply by any sample dilution factor. It is already accounted for by treating standards and samples identically. Calculate SiO_2 mass for the extract volume (40 mL).

3.1.10 Calculate SiO_2-content of sediment by dividing mass by sample weight. Report units as mg SiO_2/g DW.

Standards:

3.1.11 Make up five SiO_2 standards and a reagent blank in 50 mL of 1% Na_2CO_3.
Suggested range is 10-50 mg/L for BOX CORES and 2-20 mg/L for PONARS.
Create standards from primary SiO_2 stock of 1000 mg/L.
Make standards in designated 50 mL polypropylene tubes.

3.1.12 Standards get diluted identically to samples so there is no dilution factor for samples. Dilute 1 mL of each standard with 0.19 mL 1N HCl and 10 mL DDW. NB: Standards are not heated.

3.2 Glassware

Standards are created in polypropylene centrifuge tubes which have been shown to be free of contaminants. Tubes are dedicated for each specific standard concentration. Tubes are acid washed with 10% HCl and rinsed 6 times with DDW between each use. Sediment samples are extracted in new, used once only, polypropylene centrifuge tubes which have been shown to be free of contaminants. Extracts are neutralized and diluted in Falcon polypropylene test tubes which have been shown to be free of contaminants and then analyzed from these tubes

3.3 Waste

All sample wastes are collected and neutralized prior to disposal down the drain. There are no known toxic wastes generated by these procedures which would require special handling and disposal.