

# LABORATORY ANALYTICAL PROTOCOL

Terrestrial Group

SBC-LTER

University of California, Santa Barbara

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## SAMPLE PREPARATION

Initial processing in the laboratory consists of obtaining a filtered aliquot from the unfiltered sample if a separate field-filtered sample is not collected. A 47mm Gelman A/E 1-micron glass fiber filter is used in both the laboratory and field. Stormflow samples with high sediment loads are centrifuged before filtration. Separate aliquots from the filtered sample are removed for dissolved ionic nutrient analysis ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ ) and for total dissolved nitrogen and phosphorus analysis (TDN and TDP). Fresh samples are stored in 14 ml screw cap vials at 4° C. Whenever possible analyses is carried out within 2 day of filtering. The NaOH-persulfate TDN and TDP reagent is added immediately. Digestion is done within one week, and samples are stored at room temperature for up to 2 months before analysis. Some precipitates are observed after digestion so samples are shaken horizontally for 24 hours before analysis, but not all precipitate dissolves. Remaining portions of the unfiltered samples are archived. 50 ml of filtered sample is archived at 4° C for future needs.

## INONIC NUTRIENT ANALYSES

All ionic nutrient analyses were conducted in the Schimel Laboratory unless otherwise indicated. Dissolved  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  are quantified on a Lachat Flow Injection Analyzer (Lachat Instruments, 2000). TDN and TDP are determined after persulfate oxidation followed by measurement of  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ . Manifold diagrams and reagent concentrations will be stored on the SBC-LTER website ((SBC-LTER, 2003)). Specifics are listed below.

### *Ammonium ( $\text{NH}_4^+$ ) (uM):*

$\text{NH}_4^+$  is measured by adding base to the sample stream, which converts  $\text{NH}_4^+$  to  $\text{NH}_3$ . This diffuses across a Teflon® membrane (Willason et al., 1986) and into phenol red pH indicator. Basic peaks are detected at 570 nm (Grasshoff, 1976)

### *Nitrate ( $\text{NO}_3^-$ ) (uM):*

$\text{NO}_3^-$  is measured using a standard Griess-Ilosvay reaction after Cd reduction ((USEPA, 1983)).

### *Phosphate ( $\text{PO}_4^{3-}$ ) measured as Soluble Reactive Phosphorus (SRP) (uM):*

$\text{PO}_4^{3-}$  is measured after reaction with ammonium molybdate and antimony potassium tartrate. This complex is reduced by ascorbic acid with heating (45° C) and the absorbance measured at 880 nm (Grasshoff, 1976).

### ***Total Dissolved Nitrogen (TDN) and Phosphorus (TDP) ( $\mu\text{M}$ ):***

TDN and TDP is measured after digestion with persulfate and heating in screw-cap glass tubes (Valderrama, 1980). Digest standards are made by adding  $\text{NH}_4^+$  plus  $\text{PO}_4^{3-}$  solutions and digesting as samples. Blanks are digested MilliQ water. Detection limit is about  $0.3 \mu\text{M}$  and accuracy is  $\pm 10\%$  for P. Detection limit is about  $0.5 \mu\text{M}$  and accuracy is  $\pm 10\%$  for N.

## **PARTICULATE ANALYSES**

Two particulate samples are collected on 25mm Gelman A/E 1-micron filters: one is used for carbon, hydrogen and nitrogen analysis (CHN), the other for phosphorus. The bottle is well shaken then rapidly pipetted or poured into a graduate cylinder cut off at an appropriate volume (eg. 25, 50, 100 ml). Volume is selected so that particulates significantly darken the filter without creating a sediment cake. A third filter is periodically collected for C and N stable isotope abundance analysis.

### ***Particulate Carbon (PC) and Nitrogen (PN):***

Particulate carbon and nitrogen are measured by combustion of filtered samples using a Control Equipment Corp (now Exeter Analytical) CHN analyzer (MSI Analytical Lab), operated under the manufacturer's recommended conditions. Results are provided initially in micrograms of the element, and converted to umoles/L by dividing by the atomic weight and by the volume of sample filtered. Instrument precision is  $\pm 1\mu\text{g}$ , and detection limits are about  $2\mu\text{g}$ . Lower detection limits, however, depend on the variability of filter blanks, which typically run about  $\pm 5\mu\text{g}$ . Accuracy of measurements is monitored by analysis of one or more control samples (pure organic compounds of known composition) with each sample batch, and is better than  $\pm 0.3\%$ , or  $\pm 3\mu\text{g}$ , whichever is greater. QA/QC procedures (full document available from MSI Analytical Laboratory) include periodic re-analysis of calibration standards during each sample batch run, and post-run evaluation of results for validity.

### ***Particulate Phosphorus (PP):***

Particulate phosphorus is obtained by dry combustion ( $550^\circ\text{C}$  for 2 hours), followed by a digestion of the combusted filter with boiling in HCl (Melack Laboratory). Digested samples are stored for up to 4 months in 14 ml screw cap plastic vials. Digests are neutralized with NaOH and afterwards the digest is assayed for  $\text{PO}_4^{3-}$ . The method showed little sensitivity to sample pH between 2 and 11, as samples are poorly buffered. Detection limit is about  $1.0 \mu\text{M}$  and accuracy is  $\pm 10\%$ .

## **SEDIMENT ANALYSES**

Samples for the gravimetric determination of suspended solids are collected in separate 250 ml polypropylene centrifuge bottles if the loading is high enough for analysis by evaporation, or in larger quantities for filtration through tarred 47 mm Gelman A/E filters.

### ***Total Suspended Solids (TSS):***

Total suspended solids (TSS) is obtained by filtering a known volume of well mixed sample onto a tarred 47mm Gelman A/E filter. After drying at 105°C for 2 hours, the weight of the filter plus residue is obtained (Melack Laboratory). Typical standard deviation of the test methodology is 5.3, Method B (ASTM-D 3977-97, 1997).

### ***Suspended Sediment Concentration (SSC):***

Suspended sediment concentration samples are collected in tarred 250 ml polypropylene centrifuge bottles and weighted. After allowing the sample to settle (or centrifuging) and decanting the clear water, the bottles are first heated at a temperature slightly below boiling until all visible water is gone and then at 105° C for 2 hours. The dried bottles are reweighed and SSC calculated in accordance with ASTM D 3977-97 (Method A). Typical standard deviation of the test methodology is 36.8. Samples with inadequate sediment content for reliable SSC results are filtered in their entirety and analyzed with TSS methodology.

## **MISCELLANEOUS ANALYSES**

Specific conductance (or electrical conductivity) of unfiltered water is measured on all samples with a conductivity bridge (cell constant = 0.1) and readings corrected to 25° C; a circa 1400 µS/cm standard is used for calibration daily. Gran titrations for ANC and pH measurements are done on specifically collected unfiltered samples using a digital pH meter and a Ross Orion combination electrode (Wetzel et al., 1979). On occasion filtered samples are used for ion analysis: chloride, nitrate and sulfate by ion chromatography (Dionex model 2010i or DX500); calcium, magnesium, sodium and potassium by flame atomic-adsorption spectroscopy (Varian model AA6 or Spectraa 400); and silica colorimetrically (molybdo-silicate method; (Strickland et al., 1972)) on a Lachat Auto Analyzer (detection limit 0.5 µM). The average analytical error for anion and cation concentrations above 10 µeq/L is 3 %, 5% for concentrations below 2 µeq/L (Melack Laboratory).

### **Schimel Laboratory: Instrument Operation, Standards and QA/QC**

The Lachat AE Ion Analyzer is set up with a pump rate setting at 50 rpm and a cycle period 50 sec. All reagents and standards are made with polished MilliQ water (12-18 Mohm). Instrument calibration standards are made fresh every 2 days from 5 mM mixed standard (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup>). Calibrations are performed twice before analyzing samples. Slopes needed to be within 10-20% of values obtained under similar analytical conditions, and within 5% of each other before samples are analyzed. Three blanks are obtained by flushing MilliQ (or comparable) system for 2 minutes and then filling 3 lachat tubes. Blanks are run after every calibration, and the average is deducted from analyzed values.

A calibration standard is reanalyzed after every 10 samples (“check standard”) and the instrument is recalibrated if it drifted beyond 5% of calibration value. Sometimes recalibration is not practical and sample values are corrected post-run if check standard is beyond 5%. Detection limit is about 0.3  $\mu\text{M}$  for  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$ , about 0.5  $\mu\text{M}$  for  $\text{NO}_3^-$ , and accuracy is about  $\pm 5\%$ . Samples off scale for any channel are diluted with DI water and reanalyzed.

In addition to fresh instrument standards, QC standards mixed from independent primary standards are analyzed most days. These primary standards are also NIST-traceable or mixed directly from ACS-grade crystals. Accuracy between standards is generally within 10%, and sample values are not corrected for differences.

### **Delay Experiment in Sample Analysis**

Our goal is to analyze ionic nutrient samples ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ ), and begin the digestion of total dissolved nitrogen and phosphorus samples, within 48 hours. We are able to meet this limit for most of the baseflow samples. However, during large storm events, when high sediment loads prevent field-filtering and the laboratory is inundated with hundreds of unfiltered samples, the 48 hour limit is often exceeded, often by 1 to 5 days. In an experiment to evaluate the effect of delay, three types of samples were collected from six streams with widely varying nutrient chemistry: (1) field-filtered samples, analyzed in duplicate within 12 hours to establish base nutrient concentrations; (2) a laboratory filtered sample, filtered on the day of collection, stored at 4° C, and repeatedly re-analyzed after delays of from 1 to 14 days; and (3) an unfiltered sample, stored at 4° C, sub-samples of which were repeatedly filtered and analyzed after similar delays. Numerous duplicate and distilled deionized water samples provided quality assessment and control. The average error (the combined error of processing, delay, instrument calibration and analysis) for nitrate was 5 to 10 % (the higher percentage error in the second week of delay), 10 % for phosphate, and 20 % for ammonium. Samples filtered within two days showed virtually no variation in nitrate and phosphate from base values, while ammonium was usually within 10 %. Our conclusion is that probable delays in sampling processing and analysis do not meaningfully affect results, except possibly in the case of ammonium, where an appreciable number of unfiltered samples, delayed for more than 2 days, showed many-fold gains in concentration.

### **REFERENCES**

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Table 1 of analytical conditions used in the Schimel Laboratory.

	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>
Sample Loop	1 m x 1mm id	25 cm x 0.8 mm id	75 cm x 1mm id
Calibration:	1-50 uM	1-400 uM	1-30 uM
Standards (uM)	0,1,2,5,10,20,30,50	1, 2, 5, 10, 20, 30, 50, 100, 200, 400 (NO <sub>3</sub> ) 0, 20, 100, 300, 400, 600 (TDN)	0,1,2,5,10,20,30 (SRP) 0, 2, 10, 30, 40, 60 (TDP)
Typical slope	1.5-2.5	0.45-.56	0, 0.5, 1, 5, 10, 15, 20 (PP) 4.5-5.0
Minimum CC	0.98	0.998	0.998
Primary Standard	0.1 M diluted to 5 mM	0.1 M diluted to 5 mM	0.0105 M diluted to 5 mM
Reactor Assembly	Diffusion block, 16 cm pathlength, ~ 1.5 s residence	6 cm od HD glass tube, 15 cm long. 20 mesh copperized Cd	Water bath 115 deg F
Reagents:			
Baseline	Water	Water	Water (SRP,TDP) 0.5 M NaCl (PP)
Wavelength (nm)	540	510	620?
Total Dissolved N & P Notes		Range to 600 uM	Range to 60 uM
Partic. P Notes			
Remarks			

