

Santa Barbara Coastal Long Term Ecological Research (SBC-LTER) Project Laboratory Analytical Protocol

Sample Preparation

- The initial processing of stream samples was done in the Melack Laboratory of UC Santa Barbara.
- This 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.
- Storm samples with high sediment loads are centrifuged before filtration.
- Separate aliquots from the filtered sample are removed for dissolved ionic nutrient analysis (NH_4^+ , NO_3^- and 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 are carried out within 2 day of filtering.
- For TDN and TDP analyses, NaOH-persulfate 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.

Ionic Nutrient Analyses

- All ionic nutrient analyses are conducted in the UC Santa Barbara Schimel Laboratory unless otherwise indicated.
- Dissolved NH_4^+ , NO_3^- and PO_4^{3-} concentrations are measured on a Lachat Automated Ion Analyzer (Hach Company, Loveland, CO, USA).
- TDN and TDP are determined after persulfate oxidation followed by measurement of NO_3^- and PO_4^{3-} .
- Manifold diagrams and reagent concentrations are stored on the SBC-LTER website (<http://sbc.lternet.edu/>).
- Specifics are listed in Table 1 below.

I. Ammonium (NH₄⁺)

- NH₄⁺ is measured by adding base to the sample stream, which converts NH₄⁺ to NH₃ (Lachat Instruments Inc., 1995).
- This solution diffuses across a Teflon® membrane (Willason and Johnson, 1986) into phenol red pH indicator and peaks are detected at 570 nm.
- The detection limit is 0.5 μM, sensitivity is ± 0.2 μM and accuracy is ± 5%.

II. Nitrate (NO₃⁻)

- NO₃⁻ is measured using a standard Griess-Ilosvay reaction after cadmium reduction (USEPA, 1983; Lachat Instruments Inc., 1996a).
- The detection limit is 0.5 μM, sensitivity is ± 0.2 μM and accuracy is ± 5%.

III. Phosphate (PO₄³⁻) measured as Soluble Reactive Phosphorus (SRP)

- PO₄³⁻ is measured after reaction with ammonium molybdate and antimony potassium tartrate (Lachat Instruments Inc., 1996b).
- This complex is reduced by ascorbic acid with heating (45° C) and the absorbance measured at 880 nm (Grasshoff, 1976).
- The detection limit is 0.3 μM, sensitivity is ± 0.2 μM and accuracy is ± 10%.

IV. Total Dissolved Nitrogen (TDN) and Total Dissolved Phosphorus (TDP)

- TDN and TDP are measured after digestion with persulfate and heating in screw-cap glass tubes (Valderrama, 1980).
- Digest standards are made by adding NH₄⁺ and PO₄³⁻ solutions and digesting as samples.
- Blanks are digested deionized water (conductivity of 0.4 μS).
- The detection limit is 1 μM, sensitivity is ± 0.5 μM and accuracy is ± 10% for nitrogen and phosphorous.

Particulate Analyses

- Two particulate samples are collected on 25 mm 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.

I. Particulate Carbon (PC) and Particulate Nitrogen (PN)

- PC and PN are measured by combustion of filtered samples using a Control Equipment Corp (Exeter Analytical) CHN analyzer located in the UC Santa Barbara Marine Science Institute (MSI) Analytical Laboratory, operated under the manufacturer's recommended conditions.
- Results are provided initially in micrograms of the element, and converted to $\mu\text{moles/L}$ 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.

II. Particulate Phosphorus (PP)

- PP is obtained by dry combustion (550°C for 2 hours), followed by a digestion of the combusted filter with boiling in HCl (UC Santa Barbara 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 PO_4 .
- This method shows little sensitivity to sample pH between 2 and 11, as samples are poorly buffered.
- The detection limit is about $1 \mu\text{M}$ 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.

I. Total Suspended Sediments (TSS)

- TSS are obtained by filtering a known volume of well mixed sample onto a tarred 47 mm Gelman A/E filter.
- After drying at 105°C for 2 hours, the weight of the filter plus residue is obtained on a Mettler Toledo AB104-S Analytical Balance (UC Santa Barbara Melack Laboratory).
- Methodology is described as Method B in detail (ASTM-D 3977-97, 1997).

II. Suspended Sediment Concentration (SSC):

- SSC 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).
- 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 = 1.0) and readings corrected to 25° C; a circa 1400 µS/cm standard is used for calibration daily.
- Gran titrations for acid-neutralizing capacity (ANC) and pH measurements are done on specifically collected unfiltered samples using a digital pH meter and a Ross Orion combination electrode (Wetzel and Likens, 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-absorption spectroscopy (Varian model AA6 or Spectraa 400); and silica colorimetrically (molybdo-silicate method, Strickland and Parsons, 1972) on a Lachat Ion Autoanalyzer (detection limit 0.5 µM).
- The average analytical error for anion and cation concentrations above 10 µeq/L is 3 %, and 5% for concentrations below 2 µeq/L (UC Santa Barbara Melack Laboratory).

UC Santa Barbara Schimel Laboratory: Instrument Operation, Standards and Quality Assurance/Quality Control

- The Lachat Ion Autoanalyzer is set up with a pump rate setting at 50 rpm and a cycle period of 60 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 (NH₄⁺, NO₃⁻ and PO₄³⁻).
- Calibrations are performed twice before analyzing samples. Slopes need 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 (Table 1) is reanalyzed after every 10 samples (“check standard”) and the instrument is recalibrated if it drifts beyond 5% of calibration value.
- Sometimes recalibration is not practical and sample values are corrected post-run if check standard is beyond 5%.
- The detection limit is 0.5 μM for NH_4 and NO_3 , and 0.3 μM for PO_4 with an accuracy of $\pm 5\%$ for NH_4 and NO_3 , and $\pm 10\%$ for PO_4 .
- Samples off scale for any channel are diluted with deionized 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 (NH_4^+ , NO_3^- and 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 almost no variation in nitrate and phosphate from initial values, while ammonium was usually within 10 %. Delays greater than 2 days can sometimes cause significant increases in ammonium.

References

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