

LAB 7- SOLUBLE REACTIVE PHOSPHORUS: UPTAKE AND ANALYSIS

3 April 2008

Lab Report due 13 April

The objectives of this lab are to gain familiarity with the soluble reactive phosphorus (SRP) analysis and to investigate sediment-phosphorus interactions. Work as a group with your table mates to complete this lab. It is a relatively straightforward lab!

NOTE- some of the chemicals used for the SRP analysis are caustic. Proceed carefully and take appropriate safety precautions.

Phosphorus Sorption Index

from: Bache & Williams 1971, as modified by Meyer (1979). See also Klotz (1988)

The phosphorus sorption index (PSI) is a measure of the uptake potential, or ability of sediments to sorb a large amount of inorganic phosphorus from the overlying water. It is determined by exposing sediments to a very high concentration of P and determining the amount of P removed for a 1-hr period. Specifically,

$$\text{PSI} = X/\log C$$

where X = amount of nutrient sorbed ($\mu\text{g/g}$ dry sediment) from an initial concentration of 2000 $\mu\text{g/L}$ and C = the final nutrient concentration in solution after 1 hour. High values of the index indicate that sediments can remove large amounts of nutrient from the water column. When determining the PSI for different types of sediments (e.g., different size classes, live vs. killed, collected from different locations), it is best to collect and determine PSI's for replicate samples.

1. Place ~10 g (wet weight) of sediment into a plastic sample cup. (Noah will provide information about types of sediments and number of replicates at the beginning of the lab).
2. Add 100 mL of 2000 $\mu\text{g/L}$ $\text{PO}_4\text{-P}$ -enriched filtered stream water. Shake (swirl) sample every few minutes for 1 hr.
3. After 1 hr, remove a subsample of water from the beaker, filter, and analyze for SRP.
4. Transfer sediments into a pre-weighed aluminum pan and dry at 60-70E C for 48 hrs.
5. Calculate PSI: $((2000\text{- SRP in solution after 1 hr } [\mu\text{g/L}] * 0.1\text{L}) / \text{sed dry wt [g]}) \div \log(\text{SRP in solution after 1 hr } [\mu\text{g/L}])$

Bache, B.W. and E.G. Williams. 1971. A phosphate sorption index for soils. *Journal of Soil Science* 22:289-301.

Klotz, R.L. 1988. Sediment control of soluble reactive phosphorus in Hoxie Gorge Creek, New York. *Canadian Journal of Fisheries and Aquatic Sciences* 45:2026-2034.

Lab Report-

1. Show calculations leading to your reported PSI values.
2. Graph PSI values for different sediment types
3. Provide a brief description and interpretation of your results, and what it might mean for the different streams that provided the sediments.

Soluble Reactive Phosphorus- Ascorbic Acid Method

Principle: This analysis primarily measures phosphorus in the form of phosphate (PO_4^{2-}), but some organic P may also be reactive, thus the term soluble reactive phosphorus (SRP). In acid solution, phosphate forms a yellow complex with molybdate which is slowly reduced to a blue color by ascorbic acid. Antimony speeds the reaction process.

Cautions: The molybdate solution used in this analysis is a strong acid (5 N H_2SO_4) and should be treated accordingly. **Everyone must wear safety glasses and gloves.** All surfaces (especially in and on the spectrophotometer) should be wiped clean after analysis, and all spills (no matter how small) should be cleaned up immediately.

Standards: Three standards (samples of known concentration) and a blank (dH_2O) will be used to develop a standard curve. The relationship between concentration and absorbance of the standards will then be used to determine the concentration of samples. Standards are made by diluting a small amount of concentrated stock solution into a large volume of dH_2O . Use volumetric flasks to make up standards.

Glassware etc:

- 2 volumetric flasks
- 3 beakers (1 small, 2 medium)
- 50 or 100 mL graduated cylinder
- test tubes for blank, 3 standards, samples
- 1 mL pipette
- 10 mL pipette

Procedure:

1. Calculate the amount of stock needed to make up standards. Stock solution concentration = 50 mg/L. Each group will be responsible for making two different standards, and some sharing among tables will be necessary. Standards to be made are: 0.100 mg/L, 0.500 mg/L, 1.00 mg/L, 2.00 mg/L (and don't forget you'll also have a blank for your standard curve). Use the equation $c_1v_1 = c_2v_2$ for your calculations.
2. To make a standard, fill volumetric flask $\sim 1/3$ full with dH_2O . Add stock solution then fill flask to the line. Line the bottom of the meniscus up with the mark on the neck of the flask. Proceed carefully and do not overshoot this mark!

3. Cap the flask and invert 5-6X to mix.
4. Label then fill test tubes with 10 mL of standards and samples.
5. Make ascorbic acid solution: Dissolve 0.53 g L-ascorbic acid into 30 mL milli-Q H₂O. Gently swirl and set aside.
6. Make mixed reagent: Add the following solutions in a 150- 250 mL beaker. Stir gently after the addition of each solution:
50 mL 5N H₂SO₄
5 mL potassium antimony tartrate solution
15 mL ammonium molybdate solution
7. Be sure that all ascorbic acid granules are dissolved. Pour ascorbic acid solution into the mixed reagent. It should turn a pale yellow color. If it does not, something is amiss. Try again.
8. NOTE- before doing this step- check the availability of the spectrophotometer. Once you add the combined mixed reagent to samples, you have a very limited amount of time to read them (see step #9) Add 1.6 mL combined mixed reagent and swirl the tube.
9. After 10 minutes, but before 30 minutes, read absorbance of each sample at 880 nm.