INTERNAL REPORT 160

PROCEDURES FOR CHEMICAL ANALYSES OF PLANT AND SOIL SAMPLES OREGON STATE UNIVERSITY

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Oregon State University

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INTRODUCTION

Precipitation, streamflow, throughfall, plant tissue, and soils are analyzed at the Coniferous Forest IBP-U.S. Forest Service Central Lab by several procedures.

On all samples, four cations (Na, Ca, Mg, K) are measured by atomic absorption spectrophotometry. An air-acetylene flame is used; LiO_3 is routinely added for Ca and Mg determinations.

For precipitation, streamflow, and throughfall, seven anions are determined: ammonia N, NO_3-N, NO_2-N (only checked occasionally), total N, urea, ortho-phosphate, and total phosphate. In addition, conductivity, pH, and total alkalinity are recorded. For plant samples, only total N and P are determined in addition to the cations. Soil sample analyses include Kjeldahl N, cation exchange capacity (CEC), Walkley-Black organic matter, extractable P, and pH. All plant and soil samples are oven-dried at 50°C.

EXCHANGEABLE BASES

Weigh out 2 g of soil into 125 ml polyethylene Erlenmeyer flasks and add 50 ml of ammonium acetate inot each flask and swirl to mix. Be sure soil is all wetted. Place on water bath at high setting for 1/2 hour.

Set up Buchner filtering apparatus. Wet filter paper with ammonium acetate solution for seal. Turn on vacuum. Pour soil solution from Erlenmeyer flasks onto the filter paper. Allow 50 ml of solution to pass through into flask. Add 50 ml more and allow to drip. Collect filtrate in flaska and store in refrigerator until analysis can be run on the atomic absorption machine.

Repeat this above procedure for water soluble cations with everything exactly the same except for the substitution of water (distilled) for ammonium acetate all the way through. Save the soil in the funnels intact for the following procedure.

CATION EXCHANGE CAPACITY

Wash soil from above with 50-100 ml of 95% ETOH slowly to wet and wash all the ammonium acetate from the soil. (Add only a small amount at a time). Discard the alcohol solution.

Add NaCl solution 25 ml at a time until 225 ml is obtained. Pour into Kjeldahl flasks and add 25 ml 1-N ammonium acetate and a small amount of Zn pellets. Distill into 40 ml of Boric acid on Kjeldahl machine and read titration as for nitrogen determination.

EXCHANGEABLE HYDROGEN

Triethanolamine Method

A. *Reagents*

1. Buffer solution, 0.5 N barium chloride and 0.2 N triethanolamine (TEA): Dilute 100 ml (112.6 g) of commercial TEA (specific gravity 1.125, about 8 N) to 1 ℓ with distilled water. Partially neutralize to pH 8.1 to 8.2, which requires approximately 36.0 ml of concentrated HCl. Dilute this solution to 2 ℓ with distilled water, and mix with 2 ℓ of a second solution which contains 250 g of $BaCl_2.2H_2O$. Protect the reagent solution from the CO_2 of the air.
2. Replacement solution, 0.5 N barium chloride inndilute buffer solution: Dissolve 250 g of $BaCl_2.2H_2O$ in 4 ℓ of distilled water, and mix with 10 ml of buffer solution (Reagent 1).
3. Hydrochloric acid, 0.1 N, standardized: Dissolve 8.3 ml of reagent concentrated HCl to 1 ℓ with distilled water. Standardize against 25 ml of 0.1 N sodium carbonate (Na_2CO_3) solution prepared by dissolving 5300 g of oven-dried Na_2CO_3 in distilled water in a 1-ℓ volumetric flask and dilute to volume.
4. Mixed indicator: Dissolve 0.1 g of bromcresol green and 0.02 g of methyl red indicators in 100 ml of 95% ethyl alcohol.

B. *Procedure*

1. Place 10 g of soil in a 125-ml Erlenmeyer flask, and add 25 ml of buffer solution.
2. Swirl the flask occasionally during a 30-minute period to mix the sample suspension.
3. Prepare a 250-ml suction flask fitted with a Gooch crucible which contains a moistened Whatman No. 42 paper.
4. Transfer quantitatively the sample suspension to the Gooch crucible, and use an additional 25 ml of buffer solution to remove sample from the original 125-ml Erlenmeyer flask. Adjust the filtration rate so that this filtration requires at least 30 minutes.
5. Leach the soil sample with an additional 100 ml of the replacement solution (Reagent 2) by adding repeatedly small increments of the solution to the sample contained in the crucible.
6. Add 10 drops of mixed indicator to the filtrate and titrate with standardized HCl.
7. Prepare a blank solution which contains 50 ml of buffer solution, using standardized HCl titrate to the same endpoint selected for the sample. The blank determination serves as a reference for the calculation.

C. *Calculation*

Calculate the result as follows from the volume of standardized HCl used:

Exchangeable hydrogen in meq per 100 g of soil sample =

$$\frac{(\text{Blank-sample titration in ml}) \times N \times 100}{10 \text{ g of sample}}$$

D. *Comments*

At the endpoint of the titration, the mixed indicator changes from blue-green through violet and finally to pink. Any stage of the progressive color change may be selected as the endpoint, but the blank and the samples must be titrated to the same endpoint.

Peech, et al. (pers. commun.) indicate that subtraction of the metal cations (Ca, Mg, K, Na, and Mn) from the exchange capacity as determined by the ammonium adsorption method provides an alternate approach for determining exchangeable hydrogen. The exchangeable Na content of acid soils of the humid regions is usually small, and may be neglected in the calculation. With certain soils, the quantity of exchangeable hydrogen determined by the ammonium acetate method may vary widely from thet obtained by the triethanolamine method.

ORGANIC MATTER

Walkley-Black Titration Method

A. *Reagents*

1. Potassium dichromate, 1 N: Dissolve 49.04 g $K_2Cr_2O_7$ in distilled water and make up to 1 ℓ. If this solution is carefully prepared, it will be exactly 1 N.
2. Ferrous ammonium sulfate, 0.4 N: Dissolve 159.6 g $Fe(NH_4)_2.(SO_4)_2.6H_2O$ in distilled water containing 40 ml concentrated H_2SO_4 and make up to 1 ℓ. Determine the exact normality periodically by titrating against the potassium dichromate solution.
3. O-phenanthroline ferrous sulfate complex-0.025 M solution: Obtain the prepared solution under the trade name of "Ferroin."
4. Phosphoric acid, 85 percent.
5. Sulfuric acid, concentrated not less than 96 percent.

B. *Procedure*

1. Pass the soil sample through a 0.5-mm sieve and weigh out 0.50 g of soil into a 500-ml Erlenmeyer flask.
2. Add 10 ml of potassium dichromate solution and 20 ml of concentrated H_2SO_4. Mix rapidly and thoroughly for one minute. Let stand on a sheet of asbestos for at least 20 minutes or until cool.
3. Dilute to 150 ml with water and add 10 ml of concentrated H_3PO_4. The addition of H_3PO_4 may be omitted for most routine analyses.
4. Titrate with the standardized solution of ferrous ammonium sulfate. Use six drops of the O-phenanthroline indicator. At the endpoint, the color flashes from green to reddish-brown. If the endpoint is overrun, add 0.5 ml of dichromate and titrate again with ferrous ammonium sulfate.
5. Run a blank simultaneously using the same procedure.

C. *Calculation*

Calculate the normality of the ferrous ammonium sulfate as follows:

$$N(\text{Fe solution}) = \frac{\text{ml of dichromate} \times 1.0\ N}{\text{ml of ferrous ammonium sulfate}}$$

The equivalents of dichromate that react with the soil sample are equal

to the difference in equivalents of ferrous ammonium sulfate used to titrate the blank and the sample, respectively. The factor 1.36 is derived as follows:

$$\frac{12}{4000} \times \frac{1.72}{0.76} \times \frac{100}{0.5} = 1.36^{a}$$

where 12/4000 is the milliequivalent weight of carbon, 1.72 is the factor used based on the assumption that organic matter is 58% carbon, 0.76 is the percent correcting factor, and 0.5 is the weight of the sample in grams. Calculate the result as follows from the volume and normality of $Fe(NH_4)_2.(SO_4)_2$ used:

$$(\text{Blank - titration in ml}) \times N_{(\text{Fe solution})} \times 1.36 = \%\ \text{organic matter (O.M.)}$$

Final percent O.M. must be adjusted for oven dry soil.

D. *Comments*

The wet oxidation method for determining organic matter in soil is precisely that of Walkley and Black (1934). The only modification involves the use of the O-phenanthroline in place of the diphenylamine indicator. This modification should not affect the value of the final result.

Grinding of the soil sample to pass a 0.5 mm sieve facilitates obtaining a representative subsample. In some cases, this method requires the use of a fractional gram sample so that obtaining representative subsamples may be a problem. If more than 75 tp 80% of the total dichromate reagent is reduced by the oxidizable material in the sample, the entire analysis must be repeated using a smaller sample.

The soil is digested with the dichromatic and sulfuric acid mixture under the effect of the heat of dilution. For precise results the sulfuric acid should be added rapidly and the flasks should be cooled uniformly on a sheet of asbestos. Once these steps are accomplished, variations in reaction time from 20 to 40 minutes do not appreciably affect the results.

NITROGEN

Kjeldahl Process for Digestion and Distillation

Digestion

Prepare samples of 0.5 g foliage or 1, 2, or 5 g for soil. These are to be weighed on the analytical balance and placed in the Maxi-Kjeldahl flasks. Add a Kel-Pak to the flask, then *carefully* pour 20 ml of concentrated sulfuric acid into the flask. Use the tippet flask. Place on Kjeldahl rack.

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[a]This number adjusted according to how much soil is used.

Turn Kjeldahl apparatus on to "manual" and adjust gas burners. Allow to digest for approximately 5-10 minutes, turning several times until flasks clear of white fumes. Allow to digest for one hour or ask lab technician. At end of digestion, allow to cool. Add 200 ml distilled water *slowly* to each flask to avoid acid-water reaction. Keep it turned away from your face.

Distillation

Obtain six 250-ml Erlenmeyer flasks and number. Add 40 ml 3% H_3BO_3 to each flask and place under the glass rods on the machine. Turn Kjeldahl On "automatic water control."

Into each flask *individually* add a pinch of zinc (use 3 or 4 zinc pellets) and 70 ml 40% NaOH, pouring slowly down the side of the flask and not allowing to mix. DO ONLY ONE FLASK AT A TIME and place on the burner. Swirl flask contents *after* the flask is attached to the connecting tube. After all flasks are placed on rack, light burners and watch very closely to avoid having them boil over.

Allow 150 ml to distill into Erlenmeyer flask, then lower flasks and allow to drip. Turn off burner under flask. Remove Erlenmeyer and titrate with 0.1 N H_2SO_4. Do calculations.

Safety precautions

Make sure all burners are off before starting to light one. Wear gloves when handling the sulfuric acid and NaOH. Watch sleeves around the burners. Avoid inhaling any of the fume coming off the digesting flasks.

Microkjeldahl Nitrogen

Reagents

The digestion mixture is 525 ml H_2O, 1.5 g $NaSeO_3$, 145.5 g Na_2SO_4, 12 g $CuSO_4.5H_2O$, and 600 ml Conc. H_2SO_4. Receiver solution is 3 ml 3% boric acid. Add 5 ml of digestion mixture and 6 boiling chips to sample before digestion. Allow to digest about 1 hr after turning light inside flasks. Organic matter may foam; allow more time for digestion and use lower heat. More digestion mixture may be added if necessary.

Remove flasks and add 2-3 ml distilled water. Pour into distillation flask. Attach to microkjeldahl nitrogen apparatus and add 10 ml 70% NaOH slowly until the mixture turns dark brown. Distill 5-10 ml into Boric acid receiver solution in small Erlenmeyer flasks. Titrate with 1 N HCl or H_2SO_4.

Microkjeldahl apparatus

Cool condensor tube before putting sample on for distillation. Remove cork from boiling flask whin sample is removed. Remove stopcock when finished running apparatus. Clean out distillation apparatus when finished.

Nitrate

Cd-Cu column preparation for sea water or freshwater

Glass columns were custom made by local glassblower.

Reagents

$NH_4Cl\left(\frac{175 \text{ g analytical grade}}{500 \text{ ml dist. } H_2O}\right)$. Dilute 50 ml to 2 ℓ.
HCl, 0.0015 N. Dilute 0.5 ml conc. HCl to 4 ℓ.
HCl, 2 N. Dilute 85 ml conc. HCl to 500 ml.
HNO_3, 0.3 N. Dilute 10 ml conc. HNO_3 to 500 ml.
$CuSO_4$. Dissolve 20 g $CuSO_4.5H_2O$ in 1 ℓ water.
Saturated solution of NH_4Cl.
Cadmium. Reagent grade, stick.
KNO_3 standard. Oven dry KNO_3 for 4 hr at 105-110°C. Weigh out 180 mg KNO_3 and dilute to 1 ℓ. 1 cc = 25 µg NO_3-N.

Cadmium sticks were filed to fine chips. The chips were screened to provide a usable fraction retained on a 0.5-mm screen but passing a 2-mm screen. Losses from machining and screening amount to approximately 30% of the original cadmium weight.

Cd-Cu Amalgamation

For each sea water column, use 38-40 g of screened cadmium. This will provide a packed column of approximately 25 cm in height. For each freshwater column, use 23-25 g of screened cadmium. This will provide a packed column of approximately 15-17 cm in height. Glass beads to bottom of reservoir.

Place the cadmium filings in a 250-ml separatory funnel. Add 1 ml 2 N HCl for each gram of cadmium used (i.e., 50 g cadmium filings and 50 ml 2 N HCl). Shake thoroughly for 1 min. Discard the HCl and wash 3 times with distilled water using the same volume as the HCl solution.

Place the samples, distilled water blank and standard urea samples in preheated water bath 70°C and cover the tubes with 15-ml glass beakers and maintain heat 1 to 1 1/2 hr. Remove tubes and cool in running water.

Used Fisher spect.--green filter--large round cells.
Blank read against DDW.
Samples read against blank.

Calculation

(µg urea-N/25 cc sample)(40) = µg/L, urea-N or ppb

Standardization

Weigh out 214 mg reagent grade urea and dilute to 1 ℓ. 1.0 cc = 100 µg urea-N. Dilute 5 cc to 1 ℓ for a working standard urea solution. 1 cc = 0.5 µg urea-N. Pipet 0, 1, 2, 4, and 6 ml of dilute standard to tubes. Add distilled water to 25 ml total volume. Continue the method of analysis beginning with NaCl addition.

For each column being prepared, use 4 ℓ of nitrate standard, as the conditioning solution. Wash the columns with 150-200 ml wash solution. Standardize as described in the freshwater or sea water method.

Scope

Nitrate in freshwater is quantitatively reduced to nitrite on a cadmium-copper column. The nitrite is determined by diazotization with sulfanilamide, then coupled with N-(1-napthyl)-ethylene-diamine to produce the deep red colored dye. Nitrite containing water should be analyzed for nitrite prior to column reduction of nitrate. Nitrate nitrogen is then calculated by difference. Sulfide ion at 2 ppm may interfere. It is easily removed by addition of $CdCl_2$ prior to reduction.

The method, as outlined below, will include only the necessary steps for routine NO_3-N determination. The reader will be referred to the original paper by Wood, et al. (1967) for column construction, cadmium-copper preparation and conditioning. After several months use, reconditioning of the columns follows the original procedure for column conditioning. Wood, et al. (1967) gives a detailed procedure for NO_3-N in sea water.

Equipment

1. 8-mm I.D. glass columsn with 75-80-ml reservoir and a flow control valve.
2. Cadmium-copper amalgam, 17-17.5 column height. Items (1) and (2) are found in the paper by Wood, et al. (1967).
3. Spectrophotometer of Klett Filter Photometer, for use at 535 mu (No. 54 filter, Klett). Fisher colorimeter--green filter.
4. Graduate cylinders, 100 ml.

Reagents

Sulfanilamide: Dissolve 5 g of sulfanilamide in 50 cc 12 N HCl and 300 ml water. Dilute to 500 ml.

N-(1-Napthyl)-ethylenediaminedihydrochloride: Dissolve 0.5 g of this salt in 500 ml water. The solution is reasonably stable for 1 month when stored in the refrigerator. Substitute NH_4Cl solution 500 ml saturated solution diluted 2 ℓ.

Method

The method outlined will apply to any column or group of columns used. Measure 50 ml of sample in a 100-ml beaker. Pipet 1 ml NH_4Cl to each sample and mix. If the flow rate of the columns has not been preset, check and set to a measured rate of 12-15 ml/min. Use either distilled water or wash solution to set the flow rate. Columns which havebbeen preset for flow usually maintain this flow. Experimentally, it was determined that any flow between 6-15 ml/min for the 17-cm column produced the same reduction efficiency.

Pour the sample plus NH Cl into the column reservoir. After 12-14 ml has discharged to the cylinder, pour out this first portion and keep the remainder for analysis. Do not stop the flow while rejecting the first 15 ml portion.

Blanks for this analysis are carried through the same procedure as samples. Use B-50 ml DDW plus 1 ml NH_4Cl. Blanks and standards should be run between each 3-4 samples per column. If all 4 columns are in use, 12-16 samples could be run before a set of blanks and standards are analyzed.

Carefully mix eluant of standards (50 ml plus 1 ml NH_4Cl) and sample (50 ml plus 1 ml NH_4Cl). Measure 30 ml of the reduced nitrate solution into a 50-ml graduate cylinder and pour into 125 ml Erlenmeyer flasks. Pipet 1 cc sulfanilamide, mix, and 1 cc N-(1-Napthyl)-ethylenediamine to the reduced nitrate samples and mix. After 15 min measure on the Klett Photometer using the #54 filter (green) and the long axis of the large cells. Zero the instrument with water, and read blank-zero instrument with blank and read the samples.

Before using the columns for another sample, standard or blank pass 60 ml of wash solution through each column. This procedure effectively washes the reservoir and column between samples. When the columns are left standing, they should always be covered with wash solution.

Calculation

Sample and standards are equal volume. For calculation, set up standard curve and read directly off curve. Run standards every 16 samples and blank--check with curve. If standards do not fall within standard deviation, set up new standard curve. Standard treated exactly as sample. Read ppb off curve.

Standardization

Oven-dry approximately 0.5 g reagent grade KNO for 6-12 hr at 108-110°C. Weigh out 180 mg KNO and dilute to 1 . 1 cc = 25 g NO -N. Preserve with 2 drops of chloroform, reagent grade. For dilute working standards, prepare the following solutions:

1. 4 cc strong standard diluted to 1 ℓ = 100 μg NO_3-N/ℓ
2. 4 cc strong standard diluted to 2 ℓ = 50 g NO -N/
3. 1 cc strong standard diluted to 1 ℓ = 25 g NO -N/
4. 25 cc of weak 100 μg/ℓ standard diluted to 250 ml = 10 μg NO_3-N/ℓ
5. 25 cc of weak 50 μg/ℓ standard diluted to 250 ml = 5 μg NO_3-N/ℓ
6. 10 cc of weak 50 μg/ℓ standard diluted to 250 ml = 2 μg NO_3-N/ℓ

Measure out 50 cc of each standard, add 1 cc NH_4Cl and proceed with column reduction as outlined.

Urea

Scope

Urea is normally found as a waste product of metabolism. It is often associated with nitrogen excretion where other waste products, such as ammonia, would be toxic to the host. The method shows no interference from other waste metabolites except allantoic acid which is quantitatively measured under the test conditions.

Equipment

Waterbath, capable of maintaining 70 ±1°C
Spectrophotometer.

Reagents

Phosphate-H_2SO_4: Dissolve 86 g $Na_2HPO_4.2H_2O$ or 125.6 g KH_2PO_4 in 1 ℓ concentrated H_2SO_4. Store in a glass container.

Di-acetyl-monoxime: Dissolve 5 g di-acetyl-monoxime in 100 ml of water by gentle heating. Cool and add 60 mg semicarbazide hydrochloride to the monoxime solution. This reagent is stable for 3-4 weeks. Ignore the precipitation of dissolved salts and store in the refrigerator.

$MnCl_2$: Dissolve 244.5 g $MnCl_2.2H_2O$ and 4 g KNO_3 in 500 ml water.

NaCl, 20%: Dissolve 200 g NaCl in water and dilute to 1 ℓ.

Method

Preheat the waterbath to 70 ±1°C before starting the analysis. Good temperature control is essential for method reproducibility. Mix equal volumes of the di-acetyl-monoxime and $MnCl_2$ reagents. Total mixed volume should be slightly larger than test requirements (1 cc mixed reagent per sample). Measure 25 cc of sample into 15-mm test tubes. In the following order, with mixing between each reagent add 5 cc 20% NaCl, 4 cc phosphate-H_2SO_4 reagent, and 1 -c mixed reagent (monoxime and $MnCl_2$). Place samples blank, and standards in a 70°C waterbath and cover tubes. Leave for about 1-1 1/2 hr. Remove and cool in running water. Read at 525 μm.

$$(\mu g \text{ urea-N}/25 \text{ ml sample})(40) = \mu g/l \text{ urea-N}$$

Alkalinity

Total alkalinity is expressed as mg/ℓ $CaCO_3$. A mixed indicator is used consisting of bromocresol-green 0.1 g and 0.02 g methyl red in 100 ml 95% ethanol. Use 100 ml sample and 5 drops indicator. Titrate with 0.02 N H SO and titrate to a light gray pink (changes at pH 4.6).

$$\text{mg}/\ell\ HCO_3^- = \frac{(\text{vol. acid})(\text{N of acid}) \times 6.1 \times 10^4}{\text{volume of sample}}$$

Use a standard of 0.02 N $CaCO_3$.

PHOSPHORUS (Total and Ortho)

A spectrophotometer or filter photometer suitable for measurements at 880 mu, and providing a light path of 2.54 cm (1 in) or longer should be used. All glassware used in the determination should be washed with hot 1:1 HCl and rinsed with distilled water. The acid-washed glassware should be filled with distilled water and treated with all the reagents to remove the last traces of phosphorus that might be adsorbed on the glassware. Preferably, this glassware should be used only for the determination of phosphorus and after use it should be rinsed with distilled water and kept covered until needed again. If this is done, the treatment with

1:1 HCl and reagents is only required occasionally. Commercial detergents should never be used. Also an autoclave is used in the determination.

Reagents

Sulfuric acid solution, 5 N: Dilute 70 ml of conc. H_2SO_4 with distilled water to 500 ml.

Potassium antimonyl tartrate solution: Weigh 1.3715 g $K(SbO)C_4H_4O_6$ dissolve in 400 ml distilled water in 500 ml volumetric flask, dilute to volume. Store in glass-stoppered bottle.

Ammonium molybdate solution: Dissolve 20 g (NH) $Mo_7O_{24}\cdot 4H_2O$ in 500 ml distilled water. Store in plastic bottle at 4°C.

Ascorbic acid, 0.1 M: Dissolve 1.76 g of ascorbic acid in 100 ml of distilled water. The solution is stable for about a week if stored at 4°C.

Combined reagent: Mix the above reagents in the following proportions for 100 ml of the mixed reagent: (1) 50 ml of 5 N H_2SO_4, (2) 5 ml of potassium antimonyl tartrate solution, (3) 15 ml of ammonium molybdate solution, and (4) 30 ml of ascorbic acid solution. Mix after addition of each reagent. All reagents must reach room temperature before they are mixed and must be mixed in the order given. If turbidity forms in the combined reagent, shake and let it stand for a few minutes until the turbidity disappears before proceeding. The reagent is stable for one week if stored at 4°C.

Strong-acid solution: Slowly add 310 ml conc. H_2SO_4 to 600 ml distilled water. When cool, dilute to 1 ℓ.

Ammonium persulfate
Phosphorus persulfate: Change 6 October 1971.

Stock solution: Dissolve in distilled water 0.2197 g of potassium dihydrogen phosphate, KH_2PO_4, which has been dried in an oven at 105°C. Dilute the solution to 1000 ml; 1 ml equals 0.05 mg P.

Standard solution: Dilute 10 ml of stock phosphorus solution to 500 ml with distilled water; 1 ml equals 1 μg 1 ml P.

Procedure

A. Total phosphorus

Add 1 ml of strong-acid solution to a 50-ml sample in a 125-ml Erlenmeyer flask. Add 0.5 g K persulfate (10-6-71). Boil gently on a preheated hot plate for approximately 30-40 minutes or until a final volume of about 10 ml is reached. Do not allow sample to go to dryness[a]. Alternatively, heat for 30 minutes in an autoclave at 121°C (15-20 psi). Add phenolphthalein and adjust sample to pink with IN NaOH. Bring back to colorless with one drop strong-acid solution. Cool and dilute the sample to 50 ml. Determine phosphorus as outlined in the section on orthophosphate.

[a]We are using the autoclave method.

B. Orthophosphate

Add 1 drop of phenolphthalein indicator to the 50-ml sample. If a red color develops, add strong-acid solution drop-wise to just discharge the color. Add 4 ml of combined reagent to sample and mix thoroughly. After a minimum of 10 minutes, but no longer than 30 minutes, measure the color absorbance of each sample at 700 nm with a spectrophotometer, using the reagent blank as the reference solution.

Calculation

Prepare standard curve by plotting absorbance values of standards as ordinates and the corresponding phosphorus concentrations as abscissas. Process standards and blank exactly as the samples. Run at least a blank and two standards with each series of samples. If the standards do not agree within ±2% of the true value, prepare a new calibration curve. Obtain concentration value of sample directly from prepared standard curve. Report results as P, mg/ℓ.

Precision and accuracy

In eight laboratories involving 13 analysts, using a variety of natural water samples, both salt and fresh, the standard deviation at a concentration of 0.23 mg P/ℓ was ±0.004 (AQC Laboratory). Under the same conditions, recovery was 101% (AQC Laboratory).

Extractable Phosphorus--Dilute Acid-Fluoride Method (Bray)

This method is used for all samples received from west of the Cascade Mountains.

Reagents

Ammonium fluoride, 1 N: Dissolve 37 g of NH_4P in distilled water and dilute the solution to 1 ℓ. Store the solution in a polyethylene bottle.

Hydrochloric acid, 0.5 N: Dilute 43 ml of concentrated HCl to a volume of 1000 ml with distilled water.

Extracting solution: Add 150 ml of 1 N NH_4P and 250 ml of 0.5 N HCl to 5 ℓ of distilled water. This gives a solution 0.03 N in NH_4P and 0.025 N in HCl. It will keep in glass more than 1 yr.

Ammonium vanadate--molybdate reagent.

Stock solutions--Ammonium vanadate (0.25%): Dissolve 5 g of NH_4VO_3 (meta-powder) in about 1000 ml of boiling distilled water. In a separate beaker, add 340 ml of concentrated H_2SO_4 to about 600 ml of distilled water. Combine the two solutions when cool and then dilute to 2 ℓ. Ammonium molybdate (5%): Dissolve 100 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ in about 1000 ml of warm distilled water. Cool the solution and dilute to 2 ℓ.

Work solution--Mix 0.8 ml of ammonium vanadate (0.25%) solution, 0.8 ml of ammonium molybdate (5%) solution and 1.4 ml of distilled water together for each determination to be run. Prepare this work solution fresh each time samples are run.

Standard phosphate solution: Dilute 0.4393 g of oven-dry KH_2PO_4 to 1 ℓ in a volumetric flask with extracting solution. One ml of this solution contains 100 μg of P. Prepare solutions containing 2 to 25 ppm P by diluting suitable aliquots of the solution with extracting solution.

Procedure

1. Weigh 2.9 g of soil into an extracting bottle. Add 1/4 teaspoon of acid washed into each sample. Add 20 ml of the acid fluoride extracting solution for a 1:7 soil solution ratio.
2. Shake for 1 minute and filter immediately. It is very important that the final extract be clear. A yellow-colored solution will introduce an error.
3. Place 5 ml of standard solution or of soil extract in a small clean container.
4. Add 3 ml of the vanadate molybdate work solution. Stir and then wait 30 minutes for the color to develop. Read at 420 mm on a colorimeter. The color complex which develops is stable for 24 hr or more.

Calculations

ppm P in soil sample = ppm P in soil extract x 7 ml of soil extract/g of soil.

$$\text{ppm P in soil extract} = \frac{\text{ppm P in standard solution}}{\text{O.D. reading for standard solution}} \times \text{O.D. for soil extract solution}$$

Comments

The dilute acid-fluoride method for P follows method 1-a of Bray and Kurtz. The method has been modified to use an ammonium vanadate-ammonium molybdate color-forming reagent. This modification was introduced because: (1) the larger concentration of P which could be analyzed, (2) the steps involved in developing the color are less involved, and (3) the color complex is more stable (24 hr and over). The analytical results obtained are comparable to those obtained by means of the original method.

The dilute acid-fluoride extractant tends to dissolve Ca, Al, and Fe phosphates in soil. The dissolution of Al and Fe phosphates occurs very rapidly and it probably results from the fluoride anion complexing these metal cations. For this procedure, initiation of the filtration step is advisable immediately after the brief period of extraction on the shaker. Interference in the development of the color complex occurs if appreciable amounts of Al, Fe (excess of 100 ppm), and molybdate are present. The fluoride ion also interferes in excess of 50 ppm. In this method, the effect of this interference is minimized by making up the standards in the extracting solution.

Nitric-Perchloric Acid Digestion--Total Phosphorus

Reagents

Conc. nitric acid
60% perchloric acid
Boiling chips--Teflon

Reducing solution--
71.25 g Na bisulfate
2.50 g Na sulfite
1.25 g amino-naptho-sulfonic acid] 100 ml DDW (double distilled water)

Molybdate solution--
12.8 g NH_4 molybdate
220.0 ml perchloric acid (60%)
and add DD water to make 2 ℓ

Procedure

One hundred to four hundred mg of sample (as needed to get into range of analysis) is placed into the micro-Kjeldahl flasks--two Teflon boiling chips and the sample is washed down with a small amount of DDW. Five ml conc. nitric acid is added from an acid dispenser set up on the original bottle of conc. nitric acid, kept in the hood. The samples are allowed to sit on the digestion racks (without heat) overnight. Next morning heaters are turned on and the samples are predigested with the nitric acid. When fumes have cleared the flasks, but before going to dryness, heat is turned off. Samples are cooled and washed down with a small amount of water. Then 3 ml of 60% perchloric is added and digestion is again started. When the perchloric acid starts to fume the burners are turned down and to keep fumes from leaving the flask. Fifteen minutes on this heat the samples are turned off and cooled. Then samples are transferred quantitatively to Dispos Erlenmeyer flasks and volume measured and brought to 100 ml. Samples are now ready for phosphorus, sulfate, and cation analysis. Cation analysis is done on the atomic absorption equipment. Phosphorus analysis on a bone digest.

Set up plastic (15-ml) beakers marked with sample numbers. Add 10 ml of the NH_4-molybdate-perchloric acid mixture to each beaker. Pipet 2 ml of each sample to marked beakers. Add 1 ml of reducing solution to each beaker and stir with glass rod. Allow color to develop for 20 minutes and read on colorimeter. Standards of several concentrations are set up exactly as the samples. Ten ml of molybdate-perchloric acid and 2 ml of each standard and 1 ml of the reducing solution.

Calculations

Using Klett:
Klett reading x factor = ppm in sample

$$\frac{\text{conc. of standard}}{\text{Klett reading of standard}} = \text{factor}$$

on Spec 20--Read in % transmittance
or Fisher
or Coleman
Comont % trans.→→to O.D.

$$\frac{\text{Conc. of standard}}{\text{O. D. of standard}} = \text{factor}$$

O. D. of sample x factor = ppm in sample (of P)

$$\text{ppm of phosphorus} = \frac{100 \text{ ml} \times (x \text{ ppm})}{\text{wt of sample}}$$

SILICA

Reactive Silica

Equipment

Filter colorimeter

Reagents

Na_2SiF_6 standard solution (100 μg/ml of SiO_3-Si)-671.4 mg of Na_2SiF_6 (A.R. in 400 ml H_2O heating until solution is complete. Cool--dilute 1000 ml and store in thick walled polyethylene bottle. Dilute above solution 10 times 10 ml → 100 ml = 10 μg/ml of Si for working standard.

H_2SO_4 conc.

Na_2MoO_4 solution 5%-5 g of Na_2MoO_4 in 100 ml 0.5 N H_2SO_4--allow to stand 48 hr before use. Store in refrigerator in dark polyethylene bottle. Bring to room temperature and filter if necessary before use.

$SnCl_2$ stock solution-40 g of $SnCl_2$ in 100 ml 12 N HCl (s.g. 1.18) at room temperature. Stand 24 hr before use. Protect from light in storage. Wrap in aluminum foil. Above solution diluted 1 ml → 100 ml with DDW before use. Discard remainder and make up fresh dilution each time. Five ml or 10 ml sample aliquots taken and diluted to 50 ml with DDW. Standards are done exactly the same (1 ml to 5 ml (10 μg/ml Si) diluted to 50 ml with H_2O). Range: 0.05 ppm to 1.0 ppm.

Procedure (Samples either 5 ml → 50 ml or 10 ml → 50 ml)

To tubes containing standards and sample in total volume of 50 ml add:

2 ml Na_2MoO_4 solution and mix.
Allow to stand 15 minutes.
Add 5 ml conc. H_2SO_4 and mix; allow to cool.
Add 1 ml diluted $SnCl_2$ solution and mix.
Allow to stand 15 minutes.
Read on Coleman Jr. (700 μm) 815 μm.
Read blank against DDW and record.
Read samples and standards against blank and record.
Change %T to optical density.
Sample size adjusted to come within range of instrumentation.
5 ml sample diluted → 50 ml with DDW.
All run in duplicate.

Calculations

$$\frac{\text{Conc. of standards in ppm}}{\text{Optical density of standard}} = \text{factor}$$

average factors for each conc. of standards

optical density x factor x dilution = ppm in sample

SULFUR

Turbidimetric Determination

Sulfur can be readily determined turbidimetrically. Sulfur is converted to the sulfate anion upon the addition of barium chloride, and insoluble barium sulfate is formed. In order to obtain a more uniform suspension of barium sulfate, a gel is added. The percent transmittance of the cloudy solution is then read on a spectrophotometer. From a standard curve the concentration of sulfate-sulfur is determined. The percent sulfur is then calculated.

Reagents

Barium chloride--gel solution: Dissolve 3 g Difco Bacto gelatin in 1 ℓ hot water. Let stand in refrigerator over night. Dissolve 10 g barium chloride in the gel solution. NOTE: Use at room temperature. The solution may be stored in the refrigerator and is good for one week.

Sodium sulfate stock solution: Place 4.4304 g Na_2SO_4 in a 1-ℓ volumetric flask and bring to volume with distilled water (1000 ppm sulfate - S).

Standards (0, 1.6, 3.2, 6.4, and 9.6 ppm)

Pipet 0, 8, 16, 32, and 48 ml aliquots of stock solution into 100 ml volumetric flasks. Dilute to 100 ml. Pipet 2 ml of each into Kjeldahl flasks and digest by nitric acid-perchloric acid method. Dilute digest to 100 ml.

Standard Curve

Place 5 ml standard, 5 ml water, and 0.5 ml $BaCl_2$-gel solution in a 15-ml Dispo beaker and let stand 40 minutes. Decant resulting solution into a glass cell and read percent transmittance at 470 λ at timed intervals of 20 minutes. Do not allow samples to sit any longer.

Sample solutions are the same ones used for phosphorus and cation determinations.

Place 5 ml sample solution, 5 ml water, and 0.5 ml $BaCl_2$-gel solution in a 15 ml Dispo beaker and let stand 20 minutes. Decant resulting solution into a glass cell and read % transmittance. NOTE: Use sulfanilinide (10 mg) as a running standard. Dilute digest to 200 ml. Use 5 ml solution + 15 ml water + 1 ml $BaCl_2$ for sulfanilimide standard (D.F. = 2).

SOIL pH

pH method using 1:1 soil to solution ratio and glass electrode pH meter.

Reagents

Buffer reagents may be purchased or prepared as described.
Potassium biphthalate, 0.05 M, pH 4.005 at 25°C: Dry KH-phthalate for 2 hr at 110°C. Dissolve 10.21 g of KH-phthalate in distilled water, and dilute the solution to 1 ℓ with distilled water. As a preservative, add 1 ml of chloroform or a crystal (about 10 mm in diameter) of thymol

per liter of the buffer solution.

Phosphate, 0.025 M KH_2PO_4, and 0.025 M Na_2HPO_4, pH 6.860 at 25°C: Dry the two phosphate salts for 2 hr at 110°C. Dissolve 3.4 g of KH_2PO_4 and 3.55 g of Na_2HPO_4 in distilled water, and dilute the solution to 1 ℓ with distilled water. As a preservative, add 1 ml of chloroform or a crystal (about 10 mm in diameter) thymol per liter of the buffer solution.

Borax, 0.01 M $Na_2B_4O_7.10\ H_2O$, pH 9.177 at 25°C: Dry the $Na_2B_4O_7.10\ H_2O$ for 2 hr at 110°C. Dissolve 3.81 g in distilled water and dilute the solution to 1 ℓ.

Procedure

Weigh or scoop 10 g of soil into a 3-ounce paper cup.
Add 40 ml of water and stir thoroughly.
Let stand at least 30 minutes, stirring two or three times.
Standardize the pH meter in accordance with instructions for the instrument using the prepared buffer solutions. After standardization of the instrument, rinse the electrodes with distilled water to remove the film of buffer solution.
After the soil has settled to the bottom of cup (around 15 to 30 minutes after last stirring) read the pH by placing the pH meter electrodes in the supernatant solution. Record the pH to the nearest 0.1 unit. Rinse the electrodes with distilled water prior to each pH determination, and when not in use immerse the electrodes in distilled water.

Comments

The method outlined here is a modification of method 3-26 described by Jackson (1958). The modifications are the following: (1) a 1:2 soil solution ratio is used instead of a 1:2.5 ratio, (2) the pH reading is taken in the supernatant solution instead of in the soil suspension. These modifications were made for convenience and to minimize the errors introduced by liquid junction potential and the settling of the soil particles during the time the pH measurement is being made.

The Corning model 12, pH meter is used in this laboratory. It is advisable to prepare fresh buffer solutions at least once a month, and to check the standardization of the pH meter periodically when making a series of determinations. It is convenient to operate this instrument using the expanded scale while the meter is being standardized. After it is clearly established that the instrument is standardized and functioning properly, switch the meter back to the standard scale operation for measuring the pH of soil samples.

Greweling and Peech (pers. commun.) indicate that the measured pH value may shift slightly with each change in the soil-to-water ratio used in preparation of the soil sample and that seasonal fluctuations in pH may also be anticipated. According to their results, the pH of the saturated soil paste or of the aqueous soil suspension may tent to decrease for samples collected from a given field during extremely dry periods or after heavy fertilization. During the rainy season, the pH normally shifts back to the level previously observed for the soil when in the moist, well-leached condition. Salt accumulation in soil tends to lower the soil pH determined in water,

but salt removal from the same soil by leaching may have the opposite effect. In most instances, pH fluctuations resulting from the effects mentioned should be less than 0.2 to 0.3 pH units.

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