SOIL, PLANT AND WATER REFERENCE METHODS FOR THE WESTERN REGION[1]

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PREFACE

This manual has been developed as a guide of standard analytical methods for agricultural laboratories for use in the Western Region, byt the Western Coordinating Committee on Nutrient Management. This publication an update of *Plant, Soil and Water Reference Methods for the Western Region*, 1994, (WREP 125) written by Dr. Ray Gavlak formerly of the University of Alaska, Dr. Donald Horneck of Oregon State University, and Dr. Robert O. Miller of Colorado State University. These represent accepted methods for the analysis of soil and plant samples and were selected for the express purpose of identifying common methods on which a group of agricultural laboratories analytical results can be statistically evaluated. We would like to thank all those individuals who have contributed to this manual.

The specific soil, plant and water analytical methods listed represent those analytical procedures that are recommended for use in the current North American Proficiency Testing Program organized by the Soil Science Society of America. We encourage all suggestions and comments from participating laboratories for improving this manual for future publication.

The authors would like to thank Dr. Byron Vaughan of MDS Harris Laboratory Services, Dr. Kelly Belden of the University of Wyoming for reviewing this publication.

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QUALITY ASSURANCE IN THE AGRICULTURAL LABORATORY

A Quality Assurance (QA) program Quality Control (QC) is essential for demonstrating long term performance of accuracy and precision to the laboratory clientele. By developing and implementing a QC program that not only monitors the process but provides a mechanism for improvement, the agricultural laboratory can only enhance its credibility and that of the industry.

Quality Assurance is a set of operating principles when strictly followed in the analytical laboratory will produce analytical results of known and defensible quality. It is composed of two main sub groups quality control and quality assessment. Quality control (QC) consists of analytical appraisal tools which the laboratory utilizes to verify the analytical process. A few of these are: use of control samples, recovery of known additions, analysis of external standards, use of analytical duplicates and use of maintenance or control sample charts. The process of using QC measures in the analytical laboratory is quality assessment and includes those items such as performance sample evaluations and performance audits.

The first step towards the development a QC program involves the establishment of quality control measures. The purpose of these are to monitor the analytical process(s), document statistically the precision and accuracy, and establish limits of analytical control of the method in the laboratory. The following list describes several terms used in a QC control program:

Accuracy: A combination of bias and precision of an analytical procedure, which reflects the closeness of an individual measured value to a true, correct, or assumed value.

Bias: A consistent deviation of measured values from the true value, caused by systematic errors in a procedure. Bias is assessed by measuring the recovery of known additions (spiked samples) and the recovery of internal standards and laboratory control or reference standards.

Detection Limits: The common term that encompasses various analytical detection limits. Some of the common detection limits (in increasing order of concentration detected) include the instrument detection limit (IDL), the lower limit of detection (LLD), the method detection limit (MDL), and Practical Quantitation Limit (PQL). The MDL, is the minimum concentration of a analyte that can be measured and reported with 99% confidence that has gone through the entire analytical process, including sample preparation and instrument analysis. MDL is a more useful indicator of the reported detection limit and is always larger than the IDL or LLD because it includes recovery efficiencies and concentration factors in the sample preparation. The MDL is the lowest level that can be achieved by an experienced analyst averaging at least seven trials and operating a well-calibrated instrument on one day. MDL is often estimated as three to five times the standard deviation (99.6%-99.9% confidence level) of the sample preparation blank concentration. The relation among these limits is about IDL:LLD:MDL:PQL = 1:2:4:10 . Most of the studies report one or more of the detection limits. PQL is the lowest level that can be quantified (measured) accurately (within ±10%) and reliable day-in and day-out in the lab. The PQL is routinely reported at 10 times the MDL which assures that any reported value is reliable. In certain instances it is referred as Limit of Quantitation (LOQ) or Reported Detection Limit (RDL).

Instrument Calibration Standards: Those standards prepared for the expressed purpose of calibration of an instrument for the determination of an known analyte in a unknown sample.

Laboratory Reference Standards: -A standard, usually certified, by an outside agency, used to measure the bias and precision in a procedure. Examples include the National Institute of Standards and Technology (NIST) Standard Reference Materials and the National Research Council of Canada (NRCC) reference materials (See Appendix C). Semiquanitative analytes cannot be certified.

Matrix Duplicate: An intra-laboratory split sample which is used to document the precision of a method in a given sample matrix. Duplicate analysis can also be used to detect calibration errors or "drift" and to detect sample to sample analyte carry-over contamination.

Method Blank: an analyte-free matrix to which all reagents are added in the same volumes or proportions as used in sample processing (labware, filter paper, reagents, instrumentation etc.). The method blank should be carried through the complete sample preparation and analytical procedure and is used to document contamination resulting from the analytical process. Blanks analyzed before, after, or randomly during a sample run can detect carry-over contamination.

Precision: a measure of the degree of agreement among replicate analyses of a sample (e.g., standard deviation, percent difference, or percent relative standard deviation).

Quality Control Reference Sample: a matrix matched internal control standard routinely used in the laboratory to evaluate long term accuracy and precision. An internal control sample should be randomly placed amongst each batch of unknown samples representing a minimum of 5% of the total samples.

Random Error: the deviation of any analytical value that can't be ascertained by standard statistical techniques.

Replicate: - a repeated operation occurring within an analytical procedure (sample, extraction etc.). Two or more analyses for the same constituent in an extract of a single sample constitute replicate extract analyses.

Systematic Error: is the difference between the value obtained for a characteristic and the true or conventional value which cannot be attributed to random error.

Surrogate Standard Addition: a pure element/compound added to a sample prior to analysis at varying levels for the purpose of evaluating overall efficiency of a method.

Uncertainty: all analyses have some level of inexactness in the reported value, reports may have an "estimated uncertainty" associated with reported measurements. Estimated uncertainty should be expressed in relative or absolute terms, and not as a range. In addition, reported uncertainties should reflect all knowledge of the measurement that might add to the value reported, (e.g., matrix interferences, homogeneity, blanks, etc.

The following outlines key components to the operational structure of a QA program:

Staff organization and responsibilities
Sample control and documentation procedures
Standard operating procedures for each analytical method
Analyst training requirements
Equipment maintenance procedures
Instrument calibration procedures
Internal quality control activities
Performance testing audits
Data assessment procedures
Validation and Reporting

From the operational plan both internal and external QC components are defined. Specific elements of an internal QC program: operator proficiency certification; laboratory calibration standard checks; analysis of reagent blanks; recovery of known standard additions, analysis of external supplied standards; analysis of replicates and control charts. Operator proficiency identifies the competence of the analyst performing the method. Calibration checks verify the instrument performance and quality of the instrument calibration standards. Analysis of reagent blanks document background contamination levels and the LOD and MDL. The use of recovery of known standards and analysis of external standards verify performance of the method for the analyte of interest, while duplicates provide data on precision.

The first step to establishing a QC program is verifying the MDL and PQL for the specific test methods. There are many ways to calculate the MDL, but two are in common use. The first comes from EPA 40 CFR Part 136, whereby the standard deviation (***s***) and multiply it by 3.143. The standard deviation (**s**) is derived from the analysis of a minimum of 7 standards which are not more than five (5) times the MDL concentration. The 3.143 factor comes from the "Tables of Students' T Values at the 99% confidence limit (CL)" based on seven replications. More information can be found at the following web site: http://www.dnr.state.wi.us/org/es/science/lc/download/Loddoc.pdf .

The second method comes from a publication entitled "Fundamentals of Analytical Chemistry" by Skoog and West 1982 (4^{th}). This method works well when the analyst has no estimate of what concentration to use for the standards required in EPA 40 CFR Part 136 method. A blank reagent matrix is analyzed replicated 8 times and the standard deviation (*s*) is calculated. This number is multiplied by a factor from the Student's T Values (for n =7, CL of 99% 3.143), with an additional part of the equation to correct for the number of degrees of freedom (where N1=1 and N_b=8):

$$MDL = T \times \boldsymbol{s} \sqrt{([NI+Nb]/NI*N_b)}, \quad b = \text{number of replications}$$

The second method is useful for an initial MDL study which can then be followed up using the EPA method to more precisely define the MDL. Once established, the MDL can be multiplied by 4 to establish the PQL and be provide the with the laboratory results.

With the establishment of MDLs internal quality control measures can commence. These include the use of Method Blanks, Matrix Duplicates, Surrogate Standard Addition, use of Laboratory Reference Standards, and the use of Quality Control Reference samples, also referred to as QC check samples. Method blanks are used to track instrument drift, document contamination and track sample to sample carry-over. Changes in blanks concentrations often indicate changes in reagents or cleanliness of labware or lab technique. All analytical instruments should be evaluated for inter-sample analyte carryover. This is accomplished by first the analysis of a high sample (100 x the MDL) followed by the a matrix blank. The analyte concentration of the matrix blank should be very close to that obtained after running three consecutive matrix blanks.

Matrix duplicates are two aliquots taken from the same sample and analyzed within a batch. Results are used to measure analytical precision from sample preparation through analysis for a given matrix and used to assess method and/or sample precision. A minimum of one duplicate sample per batch or 2% of the samples should be analyzed in duplicate.

Surrogate standard addition is not often used in soil analysis as most method are only semi-quantitative for the analyte of interest, such as extractable phosphorus and DTPA extractable metals. Surrogate standard addition can be used with quantitative methods such as soil nitrate, soil chloride, total organic carbon, calcium carbonate, botanical and water analyses. These are prepared by adding a predetermined quantity of stock solution of the analyte(s) being measured to a sample prior to sample extraction/digestion and analysis. The concentration of the analyte spike should be spiked at a level that will result in a final concentration that is approximately 1.5 times the unspiked concentration. A portion of the unspiked and the spiked sample are analyzed and a percent recovery is calculated. For quantitative analytical methods spike recovery should be within the range of 90 - 110% recovery.

Quality Control Reference (QCR) samples, are prepared (see Appendix E for soils) from a matrix source with analyte concentrations and precision that has been verified through repeated analysis. QCR samples are used as an independent check to track instrument performance, lab technique and the analytical process. Typically 30 analyses of the QCR sample(s) over 5 - 10 daily analysis runs are used to establish the mean ($\bar{x}$) and standard deviation (***s***) analytical value. A high quality QCR sample should have RSD ($\boldsymbol{s}/\bar{x} \times 100$) value less than 5% for analytes at concentrations 3 x MDL. A minimum of three QCR reference samples are prepared, similar in matrix to the unknown sampled being analyzed, and which range from low to high in analyte concentration. A well-founded QC program utilizes a minimum of 5% QCR samples per batch, based on batch sizes of 20-60 samples. These are placed at random to avoid positional placement bias. Typically QCR samples are analyzed in duplicate or triplicate at the beginning of a daily analysis run, and repeated again at the end of the day to verify method bias and precision.

Laboratory control charts commonly used in the analytical laboratory are: a means control chart (***X***-chart) and a range control chart (***R***-chart) constructed from replicated analyses. Control charts can be developed for laboratory control reference samples (QCR), calibration check standards (CCS) and matrix blanks. The ***X***-chart for a QCR, CCS or reagent standard are constructed from the average ($\bar{x}$) and standard deviation (***s***) of replicated analyses (n value 30). Common practice is to construct upper and lower warning limits (UWL & LWL) and upper and lower control lower limits (UCL & LCL) based on 2(***s***) and 3(***s***) limits of ($\bar{x}$) Figure 1.

By plotting daily/weekly/monthly results of the QCR, CCS and/ or blanks an analyst can identify and separate systematic error from random error. Overall trends in laboratory accuracy, improvements in precision and effects of unidentified modifications in the analytical process can be documented. It is useful measuring changes (known or unknown) in instrumentation, reagents, and analysts.

Figure 1. Example X-Chart Nitrogen, for plant nitrogen 30 days.

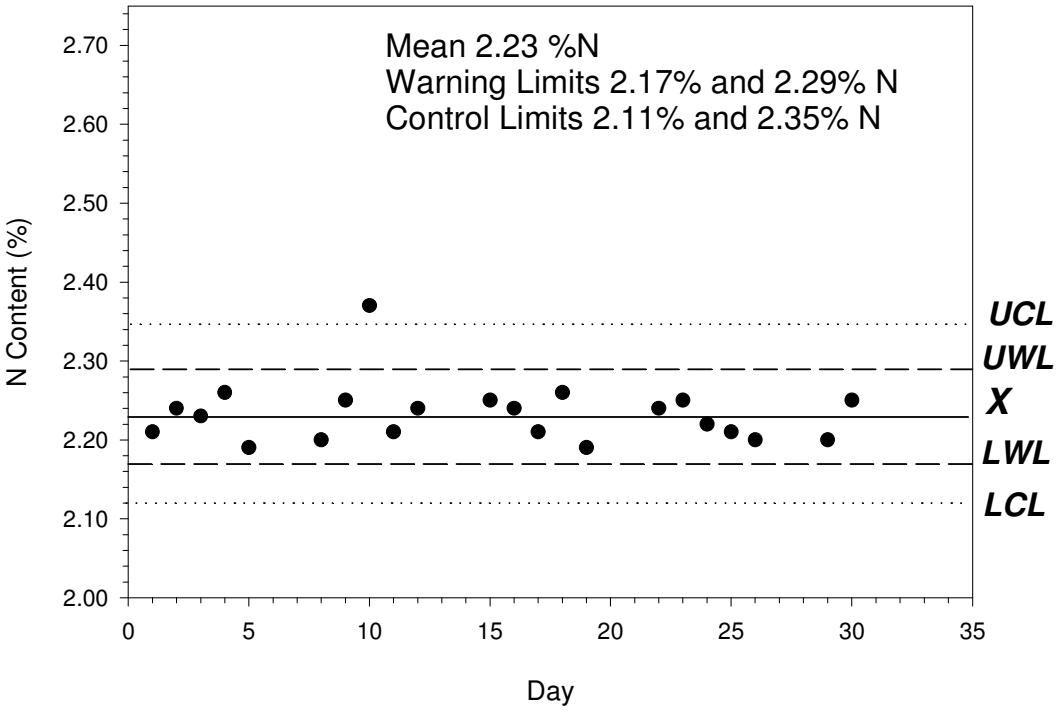
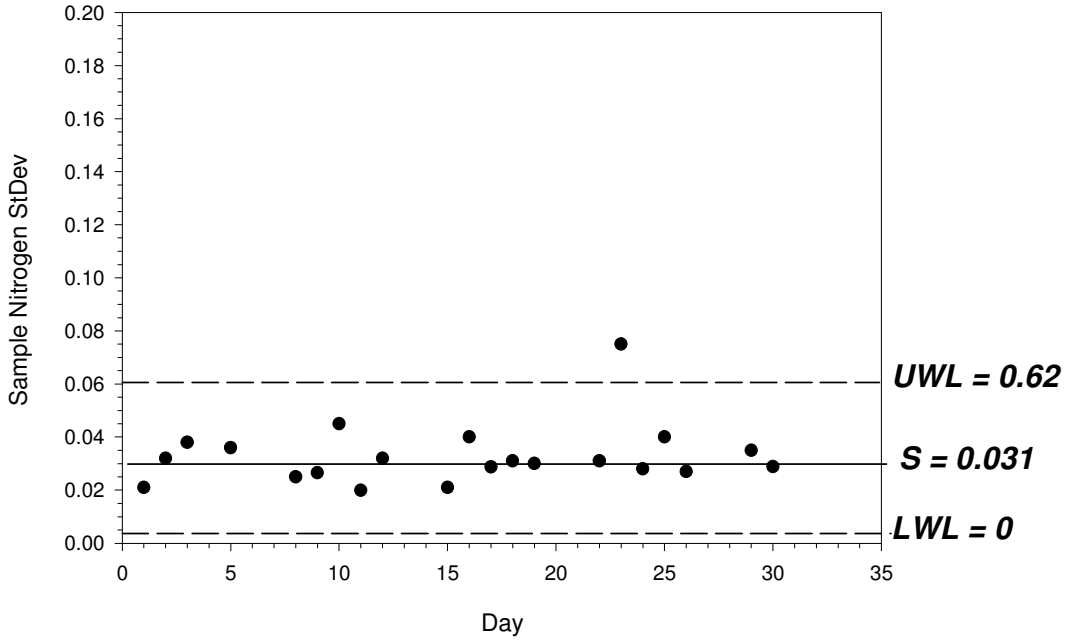


Figure 2. Example Nitrogen plant material R-chart for 30 Days.



The ***R***-chart is constructed from the standard deviation of QCR, CCS or matrix duplicate samples and can be expressed as a relative standard deviation or coefficient of variation. The ***R***-chart plots constancy of the standard deviation and therefore represents a test of variance homogeneity of the analytical method. ***R***-chart trend data also provides information on drift control.

Control charts not only document the accuracy and precision of the analytical method, but when used as a feedback mechanism can provide critical information to the analyst and laboratory manger on the influences of known and unknown modifications made to the method. As an example a laboratory may document contamination of a method reagent associated with a change in vendors or reagent lot numbers. An annual chart may identify temporal environmental variations attributed to temperature or humidity changes. It may provide documentation on the half-life of an unstable reagent. It can provide an evaluation known modifications to the analytical method such as new instrumentation, refinement of a technique, or the training of a new analyst. Overall, control charts provide critical information on the analytical process, its stability and a pivotal tool for its improvement.

External QC components involve the use of external evaluation samples of known concentration through proficiency testing programs and purchased reference standards. A listing of botanical reference standard suppliers is located in Appendix C. The use of samples of this type and performance programs provide information on laboratory bias.

Recommended QC Program Steps

1. Select the analytical method(s) and instrumentation, evaluate for performance. Evaluate inter-sample analyte carry-over, high concentration followed by matrix blank. Select analysis time based on 99% confidence of zero analyte carry-over.

2. MDL values are established for each analytical method based on EPA 40 CFR Part 136 method or that of Skoog and West (1982). PQL levels are established.

3. ***X***-chart and ***R***-charts are prepared for each calibration check standard (CCS).

4. Laboratory Reference Standards are analyzed evaluated for bias and precision. Methods with analysis values falling outside LRS confidence limits are evaluated for systematic errors.

5. Quality Control Reference (QCR) sample material is acquired and analyzed for analytes of interest six times per day over five days alongside three Laboratory Reference Standards. Mean and standard deviation is determined and ***X***-chart and ***R***-charts are prepared for each analyte.

6. Prior to each daily run each analysis method evaluated for quality. This evaluation consists of two method blanks, a minimum of three Quality Control Reference (QCR) samples each in duplicate, one Surrogate Standard Addition (where appropriate) and three Laboratory Reference Standards. All QC values (blanks, QCR and LRS) must be within tolerance limits, if not investigate bias or precision problems. During workload transition periods (after holidays or by session) going from light to heavy workloads, it may be necessary to increase QC sample frequency to verify quality assurance goals are met.

7. Unknown samples are organized in batches for analysis. These may range in size from 2 to 200 samples per batch. Each batch should include one method blank, 1- 5 unknown sample matrix duplicates and 5 % QCR samples. Method blank, duplicate and QCR samples are evaluated for QC tolerance limits based on ***X***-charts and ***R***-charts.

8. Once weekly or monthly, three QCR samples are submitted to the laboratory double-blind to evaluate QC tolerance limits based on ***X***-charts and ***R***-charts.

9. Reports are prepared for the unknown sample(s) which provide practical quantitation limit (PQL) values and precision levels of each reported analyte.

This brief overview is to familiarize the laboratory analyst/manager with the major components of a QA program. For the reader who is interested in developing or upgrading a QA program in the agricultural laboratory a number of articles and reference sources are available. The following is a brief list.

Dux, J.P. 1986. Handbook of quality assurance for the analytical chemistry laboratory. van Nostrand Reinhold Co. New York, NY.

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Analysis Calculations and Error

Errors are associated with all analytical measurements, but not all laboratory errors are monumental. There is no way to measure the " true value" of anything, the best that chemical analysis can do is to apply careful analytical technique and provide a reliable estimate based on standards and comparison of multiple measurements. With respect to the measurement one must be always cognizant of the error associated with the result. The following sections deal with the relationship between errors and the analytical measurement.

Significant Figures

The number of significant figures is the minimum number of digits needed to write a given value in scientific notation without loss of accuracy. The numbers 14.2 and 0.0142 both have three significant figures. While the numbers 1400 and 14,000,000 both have only two significant figures. The zeros are merely holding decimal places. Zeros are only significant when they occur in the middle of the number or to the right of decimal point. The number of significant figures used express a calculated result should be consistent with the uncertainty of the result.

The last significant figures in measured quantity always has some associate uncertainty or error. The minimum amount of uncertainty would be ±1 in the last digit. In general when reading the scale of an apparatus or instrument (with analog readings) one should interpolate between the markings. It is usually possible to estimate to the nearest half distance between two marks. Thus on a 50 mL burette which is graduated to 0.1 mL a technician could read the levels to the nearest 0.05 mL.

Propagation of Error

It is usually possible to estimate random error associated via a specific measurement, such as mass or temperature of a solution. Often the error is based on the operators estimate of how well the instrument was read contributing to random error. Most analytical measurements involve arithmetic operations which combine random errors. However the resultant error is not simply a sum of the individual errors because some are positive and some negative as well there is a certain amount of cancellation of errors.

For addition and subtraction the overall error can be calculated from the following equation where e_f is the overall error and e_1, e_2 and e_3 are the individual measurements:

$$e_f = \sqrt{e_1^2 + e_2^2 + e_3^2 \ldots\ldots}$$

As and example the following values were determined from mixing two reagents:

Reagent A volume:	2.76 ±0.03 mL
Reagent B volume:	1.89 ±0.02 mL
Final volume:	4.65 ± e_f

$$4.65 \pm e_f \text{ mL} = (2.76 \pm 0.03 \text{ mL} + 1.89 \pm 0.02 \text{ mL})$$

$$e_f = \sqrt{(\pm 0.03)^2 + (\pm 0.02)^2}$$

$$e_f = 0.04, \qquad 4.65 \pm 0.04 \text{ mL}$$

For multiplication and division all measurement errors need to be converted to percent relative errors (or relative errors). Thus the absolute error for the above calculation is ± 0.04, and the relative error is: 0.04 / 4.56 = 0.8%. Calculating the product or quotient error then as follows:

$$\%e_f = \sqrt{(\%e_1)^2 + (\%e_2)^2 + (\%e_3)^2 \ldots\ldots}$$

As an example the following values were determined for soil nitrate calculation:

Nitrate concentration extract: 7.5 ±0.07 mg L^{-1} NO_3-N (absolute e_f)
Soil / Extr. Dilution Factor: 5.0 ±0.07 L kg^{-1} (absolute e_f)

Nitrate concentration extract: 7.5 ±1.0% mg L^{-1} NO_3-N (relative e_f)
Soil / Extr. Dilution Factor: 5.0 ±1.4% L kg^{-1} (relative e_f)

Soil NO_3-N concentration = Extract Concentration x Dilution Factor

37.5 ±%e_f mg kg^{-1} NO_3-N = (7.5 ±1.0%) x (5.0 ±1.4%)

$$\%e_f = \sqrt{(\%0.93)^2 + (\%1.4)^2}$$

$\%e_f$ = 1.6%, 37.5 ±1.6% mg kg^{-1} NO_3-N

or 37.5 ± 0.6 mg kg^{-1} NO_3-N

Standard Calibration Curve

A majority of quantitative analytical measurements requires the construction of a standard calibration curve using known amounts of the desired element in solution. The standards should always be prepared using the same procedure as the unknowns and cover the expected concentration range of the unknown samples. It is critical that the composition of the standards be as closely matrix match as possible to that of the unknown solutions. Specific analytical determinations (i.e. boron spectrophotometric, azomethine; calcium flame emission spectrometry) require matrix masking agents or modifiers to minimize matrix problems. Typically a minimum of five to six calibration standards are used to develop the calibration curve for spectrophotometric analysis and four for ICP-AES analyses.

Typically plots of calibration data approximate a straight line; it is seldom however that all data will fall exactly on the line because of indeterminate error in the measuring process, or loss of linear response by the instrument detector. Statistics provides the best mechanism for objectively obtaining the equation for a line and specifying the uncertainty associated with its use for analyses. Two critical assumptions to the development of a calibration curve are: (1) that a definable relationship (linear, curvlinear etc.) exists between analyte concentration and the measured variable; and (2) that no significant error exists in the composition of the standards - that is the composition known with a high degree of certainty.

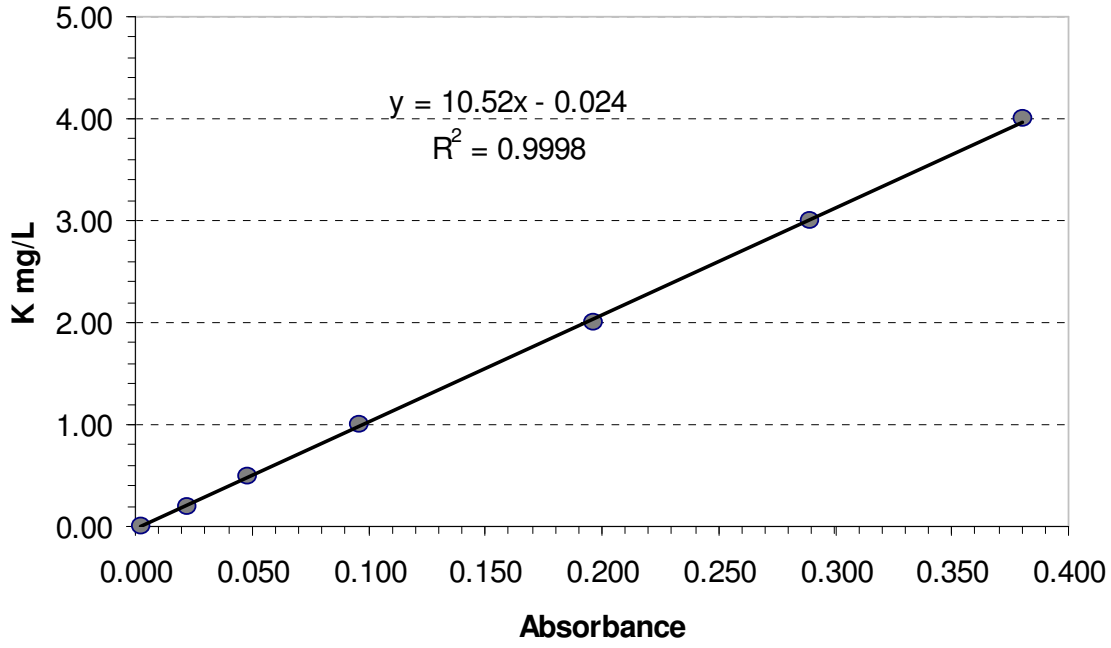
Although the mathematical equations necessary for regression analysis are readily derived, and for specific situations can be hand calculated, typically modern analytical instrumentation utilizes internal statistical software to perform standard calibration. Those laboratories with analytical instruments lacking such capabilities may rely on the use of computer spread sheet software such as Microsoft Excel, Lotus 1-2-3, Quatro Pro or statistical software packages to perform regression analyses. Examples of regression analysis calibration are shown in Table 1 and Figure 3.

All standard calibrations should be evaluated prior to computation of the unknown samples as shown in Table 1 and Figure 3. Standards showing a calculated value significantly deviating from the actual concentration indicates either an incorrect standard or incorrect instrument reading. Calculated standard values with a relative deviation exceeding 5% should be questioned and re-prepared.

Table 1. Example calibration data for potassium in 5% nitric acid-500 mg L^{-1} CsCl matrix as determined by atomic absorption spectrophotometry.

Standard Concentration K mg L^{-1}	Potassium Absorbance (AAS)	Calculated Concentration K mg L^{-1}	Deviation from Standard Value K mg L^{-1}	Relative Deviation from Standard %
0.00	0.002	0.00	0.00	-
0.20	0.022	0.21	-0.01	-5.72
0.50	0.048	0.48	0.02	3.01
1.00	0.096	0.99	0.01	1.01
2.00	0.196	2.04	-0.04	-2.10
3.00	0.289	3.02	-0.02	-0.68
4.00	0.380	3.98	0.02	0.59

Figure 3. Calibration graph for potassium standards in 5% nitric acid-500 mg L^{-1} CsCl matrix as determined by atomic absorption spectrophotometry.



Rejection of an Observation

When a set of data (from repeated analyses) contains an outlying result that appears different from the average, the decision must be made whether to retain or reject it. The choice of criteria for the rejection of the suspected result has its perils. Stringent standards making rejection difficult may result in the retention of a result that are spurious. While lenient standards making rejection of results easy, will discard measurements which rightfully belong to the data set, thus introducing bias to the data set.

Of the numerous statistical criteria available for evaluating extreme values, the *Q* test often chosen because of its simplicity. The test is based on the difference between the questionable result and its nearest neighbor which is divided by the range of the data set. The resulting *Q* test value is then compared with rejection values that are critical for a particular degree of confidence (Table 2).

Example:

Analysis of repeated analysis of soil organic matter yielded percentages of: 2.10, 2.07, 2.09, 2.09 and 2.14. The last value appears anomalous, should it be retained or rejected?

The difference between 2.10 (the nearest value) and 2.14 is 0.04%.
The range of the data is, 2.14 - 2.07, 0.07%

Thus: $Q_{exp} = \frac{0.04}{0.07} = 0.57$

For n=5 measurements, Q_{crit} is 0.64 for a 90% Confidence Interval.

Because 0.57<0.642, retention of the value is indicated.

Q test criteria must be used with good judgement. Situations do exist in which the dispersion on the data set is small and the indiscriminate application of the Q test will result in rejection of a value that should be retained. The blind application of statistical tests for decision for retention or rejection is not likely to be much more fruitful than an arbitrary decision. The application of good judgement based upon on analytical experience, and knowledge of the analysis is a sound approach. When a suspect value is found in a small set of results, the following criteria is recommended:

(1) Reexamine carefully all data for a gross error(s) which has affected the value.

(2) If possible, estimate the precision that can be reasonably expected from the analytical method to be sure that the outlying result is actually questionable.

(3) Repeat the analysis. Agreement with those that appear valid will lend support to reject the outlying result.

(4) Apply the Q test to see if the result should be retained or rejected on statistical grounds. If the Q test indicates retention, give consideration that to reporting the median rather than the mean. The median is a more robust measure and is less influenced by extreme values and moreover, the median is more likely to provide a reliable estimate of the correct value than the mean of a data set after the outlying value has been arbitrarily discarded.

Table 2. Critical values of Dixon's (r_{10}) *Q* parameter as applied to a two tailed test at three confidence levels, Rorabacher, 1991.

	Confidence Interval		
n	90 %	95 %	99%
3	0.941	0.970	0.994
4	0.765	0.829	0.926
5	0.642	0.710	0.821
6	0.560	0.625	0.740
7	0.507	0.568	0.680
8	0.468	0.526	0.634
9	0.437	0.493	0.598
10	0.412	0.466	0.568

Literature

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SATURATION PERCENTAGE S - 1.00

Saturation Paste Extract

Scope and Application

This method quantifies the soil water content of a saturated soil. At saturation all soil pore space is occupied by water and no free water collects on the surface. Salinity crop tolerance data; the relationships between cation solution concentrations and soil exchangeable cations (i.e. SAR); and soluble soil boron, are based on the saturation paste extract (U.S. Salinity Laboratory Staff, 1954 and Robbins, 1990). From the saturation paste, soil pH may be determined directly on the paste (Method S - 1.10). By extracting the liquid phase of the saturation paste under partial vacuum estimates of: electrical conductivity, EC_e (soluble salts); solution concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HBO_3, NO_3^-, SO_4^{2-}, Mn^{2+}, SeO_4^{2-}, HCO_3^-, CO_3^{2-}; and SAR can be determined. Estimates of soil water holding capacity, wilting point and texture can be made from the saturated moisture content. The method is generally reproducible within ± 12%, dependent on the soil textural class (Klages, 1984).

Equipment

1. Analytical balance: 500.0 g capacity, resolution ± 0.1 g
2. 500 mL container and cap (polypropylene container or 16 oz waxed paper cups).
3. Spatula, Blade 17.5 mm x 100 mm length.
4. Buchner filter assembly (preferably plastic) and vacuum system (capable of - 90 kPa).
5. Whatman No. 5 filter paper, or equivalent highly retentive filter paper.
6. Test tube or vial, 50 mL, polypropylene with cap.

Reagents

1. Deionized water, ASTM Type I grade. EC $<10^{-4}$ dS m^{-1}

Procedure

1. Weigh 200.0 ± 0.5 g air-dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) of known water content (P_w, %), into a 500 mL container and record total weight (See Comments #1 and #2).
2. Gradually add deionized water and mix uniformly (free of partially wetted clumps) until a saturated paste is obtained (See Comments #3 and #4). At saturation, the soil paste:

    i. Does not have free standing water on the surface of the paste.
    ii. Soil paste slides freely and cleanly off a spatula (does not apply to high clay soils, > 40% clay).
    iii. Paste will flow slightly when the container is tipped to a 45 degree angle from horizontal.
    iv. Soil surface glistens as it reflects light.
    v. Consolidates easily by tapping after a trench is formed in the paste with the flat side of a spatula (may not apply to sandy soils >70% sand).

3. Record weight, cap container and let stand for four (4) hours. Check saturation characteristics again and add soil or water as needed to obtain the desired characteristics (See Comment #5).
4. Record the mass of the soil (g) and total water (g) added.
5. After equilibration, thoroughly remix samples and determine soil pH, Method S - 1.10 (See Comment #6).
6. Transfer soil saturation paste to buchner funnel filter paper and spread evenly over surface. Apply -80 KPa vacuum and collect filtrate in test tube. Discontinue vacuum when cracks appear in soil paste. Refilter if filtrate is turbid. Determine EC_e, HCO_3^- and CO_3^{2-} with in five (5) minutes (Methods S - 1.20 and S - 1.30). Cap and retain filtrate for additional analysis (See Comments #7, #8 and #9).

Calculations

$$SP\ \% = \frac{(\text{Amount of water (g), added}) \times 100}{(\text{mass of air dry soil (g)} \times ((100 - P_w)/100)} \qquad \text{[equ. S-1.0-1]}$$

Report saturation percentage (SP) to the nearest 0.1% (See Comment #10 and #11).

Comments

1. Soil samples should not be oven-dried above 70 °C prior to extracting for soluble salts.

2. For organic soils (>16% organic matter) it is advisable to start with a 150 mL of water and add soil material.

3. Fine textured soils (> 40% clay) may puddle easily. To minimize puddling and obtain a more definite endpoint with fine-textured soils, water should be added with a minimum amount of stirring, especially in the early stages of wetting. Peat soils (>16% organic matter) will require soaking for twenty-four (24) hours. The method can be used assess greenhouse potting media.

4. Some fine textured soils swell considerably upon addition of water. In these cases, steps 2 and 3 must be repeated until the paste characteristics are stable. For salinity appraisal the paste can be extracted after four (4) hours; however, for sodic soil samples it should stand sixteen (16) or more hours. For the assessment of soil soluble boron, twenty-four (24) hours of paste equilibration is required.

5. Coarse textured soils, sandy loam and loamy sand with less than 15% clay, may not exhibit saturated paste characteristics of finer textured soils. For these soil types the relative accuracy of the method declines and should be noted when making soil comparisons.

6. If calcium carbonate precipitates are noted in the extract, dilute paste extract 1:1 with deionized water and note dilution in subsequent analysis. Samples may be refrigerated (4 °C) for storage (do not allow to freeze) for 30 days. Small quantities (200 uL) of thymol or toluene may be added to minimize the influence of microbial activity while samples are refrigerated (Carlson et al., 1971).

7. Determining saturated paste percentage alternative: take a 30 - 50 g sub sample of the paste, weigh, oven dry at 105 °C for four (4) hours, reweigh and calculate saturation percentage. Oven dry moisture values will be slightly higher than the direct method as air dry soil will retain 3-5% moisture, dependent on clay and salt content.

8. Extraction consistency is best achieved using a vacuum of -60 to -80 KPa (-0.6 to - 0.8 bars) applied for thirty (30) minutes (Jacober and Sandoval, 1970). Soils maybe centrifuged.

9. Approximately one-quarter to one-third of the water added in making the saturated paste can be recovered as extract (Loveday, 1974).

10. Soil Field Capacity (FC, 33 kPa) can be estimated from the saturation percentage as follows: SP × 0.5 ≅ FC. Soil water potential Permanent Wilting Point (PWP, 1500 kPa) can be estimated as follows: SP × 0.25 ≅ PWP. Saturation percentage is related to soil texture as follows (based on organic matter contents less than 3%):

SP (%)	Soil Texture
0 < 20	sand or loamy sand
20 - 35	sandy loam
35 - 50	loam or silt loam
50 - 65	clay loam
65 - 135	clay
> 81	organic soils

For fine-textured soils and those high in sodium (SAR > 10), SP cannot be used to estimate FC and PWP values (Reeves et al. 1954).

11. Soil saturated paste has been used to access Mn toxicity on soils from Hawaii. Concentrations of Mn greater than 0.5 - 1.0 mg L^{-1} are toxic to most crops, specifically vegetables.

Literature

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SATURATION PASTE SOIL pH S - 1.10

Scope and Application

This method semi-quantifies the soil pH of a soil saturated paste (Method S - 1.00). Soil pH is a measure of the relative acidity or alkalinity of the soil solution that is in equilibrium with the solid particles. It is a measure of the intensity of acidity or alkalinity, but does not indicate the relative buffering capacity of the soil. It is most applicable to salt-affected soils with a pH ranging from 6.0 to 9.0 (Robbins et. al. 1990). Soil pH is measured to access soil chemical properties, crop suitability, lime needs and relative nutrient availability. The method is generally reproducible within ± 0.10 pH units.

Equipment

1. pH meter, equipped with pH electrodes (indicating and reference).
2. Primary standard buffers, pH 4.00, 7.00, and 10.0.

Procedure

1. Prepare a saturation paste, as outlined in Method S - 1.00.
2. Standardize / Calibrate the pH meter: (1) rinse electrode with deionized water and place in pH 7.00 primary standard buffer and adjust as necessary; (2) rinse electrode and place in pH 4.00 primary standard buffer; (3) adjust the slope until response is ±0.05 units of expected response; and (4) check pH 7.00 primary standard buffer and adjust as necessary (See Comment #1). For high pH soils (>7.00) use pH buffers 7.00 and 10.0.
3. Insert electrode into soil paste and gently rotate the container to remove entrapped air. When the meter has stabilized record soil pH as pH_{sp} to the nearest 0.01 pH unit.
4. Remove electrode(s), rinse with deionized water and blot excess water with filter paper (See Comment #2).

Comments

1. Follow manufacturer's guidelines if meter does not read within 0.05 units of primary standards. Maintenance of combination electrodes differs from that of separate reference and glass electrodes; refer to manufacturer's instructions.

2. Store pH electrodes according to manufacturer's instructions (recommended practice is to store the electrodes in a primary standard buffer).

Literature

Rhoades, J.D. and S. Miyamoto. 1990. Testing soils for salinity. p. 299-336. *In*: R.L. Westerman (ed.) Soil testing and plant analysis. 3rd ed. SSSA, Madison, WI.

Robbins, C.W. and C.L. Wiegand. 1990. Field and laboratory measurements. p. 201-219. *In*: K.K. Tanji (ed.) ASCE manuals and Reports No. 71, Agricultural salinity, assessment, and management. American Society of Civil Engineers, 245 E. 47th St., New York.

U.S. Salinity Laboratory Staff. 1954. Saturated soil paste. Diagnosis and improvement of saline and alkali soils. Agr. Handbook 60, USDA, Washington, D.C.

Scope and Application

This method quantifies the amount of dissolved salts (mg L^{-1}) by measurement of the electrical conductivity (EC_e) of the soil saturated paste extract (Method S - 1.00). The relationship between EC_e and soluble salts is approximate due to differences in equivalent weights, ion equivalent conductivities, and relative proportions of major solutes in the paste extracts (Robbins, 1990). The EC_e measurement is sensitive to temperature and increases approximately 1.9% per °C (range 15 - 35 °C) (Rhoades, 1996). All EC_e data is normalized to 25 °C. Salt tolerance crop data is generally expressed in terms of the (EC_e) of the saturation paste extract and used to assess the potential of soluble soil salts which may limit crop productivity. The method detection limit is approximately 0.01 dS m^{-1} (mmhos cm^{-1}) and is generally reproducible within ± 7%.

Equipment

1. Conductance meter with dynamic range from 0.01 to 100 dS m^{-1} conductance, temperature compensating, 25 °C.
2. Conductance cell having a cell constant (K) appropriate to the EC of the sample being measured (see Table S -1.2 -1). Pipet-type or dip-type cell and it recommended that it be capable of measuring temperature.

Reagents

1. Deionized water CO_2-free, ASTM Type I grade. EC <10^{-4} dS m^{-1}.
2. Standard Reference Calibration Solution. Dissolve 0.7456 g KCl (previously dried at 110 °C for 2 h) in CO_2 -free deionized water and dilute to 1.0 L. At 25 ±0.1 °C a 0.010 N KCl solution will have an EC_e of 1.412 dS m^{-1} (mmhos cm^{-1}). For a 0.100 N KCl solution (7.456 g KCl diluted to 1.0 L) will have an EC_e of 12.900 dS m^{-1}. Standard EC calibration solutions are listed in Table S-1.20-A and can be purchased from a scientific supply vendor.

Procedure

1. Prepare a saturation paste, as described in Method S - 1.00, and retain extract for EC_e measurement (See Comment #1).
2. Calibrate conductance cell. Operate and adjust instrument in accordance with manufacturer's instructions (See Comments #2 and #3). Rinse conductance cell with three aliquots of 0.01 N KCl, adjust a fourth portion to 25 ±0.1 °C, measure R (where R is the measured resistance ohms) and temperature *t*. Repeat measurement of R until value is constant. Calculate cell constant K. Develop four point calibration curve.

$$K = (0.001413)\ R_{KCl})/[1+0.019(25-t)]$$

3. Rinse conductance cell with deionized water. Draw approximately 2.0 mL of soil saturation paste extract solution into conductance cell rinse and replace with a second aliquot. When the meter has stabilized record instrument reading.

Calculations

$$EC_{25} = C_x(1000)K[1 + 0.019(25 - t)]$$

Where: C_x is the instrument measured value of the sample and *t* is temperature

Report EC_e to the nearest 0.01 dS m^{-1} as EC_e 25 °C.

(See Comments #4, and #5)

Table S -1.20-A Conductivity of KCl solutions at 25 °C (Rhoades, 1996).

Concentration N	Conductivity dS m^{-1}
0.001	0.147
0.010	1.413
0.020	2.767
0.050	6.668
0.10	12.90
0.20	24.82
0.50	58.64

Comments

1. Exposure of the sample to the atmosphere may cause changes in conductivity due to loss or gain of dissolved gasses: CO_2 and NH_3-N. Freshly distilled water has a conductivity of 0.005 - 0.002 dS m^{-1} increasing after a few weeks to 0.002 -0.004 dS m^{-1}. This of special concern on samples with very low EC_e.

2. Clean platinum electrodes that are new or that are providing erratic EC readings with acid-dichromate cleaning solution. Cleaning solution: 32 mL of saturated sodium dichromate ($Na_2Cr_2O_7$) and 1L 16 M sulfuric acid. Soak electrodes 16 hours followed by three rinses of deionized water rinses. If platinum is flaked, recoat according to procedure of APHA (1985).

3. For highly saline soils (EC_e >8.0 dS m^{-1}) calibrate using 0.100 N KCl solution, EC_e 12.90 dS m^{-1}.

4. The relationship between conductivity and soluble salts is approximate due to differences in solutes, solute conductivities, and equivalent weights. The general relationship (for solutions with an EC_e range of 0.10 - 2.0 dS m^{-1}) is:

   Dissolved salt concentration (mg L^{-1}) $\cong$ 640 × EC_e, in dS m^{-1}
   Total cations (or anions) (mmolc L^{-1} or meq L^{-1}) $\cong$ 10 × EC_e, in dS m^{-1}
   Osmotic potential at 25 °C (KPa) $\cong$ 0.39 × EC_e, in dS m^{-1}

   The factor for converting EC_e to total dissolved salts (mg L^{-1}) ranges from 550 to 900 dependent on the specific anions present and their concentration. For estimating approximate total cations or anions, USDA Handbook #60, Figure 4, graphically shows this relationship for typical salt concentrations.

5. Plant tolerances to salinity (EC_e) of the soil saturated paste extract shown in Table S -1.2 - B.

Table S -1.20-B Impact of saturated paste soil salinity (EC_e) on plant growth.

dS m^{-1}	Plant salinity effects, productivity reduced 25%.
0 - 2	salinity effects negligible (field bean, carrot, onion, red clover strawberry)
2 - 4	very sensitive crops affected (spinach, lettuce, citrus, grape, alfalfa)
4 - 8	moderately salt tolerant crops affected (tomato, beet, wheat)
8 - 16	only salt tolerant crops yield satisfactory (barley, wheatgrass cotton, asparagus)
> 16	few salt tolerant crops yield satisfactory

Literature

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Robbins, C.W. and C.L. Wiegand. 1990. Field and laboratory measurements. p. 201-219. *In*: K.K. Tanji (ed.) ASCE manuals and Reports No. 71, Agricultural salinity, assessment, and management. American Society of Civil Engineers, 245 E. 47th St., New York.

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SATURATION PASTE EXTRACT ALKALINITY S - 1.30

Bicarbonate and Carbonate

Scope and Application

This method quantifies bicarbonate (HCO_3^{1-}) and carbonate (CO_3^{2-}) concentration in mmolc L^{-1} (meq L^{-1}) in the soil saturation paste extract (Method S - 1.00). It is based on titration with 0.10 N hydrochloric acid. The determination of HCO_3^{1-} and CO_3^{2-} should be made immediately due to the potential of the extract being super saturated relative to calcium carbonate ($CaCO_3$). The concentration of HCO_3^{1-} affects the solubility of calcium, the ionic strength of the extract solution and is used to calculate the adjusted SAR (Robbins, 1990 and Hanson et al. 1993). The method detection limit is approximately 0.05 mmolc L^{-1} (meq L^{-1}) and is generally reproducible within ± 10%.

Equipment

1. Titration burette 50.0 ± 0.2 mL, or automatic titrator.
2. pH meter and combination pH electrode.
3. Pipette, 2.0 ±0.05 mL and 5.0 ± 0.05 mL.
4. 50 mL beaker.
5. Magnetic stir plate and micro size (0.25 mm) Teflon coated magnetic stir bar.

Reagents

1. Deionized water, ASTM Type I grade.
2. Primary standard buffer solutions: pH 4.00, 7.00 and 10.0.
3. Standardized hydrochloric acid (HCl) solution, 0.020 N with respect to H^+ (See Comment #1).

Procedure

1. Prepare a soil saturated paste extract according to Method S - 1.00 and retain extract for carbonate and bicarbonate analysis.
2. Standardize / calibrate the pH meter: (1) rinse electrode with deionized water and place in pH 7.0 primary standard buffer and adjust as necessary; (2) rinse electrode and place in pH 4.0 primary standard buffer; (3) adjust the slope until response is ±0.05 units of expected response; and (4) and recheck standard buffers (See Comments #2 and #3).
3. Place 1.0 to 50 mL aliquot of saturation paste extract in beaker, and bring to 50 mL volume with deionized water and add magnetic stirrer. Place on stir plate and insert pH electrode (See Comment #4). Record amount of titrant needed to reach a pH of 8.3 for CO_3^{2-} and 4.5 for HCO_3^{1-} to the nearest 0.2 mL.
4. Determine the amount of HCO_3^{1-} in deionized water blank solution.

Calculations

$$CO_3^{2-} \text{ mmolc } L^{-1} = \frac{(2 \times P \times N) \times 1000}{\text{aliquot (mL)}} \qquad HCO_3^{1-} \text{ mmolc } L^{-1} = \frac{(T - (2 \times P)) \times N \times 1000}{\text{aliquot (mL)}}$$

P = number of mL of HCl of normality N to reach CO_3^{2-} inflection point, pH 8.3;
T = number of mL of HCl of normality N to reach HCO_3^{1-} inflection point, pH 4.5;
aliquot = volume of saturation paste extract sample, mL.

Comments

1. Standardized 0.020 N HCl solution can be prepared from dilution of 1.00 N HCl standard reference solution or standardized by titration of known bases (Horneck, 1989).

2. Follow manufacturer's guidelines if meter does not read within 0.05 units of primary standards. Maintenance of combination electrodes differs from that of separate reference and glass electrodes; refer to manufacturer's instructions.

3. Store pH electrodes according to manufacturer's instructions (usual recommended practice is to store the electrodes in a primary standard buffer).

Literature

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Horneck, D.A., J.M. Hart, K. Topper and B. Koespell. 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University. Ag. Expt. Station SM 89:4. p. 13.

Rhoades, J.D. 1982. Soluble salts. p. 167-178. *In:* A. L. Page et al. (ed.) Methods of soil analysis: Part 2. Agronomy Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI.

Rhoades, J.D. and S. Miyamoto. 1990. Testing soils for salinity. p. 299-336. *In*: R.L. Westerman (ed.) Soil testing and plant analysis. 3rd ed. SSSA, Madison, WI.

Robbins, C.W. and C.L. Wiegand. 1990. Field and laboratory measurements. p. 201-219. *In*: K.K. Tanji (ed.) ASCE manuals and reports No. 71, Agricultural salinity, assessment, and management . American Society of Civil Engineers, 245 E. 47th St., New York.

U.S. Salinity Laboratory Staff. 1954. Saturated soil paste. Diagnosis and improvement of saline and alkali soils. Agr. Handbook 60, USDA, Washington, D.C.

SATURATION PASTE EXTRACT SOLUBLE CHLORIDE S - 1.40

Chloride

Scope and Application

This method quantifies the concentration of chloride (mmolc L^{-1} or meq L^{-1}) in the saturation paste extract (Method S - 1.00). Chloride may be determined using an ion selective electrode (potentiometric), chloridometer or ion chromatography instrument methods. Plant tolerance to chloride can be related to its concentration in the soil saturation paste extract. The method detection limit is approximately 0.1 mmolc L^{-1} dependent on the method of analysis and is generally reproducible within ± 10%. The unit mmolc L^{-1} is the accepted scientific unit for reporting the concentration of anions and cations and is equivalent to meq L^{-1}.

Equipment

1. Solid-state chloride electrode and double junction reference electrode, chloridometer or Cl titrator.
2. pH/ion meter or millivolt meter.

Reagents

1. Deionized water, ASTM Type I grade.
3. Chloride standard, 1.0 $mmol_c$ L^{-1}: Dissolve 74.1 mg of KCl in 500 mL of deionized water and dilute to 1.0 L final volume.

Procedure

1. Prepare a soil saturated paste extract according to Method S - 1.00 and retain for chloride analysis (See Comment #1).
2. Determine the chloride concentration by ion selective electrode, chloridometer or ion chromatography. The instrument chosen will determine specific matrix modifications and sample dilutions. Adjust and operate instruments in accordance with manufacturer's instructions. Calibrate instrument using calibration solutions and determine chloride concentration of a method blank and unknown samples (See Comments #2 and #3). Report chloride concentration in saturation paste extract to the nearest 0.1 mmolc L^{-1} (See Comments #4).

Comments

1. Care must be taken to clean all labware prior to analysis. Wash all labware with 0.2 N HNO_3 and deionized water.

2. To accurately determine saturation paste chloride concentrations less than 2.0 $mmol_c$ L^{-1}, it is advisable to use standard additions techniques and potentiometric analysis (Fixen et al., 1988)

3. Samples containing chloride concentrations greater than the highest standard will require dilution.

4. Tolerance of plants to soil chloride levels in the soil saturated extract is listed in Table S-1.40-A.

Table S-1.40-A Tolerance of some plants to chloride in the soil saturated extract.

Crop	Chloride ($mmol_c\ L^{-1}$)
Alfalfa	23
Barley	90
Beets	90
Citrus (rootstock dependent)	10-25
Corn (2-8 leaf stage)	70
Cotton	50
Grapes (Thompson Seedless)	25
Tomato	39
Wheat (young)	25

Literature

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SATURATION PASTE EXTRACT SOLUBLE BORON S - 1.50

Azomethine-H Spectrophotometric / ICP-AES

Scope and Application

This procedure quantitatively determines the boron concentration in the soil saturation paste extract (Method S - 1.00). It is based on the complexation of azomethine-H with HBO_3 to form colored complex in an aqueous matrix with subsequent spectrophotometric measurement at 420 nm (Wolf, 1974). EDTA chelate is added to minimize chemical interferences. The method is readily adapted to manual or automated techniques. Boron can also be determined by Inductively coupled plasma emission spectrometry (ICP-AES) using one of three wavelengths. The method quantifies soil boron concentrations which can limit crop yield or be toxic to plant growth. The method is not applicable for assessing potential soil boron deficiencies. The method detection limit is approximately 0.10 mg L^{-1} and is generally reproducible to within ± 8%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.01 g.
2. 15 mL test tube or vial, polypropylene.
3. Pipette, 2.0 ± 0.05 mL and 3.0 ± 0.05 mL.
4. Vortex stirring device.
5. Spectrophotometer, wavelength 420 nm or ICP-AES 249.678, 249.773 or 208.959 nm.

Reagents

1. Deionized water, ASTM Type I grade.
2. Buffer-masking solution: Dissolve 250 g of ammonium acetate (reagent grade $NH_4C_2H_3O_2$), 25.0 g of disodium salt of ethylenedintrilo-teraacetic acid (Na_2-EDTA) in 400 mL of deionized water. Very slowly add 125 mL of glacial acetic acid, while stirring using a magnetic stirrer. Temporary acidic conditions may cause a slight precipitation of the EDTA salts. Continue to stir the solution until the EDTA dissolves. Do not heat the solution. Adjust the buffer to a pH of 5.4 to 5.6 with acetic acid or NH_4OH as necessary. Prepare fresh solution every two months.
3. Azomethine-H solution: Dissolve 0.9 g of azomethine-H, 2.0 g of L-ascorbic acid in 50 mL of deionized water prewarmed to 60 ^{o}C. Dilute to 100 mL and store in refrigerator. Solution is stable for forty-eight (48) hours (see comments #3 and #4).
4. Standard Boron Calibration solutions. Prepare six boron calibration standards: concentration 0.05, 0.20, 0.50,1.0, 2.0 and 4.0 mg L^{-1}, prepared in deionized water from a standard 1000 mg L^{-1} solution.

Procedure

1. Prepare a soil saturated paste according to Method S - 1.00 and allow to equilibrate twenty-four (24) hours. Retain extract for boron analysis. Boron can be determined directly using an ICP-AES instrument using wavelengths specified in Appendix A.

Spectrophotometric Analysis

1. Pipette a 2.0 mL aliquot of soil extract into a 15 mL polypropylene tube followed by 3.0 mL of the Buffer-masking solution using a pipette and stirr with vortex stirring device (See Comment #1 and #2).
2. Using a repipette add 2.0 mL of azomethine-H reagent and stir contents thoroughly. Allow the mixture to stand sixty (60) minutes.
4. Prepare standard curve following steps 4-5, substituting 2.0 mL of standard calibration solution for soil extract. A method blank is prepared in the same manner using deionized water.
5. Adjust and operate spectrophotometer instrument according to manufacture's instructions. Calibrate instrument using standard calibration solutions. Determine boron concentration of a method blank and unknown saturation paste extracts (See Comments #4 - #7).

ICP-AES Analysis

1. Adjust and operate ICP-AES instrument according to manufacture's instructions. Determine B using the 249.773 nm or 249.678 nm wavelength (see Appendix A-1) and calibrate standards of 0.02, 0.50, 1.0 and 4.0 mg L^{-1} in deionzed water matrix. Determine boron concentration of a method blank and unknown saturation paste extracts (See Comments #4 - #7).

Calculations

Calculate boron concentration of saturated paste extract from working standard curve. Report boron concentration to the nearest 0.01 mg L^{-1} of the saturation paste extract.

Comments

1. Prepare all reagents and perform all analyses in polypropylene or teflon labware. Do not use borosilicate glassware.

2. Check pipette dispensing volume,calibrate using an analytical balance.

3. EDTA chelate is added to eliminate chemical interferences from Al, Fe and Cu. Concentration of the chelate may have to be increased for soil extracts containing high concentrations of these elements.

4. The azomethine-H reagent should be added quickly so that color development is equal for all samples. A constant check must be maintained on linearity and drift of the standard curve when analyzing a large set of samples.

5. For solutions with a distinct coloration of the extract: Prepare a second solution and blank for step two of the procedure adding 1.0 mL of deionized water in place of azomethine-H solution and vortex well. The blank for this determination consists of 5.0 mL of 0.02 M $CaCl_2$ solution and 1.0 mL of buffer-masking solution.

6. For laboratories utilizing ICP-AES instrumentation it is suggested to use a rinse between samples with of 0.10 M D-sorbitol solution.

7. Plant sensitivity to saturation paste extract boron is as follows (USDA Salinity Lab., 1954):

Table S-1.50-A Tolerance of some plants to boron in the soil saturated extract.

B mg L^{-1}	Plant Sensitivity
< 0.7	safe for sensitive plants (peach, pear, plum)
0.7 - 1.5	moderately tolerant (cotton, wheat, bell pepper)
1.5 - 4.0	Toxic to all but tolerant plants (alfalfa, lettuce, sugar beet)
> 4.0	Generally toxic to all plants

Literature

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SATURATION PASTE EXTRACT **S - 1.60**
CALCIUM, MAGNESIUM, SODIUM, AND SAR
AAS ICP-AES Method

Scope and Application

This method quantitatively determines the concentration (mmolc L^{-1}, meq L^{-1}) of dissolved Ca, Mg and Na in the soil saturation paste extract (Method S - 1.00) using atomic absorption spectrometry (AAS) or Inductively coupled plasma emission spectrometry (ICP-AES). A chemical interference solution is used to minimize chemical matrix effects. The Sodium Absorption Ratio (SAR) of saturation paste extract is calculated from the concentration of these cations. The relationship between cation solution concentrations and exchangeable cations in the soil, is used to estimate exchangeable sodium percentage (ESP) from the SAR (Robbins, 1990). The method detection limit for these cations is approximately 0.02 mmolc L^{-1} on a solution basis and it is generally reproducible within ± 7%. The unit mmolc L^{-1} is the accepted scientific unit for reporting the concentration of anions and cations and is equivalent to meq L^{-1}.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.01 g.
2. Atomic Absorption Spectrophotometer (AAS) Inductively coupled plasma emission spectrometry (ICP-AES) instrument.

Reagents

1. Deionized water, ASTM Type I grade.
2. Chemical interference solution, 5000 mg L^{-1} lanthanum oxide (La_2O_3) - 2000 mg L^{-1} , cesium chloride (CsCl) solution. Dissolve: 4.691 g LaO_3 and 5.071 g CsCl in 1500 mL of deionized water and add 25.0 mL of $HClO_4$ and 25.0 mL of HNO_3 and dilute to 2000 mL.
3. Standard calibration solutions of Ca, Mg, and Na: Prepare six calibration solutions containing 0.05 - 1.3 mmolc L^{-1} of Na, 0.05 - 3.5 mmolc L^{-1} of Ca, and 0.02 - 1.6 mmolc L^{-1} for Mg prepared from 1000 mg L^{-1} standard reference solutions and dilute to volume with chemical interference solution.

Procedure

1. Prepare a soil saturated paste extract according to Method S - 1.00 and retain extract for cation analysis.
2. Dilute an aliquot of the saturated paste extract 10:1 with chemical interference solution (See Comment #1 and #2). For analysis by ICP-AES no chemical interference solution is required.
3. Adjust AAS or ICP-AES instrument according to manufacturer's instructions. Calibrate instrument using calibration solutions and determine individually cation (Ca, Mg, and Na) concentrations of saturation paste extracts and record as mg L^{-1} of analyte.

Calculations

$$[Ca]\ \text{mmolc}\ L^{-1} = \frac{Ca\ \text{mg}\ L^{-1} \times 10}{20.0\ \text{mg mmolc}^{-1}}$$

$$[Mg]\ \text{mmolc}\ L^{-1} = \frac{Mg\ \text{mg}\ L^{-1} \times 10}{12.15\ \text{mg mmolc}^{-1}}$$

$$[Na]\ \text{mmolc}\ L^{-1} = \frac{Na\ \text{mg}\ L^{-1} \times 10}{23.0\ \text{mg mmolc}^{-1}}$$

$$SAR = \frac{[Na]}{([Ca] + [Mg])/2)^{½}}$$

Report Ca, Mg, and Na concentrations to the nearest 0.1 mmolc L^{-1} and SAR to the nearest 0.1 (See Comments #3, #4, #5 and #6).

Comments

1. Saturation paste extract solutions containing greater than 750 mg L^{-1} soluble salts (> 1.2 dS m^{-1}, estimated from EC_e Method S - 1.20) will require additional dilution.

2. Cations may also be determined on the saturation paste extract using ICP-AES or ion chromatography instrumentation.

3. A measure of soil sodicity, molar proportion of cation-exchange sites occupied by sodium (Na_{exch}) can be calculated from the SAR (U. S. Salinity Laboratory, 1954). CEC can be determined using Method S-10.1 or S-10.2.

$$ESP = \frac{Na_{exch}}{CEC} \times 100 \qquad \text{[equ. S -1.6-1]}$$

$$ESP = \frac{100 \times (-0.0126 + 0.0147 \times SAR)}{(10 + (0.036 + 0.1051 \times SAR)} \qquad \text{[equ. S -1.6-2]}$$

4. Soils having an SAR greater than 13 and/or ESP > 15% are considered sodic.

5. For laboratories utilizing ICP-AES instrumentation calibrate use the 422. 673 nm wavelength for Ca, 285.213 nm for Mg, and 588.995 nm wavelength for sodium (see Appendix A) using the standards of the calibration ranges described above.

6. For samples that the HCO_3 constitutes more than 25% of the anions it may be necessary to determine the adjusted SAR. See water method W - 1.60 to calculate.

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Literature

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SATURATION PASTE EXTRACT SOLUBLE SULFATE S - 1.70

Sulfate - Turbidimetric

Scope and Application

This method quantifies the concentration of sulfate (SO_4^{2-} mmolc L^{-1} or meq L^{-1}) in the soil saturated paste extract (Method S - 1.00). The unit mmolc L^{-1} is the new accepted scientific unit for reporting the concentration of anions and cations and is equivalent to meq L^{-1}. Sulfate may be determined using turbidimetric, ion chromatography, or ICP-AES instrument methods. This method outlines the turbidimetric analysis which closely follows that described in 1992 Standard Method of the Examination of Waste Water. Sulfate is determined to evaluate anion balance in the soil saturated paste extract and estimate gypsum content . It has a method detection limit is approximately 0.02 mmolc L^{-1} and is generally reproducible within ± 7%.

Equipment

1. Magnetic stirrer.
2. Repipette dispenser calibrated to 2.0 ± 0.05 mL
3. Pipette 10.0 mL.
4. Magnetic stir plate and Teflon stir bar.
5. Nephelometer (preferred), Turbidimeter or Spectrophotometer 340 nm.

Reagents

1. Deionized water, ASTM Type I grade.
2. Turbidimetric solution. Dissolve 30.0 g of $MgCl_2 \cdot 6H_2O$; 5.0 g $CH_3COONa \cdot 3H_2O$; 1.0 g KNO_3; 20 mL acetic acid, CH_3COOH (99%) and 0.111 g Na_2SO_4, in 500 mL deionized water and add 5.0 g of powered gum acacia, or gelatin (See Comment #1) suspension agent. Dilute to 1000 mL final volume.
3. Barium chloride crystals. Parr turbidimetric grade, $BaCl_2 \cdot 2H_2O$ crystals 20 - 30 mesh. Use high purity $BaCl_2$, as low purity may result in low recovery of SO_4^{2-} (See Comment #2).
4. Standard sulfate-sulfur calibration solutions. Prepare 5.0 mmolc L^{-1} SO_4^{2-} calibration stock solution, dissolve 0.4353 g of oven dry K_2SO_4 in 500 mL of deionized water and dilute to one 1000 mL. Prepare six 100 mL calibration solutions of: 0.05, 0.1, 0.2, 0.4, 0.8, and 1.0 mmolc L^{-1} SO_4^{2-} from a 5.0 mmolc L^{-1} SO_4^{2-} solution and bring to final volume with deionized water.

Procedure

1. Prepare a soil saturated paste extract according to Method S - 1.00 and retain for sulfate analysis (See Comment #3). If the aliquot is turbid, filter prior to analysis.
2. Dilute a 10.0 mL aliquot with 10.0 mL of deionized water. Repeat using sulfate standards and method blank.
3. Add 2.0 mL of turbidimetric solution using a repipette (See Comment #4). Add magnetic stir bar and beginning stirring.
4. While stirring add 0.2 g of $BaCl_2 \cdot 2H_2O$ crystals with measuring spoon.
5. Stir for sixty (60 ± 3) seconds, then remove from stirrer and after five (5 ±0.5) minutes read absorbance with nephelometer or spectrophotometer at 340 nm (See Comment #5 and #6). Repeat with sulfate calibration solutions and method blank. Using standard calibration solutions and determine sulfate concentration of saturate paste extracts and method blank. Record as mmolc L^{-1} SO_4^{2-} of analyte in extract solution to two significant digits.

Calculations

Report soil saturated paste extract:

mmolc L^{-1} SO_4^{2-} = (mmolc L^{-1} SO_4^{2-} saturated paste extract - method blank) × (2)

(1.0 mmolc L^{-1} SO_4^{2-} = 48.03 mg L^{-1} SO_4^{2-})

Comments

1. A number of suspension agents have been reported in the literature which include: gum acacia, gelatin, glycerol, PVP-K30 (polyvinylpryrolidinone), and Tween 80 which have proven effective in turbidimetric analysis. Each of these will require experimentation and practice using SO_4-S spiking to fully refine the technique.

2. Use $BaCl_2$ specifically designated for turbidimetric determination of sulfate-sulfur. Sources: J.T. Baker Cat. Parr Turbidimetric $BaCl_2$, JT0974-5; VWR JT0974-5; and GFS Chemicals, Reagent Grade ACS #602.

3. Care must be taken to clean all labware prior to analysis. Pre-rinse all extraction flasks, turbidimetric and spectrometer cuvette in hot water followed by 0.5 N HCl rinse with deionized water.

4. Check repipette volume, calibrate using an analytical balance.

5. Samples containing SO_4^{2-} concentrations greater than the highest standard will require dilution.

6. For laboratories utilizing ICP-AES instrumentation calibrate use the 182.669 nm wavelength and calibration standards of 0.05, 0.50, 1.0, and 5.0 mmolc L^{-1} SO_4^{2-} (see Appendix A-1).

Literature

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SATURATION PASTE EXTRACT SOLUBLE NITRATE S - 1.80

Nitrate (NO_3^-)

Scope and Application

This method quantifies the concentration of nitrate (NO_3^-) (mmolc L^{-1} or meq L^{-1}) in the saturation paste extract (Method S - 1.00). Nitrate may be determined using an ion selective electrode (ISE, see Method S-3.20), ion chromatography or cadmium reduction spectrophotometric methods. This method outlines the use of the cadmium reduction spectrophotometric method (automated) outlined by (Keeney, 1982). The method detection limit is approximately 0.04 mmolc L^{-1} dependent on the method of analysis and is generally reproducible within ± 10%. Nitrate is determined to for anion balance and crop nitrogen nutrient status. The unit mmolc L^{-1} is the accepted scientific unit for reporting the concentration of anions and cations and is equivalent to meq L^{-1}.

Equipment

1. Spectrophotometer, autoanalyzer, or flow injection analyzer (FIA) instrument.

Reagents

1. Deionized water, ASTM Type I grade.
2. Standard calibration solutions of NO_3-N. Prepare six calibration standards ranging from 0.05 to 1.5 mmolc L^{-1} concentration, diluted in 0.05 N $CaCl_2$ solution prepared from 16.1 mmolc L^{-1}(1000 mg L^{-1}) NO_3^- standard solution.

Procedure

1. Prepare a soil saturated paste extract according to Method S - 1.00 and retain for nitrate analysis (See Comment #1).
2. Nitrate (NO_3^-) content of the extract is determined using a spectrophotometer, automated flow analyzer (Technicon Method No. 329-74W/A) or FIA instrument. Calibrate using standard calibration solutions and operate instrument in accordance with manufacturer instructions. Determine nitrate concentration of saturated paste extract, method blank, unknown samples and record results as mg L^{-1} of nitrate in extract solution (See Comment #2)

Calculations

Report soil saturated paste extract:

mmolc L^{-1} NO_3^- = (mmolc L^{-1} NO_3^- saturated paste extract - method blank)

(1.0 mmolc L^{-1} NO_3^- = 62.0 mg L^{-1} NO_3^-)

Comments

1. Care must be taken to clean all labware prior to analysis. Wash all labware with 0.1 N HCl and deionized water.

2. Samples containing nitrate concentrations greater than the highest standard will require dilution.

Literature

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SOIL pH (1:2) **S - 2.10**

Soil: DI Water Ratio Method

Scope and Application

This method semi-quantifies the pH of soil, using a 1:2 soil:water extract of the soil using deionized water. Soil pH is a measure of the relative acidity or alkalinity of the soil solution that is in equilibrium with the solid particles. It is a measure of the intensity of acidity or alkalinity, but does not indicate the relative buffering capacity of the soil. It is most applicable to soils with a pH ranging from 4.0 to 9.0. This method differs from the saturation paste pH (Method S - 1.10), the 1:2 ratio is 0.25 pH units higher than that obtained using the saturated paste extract. Soils containing greater than 15% organic matter may require a 1:5 or 1:10 soil :water ratio for the determination of pH. Soil pH is measured to access soil chemical properties, crop suitability, lime needs and relative nutrient availability. The method is generally reproducible within ± 0.07 pH units (Kalra, 1995). On soils with EC_e less than 0.3 dS m^{-1} the pH instrument will require longer to equilibrate.

Equipment

1. Paper cups 3 oz or 100 mL beaker.
2. Analytical balance, 250 g capacity, resolution ± 0.1 g.
3. pH meter, equipped with pH glass electrodes (indicating and reference).

Reagents

1. Deionized water, ASTM Type I grade.
2. Primary standard buffers, pH 4.0, 7.0, and 10.0.

Procedure

1. Weigh 10 ± 0.1 g of air dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) into a 100 mL beaker or 3 oz plastic cup.
2. Add 20 mL of deionized water.
3. Let stand fifteen (15) minutes, allow suspended soil particles to settle before reading pH.
4. Standardize / calibrate the pH meter: (1) rinse electrode with deionized water and place in pH 7.0 primary standard buffer and adjust as necessary; (2) rinse electrode and place in pH 4.0 primary standard buffer; (3) adjust the slope until response is ± 0.05 units of expected response; and (4) Check pH 7.0 primary standard buffer and adjust as necessary (See Comment #1). For high pH soils (> 7.0) use pH buffers 7.0 and 10.0.
5. Read the pH by placing the electrodes in the supernatant, swirling gently and read the pH once the reading is constant for fifteen (15) seconds. Report soil pH to the nearest 0.01 unit.
6. Rinse the electrodes with deionized water and blot dry between pH determinations. Do not wipe the electrode (See Comment #2 and #3).
7. When the meter is not in use, immerse the electrodes in pH 7.00 buffer.

Calculations

Record soil pH as $pH_{1:2\ H2O}$

Comments

1. Follow manufacturer's guidelines if meter does not read within 0.05 units of primary standards. Maintenance of combination electrodes differs from that of separate reference and glass electrodes; refer to manufacturer's instructions.

2. pH electrodes will equilibrate faster in fine textured soils (clay constant > 20%, See Method 14.1) than coarse textured ones and soils high in soluble salts (> 1.0 dS m^{-1}, See Method 1.10).

3. Store pH electrodes according to manufacturer's instructions (recommended practice is to store the electrodes in a primary standard buffer). Combination electrodes should be stored in a pH 4.0 buffered solution containing 5.0 g L^{-1} potassium chloride.

Literature

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Scope and Application

This method semi-quantifies the pH of soil, using a 1:2 soil:water extract of the soil using a 0.01 M $CaCl_2$ solution. Soil pH is a measure of the relative acidity or alkalinity of the soil solution that is in equilibrium with the solid particles. It is a measure of the intensity of acidity or alkalinity, but does not indicate the relative buffering capacity of the soil. It is most applicable to soils with a pH ranging from 4.0 to 9.0. This method differs from the saturation paste soil pH (Method S - 1.10) and soil pH 1:2 (Method S - 2.20) in that pH is determined using a salt suspension of 0.01 M $CaCl_2$. This method has advantages in that the pH is determined independent of the soluble salt concentration of soils, as the suspension remains flocculated errors associated with liquid junction potential are minimized. The pH is more reproducible than the 1:2 water method on soils low in soluble salts (EC_e < 0.4 dS m^{-1}). Generally soil pH obtained using 0.01 M $CaCl_2$ 1:2 ratio is similar to that of the 1:1 ratio and soil saturate paste. Soil pH is measured to access soil chemical properties, crop suitability, lime needs and relative nutrient availability. The method is generally reproducible within ± 0.06 pH units (Kalra, 1995).

Equipment

1. Paper cups 3 oz or 100 mL beaker.
2. Analytical balance, 250 g capacity, resolution ± 0.1 g.
3. pH meter, equipped with pH glass electrodes (indicating and reference).

Reagents

1. Calcium chloride, 0.01 M $CaCl_2$: Dissolve 2.940 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) with deionized water in a 2 L volumetric flask and dilute to volume. EC_e of solution should be between 2.24 and 2.40 dS m^{-1} at 25° C.
2. Primary standard buffers, pH 4.0, 7.0, and 10.0.

Procedure

1. Weigh 10 ± 0.1 g of air dry soil pulverized to pass 10 mesh serve (< 2.0 mm) into a 100 mL beaker or 3 oz plastic cup.
2. Add 20 mL of 0.01 M $CaCl_2$ solution and stir thoroughly.
3. Let stand fifteen (15) minutes, allow suspended soil particles to settle before reading pH.
4. Standardize / calibrate the pH meter: (1) rinse electrode with deionized water and place in pH 7.0 primary standard buffer and adjust as necessary; (2) rinse electrode and place in pH 4.0 primary standard buffer; (3) adjust the slope until response is ± 0.05 units of expected response; and (4) Check pH 7.0 primary standard buffer and adjust as necessary (See Comment #1). For high pH soils (> 7.0) use pH buffers 7.0 and 10.0.
5. Read the pH by placing the electrodes in the supernatant, swirling gently and read the pH once the reading is constant for fifteen (15) seconds. Report soil pH to the nearest 0.01 unit.
6. Rinse the electrodes with deionized water and blot dry between pH determinations. Do not wipe the electrode (See Comment #2, #3 and #4).
7. When the meter is not in use, immerse the electrodes in pH 7.00 buffer.

Calculations

Record soil pH as $pH_{1:2\ CaCl}$

Comments

1. Follow manufacturer's guidelines if meter does not read within 0.05 units of primary standards. Maintenance of combination electrodes differs from that of separate reference and glass electrodes; refer to manufacturer's instructions.

2. pH electrodes will equilibrate faster in fine textured soils (clay constant > 20%, See Method 14.1) than coarse textured ones.

3. Store pH electrodes according to manufacturer's instructions (recommended practice is to store the electrodes in a primary standard buffer). Combination electrodes should be stored in a pH 4.0 buffered solution containing 5.0 g L^{-1} potassium chloride.

4. The pH of the 0.01 M $CaCl_2$ solution should be between 5.5 and 5.6

Literature

Kalra, Y. 1995. Determination of pH of soils by different methods: collaborative study. J. of AOAC International. 78:310-320.

McLean, E.O. 1982. Soil pH and lime requirement. p. 199-223. *In* A.L. Page et al. (ed.) Methods of soil analysis, part 2. Agronomy Monogr. 9, 2nd ed. ASA and SSSA, Madison, WI.

Robbins, C.W. and C.L. Wiegand. 1990. Field and laboratory measurements. p. 201-219. *In*: K.K. Tanji (ed.) ASCE manuals and reports No. 71, Agricultural salinity, assessment, and management American Society of Civil Engineers, 245 E. 47th St., New York.

Hendershot, W.H., H. Lalande and M. Duquette. 1993. Soil reaction and exchangeable acidity. p. 141-144. *In*: M. R. Carter (ed.) Soil sampling and methods of analysis, Canadian Society of Soil Science, Lewis Publishers Ann Arbor, MI

Scope and Application

This method semi-quantifies the pH of soil, using a 1:1 soil:water extract of the soil using deionized water. Soil pH is a measure of the relative acidity or alkalinity of the soil solution that is in equilibrium with the solid particles. It is a measure of the intensity of acidity or alkalinity, but does not indicate the relative buffering capacity of the soil. It is most applicable to soils with a pH ranging from 4.0 to 9.0. This method differs from the saturation paste soil pH (Method S - 1.10) and soil pH 1:2 (Method S - 2.20) in that pH is determined using a 1:1 soil:water ratio and determines the pH of the supernate for a soil slurry. Generally soil pH obtained using the 1:1 ratio is 0.15 to 0.25 pH units higher than that obtained using the saturated paste extract but lower than that obtained by the 1:2 dilution. Soil pH is measured to access soil chemical properties, crop suitability, lime needs and relative nutrient availability. The method is generally reproducible within ± 0.12 pH units.

Equipment

1. Paper cups 3 oz or 100 mL beaker.
2. Analytical balance, 250 g capacity, resolution ±0.1 g.
3. pH meter, equipped with pH glass electrodes (indicating and reference).

Reagents

1. Deionized water, ASTM Type I grade.
2. Primary standard buffers, pH 4.0, 7.0, and 10.0

Procedure

1. Weigh 5.0 ± 0.1 g of air dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) into a 100 mL beaker or 3 oz plastic cup (See Comment #1).

2. Add 5.0 mL of deionized water and stir thoroughly for 5 seconds.

3. Let stand fifteen (15) minutes, allow suspended soil particles to settle before reading pH.

4. Standardize / calibrate the pH meter: (1) rinse electrode with deionized water and place in pH 7.0 primary standard buffer and adjust as necessary; (2) rinse electrode and place in pH 4.0 primary standard buffer; (3) adjust the slope until response is ±0.05 units of expected response; and (4) Check pH 7.0 primary standard buffer and adjust as necessary (See Comment #2). For high pH soils (> 7.0) use pH buffers 7.0 and 10.0.

5. Read the pH by placing the electrodes in the slurry, swirling gently and read the pH immediately. Ensure the electrode tips are in the slurry and not the supernate. Report soil pH to the nearest 0.01 unit.

6. Rinse the electrodes with deionized water and blot dry between pH determinations. Do not wipe the electrode (See Comment #3, #4 and #5).

7. When the meter is not in use, immerse the electrodes in pH 4.00 buffer.

Calculations

Record soil pH as $pH_{1:1}$

Comments

1. This follows the procedure outlined by Eckert (1989) in Recommended Chemical Tests Procedures for the North Central Region.

2. Follow manufacturer's guidelines if meter does not read within 0.05 units of primary standards. Maintenance of combination electrodes differs from that of separate reference and glass electrodes; refer to manufacturer's instructions.

3. pH electrodes will equilibrate faster in fine textured soils (clay constant > 20%, See Method S-14.1) and soils high in soluble salts (> 1.0 dS $m^{-1,}$ See Method 1.10).

4. Store pH electrodes according to manufacturer's instructions (recommended practice is to store the electrodes in a primary standard buffer). Combination electrodes should be stored in a pH 4.0 buffered solution containing 5.0 g L^{-1} potassium chloride.

5. To determine soil buffer pH add SMP (See Method S-2.50) or Woodruff buffer (See Method S-2.60) reagent to the soil slurry.

Literature

Eckert, D. J. 1988. Recommended pH and Lime Requirement Tests. p. 6-8. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. North Dakota Agr. Expt. Sta. Bulletin No. 499 (revised)..

Kalra, Y. 1995. Determination of pH of soils by different methods: collaborative study. J. of AOAC International. 78:310-320.

McLean, E.O. 1982. Soil pH and lime requirement. p. 199-223. *In* A.L. Page et al. (ed.) Methods of soil analysis, part 2. Agronomy Monogr. 9, 2nd ed. ASA and SSSA, Madison, WI.

Rhoades, J.D. 1982. Soluble salts. p. 167-178. *In:* A. L. Page et al. (ed.) Methods of soil analysis: Part 2. Agronomy Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI.

Rhoades, J.D. and S. Miyamoto. 1990. Testing soils for salinity. p. 299-336. *In*: R.L. Westerman (ed.) Soil testing and plant analysis. 3rd ed. SSSA, Madison, WI.

U.S. Salinity Lab. Staff. 1954. Saturated Soil Paste. Diagnosis and Improvement of saline and alkali Soils. Agr. Handbook 60, USDA, Washington, D.C.

Scope and Application

This method semi-quantifies the pH of soil, using a 1:1 soil:water extract of the soil using a 0.01 M $CaCl_2$ solution. Soil pH is a measure of the relative acidity or alkalinity of the soil solution that is in equilibrium with the solid particles. It is a measure of the intensity of acidity or alkalinity, but does not indicate the relative buffering capacity of the soil. It is most applicable to soils with a pH ranging from 4.0 to 9.0. This method differs from the saturation paste soil pH (Method S - 1.10) and soil pH 1:1 (Method S - 2.20) in that pH is determined using a salt suspension of 0.01 M $CaCl_2$. This method has advantages in that the pH is determined independent of the soluble salt concentration of soils, as the suspension remains flocculated errors associated with liquid junction potential are minimized. The pH is more reproducible than the 1:1 water method on soils low in soluble salts (EC_e < 0.4 dS m^{-1}). Soil pH is measured to access soil chemical properties, crop suitability, lime needs and relative nutrient availability. The method is generally reproducible within ± 0.06 pH units. Soil pH is measured to access soil chemical properties, crop suitability, lime needs and relative nutrient availability. The method is generally reproducible within ± 0.12 pH units.

Equipment

1. Paper cups 3 oz or 100 mL beaker.
2. Analytical balance, 250 g capacity, resolution ±0.1 g.
3. pH meter, equipped with pH glass electrodes (indicating and reference).

Reagents

1. Calcium chloride, 0.01 M $CaCl_2$: Dissolve 2.940 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) with deionized water in a 2 L volumetric flask and dilute to volume. EC_e of solution should be between 2.24 and 2.40 dS m^{-1} at 25° C.
2. Primary standard buffers, pH 4.0, 7.0, and 10.0.

Procedure

1. Weigh 5.0 ± 0.1 g of air dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) into a 100 mL beaker or 3 oz plastic cup (See Comment #1).
2. Add 5.0 mL of 0.01 M $CaCl_2$ solution and stir thoroughly for 5 seconds.
3. Let stand fifteen (15) minutes, allow suspended soil particles to settle before reading pH.
4. Standardize / calibrate the pH meter: (1) rinse electrode with deionized water and place in pH 7.0 primary standard buffer and adjust as necessary; (2) rinse electrode and place in pH 4.0 primary standard buffer; (3) adjust the slope until response is ±0.05 units of expected response; and (4) Check pH 7.0 primary standard buffer and adjust as necessary (See Comment #2). For high pH soils (> 7.0) use pH buffers 7.0 and 10.0.
5. Read the pH by placing the electrodes in the slurry, swirling gently and read the pH immediately. Ensure the electrode tips are in the slurry and not the supernate. Report soil pH to the nearest 0.01 unit.
6. Rinse the electrodes with deionized water and blot dry between pH determinations. Do not wipe the electrode (See Comment #2, #3 and #4).
7. When the meter is not in use, immerse the electrodes in pH 4.00 buffer.

Calculations

Record soil pH as $pH_{1:1\ CaCl}$

Comments

1. Follow manufacturer's guidelines if meter does not read within 0.05 units of primary standards. Maintenance of combination electrodes differs from that of separate reference and glass electrodes; refer to manufacturer's instructions.

2. pH electrodes will equilibrate faster in fine textured soils (clay constant > 20%, See Method S-14.1) and soils high in soluble salts (> 1.0 dS $m^{-1,}$ See Method 1.10).

3. Store pH electrodes according to manufacturer's instructions (recommended practice is to store the electrodes in a primary standard buffer). Combination electrodes should be stored in a pH 4.0 buffered solution containing 5.0 g L^{-1} potassium chloride.

4. To determine soil buffer pH add SMP (See Method S-2.50) or Woodruff buffer (See Method S-2.60) reagent to the soil slurry.

Literature

Eckert, D. J. 1988. Recommended pH and Lime Requirement Tests. p. 6-8. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. North Dakota Agr. Expt. Sta. Bulletin No. 499 (revised)..

Kalra, Y. 1995. Determination of pH of soils by different methods: collaborative study. J. of AOAC International. 78:310-320.

McLean, E.O. 1982. Soil pH and lime requirement. p. 199-223. *In* A.L. Page et al. (ed.) Methods of soil analysis, part 2. Agronomy Monogr. 9, 2nd ed. ASA and SSSA, Madison, WI.

Rhoades, J.D. 1982. Soluble salts. p. 167-178. *In:* A. L. Page et al. (ed.) Methods of soil analysis: Part 2. Agronomy Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI.

Rhoades, J.D. and S. Miyamoto. 1990. Testing soils for salinity. p. 299-336. *In*: R.L. Westerman (ed.) Soil testing and plant analysis. 3rd ed. SSSA, Madison, WI.

U.S. Salinity Lab. Staff. 1954. Saturated Soil Paste. Diagnosis and Improvement of saline and alkali Soils. Agr. Handbook 60, USDA, Washington, D.C.

Scope and Application

This method semi-quantifies the electrical conductivity ($EC_{1:1}$) of soil, using a 1:1 soil:water extract (volume to volume) of the soil using deionized water. EC is measured using a conductivity probe. This method differs from the saturation paste EC_e (Method S - 1.20), and is used as a simplified method for determining soluble salts but is less well related to field soil water composition and content. This method is not recommended for gypsiferous soils and will lead to bias high results. The EC_e measurement is sensitive to temperature and increases approximately 1.9% per °C (range 15 - 35 °C) (Rhoades, 1996). All EC_e data is normalized to 25 °C. Soil $EC_{1:1}$ is measured to access soil chemical properties, crop suitability, lime needs and relative nutrient availability. The method is generally reproducible within ± 5%.

Equipment

1. Paper cups 3 oz or 100 mL beaker.
2. 10 cm^3 scoop, volumetric.
3. Conductance cell and conductance meter with dynamic range from 0.01 to 100 dS m^{-1} conductance, temperature compensating, 25 °C.

Reagents

1. Deionized water CO_2-free, ASTM Type I grade. EC <10^{-4} dS m^{-1}.
2. Standard Reference Calibration Solution. Dissolve 0.7456 g KCl (previously dried at 110 °C for 2 h) in CO_2 -free deionized water and dilute to 1.0 L. At 25 ±0.1 °C a 0.010 N KCl solution will have an EC_e of 1.412 dS m^{-1} (mmhos cm^{-1}). For a 0.100 N KCl solution (7.456 g KCl diluted to 1.0 L) will have an EC_e of 12.900 dS m^{-1}. Standard EC calibration solutions are listed in Table S-2.30-A and can be purchased from a scientific supply vendor.

Procedure

1. Using a 10 cm^3 scoop measure two scoops of air dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) into a 100 mL beaker or 3 oz plastic cup.
2. Add 20 mL of deionized water.
3. Let stand fifteen (15) minutes, allow suspended soil particles to settle before reading $EC_{1:1}$ (See Comment #1).
4. Calibrate conductance cell. Operate and adjust instrument in accordance with manufacturer's instructions (See Comments #2, #3 and #4). Rinse conductance cell with three aliquots of 0.01 N KCl, adjust a fourth portion to 25 ±0.1 °C, measure R (where R is the measured resistance ohms) and temperature *t*. Repeat measurement of R until value is constant. Calculate cell constant K.

$$K = (0.001413)\ (R_{KCl})/[1+0.019(25 - t)]$$

5. Rinse conductance cell with deionized water. Stir conductance probe into extract solution. When the meter has stabilized record instrument reading.

Calculations

$$EC_{1:1} = C_x(1000)K[1 + 0.019(25 - t)]$$

Where: C_x is the measured C of the sample and *t* is temperature

Report $EC_{1:1}$ to the nearest 0.01 dS m^{-1} as EC_e 25 °C.

(See Comments #4, and #5)

Table S - 2.30 -A Conductivity of KCl solutions at 25 °C (Rhoades, 1996).

Concentration N	Conductivity dS m^{-1}
0.001	0.147
0.010	1.413
0.020	2.767
0.050	6.668
0.10	12.90
0.20	24.82
0.50	58.64

Comments

1. Exposure of the sample to the atmosphere may cause changes in conductivity due to loss or gain of dissolved gasses: CO_2 and NH_3-N. Freshly distilled water has a conductivity of 0.005 - 0.002 dS m^{-1} increasing after a few weeks to 0.002 -0.004 dS m^{-1}. This of special concern on samples with very low $EC_{1:1}$.

2. Clean platinum electrodes that are new or that are providing erratic EC readings with acid-dichromate cleaning solution. Cleaning solution: 32 mL of saturated sodium dichromate ($Na_2Cr_2O_7$) and 1L 16 M sulfuric acid. Soak electrodes 16 hours followed by three rinses of deionized water rinses. If platinum is flaked, recoat according to procedure of APHA (1985).

3. For highly saline soils (EC_e >8.0 dS m^{-1}) calibrate using 0.100 N KCl solution, EC_e 12.90 dS m^{-1}.

4. The conductance probe is affected by the wall of the container. This is called “field effect”. Therefore it is important to calibrate and analyze the samples and standards at the same position from the bottom and side wall of the container.

Literature

APHA (1985). Part 205. In Standard Methods for the Examination of Water and Wastewater’. 16th edn. American Public Health Association, Washington, DC.

Dahnke, W.C. and D.A. Whitney. 1988. Recommended pH and lime requirement tests. p. 32-34. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. North Dakota Agr. Expt. Sta. Bulletin No. 499 (revised).

Helrich, K. (ed.) 1990. Official methods of analysis. 15th Ed. Association of Official Analytical Chemists, Inc. Arlington, VA.

Kalra, Y and D.G. Maynard. 1991. Methods manual for forest soil and plant analysis. P. 32-36. Forestry Canada, Northwest Region Information Report NOR-X-319.

Rayment, G.E. and F.R. Higginson. 1992. Electrical conductivity. p. 15-16. *In:* Australian Laboratory Handbook of Soil and Water Chemical Methods. Inkata Press, Melbourne

Rhoades, J.D. 1996. Lime requirement. p. 417-435. *In:* J. M. Bartels et al. (ed.) Methods of soil analysis: Part 3. Chemical methods. 3rd ed. ASA and SSSA, Madison, WI. Book Series

Scope and Application

This method semi-quantifies the electrical conductivity ($EC_{1:2}$) of soil, using a 1:2 soil:water extract (volume to volume) of the soil using deionized water. EC is measured using a conductivity probe. This method differs from the saturation paste EC_e (Method S - 1.20), and is used as a simplified method for determining soluble salts but is less well related to field soil water composition and content. This method is not recommended for gypsiferous soils and will lead to bias high results. The EC_e measurement is sensitive to temperature and increases approximately 1.9% per °C (range 15 - 35 °C) (Rhoades, 1996). All EC_e data is normalized to 25 °C. Soil $EC_{1:2}$ is measured to access soil chemical properties, crop suitability, lime needs and relative nutrient availability. The method is generally reproducible within ± 8%.

Equipment

1. Paper cups 3 oz or 100 mL beaker.
2. 10 cm^3 scoop, volumetric.
3. Conductance cell and conductance meter with dynamic range from 0.01 to 100 dS m^{-1} conductance, temperature compensating, 25 °C.

Reagents

1. Deionized water CO_2-free, ASTM Type I grade. EC <10^{-4} dS m^{-1}.
2. Standard Reference Calibration Solution. Dissolve 0.7456 g KCl (previously dried at 110 °C for 2 h) in CO_2 -free deionized water and dilute to 1.0 L. At 25 ±0.1 °C a 0.010 N KCl solution will have an EC_e of 1.412 dS m^{-1} (mmhos cm^{-1}). For a 0.100 N KCl solution (7.456 g KCl diluted to 1.0 L) will have an EC_e of 12.900 dS m^{-1}. Standard EC calibration solutions are listed in Table S-2.30-A and can be purchased from a scientific supply vendor.

Procedure

1. Using a 10 cm^3 scoop measure two scoops of air dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) into a 100 mL beaker or 3 oz plastic cup.
2. Add 40 mL of deionized water.
3. Let stand fifteen (15) minutes, allow suspended soil particles to settle before reading $EC_{1:2}$ (See Comment #1).
4. Calibrate conductance cell. Operate and adjust instrument in accordance with manufacturer's instructions (See Comments #2, #3 and #4). Rinse conductance cell with three aliquots of 0.01 N KCl, adjust a fourth portion to 25 ±0.1 °C, measure R (where R is the measured resistance ohms) and temperature *t*. Repeat measurement of R until value is constant. Calculate cell constant K.

$$K = (0.001413)\ (R_{KCl})/[1+0.019(25 - t)]$$

5. Rinse conductance cell with deionized water. Stir conductance probe into extract solution. When the meter has stabilized record instrument reading.

Calculations

$$EC_{1:2} = C_x(1000)K[1 + 0.019(25 - t)]$$

Where: C_x is the measured C of the sample and *t* is temperature

Report $EC_{1:2}$ to the nearest 0.01 dS m^{-1} as EC_e 25 °C.

(See Comments #4, and #5)

Table S - 2.30 -A Conductivity of KCl solutions at 25 °C (Rhoades, 1996).

Concentration N	Conductivity dS m^{-1}
0.001	0.147
0.010	1.413
0.020	2.767
0.050	6.668
0.10	12.90
0.20	24.82
0.50	58.64

Comments

1. Exposure of the sample to the atmosphere may cause changes in conductivity due to loss or gain of dissolved gasses: CO_2 and NH_3-N. Freshly distilled water has a conductivity of 0.005 - 0.002 dS m^{-1} increasing after a few weeks to 0.002 -0.004 dS m^{-1}. This of special concern on samples with very low EC_e.

2. Clean platinum electrodes that are new or that are providing erratic EC readings with acid-dichromate cleaning solution. Cleaning solution: 32 mL of saturated sodium dichromate ($Na_2Cr_2O_7$) and 1L 16 M sulfuric acid. Soak electrodes 16 hours followed by three rinses of deionized water rinses. If platinum is flaked, recoat according to procedure of APHA (1985).

3. For highly saline soils (EC_e >8.0 dS m^{-1}) calibrate using 0.100 N KCl solution, EC_e 12.90 dS m^{-1}.

4. The conductance probe is affected by the wall of the container. This is called “field effect”. Therefore it is important to calibrate and analyze the samples at the same position from the bottom and side wall of the container.

5. Plant/crop tolerances to salinity of the soil 1:2 extract electrical conductivity ($EC_{1:2}$) are shown in Table S -2.30 - B.

Table S - 2.30-B Impact of salinity of the soil 1:2 extract electrical conductivity ($EC_{1:2}$) on plant growth.

dS m^{-1}	Plant salinity effects
< 0.4	Most crops will grow well, no injury (Pear, peach apple plum vetch, beans)
0.4 - 0.8	Very slightly saline. Yields of crops of low salt tolerance maybe reduced by 50% (Ladino clover, red clover, red fox tail, soybeans, strawberry, and orange.
0.8 - 1.6	Slightly saline. Yields of fruit and vegetable crops of medium salt tolerance maybe reduced 50% (orchard grass, birdsfoot treefoil, sunflower, corn rice sorghum, oats, cucumber, onion, carrot lettuce, bell pepper, potato, broccoli, cantaloupe, grape, and olive).
1.6 - 2.4	Moderately saline. Yield of virtually all fruit crops significantly reduced. Yield reductions of 50% may occur in the most sensitive forage and field crops. (barley, wheatgrass, cotton rape, sugarbeet, spinach, asparagus, and beets).
2.4 - 3.2	Strongly saline. Only highly salt-tolerant forage and field crops will yield satisfactorily.
> 3.2	Very strongly saline. Only a few highly salt-tolerant grasses, herbaceous plants and certain shrubs and trees will grow (salt grass, alkali sacaton)

Literature

APHA (1985). Part 205. In Standard Methods for the Examination of Water and Wastewater'. 16th edn. American Public Health Association, Washington, DC.

Dahnke, W.C. and D.A. Whitney. 1988. Recommended pH and lime requirement tests. p. 32-34. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. North Dakota Agr. Expt. Sta. Bulletin No. 499 (revised).

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Issac, R. A.(ed.) 1983. Reference Soil Test methods for the Southern Region of the United States. Southern Cooperative Series Bulletin 289. Univ. Of Georgia College of Agriculture.

Kalra, Y and D.G. Maynard. 1991. Methods manual for forest soil and plant analysis. P. 32-36. Forestry Canada, Northwest Region Information Report NOR-X-319.

Rayment, G.E. and F.R. Higginson. 1992. Electrical conductivity. p. 15-16. *In:* Australian Laboratory Handbook of Soil and Water Chemical Methods. Inkata Press, Melbourne

Rhoades, J.D. 1996. Lime requirement. p. 417-435. *In:* J. M. Bartels et al. (ed.) Methods of soil analysis: Part 3. Chemical methods. 3rd ed. ASA and SSSA, Madison, WI. Book Series

Scope and Application

Buffer pH is the measure of a soil's active and reserve acidity, i.e. buffer capacity and is used to estimate lime recommendations. The method is based on the reaction of soil buffered acidity with a chemical buffer resulting in change in the pH of the buffer. Several tests have been developed to measure lime requirement including SMP Buffer, Woodruff (1967), Mehlich (1939) and Adams & Evans (1962). The SMP (Shoemaker, McLean & Pratt, 1961) buffer tests is one of the more popular lime requirement tests used for estimating exchange acidity including that associated with exchangeable aluminum and is used predominately on soils of the northeast. Others such as the Woodruff and Mehlich method, are dependent on geographic region or preference. Standard calibration curves exist for liming based on a SMP value to a desired pH for soil groups in a geographic area. Local calibration of the method is desirable. The procedure is generally reproducible with in 0.1 pH units.

Equipment

1. Analytical balance: 100.0 g capacity, resolution ± 0.01 g.
2. Repipette dispenser(s): calibrated to 5.0 ± 0.2 mL and 10.0 ± 0.2 mL.
3. pH meter, equipped with pH electrodes (indicating and reference).
4. Primary standard buffers, pH 4.0, 7.0, and 10.0.
5. Container 50 mL (polypropylene or waxed paper).
6. Glass stirring rod.

Reagents

1. Deionized water, ASTM type I Grade.
2. Sodium Hydroxide, 0.1 N NaOH - Dissolve 4.0 g of NaOH pellets in about 500 mL deionized water. Allow to cool to room temperature and bring to 1000 mL volume.
3. SMP Buffer Solution, pH 7.5 ±0.1: Using a 1000 mL volumetric flask, completely dissolve 1.8 g of ground para-nitrophenol in 500 mL deionized water. Add 2.8 g of triethanolamine TEA (weigh rather than pipette vicious liquid). Then dissolve 3.0 g potassium chromate (K_2CrO_4), 2.0 g calcium acetate, $(CH3COO)_2Ca\ H_2O$) and 53.1 g calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in the solution. Bring to 975 mL volume with deionized water and stir with magnetic stirrer (can take up to 12 hours). Adjust solution to pH 7.50 with 0.1 N NaOH or 4 M HCl if necessary and dilute to 1000 mL volume with deionized water. CAUTION: Triethanolamine and potassium chromate are hazardous materials, consult MSDS sheet before using. Verify buffer capacity by titrating 20 mL of SMP buffer from pH 7.50 to pH 5.00 with standardized 0.1 M HCl. Should require 0.28 ± 0.005 $cmol_c$ of HCl / pH unit.
4. pH standard buffers, pH 4.0 and 7.0.

Procedure

1. Weigh 5.0 ± 0.05 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into 50 mL container. Generally, samples are placed in rows of six to accommodate continuous stirring and reading samples.
2. Add 5.0 mL of deionized water. Stir (leaving a stir rod in each sample) and allow to soak for thirty (30) minutes.
3. Add 10.0 mL of SMP Buffer Solution (See Comment #1) and stir every five (5) min during the ensuing twenty (20) minutes period.
4. Standardize / calibrate pH meter: (1) rinse electrode with deionized water and place in pH 7.0 primary standard buffer and adjust as necessary; (2) rinse electrode and place in pH 4.0 primary standard buffer; (3) adjust the slope until response is ± 0.05 units of expected response; and (4) Check pH 7.0 primary standard buffer and adjust as necessary (See Comment #2).

5. Immediately following the final stirring (twenty (20) minutes after addition of SMP buffer solution), insert the electrodes and observe the pH reading of the suspension, swirl gently and observe the subsequent reading. Continue until pH readings are constant, then record the pH reading to the nearest 0.1 unit as pH_{smp} (See Comment #3 and #4).
6. Between readings, thoroughly rinse electrodes with deionized water and pat dry. Consult Table S - 2.50-A for lime requirement.

Table S - 2.50-A. Calibrations for lime requirement for the surface 20 cm of soil using the SMP buffer pH method.

Soil SMP buffer pH	Desired Soil pH			
	7	6.5	6	Organic Soil 5.2
	Amount of 100 % $CaCO_3$ required (tons ac^{-1})			
6.8	1.1	0.9	0.8	0.6
6.7	1.8	1.6	1.3	1.0
6.6	2.4	2	1.7	1.3
6.5	3.1	2.6	2.1	1.7
6.4	4.0	3.4	2.8	2.1
6.3	4.7	4	3.3	2.5
6.2	5.4	4.6	3.7	2.9
6.1	6	5	4.1	3.2
6.0	5.8	5.7	4.7	3.6
5.9	7.7	6.5	5.3	4.1
5.8	8.3	7	5.7	4.4
5.7	9	7.6	6.2	4.7
5.6	9.7	8.2	6.7	5.2
5.5	10.4	8.8	7.2	5.5
5.4	11.3	9.6	7.8	6
5.3	11.9	10	8.2	6.3
5.2	12.7	10.7	8.7	6.7
5.1	13.6	11.5	9.2	7.1

Based on 8 inch furrow slice weighing 2.4 million pounds. From Eckert 1988.

Comments

1. Check repipette dispensing volume calibration using an analytical balance.

2. Follow manufacturer's guidelines if meter does not read within 0.05 units of primary standards. Maintenance of combination electrodes differs from that of separate reference and glass electrodes; refer to manufacturer's instructions. Store pH electrodes according to manufacturer's instructions (recommended practice is to store the electrodes in a primary standard buffer).

3. Reading soil-buffer solution pH between 20 and 25 min after the addition of the SMP buffer is necessary because the pH of the suspension will continue to decrease over time. The electrodes should be rinsed occasionally with 0.1 N HCl and deionized water when making a series of determinations to eliminate increased pH readings caused by electrode contamination. The method outlined is a modification of the method described by McLean (1982).

4. SMP Buffer solution is classified as hazardous waste and must be disposed of in a suitable manner.

Literature

Adams, F., and C.E. Evans. Evans. 1962. A rapid method for measuring lime requirement of red-yellow podzolic soils. Soil Sci. Soc. Am. Proc. 26:355-357.

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Woodruff, C.M. 1967. Crop response to lime in the Midwestern United States. In R.W. Pearson and F. Adams (eds) Soil acidity and liming. Agron. Monogr. 12, Am. Soc. Agron., Madison, WI. p. 207-231.

Scope and Application

Buffer pH is the measure of a soil's active and reserve acidity, i.e. buffer capacity and is used to estimate lime recommendations. The method is based on the reaction of soil acidity with a chemical buffer resulting in change in the pH of the buffer. Several methods have been developed to measure lime requirement including SMP Buffer, Woodruff (1967), Mehlich (1939) and Adams & Evans (1962). The SMP (Shoemaker, McLean & Pratt, 1961) and Mehlich buffer methods is one of the more popular lime requirement tests used for estimating exchange acidity including that associated with exchangeable aluminum. The Woodruff method is better suited to soils low in exchangeable aluminum with acidity associated with ammoniacal nitrogen applications. Idaho, Nebraska, Missouri and Mississippi currently use the Woodruff method to make lime recommendations. Local calibration of the method is desirable. The procedure is generally reproducible with in 0.10 pH units.

Equipment

1. Analytical balance: 100.0 g capacity, resolution ± 0.01 g.
2. Repipette dispenser(s) 10.0 ± 0.2 mL.
3. pH meter, equipped with pH electrodes (indicating and reference).
4. Primary standard buffers, pH 4.0, 7.0, and 10.0.
5. Container 50 mL (polypropylene or waxed paper).
6. Glass stirring rod.

Reagents

1. Deionized water, ASTM type I Grade.
2. pH standard buffers, pH 4.0 and 7.0.
3. Woodruff Buffer Solution: Dissolve 720 g of Calcium acetate ($Ca(CH_3COH)_2H_2O$, 11.25 g of magnesium oxide (MgO) and 144 g of para-nitrophenol into 18 L of deionized water. While stirring, continuously bubble air into the solution for (twenty-four) 24 hours. Let the solution stand for 48 hours. Syphon through a glass wool filter to another 18 L container to remove undesired precipitates. Adjust pH to 7.0 with MgO or glacial acetic acid.

Procedure

1. Weigh 10.0 ± 0.05 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into 50 mL container (See Comment #1).
2. Add 10.0 mL of deionized water. Stir (leaving a stir rod in each sample) and allow to soak for thirty (30) minutes.
3. Standardize / calibrate pH meter: (1) rinse electrode with deionized water and place in pH 7.0 primary standard buffer and adjust as necessary; (2) rinse electrode and place in pH 4.0 primary standard buffer; (3) adjust the slope until response is ±0.05 units of expected response; and (4) Check pH 7.0 primary standard buffer and adjust as necessary (See Comment #2).
4. Add 10.0 mL of Woodruff Buffer solution, stir and after thirty (30) minutes stir again and read buffer pH to the nearest 0.05 pH units and record as pH_{wd} (See Comment #3).

Calculation

Each 0.1 unit decrease in pH from 7.0 is equivalent to 1.0 meq H^+ per 100 g of soil or 1000 lbs of 100% of Calcium Carbonate Equivalent of lime per 2 million pounds of soil (See Comment #4, #5, #6 and #7).

$$\text{lbs } CaCO_3 \text{ acre}^{-1} = \frac{\text{meq } H^+}{100 \text{ lbs}} \times \frac{0.05 \text{ lbs } CaCO_3}{\text{meq}} \times \frac{2{,}000{,}000 \text{ lbs}}{\text{acre}} \quad \text{[equ. 2.6-1]}$$

Comments

1. Check repipette dispensing volume calibration using an analytical balance.

2. Follow manufacturer's guidelines if meter does not read within 0.05 units of primary standards. Maintenance of combination electrodes differs from that of separate reference and glass electrodes; refer to manufacturer's instructions. Store pH electrodes according to manufacturer's instructions (recommended practice is to store the electrodes in a primary standard buffer).

3. Stir samples immediately before reading buffer pH.

4. The working range for this procedure covers a buffer pH range of 6.0 - 6.9 units. For soils with a Woodruff buffer pH < 6.0 the procedure should be repeated using 2.5 g of soil. Thus each 0.1 pH unit change will represent 2 meq H per 100 g of soil or 2000 lbs of lime per acre.

5. Caution should be noted on coarse textured soils sands and loamy sands) this procedure will over estimate lime requirement.

6. The above procedure does not account for acidity associated with aluminum. The SMP or Mehlich lime requirement methods are more appropriate of soils high in exchangeable aluminum.

7. Woodruff Buffer solution is classified as hazardous waste and must be disposed of in a suitable manner.

Literature

Frank, Kenneth. 1997. Personal communication. University of Nebraska.

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ADAMS AND EVANS BUFFER pH S - 2.70

Lime Requirement

Scope and Application

Buffer pH is the measure of a soil's active and reserve acidity, i.e.. buffer capacity and is used to estimate lime recommendations on soils low in cation exchange capacity. The method is based on the reaction of soil buffered acidity with a chemical buffer resulting in change in the pH of the buffer. The Adams and Evans method (Shoemaker, McLean & Pratt, 1961) is one of the more popular lime requirement tests used for estimating exchange acidity including that associated with exchangeable aluminum. It is mainly used on the coastal plain soils of the mid-Atlantic states. This method can detect small differences in lime requirement where such differences may elicit large changes in pH. The procedure is generally reproducible with in 0.10 pH units.

Equipment

1. Analytical balance: 100.0 g capacity, resolution ± 0.01 g.
2. Repipette dispenser(s): calibrated to 5.0 ± 0.2 mL and 10.0 ± 0.2 mL.
3. pH meter, equipped with pH electrodes (indicating and reference).
4. Primary standard buffers, pH 4.0, 7.0, and 10.0.
5. Container 50 mL (polypropylene or waxed paper).
6. Glass stirring rod.

Reagents

1. Deionized water, ASTM type I Grade.
2. Adams-Evans Buffer Solution, pH 8.0±0.1: Dissolve 20 g of *p*-Nitrophenol, 15 g of boric acid (H_3BO_3) 74 g of potassium chloride and 10.5 g potassium hydroxide in 750 mL and dilute to 1.0 L with deionized water. Adjust pH to 8.0 with potassium hydroxide.
3. pH standard buffers, pH 4.0 and 7.0.

Procedure

1. Weigh 20.0 ± 0.1 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into 50 mL container. Generally, samples are placed in rows of six to accommodate continuous stirring and reading samples.
2. Add 20.0 mL of deionized water. Stir (leaving a stir rod in each sample) and allow to soak for sixty (60) minutes. Read soil water pH on standardized pH meter (see Method 2.10).
3. Add 20.0 mL of Adams-Evans Buffer (See Comment #1) and stir for one (1) minute, every five (5) min during the ensuing twenty (20) minutes period.
4. Standardize / calibrate the pH meter: (1) rinse electrode with deionized water and place in pH 7.0 primary standard buffer and adjust as necessary; (2) rinse electrode and place in pH 4.0 primary standard buffer; (3) adjust the slope until response is ±0.05 units of expected response; and (4) Check pH 7.0 primary standard buffer and adjust as necessary (See Comment #2).
5. Immediately following the final stirring twenty (20) minutes after addition of Adams-Evans buffer solution, insert the electrodes and observe the pH reading of the suspension, while stirring gently and observe the subsequent reading, then record the pH reading to the nearest 0.1 as pH_{ae}. Consult Table S 2.70-A to determine lime requirement.
6. Between readings, thoroughly rinse electrodes with deionized water and pat dry.

Comments

1. Check repipette dispensing volume calibration using an analytical balance.

2. Follow manufacturer's guidelines if meter does not read within 0.05 units of primary standards. Maintenance of combination electrodes differs from that of separate reference and glass electrodes; refer to manufacturer's instructions. Store pH electrodes according to manufacturer's instructions (recommended practice is to store the electrodes in a primary standard buffer).

Table S2.70-A. Calibrations for lime requirement to adjust soil pH to 6.5for the surface 20 cm of soil using the Adams-Evans Buffer method.

Soil pH (1:2) in Water	Soil pH_{ae} in Adams-Evans Buffer Solution				
	7.8	7.6	7.4	7.2	7.5
	Amount of 100 % $CaCO_3$ required (metric tons ha^{-1})				
6.3	0.49	0.98	1.48	1.97	2.46
6.1	0.87	1.74	2.61	3.48	4.35
5.9	1.17	2.34	3.52	4.69	5.86
5.7	1.42	2.84	4.26	5.68	7.1
5.5	1.63	3.26	4.88	6.51	8.14
5.3	1.81	3.61	5.42	7.23	9.03
5.1	1.97	3.93	5.9	7.86	9.83
4.9	2.11	4.22	6.33	8.44	10.54
4.7	2.25	4.49	6.74	8.99	11.23
4.5	2.4	4.79	7.19	9.58	11.98

McLean, (1982).

To convert from metric tons ha^{-1} to tons ac^{-1} multiply values by 0.446 .

Literature

Adams, F., and C.E. Evans. Evans. 1962. A rapid method for measuring lime requirement of red-yellow podzolic soils. Soil Sci. Soc. Am. Proc. 26:355-357.

McLean, E.O. 1982. Soil pH and lime requirement. p. 199-223. *In*: A.L. Page, R.H. Miller, and D.R. Keeney (ed.) Methods of soil analysis. Part 2. Agron. Monogr. 9, Am. Soc. Agron., Madison, WI.

Scope and Application

The Mehlich buffer pH is the measure of a soil's active and reserve acidity, i.e. buffer capacity and is used to estimate lime ($CaCO_3$) recommendations. The method is based on the reaction of soil buffered acidity both hydrogen and aluminum with a chemical buffer resulting in change in the pH of the buffer (Mehlich, Bowling and Hatfield, 1976). The method is particularly well suited to for determining lime requirement for neutralizing very acid soils which may be harmful to crop productivity. Calibration data presented is based on data developed by van Lierop (1990), Mehlich et al. (1976) and Ssali and Nuwamanya (1981). North Carolina Department of Agriculture uses the Mehlich Buffer pH method to make lime recommendations Local calibration of the method for lime requirement is desirable. The procedure is generally reproducible with in 0.10 pH units.

Equipment

1. Analytical balance: 100.0 g capacity, resolution ± 0.1 g.
2. Repipette dispenser(s): calibrated to 10.0 ± 0.2 mL.
3. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute (opm).
4. pH meter, equipped with pH electrodes (indicating and reference).
5. Primary standard buffers, pH 4.0, 7.0, and 10.0.
6. Container 50 mL (polypropylene or waxed paper).
7. Glass stirring rod.

Reagents

1. Deionized water, ASTM type I Grade.
2. Mehlich Buffer Reagent: dissolve 5 mL of glacial acetic acid CH_3COOH, 9.0 mL of triethanolamine [TEA: $N(CH_2 \bullet CH_2OH)_3$] or 18.0 mL of 1:1 TEA: deionized water solution for ease of deliver; 86 g NH_4Cl; and 40 g $BaCl_2 \bullet H_2O$ in 1500 mL of deionized water. Separately: dissolve 36.0 mL of sodium glycerophosphate [$(HOCH_2)_2CHOPO_3\ NA_2 \bullet 5H_20$] in 400 mL deionized water. Mix solutions while swirling vigorously, allow to cool to room temperature, then dilute to 2 L with deionized water. Check pH of buffer by mixing equal aliquots of buffer with deionized water. The pH of the 1:1 mixture should be 6.60 ±0.04. Adjust pH as necessary with glacial acetic acid of TEA stock solution.
3. Verify buffering capacity by mixing 10 mL of Mehlich buffer solution with 10 mL deionized water and 10 mL of 0.05 M HCl + 0.017 M $ALCl_3$ solution. The pH of the mixture should be 4.1± 0.05 . Prepare the 0.05 M HCl + 0.017 M $ALCl_3$ solution by dissolving 4.024 g $ALCl_3 \bullet 6H_20$ in 100 mL of 0.05 M HCl.

Procedure

1. Weigh 10.0 ± 0.1 mL of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into 50 mL container. Add 10 mL deionized water and stir sample thoroughly, allow to stand thirty (30) minutes and measure pH as described in Method S-2.20.
2. Add 10.0 mL of Mehlich buffer to soil-water suspension. Stir thoroughly with glass rod, and allow mixture to stand for one (1) hour.
3. Standardize / calibrate the pH meter: (1) rinse electrode with deionized water and place in pH 7.0 primary standard buffer and adjust as necessary; (2) rinse electrode and place in pH 4.0 primary standard buffer; (3) adjust the slope until response is ±0.05 units of expected response; and (4) Check pH 7.0 primary standard buffer (See Comment #2).
4. Measure soil-water-buffer mixture pH to the nearest 0.05 pH (See Comment #3) and record value as pH_{mel}. Determine lime requirement value for desired target pH from Equations S- 2.80-2 or S -2.80-3. Between readings, thoroughly rinse electrodes with deionized water and pat dry.

Calculations

$$\text{Soil Acidity (AC), in } cmol_c / 100\ cm^3 \text{ soil} = \frac{(6.6 - \text{Mehlich buffer pH})}{0.25} \qquad \text{[equ. S -2.80-1]}$$

Mineral soil Lime Requirement (Soil with slight to moderate tolerance of soil acidity

$$\text{Lime Requirement (tons } ac^{-1}) = 0.446 \times [(0.1) \times (AC)^2 + AC] \qquad \text{[equ. S -2.80-2]}$$

Histosols (> 16 % soil organic matter) or soils histic horizons

$$\text{Lime Requirement (tons } ac^{-1}) = 0.446 \times [-7.4 + 1.6 \times (AC)] \times 1.3 \qquad \text{[equ. S -2.80-3]}$$

Comments

1. Check repipette dispensing volume calibration using an analytical balance.

2. Follow manufacturer's guidelines if meter does not read within 0.05 units of primary standards. Maintenance of combination electrodes differs from that of separate reference and glass electrodes; refer to manufacturer's instructions. Store pH electrodes according to manufacturer's instructions (recommended practice is to store the electrodes in a primary standard buffer).

3. Mehlich Buffer solution is classified as hazardous waste and must be disposed of in a suitable manner.

Literature

Sims, Thomas J. 1996. Lime requirement. p. 491-515. *In:* J. M. Bartels et al. (ed.) Methods of soil analysis: Part 3. Chemical methods. 3rd ed. ASA and SSSA, Madison, WI. Book series No. 5.

Mehlich, A., S.S. Bowling and A.L. Hatfield. 1976. Buffer pH acidity in relation to nature of soil acidity and expression of lime requirement. Commun. Soil. Sci. Plant Anal. 7(3):253-263.

Ssali, H. And J.K. Nuwamanya. 1981. Buffer pH methods for estimation of lime requirement of tropical acid soils. Commun. Soil Sci. Plant Anal. 12:643-659.

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Scope and Application

This method involves the quantitative extraction of nitrate (NO_3-N) from soils using 2.0 N KCl. Nitrate is determined by reduction to nitrite (NO_2^--N) via a cadmium reactor, diazotized with sulfanilamide and is coupled to N-(1-Napthyl)-ethylenediamine dihydrochloride to form an azochromophore (red-purple in color) measured spectrophotometrically at 520 nm. The method is readily adapted to manual or automated techniques. The procedure outlined follows that outlined by Keeney and Nelson (1982) for determining nitrate nitrogen with a modification in which 25 mL of KCl and 5.0 g of soil are used instead of 100 mL and 10 g soil. Extending the shaking period to thirty minutes with 2.0 N KCl (Bremner et.al. 1965), permits the simultaneous extraction of ammonium and nitrate. Care must be taken to avoid contamination from filter paper and operator handling. Cadmium is a hazardous material, follow manufacturers recommendations in handling this material. Soil nitrate-nitrogen can be used to predict crop response to nitrogen fertilizers. The method detection limit is approximately 0.5 mg kg^{-1} (on a dry soil basis) and is generally reproducible ± 6%.

Equipment

1. Analytical balance,: 100.0 g capacity, resolution ± 0.01 g.
2. Repipette dispenser, calibrated to 25.0 ± 0.2 mL.
3. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute (opm).
4. Extraction vessels and associated filtration vessel.
5. Whatman No. 42 or equivalent highly retentive filter paper.
6. spectrophotometer, autoanalyzer, or flow injection analyzer (FIA) instrument.

Reagents

1. Deionized water, ASTM Type I grade.
2. Potassium chloride extracting solution, 2.0 N KCl: Dissolve 150 g of reagent grade KCl in 500 mL deionized water and dilute to a 1000 mL (See Comment #1).
3. Standard calibration solutions of NO_3-N. Prepare six calibration standards ranging from 0.1 to 20.0 mg L^{-1} concentration, diluted in 2.0 N KCl extraction solution prepared from 1000 mg L^{-1} NO_3-N standard solution.

Procedure

1. Weigh 5.0 ± 0.05 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into extraction vessel. Add 25.0 mL of 2.0 N KCl extraction reagent using repipette dispenser (See Comment #2). Include a method blank.
2. Place extraction vessel(s) on reciprocating mechanical shaker for thirty (30) minutes.
3. Filter extract (See Comment #3), refilter if filtrate is cloudy (comment #4).
4. Nitrate-N content of the extract is determined using a spectrophotometer, automated flow analyzer (Technicon Method No. 329-74W/A) or FIA instrument. Calibrate using standard calibration solutions and operate instrument in accordance with manufacturer instructions. Determine nitrate concentration of KCl extract, method blank, unknown samples and record results as mg L^{-1} of nitrate in extract solution (See Comment #5).

Calculation

NO_3-N mg kg^{-1} in soil = (NO_3-N mg L^{-1} in filtrate - method blank) × 5

Report soil nitrate concentration to the nearest 0.1 mg kg^{-1} (See Comment #6)

Comments

1. Soils may be extracted with 1.0 N KCl for the determination of nitrate only.
2. Check repipette dispensing volume calibration using an analytical balance.
3. Check filter paper supply for possible contamination of and NO_3-N. If contamination is greater than 0.2 mg L^{-1} on a solution basis, rinse filter paper with 2.0 N KCl.
4. Soil KCl extract may be stored up to three weeks if stored at 4 °C and/or with 100 uL of toluene or thymol.
5. Samples having nitrate concentrations exceeding the highest standard will require dilution and reanalysis.
6. Nitrate-nitrogen (NO_3-N) results can be expressed on a volume basis. Assuming the sample represents a 0-6 inch (0-15 cm) depth of the soil, then: NO_3-N mg kg^{-1} × 2.0 ≅ NO_3-N lbs ac^{-1}

Literature

Bremmer, J. M. and D.R. Keeney. 1965. Determination and isotopic ratio analysis of different forms of nitrogen in soils: I. Apparatus and procedure for distillation for and determination of ammonium. Soil Sci. Soc. Am. Proc. 29:504-507.

Dahnke, W.C. 1990. Testing soils for available nitrogen. p. 120-140. *In:* R.L. Westerman (ed.) Soil testing and plant analysis. Soil Sci. Soc. Am. Book series 3 ASA Madison WI.

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Keeney, D.R. and D.W. Nelson. 1982. Nitrogen - inorganic forms. *In* A.L. Page (eds.) Methods of soil analysis, part 2. Agron. Monogr. 9, 2nd ed. ASA and SSSA, Madison, WI. p. 643-698.

Scope and Application

This method quantitatively extracts nitrate from soils using an aluminum sulfate solution and subsequent determination of nitrate (NO_3) using a nitrate ion specific electrode (ISE) as explained by Dahnke (1971). The ISE determines nitrate by measuring an electrical potential developed across a thin layer of water-immiscible liquid or gel ion exchanger that is selective for NO_3. This layer of ion exchanger is held in place by a porous membrane. The NO_3-N ISE is susceptible to interferences of Cl^-, HCO_3^-, SO_4^{2-} and is sensitive to changes in solution ionic strength (i.e. high salt). The lower limit of accurate detection of NO_3-N by ISE is reported to be approximately 2.0 mg L^{-1} NO_3-N in solution thus this requires a lower soil to solution extraction ratio than the cadmium reduction method. Oien and Selmer-Olsen (1969) studied dilution ratios (soil : extract) of 1:10, 1:5, 1:2.5, 1:1.7, 1:1 and found a ratio of 1:2.5 can be used to accurately determine nitrate. Because of interferences and detection limit the ISE method is less reproducible than the cadmium-reduction method. Problems with precision have been noted by Mack and Sanderson (1971). The method detection limit is approximately 5 mg kg^{-1} (dry soil basis) and is generally reproducible ± 15%.

Equipment

1. Analytical balance: 100.0 g capacity, resolution ± 0.01 g.
2. Repipette dispenser, calibrated to 25.0 ± 0.2 mL.
3. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute (opm).
4. Whatman No. 42 or equivalent highly retentive filter paper.
5. Nitrate ion sensitive electrode.
6. pH/ion meter or pH-millivolt meter.

Reagents

1. Deionized water, ASTM type I Grade.
2. Extracting Solution: Ionic strength adjusting solution - 0.01M $Al_2(SO_4)_3$, 0.02M H_3BO_3, 0.01M Ag_2SO_4, and 0.02 M NH_2HSO_3 (sulfamic acid): Dissolve 67 g of $Al_2(SO_4)_3 \cdot 18H_2O$, 12 g of H_3BO_3, 20 g of Ag_2SO_4 and 19 g of NH_2HSO_3 in water and dilute to 10 L.
3. Standard nitrate solutions. To a 1000 mL volumetric flask, add 0.7221 g of oven dry KNO_3, make to volume with extraction solution. This gives a solution containing 100 mg L^{-1} of NO_3-N. Prepare nitrate calibration standards solution of 1.0, 2.0, 5.0, 10.0, 15.0, 20.0 and 30.0 mg L^{-1} and dilute to volume with extraction solution.

Procedure

1. Weigh 10.0 ± 0.10 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into extraction vessel. Add 25.0 mL of extraction solution using repipette dispenser (See Comment #1). Include a method blank.
2. Place extraction vessel(s) on reciprocating mechanical shaker for ten (10) minutes.
3. Filter extract (See Comment #2), refilter if filtrate is cloudy (See Comment #3.
4. Develop calibration curve for the ion selective electrode using standards.
5. Calibrate ion selective electrode/millivolt meter using standard calibration solutions and operate instrument in accordance with manufacturer instructions. Determine nitrate concentration of soil extracts, method blank, unknown samples and record results as mg L^{-1} of nitrate in extract solution (See Comment #3 and #4).

Calculation

NO_3-N mg kg^{-1} in soil = (NO_3-N mg L^{-1} in filtrate - method blank) × 2.5

Report soil nitrate concentration to the nearest 0.1 mg kg^{-1} (See Comment #5)

Comments

1. Check repipette dispensing volume calibration using an analytical balance.

2. Check filter paper supply for possible contamination of and NO_3-N. If contamination is greater than 0.2 mg L^{-1} on a solution basis, rinse filter paper with extraction solution.

3. Routinely check ISE calibration every third sample using a mid range standard. In specific instances the ISE maybe susceptible to radio frequency energy interference from surrounding electronic equipment (Carlson, 1992). For samples with $EC_e > 2$, additional Ag_2SO_4 should be added.

4. Samples having nitrate concentrations exceeding the highest standard will require dilution and reanalysis.

5. Nitrate-nitrogen (NO_3-N) results can be expressed on a volume basis. Assuming the sample represents a 0-6 inch (0-15 cm) depth of the soil, then: NO_3-N mg kg^{-1} × 2.0 ≅ NO_3-N lbs ac^{-1}

Literature

Carlson, R.M. 1992. University California Davis. Personal Communication.

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Mack, A.R. and R.B. Sanderson. 1971. Sensitivity of the nitrate-ion membrane electrode in various oil extracts. Can. J. Soil Sci. 51:95104.

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Oien, A., and Selmer-Olsen, A.R. 1969. Nitrate determination in soil extracts with the nitrate electrode. Analyst 94: pp 888-894.

Scope and Application

This method involves the quantitative extraction of nitrate (NO_3-N) from soils using 40 g (0.02 N) $CaSO_4$ solution. Nitrate is determined by reaction with chromotropic acid to form an azochromophore (yellow dye) which is measured spectrophotometrically at 420 nm (Kowalenko and Lowe,1973). The method has an interference from chloride which can be masked with addition of antimony (Sb). The procedure outlined follows that outlined by Simms and Jackson (1971). If nitrite concentrations are high (> 2.0 mg kg^{-1}) an urea-sulfite solution maybe added which converts nitrite (NO_2-N) to nitrate (NO_3-N). In addition there maybe organic interferences on soils high in organic matter (> 6.0%). Care must be taken to avoid contamination from filter paper and handling. Soil nitrate-nitrogen can be used to predict plant response to nitrogen fertilizers. The method detection limit is approximately 1.0 mg kg^{-1} (on a dry soil basis) and is generally reproducible ± 6%.

Equipment

1. Analytical balance: 100.0 g capacity, resolution ± 0.01 g.
2. Repipette dispenser(s), calibrated to 25.0 ± 0.2, 2.5 ± 0.05, and 2.5 ± 0.05 mL.
3. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute (opm).
4. Extraction vessels and associated filtration vessel.
5. S&S #597 filter paper or equivalent highly retentive filter paper.
6. Water bath 5 °C.
7. Vortex stirrer.
8. Spectrophotometer instrument.

Reagents

1. Deionized water, ASTM Type I grade.
2. Calcium Sulfate Extracting solution, 0.1 N $CaSO_4$: Dissolve 40 g of reagent grade $CaSO_4$ in 9 L deionized water and dilute to 10 L final volume (See Comment #1).
3. Antimony Interference Solution: In a 2000 mL flask add 1600 mL H_2SO_4 reagent grade. Using heat slowly dissolve 6.0 g of 100 mesh metal antimony (Sb) powder and stir until dissolved. Cool and bring to 2000 mL final volume with deionized water. Solution will require heating to 50 °C, to redissolve antimony prior to usage.
4. Chromotropic Acid solution: Dissolve 0.4 g chromotropic acid (disodium salt) in 800 mL of H_2SO_4, mix and bring to 2000 mL final volume with H_2SO_4. Store in opaque glass bottle.
5. Urea-Sulfite solution: Add 5.0 g of urea to 4.0 gm sodium sulfite to 50 mL deionized water and bring to 100 mL final volume.
6. Standard calibration solutions of NO_3-N. Prepare six calibration standards ranging from 1.0 to 20.0 mg L^{-1} concentration, diluted in calcium sulfate extraction solution prepared from 1000 mg L^{-1} NO_3-N standard solution.

Procedure

1. Weigh 5.0 ± 0.05 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into extraction vessel. Add 25.0 mL of 0.10 N $CaSO_4$ extraction reagent using repipette dispenser (See Comment #2). Include a method blank.
2. Place extraction vessel(s) on reciprocating mechanical shaker for fifteen (15) minutes.
3. Filter extract (See Comment #3), refilter if cloudy. Extract must be clear prior to analysis.
4. Place 1.0 mL of extract in test tube and place test tube in cooled water bath. Concurrently prepare NO_3-N standards with unknown samples.
5. Add 200 uL of urea-sulfite solution, and vortex mix.
6. Add 1.0 mL antimony solution, vortex stir and stand for one (1) hour in a 5 °C water bath.

7. Add 2.5 mL of chromotropic acid solution, vortex stir and under cover for thirty (30) minutes.
8. Determine absorbance on spectrophotometer at 420 nm wavelength. Calibrate using standard calibration solutions and operate instrument in accordance with manufacturer instructions. Determine nitrate concentration of calibration standards, method blank, unknown samples and record results as mg L^{-1} of nitrate in extract solution (See Comment #4 and #5).

Calculation

NO_3-N mg kg^{-1} in soil = (NO_3-N mg L^{-1} in filtrate - method blank) × 5

Report soil nitrate concentration to the nearest 0.1 mg kg^{-1} (See Comment #6)

Comments

1. Resuspend to remove undissolved $CaSO_4$. As an alternative 20g/L of $Ca(OH)_2$ can be substituted for $CaSO_4$.

2. Check repipette dispensing volume calibration using an analytical balance.

3. Check filter paper supply for possible contamination of and NO_3-N. If contamination is greater than 0.2 mg L^{-1} on a soil extract basis, rinse filter paper with $CaSO_4$ extraction solution.

4. Samples having nitrate concentrations exceeding the highest standard will require dilution and reanalysis.

5. Soil extracts contains antimony are classified as a hazardous waste and must be disposed of in a suitable manner.

6. Nitrate-nitrogen (NO_3-N) results can be expressed on a volume basis. Assuming the sample represents a 0-6 inch (0-15 cm) depth of the soil, then: NO_3-N mg kg^{-1} × 2.0 ≅ NO_3-N lbs ac^{-1}

Literature

Dahnke, W.C. 1990. Testing soils for available nitrogen. p. 120-140. *In:* R.L. Westerman (ed.) Soil testing and plant analysis. Soil Sci. Soc. Am. Book series 3 ASA Madison WI.

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Kowalenko, C.G. and L.E. Lowe. 1973. Determination of nitrates in soil extracts. Soil Sci. Soc. Am. Proc. 37:660.

Sims, J.R. and G.D. Jackson. 1971. Rapid analysis of soil nitrate with chromotropic acid. Soil Sci. Soc. Am. Proc. 35:603-606.

Scope and Application

This method involves the semiquantitative extraction of ammonium (NH_4-N) from soils using 2.0 N KCl. Ammonium is determined by spectrophotometric, diffusion-conductivity instruments or distillation techniques. The method doesn't quantitatively extract ammonium from mineral structures (i.e. nonexchangeable NH_4-N) or bound to organic compounds. The method is readily adapted to manual or automated techniques. The procedure outlined follows that outlined by Keeney and Nelson (1982) for determining nitrate nitrogen with a modification in which 25 mL of KCl and 5.0 g of soil are used instead of 100 mL and 10 g soil. Care must be taken to avoid contamination from filter paper and operator handling. Soil ammonium concentrations are generally low in mineral soils (< 10 mg kg^{-1}). The method detection limit is approximately 0.2 mg kg^{-1} (on a dry soil basis) and is generally reproducible ± 7%.

Equipment

1. Analytical balance: 100.0 g capacity, resolution ± 0.01 g.
2. Repipette dispenser, calibrated to 25.0 ± 0.2 mL.
3. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute (opm).
4. Extraction vessels and associated filtration vessel.
5. Whatman No. 42 or equivalent highly retentive filter paper.
6. Spectrophotometer, or flow injection analyzer (FIA), or distillation instruments.

Reagents

1. Deionized water, ASTM Type I grade.
2. Potassium chloride extracting solution, 2.0 N KCl: Dissolve 150 g of reagent grade KCl in 500 mL deionized water and dilute to a 1000 mL (See Comment #1).
3. Standard calibration solutions of NH_4-N. Prepare six calibration standards ranging from 0.1 to 20.0 mg L^{-1} concentration, diluted in 2.0 N KCl extraction solution prepared from 1000 mg L^{-1} NO_3-N standard solution.

Procedure

1. Weigh 5.0 ± 0.05 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into extraction vessel. Add 25.0 mL of 2.0 N KCl extraction reagent using repipette dispenser (See Comment #2). Include a method blank.
2. Place extraction vessel(s) on reciprocating mechanical shaker for thirty (30) minutes.
3. Filter extract (See Comment #3), refilter if filtrate is cloudy (comment #4).
4. Ammonium-N content of the extract is determined using a spectrophotometer, diffusion-conductivity instruments or distillation techniques using standard calibration solutions (See Comment #4 and #5). The ammonium nitrogen content of the digest solution can be determined with a rapid flow analyzer (Technicon Method No. 334-74A/A) or an flow injection analyzer (FIA). This determination can also be made using the Kjeldahl distillation method. Adjust and operate instruments in accordance with manufacturer's instructions. Determine ammonium concentration of a method blank and unknown samples.

Calculation

NH_4-N mg kg^{-1} in soil = (NH_4-N mg L^{-1} in filtrate - method blank) × 5

Report soil aluminum concentration to the nearest 0.1 mg kg^{-1} (See Comment #6)

Comments

1. Soils may be extracted with 2.0 N KCl for the simultaneous determination of nitrate (Method 3.10).

2. Check repipette dispensing volume calibration using an analytical balance.

3. Check filter paper supply for possible contamination of and NH_4-N. If contamination is greater than 0.2 mg L^{-1} on a solution basis, rinse filter paper with 2.0 N KCl.

4. It is recommended that soils extracted for aluminum be analyzed with in two (2) hours after extraction.

5. Samples having ammonium concentrations exceeding the highest standard will require dilution and reanalysis.

6. Ammonium-nitrogen (NH_4-N) results can be expressed on a volume basis. Assuming the sample represents a 0-6 inch (0-15 cm) depth of the soil, then: NH_4-N mg kg^{-1} × 2.0 ≅ NH_4-N lbs ac^{-1}

Literature

Bremmer, J. M. and D.R. Keeney. 1965. Determination and isotopic ratio analysis of different forms of nitrogen in soils: I. Apparatus and procedure for distillation for and determination of ammonium. Soil Sci. Soc. Am. Proc. 29:504-507.

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SOIL NITROGEN MINERALIZATION POTENTIAL S - 3.60

Anaerobic Method

Scope and Application

This method involves the semi quantitative extraction of minerizable ammonium nitrogen based on a anaerobic incubation as described by Keeney (1982). Minerizable nitrogen is determined by spectrophotometric, diffusion-conductivity instruments or distillation techniques. Mineralizable nitrogen is used to predict available soil nitrogen for plant response to nitrogen fertilizers. The method detection limit is approximately 0.2 mg kg-1 (on a dry soil basis) and is generally reproducible ± 7%.

Equipment

1. Analytical balance: 100.0 g capacity, resolution ± 0.01 g.
2. Repipette dispenser, calibrated to 25.0 ± 0.2 mL.
3. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute (opm).
4. Extraction vessels 125 mL and associated filtration vessel.
5. Whatman No. 42 or equivalent highly retentive filter paper.
6. Spectrophotometer, or flow injection analyzer (FIA), or distillation instruments.
7 Incubator capable of 40 ± 0.5 °C.

Reagents

1. Deionized water, ASTM Type I grade.
2. Potassium chloride extracting solution, 2.0 N KCl: Dissolve 150 g of reagent grade KCl in 500 mL deionized water and dilute to a 1000 mL.
3. Standard calibration solutions of NH_4-N. Prepare six calibration standards ranging from 0.1 to 20.0 mg L^{-1} concentration, diluted in 2.0 N KCl extraction solution prepared from 1000 mg L^{-1} NO_3-N standard solution.

Procedure

1. Weigh 20.0 ± 0.05 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into 125 mL extraction vessel. Add 25.0 mL of deionized water using repipette dispenser, stir well with a glass rod and add a 2nd aliquot of 25.0 mL of deionized water (See Comment #1 and #2).
2. Cover mouth of extraction vessel with parafilm and then plastic and tightly secure with lid.
3. Place extraction vessel(s) in incubator at 40.0 °C for 7 days (168 hours).
4 remove from incubator and add 50.0 mL of 2.0 N KCl using repipette dispenser.
5. Place extraction vessel(s) on reciprocating mechanical shaker for sixty (60) minutes
6. Filter extract (See Comment #3), refilter if filtrate is cloudy (See Comment #2).
7. Repeat using Method S-3.50 for extractable NH_4-N utilizing the same soil(s), reference soil.
8. Ammonium-N content of the extract is determined using a spectrophotometer, diffusion-conductivity instruments or distillation techniques using standard calibration solutions (See Comment #4 and #5). The ammonium nitrogen content of the digest solution can be determined with a rapid flow analyzer (Technicon Method No. 334-74A/A) or an flow injection analyzer (FIA). This determination can also be made using the Kjeldahl distillation method. Adjust and operate instruments in accordance with manufacturer's instructions. Determine ammonium concentration of a method blank and unknown samples.

Calculation

Mineralizable NH_4-N mg kg^{-1} in soil = (NH_4-N mg L^{-1} incubated - reference extract) × 5
Report mineralizable soil nitrogen concentration to the nearest 0.1 mg kg^{-1} (See Comment #6)

Comments

1. It is recommended that samples be rapidly air dried at ambient temperature immediately after sampling to minimize mineralization to nitrate.

2. Check repipette dispensing volume calibration using an analytical balance.

3. Check filter paper supply for possible contamination of and NH_4-N. If contamination is greater than 0.2 mg L^{-1} on a solution basis, rinse filter paper with 2.0 N KCl.

4. Samples having ammonium concentrations exceeding the highest standard will require dilution and reanalysis.

5. It is recommended that soils extracted for ammonium be analyzed with in two (2) hours after extraction

6. Mineralizable N (NH_4-N) results can be expressed on a volume basis. Assuming the sample represents a 0-6 inch (0-15 cm) depth of the soil, then: NH_4-N mg kg^{-1} × 2.0 ≅ NH_4-N lbs ac^{-1}

Literature

Dahnke, W.C. 1990. Testing soils for available nitrogen. p. 120-140. *In:* R.L. Westerman (ed.) Soil testing and plant analysis. Soil Sci. Soc. Am. Book series 3 ASA Madison WI.

Keeney, D.R. and D.W. Nelson. 1982. Nitrogen - inorganic forms. *In* A.L. Page (eds.) Methods of soil analysis, part 2. Agron. Monogr. 9, 2nd ed. ASA and SSSA, Madison, WI. p. 643-698.

Kowalenko, C.G. and L.E. Lowe. 1973. Determination of nitrates in soil extracts. Soil Sci. Soc. Am. Proc. 37:660.

Sims, J.R. and G.D. Jackson. 1971. Rapid analysis of soil nitrate with chromotropic acid. Soil Sci. Soc. Am. Proc. 35:603-606.

Scope and Application

This method estimates the relative bioavailability of ortho-phosphate (PO_4-P) using 0.5 N $NaHCO_3$ adjusted to pH 8.50 for soils mildly acidic to alkaline pH and is based on the method developed by Olsen et al.,1954. In the process of extraction, CO_2 from bicarbonate is driven off, pH increases and bicarbonate converts to carbonate. Thus there is lower calcium activity as calcium carbonate is formed increasing the quantity of phosphates in solution. Phosphorus content is determined spectrophotometrically at 882 nm at an acidity of 0.24 M H_2SO_4 (Rodriguez et al., 1994) by reacting with ammonium molybdate using ascorbic acid as a reductant in the presence of antimony (Murphy and Riley, 1962) using manual or automated techniques. The method has shown to be well correlated to crop response phosphorus fertilization on neutral to alkaline soils. In the Pacific Northwest and in the Northern Great Plains the method is used for the simultaneous extraction of plant available potassium, nitrate and specific cases sulfur. The method has a phosphorus detection limit of approximately 2.0 mg kg^{-1} (on a dry soil basis) and is generally reproducible to within ± 12%.

Equipment

1. Analytical balance, resolution ±0.01 g.
2. Oscillating mechanical shaker, capable of 180 oscillations per minute (opm).
3. Repipette dispenser, calibrated to 40.0 ±0.4 mL, 9.0 ±0.1 mL.
4. 125-mL plastic extraction erlenmeyer and associated filtration labware.
5. Whatman No. 1, No. 2, filter paper or equivalent.
6. Pipettes: 0.250 ±0.005 mL, 0.500 ±0.005 mL, 1.00 ±0.01 mL, 2.00 ±0.02 mL, 3.00 ±0.03 mL, 4.00 ±0.04 mL.
7. Spectrophotometer wavelength 882 nm and 2.5 cm matching spectrophotometer cuvette or automated Flow Injection Analysis system instrumentation.

Reagents

1. Deionized water, ASTM Type I grade.
2. Sodium bicarbonate extracting solution (0.5 N $NaHCO_3$ @ pH - 8.50). Dissolve 42.01 g of $NaHCO_3$ in about 900 mL of deionized water. Adjust the pH to 8.50 ±0.05 with 2.0 N NaOH before diluting with deionized water to 1,000 mL. This solution is unstable with regard to pH and should be prepared as required (See Comments #1).
3. Modified Reagent A (Watanabe and Olsen, 1965).
   - Ammonium Molybdate: Dissolve 12.0 g. of A.R. [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] in 250 mL of deionized water.
   - Antimony Potassium Tartrate: Dissolve 0.291 g. of A.R. antimony potassium tartrate [$K(SbO) \cdot C_4H_4O_6 \cdot ½ H_2O$] in 100 mL of deionized water.
   - Add both of the dissolved reagents to 1,000 mL of 5.76 N H_2SO_4 (160 mL of concentrated sulfuric acid per liter, Self and Rodriguez, 1996) mix thoroughly and make to 2,000 mL. Modified Reagent A (mixed reagent) last at least four months if it is stored in an opaque plastic bottle.
4. Reagent B, ascorbic/molybdate reagent (Watanabe and Olsen, 1965). Dissolve 1.32 g. of A.R. ascorbic acid ($C_6H_4O_6$) in 250 mL of modified Reagent A and mix well. This reagent should be prepared as required.
5. Phosphorus Calibrations Standards. From a standard solution containing 1,000 mg L^{-1} PO_4-P, prepare 100 mL of standard in 0.5N $NaHCO_3$ containing 100 mg L^{-1} PO_4-P. Then, using the 100 mg L^{-1} PO_4-P standard, prepare seven calibration solutions of 100 mL each in 0.5 N $NaHCO_3$ with PO_4-P concentrations of 0.00, 0.25, 0.50, 0.75, 1.00, 2.00, and 4.00 mg L^{-1}.

Extracting Procedure

1. Weigh 2.00 ± 0.02 g of air dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) in a 125-mL plastic extraction erlenmeyer.
2. Add 40.0 mL of 0.5 N $NaHCO_3$ extraction solution (See Comments #2, #3 and #4). Include a method blank and standard quality control samples.
3. Place extraction vessels on oscillating mechanical shaker for thirty (30) minutes.
4. Filter suspension immediately - within 1 mintue (refilter if filtrate is cloudy).

Phosphorus Analysis

1. Pipette a 3.0 mL aliquot of standard or soil extract (See Comment #5) into a 2.5 cm matching spectrometer tube.
2. Add 9.0 mL of deionized water.
3. Add 3.0 mL of Reagent B (ascorbic/molybdate reagent).
4. Adjust and operate spectrophotometer in accordance with manufacture's instructions. Read absorbance at a wavelength of 882 nm after 10 minutes of adding the Reagent B. Adjust the 0.000 absorbance using the 0.00 standard. Determine absorbance of a method blank, standards and unknown samples. Calculate phosphorus concentration for blank and unknown samples from standard curve and record phosphorus to the nearest 0.01 mg L^{-1} PO_4-P in extract solution (See Comments #6 - #8).

Calculations

Report soil bicarbonate available phosphorus to the nearest 0.1 mg kg^{-1} (See Comment #9):

Soil PO_4-P mg kg^{-1} = (PO_4-P mg L^{-1} in extract - blank) × 20

Comments

1. Storage of 0.5N $NaHCO_3$ solution can result in the reagent becoming more alkaline. Increased alkalinity of the extraction solution results in an increase of inorganic phosphorus (Olsen et al., 1954 and Cowling et al., 1987).

2. Clean all extraction and filtration labware with 0.5 N HCl and three deionized water rinses to removed potential ortho-phosphate contamination.

3. Check repipette dispensing volume, calibrate using an analytical balance.

4. Extractable phosphorus increases 0.43 expressed as mg kg^{-1}, for each degree rise in temperature between 20 and 30 °C for soils between 5 and 40 mg kg^{-1} (Olsen et al., 1954).

5. For automated FIA analysis sample extracts will require neutralization of $NaHCO_3$ and degassing to remove dissolved CO_2 prior to analysis. Specific FIA instruments have capability to remove dissolved CO_2.

6. Samples exceeding highest standard will require dilution with the extracting solution for reanalysis.
7. Potassium in the extract is can be measured by flame emission spectrophotometry by either manual or an automated system. The $NaHCO_3$ extract is combined with acidified lithium nitrate, degassed and analyzed by flame emission spectrophotometery (Schoenau and Karamanos, 1993).

8. Nitrate (NO_3-N) may also be determined directly from the Olsen extract. Extracts will require neutralization of $NaHCO_3$ and degassing to remove dissolved CO_2 prior to analysis.

9. Generally, soils having a bicarbonate available phosphorus level below 20 mg kg^{-1} will have a response to applications of phosphorus fertilizers for most crops.

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ESTIMATION OF AVAILABLE SOIL PHOSPHORUS S - 4.20

Dilute Acid-Fluoride Bray and Kurtz P-1 Method

Scope and Application

This method estimates the relative bioavailability of ortho-phosphate (PO_4-P) in acid to neutral pH soils using a dilute acid solution of pH - 2.60 that is 0.025 M HCl and 0.03 M NH_4F. In the process of extraction, phosphorus is solubilize under two different mechanisms, the strong acid increases the solubility of phosphates by protonation, whereas the fluoride lower the activity of calcium as calcium fluoride increasing the quantity of phosphates in solution. Phosphorus content is determined spectrophotometrically at 882 nm at an acidity of 0.19 M H_2SO_4 (Rodriguez et al., 1994) by reacting with ammonium molybdate using ascorbic acid as a reductant in the presence of antimony (Murphy and Riley, 1962).

The method is shown to be well correlated to crop response to phosphorus fertilization on neutral to acid soils. The method has a phosphorus detection limit of approximately 2.0 mg kg^{-1} (on a dry soil basis) and is generally reproducible within ± 10%.

Equipment

1. Analytical balance: resolution 0.01 g.
2. Reciprocating mechanical shaker, capable of 180 oscillations per minute (opm).
3. Repipette dispenser, calibrated to 20.0 ±0.2 mL, 12 ±0.1 mL.
4. 125-mL plastic extraction erlenmeyer and associated filtration vessel.
5. Whatman No. 1 filter paper or equivalent.
6. Pipettes: 0.250 ±0.005 mL, 0.500 ±0.005 mL, 1.00 ±0.1 mL, 2.00 ±0.02 mL, 3.00 ±0.03 mL.
7. 2.5 cm matching spectrophotometer cuvette.
8. Spectrophotometer, wavelength 882 nm or automated Flow Injection Analysis system.

Reagents

1. Deionized water, ASTM type I grade.
2. Hydrochloric acid 0.5 N HCL: Dilute 103 mL of concentrated HCL to a volume of 2,500 mL with deionized water.
3. Ammonium fluoride stock solution, 1.0 N NH_4F: dissolve 74.0 g of NH_4F in deionized water and dilute the solution to 2,000 mL.
4. Bray and Kurtz P-1 extracting solution (0.025 N HCl-0.03 N NH_4F @ pH - 2.60): Mix thoroughly 1,000 mL of 0.5 N HCl and 600 mL of 1.0 N NH_4F with about 18.0 L of deionized water. Adjust the pH to 2.60 ±0.05 with diluted HCl or NH_4OH before dilution to 20.0 L. This produces a solution of 0.03 N NH_4F and 0.025 N HCL. Store the solution in a polyethylene container. Check the pH before use.
5. Modified Reagent A (Watanabe and Olsen, 1965).
   - Ammonium Molybdate: Dissolve 12.0 g. of A.R. $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$ in 250 mL of deionized water.
   - Antimony Potassium Tartrate: Dissolve 0.291 g. of A.R. antimony potassium tartrate $[K(SbO) \cdot C_4H_4O_6 \cdot ½\ H_2O]$ in 100 mL of deionized water.
   - Add both of the dissolved reagents to 1,000 mL of 5.76 N H_2SO_4 (160 mL of concentrated sulfuric acid per liter, Self and Rodriguez, 1996) mix thoroughly and make to 2,000 mL. Modified Reagent A (mixed reagent) last at least four months if it is stored in an opaque plastic bottle.
6. Reagent B, ascorbic/molybdate reagent (Watanabe and Olsen, 1965). Dissolve 1.32 g. of A.R. ascorbic acid ($C_6H_4O_6$) in 250 mL of modified Reagent A and mix well. This reagent should be prepared as required. Modified Reagent A (Watanabe and Olsen, 1965).
7. Phosphorus Calibrations Standards. From a standard solution containing 1,000 mg L^{-1} PO_4-P prepare 100 mL of standard in Bray and Kurtz P-1 extracting solution containing 100 mg L^{-1} PO_4-P. Then, using the 100 mg L^{-1} PO_4-P standard, prepare six calibration solutions of 100 mL each in Bray and Kurtz P-1 extracting solution with PO_4-P concentrations of 0.00, 0.75, 1.50, 3.00, 6.00, and 12.00 mg L^{-1}.

Extracting Procedure

1. Weigh 2.00 ±0.02 g of air dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) in a 125-mL plastic extraction erlenmeyer.
2. Add 20.0 mL of Bray and Kurtz P-1 extracting solution (See Comment #2). Include a method blank.
3. Place extraction vessels on reciprocating mechanical shaker for 5 min.
4. Filter suspension immediately, refilter if filtrate is cloudy.

Phosphorus Analysis

1. Pipette 1.0 mL aliquot of standard or soil extract into a 2.5 cm matching spectrometer tube (See Comment #3).
2. Add 12.0 mL of deionized water.
3. Add 2.0 mL of Reagent B (ascorbic/molybdate reagent developing reagent).
4. Adjust and operate sprectrophotometer in accordance with manufacture's instructions. Read absorbance at a wavelength of 882 nm after ten (10) minutes of adding the Reagent B. Adjust the 0.000 absorbance using the 0.00 standard. Determine absorbance of a method blank, standards and unknown samples. Calculate phosphorus concentration for blank and unknown samples from standard curve and record phosphorus to the nearest 0.01 mg L^{-1} PO_4-P in extract solution (See Comment #4).

Calculations

Report soil Bray and Kurtz P-1 available phosphorus to the nearest 0.1 mg kg^{-1} :

soil PO_4-P mg kg^{-1} = (PO_4-P mg L^{-1} in extract - method blank) × 10

(See Comment #5).

Comments

1. This method for available P follows the procedure originally outlined by Olsen and Summers (1982). The original Bray-P1 method describes a soil extractant ratio of 1:7 and an extraction time of 60 seconds. To simplify the method a number of labs in the east and North Central United States have altered the procedure to a soil extractant ratio of 1:10 and an extraction time of five minutes.

2. Check repipette dispensing volume, calibrate using an analytical balance.

3. Extracts can be analyzed directly for phosphorus by automated FIA analysis.

4. Samples exceeding highest standard will require dilution with the extracting solution for reanalysis.

5. Generally, soils having less than 25 mg kg^{-1} will have a response to applications of phosphorus fertilizers for most crops.

Literature

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Rodriguez, J.B., J.R. Self, and P.N. Soltanpour. 1994. Optimal conditions for phosphorus analysis by the ascorbic acid-molybdenum blue method. Soil Sci.Soc. Am. J. 58:866-870.

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ESTIMATION OF AVAILABLE SOIL PHOSPHORUS S - 4.31

Dilute Double Acid, Mehlich 1 Method

Scope and Application

This method was developed by Mehlich in 1953 and estimates the relative bioavailability of ortho-phosphate (PO_4-P) on soils acid to neutral pH using a dilute double acid solution, 0.05 N HCl - 0.025 N H_2SO_4. This method is primarily for determining phosphorus in sandy soils of the eastern United States which have a cation exchange capacity (CEC) of less than 10 cmol/kg and have a pH less than 6.5. The method is applicable to simultaneous determination of: extractable potassium, calcium, magnesium, sodium, and zinc. Phosphorus content is determined spectrophotometrically at 882 nm at an acidity of 0.20 M H_2SO_4 (Rodriguez et al., 1994) by reacting with ammonium molybdate using ascorbic acid as a reductant in the presence of antimony (Murphy and Riley, 1962). The method is unsuitable for alkaline calcareous soils and those with high CEC. With specific soils the extract maybe colored. Arsenate present in the extract will produce a blue color and produce a positive interference. Phosphorus and cations may also be determined by ICP-AES instrumentation. The method is correlated to crop response to fertilizer phosphorus. The method has a phosphorus detection limit of about 1.0 kg P ha^{-1} (on a dry soil basis) and is generally reproducible within ± 8%.

Equipment

1. Soil Scoop 4 cm^3.
2. Reciprocating mechanical shaker, capable of 180 oscillations per minute (opm).
3. Repipette dispenser, calibrated to 25 ± 0.2 mL, 12 ± 0.1 mL.
4. 50-mL plastic extraction erlenmeyer and associated filtration apparatus.
5. Whatman No. 1 filter paper or equivalent.
6. Pipettes: 0.250 ±0.005 mL, 0.500 ±0.005 mL, 1.00 ±0.01 mL, 2.00 ±0.02 mL, 3.00 ± 0.03 mL.
7. 2.5 cm matching spectrophotometer cuvette.
8. Spectrophotometer, wavelength 882 nm, automated Flow Injection Analysis (FIA) and/or ICP-AES.

Reagents

1. Deionized water, ASTM Type I grade.
2. Mehlich-1 extracting solution (0.05 N HCl and 0.025 N H_2SO_4). Dilute 4 mL of concentrated HCl and 0.7 mL concentrated H_2SO_4 to 1.0 L with deionized water.
4. Modified Reagent A (Watanabe and Olsen, 1965).
   - Ammonium Molybdate: Dissolve 12.0 g. of A.R. $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$ in 250 mL of deionized water.
   - Antimony Potassium Tartrate: Dissolve 0.291 g. of A.R. antimony potassium tartrate $[K(SbO) \cdot C_4H_4O_6 \cdot ½\ H_2O]$ in 100 mL of deionized water.
   - Add both of the dissolved reagents to 1,000 mL of 5.76 N H_2SO_4 (160 mL of concentrated sulfuric acid per liter, Self and Rodriguez, 1996) mix thoroughly and make to 2,000 mL. Modified Reagent A (mixed reagent) last at least four months if it is stored in an opaque plastic bottle.
5. Reagent B, ascorbic/molybdate reagent (Watanabe and Olsen, 1965). Dissolve 1.32 g. of A.R. ascorbic acid ($C_6H_4O_6$) in 250 mL of modified Reagent A and mix well. This reagent should be prepared as required.
6. Phosphorus Calibrations Standards. From a standard solution containing 1,000 mg L^{-1} PO_4-P prepare 100 mL of standard in Mehlich 1 extracting solution containing 100 mg L^{-1} PO_4-P. Then, using the 100 mg L^{-1} PO_4-P standard, prepare six calibration solutions of 100 mL each in Mehlich 1 extracting solution with PO_4-P concentrations of 0.00, 0.75, 1.50, 3.00, 6.00, and 12.00 mg L^{-1}.

Extracting Procedure

1. Measure 5.0±0.1 g or 4.0 ±0.1 cm^3 of air dried soil pulverized to pass 10 mesh sieve(< 2.0 mm) in a 50-mL plastic erlenmeyer flask.
2. Add 25.0 mL of Mehlich 1 extracting solution (see comment # 2). Include a method blank.
3. Place extraction flask(s) on reciprocating mechanical shaker for five (5) minutes.
4. Filter suspension immediately, refilter if filtrate is cloudy.

Phosphorus Chemical Analysis

1. Pipette 1.0 mL aliquot of standard or soil extract into a 25 mL test tube (See Comment #3).
2. Add 12.0 mL of deionized water.
3. Add 2.0 mL of Reagent B (ascorbic/molybdate reagent) and stir on vortex stirrer for 30 seconds.
4. Adjust and operate spectrophotometer in accordance with manufacture's instructions. Read absorbance at a wavelength of 882 nm after ten (10) minutes of adding the Reagent B. Adjust the 0.000 absorbance using the 0.00 standard. Determine absorbance of a method blank, standards and unknown samples. Calculate phosphorus concentration for blank and unknown samples from standard curve and record phosphorus to the nearest 0.01 mg L^{-1} PO_4-P in extract solution (See Comment #4).

Calculations

Report soil Mehlich 1 extractable phosphorus to the nearest 0.1 kg ha^{-1} (lbs ac^{-1}):

soil PO_4-P kg P ha^{-1} (lbs ac^{-1}) = (PO_4-P mg L^{-1} in extract - method blank) × 10

Based on a 20 cm sampling depth (See Comment #5).

Comments

1. This method for extractable P follows the procedure originally outlined by Mehlich (1953) for soils in the southeastern United States.

2. Check repipette dispensing volume, calibrate using an analytical balance.

3. Extracts can be analyzed directly for phosphorus by automated FIA analysis.

4. Samples exceeding highest standard will require dilution with the extracting solution for reanalysis.

5. Phosphorus may also be determined directly on the extract using ICP-AES. Phosphorus concentrations, however, may differ from those determined spectrophotometrically due to organic P from hydrolysis during extraction.

6. Phosphorus recommendations are local soil-crop dependent, Table S - 4.31-A.

Table S - 4.31-A. Phosphorus recommendations for Mehlich 1 method (Issac, 1983).

Category	kg P ha^{-1}	(lbs ac^{-1}) Soil 0-20 cm
Very low	< 11	(10)
Low	11 - 33	(10 - 30)
Medium	34 - 67	(31 - 60)
High	68 - 112	(61 -100)
Very High	> 112	(> 100)

Literature

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Mehlich, A. 1953. Determination of P, Ca, Mg, K, Na, and NH4. North Carolina Soil Test Division (Mimeo 1953).

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ESTIMATION OF AVAILABLE SOIL PHOSPHORUS S - 4.33

Dilute Acid-Fluoride-EDTA Mehlich 3 Method

Scope and Application

Mehlich 3 estimates the relative bioavailability of ortho-phosphate (PO_4-P) on soils acid to neutral pH using a dilute acid-fluoride-EDTA solution of pH - 2.50 that is 0.2 N CH_3-COOH - 0.25 N NH_4NO_3 - 0.015 N NH_4F - 0.013 N HNO_3 - 0.001 M EDTA. This method is a modification of the Mehlich 2 extractant (1978) and it was developed by Mehlich in 1984. In the process of extraction, phosphorus is solubilize under two different mechanisms. The combinations of the two acids, nitric and acetic, increases the solubility of iron and aluminum phosphates by protonation, and fluoride lowers calcium activity as calcium fluoride increasing the quantity of PO_4-P in solution. Ammonium is used to exchange with potassium, calcium and magnesium and EDTA to chelate iron, manganese, zinc, and copper. Phosphorus content is determined spectrophotometrically at 882 nm at an acidity of 0.20 M H_2SO_4 (Rodriguez et al., 1994) by reacting with ammonium molybdate using ascorbic acid as a reductant in the presence of antimony (Murphy and Riley, 1962). Phosphorus and cations may also be determined by ICP-AES instrumentation. The method is unsuitable on alkaline calcareous soils. The method is shown to be well correlated to crop response to fertilizer phosphorus and applicable for the determination of extractable potassium, calcium, magnesium, sodium and micronutrients. The method has a phosphorus detection limit of about 1.0 kg P ha^{-1} (on a dry soil basis) and is generally reproducible within ± 8%.

Equipment

1. Analytical balance: 100.0 g resolution 0.01 g.
2. Reciprocating mechanical shaker, capable of 180 oscillations per minute (opm).
3. Repipette dispenser, calibrated to 20 ± 0.2 mL, 12 ± 0.1 mL.
4. 100-mL plastic extraction erlenmeyer and associated filtration vessel.
5. Whatman No. 1 filter paper or equivalent.
6. Pipettes: 0.250 ±0.005 mL, 0.500 ±0.005 mL, 1.00 ±0.01 mL, 2.00 ±0.02 mL, 3.00 ± 0.03 mL.
7. 2.5 cm matching spectrophotometer cuvette.
8. Spectrophotometer, wavelength 882 nm, automated Flow Injection Analysis (FIA) and/or ICP-AES.

Reagents

1. Deionized water, ASTM Type I grade.
2. Ammonium fluoride - EDTA stock solution (3.75 M NH_4F-0.25M EDTA): Dissolve 138.9 g of NH_4F in 600 mL of deionized water and add 73.06 g EDTA (or 93.06 g. of Na_2-EDTA.$2H_2O$), dissolve and dilute to 1000 mL.
3. Mehlich-3 extracting solution (0.2 N CH_3-COOH - 0.25 N NH_4NO_3 - 0.015 N NH_4F - 0.013 N HNO_3 - 0.001 M EDTA @ pH - 2.50 ±0.05). Dissolve 80.05 g NH_4NO_3 in about 3,000 mL of deionized water. Add 16.0 mL of 3.75 M NH_4F - 0.25 M EDTA stock solution and mix well. Add 46 mL of concentrated glacial CH_3-COOH and 3.3 mL of concentrated HNO_3 and bring to 4,000 mL final volume. The final pH should be 2.50 ±0.05.
4. Modified Reagent A (Watanabe and Olsen, 1965).
   - Ammonium Molybdate: Dissolve 12.0 g. of A.R. [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] in 250 mL of deionized water.
   - Antimony Potassium Tartrate: Dissolve 0.291 g. of A.R. antimony potassium tartrate [$K(SbO) \cdot C_4H_4O_6 \cdot ½ H_2O$] in 100 mL of deionized water.
   - Add both of the dissolved reagents to 1,000 mL of 5.76 N H_2SO_4 (160 mL of concentrated sulfuric acid per liter, Self and Rodriguez, 1996) mix thoroughly and make to 2,000 mL. Modified Reagent A (mixed reagent) last at least four months if it is stored in an opaque plastic bottle.
5. Reagent B, ascorbic/molybdate reagent (Watanabe and Olsen, 1965). Dissolve 1.32 g. of A.R. ascorbic acid ($C_6H_4O_6$) in 250 mL of modified Reagent A and mix well. This reagent should be prepared as required.
6. Phosphorus Calibrations Standards. From a standard solution containing 1,000 mg L^{-1} PO_4-P prepare

100 mL of standard in Mehlich 3 extracting solution containing 100 mg L^{-1} PO_4-P. Then, using the 100 mg L^{-1} PO_4-P standard, prepare six calibration solutions of 100 mL each in Mehlich 3 extracting solution with PO_4-P concentrations of 0.00, 0.75, 1.50, 3.00, 6.00, and 12.00 mg L^{-1}.

Extracting Procedure

1. Scoop weigh 2.0 ± 0.05 g of air dried soil pulverized to pass 10 mesh sieve(< 2.0 mm) in a 100-mL plastic erlenmeyer flask.
2. Add 20.0 mL of Mehlich 3 extracting solution (see comment # 2). Include a method blank.
3. Place extraction flask(s) on reciprocating mechanical shaker for five (5) minutes.
4. Filter suspension immediately, refilter if filtrate is cloudy.

Phosphorus Chemical Analysis

1. Pipette 1.0 mL aliquot of standard or soil extract into a 25 mL test tube (See Comment #3).
2. Add 12.0 mL of deionized water.
3. Add 2.0 mL of Reagent B (ascorbic/molybdate reagent).
4. Adjust and operate spectrophotometer in accordance with manufacture's instructions. Read absorbance at a wavelength of 882 nm after ten (10) minutes of adding the Reagent B. Adjust the 0.000 absorbance using the 0.00 standard. Determine absorbance of a method blank, standards and unknown samples. Calculate phosphorus concentration for blank and unknown samples from standard curve and record phosphorus to the nearest 0.01 mg L^{-1} PO_4-P in extract solution (See Comment #4).

Calculations

Report soil Mehlich 3 extractable phosphorus to the nearest 0.1 kg P ha^{-1}:

soil kg P ha^{-1} = (PO_4-P mg L^{-1} in extract - method blank) × 20

Based on a 20 cm sampling depth (See Comment #5).

Comments

1. This method for extractable P follows the procedure originally outlined by Mehlich (1984) for soils in the southeastern United States.

2. Check repipette dispensing volume, calibrate using an analytical balance.

3. Extracts can be analyzed directly for phosphorus by automated FIA analysis.

4. Samples exceeding highest standard will require dilution with the extracting solution for reanalysis.

5. Phosphorus may also be determined directly on the extract using ICP-AES. Phosphorus concentrations, however, may differ from those determined spectrophotometrically due to organic P from hydrolysis and particulate clays during extraction.

Literature

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ESTIMATION OF AVAILABLE SOIL PHOSPHORUS S - 4.40

AB-DTPA Soltanpour and Schwab Method

Scope and Application

This method estimates the relative bioavailability of PO_4-P, NO_3-N, K, Ca, Mg, Zn, Fe, Mn, and Cu using a 1.0 M NH_4HCO_3 - 0.005 M DTPA solution adjusted to pH - 7.60 for soils with neutral to alkaline pH. It is based on the method developed by Soltanpour and Schwab (1977) and is advantageous since it simultaneously extracts macro and micronutrients in a single extract with subsequent analysis using a spectrophotometer and ICP-AS instruments (Soltanpour, 1991). In the process of extraction, CO_2 from bicarbonate is given off, solution pH increases and bicarbonate converts to carbonate which in turn reduces calcium activity (as calcium carbonate) increasing the quantity of PO_4-P in solution. Phosphorus content is determined spectrophotometrically at 882 nm at an acidity of 0.18 M H_2SO_4 (Rodriguez et al., 1994) by reacting with ammonium molybdate using ascorbic acid as a reductant in the presence of antimony (Murphy and Riley, 1962). Nitrates are water soluble and can be analyzed from solution. Ammonium exchanges with potassium, calcium, and magnesium, and the original pH (7.60) of the AB-DTPA allows DTPA to extract and chelate iron, manganese, zinc, and copper and toxic metals. The method has shown to be well correlated to crop response to phosphorus fertilization. The method is well suited for screening mine spoils, sewage sludge amended soils and soils contaminated with potentially toxic elements (ie. As, Se, B, Cd, Ni, Pb, and Mo). The method has a phosphorus detection limit of approximately 0.50 mg kg^{-1} (on a dry soil basis) and is generally reproducible to within ± 7%.

Equipment

1. Analytical balance: resolution 0.01 g.
2. Reciprocating mechanical shaker, capable of 180 oscillations per minute (opm).
3. Repipette dispenser, calibrated to 20 ± 0.2 mL, 12 ± 0.1 mL.
4. 125-mL plastic extraction erlenmeyer and associated filtration vessel.
5. Whatman No. 1 filter paper or equivalent.
6. Pipettes: 0.250 ±0.005 mL, 0.500 ±0.005 mL, 1.00 ±0.01 mL, 2.00 ±0.02 mL, 3.00 ± 0.03mL.
7. 2.5 cm matching spectrophotometer cuvette.
8. Spectrophotometer, wavelength 520 and 882 nm, automated FIA and/or ICP-AES.

Reagents

1. Deionized water, ASTM Type I grade.
2. 0.005 M DTPA solution. Dissolve 19.70 of diethylenetriamine pentaacetic acid (DTPA) in about 9.5 L of deionized water. Since DTPA is not very soluble in water it needs about eight hrs of constant agitation for total dissolution, dilute to 10 L with deionized water. The pH of the DTPA solution is 2.40 ±0.10 and it is very stable. Store the DTPA solution in a polyethylene container.
3. AB-DTPA extracting solution (1M NH_4HCO_3 - 0.005 M DTPA @ pH - 7.60): Using a vortex stirrer, dissolve 79.06 g. of NH_4HCO_3 with about 900 mL of 0.005 M DTPA solution, after dissolution of the ammonium bicarbonate dilute to 1.0 L with 0.005 M DTPA, the pH of this solution is about 7.5, adjust the pH to 7.60 ±0.05 by agitation. This AB-DTPA solution is unstable with regard to pH, prepare only the quantity that is needed on a daily basis (Self and Rodriguez, 1996). (see comment # 1).
4. 5 M NaOH. In a plastic container with about 750 mL of deionized water, carefully dissolve 200 g. of A.R. sodium hydroxide.

5. Modified Reagent A (Watanabe and Olsen, 1965).
   - Ammonium Molybdate: Dissolve 12.0 g. of A.R. [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] in 250 mL of deionized water.
   - Antimony Potassium Tartrate: Dissolve 0.291 g. of A.R. antimony potassium tartrate [$K(SbO) \cdot C_4H_4O_6 \cdot ½ H_2O$] in 100 mL of deionized water.
   - Add both of the dissolved reagents to 1,000 mL of 5.76 N H_2SO_4 (160 mL of concentrated sulfuric acid per liter, Self and Rodriguez, 1996) mix thoroughly and make to 2,000 mL. Modified Reagent A (mixed reagent) last at least four months if it is stored in an opaque plastic bottle.
6. Reagent B, ascorbic/molybdate reagent (Watanabe and Olsen, 1965). Dissolve 1.32 g. of A.R. ascorbic acid ($C_6H_4O_6$) in 250 mL of modified Reagent A and mix well. This reagent should be prepared as required.
7. Phosphorus Calibrations Standards. From a standard solution containing 1,000 mg L^{-1} PO_4-P, prepare 100 mL of standard in AB-DTPA extracting solution containing 100 mg L^{-1} PO_4-P. Then, using the 100 mg L^{-1} PO_4-P standard, prepare seven calibration solutions of 100 mL each in AB-DTPA extracting solution with PO_4-P concentrations of 0.00, 2.5, 5.0, 10.0, 20.0, and 40.0 mg L^{-1}.

Extracting Procedure

1. Weigh 10.0 ±0.1 g of air dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) in a 125-mL plastic extraction erlenmeyer.
2. Add 20.0 mL of AB-DTPA extracting solution. Include a method blank (See Comment # 2 and # 3).
3. Place extraction vessel on mechanical shaker for fifteen (15) minutes.
4. Filter suspension immediately, refilter if filtrate is cloudy.

Phosphorus Chemical Analysis

1. Pipette 0.250 mL aliquot of standard or soil extract into a 2.5 cm matching spectrometer tube (See Comment #4).
2. Add 0.250 mL of 5 M NaOH (Self and Rodriguez, 1996).
3. Let the reaction occurs for 10 min.
4. Add 10.0 mL of deionized water (See Comment #5).
5. Add 2.0 mL of Reagent B (ascorbic/molybdate reagent).
6. Adjust and operate spectrophotometer in accordance with manufacture's instructions. Read absorbance at a wavelength of 882 nm after ten (10) minutes of adding the Reagent B. Adjust the 0.000 absorbance using the 0.00 standard. Determine absorbance of a method blank, standards and unknown samples. Calculate phosphorus concentration for blank and unknown samples from standard curve and record phosphorus to the nearest 0.01 mg L^{-1} PO_4-P in extract solution (See Comment #6).

Calculations

Report soil AB-DTPA extractable phosphorus to the nearest 0.1 mg kg^{-1} :

soil PO_4-P mg kg^{-1} = (PO_4-P mg L^{-1} in extract - method blank) × 2

(See Comment # 7).

Comments

1. pH of the extraction solution is unstable and may become effervescence in automated dispensers.

2. Clean all extraction and filtration labware with 0.5 N HCl and three deionized water rinses to removed potential ortho-phosphate contamination.

3. Cover filtration completely vessels with a plastic sheet to diminish contamination of NH_4-N to surrounding soils and water samples.

4. Extracts can be analyzed directly for phosphorus by automated FIA analysis.

5. Check pipette dispensing volume, calibrate using an analytical balance.

6. Determine nitrate on aliquot of soil extract, method S - 3.10. Determine K, Ca, Mg, Fe, Mn, Zn and Cu by AAS or IAP-AS. The use of Legere teflon nebulizer (Burtec Instrument Corporation, Delmar, New York) facilities the analysis of solutions high in dissolved solids by IAP-AS analysis.

7. Samples exceeding highest standard will require dilution with AB-DTPA for reanalysis.

Literature

Murphy,J., and J.P. Riley. 1962. A modified single solution method for determination of phosphates in natural waters. Anal. Chim. Acta 27:31-36.

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EXTRACTABLE SOIL PHOSPHORUS MODIFIED MORGAN S - 4.50

Dilute Acid Method

Scope and Application

This method estimates the relative bioavailability of ortho-phosphate (PO_4-P) in acid to neutral soils with cation exchange capacities of less than 20 meq/100 g using a buffer solution of pH 4.80. This method, first proposed by Morgan (1941), was described in detail by Lunt et al. (1950) later by Greweling and Peech (1965). The Morgan extracting reagent is a well buffered solution of 0.52 M CH_3-COOH-0.73 M CH_3-COONa @ pH - 4.80. Phosphorus is determined spectrophotometrically at 882 nm at an acidity of 0.21 M H_2SO_4 (Rodriguez et al., 1994) by reacting with ammonium molybdate using ascorbic acid as a reductant in the presence of antimony (Murphy and Riley, 1962). The method is used on soils of Northeast and Pacific Northwest and can be used for K and other cations. The method has a phosphorus detection limit of approximately 1.0 mg kg^{-1} PO_4-P (on a dry soil basis) and is generally reproducible within ±8%.

Equipment

1. Analytical balance: resolution 0.01 g.
2. Reciprocating mechanical shaker, capable of 180 oscillations per minute (opm).
3. Repipette dispenser, calibrated to 20 ± 0.2 mL, 12 ± 0.1 mL.
4. 125-mL plastic extraction erlenmeyer and associated filtration vessel.
5. Whatman No. 1 filter paper or equivalent.
6. Pipettes: 0.250 ±0.005 mL, 0.500 ±0.005 mL, 1.00 ±0.01 mL, 2.00 ±0.02 mL, 3.00 ± 0.03mL.
7. 2.5 cm matching spectrophotometer cuvette.
8. Spectrophotometer, wavelength 882 nm or automated Flow Injection Analysis (FIA) system.

Reagents

1. Deionized water, ASTM Type I grade.
2. Morgan Extracting Reagent (0.52 M CH_3-COOH - 0.73 M CH_3-COONa @ pH - 4.80). Dissolve 100 g sodium acetate (CH_3-COONa· $3H_2O$) in about 900 mL pure water. Add 30 mL glacial acetic acid (CH_3-COOH), adjust the pH to 4.80 ±0.05 with diluted acetic acid or NaOH, and dilute to 1,000 mL with deionized water.
3. Modified Reagent A (Watanabe and Olsen, 1965).
   - Ammonium Molybdate: Dissolve 12.0 g. of A.R. [$(NH_4)_6Mo_7O_{24}$ · $4H_2O$] in 250 mL of deionized water.
   - Antimony Potassium Tartrate: Dissolve 0.291 g. of A.R. antimony potassium tartrate [K(SbO) · $C_4H_4O_6$ · ½ H_2O] in 100 mL of deionized water.
   - Add both of the dissolved reagents to 1,000 mL of 5.76 N H_2SO_4 (160 mL of concentrated sulfuric acid per liter, Self and Rodriguez, 1996) mix thoroughly and make to 2,000 mL. Modified Reagent A (mixed reagent) last at least four months if it is stored in an opaque plastic bottle.
4. Reagent B, ascorbic/molybdate reagent (Watanabe and Olsen, 1965). Dissolve 1.32 g. of A.R. ascorbic acid ($C_6H_4O_6$) in 250 mL of modified Reagent A and mix well. This reagent should be prepared as required.
5. Phosphorus Calibrations Standards. From a standard solution containing 1,000 mg L^{-1} PO_4-P, prepare 100 mL of standard in Morgan extracting solution containing 100 mg L^{-1} PO_4-P. Then, using the 100 mg L^{-1} PO_4-P standard, prepare six calibration solutions of 100 mL each in 0.5 N Morgan solution with PO_4-P concentrations of 0.00, 0.75, 1.50, 3.00, 6.00, and 12.00 mg L^{-1}.

Extracting Procedure

1. Weigh 5.00 ± 0.05 g of air dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) in a 125 mL extraction vessel.
2. Add 25.0 mL of Morgan extraction solution (See comment #1 and #2). Include a method blank.
3. Place extraction vessel(s) on mechanical shaker for thirty (30) minutes.

4. Filter suspension immediately, refilter if filtrate is cloudy.

Phosphorus Chemical Analysis

1. Pipette 1.0 mL aliquot of standard or soil extract into a 2.5 cm matching spectrometer tube (see comment # 3).
2. Add 12.0 mL of deionized water (see comment # 4).
3. Add 2.0 mL of Reagent B (ascorbic/molybdate reagent).
4. Adjust and operate sprectrophotometer in accordance with manufacture's instructions. Read absorbance at a wavelength of 882 nm after ten (10) minutes of adding the Reagent B. Adjust the 0.000 absorbance using the 0.00 standard. Determine absorbance of a method blank, standards and unknown samples. Calculate phosphorus concentration for blank and unknown samples from standard curve and record phosphorus to the nearest 0.01 mg L^{-1} PO_4-P in extract solution (See Comment #5).

Calculations

Report modified Morgan extractable soil phosphorus to the nearest 0.1 mg kg^{-1} :

soil PO_4-P mg kg^{-1} = (PO_4-P mg L^{-1} in extract - method blank) × 5
(See Comment # 6).

Comments

1. Soils may be stored in an air-dry condition for several months with no effect on extractable P.
2. The soil extracts should not be stored for more than 24 hours after extraction.
3. Extracts can be analyzed directly for phosphorus by automated FIA analysis.
4. Check repipette dispensing volume, calibrate using an analytical balance.
5. Samples exceeding highest standard will require dilution with Morgan extractant for reanalysis.
6. Accurate fertilizer recommendations for phosphorus must be based on field response data conducted under local soil-climate-crop conditions. Interpretations will vary, depending on soil characteristics crops and yield potential.

Literature

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EXTRACTABLE SOIL PHOSPHORUS MODIFIED KEWLONA S - 4.60

Acetic Acid - NH_4F Method

Scope and Application

This method estimates the relative bioavailability of ortho-phosphate (PO_4-P) in mildly acid to alkaline soils. This method was developed in Alberta Canada by Ashworth and Mrazek (1989) and is a modification of the of the procedure described by van Lierop, 1988. The original bases of the method is on the work of Bray and Kurtz (1945). The Modified Kewlona extracting reagent is a well buffered solution of (0.015 N NH_4F - 0.50 M CH_3-COOH - 1.0 M CH_3-COO NH_4 . Phosphorus is determined spectrophotometrically at 882 nm at an acidity of 0.19 M H_2SO_4 (Rodriguez et al., 1994) by reacting with ammonium molybdate using ascorbic acid as a reductant in the presence of antimony (Murphy and Riley, 1962). The method is used on soils of Western Canada and the Northern Rocky Mountain States and can be used for the simultaneous determination of K . The method has a phosphorus detection limit of approximately 1.0 mg kg^{-1} (on a dry soil basis) and is generally reproducible within ±10%.

Equipment

1. Analytical balance 100.0 resolution 0.01 g.
2. Reciprocating mechanical shaker, capable of 180 oscillations per minute (opm).
3. Repipette dispenser, calibrated to, 12.0 ± 0.1 mL, 50.0 ± 0.2 mL.
4. 125-mL plastic extraction erlenmeyer and associated filtration vessel.
5. Whatman No. 1 filter paper or equivalent.
6. Pipettes:, 1.00 ±0.01 mL, 2.00 ±0.02 mL.
7. 2.5 cm matching spectrophotometer cuvette.
8. Spectrophotometer, wavelength 882 nm or automated Flow Injection Analysis (FIA) system.

Reagents

1. Deionized water, ASTM Type I grade.
2. Modified Kewlona extracting reagent (0.015 N NH_4F - 0.50 M CH_3-COOH - 1.0 M (CH_3-COO NH_4) . Ammonium fluoride stock solution (1.00 N NH_4F): Dissolve 5.54 g of NH_4F in 1500 mL of deionized water. Add 192g ammonium Acetate (CH_3-COO NH_4) and 143.7 mL Glacial Acetic Acid (CH_3-COOH) and dilute to 10.0 L final volume.
3. Modified Reagent A (Watanabe and Olsen, 1965).
   - Ammonium Molybdate: Dissolve 12.0 g. of A.R. [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] in 250 mL of deionized water.
   - Antimony Potassium Tartrate: Dissolve 0.291 g. of A.R. antimony potassium tartrate [$K(SbO) \cdot C_4H_4O_6 \cdot ½ H_2O$] in 100 mL of deionized water.
   - Add both of the dissolved reagents to 1,000 mL of 5.76 N H_2SO_4 (160 mL of concentrated sulfuric acid per liter, Self and Rodriguez, 1996) mix thoroughly and make to 2,000 mL. Modified Reagent A (mixed reagent) last at least four months if it is stored in an opaque plastic bottle.
4. Reagent B, ascorbic/molybdate reagent (Watanabe and Olsen, 1965). Dissolve 1.32 g. of A.R. ascorbic acid ($C_6H_4O_6$) in 250 mL of modified Reagent A and mix well. This reagent should be prepared as required.
5. Phosphorus Calibrations Standards. From a standard solution containing 1,000 mg L^{-1} PO_4-P, prepare 100 mL of standard in Morgan extracting solution containing 100 mg L^{-1} PO_4-P. Then, using the 100 mg L^{-1} PO_4-P standard, prepare six calibration solutions of 100 mL each in Modified Kewlona solution with PO_4-P concentrations of 0.00, 0.75, 1.50, 3.00, 6.00, and 12.00 mg L^{-1}.

Extracting Procedure

1. Weigh 5.00 ± 0.05 g of air dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) in a 125 mL extraction vessel.
2. Add 50.0 mL of Morgan extraction solution (See comment #1 and #2). Include a method blank.

3. Place extraction vessel(s) on a reciprocating mechanical shaker for thirty (30) minutes.
4. Filter suspension immediately, refilter if filtrate is cloudy.

Phosphorus Chemical Analysis

1. Pipette 1.0 mL aliquot of standard or soil extract into a 2.5 cm matching spectrometer tube (see comment # 3).
2. Add 12.0 mL of deionized water (see comment # 4).
3. Add 2.0 mL of Reagent B (ascorbic/molybdate reagent).
4. Adjust and operate sprectrophotometer in accordance with manufacture's instructions. Read absorbance at a wavelength of 882 nm after ten (10) minutes of adding the Reagent B. Adjust the 0.000 absorbance using the 0.00 standard. Determine absorbance of a method blank, standards and unknown samples. Calculate phosphorus concentration for blank and unknown samples from standard curve and record phosphorus to the nearest 0.01 mg L^{-1} PO_4-P in extract solution (See Comment #5).

Calculations

Report Modified Kewlona extractable soil phosphorus to the nearest 0.1 mg kg^{-1} :

soil PO_4-P mg kg^{-1} = (PO_4-P mg L^{-1} in extract - method blank) × 10 (See Comment # 6).

Comments

1. Soils may be stored in an air-dry condition for several months with no effect on extractable P.

2. The soil extracts should not be stored for more than 24 hours after extraction.

3. Extracts can be analyzed directly for phosphorus by automated FIA analysis.

4. Check repipette dispensing volume, calibrate using an analytical balance.

5. Samples exceeding highest standard will require dilution with Morgan extractant for reanalysis.

6. Accurate fertilizer recommendations for phosphorus must be based on field response data conducted under local soil-climate-crop conditions. Interpretations will vary, depending on soil characteristics crops and yield potential. Potassium extracted by the Modified kewlona extract is equivalent to that extracted by the ammonium acetate method (S - 5.10).

Literature

Ashworth J. And K. Mrazek. 1989. The acetic-fluoride method for available phosphorus and potassium. Proceedings Alberta Soil Science Workshop 26:178-182.

Bray, R.H. and L.T. Kurtz 1945. Determination of total, organic and available phosphorus in soil. Soil Sci. 59:39-45.

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Rodriguez, J.B., J.R. Self, and P.N. Soltanpour. 1994. Optimal conditions for phosphorus analysis by the ascorbic acid-molybdenum blue method. Soil Sci.Soc. Am. J. 58:866-870.

Self, J.R. and J.B. Rodriguez. 1996. Laboratory manual for soil and plant chemical analysis. Soil, Water, and Plant Testing Laboratory, Colorado State University.

Van Lierop, W. 1988. Determination of available phosphorus in acid and calcareous soils with the Kewlona multi-element extractants. Soil Sci. 146:284-291.

Watanabe, F.S., and S.R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soil. Soil Sci. Soc. Amer. Proc. 29:677-678.

Scope and Application

This method determines phosphorous sorption capacity of a soil based on a method adapted from Sharpley et al, 1981, and is valid across soil types and soil pH. Phosphorous in soil solution can either be adsorbed, sorbed, precipitated or left in solution. This method does not distinguish between the mechanisms that result in the disappearance of P from the soil solution, but is used to differentiate between a soil's capacity to sorb P. Soil is dosed with increasing concentrations of phosphorus and allowed to equilibrate for twenty-four hours. Soil solution phosphorus determined spectrophotometrically or by inductively coupled plasma (ICP-AES). Both spectrophotometric and ICP methods are suitable and can be interchangeable especially at high soil solution P concentrations Graphically, equilibrium soil solution P concentrations and/or P sorbed can be depicted as a function of P additions to the soil. This information can be useful agronomically to help with P in solution for plant uptake as well as environmentally to determine soil P loading rates.

Equipment

1. Analytical balance: resolution 0.01 g.
2. Reciprocating mechanical shaker, capable of 180 oscillations per minute (opm).
3. Repipette dispenser, calibrated to 40.0 ±0.2 mL, 12.0 ±0.1 mL.
4. 50 mL polypropylene centrifuge tubes.
5. Whatman No. 2 filter paper or equivalent.
6. Pipettes: 0.250 ±0.005 mL, 0.500 ±0.005 mL, 1.00 ±0.01 mL, and 2.00 ±0.02 mL.
7. 2.5 cm matching spectrophotometer cuvette.
8. Spectrophotometer, wavelength 882 nm or automated Flow Injection Analysis (FIA) system or Inductively coupled plasma (ICP-AES).

Reagents

1. Deionized water, ASTM Type I grade.
2. Standard phosphorus (PO_4-P) solutions, - 0.0, 1,0, 5.0, 10, 25 ,50, 100, 500, 1000 and 2000 mg L^{-1} PO_4-P . Prepare 2.0 Liters of 1000 and 2000 mg L^{-1} solutions by weighing 8.7768 g and 17.5536 g of KH_2PO_4, respectively. From this 2000 mg L^{-1} solution make 1.0 liter of 1.0, 5.0, 10, 25 100 and 500 mg L^{-1} P solutions. Store in HDPE bottles. Shelf life of these solutions is limited, 30 days, refrigerate until needed.
3. Hydrochloric acid 0.5 N HCL: Dilute 103 mL of concentrated HCL to a volume of 2,500 mL with deionized water.
4. Modified Reagent A (Watanabe and Olsen, 1965).
   - Ammonium Molybdate: Dissolve 12.0 g. of A.R. $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$ in 250 mL of deionized water.
   - Antimony Potassium Tartrate: Dissolve 0.291 g. of A.R. antimony potassium tartrate $[K(SbO) \cdot C_4H_4O_6 \cdot ½\ H_2O]$ in 100 mL of deionized water.
   - Add both of the dissolved reagents to 1,000 mL of 5.76 N H_2SO_4 (160 mL of concentrated sulfuric acid per liter, Self and Rodriguez, 1996) mix thoroughly and make to 2,000 mL. Modified Reagent A (mixed reagent) last at least four months if it is stored in an opaque plastic bottle.
5. Reagent B, ascorbic/molybdate reagent (Watanabe and Olsen, 1965). Dissolve 1.32 g. of A.R. ascorbic acid ($C_6H_4O_6$) in 250 mL of modified Reagent A and mix well. This reagent should be prepared as required.

6. Phosphorus Calibrations Standards. From a standard solution containing 1,000 mg L^{-1} PO_4-P prepare 100 mL of standard in deionized water 100 mg L^{-1} PO_4-P. Then, using the 100 mg L^{-1} PO_4-P standard, prepare six calibration solutions with PO_4-P concentrations of 0.00, 0.75, 1.50, 3.0, 6.0, and 12.0 mg P L^{-1}. ICP calibration standards: 1.0, 10.0, 100.0, 500 mg P L^{-1}

Extracting Procedure

1. Weigh 1.00 ±0.01 g of air dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into thirty 50-mL plastic centrifuge tubes.
2. Add 40.0 mL of P solutions (0-2000 mg L^{-1}) in triplicate. There will be three centrifuge tubes each with 0, 1, 5,10, 25, 50, 100, 500, 1000 and 2000 mg L^{-1} P solutions. A repipetter set to 40 mL is used and moved from solution to solution. Start with low concentrations and proceeds to higher concentrations
3. Place centrifuge tubes on their side on reciprocating mechanical shaker, 24 hours continuously.
4. Filter suspension with Whatman 2 or equivalent, refilter if filtrate is cloudy. A centrifuge can also be used to clear the solutions.

Phosphorus Chemical Analysis

Spectrophotometric Analysis

1. Pipette 1.0 mL aliquot of standard or soil extract into a 2.5 cm matching spectrometer tube (See Comment #2).
2. Add 12.0 mL of deionized water.
3. Add 2.0 mL of Reagent B (ascorbic/molybdate reagent developing reagent).
4. Adjust and operate spectrophotometer in accordance with manufacture's instructions. Read absorbance at a wavelength of 882 nm after ten (10) minutes of adding the Reagent B. Adjust the 0.000 absorbance using the 0.00 standard. Determine absorbance of a method blank, standards and unknown samples. Calculate phosphorus concentration for blank and unknown samples from standard curve and record phosphorus to the nearest 0.01 mg L-1 PO_4-P in soil solution (See Comment #2). Dilutions are unpredictable and difficult. Add 10.0 mL of deionized water.

ICP-AES Analysis

1. Alternately, analyze solution on a calibrated ICP for total P in solution. It is recommended that solutions be analyzed by colorimetric analysis until dilutions need to be made (12 mg L^{-1}), at least 0 - 10 mg L^{-1} P solution additions. Because dilutions can get very difficult to do and predict, the ICP can be used for all solution values above 10 mg L^{-1}. Organic P additions are negligible at these soil solution P concentrations. Pipette 1.0 mL aliquot of standard or soil extract into a 2.5 cm matching spectrometer tube (See Comment #2).

Calculations

Develop a spreadsheet for the following calculations and graphs (Table S 4.70-A).

Report soil solution concentrations in mg L^{-1} P to the nearest 0.01 mg L^{-1} :

mg P added per g of soil = (p solution concentration, 0 - 2000 mg P L^{-1}) x (0.04)
mg P in solution = (measured P in solution) x (0.04)
mg P sorbed per g soil = (mg P added per g soil) – (mg P in solution)

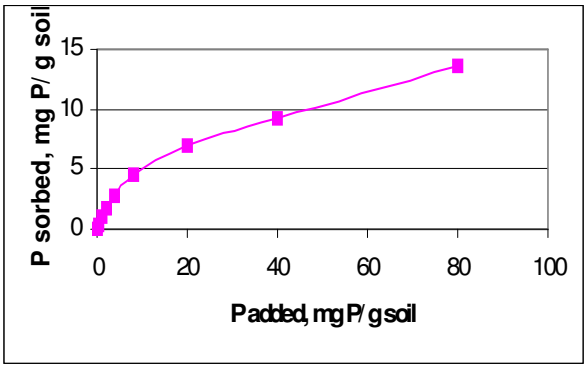
Table S 4.70-A. Example table of P sorption calculation.

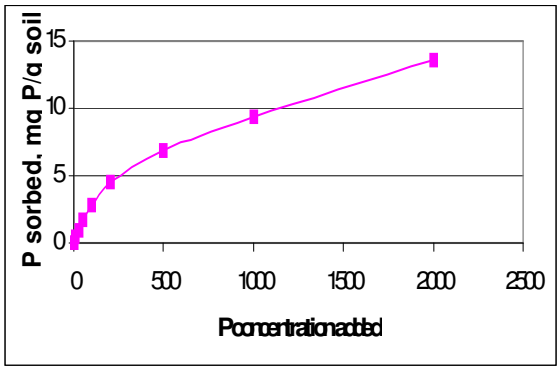
Initial P solution, mg L^{-1} (ppm)	P added, mg P g^{-1} soil	Final P, mg L^{-1}	P in solution, mg	P sorbed, mg P g^{-1} soil
0	0.00	0.0267	0.001	0.00
1	0.04	0.04	0.002	0.038
5	0.41	0.08	0.003	0.39
10	1.0	0.627	0.025	0.97
25	2.0	6.94	0.277	1.72
50	4.0	28.23	1.13	2.87
100	8.0	88.03	3.52	4.48
500	20	327	13.1	6.91
1000	40	767	30.7	9.31
2000	80	1660	66.4	13.6

Graph the Data

The following is an example of how the data can be graphed. Calculate averages for each of the replicated concentrations. Units can be changed to lbs P ac^{-1}. Graphs will commonly have dual Y axis one with units such as P sorbed in mg P g^{-1} soil and the corresponding Y axis will have P sorbed in lbs per ac^{-1} furrow slice (assumption acre furrow slice 2,000,000 lbs). Soils with Bray P1 greater than 150 mg kg^{-1} and bicarbonate P greater than 75 mg kg^{-1} may be saturated with respect to P, at solution levels less than 10 mg L^{-1}. Saturated soils will have more P in solution than was added. For example a saturated soil may have 1 - 5 mg L^{-1} of the 0 mg L^{-1} P solution and 10 - 15 mg L^{-1} for the 10 mg P L^{-1} solution. Specific soils will not sorb P past the 100 mg P L^{-1} solution, while others will not saturate until the 2000 mg P L^{-1} addition Some soils will exhibit multi-mechanistic curves, others are more simplistic (See Figure.S-4.70-1).

Figure S-4.70-1. Examples of P sorption, by P add g^{-1} of soil and P concentration added, mg L^{-1}.





Interpretation

The principle interpretation is to rank the relative differences of soils to sorb P and to calculate P loading to achieve a particular soil solution level. A soil solution level of 0.20 - 0.30 mg L^{-1} PO_4-P is considered adequate for plant growth. Environmentally acceptable soil solution phosphorus levels are yet to be specified.

Comments

1. Extracts can be analyzed directly for phosphorus by automated FIA analysis.

2. Samples exceeding highest calibration standard will require dilution with the extracting solution for re-analysis.

Literature

Murphy,J., and J.P. Riley. 1962. A modified single solution method for determination of phosphates in natural waters. Anal. Chim. Acta 27:31-36.

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EXTRACTABLE POTASSIUM, CALCIUM, MAGNESIUM, AND SODIUM S - 5.10

Ammonium Acetate Method

Scope and Application

This method semiquantitatively determines the amount of soil plant available K, Ca, Mg, and Na residing on the soil colloid exchange sites by displacement with ammonium acetate solution buffered to pH 7.0. Cation concentrations are determined using atomic emission (AES), absorption spectrometry (AAS) or ICP-AES instrumentation. A chemical interference solution is used to minimize chemical matrix effects. It is based on a modification of the procedure outlined by Knudsen et al. (1982) for exchangeable K. Generally, these cations are associated with the exchange sites. The exception are soils that have high soluble salts and are saline, which requires a special preanalysis treatment. The method doesn't correct for calcium and magnesium extracted as free carbonates or gypsum. In the northern Great Plains the method has been used to determine available sulfur. The method detection limit is approximately of 25 mg kg^{-1} (on a dry soil basis) and is generally reproducible ± 7%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.01 g.
2. Reciprocating horizontal mechanical shaker, 180 oscillations per minute (opm).
3. Repipette dispenser, calibrated to 25.0 ± 0.2 mL.
3. Extraction vessel and associated filtration vessel.
4. Whatman No. 42, No. 2 or equivalent highly retentive filter paper.
5. Atomic Emission/Absorption Spectrophotometer (AAE) (AAS) or ICP-AES.

Reagents

1. Deionized water, ASTM Type I grade.
2. Ammonium acetate (1.0 N) extraction solution neutral @ pH 7.0: Add 570 mL of glacial acetic acid CH_3COOH (99%) to 8000 mL of deionized water. Add 680 mL of concentrated ammonium hydroxide adjust pH to 7.0 with 3.0 N glacial acetic acid or 3.0 N ammonium hydroxide and dilute to 10 L final volume. Check solution for possible contamination of K, Na, Ca, and Mg (see comment #1).
3. Chemical interference solution: Dissolve 5000 mg L^{-1} lanthanum oxide (La_2O_3) and 2000 mg L^{-1} cesium chloride solution: Dissolve 4.691 g La_2O_3 and 5.071 g CsCl in 1.5 L of deionized water and add 25 mL of $HCLO_4$ and 25 mL of HNO_3 and dilute to 2000 mL. Check solution for contamination of K, Na and/or other cations.
4. Standard calibration solutions of K, Ca, Mg and Na. Prepare six calibration standard solutions of 1.0 - 20 mg L^{-1} of K, 1.0 - 70 mg L^{-1} of Ca, and 0.50 - 20 mg L^{-1} for Mg and Na prepared from 1000 mg L^{-1} stock solutions. Dilute calibration solutions with a solution containing the chemical interference solution and 0.04 N ammonium acetate solution.

Procedure

1. Weigh 2.50 ± 0.05 g of air dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into a 120 mL extraction vessel (See Comment #2). Add 25.0 mL of NH_4OAc extraction solution and place on orbital mechanical shaker for thirty (30) minutes (See Comment #3). Include a method blank.
2. Filter, refilter if filtrate is cloudy (See Comment #4 and #5).
3. Dilute 1:25 an aliquot of the soil extract with the chemical interference solution.
4. Adjust and operate AAE, AAS or ICP-AES instrument with six standards in accordance with manufacturer's instructions. Calibrate using prepared calibration solutions. Determine K, Mg, Ca and Na concentration of extract, of method blank, and record results as mg L^{-1} of cation in solution.

Calculations

Report as mg kg^{-1} of K, Ca, Mg and Na to the nearest 1 mg kg^{-1} (See Comment #7 and #8):

mg kg^{-1} cations in soil = (mg L^{-1} cation in solution - method blank) × 25 × 10

Comments

1. Extraction solution should be prepared monthly, subject to microbial growth.

2. Soils high in soluble salts (EC_e > 1 dS m^{-1}) should be washed with deionized water before adding extraction with ammonium acetate to reduce potential errors of soluble salts. This can be accomplished by adding 50 mL of deionized water to 2.5 g of soil, placed in a 50 mL centrifuge tube and centrifuge at 5000 rpm for thirty (30) minutes and decanting excess water followed by the standard analysis protocol. For soils having pH > 7.4 and calcium carbonate> 0.5%, should be extracted with ammonium acetate pH 8.5 to avoid the dissolution of calcium carbonate.

3. Check repipette dispensing volume, calibrate using an analytical balance.

4. Extracts may be stored for one week under refrigeration.

5. Filter paper should be checked periodically (monthly) for possible contamination of alkali metals. If contamination is > 5 mg kg^{-1} on a soil basis select an alternative supply of filter paper.

6. Results may be expressed as meq 100 g^{-1} or cmol kg^{-1}. Divide concentration of K, Ca, Mg and Na by 391 mg, 200 mg, 121.5 mg, and 230 mg respectively.

7. Generally, soils having less than 100 mg kg^{-1} potassium will respond to applications of potassium fertilizers for most crops.

Literature

Doll, E.C. and R. E. Lucas. 1973. Testing soil for potassium, calcium and magnesium. p 133-152. *In:* L.M. Walsh and J.D. Beaton. (ed.) Soil testing and plant analysis. SSSA Madison, WI.

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Munter, R. 1988. Laboratory factors affecting the extractability of nutrients. p. 8-10. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region, North Dakota Agricultural Experiment Station Bulletin No. 499 (revised).

EXTRACTABLE POTASSIUM, CALCIUM, MAGNESIUM, AND SODIUM S - 5.11

Ammonium Acetate Method - Buffer 8.5

Scope and Application

This method semiquantitatively determines the amount of soil plant available K, Ca, Mg, and Na residing on the soil colloid exchange sites by displacement with ammonium acetate solution buffered to pH 8.5. Cation concentrations are determined using atomic emission (AES), absorption spectrometry (AAS) or ICP-AES instrumentation. A chemical interference solution is used to minimize chemical matrix effects for the AES method. It is based on a modification of the procedure outlined by Knudsen et al. (1982) for exchangeable K but is buffered to pH 8.5 to minimize dissolution of calcium carbonate ($CaCO_3$). Generally, these cations are associated with the exchange sites. The exception are soils that have high soluble salts and are saline, which requires a special preanalysis treatment. The method doesn't correct for calcium and magnesium extracted as free carbonates or gypsum. In the northern Great Plains the method has been used to determine available sulfur. The method detection limit is approximately of 25 mg kg^{-1} (on a dry soil basis) and is generally reproducible ± 7%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.01 g.
2. Reciprocating horizontal mechanical shaker, 180 oscillations per minute (opm).
3. Repipette dispenser, calibrated to 25.0 ± 0.2 mL.
3. Extraction vessel and associated filtration vessel.
4. Whatman No. 42, No. 2 or equivalent highly retentive filter paper.
5. Atomic Emission/Absorption Spectrophotometer (AAE) (AAS) or ICP-AES.

Reagents

1. Deionized water, ASTM Type I grade.
2. Ammonium acetate (1.0 N) extraction solution neutral @ pH 8.50: Add 570 mL of glacial acetic acid CH_3COOH (99%) to 8000 mL of deionized water. Add 680 mL of concentrated ammonium hydroxide adjust pH to 8.5 with 3.0 N glacial acetic acid or 3.0 N ammonium hydroxide and dilute to 10 L final volume. Check solution for possible contamination of K, Na, Ca, and Mg (see comment #1).
3. Chemical interference solution: Dissolve 5000 mg L^{-1} lanthanum oxide (La_2O_3) and 2000 mg L^{-1} cesium chloride solution: Dissolve 4.691 g La_2O_3 and 5.071 g CsCl in 1.5 L of deionized water and add 25 mL of $HCLO_4$ and 25 mL of HNO_3 and dilute to 2000 mL. Check solution for contamination of K, Na and/or other cations.
4. Standard calibration solutions of K, Ca, Mg and Na. Prepare six calibration standard solutions of 1.0 - 20 mg L^{-1} of K, 1.0 - 70 mg L^{-1} of Ca, and 0.50 - 20 mg L^{-1} for Mg and Na prepared from 1000 mg L^{-1} stock solutions. Dilute calibration solutions with a solution containing the chemical interference solution and 0.04 N ammonium acetate solution.

Procedure

1. Weigh 2.50 ± 0.05 g of air dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into a 120 mL extraction vessel (See Comment #2). Add 25.0 mL of NH_4OAc extraction solution and place on orbital mechanical shaker for thirty (30) minutes (See Comment #3). Include a method blank.
2. Filter, refilter if filtrate is cloudy (See Comment #4 and #5).
3. Dilute 1:25 an aliquot of the soil extract with the chemical interference solution.
4. Adjust and operate AAE, AAS or ICP-AES instrument with six standards in accordance with manufacturer's instructions. Calibrate using prepared calibration solutions. Determine K, Mg, Ca and Na concentration of extract, of method blank, and record results as mg L^{-1} of cation in solution.

Calculations

Report as mg kg^{-1} of K, Ca, Mg and Na to the nearest 1 mg kg^{-1} (See Comment #7 and #8):

mg kg^{-1} cations in soil = (mg L^{-1} cation in solution - method blank) × 25 × 10

Comments

1. Extraction solution pH should be monitored daily.

2. Soils high in soluble salts (EC_e > 1 dS m^{-1}) should be washed with deionized water before adding extraction with ammonium acetate to reduce potential errors of soluble salts. This can be accomplished by adding 50 mL of deionized water to 2.5 g of soil, placed in a 50 mL centrifuge tube and centrifuge at 5000 rpm for thirty (30) minutes and decanting excess water followed by the standard analysis protocol. For soils having pH > 7.4 and calcium carbonate> 0.5%, should be extracted with ammonium acetate pH 8.5 to avoid the dissolution of calcium carbonate.

3. Check repipette dispensing volume, calibrate using an analytical balance.

4. Extracts may be stored for one week under refrigeration.

5. Filter paper should be checked periodically (monthly) for possible contamination of alkali metals. If contamination is > 5 mg kg^{-1} on a soil basis select an alternative supply of filter paper.

6. Results may be expressed as meq 100 g^{-1} or cmol kg^{-1}. Divide concentration of K, Ca, Mg and Na by 391 mg, 200 mg, 121.5 mg, and 230 mg respectively.

7. Generally, soils having less than 100 mg kg^{-1} potassium will respond to applications of potassium fertilizers for most crops.

Literature

Doll, E.C. and R. E. Lucas. 1973. Testing soil for potassium, calcium and magnesium. p 133-152. *In:* L.M. Walsh and J.D. Beaton. (ed.) Soil testing and plant analysis. SSSA Madison, WI.

Knudsen, D., G.A. Peterson and P.F. Pratt. 1982. Lithium, sodium and potassium. *In*: A.L. Page (ed) Methods of soil analysis Part 2. Agronomy Monograph 9. 2nd ed. ASA and SSSA, Madison WI.

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POTASSIUM FIXATION TEST S - 5.20

Incubation Method

Scope and Application

This method semiquantitatively determines the amount of soil K fixation potential associated with illitic clay soils. Potassium is added to soil, then dried and incubated for seven days. Potassium concentration is determined using atomic emission (AES), absorption spectrometry (AAS) and inductively Coupled Plasma (ICP) instrumentation and fixation capacity calculated based on the amount of K recovered. It is based on a modification of the procedure first proposed by Cassman et al. (1982) and that developed at the University California Davis for evaluating the fixation of potassium for soils of the San Joaquin valley of California. Data for cotton in California indicates that the test is useful for predicting K response on sub soils which have a high K fixation potential Miller et al. (1997). Generally, the method is reproducible within ± 7% K on a given soil, or 3 - 5% on a soil K fixation basis.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.01 g.
2. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute (opm).
3. Repipette dispenser, calibrated to 25.0 ± 0.2 mL.
3. 50 mL graduated centrifuge tube.
4. Whatman No. 42, No. 2 or equivalent highly retentive filter paper.
5. Atomic Emission/Absorption Spectrophotometer (AES) or (AAS).

Reagents

1. Deionized water, ASTM Type I grade.
2. KNO_3 0.01 N solution: dissolve 1.011 g of potassium nitrate in 1.0 L of deionized water.
3. $CaCl_2$, 0.01 N solution: dissolve 2.94 g of $CaCl_2 \cdot 2H_2O$ in 1.0 L of deionized water.
4. NH_4Cl 3.0 N solution: dissolve 160.3 g of ammonium chloride in 1.0 L of deionized water.
5. Standard calibration solutions of K. Prepare two (2) sets of six (6) calibration standard solutions of 1.0 - 20 mg L^{-1} of K from 1000 mg L^{-1} stock solutions prepared in matrix of 0.01 $CaCl_2$ and cesium chloride (5.071 g CsCl in 2.0 L of deionized water); and a second set of K calibration standards in a matrix of 1.0 NH_4Cl and cesium chloride (5.071 g CsCl in 2.0 L of deionized water).

Procedure

1. Weigh 3.00 ± 0.05 g of air dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into a 50 mL volumetrically marked centrifuge tube.
2. Add 3.0 mL 0.01 N KNO_3 into the centrifuge tube. Allow to air dry 48 hours. Preferred method is to place in high draft hood for forty-eight (48) hours at room temperature, avoid heating above 30° C. Include two centrifuge tubes containing only 3.0 mL of 10mM KNO_3 as a control (See Comment #1).
3. When dry, add 30 mL of 0.01 M $CaCl_2$ solution and 100 uL of toluene. Cap and place horizontally on a reciprocating shaker for thirty (30) minutes (See Comment #2).
4. Incubate for seven (7) days at 25°C, shaking for 30 minutes daily.
5. After seven (7) days, shake thirty (30) minutes, then centrifuge 2000 rpm until supernatant is clear. Remove a 10.0 mL aliquot of supernatant and determine K concentration using matrix matched standards (prepared in 0.01 M $CaCl_2$ solution) by AAS or AES.
6. Add 10 mL of 3.0 N NH_4Cl, and shake for 30 minutes. Centrifuge and determine K concentration in supernatant using matrix matched (1.0 N NH_4Cl and 0.01 M $CaCl_2$) standards by atomic absorption. Use control solution to verify 100% K recovery of KNO_3 control or as substitution of added K value of 1170.

7. Adjust and operate AES or AAS instrument with six standards in accordance with manufacturer's instructions. Calibrate using prepared calibration solutions. Determine K, concentration of extract, of method blank, and record results as mg L^{-1} of cation in solution.

Calculations

Calculation of % K Fixation:

$$\frac{1170 - [(10 \times \text{mg } L^{-1} \text{ K in 0.01 M } CaCL_2 \text{ supernatant}) + (30 \times \text{mg } L^{-1} \text{ K in } NH_4Cl)] \times 100}{1170}$$

Note: Negative values indicate soil has released more K than was fixed.
Positive values indicate soil mineral fixation of potassium (see Comment #3).

Comments

1. Check repipette dispensing volume, calibrate using an analytical balance.

2. Toluene is added to prevent microbial growth.

3. Generally, cotton grown in California on subsoils (depths 5-15 inches) with fixation potential greater than 60%, are highly response to K fertilizers with respect to lint yields.

Literature

Cassman, K.G., D.C. Bryant and B.A. Roberts 1990. Comparison of soil test methods for predicting cotton response to soil fertilizer potassium on potassium fixing soils. Commun. in Soil Sci. Plant Anal. 21(13-16), 1727-1743.

Miller, R.O., B.L. Weir, B. Roberts, R. Vargas, D. Munk, S. Wright, D. Munier, R. Travis and M. Keeley. 1997. Potassium in fertility in cotton production systems. UC Extension Publication #21562.

Munter, R. 1988. Laboratory factors affecting the extractability of nutrients. p. 8-10. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region, North Dakota Agricultural Experiment Station Bulletin No. 499 (revised).

EXTRACTABLE ZINC, MANGANESE, IRON AND COPPER S - 6.10

DTPA Extraction

Scope and Application

The DTPA (diethylenetriaminepentaacetic acid) micronutrient extraction method, developed by Lindsay and Norvell (1978), is a nonequilibrium extraction for estimating the potential soil bioavailability of Zn, Cu, Mn, and Fe for neutral and calcareous soils. Analyte concentrations are determined by atomic absorption spectrometry (AAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES). The quantity of micronutrient and trace metals extracted, are affected by solution pH, soil extraction ratio, shaking time, extraction time, and extractant concentration. The method has shown to be well correlated to crop response to zinc and copper fertilizers. It also shows potential for monitoring Cd, Ni and Pb in soils receiving sludge applications. The method detection limit is approximately 0.1 mg kg^{-1} for Zn, Cu, Mn, and Fe (on a dry soil basis) and is generally reproducible ± 10%, except for Fe which is approximately ± 15%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.01 g.
2. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute (opm).
3. Repipette dispenser, calibrate to 20.0 ± 0.1 mL.
4. Extraction vessel and associated filtration vessel.
5. Whatman No. 42 or No. 2, or equivalent medium retentive filter paper.
6. Atomic Absorption Spectrometer (AAS) or ICP-AES instrumentation.

Reagents

1. Deionized water, ASTM Type I grade.
2. DTPA extraction solution, 0.005 M: Dissolve 0.005 M DTPA (diethylenetriaminepentaacetic acid), 0.01 M $CaCl_2$ and 0.10 M Triethanolamine (TEA) adjusted to pH 7.3. For 10 L solution, dissolve 19.67 g DTPA and 149.2 g of TEA in 5 L deionized water. Add 14.69 g of $CaCl_2 \cdot 2H_2O$ to 5 L of deionized water (See Comments #1 and #2) and add DTPA-TEA mixture and adjust the pH to 7.3 ± 0.05 using 1.0 N HCl (approximately 41.5 mL of concentrated HCl are required).
3. Standard calibration solutions, Zn, Cu, Mn, and Fe. Prepare six calibration of 100 mL solutions of Zn, Cu, Mn, and Fe diluted in DTPA extraction solution from 1000 mg L^{-1} standard solutions. Concentration ranges: 0.1 - 5.0 mg L^{-1} for Zn; 0.1 10 mg L^{-1} for Mn and Fe; and 0.05 - 4.0 mg L^{-1} for Cu.

Procedure

1. Weigh 10.0 ± 0.2 g of air dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into 120 mL extraction vessel and add 20.0 mL of DTPA extraction reagent (See Comments #3 and #4). Include a DTPA method blank.
2. Place extraction vessel(s) on reciprocating mechanical shaker for two (2) hours at 25 °C, and 180 oscillations per minute.
3. Immediately filter (See Comment #5), refilter if filtrate is cloudy.
4. Adjust and operate AAS instrument in accordance with manufacturer's instructions. Calibrate instrument using standard calibration solutions and determine individually (Zn, Cu, Mn and Fe) concentration of DTPA extracts, method blank and record as mg L^{-1} of analyte in solution. (See Comments #6 and #7).

Calculations

Report as mg kg^{-1} Mn and Fe in the soil to the nearest 0.1 mg kg^{-1}, report Zn and Cu in the soil to the nearest 0.01 mg kg^{-1};

mg kg^{-1} in soil = (mg L^{-1} in extract - method blank) × 2.0

(See Comment #8)

Comments

1. Calcium chloride is added to the DTPA solution to prevent dissolution of calcium carbonate. This prevents dissolution of zinc present in calcium carbonate minerals.

2. TEA is added to buffer the DTPA solution and prevent excess dissolution of trace metals which is highly pH dependent. Check solution pH bimonthly and adjust as needed.

3. Check repipette dispensing volume, calibrate using an analytical balance.

4. Precautions should be taken to avoid potential contamination by: (1) rinsing all labware in a solution of 0.5 N HCl; (2) reagent solutions made in ASTM Type I water; and (3) filter paper checked for potential contamination.

5. Filtering should commence immediately after shaking, and should cease 15 within minutes after starting. Continued filtering will increase the amount of micro nutrient extracted.

6. Metal concentrations exceeding highest standard will require dilution for analysis.

7. Manganese concentrations will likely increase over time on soils stored for periods greater than six (6) months, the result of high oxidation. This can be minimized by storing soils in air-tight containers.

8. Soils having DTPA extractable zinc less than 0.80 mg kg^{-1} are generally responsive to zinc fertilizer for sensitive crops such as corn. Soils having DTPA extractable copper less than 0.60 mg kg^{-1} are generally responsive to copper fertilizer for sensitive crops such as wheat.

Literature

Horneck, D. A.. J. M. Hart, K. Topper and B. Koespell. 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University. Ag. Expt. Station SM 89:4.

Liang, J. and R.E. Karamanos. 1993. Soil reaction and exchangeable acidity. p. 87-90. *In*: M. R. Carter (ed.) Soil sampling and methods of analysis, Canadian Society of Soil Science, Lewis Publishers Ann Arbor, MI.

Lindsay, W. L. and W. A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci. Soc. Amer. J. 42:421-428.

Whitney, D. 1988. Micronutrient soil test for zinc, iron, manganese, and copper. p. 20-22. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. North Dakota Agricultural Experiment Station Bulletin 499 (revised).

EXTRACTABLE ZINC, MANGANESE, IRON, COPPER & BORON S - 6.12

DTPA - Sorbitol Extraction

Scope and Application

The DTPA - Sorbitol (diethylenetriaminepentaacetic acid) micronutrient extraction method, is based on that developed by Lindsay and Norvell (1978), is a nonequilibrium extraction for estimating the potential soil bioavailability of Zn, Cu, Mn, Fe and B for mildly acid to calcareous soils. The addition of Sorbitol is added for the chelate extraction of boron. Analyte concentrations are determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). The quantity of micronutrient and trace metals extracted, are affected by solution pH, soil extraction ratio, shaking time, extraction time, and extractant concentration. The method has shown to be well correlated to crop response to zinc and copper fertilizers. It also shows potential for monitoring Cd, Ni and Pb in soils receiving sludge applications. The method detection limit is approximately 0.1 mg kg^{-1} for Zn, Cu, Mn, and Fe and 0.05 mg kg^{-1} for B (on a dry soil basis). It is generally reproducible ± 10%, except for Fe which is approximately ± 15%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.01 g.
2. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute (opm).
3. Repipette dispenser, calibrate to 20.0 ± 0.1 mL.
4. Extraction vessel and associated filtration vessel - 50mL volume.
5. Whatman No. 42 or No. 2, or equivalent medium retentive filter paper.
6. ICP-AES, inductively coupled plasma atomic emission spectrometry (ICP-AES).

Reagents

1. Deionized water, ASTM Type I grade.
2. 5% sodium hypochlorite solution.
3. DTPA-Sorbitol extraction solution, 0.005 M. Rinse all labware with 5% sodium hypochlorite solution. Dissolve 0.005 M DTPA (diethylenetriaminepentaacetic acid), 0.01 M $CaCl_2$ and 0.10 M Triethanolamine (TEA) adjusted to pH 7.3 and 0.20M of Sorbitol. For 10 L solution, dissolve 19.67 g DTPA and 149.2 g of TEA in 5 L deionized water. Add 14.69 g of $CaCl_2 \cdot 2H_2O$ to 5 L of deionized water (See Comments #1 and #2), 364.4g Sorbitol (Sigma, S-1876, D-Sorbital, 98% minimum) and add DTPA-TEA mixture and adjust the pH to 7.30 ± 0.05 using 1.0 N HCl (approximately 41.5 mL of concentrated HCl are required).
4. Standard calibration solutions, Zn, Mn, Fe, Cu and B. Prepare six calibration solutions of 100 mL volume of Zn, Cu, Mn, and Fe diluted in DTPA-Sorbitol extraction solution from 1000 mg L^{-1} standard solutions. Concentration ranges: 0.1 - 10.0 mg L^{-1} for Zn; 1.0 - 30 mg L^{-1} for Mn and Fe; 0.05 - 4.0 mg L^{-1} for Cu; and 0.02 - 5.0 mg L^{-1} for B.

Procedure

1. Weigh 10.0 ± 0.2 g of air dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into 50 mL extraction vessel and add 20.0 mL of DTPA - Sorbitol extraction reagent (See Comments #3 and #4). Include a DTPA method blank.
2. Place extraction vessel(s) on reciprocating mechanical shaker for two (2) hours at 25 °C, and 180 oscillations per minute.
3. Immediately filter, refilter if filtrate is cloudy (See Comment #5).

4. Adjust and operate ICP-AES instrument in accordance with manufacturer's instructions. Calibrate instrument using standard calibration solutions and determine individually (Zn, Mn, Cu, Fe and B) concentration of DTPA-Sorbitol extracts, method blank and record as mg L^{-1} of analyte in solution. (See Comments #6 and #7).

Calculations

Report as mg kg^{-1} Mn and Fe in the soil to the nearest 0.1 mg kg^{-1}, report Zn, Cu and B in the soil to the nearest 0.01 mg kg^{-1};

mg kg^{-1} in soil = (mg L^{-1} in extract - method blank) × 2.0

(See Comment #8 and #9)

Comments

1. Calcium chloride is added to the DTPA solution to prevent dissolution of calcium carbonate. This prevents dissolution of zinc present in calcium carbonate minerals.

2. TEA is added to buffer the DTPA solution and prevent excess dissolution of trace metals which is highly pH dependent. Check solution pH bimonthly and adjust as needed.

3. Check repipette dispensing volume, calibrate using an analytical balance.

4. Precautions should be taken to avoid potential contamination by: (1) rinsing all labware in a solution of 0.5 N HCl; (2) reagent solutions made in ASTM Type I water; and (3) filter paper checked for potential contamination.

5. Soil DTPA-Sorbitol suspension filters slowly. Filtering should commence immedately after shaking, and should cease 15 within minutes after starting. Continued filtering will increase the amount of micro nutrient extracted.

6. Metal concentrations exceeding highest standard will require dilution for analysis.

7. Manganese concentrations will likely increase over time on soils stored for periods greater than six (6) months, the result of oxidation. This can be minimized by storing soils in air-tight containers.

8. Soils having DTPA extractable zinc less than 0.8 mg kg^{-1} are generally responsive to zinc fertilizer for sensitive crops such as corn. Soils having DTPA extractable copper less than 0.6 mg kg^{-1} are generally responsive to copper fertilizer for sensitive crops such as wheat.

9. The DTPA-Sorbitol extraction method extracts 5-8% less Zn, Mn, Fe and Cu than the standard DTPA extraction procedure (S-6.10). For specific soils with greater than 4% SOM, this method will extract 60% Mn of the extracted by the DTPA procedure (S-6.10). Generally DTPA-Sorbitol extracts 10% less B than the hot-water method (S-7.10).

Literature

Geasting, W.D. and P.N. Soltanpour. 1981. Boron analysis in soil extracts and plant tissue by plasma emission spectroscopy. Commun. Soil Sci. Plant Anal. 12(8):733-742.

Horneck, D. A.. J. M. Hart, K. Topper and B. Koespell. 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University. Ag. Expt. Station SM 89:4.

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Lindsay, W. L. and W. A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci. Soc. Amer. J. 42:421-428.

Vaughan, B. and J. Howe. 1994. Evaluation of boron chelates in extracting soil boron. Commun. Soil Sci. Plant Anal. 25(7&8) 1071-1084.

Whitney, D. 1988. Micronutrient soil test for zinc, iron, manganese, and copper. p. 20-22. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. North Dakota Agricultural Experiment Station Bulletin 499 (revised).

HCL EXTRACTABLE ZINC S - 6.20

0.10 M HCl Extraction

Scope and Application

The 0.1M HCl method estimates potential soil bioavailability of Zn by semiquantitatively extracting Zn from acid to neutral soils. The quality of acid-soluble zinc serves as an index of plant available zinc. The method is not suitable to alkaline soils containing calcium carbonates. For alkaline soils Nelson, Boawn and Veits (1959) recommend using titratable alkalinity as a correction for the acid-soluble zinc index. Analyte concentrations are determined by atomic absorption spectrometry (AAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES). The quantity of Zn extracted, is affected by soil properties, extraction ratio, and extraction time. Procedural differences are known to exist among laboratories and are recognized in inter-laboratory exchange programs. The method detection limit is approximately 0.2 mg kg^{-1} for Zn (on a dry soil basis) and is generally reproducible ± 10%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.01 g.
2. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute (opm).
3. Repipette dispenser, calibrate to 20.0 ± 0.1 mL.
4. 50 mL Erlenmeyer extraction vessel and associated filtration vessel.
5. Whatman No. 2, or equivalent medium retentive filter paper.
6. Atomic Absorption Spectrometer (AAS) or ICP-AES instrument.

Reagents

1. Deionized water, ASTM Type I grade.
2. 0.1 M HCl extraction solution: Add 300 mL of deionized 6 M HCl to approximately 10 L of deionized water and bring to 18.0 L final volume.
3. Standard calibration solutions Zn. Prepare six calibration of 100 mL solutions of Zn extraction solution from 1000 mg L^{-1} standard solutions. Concentration range: 0.1 - 2.0 mg L^{-1} for Zn.

Procedure

1. Weigh 5.0 ±0.1 g of air dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into 50 mL Erlenmeyer flask and add 20.0 mL of 0.1 M HCl extraction reagent (See Comments #1 and #2). Include a 0.1 M HCl method blank.
2. Place extraction vessel(s) on reciprocating horizontal mechanical shaker for thirty (30) minutes at 25 °C, and 180 oscillations per minute.
3. Immediately filter into 30 mL polypropylene flasks, refilter if filtrate is cloudy.
4. Adjust and operate AAS instrument in accordance with manufacturer's instructions. Calibrate instrument using standard calibration solutions and determine Zn concentration of extracts, method blank and record as mg L^{-1} of analyte in solution (See Comments #3).

Calculations

Report as mg kg^{-1} Zn to the nearest 0.1 mg kg^{-1}:

$$\text{mg kg}^{-1} \text{ in soil} = (\text{mg L}^{-1} \text{ in extract} - \text{method blank}) \times 4$$

Comments

1. Check repipette dispensing volume, calibrate using an analytical balance.

2. Precautions should be taken to avoid potential contamination by: (1) rinsing all labware in a solution of 0.5 N HCl; (2) reagent solutions made in ASTM Type I water; and (3) filter paper checked for potential contamination.

3. Concentrations exceeding highest standard will require dilution for analysis.

Literature

Brown, J.R., J. Garrett, and T.R. Fisher. 1997. Soil testing in Missouri. University of Missouri-Columbia, Extension Division. Extension Circular 923.

Nelson, J.L., L.C. Boawn, and F.G. Viets, Jr. 1959. A method for assessing zinc status of soils using acid extractable zinc and "titratable alkalinity" values. Soil Sci. 88:275-283.

Sorensen, R.C., D.D. Oelsligle, and D. Knuden. 1971. Extraction of Zn, Fe, and Mn from soils with 0.1 M hydrochloric acid as affected by soil properties, solution, soil ratio; and length of extraction period. Soil Sci. 11:352-359.

Tucker, T.C. and L.T. Kurtz. 1955. A comparison of several chemical methods with the bio-assay procedure for extracting zinc from soils. Soil Sci. Amer. Proc. 19:477-481.

Whitney, D. 1988. Micronutrient soil test for zinc, iron, manganese, and copper. p. 20-22. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. North Dakota Agricultural Experiment Station Bulletin 499 (revised).

EXTRACTABLE SOIL BORON, HOT-WATER S - 7.10

Azomethine-H Spectrophotometric / ICP-AES Method

Introduction

This method estimates the relative bioavailability of boron in soils using 0.02 M $CaCl_2$ in conjunction with hot water which facilitates the dissolution of boron. The extraction of boron is based on that described by Bingham (1982) and adapted for the use of plastic bags as described by Mahler et al. (1983). Boron is determined spectrophotometrically by complexation of HBO_3 with azomethine-H to which forms a colored complex. Subsequent measurement is at 420 nm (Horneck et al., 1989). This method is used to access crop potential deficiencies of boron. It differs from the saturation paste soluble boron concentration (Method S - 1.40) which assesses possible supra-optimal concentrations of soil boron. The method detection limit is approximately 0.2 mg kg^{-1} (on a dry soil basis) and is generally reproducible ± 12%.

Equipment

1. Analytical balance: 1000 g capacity, and minimum ± 0.01 g.
2. Plastic bags, sealable, 500 mL, 4 mil thickness.
3. Repipette dispenser, calibrated to 30.0 ± 0.3 mL.
4. Pipette 1.00 ± 0.05 and 4.00 ± 0.1 mL
5. Hot Plate and 4 liter beaker.
6. Vortex stirrer.
7. Whatman No. 42 or No. 2, or equivalent highly retentive filter paper.
8. Polypropylene sample tube (See Comment #1).
9. Spectrophotometer or automated Flow Injection Analysis (FIA) system, 420 nm.

Reagents

1. Deionized water, ASTM Type I grade.
2. Calcium chloride extraction solution, 0.02 M: Dissolve 2.84 g of $CaCl_2 \cdot 2H_2O$ into 750 mL deionized water and dilute to 1000 mL.
3. Buffer masking agent: Dissolve completely 250 g ammonium acetate (reagent grade $NH_4C_2H_3O_2$), 25 g disodium salt of ethylenedinitrilo-tetraacetic acid (Na_2-EDTA), and 10 g disodium salt of nitrilotriacetic acid (Na_2-NTA) in 400 mL deionized water in a 1 L beaker using a magnetic stirrer. Add 125 mL glacial acetic acid very slowly, while stirring. The temporary acidic conditions may cause a slight precipitation of the EDTA salts, stir solution until all the EDTA redissolves. Do not heat the solution. Adjust the buffer to a pH of 5.4 to 5.6 with acetic acid or NH_4OH. Prepare fresh solution every two months (See Comment #2).
4. Azomethine-H solution: Dissolve 0.9 g azomethine-H reagent (Pierce Chemical Co., Rockford, IL) and 2.0 g L-ascorbic acid ($C_6H_8O_6$) in 50 mL of deionized water prewarmed to 60 °C. A hot water bath facilitates dissolution. Dilute to 100 mL volume with deionized water and store in refrigerator. Solution is stable for forty-eight (48) hours.
5. Boron standard calibration solutions. Prepare five boron standards ranging from 0.1 to 4.0 mg L^{-1} boron from 1000 mg L^{-1} standard solution and diluted to volume with 0.02 M $CaCl_2$.

Procedure

1. Weigh 15.0 ± 0.1 g of air dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) and place in plastic bag. Add 30 mL of 0.02 M $CaCl_2$ reagent using repipette dispenser (See Comment #3) and close bag. Include a method blank.
2. Place plastic bags into 4 L beaker containing boiling water and leave for ten (10) minutes.
3. Remove plastic bags, cool one (1 ± 0.1) minute and filter. Refilter if extract is cloudy.
4. Pipette 4.0 mL of soil extract into a 12 mL polyethylene sample tube.

5. Add 1.0 mL of buffer masking agent and stir with a vortex stirrer.
6. Add 1.0 mL of azomethine-H solution, vortex stir and allow to stand for sixty (60) minutes but no longer than three (3) hours (See Comments #4 and #5).
7. Prepare standard curve following steps 4 - 6, substituting 4.0 mL of standard calibration solution for soil extract. A method blank is prepared in the same manner using 4.0 mL $CaCl_2$ extracting solution instead of the soil extract.
8. Read sample absorbance on a spectrophotometer set at 420 nm of a method blank, unknown samples and record results as mg L^{-1} of B in the solution extract (See Comments #6 and #7).

Calculations

Report as mg kg^{-1} boron in the soil to the nearest 0.1 mg kg^{-1}:

B mg kg^{-1} in soil = (mg L^{-1} B in extract - method blank) × 2

Comments

1. Teflon or polypropylene labware is recommended to minimize the potential of boron contamination from boro silicate glass.

2. The EDTA and NTA chelates eliminate interferences from Al, Fe, and Cu. The concentration of these chelates should be effective for levels of these elements commonly found in soil extracts.
3. Check pipette and repipette dispensers delivery volume, recalibrate using an analytical balance.

4. The azomethine-H should be added quickly so color development is equal for all tubes. A constant check must be maintained on linearity and drift of standards when analyzing a large number of samples.

5. For soil samples with a yellow extract: Prepare a second sample solution and blank following steps 4 and 5. Add 1.0 mL of deionized water in place of azomethine-H solution. The blank for this determination consists of 5.0 mL $CaCl_2$ extracting solution and 1.00 mL buffer masking agent.

6. Boron may also be determined directly on the hot-water extract using ICP-AE using 249.699 or 208.14 nm wavelengths.

7. Dilution will be required on samples having boron concentrations exceeding the highest standard.

Literature

Bingham, F.T. 1982. Boron. p.431-446. *In*: A.L. Page et al. (ed.) Methods of soil analysis, Part 2. Agronomy Mongr. 9. 2nd ed. ASA and SSSA, Madison, WI.

Horneck, D. A.. J. M. Hart, K. Topper and B. Koespell. 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University. Ag. Expt. Station SM 89:4.

Mahler, R.L. D.V. Naylor and M.K. Fredickson. 1983. Hot water extraction of boron from soils using sealed plastic pouches. unpublished, Univ. of Idaho. 12.

Scope and Application

The Total Kjeldahl Nitrogen (TKN) method is based on the wet oxidation of soil organic matter using sulfuric acid and digestion catalyst and conversion of organic nitrogen to ammonium nitrogen. Ammonium is determined using spectrophotometric, diffusion-conductivity or distillation techniques. The method is readily adapted to manual or automated techniques. The procedure does not quantitatively digest nitrogen from heterocyclic compounds (bound in a carbon ring), oxidized compounds such as nitrate, or ammonium from within mineral lattice structures. The method has a detection limit of approximately 0.020% N and is generally reproducible within ± 8%.

Equipment

1. Analytical balance: 250 g capacity minimum ± 0.1 mg.
2. Acid fume hood.
2. Volumetric digestion tubes, 75 mL and digestion heating block (400 °C).
3. Repipette dispenser, calibrated to 3.0 ± 0.2 mL.
4. Spectrophotometer, diffusion-conductivity instrument or distillation apparatus.

Reagents

1. Deionized water, ASTM Type I grade.
2. Sulfuric acid, concentrated - reagent grade.
3. Digestion Catalyst (K_2SO_4, $CuSO_4$, and SeO: ratio 100:10:1), Kjel-tab.
4. Standard Calibration solutions (NH_4-N). Prepare six working standards of ammonium, concentration range 0.1 - 40 mg L^{-1}, made from 1000 mg L^{-1} ammonium nitrogen standard solution and diluted to volume with 12 % sulfuric acid (v/v).

Procedure

1. Weight 1.000 ± 0.005 g of air dry soil to pass 10 mesh sieve (< 2.0 mm) into a 75.0 mL volumetric digestion tube (See Comment #1). Include a method blank.
2. Add digestion catalyst, (200 mg of mixed catalyst or Kjel-tab) and 3.0 mL of concentrated sulfuric acid (See Comment #2 and #3). Note: it is essential that all dry material be completely moistened by acid and well mixed to insure complete digestion.
3. Place tubes on a digestion block at 150 °C and after thirty (30) minutes raise to 350 °C for two (2) hours or until samples are completely digested. At completion, mineral soils will be whitish-gray and organic soils blue-green in color.
4. Remove samples from block and place under fume hood for 5-10 minutes. Add 10-20 mL of deionized water using a wash bottle to each tube to prevent hardening and crystal formation. Dilute digest to volume with deionized water, cap, invert three times, and allow digest to clear.
5. Determine digest ammonium concentration using the spectrophotometric, diffusion-conductivity instruments or distillation techniques using standard calibration solutions (See Comment #4 and #5). The ammonium nitrogen content of the digest solution can be determined with a rapid flow analyzer (Technicon Method No. 334-74A/A) or an flow injection analyzer (FIA). This determination can also be made using the Kjeldahl distillation method. Adjust and operate instruments in accordance with manufacturer's instructions. Determine ammonium concentration of a method blank, unknown samples and record ammonium concentration as mg L^{-1} of NH_4-N in soil digest.

Calculations

Report total Kjeldahl nitrogen results to the nearest 0.001% as:

$$\% N = \frac{(\text{mg } L^{-1} \text{ } NH_4\text{-N in digest - method blank}) \times (0.075) \times (100)}{\text{(Sample size mg)}} \text{[equ. S -8.10-1]}$$

Comments

1. Use 250 mg of soil if sample is greater than approximately 10% organic matter.

2. Check repipette dispenser delivery volume, recalibrate using an analytical balance.

3. When adding reagents to vessels always wear protective clothing (i.e. eye protection, lab coat, disposable lab gloves, and shoes). Always handle reagent and digestion labware in hoods capable of high air flow, 100 cfm.

4. The Kjeldahl method outline by Bremmer and Mulvaney (1982) is modified eliminating the water from the digestion step.

5. Kjeldahl soil acid digest is classified as hazardous waste and must be disposed of in a suitable manner.

Literature

Bremmer, J. M. and C. S. Sulvaney. 1982. Total nitrogen p. 595-624. *In*: A.L. Page et al. (eds.) Methods of soil analysis, part 2. Agron. Mongr. 9. 2nd ed. ASA and SSSA, Madison, WI.

Carlson, R.M. 1978. Automated separation and conductiometric determination of ammonia and dissolved carbon dioxide. Anal. Chem. 48:1528-1531.

Horneck, D. A.. J. M. Hart, K. Topper and B. Koespell. 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University. Ag. Expt. Station SM 89:4.

SOIL ORGANIC MATTER S - 9.10

Walkley-Black Titration Method

Scope and Application

This method quantifies the amount of oxidizable soil carbon as determined by reaction with $Cr_2O_7^{2-}$ and sulfuric acid. The remaining unreacted dichromate is titrated with $FeSO_4$ using Ortho-phenanthroline as an indicator and organic carbon calculated by difference. The method is based upon that described by Mebius (1960) and is an estimate since not all the organic carbon present is oxidized. Soil organic matter values are used to estimate potential nitrogen mineralization, for pesticide management and for crop production management. Chromium disposal costs have forced many laboratories to consider Loss on Ignition (LOI, see Method 9.20) as a means for estimating soil organic matter content. The method detection limit is approximately 0.10% and is generally reproducible to with in ± 8%.

Equipment

1. Analytical Balance: 100 g capacity, resolution ± 0.001 g.
2. Erlenmeyer flask 125 mL and 250 mL beaker.
3. Buchner funnel 11 cm.
4. Whatman No. 42 filter paper 11 cm, or equivalent highly retentive filter paper.
5. Repipette dispenser(s), calibrated to 5.0 ± 0.1, 10.0 ± 0.2 and 15.0 ± 0.2 mL.
6. 50 mL burette with graduations of 0.1 mL.
7. Magnetic stir plate and microsize teflon coated magnetic stir bar.

Reagents

1. Deionized water, ASTM Type I grade.
2. Potassium dichromate solution, 1.0 N solution: Dissolve 49.04 g of $K_2Cr_2O_7$ (dried at 105 °C) in deionized water and dilute to 1000 mL.
3. Ferrous sulfate heptahydrate solution, 0.5 N: Dissolve 140 g of $FeSO_4 \cdot 7H_2O$ in 500 mL of deionized water, add 15.0 mL of concentrated 16 N H_2SO_4 and dilute to 1000 mL. (See Comment #1).
4. Concentrated sulfuric acid solution (16 N).
5. Ortho-phenanthroline-ferrous complex solution, 0.025 M. Dissolve 3.71 g of O-phenanthroline monohydrate and 1.74 g of $FeSO_4 \cdot 7H_2O$ in deionized water and dilute to 250 mL. Store in plastic bottle (See Comment #2).

Procedure

1. Weigh 0.500 ± 0.005 g of air dry soil pulverized to pass 40 mesh sieve (< 0.42 mm) (See Comment #3) into 125 mL erlenmeyer flask. Include a blank solution.
2. Using repipette dispenser add 5.0 mL of 1.0 N $K_2Cr_2O_7$ solution to the flask containing soil. Include a blank flask (See Comment #4).
3. Using a repipette add rapidly 10.0 mL of concentrated H_2SO_4 acid, directing the stream of liquid into the center of soil suspension. Immediately swirl for one (1) minute, cool on a heat resistant surface for thirty (30) minutes and add 100 mL of deionized water.
4. Filter the suspension into 250 mL beaker and refilter if filtrate is cloudy (See Comment #5).

5. Add 0.30 mL of Ortho-phenanthroline-ferrous complex 0.025 M indicator solution. Titrate the solution with 0.50 N ferrous sulfate from 25 mL buret (See Comment # 6). As the endpoint is approached, the solution takes on a greenish cast and changes to dark blue green. An additional 0.20 mL of indicator may be used to sharpen the endpoint. At this point, add the ferrous sulfate drop by drop until the color changes sharply form blue to orange red (maroon in reflected light) and record amount (mL) of ferrous sulfate solution used (See Comment #7).

Calculations

$$\text{Normality (N) } FeSO_4 = \frac{\text{mL } K_2Cr_2O_7 \times \text{N } K_2Cr_2O_7}{\text{mL } FeSO_4}$$

$$\text{meq } FeSO_4 = \text{mL } FeSO_4 \times \text{N } (FeSO_4)$$

$$\text{Organic Carbon (\%)} = \frac{((\text{meq } K_2Cr_2O_7 - \text{meq } FeSO_4) \times 0.003 \times 100}{\text{sample dry weight}} \times 1.33 - \text{blank}$$

$$\text{Organic Carbon (\%)} = \frac{((5 - \text{meq } FeSO_4) \times 0.399) - \text{blank}}{\text{sample dry weight}} \quad \text{(See Comment \#8)}$$

$$\text{Organic Matter (\%)} = 1.72 \times \text{Organic Carbon \%}$$

Comments

1. Allow solution to cool to room temperature before diluting to standard volume. Store in pyrex bottle.

2. N-phenylanthranilic acid can be substituted as an indicator: dissolve 0.100 g of N-phenylanthranilic acid and 0.107 g of Na_2CO_3 in deionized water and dilute to 100 mL. The endpoint proceeds rapidly from violet to gray to bright green.

3. For soils containing greater than 8 mg of organic carbon reduce sample size. Soils should be pulverized to pass 30 mesh sieve (0.5 mm).

4. When adding reagents to vessels always wear protective clothing (i.e. eye protection, lab coat, disposable lab gloves, and shoes). Always handle reagent and digestion labware in hoods capable of high air flow, 100 cfm.

5. The filtrate is classified as a hazardous waste and must be disposed of in a suitable manner.

6. Flush burette with 0.5 N ferrous sulfate solution before titration, as it is light sensitive.

7. If more than 75% of the dichromate is reduced, repeat with smaller sample size. Samples containing large amounts of manganese or carbonates may give erroneous results and require pretreatment with 0.1 N HCl to remove.

8. The value of 1.33 represents an average correction factor since the dichromate does not completely oxidize all soil organic carbon. The value may be replaced by a more suitable value found through experimentation. Multiply organic carbon value by 1.72 to calculate organic matter (%).

Literature

Mebius, L.J. 1960. A rapid method for the determination of organic carbon in soil. Anal. Chim. Acta. 22:120-124.

Janitzky, P. 1986. Cation exchange capacity. p. 34. *In*: M.J. Singer and P. Janitzky (ed.) Field and laboratory procedures used in a soil chromosequence study. U.S. Geological Survey Bulletin 1648.

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Nelson, D.W. and L. E Sommers. 1975. A rapid and accurate procedure for estimation of organic carbon. Soil Proc. Indiana Acad. Sci. 84:456-462.

Schulte, E. E. 1988. Recommended soil organic matter tests. p. 29-31. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. North Dakota Agricultural Experiment Station Bulletin 499 (revised).

SOIL ORGANIC MATTER S - 9.20

Loss On Ignition Method

Scope and Application

This method semiquantifies the amount of oxidizable organic matter as determined by the gravimetric weight change associated with high temperature oxidation of soil organic matter in a muffle furnace. The method is based that described by Storer (1984) and is an estimate. As a result of chromium hazardous waste disposal costs associated with Walkley-Black method (S - 9.00), many laboratories have chosen to switch to loss on ignition as a means for estimating soil organic matter content. The LOI method is poorly correlated with the Walkley-Black method for soils containing less than 3% organic matter. Soil organic matter values are used to estimate potential nitrogen mineralization, pesticide management and for crop production management. The method is generally reproducible within ± 20%.

Equipment

1. Analytical balance: 100 g capacity, resolution ± 0.001 g.
2. High temperature, crucibles 20 cc capacity.
3. Drying oven, 105 ºC.
4. Dessicator, containing desiccating agent.
5. Muffle furnace capable of heating to 360 ºC.

Reagents

1. Calcium carbonate, reagent grade (See Comment #1).

Procedure

1. Weigh 10.0 ± 1.0 g of air dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) into a preweighed crucible (record to the nearest ± 0.001 g). Prepare a crucible containing calcium carbonate (See Comment #1).
2. Place in drying oven for two (2) hours at 105 ºC. Place in dessicator for one (1) hour.
3. Record crucible + soil as Initial wt to the nearest ± 0.001 g.
4. Heat in muffle furnace to 360 ºC for two (2) hours (after temperature reaches 360 ºC).
5. Place in drying oven at 105ºC for one (1) hour and place in dessicator for one (1) hour.
6. Record crucible + soil as Final wt sample weight to the nearest ± 0.001 g.

Calculations

$$\text{LOI \%} = \frac{(\text{Initial wt at 105 ºC} - \text{Final wt. at 105 ºC}) \times 100}{(\text{Initial wt at 105 ºC} - \text{crucible wt})} \quad \text{[equ. S -9.20-1]}$$

Estimation of organic matter by LOI is done by regression analysis with organic matter. Select fifty soils covering a range in organic matter expected, determine organic matter based on Walkley-Black method (Method S - 9.10) and LOI value. Use this equation to convert LOI values to estimated percent organic matter (See Comment #2).

Comments

1. Calcium carbonate is included as a method standard to evaluate potential loss of carbonates of alkali metals. If appreciable losses (>0.05% weight change) are noted check temperature calibration of the muffle furnace, reduce oven temperature 10 ^{o}C and repeat.

2. The regression slope for organic matter for the LOI method on the Walkley-Black method ranges from 0.68 to 1.04 for soils reported in the literature. Regression intercept values range from -0.04 to -0.36, (Schultee and Hopkins, 1996).

Literature

Schulte, E.E. 1988. Recommended soil organic matter tests. p. 29-31. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. North Dakota Agricultural Experiment Station Bulletin 499 (revised).

Schulte, E.E. and B.G. Hopkins. 1996. Estimation of soil organic matter by weight Loss-On-Ignition. P. 21-32. *In*: Soil Organic matter: Analysis and Interpretation. (ed.) F.R. Magdoff, M.A. Tabatabai and E.A. Hanlon, Jr. Special publication No. 46. Soil Sci. Soc. Amer. Madison, WI.

Storer, D. A. 1984. A simple high sample volume ashing procedure for determining soil organic matter. Commun. Soil Sci. Plant Anal. 15:759-772.

TOTAL NITROGEN AND ORGANIC CARBON S - 9.30

Combustion Method

Scope and Application

This method quantitatively determines the amount of organic carbon in soil materials by combustion of the sample in an O_2 environment using an automated resistance furnace and with subsequent quantification of nitrogen by thermalconductivity detector and CO_2 using an infrared or conductivity detector. For very low nitrogen analysis (< 0.05%)specific instruments are available with chemi-luminescence detectors. Soils with a pH >7.4 (method S - 2.10) and containing inorganic carbon (carbonates), organic carbon is determined by the difference between total carbon by combustion minus the quantity of inorganic carbon as determined by Method S - 13.10 or S - 13.20. The method for specific instruments requires that soils be pulverized to pass 60 mesh sieve to ensure homogeneity. It is based on the method originally described by Dumas whereby soil samples encased in tin (Sn) foil, are ignited in a furnace in excess of 1000 ºC, in a helium and oxygen environment in a quartz combustion tube. The combustion gas is passed through a catalyst (instrument manufacturer dependent) to complete conversion of CO to CO_2, scrubbed of moisture and CO_2 determined by an infrared detector andfor nitrogen by thermalconductivity detector. Specific instruments provide for the simultaneous determination of H or S. Total nitrogen and organic carbon is used to assess nutrient mineralization, water infiltration, soil structure and absorption or deactivation of agricultural chemicals. The method has a detection limit of 0.03% N and 0.02% TOC (dry sample basis instrument dependent) and is generally reproducible to with in ±7.0% for nitrogen and ±5.0% for organic carbon..

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.1 mg.
2. Total Nitrogen Analyzer: Leco CHN-1000, CNS-2000, Elmentar, Carl-Erba, Perkin-Elmer or Elementar, with a resistance furnace with infrared and/or thermal conductivity detector and operating supplies.
3. Tin foil encapsulating capsules (See Comment #1).
4. Desiccator, containing a desiccating agent.

Reagents

1. Compressed Oxygen, 99.99% purity.
2. Helium carrier gas 99.99% purity.
3. Total Organic Carbon calibrations standards: EDTA, 9.57% ± 0.05% N; sulfanilic acid ($C_6H_7NO_3S$) 41.6%C; Leco part number 502-203 soil, 2.75% ±0.09 %C; and Leco part number 502-062 soil, 0.85% ±0.05%C.

Procedure

1. Determine the soil moisture content (See comment #2).
2. Weigh of air dry soil (quantity is instrument dependent) pulverized to pass a 30 mesh sieve (< 250 *u*m) (See Comments #3 and #4 #5) and place in into a tarred tin foil container, encapsulate, close and record sample weight to the nearest 0.1 mg.
3. Initialize the instrument following manufacturers suggested protocol. Conduct a system leak check on combustion system. Perform blank stabilization test, analyze consecutive blanks until the blanks stabilize at a constant value (See Comment #6).
4. Adjust and operate the instrument according to manufacturer instructions using calibration standards. Enter sample dry matter content and analyze unknown sample for total nitrogen. Report results to the nearest 0.001% carbon (See Comment #7).

Calculations

Report total nitrogen results to the nearest 0.001%

Report total organic carbon results to the nearest 0.01%

Comments

1. Tin (Sn) foil capsules is utilized as combustion catalyst. Capsules can be obtained from the manufacture's, after market vendors, or fabricated from sheets of tin foil material.

2. Samples limited in material, should be dried over phosphorus pentoxide or magnesium perchlorate for forty-eight (48) hours and analyzed with no correction for moisture content or reported on as received basis.

3. Sample particulate material must be ground to pass a 30 mesh sieve (< 600 *u*m) for macro analysis instruments which require sample sizes in access of 250 mg (i.e. LECO, CHN-2000 and Elementar) in order to assure adequate sample homogeneity. For instruments utilizing a sample sizes 5-10 mg (Carlo-Erba and Perkin-Elmer) samples must be ground to pass 100 or 140 (106-150 *u*m) mesh sieve.

4. Soils containing free carbonates must be analyzed for free calcium carbonate according to Methods S - 13.10 or S- 13.20 for the determination of inorganic carbon. Total organic carbon is calculated by subtracting the inorganic carbon from the total carbon value determined by the combustion instrument.

5. Sample weight may be entered into instrument software using a balance interface.

6. All soil calibration samples should be: (1) checked for homogeneity; and (2)nitrogen and organic carbon content verified using standard addition techniques using two chemical standards such as EDTA and sulfanilic acid. A quality control certified reference sample (NIST SRM 2704, 3.348% ± 0.10% C) is available from the National Institute of Standards and technology, see appendix B.

7. To convert total soil organic carbon (%C) to soil organic matter (SOM by Walkely -Black method, S - 9.10), multiply %C by 1.724 to estimate soil organic matter. The conversion factor ranges from 1.6 to 2.5 dependent on the soil and cropping system management.

Literature

Nelson, D.W. and L.E. Sommers. 1996. Total carbon, organic carbon and organic matter. p. 961-1010. *In:* J.M. Bartels et al. (ed.) Methods of soil analysis: Part 3 Chemical methods. (3rd.ed.) ASA and SSSA, Madison, WI. Book series no. 5

McGeehan, S.L. and D.V. Naylor. 1988. Automated instrumental analysis of carbon and nitrogen in plant and soil samples. Commun. in Soil Sci. Plant Anal. 19:493-50-5.

Sheldrick, B.H. 1986. Test of the LECO CHN-600 Determinator for soil carbon and nitrogen analysis. Can. J. Soil Sci. 66:543-545.

Schepers, J.S. D.D. Francis, and M.T. Thompson. 1989. Automated total nitrogen of soil and plant samples. Commun. in Soil Sci. Plant Anal. 20:949-959.

Tiessen, H., J.A. Bettany, and J.W.B. Stewart 1981. An improved method of the determination of carbon in soils and soil extracts by dry combustion. Commun. Soil Sci. Plant Anal. 12(3): 211-218.

Yeomans, J.C. and J.M. Bremmer. 1991. Carbon and nitrogen analysis of soils by automated combustion techniques. Commun. in Soil Sci. Plant Anal. 22:843-850.

CATION EXCHANGE CAPACITY (CEC) S - 10.10

Ammonium Replacement Method

Scope and Application

Cation exchange capacity (CEC) is the measure of a soil to retain readily exchangeable cations which neutralize the negative charge of soils. This method involves saturation of the cation exchange sites with ammonium, equilibration, removal of the excess ammonium with ethanol, replacement and leaching of exchangeable ammonium with protons from HCl acid (Horneck, et al. 1989). This method maybe poorly suited to soils containing carbonates, vermiculite, gypsum and zeolite minerals. The procedure is time consuming and labor intensive. The speed at which samples filter depends on the strength of the vacuum applied and sample makeup. If using a water aspirated generated vacuum, some samples may never filter because of clogged filter paper. The method detection limit is approximately 1.0 cmol kg^{-1} (or meq/100 gm on a dry soil basis) and is generally reproducible within ± 10%.

Equipment

1. Analytical balance: 250 g capacity, resolution 0.01 g.
2. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute.
3. Repipette dispensers, calibrated to 20 ± 0.1, 8.0 ± 0.1 mL and 5.0 ± 0.1 mL.
4. Whatman No. 1, No. 2 or equivalent filter paper.
5. Buchner funnels vacuum flasks and source of vacuum
6. Auto analyzer or Kjeldahl distillation equipment.

Reagents

1. Deionized water ASTM Type I Grade.
2. Ammonium acetate (1.0 N) extraction solution neutral: Add 570 mL of glacial acetic acid CH_3COOH (99%) to 8000 mL of deionized water. Add 680 mL of concentrated ammonium hydroxide adjust pH to 7.0 with 3.0 N glacial acetic acid or 3.0 N ammonium hydroxide and dilute to 10 L final volume.
3. Ethanol, 95%
4. Hydrochloric acid, 0.1 N HCl - Dilute 8.3 mL of concentrated HCl reagent to 1000 mL with deionized water.
5. Standard calibration solutions of NH_4-N. Prepare six calibration standard solutions of 1.0 - 20 mg L^{-1} of NH_4-N mg L^{-1} from 1000 mg L^{-1} stock solutions. Dilute calibration solutions with 0.1 N HCl solution.

Procedure:

1. Weigh 10.0 ± 0.1 g of air dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) soil into a 125 mL Erlenmeyer flask. Add 50 mL of ammonium acetate solution (See Comment #1) and place the flask reciprocating shaker for thirty (30) minutes. Include a method blank.
2. Connect a 1 L vacuum extraction flask to a Buchner funnel fitted with a Whatman No. 5 or equivalent filter paper. Moisten the filter paper with 2 mL deionized water (See Comment #2).
3. Transfer the soil suspension into the Buchner funnel and leach the sample with 175 mL of 1 N ammonium acetate. The soil extract may be analyzed for extractable K, Ca, Mg, and Na.

4. Rinse the excess ammonium acetate from the soil sample in the Buchner funnel by leaching with a total volume of ethanol and discard the leachate. Note: Be sure to gently fill funnel to remove all excess ammonium and allow it to drain until only damp soil remains. Continue adding ethanol in this manner until 200 mL of solution has been used.
5. Change to a clean 500-mL suction flask and leach the soil sample with 225 mL of 0.1 N HCl to replace the exchangeable ammonium. Bring leachate to a final volume of 250 mL volumetric flask using deionized water.
6. The concentration of ammonium-N in the final leachate can be determined with an ALPKEM rapid flow analyzer (RF-300), which relies on ammonium to complex with salicylate to form indophenol blue (Technicon Method No. 334-74A/A). This color is intensified with sodium nitroprusside and measured at 660 nm. This determination can also be made using the Kjeldahl distillation method (See Comment #3 and #4).

Calculation

$$\text{CEC in meq per 100 g of soil} = (\text{mg L}^{-1} \text{ of } NH_4\text{-N in leachate}) \times \frac{0.25}{14} \times \frac{100}{\text{sample size (g)}}$$

mg L^{-1} NH_4-N in leachate is determined using a standard curve.

Comments

1. Check repipette dispensing volume calibration using an analytical balance.

2. Check filter paper supply for possible contamination of and NH_4-N. If contamination is greater than 0.2 mg L^{-1} on a soil extract basis, rinse filter paper with 0.1 N HCl solution.

3. Samples having ammonium concentrations exceeding the highest standard will require dilution and reanalysis.

4. This procedure used is essentially the same as that of Schollenberger (1945) except that determination of NH_4-N is done spectrophotometrically rather than Kjeldahl distillation and titration. To determine the NH_4-N content using the Kjeldahl distillation method, follow steps 1 through 5 above, then proceed to Kjeldahl distillation. Care must be taken not to allow soil to dry and crack between ethanol leaching, as this could result in incomplete removal of excess NH_4-N. A similar procedure is described by Rhoades (1982).

Literature

Horneck, D. A.. J. M. Hart, K. Topper and B. Koespell. 1989. Methods of Soil Analysis used in the soil testing laboratory at Oregon State University. Ag. Expt. Station SM 89:4.

Rhoades, J.D. 1982. Cation exchange capacity. In: A.L. Page, R.H. Miller, and D.R. Keeney (eds.) Methods of soil analysis. Part 2. Agron. Monogr. 9, Am. Soc. Agron., Madison, WI. p. 149-157.

Schollenberger, C.J. and R.H. Simon. 1945. Determination of exchange capacity and exchangeable bases in soils-ammonium acetate method. Soil Sci. 59:13-24.

Thomas, G.W. 1982. Exchangeable cations. In A.L. Page (ed.). Methods of soil analysis, Part 2, 2nd ed. Agronomy Monograph 9, American Society of Agronomy, Madison, WI.

CATION EXCHANGE CAPACITY (CEC) S - 10.20

Barium Replacement Method

Scope and Application

Cation exchange capacity (CEC) is the measure of a soil ability to retain readily exchangeable cations which neutralize the negative charge of soils. This method involves saturation of the cation exchange sites with barium, equilibration, removal of the excess barium with ethanol, replacement and leaching replacement with ammonium. Other cations such as sodium have been used as the exchanging cation with measurement by atomic absorption spectrometer (Rhoades, 1982). This method differs from that using ammonium and is more appropriate for acid soils (pH < 7). For arid soils, pH > 7.5 and high in carbonates it is recommended to use the method of Polemio and Rhoades (1977). Whatever the replacement cation, the procedure is time consuming. The speed at which samples filter depends on the strength of the vacuum applied and sample makeup. Some samples may never filter because of clogged filter paper. The method detection limit is approximately 1.0 cmol kg^{-1} (meq/100 gm) (on a dry soil basis) and is generally reproducible within ± 10%.

Equipment

1. Analytical balance: 250 g capacity, resolution 0.01 g.
2. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute.
3. Repipette dispensers, calibrated to 20 ± 0.1, 8.0 ± 0.1 mL and 5.0 ± 0.1 mL.
4. Whatman No. 1, No. 2 or equivalent filter paper.
5. Buchner funnels with vacuum flasks and source of vacuum.
6. Atomic absorption spectrophotometer (AAS) or ICP-AES instrument.

Reagents

1. Deionized water, ASTM Type I grade.
2. Barium chloride extracting solution 0.5 N: Dissolve 122 g of $BaCl_2 \cdot 2H_2O$ into deionized water and dilute to 1000 mL.
3. Ethanol, 90%
4. Ammonium acetate (1.0 N) extraction solution neutral: Add 570 mL of glacial acetic acid, CH_3COOH (99%) to 8000 mL of deionized water. Add 680 mL of concentrated ammonium hydroxide adjust pH to 7.0 with 3.0 N glacial acetic acid or 3.0 N ammonium hydroxide and dilute to 10 L final volume.
5. Standard calibration solutions of Barium. Prepare six standard solutions of 5.0 - 100 mg L^{-1} of Barium prepared from 1000 mg L^{-1} stock solutions. Dilute solutions with 1.0 N ammonium acetate solution.

Procedure

1. Weigh 5.0 ± 0.1 g of air dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) into a 125 mL Erlenmeyer flask. Add 50mL of 0.5 N Barium chloride solution (See Comment #1 and #2) and place the flask reciprocating shaker for thirty (30) minutes. Include a method blank.
2. Connect a 1 L vacuum extraction flask to a Buchner funnel fitted with a Whatman No. 5 or equivalent filter paper. Moisten the filter paper with 2 mL of deionized water.
3. Transfer the soil suspension into the Buchner funnel and leach the sample with 100 mL of 1 N barium chloride.

4. Rinse the excess barium chloride from the soil sample in the Buchner funnel by leaching with a total volume of ethanol and discard the leachate. Note: Be sure to gently fill funnel to remove all excess barium and allow it to drain until only damp soil remains. Continue adding alcohol in this manner until 200 mL of ethanol has been used.
5. Change to a clean 500-mL suction flask and leach the soil sample with 225 mL of 1.0 ammonium acetate to replace the exchangeable barium. Bring leachate to volume in a 250 mL volumetric flask using deionized water.
6. The concentration of Ba in the final leachate can be determined using atomic absorption or ICP-AES. Record barium concentration to the nearest 10 mg L^{-1} (See Comment #3 and #4).

Calculation

$$\text{CEC in meq per 100 g of soil} = (\text{mg } L^{-1} \text{ Ba in leachate}) \times \frac{0.25}{69} \times \frac{100}{\text{sample size (g)}}$$

Comments

1. For soils high in salts (EC > 4 dSm^{-1}) wash the soil with 30 mL of deionized water to remove excess salts.

2. Check repipette dispensing volume calibration using an analytical balance.

3. Samples having barium concentrations exceeding the highest standard will require dilution and reanalysis.

4. This procedure used is essentially the same as that of Rhoades (1982), except that the barium concentration is increased. For strong acid soils, pH < 6, reduce the concentration of barium to 0.2 N such that the ionic strength matches that of the soil.

Literature

Chapman, H.D. 1965 Cation exchange capacity. In C.A.Black et al. (ed.) Methods of soil analysis. Agronomy 9:891-901. Am. Soc. of Agron., Inc. Madison WI.

Polemio, M. and J.D. Rhoades. 1977. Determining cation exchange capacity: A new procedure for calcareous and gypsiferous soils. Soil Sci. Soc. Am. J. 41:524-528.

Rhoades, J.D. 1982. Cation exchange capacity. In: A.L. Page, R.H. Miller, and D.R. Keeney (eds.) Methods of soil analysis. Part 2. Agron. Monogr. 9, Am. Soc. Agron., Madison, WI. p. 149-157.

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EXTRACTABLE SOIL SULFATE-SULFUR S - 11.10

Calcium Phosphate - Turbidimetric Method

Scope and Application

This method semiquantitatively determines the amount of sulfate-sulfur (SO_4-S) in soils by extraction with $Ca(H_2PO_4)_2 \cdot H_2O$ with subsequent determination of SO_4-S by turbidimetric measurement. Calcium phosphate is utilized to supress the dissolution of organic matter and for the removal of sulfate that maybe absorbed. Turbidimetric analysis is based on the formation of $BaSO_4$ crystals in a suspension and subsequent measurement of optical density. The turbidimetric method will require practice to become proficient. It is sensitive to high concentrations of dissolved soil organic carbon which may lead to an under estimation of SO_4-S (Ajwa and Tabatabai, 1993). Sulfate-sulfur can also be determined using ion chromatography using an AS4A column. Soil SO_4-S is only an index of plant available since sulfur is also available from the mineralization of organic matter, irrigation water and atmospheric deposition. The method has a detection limit of 2.0 mg kg^{-1} (dry basis) and is generally reproducible to with in ± 15%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.01 g.
2. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute.
3. Erlenmeyer flasks, 125 mL.
4. Magnetic stirrer.
5. Whatman No. 42, S&S 597 or equivalent highly retentive filter paper.
6. Repipette dispenser calibrated to 25.0 ± 0.2 mL
7. Pipette 10.0 mL.
8. Nephelometer, Turbidimeter or spectrophotometer 420 nm filter.

Reagents

1. Deionized water, ASTM Type I grade.
2. Calcium phosphate extraction solution, 0.08 M (containing 500 mg L^{-1} PO_4-P): Dissolve 2.03 g of analytical grade $Ca(H_2PO_4)_2 \cdot H_2O$ in 1000 mL of deionized water (see Comment #1).
3. Activated Charcoal (See Comment #2). Mix 100 g of Darco G-6 activated carbon with calcium phosphate extraction solution and thoroughly wet carbon. Cap container, shake, and filter slowly through buchner funnel. Wash three times with deionized water and verify removal of SO_4-S.
4. Acid "seed" solution, 20 mg L^{-1} S in 5.8 N HCl : Dissolve 0.1087 g analytical grade K_2SO_4 in 500 mL of deionized H_2O and add 500 mL of concentrated HCl. Add Teflon coated magnetic stir bar and place on stirrer. Add 5.0 g of powered gum acacia, or gelatin (See Comment #3) suspension agent slowly add 400 mL of 40 °C deionized water, dissolve lumps and bring to 500 mL. Bring to 1000 mL final volume with acetic acid, CH_3COOH (99%), slowly stirring.
5. Barium chloride crystals. Parr turbidimetric grade, $BaCl_2 \cdot 2H_2O$ crystals 20 - 30 mesh. Use high purity $BaCl_2$, as low purity may result in low recovery of SO_4-S (See Comment #4).
6. Standard SO_4-S calibration solutions. Prepare 100 mg L^{-1} SO_4-S calibration solution, dissolve 0.5434 g of oven dry K_2SO_4 in 500 mL of deionized water and dilute to one liter. Prepare six 100 mL calibration solutions of: 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 mg L^{-1} SO_4-S and diluted to final volume with calcium phosphate extraction solution.

Procedure

1. Weigh 10.0 ± 0.1 g of air dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) into 125 mL Erlenmeyer extraction flasks (See Comment #5). Add 25 mL of calcium phosphate extraction solution using repipette dispenser and place on reciprocating mechanical shaker for thirty (30)

minutes. Include a method blank.
2. Add 0.15 g of activated charcoal and shake for an additional three (3) minutes. Repeat with 25 mL aliquot of SO_4-S calibration solutions.
3. Filter extract through paper, refilter if filtrate is cloudy.
4. Place 10.0 mL aliquot of soil extract in 25 mL flask, and add 1.0 mL of seed solution and swirl. Repeat with SO_4-S calibration solution and method blank (See Comment #6 and #7).
5. Place flask on magnetic stirrer and add 0.3 g of $BaCl_2 \cdot 2H_2O$ crystals. Stir for five (5) minutes and then read optical density (or percent transmittance) on a nephelometer, turbidimeter or spectrophotometer at 420 nm (See Comment #8). Zero optical density with deionized water. Repeat with SO_4-S calibration solutions and method blank. Using standard calibration solutions determine SO_4-S concentration of soil extracts and method blank by plotting log of transmittance versus standard concentration. Record as mg L^{-1} of analyte in extract solution to the nearest 0.5 mg L^{-1}.

Calculations

Report soil sulfate-sulfur (SO_4-S):

mg kg^{-1} = (mg L^{-1} SO_4-S in soil extract - method blank) × (2.5)

Comments

1. For acidic soils (pH < 5.5) add 10 mL of concentrated HCl to extractant solution. Acidified phosphate extraction solutions are more reliable for use on acid soils which may contain absorbed sulfate.

2. For soils containing low concentrations of SO_4-S labile organic matter may prevent the formation of barium sulfate crystals resulting in a low bias SO_4-S concentration. Labile organic matter may be removed by the addition of activated charcoal or hydrogen peroxide. For organic soils the volume of extraction (1:5) solution must be increased to account for high potential SO_4-S concentrations.

3. A number of suspension agents have been reported in the literature which include: gum acacia, gelatin, glycerol, PVP-K30 (polyvinylpryrolidinone), and Tween 80 which have proven effective in turbidimetric analysis. Each of these will require experimentation and practice using SO_4-S spiking to fully refine the technique. For use of PVP-K30 (polyvinylpryrolidinone) add 10 g to 700 mL and dilute to 1000 mL final volume.

4. Use $BaCl_2$ specifically designated for turbidimetric determination of sulfate-sulfur. Sources: J.T. Baker Cat. Parr Turbidimetric $BaCl_2$, JT0974-5; VWR JT0974-5; and GFS Chemicals Reagent Grade ACS #602.

5. Pre-rinse all extraction flasks, turbidimetric and spectrometer cuvette in hot water followed by 0.5 N HCl rinse.

6. Check repipette volume, calibrate using an analytical balance.

7. HCl is included in the turbidimetric “seed” solution to prevent the precipitation of alkali metals with carbonate and phosphate and provide nucleus for initiating precipitation.

8. Development of $BaSO_4$ suspension requires continuous mixing and development may require from 1 - 10 minutes. It is essential that time of development be equivalent for both standards and unknown samples.

Literature

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SOIL CHLORIDE S - 12.10

Calcium Nitrate Extraction

Scope and Application

This method involves the quantitative extraction of chloride (Cl) from soils using 0.1 M $Ca(NO_3)_2$. Chloride in the extract is determined spectrophotometrically by complexation with mercury(II) thiocynate. The procedure outlined follows that outlined by Fixen, et al. (1988) for determining chloride and is readily adapted to manual or automated techniques. Chloride may also be determined using ion specific electrode using 0.5 M K_2SO_4 as the extractant. Care must be taken to avoid Cl contamination from filter paper and operator handling as chloride is a typical contaminate in laboratory operations. Mercury is a hazardous material, follow manufacturers recommendations in handling this material. Soil chloride can be used to predict small grain response to Cl fertilizers. The method detection limit is approximately 2.0 mg kg^{-1} (on a dry soil basis) and is generally reproducible ± 7%.

Equipment

1. Analytical balance: 1000 g capacity, resolution ± 0.1 g.
2. Repipette dispenser, calibrated to 25.0 ± 0.2 mL.
3. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute (opm).
4. Extraction vessels and associated filtration vessel (See comment #1).
5. Whatman No. 42 or equivalent highly retentive filter paper.
6. Spectrophotometer, autoanalyzer, or flow injection analyzer (FIA) instrument.

Reagents

1. Deionized water, ASTM Type I grade.
2. Calcium nitrate extracting solution, 0.1M $Ca(NO_3)_2$: Dissolve 4.72 g of reagent grade $Ca(NO_3)_2$ into 1200 mL deionized water and dilute to a 2 L in a volumetric flask.
3. Saturated mercury (II) thiocynate [$Hg(SCN)_2$] solution. 0.75%: Add approximately 0.75 g $Hg(SCN)_2$ to 1 L of distilled water and stir overnight. Filter through Whatman No. 42 paper. Saturated solutions maybe stored for long periods of time.
4. Ferric (III) nitrate nonahydrate [$Fe(NO_3)_3 \cdot 9H_2O$] solution, Dissolve 20.2 g of $Fe(NO_3)_3 \bullet 9H_2O$ in approximately 500 mL of deionized water and add concentrate HNO_3 until the solution is almost colorless. Dilute to 1 L final volume.
5. Standard calibration solutions of Cl. Prepare six calibration standards ranging from 0.5 to 10.0 mg L^{-1} concentration, diluted in calcium nitrate extracting solution.

Procedure

1. Weigh 10.0 ± 0.1 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into extraction vessel. Add 25.0 mL of calcium nitrate extracting solution, 0.1 $Ca(NO_3)_2$ using repipette dispenser (See Comment #2). Include a method blank.
2. Place extraction vessel(s) on reciprocating horizontal mechanical shaker for fifteen (15) minutes.
3. Filter extract (See Comment #3), refilter if filtrate is cloudy.
4. Chloride content of the extract is determined using a spectrophotometer, automated flow analyzer or FIA instrument. Calibrate using standard calibration solutions and operate instrument in accordance with manufacturer instructions. Determine chloride concentration of 0.1M $Ca(NO_3)_2$ extract, method blank, unknown samples and record results as mg L^{-1} of chloride in extract solution (See Comment #4 and #5).

Calculation

Cl mg kg^{-1} in soil = (Cl mg L^{-1} in filtrate - method blank) × 2.5

Report soil chloride concentration to the nearest 0.1 mg kg^{-1} (See Comment #6)

Comments

1. Rinse all extraction labware with 0.1M $Ca(NO_3)_2$ to minimize Cl contamination of unknown samples and standards.

2. Check repipette dispensing volume calibration using an analytical balance.

3. Check filter paper supply for possible contamination of and Cl. If contamination is greater than 0.5 mg L^{-1} on a solution basis, rinse filter paper with 0.1M $Ca(NO_3)_2$.

4. Samples having chloride concentrations exceeding the highest standard will require dilution and reanalysis.

5. Chloride can also be extracted with 0.5 M K_2SO_4 and determined using potentiometric known addition methods or extracted with 0.5 M $NaHCO_3$ with subsequent analysis using ion exchange chromatography.

6. Chloride (Cl) results can be expressed on a volume basis. Assuming the sample represents a 0-6 inch (0-15 cm) depth of the soil, then: Cl mg kg^{-1} × 2.0 ≅ Cl lbs ac^{-1}

Literature

Fixen, P.E., R.H. Gelderman and J.L. Denning. 1988. Chloride Tests. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. North Dakota Agr. Expt. Sta. Bulletin No. 499 (revised).

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Keeney, D.R. and D.W. Nelson. 1982. Nitrogen - inorganic forms. p. 643-698. *In* A.L. Page (ed.) Methods of soil analysis, part 2. Agron. Monogr. 9, 2nd ed. ASA and SSSA, Madison, WI.

Scope and Application

This method involves tests for the presence of carbonates in soil materials and is also maybe known as the "*fizz*" test. The method is based on the reaction of HCl with soil carbonates and visual observation of gaseous loss of CO_2 from the sample as described by the U.S. Salinity Laboratory Staff (1954). The method is not quantitative. Soils may be categorized as slightly reactive, moderately reactive or highly reactive. The method detection limit is approximately 0.2% $CaCO_3$ equivalent (on a dry soil basis).

Equipment

1. 50 mm watchglass.
2. Pipette dispenser, calibrated to 2.0 ± 0.2 mL.

Reagents

1. Deionized water, ASTM Type I grade.
2. Hydrochloric acid (HCl), 3 N. Transfer 250 mL of concentrated HCl to 500 mL of deionized water and dilute to 1.0 L with deionized water.

Procedure

1. Place 4 - 6 g of soil on a small watchglass. Using a pipette ad sufficient water to saturate the sample (See Comment #1). Using a pipette add a few drops of 3.0 N hydrochloric acid solution. Note any effervescence that occurs. The soil may be classified as slightly, moderately or highly calcareous in accordance to the degree of effervescence.

Comments

1. Water is added to the sample to displace soil air as to avoid confusion of the loss of soil air with effervescence of lime.

Literature

Allison L.E. and C.D. Moodie. 1965. Carbonate. P. 1379-1400. *In*: C.A. Black et al. (Ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA, CSSA and SSSA, Madison, WI.

Sobeck, A.A., W. A. Schuller, J.R. Freeman, R. M. Smith, 1978 Field and Laboratory Methods to Overburden and Minesoils. U.S. Department of Commerce, National Technical Information Service.

U.S. Salinity Lab. Staff. 1954. Methods for soil characterization. p. 83-147. *In*: Diagnosis and improvement of saline and alkali soils. Agr. Handbook 60, USDA, Washington, D.C.

Scope and Application

This method involves the quantitative determination of calcium carbonate by gravimetric analysis. The method is based on the reaction of HCl with calcium carbonate and the gravimetric loss of CO_2 from the sample as described by the U.S. Salinity Laboratory Staff (1954). Major sources of error are: evaporation of water and failure to adequately degas CO_2 from the sample. Soil carbonates are measured to determine soil buffering capacity with relation to soil fertility, chemical and pedogenic processes. The method detection limit is approximately 0.2% $CaCO_3$ equivalent (on a dry soil basis) and is generally reproducible ±10%.

Equipment

1. Analytical balance: 100 g capacity, resolution ± 0.1 mg.
2. Repipette dispenser, calibrated to 25.0 ± 0.2 mL.
3. Orbital mechanical shaker, capable of 180 oscillations per minute (opm).
4. Extraction vials, 70 mL with snap-lids having 1 mm holes for gas exchange or 50 mL Erlenmeyer flask with cap (See comment #1).

Reagents

1. Deionized water, ASTM Type I grade.
2. Hydrochloric acid (HCl), 3.0 M. Transfer 250 mL of concentrated HCl to 500 mL of deionized water and dilute to 1.0 L with deionized water.
3. Calcium carbonate ($CaCO_3$), fine ground (100 or 140 mesh sieve, 106-150 *u*m), reagent grade.

Procedure

1. Weigh to the nearest 0.1 mg a 70 mL extraction vial containing 10 mL of 3.0 M HCl and record tare weight. Transfer 2.000 to 5.000 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) containing 100 to 300 mg of $CaCO_3$ equivalent, in incremental units to avoid frothing. Accurately record the weight of soil transferred to the nearest 0.1 mg. Include three calcium carbonate standards ranging from 100 to 300 mg. After the effervescence has subsided replace snap-lid and place on orbital shaker for fifteen (15) minutes. Include 3 blanks to determine water vapor loss.
2. After two (2) hours weigh vial to the nearest 0.1 mg and record the mass. (See Comment #3 and #4). Verify recovery of calcium carbonate standards, approximately 100%).

Calculation

Weight loss of CO_2 (g) = Initial weight (g) - final weight (g) (vial +stopper +acid + soil)

$$CO_3\text{-C}, \% = \left(\frac{\text{g } CO_2 \text{ lost}}{\text{g air-dry soil}}\right)(0.2727) \times 100 \qquad \text{[equ. S -13.1-1]}$$

$$CaCO_3\text{-C}, \% = \left(\frac{\text{g } CO_2 \text{ lost}}{\text{g air-dry soil}}\right)(2.273) \times 100 \qquad \text{[equ. S -13.1-2]}$$

Comments

1. Vials snap-lids should be large enough to permit gas exchange of CO_2, yet small enough to minimize loss of water vapor.

2. Soil weight should be less that 4.0 g for soils with less than 20% $CaCO_3$, 2.0 g for soils 20 to 40% $CaCO_3$.

3. Use blank subtraction to compensate for water vapor loss. If water vapor loss is > 0.003 g subtract water vapor loss (g) from g CO_2 weight loss.

4. To convert from $CaCO_3$-C, % to total inorganic carbon (TIC) multiply value by 0.12 .

Literature

Allison L.E. and C.D. Moodie. 1965. Carbonate. P. 1379-1400. *In*: C.A. Black et al. (Ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA, CSSA and SSSA, Madison, WI.

Loeppert, Richard H. And Donald L. Suarez. 1996. Carbonate and gypsum. p. 437-474. *In:* J.M. Bartels et al. (ed.) Methods of soil analysis: Part 3 Chemical methods. (3rd.ed.) ASA and SSSA, Madison, WI. Book Series no. 5

U.S. Salinity Lab. Staff. 1954. Methods for soil characterization. p. 83-147. *In*: Diagnosis and improvement of saline and alkali soils. Agr. Handbook 60, USDA, Washington, D.C.

Scope and Application

This method involves the quantitative determination of inorganic carbon by volumetric displacement. The method is based on the reaction of HCl with carbonate and the measurement of the loss of CO2 based on the equations described by Loeppert and Suarez (1996) and by Wagner et al., (1998). The method range is from 0.25 to 100% percent CaCO3 for a 20 mL serum bottle used as the reaction vessel. The method range for the 100 mL serum vial used as the reaction vessel is 2.0 to 100 percent. Soil inorganic carbon is measured to determine soil buffering capacity with relation to soil fertility, chemical and pedogenic processes and to obtain organic carbon from combustion methods that produce total carbon. The method detection limit is approximately 0.25 % CaCO3 and is reproducible within the ± 5% using the 20 mL serum bottles and 2.0% for the 100 mL serum bottles.

Equipment

1. Analytical balance: 100 g capacity, resolution ± 0.1 mg.
2. Repipette dispenser, calibrated to 2.0 mL.
3. Reaction vessels, 100 mL capacity wheaton serum bottles.
4. 0.5 dram vials (2.0 mL capacity).
5. Gray butyl rubber stoppers.(See comment #1).
6. Tear-off Aluminum serum bottle seals.
7. Hand-operated crimpers.
8. Power supply (24 volt DC. - 2 amp).
9. Digital voltage meter, capable of reading 0.01 volts resolution.
10. Pressure transducer 0-105 kPa (Setra Model 280E).

Apparatus

1. The pressure transducer is connected to the power supply with 14 gauge wire with a digital volt meter wired in line to monitor the output.
2. Attached to the base of the pressure transducer is 20 cm of tubing attached to a 18 gauge Luer-loc hypodermic needle with a particle filter in the middle to collect any reflux from reaching the pressure transducer.

Reagents

1. Deionized water, ASTM Type I grade.
2. Calcium carbonate ($CaCO_3$), fine ground (100 mesh sieve, 150 um), reagent grade.
3. Hydrochloric acid (HCl) 6N with 3% by weight ferrous chloride). Transfer 500 mL of HCl to 400 mL of deionized water and add 30 g of $FeCl_2 \cdot 4H_2O$ and bring to 1.0 L volume with deionized water.
4. Prepare $CaCO_3$ standards of 0.25, 0.50, 1, 2, 4, 8, and 15 percent by weight using laboratory sand that has been powder ground and oven dried reagent grade $CaCO_3$ for 20 mL reaction vessels for the 0-30 percent range. For soils higher than 30% $CaCO_3$, use 100 mL reaction vessels with standard concentrations of 10, 20, 30, 40, 50 and 80% $CaCO_3$.

Procedure

1. Weigh 1.00 g of soil into a 20 mL Wheaton serum bottle if soil is expected to have less than 30% $CaCO_3$ or 2.00g of soil into 100 mL Wheaton serum bottle if sample is expected to have more than 30% $CaCO_3$. Use soil screened to pass through a 2mm sieve. Place $CaCO_3$ appropriate standards into 20 mL or the 100 mL Wheaton serum bottle (See Comment #1).
2. Pipet 2.0 mL of 6.0N HCl reagent into 0.50 dram glass vial. Gently insert acid dram vial into reaction vessel with sample, but do not allow solution to mix with sample. Cap reaction vessel with gray butyl rubber stoppers and crimp with aluminum tear-off seals.
3. Shake reaction vessel vigorously to ensure that acid solution in the dram vial has mixed with the soil. Run three blanks (l.00 g of laboratory sand with acid vial) with each analysis run.
4. Prior to reading the samples on the pressure transducer (15 min), rotate the acid along the sides of the reaction vessel to ensure that soil on the sides is reacting with the acid.
5. After two (2) hours of reaction time with the acid the samples and standards are ready to read on the pressure transducer.
6. Record the voltage output to 2 decimal places. Subtract the average voltage of the blanks from the standards and samples to obtain the change in pressure due to CO_2.

Calculation

Using linear regression determine the slope (regression coefficient) and the intercept (b) of the curve of pressure change vs. the dependent variable of percent $CaCO_3$. Inorganic carbon can be obtained by dividing the formula weight of $CaCO_3$ (100) by the formula weight of carbon (12) and multiply this by the % $CaCO_3$.

$$\% \ CaCO_3 = (\text{regression coefficient}) \times (\text{delta pressure in volts}) + b.$$

$$\% \ \text{Inorganic Carbon} = \% \ CaCO_3 / 8.33 \qquad \text{[equ. S -13.3-1]}$$

$$\text{Inorganic carbon g/kg} = 10 \times (\text{Inorganic Carbon percent})$$

Comments

1. Caution should be used when there is no information available on $CaCO_3$ content of the soil as vessels may become over pressurized. Use 1.00 g of soil and the 100 mL Wheaton serum bottles as a pre-screen of of $CaCO_3$ content prior to quantitative determination using the appropriate mass and bottle. Sample size maybe increased to 2.0 g using the 20 mL Wheaton serum bottle for samples containing less than 15% $CaCO_3$ to improve method detection and precision.

2. Soils containing dolomite need longer reaction times with acid.

3. Use caution when shaking the reaction vessel (serum bottle containing sample and acid vial) as cracks in the serum bottle can occur with reuse which can weaken the glass if excessive pressure's are encountered.

4. Check 20 and 100 mL serum reaction vessels for irregularities. Discard if irregularities in glass is found.

Literature

Wagner, S.W., J.D. Hanson, Alan Olness, and W.B. Voorhees. 1998. A volumetric inorganic carbon analysis system. Soil Sci. Soc. Am. J. 62:690-693.

Loeppert, Richard H. and Donald L. Suarez. 1996. Carbonate and gypsum. p. 437-474. In: J.M. Bartels et al. (ed.) Methods of soil analysis: Part 3 Chemical methods. (3rd.ed.) ASA and SSSA, Madison, WI. Book series no. 5.

Scope and Application

This method quantitatively determines the physical proportions of three sizes of primary soil particles as determined by their settling rates in a aqueous solution using a hydrometer. Proportions are represented by stated class sizes: sand ranging from 2000 - 50 *u*m; silt ranging from 50 - 2.0 *u*m and clay < 2.0 *u*m and those stated by the USDA Soil Survey and Canadian Soil Survey Committee. Settling rates of primary particles are based on the principle of sedimentation as described by Stokes' Law and measured using a hydrometer. The use of the ASTM 152H-Type hydrometer is based on a standard temperature of 20 °C and a particle density of 2.65 g cm^{-3} and units are expressed as grams of soil per liter. For specific samples the method may require the pretreatment removal of soluble salts, organic matter, carbonates and iron oxides with subsequent dispersion using sodium hexametaphosphate (Day 1965). Corrections for temperature and for solution viscosity is made by taking a hydrometer reading of a blank solution. For further information consult Gee and Bauder (1986). Generally this method is of lower precision than the pipette or sedimentation methods and is used to determine soil texture. The method has a detection limit of 2.0% sand, silt and clay (dry basis) and is generally reproducible to within ± 8%.

Equipment

1. Analytical balance: 100 g capacity, resolution ± 0.01 g.
2. Standard hydrometer, ASTM No. 1. 152H-Type with Bouyoucos scale in g L^{-1}.
3. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute.
4. Sedimentation cylinder with 1.0 L mark 36± 2 cm from the bottom.
5. Shaker bottle 200 mL and cap (polypropylene or glass).

Reagents

1. Deionized water, ASTM Type I grade.
2. Amyl alcohol.
3. Sodium Hexametaphosphate (HMP), 5% dispersing solution. Dissolve 50.0 g Na-hexametaphosphate in 1.0 L.

Procedure

1. Weigh 40.0 ± 0.05 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into 200 mL container (See Comments #1, #2, #3 and #4). Determine oven dry soil moisture on a 2nd sample of soil.
2. Add 100 mL of HMP solution, cap and place on reciprocating horizontal shaker for sixteen (16) hours (See Comment #5).
3. Quantitatively transfer the suspension to the sedimentation cylinder and add deionized water to bring to 1.0 L final volume.
4. Allow the suspension to equilibrate to room temperature for two (2) hours.
5. Insert plunger and thoroughly mix contents, dislodging sediment from the bottom of the cylinder. Finish stirring with two or three smooth stokes. As an alternative mixing procedure stopper the cylinder and used end over end shaking for one (1) minute. Add 2 mL of amyl alcohol to the surface to suspensions covered in foam. Repeat the process and determine hydrometer reading on a blank solution and to the nearest ±0.5 g L^{-1} as R_{C1}.
6. Lower the hydrometer carefully into the suspension after thirty (30) seconds and take a reading after forty (40) seconds and record to the nearest ±0.5 g L^{-1} as R_{sand} (See Comment #6).
7. Remove the hydrometer carefully, rinse and wipe dry.
8. After six (6) hours record temperature of the suspension to the nearest ±1°C. Using the temperature correction values in Table 14.1-A determine the settling time for the 2.0 *u*m size fraction (See Comment #7). Based on time after initiation of settling, reinsert the hydrometer carefully and take a

reading and record as R_{clay} to the nearest ±0.5 g L^{-1}. Repeat the process determining hydrometer reading on a blank solution and record as R_{C2} to the nearest ±0.5 g L^{-1} (See Comments #8).

Table 14.10-A The influence of suspension temperature on the hydrometer determination of soil clay (2 *u*m) based on a particle density of 2.65 g cm^{-3} and a solution density of 0.5 g L^{-1}.

Temperature °C	Settling time for clay hours and minutes
18	8:09
19	7:57
20	7:45
21	7:35
22	7:24
23	7:13
24	7:03
25	6:53
26	6:44
27	6:35
28	6:27

Gee and Bauder (1986).

Calculations

Report results to the nearest 0.1% content (See Comment #9 and 10):

$$\text{Sand \%} = \frac{\text{(oven dry soil mass)} - (R_{sand} - R_{C1})}{\text{(oven dry soil mass)}} \times 100 \qquad \text{[equ. S -14.1-1]}$$

$$\text{Clay \%} = \frac{(R_{clay} - R_{C2})}{\text{(oven dry soil mass)}} \times 100 \qquad \text{[equ.S -14.1-2]}$$

$$\text{Silt \%} = 100 - (\text{Sand \%} + \text{Clay \%}) \qquad \text{[equ. S -14.1-3]}$$

Comments

1. The exact sample size is soil texture dependent. For fine textured soils, silts or clays, 10 - 20 g may be adequate. For coarse textured soils 60 - 100 g will be needed in order to obtain reproducible results. For moist soils dry overnight at 105 °C and correct for moisture content.

2. For soils containing carbonates ($CaCO_3$ >2.0%, see Methods 13.1 or 13.2) and/or high in soluble salts (EC_e > 2.0 dS m^{-1}) it is recommended soils be pre-treated. Place 40.0 g of soil in 250 mL centrifuge tube, add 100 mL deionized water and 10.0 mL of 1.0 M Na acetate (pH 5.0). Mix, and centrifuge for 10 min at 1500 rpm) until the supernatant is clear. Decant and wash two more times with 50 mL of deionized water.

3. For soils containing organic matter contents greater than 3.5%, after removal of carbonates, add 25 mL

of water and add 5 mL of H_2O_2 to the suspension. If excessive frothing occurs, cool and add additional H_2O_2 when reaction subsides. Heat to 90 °C when frothing ceases. Continue treatment until organic matter is oxidized (as judged by rate of reaction and bleached color).

4. For removal of Iron oxides add 150 mL to the H_2O_2 treated sample of a solution 0.3 M sodium citrate and 84 g/L sodium bicarbonate. Shake for 30 minutes to disperse the soil and add 3.0 g of sodium dithionite ($Na_2S_2O_4$). Place in water bath 80 °C and stir intermittently for 20 minutes. Remove and add 10 mL of a 10% NaCl solution, centrifuge and decant. If sample is brownish in color repeat with the sodium citrate - sodium bicarbonate step. If sample is gleyed (gray), repeat with 10% solution of NaCl, and two deionized water rinses. Proceed with HMP addition.

5. It is recommended to use horizontal reciprocating shaking for the dispersing the samples. The use of electric stirrer at high rpm may result in significant grinding of sample primary minerals.

6. It may be advantageous to take a reading at 35 seconds and a 2nd at 45 seconds an record the average as the hydrometer value is dynamic.

7. The hydrometer procedure has been used by number of laboratories. Readings are frequently made at 40 seconds and two (2) hours for the determination of sand and clay. Based on theoretical considerations the two (2) hour reading is a estimate of the 5.0 *u*m fraction and errors often exceed 10% by weight from that of actual clay (Gee and Bauder, 1979).

8. For determining sand fractions, quantitatively transfer the sediment suspension through a 270 mesh (53.0 *u*m) sieve and wash with deionized water using a wash bottle. Transfer the sand to a tared beaker, dry at 105 °C and weigh. The dried sand may be placed in nested sieves to determine individual sand fraction size analysis.

9. For soils having clay particle densities less than 2.65 g cm^{-3}, settling time will increase and for soils greater than 2.65 g cm^{-3} it will decrease, consult Gee and Bauder (1986).

10. An error of 1.0 ± g L^{-1} in the hydrometer reading results in an error of ± 2.5% for clay size Fraction and on a percentage basis translates to an error of ±12.5% for 40 g of soil containing 20% clay. For more accurate and precise measurement of silt and clay size fractions it recommended that the pipette method be used which has a precision of ±2-3%.

11. Using soil size particle analysis data, soil textural classification can be determined (Appendix E). From texture an approximate bulk density can be determined (Saxton, et al. 1986).

Literature

American Society for Testing and Materials. 1985d Standard test method for particle-size analysis of soils D 422-63 (1972). 1985 Annual Book of ASTM Standards 04.08:117-127. American Society for Testing Materials, Philadelphia.

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Gee, G. W., and J. W. Bauder. 1986. Particle-size Analysis. P. 383 - 411. *In* A.L. Page (ed.). Methods of soil analysis, Part 1, Physical and mineralogical methods. Second Edition, Agronomy Monograph 9, American Society of Agronomy, Madison, WI.

Saxton, K.E., W.J. Rawls, J.S. Romberger, and R.I. Papendick. 1986. Estimating generalized soil-water characteristics from texture. Soil Sci. Soc. Am. J. 50(4):1031-1036.

U.S. Salinity Lab. Staff. 1954. Methods for soil characterization. p. 83-147. Diagnosis and improvement of saline and alkali soils. Agr. Handbook 60, USDA, Washington, D.C.

Scope and Application

This method quantitatively determines the physical proportions of three sizes of primary soil particles as determined by their settling rates in a aqueous solution using a pipette. Proportions are represented by stated class sizes: sand ranging from 2000 - 50 *u*m; silt ranging from 50 - 2.0 *u*m and clay < 2.0 *u*m and those stated by the USDA Soil Survey and Canadian Soil Survey Committee. Settling rates of primary particles are based on the principle of sedimentation as described by Stokes' Law and measured using a pipette. For specific samples the method may require the pretreatment removal of soluble salts, organic matter, carbonates and iron oxides with subsequent dispersion using sodium hexametaphosphate (Day 1965). For further information consult Gee and Bauder (1986). Generally this method is more precise than the hydrometer method. The method has a detection limit of 0.2% sand, silt and clay (dry basis) and is generally reproducible to within ± 5%.

The Method is based on Stoke's Law: $v=g(p_s-p_l)\,x^2/18\,\eta$

Where v = velocity of fall

g = acceleration due to gravity, 980 cm s^{-2}

p_s = particle density

p_l = liquid density

x = particle diameter

η = fluid viscosity

and the following equation: $t = 18\eta h/[v=g(p_s-p_l)x^2]$

t = time for pipetting

h = depth of pipette

Equipment

1. 50 mL conical centrifuge tubes with caps
2. Analytical balance: 100 g capacity, resolution ± 0.0001 g.
3. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute.
4. Adjustable 5.0 mL pipette, pipette tip and stopper (place on pipette tip for accuracy of depth. Adjust tip to drop to a depth 2.5 cm below surface of centrifuge tube containing 40.0 mL of solution.
5. Repipette dispenser, calibrated to 50.0 ± 0.2 mL.

Reagents

1. Deionized water, ASTM Type I grade.
2. Sodium Hexametaphosphate (HMP), 0.5% dispersing solution. Dissolve 5.0 g Na-hexametaphosphate in 1.0 L.

Procedure

1. Weigh 5.0 ± 0.003 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into 40.0 mL centrifuge tube (See Comments #1, #2, #3 and #4).
2. Add 40 mL of HMP solution, cap and place on reciprocating horizontal shaker for sixteen (16) hours (See Comment #5).
3 Pre-weigh tins which have been in dessicator and record tin tare weight to nearest 0.0001 g.
4. After 16 hour shaking, hand shake centrifuge tube to disperse soil allow to settle for the required time according to Table S-14.2-A. Time begins once settling has started. Complete silt + clay fraction at time specified through entire sample set and return for clay fraction at time state in Table S-14.2-1 for clay.
5. Dispense 2.5 mL fraction of the solution in weigh tin and place in drying oven.

6. Pre-weigh tins which have been in dessicator and record tin tare weight to nearest 0.0001 g (See Comment #7).
7. Remove dried tins containing soil residue and allow to cool, dessicate and record Dry Weight to the nearest 0.0001 g. (See Comment #8 and #9).

Table S-14.2-A Sampling times for silt+clay and clay pipette method. Assumptions: Density of particles (p_s) is 2.65 g cm^{-3}; sampling depth 2.5 cm below liquid surface; g = 9.8 m s^{-2}; clay = 0.002 *u*m).

Temperature °C	Sand 100% (Silt+Clay) time (sec)	Clay Time (min)
16	11.7	122.3
18	11.2	116.4
20	10.6	110.9
22	10.2	105.7
24	9.7	101
26	9.3	96.6

Calculations

Report results to the nearest 0.1% content (See Comment #9 and 10):

[equ. S-14.2-1] Sand % = 100 - (%Silt + Clay)
= 100 - [(((Dry Weight - Tin Weight) - Blank) x 40/2.5)/5)x100]

[equ. S-14.2-2] Clay % = (((Dry Weight - Tin Weight) - Blank) x 40/2.5)/5) x 100

[equ. S-14.2-3] Silt % = (100 - %Sand) - %Clay

Comments

1. The exact sample size is soil texture dependent. For fine textured soils, silts or clays, 2.0 - 4.0 g may be adequate. For coarse textured soils 6.0 - 10.0 g will be needed in order to obtain reproducible results. For moist soils dry overnight at 105 °C and correct for moisture content.

2. For soils containing carbonates ($CaCO_3$ >2.0%, see Methods 13.1 or 13.2) and/or high in soluble salts (EC_e > 2.0 dS m^{-1}) it is recommended soils be pre-treated. Place 5.0 g of soil in 50 mL centrifuge tube, add 10 mL deionized water and 1.0 mL of 1.0 M Na acetate (pH 5.0). Mix, and centrifuge for 10 min at 1500 rpm) until the supernatant is clear. Decant and wash two more times with 50 mL of deionized water. Dry and determine particle sizes.

3. For soils containing organic matter contents greater than 3.5%, after removal of carbonates, add 10 mL of water and add 5 mL of H_2O_2 to the suspension. If excessive frothing occurs, cool and add additional H_2O_2 when reaction subsides. Heat to 90 °C when frothing ceases. Continue treatment until organic matter is oxidized (as judged by rate of reaction and bleached color).

4. For removal of Iron oxides add 20 mL to the H_2O_2 treated sample of a solution 0.3 M sodium citrate and 84 g/L sodium bicarbonate. Shake for 30 minutes to disperse the soil and add 0.40 g of sodium dithionite ($Na_2S_2O_4$). Place in water bath 80 ºC and stir intermittently for 20 minutes. Remove and add 1.5 mL of a 10% NaCl solution, centrifuge and decant. If sample is brownish in color repeat with the sodium citrate - sodium bicarbonate step. If sample is gleyed (gray), repeat with 10% solution of NaCl, and two deionized water rinses. Proceed with HMP addition.

5. It is recommended to use horizontal reciprocating shaking for the dispersing the samples. The use of electric stirrer at high rpm may result in significant grinding of sample primary minerals.

6. For determining sand fractions, quantitatively transfer the sediment suspension through a 270 mesh (53.0 *u*m) sieve and wash with deionized water using a wash bottle. Transfer the sand to a tared beaker, dry at 105 °C and weigh. The dried sand may be placed in nested sieves to determine individual sand fraction size analysis.

7. For soils having clay particle densities less than 2.65 g cm^{-3}, settling time will increase and for soils greater than 2.65 g cm^{-3} it will decrease, consult Gee and Bauder (1986).

8. An error of 0.001±g in the dry weight of the pipette sample results in an error of ± 0.32% for clay size fraction.

9. Using soil size particle analysis data, soil textural classification can be determined (Appendix E). From texture an approximate bulk density can be determined (Saxton, et al. 1986).

Literature

American Society for Testing and Materials. 1985d Standard test method for particle-size analysis of soils D 422-63 (1972). 1985 Annual Book of ASTM Standards 04.08:117-127. American Society for Testing Materials, Philadelphia.

Day, P.R. 1965. Particle fractionation and particle-size analysis p. 545-567. *In* C.A. Black et al (ed.) Methods of soil analysis, Part 1. Agronomy 9:545-567.

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Saxton, K.E., W.J. Rawls, J.S. Romberger, and R.I. Papendick. 1986. Estimating generalized soil-water characteristics from texture. Soil Sci. Soc. Am. J. 50(4):1031-1036.

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Scope and Application

This method involves the semiquantitative extraction of exchangeable aluminum from soils using 2.0 N KCl. Aluminum is determined by Inductively Coupled Plasma spectrometry (ICP-AES). The method doesn't quantitatively extract aluminum from mineral structures or bound to organic compounds. Care must be taken to avoid contamination from filter paper and operator handling. Soil aluminum concentrations are generally low in mineral soils (< 1.0 mg kg^{-1}), with the exception of soils with a soil:water (1:1) pH less than 5.40 (Method S - 2.30). The method detection limit is approximately 0.5 mg kg^{-1} (on a dry soil basis) and is generally reproducible ± 10%.

Equipment

1. Analytical balance: 1000 g capacity, resolution ± 0.01 g.
2. Repipette dispenser, calibrated to 25.0 ± 0.2 mL.
3. Reciprocating horizontal mechanical shaker, 180 oscillations per minute (opm).
4. Extraction vessels and associated filtration vessel.
5. Whatman No. 42 or equivalent highly retentive filter paper.
6. Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).

Reagents

1. Deionized water, ASTM Type I grade.
2. Potassium chloride extracting solution, 1.0 N KCl: Dissolve 75 g of reagent grade KCl in 500 mL deionized water and dilute to a 1000 mL (See Comment #1).
3. Standard calibration solutions of NH_4-N. Prepare five calibration standards ranging from 0.1 to 50.0 mg L^{-1} concentration, diluted in 1.0 N KCl extraction solution prepared from 1000 mg L^{-1} Al standard solution.

Procedure

1. Weigh 5.0 ± 0.05 g of air-dried soil pulverized to pass 10 mesh sieve (< 2.0 mm) into extraction vessel. Add 25.0 mL of 1.0 N KCl extraction reagent using repipette dispenser (See Comment #2). Include a method blank.
2. Place extraction vessel(s) on reciprocating mechanical shaker for thirty (30) minutes.
3. Filter extract (See Comment #3), refilter if filtrate is cloudy (comment #4).
4. Aluminum content of the extract is determined using a Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Suggested wavelengths are: 309.271, 396.152 and 237.335 nm. Adjust and operate instrument in accordance with manufacturer's instructions. Determine aluminum concentration of a method blank reference checks and unknown samples (See Comment #5).

Calculation

$$\text{Al mg kg}^{-1} \text{ in soil} = (\text{Al mg L}^{-1} \text{ in filtrate} - \text{method blank}) \times 5$$

Report soil Al concentration to the nearest 0.1 mg kg^{-1} (See Comment #5)

Comments

1. Soils may be extracted with 1.0 N KCl for the simultaneous determination of nitrate (Method 3.10).

2. Check repipette dispensing volume calibration using an analytical balance.

3. Check filter paper supply for possible contamination of and Al. If contamination is greater than 0.2 mg L^{-1} on a solution basis, rinse filter paper with 1.0 N KCl.

4. It is recommended that soils extracted for aluminum be analyzed with in two (2) hours after extraction.

5. Samples having aluminum concentrations exceeding the highest standard will require dilution and reanalysis.

Literature

Bertsch, P.M. and P.R. Bloom. 1996. Aluminum. p. 517-550. *In:* J.M. Bartels et al. (ed.) Methods of soil analysis: Part 3 Chemical methods. (3rd.ed.) ASA and SSSA, Madison, WI. Book series no. 5

Keeney, D.R. and D.W. Nelson. 1982. Nitrogen - inorganic forms. *In* A.L. Page (eds.) Methods of soil analysis, part 2. Agron. Monogr. 9, 2nd ed. ASA and SSSA, Madison, WI. p. 643-698.

Scope and Application

The method semi-quantitatively determines the concentration of Al, Sb, As, Ba, Be, Cd, Ca, Cr, Co, Cu, Fe, Pb, P, Mg, Mn, Mo, Na, Ni, K, Se, Ag, Na, Th, Ti, V, and Zn in soil materials utilizing a nitric acid extraction/dissolution in conjunction with heating on a hot plate. This method closely follows that outline din EPA method 3050A. Digest analyte concentrations are determined by atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). Phosphorus, S, and B, analyses require an ICP-AES with a vacuum spectrometer. Potassium, Ca, Mg, Na, Zn, Cu, Mn, and Fe can be analyzed by AAS or ICP-AES. Nitric acid digests may not provide 100% recovery of Al, Si, Fe, and Se. The method has a detection limit of approximately 0.01% for P, K, Ca, and Mg and 0.2 mg kg^{-1} for B, Zn, Cu, Fe, Mn and Mo (sample dry basis). The method can also be used for the determination of trace-elements (Co, Cd, Ni, Pb, etc.) and is generally reproducible within ± 7.0% for all analytes.

Equipment

1. Analytical balance: 100.0 g capacity, resolution ± 0.1 mg.
2. Hot plate system, capable of 150 ºC.
3. Repipette dispensers, calibrated to 0.5 ± 0.05 mL and 2.0 ± 0.08 mL
4. Polypropylene or teflon digest beaker, 50 mL volume.
5. Atomic Absorption Spectrophotometer (AAS) and/or Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), vacuum or purged system.

Reagent

1. Deionized water, ASTM Type II grade.
2. Micro® clean detergent.
3. Concentrated nitric acid, trace metal grade, 12 N.
4. Concnetrated Hydrochloric Acid, ACS reagent grade.
5. Standard Calibration solutions of Al, Sb, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, P, Mg, Mn, Hg, Mo, Ni, K, S, Se, Ag, Na, Sr, Th, Ti, V, and Zn. Prepare five multielement standards: of K, Ca, Mg ranging from 5 - 500 mg L^{-1}; P, S, and Na ranging from 1.0 - 100 mg L^{-1}; and B, Zn, Mn, Fe, Mo and Cu, ranging from 0.10 - 10.0 mg L^{-1}. Dilute standard calibration solutions with 5 % nitric acid.

Procedure

1. Weigh 1000 ± 5.0 mg of of air-dried soil pulverized to pass 20 mesh sieve (< 0.80 mm) soil material (See Comment #1, #2 and #3) and place in appropriate microwave vessel. For hydrocabon contaminated soils use no more than 200.0 mg. Include a method blank.
2. Using repipettes add 9.0 ± 0.1 mL of trace metal grade concentrated nitric acid and 3.0 ± 0.1 mL of concentrated hydrochloric acid. Ensure that the sample is completely wetted by the reagents.
3. Place digestion beaker on hot plate at 120 ºC for 4 hours and allow to digest.

6. Quantitatively transfer the sample to polypropylene labware. Samples containing suspended particulates will require centrifugation or filtering.
7. Quantitatively transfer the contents of the digestion vessel into the centrifuge tube, dilute to 15 mL final volume, cap centrifuge tube, invert three times and store (See Comment #5, #6 and #7).
8. Elemental analysis of soil digests can be made using atomic absorption spectrometry (AAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES), or other methodologies. The method chosen will determine specific matrix modifications, calibration standards used, and the need for instrument specific sample preparations and dilutions. Determination of trace elements by ICP-AES (Co, Cd, Ni, Mo, Pb) maybe facilitated by the use of an ultrasonic nebulizer (Soltanpour, 1996). Adjust and operate instruments in accordance with manufacturer's instructions. Calibrate instrument using calibration solutions. Determine the analyte concentrations of a method blank, unknown samples and record concentrations in mg L^{-1}.

Calculations

Report Elemental constituents to the nearest 3 significant digits as mg kg^{-1}:

$$\text{Analyte Content} = \frac{(\text{mg } L^{-1} - \text{method blank}) \times (30) \times (0.0001)}{\text{Sample Mass (mg)}}$$

Comments

1. Teflon PFA digestion vessel (liners) should be cleaned according to the following procedure: (1) soak liners in 1% solution of labware detergent for one hour; (2) rinse vessels in tap water; (3) rinse in solution of 0.5 N HCl; (4) three deionized water rinses (ASTM Type I grade); and (5) dry for one hour at 80 °C. Do not brush containers to clean.

2. Sample material must be ground to pass a 20 mesh screen (< 0.40 mm opening), to ensure homogeneity.

3. Check repipette dispensing volume, calibrate using an analytical balance.

4. When adding reagent to vessels always wear protective clothing (i.e. eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of high air flow, 100 cfm.

5. Centrifuging may be necessary to clear the digest.

6. Samples having analyte concentrations exceeding the highest standard will require dilution and reanalysis.

7. Place 3.0 mL of concentrate Micro® clean detergent (Baxter Scientific) in digestion vessel and allow to stand 30 minutes, rinse out any particulate, and finish cleaning according to set vessel cleaning procedure.

Literature

Kalra, Y. P., D. G. Maynard, and F. G. Radford. 1989. Microwave digestion of tree foliage for multi-element analysis. Can. J. For. Res. 19:981-985.

Kingston, H. M. and L. B. Jassie. 1986. Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. Anal Chem. 58:2534-2541.

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Stripp, R. A. and D. Bogen. 1989. The rapid decomposition of biological materials by using microwave acid digestion bomb. J. Anal. Toxic. 13:57-59.

Scope and Application

The method semi-quantitatively determines the concentration of Al, Sb, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, P, Mg, Mn, Hg, Mo, Ni, K, S, Se, Ag, Na, Sr, Ti, V, and Zn in soil materials utilizing a nitric acid/hydrochloric acid extraction/dissolution in conjunction with microwave heating in closed teflon vessels. This method closely flows that outlined in EPA method 3051A. Digest analyte concentrations are determined by atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). Potassium, Ca, Mg, Na, Zn, Cu, Mn, and Fe can be analyzed by AAS or ICP-AES. Microwave nitric acid/hydrogen peroxide digests may not provide 100% recovery of all metals. The method has a detection limit of approximately 1.0 mg kg^{-1} mg for Al, Ca, Fe, K, Mg, Na, P, and S; and 0.01 mg kg^{-1} for Sb, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Pb Mn, Hg, Mo, Ni, Se, Ag, Na, Sr, Ti, V, and Zn (sample dry basis). The method is generally reproducible within ± 7.0% for all analytes.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.1 mg.
2. Microwave digestion system and teflon double wall digestion vessels equipped with a controlled pressure relief mechanism and temperature and/or pressure feedback control (See Microwave Calibration, Comment #1).
3. Repipette dispensers, calibrated to 0.5 ± 0.05 mL and 2.0 ± 0.08 mL
4. Polypropylene centrifuge tube with cap, 15 mL graduated.
5. Atomic Absorption Spectrophotometer (AAS) and/or Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), vacuum or purged system.

Reagent

1. Deionized water, ASTM Type II grade.
2. Micro® clean detergent.
3. Concentrated nitric acid, trace metal grade, 12 N.
4. Concentrated hydrochloric acid.
5. Standard Calibration solutions of Al, Sb, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, P, Mg, Mn, Hg, Mo, Ni, K, S, Se, Ag, Na, Sr, Ti, V, and Zn. Prepare five multi element standards: Dilute standard calibration solutions with 5% nitric acid.

Procedure

1. Weigh 500± 5.0 mg of air-dried soil pulverized to pass 20 mesh sieve (< 0.800 mm) soil material (See Comment #2, and #3) and place in appropriate microwave vessel. For oil contaminated soils use no more than 200.0 mg. Include a method blank.
2. Using repipettes add 9.0 ± 0.1 mL of trace metal grade concentrated nitric acid and 3.0 ± 0.1 mL concentrated hydrochloric acid (See Comments #4 and #5). Ensure that the sample is completely wetted by the reagents.
3. Place digestion vessel in outer body shell, cap and allow the sample and reagents to predigest for thirty (30) minutes.
4. Close vessel according to manufacturers directions and connect appropriate temperature and pressure sensors.

5. Method is performance is designed to achieve a sample temperature of 175 ± 5 °C for in approximately 5.5 ± 0.25 minutes and remain at 175 °C% for 4.5 minutes for a total digestion time of ten (10) minutes. Adjust microwave temperature interlocks to achieve these desired limits. The pressure should peak between five (5) and ten (10) minutes for most samples. At the end of microwave digestion allow vessels to cool for five (5) minutes, remove and allow to cool to room temperature (optional - place in freezer to cool for thirty minutes).
6. Carefully uncap and vent each vessel in a chemical fume hood. Quantitatively transfer the sample to an acid-cleaned bottle. Samples containing suspended particulates will require centrifugation or filtering.
7. Quantitatively transfer the contents of the digestion vessel into the centrifuge tube, dilute to 15 mL final volume, cap centrifuge tube, invert three times and store (See Comment #8, #9 and #10).
8. Elemental analysis of soil digests can be made using atomic absorption spectrometry (AAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES), or other methodologies. The method chosen will determine specific matrix modifications, calibration standards used, and the need for instrument specific sample preparations and dilutions. Determination of trace elements by ICP-AES (Co, Cd, Ni, Mo, Pb) maybe facilitated by the use of an ultrasonic nebulizer (Soltanpour, 1996). Adjust and operate instruments in accordance with manufacturer's instructions. Calibrate instrument using calibration solutions. Determine the analyte concentrations of a method blank, unknown samples and record concentrations in mg L^{-1}.

Calculations

Report elemental constituents to the nearest 3 significant digits as mg kg^{-1}:

$$\text{Analyte Content} = \frac{(\text{Digest mg } L^{-1} - \text{Method Blank}) \times (\text{Final Digest Dilution Volume in Liters})}{(\text{Sample Mass (mg)}) \times 1000}$$

Comments

1. Microwave Calibration: Place 1.0 ± 0.1 kg of deionized water in teflon beaker and determined water temperature to the nearest ± 0.5 °C. Microwave at 40% power for two (2.0 ± 0.01) minutes, vigorously stir solution for thirty (30) seconds and record temperature to ± 0.5 °C. Repeat using successive microwave power settings of 50%, 60%, 70%, 80%, 90%, 95%, 100% with a fresh aliquot of deionized water each time. Absorbed power in watts (P) can be calculated using the change in temperature (ΔT according to Equation S-16.1-1:

$$P = (\Delta T) \times (34.86) \qquad \text{[equ. S-16.2-1]}$$

   Plot microwave calibration function of applied energy as a percent versus absorbed power in watts. Verify and check microwave calibration every three months.

2. Teflon PFA digestion vessel (liners) should be cleaned according to the following procedure: (1) soak liners in 1% solution of labware detergent for one hour; (2) rinse vessels in tap water; (3) rinse in solution of 0.5 N HCl; (4) three deionized water rinses (ASTM Type I grade); and (5) dry for one hour at 80 °C. Do not brush containers to clean.

3. Check repipette dispensing volume, calibrate using an analytical balance.

4. When adding reagent to vessels always wear protective clothing (i.e. eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of high air flow, 100 cfm.

5. Some materials containing carbonates or organic matter may react violently and result in rupture seal failure. When digesting these materials reduce sample mass to 200 mg of sample material. If a vigorous reaction is noted, allow sample to predigest uncapped until reaction ceases.

6. Inspect vessel rupture seal in the cap for replacement.

7. Follow microwave manufacturer's instructions for microwave power calibration. Applying excessive microwave power may result in rupture seal or vessel failure.

8. Centrifuging may be necessary to clear the digest.

9. Samples having analyte concentrations exceeding the highest standard will require dilution and reanalysis.

10. Place 3.0 mL of concentrate Micro® clean detergent (Baxter Scientific) in digestion vessel and allow to stand 30 minutes, rinse out any particulate, and finish cleaning according to set vessel cleaning procedure.

Literature

Kingston, H. M. and L. B. Jassie. 1986. Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. Anal Chem. 58:2534-2541.

Kingston, H. M. and Lois B. Jassie. 1988. Monitoring and predicting parameters in microwave dissolution. p. 97-148. *In:* M.H. Kingston and L.B. Jassie (ed.) Introduction to microwave sample preparation. American Chemical Society, Washington DC.

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BOTANICAL ANALYSIS METHODOLOGIES

DETERMINATION OF DRY MATTER CONTENT OF BOTANICAL MATERIALS B - 1.10

Gravimetric Moisture

Scope and Application

This method quantitatively determines the dry matter percentage in botanical materials based on the gravimetric loss of free water associated with heating to 105 °C for a period of two hours. The method is destructive with respect to the sample. Dry matter fraction is used to correct the sample element concentration to an absolute dry matter basis. The method does not remove molecular bound water and is generally reproducible within ± 37%.

Equipment

1. Analytical balance: 250 g capacity, resolution ±0.001 g.
2. Aluminum weight dish with handle.
3. Drying oven, preheated to 105 °C.
4. Desiccator, containing a desiccating agent.

Procedure

1. Weigh approximately 2 g of air dry botanical sample material into a tared aluminum weigh pan (preweighed to nearest 0.001 g) and record moist sample weight to the nearest 0.001 g.
2. Place sample and weigh pan in drying oven for a minimum of two (2) hours.
3. Remove and place pan in desiccator for one (1) hour.
4. Weigh sample and pan on balance, weigh and record mass to nearest 0.001 g.
5. Dispose of sample (see comment #1).

Calculation

$$\text{Sample dry matter } \% = \left(1 - \frac{\text{(Sample moist wt.) - (sample dry wt. - pan tare wt.)}}{\text{(Sample dry weight - pan tared weight)}}\right) \times 100$$

Report dry matter content to the nearest 0.1 %.

Comment

1. Drying samples at 105 °C may volatilize some carbon, nitrogen and sulfur compounds. Therefore, material used for moisture content should not be used for inorganic analysis.

Literature

Reuter, D.J., J.B. Robinson, K.I. Peverill and G.H. Price. 1986. Guidelines for collecting, handling and analyzing plant materials. p. 11-35 *In*: D.J. Reuter and J.B. Robinson (ed.) Plant analysis an interpretation manual. Inkata Press, Melbourne, Australia.

Smith, Morris and J. Benton Storey. 1976. The influence of washing procedure on surface removal and leaching of certain elements from trees. Hort. Sci. 14:718-719.

TOTAL KJELDAHL NITROGEN DETERMINATION IN BOTANICAL MATERIALS B - 2.10

Micro-Kjeldahl

Scope and Application

The Kjeldahl method quantitatively determines the amount of nitrogen (ammonium and protein) in botanical materials based on the wet oxidation of organic matter using sulfuric acid and digestion catalyst and conversion of nitrogen to ammonium (Issac and Johnson, 1976). Ammonium may be determined by distillation into boric acid and titration (Jones, 1989); spectrophotometric measurement (automated or manual); or diffusion-conductivity (Carlson, 1978). The method does not quantitatively recover nitrogen from heterocyclic rings (such as nicotinic acid) or from oxidized forms such as nitrate and nitrite. The Kjeldahl digest can be used for the determination of plant total phosphorus. The method is used to assess plant nitrogen sufficiency levels (Chapman and Pratt, 1961). The method detection limit is approximately 0.05% nitrogen (dry sample basis) and is generally reproducible within ± 8%.

Equipment

1. Analytical balance: 100 g capacity, resolution ± 0.1 mg.
2. Acid fume hood and digestion heating block (400 °C).
3. Volumetric digestion tubes, 75 mL.
4. Repipette dispenser, calibrated 3.0 ± 0.1 mL.

Reagents

1. Deionized water, ASTM Type I grade.
2. Digest catalyst accelerator: prepared by mixing (100:10:1) 100 g potassium sulfate (K_2SO_4), 10 g anhydrous copper sulfate ($CuSO_4$), and 1.0 g selenium (Se) metal powder. This can be purchased as a prepared material under the brand name Kjel-tab, distributed by various chemical suppliers.
3. Concentrated sulfuric acid (H_2SO_4), reagent grade.
4. 30% hydrogen peroxide (H_2O_2); use fresh, as this material rapidly decomposes.
5. Standard calibration solutions of NH_4-N. Prepare six calibration standards ranging from 0.2 to 40.0 mg L^{-1} concentration, diluted with 4% (v/v) sulfuric acid, prepared from 1000 mg L^{-1} ammonium nitrogen standard solution.

Procedure

1. Determine the moisture content of the botanical material on a sub sample, Method P - 1.10.
2. Weigh 250.0 ± 5.0 mg of air dried botanical material (See Comment #1) and place in into a 75 mL volumetric digestion tube (50 ml or 100 mL digestion tubes may be substituted). Include a method blank.
3. Add Kjel-tab and 6.0 mL of concentrated sulfuric acid (See Comments #2 and #3).
4. Mix on a vortex stirrer fifteen (15) seconds to thoroughly wet the sample with acid. Note: it is essential that all dry sample material be completely moistened by acid and well mixed to insure complete digestion.
5. Place the digestion tube on a digestion block, preheated to 370 °C for thirty (30) seconds or long enough to achieve complete botanical material breakup.
6. Remove from the digestion block and carefully (*slowly)* add 2-5 mL of 30% hydrogen peroxide in 1 mL increments to each digestion tube until digests begin to clear. Because this reaction takes place very rapidly, slow additions avoid excessive foaming.
7. Place the digestion tube back on the digestion block maintained at 370°C for two (2) hours. If excessive foaming occurs, remove from heat, cool two (2) minutes and add an additional 1-2 mL of hydrogen peroxide. At completion, a blue-green color may persist.

8. Remove samples from block and leave under fume hood for 5-10 minutes. Then add 10-20 mL of deionized water using a wash bottle to each tube to prevent hardening and crystal formation. Dilute digestion tubes to volume with deionized water, cap, and invert three times.
9. Sample digests can be analyzed for ammonium nitrogen by three standard methods: They are conventional ammonium distillation into boric acid and titration (Jones, 1989); spectrophotometric determination of ammonium (automated or manual); or diffusion-conductivity method of Carlson (1978). Determine ammonium concentration of a method blank, unknown samples and record results as mg L^{-1} of NH_4-N in the digest (See Comment #4 and #5).

Calculations

Report total Kjeldahl nitrogen results to the nearest 0.01%:

$$\% \text{ N} = \frac{(\text{mg L}^{-1}\ \text{NH}_4\text{-N in digest - method blank}) \times (0.075) \times (100)}{(\text{Sample size, mg}) \times (\text{Dry Matter Content (\%)} / 100)}$$

Comments

1. Use 500 mg of sample if nitrogen content is less than 0.800%.

2. Check repipette dispenser delivery volume, recalibrate using an analytical balance.

3. When adding reagent to vessels and handling digests always wear protective clothing (i.e. eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of high air flow, 100 cfm.

4. Samples having NH_4-N concentrations exceeding the highest standard will require dilution and reanalysis.

5. Sulfuric acid digest containing selenium is classified as a hazardous waste and must be disposed of in a suitable manner.

Literature

Carlson, R.M. 1978. Automated separation and conductiometric determination of ammonia and dissolved carbon dioxide. Anal. Chem. 48:1528-1531.

Carlson, R.M., R.I. Cabrera, J.L. Paul, J. Quick, and R.Y. Evans. 1990. Rapid direct measurement of ammonium and nitrate in soil and plant tissue extracts. Comm. in Soil Sci. Plant Anal. 21:1519-1529.

Chapman, H.D. and P.F. Pratt. 1961. Methods of analysis for soils, plants and waters, Priced Publication 4034. Berkeley: University of California, Division of Agriculture Sciences.

Issac, R.A. and W.C. Johnson. 1976. Determination of total nitrogen in plant tissue. J. of Assoc. of Off. Anal. Chem. 59:98-100.

Jones, J.B. 1989. Plant analysis techniques. Athens, GA: Benton Laboratories, Inc. p. 16-18.

Scope and Application

This method quantitatively determines the amount of nitrogen in all forms (ammonium, nitrate, protein and heterocyclic nitrogen such as nicotinic acid) in botanical materials using a resistance furnace and a thermal conductivity detector. It is based on the method originally described by Dumas and later modified by Sweeny (1989) whereby botanical samples, encased in tin (Sn) foil or another material, are ignited in a resistance furnace at approximately 900 °C, in helium and oxygen environment in a quartz combustion tube. An aliquot of the combustion gas is passed through a copper catalyst to remove oxygen and convert nitrous oxides to N_2, passed over absorber columns to remove moisture and carbon dioxide, and nitrogen content determined by thermal conductivity. Similar instruments have the capability of the simultaneous analysis of carbon or sulfur. The method is used to assess plant nitrogen sufficiency levels. The method has a detection limit of 0.10% nitrogen (dry sample basis) and is generally reproducible to with in ± 5.0%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.1 mg.
2. Total Nitrogen Analyzer: Leco, Carl-Erba, Elementar and Perkin-Elmer with resistance furnace with thermal conductivity detector and operating supplies.
3. Tin (Sn) foil encapsulating cups.
4. Desiccator, containing a desiccating agent.

Reagent

1. Compressed Oxygen gas, 99.995 % purity.
2. Helium carrier gas, 99.995 % purity.
3. Nitrogen calibrations standards: EDTA, 9.57% ± 0.05% N; Glycine p-toluelene sulfonate ($C_9H_{13}O_6SN$), 5.66 ± 0.05% N; highly purified acetanilide (C_8H_6NO) 10.37 ± 0.05% N; and Leco calibration standard (PN 502-055), 2.40 ± 0.03% N.

Procedure

1. Determine the moisture content of the botanical material, Method P - 1.10.
2. Weigh 150.0 ± 5.0 mg of air dried botanical material pulverized to pass 40 mesh sieve (See Comments #1 and #2) and place in into a tared tin foil container (instrument specific), encapsulate, and record sample weight to the nearest 0.1 mg (See Comment #3).
3. Initialize the instrument following manufacturers suggested protocol. Conduct a system leak check on combustion system. Perform blank stabilization test, analyze consecutive blanks until the blanks stabilize at a constant value.
4. Adjust and operate the instrument according to manufacturer instructions using calibration standards (provided by manufacturer or obtained commercially). Enter sample dry matter content and analyze unknown sample for total nitrogen. Report results to the nearest 0.001% nitrogen. (See Comment #4 and #5).

Calculation

Report sample nitrogen concentration to the nearest 0.001%

Comments

1. Samples limited in material, should be dried over phosphorus pentoxide or magnesium perchlorate for forty-eight (48) hours and analyzed with no correction for moisture content or reported on as received basis.

2. Sample particulate must be ground to pass a 40 mesh screen (< 0.40 mm) in order to assure adequate sample homogeneity for instruments utilizing sample sizes in excess of 150 mg. For instruments utilizing sample sizes 5 - 10 mg (Carl-Erba, and Perkin-Elmer) it is recommended that samples be finely ground to pass a 60 mesh sieve (<250 um) prior to analysis to ensure homogeneity. For the Elementar instrument sample size maybe increased to 1000 mg.

3. Sample weight may be entered into instrument software using a balance interface.

4. Nitrogen content as determined by automated combustion method are generally slightly greater than values determined by the Kjeldahl (TKN) method. The TKN method may have incomplete recovery from oxidized forms of nitrogen and that in heterocylic rings.

5. Instruments may have the capability for the simultaneous analysis of sulfur, carbon and hydrogen.

Literature

McGeehan, S.L. and D.V. Naylor. 1988. Automated instrumental analysis of carbon and nitrogen in plant and soil samples. Comm. in Soil Sci. Plant Anal. 19:493-50-5.

Sheldrick, B.H. 1986. Test of the LECO CHN-600 Determinator for soil carbon and nitrogen analysis. Can. J. Soil Sci. 66:543-545.

Shepers, J.S. D.D. Francis, and M.T. Thompson. 1989. Automated total nitrogen of soil and plant samples. Comm. in Soil Sci. Plant Anal. 20:949-959.

Sweeney, Rose A. 1989. Generic combustion method for determination of crude protein in feeds: Collaborative study. J. Assoc. Off. Anal. Chem. 72:770-774.

Yeomans, J.C. and J.M. Bremmer. 1991. Carbon and nitrogen analysis of soils by automated combustion techniques. Comm. in Soil Sci. Plant Anal. 22:843-850.

TOTAL SULFUR IN BOTANICAL MATERIALS B - 2.30

Automated Combustion Method

Scope and Application

This method quantitatively determines the amount of sulfur in all forms (sulfate, sulfite, and protein) in botanical materials using a resistance furnace and a thermal conductivity detector. It is based on the method originally described by Dumas and later modified by Sweeny (1989) whereby botanical samples, encased in tin (Sn) foil, are ignited in a resistance furnace at approximately 900 °C, in helium and oxygen environment in a quartz combustion tube. An aliquot of the combustion gas is passed through a copper catalyst to remove oxygen and other combustion products, passed over absorber columns to remove moisture and carbon dioxide, and nitrogen content determined by thermal conductivity. Similar instruments have the capability of the simultaneous analysis of carbon or nitrogen. The method is used to assess plant sulfur sufficiency levels. The method has a detection limit of 0.10% sulfur (dry sample basis) and is generally reproducible to with in ± 7.0%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.1 mg.
2. Total Sulfur Analyzer: Leco, CNS-2000 or Carl-Erba with combustion furnace with detector and operating supplies.
3. Desiccator, containing a desiccating agent.

Reagent

1. Compressed Oxygen gas, 99.995 % purity.
2. Helium carrier gas, 99.995 % purity.
3. Sulfur calibrations standard: Glycine p-toluelene sulfonate ($C_9H_{13}O_6SN$), 12.2 ± 0.05% S.

Procedure

1. Determine the moisture content of the botanical material, Method P - 1.10.
2. Weigh 1000.0 ± 5.0 mg of air dried botanical material pulverized to pass 40 mesh sieve (See Comments #1 and #2) and place in into a tared tin foil container, encapsulate, and record sample weight to the nearest 1 mg (See Comment #3).
3. Initialize the instrument following manufacturers suggested protocol. Conduct a system leak check on combustion system. Perform blank stabilization test, analyze consecutive blanks until the blanks stabilize at a constant value.
4. Adjust and operate the instrument according to manufacturer instructions using calibration standards (provided by manufacturer or obtained commercially). Enter sample dry matter content and analyze unknown sample for total sulfur. Report results to the nearest 0.001% sulfur. (See Comment #4).

Calculation

Report sample sulfur concentration to the nearest 0.01%

Comments

1. Samples limited in material, should be dried over phosphorus pentoxide or magnesium perchlorate for forty-eight (48) hours and analyzed with no correction for moisture content or reported on as received basis.

2. Sample particulate must be ground to pass a 40 mesh screen (< 0.40 mm) in order to assure adequate sample homogeneity for instruments utilizing sample sizes in excess of 150 mg. For instruments utilizing sample sizes 5 - 10 mg (Carl-Erba) it is recommended that samples be finely ground to pass a 60 mesh sieve (<250 um) prior to analysis to ensure homogeneity.

3. Sample weight may be entered into instrument software using a balance interface.

4. Sulfur content as determined by automated combustion method are generally of greater precision than other methods using acid digestion of analysis.

Literature

Beaton, J.D., G.K. Burns and J. Platou. 1968. Determination of sulfur in soils and plant material. Technical Bulletin No. 14, The Sulfur Institute, Washington D.C. 1968.

Sheldrick, B.H. 1986. Test of the LECO CHN-600 Determinator for soil carbon and nitrogen analysis. Can. J. Soil Sci. 66:543-545.

Shepers, J.S. D.D. Francis, and M.T. Thompson. 1989. Automated total nitrogen of soil and plant samples. Comm. in Soil Sci. Plant Anal. 20:949-959.

Sweeney, Rose A. 1989. Generic combustion method for determination of crude protein in feeds: Collaborative study. J. Assoc. Off. Anal. Chem. 72:770-774.

Yeomans, J.C. and J.M. Bremmer. 1991. Carbon and nitrogen analysis of soils by automated combustion techniques. Comm. in Soil Sci. Plant Anal. 22:843-850.

EXTRACTABLE POTASSIUM, NITRATE, AMMONIUM ORTHO-PHOSPHATE, AND CHLORIDE OF BOTANICAL MATERIALS

B - 3.10

2% Acetic Acid Extraction

Scope and Application

The method semiquantifies the concentration of potassium (K), ammonium (NH_4-N), nitrate (NO_3-N), ortho-phosphate (PO_4-P) and chloride (Cl) in botanical materials by extraction with a 2% acetic acid solution. Dilute acetic acid does not quantitatively extract these ions from botanical tissue. Potassium is determined by AES or AAS; ammonium spectrophotometrically; nitrate is determined spectrophotometrically at 520 nm by the Griess-Ilasvay method (cadmium reduction); ortho-phosphate in the extract is determined spectrophotometrically at 880 nm by reacting with paramolybdate; and chloride is determined by coulometric titration or ion selective electrode. The method has been used primarily to determine K, NH_4-N, NO_3-N, PO_4-P, and Cl for assessing plant fertility and chloride status (Johnson and Ulrich, 1959; Chapman and Pratt, 1961). The method can also be used to determine sulfate sulfur. Generally the method detection limit is approximately 10 mg kg^{-1} (sample dry basis) and is generally reproducible to within ± 10.0%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.1 mg.
2. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute.
3. 250 mL extraction vessel with cap and filtration container.
4. Repipette dispenser calibrated to 50.0 ± 0.2 mL.
5. Whatman No. 2V 11 cm filter paper or equivalent highly retentive paper.
6. Spectrophotometer instrument, 520 and 660 nm.
7. Atomic Emission Spectrometer (AES) or Atomic Absorption Spectrometer (AAS) instrumentation.
8. Coulometric titrator or chloride ion selective electrode.

Reagents

1. Acetic acid extraction solution: Dilute 20 mL acetic acid, CH_3COOH (99%), in 50 mL deionized water and dilute to 1.0 L. Care must be taken to use high purity acetic acid to avoid nitrate and chloride contamination.
2. Standard calibration solutions of K, NH_4-N, NO_3-N, PO_4-P and Cl. Prepare multiple calibration standards according to specific method and manufacturer's specifications prepared from 1000 mg L^{-1} standard solution and diluted to final volume with 2% acetic acid.

Procedure

1. Determine the moisture content of the botanical material, Method P - 1.10.
2. Weigh out 200.0 ± 1.0 mg of air dried botanical material (See Comments #1 and #2) and place in 250 mL extraction vessel. Include a method blank.
3. Add 50.0 ± 0.2 mL of 2% acetic acid extraction solution and place on reciprocating mechanical shaker for thirty (30) minutes (See Comment #3). Include a method blank.
4. Filter, refilter if filtrate is cloudy (See Comments #4, #5, #6 and #7) and retain for analysis.

5. Analysis:

   i. For the determination of K, analyze for K using Atomic Emission Spectrometer (AES) or Atomic Absorption Spectrometer (AAS).
   ii For the determination of NH_4-N, analyze for NH_4-N using by spectrophotometric, diffusion-conductivity instruments or distillation techniques.
   iii. For determination of NO_3-N, analyze using spectrophotometric Method S - 3.10 or method 418-C or 418-F for NO_3-N listed in "Standard Method for the Analysis of Waste Water", 1985. Record concentration in mg L^{-1} NO_3-N in extract.
   iv. For determination of PO_4-P, analyze using spectrophotometric Method S - 4.10 or method 424-F in "Standard Method for the Analysis of Waste Water", 1985. Record concentration in mg L^{-1} PO_4-P in extract.
   v. For determination of Cl, analyze for Cl according to Method S - 1.40 or method 407-B listed in "Standard Method for the Analysis of Waste Water", 1985. Record concentration in mg L^{-1} Cl in extract.

Calculations

Report K and Cl in sample as to the nearest 0.01 %:

$$\% = \frac{(\text{mg } L^{-1} \text{ K or } NH_4\text{-N in extract - method blank}) \times (0.025)}{(\text{Sample size, mg}) \times (\text{Dry Matter Content (\%)} / 100)}$$

Report NH_4-N, NO_3-N, and PO_4-P in sample as to the nearest 10 mg kg^{-1}:

$$\text{mg kg}^{-1} = \frac{(\text{Extract analyte Conc. mg } L^{-1} \text{ - method blank}) \times (250)}{\text{Dry matter content (\%)} / 100}$$

Comments

1. Botanical materials must be ground to pass 40 mesh screen (< 0.425 mm) in order to insure adequate sample homogeneity.

2. Sample mass may be adjusted in accordance with expected analyte concentrations. For materials containing < 500 mg kg^{-1} NH_4-N or NO_3-N increase sample size to 500 mg.

3. Check repipette dispensing volume, calibrate using an analytical balance.

4. Check filter paper supply for possible contamination of analytes. If significant contamination is found (> 10 mg kg^{-1} on a sample basis), rinse filter paper with acetic acid extraction solution or filter extract with serum separator tubes.

5. Acetic acid extracts may be stored for up to 14 days, if stored at 4°C and/or with 100 uL of toluene or thymol.

6. Extracts may be retained for analysis of total potassium, ammonium nitrogen and sulfate-sulfur.

7. Samples having K, NH_4-N, NO_3-N, PO_4-P and Cl concentrations exceeding the highest standard will require dilution and reanalysis.

Literature

Carlson, R.M., R.I. Cabrera, J.L. Paul, J. Quick, and R.Y. Evans. 1990. Rapid direct determination of ammonium and nitrate in soil and plant tissue extracts. Comm. in Soil Sci. Plant Anal. 21:1519-1529.

Chapman, H.D. and P.F. Pratt. 1961. Methods of analysis for soils, plants and waters, Priced Publication 4034. Berkeley: University of California, Division of Agriculture Sciences.

Johnson, C.M. and A. Ulrich. 1959. Analytical methods for use in plant analysis. Bulletin 766. Berkeley: University of California, Agricultural Experiment Station. p. 26-78.

Standard Method of the Examination of Waste Water. 1985. p. 265-297. A.H. Franson. (ed.) 16th ed. American Public Health Association, American Water Works Association, and Water Pollution Control Federation.

EXTRACTABLE NITRATE OF BOTANICAL MATERIALS B - 3.20

Ion Selective Electrode

Scope and Application

The method semiquantifies the concentration of nitrate (NO_3-N), in botanical materials by extraction with an aluminum sulfate solution and subsequent determination by ion-selective electrode (ISE). The ISE determines NO_3-N by measuring an electrical potential developed across a thin layer of water-immiscible liquid or gel ion exchanger that is selective for NO_3 ions. This layer of ion exchanger is held in place by a porous membrane. The ISE is susceptible to interferences of Cl^-, HCO_3^-, SO_4^{2-} and is sensitive to changes in solution ionic strength (i.e. high salt). Problems with precision have been noted by Mack and Sanderson (1971) and Miller, Amacher and Dellavalle (1996). The method has been used primarily to determine NO_3-N for assessing plant nitrogen fertility (Johnson and Ulrich, 1959; Chapman and Pratt, 1961). Generally the method detection limit is approximately 200 mg kg^{-1} (sample dry basis) and is generally reproducible to within ± 18.0%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.001 g.
2. Repipette dispenser, calibrated to 25.0 ± 0.2 mL.
3. Reciprocating horizontal mechanical shaker, capable of 180 oscillations per minute.
4. Whatman No. 2V 11 cm filter paper or equivalent highly retentive paper.
5. Nitrate ion sensitive electrode.
6. pH/ion meter or pH-millivolt meter.

Reagents

1. Deionized water, ASTM type I Grade.
2. Extracting Solution: Ionic strength adjusting solution - 0.01M $Al_2(SO_4)_3$, 0.02M H_3BO_3, 0.01M Ag_2SO_4, and 0.02 M NH_2HSO_3 (sulfamic acid): Dissolve 67 g of $Al_2(SO_4)_3 \cdot 18H_2O$, 12 g of H_3BO_3, 20 g of Ag_2SO_4 and 19 g of NH_2HSO_3 in water and dilute to 10 liters.
3. Standard nitrate solutions. To a 1000 mL volumetric flask, add 0.7221 g of oven dry KNO_3; make up to volume with extracting solution. This gives a solution containing 100 mg L^{-1} of NO_3-N. Prepare nitrate calibration standards from extraction solution of 5.0, 10.0, 15.0, 20.0, 30.0, and 50.0 mg L^{-1}.

Procedure

1. Determine the moisture content of the botanical material, Method P - 1.10.
2. Weigh out 500.0 ± 1.0 mg of air dried botanical material (See Comments #1, #2, #3) and place in 50 mL extraction vessel.
3. Add 25.0 ± 0.2 mL of extracting solution and place on reciprocating mechanical shaker for thirty (30) minutes. Include a method blank.
4. Filter extract, refilter if filtrate is cloudy and retain for analysis.
5. Calibrate ion selective electrode/millivolt meter using standard calibration solutions and operate instrument in accordance with manufacturer instructions. Develop calibration curve for the ion selective electrode using standards. Determine nitrate concentration of plant sample and record results as mg L^{-1} of nitrate in extract solution (See Comment #4 and #5).

Calculations

Report mg of NO_3-N in sample as to the nearest 10 mg kg^{-1}:

$$\text{mg kg}^{-1} = \frac{(\text{NO}_3\text{-N in extract mg L}^{-1} - \text{method blank}) \times (50)}{\text{Dry matter content (\%)} / 100}$$

Comments

1. Botanical materials must be ground to pass 40 mesh screen (< 0.425 mm) in order to insure adequate homogeneity.

2. Sample mass may be adjusted in accordance with expected analyte concentrations. For materials containing < 500 mg kg^{-1} NO_3-N increase sample size to 1000 mg.

3. Check repipette dispensing volume, calibrate using an analytical balance.

4. Routinely check ISE calibration every third sample using a mid range standard. In specific instances the ISE maybe susceptible to radio frequency energy from surrounding electronic equipment (Carlson, 1992).

5. Samples having nitrate concentrations exceeding the highest standard will require dilution and reanalysis.

Literature

Baker, A.S. and R. Smith. 1969. Extracting solution for potentiometric determination of nitrate in plant tissue. J. of Agric. and Food Chem. 17:1284-1287.

Carlson, R.M. 1992. Personal Communication.

Chapman, H.D. and P.F. Pratt. 1961. Methods of analysis for soils, plants and waters, Priced Publication 4034. Berkeley: University of California, Division of Agriculture Sciences.

Dahnke, W.C. 1971. Use of the nitrate specific ion electrode in soil testing. Comm. in Soil Sci. and Plant Anal. 2(2):73-84.

Mack, A.R. and R.B. Sanderson. 1971. Sensitivity of the nitrate-ion membrane electrode in various oil extracts. Can. J. Soil Sci. 51:95104.

Milham, P.J., A.S. Awad, R.E. Paul and J.H. Bull. 1970. Analysis of plants, soils, and waters for nitrate by using an ion-selective electrode. Analyst 95:751-759.

Miller, R.O., J. Amacher and N. Dellavalle. 1996. A Proficiency Testing Program for the agricultural laboratory inudstry, results of the 1994 program. Comm. in Soil Sci. and Plant Anal. 27(3&4):451-461.

EXTRACTABLE SULFATE-SULFUR OF BOTANICAL MATERIALS B - 3.40

Acetic Acid / Barium Turbidimetric Method

Scope and Application

This method quantitatively determines the amount of sulfate-sulfur (SO_4-S) in botanical materials by extraction with a solution of 2% acetic acid with subsequent determination by turbidimetric analysis. The method may not quantitatively extract SO_4-S on some botanical materials which have a high anion exchange capacity. Turbidimetric analysis is based on the formation of $BaSO_4$ particulates in a suspension and subsequent measurement of optical density. The turbidimetric will require practice to become proficient with the method. Sulfate-sulfur analysis can be used for the diagnosis of sulfur deficiency in specific crops (ie. alfalfa, sugar beet, clover). The method has a detection limit of 20 mg kg^{-1} (dry basis) and is generally reproducible to with in ± 12%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.1 mg.
2. Reciprocating horizontal mechanical shaker, Eberbach, capable of 180 oscillations per minute.
3. Boro-silicate test tube 16 x 150 mm with cap.
4. Repipette dispenser(s) calibrated to 10.0 ± 0.1 mL and 3.0 ± 0.05 mL
5. Serum separator tubes, (PN-02-657-3, 16 x 150 mm, Fisher Sci. Co.).
6. Pipette 10.0 mL.
7. Nephelometer (preferred), turbidimeter or spectrophotometer 420 nm.

Reagents

1. Deionized water, ASTM Type I grade.
2. Acetic acid extraction solution, 2% (v/v): Dilute 20 mL acetic acid, CH_3COOH (99%), in 50 mL deionized water and dilute to 1000 mL. Verify purity of acetic acid, sulfate-sulfur content should be less than 1.0 mg L^{-1}.
3. Barium chloride / turbidimetric solution. Dissolve 60 g $BaCl_2$ in 600 mL deionized water and add 150 mL of Tween 80 suspension agent. Shake vigorously let stand 24 hours. Dissolution of Tween 80 maybe facilitated by heating. (See Comment #1 and #2).
4. Acidification solution, 6 N HCL. Mix 150 mL of concentrated HCl with 150 mL deionized water. Add 1.5 mL of 2000 mg L-1 SO_4-S stock solution.
5. Standard sulfate-sulfur solutions, 2000 mg L^{-1}. Dissolve 2.7176 g of oven dried potassium sulfate (K_2SO_4) with deionized water and dilute to 250 mL final volume. Prepare sulfate-sulfur calibration solutions of concentration: 1.0, 2.0, 5.0, 10, 20, and 40 mg L^{-1} and dilute to volume with 2 % acetic acid extraction solution.

Procedure

1. Determine the moisture content of the botanical material, Method 1.10.
2. Weigh out 100.0 ± 1.0 mg of air dried botanical material (See Comment #3 and #4) and place in 16 x 150 mm test tube.
3. Add 10.0 ± 0.1 mL of 2 % acetic acid extraction solution and place on reciprocating mechanical shaker for thirty (30) minutes (See Comment #5). Include an extract blank and quality control samples.
4. Filter, using serum separator, refilter if filtrate is cloudy and retain for analysis.
5. Pipette 5.0 mL of standard sample into 10 mL test tube. Repeat using standard sulfate-sulfur solutions and quality control samples.

6. Add 1.5 mL of acidification solution.
7. Add 3 mL of barium chloride/ turbidimetric solution, swirl gently to mix. Let stand for five (5) minutes and repeat samples.
8. Calibrate nephelometer using standard calibration solutions and operate instrument in accordance with manufacturer instructions. Develop calibration curve using standards. Determine sulfate-sulfur concentration in extract solution and record to the nearest 0.5 mg L^{-1} (See Comment #6).

Calculations

1. Report plant sulfate-sulfur (SO_4-S):

$$mg\ kg^{-1} = \frac{(mg\ L^{-1}\ SO_4\text{-S in extract - method blank}) \times (DF)}{(\text{Dry Matter Content (\%)} / 100)}$$

Dilution Factor (DF) = 100

Comments

1. Use $BaCl_2$ specifically designated for turbidimetric determination of sulfate-sulfur. Sources: J.T. Baker Cat. Parr Turbidimetric $BaCl_2$, JT0974-5; VWR JT0974-5; and GFS Chemicals Reagent Grade ACS #602.

2. A number of suspension agents have been reported in the literature which include: gum acacia, gelatin, glycerol, PVP-K30 (polyvinylpryrolidinone), and Tween 80 which have proven effective in turbidimetric analysis. Each of these will require experimentation and practice using SO_4-S spiking to fully refine the technique. For use of PVP-K30 (polyvinylpryrolidinone) add 10 g to 700 mL and dilute to 1 L final volume.

3. Botanical materials must be ground to pass a 40 mesh screen (< 400 uM) in order to insure adequate homogeneity.

4. Sample size maybe adjusted in accordance with expected SO_4-S concentrations. For materials containing < 250 mg kg^{-1} SO_4-S use 200 mg of sample material and 10.0 mL of acetic acid extraction solution using serum separator tubes.

5. Check Repipette volume, calibrate using an analytical balance.

6. Samples having SO_4-S concentrations exceeding the highest standard will require dilution and reanalysis.

Literature

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Hoeft, R.G.. 1982. Diagnosis of sulfur deficiency, Soil and plant analyst's seminar. American Society of Agronomy Meetings, December 1, 1982.

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PHOSPHORUS, POTASSIUM, CALCIUM, MAGNESIUM, SODIUM, BORON ZINC, MANGANESE, IRON, COPPER AND MOLYBDENUM OF BOTANICAL MATERIALS **B - 4.10**

Dry Ash

Scope and Application

The method quantitatively determines the concentration of P, K, Ca, Mg, Na, B, Zn, Mn, Fe, Cu and Mo in botanical materials utilizing a high temperature dry oxidation of the organic matter and dissolution of the ash with hydrochloric acid. Digest analyte concentrations are determined by atomic absorption spectrometry (AAS) and/or inductively coupled plasma atomic emission spectrometry (ICP-AES). Analysis of P and B may be conducted using spectrophotometric methods. The procedure is not quantitative for sulfur and other elements which are easily volatilized (i.e. Se, As, Hg). Ashing temperatures exceeding 500 °C will result in poor recoveries of Al, B, Cu Fe, K and Mn (Issac and Jones, 1972). Results for boron may be inconsistent due to volatilization and desorption in the muffle furnace. The method detection limit is approximately 0.04 % for P, K, Ca, and Mg and 4.0 mg kg^{-1} for B, Zn, Mn, Fe, and Cu. The method is generally reproducible within ± 10%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 1 mg.
2. Porcelain crucibles, 30 cc capacity.
3. Muffle furnace capable of 500 °C.
4. Repipette, 10.0 ± 0.2 mL.
5. Volumetric labware, 50 mL, plastic.
6. Atomic Absorption Spectrophotometer (AAS) and/or Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES).

Reagents

1. Deionized water, ASTM Type I grade.
2. 1.0 N HCl solution, prepared by mixing 83.5 mL concentrated HCl and diluting to 1.0 L.
3. Standard calibration solutions of P, K, Ca, Mg, Na, B, Zn, Mn, Cu, and Fe. From 1000 mg L^{-1} reference solutions: Prepare five multi-element standards: of K, Ca, Mg ranging from 5.0 - 500 mg L^{-1}; P and Na ranging from 1.0 - 100 mg L^{-1}; and B, Zn, Mn, Fe, and Cu, ranging from 0.10 - 10.0 mg L^{-1}. Dilute standard calibration solutions with 0.1 N HCl.

Procedure

1. Determine the moisture content of the botanical material, Method P - 1.10.
2. Weigh 1000 ± 5.0 mg of plant material into a porcelain crucible. Include a method blank. (See Comments #1 and #2).
3. Place crucible in a muffle furnace and ramp temperature to 500 °C over two (2) hours. Ash samples for four (4) hours at 500 °C (See Comment #3).
4. Allow to cool to room temperature in muffle furnace, slowly open door and remove ashed samples. Take caution not to disturb sample ash while transferring from furnace.
5. Dissolve ash with 10.0 mL of 1.0 N HCl solution (See Comments #4 and #5). Dissolution of ash may be facilitated by heating.
6. Quantitatively transfer the contents of the crucible into a 50.0 mL volumetric flask, dilute to volume with deionized water, cap and invert three times.

7. Elemental analysis of plant digests can be made using atomic emission spectrometry (AES) atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and/or other methodologies (See Comment #6 and #7). The method chosen will determine specific matrix modifications, calibration standard range and the need for instrument specific sample preparations and dilutions. Determination of trace elements by ICP-AES (Co, Cd, Ni, Mo, Pb) maybe facilitated by the use of an ultrasonic nebulizer (Soltanpour, 1996). Adjust and operate instruments in accordance with manufacturer's instructions. Calibrate instrument using standard calibration solutions. Determine the analyte concentrations of a method blank, unknown samples and record analyte concentrations in mg L^{-1}.

Calculations

For P, K, Ca, Mg and Na report results to the nearest 0.001%:

$$\% \text{ analyte} = \frac{(\text{mg } L^{-1} - \text{method blank}) \times (50) \times (0.0001)}{\text{Dry matter (\%)}/100}$$

For Mn and Fe report results to the nearest 1 mg kg^{-1}; B, Zn and Cu the nearest 0.1 mg kg^{-1}:

$$\text{mg kg}^{-1} \text{ analyte} = \frac{(\text{mg } L^{-1} - \text{method blank}) \times (50)}{\text{Dry matter (\%)} / 100}$$

Comments

1. Labware should be cleaned (1) soak crucibles in 1% solution of labware detergent for one hour; (2) rinse vessels in tap water; (3) rinse in solution of 0.5 N HCl; (4) three deionized water rinses (ASTM Type I grade); and (5) dry for one hour at 80 °C.

2. Sample material must be ground to pass a 40 mesh screen (< 0.40 mm opening), to ensure homogeneity.

3. Ashing temperatures are not to exceed 500 °C to avoid potential volatilization of Al, B, Cu, K, and Mn.

4. Check pipette dispensing volume, calibrate using an analytical balance.

5. When adding reagent to vessels and handling digests always wear protective clothing (i.e. eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of high air flow, 100 cfm.

6. Centrifuging may be necessary to clear the digest.

7. Samples having analyte concentrations exceeding the highest standard will require dilution and reanalysis.

Literature

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Issac, R.A. and J.B. Jones Jr. 1972. Effects of various dry ashing temperatures on the determination of 13 elements in five plant tissues. Comm. Soil Sci. Plant Anal. 3:261-269.

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Soltanpour, P.N, G.W. Johnson, S.M. Workman, J.B. Jones and R.O. Miller. 1996. Inductively coupled plasma emission spectrometry and inductively coupled plasma-mass spectrometry. p. 91-139. *In:* J. M. Bartels et al. (ed.) Methods of soil analysis: Part 3 Chemical methods. 3rd.ed. ASA and SSSA, Madison, WI. Book series no. 5.

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PHOSPHORUS, POTASSIUM, SULFUR, CALCIUM, MAGNESIUM SODIUM, ZINC, MANGANESE, COPPER, IRON AND MOLYBDENUM OF BOTANICAL MATERIALS **B - 4.20**

Nitric/Perchloric Acid Digest, Wet Ashing Open vessel

Scope and Application

The method quantitatively determines the concentration of P, S, K, Ca, Mg, Na, Al, Zn, Mn, Fe, Cu and Mo in botanical materials utilizing a nitric-perchloric acid digestion of organic matter in conjunction with external heating. Digest analyte concentrations are determined by atomic absorption spectrometry (AAS) and/or inductively coupled plasma atomic emission spectrometry (ICP-AES). Analysis of P and S may be conducted using spectrophotometric and turbidimetric methods, respectively. The method requires predigestion with HNO_3, followed by addition of $HClO_4$ and digestion at high temperatures. Extreme caution is to be followed when using perchloric acid (Schilt, 1979) which may react violently with untreated organic materials and result in an explosion. A special hood is required to handle perchloric acid fumes. Reflux funnels are placed over the digestion tubes to reduce volatilization and minimize oxygen. Alternatives to the use of perchloric acids using hydrogen peroxide have been reported (Haung and Schulte, 1985; Havlin and Soltanpour, 1980). The method can also be used for the determination of trace-elements (Pb, Ni, Cd, etc). Generally the method detection limit is approximately 0.02% for P, S, K, Ca, Mg and Na; and 0.5 mg kg^{-1} (sample dry basis) for Al, Zn, Mn, Fe and Cu. Generally reproducible is within ± 7.0%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.1 mg.
2. Block Digester (400 °C) and perchloric hood.
3. 50 mL volumetric digestion tubes and 25 mm reflux funnels.
4. Repipette dispensers, calibrated to 6.0 ± 0.05 mL and 2.0 ± 0.01 mL
5. Volumetric labware, 25 mL.
6. Atomic Absorption Spectrophotometer (AAS) and/or Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), vacuum system.

Reagents

1. Deionized water, ASTM Type I grade.
2. Concentrated nitric acid, reagent grade.
3. 70% perchloric acid, reagent grade.
4. Standard calibration solutions of P, K, S, Ca, Mg, Na, Al, Zn, Mn, Cu, Fe and Mo. Prepare five multi-element standards: of K, Ca, Mg ranging from 5 - 500 mg L^{-1}; P, S and Na ranging from 1.0 - 100 mg L^{-1}; and Al, Zn, Mn, Fe; and Cu and Mo , ranging from 0.02 - 10.0 mg L^{-1}. Dilute standard calibration solutions with 5% HNO_3 and 1% $HCLO_4$ by volume.

Procedure

1. Determine the moisture content of the botanical material, Method P - 1.10.
2. Weigh 500.0 ± 0.5 mg of sample into a 50 mL volumetric digestion tube (See Comment #1 and #2). Include a method blank.
3. Using a repipette add 6.0 mL nitric acid, a boiling chip (teflon or glass) and swirl to thoroughly wet the sample (See Comment #3, #4 and #5).

4. Place 25 mm reflux funnels over the samples and allow to predigest at room temperature for sixty (60) minutes (16 h preferred).
5. Place the digestion tubes on a digestion block for sixty (60) minutes at 150 ºC.
6. Remove, cool to room temperature and using a repipette slowly add 2.0 mL of $HClO_4$ through the funnels.
7. Place the tubes in the block at 215 ºC for two (2) hours, after the HNO_3 fumes have evolved.
8. Remove the funnels ten (10) minutes before the end of the digestion.
9. Remove the tubes from the digestion block, cool twenty (20) minutes in a hood, and add 10 mL of deionized water on a hot plate (90 ºC).
10. Mix, using a vortex stirrer, cool and dilute to final volume. Filtering or centrifuging may be necessary to remove all particulate matter in the digest prior to analysis. Quantitatively transfer contents of digestion tube into a 25 mL volumetric flask.
11. Elemental analysis of plant digests can be made using atomic emission spectrometry (AES) atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and/or other methodologies. The method chosen will determine specific matrix modifications and the need for instrument specific sample preparations and dilutions. Determination of trace elements by ICP-AES (Cu, Mo) maybe facilitated by the use of an ultrasonic nebulizer (Soltanpour, 1996). Adjust and operate instruments in accordance with manufacturer's instructions. Calibrate instrument using calibration solutions and record concentration of analytes as mg L^{-1} (See Comments #6, #7 and #8). Determine the analyte concentrations of a method blank, unknown samples and record concentrations in mg L^{-1}.

Calculations

Report P, K, S, Ca, Mg and Na results to the nearest 0.001%:

$$\% \text{ analyte} = \frac{(\text{mg } L^{-1} - \text{method blank}) \times (50) \times (0.0001)}{\text{Dry matter (\%)} / 100}$$

Report Mn Al and Fe results to the nearest 1 mg kg^{-1}; Zn and Cu the nearest 0.1 mg kg^{-1}:

$$\text{mg kg}^{-1} \text{ analyte} = \frac{(\text{mg } L^{-1} - \text{method blank}) \times (50)}{\text{Dry matter (\%)} / 100}$$

Comments

1. Labware cleaning: (1) soak digestion tubes in 1% solution of laboratory detergent for one hour; (2) rinse vessels in tap water; (3) rinse in solution of 0.5 N HCl; (4) three deionized water rinses (ASTM Type I grade); and (5) dry for one hour at 80 ºC.

2. Sample material must be ground to pass a 40 mesh screen (< 0.40 mm opening), to ensure homogeneity.

3. When adding reagent to vessels and handling digests always wear protective clothing (i.e. eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of high air flow, 100 cfm.

4. It is essential that the entire sample be pretreated with nitric acid to ensure at least partial oxidation of the organic matter before the addition of perchloric acid. Caution: the use of perchloric acid in the presence of untreated organic matter can lead to rapid oxidation of the sample and a possible explosion (Blanchar, 1986).

5. Check repipette dispensing volume, calibrate using an analytical balance.

6. The method may not be quantitative for potassium since this alkali metal may form a precipitate with perchlorate.

7. Samples having analyte concentrations exceeding the highest standard will require dilution and reanalysis.

8. Increase sample mass to 2.00 g for the determination of trace metals, such as Ba, Cd, Cr, Mo, and Sr to 1000 mg and adjust dilution factor to 12.5.

Literature

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PHOSPHORUS, POTASSIUM, SULFUR, CALCIUM, MAGNESIUM SODIUM, ZINC, MANGANESE, COPPER, IRON AND MOLYBDENUM OF BOTANICAL MATERIALS **B - 4.25**

Nitric/Hydrogen Peroxide, Wet Ashing Open vessel

Scope and Application

The method quantitatively determines the concentration of P, S, K, Ca, Mg, Na, Al, Zn, Mn, Fe, Cu and Mo in botanical materials utilizing a nitric-hydrogen peroxide digestion of organic matter in conjunction with external heating and closely follows the methods described by Haung and Schulte, 1985 and Havlin and Soltanpour, 1980. Caution is to be followed when adding using H_2O_2 will react violently with hot untreated organic materials. Digest analyte concentrations are determined by atomic absorption spectrometry (AAS) and/or inductively coupled plasma atomic emission spectrometry (ICP-AES). Analysis of P may be conducted using spectrophotometric methods. The method can also be used for the determination of trace-elements (Pb, Ni, Cd, etc). Generally the method detection limit is approximately 0.02% for P, S, K, Ca, Mg and Na; and 0.5 mg kg^{-1} (sample dry basis) for Al, Zn, Mn, Fe and Cu. Generally reproducible is within ± 6.0%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.1 mg.
2. Block Digester (150 ºC) and fume hood.
3. 20 mL volumetric digestion tubes and 25 mm reflux funnels.
4. Repipette dispensers, calibrated to 6.0 ± 0.05 mL and 1.0 ± 0.01 mL
5. Volumetric labware, 25 mL.
6. Atomic Absorption Spectrophotometer (AAS) and/or Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), vacuum system.

Reagents

1. Deionized water, ASTM Type I grade.
2. Concentrated nitric acid, reagent grade.
3. 30% hydrogen peroxide, reagent grade.
4. Standard calibration solutions of P, K, S, Ca, Mg, Na, Al, Zn, Mn, Cu, Fe and Mo. Prepare five multi-element standards: of K, Ca, Mg ranging from 5 - 500 mg L^{-1}; P, S and Na ranging from 1.0 - 100 mg L^{-1}; and Al, Zn, Mn, Fe; and Cu and Mo , ranging from 0.02 - 10.0 mg L^{-1}. Dilute standard calibration solutions with 5% HNO_3 and 1% $HCLO_4$ by volume.

Procedure

1. Determine the moisture content of the botanical material, Method P - 1.10.
2. Weigh 500.0 ± 0.5 mg of sample into a 20 mL volumetric digestion tube (See Comment #1 and #2). Include a method blank.
3. Using a repipette add 6.0 mL nitric acid and swirl to thoroughly wet the sample (See Comment #3, #4 and #5).
4. Allow to predigest at room temperature for ten (10) minutes.
5. Place the digestion tubes on a digestion block for ten (10) minutes at 80 ºC.
6. Remove, cool for two (2) minutes and then add 2.0 mL of 30% H_2O_2 solution via a pipette in two separate aliquots of 1.0 mL each (See Comment #6)
7. Place the tubes in the block at 130 ºC for one (1) hour, or until total digest volume is reduced to approximately 2.0 - 3.0 mL .
8. Mix, using a vortex stirrer, cool and dilute to final volume. Filtering or centrifuging may be necessary to remove all particulate matter in the digest prior to analysis. Quantitatively transfer contents of digestion tube into a 25 mL volumetric flask.

9. Elemental analysis of plant digests can be made using atomic emission spectrometry (AES) atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and/or other methodologies. The method chosen will determine specific matrix modifications and the need for instrument specific sample preparations and dilutions. Determination of trace elements by ICP-AES (Cu, Mo) maybe facilitated by the use of an ultrasonic nebulizer (Soltanpour, 1996). Adjust and operate instruments in accordance with manufacturer's instructions. Calibrate instrument using calibration solutions and record concentration of analytes as mg L^{-1} (See Comments #6, #7 and #8). Determine the analyte concentrations of a method blank, unknown samples and record concentrations in mg L^{-1}.

Calculations

Report P, K, S, Ca, Mg and Na results to the nearest 0.001%:

$$\% \text{ analyte} = \frac{(\text{mg } L^{-1} - \text{method blank}) \times (50) \times (0.0001)}{\text{Dry matter (\%)} / 100}$$

Report Mn Al and Fe results to the nearest 1 mg kg^{-1}; Zn and Cu the nearest 0.1 mg kg^{-1}:

$$\text{mg kg}^{-1} \text{ analyte} = \frac{(\text{mg } L^{-1} - \text{method blank}) \times (50)}{\text{Dry matter (\%)} / 100}$$

Comments

1. Labware cleaning: (1) soak digestion tubes in 1% solution of laboratory detergent for one hour; (2) rinse vessels in tap water; (3) rinse in solution of 0.5 N HCl; (4) three deionized water rinses (ASTM Type I grade); and (5) dry for one hour at 80 °C.

2. Sample material must be ground to pass a 40 mesh screen (< 0.40 mm opening), to ensure homogeneity.

3. When adding reagent to vessels and handling digests always wear protective clothing (i.e. eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of minimum air flow 100 cfm.

4. It is essential that the entire sample be pretreated with nitric acid to ensure at least partial oxidation of the organic matter

5. Check repipette dispensing volume, calibrate using an analytical balance.

6. The addition of H_2O_2 solution will cause a vigorous reaction which may froth and rise in the digestion tube. If the froth volume expands to more than 2X of the initial digest volume, extend the cooling time to three or four minutes to minimize frothing.

7. Samples having analyte concentrations exceeding the highest standard will require dilution and reanalysis.

8. Increase sample mass to 2.00 g for the determination of trace metals, such as Ba, Cd, Cr, Mo, and Sr to 1000 mg and adjust dilution factor to 12.5.

Literature

Chapman, H.D. and P.F. Pratt. 1961. Methods of analysis for soils, plants, and waters. University of California Berkeley, Division of Agriculture Sciences. Priced Publication 4034.

Haung, C.L. and E.E. Schulte. 1985. Digestion of plant tissue for analysis by ICP emission spectroscopy. Comm. Soil Sci. Plant Anal. 16: 945-958.

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Schilt, Alfred A. 1979 Perchloric acid and perchlorates. Fedrich Smith Chemical Company. Columbus, OH.

Soltanpour, P.N, G.W. Johnson, S.M. Workman, J.B. Jones and R.O. Miller. 1996. Inductively coupled plasma emission spectrometry and inductively coupled plasma-mass spectrometry. p. 91-139. *In:* J. M. Bartels et al. (ed.) Methods of soil analysis: Part 3 Chemical methods. 3rd.ed. ASA and SSSA, Madison, WI. Book series no. 5.

Tolg, G. 1974. The basics of trace analysis. P. 698-710. *In:* E. Korte (ed.) Methodium Chimicun. Vol 1 Analytical Methods. Part B. Micromethods, biological methods, quality control, automation. Academic Press, New York.

Zasoski, R.J. and R.G. Burau. 1977. A rapid nitric perchloric acid digestion method of multielement tissue analysis. Comm. Soil Sci. Plant Anal. 3:425-436.

PHOSPHORUS, POTASSIUM, SULFUR, CALCIUM, MAGNESIUM, SODIUM, ALUMINUM, BORON, ZINC, MANGANESE, IRON, COPPER, AND MOLYBDENUM OF BOTANICAL MATERIALS **B - 4.30**

Microwave Digestion/Dissolution Closed Vessel

Scope and Application

The method quantitatively determines the concentration of P, S, K, Ca, Mg, Na, Al, B, Zn, Mn, Fe, Cu and Mo in botanical materials utilizing a nitric acid/hydrogen peroxide digestion in conjunction with microwave heating in closed teflon vessels. Digest analyte concentrations are determined by atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). The digestion is based on the method described by Kingston et al. (1986) using nitric acid and modified by Sah and Miller (1992) using nitric acid and hydrogen peroxide. The digest method is incomplete relative to the total oxidation of organic carbon. Phosphorus, S, and B, analyses require an ICP-AES with a vacuum spectrometer. Potassium, Ca, Mg, Na, Al, Zn, Cu, Mn, and Fe can be analyzed by AAS or ICP-AES. Microwave nitric acid/hydrogen peroxide digests may not provide 100% recovery of Al, Si, and Se. The method has a detection limit of approximately 0.01% for P, K, Ca, and Mg and 0.2 mg kg^{-1} for B, Zn, Cu, Fe, Mn and Mo (sample dry basis). The method can also be used for the determination of trace-elements (Co, Cd, Ni, Pb, etc.) and is generally reproducible within ± 7.0% for all analytes.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.1 mg.
2. Microwave digestion system and teflon double wall digestion vessels (equipped with 200 psi relief seals)(See Microwave Calibration, Comment #1).
3. Repipette dispensers, calibrated to 0.5 ± 0.05 mL and 2.0 ± 0.08 mL
4. Polypropylene centrifuge tube with cap, 15 mL graduated.
5. Atomic Absorption Spectrophotometer (AAS) and/or Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), vacuum system.

Reagent

1. Deionized water, ASTM Type I grade.
2. Micro® clean detergent.
3. Nitric Acid, trace metal grade, 12 N.
4. Hydrogen peroxide 30% solution.
5. Standard Calibration solutions of P, S, K, Ca, Mg, Na, Al, B, Zn, Mn, Fe, Cu and Mo. Prepare five multielement standards: of K, Ca, Mg ranging from 5 - 500 mg L^{-1}; P, S, and Na ranging from 1.0 - 100 mg L^{-1}; and B, Zn, Al, Mn, Fe, Mo and Cu, ranging from 0.0.25 - 10.0 mg L^{-1}. Dilute standard calibration solutions with 5 % nitric acid.

Procedure

1. Determine the moisture content of the botanical material on a sub sample, Method 1.10.
2. Weigh 250 ± 5.0 mg of dry botanical material (See Comment #2, #3 and #4) and place in 120 mL teflon digestion vessel. Include a method blank. For samples requiring Mo and Cu analyses, sample size should be increased to 500 ± 5.0 mg (dilution factor 30:1).
3. Using repipettes add 0.50 mL of trace metal grade concentrated nitric acid and 2.00 mL of 30% hydrogen peroxide to each vessel (See Comments #5 and #6). Ensure that the sample is completely wetted by the reagents.
4. Place digestion vessel in outer body shell, cap and allow the sample and reagents to predigest for thirty (30) minutes.

5. Close vessel (See Comments #7 and #8) relief valves and place samples (twelve vessels) in the microwave. Set microwave program four (4) minutes of 296 watts power and eight (8) minutes of 565 watts power based on microwave absorbed power calibration (See Comment #1).
6. At completion remove samples and place in hood to cool (optional - place in freezer to cool for thirty (30) minutes). In a hood vent vessels by rotating release valve 1/2 revolution. Vent until vessel is completely depressurized. Remove cap, rinse cap into vessel with deionized water.
7. Quantitatively transfer the contents of the digestion vessel into the centrifuge tube, dilute to 15 mL volume, cap centrifuge tube, invert three times and store (See Comment #9, #10 and #11).
8. Elemental analysis of plant digests can be made using atomic emission spectrometry (AES) atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), or other methodologies. The method chosen will determine specific matrix modifications, calibration standards used, and the need for instrument specific sample preparations and dilutions. Determination of trace elements by ICP-AES (Co, Cd, Ni, Mo, Pb) maybe facilitated by the use of an ultrasonic nebulizer (Soltanpour, 1996). Adjust and operate instruments in accordance with manufacturer's instructions. Calibrate instrument using calibration solutions. Determine the analyte concentrations of a method blank, unknown samples and record concentrations in mg L^{-1}.

Calculations

Report P, K, S, Ca and Mg results to the nearest 0.001%:

$$\% \text{ analyte} = \frac{(\text{mg } L^{-1} - \text{method blank}) \times (DF) \times (0.0001)}{\text{Dry matter (\%)} / 100}$$

Report Al, Mn and Fe results to the nearest 1 mg kg^{-1}; B, Al, Zn, Cu and Mo the nearest 0.1 mg kg^{-1}:

$$\text{mg kg}^{-1} \text{ analyte} = \frac{(\text{mg } L^{-1} - \text{method blank}) \times (DF)}{\text{Dry matter (\%)} / 100}$$

Dilution Factor (DF) : for sample weights of 250 mg is 60, for 500 mg the value is 30

Comments

1. Microwave Calibration: Place 1.0 ± 0.1 kg of deionized water in teflon beaker and determined water temperature to the nearest ± 0.5 °C. Microwave at 40% power for two (2.0 ± 0.01) minutes, vigorously stir solution for fifteen (30) seconds and record temperature to ± 0.5 °C. Repeat using successive microwave power settings of 50%, 60%, 70% 80%, 90%, 95%, 100% with a fresh aliquot of deionized water each time. Absorbed power in watts (P) can be calculated using the change in temperature according to Equation P-4.30-1:

$$P = (\Delta T) \times (34.86) \qquad \text{[equ. B-4.30-1]}$$

Plot microwave calibration function of applied energy as a percent versus absorbed power in watts. Verify and check microwave calibration every three months.

2. Teflon PFA 120 mL digestion vessel liners should be cleaned according to the following procedure: (1) soak liners in 1% solution of labware detergent for one hour; (2) rinse vessels in tap water; (3) rinse in solution of 0.5 N HCl; (4) three deionized water rinses (ASTM Type I grade); and (5) dry for one hour at 80 °C. Do not brush containers to clean.
3. Sample material must be ground to pass a 40 mesh screen (< 0.40 mm opening), to ensure homogeneity.

4. Botanical materials which may be high in starch (i.e. cereal flours) may react violently and result in rupture seal failure. When digesting these materials reduce sample mass to 200 mg of sample material. Examine digest for undecomposed sample material. Redigest sample if: (1) significant residual particulate are noted in the digest or (2) the sample shows significant discolorization (i.e. gray or black, etc.).

5. Check repipette dispensing volume, calibrate using an analytical balance.

6. When adding reagent to vessels always wear protective clothing (i.e. eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of high air flow, 100 cfm.

7. Inspect vessel rupture seal in the cap for replacement. Samples with ruptured seals will require redigestion.

8. Follow microwave manufacturer's instructions for microwave power calibration. Applying excessive microwave power may result in rupture seal or vessel failure.

9. Centrifuging may be necessary to clear the digest.

10. Samples having analyte concentrations exceeding the highest standard will require dilution and reanalysis.

11. Place 3.0 mL of concentrate Micro® clean detergent (Baxter Scientific) in digestion vessel and allow to stand 30 minutes, rinse out any particulate, and finish cleaning according to set vessel cleaning procedure.

Literature

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Kingston, H. M. and Lois B. Jassie. 1988. Monitoring and predicting parameters in microwave dissolution. p. 97-148. *In:* M.H. Kingston and L.B. Jassie (ed.) Introduction to microwave sample preparation. American Chemical Society, Washington DC.

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TOTAL SELENIUM IN BOTANICAL MATERIALS B - 6.10

Perchloric Digest, Fluorimetric Analysis

Scope and Application

This method, quantitative for selenium, is based on the wet oxidation of selenium from organic carbon materials and inorganic selenium compounds utilizing nitric and Perchloric acids. Selenium in the digest is reduced from selenate to selenite (IV), and determined by fluorometric or ICP-AES hydride analysis. Fluorescence determination by is carried our by complexation of selenium with DAN (2,3-Diaminonaphthalene) to form Se-DAN piazselenol which is measured by fluorescence based on the method described by Koh and Benson (1983). ICP-AES hydride is based on the complexation of Se with $NaBH_4$ to form SeH_2 and subsequent measurement at 196.026 nm. The fluorometric procedure is capable of measuring 0.5 ug L^{-1} of selenium in an aqueous solution and based on a 25 x dilution factor 12.5 ug kg^{-1} on a dry sample basis. It is generally reproducible to within ± 10.0%.

Equipment

1. Digestion Block, 40 place, temperature operation 25 - 400 ºC, timer controlled.
2. Digestion tubes, pyrex 25 x 200 mm with volumetric graduations at 12.5, 25.0, 35.0 and 50.0 mL.
3. Polypropylene tubes 12 x 75 mm, 5.0 mL volume.
4. Mechanical reciprocating shaker, Erberbach capable of 80 oscillations per minute.
5. Vortex Stirrer.
6. Ultrasonic water bath, adjustable temperature 30 - 90 ºC.
7. Repipette Dispenser(s), calibrated to 5.0 and 1.0 mL.
8. Pipettes, 1.00 mL and 2.00 mL.
9. Florescence detector, 379 nm excitation wavelength and emission wavelength of 519 nm) or ICP-AES with hydride generation.

Reagents

1. Deionized water, ASTM Type I grade.
2. Concentrated nitric acid, trace metal grade.
3. Concentrated perchloric acid.
4. Microclean solution 2% by volume (Baxter Sci. PN- C6286-6).
5. 1.0 % solution of DAN (2,3-Diaminonaphthalene). Dissolve 0.1 g of 2,3-Diaminonaphthalene (Sigma Chemical) in 100 mL of 0.1 N HCl and mixing 100 mL of solution with 100 mL of cyclohexane and shaking in a seperatory funnel for one (1) minute. Discard nonaqueous phase and repeat three times. Purified DAN solution is stable for thirty (30) days.
6. Cyclohexane, reagent grade.
7. Disodium ethylenediaminetetacetic acid (EDTA) solution, 0.0025 M. Dissolve 37.224 g of Na_2EDTA into 600 mL deionized water and make to 1.0 L. Withdraw 50 mL of 0.10 M solution and dilute to 2000 mL for 0.0025 M solution.
8. Standard selenium calibration solutions. Prepare ten selenium calibration solutions prepared in HCl digest, concentration: of 0,1 2, 3, 4, 5, 10, 20, 25, and 50 ug L^{-1} prepared from 1000 ug L^{-1} standard reference solution purchased from Inorganic Ventures.

Procedure

1. Determine the moisture content of the botanical material on a subsample , Method 1.10.
2. Place 1.0 mL of selenium standard solutions (0,1,2,3,4,5,10,20,25,50 ug L^{-1} Se) into 25 x 200 mm Digestion tube. Digestion tubes should be prewashed with 2% solution of Microclean® and rinsed with three times with 18 Megohm deionized water.
3. Weigh out 500 ± 5 mg of botanical material (See Comment #1) and place in 25 x 200 mm pyrex digestion tube.

4. Add a boiling bead, 5.0 mL of concentrated trace metal nitric acid and 2.0 mL of concentrated perchloric acid to all tubes (See Comment #2 #3 and #4). Allow to predigest for one hour.
5. Place digestion tubes on digester block pre-heated to 150ºC for 90 min at 150ºC and 60 min at 210ºC. At completion of digestion tubes can be covered and allowed to sit up to seven days.
6. Remove tubes and allow to cool, add 1.0 mL concentrated HCl.
7. Place digestion tubes on digestion block pre-heated to 95ºC for 15 min. Remove tubes from the block and allow to cool. Bring to 12.5 mL total volume with 0.0025M EDTA. Samples should be analyzed in forty-eight (48) hours.
8. Samples maybe analyzed directly for Se by ICP-AES hydride. See Step #9 for Fluorimetric analysis Determine Se concentration of digest standards, blank and samples, record as ug L^{-1} of Se to three significant digest (See Comment #5 and #6)..
9. Add 1.0 mL of digested sample and 1.0 mL of a 1% DAN solution to 5 mL Polypropylene tubes. Place polypropylene tubes and set rack in ultrasonic water bath that is set to 60ºC for 30 min.
10. Remove from water bath and add 2.0 mL of cyclohexane to each tube, cap, and shake for 15 min.
11. Proceed with Fluorimetric analysis at 379 nm excitation wavelength and emission wavelength of 519 nm. Analysis may proceed by manual or automated methods. Determine Se concentration of digest standards, blank and samples, record as ug L^{-1} Se to three significant digits (See Comment #5 and #6).

Calculations

Report plant selenium concentrations to three significant digits as:

$$\text{Se ug kg}^{-1} = \frac{(\text{ug L}^{-1} \text{ in digest }) \times (25)}{(\text{Dry Matter \%})/100}$$

Comments

1. Botanical materials must be ground to pass a 40 mesh screen (< 400 uM) in order to assure adequate sample homogeneity.

2. When adding reagent to vessels always wear protective clothing (i.e. eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of high air flow, 100 cfm.

3. Check Repipette volume, calibrate using an analytical balance.

4. Check nitric and perchloric acids for possible contamination of Se.

5. Samples having Se concentrations exceeding the highest standard will require dilution and reanalysis.

6. Nitric-Perchloric acid - selenium digests and cyclohexane waste are classified as hazardous waste and must be disposed of in a suitable manner.

Literature

Handelman, G.J., P. Kosted, S. Short, and E. A. Dratz. 1989. Determination of selenium in human blood by high-performance liquid chromatography with fluorescence detection.

Koh, Tee-Siaw and T.H. Benson. 1983. Critical re-appraisal of fluorometric method for determination of selenium in biological materials. J. Assoc of Off. Anal. Chem. 66:918-926.

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WATER ANALYSIS METHODOLOGIES

Scope and Application

This method quantifies water pH and is a measure of the relative acidity or alkalinity of the solution that is in equilibrium. It is a measure of the intensity of acidity or alkalinity, but does not indicate the relative buffering capacity of water. pH is measured to access irrigation water chemical properties, crop and soil suitability. The method is generally reproducible within ± 0.05 pH units.

Equipment

1. pH meter, equipped with pH electrodes (indicating and reference).
2. Primary standard buffers, pH 4.0, 7.0, and 10.0.

Procedure

1. Standardize / Calibrate the pH meter: (1) rinse electrode with deionized water and place in pH 7.0 primary standard buffer and adjust as necessary; (2) rinse electrode and place in pH 4.0 primary standard buffer; (3) adjust the slope until response is ±0.05 units of expected response; and (4) check pH 7.0 primary standard buffer and adjust as necessary (See Comment #1). For high pH soils (>7.0) use pH buffers 7.0 and 10.0.
2. Insert electrode into the water. When the meter has stabilized record soil pH to the nearest 0.01 pH unit.
3. Remove electrode(s), rinse with deionized water and blot excess water with filter paper (See Comment #2).

Comments

1. Follow manufacturer's guidelines if meter does not read within 0.05 units of primary standards. Maintenance of combination electrodes differs from that of separate reference and glass electrodes; refer to manufacturer's instructions.

2. Store pH electrodes according to manufacturer's instructions (recommended practice is to store the electrodes in a primary standard buffer).

Literature

Rhoades, J.D. and S. Miyamoto. 1990. Testing soils for salinity. p. 299-336. *In*: R.L. Westerman (ed.) Soil testing and plant analysis. 3rd ed. SSSA, Madison, WI.

Robbins, C.W. and C.L. Wiegand. 1990. Field and laboratory measurements. p. 201-219. *In*: K.K. Tanji (ed.) ASCE manuals and Reports No. 71, Agricultural salinity, assessment, and management. American Society of Civil Engineers, 245 E. 47th St., New York.

U.S. Salinity Laboratory Staff. 1954. Saturated soil paste. Diagnosis and improvement of saline and alkali soils. Agr. Handbook 60, USDA, Washington, D.C.

Scope and Application

This method quantifies the amount of dissolved salts (mg L^{-1}) by measurement of the electrical conductivity (EC_e) of the water solution. The relationship between EC_e and soluble salts is approximate due to differences in equivalent weights, ion equivalent conductivities, and relative proportions of major solutes in the solution (Robbins, 1990). The EC_e measurement is sensitive to temperature and increases approximately 1.9% per °C (range 15 - 35 °C) (Rhoades, 1996). All EC_e data is normalized to 25 °C. Salt tolerance crop data is generally expressed in terms of the (EC_e) and used to assess the potential of soluble salts which may limit crop productivity. The method detection limit is approximately 0.01 dS m^{-1} (mmhos cm^{-1}) and is generally reproducible within ± 7%.

Equipment

1. Conductance meter with dynamic range from 0.01 to 100 dS m^{-1} conductance, temperature compensating, 25 °C.
2. Conductance cell having a cell constant (K) appropriate to the EC of the sample being measured (see Table S -1.20 -1). Pipet-type or dip-type cell is recommended that it be capable of measuring temperature.

Reagents

1. Deionized water CO_2-free, ASTM Type I grade. EC $<10^{-4}$ dS m^{-1}.
2. Standard Reference Calibration Solution. Dissolve 0.7456 g KCl (previously dried at 110 °C for 2 h) in CO_2 -free deionized water and dilute to 1.0 L. At 25 ±0.1 °C a 0.010 N KCl solution will have an EC_e of 1.412 dS m^{-1} (mmhos cm^{-1}). For a 0.100 N KCl solution (7.456 g KCl diluted to 1.0 L) will have an EC_e of 12.900 dS m^{-1}. Standard EC calibration solutions are listed in Table S-1.20-1 and can be purchased from a scientific supply vendor.

Procedure

1. Calibrate conductance cell. Operate and adjust instrument in accordance with manufacturer's instructions (See Comments #2 and #3). Rinse conductance cell with three aliquots of 0.01 N KCl, adjust a fourth portion to 25 ±0.1 °C, measure R (where R is the measured resistance ohms) and temperature *t*. Repeat measurement of R until value is constant. Calculate cell constant K.

$$K = (0.001413)\ R_{KCl})/[1+0.019(25-t)]$$

2. Rinse conductance cell with deionized water. Draw approximately 2 mL of soil saturation paste extract solution into conductance cell, rinse and replace with a second aliquot. When the meter has stabilized, record instrument reading.

Calculations

$$EC_{25} = C_x(1000)K[1 + 0.019(25 - t)]$$

Where: C_x is the instrument measured value of the sample and *t* is temperature

Report EC_e to the nearest 0.01 dS m^{-1} as EC_e 25 °C.

(See Comments #4, and #5)

Table S -1.20-1 Conductivity of KCl solutions at 25 °C (Rhoades, 1996).

Concentration N	Conductivity dS m^{-1}
0.001	0.147
0.010	1.412
0.020	2.767
0.050	6.668
0.10	12.90
0.20	24.82

Comments

1. Exposure of the sample to the atmosphere may cause changes in conductivity due to loss or gain of dissolved gasses: CO_2 and NH_3-N. Freshly distilled water has a conductivity of 0.005 - 0.002 dS m^{-1} increasing after a few weeks to 0.002 -0.004 dS m^{-1}. This of special concern on samples with very low EC_e.

2. Clean platinum electrodes that are new or that are providing erratic EC readings with acid-dichromate cleaning solution. Cleaning solution: 32 mL of saturated sodium dichromate ($Na_2Cr_2O_7$) and 1L 16 M sulfuric acid. Soak electrodes 16 hours followed by three rinses of deionized water. If platinum is flaked, recoat according to procedure of APHA (1985).

3. For highly saline soils (EC_e >8.0 dS m^{-1}) calibrate using 0.100 N KCl solution, EC_e 12.90 dS m^{-1}.

4. The relationship between conductivity and soluble salts is approximate due to differences in solutes, solute conductivities, and equivalent weights. The general relationship (for solutions with an EC_e range of 0.10 - 2.0 dS m^{-1}) is:

   Dissolved salt concentration (mg L^{-1}) $\cong$ 640 × EC_e, in dS m^{-1}
   Total cations (or anions) (mmolc L^{-1} or meq L^{-1}) $\cong$ 10 × EC_e, in dS m^{-1}
   Osmotic potential at 25 °C (KPa) $\cong$ 0.39 × EC_e, in dS m^{-1}

   The factor for converting EC_e to total dissolved salts (mg L^{-1}) ranges from 550 to 900 dependent on the specific anions present and their concentration. For estimating approximate total cations or anions, USDA Handbook #60, Figure 4, graphically shows this relationship for typical salt concentrations.

Literature

APHA (1985). Part 205. In Standard Methods for the Examination of Water and Wastewater'. 16th edn. American Public Health Association, Washington, DC.

Hanson, Blaine, Stephen R. Grattan, and Allan Fulton. 1993. Agricultural salinity and drainage. University of California irrigation program, Univ. California Davis.

Helrich, K. (ed.) 1990. Official methods of analysis. 15th Ed. Association of Official Analytical Chemists, Inc. Arlington, VA.

Rayment, G.E. and F.R. Higginson. 1992. Electrical conductivity. p. 15-16. *In:* Australian Laboratory Handbook of Soil and Water Chemical Methods. Inkata Press, Melbourne.

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Determination: 1985. Method 205 Conductivity. p. 76-78. *In:* A.H. Franson (ed.) Standard methods for the examination of waste water. 16th ed. American Public Health Association, American Water Works Association and Water Pollution Control Federation.

U.S. Salinity Laboratory Staff. 1954. Saturated soil paste. Diagnosis and improvement of saline and alkali soils. Agr. Handbook 60, USDA, Washington, D.C.

Scope and Application

This method quantifies bicarbonate (HCO_3^{1-}) and carbonate (CO_3^{2-}) concentration in mmolc L^{-1} (meq L^{-1}) in the solution. It is based on titration with 0.10 N hydrochloric acid. The determination of HCO_3^{1-} and CO_3^{2-} should be made immediately due to the potential of the extract being super saturated relative to calcium carbonate ($CaCO_3$). The concentration of HCO_3^{1-} affects the solubility of calcium, the ionic strength of the extract solution and is used to calculate the adjusted SAR (Robbins, 1990 and Hanson et al. 1993). The method detection limit is approximately 0.05 mmolc L^{-1} (meq L^{-1}) and is generally reproducible within ± 10%.

Equipment

1. Titration burette 50.0 ± 0.2 mL, or automatic titrator.
2. pH meter and combination pH electrode.
3. Pipette, 2.0 ±0.05 mL and 5.0 ± 0.05 mL.
4. 50 mL beaker.
5. Magnetic stir plate and micro size (0.25 mm) Teflon coated magnetic stir bar.

Reagents

1. Deionized water, ASTM Type I grade.
2. Primary standard buffer solutions: pH 4.00, 7.00 and 10.0.
3. Standardized hydrochloric acid (HCl) solution, 0.020 N with respect to H^+ (See Comment #1).

Procedure

1. Standardize / calibrate the pH meter: (1) rinse electrode with deionized water and place in pH 7.0 primary standard buffer and adjust as necessary; (2) rinse electrode and place in pH 4.0 primary standard buffer; (3) adjust the slope until response is ±0.05 units of expected response; and (4) and recheck standard buffers (See Comments #2 and #3).
2. Place 1.0 to 50 mL aliquot of water sample in beaker, and bring to 50 mL volume with deionized water and add magnetic stirrer. Place on stir plate and insert pH electrode (See Comment #4). Record amount of titrant needed to reach a pH of 8.3 for CO_3^{2-} and 4.5 for HCO_3^{1-} to the nearest 0.2 mL.
3. Determine the amount of HCO_3^{1-} in deionized water blank solution.

Calculations

$$CO_3^{2-}\ \text{mmolc}\ L^{-1} = \frac{(2 \times P \times N) \times 1000}{\text{aliquot (mL)}} \qquad HCO_3^{1-}\ \text{mmolc}\ L^{-1} = \frac{(T - (2 \times P)) \times N \times 1000}{\text{aliquot (mL)}}$$

P = number of mL of HCl of normality N to reach CO_3^{2-} inflection point, pH 8.3;
T = number of mL of HCl of normality N to reach HCO_3^{1-} inflection point, pH 4.5;
aliquot = volume of sample, mL.

Comments

1. Standardized 0.020 N HCl solution can be prepared from dilution of 1.00 N HCl standard reference solution or standardized by titration of known bases (Horneck, 1989).

2. Follow manufacturer's guidelines if meter does not read within 0.05 units of primary standards. Maintenance of combination electrodes differs from that of separate reference and glass electrodes; refer to manufacturer's instructions.

3. Store pH electrodes according to manufacturer's instructions (usual recommended practice is to store the electrodes in a primary standard buffer).

Literature

Hanson, Blaine, Stephen R. Grattan, and Allan Fulton. 1993. Agricultural salinity and drainage. University of California Irrigation Program, Univ. California Davis.

Horneck, D.A., J.M. Hart, K. Topper and B. Koespell. 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University. Ag. Expt. Station SM 89:4. p. 13.

Rhoades, J.D. 1982. Soluble salts. p. 167-178. *In:* A. L. Page et al. (ed.) Methods of soil analysis: Part 2. Agronomy Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI.

Rhoades, J.D. and S. Miyamoto. 1990. Testing soils for salinity. p. 299-336. *In*: R.L. Westerman (ed.) Soil testing and plant analysis. 3rd ed. SSSA, Madison, WI.

Robbins, C.W. and C.L. Wiegand. 1990. Field and laboratory measurements. p. 201-219. *In*: K.K. Tanji (ed.) ASCE manuals and reports No. 71, Agricultural salinity, assessment, and management. American Society of Civil Engineers, 245 E. 47th St., New York.

U.S. Salinity Laboratory Staff. 1954. Saturated soil paste. Diagnosis and improvement of saline and alkali soils. Agr. Handbook 60, USDA, Washington, D.C.

Scope and Application

This method quantifies the concentration of chloride (mmolc L^{-1} or meq L^{-1}) in the solution. Chloride may be determined using an ion selective electrode (potentiometric), chloridometer or ion chromatography instrument methods. Plant tolerance to chloride can be related to its concentration in irrigation water. The method detection limit is approximately 0.1 mmolc L^{-1} dependent on the method of analysis and is generally reproducible within ± 10%. The unit mmolc L^{-1} is the accepted scientific unit for reporting the concentration of anions and cations and is equivalent to meq L^{-1}.

Equipment

1. Solid-state chloride electrode and double junction reference electrode, chloridometer or Cl titrator.
2. pH/ion meter or millivolt meter.

Reagents

1. Deionized water, ASTM Type I grade.
3. Chloride standard, 1.0 mmolc L^{-1}: Dissolve 74.1 mg of KCl in 500 mL of deionized water and dilute to 1.0 L.

Procedure

1. Determine the chloride concentration by ion selective electrode, chloridometer or ion chromatography. The instrument chosen will determine specific matrix modifications and sample dilutions. Adjust and operate instruments in accordance with manufacturer's instructions. Calibrate instrument using calibration solutions and determine chloride concentration of a method blank and unknown samples (See Comments #1, #2 and #3). Report chloride concentration in water sample to the nearest 0.1 mmolc L^{-1}.

Comments

1. Care must be taken to clean all labware prior to analysis. Wash all labware with 0.2 N HNO_3 and deionized water.

2. To accurately determine saturation paste chloride concentrations less than 2.0 mmolc L^{-1}, it is advisable to use standard additions techniques and potentiometric analysis (Fixen et al., 1988)

3. Samples containing chloride concentrations greater than the highest standard will require dilution.

Literature

Fixen, P.W., R.H. Gelderman and J.L. Denning. 1988. Chloride tests. *In:* W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. North Dakota Agr. Expt. Sta. Bull. No. 499 (revised).

Horneck, D. A.. J. M. Hart, K. Topper and B. Koespell. 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University. Ag. Expt. Station SM 89:4.

Rhoades, J.D. 1982. Soluble salts. p. 167-178. *In:* A. L. Page et al. (ed.) Methods of soil analysis: Part 2. Agronomy Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI.

Soil Improvement Committee, California Fertilizer Association. 1985. Western Fertilizer Handbook. 7th edition. Interstate Printers and Publishers, Inc. Danville, IL.

SOLUBLE BORON W - 1.50

Boron, Azomethine-H Spectrophotometric, ICP-AES

Scope and Application

This procedure quantitatively determines the boron concentration in water. It is based on the complexation of azomethine-H with HBO_3 to form colored complex in an aqueous matrix with subsequent spectrophotometric measurement at 420 nm (Wolf, 1974). EDTA chelate is added to minimize chemical interferences. Boron can also be determined by Inductively coupled plasma emission spectrometry (ICP-AES) using one of three wavelengths. The method is readily adapted to manual or automated techniques. The method quantifies water soluble boron concentrations which can limit crop yield or be toxic to plant growth. The method detection limit is approximately 0.10 mg L^{-1} and is generally reproducible to within ± 8%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.01 g.
2. 15 mL test tube or vial, polypropylene.
3. Pipette, 2.0 ± 0.05 mL and 3.0 ± 0.05 mL.
4. Vortex stirring device.
5. Spectrophotometer, wavelength 420 nm or ICP-AES 249.678, 249.773 or 208.959 nm.

Reagents

1. Deionized water, ASTM Type I grade.
2. Buffer-masking solution: Dissolve 250 g of ammonium acetate (reagent grade $NH_4C_2H_3O_2$), 25.0 g of disodium salt of ethylenedintrilo-teraacetic acid (Na_2-EDTA) in 400 mL of deionized water. Very slowly add 125 mL of glacial acetic acid, while stirring using a magnetic stirrer. Temporary acidic conditions may cause a slight precipitation of the EDTA salts. Continue to stir the solution until the EDTA dissolves. Do not heat the solution. Adjust the buffer to a pH of 5.4 to 5.6 with acetic acid or NH_4OH as necessary. Prepare fresh solution every two months.
3. Azomethine-H solution: Dissolve 0.9 g of azomethine-H, 2.0 g of L-ascorbic acid in 50 mL of deionized water prewarmed to 60 °C. Dilute to 100 mL and store in refrigerator. Solution is stable for forty-eight (48) hours (see comments #3 and #4).
4. Standard Boron Calibration solutions. Prepare six boron calibration standards: concentration range 0.10 - 4.0 mg L^{-1}, prepared in deionized water from a standard 1000 mg L^{-1} solution.

Procedure

1. Pipette a 2.0 mL aliquot of water into a 15 mL polypropylene tube followed by 3.0 mL of the Buffer-masking solution using a pipette and stir with vortex stirring device (See Comment #1 and #2).
2. Using a repipette add 2.0 mL of azomethine-H reagent and stir contents thoroughly. Allow the mixture to stand sixty (60) minutes.
3. Prepare standard curve following steps 4-5, substituting 2.0 mL of standard calibration solution for water. A method blank is prepared in the same manner using deionized water.
4. Adjust and operate spectrophotometer instrument according to manufacture's instructions. Calibrate instrument using standard calibration solutions. Determine boron concentration of a method blank and unknown water samples (See Comments #4 and #5). For laboratories utilizing ICP-AES instrumentation calibrate using the 249.773 nm or 249.678 nm wavelength and 0.05, 0.50, 1.0 and 4.0 mg L^{-1} calibration standards for boron determination (see Appendix A-1).

Calculations

Calculate boron concentration of water sample from working standard curve. Report boron concentration to the nearest 0.01 mg L^{-1}.

Comments

1. Prepare all reagents and perform all analyses in polypropylene or Teflon labware. Do not use borosilicate glassware.

2. Check pipette dispensing volume, calibrate using an analytical balance.

3. EDTA chelate is added to eliminate chemical interferences from Al, Fe and Cu. Concentration of the chelate may have to be increased for soil extracts containing high concentrations of these elements.

4. The azomethine-H reagent should be added quickly so that color development is equal for all samples. A constant check must be maintained on linearity and drift of the standard curve when analyzing a large set of samples.

5. For water samles with a distinct coloration prepare a second solution and blank for step two of the procedure adding 1.0 mL of deionized water in place of azomethine-H solution and vortex well. The blank for this determination consists of 5.0 mL of 0.02 M $CaCl_2$ solution and 1.0 mL of buffer-masking solution.

Literature

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Horneck, D.A., J.M. Hart, K. Topper and B. Koespell. 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University. Ag. Expt. Station SM 89:4.

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U.S. Salinity Lab. Staff. 1954. Diagnosis and Improvement of saline and alkali soils. Agr. Handbook 60, USDA, Washington, D.C.

Scope and Application

This method quantitatively determines the concentration (mmolc L^{-1}, meq L^{-1}) of dissolved Ca, Mg and Na in water using absorption spectrometry (AAS) or Inductively coupled plasma emission spectrometry ICP-AES. A chemical interference solution is used to minimize chemical matrix effects. The Sodium Absorption Ratio (SAR) of saturation paste extract is calculated from the concentration of these cations. The relationship between cation solution concentrations and exchangeable cations in the soil, is used to estimate exchangeable sodium percentage (ESP) from the SAR (Robbins, 1990). The method detection limit for these cations is approximately 0.02 mmolc L^{-1} on a solution basis and it is generally reproducible within ± 7%. The unit mmolc L^{-1} is the accepted scientific unit for reporting the concentration of anions and cations and is equivalent to meq L^{-1}.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.01 g.
2. Atomic Absorption Spectrophotometer (AAS) instrument.

Reagents

1. Deionized water, ASTM Type I grade.
2. Chemical interference solution, 5000 mg L^{-1} lanthanum oxide (La_2O_3) - 2000 mg L^{-1}, cesium chloride (CsCl) solution. Dissolve: 4.691 g LaO_3 and 5.071 g CsCl in 1500 mL of deionized water and add 25.0 mL of $HClO_4$ and 25.0 mL of HNO_3 and dilute to 2000 mL.
3. Standard calibration solutions of Ca, Mg, and Na: Prepare six calibration solutions containing 0.05 - 1.3 mmolc L^{-1} of Na, 0.05 - 3.5 mmolc L^{-1} of Ca, and 0.02 - 1.6 mmolc L^{-1} for Mg prepared from 1000 mg L^{-1} standard reference solutions and dilute to volume with chemical interference solution.

Procedure

1. For AAS instrumentation dilute an aliquot of the water sample 10:1 with chemical interference solution (See Comment #1 and #2).
2. Adjust AAS or ICP-AES instrument according to manufacturer's instructions. Calibrate instrument using calibration solutions and determine individually cation (Ca, Mg, and Na) concentrations, record as mg L^{-1} of analyte.

Calculations

$$[Ca]\ \text{mmolc}\ L^{-1} = \frac{Ca\ \text{mg}\ L^{-1} \times 10}{20.0\ \text{mg mmolc}^{-1}} \qquad [Mg]\ \text{mmolc}\ L^{-1} = \frac{Mg\ \text{mg}\ L^{-1} \times 10}{12.15\ \text{mg mmolc}^{-1}}$$

$$[Na]\ \text{mmolc}\ L^{-1} = \frac{Na\ \text{mg}\ L^{-1} \times 10}{23.0\ \text{mg mmolc}^{-1}} \qquad SAR = \frac{[Na]}{(([Ca] + [Mg])/2)^{½}}$$

Report Ca, Mg, and Na concentrations to the nearest 0.1 mmolc L^{-1} and SAR to the nearest 0.1 (See Comments #3and #4).

$$\text{Adjusted SAR} = \frac{[Na]}{(([Mg] + 0.215\ Ca_X\ (P_{CO2})^{1/3})^{½}}$$

Comments

1. Water samples containing greater than 750 mg L^{-1} soluble salts (> 1.2 dS m^{-1}, estimated from EC_e Method S - 1.20) will require additional dilution.

2. Cations may also be determined by ion chromatography instrumentation.

3. For laboratories utilizing ICP-AES instrumentation calibrate use the 422. 673 nm wavelength for Ca, 285.213 nm for Mg, and 588.995 nm wavelength for sodium (see Appendix A-1) using the standards of the calibration ranges described above.

4. For samples that contain HCO_3 it may be necessary to calculate the Adjusted SAR. constitutes more than 25% of the anions it may be necessary to determine the adjusted SAR. See water method W - 1.60 to calculate.

Literature

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U.S. Salinity Lab. Staff. 1954. Diagnosis and Improvement of saline and alkali soils. Agr. Handbook 60, USDA, Washington, D.C.

Sulfate - Turbidimetric

Scope and Application

This method quantifies the concentration of sulfate (SO_4^{2-} mmolc L^{-1} or meq L^{-1}) in water. The unit mmolc L^{-1} is the new accepted scientific unit for reporting the concentration of anions and cations and is equivalent to meq L^{-1}. Sulfate may be determined using turbidimetric, ion chromatography, or ICP-AES instrument methods. This method outlines the turbidimetric analysis which closely follows that described in 1992 Standard Method of the Examination of Waste Water. Sulfate is determined to evaluate anion balance. It has a method detection limit is approximately 0.02 mmolc L^{-1} and is generally reproducible within ± 7%.

Equipment

1. Magnetic stirrer.
2. Repipette dispenser calibrated to 2.0 ± 0.05 mL
3. Pipette 10.0 mL.
4. Magnetic stir plate and Teflon stir bar.
5. Nephelometer (preferred), Turbidimeter or Spectrophotometer 340 nm.

Reagents

1. Deionized water, ASTM Type I grade.
2. Turbidimetric solution. Dissolve 30.0 g of $MgCl_2 \cdot 6H_2O$; 5.0 g $CH_3COONa \cdot 3H_2O$; 1.0 g KNO_3; 20 mL acetic acid, CH_3COOH (99%) and 0.111 g Na_2SO_4, in 500 mL deionized water and add 5.0 g of powered gum acacia, or gelatin (See Comment #1) suspension agent. Dilute to 1000 mL final volume.
3. Barium chloride crystals. Parr turbidimetric grade, $BaCl_2 \cdot 2H_2O$ crystals 20 - 30 mesh. Use high purity $BaCl_2$, as low purity may result in low recovery of SO_4^{2-} (See Comment #2).
4. Standard sulfate-sulfur calibration solutions. Prepare 5.0 mmolc L^{-1} SO_4^{2-} calibration stock solution, dissolve 0.4353 g of oven dry K_2SO_4 in 500 mL of deionized water and dilute to one 1000 mL. Prepare six 100 mL calibration solutions of: 0.05, 0.1, 0.2, 0.4, 0.8, and 1.0 mmolc L^{-1} SO_4^{2-} from a 5.0 mmolc L^{-1} SO_4^{2-} solution and bring to final volume with deionized water.

Procedure

1. Dilute a 10.0 mL aliquot with 2.0 mL of deionized water. Repeat using sulfate standards and method blank.
2. Add 2.0 mL of turbidimetric solution using a repipette (See Comment #3 and #4). Add magnetic stir bar and beginning stirring.
3. While stirring add 0.2 g of $BaCl_2 \cdot 2H_2O$ crystals with measuring spoon.
4. Stir for sixty (60 ± 3) seconds, then remove from stirrer and after five (5 ±0.5) minutes read absorbance with nephelometer or spectrophotometer at 340 nm (See Comment #5 and #6). Repeat with sulfate calibration solutions and method blank. Using standard calibration solutions and determine sulfate concentration of water samples and method blank. Record as mmolc L^{-1} SO_4^{2-} of analyte in extract solution to two significant digits.

Calculations

Report water SO_4^{2-} concentration:

mmolc L^{-1} SO_4^{2-} = (mmolc L^{-1} SO_4^{2-} water - method blank) × (2)

(1.0 mmolc L^{-1} SO_4^{2-} = 48.03 mg SO_4^{2-} L^{-1})

Comments

1. A number of suspension agents have been reported in the literature which include: gum acacia, gelatin, glycerol, PVP-K30 (polyvinylpryrolidinone), and Tween 80 which have proven effective in turbidimetric analysis. Each of these will require experimentation and practice using SO_4-S spiking to fully refine the technique.

2. Use $BaCl_2$ specifically designated for turbidimetric determination of sulfate-sulfur. Sources: J.T. Baker Cat. Parr Turbidimetric $BaCl_2$, JT0974-5; VWR JT0974-5; and GFS Chemicals, Reagent Grade ACS #602.

3. Care must be taken to clean all labware prior to analysis. Pre-rinse all extraction flasks, turbidimetric and spectrometer cuvette in hot water followed by 0.5 N HCl rinse with deionized water.

4. Check repipette volume, calibrate using an analytical balance.

5. Samples containing SO_4^{2-} concentrations greater than the highest standard will require dilution.

6. For laboratories utilizing ICP-AES instrumentation calibrate use the 182.669 nm wavelength and calibration standards of 0.05, 0.50, and 5.0 mmolc L^{-1} SO_4^{2-} (see Appendix A-1).

Literature

Ajwa, H.A. And M.A. Tabatabai. 1993. Comparison of some methods for determination of sulfate in soils. Comm. Soil Sci. Plant Anal. 24(15&16) 1817-1832.

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Rhoades, J.D. and M. Clark. 1978. Sampling procedures and chemical methods in use at the U.S. Salinity Laboratory for characterizing salt-affected soils and waters. U.S. Salinity Laboratory USDA, Riverside CA. pp 11-12.

Rhoades, J.D. 1982. Soluble salts. p. 167-178. *In:* A. L. Page et al. (ed.) Methods of soil analysis: Part 2. Agronomy Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI.

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Nitrate (NO_3^-)

Scope and Application

This method quantifies the concentration of nitrate (NO_3^-) (mmolc L^{-1} or meq L^{-1}) in water. Nitrate may be determined using ion chromatography or cadmium reduction spectrophotometric methods. This method outlines the use of the cadmium reduction spectrophotometric method (automated) outlined by (Keeney, 1982). The method detection limit is approximately 0.04 mmolc L^{-1} dependent on the method of analysis and is generally reproducible within ± 10%. Nitrate is determined to evaluate the content of irrigation water and animal water supply. The unit mmolc L^{-1} is the accepted scientific unit for reporting the concentration of anions and cations and is equivalent to meq L^{-1}.

Equipment

1. Spectrophotometer, autoanalyzer, or flow injection analyzer (FIA) instrument.

Reagents

1. Deionized water, ASTM Type I grade.
2. Standard calibration solutions of NO_3-N. Prepare six calibration standards ranging from 0.05 to 1.5 mmolc L^{-1} concentration, diluted in 0.05 N $CaCl_2$ solution prepared from 16.1 mmolc L^{-1}(1000 mg L^{-1}) NO_3^- standard solution.

Procedure

1. Prepare a water sample (See Comment #1).
2. Nitrate (NO_3^-) content of the extract is determined using a spectrophotometer, automated flow analyzer (Technicon Method No. 329-74W/A) or FIA instrument. Calibrate using standard calibration solutions and operate instrument in accordance with manufacturer instructions. Determine nitrate concentration of water sample and method blank, unknown samples and record results as mg L^{-1} of nitrate in extract solution (See Comment #2)

Calculations

Report water:

mmolc L^{-1} NO_3^- = (mmolc L^{-1} NO_3^- water - method blank)

(1.0 mmolc L^{-1} NO_3^- = 62.0 mg L^{-1} NO_3^-)

Nitrate maybe reported as NO_3-N mg L^{-1}, NO_3-N mg L^{-1} = NO_3^- mmolc L^{-1} × 0.238

Comments

1. Care must be taken to clean all labware prior to analysis. Wash all labware with 0.1 N HCl and deionized water.

2. Samples containing nitrate concentrations greater than the highest standard will require dilution.

Literature

Bremmer, J. M. and D.R. Keeney. 1965. Determination and isotopic ratio analysis of different forms of nitrogen in soils: I. Apparatus and procedure for distillation for and determination of ammonium. Soil Sci. Soc. Am. Proc. 29:504-507.

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Rhoades, J.D. 1982. Soluble salts. p. 167-178. *In:* A. L. Page et al. (ed.) Methods of soil analysis: Part 2. Agronomy Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI.

TOTAL KJELDAHL NITROGEN IN WATER W - 1.90

Micro-Kjeldahl

Scope and Application

The Kjeldahl method quantitatively determines the amount of nitrogen in water based on the wet oxidation of organic matter using sulfuric acid and digestion catalyst and conversion of nitrogen to ammonium (Issac and Johnson, 1976). Ammonium may be determined by distillation into boric acid and titration (Jones, 1989); spectrophotometric measurement (automated or manual); or diffusion-conductivity (Carlson, 1978). The method does not quantitatively recover nitrogen from heterocyclic rings (such as nicotinic acid) or from oxidized forms such as nitrate and nitrite. The Kjeldahl digest can be used for the determination of water total phosphorus. The method is used to assess nitrogen content. The method detection limit is approximately 0.5 mg L^{-1} and is generally reproducible within ± 10%.

Equipment

1. Analytical balance: 100 g capacity, resolution ± 0.1 mg.
2. Acid fume hood and digestion heating block (400 °C).
3. Volumetric digestion tubes, 75 mL.
4. Repipette dispenser, calibrated 3.0 ± 0.1 mL.

Reagents

1. Deionized water, ASTM Type I grade.
2. Digest catalyst accelerator: prepared by mixing (100:10:1) 100 g potassium sulfate (K_2SO_4), 10 g anhydrous copper sulfate ($CuSO_4$), and 1.0 g selenium (Se) metal powder. This can be purchased as a prepared material under the brand name Kjel-tab, distributed by various chemical suppliers.
3. Concentrated sulfuric acid (H_2SO_4), reagent grade.
4. 30% hydrogen peroxide (H_2O_2); use fresh, as this material rapidly decomposes.
5. Standard calibration solutions of NH_4-N. Prepare six calibration standards ranging from 0.2 to 40.0 mg L^{-1} concentration, diluted with 4% (v/v) sulfuric acid, prepared from 1000 mg L^{-1} ammonium nitrogen standard solution.

Procedure

1. Place 25.0 ± 0.5 mL of water sample (See Comment #1) into a 75 mL volumetric digestion tube (50 ml or 100 mL digestion tubes may be substituted). Include a method blank.
2. Add Kjel-tab and 6.0 mL of concentrated sulfuric acid (See Comments #2 and #3).
3. Mix on a vortex stirrer fifteen (15) seconds to thoroughly mix the sample with acid.
4. Place the digestion tube on a digestion block, preheated to 90 °C for one hour.
5. Remove from the digestion block and carefully (*slowly)* add 2-5 mL of 30% hydrogen peroxide in 1 mL increments to each digestion tube until digests begin to clear. Because this reaction takes place very rapidly, slow additions should avoid excessive foaming.
6. Place the digestion tube back on the digestion block and maintained at 95°C for two (2) hours or until all water has been lossed through volatilization. Proceed with heating to 370 °C for two hours. At completion, a blue-green color may persist.
7. Remove samples from block and leave under fume hood for 5-10 minutes. Then add 10-20 mL of deionized water using a wash bottle to each tube to prevent hardening and crystal formation. Dilute digestion tubes to volume with deionized water, cap, and invert three times.

8. Sample digests can be analyzed for ammonium nitrogen by three standard methods: They are conventional ammonium distillation into boric acid and titration (Jones, 1989); spectrophotometric determination of ammonium (automated or manual); or diffusion-conductivity method of Carlson (1978). Determine ammonium concentration of a method blank, unknown samples and record results as mg L^{-1} of NH_4-N in the digest (See Comment #4 and #5).

Calculations

Report total Kjeldahl nitrogen results to the nearest 1.0 mg L^{-1} :

$$\% N = (\text{mg } L^{-1} NH_4\text{-N in digest - method blank}) \times (0.075) \times (40)$$

Comments

1. Use 50.0 mL of sample if nitrogen content is less than 10 mg L^{-1}.

2. Check repipette dispenser delivery volume, recalibrate using an analytical balance.

3. When adding reagent to vessels and handling digests always wear protective clothing (i.e. eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of high air flow, 100 cfm.

4. Samples having NH_4-N concentrations exceeding the highest standard will require dilution and reanalysis.

5. Sulfuric acid digest containing selenium is classified as a hazardous waste and must be disposed of in a suitable manner.

Literature

Carlson, R.M. 1978. Automated separation and conductiometric determination of ammonia and dissolved carbon dioxide. Anal. Chem. 48:1528-1531.

Carlson, R.M., R.I. Cabrera, J.L. Paul, J. Quick, and R.Y. Evans. 1990. Rapid direct measurement of ammonium and nitrate in soil and plant tissue extracts. Comm. in Soil Sci. Plant Anal. 21:1519-1529.

Chapman, H.D. and P.F. Pratt. 1961. Methods of analysis for soils, plants and waters, Priced Publication 4034. Berkeley: University of California, Division of Agriculture Sciences.

Issac, R.A. and W.C. Johnson. 1976. Determination of total nitrogen in plant tissue. J. of Assoc. of Off. Anal. Chem. 59:98-100.

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TOTAL WATER PHOSPHORUS W - 2.00

Open Vessel Digestion and Dissolution

Scope and Application

The method semi-quantitatively determines the concentration of phosphorus in water samples utilizing a nitric acid hydrogen peroxide extraction/dissolution in conjunction with microwave heating in closed teflon vessels. This method closely follows that outline din EPA method 3050A. Digest analyte concentrations spectrophotometric methods or inductively coupled plasma atomic emission spectrometry (ICP-AES). The method can also be used for the determination of trace-elements (K, Ca, Mg, Na, S, Mn, Fe, and Cu). It has a detection limit of 0.05 mg and is generally reproducible within ± 7.0%.

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.1 mg.
2. Hot plate system.
3. Repipette dispensers, calibrated to 0.5 ± 0.05 mL and 2.0 ± 0.08 mL
4. Polypropylene or teflon digestion tube with cap, 50 mL graduated.
5. Atomic Absorption Spectrophotometer (AAS) and/or Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), vacuum or purged system.

Reagent

1. Deionized water, ASTM Type II grade.
2. Concentrated nitric acid, trace metal grade, 12 N.
3. Concentrated hydrochloric acid.
4. Standard Calibration solutions of P ranging from 0.05 - 10.0 mg L^{-1}. Dilute standard calibration solutions with 5 % nitric acid.

Procedure

1. Place 10.0 mL ± 0.2 mL of water (See Comment #1, #2 and #3) in a 50 mL digestion polypropylene digestion vessel. Include a method blank.
2. Using repipettes add 9.0 ± 0.1 mL of trace metal grade concentrated nitric acid and 1.0 ± 0.1 mL of concentrated hydrochloric acid (See Comments #4 and #5).
3. Place digestion vessel in digestion block and heat to 90 °C for two (2) hours or until all water has volatilized. Continue heating at 120 °C for thirty (30) minutes.
4. Cool and dilute to final volume of 20 mL
5. Determine phosphorus content using Spectrophotometric analysis at 880 nm using method ( )or by ICP-AES. Adjust and operate instruments in accordance with manufacturer's instructions. Calibrate instrument using calibration solutions. Determine the analyte concentrations of a method blank, unknown samples and record concentrations in mg L^{-1}.

Calculations

Report phosphorus to the nearest 2 significant digits as mg L^{-1}:

$$\text{Phosphorus Content mg } L^{-1} = (\text{mg } L^{-1} - \text{method blank}) \times (0.5)$$

Comments

1. Teflon PFA digestion vessel (liners) should be cleaned according to the following procedure: (1) soak liners in 1% solution of labware detergent for one hour; (2) rinse vessels in tap water; (3) rinse in solution of 0.5 N HCl; (4) three deionized water rinses (ASTM Type I grade); and (5) dry for one hour at 80 °C. Do not brush containers to clean.

2. Check repipette dispensing volume, calibrate using an analytical balance.

3. When adding reagent to vessels always wear protective clothing (i.e. eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of high air flow, 100 cfm.

4. Samples having analyte concentrations exceeding the highest standard will require dilution and reanalysis.

5. Place 3.0 mL of concentrate Micro® clean detergent (Baxter Scientific) in digestion vessel and allow to stand 30 minutes, rinse out any particulate, and finish cleaning according to set vessel cleaning procedure.

Literature

Kalra, Y. P., D. G. Maynard, and F. G. Radford. 1989. Microwave digestion of tree foliage for multi-element analysis. Can. J. For. Res. 19:981-985.

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APPENDIX A

Table A-1. Suggested wavelengths and estimated detection limits for elements by ICP-AES.

Element	Wavelength (nm)	Detection Limit ug/L
Al	309.271	20
	396.152	30
As	193.696	50@
	197.197	80@
B	249.773	5
	249.678	6
	208.959	10
Ba	455.403	1
	493.408	2
Ca	396.847	0.5
	422.673	10
	317.933	10
Cd	214.438	3
	226.502	3
Co	238.892	6
	237.862	10
Cr	205.552	6
Cu	324.754	5
	224.700	8
Fe	238.204	5
	259.940	6
K	404.721	43@
Mg	279.553	0.2
	285.213	2
Mn	257.610	1
Mo	202.030	8
	203.844	10
Na	588.995	30
Ni	221.647	10
P	213.618	80
	178.292	20*
Pb	220.353	40
S	180.669	90*
	181.979	90*
Se	196.026	80@
Zn	213.856	2
	202.548	4

@ Sensitive to specific instrument and operation conditions.
* Requires vacuum or purged spectrometer.

Soltanpour, P.N, G.W. Johnson, S.M. Workman, J.B. Jones and R.O. Miller. 1996. Inductively coupled plasma emission spectrometry and inductively coupled plasma-mass spectrometry. p. 91-139. *In:* J. M. Bartels et al. (ed.) Methods of soil analysis: Part 3 Chemical methods. 3rd.ed. ASA and SSSA, Madison, WI. Book series no. 5.

APPENDIX B

Table B-1. Standard Reference Solutions for Elemental Analysis.

Ag	Silver Standard Solution, 1000 mg L^{-1}: dissolve 1.9080 g analytical-grade KCl in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
Al	Aluminum Standard Solution, 1000 mg L^{-1}: dissolve 8.9481 g analytical-grade $AlCl_3 \cdot 6\ H_2O$ in 500 mL of 2 M HCl and dilute to 1.0 L in a volumetric flask.
As	Arsenic Standard Solution, 1000 mg L^{-1}: dissolve 1.3203 g analytical-grade As_2O_3 in 500 mL of 8 M HNO_3 and dilute to 1.0 L in a volumetric flask.
B	Boron Standard Solution, 1000 mg L^{-1}: weigh 5.7195 mg of oven dry analytical-grade boric acid (HBO_3) and dilute to 1.0 L volumetric flask with deionized water.
Ba	Barium Standard Solution, 1000 mg L^{-1}: dissolve 1.1516 g analytical-grade $BaCl_2$ in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
Br	Bromine Standard Solution, 1000 mg L^{-1}: dissolve 1.487 g analytical-grade KBr in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
Ca	Calcium Standard Solution, 500 mg L^{-1}: dissolve 1.249 g analytical-grade $CaCO_3$ in 1:1 HCl and evaporate to dryness on a hot plate. Dissolve the residue with deionized water and bring to 1.0 L in a volumetric flask.
Cd	Cadmium Standard Solution, 1000 mg L^{-1}: dissolve 1.0000 g analytical-grade Cd metal in 1000 mL of 4 M HNO_3 in a volumetric flask.
Cl	Chloride Standard Solution, 1000 mg L^{-1}: dissolve 1.648 g analytical-grade NaCl in 500 mL of deionized water and dilute to 100 mL in a volumetric flask.
Co	Cobalt Standard Solution, 1000 mg L^{-1}: dissolve 4.0373 g analytical-grade $CoCl_2 \cdot 6H_2O$ 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
Cr	Chromium Standard Solution, 1000 mg L^{-1}: dissolve 5.1244 g analytical-grade $CrCl_3$ ($6H_2O$) in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
Cu	Copper Standard Solution, 1000 mg L^{-1}: dissolve 1.000 g analytical-grade Cu metal in 50 mL of solution of 50% concentrated HNO_3 and 50% HCl and dilute to 1.0 L in a volumetric flask with deionized water.
Fe	Iron Standard Solution, 1000 mg L^{-1}: dissolve 1.000 g analytical-grade Fe in 50 mL of 50% HCl solution and dilute to 1.0 L in a volumetric flask.
Hg	Mercury Standard Solution, 1000 mg L^{-1}: dissolve 1.3535 g analytical-grade $HgCl_2$ in 500 mL of deionized water + 1 g ($(NH_4)_2S_2O_8$) and dilute to 1.0 L in a volumetric flask.
K	Potassium Standard Solution, 1000 mg L^{-1}: dissolve 1.9067 g analytical-grade KCl in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.

Li	Lithium Standard Solution, 1000 mg L^{-1}: dissolve 6.1092 g analytical-grade LiCl in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
Mg	Magnesium Standard Solution, 1000 mg L^{-1}: dissolve 1.6581 g analytical-grade MgO in 1000 mL of 0.5 M HCl in a volumetric flask.
Mn	Manganese Standard Solution, 1000 mg L^{-1}: dissolve 1.5825 g analytical-grade MnO_2 in 1000 mL of 4M HNO_3 L in a volumetric flask.
Mo	Molybdenum Standard Solution, 100 mg L^{-1}: dissolve 0.15003 g analytical-grade molybdenum trioxide (MoO_3) in 10 mL of 0.1 N Sodium hydroxide (NaOH), make slightly acid with HCl and dilute to 1.0 L with deionized water.
NO_3-N	Nitrate-Nitrogen Standard Solution, 100 mg L^{-1}: dissolve 0.7218 g analytical-grade oven dry KNO_3 in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
Na	Sodium Standard Solution, 1000 mg L^{-1}: dissolve 2.5421 g analytical-grade KCl in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
P	Phosphorus Standard Solution, 1000 mg L^{-1}: dissolve 4.393 g oven dry analytical-grade KH_2PO_4 in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
PO_4-P	Phosphate-Phosphorus Standard Solution, 100 mg L^{-1}: dissolve 0.7218 g analytical-grade oven dry KH_2PO_4 in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
Pb	Lead Standard Solution, 1000 mg L^{-1}: dissolve 2.6758 g analytical-grade $Pb(NO_3)_2$ in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
S	Sulfur Standard Solution, 500 mg L^{-1}: dissolve 2.717 g analytical-grade oven dry K_2SO_4 in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
SO_4-S	Sulfate-Sulfur Standard Solution, 500 mg L^{-1}: dissolve 2.717 g analytical-grade oven dry K_2SO_4 in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
Se	Selenium Standard Solution, 1000 mg L^{-1}: dissolve 1.4053 g analytical-grade SeO_2 in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
Sn	Tin Standard Solution, 1000 mg L^{-1}: dissolve 1.9010 g analytical-grade $SnCl_2 \cdot 2H_2O$ in 1000 mL of 4 M HCl in a volumetric flask.
Sr	Strontium Standard Solution, 1000 mg L^{-1}: dissolve 2.4152 g analytical-grade $Sr(NO_3)_2$ in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.
Zn	Zinc Standard Solution, 1000 mg L^{-1}: dissolve 4.5506 g analytical-grade $Zn(NO_3)_2 \cdot 6H_2O$ in 500 mL of deionized water and dilute to 1.0 L in a volumetric flask.

Ward, A.F. 1978. Stock Standard Preparation. Jarrell-Ash Plasma Newslett. 1(2):14-15.

Soltanpour, P.N, G.W. Johnson, S.M. Workman, J.B. Jones and R.O. Miller. 1996. Inductively coupled plasma emission spectrometry and inductively coupled plasma-mass spectrometry. p. 91-139. *In:* J. M. Bartels et al. (ed.) Methods of soil analysis: Part 3 Chemical methods. 3rd.ed. ASA and SSSA, Madison, WI. Book series no. 5.

APPENDIX C

Table C-1. Sources of Botanical Reference Materials for use in Laboratory Quality Control Programs.

ID	Source
AAFC	Dr. M. Ihnat, Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario K1A 0C6, Canada.
AIMM	Faculty of Physics and Nuclear Techniques, University of Mining and Metallurgy, Al Mickiewicza 30, 30-059 Krakow, Poland.
ALP	Agricultural Laboratory Proficiency Program, Soil and Crop Sciences Dept, Colorado State University, Fort Collins, CO, 80550
ARC	Food Research Institute, Laboratory of Food Chemistry, Agricultural Research Centre of Finland, SF-31600 Jokioinen, Finland.
BCR	Institute of Reference Materials and Measurements (IIRMM) Retieseweg, B-2440 Geel, Belgium.
BOWEN	H.J.M. Bowen, West Down, West Street, Winterborne Kingston, Dorset DT11 9AT, Great Britain.
CANMET	Canadian Certified Reference Materials Project, Canada Centre for Mineral and Energy Technology, Natural Resources Canada, 555 Booth Street Ottawa, Ontario K1A 0G1, Canada.
IAEA	Analytical Quality Control Services, International Atomic Energy Agency, P.O. Box 100, A-1400 Wein, Austria.
ICHTJ	Commission of Trace Analysis of the Committee for Analytical Chemistry of the Polish Academy of Sciences, Department of Analytical Chemistry, Institute of Nuclear Chemistry and technology, ul. Dorodna 16, 03-195 Warszawa, Poland.
LIVSVER	Chemistry Division 2, Swedish National Food Administration, P.O. Box 622, 5-751 26 Uppsala, Sweden.
NIES	Division of Environmental Chemistry, National Institute for Environmental Studies, 16-2 Onagowa, Tsukuba, Ibaraki 305, Japan.
NIST	Standard Reference Materials Program, National Institute of Standards and Technology, Room 204 Building 202, Gaithersburg, MD 20899, USA.

Ihnat, Milan. 1993. Soil reaction and exchangeable acidity. p. 247-262. *In*: M. R. Carter (ed.) Soil sampling and methods of analysis, Canadian Society of Soil Science, Lewis Publishers Ann Arbor, MI.

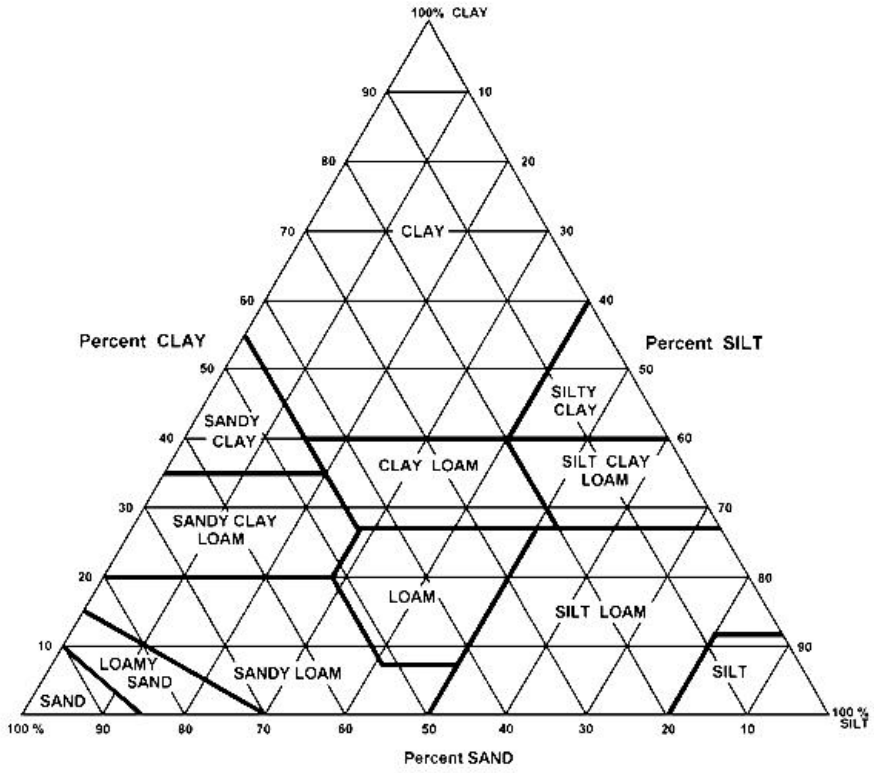
APPENDIX D

Table D-1. US Standard Testing Sieve sizes and openings.

Sieve Wire Mesh Size	Sieve Opening mm
4	4.75
5	4.00
6	3.35
7	2.80
8	2.36
10	2.00
12	1.70
14	1.40
16	1.18
18	1.00
20	0.85
25	0.710
30	0.600
35	0.500
40	0.425
45	0.355
50	0.300
60	0.250
70	0.212
80	0.180
100	0.150
120	0.125
140	0.106
200	0.075
230	0.630
270	0.530
325	0.045

Appendix E

Figure E-1. USDA Soil textural classification.

1. 

For more information on the relating soil texture to bulk density a calculator is available at the pedoshere web site at: http://www.pedosphere.com/resources/bulkdensity/worktable_us.cfm

Saxton, K.E., W.J. Rawls, J.S. Romberger, and R.I. Papendick. 1986. Estimating generalized soil-water characteristics from texture. Soil Sci. Soc. Am. J. 50(4):1031-1036**.**

Appendix F

Preparation of a Quality Control Reference Soil

A key to good laboratory quality control / quality assurance program is high quality control reference (QCR) samples. Use of a well prepared QCR soil assists a laboratory track and improve analytical performance. Development of an in-house laboratory QCR soil has two requirements: (1) chemical and physical parameters which reflect typical ranges encountered during daily analytical operation; (2) a well homogenized material in which the mean and variance can be well characterized. QCR soils should bracket the laboratory's working analytical range. Typically, soil analysis values in the low and medium range are more important than a high range soil because of the agronomic significance. High range QCR soils may have greater importance for environmental issues. When collecting a QCR soil locate a site where material can easily be collected and sufficient quantity can be obtained to be utilized over 1-2 years.

Collection, preparation and storage of QCR soil requires specific steps to ensure homogeneity and high quality over a extended period of time. Collect soil from a defined area of 200 to 600 square feet where soil type, slope, and crop residue are as homogenous as possible. The depth of collection should be limited to 4-8 inches as not to create depressional area in the field and not add variation associated with depth. Coarse fragments and crop residue (stones, root crowns, stalks, leaves and etc.) should be discarded. It is suggested that soils be air dried on large tarp (20 x 40) in thin layers 0.25 - 0.50 inches thick. While drying soil homogeneity can be enhanced by pulling on the tarp corners to the center form a pile in the center of the tarp and then using a fine rake, redistributing the soil over the tarp surface. This process should be repeated at least three times. Additional fine raking (0.30 inch spacing) rake can remove medium gravel and other crop residue. Occasionally, It may be necessary to crush large soil aggregates (> 0.5 inches diameter). Crushing should occur before the soil is completely dry. Avoid over drying as soil below 2-3% moisture increases soil aggregate resilience and increases fine dust aerosols during processing.

Standard soil analysis requires soils to be pulverized or crushed to pass a 2.0 mm (10 mesh) screen. Although this is sufficient for routine soil analysis, it is not for high quality QCR soils. Coarse textured QCR soils (sandy loams, loamy sands) should be pulverized and screened to pass 1.0 mm with medium and fine textured soils (loams, silt loams, clay loams) screened to pass 0.8 mm or finer. Removal of the coarse soil fractions increases soil uniformity and therefore analytical homogeneity. Finer QCR soil material (screened to pass 0.50 mm opening) maybe necessary for specific analytical methods utilizing less than one gram of soil material, e. g. total nitrogen, total organic carbon.

After sampling, drying, pulverizing and screening the QCR soil is blending. Small QCR soil quantities can be prepared using a rotating barrel such as a lapidary tumbler. Larger quantities (> 5 kg) require blending using a cement mixer or a large rotating drum. Rotating barrels, (such as a cement mixer), are prone to stratification of particles. Therefore it is essential to screen the QCR soil to prior to final blending.

Storage can influence the stability of a QCR soil. Many laboratories divide QCR soils into one to two kilogram quantities and store them in a zip-lock type bag. This keeps particle separation to a minimum. Bags can then be placed into a large storage container such as plastic barrels with lids. These barrels should be stored where humidity and temperature fluctuations are kept at a minimum, usually somewhere in the laboratory. When a "new" bag is taken from the barrel it should be remixed prior to its use in the laboratory.

Development of QCR standards for the soil is performed by replicated analysis along side of an already well established QCR soil. The prospective QCR soil is analyzed at least 30 times over the coarse of several days and based on repeated analysis, the mean and standard deviation of individual analytes are established. Suggested ranges of RSD (relative standard deviation) for QCR soils representing good homogeneity for a limited group of soil analyses is listed in Table F-1.

Generally acceptable homogeneity for, nitrate-nitrogen, extractable soil P (Bray, Mehlich and Olsen methods), and extractable K is less than 5% of the mean. Acceptable homogeneity for extractable sulfate sulfur, micronutrients, soil organic matter and cation exchange capacity is 10% of the mean. Note that RSD values less than 10% are very useful in tracking analytical quality, while higher RSD may have limited usefulness.

Table F-1. Suggested maximum acceptable levels of homogeneity for laboratory QCR soils.

Soil Analysis	**RSD (%)** [1]
pH (1:1, 1:2)	1.5
Buffer pH (all methods)	1.5
NO_3-N (Cadmium Reduction)	5
PO_4-P (Bray, Olsen, Mehlich-1 Mehlich-3)	5
K (NH_4-OAc, Mehlich-3)	5
Ca, Ma and Na (NH_4-OAc, Mehlich-3)	7
SO_4-S (Calcium-Phosphate, Mehlich-3)	7
Zn, Mn, Fe & Cu (DTPA and Mehlich-3)	7
Hot Water B and Mehlich 3	7
Organic Matter (WB and LOI)	7
CEC (cation replacement)	8
Sand Silt and Clay (hydrometer)	5

[1] Values based on RSD of 30 replicates.