Methods of Phosphorus Analysis

for Soils, Sediments, Residuals, and Waters

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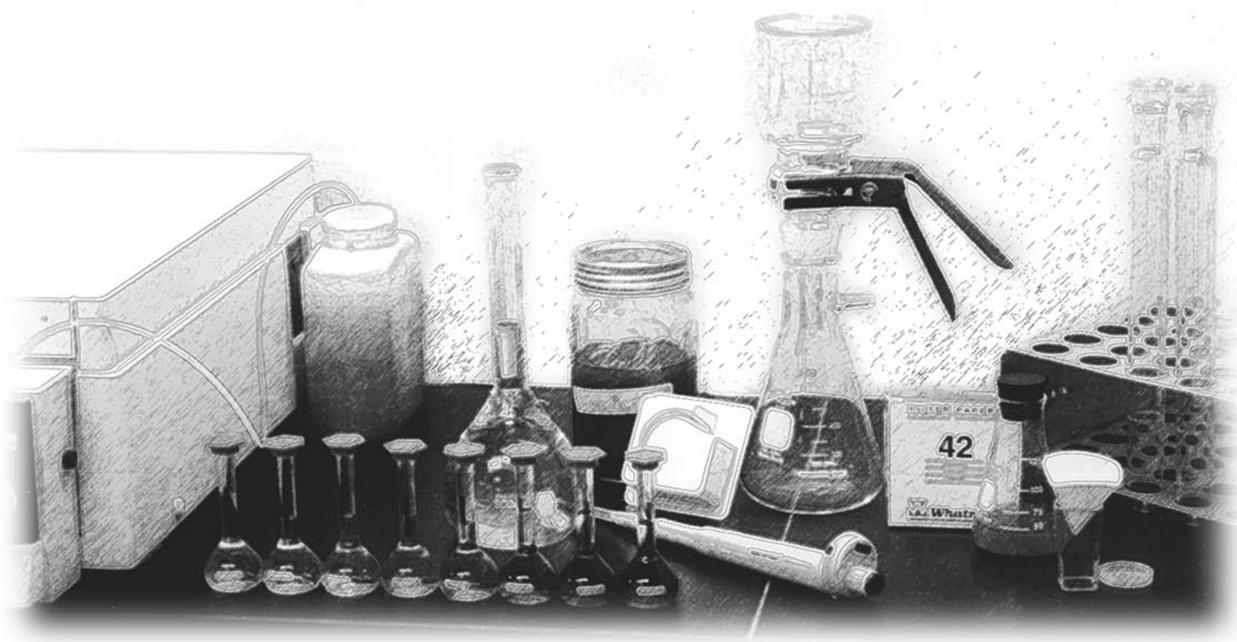

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This bulletin, from the joint Southern Extension/Research Activity - Information Exchange Group (SERA-IEG) 17, included extension specialists and research scientists. It is being published with the approval of the Southern Association of Agricultural Experiment Station Directors (SAAESD) and the Association of Southern Region Extension Directors (ASRED). Under the procedure of cooperative publications, it becomes in effect, a separate publication for each of the cooperating stations.

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Abstract

The relative contribution of phosphorus (P) from agricultural nonpoint sources to surface water quality problems has increased in recent years as point sources of P have been reduced significantly. Phosphorus contributes to eutrophication, which restricts water use for fisheries, recreation, industry, and human consumption due to increased growth of undesirable algae and aquatic weeds, followed by oxygen shortages as the biomass decomposes. The increased attention on P has increased the demand for information on methods of analysis for soil, water, and residual materials for environmentally relevant forms of P. The purpose of this publication is to present these methods in a single document. Previously, the methods have appeared across a wide variety of documents or only in the scientific literature. It is not the intent of this publication to define a uniform set of recommended methods for agronomic soils tests, water, or residual materials. The methods presented here are intended solely to provide a set of uniform testing methods for environmental scientists working across an enormous range of soil and climatic conditions, with the hope that comparable methods may lead to improved communication and understanding of this complex issue.

FOREWARD

As scientists focus on the fate of phosphorus applied to agricultural lands, it has become increasingly clear that a standard set of soil testing methods is needed to enable uniform comparison of results across county, state, regional, and even national boundaries.

By contrast, soil testing developed with a high priority on meeting local needs. As a result, many local variations in extractants and laboratory procedures have been made to achieve timely analysis and improved correlation of soil test results with plant responses within well-defined regions. Over time, enormous amounts of information on individual soils, crops and extractants have been developed using these localized modifications and laboratory methods. Soil testing labs cannot easily change from one extractant to another. The cost of repeating these calibration experiments for many soils and crops is prohibitively expensive, and the changes would initially preclude users from comparing results across years. Even so, a set of standard reference methods can be useful for laboratories wishing to consider a new analysis for a particular element, and for comparing results across laboratories. In 1992, SERA-IEG-6 selected 15 reference procedures for soil testing laboratories in the southern region. Criteria for selection included the accuracy of the method in predicting crop responses, and general acceptability by workers in the soil testing field.

This publication in no way attempts to define a uniform set of recommended methods for agronomic soil tests. The methods presented here are intended solely to provide a set of uniform testing methods for environmental scientists working across an enormous range of soil and climatic conditions, with the hope that comparable methods may lead to improved communication and understanding of this complex issue.

For more information on agronomic soil testing methods, and the source of many of the procedures described here, the reader should refer to the recent bulletins compiled by the various regional committees working on nutrient analysis of soils, plants, water, and waste materials (SERA-IEG-6, NRC-13 and NEC-67).

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Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters: Introduction

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Point sources of water pollution have been reduced significantly since the late 1960s due to their relative ease of identification, legislation, and advances in pollution control technology. Consequently, the relative contribution of agricultural nonpoint sources to remaining water quality problems has increased. Of the water quality issues that remain, a recent EPA survey has identified eutrophication as the single largest problem in surface water quality.

Eutrophication restricts water use for fisheries, recreation, industry, and drinking, due to increased growth of undesirable algae and aquatic weeds, followed by oxygen shortages as the biomass decomposes. Also, many drinking water supplies throughout the world undergo periodic massive surface blooms of cyanobacteria. These blooms contribute to a wide range of water-related problems, including summer fish kills, unpalatability of drinking water, and formation of trihalomethane, a known carcinogen, during water chlorination. Recent outbreaks of the dinoflagellate *Pfiesteria piscicida* in the eastern U.S. have also been linked to excess nutrients in affected waters. Neurological damage in people exposed to the highly toxic volatile chemical produced by this dinoflagellate has dramatically increased public awareness of eutrophication and the need for solutions. In most cases, phosphorus (P) accelerates the eutrophication of fresh waters. Consequently, controlling algal blooms and eutrophication mainly requires reducing P inputs to surface waters.

The purpose of this manual is to present methods for analysis of soil, water, and residual materials for environmentally relevant P forms in a single document. Previously, these methods appeared separately in methods publications for soil or water or have only appeared in the scientific literature. Commercial and research laboratories today must deal with the analysis of a wider range of sample types for more diverse agronomic and environmental uses. This has caused confusion over selection of the most appropriate method for a specific need and can lead to inappropriate recommendations for P management. Thus, there is an urgent need for a publication containing all of the currently available procedures for P analysis.

The mainstay of P analysis for all solution types has been use of colorimetric procedures, most notably from Murphy and Riley (1962). Colorimetric procedures are sensitive, reproducible, and lend themselves to automated analysis. In addition, the methods can accommodate water samples, digest solutions, and extracts. The basic Murphy and Riley procedure is presented in Sharpley (2000) in this bulletin. Variations in the procedure are incorporated into other sections, despite the appearance of redundancy. Modifications to the procedures are often method-specific.

Inductively coupled plasma (ICP) spectrophotometry can also be used for P determination. The use of ICP has increased as the use of multi-element soil extractants becomes more popular. Results from colorimetric analyses are not always directly comparable to those from ICP because ICP estimates the total amount of P in solution, while the colorimetric procedures measure P that can react with the color developing reagent.

Nomenclature for forms of P in soil, water, or residual materials varies in the literature, particularly for operationally-defined forms of P in water samples. Table 1 presents an abbreviated description of forms of P in runoff or drainage water that have been used in the literature and that we propose as a standardized terminology. Phosphorus forms in soils are also difficult to standardize with any reasonable consensus, due to the number of different disciplines involved (e.g., soil scientists, agronomists, limnologists, hydrologists). Thus, beyond using total soil P, we strongly encourage the use of specific chemical terminology (e.g., water extractable, $CaCl_2$ extractable, 0.1 *M* NaOH extractable, Mehlich extractable P, etc.), which has been clearly defined. Any other terminology, which may be used in conclusions and interpretations (e.g., desorbable, available, bioavailable, sorbed P etc.), must also be clearly defined.

Traditionally, extractable P has been used by soil testing laboratories to describe the amount of P in soil available for crop uptake and to determine the probability of crop response to added P, and thereby fertilizer P requirements. Bioavailable P is often used to describe P in soil or sediment that is available for uptake by algae or macrophytes in surface waters. Occasionally, bioavailable P is used to describe the availability of soil P to plants. There are also a large number of soil P extraction methods that have been designed to account for various soil types and mechanisms controlling the chemistry of soil P. For example, numerous soil extractants are available for acid soils, where Al and Fe dominate P chemistry, and basic or calcareous soils, where Ca dominates soil P reactions.

Clearly, there is a potential for confusion by the uninitiated. Hence it is essential to accurately define how P was measured in soil or water samples to avoid potential misinterpretations or inappropriate recommendations. This publication documents in detail the analytical methods available, their recommended uses, and some information on interpretation.

References:

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

Sharpley, A.N. 2000. Bioavailable phosphorus in soil. *In* G.M. Pierzynski (ed.), Methods for Phosphorus Analysis for Soils, Sediments, Residuals, and Waters. Southern Cooperative Series Bulletin No. 396, p. 39-45.

Table 1. Proposed standardization of terminology for forms of P in runoff and drainage water.

Phosphorus Form	**Abbreviation**	**Example Methodology**†
Total Phosphorus *Total amount in dissolved and particulate phases*	TP	Digestion of unfiltered water sample -Kjeldahl procedure -Acid ammonium persulfate -Perchloric acid
Total Dissolved Phosphorus *Dissolved inorganic (ortho P) and organic P*	TDP	Acid persulfate digestion of unfiltered sample
Dissolved Orthophosphate *Immediately algal available*	DP	Murphy and Riley on filtered sample
Bioavailable Phosphorus *Dissolved ortho P and a portion of particulate P that is algal available*	BAP	Extraction of unfiltered sample with -NaOH -NaC1 Anion exchange resin -Ammonium fluoride -Iron-oxide filter paper strips
Molybdate Reactive Phosphorus *Dissolved ortho P and acid extractable particulate P (possibly algal available)*	MRP	Murphy and Riley colorimetric analysis of an unfiltered sample
Particulate Phosphorus *Inorganic and organic P associated with or bound to eroded sediment*	PP	By difference = [TP - TDP]
Dissolved Organic Phosphorus‡ *Includes polyphosphates and hydrolyzable phosphates*	DOP	By difference = [TDP - DP]

† Not an inclusive list of appropriate methods that can be used. Filtered samples are defined as that passing through a 0.45µm filter.

‡ If dissolved organic P constitutes more than 25% of TDP, then measuring polyphosphates and hydrolyzable phosphates may be necessary.

Soils and Sediments

Soil Test Phosphorus: Principles and Overview

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Principles of Soil Testing for Phosphorus:

Soil testing for phosphorus (P) has been formally conducted in the United States since the late 1940s and is now a well-established agronomic practice. The fundamental goal of soil P testing has always been to identify the "optimum" soil test P concentration required for plant growth. The need for additional fertilization or manuring, and the economic return on an investment in fertilizer P, could then be predicted. Sims et al. (1998) stated that other objectives of soil P testing have been to: (i) "index" the P supplying capacity of soils, thus estimating the time before fertilization would again be required; (ii) group soils, in terms of the likelihood of an economic response to P, based on their physical and chemical properties; and, (iii) most recently, to identify when soils are sufficiently excessive in P to contribute to nonpoint source pollution of surface waters. Bray (1948) proposed that an acceptable agronomic soil P test should have the following characteristics:

- The soil test should extract all or a proportionate amount of the plant-available P from soils with differing chemical and mineralogical properties.
- The soil test should be accurate and rapid.
- The P extracted by the soil test should be well correlated with plant P concentration, plant growth, and the response of the plant to added P in fertilizers or manures.
- The soil test should accurately detect differences in soil P concentrations caused by previous fertilization or manuring.

The major steps involved in a soil P testing program are outlined in Table 1 (from Sims et al., 1998). From an agronomic perspective, if these steps are followed, soil P management will be successful and economically beneficial. However, if the goal of soil P testing is to assess the potential environmental impact of soil P, a thorough re-analysis of each step in the soil testing process, from sample collection to interpretation of results should be conducted. Several recent reviews address the principles and practices involved in environmental soil testing for P (Sibbesen and Sharpley, 1997; Sims, 1993; Sims, 1997; Sims, 1998; Sims et al., 2000).

The purpose of the following sections is to provide an overview of the four soil test P methods most commonly used in the United States and Canada today (Bray and Kurtz P-1, Mehlich 1, Mehlich 3, and Olsen P). Detailed descriptions of the laboratory methods and analytical procedures used to determine P by these methods are provided in other references (Carter, 1993; Frank, et al., 1998; Kuo, 1996; SERA-IEG-6, 1992; Sims and Wolf, 1995; SPAC, 1992). Finally, Table 2 lists other soil test P methods now used domestically and in other countries, and provides references for each method.

Table 1. Basic components in a soil testing program.

Soil Testing Component	Definition and General Considerations
Soil Sampling	*Collection of a sample that accurately represents the area of interest is the first step in an effective soil testing program. Soil samples are normally collected from the "topsoil" or "plow layer" (0-20 cm depth), although this may vary with type of crop and intent of the test. In most cases ~20-25 individual soil cores are collected from a field that is no larger than 10-15 hectares. These cores are then composited to produce one sample that is submitted to the laboratory for analysis. Soil sampling patterns should reflect natural differences in soils (e.g., soil series) and any management practices or historical activities likely to affect soil test results (e.g., crop rotation, manuring, tillage practice).*
Soil Sample Handling and Preparation	*Care should be taken during soil sample handling to avoid contamination from sampling and mixing devices. After collection, soil samples should be submitted as soon as possible to the laboratory where they are normally air-dried and ground or crushed to pass a 2mm sieve prior to analysis. Providing as much information as possible with the sample (e.g., previous fertilizer use, intended management plans, soil series) helps to ensure an accurate recommendation.*
Soil Sample Analysis	*From an agronomic perspective, the purpose of soil analysis is to chemically "extract" the amount of nutrient from the soil that is proportional to that which will be available to the crop during the growing season. Since many different soil testing methods exist (see Table 2 for an overview of soil testing methods for P), it is vital that the analytical procedures selected are appropriate to the geographic region of interest and for the intended use of the soil.*
Interpretation of Analytical Results	*The ultimate goal of soil testing is to provide the user with a recommendation as to the likelihood that the application of nutrients in fertilizers or manures will provide a profitable increase in crop response. Recommendations based on soil testing results are developed using crop response data that have been obtained within a state or region with similar soils, cropping systems, and climatic conditions. Therefore, it is important to submit samples to a laboratory that is familiar with the crops to be grown and the soils and management practices that will be used.*

Table 2. Other soil P tests used in the United States and Europe.

Soil Phosphorus Test	Reference
Ammonium bicarbonate - DTPA (AB-DTPA): The AB-DTPA soil test [1*M* NH_4HCO_3 + 0.005 *M* DTPA (Diethylenetriaminepentaacetic acid)] adjusted to pH 7.6 was developed as a multi-element soil test extractant for the western U.S. It is well correlated with Olsen P ($NaHCO_3$-P) and best suited for laboratories desiring to simultaneously analyze P, K, Ca, Mg, Cu, Fe, Mn, and Zn in neutral and calcareous soils.	Kuo (1996) Soltanpour and Schwab (1977)
Morgan's and Modified Morgan's: The Morgan's (0.72 *M* NaOAc + 0.52 *M* CH_3COOH) and the Modified Morgan's (0.62 *M* NH_4OH + 1.25 *M* CH_3COOH) soil test P extractants are mainly used in a few states in the northeastern and northwestern United States and some European countries (e.g. Ireland). These tests are best suited for acidic soils with cation exchange capacities < 20 cmol/kg.	Lunt et al. (1950) Morgan (1941) McIntosh (1969) SPAC (1992)
Ammonium lactate - acetic acid (AL-AA): The AL-AA soil test for P is used in several western European countries, with some countries substituting calcium lactate for ammonium lactate. The AL-AA solution is buffered at an acidic pH (3.75) and extracts P from Al and Fe bound forms by complexation with lactic acid.	Egner et al. (1960) Houba et al. (1997)
The P_i soil test (Iron-Oxide Impregnated Paper) The P_i soil test is fundamentally different from other soil tests in that it does not chemically extract P from soils; rather it removes P by sorption from solution onto a filter paper strip coated with Fe oxide. This facilitates desorption of available P from soil colloids. The P_i soil test has been reported to effectively measure plant available P and P susceptible to loss in runoff that is biologically available to algae and other water plants.	Chardon et al. (1996) Menon et al. (1997) Meyers et al. (1995) Kuo (1996)
Water and Dilute Salt Solutions: Deionized water and 0.01*M* $CaCl_2$ are used to extract soluble and readily desorbable P from soils. Tests such as these usually extract the most labile forms of soil P and much less P than the quantities extracted by the acidic and basic extractants commonly used by most soil testing laboratories. Water usually extracts more P than 0.01*M* $CaCl_2$ because Ca^{+2} enhances P sorption by soils. Recently, 0.01*M* $CaCl_2$ has been proposed as an effective multi-element, universal soil test (Houba, et al., 1997).	Houba et al. (1997) Kuo (1996) Sissingh (1971)

References:

Bray, R.H. 1948. Correlation of soil tests with crop response to fertilizers and with fertilizer requirement. p. 53-86. *In* H.B. Kitchen (ed.) Diagnostic techniques for soils and crops. Am. Potash Inst., Washington, D. C.

Carter, M.R. (ed.) 1993. Soil Sampling and Methods of Analysis. Can. Soc. Soil Sci., Ottawa, Ontario, Canada.

Chardon, W.J., R.G. Menon, and S.H. Chien. 1996. Iron oxide impregnated filter paper (Pi test): A review of its development and methodological research. Nutr. Cycl. Agroecosystems 46:41-51.

Egner, H., H. Riehm, and W.R. Domingo. 1960. Untersuchungen uber die chemishe bodenanalyse als grundlage fur die beurteilung des nahrstoffzustandes der boden. II. Chemische extraktions-methoden zu phosphor – and kalimbestimmung kungl. Lantbrukshoegsk. Ann. 26:204-209.

Houba, V.J.G., J.J. van der Lee, and I. Novozamski. 1997. Soil analysis procedures. Dep. of Soil Sci. Plant Nutr., Landbouwuniversiteit, Wageningen Agric. Univ., Wageningen, the Netherlands.

Frank, K., D. Beegle, and J. Denning. 1998. Phosphorus. p. 21-30. *In* J.R. Brown (ed.) Recommended Chemical Soil Test Procedures for the North Central Region. North Central Reg. Res. Publ. No. 221 (revised).

Kuo, S. 1996. Phosphorus. p. 869-919. *In* D. L. Sparks. (ed.) Methods of Soil Analysis: Part 3- Chemical Methods. SSSA, Madison, WI.

Lunt, H. A., C. L.W. Swanson, and H.G.M. Jacobson. 1950. The Morgan Soil Testing System. Bull. No. 541, Conn. Agr. Exp. Stn., New Haven, CT.

McIntosh, J. L. 1969. Bray and Morgan soil test extractants modified for testing acid soils from different parent materials. Agron. J. 61:259-265.

Menon, R.G., S.H. Chien, and W.J. Chardon. 1997. Iron oxide impregnated filter paper (Pi test): II. A review of its application. Nutr. Cycl. Agroecosystems 47:7-18.

Morgan, M.F. 1941. Chemical soil diagnosis by the universal soil testing system. Conn. Agric. Exp. Stn. Bull. No. 450.

Meyers, R.G., G.M. Pierzynski, and S.J. Thien. 1995. Improving the iron oxide sink method for extracting soil phosphorus. Soil Sci. Soc. Am. J. 59:853-857.

SERA-IEG-6 (Southern Extension Research Activity - Information Exchange Group) 1992. Donohue, S.J. (ed.) Reference Soil and Media Diagnostic procedure for the southern region of the United States. So. Coop. Series Bulletin 374. Va. Agric. Exp. Station, Blacksburg, VA.

Sibbesen, E., and A.N. Sharpley. 1997. Setting and justifying upper critical limits for phosphorus in soils. *In* H. Tunney et al., (ed.) Phosphorus Loss from Soil to Water. CAB International, London.

Sims, J.T. 1993. Environmental soil testing for phosphorus. J. Prod. Agric. 6:501-507.

Sims, J.T. 1997. Phosphorus soil testing: Innovations for water quality protection. p. 47-63. Proc. 5th Intl. Symp. Soil and Plant Analysis. Minneapolis, MN.

Sims, J.T. (ed.) 1998. Soil testing for phosphorus: Environmental uses and implications. So. Coop. Series Bull. No. 389. Univ. Delaware, Newark, DE.

Sims, J.T., and A.M. Wolf. (ed.) 1995. Recommended soil testing procedures for the Northeastern United States. (2nd ed.). Bull. No. 493. Univ. Delaware, Newark, DE.

Sims, J.T., A.C. Edwards, O.F. Schoumans, and R.R. Simard. 2000. Integrating soil phosphorus testing into environmentally based agricultural management practices. J. Environ. Qual. In Press.

Sims, J.T., S.C. Hodges, and J. Davis. 1998. Soil testing for phosphorus: Current status and uses in nutrient management programs. p.13-20. *In* Sims, J.T. (ed.) 1998. Soil testing for phosphorus: Environmental uses and implications. So. Coop. Series Bull. No. 389. Univ. Delaware, Newark, DE. (http://www.soil.ncsu.edu/sera17/)

Sissingh, H.A. 1971. Analytical technique of the Pw method used for the assessment of the phosphate status of arable soils in the Netherlands. Plant Soil 34:438-446.

SPAC (Soil and Plant Analysis Council). 1992. Handbook on reference methods for soil analysis. Georgia Univ. Stn., Athens, GA.

Soltanpour, P.N. and A.P. Schwab. 1977. A new soil test for simultaneous determination of macro and micro-nutrients in alkaline soils. Commun. Soil Sci. Plant Anal. 8:195-207.

Sample Collection, Handling, Preparation, and Storage

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Sample collection:

The collection of a representative and reliable soil sample for phosphorus (P) analysis requires predetermination of sampling depth, position relative to nutrient application patterns, and sampling intensity within the field. The appropriate soil sampling depth is dependent upon the planned interpretation of the analytical data. If investigation of P distribution or concentration with depth is a specified research objective, three factors must be considered when determining the appropriate sampling depth: 1) influence of changes in soil morphology with depth (i.e., horizonation); 2) influence of surface soil management (e.g., tillage); and 3) necessity to maintain sample collection depth uniformity across numerous sites.

Sample collection depth based on observed morphological horizon depths is quite useful when attempting to associate soil P measurements with soil physical properties. This technique may generate very reliable data for a particular, well-defined location, but this laborious task is not very practical when a research project focuses on more than a few soils or when the data will be subjected to broader, perhaps watershed-scale, interpretation.

Depth of tillage will dramatically impact soil P distribution with depth. Tillage depth is seldom constant across a given field. Sampling depths should include soil collected from a depth confidently within the tillage zone and excluding soil from below the tillage zone. A second transitional depth should be collected that is expected to be variably affected by tillage and includes the lower tillage boundary. Deeper sampling depths should not be directly impacted by physical tillage activity.

Relating soil physical and chemical properties to the potential for P transport with surface runoff water requires a different approach to soil sample collection. Sharpley (1985) studied five soils of varying physical and chemical properties and found that effective depth of interaction between surface soil and runoff ranged from 2 to 40 mm. The effective depth of interaction varied by soil type, surface slope, rainfall intensity, and crop residue. For most agricultural soils, samples collected to a depth of 20 mm would accurately define the effective depth of runoff interaction generated by moderate to high rainfall intensity (< 50 mm/h). For medium to coarse textured soils on steeper slopes (>12 %) that are subjected to high intensity rainfall (> 100 mm /h), soils should be sampled to a depth of 40 mm in order to more accurately relate the potential for P transport with surface runoff to soil physical and chemical properties.

Recommended soil sampling intensity is usually between 10 and 30 subsamples per composite sample (Whitney et al., 1985; Kitchen et al., 1990; Coale, 1997). A single composite sample may represent a single research plot or an entire production field, but generally not more than 10 ha.

Discrete nutrient application patterns in a field can increase the complexity of appropriate soil sample collection procedures. In a review of positional P availability resulting from band application of fertilizer P, Sharpley and Halvorson (1994) stated that collection of 15 random samples (Ward and Leikam, 1986; Shapiro, 1988) to 30 random samples (Hooker, 1976) were adequate to reflect crop P availability in conventionally tilled fields where previous P fertilizer bands exist. For no-till or minimum-till soils containing residual P fertilizer bands in which the location of the P bands is known, sampling to include one “in-the-band” soil sample for every 20 “between-the-band” samples for 76 cm band spacing, and one “in-the-band” sample for every 8 “between-the-band” samples for 30 cm band spacing, will accurately reflect the mean soil P status of the field (Kitchen et al., 1990). Twenty to 30 subsamples per composite are adequate. When the location of the P bands is not known, collection of 20 to 30 subsamples per composite is also adequate but paired subsamples should be collected where the location of the first subsample of the pair is completely random and the second subsample of the pair is located 50% of the band-spacing distance from the first, perpendicular to the band direction (Kitchen et al., 1990).

Sample Handling, and Preparation and Storage:

Air-drying should be satisfactory for investigations into relative changes in soil P concentrations in response to imposed treatments or for routine comparative P analyses. Soil samples should be air-dried (25 to 30^{o}C) and crushed to pass a 2 mm sieve. Air-dried and crushed soil samples are stable at room temperature. Air-drying may not be suitable for determination of the absolute quantity of the various P fractions in soils. Air-drying may artificially elevate the quantity of soluble reactive P above *in situ* conditions. Bartlett and James (1980) studied P solubility in the surface soil of a loamy fine sand and found water-soluble P concentrations to be five times higher in air-dried samples (~30 mg P/ L) than in samples stored at field moisture (~5 mg P/ L). The effect of air-drying was only partially reversed by rewetting and incubating the air-dried soil for one month (~20 mg P /L). Water-soluble P in rewetted soil samples that had previously been air-dried was shown to decrease during three months of storage at 20^{o}C (Bartlett and James, 1980). For quantitative characterization studies, soil and sediment samples should be stored at field moisture content under refrigeration, between 0 and 4^{o}C. Soil and sediment samples should not be stored frozen (<0^{o}C), because the water-soluble proportion of total P increases after freezing (Mack and Barber, 1960). Mixing moist soil samples to achieve homogeneity is difficult, and careful attention should be paid to ensure thorough mixing prior to subsampling. Moist soils are also difficult to sieve, but large particles (> 2mm) should be removed from the sample prior to analysis.

References:

Bartlett, R. and B. James. 1980. Studying dried, stored soil samples - some pitfalls. Soil Sci. Soc. Am. J. 44:721-724.

Coale, F. J. (ed.) 1997. Chesapeake Bay region nutrient management training manual. USEPA Chesapeake Bay Program, Annapolis, MD.

Hooker, M.L. 1976. Soil sampling intensities required to estimate available N and P in five Nebraska soil types. MS Thesis, Univ. Nebraska, Lincoln, NE.

Kitchen, N.R., J.L. Havlin, and D.G. Westfall. 1990 Soil sampling under no-till banded phosphorus. Soil Sci. Soc. Am. J. 54:1661-1995.

Mack, A.R. and S.A. Barber. 1960. Influence of temperature and moisture on soil phosphorus. I. Effect on soil phosphorus fractions. Soil Sci. Soc. Am. Proc. 24:381-385.
Shapiro, C.A. 1988. Soil sampling fields with a history of fertilizer bands. Soil Sci. News, Nebraska Coop. Ext. Serv., Vol. 10, No. 5.
Sharpley, A.N. 1985. Depth of surface soil-runoff interaction as affected by rainfall, soil slope, and management. Soil Sci. Soc. Am. J. 49:1010-1015.
Sharpley, A.N. and A.D. Halvorson. 1994. The management of soil phosphorus availability and its impact on surface water quality. p. 7-90. *In* R. Lal and B. A. Stewart (ed.) Soil processes and water quality. Advances in Soil Science. Lewis Publishers, Boca Raton, FL.
Ward, R. and D.F. Leikman. 1986. Soil sampling techniques for reduced tillage and band fertilizer application. *In* Proc. Great Plains Soil Fertility Workshop. March 4-5, 1986. Denver, CO.
Whitney, D.A., J.T. Cope, and L.F. Welch. 1985. Prescribing soil and crop nutrient needs. p. 25-52. *In* O. P. Engelstad (ed.) Fertilizer technology and use. 3rd ed. SSSA, Madison, WI.

Soil Test Phosphorus: Bray and Kurtz P-1

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Introduction:

The Bray and Kurtz P-1 soil test phosphorus (P) method was developed by Roger H. Bray and Touby Kurtz of the Illinois Agricultural Experiment Station in 1945 and is now widely used in the Midwestern and North Central United States (Bray and Kurtz, 1945; Frank et al., 1998). Phosphorus extracted by the Bray and Kurtz P-1 method has been shown to be well-correlated with crop yield response on most acid and neutral soils in these regions. For acid soils, the fluoride in the Bray and Kurtz extractant enhances P release from aluminum phosphates by decreasing Al activity in solution through the formation of various Al-F complexes. Fluoride is also effective at suppressing the re-adsorption of solubilized P by soil colloids. The acidic nature of the extractant (pH 2.6) also contributes to dissolution of available P from Al, Ca, and Fe-bound forms in most soils. The Bray soil test is not suitable for:

- clay soils with a moderately high degree of base saturation,
- silty clay loam or finer-textured soils that are calcareous or have a high pH value (pH > 6.8) or have a high degree of base saturation,
- soils with a calcium carbonate equivalent > 7% of the base saturation, or
- soils with large amounts of lime (> 2% $CaCO_3$).

In soils such as these, the acidity of the extracting solution can be neutralized unless the ratio of extractant:soil is increased considerably. Additionally, CaF_2, formed from the reaction of soluble Ca^{+2} in the soil with F- added in the extractant, can react with and immobilize soil P. Both types of reactions reduce the efficiency of P extraction and result in low soil test P values. Finally, the Bray and Kurtz extractant can dissolve P from rock phosphates, therefore it should not be used in soils recently amended with these materials, as it will overestimate available P. A Bray and Kurtz P-1 value of 25 to 30 mg P/kg soil is often considered optimum for plant growth, although Holford (1980) reported lower critical values for highly buffered soils.

Equipment:

1. No. 10 (2 mm opening) sieve
2. Standard 1 g and 2 g stainless steel soil scoops
3. Automatic extractant dispenser, 25 mL capacity
4. Extraction vessels, such as 50 mL Erlenmeyer flasks, and filter funnels (9 and 11 cm) and racks
5. Rotating or reciprocating shaker with a capability of 200 excursions per minute (epm)
6. Whatman No. 42 or No. 2 (or equivalent) filter paper, 9 to 11 cm. (Acid resistant filter paper may be needed if using an automated method for determining P concentration by intensity of color. Bits of filter paper may cause an obstruction in the injection valves.)

Reagents:

1. Bray and Kurtz P-1 Extracting Solution (0.025 M HCl in 0.03 M NH4F): Dissolve 11.11 g of reagent-grade ammonium fluoride (NH4F) in about 9 L of distilled water. Add 250 mL of previously standardized 1M HCl and make to 10 L volume with distilled water. Mix thoroughly. The pH of the resulting solution should be pH 2.6 ± 0.05. The adjustments to pH are made using HCl or ammonium hydroxide (NH4OH). Store in polyethylene carboys until use.

Procedure:

1. Scoop or weigh 2 g of soil into a 50 mL Erlenmeyer flask, tapping the scoop on the funnel or flask to remove all of the soil from the scoop.
2. Add 20 mL of extracting solution to each flask and shake at 200 or more epm for five minutes at a room temperature at 24 to 27^{o}C
3. If it is necessary to obtain a colorless filtrate, add 1 cm^3 (~200 mg) of charcoal (DARCO G60, J. T. Baker, Phillipburg, NJ) to each flask.
4. Filter extracts through Whatman No. 42 filter paper or through a similar grade of paper. Refilter if extracts are not clear.
5. Analyze for P by colorimetry or inductively coupled plasma emission spectroscopy using a blank and standards prepared in the Bray P-1 extracting solution.

Calculations:

Bray and Kurtz P-1 Extractable phosphorus is calculated as

$$\text{Bray and Kurtz P-1 Extractable P (mg P/kg soil)} = \frac{C_P \text{ x } [0.020 \text{ L extract}]}{0.002 \text{ kg soil}}$$

where

C_P = Concentration of P in Bray and Kurtz P-1 extract, in mg/L .

References:

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

Frank, K.D. Beegle, and J. Denning. 1998. Phosphorus. p. 21-30. *In* J. R. Brown (ed.) Recommended Chemical Soil Test Procedures for the North Central Region. North Central Reg. Res. Publ. No. 221 (revised).

Holford, I.C.R. 1980. Greenhouse evaluation of four phosphorus soil tests in relation to phosphate buffering and labile phosphate in soils. Soil Sci. Soc. Am. J. 44:555-559.

Soil Test Phosphorus: Mehlich 1

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Introduction:

The Mehlich 1 soil test for phosphorus (P), also known as the dilute double acid or North Carolina extractant, was developed in the early 1950s by Mehlich and his co-workers (Mehlich, 1953; Nelson et al. 1953). In the United States the Mehlich 1 procedure is primarily used in the southeastern and mid-Atlantic states as a multi-element extractant for P, K, Ca, Mg, Cu, Fe, Mn, and Zn. The Mehlich 1 extracts P from aluminum, iron, and calcium phosphates and is best suited to acid soils (pH < 6.5) with low cation exchange capacities (< 10 cmol/kg) and organic matter contents (< 5%). Kuo (1996) reported that the Mehlich 1 soil test was unreliable for calcareous or alkaline soils because it extracts large amounts of nonlabile P in soils with pH > 6.5, soils that have been recently amended with rock phosphate, and soils with high cation exchange capacity (CEC) or high base saturation. In soils such as these the acidity of the Mehlich 1 solution is neutralized, reducing the capability of the dilute acid to extract P. Similar reductions in P extraction efficiency have been attributed to clay and hydrous aluminum and iron oxides (Nelson et al., 1953; Lins & Cox, 1989).

A Mehlich 1 P value of 20 to 25 mg P/kg soil for the Mehlich-1 test is generally considered to be optimum for plant growth, although this may vary slightly between soil types and cropping systems. For instance, Kamprath and Watson (1980) stated a Mehlich-1 P of 20 to 25 mg P/kg soil is adequate for plants grown in sandy soils but only 10 mg P/kg soil is required for fine-textured soils, a point supported by the work of Lins and Cox (1989).

Equipment:

1. No. 10 (2 mm opening) sieve
2. Automatic extractant dispenser, 25 mL capacity (If preferred, pipettes are acceptable.)
3. Standard 5 cm^3 and 1 cm^3 stainless steel soil scoops
4. Extraction vessels, such as 50 mL Erlenmeyer flasks, and filter funnels (9 and 11 cm) and racks
5. Reciprocating or rotary shaker, capable of at least 180 epm (excursions per minute)
6. Whatman No. 42 or No. 2 (or equivalent) filter paper, 9 to 11 cm. (Acid resistant filter paper may be needed if using an automated method for determining P concentration by intensity of color. Bits of filter paper may cause an obstruction in the injection valves.)

Reagents:

1. Mehlich 1 Extracting Solution (0.0125 M H2SO4 + 0.05 M HCl). Also referred to as dilute double acid or the North Carolina Extractant. Using a graduated cylinder, add 167 mL of concentrated HCl (12M) and 28 mL of concentrated H2SO4 (18M) to ~35 L of deionized water in a large polypropylene carboy.

Make to a final volume of 40 L by adding deionized water. Mix well by bubbling air through the solution for 3 hours.

Procedure:

7. Weigh 5.0 g (or scoop 4 cm^3) of sieved (< 2 mm), air-dried soil into a 50 mL extraction flask.
8. If it is necessary to obtain a colorless filtrate, add 1 cm3 (~200 mg) of charcoal (DARCO G60, J. T. Baker, Phillipburg, NJ) to each flask.
9. Add 20 mL of the Mehlich 1 extracting solution and shake for five minutes on a reciprocating shaker set at a minimum of 180 epm at a room temperature at 24 to 27^{o}C.
10. Filter through a medium-porosity filter paper (Whatman No. 2 or equivalent).
11. Analyze for P by colorimetry or inductively coupled plasma emission spectroscopy using a blank and standards prepared in the Mehlich 1 extracting solution.

Calculations:

Mehlich 1 Extractable P (mg P/kg soil) =
[Concentration of P in Mehlich 1 extract, mg/L] x [0.020 L extract ÷ 0.005 kg soil]

References:

Kamprath, E.J. and M.E. Watson. 1980. Conventional soil and tissue tests for assessing the phosphorus status of soils. p. 433-469. *In* F. E. Khasawneh et al. (ed.) The role of phosphorus in agriculture. ASA, CSSA, and SSSA, Madison, WI.

Kuo, S. 1996. Phosphorus. p. 869-919. *In* D. L. Sparks. (ed.) Methods of Soil Analysis: Part 3- Chemical Methods. SSSA, Madison, WI.

Lins, I.D.G. and F.R. Cox. 1989. Effects of extractant and selected soil properties on predicting the optimum phosphorus fertilizer rate for growing soybeans under field conditions. Commun. Soil Sci. Plant Anal. 20:310-333.

Mehlich, A. 1953. Determination of P, Ca, Mg, K, Na, and NH_4. North Carolina Soil Test Division (Mimeo). Raleigh, NC.

Nelson, W. L., A. Mehlich, and E. Winters. 1953. The development, evaluation, and use of soil tests for phosphorus availability. Agronomy 4:153-158.

Soil Test Phosphorus: Mehlich 3

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Introduction:

The Mehlich 3 soil test was developed by Mehlich in 1984 as an improved multi-element extractant for P, K, Ca, Mn, Cu, Fe, Mn, and Zn (Mehlich, 1984). Today, the Mehlich 3 test is used throughout the United States and Canada because it is well suited to a wide range of soils, both acidic and basic in reaction. The Mehlich 3 extractant was selected by workers in the southern region as the standard reference procedure for soil test P determination (Tucker, 1992). The Mehlich 3 is similar in principle to the Bray and Kurtz P-1 test because it is an acidic solution that contains ammonium fluoride. Acetic acid in the extractant also contributes to the release of available P in most soils. It is more effective than the Mehlich 1 soil test at predicting crop response to P on neutral and alkaline soils because the acidity of the extractant is neutralized less by soil carbonates (Tran and Simard, 1993). Several studies showed that the Mehlich 3 soil test is highly correlated with P extracted from soils by the Bray and Kurtz P-1, Mehlich 1, and Olsen P methods (Sims, 1989; Tran et al., 1990; Wolf and Baker, 1985).

A Mehlich 3 value of 45-50 mg P/kg soil is generally considered to be optimum for plant growth and crop yields, higher than the critical values used for other standard soil P tests such as the Bray and Kurtz P-1, Mehlich 1, and Olsen P.

Equipment:

1. No. 10 (2 mm opening) sieve
2. Standard 1 cm^3 , 2 cm^3 (or 2.5 cm^3) stainless steel soil scoops
3. Automatic extractant dispenser, 25 mL capacity
4. Extraction vessels, such as 50 mL Erlenmeyer flasks, and filter funnels (9 and 11 cm) and racks
5. Rotating or reciprocating shaker with a capability of 200 excursions per minute (epm)
6. Whatman No. 42 or No. 2 (or equivalent) filter paper, 9 to 11 cm. (Acid resistant filter paper may be needed if using an automated method for determining P concentration by intensity of color. Bits of filter paper may cause an obstruction in the injection valves.)

Reagents:

1. Mehlich 3 Extracting Solution: (0.2 M CH3COOH, 0.25 M NH4NO3, 0.015 M NH4F, 0.013 M HNO3, 0.001 M EDTA [(HOOCCH2)2NCH2CH2N (CH2COOH)2]. Prepare as follows:

Ammonium fluoride (NH4F) and EDTA stock solution (3.75 M NH4F:0.25 M EDTA)

2. Add 1,200 mL of distilled water to a 2 L volumetric flask.
3. Add 277.8 g of NH4F and mix well.
4. Add 146.1 g EDTA to the solution.

5. Make solution to 2 L, mix well and store in plastic (stock solution for 10,000 samples).

Mehlich 3 extractant preparation

6. Add 8 L of distilled water to a 10 L carboy.
7. Dissolve 200 g of ammonium nitrate (NH4NO3) in the distilled water.
8. Add 40 mL NH4F-EDTA stock solution and mix well.
9. Add 115 mL glacial acetic acid (99.5%, 17.4 M).
10. Add 8.2 mL of concentrated nitric acid (HNO3, 68 to 70 %, 15.5 M).
11. Add distilled water to 10 L final volume and mix well (enough extractant for 400 samples), final pH should be 2.5 ± 0.1.

Procedure:

1. Scoop or weigh 2.0 g of soil into a 50 mL Erlenmeyer flask, tapping the scoop on the funnel or flask to remove all of the soil from the scoop. Where disturbed bulk density of soil varies significantly from 1.0 g cm^3, record both weight and volume of samples. (Standard 2.5 cm^3 scoops may also be used, but a 1:10 soil:extractant volumetric ratio should be maintained)
2. Add 20 mL of extracting solution to each flask and shake at 200 or more epm for five minutes at a room temperature at 24 to 27^{o}C.
3. If it is necessary to obtain a colorless filtrate, add 1 cm^3 (~200 mg) of charcoal (DARCO G60, J. T. Baker, Phillipburg, NJ) to each flask.
4. Filter extracts through Whatman No. 42 filter paper or through a similar grade of paper. Refilter if extracts are not clear.
5. Analyze for P by colorimetry or inductively coupled plasma emission spectroscopy using a blank and standards prepared in the Mehlich 3 extracting solution.

Calculations:

Mehlich 3 Extractable P (mg P/kg) =
[Concentration of P in Mehlich 3 extract, mg P/L] x [0.020 L extract ÷ 0.002 kg soil]

References:

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of the Mehlich 2 extractant. Commun. Soil Sci. Plant Anal. 15:1409-1416.

Tucker, M.R., 1992. Determination of phosphorus by Mehlich 3 extractant. *In* Donohue, S.J. (ed.) Reference Soil and Media Diagnostic procedure for the southern region of the United States. So. Coop. Series Bulletin 374. Va. Agric. Exp. Station, Blacksburg, VA. p. 9-12.

Sims, J. T. 1989. Comparison of Mehlich 1 and Mehlich 3 extractants for P, K, Ca, Mg, Mn, Cu, and Zn in Atlantic Coastal Plain soils. Commun. Soil Sci. Plant Anal. 20:1707-1726.

Tran, T. Sen and R.R. Simard. 1993. Mehlich 3 extractable elements. p. 43-49. *In* M.R. Carter (ed.) Soil Sampling and Methods of Analysis. Can. Soc. Soil Sci., Ottawa, Ontario.

Tran, T. Sen, M. Giroux, J. Guilbeault, and P. Audesse. 1990. Evaluation of Mehlich 3 extractant to estimate available P in Quebec soils. Commun. Soil Sci. Plant Anal. 21:1-28.

Wolf, A.M. and D.E. Baker. 1985. Comparison of soil test phosphorus by the Olsen, Bray P1, Mehlich 1 and Mehlich 3 methods. Commun. Soil Sci. Plant Anal. 16:467-484.

Soil Test Phosphorus: Olsen P

J. Thomas Sims, University of Delaware

Introduction:

The "Olsen P" or sodium bicarbonate soil test phosphorus (P) method was developed by Sterling R. Olsen and co-workers in 1954 (Olsen et al., 1954) to predict crop response to fertilizer P inputs on calcareous soils. It is primarily used in the North Central and western United States. The Olsen P method is best suited for calcareous soils, particularly those with > 2% calcium carbonate, but has been shown in some research to be reasonably effective for acidic soils (Fixen and Grove, 1990). The method is based on the use of the HCO_3^-, CO_3^{-3} and OH^- in the pH 8.5, 0.5*M* $NaHCO_3$ solution to decrease the solution concentrations of soluble Ca^2 by precipitation as $CaCO_3$ and soluble Al^{3+} and Fe^{+3} by formation of Al and Fe oxyhydroxides, thus increasing P solubility. The increased surface negative charges and/or decreased number of sorption sites on Fe and Al oxide surfaces at high pH levels also enhance desorption of available P into solution.

An Olsen P value of 10 mg P/kg is generally considered to be optimum for plant growth. This is lower than the critical values used for the Bray and Kurtz P-1, Mehlich 1 and Mehlich 3 soil tests because the Olsen extractant removes less P from most soils than these acidic extractants. Kuo (1996) stated that proper interpretation of Olsen P results for soils with diverse properties requires some information on soil P sorption capacity. Similarly, Schoenau and Karamanos (1993) cautioned against use of the Olsen test to compare P availability in soils with large differences in P chemistry.

Equipment:

1. No. 10 (2 mm opening) sieve
2. Standard 1 g and 2 g stainless steel soil scoops
3. Automatic extractant dispenser, 25 mL capacity
4. Extraction vessels, such as 50 mL Erlenmeyer flasks, and filter funnels (9 and 11 cm) and racks
5. Rotating or reciprocating shaker with a capability of 200 excursions per minute (epm)
6. Whatman No. 42 or No. 2 (or equivalent) filter paper, 9 to 11 cm. (Acid resistant filter paper may be needed if using an automated method for determining P concentration by intensity of color. Bits of filter paper may cause an obstruction in the injection valves.)

Reagents:

1. Olsen P Extracting Solution (0.5*M* $NaHCO_3$, pH 8.5): Dissolve 420 g commercial-grade sodium bicarbonate ($NaHCO_3$) in distilled water and make to a final volume of 10 L. Note that a magnetic stirrer or electric mixer is needed to dissolve the $NaHCO_3$. Adjust extracting solution pH to 8.5 with 50% sodium hydroxide.

Procedure:

1. Scoop or weigh 1 g of soil into a 50 mL Erlenmeyer flask, tapping the scoop on the funnel or flask to remove all of the soil from the scoop.
2. Add 20 mL of extracting solution to each flask and shake at 200 or more epm for 30 minutes at a room temperature at 24 to 27^0C
3. If it is necessary to obtain a colorless filtrate, add 1 cm^3 (~200 mg) of charcoal (DARCO G60, J. T. Baker, Phillipburg, NJ) to each flask.
4. Filter extracts through Whatman No. 42 filter paper or through a similar grade of paper. Refilter if extracts are not clear.
5. Analyze for P by colorimetry or inductively coupled plasma emission spectroscopy using a blank and standards prepared in the Olsen P extracting solution.

Calculations:

Olsen Extractable P (mg P/kg soil) =
[Concentration of P in Olsen extract, mg/L] x [0.020 L extract ÷ 0.001 kg soil]

References:

Fixen, P.E. and J.H. Grove. 1990. Testing soils for phosphorus. p. 141-180. *In* R.L. Westerman (ed.) Soil Testing and Plant Analysis. SSSA, Madison, WI.

Kuo, S. 1996. Phosphorus. p. 869-919. *In* D.L. Sparks. (ed.). Methods of Soil Analysis: Part 3- Chemical Methods. SSSA, Madison, WI.

Olsen, S.R., C.V. Cole, F.S. Watanabe, and L.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. U.S. Government Printing Office, Washington D.C.

Schoenau, J.J. and R.E. Karamanos. 1993. Sodium bicarbonate extractable P, K, and N. p. 51-58. *In* M. R. Carter (ed.) Soil Sampling and Methods of Analysis. Can. Soc. Soil Sci., Ottawa, Ontario.

A Phosphorus Sorption Index

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Introduction:

The phosphorus (P) sorption capacity of soils is typically determined by the use of batch equilibrium experiments that are used to generate sorption isotherms. These isotherms are plots of the amount of P adsorbed from several solutions of known initial concentration vs. the P concentration at equilibrium for each solution. For example, Nair et al., (1984) proposed, based on an interlaboratory comparison study, a standard approach to construct P sorption isotherms, using a soil:solution ratio of 1:25 (w:v), six initial P concentrations (as KH_2PO_4 in a 0.01*M* $CaCl_2$ matrix), and a 24 h equilibration period Results from sorption isotherms can be used to calculate P sorption maxima and P bonding energies for soils with different properties and/or as influenced by cultural practices, such as crop rotation, tillage, and manuring.

While useful for agronomic and environmental characterization of the P sorption capacity of soils, P sorption isotherms are too time-consuming, complicated, and expensive for routine use. To overcome these obstacles Bache and Williams (1971) developed a "P Sorption Index" (PSI) that could rapidly determine soil P sorption capacity. They evaluated 12 approaches and found that a PSI derived from a single-point isotherm (P sorbed from a single solution containing 50 µmol P/g soil) was easy to use and well correlated with the P sorption capacity of 42 acid and calcareous soils from Scotland (r=0.97***). Other researchers have used the PSI, or modified versions, and shown it to be well correlated with soil P sorption capacity determined from complete sorption isotherms for soils of widely varying chemical and physical properties (Mozaffari and Sims, 1994; Sharpley et al., 1984; Simard et al., 1994). In most cases these researchers have maintained the original ratio of added P to soil (1.5 g/kg), but have slightly changed the soil:solution ratio, background electrolyte, and/or shaking time. Most of these modifications have not affected the correlations between P sorption capacity estimated from the PSI and that determined by a full sorption isotherm. The procedure described below is based on Bache and Williams (1971). Details on other approaches are available in the references cited above.

Equipment:

1. Centrifuge and 50 mL polyethylene centrifuge tubes.
2. Shaker (end-over-end shaker preferred to ensure thorough mixing of soil and sorption solution).
3. Millipore filtration apparatus (0.45-µm pore size filters) and vacuum flasks.
4. 50 mL screw-top test tubes.

Reagents:

1. *<u>Phosphorus Sorption Solution</u>* (75 mg P/L): Dissolve 0.3295 g of monobasic potassium phosphate (KH_2PO_4) in 1 L of deionized H_2O. Store in refrigerator until use.

Procedure:

1. Weigh 1.00 g of air-dried, sieved (2 mm) soil into a 50 mL centrifuge tube.
2. Add 20 mL of the 75 mg P/L sorption solution to the centrifuge tube. (Note: This provides a ratio of 1.5 g P /kg soil). Add two drops of toluene or chloroform to inhibit microbial activity.
3. Place the tubes in the end-over-end shaker and shake for 18 h at 25±2^{o}C.
4. Centrifuge the samples at 2000 rpm for 30 minutes.
5. Using the Millipore filtration apparatus, 0.45-µm filters, and large vacuum flasks, filter the centrifugate into 50 mL screw-top test tubes within the flask.
6. Measure P concentration in the centrifugate colorimetrically or by inductively coupled plasma emission spectroscopy (ICP-AES).

Calculations:

The PSI has usually been calculated as follows, although some studies have shown that expressing PSI directly in mg/kg is acceptable.

$$\text{PSI (L kg}^{-1}) = \frac{X}{\log C}$$

where:

$$X = \text{P sorbed (mgP/kg)} = \frac{(75\text{ mg P/L - P}_f)\text{ x }(0.020\text{ L})}{(0.001\text{ kg soil})}$$

C = P concentration at equilibrium (mg/L),

and

P_f = Final P concentration after 18 h equilibration (in mg/L).

References:

Bache, B.W., and E.G. Williams. 1971. A phosphate sorption index for soils. J. Soil Sci. 22:289-301.

Mozaffari, P.M., and J.T. Sims. 1994. Phosphorus availability and sorption in an Atlantic Coastal Plain watershed dominated by intensive, animal-based agriculture. Soil Sci. 157:97-107.

Nair, P.S., T.J. Logan, A.N. Sharpley, L.E. Sommers, M.A. Tabatabai, and T.L. Yuan. 1984. Interlaboratory comparison of a standardized phosphorus adsorption procedure. J. Environ. Qual. 13:591-595.

Sharpley, A.N., S.J. Smith, B.A. Stewart, and A.C. Mathers. 1984. Forms of phosphorus in soils receiving cattle feedlot waste. J. Environ. Qual. 13:211-215.

Simard, R.R., D. Cluis, G. Gangbazo, and A. Pesant. 1994. Phosphorus sorption and desorption indices for soils. Commun. Soil Sci. Plant Anal. 25:1483-1494.

Determination of Water- and/or Dilute Salt-Extractable Phosphorus

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Introduction:

Many methods exist to determine the various forms of soil phosphorus (P). Early interests in examining soil P were primarily based on determining the quantity of supplemental P needed to adequately meet the needs of crops. The method for using distilled water as an extractant to determine P needs of plants was examined in a paper by Luscombe et al. (1979). They found a good correlation between the concentration of water-extractable P and dry matter yield responses in ryegrass.

There is now a national focus on examining excessive P buildup in the soil and consequent excessive P concentrations in runoff from agricultural land. Many studies have examined methods that best correlate soil P levels to concentrations of P in runoff (Sharpley, 1995; Pote et al., 1996). The study conducted by Pote et al. (1996) found an excellent correlation between water extractable soil test P and dissolved reactive P concentrations in runoff.

One criticism of various other extractants is that they are either more acid or alkaline than the soil solution. Therefore, a portion of P extracted is actually of low availability. For example, extractants such as Mehlich 3, which contain strong acids, would be expected to dissolve calcium phosphates. Also, due to the specific chemical nature of many extractants, their use is limited to specific soil types. Using distilled water or 0.01 *M* $CaCl_2$ overcomes these criticisms (Pote et al., 1995).

The following methods are variations of the method described by Olsen and Sommers (1982) for determination of water-soluble P in soils.

Equipment:

1. Shaker (reciprocating or end-over-end).
2. Centrifuge.
3. Centrifuge tubes (40 mL).
4. Filtration apparatus (0.45 μm pore diameter membrane filter, or Whatman No. 42).
5. Spectrophotometer with infrared phototube for use at 880 nm.
6. Acid washed glassware and plastic bottles: graduated cylinders (5 mL to 100 mL), volumetric flasks (100 mL, 500 mL, and 1000 mL), storage bottles, pipets, dropper bottles, and test tubes or flasks for reading sample absorbance.

Reagents:

1. Concentrated hydrochloric acid (HCl).
2. Reagents used for ascorbic acid technique for P determination, Murphy and Riley (1962).
3. *M* calcium chloride ($CaCl_2$).
4. Chloroform.

Extraction Procedure - Deionized Water:

Weigh out 2 g of soil (dried in a forced-draft oven at 60°C for 48 hours, sieved through a 2-mm mesh sieve) into a 40 mL centrifuge tube. Add 20 mL of distilled water and shake for one hour. Centrifuge at 6,000 rpm for 10 minutes. Filter the solution through a 0.45 μm membrane filter. Acidify to pH 2.0 with HCl to prevent precipitation of phosphate compounds (approximately 2 days of concentrated HC1). Freeze the sample if it is not going to be analyzed that day. Previous articles have noted that hydrolysis of condensed phosphates can occur when the solution is acidified (Lee et al., 1965). Also, at this pH level, there is the possibility of flocculation of organics. However, it is vital to ensure that the P remains in solution, therefore, we consider the negative effects of acidification minimal.

Extraction Procedure - 0.01*M* $CaCl_2$:

Weigh out 1 g of dry soil into a 40 mL centrifuge tube. Add 25 mL of 0.01 *M* $CaCl_2$ (you can add 2 drops of chloroform to inhibit microbial growth if desired) and shake for one hour on a reciprocating shaker. Centrifuge at 4000 rpm for 10 minutes. Filter solution through Whatman No. 42 filter paper.

Analysis:

For determining water or dilute salt extractable P in soil, any spectrophotometer with an infrared phototube for use at 660 or 882 nm can be used. Also, samples can be analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES), which will measure total dissolved P.

Calculations:

Water- or Dilute salt-extractable P (mg P/kg soil) =
[Concentration of P in extract, mg/L] x [Volume of extractant, L ÷ mass of soil, kg]

Comments:

It should be mentioned that some studies have shown that concentrations of P in $CaCl_2$ extracts can be one-third to one-half that of water extracts (Olsen and Watanabe, 1970; Soltanpour et al., 1974). Concentrations of Ca were less in the water extracts, as compared with the $CaCl_2$ extracts, which resulted in higher P concentrations in the water extracts. Higher concentrations of Ca in the extracting solution may precipitate calcium phosphate, lowering the P levels in solution.

References:

Lee, G.R., N.L. Clesceri, and G.P. Fitzgerald. 1965. Studies on the analysis of phosphates in algal cultures. Internat. J. Air Water Poll. 9:715-722.

Luscombe, P.C., J.K. Syers, and P.E.H. Gregg. 1979. Water extraction as a soil testing procedure for phosphate. Commun. Soil Sci. Plant Anal. 10:1361-1369.

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

Olsen, S.R., and L.E. Sommers. 1982. Phosphorus. P. 403-430 *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agronomy Monogr. 9. ASA and SSSA, Madison, WI.

Olsen, S.R., and F.S. Watanabe. 1970. Diffusive supply of phosphorus in relation to soil textural variations. Soil Sci. 110:318-327.

Pote, D.H., T.C. Daniel, P.A. Moore, Jr., A.N. Sharpley, D.R. Edwards, and D.J. Nichols. 1995. Phosphorus: relating soil tests to runoff concentrations across five soil series. Agronomy Abstracts, p. 294, Am. Soc. Agron., Madison, WI.

Pote, D.H., T.C. Daniel, A.N. Sharpley, P.A. Moore, Jr., D.R. Edwards, and D.J. Nichols. 1996. Relating extractable soil phosphorus to phosphorus losses in runoff. Soil Sci. Soc. Am J. 60:855-59.

Sharpley, A.N. 1995. Dependence of runoff phosphorus on extractable soil phosphorus. J. Environ. Qual. 24:920-926.

Soltanpour, P.N., F. Adams, and A.C. Bennett. 1974. Soil phosphorus availability as measured by displaced soil solutions, calcium chloride extracts, dilute-acid extracts, and labile phosphorus. Soil Sci. Soc. Am. Proc. 38:225-228.

Phosphorus Extraction with Iron Oxide-Impregnated Filter Paper (P_i test)

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Introduction:

The availability of phosphorus (P) in soil or surface water for biota (e.g. plants or algae) has been studied extensively, and numerous tests for available P have been developed and used. These tests can roughly be divided into four categories: (1) shaking with acid solutions which dissolve P compounds or with (buffered) alkaline solutions which displace P from the soil; (2) measuring exchangeable P, using ^{32}P; (3) shaking with dilute salt solutions or water, which simulate the soil solution, and (4) as (3), with a sink added, acting more or less analogous to the withdrawing behavior of a plant root.

The use of resin beads as a sink for P was introduced by Amer et al. (1955). Stronger sinks for P were developed by Hsu and Rich (1960) and by Robarge and Corey (1979) who affixed hydroxy-Al to a cation exchange resin. Since the use of these resins is laborious, it has not developed into a practical method (T.C. Daniel, pers. communication). Iron (hydr)oxide impregnated filter paper (FeO paper, also known as P_i paper or HFO paper) was initially developed for soil chemical studies in the late '70s. Later, it was introduced for plant availability studies as a simpler alternative for resin beads. A water extraction procedure is used for fertilizer recommendations in the Netherlands. In tropical soils this method often results in very low amounts of extracted P, causing analytical problems. Therefore, FeO paper was added as a sink during the extraction. However, since the use of water as an extractant allowed soil dispersion with resulting contamination of the FeO paper with soil particles, 0.01 *M* $CaCl_2$ was chosen as an alternative for water. Although the description of the preparation of the FeO paper and its application was only published in an internal report (Sissingh, 1983), its use became widespread. The application for plant availability studies was reviewed by Menon et al. (1990, 1997), and the use for water-quality studies was described by Sharpley et al. (1995). For long-term desorption studies, an alternative method was developed using a FeO-suspension in a dialysis bag (Lookman et al., 1995). The present paper is mainly based on Chardon et al. (1996), in which studies on the various aspects of both preparation and use of the FeO paper are reviewed in a historical perspective.

Principle of the method:

Filter paper is covered with a precipitate of amorphous iron(hydr)oxides (FeO). When a soil is shaken in $CaCl_2$ to which a strip of this FeO paper is added, P will first desorb from the soil, then adsorb onto the FeO-strip and new P will desorb from the soil. During shaking, the desorbable fraction of soil P will thus be (partly) depleted. During shaking the strip is protected against erosion by soil particles via a polyethylene screen. After shaking the strip is taken out and adhering soil particles are removed by rinsing with distilled water using an air-brush. The FeO on the paper with the P adsorbed onto it is dissolved in H_2SO_4 and P is determined in the acidic solution.

Equipment:

1. 15-cm discs of ash-free, hard filter paper (e.g. Schleicher & Schuell 589 red ribon or Whatman No. 50)
2. Tweezers
3. Immersing baths
4. Polyethylene shaking bottles (100 mL)
5. Polyethylene screen (925 µm openings)
6. Shaking apparatus, end-over-end
7. Air brush

Reagents:

1. Acidified FeCl3 solution: completely dissolve 100 g FeCl3 in 110 mL concentrated HCl and dilute with distilled water to 1 L.
2. 5 % NH4OH: dilute 200 mL NH4OH (25%) to 1 L with distilled water.
3. 0.01 M CaCl2: stock solution 0.1 M: dissolve 14.7 g CaCl2.2 H2O in distilled water and dilute to 1. L; reagent 0.01 M: dilute the stock solution tenfold with distilled water.
4. 0.1 M H2SO4: stock solution 2.5 M: add 140 mL of concentrated H2SO4 to 750 mL distilled water, cool and dilute with distilled water to 1 L; reagent 0.1 M, dilute 40 mL of 2.5 M H2SO4 to 1 L with distilled water.
5. Distilled/deionized water

Procedures:

Preparation of FeO paper

1. Immerse the filter paper in acidified FeCl3, using tweezers, for at least 5 minutes.
2. Let the paper drip dry at room temperature for 1 h.
3. Pull the paper rapidly and uninterrupted through a bath containing 2.7 M NH4OH to neutralize the FeCl3 and produce amorphous iron (hydr)oxide (ferrihydrite, denoted as FeO).
4. Rinse the paper with distilled water to remove adhering particles of FeO.
5. After air drying, cut the paper into strips with a (reactive) surface of40 cm2 (generally 2 by 10 cm).

Shaking soil suspension with FeO strip added

1. Add 40 mL 0.01 M CaCl2 to 1 g of soil in a 100 mL bottle; add one strip protected by polyethylene screen, in a fixed position, at room temperature.
2. Shake on a reciprocating shaker at a speed of 130 excursions/min, or at 4 rpm end-over-end, for 16 h.
3. Take out the strip, thoroughly rinse with distilled water to remove adhering soil particles using an air brush, and remove adhering water.

Determination of P extracted by FeO paper

Dissolve the FeO with adsorbed P by shaking 1 h in 40 mL 0.1 *M* H_2SO_4 and determine P in the acidic extract with colorimetry or by inductively coupled plasma spectrophotometry.

Calculations:

The FeO-extractable P content of a soil, also called P_i-value, is expressed as mg P/kg soil, and can be calculated as:

$$\mathbf{P_i\ value} = \frac{\mathbf{C_p V}}{\mathbf{W}}$$

where:

C_p = P concentration in H_2SO_4, mg/L,
V = volume of H_2SO_4, L,
W = mass of soil used, kg.

Comments:

The method described above can be used as a standard method to estimate soil plant-available P content. In case total desorbable P is studied one can use more FeO-strips during shaking, increase the shaking time, or the amount of FeO on a strip by using a higher concentration of $FeCl_3$ (Chardon et al., 1996). When long-term desorption kinetics is studied the shaking time can be increased, the paper can be refreshed e.g. daily (Sharpley, 1996), or the technique with an FeO-filled dialysis membrane can be used (Freese et al., 1995, Lookman et al., 1995). Myers et al. (1997) described the use of 5.5 cm diameter filter paper circles, which eliminates the need for cutting strips.

As discussed in detail in Chardon et al. (1996) soil particles adhering to the strip when the FeO on the strip is dissolved in H_2SO_4 may give erroneous results, since P from the soil particles can also dissolve in the acid as if it was desorbed. The use of a nylon screen around the strip during shaking and an air-brush after shaking to clean the strip (Whelan et al., 1994) will strongly reduce this risk. Since temperature influences P desorption it is recommended to perform the procedure at a constant temperature in order to get reproducible results.

References:

Amer, F., D.R. Bouldin, C.A. Black, and F.R. Duke. 1955. Characterization of soil phosphorus by anion exchange resin adsorption and P^{32}-equilibration. Plant Soil 6: 391-408.

Chardon, W.J., R.G. Menon, and S.H. Chien. 1996. Iron oxide-impregnated filter paper (P_i test): a review of its development and methodological research. Nutr. Cycl. in Agroecosyst. 46:41-51.

Freese, D., R. Lookman, R. Merckx, and W.H. van Riemsdijk. 1995. New method for assessment of long-term phosphate desorption from soils. Soil Sci. Soc. Am. J. 59:1295-300.

Hsu, P.S. and C.I. Rich. 1960. Aluminum fixation in a synthetic cation exchanger. Soil Sci. Soc. Am. Proc. 24: 21-25.

Lookman, R., D. Freese, R. Merckx, K. Vlassak, and W.H. van Riemsdijk. 1995. Long-term kinetics of phosphate release from soil. Environ. Sci. Technol. 29: 1569-1575.

Menon, R.G., S.H. Chien, and L.L. Hammond. 1990. Development and evaluation of the Pi soil test for plant-available phosphorus. Commun. Soil Sci. Pl. Anal. 21: 1131-1150.

Menon, R.G., S.H. Chien, and W.J. Chardon. 1997. Iron hydroxide-impregnated filter paper (P_i test): II. A review of its application. Nutr. Cycl. in Agroecosyst. 47:7-18.

Myers, R.G., G.M. Pierzynski, and S.J. Thien. 1997. Iron oxide sink method for extracting soil phosphorus paper preparation and use. Soil Sci. Soc. Am. J. 61:1400-1407.

Robarge,W.P. and R.B. Corey. 1979. Adsorption of phosphate by hydroxy-aluminum species on a cation exchange resin. Soil Sci. Soc. Am. J. 43: 481-487.

Sharpley, A.N., J.S. Robinson, and S.J. Smith. 1995. Bioavailable phosphorus dynamics in agricultural soils and effects on water quality. Geoderma 67: 1-15.

Sharpley, A.N. 1996. Availability of residual phosphorus in manured soils. Soil Sci. Soc. Am. J. 60:1459-1466.

Sissingh, H.A. 1983. Estimation of plant-available phosphates in tropical soils. A new analytical technique. Haren, Netherlands, Inst Soil Fertility Research.

Whelan, B.R., K. Wittwer, S.P. Roe, N.J. Barrow, and R.G.V. Bramley. 1994. An air brush and water to clean the iron oxide strips in the Pi soil test for phosphorus. Trans. 15th Int. Congress Soil Sci., Acapulco, Mexico, 1994, vol 5b pp 166-167.

Determination of the Degree of Phosphate Saturation In Non-Calcareous Soils

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Introduction:

The transport of phosphorus (P) by leaching, erosion and surface runoff from agricultural soils can contribute to the eutrophication of surface waters. In flat areas with shallow groundwater tables, like many areas in the Netherlands, leaching can be an important transport pathway. In order to quantify the eutrophication risk of agricultural land in areas with intensive livestock production in the Netherlands (non-calcareous sandy soils), the degree of P saturation of soils has been introduced as a simple index (Breeuwsma and Schoumans, 1987; Breeuwsma et al., 1995). The degree of P saturation (DPS) is defined as the ratio between the amount of phosphate accumulated in soils to a critical depth (P_{act}) and the maximum phosphate sorption capacity (PSC) of the soil to that depth. The relationship is described by:

$$DPS = \frac{P_{act}}{PSC} * 100$$

Eq. (1)

where

DPS = degree of phosphate saturation (%),
P_{act} = actual amount of sorbed phosphate to the critical depth (mmol/kg), and
PSC = maximum phosphate sorption capacity to critical depth (mmol/kg)

In the Netherlands the mean highest groundwater level (MHW) is used as a critical depth. The phosphate sorption capacity of soils depends on soil characteristics (e.g. aluminium, iron, clay, lime and organic matter). In acid to neutral soils fixation of P mainly takes place with reactive forms of Fe and Al (as hydroxides and Al and Fe bound to the organic matter). These reactive forms of Fe and Al can be extracted from soil samples (Beek, 1978; Schwertmann, 1964) by shaking at a 1:20 weight to volume ratio with a solution of oxalic acid and ammonium oxalate having a nearly constant pH of 3. The phosphate sorption capacity of non-calcareous sandy soils can be assessed by (Schoumans et al., 1986; Van der Zee, 1988):

$$PSC = \sum_{i=1}^{n} 0.5(Al_{ox} + Fe_{ox})_i * \rho_{d,i} .* L_i$$

Eq. (2)

where

Al_{ox} = oxalate extractable aluminium of soil layer i (mmol/kg),
Fe_{ox} = oxalate extractable iron of soil layer i (mmol/kg),
$\rho_{d,i}$ = dry bulk density of soil layer i (kg/m),
L_i = thickness of soil layer i (m), and
n = amount of observed layers.

The amount of P which is bound to the reactive amount of Al and Fe comes into solution with the oxalate extraction. Therefore, the actual amount of sorbed P can be calculated by means of:

$$P_{act} = \sum_{i=1}^{n} P_{ox,i} * \rho_{d,i} .* L_i$$

Eq. (3)

where
P_{ox} = oxalate extractable P of soil layer i (mmol/kg).

If the dry bulk densities of the observed layers (from the soil surface to the reference depth) are identical, or a soil sample has been taken over the complete depth (on volume basis), the degree of P saturation can be calculated by the mean contents of P_{ox}, Al_{ox} and Fe_{ox} (in mmol/kg) over the observed depth:

$$DPS = \frac{P_{ox}}{0.5(Al_{ox} + Fe_{ox})} * 100$$

Eq. (4)

Based on desorption characteristics of non-calcareous sandy soils, Van der Zee et al. (1990) have show that at a degree of P saturation of 25% the P concentration in pore water will become higher than 0.1 mg/L ortho-P at the long term (after redistribution of the P front in the soil). In the Netherlands this concentration is used as a target level at the mean highest water table.

A disadvantage of the definition of the phosphate saturation degree is that this parameter depends on the phosphate sorption capacity of the soil (Equation 1), which varies from layer to layer and which is in most situations assessed (e.g., for non-calcareous sandy soils by means of 0.5 (Al_{ox} + Fe_{ox})). In order to omit this assessment of the phosphate sorption capacity also an independent P saturation index (PSI) can be used:

$$PSI = \frac{P_{ox}}{Al_{ox} + Fe_{ox}}$$

Eq. (5)

Reagents:

1. Extraction solution (pH = 3). Dissolve 16.2 g of ammonium oxalate monohydrate. $(COONH_4)_2.H_20$ and 10.8 g of oxalic acid dihydrate, $(COOH_2).2H_2O$ in water in a 1000 mL volumetric flask. The pH of this solution must be 3.0 ± 0.1.
2. Hydrochloric acid. 1 *M*. Dilute 83 mL of concentrated hydrochloric acid, HCl (ρ= 1.19 g/cm^3), with water to volume of 1000 mL.
3. Hydrochloric acid. 0.01 *M*. Dilute 10 mL of 1*M* hydrochloric acid with water to volume of 1000 mL.
4. Standard Fe solution. 1000 mg/L

5. Standard Al solution. 1000 mg/L
6. Standard P solution. 500 mg/L. Dissolve 2.1950 g of potassium dihydrogen phosphate (KH_2PO_4) in water in a volumetric flask of 1000 mL and dilute to 1000 mL with water.

Procedure:

The method, which is described below, is a summary of the Dutch norm (NEN 5776). Weigh 2.5 (± 0.01) g of air-dry soil (< 2 mm) in a dry, 100 mL polyethene bottle. Add with a dispenser 50 mL of the oxalate extraction solution (1) and close the bottle. Prepare two blanks and take three reference samples. Shake at 180 excursions/min on a reciprocating shaker for 2 hours in a darkened conditioned room at constant temperature (20 °C). Filter the extracts through a fine filter paper (high quality). Discard the first three mL of the filtrate and collect the remainder in a 100 mL polyethene bottle. Pipet 10 mL of the soil extracts in flasks. Add 40 mL of 0.01 *M* HCl-solution (3) and mix. Measure the concentration of P, Al and Fe within one week with the ICP-AES.

Pipet 0, 2.5, 10.0, 25.0 and 50.0 mL of each standard element solution ((4), (5) and (6)) in a volumetric flask of 1000 mL. Add 10 mL of (1*M* HCl) and 200 mL of (extraction solution) and mix. Dilute to 1000 mL with water. This standard series contains 0, 1.25, 5.0, 12.5 and 25.0 mg/L P and 0, 2.5, 10.0, 25.0 and 50.0 mg/L Al and Fe.

Comments:

The extraction should be performed in dark because the extraction solution (1) partially reduces the poorly soluble iron(lll) ions to the much more soluble iron(ll) ions and light influences the reducing action of oxalic acid.

The soil filtrates should be stored in a refrigerator if they are not used directly for analysis.

Calculation:

$$P_{ox} = \frac{(a-b)*0.05}{m*30.97},$$

$$Fe_{ox} = \frac{(a-b)*0.05}{m*55.85}, \text{ and}$$

$$Al_{ox} = \frac{(a-b)*0.05}{m*26.98}$$

where:

P_{ox}, Fe_{ox}, Al_{ox}	= content of P, Fe and Al of the air-dry soil sample in mmol/kg
a	= concentration of P, Fe, Al in the soil extraction solution in mg/L

b = concentration of P, Fe, Al in the blank extraction solution in mg/L
m = air-dry soil sample weight in grams.

$$PSI = \frac{P_{ox}}{Al_{ox} + Fe_{ox}}$$

$$DPS = 200\ PSI$$

Comments:

Since the calculation of the results of soil analysis are generally expressed on an "oven-dry" basis, the moisture content of "air-dry" soil should be determined shortly before soil analysis and the appropriate correction made.

References:

Breeuwsma, A. and O.F. Schoumans, 1987. Forecasting phosphate leaching on a regional scale. *In*: Proceedings of the International Conference on Vulnerability of soil and groundwater to pollutants on March 30 to April 3, 1987 at Noordwijk aan Zee. (Eds. W. van Duijvenboode and H.G. van Waegeningh). The Hague: TNO Committee on Hydrological Research, The Hague, Proceedings and Information No. 38.

Breeuwsma, A., J.G.A. Reijerink, O.F. Schoumans, 1995. Impact of manure on accumulation and leaching of phosphate in areas of intensive livestock farming: IN: Animal Waste and the Land-Water Interface (Ed. K. Steele). Lewis publishers, New York, pp. 239-249.

Beek, J.,1978. Phosphate retention by soil in relation to waste disposal. Ph. D. Thesis, Wageningen, The Netherlands.

NEN 5776, Soil. Determination of iron, aluminum and phosphorus in an ammonium oxalate-oxalic acid extract for estimation of the saturation with phosphate. Netherlands Normalization Institute (NNI), Delft, The Netherlands.

Schwertmann, D. 1964, Differentierung der Eisenoxide des Badens durch photochemische Extraction mit saurer Ammoniumoxalaat-Lösung. Z.Pfl. Ern. Duengung 105. 194-202

Schoumans, O.F., W. de Vries and A. Breeuwsma, 1986. A phosphate transport model for application on regional scale. Winand Staring Centre for Integrated Land, Soil and Water Research, Wageningen, The Netherlands, rapport 1951 (in Soil Survey series).

van der Zee, S.E.A.T.M., 1988. Transport of reactive contaminants in hetereogeneous soil systems. Ph. D. Thesis, Agricultural University, Wageningen, The Netherlands.

van der Zee, S.E.A.T.M., W.H. van Riemsdijk, and F.A.M. de Haan, 1990. Protocol phosphate saturated soils. Department of soil science and plant nutrition, Agricultural University, Wageningen, The Netherlands.

Phosphorus Sorption Isotherm Determination

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Introduction:

Phosphorus (P) retention by soils is an important parameter for understanding soil fertility problems, as well as for determining the environmental fate of P. The P adsorption capacity of a soil or sediment is generally determined by batch-type experiments in which soils or sediments are equilibrated with solutions varying in initial concentrations of P. Equations such as the Langmuir, Freundlich and Tempkin models have been used to describe the relationship between the amount of P adsorbed to the P in solution at equilibrium (Berkheiser et al., 1980; Nair et al., 1984).

Advantages of the batch technique include: the soil and solution are easily separated, a large volume of solution is available for analysis, and the methodology can be easily adapted as a routine laboratory procedure. Disadvantages include difficulties in measuring the kinetics of the sorption reaction and optimizing the mixing of solution and soil without particle breakdown (Burgoa et al. 1990). Despite the disadvantages, the batch technique has been, and still is, widely used to describe P sorption in soils and sediments.

Nair et al. (1984) noted that P sorption varies with soil/solution ratio, ionic strength and cation species of the supporting electrolyte, time of equilibration, range of initial P concentrations, volume of soil suspension to head space volume in the equilibration tube, rate and type of shaking, and type and extent of solid/solution separation after equilibration. Although most researchers use a similar basic procedure for measuring P adsorption, there is considerable variation observed among studies with regard to the above parameters. This variation often makes comparisons of results among studies difficult. Thus, Nair et al. (1984) proposed a standard P adsorption procedure that would produce consistent results over a wide range of soils. This procedure was evaluated, revised, tested among laboratories and was eventually proposed as a standardized P adsorption procedure. This procedure as described below is proposed as the standard procedure recommended by the SERA-IEG 17 group.

Equipment:

1. Shaker: End-over-end type
2. Filter Apparatus: Vacuum filter system using 0.45 or 0.2 μm filters
3. Equilibration tubes: 50 mL or other size to provide at least 50% head space
4. Spectrophotometer: Manual or automated system capable of measuring at 880 nm

Reagents:

1. Electrolyte: 0.01 *M* $CaCl_2$, unbuffered
2. Microbial inhibitor: Chloroform
3. Inorganic P solutions: Selected concentrations as KH_2PO_4 or NaH_2PO_4 (in 0.01 *M* $CaCl_2$ containing: 20 g/L chloroform)

Procedure:

1. Air-dry soil samples and screen through a 2 mm sieve to remove roots and other debris.
2. Add 0.5 to 1.0 g air-dried soil to a 50 mL equilibration tube.
3. Add sufficient 0.01 *M* $CaCl_2$ solution containing 0, 0.2, 0.5, 1, 5, and 10 mg P/L as KH_2PO_4 or NaH_2PO_4, to produce a soil:solution ratio of 1:25. The range of P values could vary from 0 to 100 mg P/L (0, 0.01, 0.1, 5, 10, 25, 50 and 100 mg P/L) and the soil/solution ratio could be as low as 1:10 depending on the sorbing capacity and the P concentrations of the soils in the study.
4. Place equilibration tubes on a mechanical shaker for 24 h at 25 ± 1 °C.
5. Allow the soil suspension to settle for an hour and filter the supernatant through a 0.45 μm membrane filter.
6. Analyze the filtrate for soluble reactive P (SRP) on a spectrophotometer at a wavelength of 880 nm.

Calculations and Recommended Presentation of Results:

Two of the often used isotherms are the Langmuir and the Freundlich isotherms; the Langmuir having an advantage over the Freundlich in that it provides valuable information on the P sorption maximum, S_{max} and a constant k, related to the P bonding energy.

The Langmuir equation

The linearized Langmuir adsorption equation is:

$$\frac{C}{S} = \frac{1}{kS_{\max}} + \frac{C}{S_{\max}}$$

where:

$S = S' + S_o$, the total amount of P retained, mg/kg
S' = P retained by the solid phase, mg/kg
S_o = P originally sorbed on the solid phase (previously adsorbed P), mg/kg
C = concentration of P after 24 h equilibration, mg/L
S_{max} = P sorption maximum, mg/kg, and
k = a constant related to the bonding energy, L/mg P.

The Freundlich equation

The linear form is: log S = log K + n log C

where:

K is the adsorption constant, expressed as mg P/kg,
n is a constant expressed as L/kg, and
C and S are as defined previously.

A plot of log S against log C will give a straight line with log K as the intercept, and n as the slope.

Previously adsorbed P (also referred to as native sorbed P)

Adsorption data should be corrected for previously adsorbed P (S_o). For the calculation of previously sorbed P, Nair et al. (1984) used isotopically exchangeable P (Holford et al., 1974) prior to calculations by the Langmuir, Freundlich and Tempkin procedures. Other procedures used to calculate the previously adsorbed P include oxalate-extractable P (Freese et al., 1992; Yuan and Lavkulich, 1994), anion-impregnated membrane (AEM) technology (Cooperband and Logan, 1994) and using the least squares fit method (Graetz and Nair, 1995; Nair et al., 1998; Reddy et al., 1998). Sallade and Sims (1997) used Mehlich 1 extractable P as a measure of previously sorbed P.

Investigations by Villapando (1997) have indicated a good agreement among native sorbed P values estimated by the least squares fit method, oxalate extractions, and the AEM technology. At this point, it appears that selection of the method for determination of native sorbed P would depend on the nature of the soils in the study and reproducibility of the results.

The procedure for calculation of S_o using the least square fit method is based on the linear relationship between S' and C at low equilibrium P concentrations. The relationship can be described by

$$S' = K'C - S_o$$

where

K' = the linear adsorption coefficient, and

all other parameters are as defined earlier

(Note: It is recommended that the linear portion of the isotherm has an r^2 value 0.95 or better).

Equilibrium P Concentration

The "equilibrium P concentration at zero sorption" (EPC_0) represents the P concentration maintained in a solution by a solid phase (soil or sediment) when the rates of P adsorption and desorption are the same (Pierzynski et al., 1994). Values for EPC_0 can be determined graphically from isotherm plots of P sorbed *vs*. P in solution at equilibrium. From the calculations given above, EPC_0 is the value of C when $S' = 0$.

Comments:

The above procedure was developed to provide a standardized procedure with a fixed set of conditions that could be followed rigorously by any laboratory. The procedure uses a low and narrow range of dissolved inorganic P concentrations because these are the concentrations likely to be encountered in natural systems and because higher concentrations may result in precipitation of P solid phases. However, higher concentrations of P (up to 100 mg/L) and/or lower soil:solution ratios (1:10) have been used for isotherm determinations on soils and sediments (Mozaffari and Sims, 1994; Sallade and Sims, 1997; Nair et al., 1998; Reddy et al., 1998). A 0.01 *M* KC1 solution may be used as the background electrolyte to avoid precipitation of Ca in neutral and alkaline soils.

Toluene and chloroform have been shown to increase the dissolved P concentration in the supernatant, apparently due to lysis of microbial cells, and thus, some researchers do not try to inhibit microbial growth (Reddy et al., 1998).

Most adsorption studies are conducted under aerobic conditions, however, with certain studies it is more appropriate to use anaerobic conditions, as they more closely represent the natural environments of the soils or sediments. Reddy et al. (1998) preincubated sediment/soil samples in the dark at 25^{o}C under a N_2 atmosphere, to create anaerobic conditions. Adsorption experiments were then conducted, performing all equilibrations and extractions in an O_2-free atmosphere.

References:

Berkheiser, V.E., J.J. Street, P.S.C. Rao, and T.L. Yuan. 1980. Partitioning of inorganic orthophosphate in soil-water systems. CRC Critical Reviews in Environmental Control. 179-224.

Burgoa, B., R.S. Mansell, and D. Rhue. 1990. Effects of solids concentrations upon non-equilibrium phosphorus sorption in aqueous soil suspensions. Soil and Crop Sci. Soc. Fla. Proc. 49:60-66.

Cooperband, L.R., and Logan. 1994. Measuring in situ changes in labile soil phosphorus with anion-exchange membranes. Soil Sci. Soc. Am. J. 58:105-114.

Freese, D., S.E.A.T.M. van der Zee, and W.H. van Riemsdijk. 1992. Comparison of different models for phosphate sorption as a function of the iron and aluminum oxides of soils. J. Soil Sci. 43:729-738.

Graetz, D.A., and V.D. Nair. 1995. Fate of phosphorus in Florida Spodosols contaminated with cattle manure. Ecol. Eng. 5:163-181.

Holford, I.C.R, R.W.M. Wedderburn, and G.E.G. Mattingly. 1974. A Langmuir two surface equation as a model for phosphate adsorption by soils. J. Soil Sci. 25:242-255.

Mozaffari, M. and J.T. Sims. 1994. Phosphorus availability and sorption in Atlantic Coastal Plain watershed dominated by animal-based agriculture. Soil Sci. 157:97-107.

Nair. P.S., T.J. Logan, A.N. Sharpley, L.E. Sommers, M.A. Tabatabai, and T.L. Yuan. 1984. Interlaboratory comparison of a standardized phosphorus adsorption procedure. J. Environ. Qual. 13:591-595.

Nair, V.D., D.A. Graetz, and K.R. Reddy. 1998. Dairy manure influences on phosphorus retention capacity of Spodosols. J. Environ. Qual. 27:522-527.

Pierzynski, G.M., J.T. Sims and G.F.Vance. 1994. Soils and Environmental Quality. Lewis Publishers, Boca Raton, FL.

Sallade, Y.E., and J.T. Sims. 1997. Phosphorus transformations in the sediments of Delaware’s agricultural drainageways: I. Phosphorus forms and sorption. J. Environ. Qual. 26:1571-1579.

Reddy, K.R., G.A. O’Connor, and P.M. Gale. 1998. Phosphorus sorption capacities of wetland soils and stream sediments impacted by dairy effluent. J. Environ. Qual. 27:438-447.

Villapando, R.R. 1997. Reactivity of inorganic phosphorus in the spodic horizon. Ph.D. Dissertation, University of Florida, Gainesville, Florida. (Diss. Abstr. Vol. 58. No. 7B). 258pp.

Yuan, G., and L.M. Lavkulich. 1994. Phosphate sorption in relation to extractable iron and aluminum in Spodosols. Soil Sci. Soc. Am. J. 58:343-346.

Bioavailable Phosphorus in Soil

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Introduction:

Biologically available P (BAP) has been operationally defined as "..the amount of inorganic P, a P-deficient algal population can utilize over a period of 24 h or longer" (Sonzogni et al., 1982). The amount of P in soil, sediment, and water that is potentially available for algal uptake (bioavailable P) can be quantified by algal assays, which require up to 100-d incubations (Miller et al., 1978). Thus, more rapid chemical extractions, such as those using NaOH (Butkus, et al., 1988; Dorich et al., 1980), NH_4F (Porcella et al., 1970), ion exchange resin (Huettl et al., 1979) and citrate-dithionite-bicarbonate (Logan et al., 1979), have been used routinely to estimate bioavailable P. The weaker extractants (NH_4F and NaOH) and short-term resin extractions may represent P that could be utilized by algae in the photic zone of lakes under aerobic conditions. In contrast, the more severe extractants (citrate-dithionite-bicarbonate) represent P that may become bioavailable under the reducing conditions found in the anoxic hypolimnion of stratified lakes.

Sharpley et al. (1991) showed that when using a wide solution:soil ratio (500:1), 0.1 *M* NaOH extractable P (NaOH-P) was closely related to the growth of several algal species. However, the complexity of algal assay and chemical extraction methods often limits their use by soil testing laboratories. For example, long assay incubation (7 to 100 d) and chemical extraction times (> 16 hr), as well as large solution volumes (> 500 mL) are particularly inconvenient. As the amount of P extracted depends on ionic strength, cationic species, pH, and volume of the extractant used (Hope and Syers, 1976; Sharpley et al., 1981), these limitations will be difficult to overcome. Questions also have been raised as to the validity of relating the form or availability of P extracted by chemical solutions to P bioavailability in the aquatic environment. As a result, P sink approaches have been developed to estimate BAP in soil, sediment, and water.

P-Sink Approaches:

The concept of exposing the soil to a P-sink has merit toward the goal of assessing soil, sediment, and water BAP (i.e., available to plants and algae) for both agronomic and environmental goals. Presumably, this would allow only P that was able to respond to such a sink to be measured, which is analogous to a root acting as a sink in the soil or to the concentration gradient that exists when a small quantity of sediment is placed in a large volume of water. The analogy of a root is not entirely accurate because root exudates and mycorrhizae fungi can alter P availability in the rhizosphere such that the root does not behave as a pure sink. Still, P-sinks are likely the closest manifestation of the root environment that are available. Some authors assume that the sink maintains extremely low P concentrations in the aqueous media employed and can be considered an "infinite P-sink" in the sense that P release by the soil is clearly the rate-limiting step (Sibbesen, 1978; van der Zee et al., 1987; Yli-Halla, 1990). For anion-exchange resins used at low resin:soil ratios, this relationship cannot be assumed (Barrow and Shaw, 1977; Pierzynski, 1991) and is not necessary for the assessment of bioavailable P.

Iron-oxide-Impregnated Paper

Another P sink that has received attention is Fe-oxide impregnated filter paper, which has successfully estimated plant available P in a wide range of soils and management systems (Menon et al., 1989; 1990, Sharpley, 1991). Also, Sharpley (1993) observed that the Fe-oxide strip P content of runoff was closely related to the growth of several algal species incubated for 29-d with runoff as the sole source of P. As the resin membranes and Fe-oxide strips act as a P sink, they simulate P removal from soil or sediment-water samples by plant roots and algae. Thus, they have a stronger theoretical justification for use over chemical extractants to estimate bioavailable P. These methods have potential use as environmental soil P tests to identify soils liable to enrich runoff with sufficient P to accelerate eutrophication. The Fe-oxide impregnanted filter paper procedure was described in the section by Chardon (2000) in this bulletin and will not be described further here.

Anion-exchange Resins

The use of anion-exchange resins is the most common P-sink approach for assessing available inorganic P in soils. The procedure typically involves the use of chloride-saturated resin at a 1:1 resin-to-soil ratio in 10 to 100 mL of water or weak electrolyte for 16 to 24 h (Amer et al., 1955; Olsen and Sommers, 1982). Correlations between plant response and resin-extractable P are comparable or superior to correlations with chemical extraction methods (Fixen and Grove, 1990).

Ion-exchange Resin-Impregnated Membranes

A similar approach using ion-exchange resin impregnated membranes has been investigated by several researchers (Abrams and Jarrell, 1992; Qian et al., 1992: Saggar et al., 1992). Impregnation of the resin onto a plastic membrane facilitates separation of the resin beads from the soil and may eliminate the soil grinding step. Also, an extraction time as short as 15 min can be used without reducing the accuracy of predicted P availability for a wide range of soils (Qian et al., 1992). In pot studies, the resin membranes have provided a better index of P availability than conventional chemical extraction methods for canola (Qian et al., 1992) and ryegrass (Saggar et al., 1992). It is likely that the utility of the resin membranes will make the use of loose resin obsolete.

Ion exchange membranes have the potential to estimate P availability in aquatic as well as soil environments. Edwards et al. (1993) used ion exchange membranes to obtain in-situ estimates of the chemical composition of river water for two Scottish watersheds. It was suggested that direct multi-element analysis by X-ray fluorescence of ions retained on the membranes removes the need for sample storage or filtration, both of which can be sources of potential contamination and error. Thus, the membranes can provide useful information in addition to that obtained by conventional sampling (Edwards et al., 1993).

Soil Sampling:

Soil sampling protocol for environmental concerns should be re-evaluated since the primary mechanism for P transport from most agricultural soils is by surface runoff and erosion. Although most samples submitted to soil testing laboratories are obtained from 0 to 20 cm, the zone of interaction of runoff waters with most soils is normally less than 5 cm. Consequently, environmental soil sampling should reflect this shallower depth of soil influencing runoff P. Hence, environmental soil samples should, in general, be taken from no deeper than 5 cm. This protocol is compatible with sampling of no-till fields, currently recommended by extension specialists in several states, where the traditional 0- to 20-cm depth is split into two or three increments. Thus, on soils identified as vulnerable to P loss in runoff, the surface increment could be analyzed for environmental interpretation and all increments integrated for agronomic interpretations.

Equipment:

The following equipment is needed to conduct BAP extraction of soil and analysis for P:

1. Resin membrane, anion exchange.
2. End-over-end shaker - used to equilibrate sample and sink
3. Volumetric flasks - usually 25 or 50 mL volume
4. Pipets to aliquot samples and color reagents
5. Spectrophotometer to determine P concentration in the color developed reagent with sample.

Reagents:

Resin membranes

1. Hydrochloric acid to extract P from the membranes - 1.0 *M* HCl (166 mL concentrated HCl in 2 L)

Murphy and Riley Molybdenum Blue Color Reagent

1. Murphy and Riley Reagent A:
   a. 1. Mix 1500 mL H_2O and 125 mL H_2SO_4 and allow to cool down before adding molybdate and tartrate
   b. Add 10.66 g ammonium molybdate
   c. Add 50 mL antimony potassium tartrate
   d. Make the solution up to 2 L
   e. Store in refrigerator
2. Murphy and Riley Reagent B:
   a. Dissolve 42 g ascorbic acid in 1 L
   b. Store in refrigerator
3. Murphy and Riley Reagent
   The color development reagent is made up by mixing nine parts of reagent A and 1 part of reagent B in a measuring cylinder. Each sample in a 25 mL volumetric flask requires 5 mL of this reagent. As it takes time to make up the Murphy and Riley reagent and some of the reagents are expensive (e.g., ammonium molybdate), only make up what is needed for the day. Also, solutions A and B, once mixed, will not keep for more than a day. For example, if you have 20 samples to run this will require at least 100 mL of color reagent plus standards and some for reruns. Thus, 250 mL of color reagent should be mixed, and this will require 225 mL of reagent A and 25 mL of reagent B.
4. Neutralizing Reagents:
   a. p-nitrophenol indicator (pnp - yellow): mix 1.5 g p-nitrophenol in 500 mL of deionized distilled water on a magnetic stirrer until dissolved. Filter the solution to remove any undissolved residue.

   b. 4 *M* NaOH: 160 g NaOH in 1 L
   c. 0.1 *M* H_2SO_4: 11.1 mL conc. H_2SO_4 in 2 L
5. Solution Neutralizing
   a. Add one drop of pnp indicator to an appropriate aliquot of the filtered solution on which P is to be measured in a volumetric flask.
   b. Add 4 *M* NaOH to solution drop-wise until solution just turns yellow.
   c. Add 0.1 *M* H_2SO_4 drop-wise until solution just turns back to clear, the solution is now neutral and the Murphy and Riley reagent can be added.

Resin Strip Procedure:

1. Anion exchange resin sheets are cut into 2 x 2 cm squares and are stored in propylene glycol. Wash the resin squares in distilled water to remove all the propylene glycol. If not already saturated with an anion, saturation with $C1^-$, HCO_3^- or acetate may be necessary. They are now ready for use.
2. Phosphorus is extracted from soil or sediment by shaking a 1-g sample and one resin membrane square in 40 mL of deionized distilled water end-over-end for 16 hours at 25^o C.
3. Remove the resin membrane square and wash thoroughly with distilled water until all soil particles are removed.
4. The BAP content of runoff can also determined by shaking 50 mL of an unfiltered runoff sample with one resin membrane square for 16 hours. Smaller runoff sample volumes should be used if P concentrations are expected to be high (>1 or 2 mg/L) and made up to 50 mL with distilled water.
5. Phosphorus retained on the resin membrane square is removed by shaking the square end-over-end with 40 mL of 1 *M* HCl for 4 hours. Remove square and rinse with distilled water. Retain the HCl desorption solution for analysis. Repeat this step. Do not mix the first and second desorption solutions.
6. Measure the P concentration of the two solutions separately. The total amount of P desorbed from the resin membrane square is the sum of the amounts in the two solutions.

Calculations:

Resin extractable P (mg P/kg) =
[Concentration of P in 1 *M*HCl, mg/L] x [0.04 L ÷ 0.001 kg]

Resin BAP in runoff (mg P/L) = [concentration of P in 1 *M* HC1, mg/L] x [0.04L ÷ volume of runoff, L]

References:

Abrams, M.M., and W.M. Jarrell. 1992. Bioavailability index for phosphorus using nonexchange resin impregnated membranes. Soil Sci. Soc. Am. J. 56:1532-1537.

Amer, F., D.R. Bouldin, C.A. Black, and F.R. Duke. 1955. Characterization of soil phosphorus by anion exchange resin and adsorption by P-32 equilibration. Plant Soil 6:391-408.

Barrow, N.J., and T.C. Shaw. 1977. Factors affecting the amount of phosphate extracted from soil by anion exchange resin. Geoderma 18:309-323.

Butkus, S.R., E.B. Welch, R.R. Horner, and D.E. Spyridakis. 1988. Lake response modeling using biologically available phosphorus. J. Water Pollut. Cont. Fed. 60:1663-1669.

Chardon, W.J. 2000. Phosphorus extraction with iron oxide-impregnated filter paper (P; test). *In* G.M. Pierzynski (ed.), Methods for Phosphorus Analysis for Soils, Sediments, Residuals, and Waters. Southern Cooperative Series Bulletin No. 396, p. 27-30.

Dorich, R.A., D.W. Nelson, and L.E. Sommers. 1980. Algal availability of sediment phosphorus in drainage water of the Black Creek watershed. J. Environ. Qual. 9:557-563.

Edwards, T., B. Ferrier, and R. Harriman. 1993. Preliminary investigation on the use of ion-exchange resins for monitoring river water composition. Sci. of Total Environ. 135:27-36.

Fixen, P.E., and J.H. Grove. 1990. Testing soils for phosphorus. p. 141-180. *In* R.L. Westerman (ed.) Soil testing and plant analysis. 3rd ed. SSSA Book Ser. 3. SSSA, Madison, WI.

Hope, G.D., and J.K. Syers. 1976. Effects of solution to soil ratio on phosphate sorption by soils. J. Soil Sci. 27:301-306.

Huettl, P.J., R.C. Wendt, and R.B. Corey. 1979. Prediction of algal available phosphorus in runoff suspension. J. Environ. Qual. 4:541-548.

Logan, T.J., T.O. Oloya, and S.M. Yaksich. 1979. Phosphate characteristics and bioavailability of suspended sediments from streams draining into Lake Erie. J. Great Lakes. Res. 5:112-123.

Menon, R.G., L.L. Hammond, and H.A. Sissingh. 1989. Determination of plant-available phosphorus by the iron hydroxide-impregnated filter paper (P_i) soil test. Soil Sci. Soc. Am. J. 52:110-115.

Menon, R.G., S.H. Chien, L.L. Hammond, and B.R. Arora. 1990. Sorption of phosphorus by iron oxide-impregnated filter paper (P_i soil test) embedded in soils. Plant Soil 126:287-294.

Miller, W.E., J.C. Greene, and T. Shiroyarna. 1978. The *Selenastrum capricornutum* Printz algal assay bottle test and data interpretation protocol. U.S. EPA, Tech. Rep. EPA-600/9-78-018. USEPA, Covallis, OR.

Olsen, S.R., and L.E. Sommers. 1982. Phosphorus. p. 403-429. *In* A.L. Page et al. (eds.), Methods of soil analysis. Agronomy No. 9, Part 2, 2nd ed., Am. Soc. of Agronomy, Madison, WI.

Pierzynski, G.M. 1991. The chemistry and mineralogy of phosphorus in excessively fertilized soils. Critical Reviews in Environ. Control 21:265-295.

Porcella, D.B., J.S. Kumazar, and E.J. Middlebrooks. 1970. Biological effects on sediment-water nutrient interchange. J. Sanit. Eng. Div., Proc. Am. Soc. Civil Eng. 96:911-926.

Qian, P., J.J. Schoenau, and W.Z. Huang. 1992. Use of ion exchange membranes in routine soil testing. Commun. Soil Sci. Plant Anal. 23:1791-1804.

Saggar, S., M.J. Hedley, R.E. White, P.E.H. Gregg, K.W. Perrot, and I.S. Conforth. 1992. Development and evaluation of an improved soil test for phosphorus. 2. Comparison of the Olsen and mixed cation-anion exchange resin tests for predicting the yield of ryegrass growth in pots. Fert. Res. 33:135-144.

Sharpley, A.N. 1991. Soil phosphorus extracted by iron-aluminum-oxide-impregnated filter paper. Soil Sci. Soc. Am. J. 55:1038-1041.

Sharpley, A.N. 1993. An innovative approach to estimate bioavailable phosphorus in agricultural runoff using iron oxide-impregnated paper. J. Environ. Qual. 22:597-601.

Sharpley, A.N., W.W. Troeger, and S.J. Smith. 1991. The measurement of bioavailable phosphorus in agricultural runoff. J. Environ. Qual. 20:235-238.

Sharpley, A.N., L.R. Ahuja, M. Yamamoto, and R.G. Menzel. 1981. The kinetics of phosphorus desorption from soil. Soil Sci. Soc. Am. J. 45:493-496.

Sibbesen, E. 1978. An investigation of the anion-exchange resin method for soil phosphate extraction. Plant Soil 50:305-321.

Sonzogni, W.C., S.C. Chapra, D.E. Armstrong, and T.J. Logan. 1982. Bioavailability of phosphorus inputs to lakes. J. Environ. Qual. 11:555-563.

van der Zee, S.E.A.T.M., L.G.J. Fokkink, and W.H. van Riemsdijk. 1987. A new technique for assessment of reversibly adsorbed phosphate. Soil Sci. Soc. Am. J. 51:599-604.

Yli-Halla, M. 1990. Comparison of a bioassay and three chemical methods for determination of plant-available P in cultivated soils of Finland. J. Agric. Sci. Finl. 62:213-319.

Total Phosphorous in Soil

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Introduction:

There have been many methods developed to extract and analyze total phosphorus (P) in soil (Bray and Kurtz, 1945; Muir, 1952; Jackson, 1958; Syers et al., 1968; Sommers and Nelson, 1972; Dick and Tabatabai, 1977; Olsen and Sommers, 1982; Bowman, 1988). Two of the more commonly used and most recognizable methods of P extraction are sodium carbonate (Na_2CO_3) fusion and acid digestion. Of these methods, Na_2CO_3 fusion is thought to give more reliable results (Syers et al., 1967; Syers et al., 1968; Sherrell and Saunders, 1966; Sommers and Nelson, 1972). Underestimation of total P by acid digestion is thought to be due to inability of these methods to extract P from apatite inclusions (Syers et al., 1967). The ability of an acid digestion to extract P from inclusions depends upon the acid or combination of acids used. Syers et al. (1967) showed that the effectiveness of extraction generally followed the order: fusion > HF digestion > $HClO_4$ digestion > *N* H_2SO_4 > ignition.

In recent years, more rapid methods for determining total P in soils have been developed (Sommers and Nelson, 1972; Dick and Tabatabai, 1977; Bowman, 1988). Methods developed by Sommers and Nelson (1972) and Bowman (1988) are variations of standard $HClO_4$ digestion methods. These methods were shown to give a similar degree of underestimation of total P as standard $HClO_4$ digestion methods. Dick and Tabatabai (1977) proposed an alkaline oxidation method using sodium hypobromite (NaOBr). This method was shown to give results 1% higher than those found by $HClO_4$ digestion. However, the method still underestimated total P by 4% when compared to results from Na_2CO_3 fusion.

The methods discussed here are very silimar to Na_2CO_3 fusion and $HClO_4$ digestion as described by Olsen and Sommers (1982) in *Methods of Soil Analysis - Part 2*, and the alkaline oxidation method developed by Dick and Tabatabai (1977).

Fusion Method (Olsen and Sommers (1982)):

Reagents

1. Anhydrous sodium carbonate (Na_2CO_3)
2. 4.5 *M* H_2SO_4
3. 1 *M* H_2SO_4
4. Ammonium paramolybdate [$(NH_4)_6Mo_7O_{24} \cdot H_2O$]. Prepare by dissolving 9.6 g of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in distilled water under heat. After solution has cooled, dilute solution volume to 1 L with distilled water.
5. 2 *M* H_2SO_4
6. Ascorbic acid. Prepare by dissolving 10 g of ascorbic acid in 80 mL of distilled water, and dilute solution volume to 100 mL with distilled water. Store reagent at 2°C. Make fresh solution when noticeable color develops.
7. Potassium antimony tartrate ($KSbO \cdot C_4H_4O_6$). Prepare by dissolving 0.667 g of $KSbO \cdot C_4H_4O_6$ in 250 mL of distilled water.
8. Mixed reagent. Mix 1:1 ratio of ascorbic acid and antimony reagents prior to use. Prepare a fresh solution as required.

Procedure

Place a mixture of 1.0 g of finely ground (100 mesh), air-dried soil and 4-5 g of Na_2CO_3 in a Pt crucible. For soils high in Fe, use 0.5 g of soil. Place 1 g of Na_2CO_3 on top of the mixture. Drive off moisture from mixture by gently heating with a Meeker burner. Place a lid on the crucible so that approximately one fifth of the crucible remains open. Apply heat with a low flame for 10 min so the mass fuses gently. Adjust heat of Meeker burner to full, and heat mass for 15 to 20 min. To provide an oxidizing environment for this step, lift the lid of the crucible periodically. Do not allow the reduced portion of the flame to come in contact with the crucible. Remove crucible from flame. Rotate crucible as it cools so to deposit the melt thinly onto the walls of the crucible. After the crucible has cooled, gently roll it between your hands to facilitate the removal of the melt. Remove the melt with 30 mL of 4.5 *M* H_2SO_4, using care to avoid loss by effervescence. Place crucible and lid in a beaker containing 25 mL of 1 *M* H_2SO_4, and heat contents to a boil. Transfer the solution from the beaker and the solution from the melt to a 250 mL volumetric flask. Dilute the solution to volume using distilled water. Allow sediment to settle. Remove an aliquot of clear supernatant solution for total P analysis by the ascorbic acid method.

To analyze for total P, transfer aliquots (2 mL) into 50 mL volumetric flasks (for samples containing <150 mg of P). With 1 *M* Na_2CO_3, adjust pH of the aliquot to 5 using *p*-nitrophenol indicator. Add 5 mL of 2 *M* H_2SO_4 and 5 mL of ammonium paramolybdate reagent and mix. Add 4 mL of the mixed reagent and mix contents of the flask. Bring to 50 mL volume with distilled water and mix thoroughly. Reduction is completed and maximum color intensity develops in 10 min, and color is stable for 24 hours. The absorption maximum of the blue color formed in the presence of Sb is at 890 nm (Harwood et al., 1969)

Comments

The method for color development was described by Harwood et al. (1969) and is a variation of the method proposed by Murphy and Riley (1962). By increasing amount of antimony added, Harwood et al. (1969) found that the range of the calibration curve could be extended. This modification of the Murphy and Riley (1962) method was found to increase the upper limit of the calibration curve from 50 mg P/50ml sample to 150 mg P/50ml sample.

It should be noted that presence of arsenic in the form of AsO_4 in soil samples gives the same blue color as phosphate. To eliminate this problem, AsO_4 can be reduced to AsO_3 using a $NaHSO_3$ solution as described in the following digestion method (Olsen and Sommers, 1982).

Calculations

Total P, mg/kg =

[Concentration of P in initial 250 mL dilution, mg/L] x [0.25 L ÷ mass of soil, kg]

Digestion Method (Olsen and Sommers (1982)):

Reagents

1. 60% Perchloric acid ($HClO_4$)
2. Ammonium paramolybdate-vanadate. Prepare by dissolving 25 g of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 400 mL of distilled water, and by dissolving ammonium metavanadate (NH_4VO_3) in 300 mL of boiling distilled water. Cool vanadate solution, and add 250 mL of conc. HNO_3. Cool NH_4VO_3-HNO_3 solution to room temperature before adding $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ solution. Dilute the mixed solution to 1 L with distilled water.
3. Standard phosphate solution. Prepare by dissolving 0.4393 g of oven-dried potassium dihydrogen phosphate (KH_2PO_4) in distilled water. Dilute solution to 1 L with distilled water. Standard solution contains 100 mg P/L.
4. Sodium hydrogen sulfite ($NaHSO_3$). Prepare by dissolving 5.2 g of reagent grade $NaHSO_3$ in 100 mL of 0.5 *M* H_2SO_4. Prepare reagent weekly.

Procedure

In a 250 mL volumetric or Erlenmeyer flask, mix 2.0 g of finely ground soil (<0.5 mm) with 30 mL of 60% $HClO_4$. Digest the soil and acid mixture at a few degrees below the boiling point on a hot plate in a perchloric hood until the dark color from organic matter disappears. Continue to heat at the boiling temperature for 20 min longer. Heavy white fumes will appear, and the insoluble material will become like white sand. If any black particles stick to the side of the flask, add 1 or 2 mL of $HClO_4$ to wash down the particles. If the sample is high in organic matter it may be necessary to add 20 mL of HNO_3 and heat to oxidize organic matter before adding $HClO_4$. Total digestion time is approximately 40 min. Cool the mixture before bringing the volume up to 250 mL with distilled water. Mix the contents of the flask, and then allow sediment to settle.

To analyze for total P, transfer aliquots into 50 mL volumetric flasks (for samples containing between 0.05 to 1.0 mg of P). Add 10 mL of the ammonium paramolybdate-vanadate reagent, and bring the volume of the flask up to 50 mL using distilled water. The optical density of the sample can be measured after 10 min at wavelengths between 400 to 490 nm. The optical density of a reagent blank should be subtracted from the optical density readings of the samples.

To reduce AsO_4^{-3} to AsO_3^{-3}, add 5 mL of $NaHSO_3$ solution to the aliquot. Then partially immerse the 50 mL volumetric flasks in a water bath, and digest the solution for 30 min (20 min after temperature reaches 95^{o}C). An alternative procedure is to allow the solution to stand for 4 hours at room temperature.

Calculations

Total P, mg/kg =

[Concentration of P in initial 250 mL dilution, mg/L] x [0.25 ÷ mass of soil, kg]

Alkaline Oxidation Method (Dick and Tabatabai (1977)):

Reagents

1. Sodium hypobromite solution (NaOBr-NaOH). Prepare by slowly adding 3 mL of bromine (0.5 mL/min) to 100 mL of 2 *M* NaOH under constant stirring. Prepare reagent immediately prior to use.
2. 90 % formic acid
3. 2.5 *M* H_2SO_4
4. Ammonium molybdate -Antimony potassium tartrate solution. Prepare by dissolving 12 g of ammonium molybdate in 250 mL of distilled water, and dissolving 0.2908 g of antimony potassium tartrate in 100 mL of distilled water. Add both solutions to 1 L of 2.5 *M* sulfuric acid, and dilute volume to 2 L with distilled water. Store reagent in a cool place, in a dark Pyrex glass bottle.
5. Ascorbic acid. Prepare by dissolving 1.056 g of ascorbic acid in 200 mL of ammonium molybdate - antimony reagent. Prepare reagent daily.
6. Standard phosphate solution. Prepare by dissolving 0.2195 g of potassium dihydrogen phosphate (KH_2PO_4) in distilled water. Dilute solution to 1L with distilled water. Standard solution contains 50 mg P/L.

Procedure

Place a 100 to 200 mg sample of finely ground, air-dried soil in a 50 mL boiling flask. Add 3 mL of sodium hypobromite solution to the flask, and swirl flask for a few seconds to mix contents. Allow flask to stand for 5 min. Swirl flask again and place it in a sand bath adjusted to 260 to 280^{o}C. The sand bath should be situated in a hood. Heat flask until contents evaporate to dryness. Evaporation time is 10 to 15 min. After evaporation, continue to heat for an additional 30 min. Remove flask from sand bath, and allow it to cool for 5 min. Then add 4 mL of distilled water and 1 mL of formic acid. Mix contents before adding 25 mL of 0.5 *M* H_2SO_4. Stopper flask and mix contents. Transfer mixture to a 50 mL plastic centrifuge tube and centrifuge sample at 12,000 rpm for 1 min.

To analyze for total P, transfer aliquots of 1 to 2 mL into 25 mL volumetric flasks. Add 4 mL of ascorbic acid reagent, and bring solution up to volume with distilled water. Stopper flask and mix solution. Allow solution to stand for 30 min for color development. Optical density of sample should be measured at a wavelength of 720 nm.

Comments

This method does not require neutralization of the 1 to 2 mL of aliquot, however, longer time (30 min) is needed for full color development.

The sodium hypobromite (NaOBr-NaOH) reagent should be prepared just prior to use. The reagent should be made in a fume hood. Formic acid added after the hypobromite treatment will destroy any residual hypobromite remaining after oxidation of the sample.

Calculations

Total P, mg/kg =

[Concentration of P in initial formic acid/H_2SO_4 solution, mg/L] x [0.03 L ÷ mass of soil, kg]

References:

Bowman, R.A. 1988. A rapid method to determine total phosphorus in soils. Soil Sci. Soc. Am. J. 52:1301-1304.

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

Dick, W.A., and M.A. Tabatabai. 1977. An alkaline oxidation method for determination of total phosphorus in soils. Soil Sci. Soc. Am. J. 41:511-514.

Harwood, J.E., R.A. van Steenderen, and A.L. Kuhn. 1969. A rapid method for orthophosphate analysis at high concentrations in water. Water Res. 3:417-423.

Jackson, M.L. 1958. Soil chemical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Muir, J.W. 1952. The determination of total phosphorus in soil. Analyst 77:313-317.

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

Olsen, S.R., and L.E. Sommers. 1982. Phosphorus. pp. 403-430. *In*: A.L. Page. R.H. Miller, and D.R. Keeney (eds.), Methods of Soil Analysis. 2nd ed. Agronomy Series No.9, Part 2. Soil Science Society of America, Inc., Madison, WI.

Sherrell, C.G., and W.M.H. Saunders. 1966. An evaluation of methods for the determination of total phosphorus in soils. N.Z.J. Agric. Res. 9:972-979.

Sommers, L.E., and D.W. Nelson. 1972. Determination of total phosphorus in soils: a rapid perchloric acid digestion procedure. Soil Sci. Soc. Amer. Proc. 36:902-904.

Syers, J.K., J.D.H. Williams, A.S. Campbel, and T.W. Walker. 1967. The significance of apatite inclusions in soil phosphorous studies. Soil Sci. Soc. Amer. Proc. 31:752-756.

Syers, J.K., J.D.H. Williams, and T.W. Walker. 1968. The determination of total phosphorus in soils and parent materials. N.Z.J. Agric. Res. 11:757-762.

Phosphorus Fractionation

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Introduction:

The chemistry of phosphorus (P) in soils is complicated. Inorganic P can react with Ca, Fe and Al to form discrete phosphates, and organic P can be in different forms with varying resistance to microbial degradation. To investigate the forms of inorganic P (P_i) and transformations of applied P fertilizers, the fractionation procedure of Chang and Jackson (1957) has been widely used. Subsequent studies indicated that various extractants were not as specific as first envisioned. For example, retention of P by CaF_2 formed from $CaCO_3$ during ammonium flouride (NH_4F) extraction affects results when the Chang and Jackson method is used with calcareous soils and sediments. Since its development, modifications made by Williams et al. (1967), Smillie and Syers (1972), Peterson and Corey (1966), and Fife (1962) have improved extractability and allowed for use with calcareous soils. The original fractionation procedures and the most important modifications were summarized by Kuo (1996). The P_i fractionation in this paper is primarily based on the Kuo (1996) fractionation scheme.

Soil organic P (P_o) consists of inositol phosphates, phospholipids, nucleic acids, phosphoproteins, and various sugar phosphates, as well as a significant number of compounds that have not been identified. Organic P tied up in microbial biomass consists of nucleic acids, inositol phosphates, and polyphosphates. Microbial biomass P usually represents a small fraction of the total P in soil, and rapidly turns over to supply inorganic P to plant roots (Tate, 1984). Quantification of the various known P_o compounds in soil has been described in several studies (Anderson, 1967; Halstead and Anderson, 1970; Stott and Tabatabai, 1985) and is advocated by Kuo (1996) as a means of fractionating soil P_o. An alternative method for characterizing soil P_o fractions involves the use of acid and alkaline extractants that separate the various fractions based on the type and strength of P_o physicochemical interactions with other soil components (Bowman and Cole, 1978; Hedley et al., 1982; Cross and Schlesinger, 1995). The most common extractants are 0.5 *M* sodium bicarbonate ($NaHCO_3$) and various concentrations of hydrochloric acid (HCl) and sodium hydroxide (NaOH). The fractionation scheme involves a sequence of extractions that separates soil P_o into labile, moderately labile, and nonlabile fractions. In recent years, this scheme has been widely used to evaluate P_o turnover in diverse soils under varying management (Hedley et al., 1982; Sharpley and Smith, 1985; Ivanoff et al., 1998). The qualitative and quantitative information provided by the fractionation data is useful for agronomic and water quality issues.

Fractionation of Inorganic Phosphorus:

Principles

The fractionation procedures are based on the differential solubilities of the various inorganic P forms in various extracts. Ammonium chloride (NH_4Cl) is used first to remove soluble and loosely bound P, followed by separating Al-P from Fe-P with (NH_4F), then removing Fe-P with NaOH. The reductant-soluble P is removed with CDB (sodium citrate-sodium dithionite-sodium bicarbonate) extraction. The Ca-P is extracted with sulfuric acid (H_2SO_4) or HCl since Ca-P is insoluble in CDB. Since NH_4F reacts with $CaCO_3$ to form CaF_2 in calcareous soils, which will precipitate soluble P and reduce the effectiveness of NH_4F to extract P, the NH_4F extraction is omitted for calcareous soils.

Equipment

1. Shaker
2. Centrifuge and 100-mL centrifuge tubes
3. Hot water bath
4. Spectrophotometer

Reagents

1. 1 *M* ammonium chloride (NH_4Cl). Dissolve 53.3 g of NH_4Cl in 1 L deionized water
2. 0.5 *M* ammonium fluoride (NH_4F) pH 8.2. Dissolve 18.5 g of NH_4F in 1 L deionized water and adjust pH to 8.2 with 4 *M* NH_4OH.
3. 2 *M* and 0.1 *M* sodium hydroxide (NaOH). Dissolve 80 g and 4.0 g respectively of NaOH in 1 L deionized water.
4. 0.1 *M* NaOH + 1 *M* NaCl. Dissolve 4.0 g of NaOH and 58.5 g of NaCl in 1 L deionized water.
5. Saturated NaCl. Add 400 g of NaCl to 1 L deionized water.
6. 0.25 *M* sulfuric acid. Dilute 14 mL of concentrated H_2SO_4 to 1 L with deionized water.
7. 2 *M* hydrochloric acid. Dilute 168 mL of concentrated HCl to 1 L with deionized water.
8. 0.3 *M* sodium citrate. Dissolve 88.2 g of $Na_3C_6H_5O_7 \cdot 2H_2O$ in 1 L deionized water.
9. 1 *M* sodium bicarbonate. Dissolve 84 g of $NaHCO_3$ in 1L deionized water.
10. 0.8 *M* boric acid. Dissolve 50 g of H_3BO_3 in 1 L deionized water.
11. Sodium dithionite reagent grade.
12. 0.25% p-nitrophenol. Dissolve 0.25 g of p-nitrophenol in 100 mL of deionized water.

Procedures for Noncalcareous Soils (flow chart in Fig. 1)

1. Add 1.0 g (<2 mm) of soil and 50 mL of 1*M* NH_4Cl to a 100 mL centrifuge tube and shake for 30 min to extract the soluble and loosely bound P. Centrifuge and decant the supernatant into a 50-mL volumetric flask and bring to volume with deionized water (extract A).
2. Add 50 mL of 0.5 *M* NH_4F (pH 8.2) to the residue and shake the suspension for 1 h to extract aluminum phosphates. Centrifuge and decant the supernatant into a 100-mL volumetric flask (extract B).
3. Wash the soil sample twice with 25-mL portions of saturated NaCl and centrifuge. Combine the washings with extract B and bring to volume. Add 50 mL of 0.1 *M* NaOH to the soil residues and shake for 17 h to extract iron phosphate. Centrifuge and decant the supernatant solution into a 100-mL volumetric flask (Extract C). Wash the soil twice with 25-mL portions of saturated NaCl and centrifuge. Combine the washings with extract C and bring to volume.

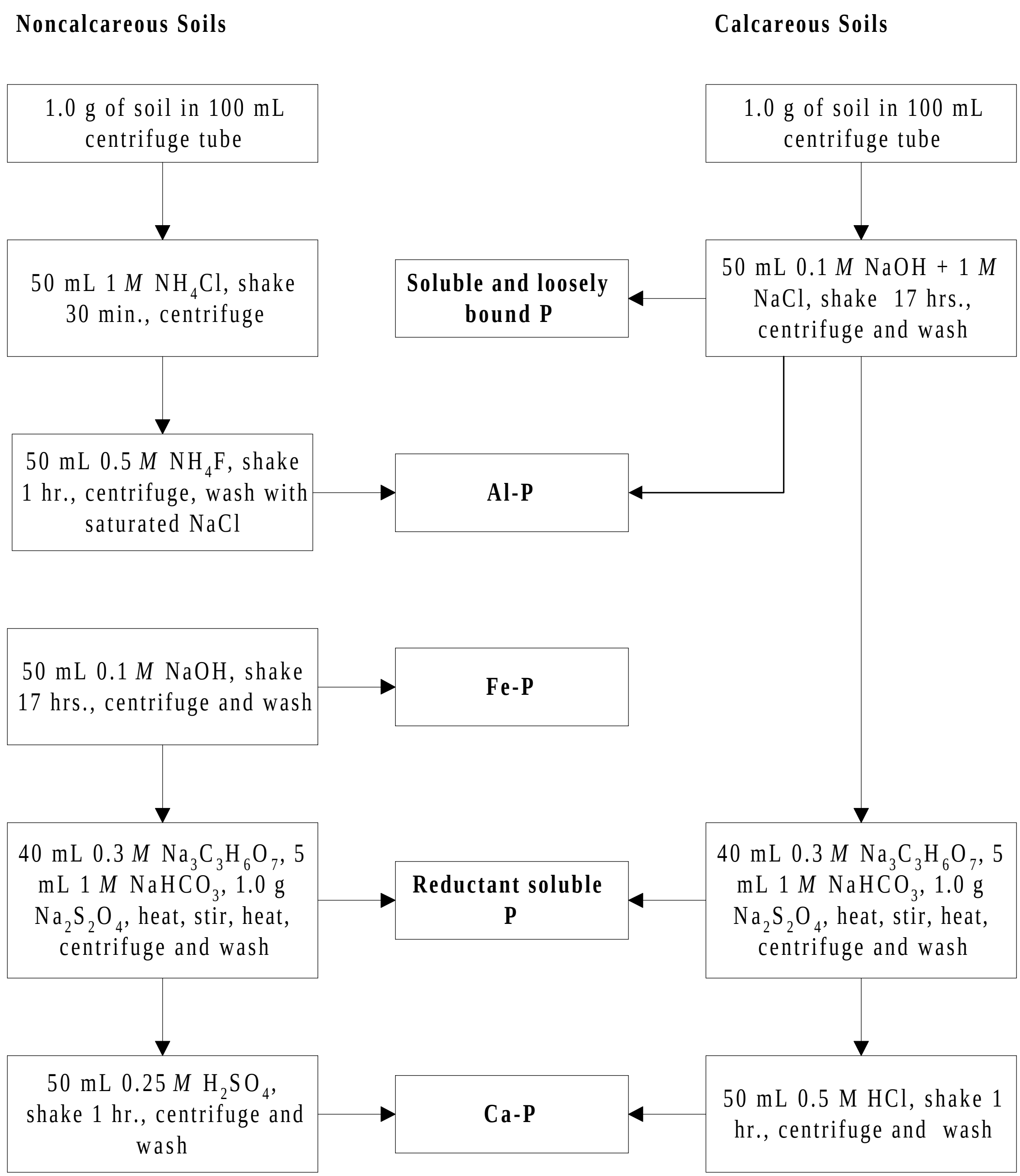

Figure 1. Sequential fractionation scheme for inorganic P.

4. Add 40 mL of 0.3 *M* $Na_3C_6H_5O_7$ and 5 mL of 1 *M* $NaHCO_3$ to the residue and heat the suspension in a water bath at 85 °C.
5. Add 1.0 g of $Na_2S_2O_4$ (sodium dithionate) and stir rapidly to extract reductant-soluble P. Continue to heat for 15 min and then centrifuge. Decant the supernatant solution into a 100-mL volumetric flask (extract D). Wash the soil twice with 25-mL portions of saturated NaCl and centrifuge. Combine the washings with extract D, and dilute D to volume. Expose extract D to air to oxidize $Na_2S_2O_4$.
6. Add 50 mL of 0.25 *M* H_2SO_4 to the soil residue and shake for 1 h. Centrifuge the suspension for 10 min and decant the supernatant into a 100-mL volumetric flask (extract E). Wash the soil twice with 25-mL portions of saturated NaCl, and centrifuge. Combine the washings with the extract E and dilute to volume.
7. Transfer an aliquot containing 2 to 40 μg P from each of extracts A, B, C, D, and E to separate 50-mL volumetric flasks. Add some deionized water and five drops of p-nitrophenol indicator to the volumetric flasks containing extracts C and E, and adjust the pH with 2 *M* HCl or 2 *M* NaOH until the indicator color just changes. Add 15 mL 0.8 *M* H_3BO_3 to the volumetric flask containing extract B. Phosphorus concentrations in the various solutions can be determined using the ascorbic acid method (Murphey and Riley, 1962). Prepare P standards that contain the same volume of extracting solution as in the extracts.

Calculations

The amount of P in each fraction is calculated using the following equation:

P concentration in given fraction (mg/kg) =
[Conc. of P (mg/L)] x [Volume of extractant (L) ÷ mass of soil (kg)]

Procedures for Calcareous Soils (flow chart in Fig. 1)

1. Add 1.0 g (<2 mm) of soil and 50 mL of 0.1 *M* NaOH + 1 *M* NaCl, shake for 17 h. Centrifuge and decant the supernatant solution into a 100-mL volumetric flask (extract A). Wash the soil twice with 25-mL portions of saturated NaCl and centrifuge. Combine the washings with extract A and bring to volume.
2. Add 40 mL of 0.3 *M* $Na_3C_6H_5O_7$ and 5 mL of 1 *M* $NaHCO_3$ to the residue and heat the suspension in a water bath at 85°C. Add 1.0 g of $Na_2S_2O_4$ (sodium dithionate) and stir rapidly. Continue to heat for 15 min and centrifuge. Decant the supernatant solution into a 100-mL volumetric flask (extract B). Wash the soil twice with 25-mL portions of saturated NaCl and centrifuge. Combine the washings with extract B, and dilute B to volume. Expose extract B to air to oxidize $Na_2S_2O_4$.
3. Add 50 mL of 0.5 *M* HCl to the soil residue and shake for 1 h. Centrifuge the suspension, and decant the supernatant into a 100-mL volumetric flask (Extract C). Wash the soil twice with 25-mL portions of saturated NaCl, and centrifuge. Combine the washings with the extract C and dilute to volume.
4. Transfer an aliquot containing 2 to 40 μg P from each of extracts A, B, and C to separate 50-mL volumetric flasks. Add some deionized water and five drops of p-nitrophenol indicator to each of the volumetric flasks containing extracts A and C and adjust the pH with 2 *M* HCl or 2 *M* NaOH until the indicator color just

changes from yellow to colorless for extracts A and C. P concentrations of various fractions can be determined using the ascorbic acid method (Murphey and Riley, 1962). Prepare P standards that contain the same volume of extracting solution as in the extracts.

Calculations

The amount of P in each fraction can be calculated using the following equation:

P concentration in given fraction (mg/kg) =
[Conc. of P (mg/L)] x [Volume of extractant (L) ÷ mass of soil (kg)]

Fractionation of Organic Phosphorus:

Principles

In general, the fractionation scheme follows the procedures developed by Bowman and Cole (1978) and modified by Sharpley and Smith (1985) and Ivanoff et al. (1998). Organic P in both calcareous and noncalcareous soils is fractionated into a labile pool, a moderately labile pool, and a nonlabile pool. The labile pool is extracted with 0.5*M* $NaHCO_3$ at pH 8.5. The extracted P includes both P_o and P_i in soil solution and sorbed on soil colloids. If desired, microbial biomass P in the soil can be determined at this point, via a chloroform ($CHCl_3$) fumigation technique (Hedley and Stewart, 1982). The moderately labile pool is extracted with 1.0 *M* HCl, followed by 0.5 *M* NaOH. The NaOH extract is acidified with concentrated HCl to separate the nonlabile fraction (humic acid fraction) from the moderately labile fraction (fulvic acid fraction). Finally, the highly resistant, nonlabile fraction is determined by ashing the residue from the NaOH extraction at 550°C for 1 h, followed by dissolution in 1.0 *M* sulfuric acid (H_2SO_4). The complete soil P_o fractionation scheme is shown in Figure 2. In all cases, P concentration in the extracts is determined colorimetrically by the phospho-molybdate method of Murphy and Riley (1962). Acid or alkaline extracts are neutralized prior to P determinations. Organic P in the extracts is calculated from the difference between total P and P_i. Total P in the extracts is measured after an aliquot is digested with 2.5 *M* H_2SO_4 and potassium persulfate ($K_2S_2O_8$), according to the method of Bowman (1989), as modified by Thien and Myers (1992).

Equipment

1. Reciprocating shaker
2. Centrifuge and 100 mL tubes
3. Hot plate
4. Muffle furnace
5. Spectrophotometer

Reagents

1. 0.5 *M* sodium bicarbonate solution. Dissolve 42 g of $NaHCO_3$ in 1 L deionized water. Adjust the pH of this solution to 8.5 with 1 *M* NaOH (40 g of NaOH in 1 L deionized water). Avoid exposure of solution to air. Prepare fresh solution if

solution has been stored more than 1 month in a glass container. Solution can be stored >1 month in polyethylene, but pH should be checked each month.

2. *p*-nitrophenol indicator. Dissolve 0.25 g of *p*-nitrophenol in 100 mL of deionized water.
3. 2 *M* and 1 *M* hydrochloric acid. Dilute 168 mL and 84 mL, respectively, of concentrated HCl to 1 L with deionized water.
4. Phospho-molybdate reagents. Dissolve 12 g of ammonium paramolybdate $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$ in 250 mL of deionized water. Dissolve 0.2908 g of potassium antimony tartrate $(KSbO \cdot C_4H_4O_6)$ in 100 mL of deionized water. Add these solutions to 1 L of 2.5 *M* H_2SO_4 (141 mL of concentrated H_2SO_4 diluted to 1 L), mix thoroughly, and after cooling, dilute to 2 L with deionized water. Store solution (Reagent A) in a dark, cool place. To prepare reagent B, dissolve 1.056 g of L-ascorbic acid $(C_6H_8O_6)$ in 200 mL of Reagent A, and mix. Reagent B should be prepared as needed, because it must be used within 24 h.
5. 2.5 *M* sulfuric acid. Dilute 140 mL of concentrated H_2SO_4 to 1 L with deionized water.
6. 2 *M* and 0.5 *M* sodium hydroxide. Dissolve 80 g and 20 g, respectively, of NaOH in 1 L deionized water.
7. Potassium persulfate $(K_2S_2O_8)$ – reagent grade
8. Chloroform $(CHCl_3)$ – ethanol free, reagent grade

Procedures

Labile Organic P:

Weigh duplicate 1.0 g (oven-dry weight basis) samples of sieved (2 mm), field-moist soil into two 100 mL centrifuge tubes. To one tube, add 50 mL of 0.5 *M* $NaHCO_3$ and place sample horizontally on a reciprocating mechanical shaker for 16 h. At the end of the extraction period, centrifuge sample at 7000 rpm for 15 min and filter supernatant through Whatman No. 41 quantitative paper into a 50-mL volumetric flask. Bring to volume with deionized water and mix well.

To determine labile P_i, transfer an aliquot containing 2 to 40 μg P to a 50-mL volumetric flask, add five drops of p-nitrophenol indicator to the flask and adjust the pH with 2 *M* HCl until the indicator color just changes from pale yellow to colorless. Add approximately 40 mL of deionized water to the flask, followed by 8 mL of Reagent B. Bring to volume with deionized water, and mix well. After 20 min., determine P concentration on a calibrated spectrophotometer at 880 nm. A blank containing the 0.5 *M* $NaHCO_3$ extracting solution should be analyzed with the sample.

To determine total labile P in the extract, add 0.5 g of $K_2S_2O_8$ with a calibrated scoop to a 25-mL volumetric flask, transfer an appropriate aliquot (usually 1 to 5 mL, depending on P concentration) of the extract into the flask, and add 3 mL of 2.5 *M* H_2SO_4. Digest sample on a hot plate at >150°C for 20 to 30 min. Digestion is complete after vigorous boiling subsides. Cool sample, add 5 mL of deionized water. After mixing, add five drops of p-nitrophenol indicator to the flask and adjust the pH with 5 *M* NaOH. Add approximately 10 mL of deionized water to the flask, followed by 4 mL of Reagent B. Bring to volume with deionized water, and mix well. After 20 min., determine P concentration on a calibrated spectrophotometer at 880 nm.

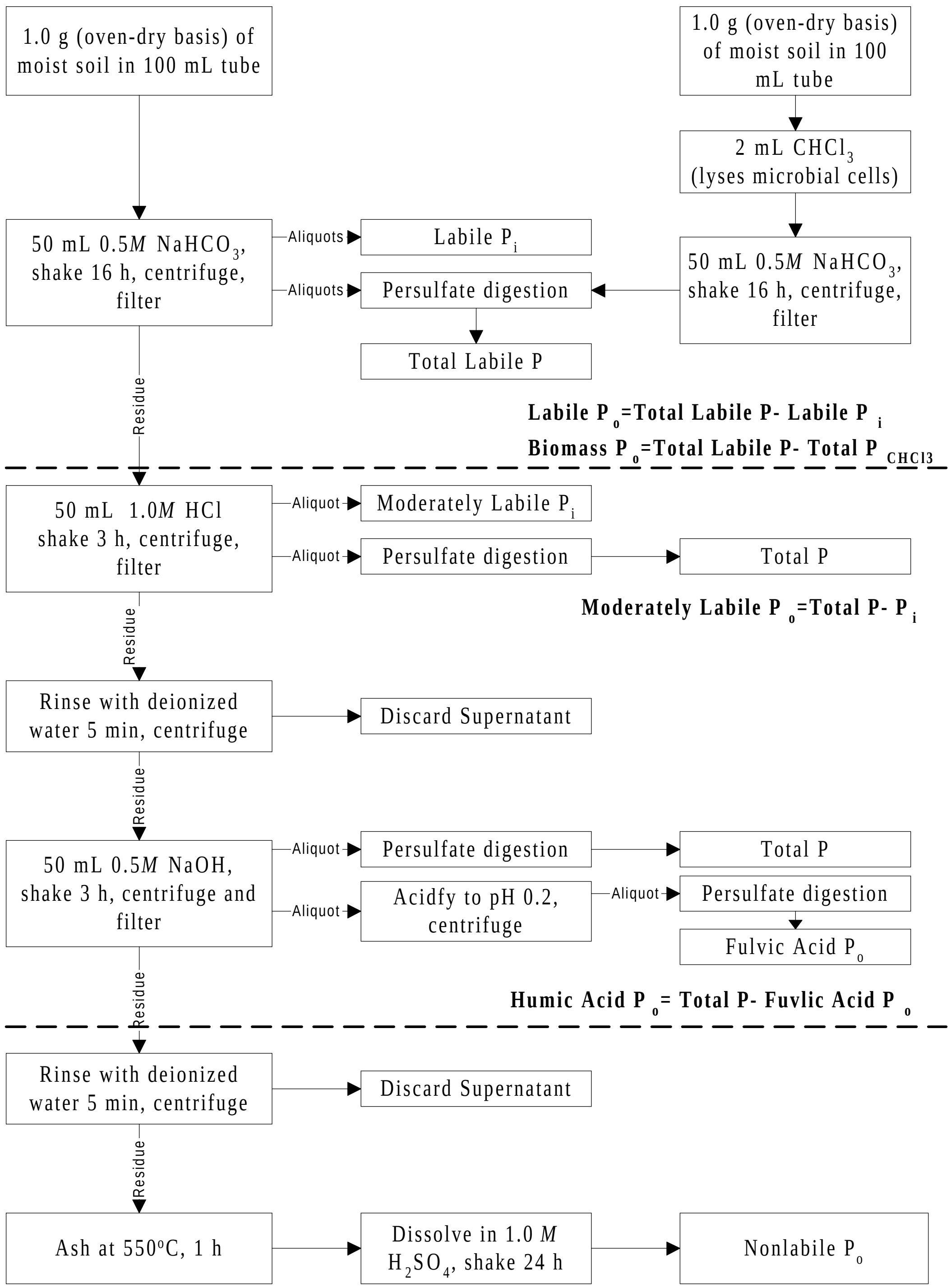

Figure 2. Sequential fractionation scheme for organic P.

The difference between total labile P by persulfate oxidation and labile P_i gives an estimate of labile P_o. The P_i analysis should be performed as soon as possible after the soil extraction to minimize hydrolysis of P_o.

To estimate P associated with soil microbial biomass, treat the second duplicate weighed sample with 2 mL of ethanol-free $CHCl_3$. Cover the uncapped tubes loosely with paper towels and place under a fume hood for 24 h. At the end of this period, extract samples with 0.5 *M* $NaHCO_3$ as previously described. The difference between the amounts of total labile P in the $CHCl_3$-treated and untreated duplicate soil samples determines biomass P that originated from lysed microbial cells.

<u>Moderately Labile Organic P:</u>

A two-step process is required to determine moderately labile P_o. Add 50 mL of 1 *M* HCl to the residue from the labile P extraction and place sample on a reciprocating mechanical shaker for 3 h. An aliquot of 1 *M* HCl should be used to rinse residue from filter paper used in the labile P extraction. After 3 h, centrifuge sample at 7000 rpm for 15 min and filter supernatant through Whatman No. 41 quantitative paper into a 50-mL volumetric flask. Bring to volume with deionized water and mix well. Determine total P and P_i in the extract as previously described. Any P_o extracted in the 1 *M* HCl is considered part of the moderately labile P fraction.

Rinse the residue from the HCl extraction with deionized water, shake for 5 min centrifuge, and discard the supernatant solution. Add 50 mL of 0.5 *M* NaOH to the residue and shake sample for 16 h. At the end of the extraction time, centrifuge sample at 7000 rpm for 15 min. The supernatant contains both moderately labile P_o (fulvic acid P) and nonlabile P_o (humic acid P). To separate these fractions, remove an aliquot of the NaOH extract and acidify to pH 0.2 with concentrated HCl. At this pH, humic acids precipitate, and fulvic acids remain in solution. Centrifuge acidified sample at 7000 rpm for 15 min. Determine total P in both the NaOH extract and the acidified sample as previously described. Total P in the acidified sample is a measure of fulvic acid P. Estimate humic acid P by subtracting fulvic acid P from the total P measured in the NaOH extract (Figure 2).

<u>Nonlabile Organic P:</u>

To determine highly-resistant, nonlabile P_o, rinse the residue from the NaOH extraction with deionized water, shake for 5 min., centrifuge, and discard the supernatant solution. Place the residue in a crucible and ash at 550°C for 1 h. Dissolve ash by shaking in 1 *M* H_2SO_4 for 24 h, and measure P in solution as previously described.

Calculations

The amount of P in each fraction can be calculated using the following equation:

P concentration is given fraction (mg/kg)=

[Conc. of P (mg/L)] x [volume of extractant (L) ÷ mass of soil (kg)]

Comments:

It should be remembered that P fractionation schemes are operationally defined. It is difficult to identify which discrete P_o compounds are extracted with each step. Moreover, hydrolysis of some P_o compounds by 1 *M* HCl or 0.5 *M* NaOH, sorption of labile P, and heterogeneity of soil particles within a sample may limit the accuracy of this fractionation procedure.

References:

Anderson, G. 1967. Nucleic acids, derivatives, and organic phosphates. p. 67-90. *In*: A.D. McLaren and G.H. Peterson (ed.). Soil Biochemistry. Marcel Dekker, New York.

Bowman, R.A., and C.V. Cole. 1978. An exploratory method for fractionation of organic phosphorus from grassland soils. Soil Sci. 125:95-101.

Bowman, R.A. 1989. A sequential extraction procedure with concentrated sulfuric acid and dilute base for soil organic phosphorus. Soil. Sci. Soc. Am. J. 53:362-366.

Chang, S.C., and M.L Jackson. 1957. Fractionation of soil phosphorus. Soil Sci. 84:133-144.

Cross, A.F., and W.H. Schlesinger. 1995. A literature review of the Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. Geoderma. 64:197-214.

Fife, C.V. 1962. An evaluation of ammonium fluoride as a selective extractant for aluminum-bound soil phosphate: detailed study of soils. Soil Sci. 96:112-120.

Halstead, R.L., and G. Anderson. 1970. Chromatographic fractionation of organic phosphates from alkali, acid, and aqueous acetylacetone extracts of soils. Can. J. Soil Sci. 50:11-119.

Hedley, M.J., J.W.B. Stewart, and B.S. Chauhan. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46:970-976.

Ivanoff, D.B., K.R. Reddy, and S. Robinson. 1998. Chemical fractionation of organic phosphorus in selected histosols. Soil Sci. 163: 36-45.

Kuo, S. 1996. Phosphorus. *In*: D.L. Sparks (ed.) Methods of soil analysis. Agronomy 9. ASA-SSSA, Madison, WI.

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

Peterson, G.W., and R.B. Corey. 1966. A modified Chang and Jackson procedure for routine fractionation of inorganic soil phosphates. Soil Sci. Soc. Amer. Proc. 30:563-565.

Sharpley, A.N., and S.J. Smith. 1985. Fractionation of inorganic and organic phosphorus in virgin and cultivated soils. Soil Sci. Soc. Am. J. 49:127-130.

Smillie, G.W., and J.K. Syers. 1972. Calcium chloride formation during extraction of calcareous soil with floride: II. Implications to the Bray-1 test. Soil Sci. Soc. Am. Proc. 36:25-30.

Stott, D.E., and M.A. Tabatabai. 1985. Identification of phospholipids in soils and sewage sludges by high performance liquid chromatography. J. Environ. Qual. 14:107-110.

Tate, K.R. 1984. The biological transformation of P in soil. Plant Soil. 76:245-256.

Thien, S.J., and R. Myers. 1992. Determination of bioavailable phosphorus in soil. Soil Sci. Soc. Am. J. 56:814-818.

Williams, J.D.H., J.K.Syers, and T.W. Walker. 1967. Fractionation of soil inorganic phosphorus by a modification of Chang and Jackson's procedure. Soil Sci. Soc. Amer. Proc., 31:736-739.

Phosphorus Fractionation in Flooded Soils and Sediments

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Introduction:

Phosphorus (P) chemistry in soils and sediments is greatly influenced by the oxidation-reduction status (redox potential). Under oxidized conditions, ferric and manganic oxides and hydroxides are important adsorption sites for P. In addition, ferric and manganic phosphate minerals, such as strengite ($FePO_4 \cdot 2H_2O$), and trivalent Mn phosphate ($MnPO_3 \cdot 1.5H_2O$) can form and persist under oxidized conditions. However, under reducing conditions these minerals are unstable, resulting in dissolution and release of soluble P into the soil solution (Patrick et al., 1973; Emerson, 1976; Emerson and Widmer, 1978; Boyle and Lindsay, 1986; Moore and Reddy, 1994).

Since Fe and Mn phosphate mineral formation is controlled by the redox potential of the soil or sediment, it is important that soil and sediment samples that are collected under reduced conditions are handled appropriately during P fractionation to get an accurate picture of the P status. Allowing anaerobic sediments to become oxidized results in the rapid conversion of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}). Within a very short time period (seconds to minutes), solid phase $Fe(OH)_3$ precipitates out of solution. Fresh ferric hydroxide precipitates have tremendous P sorption capacities, and they can cause the soluble P levels in the porewater to be reduced by orders of magnitude in minutes. To avoid this, samples should be maintained under anerobic conditions during the initial phases of P fractionation.

Sequential extraction schemes for P (phosphorus fractionation) have been employed by various workers over the past 60 years, yet this is not an exacting science (Dean, 1938; Williams, 1950; Chang and Jackson, 1957; Williams et al., 1967; Chang et al., 1983). It must be kept in mind that these are rather crude methods, with many extractants causing the dissolution of more than one type of P solid phase. For example, sodium hydroxide is often used to extract Al and Fe-bound P (van Eck, 1982; Hieltjes and Lijklema, 1980). However, this compound will also extract organic P fractions, particularly in soils that have been heavily manured in the past. Hence, authors must be aware of the pitfalls and fallibility of the methods we are outlining, and use them only when they are the best procedure available.

Materials:

1. PVC or plexiglas cylinder for taking cores
2. Purified N_2
3. Glove bag
4. Vacumn pump
5. Centrifuge and 250 mL centrifuge tubes with caps equipped with rubber septums

Reagents:

1. Deionized water
2. 1 M KCl

3. 0.1 M NaOH
4. 0.5 M HCl
5. Concentrated HCl (trace metal grade)

Method:

The P fractionation procedure described below is similar to that of van Eck (1982) as modified by Moore and Reddy (1994).

Sampling:

Flooded soil or sediment samples can be taken with a PVC or plexiglas cylinder. The coring device should be beveled from the outside so that it can be inserted into the sediment. Under certain conditions, such as in salt marshes or rice fields, it may be necessary to pound on the coring device with a hammer to reach the desired sampling depth.

The sample can be returned to the lab in the sampling cylinder or it can transferred to a pre-weighed centrifuge tube. If it is to be transported in the cylinder, then a rubber stopper should be placed on the bottom of the core to hold the sediment in place. It is a good idea to tape the stopper in place. If the sample is taken under flooded conditions, leave some of the floodwater on top of the sample. If samples have been taken from a lake bottom, then the entire headspace should be filled with lake water and a stopper should be placed on the top of the cylinder as well. This reduces the amount of shaking and minimizes disturbance of the sediment/water interface.

If samples are taken in flooded or saturated agricultural fields, transfer them directly into a 250 mL polycarbonate centrifuge tube. It is important that the sampling corer have an inside diameter slightly smaller than the inside diameter of the centrifuge tube. To take the sample, simply push the corer into the sediment to the desired depth (e.g., 10 cm). It may be difficult to remove the core from the sediment without disturbing the sample. It may be necessary to hold the sediment in place from underneath the core (usually by hand) when pulling the core out of the ground to prevent the soil from falling out. If the soil is relatively fine textured the core can be rocked side to side and removed. Once the core has been removed from the sediment, pour the water off and place the cylinder over the mouth of the centrifuge tube. If the sample is from a coarse textured soil, it will fall into the tube. However, when clay contents are high, it will adhere to the sampling core. In this case it is necessary to have a ramrod with a rubber stopper (outside diameter slightly smaller than sampling cylinder's inside diameter) to force the sample into the centrifuge tube.

After the sample is in the centrifuge tube, tap the tube on a hard surface (palm of your hand) to allow any entrained air bubbles to escape to the surface. If these air bubbles are not removed, then the sample will become oxidized.

Next, screw the lid onto the centrifuge tube and insert a 12 gauge needle through the rubber septum in the tube's top. Insert another 12 gauge needle that is connected via tygon tubing to the N_2 gas cylinder and begin purging. Purge the headspace for 5-10 minutes with N_2 at a pressure of about 10 psi. This pressure, coupled with the needle size, will result in a loud hissing sound; absence of the sound may mean the needle is clogged with sediment. Extra needles should be taken into the field in case this happens.

After purging the sample, remove the needle not connected to the N_2 first, then the other needle. This allows a positive pressure of N_2 on the sample, so if the container leaks, the leak will be outward.

If the samples are to be processed in less than two days, refrigeration is not required. For longer periods, the samples should be put on ice to slow down biological activity. It should be noted that many plastics, like polycarbonate, allow slow diffusion of oxygen. If samples are stored for months in the refrigerator, the sediment along the walls of the tubes will change color to red and orange, as oxygen enters the tube and oxidizes iron. If this happens, the sample should not be used.

Water-Soluble P:

The first fraction of P to be extracted from the sample is water-soluble P. If the sample was taken in intact sediment cores and the researcher desires to obtain a depth distribution of P in the core, then a glove bag is needed. Place the top of the core into the glove bag. Also place any supplies (spatula, purged centrifuge tubes, syringes, etc.) into the bag. Fill the bag with N_2 gas, and empty it two or three times to make sure it is oxygen-free. Use a ramrod with rubber stopper (plunger) as described above to slowly push the sediment to the surface. Using the spatula, take the first sample to the desired depth [it helps to have the depth increment (e.g., 5 cm) marked on the plexiglas corer]. After the sediment has been placed in the tube, tap the tubes to get rid of bubbles. Then push the plunger upward another 5 cm (or whatever depth is desired). Repeat this process until all of the samples are in the tubes.

Open the glove bag and purge the headspace in the centrifuge tube as described earlier. The headspace should be anerobic, if the glove bag worked correctly. However, trace quantities of O_2 can cause problems, so this extra step is warranted. If the samples were transferred to centrifuge tubes in the field, purge them in the laboratory immediately prior to centrifugation to make certain the headspace is oxygen-free.

First, record the weight of the tube plus sediment. Since the weight of the tube was recorded earlier, the wet sediment weight will be known. Centrifuge the samples at 7500 rpm for 20 minutes. At this point the samples are most susceptible to oxidation, since the porewater is separated from the soil. Hence, do not open the centrifuge tubes unless you are ready to filter immediately.

It is preferable to filter the samples quickly, so vacuum filtration is strongly recommended, using a 0.45 μm membrane filter. Turn on the pump and quickly open and pour the soil solution onto the filter. It should filter in a few seconds. Quickly pour the supernatant into a plastic sample container and acidify with concentrated HCl to pH 2. It is mandatory that the water-soluble sample be acidified. Otherwise, when the sample oxidizes, soluble iron will precipitate soluble P, as discussed earlier.

If pH measurements are to be taken, do not filter all of the sample. Using a 60 mL syringe, remove a suitable aliquot of the porewater for pH measurement. Hold the syringe upright and get rid of any air bubbles. Keep the sample in the syringe until pH is measured. Flooded soil/sediment samples have a high partial pressure of CO_2 (often greater than 5%). If degassing occurs prior to pH measurement, the pH will often change by one to two units.

The acidified, filtered sample for water-soluble P can be analyzed by several methods. If the Murphy-Riley method is used, then the anaylses can be referred to as soluble reactive P. It is considered soluble since it passed through 0.45 μm membrane, and reactive since it reacted with the reagents in the Murphy-Riley method.

The residual sediment from the water-soluble fraction will be used for the remaining fractionation. Hence, after the porewater has been removed, screw the lid back on the tube and purge with N_2 to maintain anaerbic conditions.

Loosely Sorbed P:

Various salts have been used in the past for *loosely sorbed P*. van Eck (1982) utilized NH_4Cl for this purpose. However, in many studies focusing on P, it is also desirable to measure the amount of inorganic N present as ammonium. Hence, Moore and Reddy (1994) utilized KC1 for this fraction, so that exchangeable NH_4 (and exchangeable metals minus K) could be determined on one sample.

After the porewater has been removed for water-soluble P, the tubes should be weighed to determine how much water was removed from the sample. Next, the tubes are placed into a glove bag and purged with N_2 gas as described above. The sediment in the tubes should then be homogenized with a spatula, and a subsample (approximately 1 gram dry weight) should be transferred into another pre-weighed centrifuge tube. Another subsample will be taken for moisture content, so that the exact weight of the sample for P fractionation is known. While still in the glove bag, add 20 mL of de-aerated 1 *M* KCl to the tubes. When the tubes are removed from the glove bag, purge again with N_2 gas to ensure the headspace is oxygen-free.

Shake the tubes for 2 h on reciprocating shaker, then centrifuge at 7,500 rpm for 20 minutes, and quickly filter through 0.45 μm filters as described above. The supernatant should be acidified to pH 2 with concentrated HCl. The sample can then be analyzed by the Murphy-Riley method. This fraction is *loosely sorbed P*.

After this fraction has been taken, precautions to maintain anaerobic conditions are no longer needed. Decant excess KCl, then weigh again. Weights of each successive fraction are needed to calculate the entrained liquid (containing soluble P) from the prior extraction.

Aluminum and Iron-bound P:

The residual sediment from the KCl extraction will be utilized “as is” for the next extraction (with NaOH). Add 20 mL of 0.1 *M* NaOH to the sample, and shake for 17 hours. Then centrifuge at 7500 rpm and filter through 0.45 μm membrane filters. Analyze using the Murphy-Riley method. This fraction is referred to as *Al and Fe-bound P*.

It should be noted that some researchers will split this sample and digest half of the sample prior to analysis with Murphy-Riley. The difference between the undigested NaOH sample and the digested NaOH sample is referred to as "organic-bound P."

Calcium-bound or Apatite P:

After removing excess NaOH and weighing the previous sample, add 20 mL of 0.5 *M* HCl and shake for 24 h. If the sediment contains free carbonates, open the samples during the first 15 minutes or so to relieve the pressure from CO_2 buildup. After they have shaken for 24 hours, filter through 0.45 μm membrane filters, and analyze using the

Murphy-Riley method. This aliquot is referred to as *Ca-bound P*, but may also contain some organic P.

Residual P:

The remaining sample can then be analyzed for total P using a nitric-perchloric acid digestion or other suitable method. This is simply referred to as residual P, since it probably contains some Al and Fe-bound P, as well as organic P. Residual P can also be calculated by measuring total P on the original sample and subtracting the various fractions.

References:

Boyle, F.W., Jr. and W.L. Lindsay. 1986. Manganese phosphate equilibrium relationships in soils. Soil Sci. Soc. Am. J. 50:588-593.

Chang, S.C. and M.L. Jackson. 1957. Fractionation of soil phosphorus. Soil Sci. 84:133-144.

Chang, A.C., A.L. Page, F.H. Sutherland, and E. Grgurevic. 1983. Fractionation of phosphorus in sludge-affected soils. J. Environ. Qual. 12:286-290.

Dean, L.A. 1938. An attempted fractionation of the soil phosphorus. J. Agric. Sci. 28:234-246.

Emerson, S. 1976. Early diagenesis in anaerobic lake sediments: Chemical equilibria in interstitial waters. Geochim. Cosmochim. Acta 40:925-934.

Emerson, S. and G. Widmer. 1978. Early diagenesis in anaerobic lake sediments: II. Thermodynamic and kinetic factors controlling the formation of iron phosphates. Geochim. Cosmochim. Acta 42:1307-1316.

Hieltjes, A.H.M and L. Lijklema. 1980. Fractionation of inorganic phosphates in calcareous sediments. J. Environ. Qual. 9:405-407.

Moore, P.A., Jr. and K.R. Reddy. 1994. Role of Eh and pH on phosphorus geochemistry in sediments of Lake Okeechobee, Florida. J. Environ. Qual. 23:955-964.

Murphy, J. and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27:31-36.

Patrick, W.H., Jr., S. Gotoh, and B.G. Williams. 1973. Strengite dissolution in flooded soils and sediments. Science 179:564-565.

Williams, C.H. 1950. Studies on soil phosphorus: I. A method for the partial fractionation of soil phosphorus. J. Agric. Sci. 40:233-242.

Williams, J.D.H., J.K. Syers, and T.W. Walker. 1967. Fractionation of soil inorganic phosphate by a modification of Chang and Jackson's procedure. Soil Sci. Soc. Am. Proc. 31:736-739.

van Eck, G.T.M. 1982. Forms of phosphorus in particulate matter from the Hollands Diep/Haringvliet, the Netherlands. Hydrobiologia 91:665-681.

Determination of Phosphorus Retention and Flux in Soil

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Introduction:

In soils and sediments, physicochemical and biological processes act jointly to control the amount of phosphorus (P) that is in solution. The soluble reactive P fraction is taken up by plants, sequestered in soil, or disperses in the surrounding environment. Although the primary mechanism for environmental transport of P from agricultural soils is by erosion and surface runoff, specific instances of subsurface movement have been reported (Heckrath et al., 1995; Eghball et al., 1996; Gachter et al., 1998). Agricultural P inputs to nearby surface waters have been associated with toxic algal blooms and the depletion of oxygen in aquatic systems. An improved understanding of P retention and transport mechanisms is needed to develop management practices to mitigate P transport and inputs to surface waters.

Typical methods used for assessing the environmental behavior of native and added P in terrestrial and aquatic ecosystems include procedures for measuring the retention capacity of soils and sediments and the associated kinetic parameters. Phosphorus retention has been commonly determined by batch equilibrium methods in which soil or sediment samples are agitated with P solutions of known concentrations (Graetz and Nair, 2000, this publication). The suspension is equilibrated for a sufficient time to achieve apparent equilibrium in the system. The advantages and disadvantages of the technique have been extensively reviewed (Green and Karickoff, 1990; Sparks et al., 1996).

Flow methods have also been used to study water and dissolved solute movement, the retention and desorption processes, for P in particular (Rao et al., 1979; van Riemsdijk and van der Linden, 1984; Miller et al., 1989; Beauchemin et al., 1996). Flow methods are open systems where solute and the reaction products with soil and sediment constituents are removed, minimizing re-adsorption, secondary precipitation reactions, or inhibition of desorption. Important parameters include water flux, chemical and hydrodynamic dispersion, sorption, exchange and desorption characteristics, and transformation rates coefficients.

Applications:

A flow displacement approach facilitates the simulation of the dynamic sorption-desorption, transformations, and transport of P in the soil and water system. Displacement studies provide insights in the kinetics of P release and physical and chemical non-equilibrium conditions that may influence nutrient mineralization and transport in soil. Columns experiments have been conducted to study the miscible displacement of organic chemicals (Green and Corey, 1971; Rao et al., 1979; Dao et al., 1980; Wagenet and Rao, 1990) and for PO_4-P in particular (van Riemsdijk and van der Linden, 1984; Miller et al., 1989; Chen et al., 1996). Breakthrough curves yield characteristics of the adsorption-desorption non-equilibrium and soil-solvent-solute interactions (Green and Karickoff, 1990; Chen et al., 1996).

Materials and Equipment:

1. Columns made from stainless steel, glass, or PVC tubings of known inner diameter (ID) ranging from 5 to 100 mm and length ranging from 100 to 300 mm.
2. A Mariott bottle setup to achieve a constant hydraulic head above column intake for steady-state flow.
3. A fraction collector, operating on a time- or volume-based mode.
4. A spectrophotometer for manual or automated P analysis.

Reagents:

1. A P-free nutrient solution. Deionized water or a 0.01M CaCl2 solution. Dissolve 1.47 g of CaCl2. 2 H2O in deionized water and dilute to 1 L mark.
2. A solution of known Br- concentration (10 mg Br/L). Dissolve .0149 g of KBr per L.
3. A solution of known P concentration (10 mg P/L). Dissolve .056 g of K2HPO4 per L.
4. A microbial growth inhibitor, such as acetone or chloroform (20 g/L of influent).

Procedures:

Soil/Sediment columns

Either obtain intact soil cores or pack a column with uniformly mixed soil/sediment materials at overall density of 1.2-1.3 Mg m^{-3}. The lower end of the column should be fitted with a fritted glass porous plate and a drainage port. To minimize mixing at the soil-porous plate interface, keep the pore size in the end-plate assembly as small as possible.

P sorption

Deliver from a Mariott bottle setup to achieve a constant hydraulic head above the column intake for steady-state flow. Collect effluent with a fraction collector. Acidify effluent fraction and analyze for P concentrations.

P desorption

Upon achieving a steady-state outflow P concentration, substitute a 0.01*M* $CaCl_2$ solution, or P-free nutrient solution as the influent to study P desorption from the soil/sediment column. It is important to be able to switch rapidly from one solution to the other and minimize mixing of the two solutions at the influent assembly. Collect fractions of the effluent as previously, and analyze for P concentrations.

Analysis of P in effluent

Filter effluent through a 0.45-µm membrane to remove any particulate matter, and acidify with HCl (<pH 2). Determine phosphorus concentrations of effluent samples using spectrophotometric (Alpkem, 1994), inductively-coupled plasma atomic-emission spectroscopic (Soltanpour et al., 1979), or ion chromatographic (Nieto and Frankenberger, 1985) methods.

Calculations:

Plot P concentrations against either time or volume of effluent to obtain an effluent or breakthrough curve (BTC). The analysis of BTCs is greatly facilitate by expressing P concentrations as relative or reduced concentration (C/C_o) and the effluent volume as dimensionless pore volumes. Calculate the number of pore volume (V/V_o) by dividing the amount of effluent by the liquid capacity of the column (V_o). The latter can be calculated either as

(i) $V_o = AL\,q$

where:
A = column cross-section area,
L = length, and
q = volumetric water content,

or

(ii) from the difference in the initial dry weight of the column and the weight of the saturated column at the end of the experiment.

The retardation of P, R_{phos}, relative to the movement of the water front is the measure of interaction between soil and P. In simplest terms, the value of V/V_o at $C/C_o = 0.5$ is an approximation of R_{phos}.

As pore geometry is unique for each soil column, a BTC for a non-reacting water tracer is also obtained, providing a reference R and a measure of pore water velocity. A potassium bromide (KBr) influent solution is used to obtain a Br^- breakthrough curve. The ratio of R_{phos} to R_{br} will yield the retardation factor for P. As needed, the sorption coefficient is determined from the following relationship between R and K when sorption is linearly related to solute solution-phase concentrations (e.g. at low solute concentrations),

$$R = 1 + \left(\frac{r}{q}\right)K$$

where:
r = soil bulk density, and
q = volumetric water content.

Comments:

The breakthrough of Br^- is also determined in the effluent using potentiometric (Frankenberger et al., 1996) or ion-chromatographic method (Dao, 1991; Tabatabai and Frankenberger, 1996). Organic water tracers such as fluoro-benzoates have also been used in many water movement studies (Bowman, 1984). Multiple tracers can be used simultaneously, and relatively lower concentrations of tracers are needed as lower detection and quantification limits are attainable with liquid-chromatographic techniques.

Graphical curve-fitting methods and numerical least-squares procedures are available to conveniently obtain estimates of retardation factor and dispersion coefficient for constant concentration and pulse-type effluent curves (van Genuchten, 1980; Parker and van Genuchten, 1984). Calculated effluent curves are based on an equation that approximates closely the analytical solutions of the advective-dispersive transport equation (Danckwerts, 1953),

$$C/C_0 = \frac{1}{2} erfc\left[\frac{Rx - \upsilon t}{2(DRt)^{1/2}}\right]$$

that, when x = L (column length) reduces to

$$C/C_0 = \frac{1}{2} erfc\left[\left(\frac{P}{4R\left(\frac{V}{V_0}\right)}\right)^{1/2} * \left(R - \frac{V}{V_0}\right)\right]$$

where

the Peclet number, $P = \upsilon L/D$,
R = retardation factor,
υ = pore water velocity, and
erfc = the error function complement.

The sum of squares of the residuals between observed and calculated effluent relative concentrations are minimized with iterative optimization of R and the Peclet number (or indirectly the dispersion coefficient D).

Constant-volume solvent delivery pumps can be used for the metering of the influent solutions. Maintaining constant flow conditions is essential in displacement studies of extended duration. Programmable pumps can be used to study steady state or transient flow regimes. Transport studies under unsaturated conditions are performed by the inclusion of a vacuum chamber surrounding the column bottom and the fraction collector.

References:

Alpkem. 1994. Ortho-phosphate in soil extracts. *In*: Flow Solution III Adv. Tech. Flow Anal., College Station, TX

Beauchemin, S., R.R. Simard, and D. Cluis. 1996. P sorption-desorption kinetics of soil under contrasting land uses. J. Environ. Qual. 25:1317-1325.

Bowman, R.S. 1984. Evaluation of some new tracers for soil water studies. Soil Sci. Soc. Am. J. 48:987-993.

Chen, J.S., R.S. Mansell, P. Nkedi-Kizza, and B.A. Burgoa. 1996. P transport during transient, unsaturated water flow in an acid sandy soil. Soil Sci. Soc. Am. J. 60:42-48.

Danckwerts, P.V. 1953. Continuous flow systems. Chem. Engineer. Sci. 2:1-13.

Dao, T.H., T. L. Lavy, and R. Sorensen. 1980. Atrazine degradation and residue distribution in soil. Soil Sci. Soc. Am. J. 43:1129-1134.

Dao, T.H. 1991 Field decay of wheat straw and its effects on metribuzin and S-ethyl metribuzin sorption and elution from crop residues. J. Environ. Qual. 20:203-208.

Eghball, B., G.D. Binford, and D.D. Baltensperger. 1996. P movement and adsorption in a soil receiving long-term manure and fertilizer application. J. Environ. Qual. 25:1339-1343.

Eghball, B., G.D. Binford, and D.D. Baltensperger. 1996. Phosphorus movement and adsorption in a soil receiving long-term manure and fertilizer application. J. Environ. Qual. 25:1339-1343

Frankenberger, Jr., W.T., M.A. Tabatabai, D.C. Adriano, and H.E. Doner. 1996. Bromine, chlorine, and fluorine. p. 833-867. *In*: Sparks, D.L. (ed). Methods of soil analysis. Part 3. Chemical methods. Soil Sci. Soc. Am. Book Series No. 5. Soil Sci. Soc. Am., Madison, WI.

Gachter, R., J.M. Ngatiah, and C. Stamm. 1998. Transport of phosphate from soil to surface waters by preferential flow. Environ. Sci. Tech. 32:1865-1869.

Graetz, D.A., and V.D. Nair. 2000. Phosphorus sorption isotherm determination. p. 13-16 *In*: Pierzynski, G.M. (ed) Methods of P analysis for soils, sediments, residuals, and waters. SERA-IEG 17 South. Cooperative Series Bulletin No. 396, p. 35-38.

Green, R.E., and J.C. Corey. 1971. Pesticide adsorption measurement by flow equilibration and subsequent displacement. Soil Sci. Soc. Am. Proc. 35:561-565.

Green, R.E., and S.W. Karickhoff. 1990. Sorption estimates for modeling. p. 79-101. *In*: Cheng, H.H. (ed) Pesticides in the soil environment: Processes, impacts, and modeling. Soil Sci. Soc. Am. Book Series No. 2. Soil Sci. Soc. Am., Madison, WI.

Heckrath, G., P.C. Brooks, P.R. Poulton, K.W.T. Goulding. 1995. Phosphorus leaching from soils containing different P concentrations in the Broadbalk Experiment. J. Environ. Qual. 24:904-910.

Miller, D.M., M.E. Sumner, and W.P. Miller. 1989. A comparison of batch and flow-generated anion adsorption isotherms. Soil Sci. Soc. Am. J. 53:373-380.

Nieto, K.F., and W.T. Frankenberger, Jr. 1985. Single ion chromatography: I. Analysis of inorganic anions in soils. Soil Sci. Soc. Am. J. 49:587-592.

Parker, J.C., and M. Th. Van Genuchten. 1984. Determining transport parameters from laboratory and field tracer experiments. VA Agric. Exp. Stn. Bull. 84.

Rao, P.S.C., J.M. Davidson, R.E. Jessup, and H.M. Selim. 1979. Evaluation of conceptual models for describing kinetics of adsorption-desorption of pesticides during steady-state flow in soils. Soil Sci. Soc. Am. J. 43:22-28.

Soltanpour, P.N., S.M. Workman, and A.P. Schwab. 1979. Use of inductively coupled plasma spectrometry for the simultaneous determination of macro- and micronutrients in NH_4HCO_3-DPTA extracts of soils. Soil Sci. Soc. Am. J. 43:75-78.

Sparks, D.L., S.C. Fendorf, C.V. Toner IV, and T.H. Carski. 1996. Kinetic methods and measurements. p.1275-1307. In: Sparks, D.L. (ed). Methods of soil analysis. Part 3. Chemical methods. Soil Sci. Soc. Am. Book Series No. 5. Soil Sci. Soc. Am., Madison, WI.

Tabatabai, M.A., and W.T. Frankenberger, Jr. 1996. Liquid chromatography. p. 225-245. *In*: Sparks, D.L. (ed). Methods of soil analysis. Part 3. Chemical methods. Soil Sci. Soc. Am. Book Series No. 5. Soil Sci. Soc. Am., Madison, WI.

van Genuchten, M. Th. 1980. Determining transport parameters from solute displacement experiments. Res. Rep. 118 US Salinity Lab., Riverside, CA.

van Riemsdijk, V.H., and A.M.A. van der Linden, 1984. Phosphate sorption in soils: II. Sorption measurement technique. Soil Sci. Soc. Am. J. 48:541-544.

Wagenet, R. J., and P.S.C. Rao. 1990. Modeling pesticide fate in soils. p. 351-399. *In*: Cheng, H.H. (ed) Pesticides in the soil environment: Processes, impacts, and modeling. Soil Sci. Soc. Am. Book Series No. 2. Soil Sci. Soc. Am., Madison, WI.

Residual Materials

Sampling Techniques for Nutrient Analysis of Animal Manures

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Introduction:

Nutrient concentrations vary in most wastes. A review of samples analyzed by the North Carolina Department of Agriculture and Consumer Services Agronomic Division showed the available nitrogen in animal waste varies greatly. For example, in swine lagoon liquids, nitrogen can range from 3 to 73 mg/L, in dairy slurry the range is 12 to 30,000 mg/L, and in a lagoon on a poultry operation with a liquid waste management system the range is 12 to 39,000 mg/L. This is a broad range of nutrient levels with the maximum and minimum values differing by more than a hundredfold. These numbers should send a clear message to users of animal waste: Average nutrient estimates may be suitable for the purposes of developing a waste management plan, but these averages are not adequate for calculating proper application rates.

Proper sampling is the key to reliable waste analysis. No analytical method, statistical calculation or laboratory quality control program can generate meaningful data from a poorly representative sample. If the waste product to be analyzed is entirely homogenous, then a single sample, no matter how small in weight or volume or where it is taken, would be completely representative of the product (Chai, 1996). But, since animal wastes are inherently heterogeneous, proper sampling techniques are critically important. Reliable samples typically consist of material collected from a number of locations around the lagoon or waste storage structure. The sampling methodology described herein has been adapted from a North Carolina Cooperative Extension Publication – Waste Analysis (Zublena and Campbell, 1993) developed to educate farmers on the proper techniques for waste sampling. The North Carolina Department of Environment and Natural Resources has adopted the procedures as guidance for sampling to meet monitoring conditions in the permits issued to confined animal feeding operations.

Obviously, sampling methods vary according to the type of waste. This publication will address liquid wastes and solids. The liquid waste section will address lagoon liquid (effluent) and slurries. The solid waste section will address waste products such as dairy dry stacks and poultry litter.

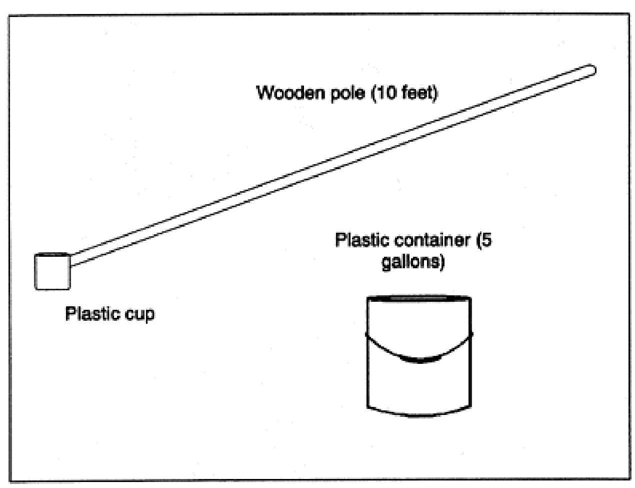

Figure 1. Liquid waste sampling device.

Liquid Wastes:

Lagoon Liquid

Premixing the surface liquid in the lagoon is not needed, provided it is the only waste component that is being pumped for land application.

Farms where multistage lagoon systems exist should have the samples collected from the lagoon they intend to pump for crop irrigation.

Samples should be collected using a clean, plastic container similar to the one shown in Figure 1. Galvanized containers should never be used for collection, mixing, or storage due to the risk of contamination from metals, such as Zn. A 500 mL sample of material should be taken from at least eight sites around the lagoon and then mixed in the larger clean, plastic container. Waste should be collected at least 2 m from the edge of the lagoon at a depth equivalent to that of the irrigation intake line in the lagoon, usually about 15 cm deep. Floating debris and scum should be avoided. A 500-mL subsample of the mixed material should be sent to the laboratory.

Liquid Slurry

Waste materials applied as a slurry from a pit or storage pond should be mixed prior to sampling. If mixing occurs prior to sampling, the liquid sampling device pictured in Figure 1 can be used. If a storage structure without agitation is sampled, use the composite sampling device as shown in Figure 2. Waste should be collected from approximately eight areas around the pit or pond and mixed thoroughly in a clean, plastic container. A 6-foot section of 1- to 2-inch plastic pipe can also be used: Extend the pipe into the pit; pull up the ball plug (or press your thumb over the end to form an air lock); remove the pipe from the waste; and release the air lock to deposit the waste in the plastic container.

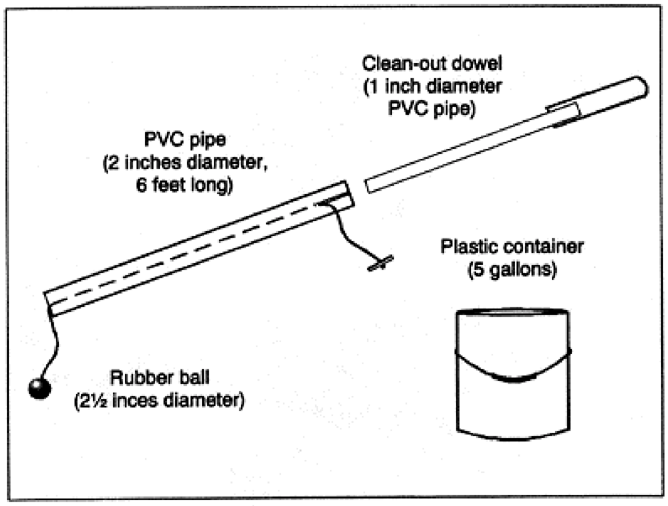

Figure 2. Composite sampling device.

Collect about a 500-mL subsample in a clean plastic container for transport to the laboratory for analysis. The sample should not be rinsed into the container, since doing so skews the measured nutrient analysis relative to the analysis of the actual collected sample. However, if water is typically added to the waste prior to land application to aid in agitation and pumping, a proportionate quantity of water should be added to the collected sample prior to analysis.

Whether sampling lagoon liquids or slurries, certain procedures are similar. All liquid waste samples collected and submitted for analysis should be placed in a sealed, clean, plastic container for storage and transport to the laboratory. Glass is not recommended due to potential damage to the container during transport. Samples should be tightly sealed as soon as possible. Some headspace should be left in the container to allow for some expansion of gases, lowering the potential for the container to rapidly erupt when opened in the laboratory. However, headspace should not exceed 2.5cm in order to minimize the potential for off-gassing of ammonia from solution. Samples that cannot be shipped on the day they are collected should be refrigerated. The most frequent changes in waste samples, be it solid liquid or sludge, are volatile losses, biodegradation, oxidation and reduction. Low temperatures reduce biodegradation and sometimes volatile losses, but freezing liquid samples can cause degassing (Bone, 1988). Anaerobic samples must not be exposed to air for significant periods of time.

Solid Wastes

Dry Stacks

Solid waste samples should represent the average moisture content of the waste. A 500 cm^3 sample is recommended. Samples should be taken from approximately eight different areas in the waste, placed in a clean, plastic container, and thoroughly mixed. Approximately 500 cm^3 of the mixed sample should be placed in a plastic bag, sealed, and analyzed as soon as possible. Samples stored for more than two days should be refrigerated. Figure 3 shows a device for sampling solid waste.

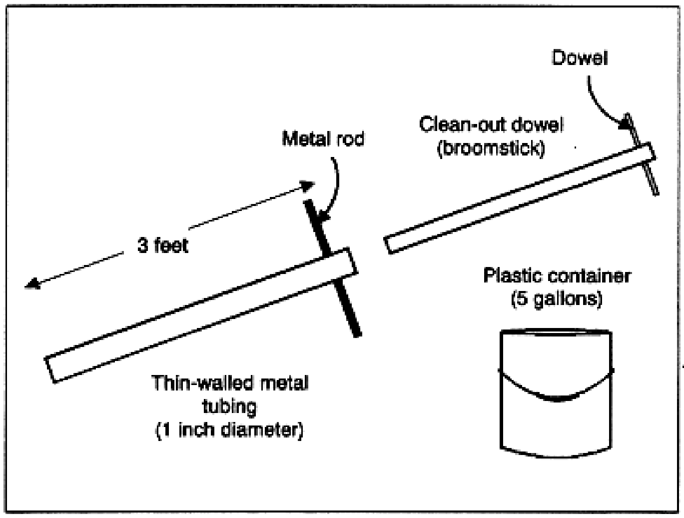

Figure 3. Solid-waste sampling device.

Poultry Litter

If collecting poultry litter from a stockpile or dry litter storage shed, follow the procedure for *Dry Stacks*. If sampling directly from the house, samples should be taken from approximately 20 to 30 different areas in the house. The samples should be placed in a clean, plastic container and thoroughly mixed. When sampling, be careful to get a representative sample. The number of samples taken from around the waterers, feeders, and brooders should be proportionate to the area occupied by each. Sample only to the depth the house will be cleaned, avoiding collecting soil from underneath the litter. Litter from broiler breeder houses should be sampled after the slats are removed and the manure and litter have been mixed. Approximately 500 cm^3 of the mixed sample should be placed in a plastic bag, sealed, and analyzed as soon as possible. Samples stored for more than two days should be refrigerated.

References:

Bone, L.T. 1988. Preservation techniques for samples of solids, sludges and non-aqueous liquids. p. 409 *In* L.H. Keith (ed.) Principals of environmental sampling. Am.. Chem.. Soc. Washington, D.C.

Chai, E.Y., 1996. A systematic approach to representative sampling in the environment. p. 33-44. *In* J.H. Morgan (ed) Sampling environmental media, ASTM STP 1282. Am. Soc. For Testing and Materials.

Zublena, J.P. and C.R. Campbell. 1993. SoilFacts: Waste analysis. North Carolina Cooperative Extension publication series. AG-439-33.

Determining Water-Soluble Phosphorus in Animal Manure

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Introduction:

There are no “standard methods” for many of the tests dealing with solid animal wastes. Therefore, the analyses of animal waste are usually modifications of “standard methods” for other substances (Overcash et al., 1975). The procedure for determining water-soluble P in animal manure is a modification of a method used to determine water-soluble P in soils (Olsen and Sommers, 1982). This method was originally developed for soils to examine the chemical composition of the soil solution that surrounds plant roots (Adams, 1974). The modification of this method presented here was utilized by Moore and Miller (1994).

Sampling:

The composition of animal manure varies greatly with location in the production facilities. To adequately describe the chemical and/or microbial composition of the manure, proper sampling techniques are needed. The following example explains how to obtain representative samples: (1) Divide the production facility to be sampled into three zones. If the buildings run in an east-west direction, then divide them into the northern third, middle third, and southern third. (2) Start in one zone and, while walking down the length of the building in a zigzag pattern, take about 10-15 samples, and place them in a plastic bucket. Note: For dryer materials (poultry litter), a soil probe works well. Due to the consistency of manure in dairy or swine facilities, a small shovel is more appropriate. (3) Make sure that if sampling inside a production facility, some of the sample (a representative portion) comes from under the feeders and waterers. The sample should be taken from the surface to just above the floor (until the resistance of the manure does not allow you to easily push the sampler in). (4) Mix the contents of the bucket well and pour about 100 g of the sample into a labeled freezer bag or plastic container. (5) Repeat this process in the other two zones of the facility.

Equipment:

1. Shaker (reciprocating or end-over-end)
2. Centrifuge
3. Centrifuge tubes (250 mL)
4. Filtration apparatus (0.45-µm pore diameter)
5. Spectrophotometer with infrared phototube for use at 880 nm
6. Acid-washed glassware and plastic bottles: graduated cylinders (5 mL to 100 mL), volumetric flasks (100 mL, 500 mL, and 1000 mL), storage bottles, pipets, dropper bottles, and test tubes or flasks for reading sample absorbance.

Reagents:

1. Concentrated hydrochloric acid (HCl)

2. Reagents used for ascorbic acid technique, Murphy-Riley (1962)

Procedure:

Weigh 20 g of fresh manure into a 250 mL centrifuge tube. Manure is not as homogeneous as soils. Therefore, a large sample is needed to get a good representation of the material. Add 200 mL of distilled water and shake for two hours. This ratio of 20 g manure to 200 mL distilled water leaves sufficient room in the centrifuge tube for proper shaking. Centrifuge at 6,000 rpm for 20 minutes. Filter the solution through a 0.45 μm membrane filter. Acidify to pH 2.0 with HCl to prevent precipitation of phosphate compounds (normally add about 5 drops of concentrated HCl per 20 mL). Freeze the sample if it is not going to be analyzed that day. Previous articles discussing the colorimetric determination of P have noted that hydrolysis of condensed phosphates can occur when the solution is acidified or in contact with acid for extended periods of time (Lee et al., 1965). Also, at this pH level, there is the possibility of flocculation of organics. However, it is necessary to make the sample solution as stable as possible, especially when there is a delay between the extraction process and actual analysis. It is vital to ensure that P remains in solution. Therefore, the negative effects of acid addition are often considered minimal.

In order to calculate the amount of soluble P per kilogram of dry manure, the water content of the manure should be measured. On the same day the manure is extracted, weigh out another subsample (approximately 10 g) into a pre-weighed metal container and dry in a forced draft oven at 60^0C for 48 hours.

Analysis:

For determining water-soluble P in animal manure, analyze the samples with a Technicon Auto-Analyzer (Technicon 1976) using the Murphy-Riley method (1962). Since this method does not quantify all the P in solution, it is referred to as "reactive" P. Anything that passes through a 0.45 μm filter is referred to as "soluble" P. Hence with this method you are determining soluble reactive P (SRP). This form of P is the most available for uptake by algae and higher plants.

Other methods of P analysis can be used. Any spectrophotometer with an infrared phototube for use at 880 nm can be used. Also, samples can be analyzed using an inductively coupled plasma-atomic emission spectrometry (ICP-AES) which will also measure dissolved P. Therefore filtered samples are used to determine total dissolved P (TDP) with ICP-AES.

Calculations:

It is preferred to report P concentrations on a dry weight basis (mg P/kg dry manure)

Manure P conc. (mg/kg) =
[P conc. in extract (mg/L)] x [Extractant volume (L) ÷ Mass of dry manure (kg)]

If presenting on an "as is" basis:

Manure P conc. (wet basis) (mg/kg) =
[P conc. in extract (mg/L)] x [Extractant volume (L) ÷ Mass of wet manure (kg)]

Comments:

It can be difficult to filter manure extracts (particularly swine and dairy manure). To improve the filter process first try increasing the centrifuge speed from 6,000 to 8,000 rpm or higher (be sure to note the maximum rpm your centrifuge tubes can withstand). Also, samples can be prefiltered though a glass fiber filter to prepare them for 0.45 μm membrane filtration. If filtering is still difficult, manure-to-water ratios can be increased (from 1:10 to 1:15 or 1:20).

References:

Adams, F. 1974. Soil solution. P. 441-481. In E.W. Carson (ed.) The plant root and its environment. University Press of Virgnia, Charlottesville.

Lee, G.F., N.L. Clesceri, and G.P. Fitzgerald. 1965. Studies on the analysis of phosphates in algal cultures. Internat. J. Air Water Poll. 9:715-722.

Moore, P.A., Jr., and D.M. Miller. 1994. Decreasing phosphorus solubility in poultry litter aluminum, calcium, and iron amendments. J. Environ. Qual. 23:325-330.

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

Olsen, S.R. and L.E. Sommers. 1982. Phosphorus. p. 403-430 In A.L. Page et al. (Ed.) Methods of soil analysis. Part 2. 2nd ed. Agronomy Monogr. 9. ASA and SSSA, Madison, WI.

Overcash, M.R., A.B., Hashimoto, D.L. Reddell, and D.L. Day. 1975. Evaluation of chemical analysis for animal wastes. *In* Standardizing Properties and Analytical Methods Related to Animal Waste Research. ASAE SP-0275, ASAE, St Joseph, MI, pp.335-55.

Technicon. 1976. Individual/simultaneous determination of nitrogen and/or phosphorus in BD digest. Industrial method no. 329-74W/A. Technicon Industrial Systems, Tarrytown, NY.

Total Phosphorous in Residual Materials

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Introduction:

A review of literature pertaining to the analysis of total P in residual materials shows the use of varied methods. Many methods employed are the same as those used in the determination of total P in soil, such as $NaHCO_3$ fusion and alkali oxidation as described by Olsen and Sommers (1982) and Dick and Tabatabai (1977), respectively (Wen et al., 1997; Harris et al., 1994). In this case, it is important that the selected method effectively oxidizes the organic matter of the residual material, since this component may contain P.

The methods discussed here are perchloric acid digestion, nitric acid-sulfuric acid digestion, and persulfate oxidation used in conjunction with colormetric methods for determination of total P as described by APHA (1989), and a rapid perchloric acid digestion for analysis of total P by ion chromatography developed by Adler (1995). These methods have been developed for the organic materials found in wastewater and other types of residual materials.

Perchloric Acid Digestion (APHA (1989)):

Reagents

1. Concentrated HNO_3
2. 70-72% $HClO_4$ reagent grade
3. 6 *M* NaOH
4. Methyl orange indicator solution
5. Phenolphthalein indicator aqueous solution

Procedure

Add a known volume of a well-mixed sample to a 125 mL Erlenmeyer flask, and acidify to a methyl orange endpoint (from orange to red) with concentrated HNO_3. Add 5 mL more of HNO_3. Evaporate solution to 15 to 20 mL on a steam bath or hot plate. Add 10 mL each of concentrated HNO_3 and $HClO_4$ to the flask. Be sure to cool the flask before each addition. After adding a few boiling chips, heat flask on a hot plate, and evaporate until dense, white fumes of $HClO_4$ appear. If the solution is not clear, cover the flask with a watch glass and keep solution barely boiling until it clears. If necessary, 10 mL more of concentrated HNO_3 can be added to aid oxidation. Cool the digested solution, and add 1 drop of phenolphthalein indicator solution. Then add 6 *M* NaOH until solution turns pink in color. If necessary, filter the neutralized solution to remove particulate material. Wash the filter liberally with distilled water. Bring the volume of the solution to 100 mL with distilled water.

To determine total P, use one of the colormetric methods discussed in the colormetric methods section of this chapter. Please note that choice of colormetric method depends on the concentration range of orthophosphate in the sample. The vanadomolybdophosphoric acid method can be used for samples that range between 1 to 20 mg P/L. The ascorbic acid method can be used for samples that range between 0.01 to 6 mg P/L.

Comments

The perchloric digestion method is recommended for samples that are difficult to digest.

Caution must be taken when mixing $HClO_4$ with organic materials. To avoid a violent reaction:

1. Do not add $HClO_4$ to a hot solution containing organic matter.
2. Begin digestion of sample containing organic material with HNO_3 first, then complete digestion with HNO_3 and $HClO_4$ mixture.
3. Only use a fume hood designed for $HClO_4$ use.
4. Do not allow solution to evaporate to dryness.

Nitric Acid and Sulfuric Acid Digestion (APHA (1989)):

Reagents

1. Concentrated H_2SO_4
2. Concentrated HNO_3
3. Phenolphthalein indicator aqueous solution
4. 1 *M* NaOH

Procedure

Add a known volume of a well-mixed sample to a micro-kjeldahl flask. Add 1 mL of concentrated H_2SO_4 and 5 mL of concentrated HNO_3. Digest the solution to a volume of 1 mL, and then continue digestion until solution becomes colorless to remove HNO_3. Cool solution, then add 20 mL of distilled water. Add 1 drop of phenolphthalein indicator solution and add 1 *M* NaOH to the solution until a faint pink color is reached. If necessary, filter neutralized solution to remove particulate material. Wash filter liberally with distilled water. Bring the volume of the solution to 100 mL with distilled water.

To determine total P use one of the colormetric methods discussed in the colormetric methods section of this chapter. Please note that choice of colormetric method depends on the concentration range of orthophosphate in sample. The vanadomolybdophosphoric acid method can be used for samples that range between 1 to 20 mg P/L. The ascorbic acid method can be used for samples that range between 0.01 to 6 mg P/L.

Comments

Nitric acid and sulfuric acid digestion is recommended for most samples.

Persulfate Oxidation Method (APHA (1989)):

Reagents

1. Sulfuric acid solution (H_2SO_4). Prepare by adding 300 mL of concentrated H_2SO_4 to 600 mL of distilled water. Dilute solution to 1 L with distilled water.
2. Ammonium persulfate ($(NH_4)_2S_2O_8$) solid or potassium persulfate ($K_2S_2O_8$) solid
3. 1 *M* (NaOH)
4. Phenolphthalein indicator aqueous solution

Procedure

Add 50 mL of a well-mixed sample (or any other suitable volume) to a flask. Add 1 drop of phenolphthalein indicator solution. If a red color develops, add H_2SO_4 solution dropwise until color disappears. Then add 1 mL of H_2SO_4 solution and either 0.4 g of $(NH_4)_2S_2O_8$ or 0.5 g of $K_2S_2O_8$. Boil the solution gently on a preheated hot plate for 30 to 40 min or until a final volume of 10 mL is reached. Those samples containing organophosphorous may take as much as 1.5 to 2 hr for complete digestion. Cool solution and dilute to 30 mL with distilled water. Add 1 drop of phenolphthalein indicator solution. Then add 1 *M* NaOH until solution turns a faint pink color. Heat the solution for 30 min in an autoclave or a pressure cooker at 98 to 137 kPa, then cool the solution. Add 1 drop of phenolphthalein indicator solution. Then add 1 *M* NaOH until solution turns a faint pink color. Bring the volume of the sample to 100 mL with distilled water. If a precipitate forms, do not filter. The precipitate will redissolve during the colormetric method used to determine total P. Mix solution well before further subdivision of the sample.

To determine total P use one of the colormetric methods discussed in the colormetric methods section of this chapter. Please note that choice of colormetric method depends on the concentration range of orthophosphate in sample. The vanadomolybdophosphoric acid method can be used for samples that range between 1 to 20 mg P/L. The ascorbic acid method can be used for samples that range between 0.01 to 6 mg P/L.

Comments

Though the persulfate digestion method is a simple method, it may be prudent to check this method against one of the other methods described in this chapter.

COLORIMETRIC METHODS

Vanadomolybdophosphoric Acid Method:

Reagents

1. Phenolphthalein indicator aqueous solution
2. 6 *M* HC1 or similar strength solution of H_2SO_4 or HNO_3.
3. Activated carbon (Darco G60 or equivalent). Rinse with distilled water to remove fine particulate material.
4. Vanadate-molybdate reagent. Prepare solution A by dissolving 25 g ammonium molybdate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$) in 300 mL of distilled water. Prepare solution B by dissolving 2.5 g of ammonium metavanadate (NH_4VO_3) by heating to boil in 300 mL of distilled water. Cool solution and then add 330 mL conc. HCl. Cool solution B to room temperature. Pour solution A into solution B, mix, and then dilute to 1 L with distilled water.
5. Standard P solution. Prepare by dissolving 219.5 mg of anhydrous KH_2PO_4 in distilled water. Dilute solution to 1 L with distilled water. (1.00 mL = 50.00 μg PO_4-P).

Procedure

If sample pH is greater than 10, add 1 drop of phenolphthalein indicator solution to 50 mL sample and add 6 *M* HC1 drop until the indicator changes color. Dilute sample to 100 mL. To remove excess color, shake sample with 200 mg of activated carbon for 5 min in an Erlenmeyer flask. Place 35 mL or less of sample in a 50 mL volumetric flask. Add 10 mL of vanadate-molybdate solution to the flask and dilute the contents to 50 mL with distilled water. To prepare a blank, add 35 mL of distilled water to a 50 mL volumetric flask in place of sample. Prepare a standard curve by using suitable volumes of standard solution in place of sample. Add standard solution to a 50 mL volumetric flask. Add 10 mL of vanadate-molybdate solution to the flask and dilute the contents to 50 mL with distilled water. After 10 min or more read absorbance of sample against blank. For solutions with 1-5 mg P/L, 2-10 mg P/L, or 4-18 mg P/L measure absorbence at 400, 420, or 470 nm, respectively.

Calculations

To calculate mg P/L:

mg P/L = [mg P (in 50 mL final volume) x 1000] ÷[sample volume (mL)]

Comments

Check activated carbon for P. Phosphorus in the activated carbon can result in high reagent blanks.

Use acid-washed glassware for determining low concentrations of P. Wash glassware with a P-free detergent, then clean all glassware with hot, diluted HCl and rinse well with distilled water. For a P range of 1.0 to 5.0 mg P/L use a filter wavelength of 400 nm for the spectrophotometer. For a range of 2.0 to 10 mg P/L use a filter wavelength of 420 nm for the spectrophotometer. For a P range of 4.0 to 18 mg P/L use a filter wavelength of 470 nm for the spectrophotometer.

Ascorbic Acid Method:

Reagents

1. 2.5 *M* sulfuric acid (H_2SO_4). Prepare by diluting 5 mL of concentrated H_2SO_4 into 500 mL of distilled water.
2. Potassium antimonyl tartrate solution ($K(SbO)C_4H_4O_6 \cdot 1/2H_2O$). Prepare by dissolving 1.3715 g $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$ in 400 mL of distilled water in a 500 mL volumetric flask, and dilute to volume with distilled water. Store reagent in a glass-stoppered bottle.
3. Ammonium molybdate solution ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$). Prepare by dissolving 20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 mL of distilled water. Store reagent in a glass-stoppered bottle.
4. 0.01*M* ascorbic acid. Prepare by dissolving 1.76 g of ascorbic acid in 100 mL of distilled water. Reagent is stable for approximately 1 week at 4^oC.
5. Mixed reagent. Prepare by mixing 50 mL 5*N* H_2SO_4, 5 mL potassium antimonyl tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. Mix after addition of each reagent. Be sure that all reagents are at room temperature before mixing, and be sure to mix in the order given. If turbidity

forms during the combination of reagents, shake and allow to stand until turbidity disappears before continuing.

6. Stock P solution. Prepare by dissolving 219.5 mg of anhydrous KH_2PO_4 in distilled water. Dilute solution to 1 L with distilled water. (1.00 mL = 50.00 μg PO_4-P).
7. Standard P solution. Prepare by diluting 50 mL of stock P solution to 1000 mL of distilled. (1.00 mL = 2.50 μg PO_4-P).

Procedures

Pipet 50 mL of sample into a clean, dry test tube or a 125 mL Erlenmeyer flask. Add 1 drop of phenolphthalein indicator solution, if a pink color develops add 2.5*M* H_2SO_4 dropwise to the solution. Add 8.0 mL of mixed reagent to the solution and mix thoroughly. Prepare a standard curve by using suitable volumes of standard solution in place of sample. Use a series of 6 standard solutions within the approximate range of 0.01 to 2.0 mg P/L. After 10 min and before 30 min measure absorbance at 880 nm. Use a reagent blank as a reference solution.

Calculations

To calculate mg P/L:

mg P/L = [mg P (in approximately 58 mL final volume) x 1000] ÷ [sample volume (mL)]

Comments

For a P range of 0.30 to 2.0 mg P/L use a light path of 0.5 cm for the spectrophotometer. For a range of 0.15 to 1.3 mg P/L use a light path of 1.0 cm for the spectrophotometer. For a range of 0.01 to 0.25 mg P/L use a light path of 5.0 cm for the spectrophotometer.

Rapid Perchloric Acid Digestion for Analysis by Ion Chromatography (Adler (1995)):

Reagents

1. 70 % HNO_3
2. 70-72% $HClO_4$ reagent grade
3. 30% H_2O_2 solution

Procedures

Add 200 mg (dry wt.) of the sample to a graduated 50 mL digestion (N.P.N.) tube. Add 1.0 mL of each HNO_3 and $HClO_4$ to the tube. Place tube into a 300°C preheated aluminum digestion block and digest at boiling point until the HNO_3 has boiled off (10 min). This is indicated by the subsidence of boiling. Then add 1.0 mL of H_2O_2 to the solution and continue digestion for another 20 min. Dilute the solution to 25 mL with double deionized water, vortex, and filter solution through a 0.2 mm Gelman ion chromatography acrodisc. The sample can then be further diluted for analysis by ion chromatography.

Dilution of sample for determining total P depends upon the column setup for ion chromatography. Adler (1995) found that a 1:10 dilution of the sample is suitable when both Dionex IonPac-AG4A and AS4A columns are used. A 1:50 dilution of the sample must be used when only an Dionex IonPac-AG4A column is used. The eluent for either column setup should be 1.80 mM Na_2CO_3 and 1.70 mM $NaHCO_3$ at a flow rate of 2.0 mL/min. The regenerant for the suppressor should be 12.5 mm H_2SO_4 at a flow rate of 3 mL/min. The sample loop volume should be 50 μL. Use standards containing equivalent concentrations of $HClO_4$ as digested samples to develop a 3 point standard curve.

Comments

Adler (1995) found that the addition of an IonPac-AG4A guard column aided in better separation of peaks of PO_4 and SO_4 in a $HClO_4$ matrix, and that all ions were eluted in less than 10 min. This also allows for up to 75% reduction in run time and the use of organic solvents can be avoided.

References:

Adler, P.R. 1995. Rapid perchloric acid digestion methods for analysis of phosphorus and sulfur in aquacultural waterwater and biosolids by ion chromatography. Commun. Soil Sci. Plant Anal. 26:85-90.

APHA. 1989. Standard Methods for the Examination of Water and Wastewater, 17th edition. American Public Health Association, Washington, D.C.

Dick, W.A., and M.A. Tabatabai. 1977. An alkaline oxidation method for determination of total phosphorus in soils. Soil Sci. Soc. Am. J. 41:511-514.

Harris, W.G., H.D. Wang, and K.R. Reddy. 1994. Dairy manure influence on soil and sediment compostion: implications for phosphorus retention. J. Environ. Qual. 23:1071-1081.

Olsen, S.R. and L.E. Sommers. 1982. Phosphorus. pp. 403-430. In: A.L. Page, R.H. Miller, and D.R. Keeney (eds.), Methods of Soil Analysis. 2nd ed. Agronomy Series No. 9, Part 2. SoilScience Society of America, Inc., Madison, WI.

Wen, G., T.E. Bates, R.P. Voroney, J.P. Winter, M.P. Schellenbert. 1997. Comparison of phosphorus availability with application of sewage sludge, sludge compost, and manure compost. Commun. Soil Sci. Plant Anal. 28:1481-1497.

Water

Sample Collection, Handling, Preparation and Storage

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Introduction:

Interfacing between the field, laboratory and chemical analysis is critical in determining the forms of phosphorus (P) present in soil-water samples. Collection, handling, preparation and storage procedures play a key role the operational definitions of P forms (Rowland and Haygarth, 1997) and the lack of a standard protocol can introduce serious bias into the precision and accuracy of the determination of the P forms (Haygarth et al., 1995). It is therefore essential to adhere to sensible protocols.

Nomenclature:

To understand the problems of collection, handling, preparation, and storage it is first necessary to consider the definitions and nomenclature of P forms which may be determined in a water sample: Collection, handling, preparation, and storage can directly affect the analytical endpoint. Forms of P in water attract differing and confusing nomenclature, and a systematic and logical means of classification is required. Some fraction of the total P content of any water has previously been classified by names which define the P in terms of filtration, and subsequently chemical (i.e., Murphy and Riley (1962) molybdenum (Mo) blue reaction) methodologies (Haygarth et al., 1998). Filtration is strictly a physically based definition of the carrier rather than P form, but has been used to define the difference between “soluble” or “dissolved” and “particulate” forms. However, any classification of nomenclature based on “dissolved,” “soluble,” or “particulate” is potentially flawed, because (a) different laboratories use different filter sizes and (b) P can be associated with a continuum of <0.45 µm sized particles/colloids, and samples vary widely in size distribution of particulate/colloidal material (De Haan et al., 1984; Haygarth et al., 1997).

There are similar problems with chemically based definitions. Traditionally the Murphy-Riley method has been the standard, but this has been subject to many modifications, and there are also uncertainties about what forms of P are determined. More recently, ion chromatography and inductively coupled plasma techniques have become more popular, but these determine different forms of P than the Murphy-Riley reaction. Users therefore need to be aware that P forms are very much methodology defined, and the problem is identifying exactly what P forms are determined by each method, and ultimately finding a system of nomenclature to incorporate these difficulties. Because of this, methodology definitions should be used in the nomenclature where possible.

Reactive P is defined as that which is readily determined analytically by the Mo blue reaction (Murphy and Riley, 1962). This is a very specific color reaction that determines orthophosphate, but the conditions prior to determining the blue color can change the composition of the sample. This means that Mo blue methodology is prone to overestimating P, in comparison to chromatographic determinations (Denison et al., 1998; Edwards and Withers, 1998), because the procedure may also determine loosely bound inorganic/organic forms of P, by either acid-enhanced hydrolysis (Tarapchak, 1993) or hydrous ferric oxide-orthophosphate. The reaction is also vulnerable to interferences with silica (Ciavatta et al., 1990). Conversely, a sample that requires digestion prior to analysis should be called unreactive P. Unreactive P will contain organic forms and some condensed forms of P, such as polyphosphates (Ron Vaz et al., 1993). Therefore any attempt to classify P as "orthophosphate," "organic," or "inorganic" in context with Murphy-Riley chemistry is technically incorrect, as it relies on the Mo-reaction. Methodology defined terms for describing the P chemistry with the Murphy-Riley reaction are therefore "reactive P"(RP), "unreactive P"(UP) and "total P"(TP) (i.e., reactive + unreactive, occurring after an appropriate method of digestion, or measured directly in an Inductively Coupled Plasma (ICP) system). Thus RP, UP or TP are the three prefixes of the suggested nomenclature.

Similarly, a systematic nomenclature for filtration is proposed, to be used as a suffix after chemical form, which removes ambiguity associated with terms like "soluble," "dissolved" or "particulate," all of which are non-exacting and subjective. Samples are defined specifically according to filter size, with a suffix denoting the pore size (in microns) of the filter used in parenthesis (e.g., <0.45 or >0.45). Therefore the established system of classifying dissolved reactive P (DRP) would be replaced by RP(<0.45). Where a sample was not subjected to filtration, the suffix (unf) is used. Figure 1 provides a visual summary of this nomenclature. Ultimately, researchers can expand and adapt this methodology-defined nomenclature to include other analytical methods such as ICP or ion chromatography.

Background:

An idealized and all-encompassing methodology for sample collection is impossible to prescribe because it depends on circumstances and samples. Sampling designs must be systematic, defensible and hypothesis-driven and therefore random and non-orthogonal sample collection programs are not advisable. Types of soil water samples may vary from (1) soil extracts determined in some type of laboratory batch procedure, (2) suction cup samples that draw water under tension and, (3) flowing or standing waters. Soil extractions are considered in other chapters, but it is necessary to be aware that storing and sieving soil samples has been found to have a marked affect on resulting extractable soil P characteristics (Chapman et al., 1997). Suction cup samplers present uncertainties because they draw water under tension, which may not be representative of "mobile" soil water. Users of these techniques need to be aware of these limitations. When sampling flowing waters from soils, there are three types of procedures: grab samples, flow proportional samples and continuous (regular) samples (Haygarth et al., 1998; Lennox et al., 1997). Flow proportional or continuous regular sampling provides a truer estimate if determining export coefficients is the aim, whereas grab samples can be used for comparative studies of spatial differences at one time.

Phosphorus is vulnerable to transformations during handling and storage, and there have been many publications suggesting recommended handling strategies (Annett and D'Itri, 1973; Bull et al., 1994; Gilmartin, 1967; Haygarth et al., 1995; Henriksen, 1969; Heron, 1962; Krawczyk, 1975; Mackereth et al., 1989). Changes can occur in the long term (Bull et al., 1994) and short term (Haygarth et al., 1995) and can be classified into two types: removal or transformation.

Removal (or "apparent" removal) occurs by sorption to vessel wall (Latterell et al., 1974) or precipitation. All forms of P can potentially suffer from removal by sorption/precipitation reactions (indirectly affected by pH, redox, DOC, Ca, Al and Fe content) with container walls. Colloid and particulate content of the water will also provide surfaces for sinks and sources of P. Storage vessel size and material are critical at regulating the extent of removal by sorption (Annett and D'Itri, 1973; Haygarth et al., 1995). Freezing of samples is known to reduce losses by sorption, but is not recommended because it causes transformations to occur (Johnson et al., 1975).

Transformation occurs by either chemical or biological mechanisms (Fitzgerald and Faust, 1967; Heron, 1962). The sensitivity to transformations in storage brought about by microbial mineralization/immobilization, hydrolysis and cell lysis generally increase with the complexity of analysis and fractionation performed. For example, analysis for total P will only be vulnerable to sorption/desorption interactions (see Figure 1) with vessel walls whereas filtered, Mo reactive and unreactive forms of P are also vulnerable to transformations and therefore may require a more stringent sample treatment. The presence/absence of chemical or biological preservative has been shown to affect transformations and Krawczyk (1975) demonstrated that $HgCl_2$ at an equivalent concentration of 400 mg/L suppressed microbial transformations, but has the disadvantage of suppressing Mo-blue color reaction in flow-injection systems (Haygarth et al., 1995). Other preservatives, such as chloroform, iodine and weak H_2SO_4 solutions have been described (Chakrabarti et al., 1978; Fishman et al., 1986; Murphy and Riley, 1959), but these techniques can (1) kill microbial populations – releasing reactive P, and (2) hydrolyse organic/polyphosphate P. Removal of light and reduction of temperature has been shown to have a direct effect on transformations (Haygarth et al., 1995). Freezing as a method of preventing transformation is not advisable because it ruptures cells and releases P from microorganisms into the soluble phase (Nelson and Romkens, 1972).

Sizes and types of filters affect the concentrations of P determined (Haygarth et al., 1997) and, although threshold sizes used vary from 0.2 to 0.5 µm, 0.45 µm cellulose-nitrate-acetate (CNA) filters are most common. Pressure of filtration affects the gas propensity for particle retention. The relationship between soluble and particulate P is not fixed, but depends on the subsequent storage time and conditions. Samples with a high particulate content may tend to block filters during filtration.

Recommended 'Best Practice':

Since the range and permutations of sample type, experimental conditions and requirements are very high, we are reluctant to recommend a stringent "best procedure." One of the key conclusions of Haygarth et al. (1995) was that the range of conditions and recommendations by researchers vary in response to different types of sample. For example, a suction cup sample from a chalk soil with a high Ca content may require a different set of storage conditions than a sample of drainage water from plots recently treated with cattle slurry: The former may be vulnerable to removal by Ca-P precipitation, while the sample influenced by slurry may be more vulnerable to microbial transformations. Further, since the kinetics of change during storage are extremely

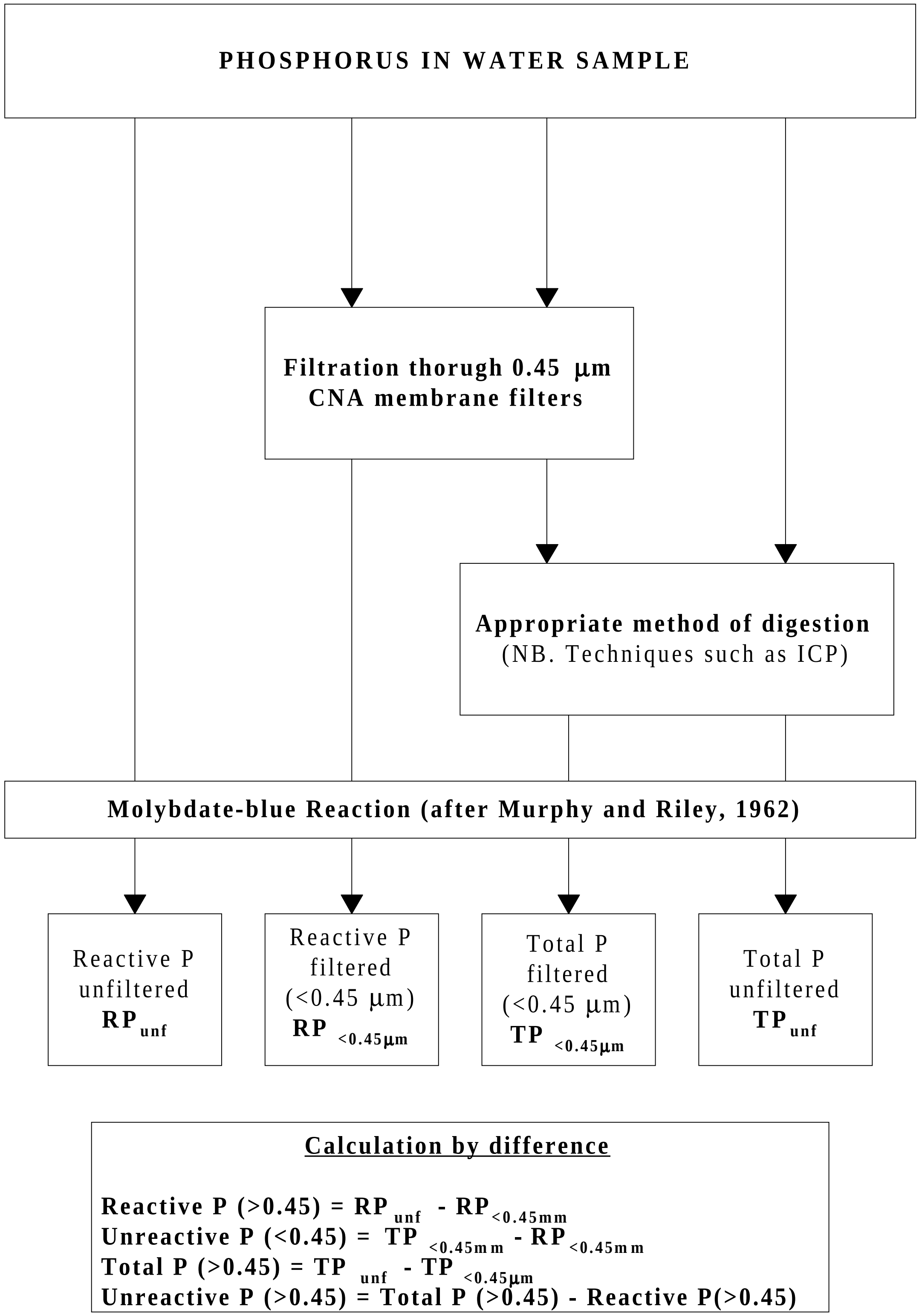

Figure 1. Operationally defined forms of P in water samples. CNA = cellulose-nitrate-acetate, ICP=inductively coupled plasma.

variable between water samples, uniform procedures may not help as a broad-brush recommendation for all samples. On this cautionary note, we therefore recommend a "best practice" rather than a "best procedure." Researchers must be aware of the potential hazards and be ready to adapt the procedures to suit their particular circumstances.

Equipment:

With field sampling, ceramic suction cups may have a tendency to sorb P, whereas PTFE samplers may present less of a problem. Storage vessels made with PTFE may minimize sorption, but the removal effects of using polyethylene are only slightly worse (Haygarth et al., 1995). Sample bottles should be as large as is practicable because this reduces the surface area to volume ratio, with volumes >100 mL most effective for minimizing changes. Researchers need to consider whether bottles should be washed (e.g., 10% v/v H_2SO_4 or in a P free detergent such as Decon) and if so, how often and the appropriate rinsing procedure. If bottles are to be used again perhaps it may be more appropriate to store them in clean water. Filtration usually should be through 0.45-µm CNA membranes, according to the water industry standards.

Reagents:

No chemical preservatives should be used, as these change microbial populations, which affect the forms of P determined. In extreme circumstances, with waters that are particularly vulnerable to transformations, researchers may wish to consider the relative advantage of using a 0.22-µm CNA membrane to sterilize by filtration.

Procedure:

1. When sampling, three bottle fills should be discarded and the fourth sample retained, in order to "condition" the bottle. This may be difficult to achieve with an autosampler.
2. Samples must be rapidly transferred to the laboratory and stored in a refrigerator at 4^oC.
3. The pressure of filtration should not ordinarily exceed 60 cm /Hg (80 kPa). All filtration should be undertaken within 12 h. Filters should be pre-washed with deionized water, conditioned with sample, and both these eluent solutions discarded.
4. For samples that are vulnerable to transformation (such as those for reactive/unreactive P), the total time between sampling and analytical determination should not be greater than 24 h. Researchers should be aware of the potential for transformations to occur when samplers store storm samples at remote sites.
5. For samples only vulnerable to removal (such as those for total P determination), the total time between sampling and analysis can be longer than 24 h, most ideally stored at 4^oC. It is recommended that if samples must be stored for TP, where digestions are needed, they should be readily pipetted into bottles for digestion prior to storage to minimize problems of sorption to bottle walls.

Comments:

The wide range of sample properties means that it is difficult to set a standard protocol and the above recommendations must be interpreted in this context. For example, there may be a need for setting different protocols for different extremes of particulate content or electrolyte concentration. The main principle behind sampling is minimal disturbance and rapid transfer to the analytical end point. There is a need to be aware of the varying methodological definitions of P, controlled by the analytical methods. Recognize that storage starts in the field – perhaps in the suction cup collection vessel or in an autosampler bottle, so this must be borne in mind in adopting a best practice. Quality control and quality assurance schemes, which use real samples, are to be encouraged and adopted.

References:

Annett, C.S., and F.M. D'Itri. 1973. Preservation of total soluble phosphorus. *In* "Proceedings 16th Conference Great Lake Research," pp. 214-220. International Association Great Lakes Research.

Bull, K. R., K.H. Lakhani, and A.P. Rowland. 1994. Effects of chemical preservative and temperature storage conditions on cations and anions in natural water. Chemistry and Ecology 9: 47-62.

Chakrabarti, C. L., K.S. Subramanian, J.E. Sueiras, and D.J. Young. 1978. Presevation of some anionic species in natural waters. J. Amer. Water Works Assoc. October 1978, 560-565.

Chapman, P.J., C.A. Shand, A.C. Edwards, and S. Smith. 1997. Effect of storage and seiving on the phosphorus composition of soil solution. Soil Sci. Soc. Am. J. 61:315-321.

Ciavatta, C., L.V. Antisari, and P. Sequi. 1990. Interference of soluble silica in the determination of orthophosphate-phosphorus. J. Environ. Qual. 19:761-764.

De Haan, H., T. De Boer, J. Voerman, A.J. Kramer, and J.R. Moed. 1984. Size classes of "dissolved" nutrients in shallow, alkaline, humic and eutrophic Tjeukemeer, The Netherlands, as fractionated by ultrafiltration. Verh. Int. Ver., Theor. Angew. Limnol. 22:876-881.

Denison, F.H., P.M. Haygarth, W.A. House, and A.W. Bristow. 1998. The measurement of dissolved phosphorus compounds: Evidence for hydrolysis during storage and implications for analytical definitions in environmental analysis. International Journal of Environmental Analytical Chemistry. 69:111-123.

Edwards, A.C., and P.J.A. Withers. 1998. Soil phosphorus management and water quality: A UK perspective. Soil Use Manage. 14:124-130.

Fishman, M.J., L.J. Schroder, and M.W. Shockey. 1986. Evaluation of methods for preservation of water samples for nutrient analysis. International Journal of Environmental Studies 26:231-238.

Fitzgerald, G.P., and S.L. Faust. 1967. Effect of water sample preservation methods on the release of phosphorus from algae. Limnol.and Oceanog. 12:332-334.

Gilmartin, M. 1967. Changes in inorganic phosphate concentration occurring during seawater sample storage. Limnol. and Oceanog. 12:325-328.

Haygarth, P.M., C.D. Ashby, and S.C. Jarvis. 1995. Short term changes in the molybdate reactive phosphorus of stored soil waters. J. Environ. Qual. 24:1133-1140.

Haygarth, P.M., L. Hepworth, and S.C. Jarvis. 1998. Forms of phosphorus transfer in hydrological pathways from soil under grazed grassland. European J. Soil Science 49:65-72.

Haygarth, P. M., M.S. Warwick, and W.A. House. 1997. Size distribution of colloidal molybdate reactive phosphorus in river waters and soil solution. Water Res. 31:439-442.

Henriksen, A. 1969. Preservation of water samples for phosphorus and nitrogen determination. Vatten 25:247-254.

Heron, J. 1962. Determination of phosphate in water after storage in polyethylene. Limnol. and Oceanog. 7:316-321.

Johnson, A.H., D.R. Bouldin, and G.W. Hergert. 1975. Some obsevations concerning preparation and storage of stream samples for dissolved inorganic phosphate analysis. Water Resourc. Res. 11:559-562.

Krawczyk, D.F. 1975. Preservation of wastewater effluent samples for forms of nitrogen and phosphorus. Philadelphia, 152-163. *In* “Water Quality Parameters,” pp. 152-163. American Society for Testing and Materials.

Latterell, J.J., D.R. Timmons, R.F. Holt, and E.M. Sherstad. 1974. Sorption of orthophosphate on the surface of water sample containers. Water Resourc. Res. 10:865-889.

Lennox, S.D., R.H. Foy, R.V. Smith, and C. Jordan. 1997. Estimating the contribution from agriculture to the phosphorus load in surface water. *In* “Phosphorus Loss from Soil to Water” (H. Tunney, O.T. Carton, P.C. Brookes and A.E. Johnston, eds.). CAB International, Oxford.

Mackereth, F.J.H., J. Heron, and J.F. Talling. 1989. “Water Analysis,” Titus Wilson and Sons Ltd., Kendal.

Murphy, J., and J.P. Riley. 1959. The storage of sea-water samples for the determination of dissolved inorganic phosphate. Anal. Chim. Acta 14:318-319.

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

Nelson, D.W., and M.J.M. Romkens. 1972. Suitability of freezing as a method of preserving runoff samples for analysis of soluble phosphate. J. Environ. Qual. 1:323-324.

Ron Vaz, M.D., A.C. Edwards, C.A. Shand, and M.S. Cresser. 1993. Phosphorus fractions in soil solution: Influence of soil acidity and fertilizer additions. Plant Soil 148:179-183.

Rowland, A.P., and P.M. Haygarth. 1997. Determination of total dissolved phosphorus in soil solutions. J. Environ. Qual. 26: 410-415.

Tarapchak, S.J. 1993. Soluble reactive phosphorus in lake water: Evidence for molybdate enhanced hydrolysis. J. Environ. Qual. 12:105-108.

Analyzing for Dissolved Reactive Phosphorus in Water Samples

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Introduction:

Dissolved reactive P (DRP), sometimes called soluble reactive P, refers to the P fraction that passes through a 0.45-µm-pore-diameter membrane filter and responds to the molybdate colorimetric test without preliminary hydrolysis or oxidative digestion of the water sample. It is largely a measure of dissolved orthophosphate, the form of P most readily available to aquatic plants, and thus is often considered the most critical P fraction contributing to accelerated eutrophication of surface waters. Although filtration through a 0.45-µm pore diameter membrane filter may not completely separate dissolved and suspended forms of P, this method can be easily replicated. Therefore, it provides a convenient technique for clearly defining the analytical separation of the dissolved and suspended P fractions.

Development of the molybdate colorimetric test for ortho-P in water samples was based on the observation that ammonium molybdate and potassium antimony tartrate react with dilute ortho-P solutions in an acid medium to form an antimony-phospho-molybdate complex. Reduction of this complex by ascorbic acid gives it an intense blue color that is proportional to the ortho-P concentration. Early prototypes of this colorimetric technique have been used for more than 60 years to determine P concentrations. Ammon and Hinsberg (1936) reported using ascorbic acid to reduce phosphomolybdic acid to molybdenum blue as a method of analyzing for P and As. Greenfield and Kalber (1954) suggested using the technique for analysis of sea water. Murphy and Riley (1958) recommended altering the method to provide a single reagent for phosphate determination in sea water, but their initial modified technique required 24 h at room temperature or 30 min at 60^{o}C for full color development. The higher temperature or long time period required for color development raised concerns because either condition may allow hydrolysis of some organic P compounds to orthophosphate. Therefore, Murphy and Riley (1962) revised the method again when they found that adding antimony (as potassium antimonyl tartrate) to the reagent caused full color development in 10 min at room temperature. The basic procedure has changed little since 1962, but it has been modified for use on autoanalyzers.

For the procedure as described below, the minimum detectable P concentration is approximately 10 µg/L.

Equipment:

1. Filtration apparatus (0.45-µm pore diameter)
2. Photometer - Spectrophotometer with infrared phototube for use at 880 nm and providing a light path of at least 2.5 cm <u>or</u> a filter photometer with a red color filter and a light path of at least 0.5 cm. For light path lengths of 0.5, 1.0, and 5.0 cm, the P ranges are 0.3-2.0, 0.15-1.30, and 0.01-0.25 mg/L, respectively.

3. Acid-washed glassware and plastic bottles: graduated cylinders (5 mL to 100 mL measurements), volumetric flasks (100 mL, 500 mL, and 1000 mL), storage bottles (including dark glass-stoppered, and opaque plastic), pipets, eye droppers, and test tubes or flasks for reading sample absorbance

Reagents:

1. 2.5 *M* H_2SO_4. Slowly add 70 mL of concentrated H_2SO_4 to approximately 400 mL of distilled water in a 500 mL volumetric flask. After the solution has cooled, dilute to 500 mL with distilled water, mix, and transfer to a plastic bottle for storage.
2. Ammonium molybdate solution. Dissolve 20 g of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 mL of distilled water. Store in a plastic bottle at 4^oC.
3. Ascorbic acid, 0.1 *M*. Dissolve 1.76 g of ascorbic acid in 100 mL of distilled water. The solution is stable for about a week if stored in an opaque plastic bottle at 4^oC.
4. Potassium antimonyl tartrate solution. Using a 500 mL volumetric flask, dissolve 1.3715 g of $K(SbO)C_4H_4O_6 \cdot 1/2\ H_2O$ in approximately 400 mL of distilled water, and dilute to volume. Store in a dark, glass-stoppered bottle.
5. Combined reagent. When making the combined reagent, all reagents must be allowed to reach room temperature before they are mixed, and they must be mixed in the following order. To make 100 mL of the combined reagent:
   a. Transfer 50 mL of 2.5 *M* H_2SO_4 to a plastic bottle.
   b. Add 15 mL of ammonium molybdate solution to the bottle and mix.
   c. Add 30 mL of ascorbic acid solution to the bottle and mix.
   d. Add 5 mL of potassium antimonyl tartrate solution to the bottle and mix. If turbidity has formed in the combined reagent, shake and let stand for a few min until turbidity disappears before proceeding. Store in an opaque plastic bottle. The combined reagent is stable for less than 8 h, so it must be freshly prepared for each run.
6. Stock phosphate solution. Using a 1000 mL volumetric flask, dissolve 219.5 mg anhydrous KH_2PO_4 in distilled water and dilute to 1000 mL volume; 1 mL contains 50 µg of P.
7. Standard P solutions. Prepare a series of at least six standard P solutions within the desired P range by diluting stock phosphate solution with distilled water.
8. Phenolphthalein indicator solution.

Procedure:

1. Filter sample through a membrane filter (0.45-µm pore diameter). Hard-to-filter samples can be prefiltered through a glass fiber filter to prepare them for membrane filtration.
2. Pipet 50.0 mL of sample into a clean, dry test tube or flask. Add 1 drop (0.05 mL) of phenolphthalein indicator and mix. If a red color develops, add just enough drops of 2.5 *M* H_2SO_4 to remove the color. Add 8.0 mL of combined reagent and mix thoroughly. Wait at least 10 min (but no more than 30 min) before measuring

the absorbance of each sample at 880 nm, using reagent blank as the reference solution.

3. Natural color of water should not interfere at the high wavelength used in this procedure. However, if the water samples are turbid or strongly colored, prepare a blank by adding all reagents except potassium antimonyl tartrate and ascorbic acid to a water sample. To obtain the actual absorbance of each sample, subtract absorbance of the blank from the sample's measured absorbance.
4. Prepare a calibration curve from the series of at least six standard P solutions within the desired P range. Use a distilled water blank with the combined reagent when making the photometric readings for a calibration curve, and plot absorbance vs. P concentration to obtain a straight line passing through the origin. Each set of samples should include at least one P standard to assure accuracy of the results.

Comments:

Arsenate concentrations as low as 0.1 mg/L can interfere with the P determination by reacting with the molybdate reagent to produce a blue color. Hexavalent chromium and NO_2^- at 1 mg/L can interfere to give results about 3% low, and at 10 mg/L give results 10-15% low.

If an autoanalyzer is being used for this procedure, the following adjustment in reagent preparation is recommended: When making potassium antimonyl tartrate solution, 1.5 g of $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$ should be dissolved in distilled water to make 500 mL of solution.

References:

Ammon, R., and K. Hinsberg. 1936. Colorimetric determination of phosphorus and arsenic with ascorbic acid. Hoppe-Seyl. Z. 239:207.

Greenfield, L.J., and F.A. Kalber. 1954. Inorganic phosphate measurement in sea water. Bull. Marine Sci. Gulf Caribbean 4:323-335.

Murphy, J., and J.P. Riley. 1958. A single-solution method for the determination of soluble phosphate in natural waters. J. Marine Biol. Assoc. UK 37: 9.

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

Analyzing for Total Phosphorus and Total Dissolved Phosphorus in Water Samples

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Introduction:

Dissolved orthophosphate is the form of P most readily available to aquatic plants, but numerous studies have shown that other forms of P can be hydrolyzed to the orthophosphate form in wastewater-treatment facilities and in natural waters. Therefore, when assessing the long-term potential for accelerated eutrophication of surface water due to P loading, many researchers and watershed managers want to know the total P concentration (regardless of P form) in water samples.

Polyphosphates and phosphates bound to organic substances do not react with the molybdate reagent used for colorimetric P analysis. Therefore, analysis for total P content of water samples requires that all condensed and organic P compounds, including particulate P, first be converted (hydrolyzed) to orthophosphate so they can be determined colorimetrically. This is accomplished by digesting the sample in strong acid at high temperature to oxidize the organic matter and release P as orthophosphate. Published methods for accomplishing the digestion process have been available for many decades. Improved methods have been developed, but all of them use heat and/or various strong acids, sometimes in combination with strong oxidizing reagents. For example, the wet ashing digestion method (using concentrated HNO_3 and H_2SO_4) described by Peters and Van Slyke (1932) was considered reliable, but was very time-consuming, so other researchers developed faster digestion procedures. Perchloric acid digestion, described by Robinson (1941), is still considered a standard method for total P analysis, but it is time-consuming and dangerous because heated mixtures of $HClO_4$ and organic matter may explode violently. Therefore, other digestion methods (listed below) are usually preferred.

To determine the total dissolved P fraction, the particulate P is separated by filtering the water sample through a 0.45 µm pore diameter membrane filter before beginning the digestion procedure. To determine total P (dissolved + particulate), an unfiltered sample is shaken (to suspend the particulate matter) just before measuring the subsample for digestion.

Sulfuric Acid - Nitric Acid Digestion Method:

Equipment

1. Digestion rack. Digestion racks designed for micro-Kjeldahl digestions can be used, but need to include a provision for withdrawal of fumes. A digestion rack heated by either gas or electricity is suitable.
2. Micro-Kjeldahl flasks.
3. Acid-washed graduated cylinders, pipets, eye droppers, and 100 mL volumetric flasks.
4. Any additional equipment required for colorimetric determination of P in the digested sample solution (described in the Dissolved Reactive P section).

Reagents

1. Concentrated H_2SO_4
2. Concentrated HNO_3
3. Phenolphthalein indicator aqueous solution
4. 1 *M* NaOH
5. Any additional reagents required for colorimetric determination of P in the digested sample solution (described in the Dissolved Reactive P section)

Procedure

1. Transfer a measured volume of sample into a micro-Kjeldahl flask. We recommend a volume of at least 25 mL if adequate sample is available. Larger volumes can be used, but they require a longer digestion time.
2. Add 1 mL of concentrated H_2SO_4
3. Add 5 mL of concentrated HNO_3
4. Digest to a volume of 1 mL and then continue digesting until the solution becomes colorless (to remove the HNO_3)
5. Cool the flask and add approximately 20 mL of distilled water.
6. Add 1 drop (0.05 mL) of phenolphthalein indicator and mix.
7. Add drops of 1 *M* NaOH until the sample solution acquires a faint pink tinge.
8. Transfer the neutralized solution (if necessary, filtering to remove turbidity or particles) into a 100-mL volumetric flask. If a filter is used, be sure to add distilled-water filter washings to the flask.
9. Adjust sample volume to 100 mL with distilled water.
10. Use the molybdate colorimetric test (described in previous chapter on Dissolved Reactive P) to determine the P content of the digested solution.
11. To prepare the calibration curve, carry a series of standards through the digestion process. **Do not use standards that have not been digested.**

Persulfate Digestion Method:

Equipment

1. Hot plate with adequate heating surface. An autoclave or pressure cooker capable of developing 98 - 137 kPa may be used instead of a hot plate.
2. Acid-washed graduated cylinders, pipets, eye droppers, and volumetric flasks (100 mL and 1000 mL).
3. Any additional equipment required for colorimetric determination of P in the digested sample solution (described in the Dissolved Reactive P section).

Reagents

1. Phenolphthalein indicator solution
2. Sulfuric acid solution. Transfer approximately 600 mL of distilled water to a 1000 mL volumetric flask. Slowly (and carefully) add 300 mL of concentrated H_2SO_4. After the solution has cooled, dilute to 1000 mL with distilled water and mix.
3. Ammonium persulfate, $(NH_4)_2S_2O_8$ solid or potassium persulfate, $K_2S_2O_8$ solid.
4. 1M NaOH
5. Any additional reagents required for colorimetric determination of P in the digested sample solution (described in the Dissolved Reactive P section)

Procedure

1. Thoroughly mix the sample, and measure a suitable portion (50 mL is recommended) into a flask.
2. Add 1 drop (0.05 mL) of phenolphthalein indicator and mix. If a red color develops, add just enough drops of H_2SO_4 to remove the color.
3. Add 1 mL of H_2SO_4 solution.
4. Add either 0.4 g of solid $(NH_4)_2S_2O_8$ or 0.5 g of solid $K_2S_2O_8$ and mix.
5. Boil the sample solution gently on the preheated hot plate for at least 30-40 min or until the volume is reduced to 10 mL. Some organophosphorus compounds may require 2 h for complete digestion.
6. Cool the solution, and dilute to approximately 30 mL with distilled water.
7. Add 1 drop (0.05 mL) of phenolphthalein indicator.
8. Add drops of 1M NaOH until the sample solution is neutralized (acquires a faint pink tinge).
9. Dilute to 100 mL volume with distilled water. If a precipitate forms, **do not filter**, but shake well for any subdividing of the sample. The precipitate redissolves during the colorimetric test due to increased acidity.
10. Use the molybdate colorimetric test (described in previous chapter on Dissolved Reactive P) to determine the P content of the digested solution.
11. To prepare the calibration curve, carry a series of standards through the digestion process. **Do not use standards that have not been digested.**

Kjeldahl Digestion Method:

The Kjeldahl digestion procedure also converts condensed and organic P compounds, including particulate P, to orthophosphate. Therefore, if the water samples are being digested by the Kjeldahl method to determine their total Kjeldahl nitrogen content, then total P can also be measured (without further digestion) by simply using the molybdate colorimetric test (described in the previous chapter on Dissolved Reactive P) to determine the P content of the digested solution. To prepare the calibration curve, carry a series of standards through the Kjeldahl digestion process.

Calculations:

For any of the three digestion methods listed above, always use the correct dilution ratio when calculating the total P concentration in the original sample. For example, if a 50-mL sample is used, and the sample is diluted to a final volume of 100 mL following the digestion procedure, then the measured concentration should be multiplied by 2 to obtain the concentration in the original water sample.

$$\text{Total P (mg/L)} = \text{P concentration in analyzed solution (mg/L)} \times \frac{\text{TotalDilutedVolume(mL)}}{\text{OriginalSampleVolume(mL)}}$$

Comments:

The sulfuric acid - nitric acid digestion method is recommended for most samples. The persulfate digestion method is much simpler to use and usually gives excellent recovery rates, but when digesting potentially difficult samples, it should probably be checked against the sulfuric acid - nitric acid digestion and adopted if identical recoveries are obtained.

References:

Peters, J.P., and D.D. Van Slyke. 1932. Quantitative Clinical Chemistry. Vol II. Methods. The Williams and Wilkins Co., Baltimore, MD.

Robinson, R.J. 1941. Perchloric acid oxidation of organic phosphorus in lake waters. Ind. Eng. Chem. Anal. (Ed.)13:465-466.

Using the Iron Oxide Method to Estimate Bioavailable Phosphorus in Runoff

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Introduction:

The use of iron-oxide (FeO) coated paper to test soil was first reported by Sissingh (1983), who wanted to develop a soil phosphorus (P) test that would estimate plant-available P in tropical soils without mobilizing other forms of phosphates. A strip of filter paper impregnated with iron hydroxide functioned as a P sink and adsorbed mobile P from solution, so Sissingh (1983) called the analyzed P, the *Pi value* (*i* referring to *i*ron hydroxide). Interest in the method was soon extended to a wider range of soils (Menon et al., 1989). The test has an advantage over standard soil P tests because the FeO paper functions as an ion sink and doesn't react with soil as do chemical extractants. A unique feature of the FeO method rests in its inherent preferential selectivity of FeO for P ions over all other anions found in soil, except OH (Menon, 1993; van der Zee et al., 1987).

The FeO test has been identified by quite a number of different terms in various papers and publications, e.g., *Pi test, Fe-oxide strip method, and* P_i *test* (Chardon et al., 1997; Perrot and Wise, 1993; Sharpley, 1993a). To avoid confusion, P extraction by FeO-coated paper will be called the FeO method, and the P extracted will be called FeO-P.

Interest in applying the FeO method to agricultural runoff has been developing recently in an effort to assess the potential of P in runoff to stimulate freshwater eutrophication. The bioavailable P content (BAP) of dilute runoff sediment assessed by the FeO method was related (r^2 = 0.63-0.96) to the growth of P-starved algae (*Selanastrum capricornutum)* (Sharpley, 1993a). Additional work showed that FeO-P from runoff sediment was related (P > 0.001) to algal growth in *Anabaena, Ankistrodesmus,* and *Euglena* (Sharpley, 1993b). The FeO method has the unique capability of differentiating soluble inorganic P from FeO-P in sediment of runoff. The sediment FeO-P is called bioavailable particulate P (BPP) and is calculated according to

$$\text{BPP} = \text{total BAP} - \text{SP} \qquad [1]$$

where total BAP is total FeO-P from unfiltered runoff, and SP is soluble inorganic P in filtered runoff (0.45-µm filter).

The FeO method has a stronger theoretical justification for estimating P availability of soil and runoff for plants and algae than do chemical methods (Sharpley, 1993a). The rationale for this theoretical justification lies in the mechanism of P adsorption onto the FeO-coated paper. Such adsorption closely simulates that of plants and algae and thereby gives an estimation of BAP, whereas chemical methods may mobilize additional forms of P which are not available to plants or algae. Therefore, the FeO method is an additional tool used to assess the potential for runoff to increase fresh-water eutrophication.

In the past, filter paper with large pores up to 20 to 25 µm sometimes was used to make FeO paper, however, there is less tendency for soil particles to become lodged in papers with small pores, e.g. < 5.0 µm, so small-pore paper is now recommended

(Chardon et al., 1997). Traditionally, filter paper circles with a 15-cm diameter were coated with FeO by immersing them first in a $FeCl_3$ solution, then after drying, they were immersed in an NH_4OH solution (van der Zee, et al. 1987). After drying they were cut into strips, often 2 x 10 cm--from whence came the term *strip-P*.

Recently, filter circles with a 5.5 cm diameter have been used to make the FeO papers instead of cutting strips from the larger circles (Myers et al., 1995, 1997). The surface area of the 5.5-cm circles exceeds that of the traditional 2 x 10-cm strips by about 20%; however, the primary reason for using circles instead of strips is to eliminate the need for cutting strips. Within a 12 h shaking time, each 5.5-cm FeO circle has adequate adsorption capacity to remove 99% of the P in a solution containing 16.1 µm P (Myers et al., 1997). van der Zee et al. (1987) reported similar results with adsorption of 18 µmol P after shaking one 2 x 10-cm strip for 20 h.

Holding the FeO paper in a fixed orientation during shaking helps to prevent soil particles from lodging in the pores of the paper and contaminating it (Myers et al., 1995; 1997). Although runoff aliquots usually contain much less than 1.0 g of sediment, the amount of soil used in soil extraction, stabilization of each FeO paper between polyethylene screens is still recommended for analysis of runoff samples, some of which can contain substantial quantities of sediment. Holding the screens in a fixed orientation during shaking also prevents the FeO papers from sticking to the walls of the shaking vessel, as often occurs when the papers are allowed to shake freely in solution. Such sticking could reduce adsorption effectiveness of the FeO paper.

A solution of 0.01 *M* $CaCl_2$ is used as the shaking matrix for the FeO paper and soil because deionized water has the tendency to disperse soil, which may then lodge in the pores of the filter paper (Sissingh, 1983). This may lead to errors in P analysis (Myers et al., 1995); however, runoff has been extracted by the FeO method without addition of any $CaCl_2$ (Sharpley, 1993a). We have found that FeO-P from runoff made with 0.01 *M* $CaCl_2$ was the same as that from duplicate runoff samples shaken without $CaCl_2$ (data unpublished), but similar results may not always hold true for every type of runoff in every location. The potential for significant contamination of FeO papers by not amending the runoff with $CaCl_2$ during shaking may depend upon the clay content of the sediment and the P content of the clay as well as the amount of sediment in the runoff.

Equipment:

1. End-over-end shakers have been used for the FeO method (Sissingh, 1983; Sharpley, 1993). Reciprocating shakers have also been used (Menon et al., 1989; Myers et al., 1997).
2. 2 L beaker
3. 118-mL wide-mouthed glass bottles
4. 125-mL Erlenmeyer flasks
5. 50-mL Erlenmeyer flasks
6. Spectra/Mesh polyethylene screens (925 µm, Spectra/Mesh filters, Fisher Co., St. Louis; Fisher cat. no. 08-670-175)
7. Parafilm

Reagents:

1. 0.65 *M* $FeCl_3 \cdot 6H_2O$ + 0.6 *M* HCl
2. 2.7 *M* NH_4OH
3. 0.2 *M* H_2SO_4
4. Reagents used for the Murphy and Riley (1962) colorimetric procedure

Procedure:

We use hardened 5.5 cm circles of Whatman no. 50 filter paper for making the FeO paper (Myers et al., 1997). Briefly, we immerse the papers, one by one, in 0.65 *M* $FeCl_3 \cdot 6H_2O$ containing 50 mL of concentrated HCl per liter of solution, and leave them in the container overnight. Chardon et al. (1997) recommend acidification of the $FeCl_3$ solution if the papers are to be stored, thus we acidify with HCl. After air-drying the papers on a rack, they are immersed in 2.7 *M*NH_4OH for 30 s and then allowed to drain for 15 s before thoroughly rinsing in two containers of clean distilled water. They are placed in a bucket of clean water for 1 h to permit dissipation of any residual ammonia. The papers are then ready to use immediately or they can be dried for later use. For further details on paper preparation, see Myers et al. (1997).

Polyethylene screens are cut approximately 9 cm in diameter from Spectra/Mesh filters. These screens are used to enclose each FeO paper during shaking (Myers et al., 1997). One FeO paper is placed between two of these screens held together by a plastic clamp, making a paper-screen assembly to insert into the shaking bottle.

We have followed the traditional FeO method for determining BAP in runoff (Sharpley, 1993a), except that we use a total shaking volume of 80 mL. We add 50 mL of runoff plus 30 mL of deionized water. When 80 mL of solution is shaken in 118-mL bottles orientated horizontally and end-to-end, the shaking action completely rinses the sides and top of the bottles with each excursion of the reciprocating shaker. If shaking action is adequate in some other type of shaking vessel, the total volume of solution is optional and discretionary. Also, for runoff with low levels of FeO-P, 80 mL of runoff may be used without adding any water.

The FeO paper-screen assembly is inserted, clamp end first, into the bottle containing runoff. Cover the bottles tightly with a layer of Parafilm, and then screw the closures on tightly to seal. The bottles are shaken on a reciprocating shaker for 16 h at a speed of 125 to 135 excursions/min. Shaking speed can be increased, if needed, to increase mixing.

After a 16 h shaking period, we remove the papers from the screens and rinse each paper under a stream of deionized water for a few seconds. The papers are coiled and placed in the neck of a 125-mL Erlenmeyer flask where they may either be left to dry or pushed to the bottom and extracted immediately. Extract the P from the papers by adding 50 mL of 0.2 *M*H_2SO_4 to flasks and shaking them 1 h at 100 to 125 excursion/min. An aliquot of the H_2SO_4 solution is analyzed for P using the Murphy and Riley (1962) after neutralization of acidity. For neutralization, phenolphthalein color indicator gives a clear end point in the FeO solution. Duplicate, or triplicate, control FeO papers, without any soil or runoff, are also shaken and extracted to correct for any P contained in reagents and water. For further details on the FeO procedure described above, see Myers et al. (1995, 1997).

Calculations:

The Murphy and Riley (1962) method of P analysis gives FeO-P in µg P/mL. If data for FeO-P are presented in units of µg/L then the appropriate calculations for BAP are:

Total BAP (µg/L) = [volume of H_2SO_4 (L) x P in H_2SO_4 (µg/L)] ÷ [volume of runoff sample extracted with FeO (L)]

where total BAP is the total bioavailable P in the runoff, and H_2SO_4 is 50 mL of 0.2 *M*H_2SO_4 used to extract P from each FeO paper. Calculations for bioavailable particulate P (BPP), the FeO-P associated with the sediment, are given in Eq. 1 above.

Comments:

Algae use only the orthophosphate form of P; however, organic forms of P can undergo mineralization and also become available (Correll, 1998). Thus, organic P can be considered a latent source of BAP. Some discussion has been focused on methods to limit hydrolysis of organic P adsorbed onto FeO paper (Robinson and Sharpley, 1994); however, it appears that such adsorption and hydrolysis of organic P is not a problem in using the FeO method to estimate BAP because organic P may be justifiably classified as latent BAP which may be mineralized at any time and thereby become immediately available for algal uptake.

References:

Chardon, W.J., R.G. Menon, and S.H. Chien. 1997. Iron oxide impregnated filter paper (P_i test): A review of its development and methodological research. Nutr. Cycl. Agroecosyst. 46:41-51.

Correll, D.L. 1998. The role of phosphorus in the eutrophication of receiving waters: A review. J. Environ. Qual. 27:261-266.

Menon, R.G. 1993. The P_i test for evaluating bioavailability of phosphorus. p. 58-67. *In* K.B. Hoddinott and T.A. O'Shay (ed.) Application of agricultural analysis in environmental studies. ASTM STP 1162, Philadelphia, PA.

Menon, R.G., L.L. Hammond, and H.A. Sissingh. 1989. Determination of plant-available phosphorus by the iron hydroxide-impregnated filter paper (P_i) soil test. Soil Sci. Soc. Am. J. 53:110-115.

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

Myers, R.G., G.M. Pierzynski, and S.J. Thien. 1995. Improving the iron oxide sink method for extracting soil phosphorus. Soil Sci. Soc. Am. J. 59:853-857.

Myers, R.G., G.M. Pierzynski, and S.J. Thien. 1997. Iron oxide sink method for extracting soil phosphorus: Paper preparation and use. Soil Sci. Soc. Am. J. 61:1400-1407.

Perrott, K.W., and R.G. Wise. 1993. An evaluation of some aspects of the iron oxide-impregnated filter paper (Pi) test for available soil phosphorus with New Zealand soils. N. Z. J. Agric. Res. 36:157-162.

Robinson, J.S., and A.N. Sharpley. 1994. Organic phosphorus effects on sink characteristics of iron-oxide-impregnated filter paper. Soil Sci. Soc. Am. J. 58:758-761.
Sharpley, A.N. 1993a. An innovative approach to estimate bioavailable phosphorus in agricultural runoff using iron oxide-impregnated paper. J. Environ. Qual. 22:597-601.
Sharpley, A.N. 1993b. Estimating phosphorus in agricultural runoff available to several algae using iron-oxide paper strips. J. Environ. Qual. 22:678-680.
Sissingh, H. A. 1983. Estimation of plant-available phosphates in tropical soils. A new analytical technique. Nota 235. Inst. for Soil Fertility Res., Haren, the Netherlands.
van der Zee, S.E.A.T.M., L.G.J. Fokkink, and W.H. van Riemsdijk. 1987. A new technique for assessment of reversibly adsorbed phosphate. Soil Sci. Soc. Am. J. 51:599-604.