A3769

Recommended Methods of Manure Analysis

Editor: John Peters — University of Wisconsin-Madison

Sherry Combs — University of Wisconsin-Madison
Bruce Hoskins — University of Maine
Jan Jarman — Minnesota Department of Agriculture
John Kovar — USDA-ARS
Maurice Watson — Ohio State University
Ann Wolf — Penn State University
Nancy Wolf — University of Arkansas

Contents

Recommended Methods of Manure Analysis

Introduction

The benefits of applying livestock manure to crops have been recognized for centuries. Nutrient composition of manure varies with a number of factors, including animal type, bedding, ration, storage/handling, environmental conditions, field application method, and age of manure. These factors certainly present sampling and analysis challenges. In addition, the chemical form and amount of each nutrient varies between fecal and urine fractions.

Nutrient values can be assigned by using estimated "book" or average available N, P_2O_5 and K_2O contents. However, testing manure may better indicate how animal management and other factors actually affect nutrient content. In fact, many state nutrient management programs now require manure testing as part of farm nutrient management.

Using good sampling technique is critical for having confidence in manure nutrient analysis results. Appropriate sample handling and laboratory methods are also important to ensure producers have confidence in test results. However, quantifying the nutrient value of applied manure remains a complex challenge. This information is needed to better manage manure as a nutrient asset.

Work on the development of this multi-regional publication began in 1996 following a joint meeting of regional soil testing workgroups in Raleigh, North Carolina. Earlier in that year, a sample exchange was conducted with NCR-13, SERA-6 and NEC-67 laboratories. Results from that sample exchange were presented at the Raleigh meetings and sparked interest in joining efforts to develop a manure testing manual, which could be used in all regions. This document is the result of the work of this multi-regional committee in developing a reference document for sampling and testing livestock manure.

—John Peters

Note: Reference to commercial products or manufacturers' names throughout this publication does not constitute an endorsement by the authors. When this type of information is listed, it is only done to give the reader an indication of the relative type of equipment, chemicals and supplies that are required.

The authors wish to thank the following individuals for their assistance in developing this manual:

Doug Beegle — Penn State University
Michelle Campbell — Minnesota Dept. of Agriculture
Jerry Floren — Minnesota Dept. of Agriculture
Steve Offer — University of Wisconsin-Madison
Louise Ogden — Minnesota Dept. of Agriculture

Unit I **Sampling Livestock Waste for Analysis**

John Peters and Sherry Combs

1. Introduction

There are essential pieces of information required to determine the proper application rate and nutrient credits for livestock waste to meet crop needs. These include the acreage of the field, capacity of the spreader and nutrient concentration of the manure. Nutrient concentration can be assigned by using estimated "book" or average available N, P_2O_5 and K_2O concentrations. However, testing manure may better indicate how factors such as animal and manure management affect manure nutrient content. Using good sampling technique is critical for maintaining confidence in manure nutrient analysis results. Appropriate sample handling and laboratory methods are also important to ensure accurate results.

2. Sampling livestock waste

Data in the livestock waste facilities handbook (MWPS-18, 2000) provides "typical" or average nutrient contents for manure from several types of animals. These values probably give an acceptable estimate for "typical" producers, especially if current sampling methods used do not represent the pit, pack or gutter adequately. However, an analysis of a well-sampled system may give a better estimate of manure nutrient concentrations for individual farms than book values, especially if herd and manure management are not "typical." The MWPS total nutrient estimates are compared in table 1 to actual manure analysis of 51 farms in Minnesota (Wagar et al., 1994) and from 1959 manure samples submitted to the University of Wisconsin Soil and Forage Analysis Laboratory between 1998–2001 (Combs, 1991). On average, the actual farm values compare

Table 1.
Comparison of analyzed manure total nutrient concentrations to "typical" nutrient concentrations

Animal Type	System	Nutrient	Minnesota*		Wisconsin**			MWPS***
			Avg.	Range	Avg.	s.d.	Range	Avg.
					lbs/1000 gal			
Dairy	Liquid	N	29	10-47	22	9	1-73	31
		P_2O_5	15	6-28	9	7	1-118	15
		K_2O	24	11-38	20	11	1-114	22
					lbs/t			
Dairy	Solid	N	13	7-25	12	10	2-97	9
		P_2O_5	6	3-13	6	7	1-78	4
		K_2O	8	2-18	8	7	1-60	7
					lbs/1000 gal			
Swine	Liquid	N	48	7-107	34	20	1-91	28
		P_2O_5	28	3-64	16	12	1-60	24
		K_2O	21	7-51	20	12	2-70	23

*Nutrient levels in manure samples taken from 51 farms.

**Nutrient levels in 799 solid/semi-solid dairy, 746 dairy liquid and 414 liquid swine manure samples submitted to the University of Wisconsin Soil and Forage Analysis Lab, 1998-2001.

****Livestock Waste Facilities Handbook* (MWPS-18, 2000)

well to the MWPS estimates. Note, however, that the actual analysis values range widely from the MWPS estimates, indicating poor sampling, management or other on-farm differences. Lindley et al. (1988) also found actual manure analysis values to be highly variable and ranged from 50 to 100% of published values.

2.1 Technique

In virtually any type of agricultural analytical work the results are greatly influenced by sampling. For solid manure, it is generally recommended to sample from loaded spreaders rather than from the actual manure pack. A Wisconsin study (Peters and Combs, 1998) showed that even when well-trained professionals sampled dairy manure, variability was much higher when samples were collected directly from the barnyard and pack compared to those collected from the loaded spreader. The data also indicated that taking several samples would help minimize potential variability.

In this same study, several samples of liquid manure were taken from a thoroughly agitated lagoon while being pumped into a spreader tank. The results of multiple samples taken by different individuals from a well-agitated liquid dairy manure lagoon indicate that variability is much lower than in the solid manure/barnyard system.

Variability can exist among different samplings even when they are taken by the same individual under ideal conditions. This occurred when samples of liquid and semi-solid dairy manure were collected. Five-gallon samples were mixed as thoroughly as possible before being split into twenty-four subsamples. The results indicate that the variability between liquid samples was quite low, but higher with semi-solid dairy samples. This was particularly apparent with total N and dry matter measurements (Peters and Combs, 1998).

2.2 Time

An evaluation of long-term sampling of solid/semi-solid manure showed little variability occurred in nutrient concentration over a three-year period at the University of Wisconsin Arlington Agricultural Research Station (Combs, 1991). Sampling a stanchion barn periodically for three years showed that all samples had similar total nutrient values. The least variation occurred for N while most variation was associated with K. These results seem to indicate that with good representative sampling and no significant change in herd management, consistent results, even for solid manure, are possible.

On the other hand, results from sampling solid manure in a poultry-laying barn at the University of Wisconsin Arlington Agricultural Research Station indicated inconsistent results over time (Peters and Combs, 1998). These poultry manure samples taken from the same barn approximately five months apart show a significant difference in all parameters measured. This could be partially a result of seasonal changes in the feed ration, feed contamination or differences in individual sampling technique. Commonly, five to six batches of birds are grown out before the litter is removed. Poultry houses are normally sampled when the last batch of birds is removed from the house, since the nutrient content in poultry litter will change over time. Therefore, sampling earlier is not recommended.

Due to these variations over time, manure nutrient concentration values used to determine field nutrient credits should ideally be based on long-term farm averages, assuming herd and manure management practices have not changed significantly. If an established baseline level does not exist for a farm, manure testing needs to be done frequently and consistently to develop a historic record that spans at least two–three years. Preferably, manure sampling and analysis should be done just prior to land application, with the time of year noted to monitor potential seasonal variability.

2.3 Storage management

The segregation of manure that occurs in liquid storage requires that special care be taken to ensure that a homogeneous mix is sampled. In a Minnesota study, manure agitated for 2–4 hours before application had highly consistent results for total N, P, K concentrations and percent solids when individual tanks (first to last) were analyzed (Wagar et al., 1994). Samples taken at various stages during the storage system emptying process at Wisconsin also showed very little variability providing the material was thoroughly agitated (Peters and Combs, 1998).

3. Sampling recommendations

The number of manure samples tested by public and private labs has increased from approximately 6,220 in 1988 to almost 16,000 in 1996 (Soil, Plant and Animal Waste Analysis Status Report, 1992-96). However, the majority of animal producers still do not sample manure. Reasons for not doing so include sample heterogeneity and the inherent difficulty of taking a representative sample.

Several states have developed guidelines for sampling manure to minimize the sample heterogeneity problem. This information was used to help develop the sampling guidelines presented here. It is generally not recommended to attempt to sample bedded packs or unagitated liquid manure storage facilities. In fact, using nutrient analysis results from poorly sampled systems will not improve the accuracy in estimating N or P loading to a field and may in fact be detrimental.

Taking an adequate number of subsamples is critical for getting a good estimate of nutrient value. In order to characterize N content of a beef manure stockpile within 10%, it took a Colorado State researcher 17 subsamples (Successful Farming, August 1998). However, getting that level of accuracy for P required 20 subsamples and for K it required 30.

4. Recommended procedures for sampling livestock waste for analysis

Recommended procedures for sampling liquid and solid waste are given below. Producers may choose from these methods as appropriate.

4.1 Solid manure—dairy, beef, swine, poultry

Obtain a composite sample by following one of the procedures listed below. Also, one method of mixing a composite sample is to pile the manure and then shovel from the outside to the inside of the pile until well mixed. Fill a one-gallon plastic heavy-duty zip lock bag approximately one-half full with the composite sample, squeeze out excess air, close and seal. Store sample in freezer if not delivered to the lab immediately.

1. **Sampling while loading—***Recommended method for sampling from a stack or bedded pack*. Take at least five samples while loading several spreader loads and combine to form one composite sample. Thoroughly mix the composite sample and take an approximately 1-lb. subsample using a one-gallon plastic bag. *Sampling directly from a stack or bedded pack is not recommended.*
2. **Sampling during spreading**—*Spread tarp in field and catch the manure from one pass.* Sample from several locations and create a composite sample. Thoroughly mix composite sample together and take a one-pound subsample using a one-gallon plastic bag.
3. **Sampling daily haul**—*Place a five-gallon pail under the barn cleaner 4–5 times while loading a spreader.* Thoroughly mix the composite sample together and take a one-pound subsample using a one-gallon plastic bag. Repeat sampling 2–3 times over a period of time and test separately to determine variability.
4. **Sampling poultry in-house**—*Collect 8–10 samples from throughout the house to the depth the litter will be removed.* Samples near feeders and waterers may not represent the entire house and subsamples taken near here should be proportionate to their space occupied in the whole house. Mix the samples well in a five-gallon pail and take a 1-lb. subsample; place it in a one-gallon zip lock bag.
5. **Sampling stockpiled litter**—Take 10 subsamples from different locations around the pile at least 18 inches below the surface. Mix in a 5-gallon pail and place a 1-lb. composite sample in a gallon zip lock bag.

4.2 Liquid manure—dairy, beef, swine

Obtain a composite following one of the procedures listed below and mix thoroughly. Using a plunger, an up-and-down action works well for mixing liquid manure in a 5-gallon pail. Fill a one-quart plastic bottle not more than three-quarters full with the composite sample. Store sample in freezer if not delivered to the lab immediately.

1. **Sampling from storage**—Agitate storage facility thoroughly before sampling. Collect at least five samples from the storage facility or during loading using a five-gallon pail. Place a subsample of the composite sample in a one-quart plastic container. *Sampling a liquid manure storage facility without proper agitation (2-4 hrs. minimum) is not recommended due to nutrient stratification, which occurs in liquid systems. If manure is sampled from a lagoon that was not properly agitated, typically the nitrogen and potassium will be more concentrated in the top liquid, while the phosphorus will be more concentrated in the bottom solids.*
2. **Sampling during application**—Place buckets around field to catch manure from spreader or irrigation equipment. Combine and mix samples into one composite subsample in a one-quart plastic container.

4.3 Sample identification and delivery

Identify the sample container with information regarding the farm, animal species and date. This information should also be included on the sample information sheet along with application method, which is important in determining first year availability of nitrogen.

Keep all manure samples frozen until shipped or delivered to a laboratory. Ship early in the week (Mon.–Wed.) and avoid holidays and weekends.

5. References

1. Combs, S. M. 1991. Effects of herd management and manure handling on nutrient content: A lab summary. New Horizons in Soil Sci. No. 5-91. Dept. Soil Sci., University of Wisconsin-Madison, WI.
2. Lindley, J. A., D. W. Johnson and C. J. Clanton. 1988. Effects of handling and storage Systems. on manure value. ASAE. Applied Engr. Agric. 4(3), 246-252.
3. MWPS Livestock waste facilities handbook. Handbook #18, 2000. Midwest Plan Service. Ames, Iowa.
4. Peters, J. B. and S. M. Combs. 1998. Variability in Manure Analysis as Influenced by Sampling and Management. New Horizons in Soil Sci. No. 6-98. Dept. Soil Sci., University of Wisconsin-Madison, WI.
5. Peters, J. B. and S. M. Offer. 2002. Laboratory data summary, unpublished. UW Soil and Forage Analysis Laboratory, May, 2002.
6. Wagar, T., M. Schmitt, C. Clanton and F. Bergsrua. 1994. Manure sampling and testing. FE-6423-B. University of Minnesota, Extension Service.

Unit II **Laboratory Quality Assurance Program**

Bruce Hoskins

1. Introduction

Quality assurance (QA) is an essential component of laboratory operation. It ensures consistent quality of analytical results through the application and documentation of appropriate quality control and quality assessment procedures. This serves the dual purpose of promoting client confidence in analytical results and documenting analytical uncertainty.

Quality control (QC) is comprised of laboratory practices undertaken specifically to achieve accurate and reliable analytical results. Quality assessment is comprised of those procedures undertaken to monitor and document the effectiveness of quality control practices. A regular assessment of quality control documents both accuracy (closeness to the known or expected value) and precision (repeatability of multiple results for the same sample) (Garfield, 1991). Accuracy and precision together characterize analytical uncertainty.

A formal QA plan can be a useful foundation document from which to derive quality control and assessment guidelines for all methods run within a lab operation. In addition to QC guidelines, a QA plan should contain a laboratory mission statement, overall QA objectives, an organizational chart, a code of ethics, training and safety practices and procedures. A complete listing of QA plan components can be found in EPA SW-846 (EPA, 1986).

An efficient QA program will initially add a small amount of overhead to any laboratory operation. This investment should be more than offset by an improved ability to pinpoint problems earlier, resulting in less repeat analysis and a streamlining of lab operations. Many funding agencies now require a documented QA program, as well as accuracy and precision statistics as part of any report on routine nutrient analysis of soils and biological materials. Reporting accuracy and precision statistics can improve confidence in and satisfaction with analytical results for all clients.

The relative cost/benefit ratio of individual QC practices or techniques should be considered when implementing or modifying a QA program (Garfield, 1991). The scale of a QA program should reflect the end-use of the analytical results. Specific QA program components and guidelines should be determined within each laboratory, with input from as many laboratory personnel, clients and other constituents as possible. This section does not include a complete listing of all possible QA program components, but is intended to address common operational problems and practices affecting analytical accuracy and precision in routine nutrient analysis. For other viewpoints on and approaches to quality assurance and a more complete listing of QA/QC program components and techniques, the following references are highly recommended:

1. Quality Assurance of Chemical Measurements by J.K. Taylor. 1987.
2. Quality Assurance Quality Control Guidelines for Forage Laboratories by Thiex, Torma, Wolf, and Collins. 1999.
3. Association of American Feed Control Officials Quality Assurance/Quality Control Guidelines for State Feed Laboratories by Ogden, Kane, Knapp, Thiex, and Torma. 1998.

It is not the intention of this manual to mandate rigid QA/QC standards for all laboratories. The accuracy and precision specifications listed here are considered acceptable and attainable for all laboratories running routine manure nutrient analyses. Higher standards are almost always attainable and there should be continuing effort to provide the best quality analytical results possible from the resources available.

2. Components of a quality control program

A good quality control program includes documentation, training and implementation of good laboratory practices and procedures. Attention to detail and consistent execution are paramount to quality analytical results.

Documentation of standard operating procedure (SOP) is one of the most important components of a QC program, since this is where most quality control practices are specified. An SOP should be specific to an individual process or area of responsibility within a laboratory operation.

Sample receipt, login, preservation, holding time and tracking should be detailed in one or more SOPs. The importance of sample handling procedures and sample order verification is often overlooked, even though they are both common sources of errors. Other areas of operation deserving individual SOPs are:

- lab ware cleaning/decontamination/storage,
- sample preparation, analytical procedures,
- reference material choice/storage/disposal,
- standard solution preparation and verification,
- data acquisition/reduction/archival, data validation and report generation,
- sample archival and disposal.

(Thiex, N., L. Torma, M. Wolf, and M. Collins, 1999).

Analytical procedure SOPs should contain a detailed description of all method-specific steps in sample preparation, extraction or digestion, and solution analysis. Also include calibration solution preparation and instrument setup, operation, or maintenance applicable to the method. Quality assessment methods, frequency, control limits, and failure actions should be delineated within each method SOP. Control limits for accuracy and precision should be specific to the method and the analytes being evaluated. If assessment results fall outside these limits, QC failure actions should be specified. Failure actions should address known or common problems and can range from checking for plugged sample delivery tubes to a complete rerun of digestion and analysis. There should also be provision or even a separate SOP for the evaluation of a method, which exhibits chronic QC failure. All analytical method SOPs should be referenced to published standard methods, to document for clients the exact methodology in use and to demonstrate method conformity and validation.

Slight alterations in testing procedures can sometimes cause substantial differences in the final results. A detailed SOP ensures that a method is executed consistently, minimizes variability in results, and helps in troubleshooting problems.

A useful practice, and one that can especially benefit new employees, is writing a summary of known sources of error for each operation or method. These include, but are certainly not limited to the examples listed in table 2. Keeping an internal log of known errors encountered over time, some of which may be peculiar to a specific apparatus or process, can be an invaluable tool in preventing or troubleshooting problems in the lab. A separate log should also be kept for each instrument, which includes a routine maintenance schedule and a listing of maintenance problems and service calls. Logs promote continuity within a succession of technicians or operators over time for any process or individual instrument.

3. Assessment of quality control

Quality assessment should be considered an integral component of a QC program. It is considered separately here, since its purpose is to check the effectiveness of the other program components. Quality assessment is the systematic measurement and documentation of bias, accuracy and precision. It is used to determine if an analytical process is in statistical control and in compliance with QA program guidelines.

3.1 Measuring and documenting bias

The most common technique used to detect and quantify analytical bias is the inclusion of process or reagent blanks. One or more empty sample containers are carried through the entire preparation process, with the same reagents added and final dilution applied. Blank solutions are analyzed with actual samples, using the same calibration. Blanks should be run at regular intervals with each batch of samples to determine if any analyte concentration is consistently above method detection limits (MDL) and also to determine the variability of blank content. Blanks are more likely to be significant for those analytes present at relatively low concentrations, as in trace element or micronutrient analysis.

Including blanks quantifies any addition to the prepared samples or solutions from containers, reagents or the laboratory environment. A consistent blank value (if the source cannot be eliminated), should be subtracted from the concentration values for that analyte in the samples run in association with the blanks. Blank subtraction is used to correct for systematic sources of contamination, not random ones. In this way, systematic bias in the process can be corrected to improve accuracy.

Groups of process or reagent blanks can also be used to calculate detection and quantification limits for each analyte, typically defined as 3 times and 10 times the standard deviation of the blank values, respectively, for each analyte (Taylor, 1987; Thiex, N., L. Torma, M. Wolf, and M. Collins, 1999). Blanks

Table 2. Known sources of error in manure testing

Source of error	Corrective action
Variable or heterogeneous samples.	Homogenize thoroughly prior to sub-sampling. Use larger sample size. Run replicate analysis.
Sample carryover on digestion vessels or extraction between other apparatus.	Decontaminate equipment with cleaning solution between uses.
Contamination of samples or equipment by lab environment.	Store samples, reagents, and equipment separately.
Samples weighed, processed, or analyzed out of order.	Verify sample IDs during subsampling. Run known reference samples at regular intervals.
Inaccurate calibration solution content.	Check new cal standards against old. Run an independent check sample to verify standards.
Mismatch between sample and calibration solution matrices.	Make up calibration solutions in digestion matrix or method blank solution. Use instrument internal standard(s) if applicable and available.
Drift in instrument response.	Use frequent calibration or drift checks.
Blank values substantially above detection limit.	Use high-purity reagents and deionized water. Decontaminate sample containers between uses.
Poor instrument sensitivity or response.	Optimize all operating parameters. Check for obstructions in sample delivery system.
Transcription errors, faulty data handling.	Automate data transfer, verify manual input.

should be run at relatively high frequency until valid mean and standard deviation statistics can be generated and a determination made as to whether blank values are consistent within an analytical process. Blank values should also be checked at increased frequency after any change in procedure or reagents.

Matrix spiking is another technique used to measure bias, where total content is being measured. A sample is supplemented with a known amount of the analytes under scrutiny before it is digested. Analyte concentrations in the spiked sample are compared with those in the same sample run without spiking, with the percent recovery calculated. This technique helps to determine bias in analyte recovery due to sample matrix interferences, incomplete digestion, or volatilization loss. Matrix spikes are appropriate when determining total content of P, K, Mg, Ca, or micronutrients by acid digestion or dry ashing. They are *not* appropriate in methods where nutrient content is measured by partial extraction (Delavalle, 1992).

3.2 Documenting accuracy

Accuracy of analytical results is documented by analyzing reference samples of known content. A reference sample should be as similar as possible to the routine samples being tested. Several standard reference materials (SRMs) can be purchased from commercial or government sources. Table 3 lists several currently available materials, with guaranteed or provisional contents.

SRMs typically have a certified analysis (with a range of uncertainty) of the elemental content for several analytes. Accuracy control limits can be set to the listed uncertainty range or control limits can be set as a fixed range of percent recovery of the certified content for each analyte. Analysis of an SRM is considered the most unbiased way to document accuracy in a laboratory QA program (Delavalle, 1992). Often, it is necessary to use an SRM, which is similar, but not exactly the same matrix, as sample unknowns. At present, there are no certified reference manure samples available. A reference plant tissue, soil or sludge is an appropriate SRM for checking the accuracy of many manure analyses.

Table 3. Suitable standard reference materials for manure analysis

Company or Agency	Material ID	Analytes
SCP Science 348 Route 11 Champlain NY 12919-4816 800-361-6820 www.scpscience.com	CP-1 Compost BE-1 Sewage Sludge	N P K Mg Ca Cu Fe Mn Zn Na pH B Ca Cu Fe K Mg Mn Na P Zn
Environmental Research Assoc 5540 Marshall St Arvada CO 80002 800-372-0122 www.eraqc.com	 Catalog # 545 - Sludge Catalog # 160 - Sewage Sludge Catalog # 542 - Soil	 TKN P NH_3 Ca Cu Fe Mg Na Zn TKN P NH_3
Ultra Scientific 250 Smith St North Kingstown RI 02852 800-338-1755 www.ultrasci.com	Catalog # IRM005 - Sludge Amended Soil Catalog # IRM007 - Sewage Sludge (POTW)	Ca Cu Fe Mg Mn P K Na Zn B Ca Cu Fe Mg Mn K Na Zn
Resource Technology Corp. 2931 Soldier Springs Rd Laramie WY 82070 800-576-5690 www.rt-corp.com	Catalog # CRM007-040 Sewage Sludge Catalog # CRM018-050 Sewage Sludge	B Ca Cu Fe Mg Mn K Na Zn B Ca Cu Fe Mg Mn K Na Zn
US Dept of Commerce National Institute of Standards & Technology Building 202, Room 204 Gaithersburg MD 20895 301-975-6776 www.nist.gov	SRM2781 - Domestic Sludge SRM2782 - Industrial Sludge SRM1515 - Apple Leaves	Ca Cu Fe Mg P K Na Zn Ca Fe Mg P K Na N P K Ca Mg B Cu Fe Mn Zn

A supplement to purchased SRMs is enrollment in one or more proficiency testing (PT) programs. In these programs, identical samples are sent to all cooperating laboratories, which analyze them according to specified methods and protocols. Accuracy of analytical results for manure testing methods, which may not be available from purchased SRMs, can be obtained in this way. Typically, median and mean absolute deviation (MAD) statistics are reported for each analyte and for each method, based on the data returned by participating labs. Any results from a contributing lab, which are outside acceptable control limits, are flagged on the report to that lab. While median values from PT reports do not constitute a certified or guaranteed analysis, values obtained from several laboratory sources can be considered closer to the "true" values than results derived solely from one laboratory.

A Manure Proficiency Testing program is currently available through the Minnesota Department of Agriculture (90 West Plato Blvd, St Paul MN 55107-2094 or http://www.mda.state.mn.us). Compost has a similar matrix to many manures and is tested for many common parameters using identical protocols. A Compost Analysis Proficiency testing program (CAP) is available through the US Composting Council (contact: USU Analytical Lab, Utah State University, Logan UT 84322 or **http://tmecc.org/cap/contact.html).**

Samples from PT programs are high quality and can be stabilized by refrigeration as necessary. Median values can be used in lieu of certified content and any remaining sample used as a surrogate SRM to document accuracy. This is especially useful where no purchased SRM of similar matrix and/or concentration range is available.

Accuracy of solution analysis is documented using a quality control check sample (QCCS). A QCCS is a solution of known content, which is derived from a separate or independent source from the calibration standards. QCCS solutions can be made in-house using separate stock solutions or they may be purchased ready-made from many scientific supply vendors. The contents of a QCCS should be within the normal range of sample unknowns for all analytes. It serves as an independent verification of the calibration standards and can also be used as a calibration drift check. Accuracy control limits for a QCCS are set as a fixed range of percent recovery of known content for each analyte.

3.3 Documenting precision

Precision of analytical results is measured through replicate testing of routine samples or by repeated analysis of internal reference samples. Replicate analysis involves two or more analyses of a routine sample unknown at some specified frequency, such as every tenth or every twentieth sample. Precision control limits are based on relative percent difference (RPD) between replicates for each analyte. A relatively high frequency of replication should be used initially. Replication frequency can be reduced after the minimum number of replicates has been generated to produce valid statistics (see R-Chart) and when QC precision standards for the method are being met. Replicate analysis is especially useful where appropriate reference samples are unavailable (Garfield, 1991) or where reference samples are available, but matrix and concentration range mismatch is a concern (Delavalle, 1992).

An alternative or supplement to replicate analysis is to run internal reference sample(s). An internal reference is typically a large, stabilized (dried or refrigerated) sample, subsamples of which are run with each batch of sample unknowns. Precision control limits are derived from the standard deviation from the mean of these repeated measurements. Bulk samples can be prepared relatively easily and with minimal expense. It is important that the reference sample be thoroughly homogenized before each use to prevent sample stratification. The content of an internal reference sample can be verified by running it in the same batch with one or more SRMs. Internal reference samples are often used in this way as a surrogate SRM for daily accuracy checking. Internal reference samples are often the primary daily QC assessment used in lab operations.

By routinely running replicates or internal check samples and occasionally running SRM or PT samples, statistical control of both precision and accuracy can be adequately and economically documented (Delavalle, 1992).

3.4 Known vs. blind checks

Quality assessment samples can be run with the full knowledge of the technical staff or as single or double blind samples. Check samples of known composition run at known intervals can be used by technicians to monitor the quality of analytical results as they are being produced. A blind sample is known to the technical staff as a check sample, but the composition is unknown. A double blind sample is completely unknown to the technical staff and is used to eliminate any possible bias in the results, from knowing the location or composition of the check sample. Blind and double blind samples are best reserved for formal performance audits (Taylor, 1987, EPA, 1986).

4. Statistical control and control charts

Descriptive statistics used in quality assessment can be presented in a variety of ways. Accuracy is measured in terms of the deviation or relative deviation of a measured value from the known or certified value. Precision is presented in terms of standard deviation (SD) from the mean of repeated measurements on the same sample or in terms of relative percent difference (RPD) between replicate analyses of the same sample. Together, accuracy and precision document the systematic and random errors which constitute analytical uncertainty in all laboratory results.

Accuracy and precision statistics are the performance criteria used to determine if a methodology is in “statistical control”; that is, whether method control limit standards are being met daily and over the long term. Check sample statistics can also be used by technicians and managers as daily decision-making tools during sample analysis to determine if expected results are being generated and if the analytical system is functioning properly at any given time. Determining that a problem exists at the time it is happening can save a great deal of time in running samples over again at a later date (Delavalle, 1992).

4.1 X-charts

Quality assessment statistics can be presented graphically, through control charts, for ease of interpretation. X-charts can be used to present both accuracy and precision data. Repeated measurements of external or internal reference samples are graphed on a timeline. A minimum of seven measurements is needed, though 15 are recommended for valid statistical calculations (Taylor, 1987). Superimposed on the individual results is the cumulative mean (in the case of an internal reference sample) or the known content (in the case of an external SRM or PT sample). Upper and lower warning limits (UWL & LWL) are calculated as +/- 2 SD and upper and lower control limits (UCL & LCL) are calculated as +/- 3 SD (figure 1). In a normally distributed sample population, +/- 2 SD represents a 95% confidence interval (CI) and +/- 3 SD corresponds approximately to a 99% CI.

An individual value between UWL and UCL or LWL and LCL is considered acceptable, though two or more in a row are unacceptable. A single value outside UCL or LCL is considered unacceptable. If statistical control is considered unacceptable based on either standard, all routine sample unknowns run since the last check sample, which was in control, should be rerun. Check sample results which fall within the warning limits, but which are exhibiting a trend toward the UWL or LWL can signal a potential problem in the process, which needs to be addressed (Delavalle, 1992). X-charts are especially useful as a day-to-day tool to monitor ongoing or emerging problems.

4.2 R-charts

Another useful graphical tool is the R-chart or range chart. When two or more replicate analyses are run on a routine sample or a reference sample, the difference between the lowest and highest values in a set of replicates (or just the difference between replicates when there are only two) is called the replicate range. The R-chart maps individual replicate ranges for a given analyte over time. The replicated samples should ideally be within a limited total range of concentration, well above MDL, within the same process or method (Delavalle, 1992). A cumulative mean range is calculated and superimposed on the individual range values. Warning and control limits are calculated as 2.512 times and 3.267 times the mean range, respectively (Taylor, 1987). Since replicate ranges are absolute, only one warning and control limit are displayed (figure 2). Since R-chart data consist solely of replicate ranges, they can only be used to document precision. A minimum of 15 replicated samples is recommended for producing an R-chart (Taylor, 1987).

Figure 1. Example X-Chart

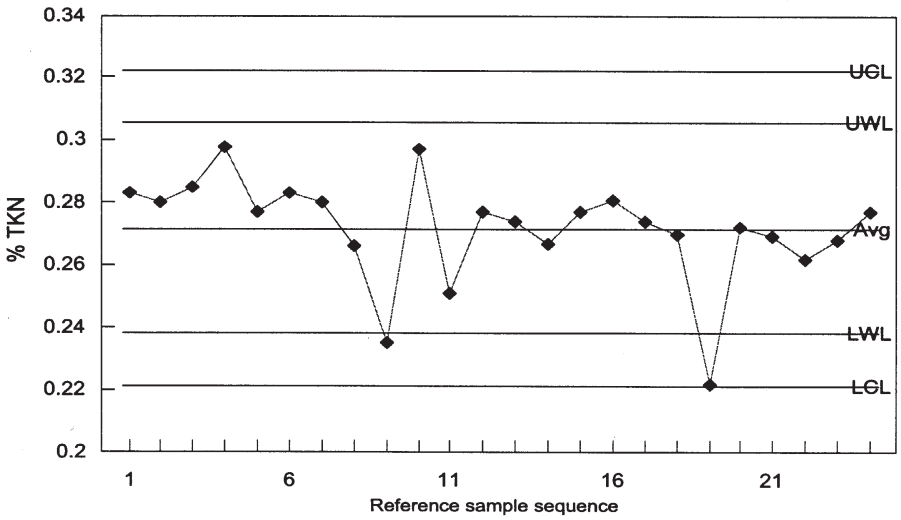
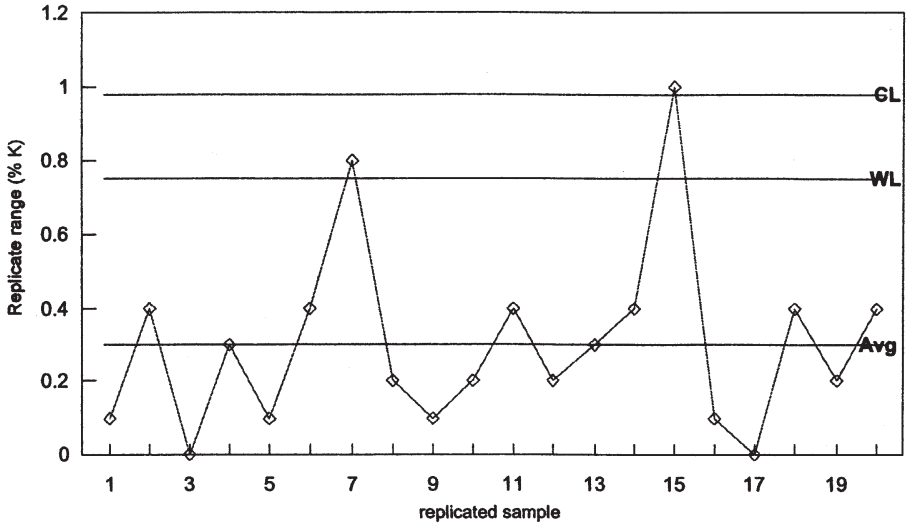


Figure 2. Example R-Chart



4.3 Establishing control limits

Since warning and control limits are calculated from cumulative statistical data, new quality control assessments are always viewed relative to past performance. Cumulative statistics effectively characterize the inherent capability of a laboratory to execute a given methodology. Realistic QC standards for accuracy and precision in any lab must take this capability into account. The first step should be to define *attainable* accuracy and precision within the normal range of sample content (Taylor, 1987). When attainable standards are determined, they should be used to maintain consistent analytical quality over time. Allowance must be made for decreased accuracy and precision and increased analytical uncertainty as an analyte approaches MDL.

The accuracy and precision levels specified within the methods in this manual are considered to be attainable for routine analysis of manures. These should serve only as a starting point. Each laboratory should individually determine acceptable control limits and standards internally. These QA/QC standards should be re-evaluated when methodologies are changed or modified and as analytical capabilities are improved.

4.4 Reporting

Accuracy and precision statistics should be documented and updated daily, both for individual analytical jobs and cumulatively for any given method on an on-going basis. Accuracy and precision statistics can be reported only on demand or on a routine basis, depending on the client and the end-use of the results.

For some projects or clients, replicate analysis and reference sample statistics are required to be reported along with or as a supplement to the analytical results. Precision is typically documented by reporting replicate percent differences (RPDs) from replication of the client samples. For replicated samples, the mean of replicate analyses is usually reported as the measured content. Accuracy is documented by reporting the result of SRM(s) run in the same batch with the client samples. QC standards for acceptable RPD and SRM acceptable ranges should be listed for comparison.

Even when not required, a simple summary of typical precision or analytical uncertainty can be listed or sent with the report for a routine nutrient analysis of manure. A listing of 95% CI ranges for the reported analytes can be taken from the cumulative statistics for a reference sample or samples, run over the past several months or years. This simple listing of expected uncertainties can greatly improve client understanding and confidence in the quality of the analytical results.

5. References

1. Delavalle, N. B. 1992. Handbook on Reference Methods for Soil Analysis. Quality Assurance Plans for Agricultural Testing Laboratories: 18–32. Soil and Plant Analysis Council, Inc. Athens, GA.
2. EPA, 1986. Test Methods for Evaluating Solid Waste, SW-846, vol 1A. Chapter one: Quality Control.
3. Garfield, F. M. 1991. Quality Assurance Principles for Analytical Laboratories. Association of Official Analytical Chemists. Arlington, VA.
4. Ogden, L., P. Kane, P. Knapp, N. Thiex, L. Torma, 1998. Association of American Feed Control Officials Quality Assurance/Quality Control Guidelines for State Feed Laboratories. Association of American Feed Control Officials.
5. Schumacher, B.A., A.J. Neary, C.J. Palmer, D.G. Maynard, L. Pastorek, I.K. Morrison, and M. Marsh, 1995. Laboratory Methods for Soil and Foliar Analysis in Long-Term Environmental Monitoring Programs. Chapter 2: Good Laboratory Practices. EPA/600/R-95/077.
6. Taylor, J. K. 1987. Quality Assurance of Chemical Measurements. Lewis Publishers, Inc. Chelsea, MI.
7. Thiex, N., L. Torma, M. Wolf, and M. Collins, 1999. Quality Assurance Quality Control Guidelines for Forage Laboratories. National Forage Testing Association.

Unit III **Laboratory Procedures**

1. Sample handling

Bruce Hoskins

1.1 Introduction

An active microbial population and the volatility of many constituents can make animal manure a very unstable and analytically difficult material. Sample stabilization, storage, and handling can all have an important impact on analytical results. Many manures are highly variable, heterogeneous, and difficult to subsample reproducibly—at the farm or in the laboratory. Animal manure can also pose potential problems with regard to laboratory safety and sample disposal.

1.2 Recommended sample handling protocols

1. Biohazards and laboratory safety

Animal manures may contain disease pathogens and parasitic organisms that can pose a health risk to humans under certain circumstances. Common exposure pathways are through dust inhalation or through hand-mouth contact (Clemson University, 2000) (Standard Methods, 1995).

Most known microbiological hazards associated with manure handling are classified at Biosafety level 2 (Health Canada, 2001). At this hazard level, recommended safety equipment consists of the same personal protective equipment (PPEs) normally used in a chemical laboratory (lab coats, gloves, and eye protection). For those processes that generate dust or other aerosols, such as grinding or weighing dried manures, a Biological Safety Cabinet is recommended to contain any airborne particulates. In addition, cleaning grinders or other contaminated equipment should be done using a HEPA vacuum and drying ovens should be vented to the outside (Clemson University, 2000).

Any laboratory analyzing animal manures should coordinate with their Department of Environmental Safety or Industrial Hygienist to ensure that all prudent safety precautions are in place. Laboratory personnel should be trained with regard to potential hazards and in the use of PPEs and other appropriate equipment with respect to manure analysis.

2. Sample receiving, examination, and transfer

Examine all manure samples when they arrive at the laboratory. Document on the report any sample that is not in a sealed plastic or glass container or that shows evidence of leakage or sample loss. All semi-solid and liquid samples will segregate during shipping. Most sample loss from leakage will be predominantly water rather than solids, rendering dry matter (and other) analysis inaccurate.

If a manure sample is not in a sealed plastic or glass container, it should immediately be transferred to an appropriate secondary container for storage and archival in the lab. If the original sample size exceeds that of the secondary container, it should be homogenized before transfer. To minimize subsampling error, transfer as much of the original sample as possible to the secondary container while leaving sufficient headspace for freezing. If the sample arrives frozen it should be thawed to room temperature to facilitate subsampling and transfer. Secondary containers can be made of glass or plastic, should be of convenient size for storage, should seal sufficiently to prevent evaporative loss of moisture or other leakage, and should be able to sustain freezing.

3. Sample stabilization and storage

All manure samples should immediately be refrigerated at 4˚C upon receipt to retard microbial activity and volatilization losses (US Compost Council, 2000) (Standard Methods, 1995). Total refrigerated storage time before analysis should not exceed the holding times listed below. If a manure sample cannot be analyzed for one or more of the listed parameters within the specified time limit, it should be frozen at -18˚C to suspend microbial and chemical activity. The manure should then be sampled for analysis immediately after thawing to room temperature.

4. Sample holding times

Suggested maximum holding times for various analyses are listed below. The sample constituents most sensitive to holding time are those

Table 4. Maximum holding times for manure at 4° C before specific analyses.

pH	7 days
Dry matter/Total solids	7 days
Total nitrogen/Kjeldahl nitrogen	7 days
Ammonia nitrogen	7 days
Electrical conductivity	6 months
Minerals— Total P, K, Ca, Mg, Cu, Zn	6 months

that are prone to evaporative/volatilization loss or microbial/chemical transformations in storage.

5. Homogenizing and subsampling

Thoroughly mix and homogenize the entire sample before subsampling. Liquid samples can often be shaken within and poured from the original container, provided the solids stay uniformly suspended. Liquid and semi-solid samples without straw or hay bedding or other long-fiber material can be pulverized and mixed in a blender to a uniform consistency (Thiex, personal communication). Solid manures or manures with coarse bedding can be chopped, divided, recombined, and mixed with a spatula to minimize heterogeneity prior to subsampling.

An alternative for coarse or heterogeneous manures is to process very large subsamples in two stages. Up to ½ the original sample can be partially or completely dried, ground, homogenized, and subsampled again for some analyses (Undersander, Mertens, and Thiex, 1993) (Thiex, personal communication). This technique is not recommended for the analysis of total nitrogen, ammonium nitrogen, or other constituents that may be volatilized or transformed during the drying process. Subsampling for the analysis of these unstable constituents should be done directly from the as-received sample. Analysis run on partially dried material will also require additional corrections to both an oven-dried and as-received basis, based on the measured moisture loss during partial drying. If two-stage subsampling is conducted, a representative portion of the original sample should always be maintained for direct subsampling and archival.

In general, the larger the subsample for any analysis, the more representative it will be and the higher the precision of the results. Any specific subsampling instructions for an individual analysis will be provided in the respective chapter or section.

6. Archiving and disposal

After analysis, manures can be archived by freezing at -18˚C. Additional or repeat analysis can be done immediately after thawing to room temperature.

Sample disposal will depend on local or institutional regulations. The safest way to prepare samples for disposal is to sterilize by autoclaving. Samples autoclaved for 30 minutes under pressure at 121˚C are safe for disposal in the normal waste stream (Standard Methods, 1995). Alternatively, manure samples may be incinerated prior to disposal.

1.3 References

1. Clemson University Department of Environmental Health & Safety Memo. 2000. Clemson SC.
2. Health Canada, Health Protection Branch—Laboratory Centre for Disease Control.
3. http://www.hc-sc.gc.ca/hpb/lcdc/biosafty/msds/index.html. Material Safety Data Sheets 23, 28, 32, 43, 48, 82, 95, 96, 103, 124, 135, 153. Accessed in June, 2001.
4. Standard Methods for the Examination of Water and Wastewater, 19th edition. 1995. Pp. 1-22 to 1-24, 1-40.
5. Thiex, Nancy, 2002. Personal communication. SOP for Sample Preparation of Liquid Manure. SOP for Sample Preparation of Solid Manure. Olson Biochemistry Laboratories, South Dakota State University.
6. Undersander, D., D.R. Mertens, and N. Thiex. 1993. Forage Analysis Procedures. National Forage Testing Association, Omaha.
7. United States Composting Council. 2000. Test Methods for the Examination of Composting and Compost (draft). Sample Collection and Laboratory Preparation - Field Sampling of Compost Materials. Section 02.01-5.

2. Dry matter analysis

Bruce Hoskins, Ann Wolf and Nancy Wolf

2.1 Introduction

Dry matter (DM) content can be important in determining the handling characteristics and relative nutrient content of manure. Accurate determination of dry matter is also important because of its effect on analytical results converted from a dry weight to a wet weight basis. Since many analyses are performed on dried material, but are reported on a wet sample or "as received" basis, errors in determining dry matter content will be manifested through all converted values. Many labs use the dry matter subsample for subsequent analysis; therefore subsample quantity, drying time and temperature are often dictated by the requirements for those additional analyses.

2.1.1 Method variability

Recent manure analysis surveys and sample exchange programs point out the wide range of methodologies employed by different laboratories in the public and private sectors. A manure sample exchange done by the Minnesota Department of Agriculture in 1996 consisted of four manure samples (all <10% DM) sent to 17 private laboratories in the region. Only analytical results were available, with no specific procedures reported. The inter-laboratory comparison of dry matter results showed coefficients of variation (CV) ranging from 12.8–22.2% for individual manure samples (Jarman, MN Dept of Ag, 1996).

A multi-regional manure sample exchange, conducted at 14 state university laboratories in 1996 consisted of two liquid manures and three dried and ground manures. A survey of methods used by participating laboratories was also done. Drying temperatures ranged from 50°C to 110°C. Documented drying times ranged from 16 to 24 hours. The CVs among laboratories for dry matter content of each of the manures ranged from 2.2–9.0% (Combs & Peters, 1996).

2.1.2 Method validation

To explore sources of variability, parallel studies were undertaken at the University of Arkansas, Penn State University, and the University of Maine. Specific effects studied included species of origin, dry matter content, subsample size, drying time, drying temperature, and drying vessels. Multiple samples from each laboratory's sample stream were chosen to include solid cow, liquid cow, liquid swine and solid poultry manures. Dry matter contents ranged from 2.3–84.0%. Manure with 15% or more dry matter was considered solid for the purpose of the study.

Samples were dried at 50°C, 70°C and 110°C for 6, 16, 24 and 48 hours each. A range of subsample sizes (all <2g DM) were selected for each time and temperature. All samples were replicated at each time and temperature. Replicate percent difference (RPD) was calculated to document sample variability and analytical precision. Forced-draft ovens were used in each laboratory. Containers consisted of aluminum boats, ceramic crucibles, beakers and digestion flasks. Minimum drying time for each temperature was determined when no significant increase in apparent moisture content was seen at the next higher time interval. Residual moisture content after achieving constant weight at 50°C and 70°C was determined by comparison with the 110°C moisture content (Wolf, Wolf, & Hoskins, 1997). Relevant findings were as follows:

1. Liquid samples in containers with restricted tops took up to twice as long to completely dry as they did in low-sided containers.

2. Residual moisture contents were insignificant for samples dried at 50°C and 70°C, compared to 110°C, for all but poultry manures.

3. Sample size and drying temperature had a significant effect on drying time of all manures.

4. Sample sizes up to 5g fresh wt needed only 6 hr at 110°C or 70°C and 16 hr at 50°C to dry completely.

5. Sample sizes of 10–20g fresh wt required 16 hr at 110°C and 24 hr at 70°C to dry completely, but often did not dry completely even after 48 hr at 50°C.

6. Subsample variability and analytical precision were very dependent on the type of manure and the magnitude of the numerical results.

a) Relative errors were smallest for moisture content of liquid manures.

b) Relative errors were greatest for *dry matter content* of liquid manures.

c) Consistently high RPDs were seen with poultry manures, due to difficulty in homogenizing the sample before subsampling.

2.2 Principle of the method

1. Dry matter content can be defined as the material remaining after water is completely evaporated from the sample. Drying is considered complete when the sample weight remains constant (< 0.1% DM change) with at least 6 hr additional drying time.

2. Advantages: 1) A simple procedure, requiring routine reusable apparatus, commonly available in all analytical labs. **2)** No caustic reagents are used.
3) Only minimal sample handling is needed.
4) Drying can be done overnight, requiring no technician time.

3. Disadvantages: 1) Semi-solid and liquid samples will segregate in the container during shipping and storage, which can lead to unrepresentative subsampling for dry matter determination. **2)** subsample size can greatly affect the precision of the analysis, especially with high solids manures. **3)** subsample size will also impact the time necessary for thorough drying. **4)** Drying time can be affected by the size and shape of the drying vessel, the oven temperature, and whether the oven is a static air or forced-draft type. **5)** Drying at too high a temperature can lead to thermal breakdown and volatile loss of some organic constituents (Mills and Jones, 1996).

4. Caution: Complete or partial drying is not compatible with subsequent analysis of the same subsample for total N, ammonium N, or other constituents which may be volatilized or chemically or biologically transformed during drying.

2.3 Apparatus

Drying ovens—should be forced-draft or capable of circulating air. Ovens, which cannot circulate air, will lengthen drying times beyond those recommended in these guidelines. Temperature adjustment should be accurate within ±5°C and should be verified with an accurate thermometer kept in the oven exhaust port or in a small container of silica inside the oven.

Balance—should be capable of at least 0.01 g resolution. Accuracy should be checked daily with reference weights.

Drying vessels —should be low-sided containers such as weighing boats, pans, or crucibles, capable of withstanding 110°C. Vessels with restricted top openings or high sides, such as flasks or beakers, will restrict air movement and lengthen drying times beyond those recommended in these guidelines. Vessels should be clean of material from previous samples, but need not be decontaminated for dry matter analysis unless subsequent elemental analysis is to be run on the same subsample.

Desiccators—should be large enough to hold all samples from a normal batch run. Any type of desiccant is acceptable. Gypsum or silica gel are the most common. It is recommended that the desiccant contain dye, which shows a color change when saturated with moisture, to effectively signal when the desiccant should be replaced.

2.4 Procedure

Note: Specifications in this procedure are for the complete drying of manure samples as received. Partial drying for two-stage subsampling (see Sample Handling section) requires two separate dry matter determinations—one for partial drying of a large subsample and a second determination to complete dryness on a smaller subsample of the partially dried and ground manure. (Undersander, Mertens, and Thiex,1993).

1. Subsampling and sample size

1.1 Thoroughly mix and homogenize the entire sample before subsampling, as specified in the Sample Handling section. Minimize contact time with the open air to avoid moisture loss from evaporation before taking the wet sample weight.

1.2 Place subsample in an open container such as a weigh boat, open pan, or ceramic crucible.

1.3 Suggested fresh sample size for both liquid and solid manures depends on the drying temperature and the desired drying time (see table 5). Larger subsamples will be more representative, especially for high DM or heterogeneous manures. subsample size should be as large as possible without exceeding those listed in the table below and without exceeding drying vessel capacity.

2. Times and temperatures

2.1 Liquid manures, semi-solid manures, and solid cow manures can be dried to constant weight at 50°C or 70°C with no significant residual moisture content when compared to samples dried at 110°C.

2.2 For the most accurate determination of DM, poultry manures should be dried at 110°C. Poultry manures dried to constant weight at 50°C or 70°C will have a small but significant residual moisture content when compared to samples dried at 110°C.

2.3 Recommended drying times and temperatures for samples of varying sizes are shown in table 5 below.

3. Weighing

3.1 Record empty weight of container to nearest 0.01 g.

3.2 Add wet sample as specified for the temperature and drying time desired. Record weight of container plus wet sample to nearest 0.01 g.

3.3 Dry in forced-draft oven for the time specified for oven temperature and sample weight, or until weight remains constant (<0.1% DM change) after 6 hr additional drying time.

3.4 Remove sample from drying oven and allow to cool in a desiccator with active desiccant. Record weight of container plus dried sample to nearest 0.01 g.

Table 5. Maximum fresh sample size for dry matter determination in open vessels

	Drying temperature		
Drying Time	**50°C**	**70°C**	**110°C**
6 hr	Not recommended	5 g	10 g
16 hr	5 g	10 g	20 g
24 hr	10 g	20 g	20 g

2.5 Calculations

1. Percent moisture content is measured as the weight lost during drying and is expressed as a percentage of the as received or undried sample:

$$\% \text{ Moisture} = \frac{[(\text{weight undried sample + container}) - (\text{weight dry sample + container})] \times 100}{[(\text{weight undried sample + container}) - (\text{weight empty container})]}$$

2. Percent dry matter is measured as the remaining weight of sample, after drying, and is expressed as percentage of the as received or undried sample:

$$\% \text{ Dry Matter} = \frac{[(\text{weight dry sample + container}) - (\text{weight empty container})] \times 100}{[(\text{weight undried sample + container}) - (\text{weight empty container})]}$$

3. Dry matter or moisture can be calculated from the other as the complement of 100 % original content:

$$\% \text{ Dry Matter} = 100 - \% \text{ Moisture}$$
$$\% \text{ Moisture} = 100 - \% \text{ Dry Matter}$$

2.6 Quality control

1. Precision

1.1 All solid and semi-solid manure analysis should be replicated. Control limit: 2% (DM or moisture) difference. Failure action: run larger and more representative subsamples, with a longer drying time to compensate.

1.2 For liquid manures (<15% DM), replicate one in every 10 samples (or 1 per batch). For semi-solid manures with 5-15% DM, absolute differences should not exceed 1.0% (DM or moisture). For liquid manures with < 5% DM, absolute differences between replicates should not exceed 0.2% (DM or moisture). Failure action: dry samples an additional 6 hr to assure complete drying and recheck. If control limit still exceeded, rerun all samples since last successful replicate.

2. Accuracy

2.1 Option 1: For 1 in every 20 samples (solid or liquid) or 1 per batch, after initial DM determination at routine time and temperature, continue to dry at 110°C for an additional 24 hr to determine residual moisture content. To eliminate subsampling variability, this should be done to the same subsample used in the initial determination. Control limits are 1% residual moisture in solid manures (>15% DM), 0.5% residual moisture in semi-solid manures (5-15 % DM), and 0.1% residual moisture in liquid manures (<5% DM). Failure action: increase drying temperature and/or time and rerun all samples in the batch.

2.2 Option 2: Run 1 Compost Analysis Proficiency (CAP) compost or 1 North American Proficiency Testing (NAPT) plant sample for every 20 samples or 1 per batch. Control limit: median ± 4*MAD, obtained from CAP or NAPT reported values. Failure action: increase drying temperature and/or time and rerun all samples in the batch.

2.7 References

1. Combs, S.M. and Peters, J.B. 1996. Manure Variability: A Sampling and Laboratory Analysis Survey. Presented at joint NCR-13/SERA-6/NEC-67 meeting. Raleigh, NC.
2. Jarman, J. 1996. Report on Manure Sample Exchange. Minnesota Dept. of Agriculture.
3. Mills, H.A. and J.B.Jones. 1996. Plant Analysis Handbook II. MicroMacro Publishing Inc. p 117.
4. Undersander, D., D.R. Mertens, and N. Thiex. 1993. Forage Analysis Procedures. National Forage Testing Association, Omaha
5. United States Composting Council. 2000. Test Methods for the Examination of Composting and Compost (draft). Physical Examination - Total Solids and Moisture. Section 03.09.
6. Wolf, N.; Wolf, A.; and Hoskins, B. 1997. Dry Matter Analysis Methods. A report to the Manure Testing Manual Joint Committee NCR-13/SERA-6/NEC-67.

3. Total nitrogen

Maurice Watson, Ann Wolf and Nancy Wolf

3.1 Introduction

The determination of total nitrogen in manure is extremely important if manure is to be used as a nutrient source for plants. The wet Kjeldahl method has been used for more than a hundred years to determine the concentration of N in various materials (Kjeldahl, 1883; Scarf, 1988). It is usually denoted as Total Kjeldahl Nitrogen (TKN) and is an approximation of total nitrogen.

The dry combustion Dumas method (Dumas, 1831), although older than the Kjeldahl method, has not been as widely adopted. However, because of the development of computer automated instrument systems that allow for more precise control of gases and improved instruments designed to handle more samples, the Dumas method has gained favor recently.

These two methods have been used predominantly for the analysis of plant tissue and have been adapted for the determination of N in manure. They were compared on various materials under routine laboratory operations (Watson and Galliher, 2001). Because of the various kinds of manures a laboratory receives, the laboratory should do an evaluation of these methods to make sure they are performing correctly. Nitrogen analysis has remained difficult and expensive compared to the analysis of many other elements, but with the advent of modern computers and microchip technology, both of these methods are easier to use than in the past. The selection of the method depends on many factors, of which cost, safety and ease of operation are paramount.

3.2 Total Kjeldahl nitrogen

1. Principle of the method

1.1 The main objective of the Kjeldahl method is to convert the nitrogen contained in materials to the ammonium form of nitrogen and then determine the concentration of ammonia-N. (The ammonia-N measurement is discussed in the following section.)

Concentrated sulfuric acid, catalysts and salts are used in the Kjeldahl method to convert organically bound N to ammonium (NH4). The addition of the catalyst aids the chemical conversion while the addition of the salts elevates the temperature of the acid-sample mixture, speeding up the digestion. Catalysts that have been used in the digestion process are mercury (Hg), copper (Cu), selenium (Se), chromium (Cr) and titanium (Ti) (Simonne et al., 1993). Mercury is the most effective catalyst. However, because it is considered an environmental hazard it is rarely used. A mixture of Se and Cu is an effective catalyst. However, Se is also now considered an environmental hazard and precautions should be taken regarding its use. Any waste containing Se should be collected for proper disposal. Another catalyst that has been used is the combination of Ti and Cu. Copper alone can also be used, but usually a longer time is required to complete the digestion (Hoskins, 2001). The Kjeldahl method has been adapted to various scales of apparatus. These are depicted as macro-Kjeldahl, for 1-2 g sample size, and the micro-Kjeldahl, <1.0 g sample size. The macro-Kjeldahl employs large flasks and heaters, while the micro-Kjeldahl uses small flasks and heaters or a digestion block.

1.2 Advantages. The advantages of this method are: **1)** relative low cost of digestion and distillation apparatus, depending on size; **2)** macro-Kjeldahl systems can handle large sample sizes; **3)** can handle either wet or dry samples.

1.3 Disadvantages. The method requires: **1)** concentrated sulfuric acid; **2)** a catalyst; **3)** a salt to raise the temperature of digestion; **4)** a long digestion time and labor-intensive procedure with a separate measurement for ammonium after digestion; **5)** strong sodium hydroxide solution during distillation. Furthermore, standard Kjeldahl does not completely account for oxidized forms of nitrogen, such NO_3 and NO_2, nor nitrogen in heterocyclic ring compounds. However, concentrations of these compounds are negligible in manure.

2. Operational considerations

2.1 Sulfuric acid/salt ratio is critical in maintaining proper boiling temperature in the digestion step. Too much salt relative to acid can raise the digestion temperature above 400ºC, which can cause nitrogen loss through volatilization. Insufficient salt will not maintain the digestion temperature high enough to complete the recovery of organically bound nitrogen. Acid and salt dispensers should be calibrated daily to ensure accurate delivery. Prepared salt and catalyst mixtures in tablet form can be purchased from various chemical suppliers under the brand name of "Kjeltab," "Kjeltab" or "Kelmate."

2.2 During the distillation step excessive foaming can cause foam to rise through the reflux trap and into the condenser, contaminating the condenser and the receiving flask with alkali. Use an anti-foaming agent to reduce foaming.

2.3 The determination of the ammonium-N concentration in Kjeldahl digest can be accomplished by various methods. The use of distillation and titration is described in this chapter. Other ways to determine the concentration of ammonium-N are spectrophotometric (Baethegen and Alley, 1989; Isaac and Johnson, 1976), diffusion-conductivity (Carlson, 1978; Carlson et al., 1990), and ammonia electrode (Eastin, 1976).

3. Safety

3.1 Precautions should be taken when using cold or heated concentrated sulfuric acid and strong alkali solutions. Violent reactions are always possible. Be sure to wear protective clothing, hand protection and splash-proof goggles. Safety eye wash stations and safety showers should be nearby and technicians should be trained in their use. In addition, a fully stocked spill kit with neutralizers and absorbent should be available in the work area. Sulfuric acid digest is classified as a hazardous waste, regardless of the metal catalyst used, and must be disposed of accordingly.

4. Quality control and quality assurance

4.1 Carry a digestion blank through the entire digestion and measurement process. Perform replicate analysis on 10% of the samples. Replicate results should be within 10 to 15% of the mean value of the replicates. For accuracy assurance run one Standard Reference Material [NIST 1515(apple, 2.25 ± 0.19%N); NIST 2781 (domestic sludge, 4.78 ± 0.11%)]. Control limit is 95–105% recovery of nitrogen. If outside this range, rerun all samples in the same run until control level is achieved.

5. Macro-Kjeldahl (Adapted from Kane, 1998)

5.1 Apparatus

5.1.1 *Digestion rack*—with operational exhaust manifold

5.1.2 *Distillation rack*—fitted with reflux traps, water-jacket condensers, and in-flasks emitters

5.1.3 *Kjeldahl digestion flasks*—500–800 ml

5.1.4 *Erlenmeyer* —500 ml wide mouth

5.1.5 *Burettes*—for titration

5.1.6 *Two carboys*—18 L

5.1.7 *Alundum boiling chips*

5.2 Reagents

5.2.1 *Kjeldahl digestions*

5.2.1.1 *Sulfuric acid (H_2SO_4)*—concentrated

5.2.1.2 *Digestion salt mixture*—should be mixed as follows:

5.2.1.2.1 Potassium sulfate (K2SO4), 1000 g

5.2.1.2.2 Copper sulfate, anhydrous ($CuSO_4$), 32 g

5.2.1.2.3 Quantities can vary but the ratio should be constant

5.2.1.2.4 Premixes of potassium sulfate and copper sulfate can be obtained from suppliers

5.2.2 Ammonia Distillation

5.2.2.1 *Sodium hydroxide (NaOH)* —40%

5.2.2.2 *Mixed indicator*—0.033% methyl red and 0.10% bromocresol green in 95% ethanol

5.2.2.3 *Sodium hydroxide, 0.1N —* Dissolve 72.0 g NaOH in 18 L DI H_2O. Stir to dissolve. Standardize against reference H_2SO_4 (purchased). Store with ascarite trap on air intake. Restandardize each week.

5.2.2.4 *Sulfuric acid, 0.3N —* Dilute 150 ml conc H_2SO_4 in 18 L DI H_2O. Mix thoroughly and standarize against reference NaOH (purchased). Restandardize each week.

5.3 Procedure

5.3.1 Weigh approximately 5 g manure (as received) into 500 ml Kjeldahl flask. Record sample weight to 0.01 g.

5.3.2 Use a method blank with each determination

5.3.3 Add 15g salt/catalyst mixture and 20 ml of concentrated H_2SO_4 to each flask

5.3.4 Place flasks on digestion rack, turn on exhaust manifold. Digest on highest burner setting rotating flasks frequently. Continue digestion for 1 hour after mixture is clear.

5.3.5 Turn off burners and cool digestion flask for 1 hour.

5.3.6 Dilute contents with about 250 ml of distilled water. Allow to cool to room temperature.

5.3.7 Prepare Erlenmeyer receiving flasks by adding 10 ml standardized H_2SO_4, 10 drops of mixed indicator solution, and 100 ml distilled water. Place under condenser with emitter below solution level. Turn on water flow to condenser.

5.3.8 To the digestion flask, add 2-3 Alundum boiling chips, 3-4 drops of mineral oil to prevent foaming, and 70ml 40% NaOH solution. Dispense NaOH solution down the neck to avoid initial mixing. Connect flask to condenser trap and mix thoroughly before heating.

5.3.9 Heat on low to medium setting until foaming subsides, then on medium to high setting until receiving flask contains 200-250 ml total volume (100-150 ml distilled volume).

5.3.10 Titrate with standardized NaOH to greenish-blue endpoint. Record titration volume to 0.1 ml.

5.4 Calculations and reporting

$\%N(TKN) = ((B_{ml} - A_{ml})/C_g) \times Nmeq/ml \times Dmg/meq \times g/1000\ mg \times 100$

Where:
A = Volume (ml) NaOH sample titration
B = Volume (ml) NaOH method blank titration
C = Sample weight (g)
D = Milliequivalent weight of nitrogen,14 mg
N = Normality of NaOH

Report % TKN to 0.01% in manures as received (wet-weight basis).
Report results below 0.01% as below detection limit on a wet-weight basis.
Any sample with <1.0 ml titration volume to reach endpoint should be rerun using a smaller sample size.

5.5 References

1. Baethegen, W. E and M. M. Alley. 1989. A manual colorimetric procedure for measuring nitrogen in soil and plant Kjeldahl digests. Commun. Soil Sci. Plant Anal. 20:961-969.
2. Carlson, R. M. 1978. Automated separation and conductiometric determination of ammonia and dissolved carbon dioxide. Anal. Chem. 48:1528-1531.
3. Carlson, R. M., R. I. Cabrera, J. L. Paul, J. Quick, and R. Y. Evans. 1990. Rapid direct measurement of ammonium and nitrate in soil and plant tissue extracts. Commun. Soil Sci. Plant Anal. 21:1519-1529.
4. Dumas, J. B. A. 1831. Procedes de l'analyse organique. Ann. Chim. Phys. 47:198-205.
5. Eastin, E. F. 1976. Use of ammonia electrode for total nitrogen determination in plants. Commun. Soil Sci. Plant Anal. 7:477-481.
6. Kane, P. F. 1998. AOAC Method 978.02. Nitrogen (total) in Fertilizers. In: Official Methods of Analysis of AOAC International. 16th ed., P. Cunniff (ed), Gaithersburg, Md; AOAC International
7. Hoskins, B. 2001. Personal communications. University of Maine
8. Isaac, R. A. and W. C. Johnson. 1976. Determination of total nitrogen in plant tissue. J. Assoc. Off. Anal. Chem. 59:98-100.

9. Kjeldahl, J. 1883. Neue methode zur bestimmung des stickstoffs in organischem korpern. Z. Anal. Chem. 22:366-382.

10. Scarf, H. 1988. One hundred years of the Kjeldahl method for nitrogen determination. Arch. Acker. Pflanzenbau Bodenk. 32:321-332.

11. Simonne, E. H., J. B. Jones, Jr, H. A. Mills, D. A. Smittle, and C. G. Hussey. 1993. Influence of catalyst, sample weight, and digestion conditions on Kjeldahl N. Commun. Soil Sci. Plant Anal. 24:1609-1616.

12. Watson, M. E. and T. L. Galliher. 2001. Comparison of Dumas and Kjeldahl methods with automatic analyzers on agricultural samples under routine rapid analysis conditions. Commun. Soil Sci. Plant Anal. 32:2007-2019.

6. Micro Kjeldahl Analysis using a block digester *(adapted from Isaac and Johnson, 1976)*

6.1 Apparatus

6.1.1 *Block Digester*—with 75 ml calibrated digestion tubes and scrubber

6.1.2 *Acid fume hood*

6.1.3 *Analytical balance, weighing boats and nitrogen-free filter paper*

6.1.4 *Pipettes*

6.1.5 *Boiling stones*

6.1.6 *Whatman #1 filter paper*

6.1.7 *Filter funnels*

6.1.8 *AutoAnalyzer unit* —with autosampler, pump, manifold, heating bath, colorimeter and computer.

6.2 Reagents

6.2.1 *Distilled water*

6.2 2 Digestion Reagents:

6.2.2.1 *Digestion mixture*—Dissolve 6 g of selenous acid (H_2SeO_3) in 10 ml of distilled water and pour into 2.5 liter bottle of concentrated H_2SO_4. Mix well. Handle cautiously!

6.2.3 Reagents for autoanalyzer:

6.2.3.1 *Complexing mixture*—Dissolve 56 g of NaOH pellets in about 1 L of distilled water in a 4 L beaker. Stir until completely dissolved. Cool. Add 10 g of disodium EDTA and 100 g of potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$). Stir until dissolved. Transfer to a 2 L volumetric flask, cool and add 1 ml (about 20 drops) of Brij-35. Bring to volume. Add the water carefully or the Brij will foam. Filter into reagent bottle using fluted paper.

6.2.3.2 *Alkaline phenol*—Dissolve 400 g NaOH in about 1 L of distilled water in a 4 L beaker. Cool beaker by placing in cold water. Once cool, add 552 ml of phenol slowly and cool again. Transfer to a 2 L volumetric flask, add 0.5 ml of Brij-35 (about 10 drops) and bring to volume. Filter into brown bottle using fluted paper.

6.2.3.3 *Sodium hypochlorite (NAOCl)*— Add 25 ml of distilled water to 75 ml of liquid household chlorine bleach

6.2.4 Standards:

6.2.4.1 *N stock solution (1000 mg/L N)* — Dissolve 4.717 g of reagent grade ammonium sulfate ($(NH_4)_2SO_4$)) in distilled water and bring to volume in a 1 L volumetric flask. Stock will be 1000 mg/L (ppm) N.

N mg/L or ppm	ml of stock solution
0	0
25	50
50	100
75	150
100	200

6.2.4.2 *Working standards* — Dilute stock solution to make up a series of 5 standard solutions from 0 to 100 mg/L (ppm) N. Add the following amounts of the N stock solution to distilled water in 2 L volumetric flasks. Add 20 ml of concentrated H_2SO_4 to each flask and dilute to volume.

6.3 Procedure (digestion)

6.3.1 Sample preparation

6.3.1.1 Sample preparation depends on sample type. All samples should be brought to room temperature and thoroughly mixed or, if necessary, blended before analysis. Sample sizes indicated below are approximate. Actual size may vary depending on anticipated total N content.

6.3.1.2 If sample is a *pipettable slurry* pipette 1-2 ml of the well-mixed manure into a 75 ml calibrated digestion tube. Record volume taken. Rinse the pipette into the tube with a small amount of distilled water.

6.3.1.3 If sample is a *concentrated slurry* pipette 0.5-1 ml or weigh 0.5-1 g of the manure into a 75 ml calibrated digestion tube. Record sample volume or weight. Rinse the pipette or weigh boat with a small amount of distilled water into the tube to ensure quantitative transfer of sample.

6.3.1.4 If sample is *moist or dry solid*, weigh approximately 0.25-0.5 g of sample into a weigh boat or tarred filter paper. Transfer sample into tube. If using a weighing boat, rinse boat contents into tube. If using filter paper, fold filter paper with sample and place into tube. Filter paper type should have been previously tested to ensure that it contains no nitrogen.

6.3.2 Add 6 ml of the digestion mixture and a boiling stone into each tube. Swirl the tube to mix the acid and sample and allow to sit overnight.

6.3.3 Warm the digestion block to about 370-400°C. When the block is to temperature, place tubes in the block and digest for 80 minutes.

6.3.4 Remove the tubes from the block and cool under the fume hood for 30 minutes.

6.3.5 Bring sample tubes to approximate 75 ml volume and mix well. Allow samples to cool again for approximately 1 to 1½ hours and bring to final volume of 75 ml. Mix thoroughly (invert tubes 3 times) and filter through Whatman #1 (or equivalent) filter paper into auto sampler cups.

6.3.6 Prepare auto analyzer for sample analysis following manufacturer's instructions. Ensure that the level of all reagent containers is adequate to complete sample run. Calibrate using the 0-100 mg/L N standards.

6.3.7 Measure N in the digests in mg/L.

6.4 Calculations and reporting

$$\% \text{ N (wet weight basis)} = \frac{\text{N in the digest (mg/L)} \times 0.075 \text{ L} \times 100}{\text{wet weight of sample (mg)}}$$

Note: 0.075 is total volume used, L. To determine results on a dry weight basis, a separate determination of percent solids must be performed (see Method 2). Divide % N (wet weight basis) by [(% Solids/100)] to determine results on dry weight basis.

6.5 Quality control

6.5.1 Carry a digestion blank through the entire digestion and measurement process.

6.5.2 Perform replicate analysis on 10% of samples. Replicate results should be within 10-15% of the mean value of the replicates.

6.5.3 Include one standard reference material (see Laboratory Quality Assurance Program in this manual) with each batch of samples or each group of 30 samples. Results should be within limits specified with reference material.

3.3 Total nitrogen by combustion

(adapted from AOAC 990.3)

1. Introduction

The recent development of instruments capable of the classic Dumas method of combustion for the analysis of nitrogen has made this method more popular for automated, unattended, routine analysis of N in plant tissue, feeds, and soil. Different types of instruments are available. Many combustion instruments can use only a *30-150 mg* sample size in a tin foil, capsule or gel cap.

Due to this small sample size, sample homogeneity is crucial. Obtaining this needed homogeneity in manure samples can be extremely difficult with moist samples that cannot be sieved or blended. Although it has been shown that drying manure samples will decrease the nitrogen content, drying may still be considered an alternative in certain circumstances. If drying is required, microwave drying at 40˚C was found to be the better method of drying over oven drying at 40˚C, 60˚C and air-drying (Wood and Hall, 1991). Also, drying may be an alternative to low moisture manures such as poultry litter samples with initial moisture <25%. A lab study (Wolf, N., 2001) was conducted with 21 poultry litters. A dried (50˚C) and ground (2 mm sieve) 150 mg subsample was analyzed on aq LECO 428 instrument using tin foil capsules while the coarsely (14-inch) sieved as-is sample was analyzed on the LECO CN2000 instrument using a 0.7-1.0 g subsample in a ceramic boat.

All samples were done in duplicate and corrected for moisture content to compare results. Out of the 13 samples with <25% moisture, the average difference between the N analyzed as-is and the N analyzed dry but calculated to as-is basis was 2.3%. One hen manure with 20% moisture was much higher at 22% difference. For samples at 25-56% moisture, the difference averaged 15% indicating that nitrogen was underestimated in the dried samples. The %RPD of the duplicates of the dried, ground samples averaged 3.05%; for the coarsely sieved as-is samples the average %RPD was 4.13% but ranged up to 15%.

While it is recommended that manure samples be analyzed for Total Nitrogen on an as-is basis (without drying), the laboratory must determine and verify which sample preparation methods meet their instrumentation and end use needs.

The nitrogen combustion instruments claim they can be used for liquid samples with minor modifications in the sampling apparatus, the sample containers or by mixing the liquid with an inert absorbent. There have not been enough laboratories that have tried combustion instruments with liquid manure to make any recommendations about their use for this purpose.

2. Principle of the method

2.1 Based upon the Dumas method of flash combustion, a representative sample is converted to its combustion products. This is achieved by igniting the sample in an induction furnace at approximately 950-1350˚C with helium and oxygen carrier gases. The combustion gas or an aliquot of the combustion gas is passed through a Cu catalyst to remove O_2 and convert nitrous oxides to N_2. Moisture and carbon dioxide are removed with magnesium perchlorate and ascarite scrubbers, and the Total Nitrogen is detected by thermal conductivity cell.

2.2 Advantages: The advantages of this method are **1)** no concentrated acids or long digestion time, **2)** fast and automated analysis, and **3)** complete recovery of oxidized forms of N, such as NO_3 and NO_2, and heterocyclic rings unlike the Kjeldahl method.

2.3 Disadvantages: The disadvantages of this method are **1)** high cost of instrumentation, **2)** small sample size (0.100 or less) for some instruments requiring extensive homogenizing and blending to ensure a representative subsample, and **3)** high instrument maintenance, especially when analyzing liquid or high moisture samples.

3. Apparatus

3.1 *Analytical balance*—resolution to 0.1 mg

3.2 *Total Nitrogen Analyzer*—Any instrument or device designed to measure nitrogen by combustion equipped with the following conditions:

3.2.1 Furnace to maintain minimum operating temperature of 95˚C for combustion of sample in pure oxygen.

3.2.2 Isolation system of scrubbers and catalysts to isolate and convert nitrogen combustion gases to N_2.

3.2.3 Nitrogen detection system such as a thermal conductivity cell.

3.3 *Sample containers*—tin foils, tin capsules, gel caps, metal crucibles or ceramic boats depending upon type of instrument used.

4. Reagents and reference standards

4.1 *Helium*—99.996% purity

4.2 *Oxygen*—99.996% purity

4.3 *Air*—breathing quality

4.4 *Consumables*—as instrument dictates, such as reduction Cu, Ascarite, Magnesium Perchlorate, quartz wool, and steel wool.

4.5 *Pure standard organic material*—such as EDTA (9.59% N), glutamic acid (9.52%N), or acetanilide (10.36%N) for calibration.

4.6 *Sludge or manure reference sample*—Sludge or soil reference samples are commercially available from many analytical standard companies. Two known sources include: UltraScientific (800-338-1754) and Environmental Resource Associates (800-372-0122).

5. Procedure

Note: The following procedures apply to the LECO CN2000 Instrument, which uses ceramic boats as sample containers and fairly large sample size. Please see the comments section, which discusses other types of sample sizes.

5.1 Weigh 0.5 to 1.0 g well homogenized sample into a ceramic boat. If possible, analyze all manure samples in duplicate to discover problems with sample heterogeneity as indicated by % relative percent differences >10%. Record sample weight to the nearest 0.1 mg. Cover or store in dessicator to avoid moisture loss while samples are waiting to be analyzed. Include as the first sample, an EDTA calibration standard and as the second sample, the reference sludge sample. Include an EDTA calibration sample every 15-20 samples and as the last sample in the batch to check for drift in the calibration or instrument operation problems.

5.2 Initialize the instrument according to manufacturer's protocol. Conduct a leak check daily. Analyze consecutive blanks until the blanks stabilize at a constant value.

5.3 Enter sample identifications and weights into the instrument. Analyze standards and samples while monitoring instrument operation for proper gas flows, restrictions, and calibration.

5.4 After all samples in the batch are complete, check the EDTA calibration standards within the batch and perform a drift calibration if necessary. If any EDTA standard is greater than +/- 10% and instrument parameters indicate a system leak or restriction occurred within the batch, rerun all samples analyzed after the out of control EDTA.

6. Quality control

6.1 Blank correction should be performed before daily operation and anytime a reagent or carrier gas is replaced.

6.2 A calibration standard such as EDTA, glutamic acid, or acetanilide should be analyzed at the beginning and again at the end of a batch of 30 samples. The amount of standard used should be the same weight as the unknown samples. Drift correction can be applied using the two standards and must be applied when the calibration standard is > +/-5% of the known value.

6.3 Include one standard reference material with each batch of 30 samples. Results should be within limits specified for the reference material.

6.4 Perform replicate analysis on at least 10% of the samples, or every sample if homogeneity is difficult to achieve. Replicate results should be within 10-15% of the mean value of the replicates.

7. References

1. AOAC Official Methods of Analysis. Protein (Crude) in Animal Feed: Combustion Method. (990.03).

2. Forage Analysis Procedures. July 1993. National Forage Testing Association. Omaha, NE.

3. Wolf, N. 2001. Nitrogen comparison data. Unpublished. Agriculture Diagnostic Laboratory, U of AR.

4. Wood, C.W. and B.M. Hall. 1991. Impact of drying method on broiler litter analysis. Commun. Soil Sci. Plant Anal. 22:1677-1688.Total Nitrogen by Combustion.

4. Ammonium nitrogen

John Peters, Ann Wolf and Nancy Wolf

The ammonium nitrogen content of livestock manure is commonly used to help estimate the readily available nitrogen content. Because ammonium is the primary inorganic form of nitrogen in manures, the difference between total and ammonium nitrogen can be used to estimate the manure's organic nitrogen content. Some states use the ratio of ammonium or inorganic nitrogen to total nitrogen for determining first year nitrogen availability while other states use separate availability factors for ammonium and organic nitrogen for this purpose.

There are a number of methods that can be used to successfully measure the ammonium nitrogen content of both liquid and solid manures of various livestock species. The methods, which will be discussed in this chapter, include distillation/titration, colorimetric (usually automated), and electrode. The choice of which method might be most appropriate may depend on equipment availability, sample volume and other factors.

4.1 Ammonium-N determination by distillation

(adapted from AOAC 973.49 & EPA 350.2)

1. Principle of the method

1.1 A sample is diluted with 425 ml of water and distilled in the presence of heavy MgO and mossy zinc into a flask containing 50 ml of boric acid indicator solution. The indicator is then titrated using 0.1 N H_2SO_4.

1.2 Advantages: The advantages of this method are **1)** the use of fresh sample minimizes loss of exchangeable ammonium, **2)** no concentrated acids are needed, **3)** simple distillation equipment can be used, and 4) relatively safe procedure.

1.3 Disadvantages: The disadvantages of this method are **1)** Volatile alkaline compounds such as hydrazine and amines may influence results, **2)** distilling temperature needs to be monitored to prevent foaming or boiling of the sample into condenser, **3)** potential contamination from unclean distillation apparatus, and **4)** potential loss of produced ammonia if flask is not attached to condenser immediately after addition of MgO.

2. Apparatus

2.1 *Analytical balance*—accurate to ±0.001 g

2.2 *Distillation apparatus* —with variable heating mantle.

2.3 *Distillation flasks* —500-800 ml

2.4 *Graduated cylinder*—10 ml

2.5 *Plastic weighing vessels*

2.6 *Erlenmeyer flasks*—500 ml

2.7 *Burette*—50 ml, graduated at 0.1 ml intervals (or automatic titrator)

3. Reagents and materials

3.1 *Magnesium oxide (MgO)*—heavy

3.2 *Zinc, mossy, or Alundum boiling chips*—to prevent bumping

3.3 *Distilled water*

3.4 *Boric acid indicator solution*—Add 280 g of reagent-grade boric acid (H_3BO_3) to approximately 3000ml of distilled water in a 4000 ml glass beaker. Stir and heat mixture until boric acid is dissolved. Bring to 3500 ml with distilled water. Add hot solution to 20 L carboy and rinse beaker with 3500 ml distilled water. Add the rinse to carboy. Repeat with another 280 g boric acid in 3500 ml water and 3500 ml rinse. Add 164 ml of indicator solution (prepared by dissolving 0.4000 g bromocresol green and 0.0800 g methyl red in 480 ml ethanol) and swirl carboy to mix completely. Makes 14 L.

3.5 *Sulfuric acid (H_2SO_4)*—0.1 N

4. Procedure

4.1 Weigh a well-mixed sample (as received) to the nearest 0.001 g into distillation flask. Sample size should reflect approximately 1.0 to 2.0 g dry sample

4.1.1 For liquid samples, add approximately 10 ml to a 10 ml-graduated cylinder, weigh and pour contents into flask. Re-weigh cylinder and residual to determine sample mass.

4.1.2 For solid or semi-solid samples, add 3.0 to 5.0 g of sample into a plastic weighing vessel. Record weight of sample and vessel,

add sample to distillation flask and reweigh measuring vessel and residue.

4.1.3 Alternately, weigh sample using a tared vessel, pour sample into Kjeldahl flask and rinse weighing vessel with distilled water. Not recommended for samples that adhere to sides of weighing vessel.

4.2 Add 50 ml boric acid indicator to 500 ml Erlenmeyer flask and position under condenser of distillation apparatus so that the tip of the condenser is below the surface of the indicator.

4.3 Add 300 ml distilled water to distillation flask.

4.4 Add approximately 2 g heavy MgO to distillation flask. A calibrated metal scoop may be used to measure MgO.

4.5 Add approximately 10 to 15 g mossy zinc to flask.

4.6 Rinse down flask neck with 225 ml distilled water.

4.7 Immediately attach distillation flask to condenser.

4.8 Begin heating flask at a low setting until foaming subsides. Once flask begins to boil, gradually turn up burners until a steady, rolling boil is achieved.

4.9 Collect distillate until volume in collection flasks is 200 ml (about 1½ hours).

4.10 Titrate with 0.1 N sulfuric acid. The end point is a violet color partly between the initial blue and the final bright pink.

5. Calculations

5.1 Percent ammonium on a dry matter basis

$$\%\ NH4\text{-}N = \frac{(A - B) \times 14.01 \times (10 * C)}{\text{Sample wt (g)} \times \%\ DM}$$

Where:

A = Volume of H_2SO_4 titrated for sample (ml)
B = Volume of H_2SO_4 titrated for blank (ml)
C = Normality of Sulfuric Acid used

6. Quality control

6.1 Carry a blank through the distillation and measurement process.

6.2 Perform replicate analysis on 10% of samples. Replicate results should be within 10-15% of the mean value of the replicates.

6.3 If available, include one standard reference material (see Laboratory Quality Assurance Program section in this manual) with each batch of samples or each group of 30 samples. Results should be within limits specified with reference material

4.2 Ammonium-N determination by electrode

(*adapted from Standard Methods for the Examination of Water and Wastewater, Method 4500-NH3F*)

1. Principle of the method

1.1 Dissolved ammonia ($NH_{3\ (aq)}$ and NH_4^+) are converted to $NH_{3\ (aq)}$ by adding a strong base to the standards and samples and raising the pH above 11. The NH_3 concentration is measured with an ammonia selective electrode.

1.2 Advantages: The advantages of this method are that **1)** it is fairly simple to perform, **2)** the required instrumentation is relatively inexpensive, and **3)** color and turbidity do not affect the measurement.

1.3 Disadvantages: The disadvantages of this method are that **1)** amines create a positive interference and **2)** high concentrations of dissolved ions can affect the measurement, and **3)** mercury and silver, if present, can interfere by complexing with ammonia.

2. Apparatus

2.1 *Ammonia selective electrode and meter*

2.2 *Magnetic stirrer*—thermally insulated, and Teflon-coated stir bars

2.3 *Top-loading balance*

2.4 *Stainless steel spatula*

2.5 *Pipettes*

2.6 *Beakers* —150 ml

2.7 *Erlenmeyer flasks*—125 ml

2.8 *Table-top reciprocating shaker*

3. Reagents

3.1 *Distilled water*

3.2 *10 N Sodium Hydroxide (NaOH)* —Dissolve 400 g NaOH (100 g at a time) in 800 ml water in a 1 L plastic beaker in an ice or cold-water bath on a stirrer. When cool, transfer to a 1 L plastic bottle and dilute to volume with distilled water.

3.3 *Stock ammonium chloride solution*—Dry a sufficient amount of NH4Cl in the oven at 55 °C and cool in desiccator. Dissolve 7.638 g of NH4Cl in distilled water and dilute to 1 L. Store solution in refrigerator. Final solution concentration is 2000 mg/L NH4-N.

3.4 *Standard ammonium chloride solutions* — Bring 2000 mg/L NH_4-N solution to room temperature.

3.4.1 10 mg/L NH_4-N Solution: Pipette 5 ml of 2000 mg/L NH_4Cl stock solution in to a 1 L volumetric flask and bring to volume with distilled water.

3.4.2 100 mg/L NH_4-N Solution: Pipette 25 ml of 2000 mg/L NH_4Cl stock solution in to a 500 ml volumetric flask and bring to volume with distilled water.

4. Procedure

4.1 Electrometer calibration

4.1.1 Place 100 ml of each standard solution into 150 ml beakers.

4.1.2 Immerse electrode in the 10 mg/L NH_4-N standard solution and mix on magnetic stirrer. Stirring speed should be such that bubbles are not generated and should be maintained at the same rate for all standards and samples. Temperature of samples and standards should be maintained around 25°C. Add 1 ml of 10 N NaOH to raise the pH above 11. If necessary, add additional NaOH to raise pH above 11 and record volume added. Keep electrode in solution until a stable reading is obtained. Set meter reading on instrument in concentration mode to 10.0 following instrument manufacturer's instructions. Rinse the electrode with distilled water.

4.1.3 Immerse electrode in the 100 mg/L NH_4-N solution and mix on magnetic stirrer as above. Add 1 ml of 10 N NaOH solution to raise the pH above 11. Allow the instrument to stabilize and set the meter reading in concentration mode to 100.0.

4.2 Measurement of samples

4.2.1 Weigh approximately 1.0 to 2.0 g to the nearest 0.001 g of a well-mixed sample (as received) into a 125 ml Erlenmeyer flask and bring to 100 ml final volume. Record sample weight.

4.2.2 Cover flasks with stoppers or Parafilm and place on reciprocating shaker for 1 hour at 175 rpm. Remove from shaker and pour contents into 150 ml beakers

4.2.3 Immerse electrode in sample and mix with magnetic stirrer using the same stirring speed as standards. Add 1 ml of 10 N NaOH and measure pH. If necessary, add additional NaOH to raise pH above 11 and record volume of 10 M NaOH added if greater than 1 ml. Keep electrode in solution until a stable reading is obtained. If reading is outside of range of calibration curve, readjust sample size as necessary to bring the NH_3-N concentration to within the calibration range.

5. Calculations

5.1 The concentrations determined are reported on a wet weight basis in mg/kg.

Sample weight (g) 101

where C is the volume of 10 M NaOH added in excess of 1 ml.

5.2 To determine results on a dry weight basis, a separate determination of percent solids must be performed (see Method 2) Divide results (mg/kg) on a wet weight basis by (% solids/100) to determine results on a dry weight basis.

6. Quality control

6.1 Carry a blank through the extraction and measurement process.

6.2 Perform replicate analysis on 10% of samples. Replicate results should be within 10-15% of the mean value of the replicates.

6.3 Check accuracy of calibration curve with purchased ammonium standard that has certified ammonium-N value.

6.4 If available, include one standard reference material (see Laboratory Quality Assurance Program section in this manual) with each

batch of samples or each group of 30 samples. Results should be within limits specified with reference material.

7. References

1. Methods for the Examination of Water and Waste Water. 1992. Method 4500-NH3F. Ammonia-Selective Electrode Method. 18th Edition Standard, American Public Health Association, 1015 Fifteenth Street, NW, Washington, DC 20005.

4.3 Ammonium-N by colorimetry using an autoAnalyzer

(adapted from USEPA 351.2 and ISO 11732)

1. Principle of the method

1.1 Based upon the modified Berthelot reaction, NH_4^+ is chlorinated to monochloramine, which reacts with salicylate to form 5-aminosalicylate. In the presence of sodium nitroprusside as a catalyst and heat, a green colored complex is formed. Absorption of the formed complex is measured at 660 nm.

1.2 Advantages: The advantages of this method are that **1)** it is very sensitive, **2)** it is rapid and automated, and **3)** interferences (if present) can be eliminated by dilution.

1.3 Disadvantages: The disadvantages of this method are that **1)** initial instrumentation cost can be expensive, **2)** samples must be filtered or centrifuged so particulates do not clog tubing, and **3**) most efficient use is with larger batches of samples rather than individual samples due to time devoted to instrumentation warm-up and reagent preparation.

2. Apparatus

2.1 *Autoanalyzer unit* - consisting of sampler, manifold, proportioning pump, heating bath, colorimeter, and software interface or chart recorder.

3. Reagents

The following reagents pertain to a SKALAR autoanalyzer manifold to determine NH4+ in the range 0.2 -20 mg/L. Follow the manufacturers instructions for other instrumentation and/or concentration ranges. See EPA Method 351.2 in Methods of Analysis in Water and Wastewater (1978) for reagents and manifold set-up using a Technician Autoanalyzer.

3.1 *Buffer solution*—Dissolve 33 g of Potassium sodium tartrate in 800 ml deionized water. Add 24 g Sodium citrate and dissolve. Bring to 1 liter and add 3ml Brij 35 (surfactant). Adjust pH with HCl if necessary to 5.2+/-0.1.

3.2 *Sodium salicylate solution*—Dissolve 25 g sodium hydroxide in about 800 ml deionized water. Add 80 g sodium salicylate and bring to 1 liter. Mix well and store in a dark colored bottle. Solution is stable for about one week.

3.3 *Sodium nitroprusside solution*—Dissolve 1 g sodium nitroprusside in about 800 ml deionized water. Bring to 1 liter volume, mix well and store in a dark colored bottle. Solution is stable for about one week.

3.4 *Sodium dichloroisocyanurate solution*—Dissolve 2 g sodium dichloroisocyanurate in about 800 ml deionized water. Bring to 1 liter volume and mix well. The solution is stable for about one week.

3.5 *Carrier or rinsing solution*—This solution is the matrix and can be deionized water when analyzing Kjeldahl digests or liquid manures. For manure extracts, the carrier should be the extracting solution (ie: 2N KCl).

3.6 *Standards*—From stock ammonium-N solution made from ammonium chloride or from commercial 1000ppm standard, dilute appropriately with the carrier solution to obtain at least 5 standards within the concentration range.

4. Procedure

4.1 This procedure can be used for the analysis of NH_4^+ in TKN digests, liquid manures, and KCl extracts of dry or semi-solid manures. Prepare samples as described below:

4.1.1 TKN digests: (See Method 3.1 Total Kjeldahl Nitrogen in this manual.)

4.1.2 Liquid manures: Prepare subsample by filtering to remove solids which may clog small tubing of instrument.

4.1.3 KCl extract of semi-solid or solid manures: Weigh 0.5 g sample into plastic capped centrifuge tube. Add 30 ml 2N KCl and place on lateral shaker for 20 minutes. Centrifuge and/or filter solution. Previous lab studies indicated that a 1:60 manure/KCl ratio extracted the maximum amount NH_4+ in

poultry manures (<25% moisture). Little research exists which compares extraction solutions, ratios and shaking times for NH4+ extraction from manures.

4.2 Analysis of digest, liquid manure, or extract

4.2.1 Check the level of all reagent containers to ensure an adequate supply. Place all of the reagent lines in their respective containers and warm-up the instrument until a steady baseline is obtained. After a steady baseline is obtained, begin the autosampler. The five standards should be analyzed at the beginning of the sample run and again at the end of the sample run.

5. Calculations

5.1 Prepare the standard curve by plotting peak heights of the standards against the concentration values. This can be done automatically using a software interface or manually using a chart recorder. Compute the sample solution concentration by comparing the sample peak heights with the standard curve.

5.2 Samples that exceed the high standard concentration must be diluted with carrier solution and reanalyzed. The solution concentration of the dilution should be calculated from the calibration curve. Then calculations should be performed to correct for any digestion, extraction or dilution factors.

5.3 TKN calculation- wet weight basis:

$$\text{N, µg/g} = \frac{(\text{µg/ml } NH_4\text{–N in digest – method blank}) \times (\text{digest total vol, ml})}{(\text{sample weight digested-wet basis, g})}$$

%N = N µg/g x dilution factor(if performed) /10,000

5.4 Liquid manure calculation:

$$\text{N, µg/ml} = (\text{µg/ml } NH_4\text{-N in sample – method blank}) \times \text{dilution factor}$$

%N = µg/ml / 10,000

5.5 Semi-solid or dry manure calculation– wet weight basis

$$\text{N, µg/g} = \frac{(\text{µg/ml } NH_4 - \text{N in extract – method blank}) \times (\text{extract total vol ml})}{(\text{sample weight extracted- wet basis, g})}$$

%N = N µg/g x dilution factor(if performed) /10,000

5.6 To determine results on a dry weight basis, a separate determination of % solids or dry matter must be performed (see Method 2). Divide results on a wet weight basis by (%DM/100) to determine results on a dry weight basis.

6. Quality control

6.1 Carry a digestion or extraction blank through the entire digestion or extraction and measurement process.

6.2 Perform replicate analysis on 10% of the samples or at least 1 per batch. Replicate results should be within 10-15% of the mean value of the replicates.

6.3 Include one standard reference material with each batch of samples or each group of 30 samples. Results should be within limits specified for the reference material.

7. References

1. International Organization for Standardardization.. Water quality – Determination of ammonium nitrogen by flow analysis (CFA and FIA) and spectrometric determination. ISO 11732:1997.
2. Keeney, D.R. and Nelson, D.W. Nitrogen - Inorganic Forms *In* Methods of Soil Analysis, Part 2. A.L. Page (ed). 1982.
3. USEPA. Method 351.2 Nitrogen, Kjeldahl, Total. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020. Revised 1983.

5. Digestion and dissolution methods for P, K, Ca, Mg and trace elements

Ann Wolf, Maurice Watson, Nancy Wolf

5.1. Introduction

To accurately determine the concentrations of the elemental constituents in manure, it is necessary to destroy the organic matter components and make soluble the elements of interest. There are numerous methods that can be used for this purpose (Bock, 1979, Gorsuch, 1970). However, for the purpose of this manual, methods deemed relatively simple and safe are recommended for use in a routine testing laboratory. The methods that are predominantly used involve: **(1)** heating the sample to high temperature in a muffle furnace under normal atmospheric oxygen conditions and adding acid to the resulting ash (Isaac, 1998); **(2)** adding concentrated acid to the sample and then incorporating heat (U.S. EPA, 1992); and **(3)** adding concentrated acid to the sample in a sealed vessel and heating the sample with microwave energy (Kingston and Jassie, 1988; Binstock et al., 1989; U.S. EPA, 1994). Digestion can take place in open or closed vessels. Closed vessels are used if volatile elements are a concern. The objective of these methods is to remove the organic matrix and leave the elements dissolved in the solution phase.

The final elemental concentrations represent closely the "total" concentration of the element present in the manure. However, to determine the "total" element concentration, further treatment is usually necessary to remove the effect of silica complexes, which prevent complete dissolution of some elements (Bock, 1979). The methods given in this manual do not address the dissolution of silica that may be in the sample.

The selection of the method depends on several factors. These include the kind of equipment available, the ease of the digestion, kind of sample, element of interest, fume removal, contamination considerations and necessary safety precautions. These methods have been used routinely for the analysis of plant tissue, compost, and other organic matrices (Jones and Case, 1990, Thompson, et al., 2001; Bock, 1979).

5.2 Dry ashing *(adapted from—AOAC 985.01)*

1. Principle of the method

1.1 The sample is heated under normal atmospheric conditions at high temperature such that oxidation of the carbon structures occurs. Carbon, nitrogen, sulfur, and water are volatized from the sample. If the temperature is sufficiently high, other elements will also be volatized. The residual ash is acidified to ensure dissolution of the remaining elements. The concentrations of the elements, depending on the element, are determined with atomic absorption spectrophotometry (AAS) or with inductive coupled plasma emission spectroscopy (ICP-AES).

1.2 Advantages: The advantages of this method are **1)** relatively simple equipment can be used, **2)** very little handling of the sample required, **3)** concentrated acid not required, **4)** relatively safe procedure, **5)** samples can be ashed over night allowing for efficient use of technician time, and **6)** large numbers of samples can be easily handled at one time.

1.3 Disadvantages: The disadvantages of this method are **1)** critical to maintain appropriate ashing temperature, **2)** optimum ashing temperatures may be required for different kinds of samples, **3)** volatile elements can be lost depending on the ashing temperature, **4)** losses due to spray and dust, **5)** losses due to incomplete working up of the ash, **6)** possible reaction with the crucible, **7)** potential contamination from the muffle furnace lining if the furnace is not kept clean.

2. Apparatus

2.1 *Crucible with Cover*—high-form glazed porcelain, or silica-glass

2.2 *Analytical Balance*—accurate to ±0.001 g

2.3 *Muffle Furnace*—heated to 500±50°C

2.4 *Dessication Chamber and Desiccant*

2.5 *Digestion Vial*

2.6 *Volumetric Pipettes*—1, 5, 10, 20, 50 ml (TD)

2.7 *Volumetric Flask*s—100 ml and 1.0

3. Reagents and materials

3.1 *Concentrated Nitric Acid, (HNO_3)*—Reagent grade acid should be analyzed to determine level of impurities. If the method blank is less than the MDL, the acid can be used.

3.2 *Concentrated Hydrochloric Acid, (HCl)*—Reagent grade acid should be analyzed to determine level of impurities. If method blank is less than MDL, the acid can be used.

3.3 *Reagent Water*—Distilled, minimum resistively of 17 MΩ cm

4. Procedure

4.1 Dry sample—Samples must be dry before ashing or they may burst violently inside of the furnace during the ashing procedure. Weigh or pipet a well-mixed sample into a porcelain crucible to provide a sample with an estimated 0.5 to 1.0 g of dry matter. Record sample weight. For liquid or semi-solid manure samples, a high form porcelain crucible may be required to accommodate the sample size needed to provide 0.5 g of dry material. Dry sample according to the procedures specified in the Dry Matter Analysis section of this manual. Record sample weights before and after drying as specified in this method if a percent solids determination of the sample is desired.

4.2 Ash sample—Place crucibles with dried sample into the muffle furnace. Ash in muffle furnace (550°C) for 4 h. Allow sample to cool in desiccator for approximately 1 hour.

4.3 Acid digest—Dissolve ash in 10 ml HCl (1+1) and transfer quantitatively to 100 ml volumetric flask. Dilute to volume with distilled H_2O.

4.4 Elemental determination—Analyze for elements of interest using either atomic absorption spectrophotometry (AAS) or inductively coupled plasma emission spectroscopy (ICP-AES). Ensure that the samples and standards are matrix matched.

5. Calculations

5.1 The concentrations determined are reported on a wet weight basis in µg/g.

$$\text{Result from AAS or ICP} = \frac{(\mu g/ml \text{ in digest}) \times 100 \text{ ml}}{\text{Sample size (g)}}$$

If results for final report are to be in mg/kg, then µg/g = mg/kg

5.2 To determine results on a dry weight basis, divide results (µg/g) on wet weight basis by [(% solids/100)] to determine results on dry weight basis.

6. References

1. Binstock, D. A., W. M. Yeager, P.M. Grohse, and A. Gaskill. 1989. Validation of a Method for Determining Elements in Solid Waste by Microwave Digestion. In: Research Triangle Institute Technical Report Draft, RTI Project Number 321U-3579-24, November, 1989, prepared for the Office of Solid Waste, US Environmental Protection Agency, Washington, DC 20460.
2. Bock, R. 1979. A Handbook of Decomposition Methods in Analytical Chemistry. London, England; International Textbook Publishers.
3. Gorsuch, T. T. 1970. The Destruction of Organic Matter. Elmsford, NY; Pergamon Press Publishers
4. Isaac, R. A. 1998. AOAC Method 985.01. Metals and Other Elements in Plants. In: Official Methods of Analysis of AOAC International. 16th ed., P. Cunniff (ed.), Gaithersburg, Md: AOAC International.
5. Jones, J. B., Jr and V. W. Case. 1990. Sampling, handling, and analyzing plant tissue samples. p. 389-427. In: R. L. Westerman (ed.). Soil Testing and Plant Analysis. No. 3. American Book Series. SSSA. Madison, WI.
6. Kingston, H. M. and L. B. Jassie, eds. 1988. Introduction to Microwave Sample Preparation Theory and Practice. ACS Professional Reference Book Series. American Chemical Society: Washington, DC.
7. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, US EPA SW-846, 3rd ed., November 1992.

8. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, US EPA SW-846, 3rd ed., Revision September 1994.

9. Test Methods for the Examination of Composting and Compost, 2001. W. H. Thompson, P. B. Leege, P. D. Millner and M. E. Watson (eds.) U.S. Gov. Print. Office, Washington, DC (in press).

5.3 Microwave-assisted acid digestion

(adapted from EPA 3051)

1. Principle of the method

1.1 A representative sample containing up to 0.5 g dry matter is digested in 10 ml of concentrated nitric acid using microwave heating with a suitable laboratory microwave unit. The sample and acid are placed in a fluorocarbon (PFA or TFM) microwave vessel. The vessel is capped and heated in the microwave unit. After cooling, the vessel contents are filtered, centrifuged, or allowed to settle and then diluted to volume and analyzed by atomic absorption spectrophotometry (AAS) or inductively coupled plasma emission spectroscopy (ICP-AES).

1.2 Advantages: The advantages of this method are **1)** volatile elements are not lost since the digestion is performed in closed vessels, **2)** minimal potential for cross-contamination of samples, **3)** rapid digestion time, **4)** minimal handling of sample is required, and **5)** relatively safe procedure.

1.3 Disadvantages: The disadvantages of this method are **1)** expensive equipment (laboratory microwave and vessels) required, **2)** relatively small number of samples can be handled at one time, **3)** concentrated acids required, **4)** potential sample loss through venting if sample size is too large.

2. Apparatus

2.1 *Microwave digestion system*—commercially designed for laboratory use with minimum power delivery of 600 watts and with pressure and temperature monitoring. Microwave unit cavity must be corrosion resistant and well-ventilated with electronics protected against corrosion for safe operation. Unit should have a rotating turntable to insure homogenous distribution of microwave radiation within the unit. The speed of the turntable should be a minimum of 3 rpm.

Caution: Kitchen-type microwave ovens must not be used for this method due to safety concerns. **a)** When an acid such as nitric is used to assist sample digestion in microwave units with open or sealed vessels, the potential exists for the acid gases released to corrode the safety devices that prevent the microwave magnetron from shutting off when the door is opened. This can result to operator exposure to microwave energy. **b)** There is a safety concern related to the use of sealed containers without pressure relief valves in the microwave unit. Temperature is the important variable controlling the reaction. Pressure is needed to attain elevated temperatures but must be safely contained. However, many digestion vessels constructed from certain fluorocarbons may crack, burst, or explode in the unit under certain pressures. Only unlined fluorocarbon (PFA or TFM containers with pressure relief mechanisms) or containers with PFA-fluorocarbon liners and pressure relief mechanisms are considered acceptable at present.

2.2 *Fluorocarbon (PFA or TFM) digestion vessels* —(100-120 ml capacity) capable of withstanding pressures up to 7.5 ± 0.7 atm (110 ± 10 psi) and capable of controlled pressure relief at pressures exceeding 7.5 ± 0.7 atm (110 ± 10 psi).

2.3 *Pressure vessel liners and safety membranes*

2.4 *Fast delivery pipettes*—10 and 15 ml

2.5 *Volumetric flasks* — 100 ml

2.6 *Plastic storage bottles* —100 ml

2.7 *Filter funnels* — glass, plastic or disposable polypropylene

2.8 *Whatman No. 40 or 41 filter paper*

2.9 *Analytical balance*—300 g capacity minimum accuracy of ± 0.01 g.

Note: All digestion vessels and glass and plasticware must be carefully acid washed and rinsed with reagent water. If performing trace element or metal analysis a more

extensive washing procedure should be performed when switching between high concentration samples and low concentration samples for the trace analytes of interest: all digestion vessels should be cleaned by leaching with hot (1:1) hydrochloric acid (greater than 80° C but less than boiling) for a minimum of two hours followed with hot (1:1) nitric acid (greater than 80° C, but less than boiling) for a minimum of two hours and rinsed with reagent water and dried in a clean environment. This cleaning procedure should also be used whenever the prior use of the digestion vessels is unknown or cross contamination from vessels is suspected. Polymeric or glass volumetric ware and storage containers should be cleaned by leaching with more dilute acids (approximately 10% v/v) appropriate for the specific plastics used and then rinsed with reagent water and dried in a clean environment.

3. Reagents

3.1 *Concentrated nitric acid, HNO_3*—purified and certified for trace element analysis. All acids should be sub-boiling distilled where possible to minimize the blank levels due to metallic contamination. Other grades may be used provided it is first ascertained that the reagent is of sufficient purity to permit its use without lessening the accuracy of the determination. Acid should be analyzed to determine level of impurities. If the method blank is less than the method detection limit, the acid can be used.

3.2 *Reagent Water*—Distilled, minimum resistivity of 17 MW·cm

4. Procedure

4.1 Calibrate microwave equipment. Note: if the microwave unit uses temperature feedback control capable of replicating the performance specifications of the method, then the calibration procedure may be omitted. Otherwise, follow calibration procedure recommended by EPA (ref).

4.2 Weigh or pipette a well-mixed sample (as received) to the nearest 0.001 g into the fluorocarbon sample vessel equipped with a single ported cap and pressure relief valve. Adjust sample size to obtain a sample of between 0.1 to 0.5 g of solids.

4.2.1 For liquid samples, pipet 10 to 15 ml of the sample into the vessel. Record sample size. Rinse pipet with distilled water into the sample vessel and bring all vessels up to approximately 20 ml to ensure constant sample volume of vessels during the microwave digestion.

4.2.2 For solid or semi-solid samples, weigh 0.100 to 5.000g of sample into the vessel to achieve a final sample size that yields from 0.1 to 0.5 g of solids. Record sample weight. If necessary, rinse weighing boat into the vessel. Bring all samples up to approximately 5 ml to ensure equal sample volume of vessels during the microwave digestion.

4.3 Add 10 ml ± 1 ml of concentrated nitric acid in a fume hood. If a vigorous reaction occurs, allow the reaction to stop before capping the vessel. Cap the vessel and torque the cap according to the unit manufacturer's directions. Weigh the vessels to the nearest 0.001 g and place the vessels in the microwave carousel.

Caution 1: Toxic nitrogen oxide fumes may be evolved, therefore all work must be performed in a properly operating ventilation system. The analyst should also be aware of the potential for a vigorous reaction. If a vigorous reaction occurs, allow to cool before capping the vessel.

Caution 2: When digesting samples containing volatile or easily oxidized organic compounds, initially weigh a sample of no more than 0.1 g dry matter and observe the reaction before capping the vessel. If a vigorous reaction occurs, allow the reaction to cease before capping the vessel. If no appreciable reaction occurs, a sample weight containing up to 0.50 g of dry matter can be used.

4.4 Weigh the vessels to the nearest 0.001 g and place the vessels in the microwave carousel according to the manufacturer's recommended specifications. It is important that all vessels in the microwave contain the same volume of liquid. Consequently, samples for liquid manure containing a final vessel volume of 30 ml (20 ml of sample plus water and 10 ml of nitric acid) should be digested with other liquid manures. Samples for manures (semi-solid or solid) with a final

vessel volume of 15 ml (5 ml of sample plus water and 10 ml of nitric acid) should be digested with other semi-solid and solid manures.

4.5 When fewer than the recommended number of samples are to be digested, the remaining vessels should be filled with 20 ml of water and 10 ml of nitric acid (when digesting liquid manures) or with 5 ml of water and 10 ml of nitric acid (when digestion semi-solid or solid manures) to achieve the full complement of vessels. This provides an energy balance since the microwave power absorbed is proportional to the total mass in the cavity.

4.6 Irradiate each group of samples for 12 to 17 minutes. The temperature of each sample should rise to 175° C in less than 6 to 7 minutes and remain between 170° C to 180° C for the balance of the 12 to 17 minute period. While the original EPA 3051 procedure (USEPA, 1986) calls for a 10 minute digestion time (up to 175° C in less than 5.5 minutes and between 170-180° C for the balance of the 10 minutes), this procedure was for developed for samples containing a total final volume of 10 ml. Because of the larger sample volume when digesting manures, a longer digestion period may be required in some microwaves to reach the temperature specified and to ensure complete digestion of all samples.

Note: The pressure should peak at less than 6 atm for most samples. The pressure will exceed these limits in the case of high concentrations of carbonate or organic compounds. In these cases, the pressure will be limited by the relief pressure of the vessel to 7.5 ± 0.7 atm (110 ± 10 psi). All vessels should be sealed according to the manufacturer's recommended specifications.

4.7 At the end of the microwave program, allow the vessels to cool for a minimum of 5 minutes before removing them from the microwave unit. When the vessels have cooled to room temperature, weigh and record the weight of each vessel assembly. If the weight of the acid plus sample has decreased by more than 10% from the original weight, determine the reason for the weight loss and repeat the digestion process following steps 4.2 through 4.7.

4.8 Complete the preparation of the sample by carefully uncapping and venting each vessel in a fume hood. Transfer the sample to an acid-cleaned bottle. If the digested sample contains particulates which may clog nebulizers or interfere with the injection of the sample into the instrument, the sample may be centrifuged, allowed to settle or filtered.

4.8.1 *Centrifugation:* Centrifugation at 2,000-3,000 rpm for 10 minutes is usually sufficient to clear the supernatant. Dilute to a final volume of 100 ml with distilled water.

4.8.2 *Settling:* Allow the sample to stand until the supernatant is clear. Allowing a sample to stand overnight will usually accomplish this. If it does not, centrifuge or filter the sample. Sample should be diluted with distilled water to 100 ml final volume.

4.8.3 *Filtering:* Filter the sample through Whatman 40, 41 or equivalent filter paper into 100 ml volumetric flasks. Dilute to volume with distilled water.

4.9 The digest is now ready for analysis for elements of interest using either atomic absorption spectrophotometry (AAS) or inductively coupled plasma emission spectroscopy (ICP-AES). Ensure that the samples and standards are matrix matched.

5. Calculations

5.1 The concentrations determined are reported on a wet weight basis in µg/g.

$$\frac{\text{Result from AA or ICP (µg/ml in digest) x final vol (ml)}}{\text{Sample size (g)}}$$

5.2 To determine results on a dry weight basis, a separate determination of percent solids must be performed (see Method 2). Divide results (mg/kg) on wet weight basis by (% solids/100) to determine results on dry weight basis.

6. Quality control

6.1 Carry a digestion blank through the entire digestion and measurement process.

6.2 Perform replicate analysis on 10% of samples. Replicate results should be within 10-15% of the mean value of the replicates.

6.3 Include one standard reference material (see Laboratory Quality Assurance Program in this manual) with each batch of samples or each group of 30 samples. Results should be within limits specified with reference material.

7. References

1. USEPA. 1986. Method 3051. Acid Digestion of Sediments, Sludges and Soils. Test Methods for Evaluating Solid Waste. Volume 1 A: 3rd Edition. EPA/SW-846. National Technical Information Service. Springfield, VA.

5.4 Nitric and hydrochloric acid digestion with peroxide

(adapted from EPA 3050)

1. Principle of the method

1.1 A representative sample containing up to 0.5 g dry matter is digested in nitric acid and hydrogen peroxide. The digestate is then refluxed with nitric and hydrochloric acid. After cooling, the vessel contents are filtered, centrifuged, or allowed to settle and then diluted to volume and analyzed by atomic absorption spectrophotometry (AAS) or inductively coupled plasma emission spectroscopy (ICP-AES).

1.2 Advantages: The advantages of this method are **1)** equipment required is relatively inexpensive and easy to use and **2)** a large number of samples can be digested simultaneously if large hot plate is used, **3)** volatile elements are not lost.

1.3 Disadvantages: The disadvantages of this method are **1)** potential for cross-contamination of samples during refluxing, **2)** it is labor-intensive in comparison to other digestion methods, **3)** a lengthy digestion time required and **4)** concentrated acids are required.

2. Apparatus

2.1 *Hot plate*

2.2 *Conical Phillips beakers* — 250 ml

2.3 *Watch glasses*

2.4 Thermometer — covers the range of 0 to 200° C

2.5 *Fast delivery pipettes* — 10 and 15 ml

2.6 *Volumetric flasks* — 100 ml

2.7 *Plastic storage bottles* — 100 ml

2.8 *Filter funnels* — glass, plastic or disposable polypropylene

2.9 *Whatman No. 41 filter paper*

2.10 *Analytical top*—loading balance

3. Reagents

3.1 *Reagent water* — distilled, minimum resistivity of 17 MW·cm

3.2 *Concentrated nitric acid (HNO_3)*—purified and certified for trace element analysis if trace elements are of interest.

3.3 *Concentrated hydrocholoric acid (HCl)*—purified and certified for trace element analysis if trace elements are of interest.

3.4 Hydrogen Peroxide—30% H_2O_2

4. Procedure

4.1 Weigh or pipette a well-mixed sample (as received) to the nearest 0.001 g into a conical Phillips beaker. Adjust sample size to obtain a sample of approximately 0.5 g of solids.

4.2 For liquid samples, pipette 10 to 15 ml of the sample into the vessel. Record sample size. Rinse pipette with distilled water into the sample beaker.

4.3 For solid or semi-solid samples, weigh 0.100 to 5.000 g of sample into the vessel to achieve a final sample size that yields approximately 0.5 g of solids. Record sample weight. If necessary, rinse weighing boat into the beaker.

4.4 Add 10 ml of 1:1 HNO_3, mix the slurry, and cover with a watch glass. Heat the sample to 95°C on a hot plate in a hood with an exhaust fan. Reflux for 10 to 15 minutes without boiling. Allow the sample to cool, add 5 ml of concentrated HNO_3, replace the watch glass and reflux for 30 minutes. Repeat this

last step to ensure complete oxidation. Using a ribbed watch glass, allow the solution to evaporate to 5 ml without boiling, while maintaining a covering of solution over the bottom of the beaker.

4.5 After step 4.2 has been completed and the sample has cooled, add 2 ml of distilled water and 3 ml of 30 % H_2O_2. Cover the beaker with a watch glass and return the covered beaker to the hot plate for warming and to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat until effervescence subsides and cool the beaker.

4.6 Continue to add H_2O_2 in 1-ml aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged. Note: do not add more than a total of 10 ml of 30% H_2O_2.

4.7 Remove the beakers from the hot plate and add 5 ml of concentrated HCl and 10 ml of distilled water to the beakers. Return the beaker to a hot plate and reflux for an additional 15 minutes without boiling.

4.8 Dilute the sample to 100 ml and transfer to an acid-cleaned bottle. If the digested sample contains particulates which may clog nebulizers or interfere with the injection of the sample into the instrument, the sample may be centrifuged, allowed to settle or filtered.

4.9 *Centrifugation:* Centrifugation at 2,000-3,000 rpm for 10 minutes is usually sufficient to clear the supernatant. Dilute to a final volume of 100 ml with distilled water.

4.9.1 *Settling*—Allow the sample to stand until the supernatant is clear. Allowing a sample to stand overnight will usually accomplish this. If it does not, centrifuge or filter the sample. Sample should be diluted with distilled water to 100 ml final volume.

4.9.2 *Filtering*—Filter the sample through Whatman 41 or equivalent filter paper into 100 ml volumetric flasks. Dilute to volume with distilled water.

4.10 The digest is now ready for analysis for elements of interest using either atomic absorption spectrophotometry (AAS) or inductively coupled plasma emission spectroscopy (ICP-AES). Ensure that the samples and standards are matrix matched. The diluted sample has an approximate acid concentration of 5% (v/v) HCL and 5% (v/v) HNO_3.

5. Calculations

5.1 The concentrations determined are reported on a wet weight basis in µg/g.

$$\frac{\text{Result from AA or ICP (µg/ml in digest) x final vol (ml)}}{\text{Sample size (g)}}$$

5.2 To determine results on a dry weight basis, a separate determination of percent solids must be performed (see Method 2). Divide results (mg/kg) on wet weight basis by (% solids/100) to determine results on dry weight basis.

6. Quality control

6.1 Carry a digestion blank through the entire digestion and measurement process.

6.2 Perform replicate analysis on 10% of samples. Replicate results should be within 10 - 15% of the mean value of the replicates.

6.3 Include one standard reference material (see Laboratory Quality Assurance Program section of this manual) with each batch of samples or each group of 30 samples. Results should be within limits specified with reference material.

7. References

1. USEPA. 1986. Method 3050. Acid Digestion of Sediments, Sludges and Soils. Test Methods for Evaluating Solid Waste. Volume 1 A: 3rd Edition. EPA/SW-846. National Technical Information Service. Springfield, VA.

5.5 Nitric acid digestion with peroxide using a block digester

1. Principle of the method

1.1 A representative sample containing up to 0.5 to 1.0 g dry matter is digested in nitric acid and hydrogen peroxide in calibrated digestion tubes in a block digester. The hydrogen peroxide is used to enhance the reaction speed and complete digestion. After cooling, the digestion tubes are brought to volume with dilute nitric acid, hydrochloric acid or a combination of the two acids and distilled water. The solution is mixed and filtered, and ready for analysis by atomic absorption or ICP.

1.2 Advantages: The advantages of this method are **1)** digestion blocks allow very uniform temperature control, **2)** constricted digestion tubes minimizes cross contamination between samples from spattering, and **3)** calibrated digestion tubes allow samples to be brought to volume without transferring errors, and **4)** large numbers of samples (40-60) can be digested in a relatively small space.

1.3 Disadvantages: The disadvantages for this method are **1)** commercial digestion blocks and calibrated digest tubes can be expensive, **2)** adding peroxide while digest tubes on the block require special pipetting equipment, and **3)** concentrated acids are required.

2. Apparatus

2.1 *Commercial block digester or modified hot plate digestion block*

2.2 *Constricted top or Folin-Wu calibrated digest tubes*—50 or 100 ml total volume

2.3 *Fume hood*

2.4 *Jet pipet or other automatic pipette*—1–5 ml range

2.5 *Vortex mixer or stoppers to fit digest tubes*

2.6 *Filter funnels*

3. Reagents and reference standard

3.1 *Distilled water*

3.2 *Concentrated nitric acid* (HNO_3)

3.3 30% hydrogen peroxide (H_2O_2)

3.4 Sludge or manure reference sample—Sludge reference samples are commercially available from many analytical standard companies. Two known sources include: UltraScientific (800-338-1754) and Environmental Resource Associates (800-372-0122).

4. Procedure

4.1 Weigh or pipet a well-mixed sample (as received) to the nearest 0.001 g into the digest tube. Adjust sample size as needed as dependent upon the type of sample analyzed.

4.4.1 *Liquid samples:* Pour or pipet 5 to 10 ml of the sample into the tube using a hollow rod to take up liquid and suspended solids. Record sample weight. Very low solid samples may require more volume and need to be concentrated by evaporation by heating at low temperature if the digest tubes are small diameter.

4.1.2 *Solid and semi-solid samples:* Weigh enough of the homogenized sample to achieve a final sample size of approximately 0.5 g solids. Record the sample weight.

4.2 Add 5.0 ml of concentrated nitric acid and allow to stand at least one hour or overnight to prevent frothing and foaming when heat is applied.

4.3 Place digestion tube onto block digester and heat slowly to reach 60° C in 1/2 hour. Dark reddish brown fumes will begin to form in the tubes.

4.4 With the tubes still on the digester, carefully (wearing long gloves) add 1 ml of 30% hydrogen peroxide using a Jet-Pipet or other automatic pipet by directing the peroxide down the sides of the tube. Add 1 ml peroxide to all tubes then go back and repeat 2 more times for a total of 3 ml peroxide added to each tube.

4.5 If frothing or foaming occurs, carefully remove tube and swirl to clean off sides.

4.6 Raise the temperature gradually to 120°C and heat at that temperature until 2 to 3 ml of acid remains (about 2 hours). The solution should be pale yellow or clear if digestion is complete.

4.7 Remove the tubes from the digester to cool. Dilute the solution to 25, 50 or 100 ml according to the calibrated tubes with distilled water. Mix on a vortex mixer, with a Teflon rod, or by inverting the stoppered tubes.

4.8 Filter the solution through qualitative filter paper only if Na and/or S are not analytes of interest. Filter through quantitative filter paper if Na and/or S are desired.

4.9 The digest is now ready for analysis for elements of interest using either atomic absorption spectrophotometry (AAS) or inductively coupled plasma emission spectroscopy (ICP-AES). Ensure that the samples and standards are matrix matched.

5. Calculations

5.1 The concentrations determined are reported on a wet weight basis in µg/lg.

$$\frac{\text{Result from AA or ICP (µg/ml in digest) x final vol(L)}}{\text{Sample size (kg)}}$$

5.2 To determine results on a dry weight basis, a separate determination of percent solids must be performed (see Method 2). Divide results (mg/kg) on wet weight basis by (% solids/100) to determine results on dry weight basis.

6. Quality control

6.1 Carry a digestion blank through entire digestion and measurement process.

6.2 Perform replicate analysis on 10% of samples. Replicate results should be with 10-15% of the mean value of the replicates.

6.3 Include one standard reference material with each batch of samples. Results should be within.

6.4 Limits specified for the reference material.

7. References

1. Campbell, C.R. and C.O. Plank. 1991. Sample Preparation. p. 1-11. In C. Owen Plank (ed.) Plant Analysis Reference Procedures for the Southern Region of the United States. Southern Cooperative Series Bulletin #368.

2. Huang, C.L. and E.E. Schulte. 1985. Digestion of plant tissue for analysis by ICP emission spectroscopy. Commun. Soil Sci. Plant Anal. 16:943-958.

3. Tucker, M.R. 1974. A modified heating block for plant tissue digestion. Commun. Soil Sci. Plant Anal. 5:539-546.

6. Methods of determination of P, K, Ca, Mg and trace elements

John L. Kovar

6.1 Introduction

Spectrochemical methods have been used for many years to determine elemental concentrations in extracts and digests of soils, plant tissue, manures, composts, and other materials. In this section, three methods for determining elemental concentrations in manure digests are discussed. Although a number of other techniques are available, atomic absorption spectroscopy, inductively coupled plasma-atomic emission spectroscopy, and colorimetry are the methods most commonly used by public and private laboratories. Atomic absorption spectroscopy can be used to determine the concentration of most elements of interest, with phosphorus being the exception. Inductively coupled plasma-atomic emission spectroscopy is the method of choice for the analysis of manure digests. The technique has excellent sensitivity coupled with a usable linear concentration range of four to five orders of magnitude for most elements of interest. The classical colorimetric procedure for the determination of phosphorus is still used, although many laboratories have automated the procedure with either an AutoAnalyzer or flow injection system (Isaac and Jones, Jr., 1970; Shaw et al., 1988).

6.2 Atomic absorption spectroscopy

(adapted from EPA 7000a)

1. Principles of method

1.1 This section covers the determination of elemental concentrations in digest solutions by means of atomic absorption spectroscopy (AAS). The method is simple, rapid, and applicable to most elements of interest in animal manures. Detection limits vary considerably among the elements of interest (table 6.2-1), and depend on both equipment (e.g., type of spectrophotometer, the energy source, and the degree of electrical expansion of the output signal) and sample matrix. Detection limits should be established empirically for each type of matrix analyzed.

Table 6.2-1. Atomic absorption spectrophotometry detection limits in water (Wright and Stuczynski, 1996).

Element	Detection Limit mg L^{-1}
Calcium	0.01
Copper	0.02
Iron	0.03
Magnesium	0.001
Manganese	0.01
Potassium	0.01
Sodium	0.002
Zinc	0.005

1.2 In direct-aspiration AAS, a sample is aspirated and atomized in a flame. This produces free, unexcited ground-state atoms in the flame. A light beam from a hollow cathode lamp or an electrodeless discharge lamp (EDL) is directed through the flame into a monochromator, and onto a detector that measures the amount of absorbed light. A wavelength is chosen to match the absorption characteristics of the element being determined. The light energy absorbed by the atoms in the flame is a measure of the concentration of that element in the sample. This is the basis of AAS. When direct-aspiration atomic absorption techniques do not provide adequate sensitivity, specialized procedures such as graphite furnace AAS and the gaseous-hydride method for arsenic and selenium can be utilized.

1.3 Advantages: The advantages of AAS are: **1)** it is highly specific for an individual element; **2)** there is minimal spectral interference; **3)** detection limits are lower than those with flame emission for some elements; and **4)** AAS instruments are relatively easy to operate.

1.4 Disadvantages: The primary disadvantages of AAS are: **1)** chemical interferences occur for elements that form stable compounds,

and the flame is not hot enough to dissociate the molecule; **2)** ionization enhancement of the signal occurs when the flame temperature is sufficiently high to generate the removal of an electron from a metal atom, i.e., produce cations (especially Na and K); **3)** matrix interferences are caused by viscosity or specific gravity differences between the sample and standards; **4)** range of linearity of one to two orders of magnitude is much less than for ICP spectroscopy (Section 6.3); **5)** only one element can be analyzed during each run; and **6)** the method is not suitable for phosphorus analysis. Chemical interferences are the most troublesome type. Addition of lanthanum will overcome interferences in magnesium, calcium, and barium determinations. Similarly, addition of calcium to mixtures of magnesium and silica will allow accurate determinations of Mg concentrations. Complexing agents may also be used to eliminate or reduce interferences. Ionization can generally be controlled by addition of an easily-ionized element, such as Li, Na, K, or Cs, to both standard and sample solutions. Samples and standards should be monitored for viscosity differences, because this may alter aspiration rate. All metals are not equally stable in the digest, so the digest should be analyzed as soon as possible.

2. Sample handling and quality control

2.1 Refer to selected dissolution/digestion method described in Chapter 5 for specific sample handling and preparation procedures.

2.2 Replication of samples and inclusion of reference materials is performed at the digestion phase of the analysis. Included with each batch of 20 samples, is a minimum of one in-house check and one external reference sample, such as those provided by the North American Proficiency Testing (NAPT) program.

2.3 A calibration curve must be prepared each day with a minimum of a calibration blank and three standards. After calibration, the calibration curve should be verified by use of a calibration check standard that is made from a reference material or other independent standard material. The calibration reference standard should fall near the mid-range of the calibration curve and must measure within 10% of its true value for the curve to be considered valid.

2.4 If more than 10 samples per day are analyzed, the working standard curve should be verified by measuring a mid-range standard or reference standard after every 10 samples. This sample value must be within approximately 10% of the true value, or the previous ten samples should be reanalyzed.

2.5 All quality control data should be maintained and available for easy reference or inspection.

3. Apparatus

3.1 *Atomic absorption spectrophotometer*—A single- or dual-channel, single- or double-beam instrument equipped with a grating monochromator, photomultiplier detector, adjustable slits, a wavelength range of 190 nm to 800 nm, and the ability to interface with a graphical display.

3.2 *Burner*—Use the burner recommended by the instrument manufacturer. For certain elements, a separate burner for use with nitrous oxide gas is required.

3.3 *Hollow cathode lamps*—Single-element lamps are preferred, but multi-element lamps may be used. When available, EDL lamps also may be used.

3.4 *Stock standard metal solutions*—Stock standard solutions are prepared from high purity metals, oxides, or non-hygroscopic salts combined with water and reagent grade nitric or hydrochloric acids (see individual methods). Sulfuric and phosphoric acids should be avoided if possible, as they adversely affect many elements. The stock solutions are prepared at concentrations of 1,000 mg of the metal per liter. Commercially available standard solutions also can be used.

3.5 *Calibration standards*—Calibration standards are prepared by diluting the stock solutions at the time of analysis. For best results, calibration standards should be prepared each time a batch of samples is analyzed. Prepare a blank and at least three calibration standards in graduated amounts in the appropriate range of the linear part of the curve. The calibration standards should be prepared with

the same type of acid or combination of acids and at the same concentration as the samples in order to minimize matrix differences. Begin with the blank and work toward the highest standard. Repeat the operation to secure a reliable average reading for each solution.

4. Procedures

Differences among the various makes and models of atomic absorption spectrophotometers make it impractical to outline detailed instructions that apply to every instrument. Follow the manufacturer's operating instructions for a specific instrument.

4.1 After choosing the proper lamp for the analysis, allow the lamp to warm for a minimum of 15 minutes, unless operated in a double-beam mode. During this time, align the instrument, position the monochromator at the correct wavelength, select the proper monochromator slit width, and adjust the current according to the manufacturer's recommendations.

4.2 Light the flame and regulate the flow of fuel and oxidant. Adjust the burner and nebulizer flow rate for maximum percent absorption and stability.

4.3 Run a series of standards containing the element under analysis. If necessary, construct a calibration curve by plotting the concentration of the standards against absorbance. Aspirate the samples and determine the concentrations either directly or from the calibration curve. Standards must be run each time a sample or series of samples is run.

4.4 Elemental concentrations (mg L-1) determined in the digests are then used to calculate initial concentrations in the manure, according to the calculation procedure outlined for each of the dissolution and digestion methods.

6.3 Inductively coupled plasma spectroscopy

(adapted from EPA 6010a)

1. Principles of method

1.1 This section covers the determination of elemental concentrations in digest solutions by means of inductively coupled plasma-atomic emission spectroscopy (ICP-AES). This method is generally superior in accuracy, precision, detection limit, freedom from interferences, and dynamic range than other analytical methodology. Use of automated samplers and computer-assisted electronic data capture facilitates accurate and rapid analyses. With simultaneous instruments, one technician can analyze a solution for many elements in a few minutes, so that large volumes of data can quickly be generated. As with AAS, detection limits (table 6.3-1) depend on both equipment and sample matrix.

Table 6.3-1. Inductively coupled plasma – atomic emission spectrometry detection limits in water *(Soltanpour et al., 1996)***.**

Element	Detection Limit, mg L^{-1}
Boron	0.0007
Calcium	0.00002
Copper	0.0001
Iron	0.0003
Magnesium	0.00001
Manganese	0.00006
Phosphorus	0.02
Potassium	0.01
Sodium	0.0002
Zinc	0.002

1.2 To produce the plasma, argon gas is passed through a quartz torch located inside a copper induction coil that is connected to a radio frequency generator. Current flowing through the induction coil creates a magnetic field. Electrons and ions flowing through the field are accelerated within the torch. Argon gas atoms ionize after colliding with the accelerated electrons and ions.

1.3 The sample is injected into the plasma through a nebulizer system. Aerosol droplets containing the analyte are desolvated, the analyte salts/oxides are vaporized, and the analyte atomized at the high temperature region of the plasma in the vicinity of the Cu coil. At the sample aerosol flow rates typically used in ICP-AES, the high temperature and residence time combination, leads to complete sample vaporization and atomization.

1.4 Once the free compounds, atoms, and ions are formed in ICP-AES, they are in a chemically inert environment.

1.5 Advantages: The advantages of ICP-AES are: **1)** chemical interferences are minimal; **2)** four to six orders of magnitude in linearity of intensity versus concentration are attainable; **3)** rapid, multi-element analyses are possible; **4)** detection limits are equal to or lower than those of AAS for many elements; and **5)** analyses are more accurate and precise compared with other emission sources.

1.6 Disadvantages: The primary disadvantages of ICP-AES are: **1)** spectral interferences may occur, depending on the element and background; **2)** matrix interferences may be caused by viscosity or specific gravity differences between the sample and standards; **3)** the most useful spectral lines may fall out of the range of the spectrometer; **4)** the plasma, generated in argon with normal aqueous solution nebulization, may be unable to produce measurable amounts of positive ions for some analytes that could be of interest, e.g. Cl and/or S; and **5)** the initial purchase, operation, and maintenance of the equipment are expensive, relative to AAS.

2. Sample handling and quality control

2.1 Refer to selected dissolution/digestion method for specific sample handling and preparation procedures.

2.2 Replication of samples and inclusion of reference materials is performed at the digestion phase of the analysis.

2.3 Included with each batch of 20 samples, is a minimum of one in-house check, and one external reference sample, such as those provided by the NAPT program.

2.4 After every ten samples within each run, the high standard should be analyzed. If any of the calibrated elements exceeds 5% of the known value, the calibration curve is "normalized" on the zero and high standards. If any of the calibrated elements exceeds 10% of the known value, the previous set of ten samples must be re-analyzed after re-calibration.

2.5 All quality control data should be maintained and available for easy reference or inspection.

3. Apparatus

3.1 *Inductively Coupled Plasma-Atomic Emission Spectrophotometer*—sequential or simultaneous multi-element spectrophotometer equipped with 27-element capability and background correction. Suggested wavelengths for elements of interest in animal manures are given in Table 6.3-2. Refer to specific manuals for methods and procedures appropriate for ICP instruments and laboratory operation.

3.2 *Random access sample changer*—use the autosampler recommended by the particular instrument manufacturer, e.g., Gilson model 222, with five 44-position sample trays.

3.3 *Stock standard metal solutions* —stock standard solutions are prepared from high purity metals, oxides, or non-hygroscopic salts combined with water and reagent grade nitric or hydrochloric acids (see individual methods). The stock solutions are prepared at concentrations of 1,000 mg of the metal per liter. Commercially available standard solutions also can be used.

3.4 *Calibration standard*s—calibration standards are prepared by diluting the stock solutions at the time of analysis. For best results, calibration standards should be prepared each time a batch of samples is analyzed. Prepare a blank (zero standard) and a high standard in the appropriate range of the linear part of the curve. The calibration standards should be prepared with the same type of acid or combination of acids and at the same concentration as the samples in order to minimize matrix differences.

Table 6.3-2. Inductively coupled plasma—atomic emission spectrophotometry wavelength table for use with manure digests. Wavelengths (nm) for each element are listed. Soltanpour et al., 1996.

Element	Wavelength (nm)
Boron	249.77
Calcium	317.93
Copper	324.75
Iron	259.94/233.28
Magnesium	279.08
Manganese	257.60
Phosphorus	213.62
Potassium	766.49
Sodium	589.59
Zinc	213.86

4. Procedures

Differences among the various makes and models of ICP spectrophotometers make it impractical to outline detailed instructions that apply to every instrument. Follow the manufacturer's operating instructions for a particular instrument.

4.1 Transfer approximately a 10 ml aliquot of the diluted digest to 17 ml polyethylene disposable tubes. Cap the tubes firmly.

4.2 Place sample tubes in auto-sampler trays and enter sample identifiers and other test parameters into a Set-up file for automated analysis.

4.3 Create an analysis program with previously determined inter-element spectral corrections for the elements (table 6.3-2) which show interfering spectra.

4.4 Read time: three separate 10-sec. readings per sample.

4.5 After a 30-min. warm-up period of aspirating a 10% HCl rinse solution, calibrate the zero concentration point (zero standard), followed by calibration of the high standard.

4.6 Confirm the calibration by analyzing the above two calibration standards as if they are "sample" solutions. If the measured concentrations are not within 5% of set values, recalibrate.

4.7 Proceed with the analysis of samples, blanks and reference samples.

4.8 For within-run quality control, set normalization mode to read the high standard after every ten samples. Set "Normalization" limit for the high standard at 5% for one or more elements.

4.9 If the concentration for any of the calibrated elements exceeds the known linear range of the spectrometer, the sample must be diluted and rerun.

4.10 Elemental concentrations (mg L^{-1}) determined in the digests are then used to calculate initial concentrations in the manure, according to the calculation procedure outlined for each of the dissolution and digestion methods.

6.4 Colorimetric method for phosphorus

(adapted from Standard Methods for the Examination of Water and Wastewater, Method 4500-P)

1. General principles

This section covers the determination of phosphorus concentrations in digest solutions (Chapter 5) by means of colorimetric analysis. Several colorimetric procedures are available. Interferences and minimum detectable concentrations are controlled by the colorimetric method used, rather than the digestion procedure.

The reaction of phosphoric acid with molybdate (MoO_4^{2-}) forms a heteropoly molybdophosphate complex according to the reaction:

$$H_3PO_4 + 12\ H_2MoO_4 \rightarrow H_3P(Mo_3O_{10})4 + 12\ H_2O$$

The complex is yellow, and a yellow precipitate forms at high P concentrations. In the presence of vanadium (V), the yellow color intensifies. This is the basis for the vanado-molybdophosphoric acid colorimetric procedure. In the presence of reducing agents, the molybdenum (Mo) in the complex is reduced from 6^+ to 3^+ and/or 5^+, which results in the characteristic blue color. Several Mo blue colorimetric methods have been developed, varying mainly in the type of reducing agent utilized (Jackson, 1958; Murphy and Riley, 1962; Watanabe and Olsen, 1965).

The sensitivity required, stability of the colored solution, and freedom from interferences are the important considerations for choosing a method.

The vanado-molybdophosphoric acid method is least sensitive; however, its yellow color remains stable for days, and the acid concentration in the solution is not as critical for color development as is the case with other methods. The chlorostannous method is more sensitive than the vanado-molybdophosphoric acid method, but the characteristic blue color is stable for only 15 to 20 min. The ascorbic acid method is equally sensitive, with a blue color that is stable for up to 24 h. Hence, the ascorbic acid method is used more extensively.

2. Sample handling and quality control

2.1 Refer to selected dissolution/digestion method described in Section 5 for specific sample handling and preparation procedures.

2.2 Replication of samples and inclusion of reference materials is performed at the digestion phase of the analysis. Included with each batch of 20 samples, is a minimum of one in-house check and one external reference sample, such as those provided by the NAPT program.

2.3 A calibration curve must be prepared each day with a minimum of a calibration blank and four standards. After calibration, the calibration curve should be verified by use of a calibration check standard that is made from a reference material or other independent standard material. The calibration reference standard should fall near the mid-range of the calibration curve and must measure within 10% of its true value for the curve to be considered valid.

2.4 If more than 10 samples per day are analyzed, the working standard curve should be verified by measuring a mid-range standard or reference standard after every 10 samples. This sample value must be within approximately 10% of the true value, or the previous ten samples should be reanalyzed.

2.5 All quality control data should be maintained and available for easy reference or inspection.

3. Vanado-molybdophosphoric acid method

3.1 Principles of method

3.1.1 In the presence of V, molybdophosphoric acid forms yellow vanado-molybdophosphoric acid. The intensity of the yellow color is proportional to the phosphate concentration in the digest solution.

3.1.2 Advantages: 1) The yellow color remains stable for several days, and the intensity is unaffected by room temperature in the lab; **2)** the method is free from interferences by a wide range of ions in concentrations up to 1000 mg L^{-1}; and **3)** interference by arsenic (As) can be eliminated by pretreating the test solution with HBr to remove As as AsBr3 (Jackson, 1958).

3.1.3 Disadvantages: 1) The sensitivity of the method is relatively low – 0.2 mg L^{-1} with a 1-cm cell; and **2)** although the sensitivity of the method is greater at a wavelength of 400 nm, Fe^{3+} concentrations greater than 100 mg L^{-1} interfere at this wavelength, so that 470 nm must be used.

3.2 Apparatus

3.2.1 *Spectrophotometer*—for use at 400 to 490 nm. Desired sensitivity determines the wavelength to be used:

P Range, mg L^{-1}	Wavelength, nm
1.0-5.0	400
2.0-10	420
4.0-18	470

3.2.2 *Acid-washed glassware* — All glassware should be cleaned with dilute HCl and triple rinsed with distilled water. If possible, reserve the glassware for P determinations only. This will lessen the need for acid washing.

3.2.3 Volumetric pipettes or automatic pipette.

3.3 Reagents

3.3.1 *Distilled water*

3.3.2 *Ammonium molybdate $[(NH_4)_6Mo_7O_{24}]$ (Solution A)*—Dissolve 25 g of reagent grade $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$ in 400 ml of distilled water.

3.3.3 *Ammonium vanadate (NH_4VO_3) (Solution B)*—Dissolve 1.25 g of NH_4VO_3 in 300 ml of boiling water. Allow to cool to room temperature.

3.3.4 *Mixed reagent*—Transfer Solution B to a 1-L volumetric flask and slowly add 250 ml of concentrated HNO_3 (15.8 M) with rapid stirring. Allow the solution to cool to room temperature, then add entire amount of Solution A. Dilute the mixture to volume.

3.3.5 *Sulfuric acid, 3.5 M*—Slowly add 194 ml of concentrated H_2SO_4 (18 M) to distilled water and dilute to 1 L with distilled water after it has cooled to room temperature.

3.3.6 Working phosphate solution, 50 mg P L^{-1}— Dissolve 0.2197 g of oven-dried (40˚C) KH_2PO_4 in distilled water. Add 25 ml of 3.5 M H_2SO_4, and dilute to 1 L.

3.4 Procedure

3.4.1 *Sample analyses*—Pipette 35 ml or less of sample digest containing 0.05 to 1.0 mg P into a 50-ml volumetric flask. Add 10 ml vanadate-molybdate reagent, and dilute to mark with distilled water. At the same time, prepare a blank in which 35 ml distilled water is substituted for the sample. After 10 min. or more, measure absorbance of blank and samples at a wavelength of 400 to 490 nm, depending on the sensitivity required. Subtract the absorbance value of the blank from those of the samples.

3.4.2 *Preparation of calibration curve*—Prepare a calibration curve by pipetting suitable volumes of standard P solution into 50-ml volumetric flasks, and proceeding as outlined in 3.4.1 above. When Fe^{3+} is low enough not to interfere, plot a series of calibration curves for one set of standard solutions measured at various wavelengths. Analyze a least one quality control sample with each set of unknown samples.

3.4.3 Calculation of phosphorus in digest—To calculate the concentration of P in the original digest, divide the concentration value obtained from the colorimetric analysis (mg L^{-1}) by the aliquot size (35 mL or less), and then multiply by the total volume analyzed (50 mL). Phosphorus concentrations (mg L^{-1}) in the digests are then used to calculate initial concentrations in the manure, according to the calculation procedure outlined for each of the dissolution and digestion methods. See sections 5.1, 5.2, 5.3, and 5.4 of Section 5.

4. Ascorbic acid method

4.1 Principles of method

4.1.1 Ammonium molybdate and potassium antimonyl tartrate react with orthophosphate in the digest solution to form phosphomolybdic acid. This heteropoly acid is reduced to intensely-colored “molybdenum blue” by ascorbic acid. The intensity of the blue color is proportional to the phosphate concentration in the solution.

4.1.2 Advantages: 1) The blue color remains stable for several hours, and the intensity is unaffected by room temperature in the lab; **2)** compared with the vanado-molybdophosphoric acid method, the detection limit (0.01 mg L^{-1}) is more than an order of magnitude lower; **3)** interference by As can be avoided by reducing AsO_4^{3-} to AsO_3^{3-} with $NaHSO_3$ (Jackson, 1958); and **4)** automated versions of the ascorbic acid method have been developed.

4.1.3 Disadvantages: 1) Arsenates in concentrations as low as 0.1 mg As L^{-1} react with the molybdate reagent to produce a similar blue color; **2)** hexavalent chromium and nitrites interfere with color development; **3)** sample acidity affects color development; **4**) the molybdate reagent is stable for less than 24 h; and **5)** the molybdenum blue color is stable for several hours, not days.

4.2 Apparatus

4.2.1 *Spectrophotometer*—for use at 880 nm. Light path length determines the sensitivity of the method:

P Range, mg L^{-1}	Light Path, cm
0.30-2.0	0.5
0.15-1.30	1.0
0.01-0.25	5.0

4.2.2 *Acid-washed glassware*—All glassware should be cleaned with dilute HCl and triple rinsed with distilled water. If possible, reserve the glassware for P determinations only. This will lessen the need for acid washing.

4.2.3 *Volumetric pipettes or automatic pipette*

4.3. Reagents

4.3.1 *Sulfuric acid, 2.5M*—Dilute 70 ml of concentrated H_2SO_4 (*18 M*) to 500 ml with distilled water.

4.3.2 *Ammonium molybdate*—Dissolve 20 g of $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$ in 500 ml of distilled water. Store the solution in a glass-stoppered bottle.

4.3.3 *Antimony potassium tartrate* $[K(SbO)C_4H_4O_6 \cdot \frac{1}{2} H_2O]$ (1 mg Sb ml^{-1}) — Dissolve 0.2728 g of $K(SbO) \cdot C_4H_4O_6 \cdot \frac{1}{2} H_2O$ in 100 ml of distilled water.

4.3.4 *Ascorbic acid, 0.1M*—Dissolve 1.76 g of $C_6H_8O_6$ in 100 ml of distilled water. Prepare the solution on the day it is required.

4.3.5 *Mixed reagent*—Thoroughly mix 50 ml of 2.5 *M* H_2SO_4, 15 ml of ammonium molybdate solution, 30 ml of ascorbic acid solution and 5 ml of antimony potassium tartrate solution. Prepare a fresh-mixed reagent daily.

4.3.6 *p-Nitrophenol*—0.25% wt/vol.

4.3.7 *Sodium hydroxide*, *5 N*

4.3.8 *Phosphate stock solution, 50 mg P* L^{-1}—Dissolve 0.2197 g of oven-dried (40˚C) KH_2PO_4 in distilled water. Add 25 ml of 3.5 M H_2SO_4, and dilute to 1 L with distilled water.

4.3.9 *Working phosphate standard solution, 5 mg P* L^{-1}—Dilute 10 ml of the 50 mg P L^{-1} stock solution to 100 ml with distilled water.

4.4 Procedure

4.4.1 *Sample analyses*—Pipette an aliquot of sample digest containing 0.05 to 1.0 mg P into a 50-ml volumetric flask. If the aliquot contains more than 2 meq H^+, add 5 drops of 0.25% p-nitrophenol and neutralize with *5 N* NaOH. Dilute the sample to approximately 40 ml with distilled water, add 8 ml of the mixed reagent, and dilute to mark with distilled water. At the same time, prepare a blank in which 40 ml distilled water is substituted for the sample. After 10 min. or more, measure absorbance of blank and samples at a wavelength of 880 nm. Subtract the absorbance value of the blank from those of the samples.

4.4.2 *Preparation of Calibration Curve*—Prepare a calibration curve by pipetting six separate volumes of standard P solution within the phosphate ranges given in 4.2.1 above into 50-ml volumetric flasks, and proceeding as outlined in 4.4.1. Plot absorbance versus phosphate concentration to obtain a straight line passing through the origin. Analyze at least one quality control sample with each set of unknown samples.

4.4.3 *Calculation of phosphorus in digest*—To calculate the concentration of P in the original digest, divide the concentration value obtained from the colorimetric analysis (mg L^{-1}) by the aliquot size, and then multiply by the total volume analyzed (50 mL). Phosphorus concentrations (mg L^{-1}) in the digests are then used to calculate initial concentrations in the manure, according to the calculation procedure outlined for each of the dissolution and digestion methods. See sections 5.1, 5.2, 5.3, and 5.4 of Section 5.

5. References

1. American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. 4500-P Phosphorus. In: L.S. Clesceri et al. (eds.) Standard methods for the examination of water and wastewater. 20th ed. Am. Public Health Assoc., Washington, D.C.
2. Ingle, J.D., and S.R. Crouch. 1988. Spectrochemical Analysis. Prentice Hall. Englewood Cliffs, NJ.
3. Isaac, R.A., and Jones, Jr., J.B. 1970. AutoAnalyzer systems for the analysis of soil and plant extracts. pp. 57-64. In: Advances in Automated Analysis — Technicon Congress Proceedings. Technicon Corp., Tarrytown, NY.
4. Jackson, M.L. 1958. Soil chemical analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ.
5. Murphy, J., and H.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27:31-36.
6. Shaw, A., C. Karlsson, and J. Müller. 1988. An introduction to the use of flow injection analysis. Tecator AB, Höganäs, Sweden.
7. Soltanpour, P.N., G.W. Johnson, S.M. Workman, J.B. Jones, Jr., and R.O. Miller. 1996. Inductively coupled plasma emission spectrometry and inductively coupled plasma-mass spectrometry.

pp. 91-139. In: D.L. Sparks (ed.) Methods of Soil Analysis – Part 3. Chemical Methods. SSSA Book Series no. 5.

8. United States Composting Council. 2000a. Test Methods for the Examination of Composting and Compost (draft). Atomic Absorption Spectrometry. Section 04.13.
9. United States Composting Council. 2000b. Test Methods for the Examination of Composting and Compost (draft). Inductively Coupled Plasma Analysis. Section 04.14.
10. United States Environmental Protection Agency (US EPA) Method 6010A. 1992a. Inductively coupled plasma-Atomic emission spectroscopy. In: Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. SW-846, 3rd Edition.
11. United States Environmental Protection Agency Method 7000A. 1992b. Atomic absorption methods. In: Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. SW-846, 3rd Edition.
12. Watanabe, F.S., and S.R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soil. Soil Sci. Soc. Am. Proc. 29:677-678.
13. Wright, R.J. and T. Stuczynski. 1996. Atomic absorption and flame emission spectrometry. pp. 65-90. In: D.L. Sparks (ed.) Methods of Soil Analysis – Part 3. Chemical Methods. SSSA Book Series no. 5.

7. Determination of manure pH

Nancy Wolf

7.1 Introduction

The pH is a measure of the acidity or alkalinity of manure. This can be quite variable, dependent upon the feed and bedding practices associated with the animal production system.

Although maintaining proper soil pH is an important component in a sound nutrient management plan, the typical pH of the manure itself at neutral to slightly above neutral is not critical when considering nutrient loading rates because once the manure is incorporated into the soil, microbial activity very rapidly breaks down the organic matter, decreasing the pH. However, long-term manure applications can eventually increase soil pH (Kingery et al., 1994) and it is recommended that regular soil testing be performed to determine if excess manure application is creating a high pH problem. In layer hen production systems, ground limestone is added to the feed to improve egg quality. Although, this type of manure actually has some liming value, it's potential as a lime is very small and the manure should be applied for it's nutrient value and not it's liming potential (Mitchell and Donald, 1999). Similarly, coarsely ground limestone is often applied to concrete floors in dairy barns to reduce animal slippage. This relatively small amount of lime when applied to the field with the manure may help reduce the rate of soil acidification in the long term, but this value is normally not considered when determining a manure's nutrient value.

The pH of the manure directly influences the amount of ammonia volatilization. Undesirable ammonia volatilization can be reduced by decreasing the manure pH with amendments such as alum or ferrous sulfate (Moore et al., 1995).

For a liquid manure, pH can be easily and directly measured by electrode (EPA SW-846, Method 9040). However, for solid or semi-solid manure, pH must be measured in a slurry. As with soil pH, different manure to water ratios can be used: a saturation extract as with a growing media (South. Coop. Bull 289, 1983.), a 1:2 manure/water slurry as in a soil sample (South. Coop. Bull 289, 1983), or a 1:15 (approximate) manure/water slurry as in a peat (AOAC method 973.04).

The following methods outline manure pH measurement in a liquid or a solid or semi-solid manure at a 1:2 manure/water slurry. It is recommended that the ratio used for solid and semi-solid manure always be denoted with the pH result. Samples containing large amounts of hay or sawdust may require more water to create enough slurry for proper pH electrode operation. Modifications in the manure/water ratio must be denoted with the results.

7.2 Principle of the method

pH is measured potentiometrically in the undiluted liquid manure or in a 1:2 manure/water slurry for solid or semi-solid manure .

7.3 Apparatus

1. *pH/mV meter*—with a combination or dual electrode system

2. *Scoop*—10 or 20 cm3 volumetric

3. *Cup*—50 ml (glass, plastic or waxed paper)

4. *Glass stir rods*

5. *Automatic dispense*r—to deliver 20-40 ml distilled water

7.4 Reagents

1. *Buffer solutions*—pH 4.0, 6.0, 7.0 and 10.0

7.5 Procedure

1. Liquid manure

1.1 Thoroughly mix liquid manure by inverting sample bottle several times. Manure should be at room temperature.

1.2 Pour about 25 ml into a plastic cup.

1.3 Immerse pH electrode

1.4 Record pH value when the meter has stabilized.

1.5 Save the sample for the determination of EC.

2. Semi-solid or solid manure

2.1 Scoop 20 cm3 solid manure (as received) into a 50ml cup.

2.2 Add 40 ml distilled water with dispenser.

2.3 Stir well with a glass rod for 10 seconds

2.4 Allow to stand for 30 minutes.

2.5 Measure pH by immersing electrode in the supernatant solution.

2.6 Record the pH when the meter has stabilized.

2.7 Report the result as pH(water 1:2)

2.8 Save the sample for the determination of EC.

7.6 Quality control

1. Calibrate pH meter daily using pH buffers which bracket unknown samples.

2. Check calibration with third pH buffer within calibrated bracket.

3. Perform replicate analysis on 10% of samples. Replicate results should be within 10-15% of the mean.

4. Include one standard reference material with each batch of samples or each group of 30 samples.

5. Results should be within limits specified for the reference material.

7.7 References

1. AOAC Official Methods of Analysis. 1990. Method 973.04 pH of peat.

2. Kingery, W.L., C.W. Wood, D.P. Delaney, J.C. Williams, and G.L. Mullins. 1994. Impact of long-term land application of broiler litter on environmentally related soil properties. J. Environ. Qual. 23:139-147.

3. Mitchell, Charles C. and James O. Donald. 1999. The value and use of poultry manures as fertilizer. Alabama Cooperative Extension. ANR-244.

4. Moore,P.A.,Jr., T.C. Daniel, Dr. R. Edwards, and D. M. Miller. 1995. Effect of chemical amendments on ammonia volatilization from poultry litter. J. Environ. Qual. 24:293-300.

5. Southern Cooperative Series. 1983. Reference soil test methods for the southern region of the United States. South. Coop. Ser. Bull. 289. Georgia Agric. Exp. Stn., Athens, GA.

6. U.S. Environmental Protection Agency. 1986. Test methods for evaluating solid waste. USEPA report SW-846.

8. Determination of manure electrical conductivity (EC)

Nancy Wolf

8.1 Introduction

The electrical conductivity (EC) is directly related to the soluble salt content of a sample. Manure can have high EC levels due to the large amounts of salt-based minerals commonly added to feed rations. An excessive manure application just prior to planting can reduce germination or injure the seedling plants. Long term or excessive applications can lead to a build up of soluble salts in the soil (Chang et al., 1991). This is particularly a problem of medium and fine textured soils, which typically have lower infiltration rates. If adequate rainfall is unavailable to leach these excess salts out of the root zone, crop growth can be stunted and soil structure destroyed.

For a liquid manure, EC can be easily and directly measured using a conductivity cell and meter (EPA SW-846, Method 9050). However, for solid or semi-solid manure, EC must be measured in a slurry. As with Soil EC, different manure to water ratios can be used: a saturation paste extracted used for saline soils (US Salinity Lab, 1954) and with growing media (South. Coop. Bull 289, 1983) or a 1:2 manure/water slurry as in a soil sample (South. Coop. Bull 289, 1983).

The following methods outline manure EC measurement in a liquid or a semi-solid or solid manure at a 1:2 manure/water slurry. It is recommended that the ratio used for semi-solid and solid manure always be denoted with the EC result. Samples containing large amounts of hay or sawdust may require more water to create enough slurry for proper EC electrode operation. Modifications in the manure/water ratio must be denoted with the results.

8.2 Principle of the method

1. EC is measured at room temperature using a self-contained conductivity meter on the undiluted liquid manure or in the 1:2 manure/water slurry for semi-solid or solid manure.

2. The 1:2 manure/water slurry is based on a manure volume rather than on a weight basis. This avoids the need for further dilution for highly organic materials.

8.3 Apparatus

1. *Conductivity meter*—with temperature compensation and electrode.

2. *Scoop*—10 or 20 cm3 volumetric

3. *Cup*—50 ml (glass, plastic, or waxed paper)

4. *Glass stir rods*

5. *Automatic dispenser*—to deliver 20 or 40 ml distilled water.

8.4 Reagents

1. *Distilled water*

2. *Commercial standard solutions*—1000 and 10,000 micromhos/cm.

8.5 Procedure

1. Liquid manure

1.1 Thoroughly mix liquid manure by inverting sample bottle several times.

1.2 Pour about 25 ml into a plastic cup. Sample must be a room temperature.

1.3 Immerse EC electrode and temperature probe.

1.4 Record the EC value when the meter has stabilized.

2. Semi-solid or solid manure

2.1 Scoop 20 cm3 solid manure (as received) into a 50 ml cup.

2.2 Add 40 ml distilled water using the dispenser.

2.3 Stir well with a glass rod for 10 seconds.

2.4 Allow to stand for 30 minutes.

2.5 Immerse EC electrode and temperature probe.

2.6 Record the EC value as EC (water 1:2) when the meter has stabilized.

8.6 Comments

1. EC increases with increasing temperature. Thus, samples and standards should be at room temperature or temperature compensation should be made.

2. Platinum electrodes can degrade and become coated with oil and other materials. Clean and well-platinized electrodes are essential for reproducible results.

8.7 Quality control

1.Check performance of electrode and meter daily by analyzing calibration standards bracketing the unknown samples. Standards should be within 10% of the known value. If greater than 10%, clean and/or replatinize electrode.

2. Check temperature of unknown samples and standards to ensure room temperature.

3. Perform replicate analysis on 10% of samples. Replicate results should be within 10% of the mean.

4. Include one standard reference material with each batch of samples or each group of 30 samples. Results should be within limits specified for the reference material.

8.8 References

1. Chang, C., T.G. Sommerfeldt, and T. Entz. 1991. Soil chemistry after eleven annual applications of cattle feedlot manure. J. Environ. Qual. 20:475-480.
2. Southern Cooperative Series. 1983. Reference soil test methods for the southern region of the United States. South. Coop. Ser. Bull. 289. Georgia Agric. Exp. Stn., Athens, GA.
3. U.S. Environmental Protection Agency. 1986. Test methods for evaluating solid waste. USEPA report SW-846.
4. United States Salinity Laboratory Staff. 1954. Diagnosis and improvements of saline and alkali soils. U.S. Dept. Agr. Handbook 60.

Unit IV **Reporting Manure Analysis Results**

Jan Jarman and John Peters

1. Introduction

Manure analysis reports are designed to meet primary customer needs in planning land application rates or determining nutrient credits from applied manure. However, the use of different report formats, reporting units, analysis and reporting bases, conversion factors, and estimates of nutrient availability and fertilizer value often results in confusion for livestock producers and difficulty in interpreting the report. Differences in reporting conventions can also result in the perception by producers that laboratory manure analysis is inaccurate. These problems may explain why some producers do not analyze their manure on a regular basis, do not use the results properly, or fail to analyze their manure at all. The perception that manure nutrient credits are inaccurate can then lead to over-application of commercial fertilizer in order to reduce the risk of crop yield losses.

2. Considerations for reporting manure analysis results

There are three types of information usually included on manure analysis reports. The first is descriptive information about the sample and the customer, including customer identification, sample identification, description and date of analysis. The purpose of providing most of this information is self-explanatory. A more complete description of the sample is usually requested if information on nutrient availability is provided, because this is influenced by livestock species, storage and handling system, animal production phase and application method.

The second type of information provided on manure analysis reports is the actual analytical results. In reporting results, three things must be considered in addition to the accuracy of the results: reporting units, reporting basis and use of conversion factors. Typically, manure analysis results are recorded in the laboratory in units of percentage or parts per million (ppm), and then converted to the units needed by the customer to calculate application rates or nutrient credits (lbs/1000 gal for liquid manures, lbs/T for solids). Some laboratories report just percentage, some report just lbs/1000 gal or lbs/T, and others report both. Having more than one type of unit on the report form can sometimes be confusing for clients, if they are unfamiliar with how the different units are related, or which ones they need to use for nutrient management planning.

For liquid manures, conversion of percentage to lbs/1000 gal must account for the manure density, which can be estimated or determined. Laboratories vary widely as to the manure density or conversion factor used. Some use the density of water (approximately 8.33 lbs/gal), others use a higher density value (ranging from 8.4 to 9.5 lbs/gal) to account for the presence of solids, some vary the density value according to solids content, and others use measured density or specific gravity. Only infrequently is the conversion factor identified on the analysis report.

In addition, manure samples may be analyzed and results reported on either a dry matter basis or an as-is basis. If the laboratory dries and grinds manure for analysis (as is sometimes done for mineral analysis of heterogeneous solid manures), the results must then be converted to an as-is basis using the dry matter determination. Most laboratories report results on an as-is basis, regardless of analysis basis, because manure is applied “as-is.” Again, having more than one type of unit or reporting basis can be confusing for farmers, but dry matter basis results do allow direct comparison of nutrient value between two manures. For research purposes, this can be invaluable.

Reported results can be inaccurate due to calculation and typographical errors. Typical problems seen in manure nutrient analysis reporting include not accounting for dilutions, use of incorrect conversion factors, transposition of values, misplaced decimal points and switched samples.

The third type of information found on manure analysis reports is interpretive, including estimates of nutrient availability and fertilizer value, and use of results in application rate planning. Many laboratories provide estimates of nutrient availability, either in addition to or in place of the actual nutrient content of the manure. These values can be a significant source of confusion for producers. Although virtually all availability estimates are based on livestock species,

manure type (liquid or solid) and application method, considerable differences exist between values published by various State Extension Services. In addition, some laboratories use availability factors from other sources. Reported nitrogen availability estimates can differ by 30 percentage points or more for manures from the same species and using the same application method. Reported phosphorus and potassium availability estimates can range from approximately 50 to 100%. Therefore, laboratories should report the availability factors coinciding with the state where the sample was taken.

Additional confusion may arise when customers live outside the state in which the laboratory operates and are provided Extension or other availability values that differ from those used by their own state Extension Service, or when the application method used by the customer is not listed in the availability estimates provided. Also, computer programs designed to generate nutrient availability estimates may default to incorrect values if the sample information provided by the customer is insufficient for determining the correct availability factor.

In order to promote manure testing and other practices which optimize use of manure nutrients, some laboratories provide estimates of the economic or "fertilizer" value of manure nutrients on their analysis reports. Most often a dollar value is assigned to the nutrients present in the manure or estimated to be available to a crop, based on current local fertilizer prices (fertilizer *equivalent* value). However, this information is often misleading. The economic value of the nutrients in manure is equivalent to only the cost of the fertilizer being replaced on a particular field (fertilizer *replacement* value). If the producer's fields and crops need all the nutrients applied in the manure, and fertilizer and manure application costs are equal, then the fertilizer replacement value is the same as the fertilizer equivalent value. If all the nutrients in the manure are not needed on a particular field, due to low crop need or high soil test values, then the excess nutrients have little economic value to the producer. Manure application costs also vary between fields and can overshadow the fertilizer value of the nutrients. Therefore, manure nutrients have different economic values depending on the fields to which they are being applied.

3. Guidelines for reporting manure analysis results

Manure analysis reports should provide information that is easy to use and interpret, and should help fulfill the record-keeping needs of the customer. Ideally, livestock producers should be able to look at analysis reports from several different laboratories and be able to come to similar conclusions regarding application rates and nutrient credits for their manure. This may not be realistic, due to different approaches to estimating nutrient availability. Manure analysis reporting could still benefit from standardization in other areas, however. The following guidelines are suggested in order to encourage dialogue within the testing industry that will result in some level of standardization of reporting, with simplicity and ease of interpretation being the primary goals. Two example laboratory reports are given at the end of this chapter to illustrate the guidelines suggested. Any number of formats can work equally as well, as long as the information presented and the purpose for presenting it is clear to the customer.

3.1 Descriptive information

Descriptive information should include the following:

Laboratory name, mailing address, telephone number, e-mail address;

Customer name, mailing address, telephone number, e-mail address (farmer name also, if different than customer);

Sample identification (laboratory number and customer-provided identification);

Sample description (at a minimum, include livestock species, liquid or solid, manure application method; may also include storage and handling system, application timing, days until incorporation); and

Date received by the laboratory as well as date analyzed and reported.

Sample submission sheets should have spaces for customers to record the above information. The more information the customer can supply about the sample, the more assistance the laboratory can provide for interpreting the results. Having this information on the analysis report also simplifies record-keeping for the customer. For laboratories, an additional benefit of having descriptive sample information is being able to analyze cumulative data for differences in manure nutrient content between different livestock

species and different manure handling and storage systems. Currently there is very little information of this type available that is state-specific and that reflects current trends in production and management. This information would be invaluable for improving outdated 'table' values of nutrient content and nutrient availability indices.

3.2 Analysis results

Units and reporting basis: Report dry matter as percent solids, to at least the nearest 0.1%. Samples should always be analyzed for total solids content, and the results reported (rather than moisture content), even if the customer does not request it specifically. Dry matter determination is often necessary to convert the results of analyses performed on dried samples to an as-is basis. Also, most laboratories include solids in the fee charged for routine manure analysis. Reporting of dry matter or solids content also makes it easier to compare results between different samples.

Report total nitrogen (N), ammonium-nitrogen (NH_4-N), total phosphorus as phosphate (P_2O_5), total potassium as potash (K_2O) and other minerals in units of lbs/1000 gal for manures applied as liquids, and lbs/T for manures applied as solids. A strong effort should be made to obtain the desired reporting units from the client. The type of spreader being used will dictate how the results should be reported, not the dry matter content. If a particular dry matter level is used to generate reporting units, there should be an option in the computer program to over-ride this default if the sample dry matter falls outside the normal dry matter ranges for liquid and solid manures.

Report N, NH_4-N, P_2O_5 and K_2O to at least the nearest 0.1 lb/1000 gal or lb/T to provide consistency for samples with low concentrations of particular nutrients. Do not report beyond the number of significant digits that are appropriate for the analysis methods and calculations you are using. Report phosphorus and potassium as $P_2O_5$and K_2O. This is necessary to be consistent with standardized reporting of soil fertility recommendations and nutrient content of fertilizers.

Results may also be reported in units of percent or ppm, but these should be reported separately from the results reported as lbs/T or lbs/1000 gal, and clearly labeled in order to prevent confusion. It should then be indicated on the report which values should be used to calculate application rates and nutrient credits.

Results reported in units of percent or ppm should be reported on an as-is basis. Results reported on a dry matter basis can be useful for comparing results between different manures, or for generating more accurate table values for different regions. Most producers, however, will have little use for dry-matter basis results. If they are reported, they should be clearly labeled, and the relationship between different units should be indicated. Dry matter content should always be reported, to allow conversion of results to dry matter basis results, if desired.

Conversion factors: Most conversion factors are simply mathematical standards used by all laboratories. These include multiplying percentage by 20 to get lbs/T, multiplying ppm by 10^{-4} to get percent, multiplying P by 2.29 to get P_2O_5, multiplying K by 1.2 to get K_2O, and multiplying dry matter basis results by the dry matter fraction to get as-is basis results. For liquid manures, the factor used to convert percentage to lbs/1000 gal for is based on the density of the sample, and different laboratories use different factors. Some laboratories use the density of water (8.33 lbs/gal) and others use measured or estimated density values. This is done to account for the presence of solids in liquid manures and thereby improve the accuracy of the reported value. This practice is probably not justified, however.

In order to assess the affects of solids content and manure density on conversion factors and reported analysis values, 262 liquid dairy and swine manures from a variety of storage and handling systems were analyzed for density, solids content, specific gravity and total nitrogen (N) content (Jarman, 1999). The samples ranged in solids content from 0.3 to 16%. Nitrogen content in lbs/1000 gal was calculated using the density of water, measured sample density or measured specific gravity, or an estimated density of 9 lbs/gal. Calculated N content in lbs/1000 gal was similar when based on specific gravity, measured density or the density of water. Significantly larger N content values were obtained when a density value of 9.0 lbs/gal was used. Therefore, in order to standardize results between laboratories, it is recommended that the density of water (8.33 lbs/gal) be used, and percentage (as-is basis) would then be multiplied by 83.3 to obtain lbs/1000 gal.

If standardized conversion factors are used, it is not necessary to report these factors. If they are reported, caution should be used in their placement on the report. In order to streamline the report and avoid

confusion, conversion factors and calculations could be placed on the back of pre-printed reporting forms.

Accuracy of reported results: All results should be examined for transcription and other errors. The results should fall within the expected range of values for that manure type, unless unusual conditions are present. Computerized calculations should be checked for accuracy, and assumptions used in computer generation of numbers should be updated periodically. Check and verify every number on every report before it leaves the laboratory.

3.3 Interpretive information

Nutrient availability estimates: It is not likely that differences in availability estimates between State Extension Services are likely to be resolved in the near future, although some regions and groups of states are currently working towards consistency in their values. This is desirable when climate and typical storage, handling and application methods are similar. Differences in factors which affect manure nutrient availability do exist, however, between regions and states and even within states, and use of consistent values across large regions would be inappropriate. Also, some states have conducted extensive research in order to determine the most accurate availability estimates for the conditions within their states, and there is no scientifically valid reason for recommending use of other values.

The simplest solution for dealing with these issues is to report only the actual analysis values and refer the customer to their State Extension Service for assistance in determining nutrient availability, application rates and nutrient credits. However, many laboratories want to provide these services to their customers. Information about nutrient availability and how to calculate nutrient credits and application rates does help producers interpret their results, as long as the information is correct for that producer.

The ideal situation would be to provide Extension availability estimates that originate from the state in which the producer lives. Because the time and expense of setting up the computer programs required to do this for customers in several states might be prohibitive, it may be easier to provide availability factors recommended by the Extension Service in the state where the laboratory operates (usually where the laboratory does the most business). Providing availability factors (percentage of total nutrients available) rather than calculating the amounts of nutrients available, solves the problem of incorrect calculations in situations where insufficient information is provided by the customer to accurately determine the correct availability factors to use. However, providing amounts of available nutrients instead of availability factors can make it easier for the producer to interpret the results.

Regardless of the availability values provided, the actual analysis results should always be reported first, and the source of the availability values should always be stated prominently on the report, especially for the benefit of out-of-state customers. Laboratories should also check each report to ensure that the values provided (and the factors on which they are based) apply to that customer's particular sample. Due to development of new storage, handling and application methods, and the availability of their own large databases of manure nutrient content, laboratories could also help gather information to assist Extension in developing or modifying availability factors for their region.

Manure nutrient value: As stated previously, the economic value of manure nutrients is only equal to the cost of the fertilizer that is being saved on the particular fields to which the manure is applied, and must account for application costs. Unless a laboratory has access to information about fertility levels, crops being grown, manure and fertilizer rates applied and application costs for each of a customer's fields, then estimates of manure nutrient value are usually inaccurate and misleading. These estimates may provide some value to a producer as long as he or she understands what is being estimated.

4. References

1. Jarman, J.K. 1999. Fact sheet on use of liquid manure density values in reporting manure nutrient analysis results. Laboratory Certification Programs, Minnesota Department of Agriculture, St. Paul, MN.

Example laboratory report 1
(analysis results only, no interpretive information)

Laboratory Name
Laboratory Address
Tel. No. Fax No.
E-mail Address

Manure Analysis Report for: *Producer/Farm name*

Submitted by: *Customer name*
Customer address
Customer tel. no.
Customer e-mail address

Date received: *Mo/Day/Yr* Date reported: *Mo/Day/Yr*

Lab No.	*M1934*
Sample ID	*Finish*
Manure Type	*Liquid swine*
Storage Type	*Outdoor Lagoon*
Application method	*Knife injected*
Incorporation	*Immediate*
Total solids	5.5 %

ANALYSIS

Lab No.	**M1934**
Unit	**lbs/1000 gal**
Total nitrogen (N)	**39.2**
Ammonium nitrogen (NH_4-N)	**17.5**
Total Phosphorus expressed as P_2O_5	**30.0**
Total Potassium expressed as K_2O	**21.6**

Manure analysis values must be multiplied by an availability factor to obtain pounds of available nutrients per 1000 gallons of manure.

Availability factors depend on animal species and management, manure storage and handling system, application method and timing, days until manure incorporation, and other factors.

The amount of available nutrients is then multiplied by the application rate to obtain pounds of available nutrients applied per acre.

Contact your County Extension office for further information on manure nutrient availability and manure nutrient management.

Example laboratory report 2
(analysis results plus interpretive information)

Laboratory Name
Laboratory Address
Tel. No. Fax No.
E-mail Address

Manure Analysis Report for: *Producer/Farm name*

Submitted by: *Customer name*
Customer address
Customer tel. no.
Customer e-mail address

Date received: *Mo/Day/Yr* Date reported: *Mo/Day/Yr*

Lab No.	*M1934*
Sample ID	*Finish*
Manure Type	*Liquid swine*
Storage Type	*Outdoor Lagoon*
Application method	*Knife injected*
Incorporation	*Immediate*
Total solids	5.5 %

	Analysis	**1st Year** Availability **Factor**	**1st Year** Available **Nutrients**	**2nd Year** Availability **Factor**	**2nd Year** Available **Nutrients**
	lbs/1000 gal	**%**	**lbs/1000 gal**	**%**	**lbs/1000 gal**
Lab No. M1934					
Total nitrogen (N)	39.2	70	27	15	6
Ammonium nitrogen (NH_4-N)	17.5 (included in total N availability)				
Total Phosphorus expressed as P_2O_5	30.0	80	24		
Total Potassium expressed as K_2O	**21.6**	90	19		

Nutrient availability factors are those provided by the State/University Extension Service.

Nitrogen availability is based on livestock species, manure type, storage, application method and time until incorporation.

Availability of P_2O_5 and K_2O is the same for all manure types and application methods, and is only for the first year following application.

Contact your County Extension office for further information on manure nutrient availability and manure nutrient management.

Author(s):

John Peters, Director, Soil and Forage Analysis Laboratory at the University of Wisconsin–Marshfield.
Sherry M. Combs, Director, Soil and Plant Analysis Laboratory, University of Wisconsin-Madison.
Bruce Hoskins, Assistant Scientist, Analytical Lab, University of Maine, Orono, ME
Jan Jarman, Soil Scientist, formerly with Minnesota Department of Agriculture, St. Paul, MN
John L. Kovar, Soil Scientist, USDA/ARS National Soil Tilth Laboratory at Ames, Iowa
Maurice E. Watson, Soil Extension Specialist, School of Natural Resources, Ohio Agricultural Research and Development Center, The Ohio State University
Ann M. Wolf, Director, Agricultural Analytical Services Laboratory, Penn State University, University Park, PA.
Nancy Wolf, Research Associate, Agriculture Diagnostic Laboratory, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72701

Cover photos by John Peters

Produced by Cooperative Extension Publishing

University of Wisconsin-Extension, U.S. Department of Agriculture and Wisconsin counties cooperating. UW-Extension provides equal opportunities in employment and programming, including Title IX and ADA.

For more Cooperative Extension information, visit our web site: **www1.uwex.edu/ces/pubs/**

Recommended Methods of Manure Anaysis (A3769) I-2/2003