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Development of a Water-Extractable Phosphorus Test for Manure: An Interlaboratory Study

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ABSTRACT

The loss of P in runoff from agricultural land to which manure has been applied is related to water extractable P (WEP) in manure. However, a standard method to routinely measure WEP in manures has not been established and variables impacting the measurement have not been widely studied. In this investigation, the impact of manure holding times (1–22 d), WEP extract holding times (0–17 d with and without acidification), and method of P measurement on WEP were evaluated. In addition, four manure samples (one dairy, one swine, two poultry) and proposed WEP method were distributed to seven public and private laboratories to assess inter- and intralaboratory variability of WEP test results. The proposed method entailed extracting manures with water at a 1:200 ratio (manure solids/water), shaking for 60 min on a reciprocating shaker, and either filtering or centrifuging before P measurement by inductively coupled plasma atomic emission spectroscopy (ICP). Results show refrigerated (4°C) manure samples can be held up to 22 d and acidified extracts 18 d before analysis. Separation procedures (filtering vs. centrifuging) did not impact WEP measurements. While the method of P measurement (ICP vs. colorimetric) did have a significant impact on test results, the two methods were highly correlated and results within 5 to 10%. The proposed manure WEP method shows a high level of precision (relative standard deviations [RSDs] < 6.5) within laboratories, although greater variability (RSDs of 13.3–28.8) exists among laboratories when compared with other standard manure analyses, such as total P.

Accelerated eutrophication of surface waters is the most common surface water impairment in the USA (USEPA, 1996). For many watersheds, runoff from agricultural soils is responsible for elevated concentrations of P in surface waters, the chief cause of accelerated eutrophication (U.S. Geological Survey, 1999). In response to state (Coale et al., 2002) and federal water quality and nutrient management initiatives (USDA and USEPA, 1999), nearly all states have implemented guidelines for land application of manure that take into account the potential for P loss in runoff from manure-amended soils. To date, 47 states have adopted versions of the P Index to identify agricultural fields that are "critical source areas" of P to surface water (Sharpley et al., 2003). The P Index evaluates a variety of field-specific "source" and "transport" factors to rate fields on their relative vulnerability to P loss.

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Phosphorus source coefficients (PSCs), also referred to as P availability coefficients, represent a relatively novel set of factors within the P Index (Sharpley et al., 2003). Phosphorus source coefficients are quantitative indicators of the relative availability of P in mineral fertilizer or manure to be transported in runoff (Leytem et al., 2004). One approach to estimating PSCs involves direct measurement of WEP in manures to represent the potential for dissolved P losses from recently amended soils. This "worst case scenario" approach is based on the observation that concentrations of dissolved P in runoff are controlled by WEP in broadcast manures (Moore et al., 2000). Kleinman et al. (2002b) observed that concentrations of dissolved P in runoff from packed soil boxes recently broadcast with various manures were a function of the WEP concentration of the manure. Similar findings were observed by Brandt and Elliott (2003) who examined runoff P losses from soils that were broadcast with various biosolids or dairy manure.

The Pennsylvania P Index (Weld et al., 2003) includes PSCs for specific categories of manures and biosolids. At present, PSCs are fixed for individual manure and biosolid categories, based on the WEP survey conducted by Kleinman et al. (2005) and other sources of manure WEP information (Kleinman et al., 2002b; Brandt and Elliott, 2003). Ultimately, to better reflect the unique properties of individual manures, support innovations in manure management and promote consideration of manure properties in land application decisions, farmers will have the opportunity to submit their manures for WEP testing. Such testing will allow farmers to obtain a PSC for the Pennsylvania P Index that is specific to their manure.

A major obstacle to deriving PSCs from a manure WEP test has been the lack of one standard laboratory test for WEP. To be an effective environmental indicator, a WEP test must reflect differences in runoff dissolved P while also meeting reproducibility and other service laboratory analytical criteria. Kleinman et al. (2002a) showed that controlling manure-dry-matter/distilled-water ratio and length of shaking period were keys to consistently estimating WEP in manures and predicting DRP in runoff. This study was conducted to support the development of a routine WEP test for commercial laboratories by assessing factors affecting the outcome and replicability of a manure test within a single laboratory and then examining variability in the WEP test among laboratories.

Abbreviations: ICP, inductively coupled plasma atomic emission spectroscopy; PSC, phosphorus source coefficient; RSD, relative standard deviation; WEP, water-extractable phosphorus.

Table 1. Properties of manures used in study.

Manure	Solids	pH	Total N	Total P	Total K	Total Ca
	%		g kg^{-1} dry weight basis			
Swine	2.0	6.9	84.6	35.5	52.7	38.0
Dairy	8.0	7.0	28.9	6.7	22.0	19.0
Layer poultry	31.9	7.3	62.6	17.6	24.0	66.9
Broiler poultry	72.4	8.1	46.5	26.2	41.2	39.9

MATERIALS AND METHODS

Manure Sampling and Preparation

Manures with a broad range of properties (Table 1) were selected for this study to evaluate the influence of analytical variables on WEP determinations and assess interlaboratory variability. Twenty to forty liters (five to ten gallons) each of dairy (*Bos taurus*) manure, swine (*Sus scrofa* domestica L.) slurry, layer poultry (*Gallus gallus* domesticus L.) manure, and broiler poultry litter were collected. The dairy manure was from an aboveground storage tank located on a commercial dairy farm in Clearfield County, PA. The swine slurry was from finishing sows at the Penn State University Swine Center (University Park, PA) that had been washed into a holding tank and agitated before sampling. Both the broiler litter (wood shavings bedding material) and layer poultry manure were from the Penn State University Poultry Center (University Park, PA).

Immediately after collection, manure samples were mixed by hand and divided into 1-L subsamples. To maintain samples in a state similar to how they would be submitted from a farm and to reflect P solubility in manure as it is land applied, samples were not processed further (i.e., blended, chopped, or screened). One set of subsamples for each manure type was stored at 4°C at the Penn State University Agricultural Analytical Services Laboratory where tests on variables impacting soluble P measurement were performed. The remaining sample sets were frozen, packaged in insulated containers and mailed overnight to A&L Eastern Agricultural Laboratory (Richmond, VA); Agri Analysis, Inc. (Leola, PA); Brookside Laboratory (New Knoxville, OH); Clemson University Agricultural Services Laboratory (Clemson, South Carolina); North Carolina State Department of Agriculture Laboratory (Raleigh, NC); Spectrum Analytic Laboratory (Washington Courthouse, OH); and the University of Maryland Soil Testing Laboratory (College Park, MD). Manure samples were analyzed at Penn State University Agricultural Analytical Services Laboratory for percentage of solids (oven-dried at 105°C for 12–14 h), pH (1:5, volume basis, manure/water slurry), total N by combustion (Watson and Galliher, 2001) and total P, K, and Ca by microwave digestion with nitric acid (USEPA, 1986).

Evaluation of Analytical Variables Impacting Water-Extractable Phosphorus Measurements

The WEP procedure of Kleinman et al. (2002a) was followed with modifications to evaluate the impact of manure sample holding time, separation technique (filter vs. centrifuge), WEP extract holding time (with and without acidification), and P determination method (ICP vs. colorimetric). The procedure entailed weighing, in triplicate, a sample (as received basis) containing 0.5 g of solids into a 250-mL Erlenmeyer flask. Based on the percentage of solids of samples used in this study, sample weights of the as-received materials ranged from approximately 1 to 30 g. Deionized water was added to provide a final solid/solution ratio of 1:200 on a dry weight basis. Flasks were placed on an orbital shaker (60 min, 150 rpm) and either filtered through Whatman No. 40 filter paper (Whatman International Ltd., Maidstone, England) or centrifuged (10 min at 1500 × *g*) and decanted. Phosphorus in the extracts was measured within 8 h of extraction by ICP.

To evaluate the impact of manure sample holding time on WEP, triplicate samples of each manure (stored at 4°C) were extracted 5, 8, 15, and 22 d after the initial (Day 1) WEP measurement following the procedure specified above except that samples were centrifuged only. To evaluate the impact of extract acidification and holding time on WEP results, one triplicate set of extracts for each manure type was refrigerated (4°C) without acidification and a second set acidified (5 drops concentrated HCl for each 20 mL of extract) and refrigerated. P was measured by ICP in the unacidified extracts 1 d after extraction and in the acidified extracts 1, 3, 7, 10, and 17 d after extraction. In addition to ICP measurement, the P concentration in one set of acidified extracts was measured colorimetrically (Murphy and Riley, 1962). A summary of the analytical variables included in this study for the swine, dairy, layer poultry, and broiler poultry manures is presented in Table 2.

To further evaluate the effect of P measurement method on WEP results, P in WEP extracts (centrifuged, nonacidified) was measured by both ICP and colorimetric procedures on 66 manure samples (40 dairy, 12 broiler poultry, 8 swine, 3 beef cattle, 2 turkey, 1 layer poultry) submitted for routine analysis to the Penn State Agricultural Analytical Services laboratory from February 2002 through June 2002

Interlaboratory Study

Each participating laboratory was instructed to bring the frozen manure samples to room temperature and to measure WEP in triplicate as follows: (i) Determine percentage of solids content of manure, (ii) weigh, in triplicate, a sample (as received basis) containing 0.5 g of solids into a 250-mL Erlenmeyer flask or, if the sample contained <0.5% solids weigh 100 g and skip to step (iv), (iii) add deionized water to provide a final solid/solution ratio of 1:200 on a dry weight basis, (iv) place flasks on an orbital shaker (60 min, 150 rpm) and filter (Whatman No. 40) or centrifuge (10 min, 1500 × *g*), and (v) measure P in extracts by ICP. Each participating laboratory also analyzed the manure samples for total P and percentage of solids following recommended manure analysis procedures (Peters, 2003).

Statistical Analysis

Separation of means for the analytical variables impacting WEP measurements was tested with Tukey's honestly significant difference with a significance level of $p < 0.05$. Regression analysis was performed to evaluate the relationship between colorimetric and ICP determinations. Descriptive statistics were used to assess inter- and intralaboratory variability of WEP results. Intralaboratory variability was assessed from the mean RSD of the eight laboratories. All analyses were conducted using Minitab's statistical software, Release 13 (Minitab, 2000).

RESULTS AND DISCUSSION

Effect of Analytical Variables on Water-Extractable Phosphorus

Extract Separation Method

There was no significant difference ($p < 0.05$) in WEP results when samples were centrifuged or filtered through Whatman No. 40 paper filter (Table 3). While research-

Table 2. Summary of analytical variables for swine, dairy, layer poultry, and broiler poultry manures included in this study.

Days manures held before extraction†	Separation procedure	Extract acidification	Time of P measurement (days after extraction)‡	P measurement method
1	centrifuge	not acidified	0	ICP
	filter	not acidified	0	ICP
5	centrifuge	not acidified	0, 1	ICP
	centrifuge	acidified	1, 3, 7, 10, 17	ICP
		acidified	1	Color
8	centrifuge	not acidified	0	ICP
15	centrifuge	not acidified	0	ICP
22	centrifuge	not acidified	0	ICP

† All manures extracted at 1:100 (solid/solution) ratio in triplicate. Samples stored at 4°C before extraction.
‡ 0 indicates P measured same day as extracted.

ers have commonly used 0.45-μm membrane filters for WEP analysis (Self-Davis and Moore, 2000; Sharpley and Moyer, 2000), centrifugation and filtration through paper filters were selected for this study because they are more rapid and hence more highly suited for routine analysis. Kleinman et al. (2002a) found a significant increase in WEP concentration in dairy and poultry manures, although not in a swine slurry, when samples were filtered through paper (Whatman 1) instead of a 0.45-μm membrane filter. However, these differences were small and had only minimal impact on the relationship between WEP and dissolved runoff P suggesting that use of quantitative paper filters for WEP analysis is appropriate for this purpose. The results from the present study further suggest that centrifugation, a separation method not tested by Kleinman et al. (2002a), is a suitable alternative to paper filters for WEP measurement in manures.

Manure Holding Time

No significant differences in WEP results were noted for the dairy and the two poultry manures for samples held up to 22 d (refrigerated at 4°C), but significant differences were noted for the swine slurry (Table 4). For this sample, WEP analyzed on Day 1 was significantly different from analyses performed on Days 5 and 8 but not significantly different from those performed on Days 15 and 22. The swine slurry was very low in solid content (Table 1) and difficult to subsample uniformly. The solids present in this sample settled rapidly to the bottom after stirring and while efforts were made to keep the solids suspended during subsampling, maintaining a uniform suspension was difficult. Consequently, while approximately the same sample size was taken for all WEP measurements, it is possible that the actual solids content of the subsamples varied among days because of differences in subsampling technique. If this were the case, the solid/solution ratio of the WEP extracts would have varied as well. Kleinman et al. (2002a) found that the solid/solution ratio has a strong influence on WEP, with WEP increasing as the solid/solution ratio decreases (i.e., manure solids increasingly diluted).

In our study, WEP determined on Day 1 was not significantly different from determinations made on the final 2 d of analysis (Days 15 and 22). Consequently, we suspect that differences in WEP noted for Days 5 and 8 on the swine slurry are more likely related to differences in subsampling on those days and in the solid/solution ratio obtained than to actual changes in the sample over time. As will be discussed later, differences among laboratories in determining solids content of the swine slurry were much more variable than for the other manures confirming the subsampling challenge.

Extract Acidification and Holding Time

For the swine slurry, P measured (by ICP) in the unacidified WEP extract the day after extraction (Day 1) dropped significantly ($P < 0.05$) when compared with P measured the same day (Day 0, Table 5). However, no difference was noted between measurements made on Day 0 and Day 1 for the acidified swine extract nor were differences noted for the acidified extract when held for up to 17 d before measurement. In contrast to the swine slurry, acidification of the dairy and poultry manures had no impact on extract P measurements the day following extraction. While further measurements on the unacidified extracts were not continued, P measured in acidified extracts for these manures did not change from initial measurements when held for up to 17 d. Self-Davis and Moore (2000) recommend acidifying WEP extracts to avoid precipitation of calcium phosphates before P measurement. Precipitation of calcium phosphates may have contributed to the decrease in P measured in the unacidified swine slurry 1 d after the initial extraction, although this effect was not noted for the other manures. Water-extractable P extract acidification corrected this problem, and our results demonstrate that acidified extracts for the swine and other

Table 3. Effect of centrifuging versus filtering on WEP measurement.

	Swine	Dairy	Layer poultry	Broiler poultry
	WEP (g kg^{-1} dry weight basis)†			
Centrifuge	9.8 a	4.8 a	4.4 a	5.3 a
Filter	9.8 a	4.2 a	4.0 a	5.6 a

† Values followed by same letter within a column are not significantly different at $P < 0.05$. Each value represents mean of three observations.

Table 4. Effect of manure holding time on WEP measurement.

Manure holding time	Swine	Dairy	Layer poultry	Broiler poultry
d	WEP (g kg^{-1} dry weight basis)†			
1	9.8 a	4.8 a	4.4 a	5.3 a
5	8.9 b	4.2 a	4.8 a	5.2 a
8	8.8 b	4.4 a	4.2 a	5.0 a
15	9.5 ab	4.3 a	4.6 a	5.2 a
22	9.3 ab	4.2 a	4.7 a	5.2 a

† Values followed by same letter within a column are not significantly different at $P < 0.05$. Each value represents mean of three observations.

Table 5. Effect of water-extractable P (WEP) extract holding time and acidification on P measured (ICP) in WEP extract.

Extract holding time	Swine	Dairy	Layer poultry	Broiler poultry
d	P in WEP extract (mg L^{-1})†			
0‡	45.1 a	21.6 a	24.7 a	25.9 a
1 (no acid)	39.7 b	20.5 a	23.4 a	24.9 a
1	43.3 a	20.2 a	23.3 a	24.2 a
3	45.8 a	21.9 a	24.6 a	25.7 a
7	46.3 a	22.3 a	24.1 a	24.5 a
10	43.8 a	21.7 a	23.6 a	26.6 a
17	43.8 a	21.4 a	23.5 a	25.3 a

† Values followed by same letter in a column are not significantly different at $P < 0.05$. Each value represents mean of three observations.
‡ Unacidifed samples measured same day as extracted. Samples acidified (except as noted) before storage and before other measurements.

manures can be held for up to 17 d before P measurement without significantly impacting the analytical results.

Extract Phosphorus Measurement Method

For all manures with the exception of the layer poultry, P measured in the WEP extracts by the Murphy Riley colorimetric procedure was significantly higher than P measured by ICP (Table 6). Similarly, when P measured by ICP was regressed against P measured colorimetrically on 66 manure samples submitted for routine analysis to the Agricultural Analytical Services Laboratory, the slope of the curve was 0.93 indicating that P measured by ICP was, over all, approximately 7% lower than the colorimetric P measurement (Fig. 1). The greater quantity of P measured by the colorimetric method in comparison with ICP does not follow expectations based on forms of P detected by the two methods. The Murphy Riley colorimetric procedure measures primarily orthophosphate P, although some organic P compounds can be hydrolyzed by acidic Murphy Riley reagents (Dick and Tabatabai, 1977). The ICP method measures orthophosphate P plus some fraction of the organic and inorganic complexed P forms (Kuo, 1996).

While we are not aware of other studies comparing P measured by the Murphy Riley colorimetric procedure to ICP in WEP extracts, P measured in soil fertility extracts is commonly higher when determined by ICP than by the Murphy Riley procedure (Eckert and Watson, 1996; Kuo, 1996; Nathan et al., 2002; Mallarino, 2003). We suspect that the higher P measurements determined with the colorimetric procedure are a result of color interference in the WEP extracts. After centrifuging or filtration, these extracts were generally brown or amber in color and occasionally cloudy. Consequently, it is likely that the higher values obtained by the Murphy Riley procedure are a result of a higher background reading due to color interference. Despite this difference, the two methods were highly correlated ($r^2 = 0.98$, Fig. 1) and results generally within 5 to 10% of each other (Table 6, Fig. 1). Consequently, while either method is appropriate for measuring P in the WEP extracts, colorimetric P measurements may need to be corrected for background interference for accurate assessment of WEP.

Table 6. Effect of measurement method (ICP vs. colorimetric) on P measured in WEP extract.

Measurement method	Swine	Dairy	Layer poultry	Broiler poultry
	P in WEP extract (mg L^{-1})†			
ICP	43.3 a	20.2 a	23.3 a	24.2 a
Colorimetric	46.4 b	23.6 b	25.2 a	27.8 b

† Values followed by same letters in a column are not significantly different at $P < 0.05$. Each value represents mean of three observations.

Interlaboratory Comparisons of Water-Extractable Phosphorus Results

Interlaboratory Variability

Among laboratories, average WEP values (dry weight basis) ranged from 4.6 g kg^{-1} for the dairy manure to 11.6 g kg^{-1} for the swine slurry with WEP values for the two poultry samples falling in between (Fig. 2). Variability among laboratories was highest for the swine slurry. For this sample, the RSD among laboratories was 28.8% and there was more than a two-fold difference between the lowest (6.9 g kg^{-1}) and highest (16.3 g kg^{-1}) WEP value obtained. In comparison, the interlaboratory RSD for the broiler poultry was much lower (13.3%) and RSDs for the dairy and layer poultry manures were near 20%. There was a clear association between interlaboratory variability of the WEP results and the sample percentage of solids with the precision of results improving (lower RSDs) as the percentage of solids increased (Table 7). This trend was not only evident for WEP, but for the percentage of solids and the total P results as well where the interlaboratory RSDs on the swine slurry (2.0% solids) were 23.5 and 35.9%, respectively, but decreased to 0.7 and 7.7% on the broiler poultry manure (72.4% solids). These results suggest that the interlaboratory variability of these analyses is directly related to variability associated with subsampling and analyzing manures with low percentage of solids as noted earlier.

Although the interlaboratory RSD for WEP on the swine manure was comparable with that of total P, the interlaboratory WEP RSDs for the other manures were approximately two or more times greater than those for total P, a method routinely performed by manure-testing laboratories (Table 7). Commonly, the variability of an analytical method is high when it is first introduced, but decreases over time as factors contributing to analytical variability are identified and as laboratories gain proficiency in performing the given method through experience and by participation in interlaboratory sample exchanges (Wolf et al., 1996). While the interlaboratory RSDs for WEP are higher than those of total P on three of the four samples included in this study, it is likely that this variability will decrease over time as factors impacting the method variability are more clearly defined and as laboratories become more proficient with the method.

Intralaboratory Variability

Variability of results (RSDs) within each laboratory was determined from the triplicate analyses performed and the mean RSD used as the measure of intralaboratory variability. Although there was a wide range in laboratory variability, as noted from the standard deviation of the RSDs (Table 7), several trends in the data

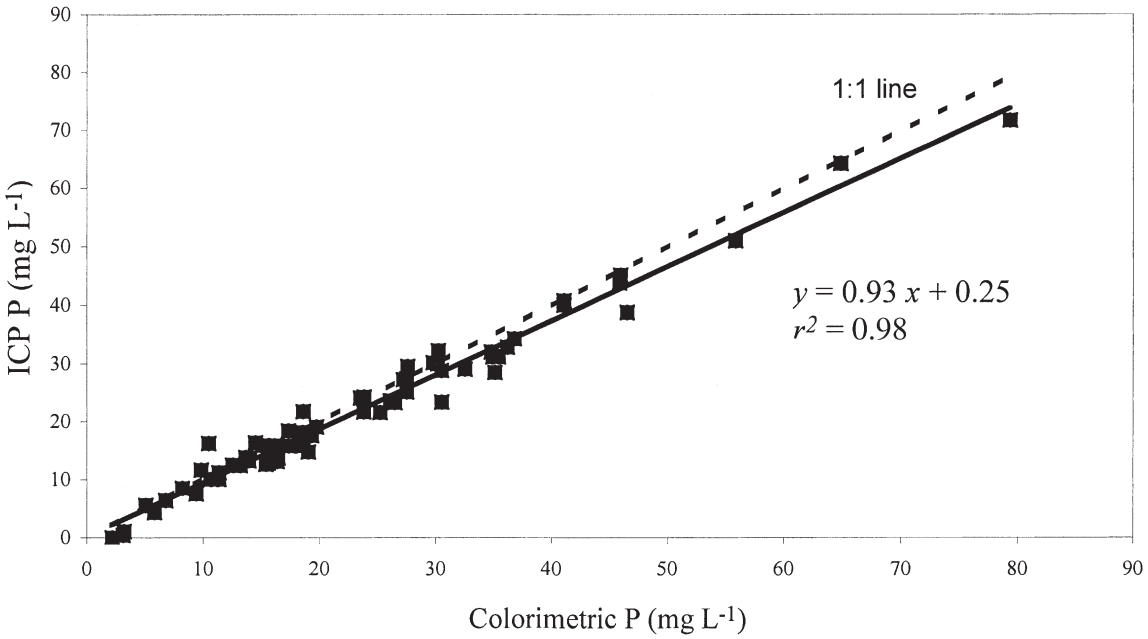

Fig. 1. Relationship between ICP and colorimetric P measurements in WEP extracts.

are evident. Unlike the variability of the WEP results among laboratories, the variability of WEP results within laboratories was not clearly associated with the percentage of solids of the samples (Table 7). Instead, WEP intralaboratory variability was fairly uniform across samples, ranging from 4.0 to 6.4. In contrast, the trend of increased variability with decreased percentage of solids was noted for the percentage of solids and total P results.

The mean variability within laboratories is lower than variability among laboratories (Table 7). This would be expected since the analyst, instrumentation, and other laboratory-specific factors that can contribute to the variability of the results among labs are constant within a given laboratory. In addition, intralaboratory variability for WEP results was either less than or only slightly higher than intralaboratory variability for total P. These results indicate that within each lab, the WEP method is being performed uniformly and at levels of precision consistent with routinely performed manure tests, but there are apparent differences in the method being followed among the laboratories that are contributing to the higher interlaboratory variability of the results.

CONCLUSIONS

This study evaluated the impact of analytical variables on WEP measurements in manure and assessed the inter- and intralaboratory variability of WEP results.

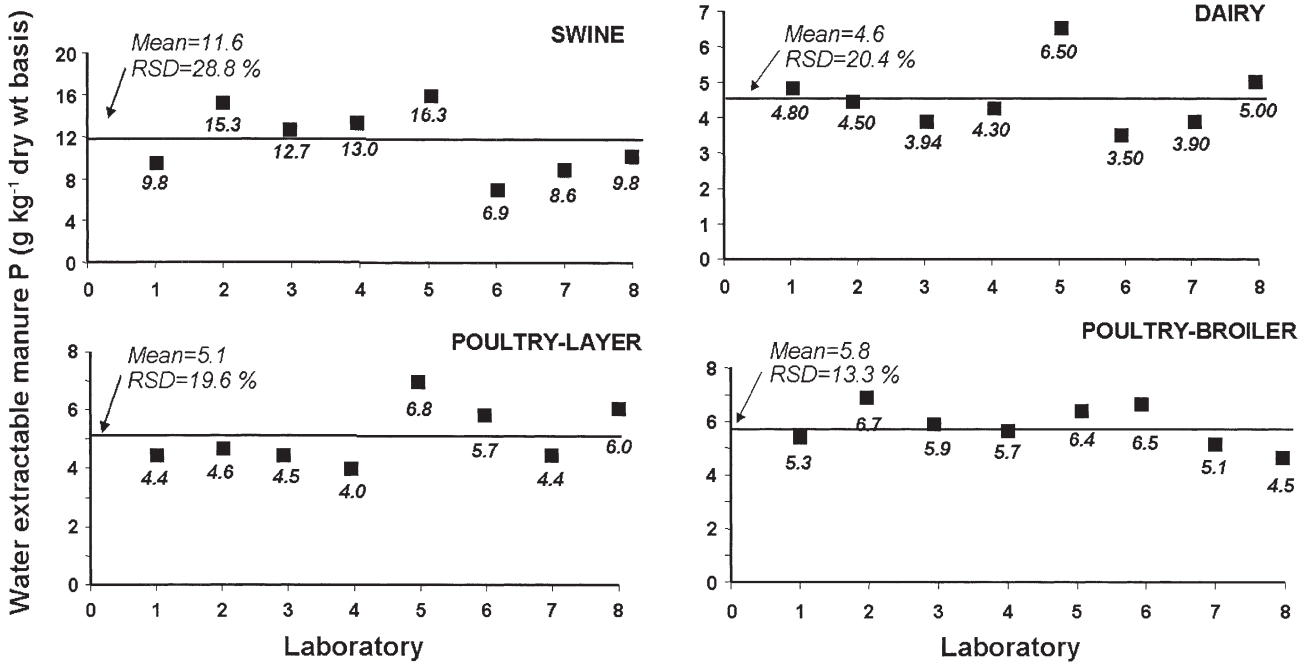

Fig. 2. Interlaboratory comparison of WEP results.

Table 7. Relative Standard Deviations among (inter) and within (intra) laboratories for percentage of solids, total P and WEP results.

		Relative standard deviations					
		% Solids		Total P (dry weight basis)		WEP (dry weight basis)	
Manure type	Solids	Interlab	Intralab†	Interlab	Intralab†	Interlab	Intralab†
	%						
Swine	2.0	23.5	11.9 (10.8)	35.9	9.5 (7.3)	28.8	4.8 (4.3)
Dairy	8.0	13.5	4.3 (4.1)	13.2	5.8 (4.0)	20.4	4.0 (5.1)
Layer poultry	31.9	1.6	2.2 (2.3)	4.8	4.2 (2.1)	19.6	6.4 (6.9)
Broiler poultry	72.4	0.7	0.4 (0.2)	7.7	2.7 (2.0)	13.3	5.0 (4.3)

† Mean RSD of eight laboratories. Value in parentheses is standard deviation.

Study results indicate that refrigerated (4°C) manure samples can be held for an extended period (22 d) before analysis. Sample extracts not analyzed the same day as extraction should be acidified and refrigerated and measured up to 18 d after extraction. Separation procedures (filtering vs. centrifuging) did not impact WEP results. While method of P measurement (ICP vs. colorimetric) did have a significant impact on test results, the two methods were highly correlated and results were generally within 5 to 10% of each other.

Precision within laboratories for the WEP analysis was relatively high, although greater variability among laboratories exists for this procedure than for other routine manure analyses such as total P. Still, the level of precision among laboratories achieved for WEP is reasonable for a new manure test and would be expected to decrease over time as laboratories perform this procedure on a more routine basis and as adjustments to the method are made based on our understanding of factors impacting the variability of the analytical results.

REFERENCES

Brandt, R.C., and H.A. Elliott. 2003. Phosphorus runoff losses from surface-applied biosolids and dairy manure. Proc. 17th Annual Residuals and Biosolids Mgmt. Conf., February 19–22, Baltimore, MD, Water Environment Federation, Alexandria, VA.

Coale, F.J., J.T. Sims, and A.B. Leytem. 2002. Accelerated deployment of an agricultural nutrient management tool: The Maryland Phosphorus Site Index. J. Environ. Qual. 31:1471–1476.

Dick, W.A., and M.A. Tabatabai. 1977. Determination of orthophosphate in aqueous solutions containing labile organic and inorganic compounds. J. Environ. Qual. 6:82–85.

Eckert, D.J., and M.E. Watson. 1996. Integrating the Mehlich 3 extractant into existing soil test interpretation schemes. Commun. Soil Sci. Plant Anal. 27:1237–1249.

Kleinman, P.J.A., A.N. Sharpley, A.M. Wolf, D.B. Beegle, and P.A. Moore, Jr. 2002a. Measuring water extractable phosphorus in manure. Soil Sci. Soc. Am. J. 66:2009–2015.

Kleinman, P.J.A., A.N. Sharpley, B.G. Moyer, and G.F. Elwinger. 2002b. Effect of mineral and manure phosphorus sources on runoff phosphorus. J. Environ. Qual. 31:2026–2033.

Kleinman, P.J.A., A.M. Wolf, A.N. Sharpley, and D.B. Beegle. and L.S Saporito. 2005. Survey of water extractable phosphorus in livestock manures. Soil Sci. Soc. Am. J. 69:701–708 (this issue).

Kuo, S. 1996. Phosphorus. p. 869–919. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 3. SSSA Book Ser. 5. SSSA, Madison, WI.

Leytem, A.B., J.T. Sims, and F.J. Coale. 2004. Determination of phosphorus availability coefficients for organic phosphorus sources: Laboratory studies. J. Environ. Qual. 33: 380-388.

Mallarino, A.P. 2003. Field calibration for corn of the Mehlich-3 soil phosphorus test with colorimetric and inductively coupled plasma emission spectroscopy determination methods. Soil Sci. Soc. Am. J. 67:1928–1934.

Minitab. 2000. Meet Minitab, release 13. Minitab, State College, PA.

Moore, P.A., Jr., T.C. Daniel, and D.R. Edwards. 2000. Reducing phosphorus runoff and inhibiting ammonia loss from poultry manure with aluminum sulfate. J. Environ. Qual. 29:37–49.

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

Nathan, M.V., A. Mallarino, R. Eliason, and R. Miller. 2002. ICP vs. colorimetric determination of Mehlich III extractable phosphorus. Abstract. 2001 International Symposium on Soil and Plant Analysis. Commun. Soil Sci. Plant Anal. 33: 2432.

Peters, J.B. 2003. Recommended methods of manure analysis [Online]. University of Wisconsin, Madison, Cooperative Extension Publication A3769. Available at http://uwlab.soils.wisc.edu/pubs/A3769.pdf. (verified 7 Dec. 2004).

Self-Davis, M.L., and P.A. Moore, Jr. 2000. Determining water soluble phosphorus in animal manure. p. 74–76. *In* G.M Pierzynski (ed.) Methods of phosphorus analysis for soils, sediments, residuals and waters. Southern Coop. Series Bull. #396, North Carolina State University, Raleigh.

Sharpley, A.N., and B. Moyer. 2000. Phosphorus forms in manure and compost and their release during simulated rainfall. J. Environ. Qual. 29:1462–1469.

Sharpley, A.N., J.L. Weld, D.B. Beegle, P.J.A. Kleinman, W.L. Gburek, P.A. Moore, and G. Mullins. 2003. Development of phosphorus indices for nutrient management planning strategies in the U.S. J. Soil Water Conserv. 58:137–152.

USDA and USEPA. 1999. Unified national strategy for animal feeding operations. Available online at http://www.epa.gov/npdes/pubs/finafost.pdf. (verified 10 Dec. 2004).

USEPA. 1986. Method 3051. Acid digestion of sediments, sludges and soils. Test methods for evaluating solid waste. Volume 1A: 3rd ed. EPA/SW-846. National Technical Information Service. Springfield, VA.

USEPA. 1996. Environmental indicators of water quality in the United States. EPA 841-R-96–002. U.S. EPA, Office of Water (4503F), U.S. Gov. Printing Office, Washington, DC.

U.S. Geological Survey. 1999. The quality of our nation's waters: Nutrients and pesticides. U.S. Geological Survey Circ. 1225. USGS Information Services, Denver, CO.

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.

Weld, J.L., D.B. Beegle, W.L. Gburek, P.J.A. Kleinman, and A.N. Sharpley. 2003. The Pennsylvania phosphorus index: Version 1. Publications Distribution Center, Pennsylvania State University, University Park, PA.

Wolf, A.M., J.B. Jones, and T. Hood. 1996. Proficiency testing for improving analytical performance in soil testing laboratories. Comm. Soil Sci. Plant Anal 27:1611–1622.