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2006

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Baum, Kristen; Pierzynski, Gary; Kleinman, Peter; Kovar, John; Maguire, Rory; Moore, Philip; and Zhang, Tiequan, "Evaluating the Influence of Storage Time, Sample-handling Method, and Filter Paper on the Measurement of Water-Extractable Phosphorus in Animal Manures" (2006). *Publications from USDA-ARS / UNL Faculty*. 545.
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Evaluating the Influence of Storage Time, Sample-handling Method, and Filter Paper on the Measurement of Water-Extractable Phosphorus in Animal Manures

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Abstract: Surface-applied manures create a potential phosphorus (P) runoff hazard, especially when unincorporated. In such cases, the concentration of water-extractable

Received 6 August 2004, Accepted 1 September 2005

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P in the manure has been correlated to soluble P concentrations in runoff. This study evaluated the influence of holding time, sample-handling procedure, and filtration method on measurement of the water-extractable P content of manures in a $3 \times 3 \times 2$ factorial arrangement of treatments. A two-way interaction between holding time and sample-handling procedure occurred for most samples. Six samples had water-extractable P concentrations that were less than or equal to dried and dried/ground treatments. Only one sample had higher water-extractable P concentrations for fresh than for dried and dried/ground treatments. When significant differences occurred as a result of the filtration method, results for Whatman No. 40 filters, with a larger pore size than $0.45 \mu\text{m}$ nitrocellulose membranes, were usually higher. There was no significant difference in the coefficient of variation across sample-handling procedures, suggesting that efforts to dry and/or grind samples were not needed. These results support the adoption of a standardized protocol for measuring water-extractable P in manures that represents the appropriate balance between the ease of implementation and the strength of the correlation to P runoff concentrations.

Keywords: Water-extractable phosphorus, runoff, manure

INTRODUCTION

With concerns growing over non point source water pollution, agriculture has been identified as a major contributor of nutrients that are causing a decline in water quality (United States Environmental Protection Agency 1996; United States Geological Survey 1999). The United States Environmental Protection Agency (USEPA) recently issued regulations that will require some concentrated animal feeding operations (CAFOs) to implement a P-based nutrient management plan for land application of manures (United States Environmental Protection Agency 2003). Widespread implementation of the P Index, a site assessment tool designed to rank fields on the basis of their relative vulnerability to runoff P losses, has heightened the need for information on P loss under different nutrient management practices.

When manures are land applied, management decisions such as timing and method of application can affect the potential P loss in runoff (Westerman and Overcash 1980; Mueller et al. 1984; Sharpley 1997, Kleinman et al. 2002a). Runoff water has a shallow interaction depth (Ahuja and Lehman 1983; Zhang et al. 1997), so the properties of the manure will significantly influence potential P loss when it is surface-applied and not incorporated (Kleinman et al. 2002a; Moore et al. 2000; Sharpley et al. 2003). Under such circumstances, studies have shown that water-extractable P in manures is well correlated with P concentrations in runoff (Kleinman et al. 2002a; Sharpley and Moyer 2000). Accordingly, the Arkansas, Pennsylvania, and New Hampshire P indices all use water-extractable P as an indicator of runoff P potential (Sharpley et al. 2003).

To date, there is no single standardized protocol for measuring water-extractable P in manures. Several protocols have been published and are in

use, but none have gained widespread adoption (Kleinman et al. 2002b; Self-Davis and Moore 2000). Many methodological factors affect the results, including sample-handling procedures, holding times, shaking times, extract to solid ratios, and filtration methods. A standardized procedure for measuring water-extractable P should be developed, which will not only yield a strong correlation between the results and P concentrations in runoff but can also be practically implemented by commercial laboratories.

A study by Kleinman et al. (2002b) examined the effects of the extract: solid ratio, shaking time, and filter paper. They found that water-extractable P measurements increased as the extract to solid ratio increased and as shaking time increased. They also found that when statistically significant differences did occur between filtration methods, water-extractable P results were higher when the more coarse paper filters were used (Whatman No. 1, Whatman International, Ltd., Maidstone, UK). After comparing these results with experimental runoff P concentrations, they concluded that the optimum shaking time needed to be at least 60 min, that no single extract to solid ratio was optimum but a fixed ratio was needed for a universal test, and that the filtration methods did not produce significantly different predictions.

This interlaboratory study contributes to the development of a standardized water-extractable P test for manures by examining methodological factors affecting water-extractable P measurement in a variety of animal manures. Specifically, this study evaluates the influence of holding time, sample-handling procedure, and filtration method.

MATERIALS AND METHODS

Six different laboratories participated in this study, collecting a combined total of 17 different 1- to 2-kg organic waste samples from various animal species (Table 1). Each sample was homogenized and divided into thirds. Two portions were stored at 4°C and analyzed after holding times of 3 and 7 days. The remaining portion was analyzed immediately (0 day holding time).

For each holding time, samples were again divided into thirds. Two of these portions were oven dried at 50°C, from which the solids content was obtained. After the portions were dried, one was ground through a 1-mm screen. This provided three different handling procedures: fresh, dried, and dried/ground. Dried and dried/ground samples for a given holding time could not be extracted at the same time as the corresponding fresh samples because of the time required for the drying process.

For the fresh subsamples, 20 g of sample was extracted with 200 mL of deionized water for a 10:1 dilution (solution to fresh solid). On the basis of moisture content, each dried and dried/ground subsample was extracted to the same solution to dry solid ratio as the corresponding fresh subsample so that the solution to dry solid ratio was fixed between fresh and dried subsamples. These steps were conducted in triplicate. Two notable exceptions

Table 1. Summary of participants and corresponding sample descriptions

Location	Investigator	Animal species	Solids content (%)	Comments	Sample ID
Agriculture & Agri-Food Canada	Tiequan Zhang	Cattle	15.1	Manure	CT1
Agriculture & Agri-Food Canada	Tiequan Zhang	Cattle	15.0	Manure	CT2
Agriculture & Agri-Food Canada	Tiequan Zhang	Cattle	16.3	Manure	CT3
Agriculture & Agri-Food Canada	Tiequan Zhang	Pig	15.5	Manure	P1
Agriculture & Agri-Food Canada	Tiequan Zhang	Pig	16.5	Manure	P2
Agriculture & Agri-Food Canada	Tiequan Zhang	Pig	17.8	Manure	P3
Kansas State University	Gary Pierzynski	Chicken	77.1	Broilers; Manure	CHB
Kansas State University	Gary Pierzynski	Chicken	25.2	Layers; Manure	CHL
Kansas State University	Gary Pierzynski	Cattle	39.0	Beef; Manure	CTB
Kansas State University	Gary Pierzynski	Cattle	38.4	Dairy; Manure	CTD
Kansas State University	Gary Pierzynski	Pig	36.3	Manure	P4
North Carolina State University	Rory Maguire	Chicken	84.3	Normal diet; Litter	CHN
North Carolina State University	Rory Maguire	Chicken	71.5	Phytase supplemented diet; Litter	CHP
USDA-ARS Fayetteville, AR	Philip Moore	Chicken	33.1	Not amended with alum; Litter	CH
USDA-ARS Fayetteville, AR	Philip Moore	Chicken	37.4	Amended with alum; Litter	CHA
USDA-ARS Ames, IA	John Kovar	Turkey	51.3	Litter	T
USDA-ARS Univ. Park, PA	Peter Kleinman	Pig	7.0	Manure	P5

to this procedure were the chicken litter samples analyzed by the North Carolina State University and USDA-ARS in Fayetteville, Arkansas. In these instances, the dried and dried/ground samples were not adjusted for moisture, resulting in different solution to dry solid ratios for fresh subsamples vs dried and dried/ground subsamples. As previously mentioned, comparisons could only be made within a sample because the dilutions varied across samples, and studies have shown that water-extractable P measurements increase as the dilution increases (Kleinman et al. 2002b). Although the experimental design of the study by Kleinman et al. (2002b) allows for comparisons to be made among manure samples, this study only allows for comparison within samples, which permits analysis of fresh samples immediately after sample collection, with no knowledge of moisture content.

Diluted sample extracts were shaken for 4 h at room temperature on an orbital, reciprocating, or end-over-end shaker at 120 oscillations per minute. Half of the diluted sample was filtered through 0.45- μ m nitrocellulose membrane filters; the other half was filtered through Whatman No. 40 filter paper, with an average particle retention greater than 8 μ m (Whatman International Ltd., Maidstone, uk). For samples analyzed at Kansas State University, the filtration of the fresh samples at time 0 was impractical because of clogging of the filters, so subsequent samples were centrifuged before filtration. All samples analyzed at the USDA-ARS in Fayetteville, Arkansas, were centrifuged as well. Two drops of concentrated HNO_3 were added to the extracts to prevent precipitation of calcium phosphates. Extracts were stored at 4°C until analysis. The P concentration in the extracts was determined colorimetrically according to the method of Murphy and Riley (1962) at all sites except North Carolina State University, which used inductively coupled plasma atomic emission spectroscopy (ICP-AES). Water-extractable P content of the manure was calculated in g/kg on a dryweight basis.

The experimental design was a $3 \times 3 \times 2$ factorial arrangement of time, sample handling, and filter paper, arranged in a completely randomized design with three replications. Analysis of variance (ANOVA) was calculated by using SAS System for Windows Version 8.0 (SAS Institute, Inc., Cary, NC). Mean separations were made by using LSD at $p = 0.05$.

RESULTS AND DISCUSSION

The amount of water-extractable P measured in the various manures depended on holding time, sample handling, and filter paper. The discussion of these results is organized by main effects and interactions, based on the ANOVA results presented in Table 2. Three manure samples—CT3, CHP, and T—had three-way interactions. A time by sample-handling interaction occurred for all but one sample, CT1, which did, however, have time and sample-handling main effects. Six samples—CT2, CHB, CTD, CHN, T, and

Table 2. ANOVA Results^a

Factor ^b	Manure sample																
	CT1 ^c	CT2	CT3	P1	P2	P3	CHB	CHL	CTB	CTD	P4	CHN	CHP	CH	CHA	T	P5
T	●	●	●	●	●	●	●	●	○	●	●	●	●	●	●	●	●
SH	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
FP	○	●	●	○	●	●	●	○	●	○	●	●	●	○	○	○	○
T X SH	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
T X FP	○	○	●	○	○	○	○	○	○	○	○	●	○	○	○	●	●
SH X FP	○	●	●	○	○	○	●	○	○	●	○	●	●	○	○	○	○
T X SH X FP	○	○	●	○	○	○	○	○	○	○	○	○	●	○	○	●	○

^a ●, Statistical significance at $p = 0.05$; ○, no statistical significance.
^bT, time; SH, sample-handling method; FP, filter paper.
^cCT1-3 are cattle manure samples from Canada; P1-3 are pig manure samples from Canada; CHB, CHL, CTB, CTD, and P4 are broiler chicken, laying hen, beef cattle, dairy cattle, and pig manure samples from Kansas State University; CHN and CHP are chicken litter samples from North Carolina State University; CH and CHA are chicken litter samples from Fayetteville, AR; T is a turkey litter sample from Ames, IA; P5 is a pig manure sample from University Park, PA. For more information, see Table 1.

P5—had time by filter paper interactions or sample handling by filter paper interactions, without a three-way interaction. Four samples—P2, P3, CTB, and P4—had filter paper main effects with no interactions involving filter paper.

MAIN EFFECTS

Only one sample, CT1, had main effects of time and sample-handling with no interactions. Water-extractable P measured at 3 days was 0.6 g/kg. This was statistically higher than at 0 or 7 days, for which averages were 0.4 g/kg and 0.5 g/kg, respectively. Fresh subsamples had a water-extractable P concentrations of 0.8 g/kg, more than that of dried or dried/ground subsamples, at 0.3 g/kg each.

For the samples with main effects due to filter paper, water-extractable P was higher with use of the Whatman No. 40 filter paper in each case. Water-extractable P for the nitrocellulose membrane filters and Whatman No. 40 filter paper was 4.9 g/kg and 5.2 g/kg for P1, 4.1 g/kg and 4.4 g/kg for P3, 1.1 g/kg and 1.2 g/kg for CTB, and 4.1 g/kg and 5.4 g/kg for P4, respectively. This finding suggests that there was a positive interference from use of the Whatman No. 40 filters when P is analyzed colorimetrically. Whatman No. 40 filters may allow passage of colloidal material that increases the measured P concentration in the extract or affects the absorption of light in the colorimetric procedure.

Two-Way Interactions

Time and sample handling affected the water-extractable P results, with a two-way interaction occurring for 13 samples (Table 3). However, there was no observable relationship between water-extractable P measurement patterns and animal species. Six samples—P2, CHL, P4, CHN, CH, and P5—had water-extractable P concentrations for fresh subsamples that were equal to or less than dried and dried/ground treatments. For CHN and CH, this could be attributed to the lower solution to dry solid extraction ratio. Most of these dried and dried/ground subsamples were stable over time but for P5, water-extractable P results decreased with time for the dried and dried/ground subsamples. For one sample, CT2, the water-extractable P measurement was higher for the fresh samples than for the dried and dried/ground samples. The remaining six samples showed no discernable trends.

Two samples, CHN and P5, had time by filter paper interactions (Table 4). For CHN, water-extractable P measurements increased with time and were consistently higher for the Whatman No. 40 filter paper. For P5, water-extractable P results increased with time and when filter paper differences occurred, values for the nitrocellulose membrane filters were higher.

Table 3. Time by sample-handling interactions for water-extractable P^a

T	SH ^b	Manure sample												
		CT2 ^c	P1	P2	P3	CHB	CHL	CTB	CTD	P4	CHN	CH	CHA	P5
-days-								g/kg						
0	D	0.2d ^d	2.4c	4.7e	5.1b	1.9b	3.2b	1.0c	1.2a	4.3ab	3.5d	1.2b	0.4bc	7.7ab
0	DG	0.3c	5.5a	5.3c	5.0b	2.0b	3.6a	1.2b	0.9c	4.6a	4.0a	1.2b	0.3c	8.4a
0	F	0.6b	4.2b	3.3f	3.7cd	2.2a	2.3d	1.3ab	1.0bc	4.7a	1.2f	0.7d	0.4bc	4.6d
3	D	0.4c	4.6b	5.0d	4.7b	1.6cd	3.5a	1.1bc	1.1ab	4.7a	3.9ab	1.3a	0.7a	6.1c
3	DG	0.3c	2.6c	5.5b	3.4de	1.2e	2.7c	1.4a	1.0bc	4.6a	3.9ab	1.2b	0.3c	7.3b
3	F	1.0a	4.5b	4.5e	4.0c	1.7c	1.4e	0.8d	0.8cd	3.8b	1.0g	0.7d	0.5b	3.7c
7	D	0.3c	5.4a	5.9a	3.2e	1.5d	3.5a	1.1bc	1.2a	4.2ab	3.7c	1.2b	0.5b	3.7c
7	DG	0.3c	5.4a	5.8a	3.2e	1.3e	3.4ab	1.1bc	1.0bc	4.4a	4.0a	1.2b	0.3c	4.6d
7	F	1.0a	5.2a	5.4bc	5.8a	1.7c	1.2e	0.9cd	1.1ab	3.5b	1.8e	0.9c	0.4bc	2.4f

^aT, time; SH, sample-handling method.^bD, dried; DG, dried/ground; F, fresh.^cCT2 is a cattle manure sample from Canada; P1-3 are pig manure samples from Canada; CHB, CHL, CTB, CTD, and P4 are broiler chicken, laying hen, beef cattle, dairy cattle, and pig manure samples from Kansas State University; CHN is a chicken litter sample from North Carolina State University; CH and CHA are chicken litter samples from Fayetteville, AR; P5 is a pig manure sample from University Park, PA. For more information, see Table 1.^dMeans within a column having the same letter are not significantly different at $p = 0.05$.

Table 4. Time by filter paper interactions for water-extractable P^a

T(d)	FP ^b	Manure sample	
		CHN ^c	P5
g/kg			
0	MF	2.6e ^d	7.0a
0	W	3.2b	6.7a
3	MF	2.8d	6.1b
3	W	3.2b	5.3c
7	MF	3.0c	3.6d
7	W	3.3a	4.0d

^aT, time; FP, filter paper.
^bMF, Nitrocellulose membrane; W, Whatman 40.
^cCHN is a chicken litter sample from North Carolina State University, and P5 is a pig manure sample from University Park, PA. For more information, see Table 1.
^dMeans within a column having the same letter are not significantly different at $p = 0.05$.

Four samples—CT2, CHB, CTD, and CHN—had a two-way interaction between sample-handling and filter paper (Table 5). The effects of sample-handling varied among the four samples. When filter paper affected the results, water-extractable P concentrations were consistently higher with use of the Whatman No. 40 filter paper, which agrees with the main effects for filter paper.

Three-Way Interactions

A three-way interaction occurred for CT3 (Figure 1a). Filter paper effects varied. Water-extractable P values were consistently less for fresh subsamples than for dried and dried/ground subsamples. In addition, water-extractable P values for the dried and dried/ground subsamples increased with time, whereas there seemed to be no time effect for fresh subsamples.

Another sample with a three-way interaction was CHP (Figure 1b). When filter paper differences occurred, water-extractable P values were higher when Whatman No. 40 filter paper was used. Dried/ground subsamples had a higher water-extractable P values than did dried subsamples. Water-extractable P values for the fresh subsamples were lowest, which could be attributed to the different solution to dry solid extraction ratio previously mentioned. Time effects varied.

Table 5. Sample handling by filter paper interactions for water-extractable P^a

SH ^b	FP ^c	Manure sample			
		CT2 ^d	CHB	CTD	CHN
g/kg					
D	MF	0.3c ^e	1.7b	1.2a	3.4d
D	W	0.3c	1.7b	1.2a	4.0b
DG	MF	0.3c	1.5d	1.0b	3.6c
DG	W	0.3c	1.6c	1.0b	4.3a
F	MF	0.8b	1.7b	0.9c	1.3e
F	W	1.1a	2.0a	1.0b	1.4e

^aSH, sample handling method; FP, filter paper.
^bD, dried; DG, dried/ground; F, fresh.
^cMF, Nitrocellulose membrane; W, Whatman 40.
^dCT2 is a cattle manure sample from Canada; CHB and CTD are broiler chicken and dairy cattle manure samples from Kansas State University; CHN is a chicken litter sample from North Carolina State University.
^eMeans within a column having the same letter are not significantly different at $p = 0.05$.

A third sample having a three-way interaction was T (Figure 1c). Filter paper effects varied Water-extractable P values were higher for the fresh subsamples than for the dried and dried/ground subsamples. Water-extractable P values for the fresh subsamples increased with time, whereas the effect of holding time for dried and dried/ground subsamples was variable.

Variability

A coefficient of variation (CV) was calculated for each sample-handling method. When averaged across all manure samples, the CV was 23.7% for fresh subsamples, 17.8% for dried subsamples, and 17.3% for dried/ground subsamples. No statistically significant differences were found between these values. A higher value was expected for fresh samples because of decreased homogeneity. The relatively large sample size may have helped reduce variability compared with dried and dried/ground samples.

CONCLUSIONS

In this study, the effect of holding time was highly variable, with no evident relationship to animal species. Thus, no generalizations can be made for an analyst needing to determine if storing fresh samples at 4°C is

Figure 1. Three-way interactions for time, sample-handling method, and filter paper. (a) CT3, (b) CHP, and (c) T. D, dried; DG, dried/ground; F, fresh; MF, nitrocellulose membrane; W, Whatman 40.

an acceptable practice or if immediate extraction is needed. In addition, water-extractable P measurements were affected by an interaction between holding time and sample-handling for most samples, but those results were also variable.

The effect of sample-handling method produced similar variability, as well. Likewise, no generalization can be made regarding decisions on sample-handling after a manure reaches the laboratory. As estimated with a coefficient of variation, there were no significant differences in variability of water-extractable P values among fresh, dried, or dried/ground samples.

When significant differences due to filter paper were found, water-extractable P measurements were usually higher from the more coarse Whatman No. 40 filters than those from the 0.45- μm nitrocellulose membrane filters, which agrees with the findings from the study by Kleinman et al. (2002b). These results suggest a positive interference may result from the use of the Whatman No. 40 filters. Filtering time is excessive with the nitrocellulose membrane filters, however, so their use may be impractical.

These findings support the adoption of a standardized protocol for water-extractable P in manure. Further studies are needed that explore correlations between the P concentrations in runoff and results of different procedures. Ultimately, a method will have to be chosen that will have a strong correlation to P concentrations in runoff but can be conducted in a practical manner.

ACKNOWLEDGMENTS

This is Contribution Number 04-339-J of the Kansas Agricultural Experiment Station, Manhattan, Kansas.

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