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Differences of Phosphorus in Mehlich 3 Extracts Determined by Colorimetric and Spectroscopic Methods

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Abstract: Mehlich 3 (M3) is a widely used extractant for evaluating plant available phosphorus (P) in soils and may be quantified using colorimetric or inductively coupled plasma (ICP) spectroscopic methods. Analysis by ICP has recently become increasingly popular in soil-testing labs primarily due to its ability to simultaneously measure multiple elements. Despite the versatility and efficiency of ICP, some laboratories hesitate to use ICP to determine P in M3 extracts because (1) fertilizer recommendations were developed using colorimetric analysis, and (2) differences have been reported between P analyzed colorimetrically and by ICP. This study documented the differences between M3 P measured colorimetrically using a flow injection autoanalyzer and by ICP (ICP-P) for approximately 6400 routine soil samples analyzed by the Soil, Water and Forage Analytical Laboratory at Oklahoma State University. Potential factors contributing to the difference in colorimetric P and ICP-P were also examined using another set of 100 well-characterized soils. Mean ICP-P was greater than colorimetric P ($p < 0.001$) for both sets of soils. Highly significant relationships existed between colorimetric P and ICP-P for the 6400 soils ($r^2 = 0.98$, $p < 0.001$) and for the 100 soils ($r^2 = 0.97$, $p < 0.001$). The relative difference between colorimetric P and ICP-P decreased exponentially with increasing (soil test P measured) colorimetrically. Significant relationships did not exist ($p > 0.05$) between the relative difference in colorimetric and ICP-P and soil properties, including soil pH, clay content, organic

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carbon content, or M3 extractable aluminum (Al), calcium (Ca), and iron (Fe). Fertilizer recommendations based on colorimetric P predicted by ICP values were highly related ($r^2 = 0.81$, $p < 0.001$) to fertilizer recommendations based on actual colorimetric P.

Keywords: Please supply

INTRODUCTION

Excess levels of soil phosphorous (P) have been linked to surface water quality degradation (Sharpley et al. 1996). Commercial fertilizer application and nitrogen-based application of animal wastes associated with confined animal feeding operations (CAFOs) have been linked to P deposition into surface waters through runoff and erosion. Furthermore, excess P in soil can reduce micronutrient solubility and impair crop production (Provin and Pitt, 1999). Soil testing is routinely used as a management tool in evaluating nutrient levels for crop production (Sen Tran et al. 1990) and environmental quality (Sims 1993). Soil test phosphorous (STP) is an estimate of P in soil that will be plant available during a growing season. Pote et al. (1996) found STP to be well correlated with dissolved P in runoff, suggesting that STP could be used in evaluation of soil P levels for environmental quality assessments. There are various STP methods using different extraction solutions, such as Mehlich 3 (M3) (Mehlich 1984), Morgan (Morgan, 1941), Bray 1 (Bray and Kurtz 1945), Olsen (Olsen et al. 1954), and ammonium bicarbonate-DTPA (Soltanpour et al. 1996). Mehlich 3 is a widely used extractant for evaluating plant available P due to its versatility. Mehlich 3 can be used for multiple-element extraction on a wide soil pH range and has been well correlated with crop production (Eckert and Watson 1996; Kuo 1996; Mehlich 1984; Sen Tran et al. 1990). Two common types of instrumentation used in measuring M3 extractable P are calorimetry based on the Murphy and Riley method (Murphy and Riley 1962) and spectroscopy using inductively coupled plasma (ICP). ICP is relatively new in comparison to colorimetric methods, but its versatility (i.e., the ability to simultaneously measure several elements) and efficiency make it a desirable instrument to use in soil testing. Despite the versatility and efficiency of ICP, some laboratories hesitate to use ICP M3P measurements for fertilizer recommendations because colorimetric P determination methods were initially used to calibrate P fertilizer recommendations by many states.

Differences between ICP and colorimetric M3P analyses have been noted by a number of studies (Eckert and Watson 1996; Eliason, Lamb, and Rehm 2002; Kuo 1996; Mallarino 2003; Nathan et al. 2002; Provin and Zhang 2002). Despite the differences noted between ICP and colorimetric M3P analyses, fertilizer recommendations based on ICP M3P analyses are used by some laboratories without any correction or field validation.

This practice could lead to misapplication of nutrients and contribute to monetary crop losses or negative environmental effects if a documented relationship between ICP and colorimetric M3P is not in use to adjust fertilizer recommendations. Concerns brought about by potentially excessive P fertilizer applications and surface water quality degradation have prompted further examination of the difference between ICP and colorimetric M3P. Texas A&M University (TAMU), Kentucky, and Georgia soil-testing laboratories have observed little or no difference between ICP and colorimetric STP measurement, while soil testing laboratories in Oklahoma and Iowa have reported ICP measurements of M3P are generally higher than colorimetric measurements (17, 18). This further suggests the need for examination of the difference between ICP and colorimetric STP in order to identify the reason for inconsistencies among laboratories.

Identifying factors contributing to the difference between ICP and colorimetric M3P could assist in relating ICP to colorimetric P and could possibly resolve inconsistencies among laboratories. Eliason (2001) and Eckert and Watson (1996) attributed the difference between ICP and colorimetric M3P to soil pH, although a pH influenced mechanism was not identified. A probable cause for such findings could be due to the pH specificity in colorimetric methods (Jackson 1958) and the shift of P species with change in pH. Organic P species in soil represent another possible explanation for the difference in ICP and colorimetric P. Kuo (1996) states that the difference in ICP and colorimetric P is likely due to organic P. The limited ability of current soil-testing methods to evaluate this statement and lack of information surrounding this topic necessitates additional examination of the relationship between organic P and the difference between ICP and colorimetric M3P. Yet another explanation for the difference in ICP and colorimetric P is filtration of samples. Kuo (1996) outlines extraction filtration to use quantitative 0.45 μm filter paper until extractant is clear. Despite this, laboratories use time and cost-effective filtration procedures (e.g., Whatman no. 2 filter paper) to expedite sample turnaround (Hehnke and Sparks 1996). Filtration with qualitative filter paper could allow P bound to particulate matter or clay to pass through filters and remain in filtrate, thus being detected by ICP but potentially not detected by Murphy and Riley calorimetry. Additionally, Eckert and Watson (1996), and Sen Tran et al. (1990) reported soil organic carbon (OC) was correlated with M3P but did not elaborate on possible mechanisms responsible for the relationship. Further examination of the relationship between OC and the difference in ICP and colorimetric M3P is needed in order to understand the influence of soil organic carbon. The objectives of this study were to (1) determine the relationship between ICP-P and colorimetric P for routine soil extracts from the state of Oklahoma, (2) determine the relationship between ICP-P and colorimetric P for soils with a wide range of physical and chemical properties, (3) to examine the possible mechanisms causing the difference between ICP-P and colorimetric P for soils with a wide range of physical and chemical properties, and (4) determine if P fertilizer recommendations for Oklahoma soils may be based on a measured ICP-P value that is converted to a colorimetric P value.

MATERIALS AND METHODS

Soil Samples and Analysis Procedures

Approximately 6400 randomly selected routine soil samples, received by the Oklahoma State University Soil, Water and Forage Analytical Laboratory (SWFAL), were analyzed from January 2004 to April 2004 for Mehlich 3 extractable P (M3P). Soils were dried at 35°C and ground to pass a 2.0-mm screen. One 2.0 cm^3 volume scoop of dried, ground soil was extracted with 20 ml of M3 extracting solution (0.2 M CH_3COOH, 0.25 *M* NH_4NO_3, 0.015 *M* NH_4F, 0.013 *M* HNO_3, and 0.001 *M* EDTA) for 5 minutes at 200 reciprocations per minute on an end-to-end Eberbach shaker (Mehlich 1984). The mixture was filtered using Fisherbrand P4 filter paper (Fisher Scientific, Pittsburgh, PA). The filtrate was analyzed colorimetrically for P (colorimetric P) at 880 nm using a Lachat Quickchem 8000 automated flow-injection analyzer (Zellweger Analytics, Milwaukee, WI, Lachat method # 10-115-01-1-A). The same filtrate was also analyzed spectroscopically (ICP-P) using a Spectra CirOs Inductively Coupled Argon Plasma Spectrometer (Spectra CirOs, ICAP, Fitchburg, MA) for M3P at 178.2 nm. Soil pH was measured in a 1 : 1 soil-to-deionized-water suspension (Thomas, 1996).

An additional collection of 100 soils consisting of 40 Oklahoma benchmark soils, 10 Iowa soils, and 50 exchange samples from the North American Proficiency Testing (NAPT) Program were also analyzed for M3P by both colorimetric and spectroscopic methods. These soils were further characterized by measuring soil pH, clay content, organic carbon content (OC), and M3 extractable Al, Ca, Fe, and potassium (K). These soils were selected to further investigate the potential factors responsible for the difference in ICP and colorimetric M3P because of their diverse soil textures, organic carbon content, P content, and point of origin. Soil pH, colorimetric, and ICP M3P were determined in the same manner as for the 6400 SWFAL soils. Elemental contents (Al, Fe, Ca, and K) were measured in the M3 extract using ICP. Clay content was determined using the hydrometer method (Gee and Bauder, 1986), while OC was determined by combustion using a LECO CN 2000 (LECO Corporation, St. Joseph, MI). The effect of filtration was also evaluated on these soils by refiltering an aliquot of filtrate from the initial qualitative filtering procedure using Whatman 0.45 μm Puradisc 25 Nyl syringe filters (Whatman Limited, England; Fisher Scientific, Pittsburgh, PA). The 0.45 μm filtered M3 extracts were analyzed using the same ICP and colorimetric methods as for the qualitatively filtered (i.e., P4 filters) extracts. Effect of filter pore size was evaluated by filtering a subset of soil samples (two medium and one fme-textured soil with high, medium, and low M3P concentrations) with Whatman 0.22 μm syringe filters (Whatman Limited, England; Fisher Scientific, Pittsburgh, PA).

Quality Assurance/Quality Control

The analyses of the 6400 routine samples included laboratory check samples for quality assurance and quality control of soil pH and P. One check sample was analyzed for every nine routine samples. Results for check samples were graphed on quality-control charts, and the control limits for check samples were the mean ±2 std. If a check sample failed control limits, then the nine samples analyzed prior to and the nine samples analyzed after the failure were reanalyzed. Standard reference samples from the NAPT were analyzed for quality assurance and quality control of clay content, organic carbon content, soil pH, P, and K for the 100 characterization soils. Reference samples were evaluated every nine samples with control limits set by NAPT. Corrective action for failures was the same as with the 6400 routine samples. Reagent blanks and reference samples were extracted and analyzed (one per nine samples) for quality assurance and quality control of metals in soil. Extracted blanks contained below detection limit concentrations of Al, Ca, Fe, and K. Detection limits for metals in soil were Al (0.20 $\mathrm{mg\,kg^{-1}}$), Ca (0.50 $\mathrm{mg\,kg^{-1}}$), Fe (0.10 $\mathrm{mg\,kg^{-1}}$), and K (6.0 $\mathrm{mg\,kg^{-1}}$).

Statistical Analysis

All analyses were conducted using PC SAS Version 8.2 (SAS Institute, 2000). To evaluate the relationship of colorimetric P to ICP-P, regressions using PROC REG (SAS Institute, 2000) were conducted for each experiment using colorimetric P as the response variable and ICP-P as the explanatory variable. To compare the regression for the two experiments, an indicator variable regression model was fit where the indicator variable was defined as 0 for the 100 soils experiment and 1 for the 6400 soils experiment. A full interactive model was initially utilized testing whether the slope estimates for the two experiments differed significantly. Differences in intercepts were also tested. Additionally, the role of pH on the colorimetric P and ICP-P relationship was investigated. A pH categorical variable was defined as low (less than 5.5), medium (greater than or equal to 5.5 and less than 7.6), and high (greater than or equal to 7.6). This variable was included as a classification variable within a fully interactive analysis of covariance (ANCOVA) model using PROC GLM (SAS Institute, 2000) and fitting a separate regression parameter for each pH classification. The slopes of these regression parameters were compared and the model sequentially reduced if higher-order factors were not significant. Paired t-tests were used to compare the means of colorimetric P and ICP-P for the two experiments (SAS Institute 2000). PROC REG (SAS Institute 2000) was used to evaluate relationships between various soil-chemical and physical properties compared to the difference in ICP and colorimetric M3P.

RESULTS AND DISCUSSION

Soil Chemical and Physical Properties

The 6400 SWFAL soils had a wide range of chemical and physical properties. Soil pH averaged 6.1 and ranged from 3.7 to 8.5 (Table 1). Phosphorus analyzed by ICP ranged from 1.00 to 277 mg kg^{-1} with colorimetric P ranging from 0.00 to 200 mg kg^{-1}. Mean ICP-P of 41.9 mg kg^{-1} was significantly greater ($p < 0.001$) than mean colorimetric P of 32.7 mg kg^{-1} (Table 1). Our findings are consistent with those reported by other researchers. Sen Tran et al. (1990) reported that analyses for P by ICP yielded higher values than colorimetric analyses in M3 extracts of 82 soils from Quebec. Additionally, Mallarino (2003) found that mean ICP M3P was greater than mean colorimetric M3P for soils from 59 Iowa locations. However, our results contrast with those of Sikora et al. (2005), who reported that colorimetric P and ICP-P were similar for routine soil samples analyzed at the University of Kentucky soil-testing laboratories. The difference between P measured by ICP and by calorimetry ranged from 0.00 mg kg^{-1} to 127 mg kg^{-1} with a median value of 8.50 mg kg^{-1} (Table 1). Our results agree well with those of the North American Proficiency Testing Program, which reported that median P measured by ICP was approximately 7.0 mg kg^{-1} greater than median P measured colorimetrically for 20 samples analyzed by several different laboratories in 2001. The ratio between ICP-P and colorimetric P for the 6400 SWFAL soils ranged from

Table 1. Summary statistics for approximately 6400 Oklahoma soils used to characterize the difference between ICP-P and colorimetric P extracted with Mehlich 3

Statistic	ICP-P (mg kg^{-1})	Colorimetric P (mg kg^{-1})	ICP and colorimetric difference[a] (mg kg^{-1})	Ratio ICP-P/color P[b]	pH
Mean	41.9	32.7	9.1	1.71	6.07
Median	25.5	17	7.00	1.42	5.90
Minimum	1.00	0.00	0.00	1.00	3.70
Maximum	277	200	127	14.0	8.50

[a] Difference = ICP-P − colorimetric P for each paired measurement.

[b] Ratio = ICP-P/colorimetric P for each paired measurement.

1.00 to 14.0 and averaged 1.28 suggesting that significant amounts of non-molybdate reactive P were present in the soils (Table 1).

The 100 soils chosen to characterize the difference between ICP-P and colorimetric P varied widely in chemical and physical properties. Soil pH ranged from 4.4 to 8.6, organic carbon (OC) from 0.27 to 15.9%, and clay from 4.0 to 66.0% (Table 2). Wide ranges of elemental content were also observed for Al, Ca, Fe, and K in the 100 characterization soils. Mean P ($54.4\,\mathrm{mg\,kg^{-1}}$) analyzed by ICP was significantly greater ($p < 0.02$) than mean P analyzed colorimetrically ($41.2\,\mathrm{mg\,kg} - 1$) (Table 2). The difference between ICP-P and colorimetric P ranged from 0.50 to $49.5\,\mathrm{mg\,kg^{-1}}$ with a median of $11.5\,\mathrm{mg\,kg^{-1}}$. The ratio between ICP-P and colorimetric P for the 100 characterization soils ranged from 0.94 to 6.00 and averaged 1.31, which is similar to the mean ratio found for the 6400 soil and similar to ratios between ICP-P and colorimetric P observed by Mallinaro (2003) in his study of Iowa soils.

Relationships between Colorimetric P and ICP-P

A highly significant relationship ($r^2 = 0.98$, $p < .0.001$) was found between colorimetric P and ICP-P for the 6400 SWFAL soils (Figure 1A). A highly significant relationship ($r^2 = 0.97$, $p < 0.001$) also existed between P determined colorimetrically and P measured by ICP for the 100 soils chosen for characterization (Figure 1B). These results are similar to those reported by other researchers who found that highly significant relationships existed between colorimetric P and P analyzed by ICP (Mallarino 2003; Sen Trans, 1990; Sikora et al. 2005). Unlike the study conducted by Sikora et al. (2005), the slopes of the regressions were not very close to one, and the *y*-intercepts were not very close to zero, clearly indicating that colorimetric P was not similar to ICP-P in our study soils (Figure 1). Similarly, Mallarino (2003) reported a slope not equal to one and a *y*-intercept not close to zero for this relationship in his Iowa study. A M3P concentration of $30\,\mathrm{mg\,kg^{-1}}$ is adequate for production of most crops (Johnson et al. 1998). However, research conducted by Oklahoma State University has shown that a field-average soil test P level of $60\,\mathrm{mg\,kg^{-1}}$ is desirable to ensure that most areas of a field have sufficient P and prevent any localized deficiencies due to field variability (Johnson et al. 1998). Therefore, nutrient utilization standards recommend that animal manure applications should not result in soil-test levels of $>60\,\mathrm{mg\,P\,kg^{-1}}$ for Oklahoma soils (Johnson et al. 1998). Thus, an additional regression analysis was conducted using soils with colorimetric $P < 60\,\mathrm{mg\,kg^{-1}}$. There was a highly significant relationship ($r^2 = 0.90$, $p < 0.001$) between colorimetric P and ICP-P for approximately 5400 soils that contained $<60\,\mathrm{mg\,P\,kg^{-1}}$ (Figure 1C). Statistical analysis between the 6400 SWFAL soils and the 100 soils chosen for characterization revealed that the slopes of the two regressions were not significantly different

Table 2. Summary statistics for the 100 characterization soils used to study the potential factors contributing to the differences between colorimetric and ICP-P

Statistic	ICP-P (mg kg^{-1})	Colorimetric P (mg kg^{-1})	ICP and colorimetric difference (mg kg^{-1})[a]	Ratio ICP-P/ color P[b]	Al (mg kg^{-1})	Ca (mg kg^{-1})	Fe (mg kg^{-1})	K (mg kg^{-1})	pH	Clay (%)	Carbon (%)
Mean	54.4	41.2	13.8	1.31	511	2050	169	350	6.40	21.7	2.06
Median	44.0	32.3	11.7	1.36	503	1690	144	260	6.13	19.1	1.49
Minimum	3.50	1.50	0.50	0.94	9.45	119	12.8	16.0	4.40	4.00	0.27
Maximum	223	187	49.5	6.00	1340	7220	447	1610	8.60	66.0	15.9

[a] Difference = ICP-P − colorimetric P for each paired measurement.

[b] Ratio = ICP-P/colorimetric P for each paired measurement.

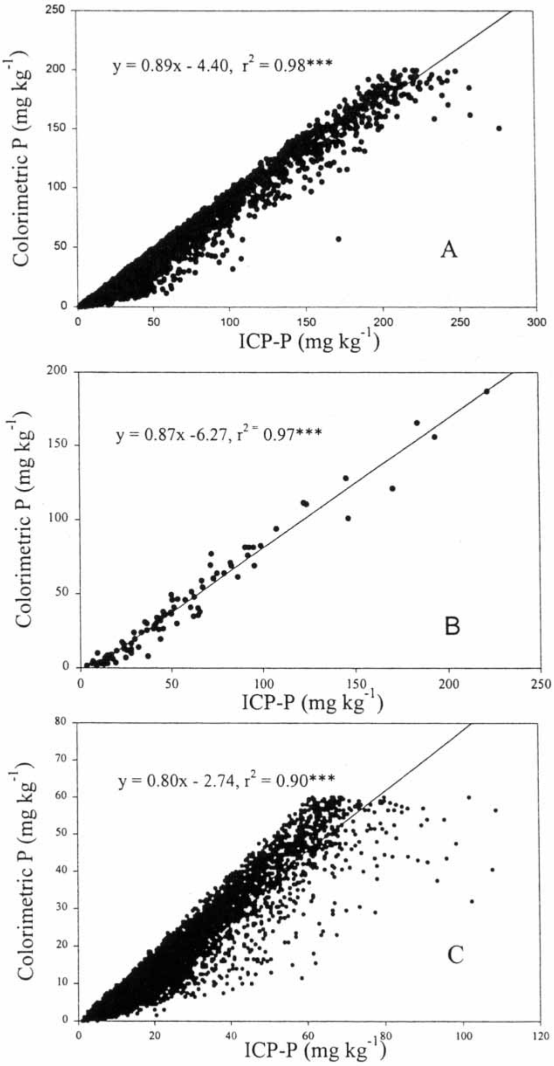

Figure 1. The relationship between Colorimetric P and ICP-P for (A) 6400 soil samples received by Oklahoma State University Soil Testing Laboratory, (B) 100 characterization soils, and (C) 5400 soils with colorimetric $P < 60\,mg\,kg^{-1}$. ***$p < 0.001$.

($p > 0.41$) but that the lower slope of 0.80 obtained for the 5400 soils with $<60\,mg\,P\,kg^{-1}$ was significantly less than the other two regressions ($p < 0.01$). The relative difference between ICP-P and colorimetric P (i.e., ratio of ICP-P/colorimetric P) decreased exponentially as colorimetic P increased (Figure 2). In other words, the relative difference is larger when

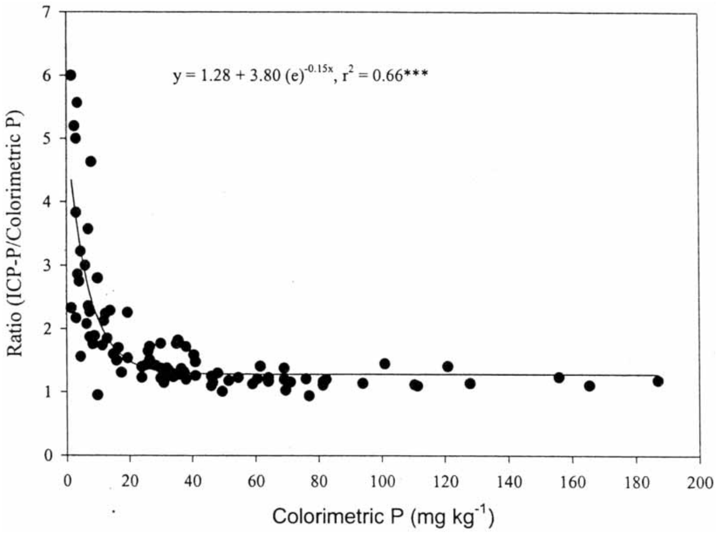

Figure 2. The relationship between the ratio of ICP-P and Colorimetric P and Colorimetric P for the 100 characterization soils. ***$p < 0.001$.

soil test P is lower, where it is critical for P fertilizer recommendations. The results of this study are similar to those reported by Mallarino (2003).

Potential Factors Contributing to the Difference between Colorimetric P and ICP-P

The identification of factors causing the difference between ICP and colorimetric M3P could assist in relating ICP to colorimetric P and could possibly resolve inconsistencies among laboratories. The difference between the M3P values generated by the two methods could be a result of nonmolybdate reactive P species extracted by M3. The Murphy and Riley method (Murphy and Riley 1962) measures molybdate reactive P (i.e., ortho P) and relies upon chemical reaction of the Mehlich 3 extract with reagents to produce a blue color. Therefore, organic P species and/or additional inorganic species aside from ortho P could fail to be detected colorimetrically due to the chemical specificity of the method (Murphy and Riley 1962). On the other hand, ICP completely ionizes the sample at high temperatures such that all species of P in solution are detected. In order to further examine the effect P speciation could have on the difference between ICP and colorimetric M3P, soil-chemical and physical properties that would likely influence the forms of P in M3 extracts were considered. Additionally, the effect of filtration was examined.

Kuo (1996) suggested filtering the extract with a 0.45 μm filter paper. To expedite sample turnaround time, commercial laboratories utilized qualitative filter papers such as Whatman no. 2 or equivalent (Helmke and Sparks 1996). Laboratory use of qualitative filter paper could contribute to the difference in ICP and colorimetric M3P by allowing P attached to colloidal particles to pass filtration and be detected by ICP, but not by the colorimetric method. For comparison, Mehlich 3 extracts were filtered with Whatman 0.45 μm filters (quantitative filters) and with Fisher P4 filters (qualitative filters) and were measured by calorimetry and spectroscopy. Mean colorimetric P of 41.1 mg kg^{-1} for extracts filtered with Whatman 0.45 μm filters was not significantly different ($p > 0.05$) from mean colorimetric P of 41.2 mg kg^{-1} in extracts filtered with Fisher P4 filters (Table 3). Additionally, mean ICP-P of 53.6 mg kg^{-1} in Whatman 0.45 μm filtered extracts was statistically equivalent ($p > 0.05$) to mean ICP-P of 54.4 mg kg^{-1} measured in extracts filtered with Fisher P4 filters (Table 3). Regression analysis also showed a highly significant relationship ($r^2 = 1.00$, $p < 0.01$) between ICP-P in extracts filtered with Whatman 0.22 μm syringe filters and ICP-P in extracts filtered with Fisher P4 filters (data not shown). These observations indicated that P adsorbed to or partially comprising colloidal material able to pass through qualitative filter paper does not significantly contribute to P detected by ICP or calorimetry.

Ortho P species that are measured colorimetrically are influenced by soil pH. Relationships between colorimetric P and ICP-P for different pH ranges are shown in Figure 3. Significant relationships between colorimetric and ICP-P ($r^2 > 0.95$, $p < 0.001$) were observed for all three pH ranges. Statistical analyses showed that the slope of 0.96 for the regression of soils with pH < 5.5 was equivalent ($p > 0.05$) to the slope of 0.88 for the regression of soils with pH > 7.5 but that the slope of 0.83 for the regression of soils with pH 5.5 to 7.5 was different ($p < 0.01$) than the regression of soils with pH > 7.5 (Figure 3). The relationship between the relative difference in ICP-P and colorimetric P (i.e., ratio of ICP-P/colorimetric P) and soil pH was also examined. A significant relationship did not exist ($p > 0.05$)

Table 3. The effect of filtration on phosphorus measured in Mehlich 3 extracts of the 100 characterization soils used to study the potential factors contributing to the differences between colorimetric P and ICP-P

	Colorimetric P (mg kg^{-1})		ICP-P (mg kg^{-1})	
Statistic	Fisher P4	Whatman 0.45 μm	Fisher P4	Whatman 0.45 μm
Mean	41.2	41.1	54.4	53.6
Median	32.2	32.4	43.9	44.1
Minimum	1.29	1.32	3.51	3.05
Maximum	187	186	223	215

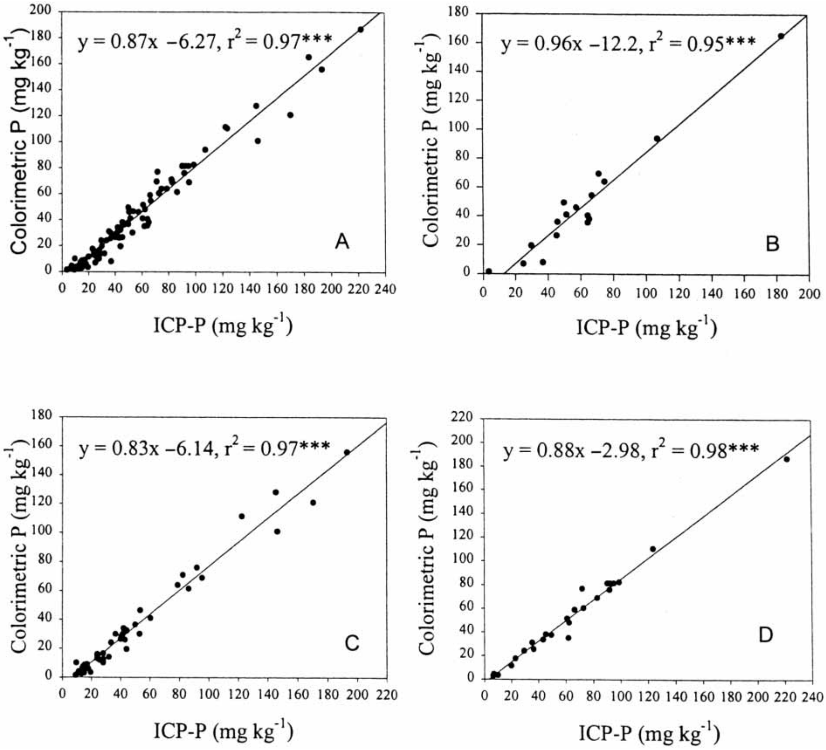

Figure 3. The relationship between Colorimetric P and ICP-P for (A) all 100 characterizations soils, (B) characterization soils with pH < 5.5, (C) characterization soils with pH 5.5 to 7.5, and (D) characterizations soils with pH > 7.5. ***$p < 0.001$.

between the relative difference in ICP-P and colorimetric P and soil pH (data not shown). These results are similar to those reported by Mallarino (2003), who found only a weak but significant negative correlation between the relative difference in ICP-P and colorimetric P and soil pH. Although Eliason (2001) and Eckert and Watson (1996) attributed the difference between ICP and colorimetric M3P to soil pH, the role of pH in relation to the difference between the two methods for both of these studies remained undetermined. While it is obvious that pH affects the relationship between colorimetric P and ICP-P (Figure 3), the role of pH in relation to the difference between the two methods remains unclear.

The effects of M3 extractable Al, Ca, and Fe concentrations in soil could have on the difference between ICP and colorimetric M3P have had little examination. The common P compounds found in soil are related to pH and often contain these three ions, thus the relevance in their examination. Despite the natural close association of these ions and P, no significant relationships ($p > 0.05$) were observed between M3 extractable Al, Ca, and

Fe concentrations and the relative difference in ICP-P and colorimetric P (Figures 4A, 4B, 4C).

The possibility that percent clay content of a soil may significantly contribute to the difference between ICP and colorimetric M3P was also investigated. This was evaluated by regression analysis of the difference in the two

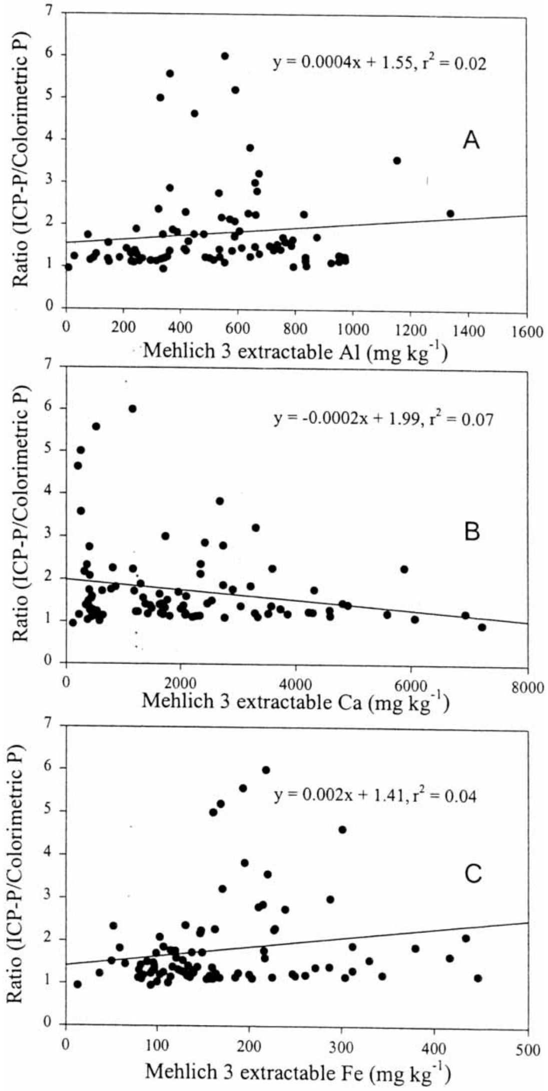

Figure 4. The relationships between the ratio of ICP-P to Colorimetric P and (A) Mehlich 3 extractable Al, (B) Mehlich 3 extractable Ca, and (C) Mehlich 3 extractable Fe for the 100 characterization soils.

M3P testing methods in relation to clay content (Figure 5A), which did not show a significant relationship ($p > 0.05$) between clay content and the relative difference in ICP-P and colorimetric P.

Documentation of the relationship between OC and the difference between ICP and colorimetric M3P is limited. It is possible that OC is a contributor or indicator of the amount of organic P not detectable by calorimetry. Despite this, there was no significant relationship ($p > 0.05$) observed

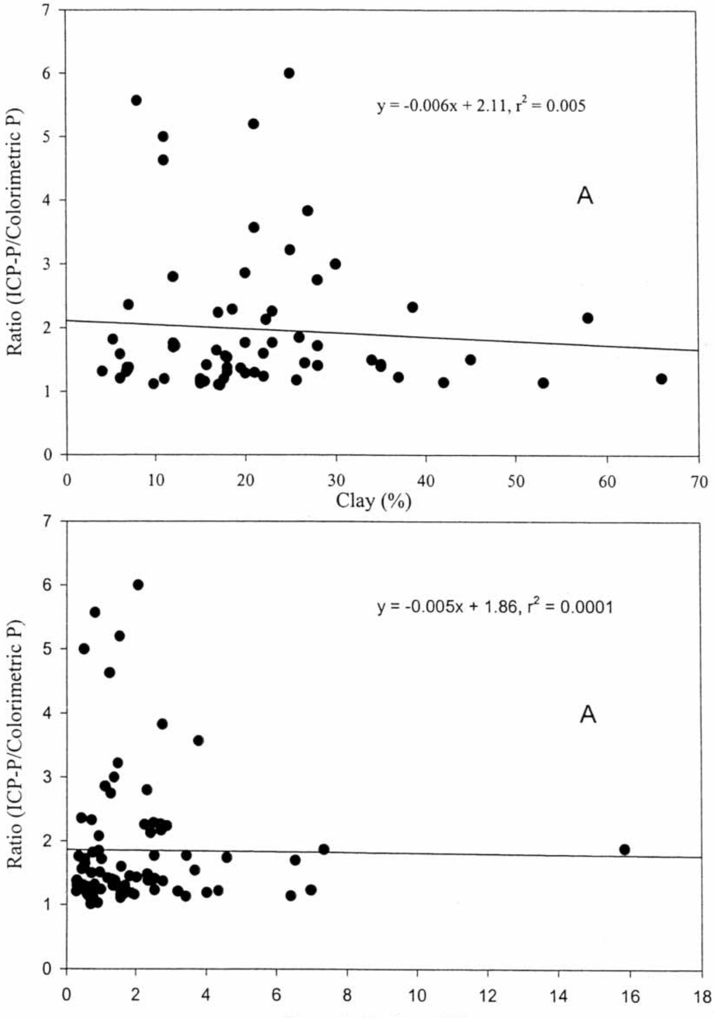

Figure 5. The relationships between the ratio of ICP-P to Colorimetic P and (A) percent clay or (B) percent organic carbon for the 100 characterization soils.

between soil OC and the relative difference in ICP-P and colorimetric P for our study, suggesting that the additional P measured by ICP was not extracted organic P or was forms of organic P not associated with measured OC (Figure 5B). Similarly, Mallarino (2003) found only a weak but significant negative correlation between OC and the relative difference in soil test P measured colorimetrically and by ICP. Eckert and Watson (1996) and Sen Tran et al. (1990) reported soil organic carbon to be correlated with M3P but did not identify the mechanism contributing to the correlation.

Kuo (1996) attributed the difference between ICP and colorimetric results to the presence of organic P in extracts. Higher concentrations of organic matter, such as manure, could contribute to organic P in an extract. ICP measurement could detect larger quantities of P in solution than calorimetry due to the difference in detection mechanisms of each method. Organic phosphorous could be detected by ICP due to the complete ionization of samples by this instrument. The same extract could produce lower M3P analysis when Murphy and Riley calorimetry is used since this method was designed to detect ortho P (Murphy and Riley, 1962). To further examine the influence of organic P species on the difference between ICP and colorimetric M3P, a group of soils was collected consisting of soils amended with varying rates of different types of animal wastes. Mehlich 3 extracts were acquired from six soils amended with poultry litter and four soils amended with beef feedlot manure. A highly significant relationship ($r^2 = 0.96$, $p < 0.001$) existed between colorimetric and ICP-P for the manured soils (Figure 6).

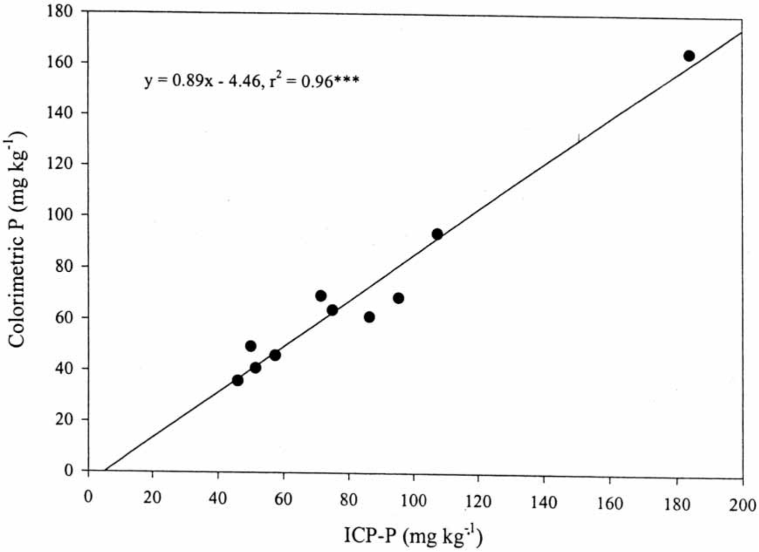

Figure 6. The relationship between Colorimetric P and ICP-P for 10 manured soils. $^{***}p < 0.001$.

Statistical analyses indicated that the slope for the manured soils of 0.89 was equivalent ($p > 0.05$) to the slope of 0.87 for the 100 characterization soils. This suggests that manure additions do not influence the difference between ICP and colorimetric M3P in such a manner that amended soils would exhibit a different relationship than unamended soils between the two methods of P analysis.

Comparison of Fertilizer Recommendations

Current P fertilizer recommendations in Oklahoma are based on colorimetric analysis. Either a new field calibration is needed if ICP-P is used or ICP-P must be converted to colorimetric P so that recommendations may be made. To evaluate if a conversion can be used, a random set of 250 soils was chosen to evaluate P_2O_5 recommendations based on actual measured colorimetric P values and based on colorimetric P values predicted from regression analysis. The 250 soils were extracted and analyzed by both calorimetry and ICP. Measured colorimetic values were used to calculate the amount of P_2O_5 required for small grain crops in Oklahoma while the ICP value obtained was used in the regression equation from Figure 1C to predict colorimetric P. The predicted colorimetric P was then used to calculate the amount of P_2O_5 required for small grain crops in Oklahoma. Results of the recommendation based on actual measured colorimetric P and on predicted colorimetric P were compared. Mean recommendations of 44.6 lbs of P_2O_5 acre^{-1} based on actual measured colorimetric P values were not significantly different ($p > 0.05$) from the mean recommendation of 43.0 lbs of P_2O_5 acre^{-1} based on predicted colorimetric P values (data not shown). A highly significant relationship ($r^2 = 0.81$, $p < 0.001$) existed between P_2O_5 recommendations based on predicted colorimetric P and recommendations based on actual colorimetric P (Figure 7). It is evident from Figure 7 that some error is associated with using the predicted colorimetric P values to make P fertilizer recommendations. However, one must remember that P_2O_5 recommendations based on the actual colorimetric P values also have errors associated with them, which is not highly evident in the fertilizer recommendation tables published by the university. The routine soil samples received by the Oklahoma State University Laboratory are composite samples, and P content may vary by as much as six-fold within a field, depending on the manner that they were collected (Zhang and Johnson, 2004). Ideally, if the P_2O_5 recommendations based on predicted colorimetric P were exactly the same as recommendations based on actual colorimetric P, the slope would equal 1.0, the y-intercept would equal 0, and the r^2 would equal 1.0. The slope and y-intercept of our regression were 0.96 and 0.36, respectively, indicating that making the fertilizer recommendation based on a measured ICP-P value that was converted to a colorimetric P value was practicable.

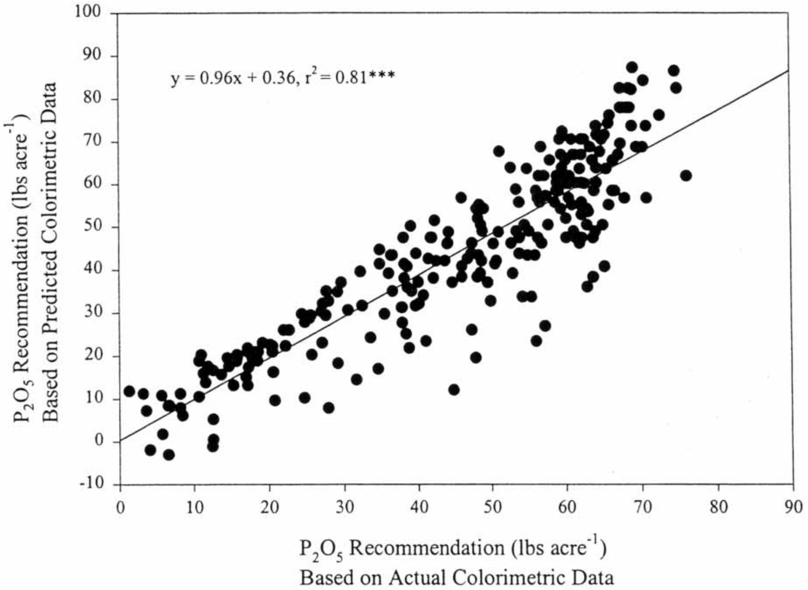

Figure 7. The relationship between P_2O_5 recommendation based on predicted colorimetric data and P_2O_5 recommendation based on actual colorimetric data. $^{***}p < 0.001$.

CONCLUSIONS

Detection of M3P by calorimetry yields lower values than detection of M3P by ICP when extraction techniques and calibration procedure such as those used in this study are implemented. ICP M3P analyses do not produce results that agree with calibrated methods of P detection for fertilizer recommendations in Oklahoma. The implications of these findings are (1) soil tests for P using ICP analysis relating to fertilizer recommendations must be calibrated by, or correlated to, plant growth, or (2) that ICP measurements of P must be converted to colorimetric P with recommendations based on a converted value. Highly significant relationships were found between colorimetric P and ICP-P for both high levels of P (i.e., $>$60.0 mg colorimetric P kg^{-1}) and for lower levels of P (i.e., $<$60.0 mg colorimetric P kg^{-1} where fertilizer recommendations would be made), suggesting that the conversion approach was a viable alternative. Additionally, fertilizer recommendations based on colorimetric P predicted by ICP values were highly related to fertilizer recommendations based on actual colorimetric P. This study has produced two useful regression equations relating colorimetric P to ICP-P. One equation (Eq. 1) is applicable to higher levels of P, which may be of an environmental concern. For example, land application of manure guidelines set by the Oklahoma Natural Resource Conservation Service for P are currently based on soil test P measured colorimetrically (NRCS, 2004), and in certain situations a conversion may be needed to convert ICP-P to colorimetic P.

$$\text{Colorimetric P} = 0.89(\text{ICP-P}) - 4.40 \qquad (1)$$

where colorimetric P and ICP-P are M3 extractable P expressed in'mg kg^{-1} soil.

Another equation (Eq. 2) is more applicable to lower levels of P, where the conversion of ICP-P to colorimetic P (0–60 mg/kg) is needed to make fertilizer recommendations.

$$\text{Colorimetric P} = 0.80(\text{ICP-P}) - 2.74 \qquad (2)$$

where colorimetric P and ICP-P are M3 extractable P expressed in mg kg^{-1} soil.

REFERENCES

Bray, R.H. and Kurtz, L.T. (1945) Determination of total, organic and available forms of phosphorous in soils. *Soil Sci.*, 59: 39–45.

Eckert, D.J. and Watson, M.E. (1996) Integrating the Mehlich-3 extractant into existing soils test interpretation schemes. In *Soil and Plant Analysis in Sustainable Agriculture and Environment*; Hood, T.M. and Jones, J.B., Jr., eds.; Marcel Dekker, Inc: New York.

Eliason, R., Lamb, J.A., and Rehm, G.W. (2001) Agronomy Abstracts. In *Colorimetric and ICP Measurement of P Extracted by M3 Procedure*; CD-ROM, ASA, CSA, and SSSA: Madison, Wisconsin.

Gee, G.W. and Bauder, J.W. (1986) In *Methods of Soil Analysis.* Part 1; Klute, A., ed.; SSSA: Madison, Wisconsin, 383–411.

Helmke, P.A. and Sparks, D.L. (1996) Lithium, potassium, rubidium, and cesium. In *Methods of Soil Analysis: Chemical Methods.* Part 3; Sparks, D.L., ed.; ASA and SSSA: Madison, Wisconsin, 551–574.

Jackson, M.L. ed.; (1958) Phosphorous determinations for soil. In *Soil Chemical Analysis*; Prentice Hall: Englewood Cliffs, New Jersey, 135–141.

Johnson, G.V., Basta, N.T., Zhang, H.A., Hattey, J.A., Raun, W.R., and Steigler, J.H. (1998) *Science-Based Animal Waste Phosphorus Management for Oklahoma*; Oklahoma State University: Stillwater, Oklahoma; Vol. 10, Production Tech. PT 98-1.

Kuo, S. (1996) Phosphorous. In *Methods of Soil Analysis: Chemical Methods Part 3*; Sparks, D.L., ed.; ASA and SSSA: Madison, Wisconsin, 869–919.

Mallarino, A.P. (2003) Field calibration for corn of the Mehlich-3 soil phosphorus test with colorimetric and inductively plasma emission spectroscopy determination methods. *Soil Sci. Soc. Am. J.*, 68: 1928–1934.

Mehlich, A. (1984) Mehlich III soil test extractant: A modification of Mehlich II extractant. *Commun. Soil Sci. Plant Anal.*, 15: 1409–1416.

Morgan, M.F. (1941) Conn. Agric. Exp. Stn. Bull. No. 450. Stors. In *Chemical Soil Diagnosis by the Universal Soil Testing System*; University of Connecticut: Connecticut.

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

Nathan, M.V., Mallarino, A., Eliason, R., and Miller, R. (2002) ICP vs. colorimetric determination of Mehlich III extractable phosphorus. *Commun. Soil Sci. Plant Anal.*, 33: 2432–2433.

Natural Resources Conservation Service. *Nutrient Management Code 590 for Oklahoma*; Oklahoma City, Oklahoma: NRCS, 2004.

Olsen, S.R., Cole, C.V., Watanabe, F.S., and Dean, L.A. (1954) *Estimation of Available Phosphorous in Soils by Extraction with Sodium Bicarbonate*; U.S. Department of Agriculture: Washington, D.C., USDA Circ. 939.

Pote, D.H., Daniel, T.C., Sharpley, A.N., Moore, P.A., Jr., Edwards, D.R., and Nicholos, D.J. (1996) Relating extractable soil phosphorus to phosphorus losses in runoff. *Soil Sci. Soc. Am. J.*, 60: 855–859.

Provin, T.L. and Pitt, J.L. (1999) Texas Agriculture Extension Service Risk Management Education Fact Sheet No. TRM-SCS-59. In *Phosphorous: Too Much and Plants May Suffer*; Agriculture Communications: College Station, Texas.

Provin, T. and Zhang, H. (2002) *The Texas and Oklahoma Soil Testing Calibration/Correlation Effort to Unify Soil Fertility Recommendations*, Proc. Great Plains Soil Fertility Conference; Denver, Colorado, 127–131.

SAS Institute. (2000) *The SAS System for Windows.* Release 8.02 . Cary, North Carolina: SAS Institute, Available at http://www.sas.com/.

Sen Tran, T., Giroux, M., Guilbeault, J., and Audesse, P. (1990) Evaluation of Mehlich 3 extractant to estimate the available P in Quebec soils. *Commun. Soil Sci. Plant Anal.*, 21: 1–29.

Sharpley, A.N., Daniel, T.C., Sims, J.T., and Pote, D.H. (1997) Determining environmentally sound soil phosphorus levels. *J. Soil Water Conservat.*, 51: 160–166.

Sikora, F.J. (2002) *Proposal for Assessing the Difference between ICP and Colorimetric P in Routine Soil Extraction from Soil Testing Laboratories in the Southern Region of the United States*; University of Kentucky: Lexington, Kentucky.

Sikora, F.J., Howe, P.S., Hill, L.E., Reid, D.C., and Harover, D.E. (2005) Comparison of colorimetric and ICP determination of P in Mehlich3 soil extracts. *Commun. Soil Sci. Plant Anal.*, 36 (7&8), in press.

Sims, J.T. (1993) Environmental soil testing for phosphorous. *J. Prod. Agric.*, 6: 501–507.

Soltanpour, P.N., Johnson, G.W., Workman, S.M., Jones, J.B., Jr., and Miller, R.O. (1996) Inductively coupled plasma emission spectrometry and inductively coupled plasma-mass spectrometry. In *Methods of Soil Analysis: Chemical Methods.* Part 3; Sparks, D.L., ed.; ASA and SSSA: Madison, Wisconsin, 91–140.

Thomas, J.S. (1996) Soil pH and soil acidity. In *Methods of Soil Analysis: Chemical Method.* Part 3; Sparks, D.L., ed.; ASA and SSSA: Madison, Wisconsin, 475–490.

Zhang, H. and Johnson, G. (2004) Oklahoma Coop. Ext. Fact. Sheet No. F-2207. In *How to Get a Good Soil Sample*; Oklahoma State University: Stillwater, Oklahoma.