Mass Spectrometric Analysis for Phosphate in Soil Extracts: Comparison of Mass Spectrometry, Colorimetry, and Inductively Coupled Plasma

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ABSTRACT
Phosphate (P) was extracted from soil samples using common soil extraction techniques: deionized water (DI) and ammonium oxalate (AmOx). Colorimetry (Murphy Riley), electrospray ionization mass spectrometry (ESI-MS), and inductively coupled plasma (ICP) were used in the P analysis. Mass spectrometry measured considerably more phosphate than colorimetric analysis following DI extraction and showed a good correlation with ICP for P analysis following AmOx extraction. These results suggest that ESI-MS analysis may detect more than just inorganic P in soil extracts, as ionization and ion transport in ESI-MS may convert organic P into detectable phosphate ions.

INTRODUCTION
Soil test P (STP) is used to determine the amount of phosphate (P) needed for crop production as well as to determine environmental risks associated with elevated levels of soil P. Phosphorus is an environmental concern, because excess P often promotes weed and algae growth in bodies of fresh water. Decomposition of the weed and algae material reduces dissolved oxygen levels, leading to odors, fish kills, and a general degradation of the aesthetic and recreational value of the environment.

Nearly all methods of determining P in soil involve extraction of P into a liquid phase. Deionized water (DI) is a commonly used extractant for P analysis, and ammonium oxalate (AmOx) is a multipurpose extractant that can be used for cation analysis and P determination. Oxalate binds to cations, pulling some of them from soil particles. An ammonium oxalate solution will extract more phosphate from soil than will DI, because the oxalate can replace the phosphate at cationic sites on soil particles. AmOx extractions are of agronomic significance, because they more closely approximate the P that is available to plants. DI extractable P is of particular environmental significance, because it is an indicator of P run-off susceptibility.
Inductively coupled plasma (ICP) optical emission spectroscopy and ascorbic acid colorimetry (Murphy Riley) are commonly used techniques for P analysis in soil extracts. DI extracts are generally analyzed for P by colorimetry or by ICP, and AmOx extracts are generally analyzed by ICP. In Murphy Riley colorimetric analysis, phosphate binds to molybdenum to form a complex that has a blue color. The intensity of the blue color and the absorption at 882 nm are proportional to the amount of phosphate available to form the complex. In ICP the sample is atomized in a plasma for elemental analysis. Analysis by ICP typically results in slightly higher values for STP than colorimetric methods, because some of the organically bound P can be unavailable to form the phosphomolybdate complex necessary for color formation, whereas this ability is not an issue in ICP analysis as the plasma releases elemental P in all P forms.

Mass spectrometric analysis of these types of soil extracts for P analysis is not a common technique. When it has been used, it has generally taken the form of ICP-MS; that is, mass spectrometric determination of elements in place of optical methods following ICP. Mass spectrometry has also been used for the analysis of speciation of phosphate compounds after a separation technique. The way in which MS compares to other methods depends on the amount of bound phosphates that are converted to inorganic phosphate during the electrospray process, because the m/z of 97 is the only value that contributes to the phosphorus count. The mass spectrometric analysis described in this manuscript is a simple electrospray ionization with a quadrupole detector used to analyze filtered extract for inorganic phosphate as an alternative to the common colorimetric and ICP methods.

Three analysis methods and two extraction techniques are investigated in this manuscript. Two combinations are not reported. The first omitted combination is colorimetric analysis of AmOx extracts, because in this lab work, it was found that the blue color did not develop in attempts at colorimetric analysis of AmOx extractions. Searches of scientific literature yielded no examples of colorimetric analysis of AmOx extracts for P. The AmOx extracts were found to be analyzed by ICP, but when colorimetric analysis was employed alternate extractants were used. The other omitted combination is ICP analysis of DI extracts. The AmOx samples were sent out for ICP analysis as a part of a different, funded research project. The DI extracts have not been afforded this opportunity. In this research, DI extracts were analyzed by colorimetry and MS, and AmOx extracts were analyzed by ICP and MS.
MATERIALS AND METHODS

Wisconsin soils tested. Selected soil samples from incubation studies by Ebeling et al. on Phosphorous Buffer Capacity (PBC) of various Wisconsin soils\textsuperscript{13} (“PBC Inc” sample set) and the effect of biosolids application on soil PBC\textsuperscript{14} (“BIO Inc” sample set) were used in this study.

Soil P extraction of Wisconsin soils. PBC Inc soil samples were extracted for soil P by a deionized water (DI) extraction method\textsuperscript{5} with a soil:extractant ratio of 1:10. Samples were shaken in 50 mL conical tubes for 60 minutes at 180 RPM on an orbital shaker. The tubes were centrifuged for 10 minutes at 2500 RPM. After centrifugation, extracts were decanted and filtered. Filtered extracts were stored at room temperature for analysis by Murphy Riley and MS.

BIO Inc soil samples were extracted for soil P by an ammonium oxalate (AmOx) extraction method\textsuperscript{5,15} with a soil:extractant ratio of 1:20. Table 1 shows the components of the ammonium oxalate extractant. Samples were shaken in 50 mL conical tubes for 2 hours in the dark at 180 RPM. The tubes were centrifuged for 20 minutes at 2000 RPM. After centrifugation, the extracts were decanted and filtered. Filtered extracts were stored in the dark at 3.0°C for analysis by ICP and MS.

Ascorbic acid colorimetric analysis using the Murphy Riley method. DI-extracted PBC Inc soils were analyzed by ascorbic acid colorimetry (Murphy Riley).\textsuperscript{16} To prepare samples, 4.0 mL Reagent B (Table 1) and 19.0 mL DI water was added to 2.0 mL of each extract. Standards consisting of 5.0 mL of each standard P solution (0.1 ppm to 1.0 ppm P), 4.0 mL Reagent B, and 16.0 mL DI water and a 0.0 ppm P standard consisting of 4.0 mL Reagent B and 21.0 mL DI water were also prepared. Samples were allowed 30 minutes for color development. The absorbance of the samples and standard solutions at 882 nm was measured with a Perkin Elmer Lambda 25 UV/VIS spectrometer.

Table 1. Components of extractants and reagents used in AmOx soil extraction and Murphy Riley method.

<table>
<thead>
<tr>
<th>Ammonium Oxalate Extractant</th>
<th>Reagent A</th>
<th>Reagent B</th>
</tr>
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<tbody>
<tr>
<td>• 700 mL 0.2 M ammonium oxalate</td>
<td>• 6 g ammonium molybdate</td>
<td>• 1.584 g L-ascorbic acid</td>
</tr>
<tr>
<td>(56.8 g ammonium oxalate monohydrate in 2 L DI)</td>
<td>• 0.146 g antimony potassium tartrate</td>
<td>• 300 mL reagent A</td>
</tr>
<tr>
<td>• approx. 535 mL 0.2 M oxalic acid</td>
<td>• 72 mL sulfuric acid</td>
<td></td>
</tr>
<tr>
<td>(36.0 g oxalic acid in 2 L DI)</td>
<td>• brought to 1 L with DI water</td>
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Inductively coupled plasma (ICP) optical emission spectroscopy. “Total elemental analysis” by ICP optical emission spectroscopy on the AmOx-extracted BIO Inc soil samples was performed by the University of Wisconsin Soil and Plant Analysis Lab (Madison, WI). The results from the P emission at 213.62 nm were used.

Electrospray ionization mass spectrometry (ESI-MS) procedure. A Varian 1200L Quadrupole MS instrument was used to analyze samples. Table 2 shows instrument specifications. Samples were loaded into a 5.0 µL sample loop using the Varian 1200L-LC-ESI Switching Valve. A DI water mobile phase was set at a flow rate of 0.1 mL/min to carry the sample to the electrospray. Varian MS Data Review software and Microsoft Office Excel 2003 were used to analyze the raw data.

MS analysis of DI-extracted PBC Inc soil samples. Two sets of standard addition samples were prepared from two DI-extracted PBC Inc soil samples: sample 6 (SA6) and sample 11 (SA11). Each standard addition set consisted of six samples. Five samples consisted of 0.7 mL soil extract, 0.2 mL standard P solution (one in each sample ranging from 3.4 to 31.6 ppm P), and 0.1 mL 350 ppm chlorate standard. The remaining sample consisted of 0.7 mL soil extract, 0.2 mL DI water, and 0.1 mL 350 ppm chlorate standard. These samples were analyzed by the MS procedure described above using instrument specifications as listed in Table 2. The concentrations of P in SA6 and SA11 were determined from the standard addition peak areas.

DI-extracted PBC Inc samples were prepared using only a chlorate internal standard. Each sample consisted of 0.9 mL soil extract and 0.1 mL 350 ppm chlorate standard. These samples were analyzed by the MS procedure described above using instrument specifications as listed in Table 2. The peak areas of these samples were then
compared to the (peak area) to (P concentration) ratio of the reference sample (SA6 or SA11) in order to determine concentrations of P.

**MS analysis of AmOx-extracted BIO Inc soil samples.** Two sets of standard addition samples were prepared from two AmOx-extracted BIO Inc soil samples: sample 3 (SA3) and sample 9 (SA9). Each standard addition set consisted of six samples. Five samples consisted of 0.8 mL soil extract and 0.2 mL standard P solution (one in each sample ranging from 3.4 to 31.6 ppm P). The remaining sample consisted of 0.8 mL soil extract and 0.2 mL DI water. No internal standard was added to the samples as the extractant itself (Ammonium Oxalate) functioned as an internal standard. These samples were analyzed by the MS procedure described above using instrument specifications as listed in Table 2. The concentrations of P in SA3 and SA9 were determined from the standard addition peak areas.

AmOx-extracted BIO Inc samples were analyzed by the MS procedure described above using instrument specifications as listed in Table 2 with no alterations made to the samples. The peak areas of these samples were then compared to the (peak area) to (P concentration) ratio of the reference sample (SA3 or SA9) in order to determine concentrations of P.

**Murphy Riley and MS analysis of percent P in phytic acid.** Phytic acid solutions ranging from 10 to 100 ppm phytic acid were prepared from a 50% weight-by-weight in water stock solution. Phytic acid solutions were stored in the dark at 3.0°C prior to analysis. Phytic acid solutions were analyzed by Murphy Riley and MS using the procedure described above and instrument specifications as listed in Table 2. Percent P was calculated by dividing the number of phosphates detected by the theoretical number of phosphates that are bound to the phytic acid rings.

**Iron (Fe) precipitation of P in phosphate solutions.** A control group and a test group were prepared for iron precipitation. The control group consisted of four samples with 1.9 mL P solution (one in each ranging from 0.1 ppm to 5.0 ppm P) and 0.1 mL 350 ppm chlorate standard. The test group consisted of four samples with 1.7 mL P solution (one in each ranging from 0.1 ppm to 5.0 ppm P), 0.1 mL 350 ppm chlorate standard, and 0.2 mL 500 ppm Fe standard. These samples were analyzed by the MS procedure described above using instrument specifications as listed in Table 2.
Standards. Phosphate standards (standard P solutions) ranging from 3.4 ppm P to 31.6 ppm P were prepared by serial dilution in DI water starting from a concentrated phosphoric acid solution. All phosphate solutions were then standardized by Murphy Riley.\(^6\)

A 350 ppm chlorate standard was prepared by dissolving 0.2567 g potassium chlorate in 500 mL DI water. This solution was used as an internal standard to correct for variability in the Varian MS instrument and to normalize ion peaks in the MS spectrum.

RESULTS AND DISCUSSION

Iron precipitation of phosphate in phosphate solutions. Iron precipitation of P in soil extracts was performed in order to determine if the MS peak at 97 m/z was due to phosphate and not another ion. Results showed that after addition of iron, the ion peak at 97 m/z was eliminated or significantly reduced. This indicates that MS was likely detecting phosphate.

Matrix effects and the use of standard addition. An attempt was made to quantify P in the soil extracts by establishing a standard curve in MS using P solutions in DI water. Peak areas for soil samples analyzed by MS would have been compared to the standard curve to determine P concentration, if there were no complicating matrix effects. Differences in matrix effects can be gauged by comparison of the slopes of the standard addition curves to the P standard curve. The slope of the P standard curve was approximately 5 times greater than the slope of the DI-extracted PBC Inc standard addition curve and 50 times greater than the slope of the AmOx-extracted BIO Inc standard addition curve. This indicates a significant matrix effect, likely due to components extracted from the soil and the ammonium and oxalate ions. Electrospray performance is very dependent on the amount and type of salts that may be in the matrix. Due to these matrix effects, two samples for each extractant were chosen for standard addition analysis. In order to minimize analysis time, the remainder of the samples were analyzed without standard addition and compared to the reference (standard addition) samples (as described above). For this to be valid, the matrix effects cannot significantly differ between soil extract samples. The reference samples in the DI-extracted PBC Inc sample set had slopes of 0.0038 and 0.0045 (normalized peak area/ppm P) for SA6 and SA11, respectively. For the AmOx-extracted BIO Inc sample set, the slopes of the reference samples were 0.0035 and 0.0051 (normalized peak area/ppm P) for SA3 and SA9, respectively. This demonstrates a difference in the intensity of signal response to phosphate due to matrix effects. The resultant discrepancy between P values based on the
different reference samples (Fig. 1 and 2) clearly indicates that in the future, the matrix effects must be controlled if one does not wish to run standard additions on every sample.

**Comparison of soil P in DI-extracted PBC Inc soils measured by Murphy Riley and MS.** Mass spectrometry measured considerably higher concentrations of P than Murphy Riley in the DI-extracted PBC Inc soil samples (Fig. 1). On average, MS measured 4200% higher when using SA6 as a reference sample and 3200% higher when using SA11. The correlation between Murphy Riley and both MS data sets was poor ($R^2 = 0.1281$). This may be because MS detects more P that is unavailable for phosphomolybdate complex formation.

**Comparison of soil P in AmOx-extracted BIO Inc soils measured by ICP and MS.** Mass spectrometry measured similar concentrations of P as ICP in the AmOx-extracted BIO Inc soil samples when using SA9 as a reference sample (Fig. 2). However, when using SA3 as a reference sample, concentrations of P measured by MS were 270% higher than the “MS - SA9” data set. The correlation of MS to ICP data was $R^2 = 0.8827$. The change in the absolute concentrations is likely due to matrix effects as mentioned above. The relatively good correlation between ICP and MS in AmOx may result from a similarity in the amount and types of phosphorus species that the techniques can detect.
Comparison of percent P in phytic acid measured by Murphy Riley and MS. Phytic acid is a six-carbon ring with a phosphate bound to each carbon. It serves as a good example of organic phosphate.

Mass spectrometry measured a higher percentage of phosphate in phytic acid than Murphy Riley. On average, MS measured 23% P in phytic acid, whereas Murphy Riley only measured 13%. These results are expected as electrospray ionization (ESI) is a much “harder” technique than Murphy Riley and is thus liable to break apart larger molecules. Although ESI is often considered a “soft” MS technique, the analyte is propelled quickly through a part of the spectrometer that is held at atmospheric pressure. The collisions of phytic acid ions with the gas molecules likely remove phosphate ions from the carbon ring. These phosphate ions are thus detected as inorganic phosphate.

It is expected that the carbon ring will be stripped of some phosphate ions during the molybdate reduction reaction in the Murphy Riley method; however, this should not produce as much inorganic P as in the MS procedure, as observed. Also, natural degradation of phytic acid to produce inorganic P is expected, even in the reagent bottle. Thus the phytic acid solution may consist of a certain percent inorganic P prior to analysis. The percent P measured by Murphy Riley may be representative of this “natural” degradation percentage.

These results support the hypothesis that mass spectrometry measures P not detected by the Murphy Riley.
CONCLUSION

Electrospray ionization mass spectrometry has promise as a technique for the analysis of phosphate in soils. The problem of varying matrix effects may be a limitation in the applicability of MS analysis for P. Future work should be invested into improving the precision of MS phosphate measurements and minimizing matrix effect variation; also, measuring P for the same soils after different extraction techniques would give a useful comparison.

The value of STP in soil extracts varies with measurement technique. Just as extraction methods operationally define STP, the method of analysis of P in those extracts has a similar impact. It appears that MS converts organic P into inorganic P and detects more phosphate than Murphy Riley analysis. With further improvements to the method, MS may be found to play an important role in providing a unique and important value of STP, such as approximating bioavailable or run-off susceptible P.

REFERENCES


