Rapid Phytate Quantification in Manures and Runoff Sediments using HPIC

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Abstract

Accurate quantification of phosphorus (P) fractions in manures and agricultural runoff is vital to understanding P dynamics in the environment. Phytate is an organic form of P, with 6 phosphate groups, which is found in manures, but is difficult to quantify due to its affinity for complex formation with aluminum (Al) and iron (Fe).

The objective of the first study was to measure if high concentrations of aluminum (Al) and iron (Fe) hinder accurate quantification of phytate in dairy manure and broiler litter when measured by high performance ion chromatography (HPIC). In this study dairy manure and broiler litter samples were spiked with Al, Fe, and phytate. Samples were alkaline extracted, acidified,cleaned up and filtered, and then phytate was analyzed with HPIC. High concentrations of Fe did not hinder phytate recovery in manure or litter samples. While phytate recovery was close to 100% at typical manure and litter Al concentrations, high concentrations of Al inhibited phytate recovery in litter samples and in some manure samples. Overall, alkaline extraction of dairy manure and broiler litter and analysis with HPIC proved to be a relatively accurate, fast and cheap within normal Al and Fe ranges, compared to the commonly used NMR method.

The developed method was then used to measure the concentration of phytate P (Pp) rather than the entire phytate molecule (6 moles Pp per mole phytate) in runoff waters and in an adsorption study. Phytate P has not been previously measured in runoff, due to the low concentrations of Pp found in runoff and the complexity Pp extraction from runoff. Runoff treatments were dairy manure with and without added Pp (DM, DM+Pp), broiler litter with and without added Pp (BL, BL+Pp), and control, with and without two levels of Pp (control, control

Pp low, control Pp high). Runoff was collected under simulated rainfall and analyzed for total and dissolved reactive P (DRP). Runoff was also separated into sediment (>0.45µm) and liquid (<0.45µm and lyophilized) fractions for Pp analysis by high performance ion chromatography (HPIC). The new method for Pp analysis recovered 70% of Pp spiked into runoff samples. Phytate P was found only on the sediment fraction of runoff and was not detected in the liquid fraction, even when it was lyophilized and extracted. This agreed with the adsorption study, which showed strong adsorption of Pp. Phytate P loss from control Pp high was significantly greater than Pp loss from control. When control Pp high was removed from analysis, Pp loss from BL, BL+Pp, DM, and DM+Pp was significantly greater than from control. Phytate P in the manures appeared to behave differently than pure Pp salts, likely to do the formation of protein-phytate and cation-phytate complexes in the manures. Phytate P had no effect on DRP in runoff for any treatment, indicating no significant release of inorganic P through competitive binding. The majority of P lost in runoff was in fractions other than DRP and Pp in the sediment fraction. Efforts to control the erosive loss of soil during rainfall events appear to be the best way to reduce total P loss, irrespective of the presence of Pp.

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Table of Contents

Table of Figures

Table of Tables

Table of Equations

Chapter 1: Literature Review

Environmental Impacts of Phosphorus

Phosphorus (P) is an essential nutrient for plant growth. Fertilization of agricultural fields with P has long been known to increase crop yield and profitability. Phosphorus has also been identified as the most common cause of eutrophication in freshwater lakes, streams, reservoirs, and in the headwaters of estuaries, such as the Chesapeake Bay (Correll, 1999). Eutrophication can lead to increased algal and plankton growth which in turn can lead to increased water treatment costs and off flavors in drinking water (Anderson et al., 2002). Ingestion of untreated water from rivers and lakes experiencing blooms can pose serious health risks to livestock and humans (Codd, 2000). Additionally, algal and plankton die-off can cause hypoxic conditions in surface waters, leading to fish kills, which can devastate fisheries and tourism (Southard et al., 2010).

Phosphorus is of special concern in the Chesapeake Bay, where excess P loads from the watershed have contributed to eutrophication. Early efforts to reduce P pollution focused on reducing pollution from discrete sources (Point-source; Daniel et al., 1998). Reduced point-source P pollution has drawn attention to diffuse sources of P pollution (nonpoint-source) especially P loss from agriculture. Agriculture has been identified as a large contributor (44%) of total P to the Chesapeake Bay, of which manure P constitutes a significant portion (USEPA, 2010). Concentrated animal feeding operations (CAFOs) in the Chesapeake Bay watershed import animal feed from other parts of the country. In the process, excess P is imported as well, in feeds and manure relative to crop uptake (Maguire et al., 2007). Land application of manure and poultry litter to meet crop nitrogen (N) needs is commonly practiced in the Chesapeake Bay watershed. However, the amount of P removed by crops is less than P applied in manure and litter in this scenario (Maguire et al., 2007). This is because the ratio of N:P in animal wastes is less than the ratio of N:P taken up by plants. Excess P leftover in the field remains after the growing season adsorbed to soil particles.

Long term application of animal wastes can cause an accumulation of soil-bound P (Sharpley et al., 1996). This P is vulnerable to loss as dissolved reactive phosphorus (DRP) and through erosion of soil particles as sediment-bound phosphorus. Efforts have been made in the last decade to reduce soil-bound P loss by implementing erosion prevention strategies, such as no-till farming, throughout the Chesapeake Bay watershed. Additional efforts have been made to increase the ratio of N:P in animal wastes through dietary modification (McGrath et al., 2005). Techniques used include additions of fungal phytase to broiler diets, isolating and breeding crops with highly available phosphorus for animal feed, and lowering supplemental P to both broiler and dairy cow diets (Raboy et al., 2000; Knowlton et al., 2004; McGrath et al., 2005). However, there is still room for improvement because P loss in runoff continues to be an issue. Robust manure P identification and quantification techniques are essential for understanding the effects that dietary and land management changes will have on both the animals and the environment (Turner, 2004).

Phosphorus Fractionation Methods in Manures and Soils

Part of the process of identifying P forms involves the fractionation of P by solubility as a proxy for environmental availability. The earliest attempts to fractionate P in soils focused on assessing inorganic P fractions (Pi; Chang and Jackson, 1957). These fractions were defined as calcium phosphate, aluminum phosphate, iron phosphate and reductant-soluble phosphate

(Chang and Jackson, 1957). Differentiation of the fractions was done by sequential extraction, where by P is partitioned based on its solubility in a series of extractants. The method used by Chang and Jackson has been modified to assess organic P (Po) in soils (Hedley et al., 1982). Po is defined by Hedley et al. (1982) as the difference between total P (TP) and molybdate-reactive P (orthophosphate). This gives a very general measure of Po, as specific Po forms such as inositol phosphates, adenosine triphosphate, and adenosine diphosphate cannot be speciated without additional analysis.

The first complete P fractionation in manure was reported by Peperzak et al. (1959), who identified Pp (*myo*-inositol hexa*kis*phosphate) as a major contributor to the acid soluble fraction of Po in manures. Investigations done by Barnett (1994), using a similar fractionation method to Peperzak et al. (1959), found that acid soluble Po contributed to 53.4% of TP for broiler litter and 7.8% to dairy manure. Barnett (1994) attributed most of the Po found in the acid soluble fraction to Pp. Other P forms constituted a much smaller percentage of TP in both cases.

Recently, sequential fractionation schemes have been developed to better understand the environmental fate of P forms in both manures and soils, following the method developed by Hedley et al. (1982). Turner and Leytem (2004), sequentially extracted manure and litter with water, $NaHCO_3$, NaOH, and HCl. Manure and litter P found in the water and $NaHCO_3$ fraction were considered to be highly soluble and easily lost as DRP in a runoff event (Turner and Leytem, 2004). Phosphorus in the NaOH fraction was assumed to be associated Fe, Al and organic matter (OM), while P in the HCl fraction was assumed to be associated with calcium (Turner and Leytem, 2004). Phosphorus extracted in the HCl and NaOH fractions was considered to be poorly water soluble and thus associated environmentally with the sediment fraction of runoff (Turner and Leytem, 2004). However, little information exists on the P compounds in sequential extracts, making predictions on the environmental fate of P in these extracts difficult (Turner and Leytem, 2004). Additionally, quantification of specific P compounds in manures allows for a mass balance approach to quantifying P needed in animal feed. The digestibility of specific P forms can be identified, allowing for dietary modifications to increase the digestibility of certain P compounds.

In an attempt to speciate Pp compounds in manures, Turner and Leytem (2004) modified Hedley's fractionation method. They separated P fractionation into two steps: extraction with $NaHCO_3$, and extraction with NaOH + ethylenediaminetetracetic acid (EDTA; alkaline extraction). The addition of EDTA improves Pp solubility by chelating polyvalent cations such as Al, Fe, and Ca, thus preventing Pp-cation complex formation. EDTA also improves analytical resolution for P species analysis by ^{31}P NMR. This much-improved fractionation method recovered 99% of TP for broiler litter and 83% of TP for beef cattle manure (Turner and Leytem, 2004).

Single step extraction methods for analytical species determination have been developed using water, $NaHCO_3$, NaOH, and NaOH+EDTA (Alkaline extraction) in manures and soils. Alkaline extraction has been found to give the highest P and Po recovery of any of the extractants. Alkaline extraction of soils recovered between 71 and 90% of soil TP (Cade-Menun and Preston, 1996). Speciation of P compounds in the alkaline extract from soils found Pp to be the dominant form of organic P, ranging from 5.5%-16% of TP (Turner et al., 2003).

Alkaline extraction of cow manure and broiler litter recovered 80% and 96% of total P, respectively (Turner, 2004). Phytate in the alkaline extract from dairy manure and broiler litter was reported to make up between 5 and 14% of total P in dairy manure and as much as 78% of total P in broiler litter (Toor et al., 2005a; Leytem et al., 2006). Given the high percentage of Pp in soils and animal wastes, especially in broiler litter, special attention is given to the origin, digestibility, and environmental fate of Pp.

Origins and Forms Pp in Soils and Manures

Phytate P is part of a class of organic compounds grouped as inositol phosphates that are widely found in nature (Turner et al., 2002). Inositol phosphates are composed of a six-carbon cyclohexanehexol (inositol) ring with up to 8 phosphoric ester groups (Turner et al., 2002; Hill and Cade-Menun, 2009). Of the inositol phosphates, inositol hexa*kis*phosphate (six phosphate groups) is the most commonly found in nature. Four stereoisomers of inositol hexa*kis*phosphate (myo, scyllo, d-chiro-, and neo) have been reported in soils with the myo-stereoisomer (phytate) being most abundant (up to 90%; McKercher and Anderson, 1968).

Phytate P (Pp) is also an important component of animal feeds. In seeds and grains, Pp is the primary storage form of P. Phytate P represents between 75% and 80%, 51% and 57%, and 61% and 80% of total P in maize, soybeans, and wheat, respectively (Lolas et al., 1976; Raboy et al., 2000). Most Pp is (>80%) is found in the germ rather than in aleurone tissues, while for small grains like wheat, the reverse is true (Raboy et al., 2001). Besides its use as P storage, Pp is an important metabolite in cellular P homeostasis and plays a key role in metabolic signaling and ATP regeneration (Raboy et al., 2000).

Phytate P found in seeds and grains is difficult for monogastric animals to digest because they lack the phytase enzymes needed to hydrolyze Pp (Toor et al., 2005b). Unhydrolyzed Pp passes through the digestive system of livestock, where it becomes part of their manure. As previously indicated, Pp content in broiler litter has been found to be as high as 78% of TP, so Pp is of high environmental concern in areas that are economically dependent on concentrated animal agriculture. Additions of fungal phytase and low Pp corn (HAP) to broiler diets, along with reductions in the TP content of broiler diets, have been found to reduce both the concentration of Pp and TP in broiler litter and hence in the environment (Toor et al., 2005b).

Initial investigations into the Pp content of dairy manure found that virtually all Pp (>99%) was hydrolyzed to Pi by ruminal phytases (Clark et al., 1986; Morse et al., 1992). Incomplete Pp recovery in these studies was likely due to metal-Pp complex formation, which hinders dilute HCl extraction of manure samples.

Using alkaline extraction, which give more complete Po recovery than acid extraction, other investigators have reported measureable concentrations of Pp in manures (Turner and Leytem, 2004; Toor et al., 2005a). Additionally, Ray et al. (2012) reported significantly greater Pp recovery in manures following alkaline extraction compared to acid extraction. This new result indicates that while Pp is largely digestible by ruminants, due to ruminal production of phytase, a measurable amount of Pp is not digested, probably due to incomplete hydrolysis caused by metal Pp complexes (He et al., 2009).

Phytate P found in soils comes primarily from plant residue, specifically waste seeds and grains, in addition to applied animal wastes (Leytem and Maguire, 2007; Raboy, 2007). Broiler litter, with its naturally high Pp content, represents an especially significant source of Pp in soils. Turner et al. (2003) suggested that the long term application of broiler litter likely leads to an accumulation of Pp in soils. It has been theorized that the formation of metal-Pp complexes hinders Pp hydrolysis. Additionally, Pp may be stabilized on the surface of clay particles, which hinders the microbial degradation of Pp to Pi. This theory has been the subject of much debate. In an 18 week incubation study done by Leytem et al. (2006), Olson P content in soils at the end of the study was found to be linearly related to soil Pp content, which indicates that Pp hydrolysis was occurring as Olson P values increased with increasing Pp content. As explained previously, microbial organisms have a strong energetic impetus to hydrolyze Pp as it is used for a variety of metabolic functions. Phytate P degradation in soils has been further substantiated by studies showing little Pp accumulation in soils which had received long term, heavy, manure applications (Dou et al., 2009).

Methods of Pp Quantification in Soils, Animal Wastes, and Sediments

Quantification of Pp in soils, animal wastes and sediments is dependent on successful extraction, separation, and detection (Turner et al., 2002). The ideal extraction, separation, and detection process would remove 100% of Pp from the media being analyzed, separate Pp from any metal or organic compounds that might hinder analysis, and detect a wide range of Pp concentrations.

Early Pp quantification methods involved the use of the ferric iron to precipitate Pp as an insoluble iron-Pp complex after acid extraction (Makower, 1970; Lolas et al., 1976). This method is of limited use in samples with low concentrations of Pp as the detection limit for this method is 100 mg Pp L^{-1} (Wheeler and Ferrel, 1971).

Chromatographic quantification methods for Pp replaced quantification by ferric iron precipitation, due in part to the limited detection range of the precipitation method. An early method by Harland and Oberleas (1977) paired Pp concentration and separation on an anion exchange resin with colorimetric P determination after digestion. This methodology was improved by Latta and Eskin (1980) who also used anion exchange for concentration and separation, but measured Pp colorimetrically with Wade's reagent. However, detection and quantification of low Pp concentrations was problematic with this method. Additionally, Pp measurements using this method may have overestimated Pp concentrations due to the inability of the method to separate eluted Pp peaks from those of the lower inositol phosphates (Phillippy and Johnston, 1985).

More recently, HPIC and high performance liquid chromatography (HPLC) have been used for Pp quantification in monogastric animal wastes, feeds, and grains. Skoglund et al. (1997) analyzed ground rye roll, pea flour, and human feces for Pp and lower inositol phosphates. Recovery of spiked Pp after acid extraction was between 95% and 104% (Skoglund et al., 1997). Leytem et al. (2008) measured Pp in broiler litter and ileal digesta using acid extraction and HPLC. Phytate measurements obtained by HPLC were compared to Pp measurements obtained by ^{31}P NMR after alkaline extraction of the same samples, with good correlation between the measures (Leytem et al., 2008). While acid extraction methods developed for use with HPIC and HPLC have given good results for Pp quantification in grain, feed, and monogastric manure, they have limited use for the quantification of Pp in soils and ruminant manures due to relatively poor Pp recovery with these media.

Better extraction of Pp in soils and manures has been accomplished in a single step using alkaline extraction followed by analysis with ^{31}P NMR (Cade-Menun and Preston, 1996; Turner et al., 2003; Turner, 2004). Extracts are frozen to -80 C, lyophilized, and redissolved in 0.9*M* NaOH (Turner, 2004). This method has been found to give accurate Pp quantifications in soils, manures, litters, and sediments. However, Pp analysis using this method is relatively time consuming, requiring between 8 and 22 hours per sample for analysis, excluding lyophilization time (Turner, 2004). This method is inappropriate for use with anion exchange column HPIC due to the presence of organic residues and the alkaline nature of the extracts that can damage analytical columns..

Ray et al. (2012) modified the alkaline extraction technique used by Turner et al. (2004) for analysis of digesta and manure samples with HPIC. Following the alkaline extraction step, the extract is acidified using both 6*M* HCl and 1.2*M* hydrofluoric acid (HF). The extracts are then filtered to remove residual organic residues (Ray et al., 2012). Overall Pp recovery by this method was reported to be ~100%, with an analysis time of 10 min (Ray et al., 2012). This method was chosen for further exploration in the current studies due to its high efficacy and speed compared to ^{31}P NMR.

Pp Adsorption Behavior

Numerous investigations have been made into Pp adsorption onto soils and crystalline and non-crystalline aluminum and iron oxides. Phytate P adsorption in soils is affected by a number of factors including pH, OM content, clay %, and amorphous Al and Fe content (Anderson et al., 1974; Shang et al., 1992; Leytem et al., 2002; Berg and Joern, 2006). Increasing soil pH has been reported to have a negative impact on Pp adsorption on non-crystalline Al and Fe precipitates (Shang et al., 1992). However, the effect of pH on Pp adsorption in soils is inconsistent. Anderson et al. (1974) reported decreased Pp adsorption on soils as pH increased while Leytem et al. (2002) reported increased Pp adsorption on soils as pH increased. There are clearly other factors impacting adsorption in such studies.

Increasing OM content in soils was reported to increase Po adsorption, especially Pp (McKercher and Anderson, 1989; Leytem et al., 2002; Berg and Joern, 2006). Similarly, increasing clay content was reported to increase Pp adsorption in both acidic and alkaline soils (McKercher and Anderson, 1989; Leytem, et al., 2002). This is probably due to the high surface area and reactivity of OM and clay.

Phytate P adsorption was also reported to increase with increasing concentrations of oxalate extractable Al and Fe in acidic soils (Leytem et al., 2002; Berg and Joern, 2006). A linear relationship was reported between P sorption and the sum of oxalate extractable Al and Fe (Freese et al., 1992). This relationship is not specific to Pp, but rather to Pi. The formation of Al and Fe-Pp compounds in soils was reported to stabilize Pp and limit Pp solubility, especially under acidic extraction conditions (Anderson et al., 1974). The precipitation of Al and Fe-Pp compounds in manures has also been reported and is likely the cause of incomplete Pp recovery using acid extraction methods (He, 2006).

Pp in Runoff

Turner et al. (2002) theorized that significant amounts of Pp may be lost from manure-amended soils during rain events resulting in runoff. Although Pp is strongly adsorbed to soil particles, the erosive loss would transfer soil-bound Pp to surface waters. This idea is supported by Suzumura and Kamatani (1995) who measured Pp in river water suspended solids. Additionally, Pp has been measured in dry runoff ditch sediments downstream of chicken houses (Hill and Cade-Menun, 2009). However, Pp has never been measured directly in runoff water from agricultural fields.

Research Statement of Purpose

Our research was designed to a) measure phytate recovery from dairy manure and broiler litter using the method developed by Ray et al. (2012) under a wide range of concentrations of Al and Fe; b) evaluate the NaOH extraction procedure for Al, Fe, and P recovery after spiking with different concentrations of Al and Fe cations; c) use the method developed by Ray et al. (2012) to investigate how Pp is partitioned between > 0.45 μm-sized sediment particles from runoff and the runoff solution passing through a 0.45 μm filter; and d) to quantify actual Pp loss to runoff water from the application of dairy manure and broiler litter to agricultural fields. This is all aimed at improving the understanding of Pp dynamics and P cycling in the environment in the context of agricultural practices.

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Chapter 2: Phytate Quantification Using HPIC in the Presence of Iron and Aluminum

Abstract

Phytate is an organic form of P that is difficult to analyze in complex matrices. To test if high concentrations of aluminum (Al) and iron (Fe) hinder accurate quantification of phytate in dairy manure and broiler litter when measured by high performance ion chromatography (HPIC), we spiked dairy manure and broiler litter samples with Al, Fe, and phytate. Samples were alkaline extracted, acidified, filtered, and then phytate spike recovery was analyzed with HPIC. High concentrations of Fe did not hinder phytate recovery in manure or litter samples. While phytate recovery was complete at typical manure and litter Al concentrations, high concentrations of Al inhibited phytate recovery in litter samples and in some manure samples. Overall, alkaline extraction of dairy manure and broiler litter and analysis with HPIC proved to be a relatively accurate, fast and cheap within normal Al and Fe ranges, compared to the commonly used NMR method.

Introduction

Long-term, high rates of land-applied manures as an agricultural practice can lead to accumulation of phosphorous (P) in surface soils and impaired water quality (Sims et al., 2000; Boesch et al, 2001). Phytate (*myo*-inositol hexakisphosphate) makes up between 5 and 14% of total P in dairy manure and as much as 78% of total P in broiler litter, and is therefore important to environmental conservation (Toor et al., 2005; Leytem et al., 2006). Phytate is the primary P form in seeds and grains, composing 60 – 80% of total P (Raboy, 1997). Phytate found in seed and grains is difficult for monogastric animals to digest and so mineral P is often added as a dietary supplement (McGrath et al., 2005). Phytate is largely digestible by ruminants because ruminal microorganisms produce the enzyme phytase (Raun et al., 1956) but some phytate is still measured in ruminant manures, probably due to incomplete hydrolysis caused by metal-phytate complexes (He et al., 2009a).

Acid extraction of broiler litter and grain samples with $1M$ hydrochloric acid (HCl) has been used for HPIC quantification of phytate (Skoglund et al., 1997). However, this extraction method yields incomplete phytate recovery in dairy manure samples (Turner and Leytem, 2004; Ray et al., 2012). Phytate recovery using HCl extraction may be inhibited in dairy manures through the formation of cation complexes of aluminum (Al), and iron (Fe), and organic complexes (McDowell et al., 2008; Ray et al., 2012).

Alkaline extraction of phytate from dairy manure with $0.25M$ sodium hydroxide (NaOH) + $50mM$ ethylenediamine tetraacetate (EDTA) for phytate quantification using ^{31}P Nuclear Magnetic Resonance Spectroscopy (NMR) gives almost complete phytate recovery (Turner, 2004; Toor et al., 2005; McDowell et al., 2008; Ray et al., 2012). The addition of EDTA as a chelating agent has made this extraction method particularly successful (Turner, 2004; Ray et al., 2012). However, alkaline extraction is inappropriate for use with HPIC because the anion exchange column may be damaged due to the alkaline nature of the extracts and the high levels of organic residues present in the extracts. Thus, quantification of phytate in alkaline extracts must be done using ^{31}P NMR. However, using ^{31}P NMR for phytate quantification is slow, requiring between 8 and 22 hours per sample (Turner et al., 2004). It is also costly and can be insensitive to low phytate concentrations.

Ray et al. (2012) modified the alkaline extraction technique used by Turner et al. (2004) for analysis of digesta and manure samples with HPIC. Following the alkaline extraction step, the extract is acidified using both 6*M* HCl and 1.2*M* hydrofluoric acid (HF). The extracts are then subjected to further cleanup to remove organic residues (Ray et al., 2012).

An appropriate P extraction technique must be able to extract the P forms associated with multivalent cations (He et al., 2009a). Two particular cations, Al and Fe, have been reported to control total P and phytate solubility in manures (McDowell and Stewart, 2005; He et al., 2006; He et al., 2009a). Therefore the objectives of this work were a) to measure phytate recovery from dairy manure and broiler litter using the method developed by Ray et al. (2012) under a wide range of concentrations of Al and Fe, and b) to evaluate the NaOH extraction procedure for Al, Fe, and P recovery after spiking with different concentrations of Al and Fe cations.

Materials and Methods

Manure Collection

Fresh dairy manure was randomly collected from 10 lactating cows (TDM) (*Bos taurus*) and composited. Cows were fed a total mixed ration (TMR) that included corn silage (*Zea mays* L.), alfalfa silage, (*Medicago* sativa), barley silage (*Hordeum vulgare* L.), and a custom grain mix. Additional fresh manure was collected from 10 nonlactating cows (GDM) grazed on a predominantly fescue pasture (*Festuca arundinacea* Schreb) with supplemental fescue hay and composited separately. Fresh broiler (*Gallus domesticus*) litter was collected and composited from a Shenandoah Valley chicken house from which the birds had been recently removed. The broiler diets included a significant fraction of corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] meal. The three composites were well mixed and frozen at -20°C in airtight containers until analysis. Additionally, 22 dried and ground dairy manure samples (field samples) originating from 11 different states were obtained from Cumberland Valley Analytical Services (Hagerstown, MD). No dietary information was available with the field samples.

Sample Analysis

The frozen manure and litter composites were thawed at room temperature, subsampled, dried at 55°C to constant weight, and ground through a 1-mm screen (Toor, et al., 2005; Ray et al., 2012). The ground samples were stored for further analysis in airtight containers. Ground samples were digested using a Kjeldhal digestion procedure from Quikchem Method 13-115-01-1-B (Lachat Instruments, Loveland, CO, USA). Total Kjeldhal P,Ca, and Fe concentrations were quantified using inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Samples were also analyzed using ICP-AES after digestion with nitric acid and perchloric acid (double acid digestion; AOAC, 1984) for purposes of comparison with the Kjeldahl procedure.

Spiking Dairy Manure and Broiler Litter with Phytic Acid, Aluminum and Iron

Phytic acid dodecasodium salt ($NaIP_6$, $C_6H_6O_{24}P_6Na_{12}$, minimum 90% purity; Sigma, St. Louis, Mo.) from rice was used to produce a phytate stock solution. Stock solution concentration was 1000 mg phytate P L^{-1} and was pH-adjusted to 7 with 1.0*M* NaOH. To simulate high levels of Al and Fe, manure and litter samples were spiked with aluminum chloride ($AlCl_3$) and ferric chloride ($FeCl_3$). Separate stock solutions of $AlCl_3$ and $FeCl_3$ were made for use with the manure and the litter samples. Solutions of $FeCl_3$ were formulated to mimic addition of 0%, 50%, 100%, 150%, and 300% of the average measured total Fe in GDM and broiler litter A. Broiler litter A was only used for spiking solutions of Fe. All other sample analysis and comparisons were done with broiler litter B (BL). Solutions of $AlCl_3$ were formulated to mimic addition of 0%, 50%, 100%, 150%, and 300% of dairy manure and broiler litter Al concentrations reported in literature (Table 1).

Dried ground samples of broiler litter and dairy manure were spiked with $AlCl_3$ or $FeCl_3$ solutions. Samples were vortex mixed for 10 seconds and then allowed to equilibrate for 24 hours at 4°C in airtight centrifuge tubes. The samples were then spiked with either the phytate solution (1000 mg L^{-1}) or H_2O to evaluate phytate recovery. Solutions were then vortex mixed for 10 seconds and allowed to equilibrate for another 24 hours at 4°C in airtight centrifuge tubes.

Extraction of Spiked and Non-spiked Manure Samples

All sample solutions were extracted with 20 mL of 0.25*M* NaOH + 50*mM* EDTA and shaken continuously for 4 h on a horizontal shaker at room temperature. Samples were centrifuged at 12,000 *g* for 15 min at 10°C. Supernatant was reserved for analysis.

For phytate analysis, 10 mL aliquots were acidified using 0.5 mL of 6*M* HCl and 0.5 mL of 1.2*M* hydrofluoric acid (HF). Acidified extracts equilibrated at 4°C overnight and were then centrifuged at 12,000 x *g* for 15 minutes at 10°C. The supernatant from each sample was passed drop-wise through a methanol-conditioned C_{18} cartridge (Waters Corp., Milford, MA). The first 2 mL of supernatant were discarded, a 0.2-µm IC filter membrane was attached, and the remaining supernatant was filtered drop wise into sample vials (Dionex Corp., Sunnyvale, CA) for HPIC analysis. Broiler litter samples were diluted with deionized distilled water (1:10) to bring the phytate concentration within in the detection limit of the HPIC.

HPIC Analysis

Chromatographic quantification of phytate in the acidified alkaline extracts was by HPIC (Dionex ICS 3000, Dionex 4x50 mm IonPac AG7 guard column, and 4x250 mm IonPac AS7 analytical column; Dionex Corp., Sunnyvale, CA) using post column reaction detection. Sample extracts were injected using a 50-µl sample loop and phytate was eluted after 7.5 min with isocratic elution (0.25 *M* HNO_3) at 1 mL min^{-1}. Eluted phytate was reacted with a 2% $HClO_4$ solution containing 0.1% $Fe(NO_3)_3$ in a post-column reactor. The phytate products were measured using an UltiMate 3000 UV-Visible light detector (Dionex Corp., Sunnyvale, CA) at 290 nm. Calibration standards (6, 30, 60 µg of P mL^{-1}) for the chromatograph were prepared using the stock phytate solution.

Statistical Analysis

All statistical analysis (analysis of variance, Student's T pairwise comparison of means, normality, and regression) was performed with JMP® Pro 10 (SAS Institute, Cary, NC, 2012). Significance was declared at $P < 0.05$. Error bars on figures represent standard deviation of the mean.

Results and Discussion

Effect of Digestion Method on Recovery of Fe, Ca and P

Total elemental quantification in organic matrixes such as dairy manure and broiler litter is frequently conducted with an open vessel nitric and perchloric acid digestion procedure (Mcbride and Spiers, 2001; Cooperband and Good, 2002). This procedure is lengthy and cannot be used for total N quantification because of interference from nitric acid. Total Kjeldhal digestion is less time-consuming and the results are suitable for both total N and P quantification, which can be used for nutrient management plans for the land application of manure and litter on agricultural fields.

ICP-AES analysis of the manures and litter after Kjeldhal digestion measured a wide range of Fe, Ca, and P concentrations (Table 2-1). Differences in polyvalent cation concentrations can affect phytate recovery due to the formation of insoluble cation-phytate complexes. Of the cations typically found in manures, Al and Fe are considered to be the most important cations for P complex formation (Jackman and Black, 1951; McDowell and Stewart, 2005). In the samples analyzed, the BL had the highest concentration of P (Table 2-1). The concentrations of P and Fe measured in the BL sample were similar to those reported by other authors (Turner, 2004; He et al., 2006). The Ca concentration in BL was slightly higher than that reported by Turner (2004).

The highest concentration of Fe was measured in GDM, while TDM had the highest concentration of Ca (Table 2-1). The concentrations of P, Ca, and Fe in the dairy manure samples were similar to those reported by others (Toor et al., 2005). Differences in Fe and Ca concentrations in the dairy manures were likely caused by dietary differences. The feed for the TMR-fed dairy cows included a mineral supplement that contained Ca (4,200 mg kg^{-1} as fed) and Fe (207 mg kg^{-1} as fed), but no added Al. The grass fed cows were allowed to graze, with Fe from ingested soil acting as a major supplement to total Fe in the cows' diet (Healy, 1972). Additionally, the ingestion of soils with low exchangeable Ca, like those found in Virginia, has been reported to reduce Ca availability in the diet of ruminant animals (Healy, 1972). These differences in Fe and Ca ingestion may account for the differences in Fe and Ca concentrations in the two dairy manures.

Measured total P content of the BL and the dairy manures was not affected by digestion method (Table 2-1). There was no significant effect of digestion method on Ca concentration for BL or GDM, but the Kjeldhal digestion extracted significantly more Ca from the TDM than did the double acid digestion (Table 2-1). Significantly more Fe was extracted from both dairy manure samples by double acid digestion than by Kjeldahl digestion (Table 2-1). There was no difference in broiler litter Fe content between the two digests (Table 2-1).

Additionally, comparisons for total elemental recovery were made between the two digestion methods above and the alkaline extraction method used for phytate quantification. Total elemental recovery from the alkaline extraction was significantly lower than was recovered by the two digestion methods except for P for both GDM and TDM and Ca for the GDM (Table 2-1).

Double acid digestion was also done on field samples to provide a wide range of manures with which to compare the elemental concentrations of GDM and TDM. The mean Al, Ca, Fe, and P concentrations for the field samples were 1.5, 20.1, 1.6, and 7.3 g kg^{-1} dry weight, respectively. The concentrations of these elements were within typical ranges (Toor et al., 2005). GDM had significantly higher Al and Fe concentrations than both the TDM and field samples. This discrepancy is probably due to the soil ingested by grass-fed cows (Healy, 1972). The concentrations of Al and Fe across all samples were positively correlated ($r^2 = 0.94$), likely because they are commonly found together in soil or soil derived systems (Fig. 2-1).

Effect of Added Fe on Phytate Recovery

Mean recovery of spiked phytate was 107% for GDM, 96% for TDM and 102% for BL (Fig. 2-2), similar to recovery in dairy manure reported by Ray et al. (2012) using the same extraction procedure. Mean phytate recovery from GDM was never below 100%, even at the highest Fe spike (Fig.2-2). Mean phytate recovery from TDM decreased slightly as Fe concentrations increased, with recovery dropping from 109% to 94% with the (Fig. 2-2), similar to results reported in literature for the phytate content of foods (Skoglund et al., 1997; Brooks and Lampi, 2001). Phytate recovery was satisfactory for both manures at all levels of Fe addition.

Recovery of phytate for BL with no added Fe was 102% (Fig. 2-2), was above 95% with addition of the 50, 100, and 150% Fe spikes, and dropped to 90% at the highest Fe spike (300%). Less Fe was spiked into the broiler litter than into the dairy manures because of lower concentrations of Fe in non-spiked broiler litter. Phytate recovery from broiler litter with this method was comparable to other methods of phytate measurement (>95% recovery in the typical range of Fe concentrations; Fig 2; Skoglund et al., 1997; Chen, 2004).

Effect of added Al on Phytate Recovery

Phytate recovery for GDM was approximately 100% at all Al spike concentrations (Fig. 2-3). This is similar to phytate recovery reported by other authors using the same method (Ray et al., 2012). Phytate recovery for TDM was 101% with the initial Al spike (50%; Fig. 2-3) and subsequent additions of Al (100%, 150%, and 300%) were associated with a drop in recovery values to 84%, 71%, and 49%, respectively (Fig. 2-3). This loss may be attributed to the formation of insoluble Al-phytate precipitates (Dao, 2003). High concentrations of Al from the spikes likely led to the formation of Al-phytate complexes. However, spiked phytate recovery losses were less for GDM, which had a naturally higher Al concentration than TDM. The reason for this is not clear. Phytate recovery for both manures remained above 90% at typical Al concentrations measured in the multi-state manure comparison.

Recovery of phytate in BL decreased with increasing Al spike concentrations. Mean phytate spike recovery for the initial metal spike (50%) was 93% while mean phytate recovery from progressively higher Al concentrations (100%, 150%, and 300%) dropped to 91%, 83% and 81%, respectively (Fig. 2-3). Overall, phytate recovery values at each Al spike concentration indicate a trend of reduced recovery. Similar to TDM, this may be caused by the formation of insoluble Al-phytate compounds, which has been reported by others (Dao, 2003; Turner, 2004).

Additionally, phytate recovery in the BL samples may have been hindered by the formation of protein-phytate complexes. At low pH (<2), Okubo et al. (1975) reported that phytate strongly complexes with soy proteins, forming insoluble precipitates. Feed given to broiler chickens typically includes a sizable fraction of soybean meal (Leytem et al., 2007). Partially digested proteins extracted in the alkaline extraction step may have formed stabile complexes with phytate after sample acidification. Ray et al. (2012) noted the possibility of a similar occurrence while using this method to extract phytate from soybean meal. However, this only appears to be an issue with the addition of high concentrations of Al. At typical Al concentrations measured in broiler litter, phytate recovery remained above 90%.

Overall recovery was greater than 90% for the broiler litter and dairy manure samples at typical Al and Fe concentrations measured in the field samples. Given the speed, relatively low cost, and minimal training requirements needed to run samples on the HPIC, this method seems to be an excellent alternative to NMR and XANES for the quantification of phytate in manures.

Recovery of Total Phosphorus in NaOH+ EDTA Extracts

Average recovery of total P from alkaline extracts of manure after spiking with phytate was 95% for GDM and 92% for the TDM (Fig. 2-4a). Total recovery of P was above 90% at all

Fe spike concentrations for both manures (Fig. 2-4a).

Recovery of total P from dairy manure was similar to values reported by others (He et al., 2009b; Ray et al., 2012). However, McDowell and Stewart (2004) reported recovery of <50% of added P with alkaline extraction of manures containing naturally high concentrations of Al (8.5 g kg^{-1}) and Fe (4.3 g kg^{-1}). Poor recovery was attributed to the formation of insoluble Fe-P and Al-P compounds (McDowell and Stewart, 2004). The uniformly high recovery of total P from TDM (relatively low in Al and Fe) and GDM (high Al and Fe similar to samples in the McDowell and Stewart study) in the current study suggests that Al and Fe concentration alone may not be good predictors of total P recovery in alkaline extracts. .

Recovery of total P from BL was less than measured for the dairy manures (Fig. 2-4a), but was in the range (64 to 96%) reported for broiler litters by Turner (2004) and He et al. (2006). Recovery of total P from BL was not affected by addition of Fe (Fig. 2-4a).

Differences in reported total P recovery between broiler litters may be attributed to variability in the Ca concentration of the litters. Broiler litters with low concentrations of Ca (similar to samples in the study by He et al.) appeared to have greater recovery than those with higher concentrations of Ca (Similar to samples in the current study and the study by Turner). This may be caused by calcium precipitation in the form of Ca-P compounds during alkaline extraction due poor solubility. This precipitation reaction can reduce total P recovery, especially in broiler litters with high concentrations of Ca (Turner, 2004 and Ajiboye et al., 2007). While Ca concentrations may play a role in total P recovery, it appeared that the effect of the Fe spike on total P recovery was small, for both the dairy manure and broiler litter samples.

The addition of Al to GDM had little effect on recovery of total P in alkaline extracts. Mean total P recovery stayed above 90%, except at the highest Al spike concentration (300%), which reduced mean total P recovery to 89% (Fig. 2-4b). For TDM, total P recovery was reduced with the addition of increasing concentrations of Al. Mean total P recovery without the addition of aluminum (0%) was 92% and was reduced with subsequent additions of Al (50%,100%, 150%, and 300%) to 82%, 81%, 78%, and 74%, respectively (Fig. 2-4b). The difference in total P recovery between the 0% and 300% Al treatments was greater than the amount of phytate P (1000 mg kg^{-1}) added to the manure samples. Given that 47-50% of the phytate spike was recovered (Fig. 2-3), the addition of Al not only hindered phytate recovery, but also hindered recovery of other forms of P. This may be due to the formation of a variety of Al-P complexes (Dao, 2003; Turner, 2004).

The addition of Al to BL had little effect on the recovery of Total P in alkaline extracts. Mean total P recovery without the addition of aluminum (0%) was 85% while the mean P recovery for the highest Al spike (300%) was 88% (Fig. 2-4b). However, as discussed earlier, phytate spike recovery decreased with increasing Al spike concentrations (Fig. 2-3). This may indicate that phytate recovery inhibition and therefore total P recovery inhibition, were taking place after the initial extraction step, possibly during the acidification process. Okubo et al. (1975) suggested that protein phytate complex formation may be encouraged when sample pH is low (<2).

Recovery of added Fe and Al in Alkaline Extracts of Dairy Manure and Broiler Litter

Total Fe recovery in the alkaline extracts of the non-spiked GDM was 10% and was 21% in the non-spiked TDM Total Fe recovery values were similar to those reported by Turner (2004) and greater than those reported by Toor et al. (2005) of 12 and 3%, respectively. Spike recovery trends for Fe spiked manure samples were similar, with recovery decreasing with increasing Fe additions (Fig. 2-5). Although measured Fe spike recovery decreased with increasing Fe spike additions, phytate recovery did not decrease (Fig. 2-2). This indicates that phytate solubility in the dairy manure samples may not be governed by the available Fe concentration in response to alkaline extraction.

Mean total Fe recovery in the alkaline extract for the non-spiked BL was 15%. This was slightly less than the recovery values that Turner (2004) reported of 19%. Mean Fe spike recovery initially increased with increasing Fe additions, peaking at 74% (150%) before falling to 70% (300%; Fig. 2-5). It appears that Fe spike recovery generally increased with the addition of higher concentrations of Fe, but this had little effect on phytate recovery. Mean phytate recovery stayed constant at around 100% (Fig. 2-2) regardless of the amount of Fe recovered from the spike (Fig. 2-5).

Mean total Al recovery for non-spiked GDM in the alkaline extract was 4% while mean total Al recovery for the TDM was 2% (Table 2-1). These are similar to total Al recovery values reported by Turner (2004) of 5% and by Toor et al. (2005) of 3%. For both manures, Al spike recovery increased with increasing Al additions, however Al spike recovery was greater for the GDM samples then for the TDM samples (Fig. 2-6). Interestingly, phytate recovery remained at or above 100% for the GDM at all Al concentrations, while it fell to almost 50% for the TMR fed dairy (Fig. 2-3). Other authors have suggested that phytate recovery depends strongly on the availability of Al (Graf, 1983; Dao, 2003). The recovery results for TDM suggest that insoluble Al-phytate complexes may be forming with the addition of Al spikes, reducing both Al and phytate recovery (Dao, 2003; Turner, 2004). However, this trend was not observed with the grass fed manure samples. The reasons for this are not clear.

Mean total Al recovery for non-spiked BL in the alkaline extract was 13%, and was significantly lower than the 46% recovery observed by Turner (2004). Al spike recovery was not significantly different for any of the Al spike concentrations. Mean recovery for the initial Al spike (50%) was 49% and was 56% for the highest Al spike (300%; Fig. 2-6). However, while Al spike recovery percentage remained constant, phytate recovery decreased with the increasing Al spike concentrations (Fig. 2-3). Additionally, total P recovery remained almost constant across all Al spike concentrations for BL (Fig. 2-4b). This may indicate that Al was not interfering with phytate extraction, but rather phytate recovery was hindered during the acidification step where protein-phytate and Al-phytate complex formation may be encouraged due to the low pH (<2) (Okubo et al., 1975). Further testing using infrared spectroscopy to analyze a dried, extracted, acidified sample for an Amide I strong vibration (Centered at 1650 wavenumbers) may be able to identify the presence of proteins as well as protein-phytate interactions (Barth and Zscherp, 2002).

Conclusions

Alkaline extraction coupled with HPIC analysis provided a rapid, low cost, and sensitive method for quantifying phytate in dairy manures and broiler litter. Addition of Fe had little effect on phytate recovery from these samples. The addition of high concentrations of Al impeded phytate recovery from BL and TDM, but phytate recovery was acceptable when Al and Fe concentrations were typical of those observed in field samples. Similarly, recovery of total P by the alkaline extraction was generally high (>82%) at Al and Fe concentrations typical of manure and broiler litter. Iron spike recovery in GDM and TDM decreased with increasing additions of Fe, while Fe spike recovery increased for BL with increasing additions of Fe, which occurred at the much lower Fe concentrations compared with those in GDM and TDM. Aluminum recovery for GDM and TDM increased with increasing additions of Al, while Al spike recovery for BL changed little with increasing additions of Al. Overall, this method of phytate quantification gave satisfactory recovery of phytate across a range of Al and Fe concentrations with minimal preparation, relatively low cost, and high sensitivity.

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Table 2-1. Amounts of total P, Al, Ca, and Fe in broiler litter and dairy manure samples quantified after Kjeldhal digestion, nitric-perchloric acid digestion (Double Acid Digestion), NaOH + EDTA extraction, and reported from literature

Sample	P	Al	Ca	Fe
	mg kg^{-1}			
	Kjeldhal digestion			
Broiler litter A	14.9b†	-	32.2a	0.8d
BL	15.7a	-	27.3b	1.1c
TDM	5.9de	-	28.0b	0.4e
GDM	6.4d	-	16.0f	3.7b
	Double acid digestion			
BL	16.0a	1.4b	25.9bc	1.1c
TDM	6.0de	1.4b	24.1cd	1.2c
GDM	6.4d	7.9a	15.2f	5.17a
	NaOH + EDTA extractible P			
BL	13.3c	0.2d	20.9e	0.2f
TDM	5.3e	0.03e	22.0de	0.1f
GDM	5.9de	0.3c	15.6f	0.4e
	Literature values			
Poultry litter §	14.9	0.3	152.0	0.8
Dairy manure ¶	5.8	2.9	20.7	1.6

† Means within columns followed by different letters are significantly different at the 0.05 probability level

§ Values from He et al., 2006

¶ Values from Toor et al., 2005

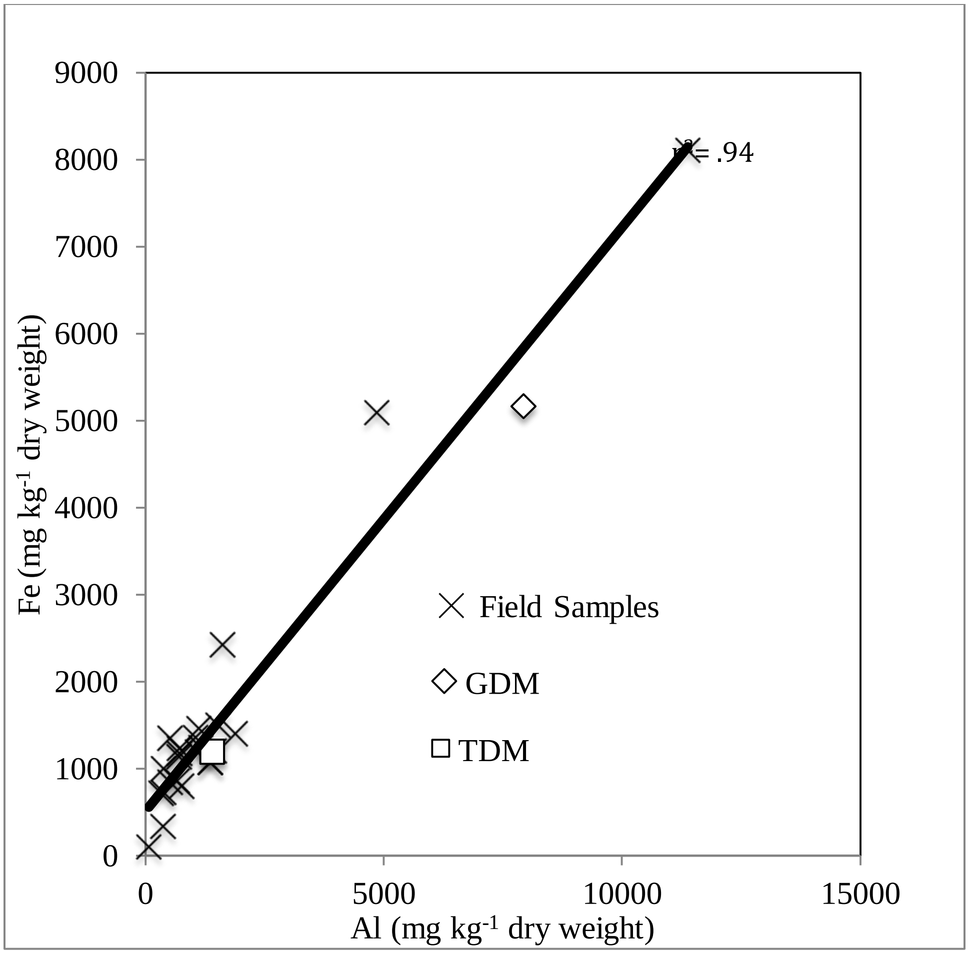

Figure 2-1. Comparison of Fe (mg kg^{-1} dry weight) to Al (mg kg^{-1} dry weight) in field samples, manure from grass fed cows (GDM) and manure from TMR fed cows, (TDM)

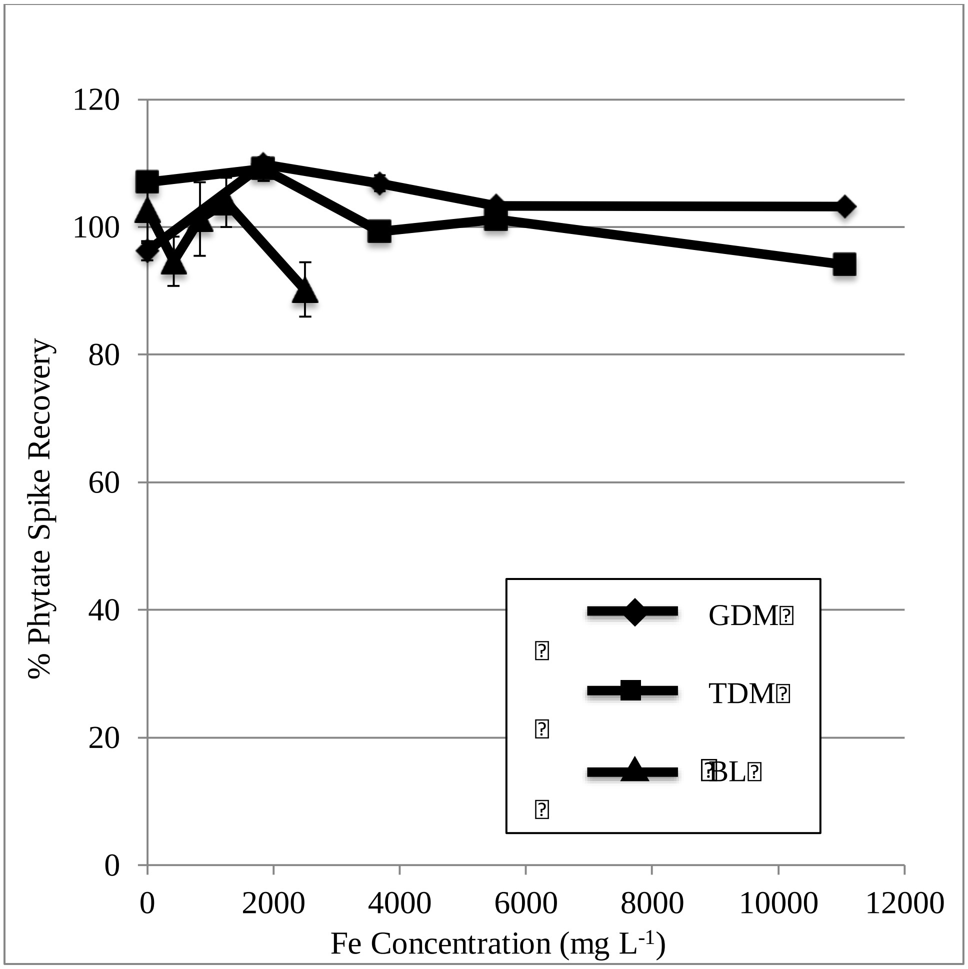

Figure 2-2. The effect of increasing additions of Fe on phytate spike recovery for manure from grass fed cows (GDM), manure from TMR fed cows, (TDM), and broiler litter (BL); line markers at 0, 50, 100, 150, and 300% Fe additions

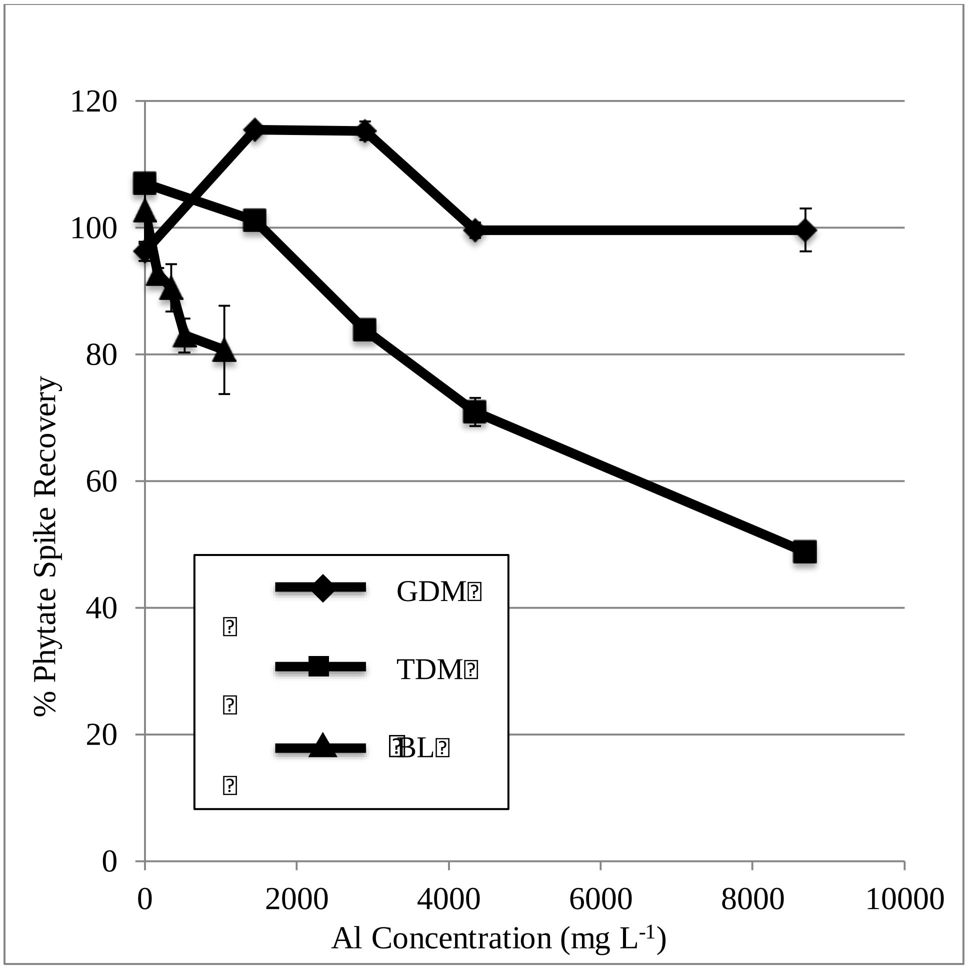

Figure 2-3. The effect of increasing additions of Al on phytate spike recovery for manure from grass fed cows (GDM), manure from TMR fed cows, (TDM), and broiler litter (BL); line markers at 0, 50, 100, 150, and 300% Al additions

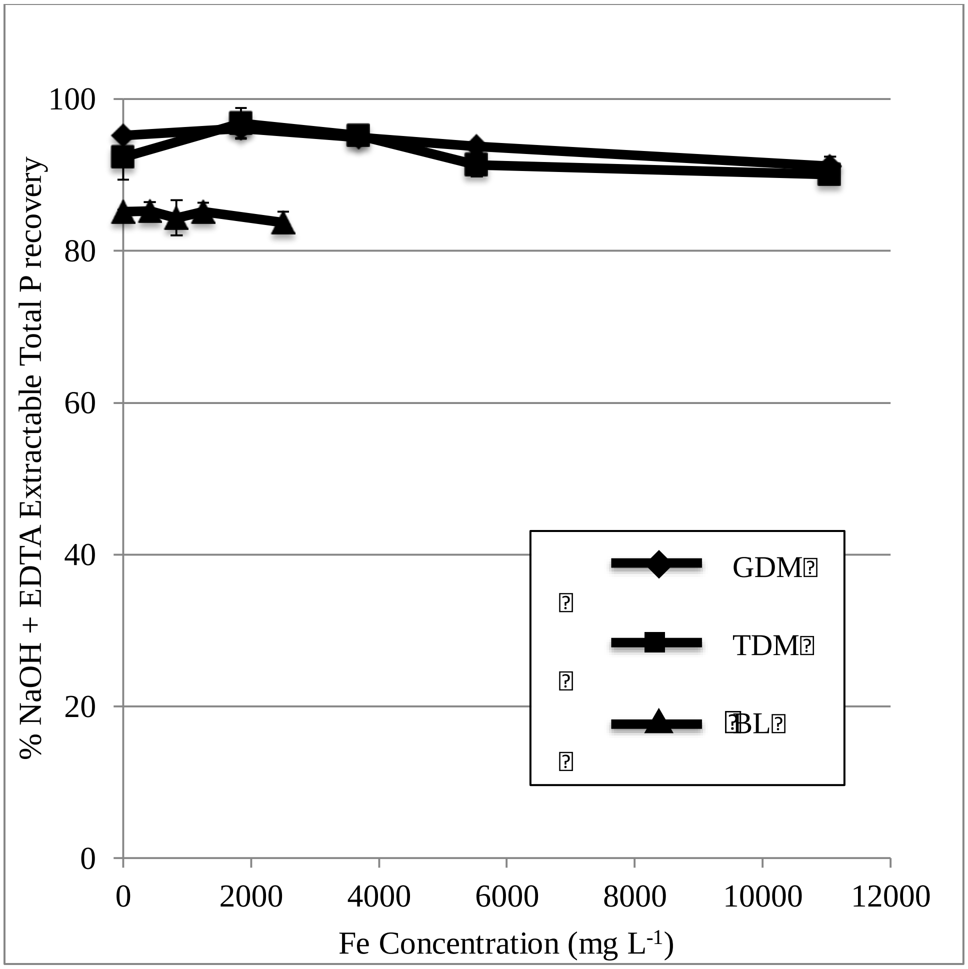

Figure 2-4a. Percentage of total P recovered in the 0.25*M* NaOH + 50 *mM* EDTA extract with increasing additions of Fe for manure from grass fed cows (GDM), manure from TMR fed cows, (TDM), and broiler litter (BL); line markers at 0, 50, 100, 150, and 300% Fe additions

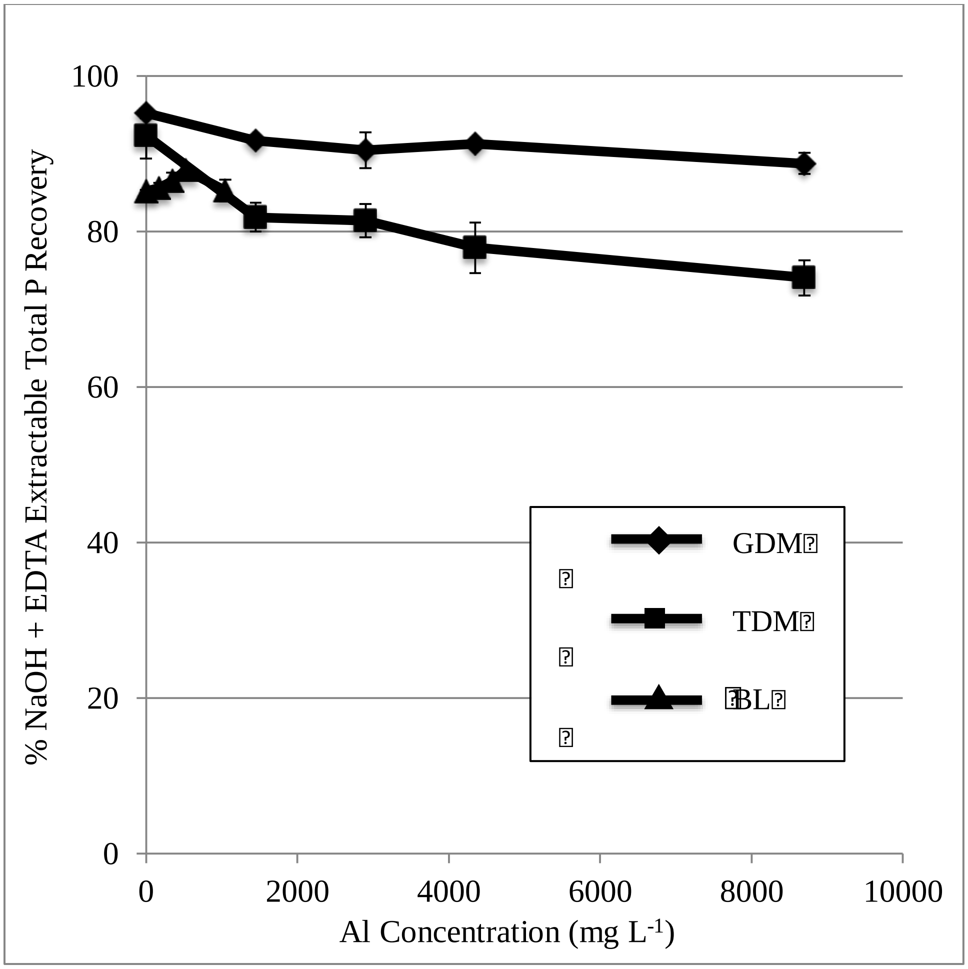

Figure 2-4b. Percentage of total P recovered in the 0.25*M* NaOH + 50 *mM* EDTA extract with increasing additions of Al for manure from grass fed cows (GDM), manure from TMR fed cows, (TDM), and broiler litter (BL); line markers at 0, 50, 100, 150, and 300% Al additions

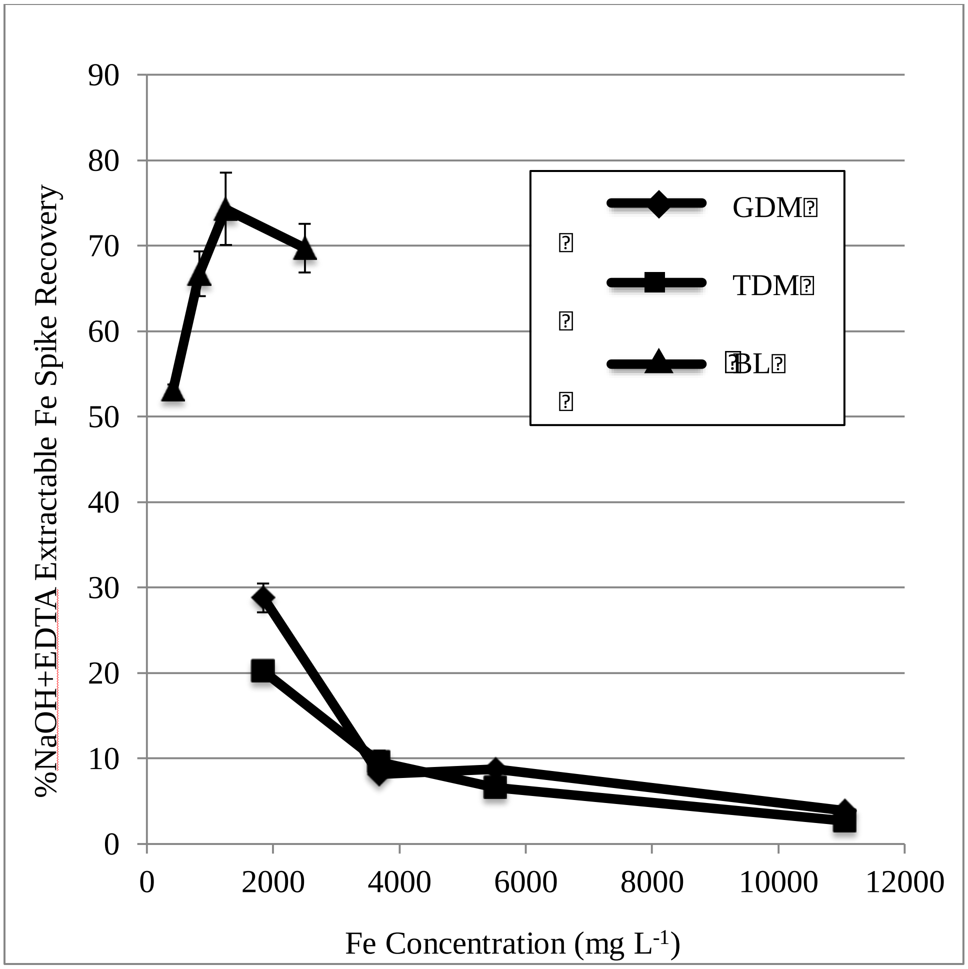

Figure 2-5. Percent Fe spike recovery in the 0.25*M* NaOH + 50 *mM* EDTA extract after Fe addition for manure from grass fed cows (GDM), manure from TMR fed cows, (TDM), and broiler litter (BL); line markers at 0, 50, 100, 150, and 300% Fe additions

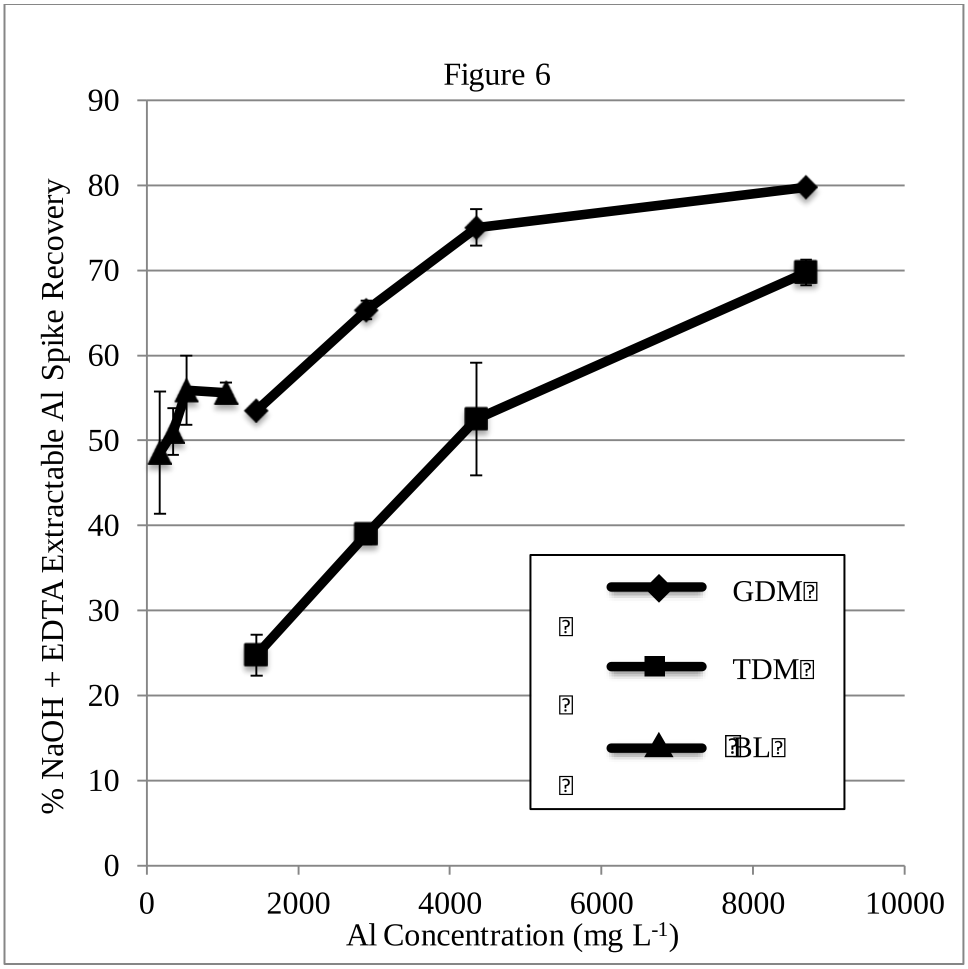

Figure 2-6. Percent Al spike recovery in the 0.25*M* NaOH + 50 *mM* EDTA extract after Al addition for manure from grass fed cows (GDM), manure from TMR fed cows, (TDM), and broiler litter (BL); line markers at 0, 50, 100, 150, and 300% Al additions

Chapter 3: Runoff Phytate Loss From Surface Applied Animal Manures

Abstract

Phytate (Pp) is a common form of organic phosphorus (P) in animal manure, but direct measurement of Pp in runoff has not been previously reported due to the difficulty of measuring it in a complex matrix. This study was conducted to measure Pp adsorption by two soils and transport of Pp in runoff using a new method. Runoff treatments were dairy manure with and without added Pp (DM, DM+Pp), broiler litter with and without added Pp (BL, BL+Pp), and control, with and without two levels of Pp (control, control Pp low, control Pp high). Runoff was collected under simulated rainfall and analyzed for total and dissolved reactive P (DRP). Runoff was also separated into sediment (>0.45µm) and liquid (<0.45µm and lyophilized) fractions for Pp analysis by high performance ion chromatography (HPIC). The new method for Pp analysis recovered 70% of Pp spiked into runoff samples. Phytate P was found only on the sediment fraction of runoff and was not detected in the liquid fraction after lyophilization. This agreed with the adsorption study, which showed strong adsorption of Pp to soil particles. Phytate P significantly increased DRP for control Pp high compared to control, indicating that inorganic P was released through competitive binding. The majority of P lost in runoff was in the sediment fraction and was in forms other than Pp. Efforts to control the erosive loss of soil during rainfall events appear to be the best way to reduce total P loss, irrespective of the presence of Pp.

Introduction

Eutrophication of surface waters leading to hypoxic conditions has become a major environmental concern throughout the United States (Sharpley et al., 1994). Phosphorous (P) surpluses in soils, built up from long-term manure applications, can cause surface water eutrophication by P loss in runoff (Correll, 1999; Maguire et al., 2007). Phosphorous loss in runoff is controlled by the release of P from surface applied manure, crop residues, and soil as water moves across the soil surface (Sharpley, 1985). This interaction takes place in an extremely thin layer, primarily in the top 5 cm of the soil surface (Sharpley et al., 1996). Additions of high P amendments like dairy manure and broiler litter to soils significantly increase P loss in runoff (Eghball and Gilley, 1999).

Phytate P (*myo*-inositol hexakisphosphate; Pp) makes up between 5% and 14% of total P (TP) in dairy manure and up to 78% of Pp of TP in broiler litter (Turner, 2004; Toor et al., 2005a). Phytate P in manures and litters originates in the grains and seeds in feed. Typically, 60 – 80 % of TP in seeds and grains is in the Pp form (Raboy, 1997). Phytate P is difficult for monogastric animals to digest without the addition of the enzyme phytase to the diet. Ruminants, whose ruminal bacteria produce phytase, typically have significantly less Pp as a percentage of TP in their manures compared to monogastric animals (Turner, 2004; Toor et al., 2005a).

The application of broiler litter and dairy manure to fields has been identified as a major source of Pp in soils (Turner et al., 2002). Phytate P is strongly bound in the soil system as it is preferentially adsorbed to soil particles over other forms of P (McKercher and Anderson, 1989; Berg and Joern, 2006). Additionally, Pp can complex with aluminum (Al) and iron (Fe) to form insoluble precipitates. Adsorbed Pp and insoluble Pp complexes are partially protected from enzymatic hydrolysis and are considered by some authors to be relatively stable in soils (Turner et al., 2005). This stability is enhanced in no-till soils that benefit from the long-term build up of organic materials and contain significant amounts of organic P, including Pp (Turner et al., 2005). Other measurable factors that lead to enhanced Pp sorption and possible accumulation are high clay content, and high oxalated extractable Al and Fe (Leytem et al., 2002; Berg and Joern, 2006).

Phytate P extraction from manures, soils, and suspended river sediments for ^{31}P NMR analysis has previously been accomplished using sodium hydroxide (NaOH) and ethylenediaminetetracetic acid (EDTA) (Turner et al., 2003; Toor et al., 2005a; Hill and Cade-Menun, 2009). This extraction method coupled with a acidification and clean up step has been used with good results (>95% recovery) for Pp analysis in manures using high performance ion chromatography (HPIC; Ray et al., 2012). However, this method for HPIC analysis, has not yet been used for Pp quantification in soils and runoff sediments.

Turner et al. (2002) theorized that significant amounts of Pp may be lost from manure amended soils during runoff. Although Pp is strong adsorbed to soil particles, the erosive loss would transfer soil bound Pp to surface waters. This idea is supported by Suzumura and Kamatani (1995) who measured Pp in river-water suspended solids. Additionally, Pp has been measured in dry runoff ditch sediments (Hill and Cade-Menun, 2009). However, Pp has never been measured directly in runoff water from agricultural fields, rather, previous investigations of Pp loss have focused on increases in dissolved reactive phosphorus (DRP) or TP in runoff waters (Penn et al., 2004; Vadas et al., 2004; Leytem et al., 2006; Tarkalson and Mikkelsen, 2007). Our objectives were a) to investigating how Pp is partitioned between > 0.45 μm sized sediment particles from runoff and the runoff solution passing through a 0.45 μm filter and b) to quantify actual Pp loss to runoff water from the application of dairy manure and poultry litter to agricultural fields.

Materials and Methods

Initial Soil Sample Analysis

For the adsorption study, samples were collected from 0-20cm of a Shottower loam (fine, kaolinitic, mesic Typic Paleudults) and a Contoe sand (loamy, mixed, semiactive, thermic Arenic Hapludults). For the rainfall simulation study, samples were collected from 0-20 cm of a Slabtown loam (fine-loamy, mixed, semiactive, mesic Aquic Paleudalfs). The three soils were sieved through a 2mm screen then analyzed for organic matter (OM), pH, particle size, and Mehlich 1 P. Organic matter was quantified by loss on ignition (g/g) (Maguire and Heckendorn, 2011). Soil pH was determined using a 1:1 ratio of soil to distilled water (v/v) after being stirred for 10 minutes (Maguire and Heckendorn, 2011). Soil particle size analysis was determined by the pipette method with wet sieving (USDA-NRCS, 2011). Mehlich 1 P was extracted at a solution to soil ratio of 1:1 (v/v) and quantified by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Maguire and Heckendorn, 2011).

Phytate P Adsorption Isotherm Experiment

The adsorption characteristics of Pp were investigated using the Shottower loam and the Conetoe loamy sand. All experiments were done in triplicate with a soil-to-solution ratio of 1:25 (g/v). Phytic acid dodecasodium salt ($NaIP_6$, $C_6H_6O_{24}P_6Na_{12}$, minimum 90% purity, Sigma, St. Louis, Mo.) from rice was used to prepare Pp solutions in deionized water with 0.015 M L^{-1} KCl with concentrations of 0, 5, 10, 25, 50, 100, 150, and 250 mg Pp L^{-1}, similar to previous Pp adsorption studies (Leytem et al., 2002). Solution pH was adjusted to pH 7 with 0.1 *M* NaOH (Leytem et al., 2002). The Pp solution was added to 1 g soil treated with chloroform and equilibrated for 24 hours at 25°C in a reciprocating shaker, then immediately centrifuged at 12,000 *g* for 15 minutes. The supernatants were filtered through a 0.2 µm IC filter membrane (EMD Millipore, Billerica, Massachusetts) and Pp was quantified chromatographically by HPIC (Dionex ICS 3000 with Dionex 4x50 mm IonPac AG7 guard column, and 4x250 mm IonPac AS7 analytical column; Dionex Corp., Sunnyvale, CA) with post column reaction detection. Soil filtrates were injected using a 50-µl sample loop and Pp was eluted after 7.5 min with isocratic elution (0.25 *M* HNO_3) at 1 mL min^{-1}. Eluted Pp was reacted with a 2% $HClO_4$ solution containing 0.1% $Fe(NO_3)_3$ in a post-column reactor. The Pp products were measured using an UltiMate 3000 UV-Visible light detector (Dionex Corp., Sunnyvale, CA) at 290 nm. Calibration standards (6, 30, 60 µg of P mL^{-1}) for the chromatograph were prepared using the stock Pp solution.

Experimental Pp adsorption isotherms were created for each soil type by calculating the amount of adsorbed Pp at 8 different initial Pp concentrations. Sorption maxima were calculated using the linear form of the Langmuir isotherm as follows:

Equation 1.

$$\frac{Ceq}{qi} = \frac{C}{B} + \frac{1}{Bk}$$

where *Ceq* is the concentration of Pp in solution and q_i is the concentration of Pp on the soil surface. The graph of $\frac{Ceq}{qi}$ is a linear plot with a slope of $\frac{1}{B}$ and a y-intercept of $\frac{1}{Bk}$.

Manure Collection for Rainfall Experiment

Fresh dairy manure was randomly collected from 30 lactating cows (*Bos taurus*) and composited for application to rainfall simulation plots. The lactating cows were fed a total mixed ration diet (TMR) that included corn silage (*Zea mays* L.), alfalfa silage, (Medicago sativa), barley silage (*Hordeum vulgare* L.), and a specialized grain mix. Fresh broiler (*Gallus domesticus*) litter was collected and composited from a chicken house from which the birds had been recently removed. The broiler diets included a significant fraction of corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] meal. The composites were sub-sampled and frozen at -20 °C in airtight containers until analysis.

Manure Analysis for Rainfall Experiment

To prepare manure for chemical analysis, frozen manure and litter composites were thawed to room temperature, subsampled, dried at 55°C to constant weight, and ground through a 1-mm screen (Toor et al., 2005a; Ray et al., 2012). Ground samples were digested using a Kjeldhal digestion procedure from Quikchem Method 13-115-01-1-B (Lachat Instruments, Loveland, CO, USA). Total P was quantified using ICP-AES.

Ground samples extracted with 20 mL of 0.25*M* NaOH + 50*mM* EDTA and shaken continuously for 4 h on a horizontal shaker at room temperature. Samples were centrifuged at 12,000 *g* for 15 min at 10°C. Aliquots of the supernatant (10 mL) were acidified using 1 ml of HCl-HF solution (6*M* HCl and 1.2*M* HF). Acidified extracts equilibrated at 4°C overnight and were then centrifuged at 12,000 x *g* for 15 minutes at 10°C. The supernatant from each sample was passed drop-wise through a methanol-conditioned C_{18} cartridge (Waters Corp., Milford, MA). The first 2 mL of supernatant were discarded, a 0.2-μm IC filter membrane was attached, and the remaining supernatant was filtered drop wise into sample vials (Dionex Corp., Sunnyvale, CA) for HPIC analysis as described above.

Rainfall Simulation Experiment Plot Setup

Rainfall simulation plots were installed on a predominantly Slabtown loam soil, 9-11% slope, following the guidelines of the National Research Project for Simulated Rainfall for Surface Runoff Studies (SERA-17, 2001). Plot areas were 1.5m by 2m and plots were installed prior to manure and litter application. To evaluate the effects of Pp on runoff, a partial factorial design was used for the treatments.

Treatments were dairy manure, with and without added Pp (DM, 306 mg Pp; DM+Pp, with an additional 435 mg Pp; dairy manure mixed with water at a ratio of 1:20 solids to liquid (g g^{-1}) to simulate dairy lagoon water), broiler litter, with and without added Pp (BL, 1357 mg Pp; BL+Pp, with an additional 8,305 mg Pp), and water, with and without added Pp (control, 0 mg Pp; control Pp low, 435 mg Pp; control Pp high, 8,305 mg Pp). DM and DM+Pp were applied by hand at a rate of 56,000 L ha^{-1} 20 minutes prior to rainfall. BL and BL+Pp was applied by hand at a rate of 6.7 Mg ha^{-1} 20 minutes prior to rainfall. Application rates of DM, DM+P, BL, and BL+Pp were designed to be similar to rates actually applied by farmers and other investigators (Sanderson et al., 2001; Schroeder et al., 2004).

Phytate P addition to the dairy manure was based on average Pp measurements reported by other investigators (435 mg Pp kg-1; Toor et al., 2005a). Phytate P addition to the broiler litter was based on average Pp measurements previously quantified by the authors in other broiler litters (5,350 mg Pp kg^{-1}) and is similar to measurements reported by other authors (6250 mg Pp kg^{-1}; Leytem et al., 2006; Rippner et al., 2013).

Simulated rainfall intensity was 70 mm hr^{-1}, and runoff collection time was 30 minutes. Total runoff was collected and weighed to determine a volume using an average density determined for each runoff treatment. Runoff was well mixed to ensure representative sediment sampling, and two 1000 ml subsamples were taken and immediately stored on ice. Subsamples for TP and ortho-P analysis were refrigerated at 4°C prior to filtration and analysis, and subsamples for Pp analysis were frozen at -20°C until analysis.

Initial Runoff Analysis

Unfiltered runoff was analyzed for TP colorimetrically using phosphomolybdate reduction after Kjeldhal digestion (QuickChem Method 10-115-01-1-C; Lachat Instruments, Loveland, CO, USA). Total solids content was determined by drying a known volume of unfiltered runoff water at 110°C to constant weight. A subsample of runoff water was vacuum filtered through a 0.45 µm filter membrane (EMD Millipore, Billerica, Massachusetts). Filtered runoff was analyzed for DRP colorimetrically using phosphomolybdate reduction (QuickChem Method 10-115-01-1-A, Lachat Instruments, Loveland, CO, USA).

Phytate P Analysis in Runoff

Sample extracts were concentrated to allow accurate Pp quantification given detection limits of the HPIC (2 mg L^{-1} extractant). Unfiltered runoff samples were thawed, mixed, and vacuum filtered with a 0.45 µm filter membrane (EMD Millipore, Billerica, Massachusetts), and retained sediment was dried to constant weight at 55°C. The sediment content of runoff samples was determined by measurement of solids trapped on the 0.45 µm filter membrane. Filtrate was collected and lyophilized for Pp determination for the liquid fraction. One sample per treatment for both the sediment and liquid fractions was spiked with 0.25 mg Pp prior to extraction to assess effectiveness (% Pp recovery) of the extraction method applied to sediment and liquid fractions. Phytate P spike concentrations were designed to be less than the Pp adsorption maximum previously determined for the Shottower loam soil, a soil found in the same field and with similar particle size distribution to the Slabtown loam that the rainfall experiments were performed on. Following alkaline extraction and acidification Pp was quantified using HPIC as described above.

Phytate P Fractionation in Runoff

To determine Pp fractionation between the solution and sediment from runoff, Pp spikes were also applied prior to filtration. Untreated runoff samples were thawed, spiked with 0.3 mL of Pp to a Pp concentration of 1 mg L^{-1}, allowed to equilibrate at 4°C for 24 hours, mixed, and vacuum filtered with a 0.45 µm filter membrane, which was dried to constant weight at 55°C. The sediment concentration of runoff samples was determined by weighing sediment trapped on the 0.45 µm filter membrane. Filtrate was collected and lyophilized for Pp determination in the liquid fraction. One sample per treatment for both the liquid and sediment fractions was spiked with 0.25 mg Pp prior to alkaline extraction to determine % Pp recovery. Phytate P from each fraction, sediment and liquid, was quantified following alkaline extraction and acidification using HPIC as described above.

Statistical Analysis

All statistical analysis (analysis of variance, Student's T pairwise comparison of means, normality, and regression) was performed with JMP® Pro 10 (SAS Institute, Cary, NC, 2012). Significance was declared at $P < 0.05$. Error bars on figures represent standard deviation of the mean.

Results and Discussion

Soil Chemical and Physical Properties

The Slabtown and Shottower soils, both classified as loams, had much higher clay contents (10% and 19%, respectively) than the Conetoe sand (4%; Table 1). Clay content is correlated with P adsorption capacity in soils, so the higher clay in the Slabtown and Shottower soils would be expected to lead to higher P adsorption capacity compared to the Conetoe soil (Fox and Kamprath, 1970; Berg and Joern, 2006). Mehlich 1 P for the Conetoe soil (63 mg kg^{-1}) was ten times greater than for the Shottower soil (6 mg kg^{-1}; Table 1). Organic matter content was greater in the Shottower (52 g kg^{-1}) and Slabtown (50 g kg^{-1}) soils than the Conetoe soil (10 g kg^{-1}; Table 1). The Shottower (9 cmol$_c$ kg^{-1}) and Slabtown (9 cmol$_c$ kg^{-1}) soils also had greater CEC than the Conetone soil (2 cmol$_c$ kg^{-1}; Table 1), which would be expected when comparing loams to sands.

Adsorption of Pp on Shottower Loam and Conetoe Sand

Phytate P adsorption maxima for the Shottower and Conetoe soils were calculated to be 1429 mg kg^{-1} and 526 mg kg^{-1}, respectively (Fig. 3-1). The linear fit of the Langmuir equation used to calculate Pp adsorption was satisfactory ($r^2 = 0.99$) for both soils. Maximum adsorption was obtained at a solution concentration 100 mg Pp L^{-1} for both soils, with no additional adsorption measured at higher solution concentrations (Fig. 3-1). Similar to results from other investigators, higher clay and OM-content were associated with higher Pp adsorption maximum

(Leytem et al., 2002; Berg and Joern, 2006). The Shottower soil used in the adsorption study was from the same field as the Slabtown soil used in the runoff study. Phytate P at concentrations typical of animal manures amended to fields with high P sorption capacity would likely be rapidly adsorbed to soil surfaces (Fox and Kamprath, 1970; Leytem et al., 2002). Sediments in runoff water from fields amended with animal manures would thus carry excess Pp to surface waters compared to un-amended soils.

Phytate P in Runoff

Phytate P loss in the runoff from control Pp high was significantly greater (6.3 mg L^{-1}) than any of the other treatments, 30 times greater than from control (0.21 mg L^{-1}; Fig. 3-2). When concentration was multiplied by runoff volume, only 2.4% of the total mass of Pp applied to control Pp high was lost in the runoff; the remaining was likely adsorbed to soil particles as reported in the adsorption isotherm experiment. Phytate P loss for the remainder of the treatments were not significantly different, though the loss from BL (0.9 mg L^{-1}) and BL+Pp (1.8 mg L^{-1}) was four times and eight times numerically higher, respectively, than the Pp loss from control (Fig. 3-2). When control Pp high was excluded from analysis, Pp loss from BL and BL+Pp was significantly greater than Pp loss from the control and control Pp low (Fig. 3-2). Broiler litter added to rainfall plots had lower Pp (874 mg Pp kg^{-1}) than has been measured in other studies (6250 mg Pp kg^{-1}; Leytem et al., 2006; 5560 mg Pp kg^{-1}; Rippner et al., 2013). When control Pp high was excluded from analysis, Pp loss from DM and DM+Pp was significantly greater than Pp loss from control (Fig. 2). Dairy manure applied as DM to the rainfall plots had a similar Pp content (352 mg Pp kg^{-1}) to manure measured by other investigators (496 mg Pp kg^{-1}; Toor et al., 2005a). Phytate P loss from BL+Pp was significantly greater than from DM, but was not significantly different from BL and DM+Pp. However, the percentage of total Pp (mg) in the runoff compared to the total mass that was applied to the plots (mg) for each respective treatment was significantly greater for DM (10.1%) and DM+Pp (8.1%) than for BL (0.6%) and BL+Pp (0.3%). These results indicate that Pp in DM and DM+Pp is more vulnerable for runoff than Pp in broiler litter. Phytate P loss from the control plots was small (0.21 mg L^{-1}), but detectable (Fig. 2). Phytate P has been previously measured in soils, suspended river sediments, and surface deposited sediments but not in runoff water (Suzumura and Kamatani, 1995; Turner et al., 2003; Hill and Cade-Menun, 2009). Our data indicates that Pp can be lost in runoff from soils, especially those amended with manure, and therefore contribute to Pp in river and lake sediments.

Phytate P Fractionation Between Runoff Sediments and Liquid

Phytate P was recovered exclusively on the sediment fraction of runoff waters from the fractionation experiment (Fig. 3-3). Mean Pp recovery from the sediment fraction was 70% of Pp spiked. Phytate P in the liquid fraction of the runoff was not detectable in the extracts (Fig. 3-3). Similar results were observed for all treatments in the runoff study, where Pp was only recovered from the sediment and was not detectable in the liquid fraction. Therefore, total Pp in the runoff study was determined by the concentration of Pp on the sediment fraction (Fig. 3-3). Phytate P is strongly adsorbed to soil particles (Fig. 3-1) and has been shown to have both a higher affinity for soils and a higher adsorption maximum than other P species (Leytem et al., 2002; Berg and Joern, 2006). Water extraction of Pp from soils is found to contain negligible concentrations of Pp (McDowell et al., 2005). It follows that little Pp would be found in the liquid fraction of runoff water.

The observation that Pp is primarily adsorbed to runoff sediment is important new information that can improve our understanding of P dynamics. No-till management has been an important practice for limiting sediment loss, and thus P loss, from agricultural lands, especially in the Chesapeake Bay watershed. Continued reduction of P loss from no-till fields may depend on the implementation of other management practices that further reduce sediment loads to surface waters rather than focusing on Pp.

Runoff DRP and TP

Dissolved reactive P is a measure of dissolved inorganic or ortho-Pin runoff water (McDowell and Sharpley, 2001). Runoff DRP concentration was significantly greater for DM and DM+Pp than for BL and BL+Pp (Fig. 3-4a). Runoff DRP concentration was also significantly greater for DM, DM+Pp, BL, and BL+Pp than for control and control Pp low (Fig. 3-4a). This was expected as manure and litter applications on fields have been shown to increase DRP compared to runoff from un-amended soil (Kleinman et al., 2002a; Kleinman et al., 2002b). Increases in runoff DRP are a concern as DRP is considered to be much more bioavailable than adsorbed P (Correll, 1998). Additions of Pp have been reported to cause the desorption of ortho P from the surface of soil in adsorption isotherm experiments, increasing DRP (Berg and Joern, 2006). When all treatments were included in analysis, the addition of high rates of Pp to the plots did not significantly increase measured DRP in runoff. However, when control, control Pp low, and control Pp high were analyzed independently, DRP loss in runoff significantly increased with increasing additions of Pp (Fig. 3-4a).

Runoff TP was significantly greater for DM (28.6 mg L^{-1}) and DM+Pp (22.5 mg L^{-1}) than for BL (9.3 mg L^{-1}) and BL+Pp (6.5 mg L^{-1}) (Fig. 3-4b). Neither sediment nor Pp loss in runoff were significantly different between the four treatments (DM, DM+Pp, BL, BL+Pp). Runoff DRP loss was significantly greater for DM and DM+Pp than BL and BL+Pp, but not enough to account for differences in TP lost in the runoff (Fig. 3-4a). Therefore, the concentration of other forms of P in the sediment fraction, such as adsorbed P, lipid-P, and DNA-P, was much greater for DM and DM+Pp than for BL and BL+Pp. Runoff TP was significantly higher for control Pp high (14.8 mg L^{-1}) than for control (3.1 mg L^{-1}) and control Pp low (3.5 mg L^{-1}) (Fig. 3-4b). Sediment losses from the three control plots accounted for most of the TP in the runoff. Though the loss of sediment from each of the three plots was not significantly different, the sediment from the control Pp high plot had significantly greater adsorbed Pp (6136 mg kg^{-1}) than either control Pp low (178 mg kg^{-1}) and control (118 mg kg^{-1}).

Ratio of Phytate P to DRP in Runoff

Ratios between Pp and DRP in runoff provide a basis to compare treatments with different amounts of added Pp and ortho P. The ratios of Pp to DRP concentrations for control, control Pp low, and control Pp high were 1.7, 1.4, 5.9, respectively (Fig. 3-5). Ratios greater than one indicate that the concentration of Pp in runoff sediment was greater than the concentration of DRP in runoff. Given the elevated ratio of Pp to DRP for even the control plot, sediment in runoff water played a greater role in P loss than the liquid fraction for control, control Pp low, and control Pp high (Fig. 3-5). This agrees with previous studies that reported sediment erosion as the primary path for P loss (McDowell et al., 2001)

As DRP loss from plots amended with DM, DM+Pp, BL, and BL+Pp was greater than Pp loss, the ratio of Pp to DRP was less than one for these treatments. The ratios of Pp to DRP for BL, BL+Pp, DM, and DM+Pp were 0.25, 0.8, 0.14, and 0.21, respectively (Fig. 3-5). Ratios of Pp to DRP for BL and BL+Pp did not indicate the displacement of ortho P by Pp. Similarly, the ratio of Pp to DRP for DM and DM+Pp did not indicate the displacement of ortho P by Pp. Ratios of Pp to DRP for BL and BL+Pp and especially for DM and DM+Pp indicate that DRP contributed significantly more to P loss than Pp (Fig. 3-5). Efforts in the last decade have been made to reduce Pp content in animal manures, especially broiler litter (McGrath et al., 2005). These efforts could decrease the ratio of Pp to DRP further.

Ratio of Pp to Total P in Runoff

For all treatments, the ratio of Pp to TP in runoff was much less than the ratio of Pp to DRP (Fig. 3-6; Fig. 3-5). Phytate P appears to contribute little to TP loss in the absence of animal manures or added Pp, indicated by the Pp:TP ratio of 0.07 for runoff from control. Compared to control, DM, and DM+Pp, runoff water from BL+Pp had a significantly greater ratio of Pp to TP (Fig. 3-6). This was expected as Pp in applied BL+Pp constituted a large percentage (22%) of applied TP, whereas Pp in applied DM and DM+Pp only constituted a small percentage (5% and 9%, respectively) of applied TP. Runoff from BL and BL+Pp had a greater Pp:TP ratio (0.10 and 0.26, respectively) than was measured in the applied BL and BL+Pp (0.04 and 0.22, respectively). Increased Pp:TP ratios in runoff sediments signify that Pp enrichment occurred in the runoff sediments from BL and BL+Pp. However, the inverse was seen for DM and DM+Pp, with the Pp:TP ratio in runoff from DM and DM+Pp (0.02 and 0.05, respectively) lower than the Pp:TP ratio in applied DM and DM+Pp (0.05 and 0.09, respectively) .This was probably due to differences in the physical and chemical properties between the two types of animal manures.

While Pp loss made up a large percentage (26%) of TP loss in runoff for BL+Pp, it was less than the loss contributed by DRP as calculated by the ratio of Pp to DRP (Fig. 3-5). Alternative application methods such as broiler litter and dairy manure injection on no-till fields has been shown to decrease DRP and TP loss (Maguire et al., 2011). Evidence from the current study suggests that those technologies may be more important than reducing Pp content in manures.

Conclusions

Runoff from agricultural fields contributes a significant amount of P to surface waters, but previous research has not been able to distinguish Pp from other forms of P. In the adsorption study, Pp was strongly adsorbed by both soils. This agrees with the runoff study, where Pp in runoff was strongly adsorbed (70%) to the sediment and Pp concentrations in the liquid fraction were not detectable. Applications of BL, DM, and high rates of Pp significantly increased Pp loss in runoff relative to the control. Additions of Pp was found to increase DRP for control Pp high compared to control, similar to what has previously been observed in adsorption isotherm studies. The ratio of Pp to DRP loss for the un-amended soil indicated that Pp loss in the sediment fraction from un-amended soils is greater than DRP loss. For treatments amended with BL, Pp made up a small fraction (10%) of TP lost from runoff. Other forms of P in runoff sediments contributed the largest fraction of P to runoff. Based on the results of the current study, implementing erosion prevention strategies would reduce both Pp loss and the loss of other forms of P in runoff sediments.

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Table 3-1. Selected chemical and physical properties of soils from the adsorption and rainfall simulation studies.

Soil Series	Soil chemical and physical properties						
	Sand	Silt	Clay	pH	Mehlich 1 P	OM	CEC
	………	%	………		mg kg^{-1}	g kg^{-1}	cmol kg^{-1}
Slabtown	46	44	10	6.3	82	50	9
Shottower	41	40	19	6.4	6	52	9
Conetoe	91	6	4	6.0	63	10	2

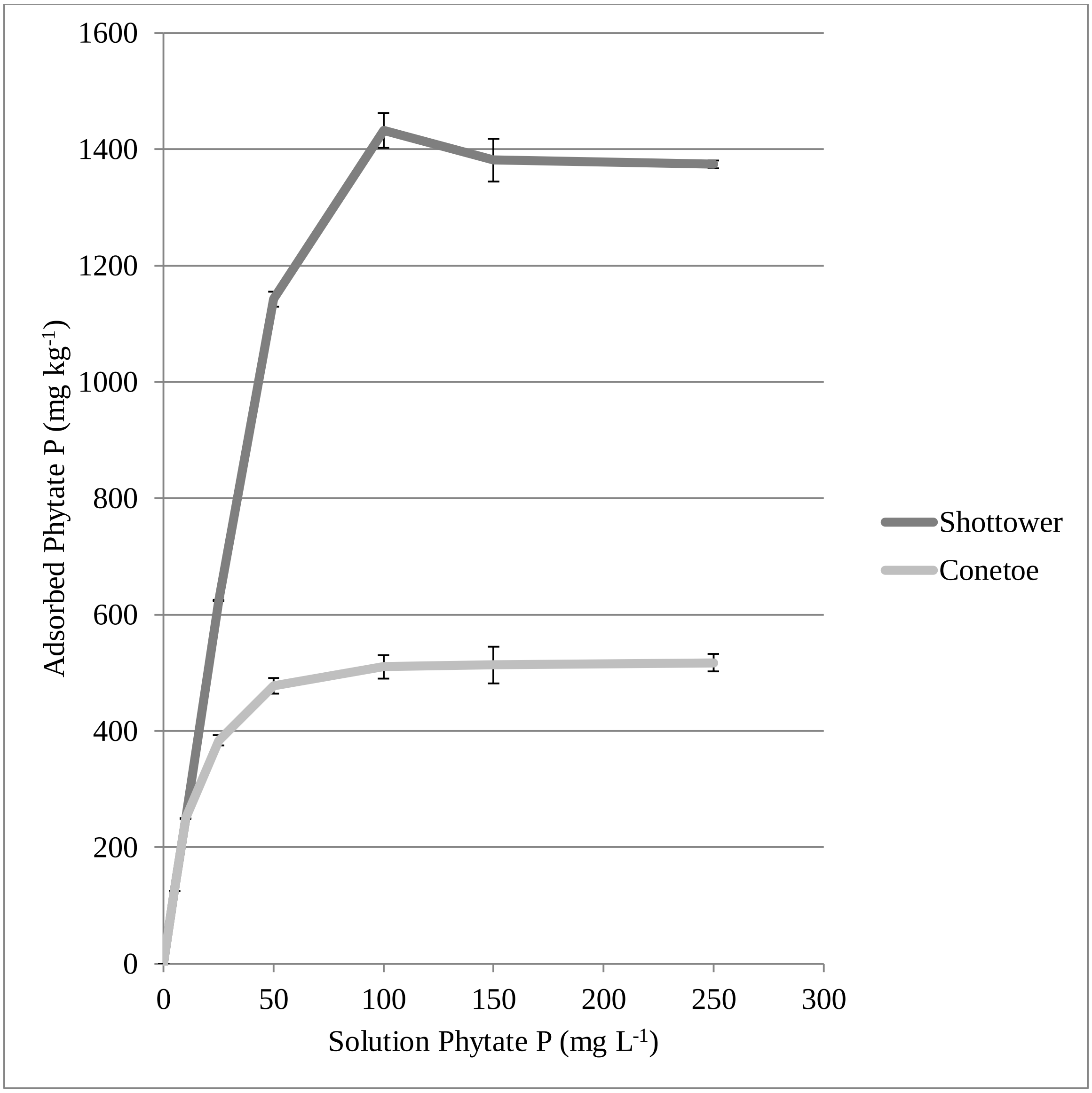

Figure 3-1. Adsorption isotherm for phytate P (Pp) on a Shottower loam and a Conetoe sand; samples were shaken for 24 hours.

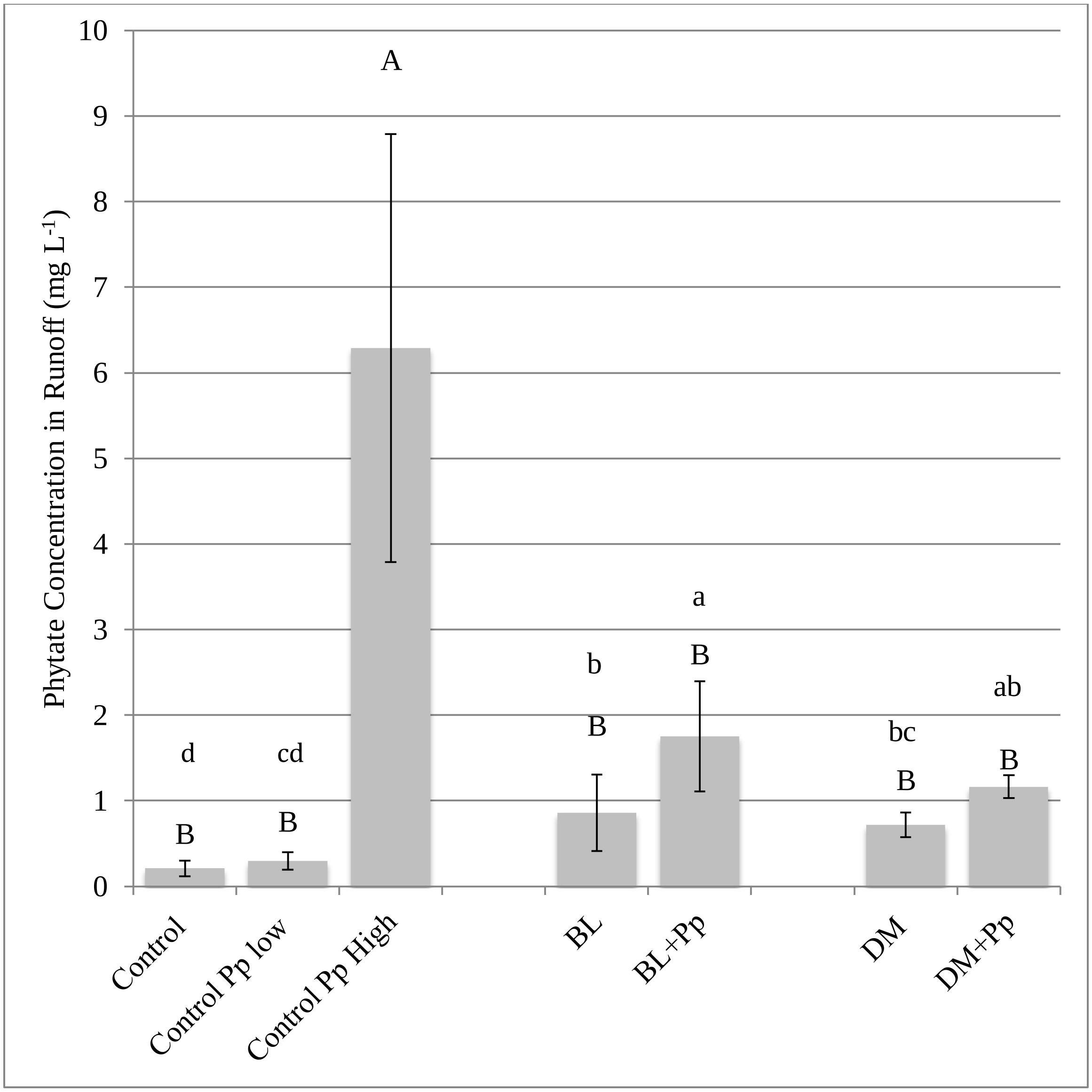

Figure 3-2. Phytate P (Pp) concentration in runoff water; Control, 0 mg Pp; control Pp low, 435 mg Pp; control Pp high, 8,305 mg Pp; BL, broiler litter, 1357 mg Pp,; BL+Pp, with an additional 8305 mg Pp; DM, Dairy manure, 306 mg Pp; DM+Pp, with an additional 435 mg Pp; Mean values with different capital letters are significantly different at $P<0.05$; Mean values with different lower case letters exclude control Pp high and are significantly different at $P<0.05$; error bars represent standard deviation from the mean.

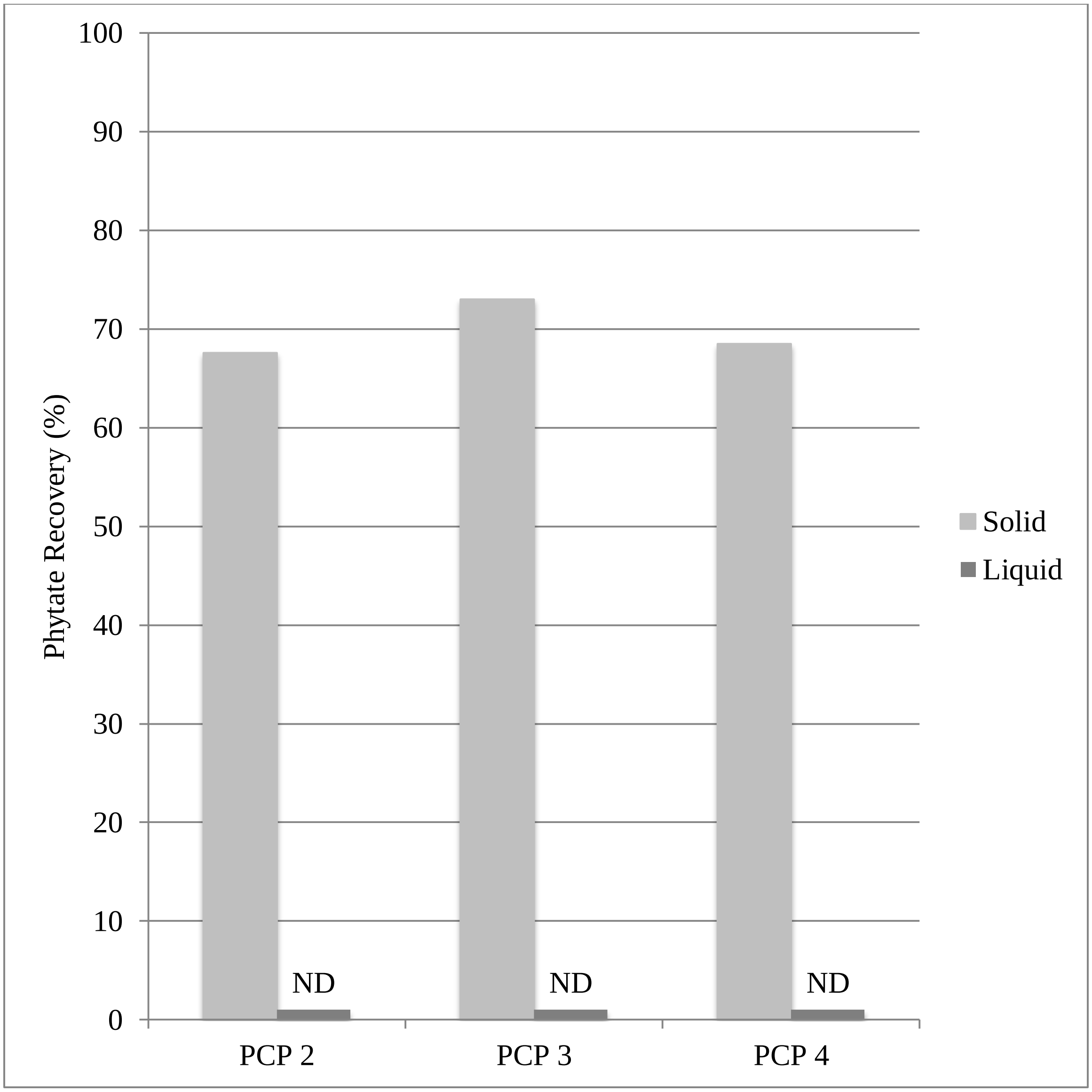

Figure 3-3. Partition of spiked phytate P (Pp), added to runoff water from each of the control plots (PCP), between the sediment and liquid fractions; Recovery from the liquid fraction was not detectable (ND)

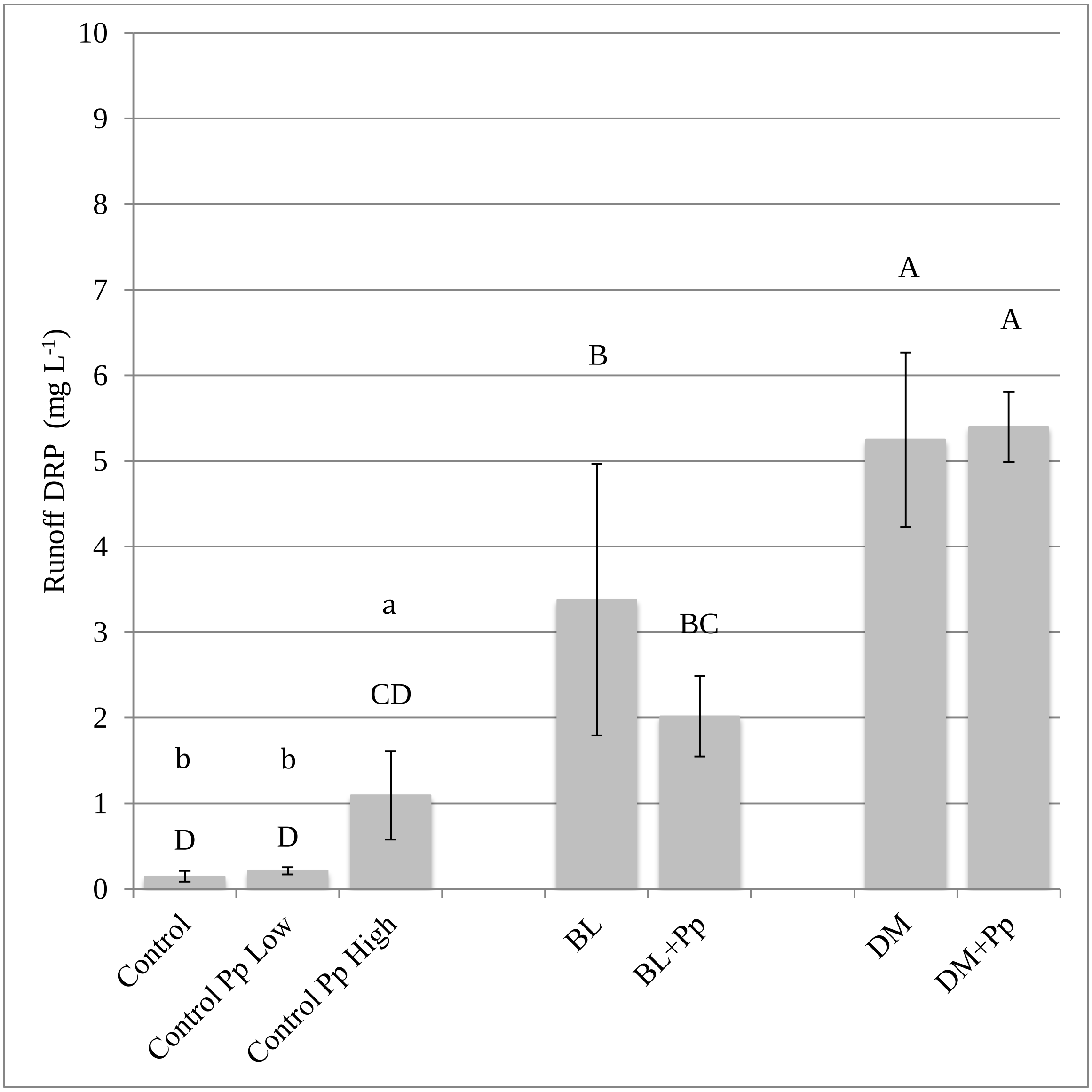

Figure 3-4a. Dissolved reactive P (DRP) concentrations (mg L^{-1}) in filtered runoff samples from plots; Control, 0 mg Pp; control Pp low, 435 mg Pp; control Pp high, 8,305 mg Pp; BL, broiler litter, 1357 mg Pp,; BL+Pp, with an additional 8305 mg Pp; DM, Dairy manure, 306 mg Pp; DM+Pp, with an additional 435 mg Pp; Mean values with different letters are significantly different at P<0.05; error bars represent standard deviation from the mean.

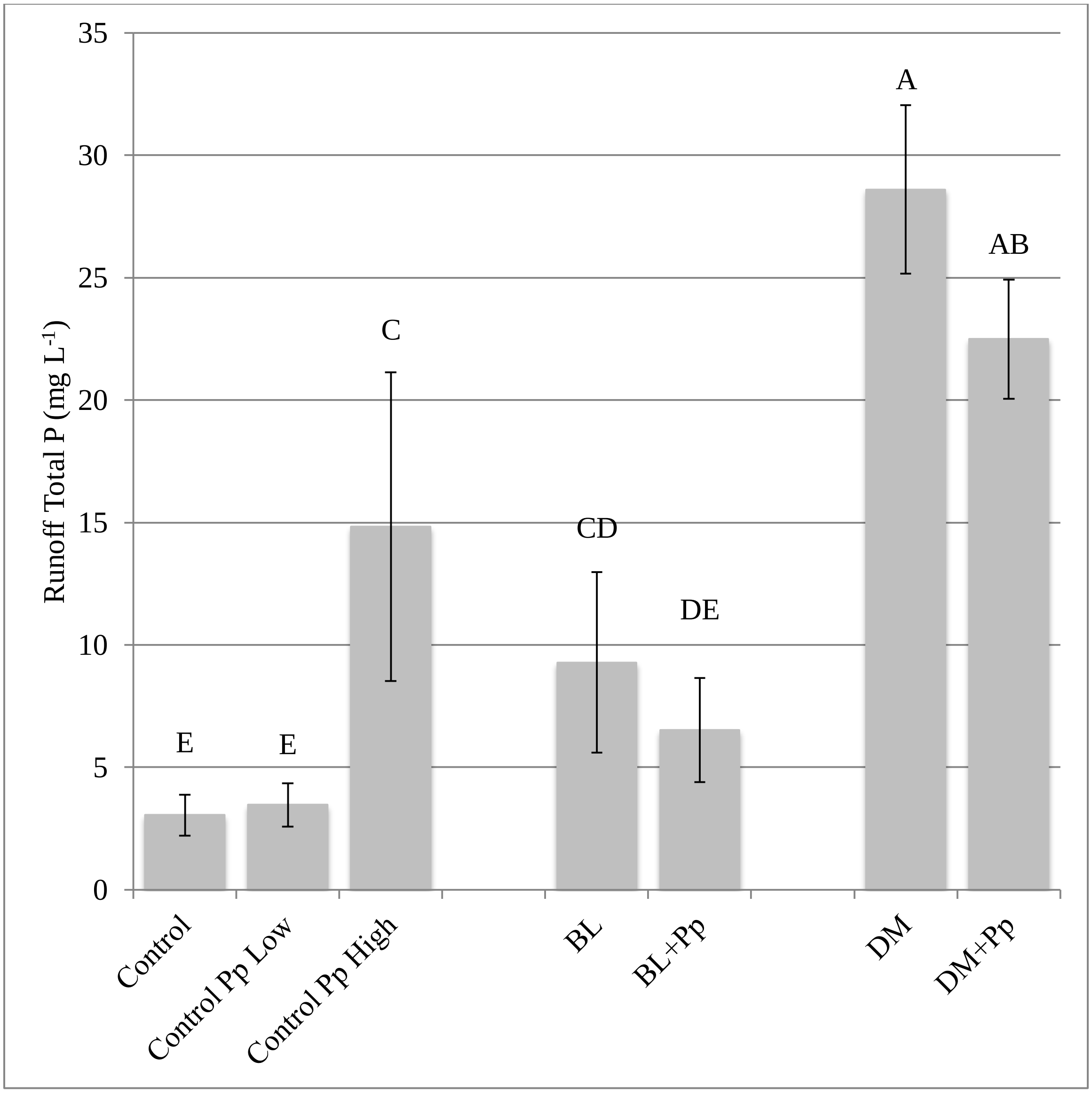

Figure 3-4b. Total P (TP) concentrations (mg L^{-1}) in plot runoff; Control, 0 mg Pp; control Pp low, 435 mg Pp; control Pp high, 8,305 mg Pp; BL, broiler litter, 1357 mg Pp,; BL+Pp, with an additional 8305 mg Pp; DM, Dairy manure, 306 mg Pp; DM+Pp, with an additional 435 mg Pp; Mean values with different letters are significantly different at $P<0.05$; error bars represent standard deviation from the mean.

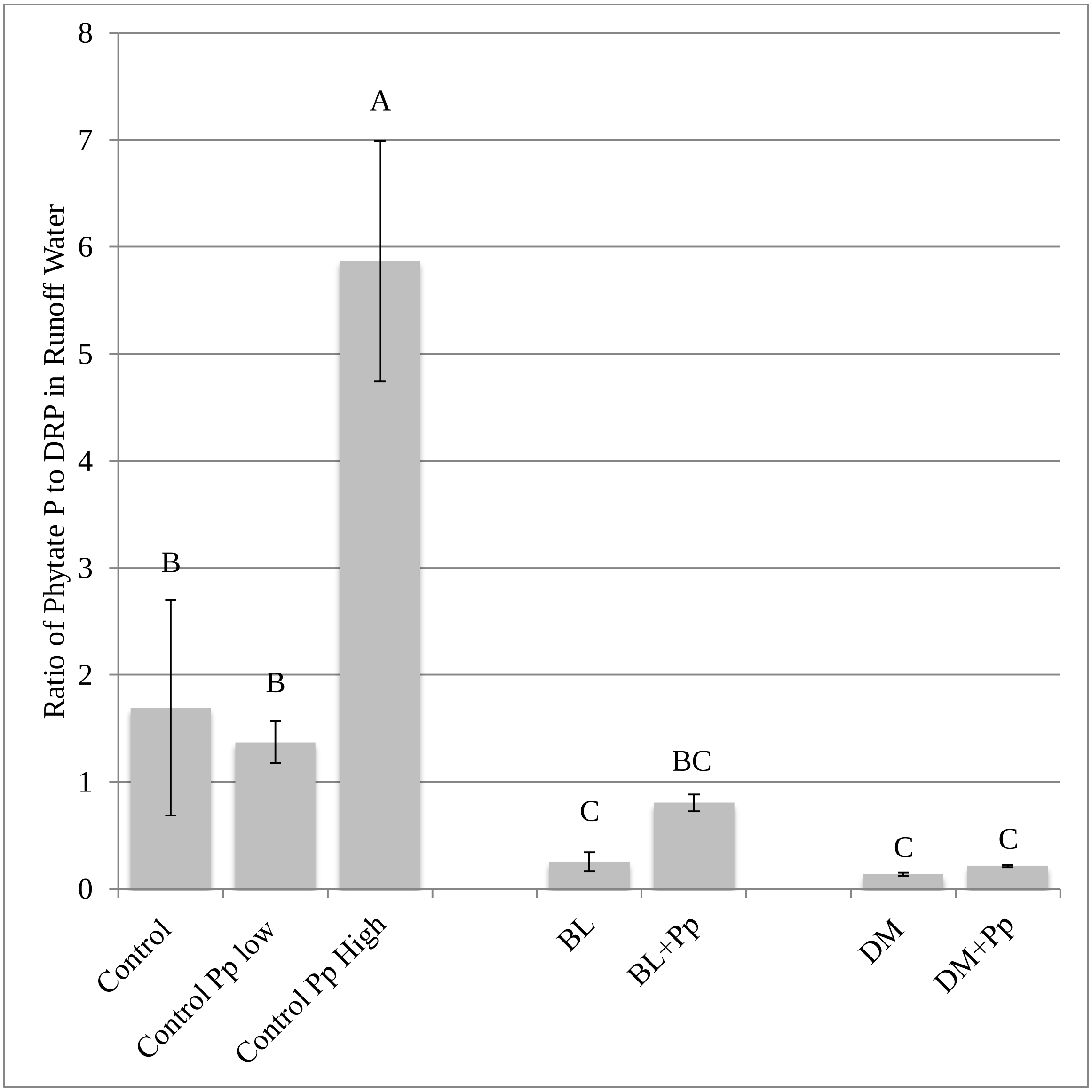

Figure 3-5. Ratio of phytate P (Pp) to dissolved reactive P (DRP) in runoff water; values greater than 1 represent Pp > DRP in runoff. Control, 0 mg Pp; control Pp low, 435 mg Pp; control Pp high, 8,305 mg Pp; BL, broiler litter, 1357 mg Pp,; BL+Pp, with an additional 8305 mg Pp; DM, Dairy manure, 306 mg Pp; DM+Pp, with an additional 435 mg Pp; Mean values with different letters are significantly different at P<0.05; error bars represent standard deviation from the mean.

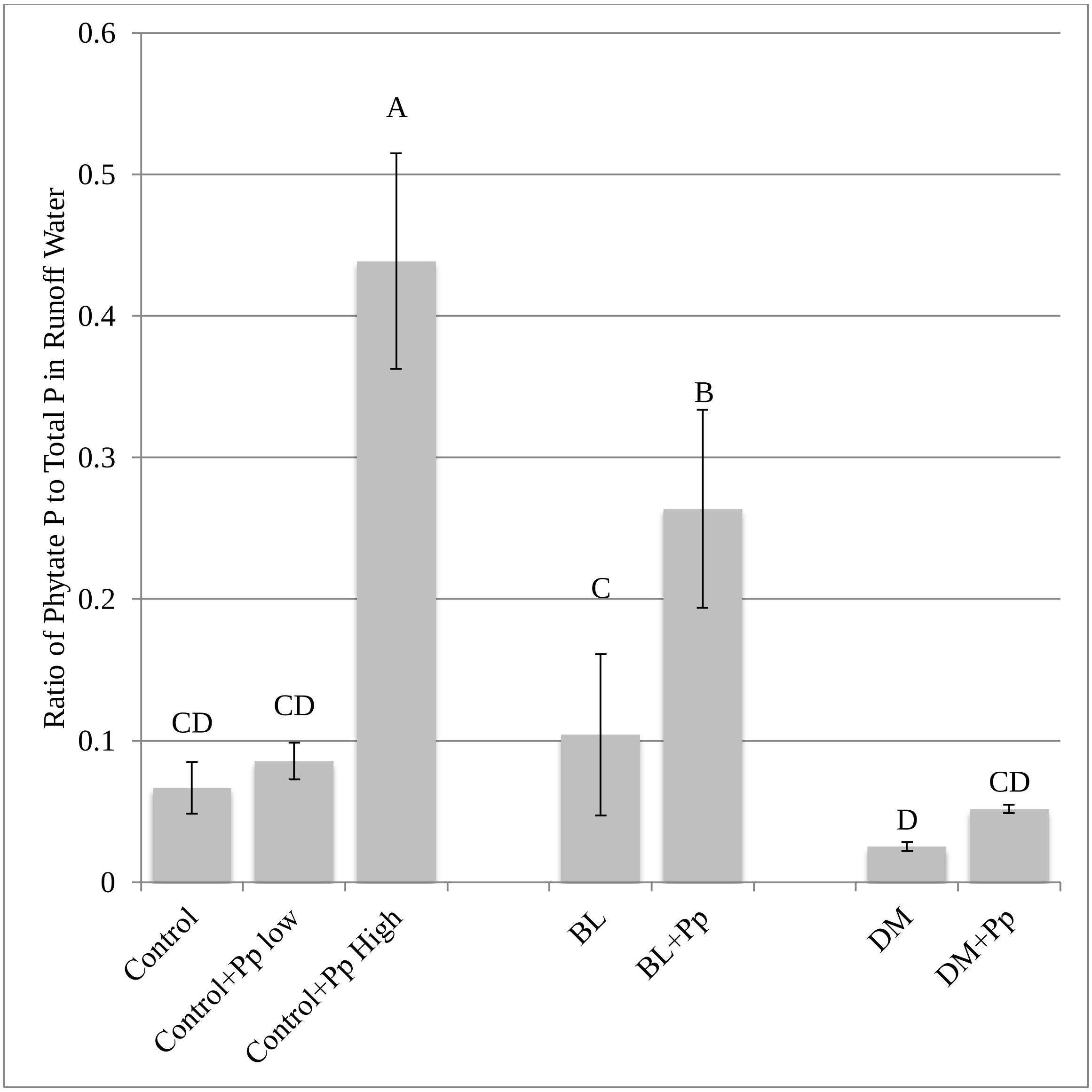

Figure 3-6. Ratio of phytate P (Pp) to total P (TP) in runoff water; Control, 0 mg Pp; control Pp low, 435 mg Pp; control Pp high, 8,305 mg Pp; BL, broiler litter, 1357 mg Pp,; BL+Pp, with an additional 8305 mg Pp; DM, Dairy manure, 306 mg Pp; DM+Pp, with an additional 435 mg Pp; Mean values with different letters are significantly different at P<0.05; error bars represent standard deviation from the mean.

Conclusions

Alkaline extraction coupled with HPIC analysis provided a rapid, low cost, and sensitive method for quantifying phytate in dairy manures and broiler litter. Addition of Fe had little effect on phytate recovery from these samples. The addition of high concentrations of Al impeded phytate recovery from BL and TDM, but phytate recovery was acceptable when Al and Fe concentrations were typical of those observed in field samples. Similarly, recovery of total P by the alkaline extraction was generally high (>82%) at Al and Fe concentrations typical of manure and broiler litter. Iron spike recovery in dairy manure decreased with increasing additions of Fe, while Fe spike recovery increased for BL with increasing additions of Fe. Aluminum recovery for dairy manure increased with increasing additions of Al, while Al spike recovery for BL changed little with increasing additions of Al. Overall, this method of phytate quantification gave satisfactory recovery of phytate across a range of Al and Fe concentrations with minimal preparation, relatively low cost, and high sensitivity.

The methods described in chapter 2 were adapted to evaluate Pp in agricultural runoff water. Runoff from agricultural fields contributes a significant amount of P to surface waters, but previous research has not been able to distinguish Pp from other forms of P. In the adsorption study, Pp was strongly adsorbed by both soils. This agrees with the runoff study, where Pp in runoff was strongly adsorbed to the sediment and Pp concentrations in the liquid fraction were not detectable. Applications of BL, DM, and high rates of Pp significantly increased Pp loss in runoff relative to the control. Additions of Pp did not significantly increase DRP for any treatment, contrary to what has previously been observed in laboratory adsorption isotherm studies. The ratio of Pp to DRP loss for the un-amended soil indicated that Pp loss in the sediment fraction from un-amended soils is greater than DRP loss. For treatments amended with

BL, Pp made up a small fraction (10%) of TP lost from runoff. Other forms of P in runoff sediments contributed the largest fraction of P to runoff. Based on the results of the current study, implementing erosion prevention strategies would reduce both Pp loss and the loss of other forms of P in runoff sediments.

Appendix

Appendix A: Elemental analysis by ICP-AES of 22 manures from 11 different states after nitric acid and perchloric acid digestion.

State	Al	Ca	Fe	Mg	P
			mg kg^{-1}		
GA	1438	19330	1207	7517	7684
GA	1524	20677	1498	7379	6182
MI	752	29520	801	8823	9364
TX	11378	3937	8109	3029	1790
TX	364	3189	340	1826	1443
MI	1123	18065	1465	9216	7036
PA	1160	20333	1240	7516	6949
NY	375	32806	728	11082	11649
VA	662	26356	860	9597	9246
FL	1890	24883	1405	9920	5800
FL	1088	23804	1189	12191	6031
FL	1046	25404	1357	12114	5059
NY	327	24297	718	9225	10572
CA	4853	21171	5094	9975	7697
WI	705	27762	1144	8385	7410
OH	1620	25900	2429	8572	9514
IN	721	26931	1176	8971	8056
OH	510	21167	849	11118	11232
OH	385	12772	1003	8092	6388
OH	717	15819	1234	6724	8405
OH	516	16658	1351	8588	9543
MI	66	2337	107	1857	2457