

**Influence of Phytase and High Available Phosphorous Corn
Diets on Solubility and Plant Uptake of P, Cu, and Zn
in Poultry Manure and Manure-Amended Soils**

by
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(ABSTRACT)

Poultry manure is a useful nutrient source but recently it has raised environmental concern due to possible P movement from P saturated soils to waterbodies. This study was conducted to determine the effects of using phytase and high available phosphorous corn diets on the solubility and plant uptake of P, Cu, and Zn in poultry manure and soils amended with manure. Five diet treatments were used in the study: 1) normal phytic acid corn and 0.135% inorganic P (NPA), 2) normal phytic acid corn, 600 units phytase, and 0.135% inorganic P (NPA+Phytase), 3) normal phytic acid corn and 0.345% inorganic P (NPA+P), 4) high available phosphorous corn and 0.135% inorganic P (HAP), 5) high available phosphorous corn, 600 units phytase, and 0.135% inorganic P (HAP+Phytase). The NPA+P diet and NPA+Phytase diets are most similar to the conventional and alternative phytase supplemented diets currently used commercially. Three Virginia soils (Groseclose, Cecil, Mahan) were amended with manure from the diet treatments at rates of 25 and 50 g/kg and P and Cu were extracted with 0.01 M CaCl₂ and Mehlich III extractant after incubation periods of 6 and 12 months. Corn was grown in a greenhouse experiment using these same Virginia soils and sand amended with 8.96 Mg/ha poultry manure from each of the five diet treatments. Poultry manure was nonsequentially extracted for determination of P, Cu, and Zn fractions. Comparing the alternative NPA+Phytase, HAP, and HAP+Phytase treatments to the conventional NPA+P treatment on an N- (nitrogen) basis all reduced both CaCl₂ and Mehlich III-soluble P concentrations (P<0.05). Comparing these same treatments on a P-basis increased P extracted with CaCl₂ 24, 26, and 37%, respectively, and P extracted with Mehlich III P 5, 4, and 9%, respectively (P<0.05). The alternative NPA+Phytase and HAP+Phytase treatments increased water-soluble Cu compared to the conventional NPA+P on both a N- and P-basis, while no differences were observed in Mehlich III solubility between these treatments (P<0.05). The alternative NPA+Phytase treatment did not differ in P and Cu in corn tissue or plant uptake when compared to the NPA or NPA+P (N- or P-basis) treatments. No difference in Zn in corn tissue was observed between these treatments on a N-basis, while NPA+Phytase was higher on a P-basis. Plant uptake of Zn was higher in the NPA+Phytase treatment compared to the NPA+P treatment on both a N- and P-basis. Addition of phytase reduced P solubility from all reagents except for CaCl₂ (P<0.05). Replacing the conventional NPA+P treatment for the alternative NPA+Phytase treatment resulted in higher Cu concentrations for all reagents except for K-pyrophosphate and nitric acid. This same replacement increased Zn extracted by water, CaCl₂, and CaNO₃, while it reduced Zn extracted by HCl, acetic acid, PbNO₃, K-pyrophosphate, and NH₄-oxalate in the light. The use of phytase decreased P solubility from manure amended soils when treatments are compared on an equal N-basis, and increased P solubility when compared on an equal P-basis. No effect on plant uptake of P or Cu occurred from the NPA+Phytase treatment.

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Chapter 1: Introduction and Literature Review

1.1. INTRODUCTION

Confined animal feeding operations have increased in popularity and have resulted in high manure application rates to agricultural land surrounding these operations. Manure applications are currently based on the nitrogen (N) needs of the crop, which have saturated soils with excessive concentrations of phosphorous (P), since the ratio of N to P in manure is considerably higher than that taken up by crops. Virginia will be required to restrict poultry waste applications to the expected P needs of the crop in 2001, which further heightens the poultry manure disposal problem. The use of phytase as a feed additive in poultry diets is becoming a widely used practice for reducing concentrations of P excreted in manure. Phytase is a cost-effective method known to reduce P excretion without affecting bird health or performance. However, many questions remain to be answered with the use of phytase. For example, does the use of phytase as a feed additive alter the chemical form of P in manure and manure-amended soils? Even more importantly it is critical to ask, is the P that remains in the manure in a form more susceptible to runoff and contributing to the P enrichment of surface waters, instead of serving to control this environmental problem? With the use of phytase becoming widespread, it is also important to determine any changes that could occur after applications are made to soil, and also on plant uptake of P, and crop yield. These same questions regarding any changes that may occur in P also apply to copper (Cu) and zinc (Zn). Though P is now the nutrient of concern, continued applications of manure could lead to toxicity from Cu and Zn. The use of high available P (HAP) corn (zea mays) in poultry diets as an effective means of reducing P excretion in manure is currently being researched and could be introduced into the poultry industry in the near future. A secondary objective of this study was to evaluate changes in P, Cu, and Zn solubility in manure and manure-amended soils from the use of HAP corn, and to determine the effects on crop uptake of these nutrients and crop yield. A previous study conducted by Lawrence (2000) sought to assess the impact of phytase and HAP corn on P, Cu, and Zn solubility in manure and changes in these elements that may have taken place in soils amended with manure over a short time period. This research continues that of

Lawrence's objectives for a longer time period as well as examining yield and uptake of P, Cu, and Zn in a greenhouse experiment from soils amended with poultry manure. Another objective was to fractionate poultry manure to determine elemental fractions of P, Cu, and Zn.

Five diet treatments were used in this study from manure collected from a feeding trial in the Department of Animal and Poultry Sciences and are as follows: A baseline diet serving as the control deficient in inorganic P (NPA), a conventional diet supplemented with inorganic P (NPA+P), an alternative diet supplemented with phytase (NPA+Phytase), an alternative diet using high available P corn in place of normal phytic acid corn (HAP), and HAP corn supplemented with phytase (HAP+Phytase).

Objective 1: Determine effects of using phytase on P solubility in soils amended with manure at a period of six months and one year, which extends the 4 day and 28 day incubation studies conducted by Lawrence (2000). Secondary objectives were to determine effects of HAP corn substitution on P solubility in manure-amended soils, and to determine the effects of both phytase and HAP substitution on Cu solubility in manure-amended soils.

Objective 2: Evaluate the effects of phytase supplementation in turkey (*Meleagris gallopavo*) rations on P, Cu, and Zn uptake and crop yield from corn grown in a greenhouse experiment in soils and sand amended with poultry manure.

Objective 3: Fractionate P, Cu, and Zn in poultry manure to determine any changes in these elements from the use of phytase. Secondary objectives were to determine changes that may arise in P, Cu, and Zn fractions from replacing normal corn with HAP corn in poultry rations.

1.2. LITERATURE REVIEW

1.2.1. Confined Animal Feeding Operations, P Disposal Problems, and the Need for Action

Phosphorous is an essential element that is crucial for plant and animal growth. It is a naturally occurring element and plays an important role in healthy seed development, developing plant root systems, the fundamental processes of photosynthesis, N fixation, and crop maturation. The total P content of healthy plant tissue is rather low, ranging from 0.2 to 0.4% of the dry matter. However, P is a critical component in assuring healthy plant development (Brady and Weil, 1999). The natural supply of P in most soils is low, and the availability of that which is present is most often not sufficient to supply adequate amounts for plant growth. Supplemental P must be added to increase crop growth and yields on soils that are low in P or soils that have been depleted of P through crop removal.

Phosphorous is a major topic of environmental concern due to application of this element at rates in excess of crop needs over extended periods of time. Nutrients (N and P) are the leading pollutants in lakes and estuaries and the third leading pollutants in rivers according to USEPA (1995). Eutrophication of most fresh waters is accelerated by inputs of P and is often the limiting nutrient. Eutrophication poses numerous health risks to humans, livestock, and aquatic communities. The outbreak of *Pfiesteria piscicida* particularly in the Chesapeake Bay tributaries is believed to be linked to nutrient enrichment (USEPA, 1996).

Confined animal feeding operations (CAFOs) have increased in popularity over the past several years. The Shenandoah Valley of Virginia is a prime example of highly concentrated livestock operations with nearly 1,000 of Virginia's 1,300 poultry farms being located in this area (1997 Virginia Census of Agriculture). The poultry industry has increased in size in the state over the past 20 years, and grain consumption has therefore increased dramatically. Corn and soybean (Glycine max) consumption by broilers has increased 250 % since 1970, and is the fastest growing sector in the state (Pelletier, 1999). The turkey sector has increased consumption of grain and soybean by 500 % over the past 20 years. Grain production in Virginia has been decreasing during

this same time period of increased consumption. Many producers cannot compete with Midwestern producers, and have taken acreage out of production. Almost 300,000 acres of land previously in corn production in VA have been taken out of production since 1970 (Pelletier, 1999).

A large deficit has occurred between grain consumption and production within the state. The average shortage of grain in Virginia over the past 10 years has been nearly 40 million bushels, with a deficit average of 41 to 58 million bushels over the past 5 years (Pelletier, 1999). The shortage of grain is continuing to rise with the increase in consumption by livestock, mainly poultry. Grain for use as animal feed must be imported from other areas, primarily the Midwest into the Shenandoah Valley to support the large number of birds produced.

A net import of nutrients mainly in the form of feed into the Valley has arisen. Thus, inputs of nutrients into the Valley far exceed the outputs, as is the case with most animal feeding operations. An average of only 30% of the P coming into farming systems in the Chesapeake Bay watershed from feed and fertilizer is removed as outputs in crops and animal products (Sharpley, 2000). These massive inputs of nutrients tend to accumulate in manure, which create a substantial imbalance in nutrients (mainly N and P) imported into and exported off the farm.

Manure applied to agricultural land is a valuable resource that can serve as an efficient fertilizer to enhance crop production. It provides essential elements as well as improving soil tilth, aeration, water-holding capacity, and decreasing the erosion potential of the soil (DCR, 1993). However, manure is often applied in excess of crop needs in many areas of intensive livestock production. In the Shenandoah Valley, the counties of Augusta, Page, and Rockingham produced an estimated 364,190 tons of poultry manure in 1997. The manure produced in these three counties alone contains enough P to supply twice the amount of P needed for grain corn production in the entire state of Virginia (Pelletier, 1999).

Manure disposal options are quite limited in areas with high densities of animal feeding operations, and result in high or excessive P levels in soils. The United States Department of Agriculture's Natural Resources Conservation Service have identified counties in the United States where more than 100% of the P needs for crop production

are available in livestock manure are correlated with areas that are predominated by high P soils (Sims et al., 2000). Most manures are bulky due to their high moisture or bedding material content, and thus have a much lower nutrient content than commercial fertilizers. Therefore, much larger amounts of manure must be applied to land than mineral fertilizer to achieve the same nutrient application rate (Sharpley et al., 1994). It is not economically feasible to transport poultry manure long distances to be applied on suitable cropland, because the cost of transporting the material more than short distances exceeds its nutrient value. Therefore, manure is applied at above optimum levels for crop production on land surrounding these farms. An inevitable consequence is the buildup of P in the soils to very high or excessive levels to which the manure is applied (Pautler and Sims, 2000).

Poultry are relatively inefficient users of P. More than 60% of P in corn and soybean meal is in the low-available phytate P form (Kornegay, 1996). This results in additional inorganic P supplements added to the diet to meet the nutrient requirements for poultry, which further exacerbates the P-enriched manure disposal problem. Approximately 75% of the P ingested by poultry is excreted in the manure because of their inability to utilize P effectively (Duval, 1996).

Nutrient management planning is a widely used strategy to manage crop nutrients for anticipated crop production, while protecting water quality in the process. A nutrient management plan (NMP) is a site-specific written plan which strives to match nutrient application to soils to ensure maximum crop yields and optimize farm profit, while reducing the risks of environmental degradation (DCR, 1993). Current NMPs are based on N, which matches application of manure or fertilizer to the crop's expected N needs. Nitrogen has traditionally been the nutrient of primary concern in establishing controls for reducing nutrient pollution. The basis behind this reasoning takes into account soil chemistry principles and the major pathways for each nutrient's transport. Nitrogen, after it is mineralized into its negatively charged nitrate form (NO_3^-), is not adsorbed by the negatively charged colloids that dominate most soils. Therefore, it can be readily leached through the soil with drainage water. Phosphorous, on the other hand, is considered to be much less mobile in soils. It is readily fixed in acid soils, which dominate the

southeastern U.S., by the formation of Al and Fe phosphates (Prasad and Power, 1997). In higher pH soils, P is retained by soil Ca carbonates.

It was traditionally thought that the only pathway of P transport to surface waters of any concern was particulate P, bound to soil particles. Erosion control measures taken to prevent this form of P from entering water bodies was thought to have retained P in the soil, and concern for movement by any other mechanisms was discarded. Research has proven that surface runoff transports dissolved P as well as sediment-bound P (Sharpley 2000). Particulate P constitutes the major pathway for movement (75-95%) from conventionally-tilled land, some of which is considered bioavailable (Sharpley et al., 1994). Dissolved P is the major mechanism of P loss from grassland or other nonerosive soils (Sharpley et al., 2000). Both sediment bound P and dissolved P are active in determining the health of the water body into which it enters, and therefore deserve careful attention for control. Several studies have indicated there is a relationship between dissolved P in runoff and soil P concentration. Galeone (1996) conducted a P transport study on a conventionally-farmed site in Pennsylvania and found that the concentration of dissolved P increased as the soil test P value increased. Since P is sorbed strongly, it tends to concentrate in a thin layer at the soil surface. When soil P is built up to high or excessive levels, the soil's capacity to bind P is decreased, therefore increasing the opportunity for dissolved P to move in surface runoff (Sims, 1993). Conservation tillage can increase the soil test P (STP) content of surface soils, if P is applied without incorporation. No-till agriculture has also been found to increase dissolved P in runoff since little soil is exposed to the surface for sorption to occur (Sharpley, 1994). Phosphorous movement has also been noted in subsurface drainage. Although results indicate P content to be small in agronomic terms ($<0.5 \text{ kg ha}^{-1} \text{ yr}^{-1}$), it still represents a portion large enough to contribute to eutrophication (Turner and Haygarth, 2000).

The conventional measures taken to control nutrient transport have created a new set of problems. Nutrient management plans based on N, along with best management practices (BMPs) installed for controlling erosion were once thought to have properly controlled both N and P transport. Since research has identified the major mechanisms of P movement, it is now very apparent that BMPs for controlling erosion only serves as an

aid in controlling P movement. Nutrient management plans based on N allow for manure to be applied based on the expected crop N needs. This rate of manure application has caused P to become oversaturated in many soils especially in areas of intensive livestock production. According to the Nutrient Management Handbook for Virginia, the average Total Kjeldahl N (TKN) value for dry poultry litter tested in Virginia is 62.58 lbs/ton, while P content is 27.11 lbs/ton, an N:P ratio in manure of 2:1 (1993). However, crop removal of N estimated for a yield of 150 bu corn used for grain is 135 lbs, while P removal is 23 lbs, with an N:P crop removal ratio of 6:1 (DCR, 1993). This rate of manure application based on the N needs of the crop represents a P overapplication of 252%. The low N:P ratio found in manure can be partly attributed to a loss of N through ammonia (NH₃) volatilization. Moore et al. (1995) found 33% of the total N content was lost by volatilization from broiler litter in six weeks. The control of NH₃ volatilization is important from an environmental standpoint as well as a health standpoint. Volatilization of NH₃ in poultry houses causes high levels of atmospheric NH₃, which is considered a serious health hazard to both workers and poultry (Moore, 1998). Continued application of manure based on N requirements has led to oversaturation of P in many soils, especially in areas such as the Shenandoah Valley, where limited land area exists for application.

Loss of P from runoff does not represent a large expense to farmers, due to the relatively low amounts lost from an agronomic standpoint. For example, P concentrations in soil solution critical for plant growth are 0.2 to 0.3 ppm. Much less P is required for the acceleration of eutrophication (0.02 ppm) in lake water (Sharpley et al., 1999). This concentration would not represent a major financial loss of fertilizer P to the farmer, but does represent a significant environmental concern. Therefore, the primary need for control of nutrient transport is from an environmental standpoint. Legislation has been passed in the Chesapeake Bay area requiring nutrient management plans to be implemented because of the existing concerns for continued application of manure on soils already high in soil test P, and the associated damage to receiving waters such as eutrophication and its links to the *Pfiesteria* spp. outbreaks. It is also important to keep in mind the fact that P is not a renewable resource, with an estimated 20,000-90,000 Tg

mineable P remaining (Sharpley et al., 2000). Finite sources of P emphasize the need for its control and proper utilization.

Maryland passed the Water Quality Improvement Act in 1998, that requires all agricultural operations with either annual incomes greater than \$2,500 or more than eight animal units (one animal unit is equivalent to 1,000 pounds live weight) to submit a N- and P-based nutrient management plan by July 1, 2004, and implement the plan by July 1, 2005 (Simpson, 1998).

House Bill 1207 was enacted by the Virginia General Assembly in 1999, to require confined poultry feeding operations with 200 or more animal units to implement P-based nutrient management plans by October 1, 2001. The Department of Conservation and Recreation in collaboration with Department of Environmental Quality must examine advances in scientific research and technology, which must include the review of land application of poultry waste, soil nutrient retention capacity, and water quality degradation by December 31, 2005. Changes in the current regulations will be adopted if the examining agencies deem necessary based upon their findings. Amendments, if any, must be implemented for those NMPs developed after December 31, 2005.

It is important to identify agricultural lands that are most vulnerable to P loss in developing P-based nutrient management plans. Sites with very high or excessive soil test P levels and/or have the most potential for P loss are of utmost concern and should be given first priority in implementing the P-based plans. The P Index system developed by Lemunyon and Gilbert (1993) identifies soils with the potential for P movement and will serve as an important management tool for implementation of NMPs. The P Index incorporates several site specific factors that are weighted based on their importance in controlling P loss, including soil test P (Coale and Olear, 1996). The P index value calculated for a given site can then be used to determine the type of nutrient management that should be implemented. For example, Vermont's P index system recommends that for sites having a P index of high or very high that nutrient management be based upon P, and those sites with a low or medium rating can have a N-based management plan (Jokela, 1999).

The restrictions being placed on farmers in the Chesapeake Bay region presents a dilemma that is not easily solved. Manure disposal is already a problem in certain concentrated areas of livestock production such as the Shenandoah Valley. With the establishment of P-based NMPs, less manure can be applied on a given area of land, which further magnifies the disposal problem. Therefore, manure will have to be transported further distances from the farm, increasing costs to the poultry grower. Supplemental fertilizer N will most likely be necessary to replace some of the N formerly supplied by the manure, which presents the farmer with an added expense.

It is critical for alternative uses of manure to be considered to help lower the financial liability of manure disposal by farmers. Several uses are potentially feasible in Virginia. Pelletier (1999) has proposed an exchange program that allows poultry producers to transport manure longer distances and backhaul corn for feed. Virginia corn producers are able to utilize the manure on corn acreage, reducing production costs, and improve corn producer prices. Other alternative uses include composting, pelletization or granulation for fertilizer, and energy generation (DCR, 1999).

Several studies have shown the use of chemical amendments to reduce the availability of P in poultry litter. Moore and Miller (1994) found that the addition of alum, quick lime, slaked lime, ferrous chloride, ferric chloride, ferrous sulfate, and ferric sulfate all reduced water soluble P levels from $>2,000 \text{ mg P kg}^{-1}$ to $<1 \text{ mg P kg}^{-1}$. Moore et al. (1998) has shown not only the decrease in soluble P with the addition of Al-sulfate, but also the decrease in NH_3 volatilization. As stated earlier, reducing NH_3 volatilization increases the N:P ratio, decreasing the P content in manure when applied on an N-basis. Poultry litter treated with coal combustion by-products have also been researched and have proven to reduce dissolved P concentrations considerably on field and pasture soils (Stout et al., 2000). However, more research is still needed to determine if there are any adverse effects of using poultry litter amendments.

1.2.2. Use of Phytase and HAP Grains

The use of enzyme additives that increase the efficiency of P in poultry diets has proven to be an effective means for reducing P in manure. Corn and soybean meal comprise the major feed sources in the United States in poultry diets (Kornegay, 1996). Greater than 60% of P in corn and soybean meal occurs in the phytate form, which has

low P availability to poultry. Phytic acid is a myoinositol 1,2,3,4,5,6-hexakis molecule (dihydrogen phosphate) and is used interchangeably with phytate. Phytic acid serves as the major storage form of P in seeds and plant tissues, with a P content of 28.2% (Ravindran, 1996). The amounts of P contained in plant phytates should be sufficient to meet the nutritional needs of poultry if this content were entirely available. However, P in the phytate form is poorly utilized by poultry because monogastric animals lack the phytase enzyme which releases P from the phytate molecule. Not only is P in phytic acid relatively unavailable, but phytate also binds with other minerals such as Ca, Zn, Fe, Mg, Mn, and Cu (Harland, 1996). Phytic acid is often referred to as the “antinutritional factor” due to its low digestibility and binding capacity which causes a reduction in bioavailability of the phytate bound components (Coelho, 1996).

It is estimated that about one-third of the phytate of plant origin contained in feed is in the nonphytate form and is considered bioavailable. This amount is not sufficient to support the dietary needs of poultry; hence, inorganic P is usually added as a feed supplement. Supplemental P usually consists of one of the many forms of Ca-phosphate, such as mono-dicalcium phosphate, dicalcium phosphate, and thermochemically-produced defluorinated phosphate. These inorganic additions, along with plant-derived P that is in the nonphytate form, are digestible by poultry. However, inorganic P supplements are not totally available either. Approximately two-thirds of plant-derived P from the feed is excreted in the manure as well as a portion of the inorganic P added as a feed supplement. This amount of P contributes highly to the associated problems of soil P buildup from additions of manure in areas of intensive livestock production. Phytase added to poultry diets breaks the phytate bonds increasing the bioavailability of plant-derived phytate P. Phosphorous in the manure can be decreased by 25 to 35% as a result of adding phytase. As a direct result of better utilization, less P will be necessary for supplementation.

Phytase is not only important in P liberation, but also has been shown to increase the bioavailability of other essential nutrients including Ca, Mg, and Zn (Gordon and Roland, 1996; Kornegay, 1996). Since phytase increases the availability of Ca in poultry, it is important to reduce the Ca supplementation when phytase is added to maintain a balance in the Ca:P ratio. Studies have shown that when Ca:P ratios are widened, Ca

reduces phytase activity (Kornegay et al., 1996; Nys et al., 1996). Maintaining a Ca:P ratio of 1.1:1 to 1.4:1 have been shown to maximize phytase activity. Since the phytase enzyme is a protein, it is degraded in the intestinal tract of poultry, and has no known adverse effects on nonruminants.

The use of Low phytic acid corn is currently being researched as another means to maximize P availability to poultry. Low phytic acid corn (also known as high available P corn) contains less phytate-P but more available P than normal corn. Pioneer Hi-Bred International, Inc. is currently testing a corn mutant that contains the same amount of total P as normal corn, but contains 54% less phytate-P in the seed (Iragavarapu, 1999). Animal feeding trials have shown a 3- to 5-fold increase in P bioavailability from low phytic acid corn. Effective utilization of P made available through low phytic acid corn by poultry has been documented. A study conducted by Saylor et al. (1999) demonstrated the improvement in body weights and feed efficiency by broilers fed high available P corn, compared to birds fed normal phytic acid corn. Kornegay and Denbow (1999) showed that high available P (HAP) corn was more available than P in the control corn, 51.2 vs. 27.4% respectively. Phosphorous availability was increased to 63% when phytase was added to the HAP corn diet. These results demonstrate that when phytase and HAP corn are used together, the effects on P improvement are additive. Total and water-soluble P concentrations in broiler litter are also reduced when either HAP corn or HAP corn with the addition of phytase is used compared to normal phytic acid corn (Sims et al., 1999). Both phytase and HAP corn have demonstrated the effectiveness in reducing P concentrations excreted in manure. Inorganic P supplementation can be reduced dramatically when either of these options are implemented. The use of HAP grains in the commercial poultry industry holds a promising future. Yields are currently too low for commercial adoption, but researchers are working on identifying the cause for obtaining these reduced yields. Low phytic acid corn is expected to reach the market in two to three years if researchers can maximize these yields to support commercial production.

Many studies have been conducted to assess P availability on sites that have received applications of poultry litter. These studies have determined the potential for loss from sediment-bound P, dissolved P in surface runoff, and also leaching potential of

P. However, very few studies have been performed using manure from the alternative phytase-amended diets or substitution of HAP grains. More research is needed to determine whether these manipulations in feed formulation alter the chemical forms of P found in the manure. Both phytase and the use of HAP grains are effective in reducing P excretion in manure because they allow for better utilization of plant P by the bird, since it is in a more available form. It is important to consider the fact that since P is more available to the bird with phytase supplementation or the use of HAP grains, the forms excreted in the manure may also be in more available forms. It is well documented that total P concentrations in manure are decreased with the use of phytase and HAP grains; however, the potential exists for the P remaining in the manure to be more soluble than forms excreted in manure from poultry fed conventional diets. If the soluble P concentrations in manure from the alternative diets are found to be higher than in manure from the conventional diets, an increased risk of environmental contamination may result. It is critical to determine any differences in solubility of P from manure that may arise from the use of these alternative diets. It is also important to determine if any chemical changes may arise after land application of manure from poultry fed phytase or HAP corn. Solubility of P could increase when manure from alternative diets are applied to soils, increasing the potential for movement to surface waters. Another question with the use of these alternative diets remains to be answered. Does the use of manure produced from phytase or HAP grain based diets have any effects on plant uptake or crop yield after application to agricultural land? It is very unlikely that any adverse effects will occur with the use of these alternative diets on crops. It is still very important to address this question since the use of phytase is already becoming a popular additive to feeds, and HAP corn is predicted to be in the mainstream in a few years.

1.2.3. Phytase and HAP Related Studies of P Solubility in Manure

Sims et al. (1999) determined the effects on broiler litter composition with the additions of microbial phytase and/or HAP corn to poultry diets. Litter from six diets were generated for use in the study. The diets used were as follows: 1&2) either normal or HAP corn with no phytase additions and control levels of nonphytate P, 3&4) either normal or HAP corn with phytase supplemented with 0.10% nonphytate P, 5&6) either normal or HAP corn with phytase supplemented with 0.20% nonphytate P.

Phytase was supplemented at 800 FTU/kg in starter diets and at 600 FTU/kg in all other diets. Four replications were used for determining water soluble and total P. Three methods were used for determining water-soluble P, but were not specified in the abstract. This paper has not yet been published in a scientific journal at the time of this writing. According to the abstract, both total and water-soluble P concentrations decreased in litter from poultry diets formulated with phytase and HAP corn. Total P concentrations in litter for diets 1-6 were: 1.43, 1.38, 1.21, 1.11, 0.97, and 0.84% respectively. Water-soluble P concentrations were: 0.24, 0.23, 0.22, 0.12, 0.09, and 0.04% respectively. Suggestions made in this paper indicated that phytase and HAP corn will decrease P buildup in areas with intensive agricultural production, and reduce the potential for P movement into surface waters by reducing soluble P concentrations in broiler litter.

Lawrence (2000) determined both water soluble and Mehlich III soluble P of poultry litter from diets with added phytase or HAP corn. Manure was collected from five diet treatments and include: 1) NPA: normal phytic acid corn supplemented with 0.135% inorganic P, 2) HAP: high available P corn supplemented with 0.135% inorganic P, 3) NPA+Phyt: normal phytic acid corn supplemented with 600 units phytase and 0.135% inorganic P, 4) HAP+Phyt: HAP corn supplemented with 600 units phytase and 0.135% inorganic P, and 5) NPA+P: normal phytic acid corn supplemented with 0.345% inorganic P (most similar to the conventional diets currently used in the poultry industry). Water soluble P was determined by extraction with 0.01 M CaCl₂. Total P content in manure decreased from 1.46% in the conventional NPA+P manure to 0.88% in the alternative NPA+Phyt manure. However, a greater percentage of water soluble P was found in the NPA+Phyt manure (78.5% of total P compared to 51.8% of total P in the conventional NPA+P diet). The NPA+Phyt manure had a lower content of water soluble P (6929 mg P/kg manure) compared to NPA+P (7568mg P/kg manure) on an equal manure weight basis, which is consistent with the results found by Sims et al. (1999). Water solubility of P was increased in the HAP and HAP+Phyt manure treatments compared to the conventional NPA+P diet, containing 79.3, 84.3, and 51.8% of total P respectively.

Lawrence (2000) also determined Mehlich III extractable nutrient levels for these manure treatments. Fewer differences in potential plant availability were noted than for water solubility, but the trends remained the same. The percentage of plant available P was slightly higher in the NPA+Phyt manure treatment (73.2% of total P) compared to the NPA+P manure treatment (67.6% of total P), and were not different ($p=0.05$ level). As seen with water soluble P, more Mehlich III P was extracted from the NPA+P treatment (9868 mg P/kg manure) than the NPA+Phyt treatment (6455 mg P/kg manure). The HAP manure treatment also did not differ from the NPA+P treatment. However, differences were observed with a higher content of plant available P in the HAP+Phyt than the conventional NPA+P treatment.

Baxter et al. (1998) used manure collected from swine fed HAP corn and phytase to determine total, phytic acid, and water soluble P levels. Since both poultry and swine are nonruminants, supplementing diets with phytase or using HAP grains allows for more efficient utilization of P for both animals. Thus the effects of P solubility in swine manure may provide useful insight in the study of P solubility in poultry manure. Total manure P excretion was decreased by 21, 23, and 41% in comparison with the control diet for the phytase, HAP corn, and HAP corn + phytase diets, respectively. Water soluble P as a percentage of total P increased in the alternative diets. The water soluble P percentages for the control, phytase, HAP corn, and HAP corn + phytase comprised 56, 64, 73, and 67% of total P, respectively. However, water-soluble P on a total mass basis were lower in the alternative diets, with the lowest concentration in the HAP corn + phytase diet.

The work of Joern et al. (1996) also compared differences in solubility of P in manure excreted from swine fed both conventional and phytase amended diets. The manure treatments were extracted fresh and after 110 days of storage with water and also with 0.5 M NaOH. Water-soluble P increased from 1 to 8% of total P in the conventional and phytase amended fresh treatments, respectively. Storage for 110 days increased water soluble P but led to a less notable difference between the two treatments, increasing from 5% to 10% of total P in the conventional and phytase treatments, respectively. Extraction with NaOH resulted in 39 and 41% base soluble P extracted as a percentage of total P for the conventional and phytase treatments respectively. Storage of the manure

for 110 days once again increased base soluble P percentages slightly with 47 and 59% of total P extracted from the conventional and phytase amended treatments, respectively.

1.2.4. Phytase and HAP Related Studies of P Solubility in Manure Treated Soils

Lawrence (2000) conducted an incubation study to simulate land application of manure from poultry fed phytase and HAP corn. Water-soluble P and Mehlich III P were determined in manure-amended soils after equilibration for 4 and 28 days. Results showed that when manure was applied on a P-based rate, water-soluble P levels were increased by 26% in the phytase amended treatment compared to the conventional treatment after 4 days equilibration. After 28 days, the phytase amended treatment contained 36% more water-soluble P than the conventional treatment. The differences between the time periods were not statistically different, although an increase in water soluble concentrations between the treatments was observed at 28 days. When manure applications to soils were applied on a N-basis, a significant 14% decrease in water-soluble P concentrations occurred in the phytase treatment compared to the conventional treatment, due to the reduction in total P content in the phytase treatment. Phosphorous extracted by Mehlich III produced a less pronounced difference between these two treatments on a P-based manure application regime. After 4 days incubation, plant availability of P was increased by only 1% in the phytase amended treatment in comparison with the conventional treatment, a statistically insignificant difference. Plant availability of P was increased by a statistically significant 4% in the phytase treatment after 28 days incubation. Application of manure on a N-basis produced a significant 24% decrease in plant available P in the alternative phytase treatment compared to the conventional treatment. It is important to note that the total manure P was decreased 41% by switching to the phytase diet.

Moore et al. (1998) conducted a study to determine the effect of HAP corn and phytase in poultry diets on runoff from fescue plots amended with broiler manure. Manure was applied at a rate of 8.96 Mg ha⁻¹ to tall fescue (*Festuca arundinacea*) plots, and simulated rainfall was used to produce two runoff events immediately after application and 7 days later. Manure was collected from five diet treatments used in the study: 1) no litter, 2) normal feed, 3) HAP corn, 4) normal feed with 500 units/kg

phytase, and 5) HAP with 500 units/kg phytase. No significant differences were observed in either total or soluble reactive P in runoff from manure treated plots. HAP corn and HAP corn with phytase did, however, lower P runoff by 22 and 26% respectively, indicating the potential for controlling P movement with the use of HAP grains.

1.2.5. Trace Metals: Cu and Zn

Although P is currently the nutrient of concern in poultry manure, trace metals are also added to poultry diets. Diets are supplemented with Cu and Zn for disease prevention, weight gain improvements, and feed conversion (Moore et al., 1998). Additions of these elements serve as an economical alternative to using antibiotics for disease prevention and growth stimulation. However, large amounts supplemented in diets pose an environmental hazard since most of these elements are excreted in the manure. As much as 95% of Cu supplemented in swine diets can be excreted in the manure, much of which is in soluble forms (Payne et al., 1988a). Since phytase is known to release elements bound to the phytate molecule, including Cu and Zn, a greater percentage of these metals could be excreted in the manure. It is important to keep this fact in mind when supplementing diets with phytase. Reducing Cu and Zn concentrations in diets may be possible, due to more effective utilization of these elements by phytase.

Several studies have shown high concentrations of trace metals, including Cu and Zn, from soils receiving long-term additions of poultry litter. These high concentrations near the soil surface suggest there is a potential for trace metal movement in surface runoff. There is limited data available on the movement of these metals from manured soils. However, Moore et al. (1998) showed that Cu and Zn concentrations in runoff water increased with increasing poultry litter application rates, and Cu levels were found in extremely high concentrations (1 mg Cu L^{-1}) in the runoff. Another concern of these soils with high concentrations of trace metals is the potential toxicity to crops. Anderson (1991) conducted a study on the long-term effects of Cu enriched swine manure application on corn production. While the Cu loadings on soils exceeded the limits set by U.S.E.P.A., Cu concentrations in the plant tissue were found to be in the normal range. Another study showed Cu and Zn had accumulated to toxic levels for plants in fields with

long-term poultry litter applications (Mitchell et al., 1992). Mobility of trace metals is dependent upon many factors, including soil pH. The study by Anderson (1999) was conducted at near neutral pH, which reduces the availability of metals. Different results may have occurred if the study had been conducted in a lower pH soil. Although Cu and Zn concentrations currently may not pose a toxicity problem on selected heavily manured fields, continued application could lead to toxicity for some crops.

1.2.6. Phytase and HAP Related Studies of Cu and Zn Solubility in Manure and Manure Amended Soils

To date, only one project (Lawrence, 2000) has been completed to determine the effects of Cu and Zn solubility in poultry manure and manure-amended soils from the use of phytase and/or HAP grains. The same procedures Lawrence (2000) used for P solubility were also used to determine the solubility of Cu and Zn in poultry manure and upon addition of manure to soils. Water solubility of Cu in the manure did not change significantly between the conventional treatment and the phytase-amended treatment. However, when these manures were applied to soils on an N-basis (and thus an equal Cu-basis), the phytase treatment contained a statistically significant 6% more water-soluble Cu than the conventional manure treatment applied to soils. When these manure treatments were applied to soils on a P-basis, the alternative phytase manure treatment produced a 92% increase in water-soluble Cu concentrations. This significant increase can be attributed to the fact that Cu loadings to soils increased by 67% after conversion to the phytase treatment on a P-basis. The use of HAP corn and HAP with the addition of phytase produced statistically significant increases in water solubility of Cu of 4 and 7%, respectively upon addition of the manure to soils.

Water solubility of Zn in the manure treatments proved to contain 11% of total Zn in the conventional manure treatment, while the phytase treatment contained 61.5% of total Zn. Zinc solubility was not determined for manure-amended soils.

Plant availability was also determined for Cu and Zn using the Mehlich III extractant. Mehlich III extraction of Cu from the manures produced a statistically significant decrease in the phytase treatment compared to the conventional manure treatment. However, when these manure treatments were applied to soils on an equal N-

basis (and thus an equal Cu basis), the concentrations were not different between the phytase and conventional manure treated soils. The plant availability of Zn also did not differ between the phytase and conventional manure treatments.

No studies to date have been published in scientific journals on the effects of crop uptake of P, Cu, or Zn, or crop yield with the addition of manure from diets fed phytase or HAP corn.

1.2.7. Procedures Applicable to Research

Very few studies have been conducted on the solubility of P, Cu, and Zn in manure and manure-amended soils from the use of phytase and HAP corn, as previously stated. However, much research has been conducted on the solubility of these elements in soils, as well as those soils that have been amended with poultry litter. Many of the procedures used in these experiments are also applicable to the studies using phytase and HAP corn.

Several researchers have fractionated P, Cu, and Zn in both soils and manure to determine most dominant forms of these elements. Fractionation allows for a more accurate analysis of the forms that are most readily bioavailable, and also those forms that are relatively insoluble and pose no threat to environmental contamination. Miller et al. (1986a) devised a nine-step sequential extraction procedure for determination of the chemical forms of trace metals in agricultural, polluted, and waste-amended soils. A sequential extraction procedure was also used by Miller et al. (1986b) to determine the forms of Cu in swine manure from diets supplemented with high levels of Cu. Fresh, freeze-dried, and oven-dried samples were fractionated. This fractionation scheme also involved sequential separation of Cu fractions with the use of nine extractants. A solution: solid ratio of 100:1 was used in all extractions. The manure and extracting solution was added to centrifuge tubes, and placed on an end-over-end shaker for the prescribed time. The samples were then centrifuged for 15 min. at 2000 relative centrifugal force. The Cu forms solubilized by the extractants include water-soluble, exchangeable, specifically bound, acid-soluble, organically-bound, amorphous and crystalline Fe-oxide, occluded, and total Cu. The Cu fractions were determined by atomic absorption spectrophotometry. Water was just as effective as salt solutions (Ca and Pb) in extracting Cu. Most of the Cu was extractable with Na-hydroxide or

potassium pyrophosphate, indicating association with organic compounds. Payne et al. (1988b) also used a sequential extraction procedure to determine forms of Cu and Zn in surface soil samples from sites receiving high levels of these elements. Chemical forms solubilized in this procedure were solution and exchangeable, specifically adsorbed, pyrophosphate-extractable, oxide-associated, and residual Cu and Zn fractions.

Determining water-soluble and plant available forms of elements are especially important in soils. The water soluble form consists of the fraction that is readily available to plant roots and is also the most environmentally available form. Plant available forms are also important in soils to determine the amount that is potentially available for uptake by plants, and thus constitutes a larger portion than water soluble forms. Many researchers have employed the use of 0.01 M CaCl_2 as an extractant for measuring water soluble forms of P, Cu, and Zn. The dilute CaCl_2 solution is sometimes used in place of water as an extracting solution because it has similar ionic strength to the natural soil solution, and it also encourages flocculation of the solids in the extracting solution (Li and Shuman, 1996b).

Mehlich III is an acidic extractant which contains a chelating agent and is often used for extracting potentially plant available forms of elements in soils. The Mehlich III extractant has been used extensively for extracting P, Cu, and Zn in a wide range of soil types (Mehlich, 1984).

Both CaCl_2 and Mehlich III extracting solutions have been used as extractants in soils amended with poultry litter. Peters and Basta (1996) determined the effects of using municipal and industrial wastes on P bioavailability from sites with significant additions of poultry and swine waste. The Mehlich III solution was used to evaluate the ability of the municipal and industrial amendments to reduce plant-available P. Plant available P was estimated by the addition of 20 mL of Mehlich III solution to 2 g soil and placed on a rotary shaker for 5 minutes. The samples were then filtered using Whatman No. 2 filter paper. The dilute solution of 0.01 M CaCl_2 was also used to determine water-soluble P levels in the amended soils. Ten mL of 0.01 M CaCl_2 was added to 5 g of soil and placed on a shaker for 15 h. Moore et al. (1998b) treated poultry litter with Al-sulfate to evaluate metal runoff on plots with treated and untreated poultry litter. Soil samples were analyzed for Mehlich III P to serve as an indicator of past manure applications, and thus

as an indicator for metal additions to the soil from the manure. Cox and Hendricks (2000) assessed the effects of clay content from sites receiving applications of poultry manure on the movement of P from surface runoff by extracting soil samples with Mehlich III. Pote et al. (1996) related soil P to losses in runoff by extracting soil that had previously received poultry litter with Mehlich III solution. Sharpley (1996) also determined Mehlich III P levels in soils which had received poultry, swine, or beef feedlot manure. The Mehlich III P content was determined by extracting 1g of soil with 10 mL of extractant.

Eghball et al. (1996) evaluated P movement in soils receiving long-term additions of manure. Water-soluble P was determined by addition of 20 mL of 0.01 M CaCl_2 to 2 g of soil in centrifuge tubes. The samples were shaken for 24 hours and centrifuged. Sallade and Sims (1997) evaluated the forms of P and P sorption capacity of sediments from drainageways in a Delaware watershed that contains one of the most densely populated poultry industries in the world. Water-soluble P was determined by extracting soil with 0.01 M CaCl_2 at a ratio of 1g soil: 25 mL solution and placed on a shaker for 24 h. Mozaffari and Sims (1996) leached coastal plain soils with 0.01 M CaCl_2 to estimate P solubility, while Li and Shuman (1996) determined the leaching ability of Zn in metal-contaminated soils. Both of these studies were conducted on soils receiving applications of poultry litter.

In estimating P fractions in soils it is imperative that dissolved P and particulate P fractions be separated since both of these fractions contribute differently to the release of P to surface waters. A popular technique for separation of dissolved and particulate P fractions is the use of a 0.45 μm filter after extraction with solutions such as CaCl_2 or Mehlich III. The dissolved forms pass through the filter and include the inorganic (reactive-P) and organic (unreactive-P) dissolved P fraction (Mullins and Hajek, 1997). Total dissolved P is determined by inductively coupled plasma spectroscopy (ICP). The dissolved reactive P fraction is molybdate reactive and is determined by the method of Murphy and Riley (Haygarth and Sharpley, 2000). The unreactive P fraction is calculated by subtracting molybdate reactive P from total dissolved P determined by ICP.

No studies using phytase or HAP grains to date have considered the effects on crop yield or uptake of P, Cu and Zn. The procedures used in other studies for

determining the effects of soil amendments on crop uptake of selected elements and crop yield serve as a useful guide in developing procedures specific to this research.

Lucas (1991) conducted a greenhouse study to assess crop response from additions of water treatment residuals on selected soils. Lucas (1991) indicated that supplemental nutrients that are intended to be nonlimiting in greenhouse experiments should be applied in excess of rates commonly recommended to ensure maximum response to the limiting nutrient under study. Nutrients should be added in excess to prevent crop response that is unrelated to the experimental variable. This study used twice the recommended rates for nonlimiting nutrients. Pots were prepared by mixing 2 kg of soil (pH adjusted) with the nonlimiting nutrients in a twin shell blender. The appropriate quantity of residual was added and the contents were remixed. Each pot was double lined with plastic bags to prevent water and nutrient loss. Pots were seeded with fescue and watered to 90% field capacity daily. Proper water content was determined by weighing each pot on a double beam balance and adding the amount of water necessary to bring moisture content to 90% of field capacity, taking into account the weight of the soil, pot, and amendments. Plant material was cut and dried at 70° C in a forced-air drying oven for two days.

Many greenhouse experiments have been conducted in the Muscle Shoals greenhouse at the Tennessee Valley Authority Experiment Station (Allen et al., 1976). Muscle Shoals uses corn in research studies more than any other crop for several reasons. It is relatively resistant to disease and insect damage, it grows well at all seasons of the year, and it responds well to several nutrients. Corn seeds were planted in pots with the use of a template. Seven corn seeds were placed on the surface of the soil with a template and punched into the soil at a depth of 2.5 cm with a large glass rod fitted with a rubber stopper. The plants were thinned 3-7 days after emergence. Water was added by the weighing method employed by Lucas (1991) described previously. The pots were watered to two-thirds of field capacity, with the weight of the pots, amendments and corn taken into account. Corn was grown for 6 to 8 weeks, with the above ground portion harvested and dried in a forced-air electric drying oven maintained at 70° C for one week. Samples were dry-ashed and P was determined by the vanadate or phosphomolybdate method, while Cu and Zn was determined by atomic absorption spectrophotometry.

Sand is often used as a growth medium to ensure that the nutrient under study is only being supplied by the soil amendment. Van der Watt et al. (1994) conducted a greenhouse experiment to assess the uptake of Cu, Mn, and Zn from soils treated with poultry litter. Plants were produced to ensure they were supplied with all nutrients in nonlimiting quantities except for the nutrients under study. Sudax (*Sorghum bicolor*) seed was sown in 500 mL plastic cups containing 600 g of washed quartz sand. The base of the cups were perforated for ease of removal to later employ the double-pot technique in which the cups will be placed on the test soils for root penetration. Nonlimiting quantities of nutrients using the Ruakara solution were used without the addition of Cu, Mn, and Zn. The Ruakara nutrient solution was developed specifically for growing plants in sand culture (Smith et al., 1983). Fourteen days after plant emergence, bases were removed and the cups were placed on the test soils containing one kg of soil. No leaching of nutrients or water was permitted. Plants were harvested 21 days after placement on the test soils. The plant material was harvested and dried at 70° C, weighed, and ground in a Wiley mill with a 1.0 mm screen. The top growth was analyzed by digestion in nitric acid. One gram of plant material was digested in 10 mL concentrated HNO₃ at 125° C in a block digester until the digests were clear and were reduced in volume to approximately 3 mL. The samples were diluted to 50 mL with deionized water and determined by AA spectrometry.

Chapter 2: INCUBATION STUDY

2.1. INTRODUCTION

This chapter seeks to determine the effects of phytase addition and HAP substitution in manure applied to soils over time. Phytase is a popular feed additive, and HAP may become mainstream in the next few years, therefore, it is important to determine any effects these additives may have on P and Cu solubility after application of poultry manure to agricultural lands. An important question yet to be answered is whether phytase and HAP amended manure will react with soil over time and produce changes in solubility of P and Cu. This study involves applying manure at two application rates to southeastern soils and incubating these soils for a period of six months and one year to determine if changes in P and Cu solubility arise after reaction with soil during this period of time.

2.2. MATERIALS AND METHODS

2.2.1. Manure Collection

The manure used in this study was collected during a feeding experiment in the Department of Animal and Poultry Sciences at Virginia Tech. The feeding experiment compared 12 dietary treatments on turkeys from 1 to 5 weeks old. Only five of these twelve dietary treatments were compared in this study. The objective of the feeding experiment was to compare P bioavailability to young turkeys fed high available phosphorous corn (HAP), normal phytic acid corn (NPA), and birds fed these same diets with the addition of microbial phytase.

Nine turkey poults were randomly assigned to each of 96 Petersime pens on the day they hatched and were fed a commercial starter diet for one week. The birds were then reduced to eight birds per pen and were started on test diets. The 12 test diets were randomly assigned to eight replicate pens for each diet in a block design. Poults were reduced again to 7 birds per pen after 2 weeks on the test diets, and were transferred to grower pens for an additional 2 weeks.

The pens containing the birds were in environmentally controlled rooms, and the birds had unlimited access to food and water. No bedding material was used in the pens, and metal trays were placed below each pen for collection of manure. The food and water was placed in the front of the pens and only manure at least 6 inches away was collected for use in this study. These trays also prevented any manure from dropping to pens below. The trays were scraped clean daily and was discarded except for the three-day manure collection for use in this study. These manure samples were collected for three consecutive days during the fourth week the birds were on the test diets. Since only five diets were to be compared, manure was collected in plastic bags from the 40 pens that were fed these diets (8 replicate pens per treatment X 5 diets= 40 pens). A total of 120 samples were collected during the 3-day period. The manure samples collected from each of the 40 pens were combined into 40, 2-gallon buckets and mixed with an electric hand drill holding a metal paint mixer. These buckets were then frozen at -10°C .

The manure collected contained approximately 80% moisture, and it was decided that the manure would be analyzed in this “wet” form. This manure was frozen when not in use to reduce any changes that may occur over the duration of the experiment. The manure in these buckets was thawed and mixed thoroughly with a long-handled plastic spoon prior to each use. Subsamples were removed and weighed before and after placement in a 110°C oven for 24 hours to determine moisture content. Manure was mixed with water at a ratio of 1g dry manure: 10 mL water for determination of pH. The wet manure was analyzed for total P, Ca, Cu, and Zn after wet digestion using nitric and perchloric acid. Phosphorous content was analyzed colorimetrically using the vanadomolybdate procedure (Kuo, 1996). Total Ca, Cu, and Zn were determined by atomic absorption spectrometry.

Manures from each of the five diet treatments were then combined for use in the soil incubation study. A total of 150 g (dry weight basis) of wet manure from each of the original 40 buckets were placed by diet treatment into five, 5-gallon plastic buckets and mixed with a long-handled plastic spoon. Subsamples were removed from each of the buckets and moisture content, pH, and total P, Ca, Cu, and Zn were again determined using the same procedures used for the original samples (Table 2.1).

Table 2.1. Manure analysis

| Diet | Dry Matter (%) | pH | % dry basis | | Mg kg ⁻¹ , dry basis | |
|-------------|----------------|------|-------------|-------|---------------------------------|-------|
| | | | P | Ca | Cu | Zn |
| NPA | 19.0 | 5.04 | 0.920 | 0.876 | 29.9 | 282.4 |
| NPA+P | 22.5 | 5.40 | 1.460 | 1.605 | 30.3 | 263.7 |
| NPA+PHYTASE | 20.1 | 4.95 | 0.862 | 0.733 | 29.6 | 246.6 |
| HAP | 19.4 | 5.23 | 0.858 | 0.767 | 32.2 | 258.6 |
| HAP+PHYTASE | 21.1 | 5.30 | 0.844 | 0.700 | 29.8 | 223.5 |

2.2.2. Diet Treatments

Table 2.2 shows the five diet treatments used in the study along with the ingredients that comprise these treatments. The same ingredients were used in all five of the diets, only differing with the type of corn used, and the addition of microbial phytase to two of the treatments. NPA corn, which is added to standard diets in the turkey industry, was added to the NPA, NPA+P, and NPA+Phytase diets. HAP corn, supplied by Optimum Quality Grains, was included in the HAP and HAP+Phytase diets. All five diets contained identical quantities of soybean meal, limestone, and a trace mineral premix. Defluorinated phosphate (PCS Phosphate Company, Inc.) was added in equal amounts, with the exception of NPA+P. The trace mineral premix was added to supply 12.5 mg Cu and 95 mg Zn per kg of feed. Starch/dextrose was added as a filler ingredient to compensate for differences in quantities of other ingredients added. The five diets contained equal amounts of all nutrients except P and Ca, in quantities at or above levels recommended by the National Research Council for turkeys in the 0 to 4 and 4 to 8 week age range (NRC, 1994).

Table 2.2: Feed Composition

| DIET | NPA corn | HAP corn | Soybean meal | Defluorinated phosphate (18% P & 32% Ca) | Limestone (38% Ca) | Natuphos 600 phytase enzyme | Trace mineral premix | Starch/Dextrose | Other ingredients | Total |
|-------------|------------------------------|----------|--------------|--|--------------------|-----------------------------|----------------------|-----------------|-------------------|--------|
| | % of each ingredient in feed | | | | | | | | | |
| NPA | 41.06 | 0.00 | 51.06 | 0.75 | 0.72 | 0.00 | 0.10 | 2.25 | 4.06 | 100.00 |
| NPA+Phytase | 41.06 | 0.00 | 51.06 | 0.75 | 0.72 | 0.10 | 0.10 | 2.15 | 4.06 | 100.00 |
| NPA+P | 41.06 | 0.00 | 51.06 | 1.92 | 0.72 | 0.00 | 0.10 | 1.08 | 4.06 | 100.00 |
| HAP | 0.00 | 41.06 | 51.06 | 0.75 | 0.72 | 0.00 | 0.10 | 2.25 | 4.06 | 100.00 |
| HAP+Phytase | 0.00 | 41.06 | 51.06 | 0.75 | 0.72 | 0.10 | 0.10 | 2.15 | 4.06 | 100.00 |

Table 2.3 shows the forms of P found in each of the five diets. Nonphytate P is the form of P considered available to the bird. Notice that after predicted phytase activity, the phytase converts a portion of the phytate P to plant-derived nonphytate P. This prediction is based upon the assumption that 600 units of phytase activity per kg feed will convert 0.100% of feed from the phytate to the plant-derived nonphytate form. Total feed P is the sum of phytate and nonphytate P. The NPA diet is considered to be a control diet in this study, and contains only 0.282% total nonphytate P. As seen from table 2.3, this level of nonphytate P is much lower than that recommended by NRC (1994). The NPA diet is not a representative diet used in the turkey industry due to these low levels of P. It does provide a basis for comparing the effects when additions of phytase, supplemental inorganic P, and HAP corn substitution are made. NPA+Phytase differs only from the NPA control diet with the addition of 600 units of phytase activity per kg of feed and allows comparisons to be made when this phytase is added. NPA+P also consists of the same ingredients as the NPA diet, the only exception is that higher levels of defluorinated phosphate was added to the NPA+P diet. The effects of additional inorganic P can be assessed using this diet treatment. The only difference between the NPA and HAP diets is the type of corn added to each diet. HAP corn was substituted for NPA corn in the HAP diet and is considered to be more available to poultry because it contains higher quantities of plant-derived nonphytate P. The HAP diet allows for comparisons to be made when HAP corn is substituted for the conventional NPA corn. HAP+Phytase is similar to the HAP diet, only differing with the addition of 600 units of phytase per kg of feed. The HAP+Phytase diet does allow for assessment of HAP substitution and phytase addition.

Table 2.3: Forms of P found in feeds and NRC recommended levels of P

| | Percentage of P in Feed | | | | Percentage of nonphytate P levels recommended by NRC | | Percentage of Total Feed P | | | | |
|-------------|-------------------------|-----------------------|-----------------------|-----------------------|--|------------------------|----------------------------|---------------------|---------------------|---------------------|---------------------|
| | Phytate P | Nonphytate P | | | Total P | Turkeys 0 to 4 wks old | Turkeys 4 to 8 wks old | Phytate P | Nonphytate P | | |
| Diet | | Plant-derived | Inorganic | Total | | | | Plant-derived | Inorganic | Total | |
| NPA | 0.269 | 0.147 | 0.135 | 0.282 | 0.551 | 0.6 | 0.5 | 48.8 | 26.7 | 24.5 | 51.2 |
| NPA+Phytase | 0.269 0.169* | 0.147 0.247 | 0.135 0.135 | 0.282 0.382 | 0.551 0.551 | 0.6 | 0.5 | 48.8 30.6 | 26.7 44.9 | 24.5 24.5 | 51.2 69.4 |
| NPA+P | 0.269 | 0.147 | 0.345 | 0.492 | 0.761 | 0.6 | 0.5 | 35.3 | 19.3 | 45.3 | 64.7 |
| HAP | 0.236 | 0.185 | 0.135 | 0.320 | 0.556 | 0.6 | 0.5 | 42.5 | 33.2 | 24.3 | 57.5 |
| HAP+Phytase | 0.236 0.136 | 0.185 0.285 | 0.135 0.135 | 0.320 0.420 | 0.556 0.556 | 0.6 | 0.5 | 42.5 24.5 | 33.2 51.2 | 24.3 24.3 | 57.5 75.5 |

* Numbers in bold represent forms of P after predicted phytase activity

The NPA+P diet is most similar to the diets currently used in the turkey industry today, because it contains close to the recommended levels of nonphytate P, as seen in Table 2.3. NPA+Phytase is similar to a commercial diet being supplemented with phytase. This diet would most likely be supplemented with additional inorganic P to reach the NRC recommended levels of nonphytate P in a commercial setting. It would not be economical for additional phytase to be included (Duval, 1996). The HAP diets are not standard diets in the turkey industry, because HAP is not commercially available. The HAP+Phytase diet does contain levels of nonphytate P which are close to the NRC recommended levels. These HAP diets may hold a promising future after more research using HAP grains has been conducted.

The five diet treatments were analyzed for P and Ca contents and are shown in Table 2.4. The samples were ground to pass a 1-mm sieve. Calcium contents in the diets were adjusted to maintain a recommended total Ca: total P ratio of 1.2 to 1.3:1.

Table 2.4. Analysis of Feed

| Diet | Analyzed Concentration (%) | | |
|-------------|----------------------------|-------|-------|
| | Dry Matter | P | Ca |
| NPA | 90.3 | 0.550 | 0.654 |
| NPA+Phytase | 91.0 | 0.556 | 0.652 |
| NPA+P | 91.1 | 0.753 | 1.057 |
| HAP | 90.8 | 0.560 | 0.667 |
| HAP+Phytase | 90.6 | 0.563 | 0.633 |

2.2.3. Soil Collection and Characterization

Three soils were collected from three different physiographic provinces in Virginia for use in this study, and are considered to be productive agricultural soils. A Groseclose loam was taken from Montgomery County located in the Ridge and Valley region and is classified as a clayey, mixed, mesic Typic Hapludult. A Cecil loam was collected from Amelia County in the Piedmont, classified as a fine, kaolinitic, thermic Typic Kanhapludult. A Mahan sandy loam was taken from Isle of Wight County in the Coastal Plain. The Mahan soil was classified as a fine, kaolinitic, thermic Typic Hapludult.

The soils were air-dried, and clods were broken up with the use of a mortar and pestle. The samples were sieved to remove coarse fragments greater than 2 mm, and were thoroughly mixed using a portable electric cement mixer. Moisture content of the air-dried soils was determined by weighing a sample before and after oven drying at 110°C for a 24-hour period. The water holding capacity of the soils was determined by estimating the amount of water held in the soils after being freely drained by using the technique of Kuo (1996). Subsamples of the three soils were extracted with Mehlich I solution and analyzed for plant-available P, K, Ca, Mg, Cu, and Zn by ICP in the Virginia Tech Soil Testing Laboratory (Donohue, 1994). The lab also determined pH (1:1 soil: water ratio) and percent organic matter (Table 2.5).

2.2.4. Manure Application to Soils and Incubation

Six manure treatments were applied to the three soils and are shown in Table 2.6. These manure treatments include HAP+Phytase, HAP, NPA+Phytase, NPA, NPA+P on P basis, and NPA+P on N basis. The NPA+P was separated into two manure treatments, on an N-basis and also on a P-basis. As seen in Table 2.6, HAP+Phyt, HAP, NPA+Phytase, NPA, and NPA+P on P basis have similar P contents, while HAP+Phyt, HAP, NPA+Phytase, NPA, and NPA+P on a N basis have similar N contents, as well as similar Cu contents. The NPA+P manure treatment on a P basis allows for equal comparisons of P content, while the NPA+P treatment on an N basis allows for comparing Cu between treatments. Notice that Zn contents vary considerably between manures. It was discovered that manure from five pens had been contaminated with Zn when the metal trays were scraped daily for collection. Scrapers were used to collect manure daily from the metal trays. The cause of the contamination is attributed to the metal trays, which may have been rusted in these pens. No comparison could be made for Zn after incubation with soils for this reason.

Two application rates of each manure treatment were applied to the soils. The low and high manure application rates were set at 56 Mg ha⁻¹ (25 ton acre⁻¹) and 112 Mg ha⁻¹ (50 ton acre⁻¹) respectively, on a dry manure basis. Normal application rates of manure up to 11.2 Mg ha⁻¹ (10 ton acre⁻¹) are recommended for Virginia farmers. These high rates of manure were selected after preliminary experiments using lower rates

| Table 2.5: pH, organic matter, and plant-available nutrient contents of soils | | | | | | | |
|---|-----|--------------------|---|----------|-----------|---------|-----|
| | pH | Organic Matter (%) | Mehlich I extractable (mg kg ⁻¹ in soil) | | | | |
| Soil Type | | | P | K | Ca | Mg | Cu |
| Groseclose | 6.7 | 3.8 | 152 (VH)* | 155 (H+) | 1086 (VH) | 91 (H) | 0.9 |
| Cecil | 6.6 | 3.1 | 57 (VH) | 176 (VH) | 753 (H-) | 83 (M-) | 0.9 |
| Mahan | 6.1 | 2.3 | 34 (H) | 112 (H) | 456 (M-) | 84 (M-) | 1.7 |

* Nutrient availability rankings: VH=very high, H=high, M=medium (Virginia Cooperative Extension)

| Table 2.6: Manure applications to soils and elemental composition* | | | | | | |
|--|--|----------------------------------|----------------------------|----------------------------|-----------------------------|-----------------------------|
| Manure Treatment | | g manure kg ⁻¹ soil** | mg P kg ⁻¹ soil | mg N kg ⁻¹ soil | mg Cu kg ⁻¹ soil | mg Zn kg ⁻¹ soil |
| Control | | 0.0 | 0.0 | 0.0 | 0.00 | 0.00 |
| NPA | | 22.8 | 190.4 | 1904.2 | 0.70 | 9.64 |
| NPA+Phytase | | 23.8 | 188.7 | 1866.7 | 0.73 | 11.50 |
| NPA+PP-basis | | 14.4 | 191.2 | 1114.0 | 0.44 | 4.22 |
| NPA+P N-basis | | 24.0 | 318.7 | 1856.7 | 0.74 | 7.03 |
| HAP | | 24.4 | 190.8 | 2082.6 | 0.70 | 9.61 |
| HAP+Phytase | | 24.9 | 188.5 | 2050.7 | 0.74 | 10.28 |

* Expressed on dry weight basis

** Represents low rate of manure application. Application rate was doubled to obtain high application rate, therefore all elemental concentrations in table are also doubled

concluded that any differences in P content between the soils treated with manure and control soils with no manure may be very small or nonexistent, due to the soils ability to adsorb P added from the manure and release P from the soil.

Manure was applied to the soils wet, as excreted from the bird. It was necessary to balance out the water content between the manures when applied to the soils since each manure treatment contained varying amounts of moisture. The large amounts of manure added to the soils in the wet form exceeded the water holding capacity calculated for the soils. It was decided to adjust soil moisture to 150% of the water holding capacity after observations containing this amount of moisture were deemed acceptable. Water was added to each container after manure and soils were mixed to bring the moisture content to 150%. Wet manure was weighed out to achieve the low and high application rates on a dry weight basis into 12 oz. plastic containers to be used for incubation. To these containers 100 g (oven-dry basis) of air-dried soil was added. The soil and manure was thoroughly mixed to ensure the manure was equally distributed throughout the container. Water was added to the cups to bring the total moisture content to 150% of the water holding capacity of the container. The control treatments received only soil, and water was added to bring the container to 150% of the water holding capacity of the cup. Airtight lids were placed on the cups to seal the container, and holes were punched in the top. Weights of each container were recorded, and the cups were then incubated at 20 °C for a period of six months and one year. Containers were weighed periodically during incubation and moisture was added to its original volume.

Seven different treatments were used (control plus the six manure treatments) with the three soils, as well as two application rates, and two incubation periods, which makes up a total of 12 different combinations of factors applied to each manure type. Two reps were used for each factor combination to bring the total number of containers used in the incubation study to 168. Two subsamples were collected from each rep for analysis for the one-year incubation period. Only one subsample was collected for analysis for the six-month incubation period.

Two extractants were selected for use with the manure-amended soils. To determine the water solubility of P and Cu, 0.01M CaCl₂ was used (Kuo, 1996). Mehlich III solution (Mehlich, 1984), which is an acidic extractant, was used in estimating the plant availability of P and Cu. Both of these solutions are standard extractants used for soils. Objectives of an earlier study were to subject the same extraction procedures to both the manures and manure-amended soils. To remain consistent with these studies, these same extractants were also used in this incubation study.

2.2.5. CaCl₂ Extraction

Moisture content of the manure-amended soil was determined by weighing a subsample before and after placement in a 65°C oven for a 24-hour period. A 10:1 ratio of extractant (mL) to manure-amended soil (g on dry basis) was used for both extractants.

The calculated quantities of soil (5 g on oven-dry basis) were weighed into 100 mL plastic centrifuge tubes. In order to account for the moisture present in the soil, 25 mL 0.02M CaCl₂ (double strength) solution was added to the centrifuge tubes. The remainder of the water was added to the tubes to achieve 50 mL 0.01M CaCl₂ solution: 5g dry manure-amended soil (10:1 ratio), taking into account the water entrained in the soil. Plastic caps sealed the centrifuge tubes and were placed on a reciprocal shaker for 1-hour and centrifuged at 2500 rpm for 10 minutes. The supernatant liquid from each tube was decanted into 60-cm³ syringes with Luerlok tips packed with 1-gram of non-reactive filter pulp in the bottom of the syringe. Nucleopore filter housings measuring 25-mm in diameter were attached to the Luerlok tips. The filter housings hold 0.45 µm Nucleopore filters. A syringe plunger was used to force the supernatant through the filter pulp in the syringe and then through the 0.45 µm filter in the filter housing. The liquid was collected in a plastic dilu-vial for analysis. The extracts were analyzed for P and Cu using inductively coupled plasma emission spectrometry (ICP). The samples were also analyzed for molybdate-reactive orthophosphate using the modified ascorbic acid method (Watanabe and

Olsen, 1965) of Murphy & Riley (1962). The Spectronic 21 D-V spectrometer was used to do the colorimetric tests with wavelength set at 880 nm.

2.2.6. Mehlich III Extraction

Moisture content of manure-amended soil was determined by weighing a subsample before and after placement in a 65 °C oven for a 24 hour period. The calculated quantities of soil (5-g on oven-dry basis) were weighed into 100 mL plastic centrifuge tubes. In order to account for the moisture present in the soil, 25 mL double strength Mehlich III solution was added to the centrifuge tubes after the remainder of the water was added to the tubes to achieve 50 mL Mehlich III solution: 5g dry manure-amended soil (10:1 ratio), taking into account the water entrained in the soil. Plastic caps sealed the centrifuge tubes and were placed on a reciprocal shaker for 5 minutes and centrifuged at 2500 rpm for 10 minutes. The same procedure used for the CaCl₂ extraction was also used for the Mehlich III extraction from this point forward. The Mehlich III samples were also analyzed colorimetrically using the same method previously described.

2.2.7. Statistical Analysis

A total of 252 extractions were performed for each extractant, for a total of 504 extractions. The following equation was used to calculate the quantity of P or Cu extracted in mg per kg of soil:

$$A \times B = C$$

A=mg element per L of extractant

B= mL extractant per g soil extracted (dry weight basis)

C= mg element extracted per kg dry soil

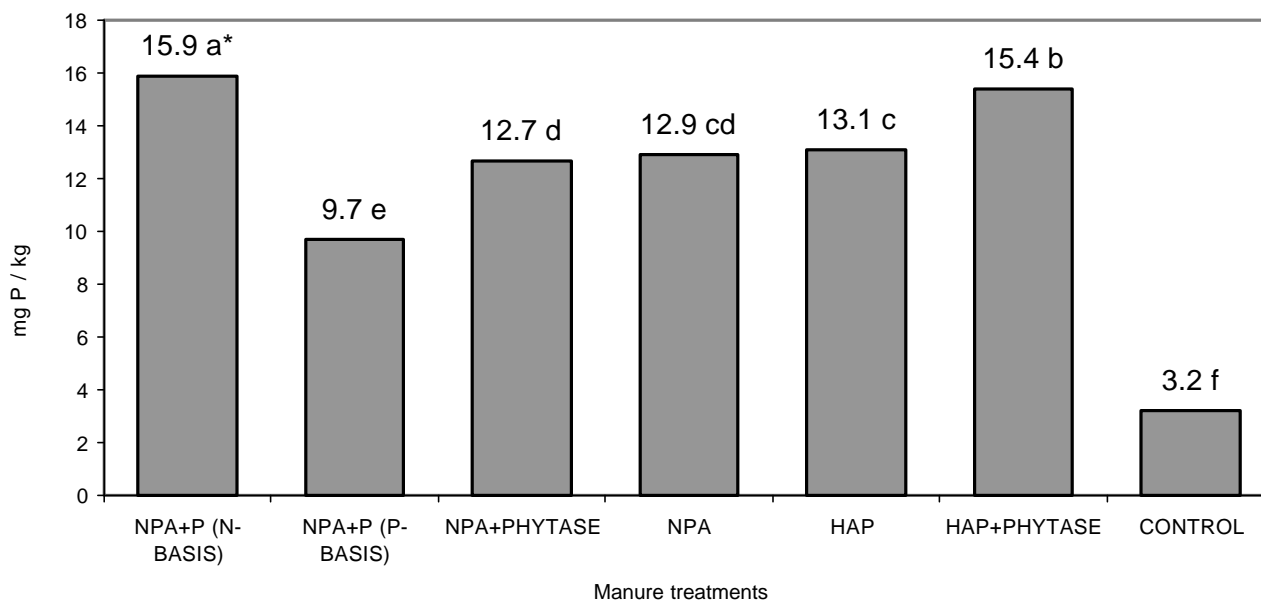
SAS Analysis of variance was performed using SAS (SAS Institute, 1996) to evaluate statistical differences between soils amended with the manures, and between manure treatments. Duncan's Multiple Range Test was also used in SAS to compare means at the alpha level of 0.05.

2.3. RESULTS AND DISCUSSION

2.3.1. Water Solubility of P

Manure-amended soils were extracted with 0.01 M CaCl₂ to determine water-soluble P concentrations. A total of 572 extractions were performed over 7 treatments, 3 soil types, 2 manure application rates, and 4 incubation time periods. Lawrence (2000) performed extractions on manure-amended soils incubated for the two shortest time periods. The results of these extractions are shown in Fig. 2.1, with the combinations of soil type, manure application rate, and time period averaged across treatments.

Fig. 2.1. Water-soluble P extracted from manure amended soils averaged across all three Virginia soils, incubation times (4 and 28 days, 6 and 12 months), and manure application rates (25 and 50 g / kg)

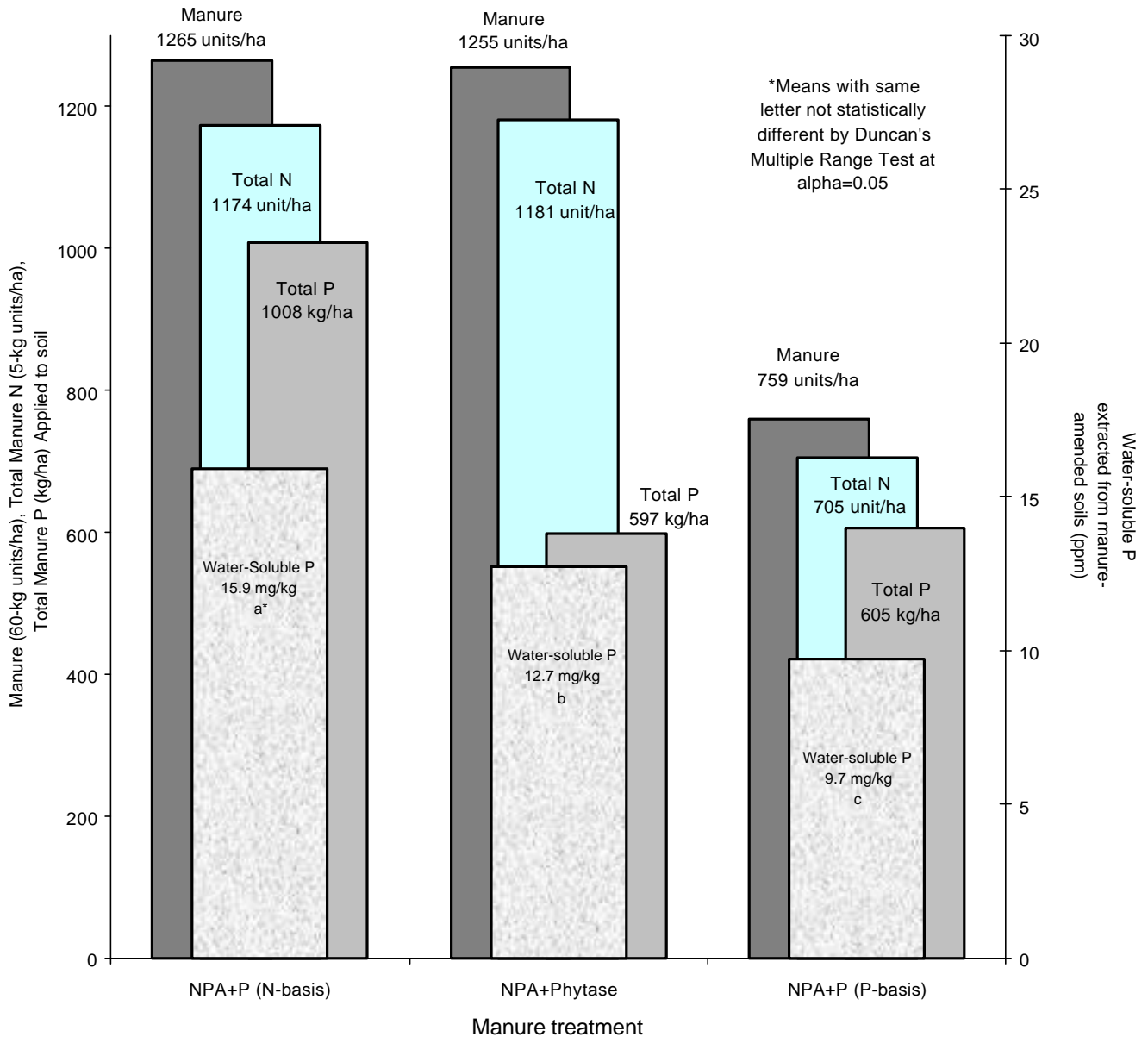


*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

The control treatments had no additions of manure. It is important to note that the NPA+P treatments were split into two treatments based on manure application rate. These treatments allow for comparison of manure treatments added to soil on an N-based rate, as well as on a P-based rate. The following treatments should be compared when interested in treatment differences that result from applying manure on an N-basis, and thus have similar N contents: NPA+P (N-basis), HAP+Phytase, HAP, NPA, and NPA+Phytase. The treatments that have

similar P contents include: HAP+Phytase, HAP, NPA, NPA+Phytase, and NPA+P (P-basis), and should be compared when one is concerned about differences between treatments upon implementation of P-based restrictions. The addition of phytase to the baseline NPA treatment does not produce a statistically significant difference in water-soluble P levels upon addition to soils. Since the NPA+P diet treatment is most similar to that currently used in the poultry industry, the key issue is considering any differences in water solubility of P between the conventional NPA+P manure treatments and the alternative phytase treatment. The alternative phytase treatment will therefore be compared to this conventional NPA+P treatment throughout this document. The baseline NPA treatment does provide for useful comparisons when determining if any differences in P solubility exist solely from the addition of phytase or substitution of HAP corn in this NPA diet. A comparison of water-soluble P extracted (as given in Fig. 2.1) from soils amended with NPA+P (N-basis), NPA+Phytase, and NPA+P (P-basis) manure treatments is illustrated in Fig. 2.2. Also given is the rate of manure, total N, and total P applied to soils from each treatment. When comparing the alternative NPA+Phytase treatment to the conventional NPA+P manure treatment on an N-basis, water-soluble P is decreased by 20% (15.9 mg P kg⁻¹ and 12.7 mg P kg⁻¹ for NPA+P on N-basis and NPA+Phytase treatments, respectively). It is important to note that although similar amounts of manure are applied to soils, 41% less total P is applied to soils from the NPA+Phytase treatment compared to the NPA+P on N-based manure application regime. When comparing the alternative phytase treatment to the conventional NPA+P treatment on a P-basis, 40% less manure is applied from the NPA+P treatment. Water-solubility of P increased by 24% in the alternative phytase treatment in comparison with the NPA+P treatment on a P-basis. The decreased content of water-soluble P in the NPA+P treatment may be attributed to increased levels of Ca present in supplemental calcium phosphate. The added Ca present in these supplements could quite possibly precipitate P in the soil, preventing CaCl₂ extraction of this bound P. This is evident when the NPA+P on the P-basis is compared to the baseline NPA treatment. The addition of Ca-phosphate to the NPA treatment decreased CaCl₂ extractability of P by a statistically significant 25%. This Ca-phosphate effect is most likely producing the marked reduction in extractability of NPA+P, in comparison with the alternative phytase treatment.

Figure 2.2. Comparison of water-soluble P concentrations from soils amended with NPA+P and NPA+Phytase manure treatments averaged across three Virginia soils, application rates (25 and 50 g/kg) and incubation periods (4 and 28 days, 6 and 12 months)

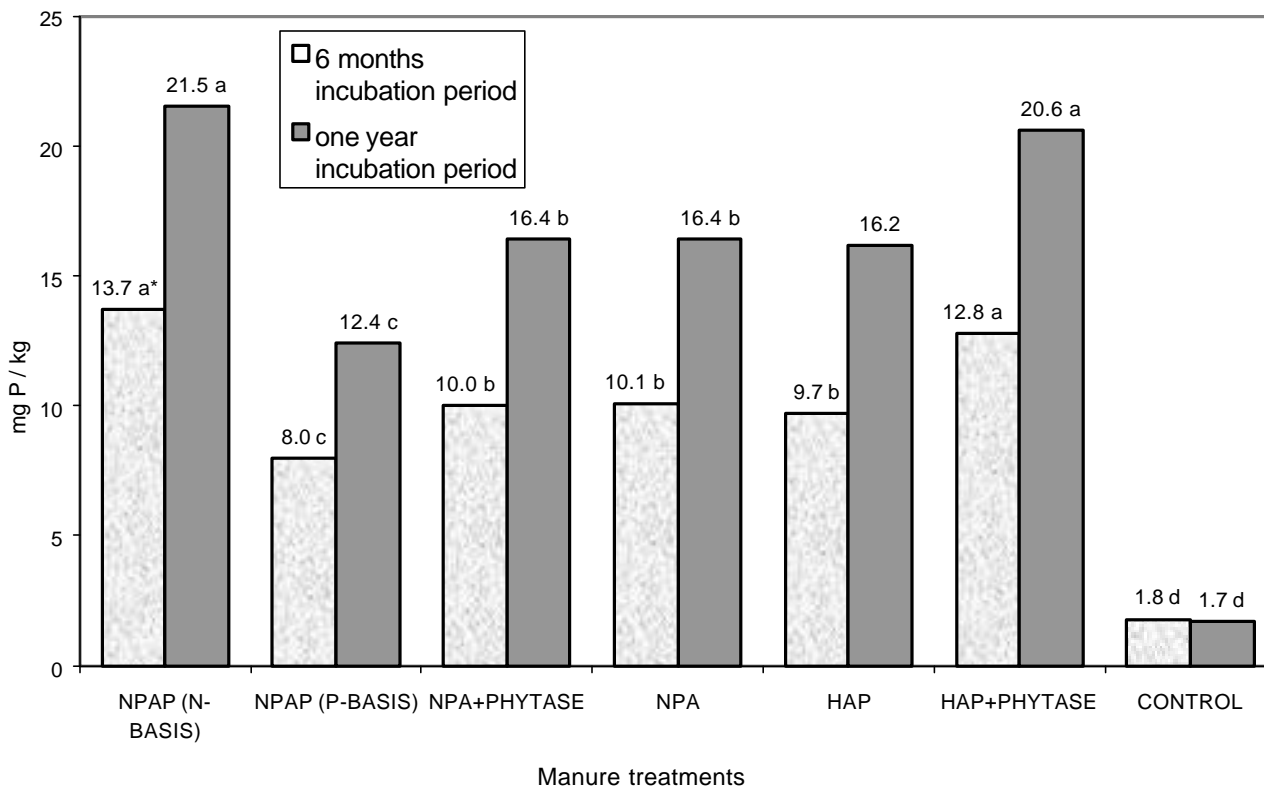


A statistically insignificant 1.5% increase in water solubility of P is produced by the replacement of HAP for NPA corn in the diets. However, a significant increase of 16% is seen when both HAP and phytase replaces the baseline NPA treatment, signifying that the effects of increased P solubility of these amendments are additive.

2.3.2. Effects of Incubation Time, Manure Application Rate, and Soil Type

Manure-amended soils were incubated for 4 days, 28 days, 6 months, and one year prior to extraction. Lawrence (2000) incubated and extracted soils for the two short time periods. The following tables in this section provide results for extractions conducted only on soils incubated for 6 months and one year. Figure 2.3 allows for comparison of treatments incubated for six months and one year averaged across soil type and application rate.

Figure 2.3. Effects of incubation time on water-soluble P concentrations extracted from manure amended soils averaged across application rates (25 and 50 g/kg) and three Virginia soils



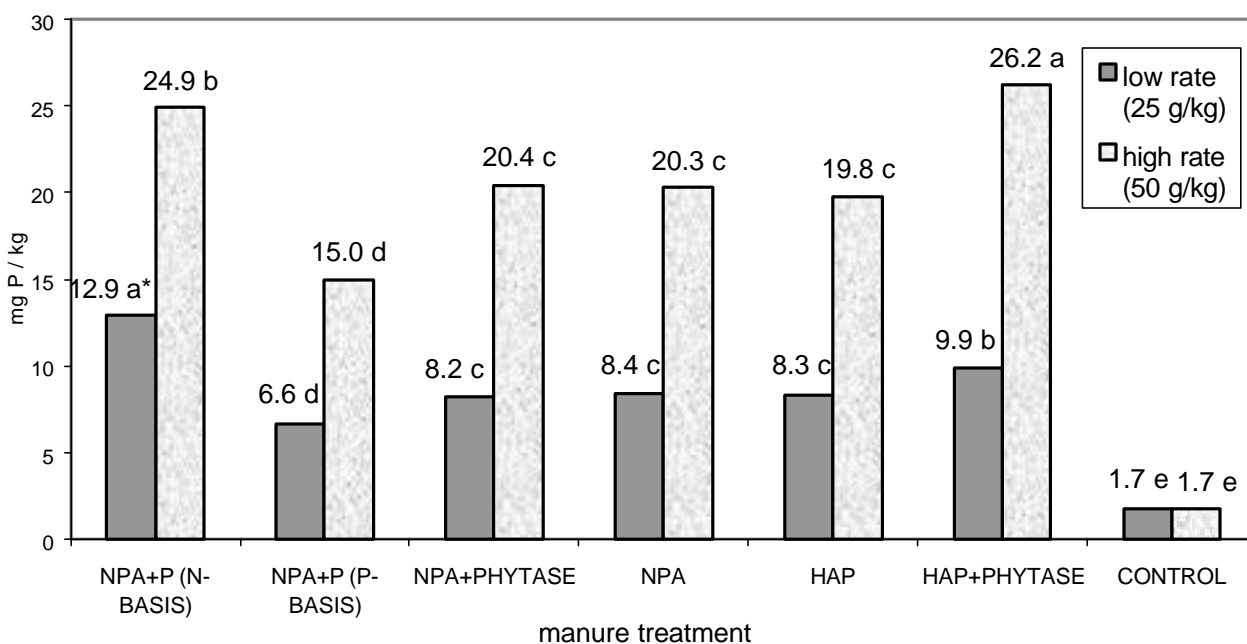
*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
 Statistical analyses were conducted separately on each of the incubation periods.

As illustrated in Fig. 2.3, incubation of soils with manure treatments for one year compared to 6 months increased water-solubility of P by at least 35% for every manure treatment. The increase in water-soluble P concentrations may be attributed to mineralization of organic P into water-soluble forms over the one-year period. Although significant increases are observed upon

increasing incubation time, the trends between manure treatments remain the same. Quantities of water-soluble P are almost identical between the NPA and NPA+Phytase treatments for both time periods, indicating there is no effect on water-solubility of P from increased incubation time due to inclusion of phytase. No statistical differences exist from substituting HAP corn for NPA corn, also indicating no effect on P solubility by extending incubation time from the use of HAP corn. Therefore, the use of amendments does not cause any notable changes in water-solubility of P to occur upon increasing the incubation time period of manure-amended soils.

Data presented in Fig. 2.4 allows for comparison of treatments for soils amended with low and high rates of manure. The results given for each treatment are averaged across soil type and incubation time (six months and one year incubations).

Fig. 2.4. Effect of manure application rate on water-solubility of P averaged across all three Virginia soils and incubation periods (6 and 12 months)



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05.

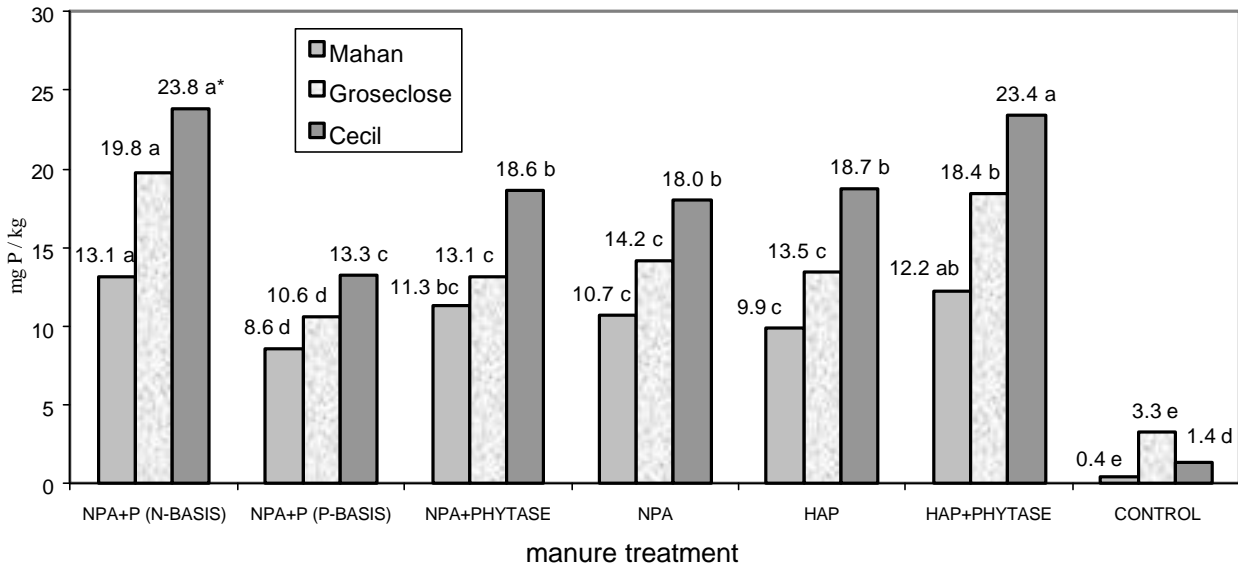
Statistical analyses were conducted separately on each of the application rates.

Water-soluble P concentrations increased an average of 57% over all treatments by increasing application rate. It is important to keep in mind that the high manure application rate was obtained by doubling the low rate. Extractable P concentrations were more than doubled by increasing application rate, but the trends in P extractability between treatments were very similar. Addition of phytase did not increase water-soluble P between the treatments by

increasing application rate. The CaCl_2 -extractable P was 2% lower in the phytase-amended diet compared to the baseline diet in the low application regime. Addition of phytase to the baseline diet did increase P solubility slightly (<1%) when manure was applied to soils at the high application rate; however, phytase addition did not alter water-solubility of P enough to create a significant difference. The substitution of HAP corn for NPA did not produce a significant difference in either the low or high application regime. The use of phytase or HAP corn did not create any significant differences in extractability of P with CaCl_2 by increasing application rate.

Three soils were used in the incubation study that are considered to be productive agricultural soils in the Southeastern United States. Water-extractable P levels from each of these three soils for each treatment averaged across application rate and incubation time are compared in Fig. 2.5. Differences in water-solubility of P between soils can be attributed to the levels of residual soil P existing in each soil. The Mahan soil contained the least amount of residual soil P, and therefore the lowest concentration of CaCl_2 -extractable P. Addition of phytase to the baseline NPA diet does not create a significant difference among all three soils. Significant increases in P solubility do exist in all three soils comparing the alternative NPA+Phytase treatment to the conventional NPA+P treatment on P-basis. The NPA+Phytase treatment contains 24% more water-soluble P than the NPA+P treatment on P-basis in the Mahan soil, and the Cecil soil contains 28% more water-extractable P. However, conversion to the alternative NPA+Phytase treatment contains only 18% more water-soluble P in the Groseclose soil, which contains the highest level of native soil P. The significant differences observed between these two treatments are once again due to the addition of inorganic P to the NPA+P treatment, causing suppression of extractable P. This difference is not due to the addition of phytase, since very similar levels result between the NPA+Phytase and baseline NPA treatment. The use of HAP corn in place of NPA corn did not produce a statistical difference in any of the three soils.

Fig. 2.5. Effect of soil type on water-solubility of P averaged across all application rates (25 and 50 g/kg) and incubation periods (6 and 12 months)



*means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05.

Statistical analyses were conducted separately on each of the application rates.

Water-soluble P levels for all twelve combinations of soil type, application rate, and incubation time (6 months and one year) are presented in Table 2.7. Notice in all twelve combinations the alternative NPA+Phytase treatment contained more water-soluble P levels than did the conventional NPA+P treatment on P-basis. Not all combinations contained significantly different concentrations of water-extractable P, between these two treatments.

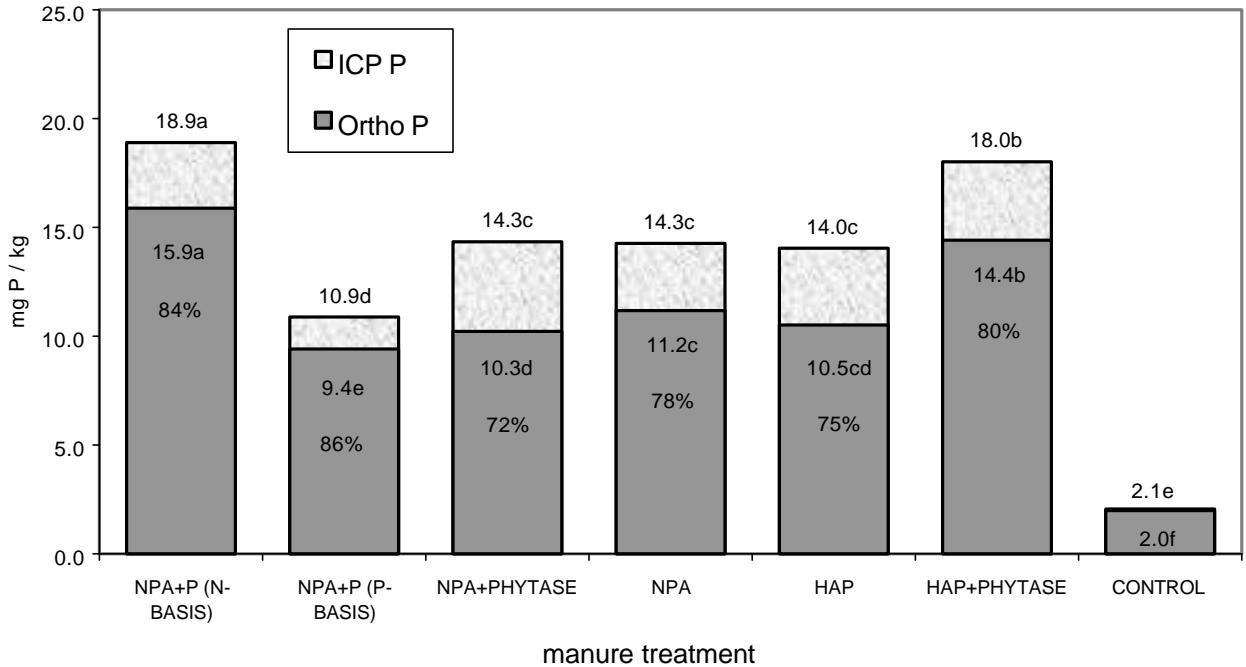
All twelve combinations were also analyzed colorimetrically for ortho-P after extraction with 0.01 M CaCl₂. The ortho-P data for each treatment averaged across all soil types, incubation periods, and application regimes are presented in Fig. 2.6. This data is shown in comparison with the water-soluble P concentrations analyzed by ICP. This ortho-P data represents the dissolved inorganic P fraction, and comprises 79% of the P fraction analyzed by ICP, averaged across all treatments. It is important to note that the dissolved inorganic P fraction is the most available form. The trends between treatments analyzed for ortho and ICP P are relatively similar. The major difference observed is that the addition of phytase to the baseline NPA treatment produces a significant 8% decrease in the ortho-P fraction, whereas phytase addition to the NPA treatment analyzed for ICP P results in the same P concentrations.

Table 2.7. Water-soluble P concentrations extracted from manure amended soils for all three Virginia soils, application rates (25 and 50 g/kg) and incubation periods (6 and 12 months)

| Low application | | | | High application | | | |
|-------------------------|-------------------------------|-------------------------|-------------------------------|-------------------------|-------------------------------|-------------------------|-------------------------------|
| 6 months incubation | | One year incubation | | 6 months incubation | | One year incubation | |
| <u>Mahan soil</u> | | | | | | | |
| <u>Manure Treatment</u> | mg P kg ⁻¹ soil | <u>Manure Treatment</u> | mg P kg ⁻¹ soil | <u>Manure Treatment</u> | mg P kg ⁻¹ soil | <u>Manure Treatment</u> | mg P kg ⁻¹ soil |
| NPA+P (N-BASIS) | 6.1a* | NPA+P (N-BASIS) | 9.7a | NPA+P (N-BASIS) | 15.6ab | NPA+P (N-BASIS) | 16.9ab |
| NPA+P (P-BASIS) | 3.6c | NPA+P (P-BASIS) | 3.7c | NPA+P (P-BASIS) | 11.9b | NPA+P (P-BASIS) | 12.3c |
| NPA+PHYTASE | 5.9a | NPA+PHYTASE | 6.4b | NPA+PHYTASE | 14.0ab | NPA+PHYTASE | 16.7ab |
| NPA | 4.1bc | NPA | 5.6bc | NPA | 12.1b | NPA | 16.5ab |
| HAP | 5.5ab | HAP | 6.0b | HAP | 12.2b | HAP | 14.1bc |
| HAP+PHYTASE | 6.1a | HAP+PHYTASE | 6.9b | HAP+PHYTASE | 17.5a | HAP+PHYTASE | 17.3a |
| CONTROL | 0.5d | CONTROL | 0.3d | CONTROL | 0.7c | CONTROL | 0.3d |
| <u>Cecil soil</u> | | | | | | | |
| <u>Manure Treatment</u> | mg P kg ⁻¹ soil | <u>Manure Treatment</u> | mg P kg ⁻¹ soil | <u>Manure Treatment</u> | mg P kg ⁻¹ soil | <u>Manure Treatment</u> | mg P kg ⁻¹ soil |
| NPA+P (N-BASIS) | 9.9a | NPA+P (N-BASIS) | 21.6a | NPA+P (N-BASIS) | 16.9b | NPA+P (N-BASIS) | 36.4b |
| NPA+P (P-BASIS) | 5.1c | NPA+P (P-BASIS) | 8.0d | NPA+P (P-BASIS) | 11.1d | NPA+P (P-BASIS) | 23.7e |
| NPA+PHYTASE | 7.4bc | NPA+PHYTASE | 12.8c | NPA+PHYTASE | 14.7c | NPA+PHYTASE | 32.1c |
| NPA | 7.8ab | NPA | 12.3c | NPA | 16.1bc | NPA | 29.8d |
| HAP | 7.0bc | HAP | 12.7c | HAP | 16.3bc | HAP | 31.6c |
| HAP+PHYTASE | 7.6b | HAP+PHYTASE | 16.9b | HAP+PHYTASE | 21.7a | HAP+PHYTASE | 38.8a |
| CONTROL | 1.2d | CONTROL | 1.5e | CONTROL | 1.2e | CONTROL | 1.5f |
| <u>Groseclose soil</u> | | | | | | | |
| <u>Manure Treatment</u> | mg P kg ⁻¹ soil | <u>Manure Treatment</u> | mg P kg ⁻¹ soil | <u>Manure Treatment</u> | mg P kg ⁻¹ soil | <u>Manure Treatment</u> | mg P kg ⁻¹ soil |
| NPA+P (N-BASIS) | 13.3a | NPA+P (N-BASIS) | 10.4a | NPA+P (N-BASIS) | 16.7a | NPA+P (N-BASIS) | 34.0a |
| NPA+P (P-BASIS) | 6.2c | NPA+P (P-BASIS) | 7.0c | NPA+P (P-BASIS) | 8.5b | NPA+P (P-BASIS) | 15.3c |
| NPA+PHYTASE | 6.9bc | NPA+PHYTASE | 7.1c | NPA+PHYTASE | 10.0b | NPA+PHYTASE | 23.6b |
| NPA | 7.2bc | NPA | 8.4bc | NPA | 9.9b | NPA | 25.8b |
| HAP | 7.4bc | HAP | 7.8bc | HAP | 8.5b | HAP | 24.8b |
| HAP+PHYTASE | 8.8b | HAP+PHYTASE | 8.8abc | HAP+PHYTASE | 13.7a | HAP+PHYTASE | 35.3a |
| CONTROL | 3.6d | CONTROL | 3.2d | CONTROL | 3.6c | CONTROL | 3.2d |

*Means with same letter not statistically different by Duncan's Multiple Range Test at alpha=0.05
Statistical analyses were conducted separately on each of the twelve combinations

Fig. 2.6. Ortho-P and ICP comparison of water-soluble P from manure amended soils averaged across three Virginia soils, application rates, incubation times



*means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

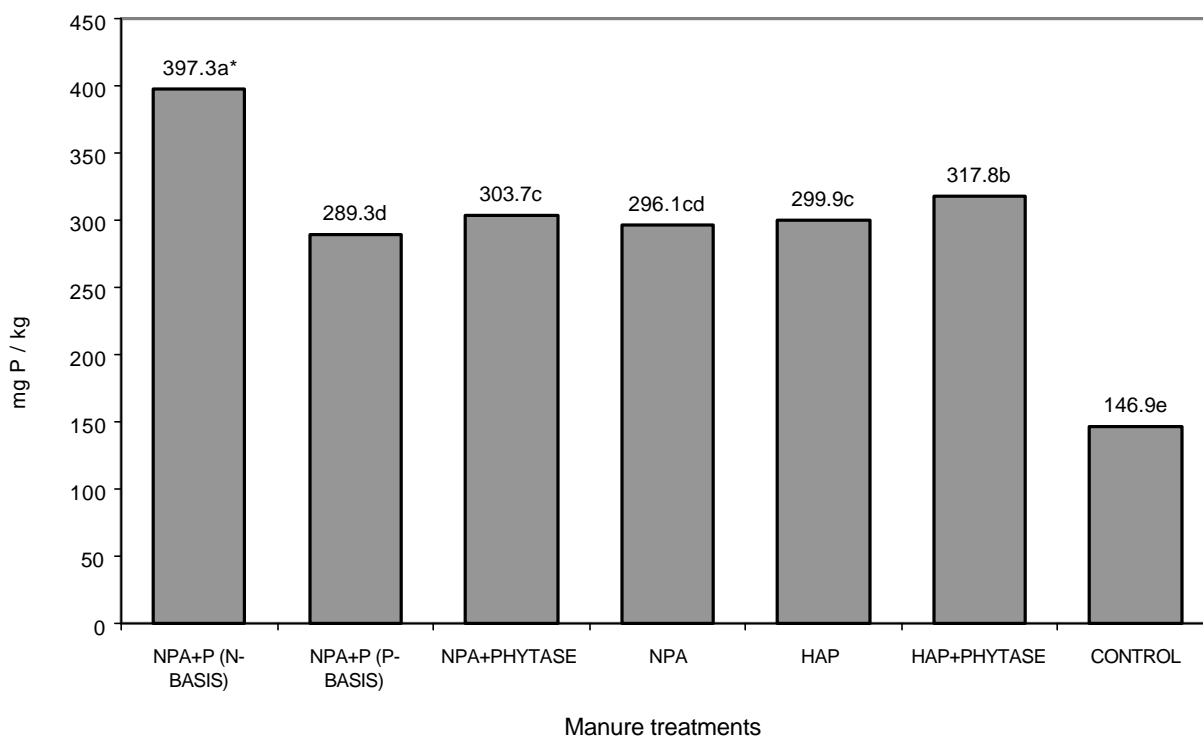
Statistical analyses were conducted separately on each of the P fractions.

2.3.3. Mehlich III-Solubility of P

The acidic Mehlich III extractant was used to determine potential plant availability of P in the manure-amended soils. An overall comparison of Mehlich III-soluble P concentrations for each of the 7 treatments averaged across 3 soil types, 2 manure application rates, and 4 incubation time periods is given in Fig. 2.7. A total of 575 extractions were performed from the seven treatments. Lawrence (2000) extracted soils incubated for the two shortest time periods. Addition of phytase to the baseline NPA treatment produced no difference in plant-available P concentrations.

The alternative NPA+Phytase treatment is compared to the conventional NPA+P treatments in Fig. 2.8. Comparing the alternative phytase treatment to the conventional treatment on an equal N-basis (and equal manure basis) produced a significant 24%

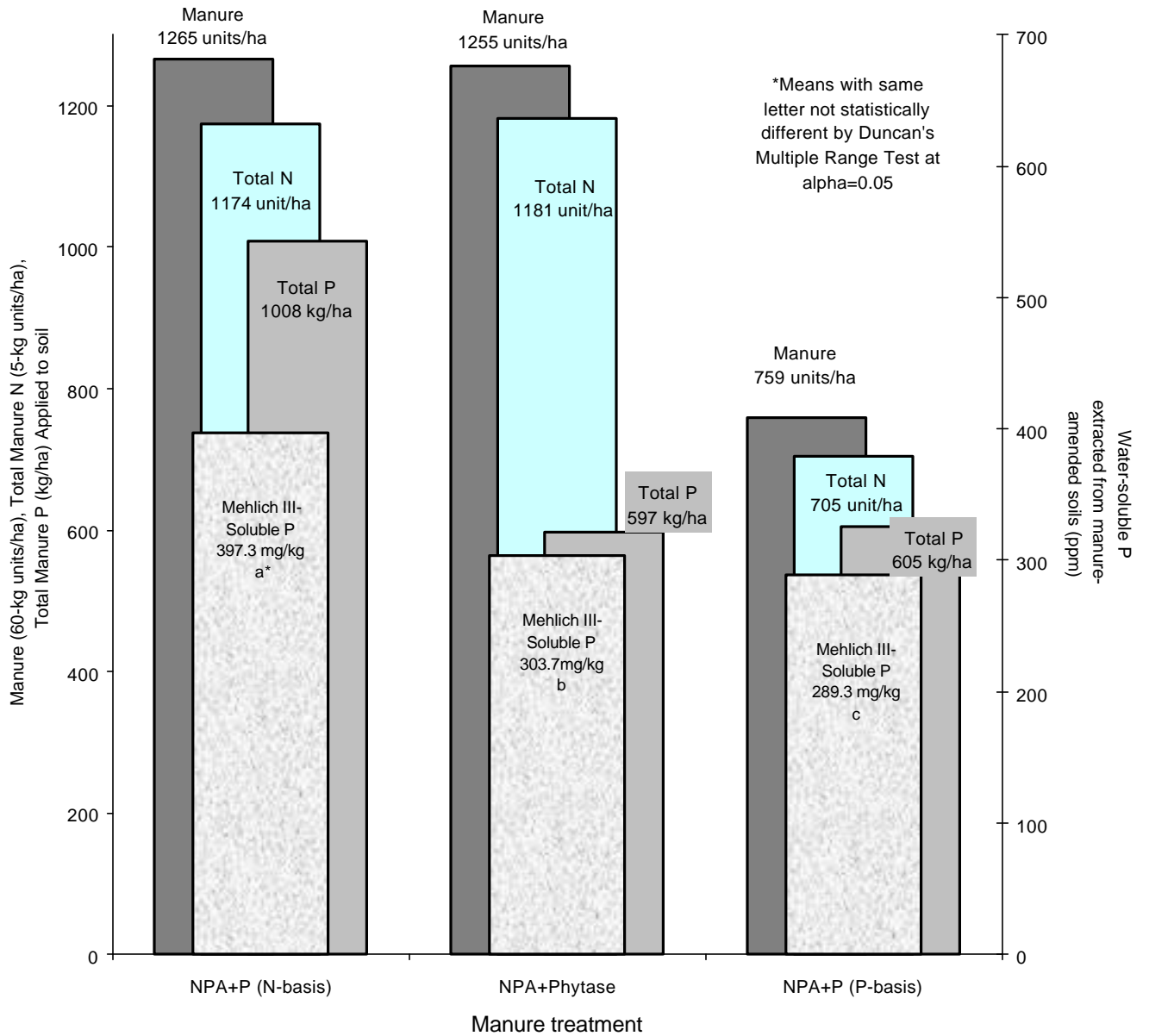
Fig. 2.7. Mehlich III-soluble P extracted from manure amended soils averaged across all three Virginia soils, incubation times (6 and 12 months), and manure application rates (25 and 50 g/kg)



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

reduction in plant-available P concentration. A statistically significant 5% increase in plant available P is observed when the phytase treatment is compared to the NPA+P treatment on an equal P-basis. This increase is less notable than the 24% increase in water-soluble P levels previously discussed. The Ca-phosphate effect which prevented P solubilization by CaCl_2 in the NPA+P treatment was decreased considerably, most likely due to the acidic extractant releasing more P into solution. The decreased effect of Ca is also evident when NPA+P is compared with the baseline NPA treatment. The addition of inorganic P to the baseline diet did not result in any statistical differences in plant-availability of P. Therefore, the Ca added as supplemental Ca-phosphate did not suppress solubilization of P by the acidic Mehlich III extractant. The substitution of HAP for NPA corn did not produce a significant increase in Mehlich III-solubility of P. The addition of phytase to the HAP treatment does produce a significant 16% increase in P

Fig. 2.8. Comparison of Mehlich III-soluble P concentrations from soils amended with NPA+P and NPA+Phytase manure treatments averaged across all three Virginia soils, incubation periods (6 and 12 months) and application rates (25 and 50 g/kg)

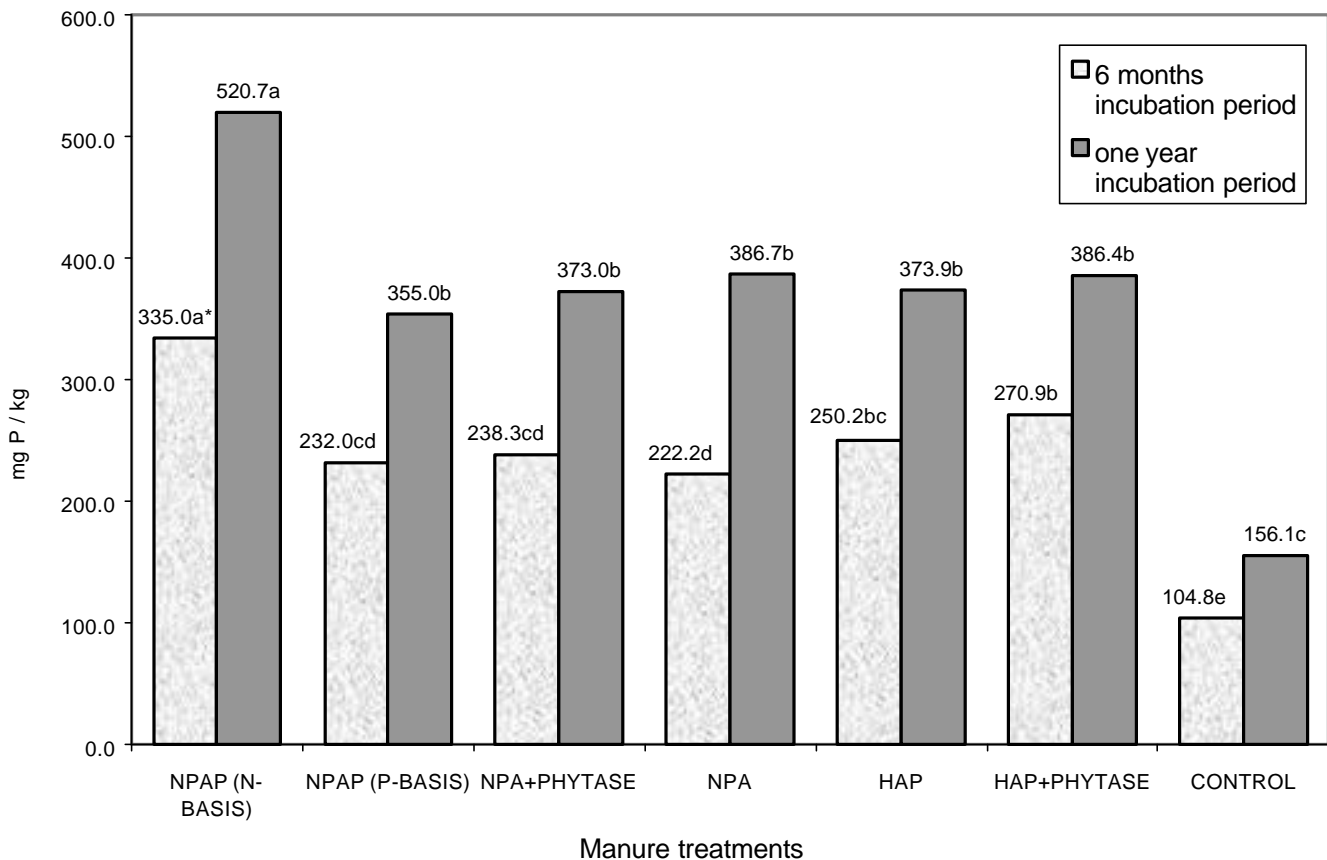


solubility compared to the baseline NPA diet, signifying when both amendments are used together, the effects of increased plant-availability of P are additive.

2.3.4. Effects of Incubation Time, Manure Application Rate, and Soil Type

A comparison of treatments extracted with Mehlich III for 6 months and one year incubation periods averaged across soil type and application rate is presented in Fig. 2.9. Extractable P concentrations increased by at least 30% for every manure-amended soil by increasing incubation time.

Fig. 2.9. Effects of incubation time on Mehlich III-soluble P concentrations extracted from manure amended soils averaged across all three Virginia soils and manure application rates (25 and 50 g/kg)

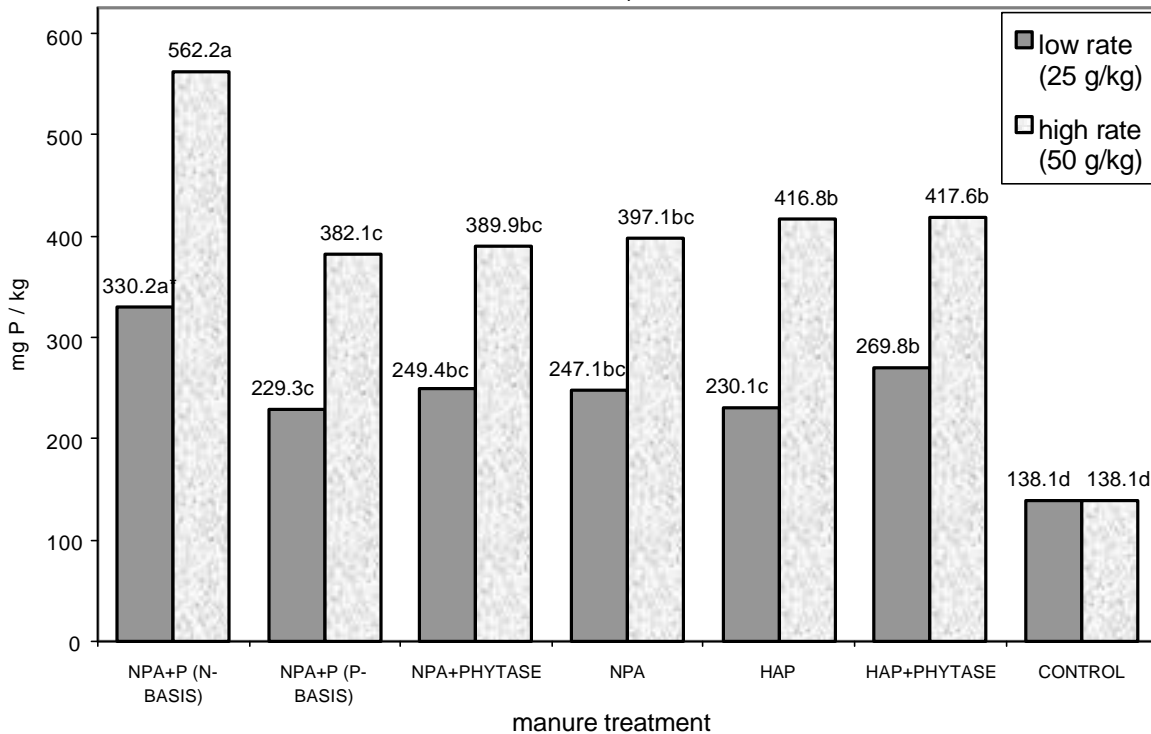


*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
 Statistical analyses were conducted separately on each of the incubation periods.

Increasing incubation time also caused changes in plant availability of P between treatments. A 4% decrease in plant available P was observed upon phytase addition to the NPA treatment after one year incubation. Substitution of HAP corn for NPA corn increased P solubility by a significant 11% when soils were extracted at 6 months. However, a 3% decline in plant available P extracted at one year was noted when HAP was substituted. These observations indeed show that changes in plant available P extracted between the alternative treatments (phytase and HAP) do occur upon increasing incubation time.

Plant-available P concentrations for treatments receiving low and high rates of manure to soils averaged across soil type and incubation time (6 months and one year) are given in Fig. 2.10. An average increase of 39% in extractable P is observed by increasing application rate of manure to soils. Note this percentage is considerably less than the 57% increase in water-soluble P observed by doubling the manure application rate to soils. Addition of phytase to the baseline NPA diet in both the low and high application regimes did not create significant differences between these two treatments, indicating the use of phytase did not cause changes in plant-availability of P. Addition of phytase in the low application regime did slightly increase solubility of P (<1%), but decreased P solubility by 2% in the high application regime. Note that the alternative phytase amended treatment contained 8% more Mehlich III-soluble P than did the conventional NPA+P (P-basis) treatment with the low application rate, but only 2% more soluble P with the high application rate. Due to the similar P levels extracted from the NPA and NPA+Phytase treatments, these differences can be attributed to the P suppression effect of the calcium phosphate supplemented in the NPA+P treatment. Substitution of HAP corn for NPA corn did not produce statistical differences in plant available P concentrations, though the HAP treatment contained 7% less plant available P with the low application rate, and 5% more extractable P with the high application rate. The use of phytase and HAP corn increased P solubility when manure was applied using the low application rate, but the high rate produced relatively no change in P solubility. No statistical differences were observed by addition of phytase to the HAP treatment for either application rate.

Fig. 2.10. Effect of manure application rate on Mehlich III-solubility of P extracted from manure amended soils averaged across three Virginia soils and incubation periods (6 and 12 months)

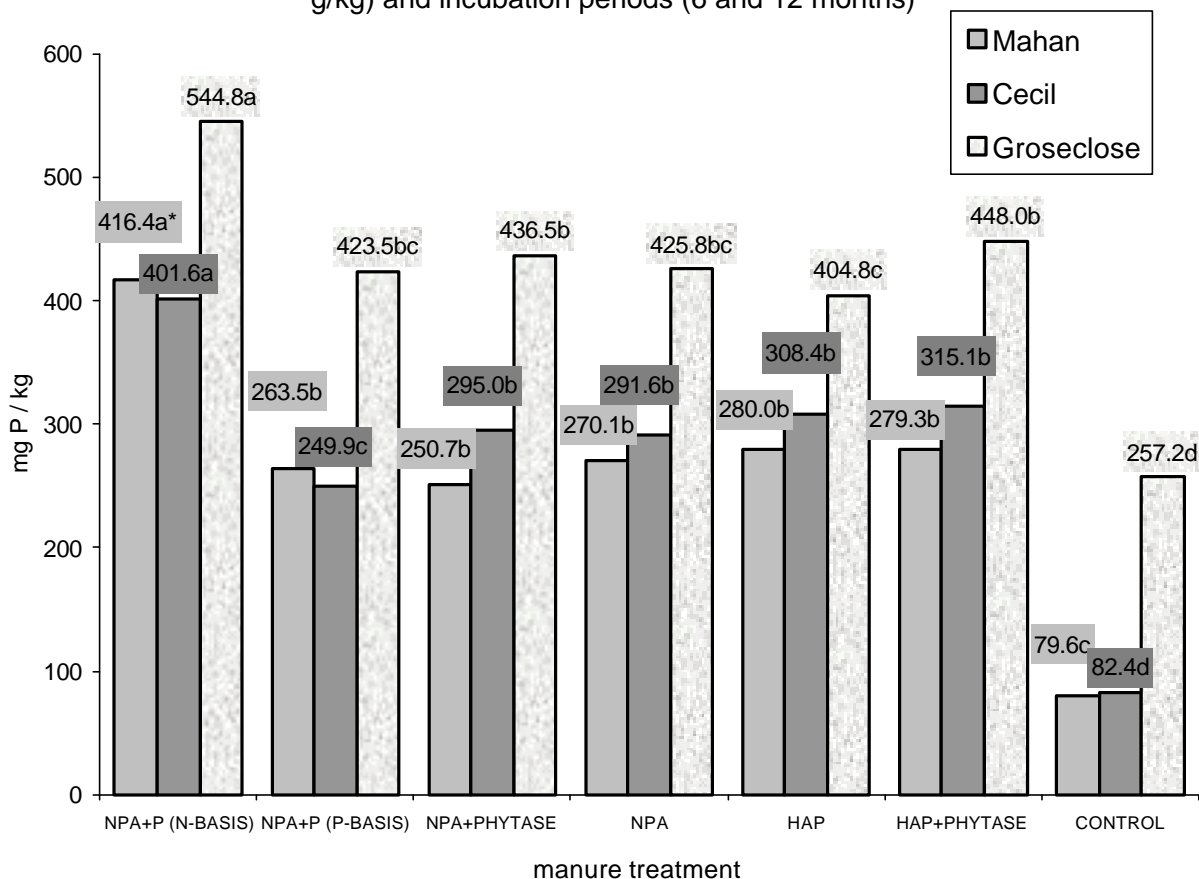


*means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

Statistical analyses were conducted separately on each of the application rates.

Plant available P extracted from each of the three soils for each treatment averaged across manure application rate and incubation time are illustrated in Fig. 2.11. Differences in extractable P do exist between the soils and can mainly be attributed to the existing concentrations of plant available P in the soils. The Mahan soil contains the lowest concentration of residual soil P, and for all treatments except for the NPA+P treatments, contains the lowest amounts of extractable P. The Mahan soil contains 3.5% more Mehlich III-soluble P compared to the Cecil soil in the NPA+P (N-basis), and 5% more in the NPA+P (P-basis). The Groseclose soil contains the highest concentrations of residual soil P, and also extractable P. Phytase addition to the baseline NPA treatment did not cause any statistical changes to occur in the three soils, and neither does the

Fig. 2.11. Effect of soil type on Mehlich III-solubility of P extracted from manure amended soils averaged across all application rates (25 and 50 g/kg) and incubation periods (6 and 12 months)



*means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

Statistical analyses were conducted separately on each of the application rates.

substitution of HAP corn for NPA corn. Changes do occur between soil type when comparing the alternative NPA+Phytase treatment to the conventional NPA+P (P-basis). For example, no statistical differences exist between these treatments in the Groseclose soil, with only a 3% increase in extractable P levels comparing the NPA+Phytase treatment (436.5 mg P kg⁻¹soil) to the NPA+P treatment on a P-basis (423.5 mg P kg⁻¹ soil). The Cecil soil is quite different, with an increase of 15%

comparing the alternative (295 mg P kg⁻¹ soil) to the conventional treatment (249.9 mg P kg⁻¹ soil). Even more interesting is the fact that the NPA+Phytase treatment contained 5% less Mehlich III-soluble P than the NPA+P treatment on a P-basis in the Mahan soil (250.7 mg P kg⁻¹ soil for NPA+Phytase vs. 263.5 mg P kg⁻¹ soil for NPA+P on a P-basis).

Mehlich III-soluble P concentrations for treatments from each of the twelve combinations of soil type, application regime, and incubation period (6 months and one year) are given in Table 2.8. The most interesting observation is the increase in P extractability in the control treatment with the increase in incubation time, which is consistent over all three soil types. Solubility of P increased 18% in the control by increasing incubation time in the Mahan, while the Groseclose and Cecil soils showed a 39 and 41% increase in Mehlich III-extractable P, respectively. This increase may be due to a mineralization of residual soil P over the longer incubation period. Many changes between treatments have taken place when all twelve combinations are compared. However, similar trends are observed with the addition of phytase to the baseline NPA diet. In all cases except the Groseclose high application rate, incubated for six months, the addition of phytase did not create a significant difference between these two treatments. Conversion of NPA to HAP corn does not produce significant differences in any of the twelve combinations of soil type, incubation period, and application regime.

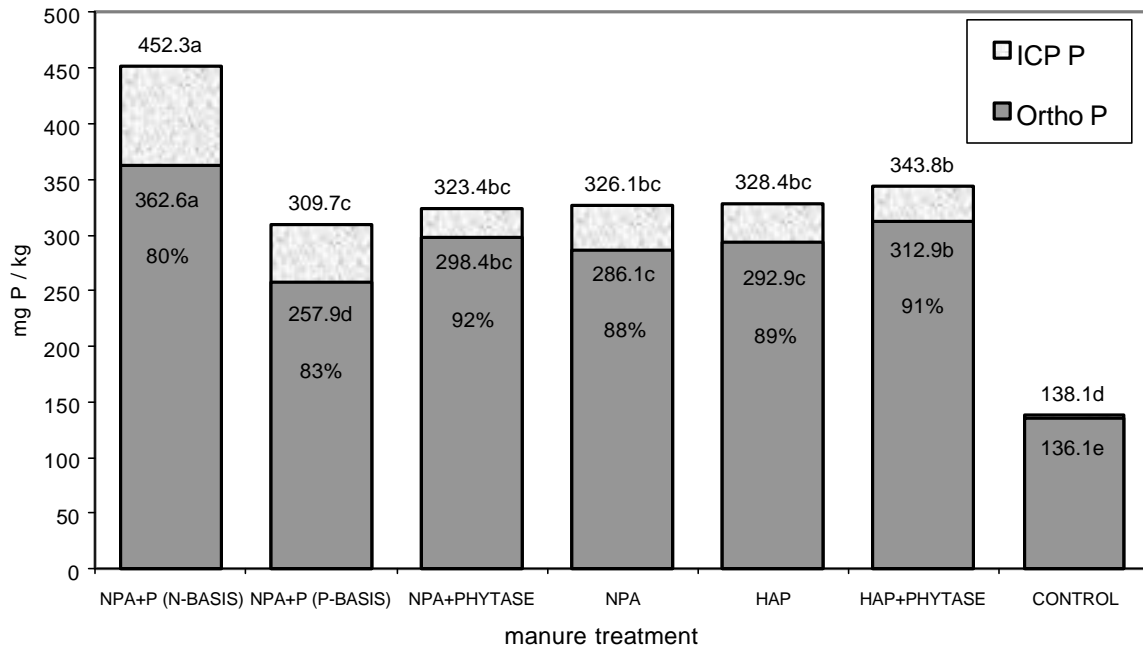
Dissolved inorganic P fraction analyzed colorimetrically is compared to total dissolved P analyzed by ICP extracted with Mehlich III, averaged across all soil types, incubation periods, and application rates in Fig. 2.12. The ortho-P fraction comprises 87% of the total dissolved P averaged across all treatments. Subtraction of dissolved inorganic P from total dissolved P yields the dissolved organic P fraction. Interesting changes between treatments have taken place comparing the ortho-P fraction to the P fraction analyzed by ICP. Addition of inorganic P to the baseline NPA treatment on a P-basis, reduces ortho-P by a significant 10%, while a statistically insignificant 5% reduction in extractable P is observed with the addition of inorganic P to the NPA treatment analyzed by ICP. The NPA+Phytase treatment produces a significant 14% increase in ortho-P compared to the NPA+P (P-basis) treatment. These treatments are not significantly different in P analyzed by ICP.

Table 2.8. Mehlich III-soluble P concentrations extracted from manure amended soils for all three Virginia soils, application rates (25 and 50 g/kg) after 6 months and one year incubation periods.

| Low application | | | | High application | | | |
|----------------------------|--------------------------------------|----------------------------|--------------------------------------|----------------------------|--------------------------------------|----------------------------|--------------------------------------|
| <u>6 months incubation</u> | | <u>One year incubation</u> | | <u>6 months incubation</u> | | <u>One year incubation</u> | |
| <u>Mahan soil</u> | | | | | | | |
| <u>Manure Treatment</u> | <u>mg P kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg P kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg P kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg P kg⁻¹ soil</u> |
| NPA+P (N-BASIS) | 136.8ab* | NPA+P (N-BASIS) | 334.1a | NPA+P (N-BASIS) | 458.1a | NPA+P (N-BASIS) | 597.0a |
| NPA+P (P-BASIS) | 115.3ab | NPA+P (P-BASIS) | 204.1b | NPA+P (P-BASIS) | 339.5b | NPA+P (P-BASIS) | 321.0b |
| NPA+PHYTASE | 101.3b | NPA+PHYTASE | 247.9b | NPA+PHYTASE | 282.4b | NPA+PHYTASE | 296.6b |
| NPA | 84.8b | NPA | 232.0b | NPA | 291.5b | NPA | 379.5b |
| HAP | 97.3b | HAP | 226.0b | HAP | 368.2ab | HAP | 337.3b |
| HAP+PHYTASE | 203.3a | HAP+PHYTASE | 232.2b | HAP+PHYTASE | 368.8ab | HAP+PHYTASE | 327.1b |
| CONTROL | 70.1b | CONTROL | 85.5c | CONTROL | 70.1c | CONTROL | 85.5c |
| <u>Groseclose soil</u> | | | | | | | |
| <u>Manure Treatment</u> | <u>mg P kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg P kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg P kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg P kg⁻¹ soil</u> |
| NPA+P (N-BASIS) | 324.3a | NPA+P (N-BASIS) | 508.1a | NPA+P (N-BASIS) | 425.7a | NPA+P (N-BASIS) | 751.3a |
| NPA+P (P-BASIS) | 179.3c | NPA+P (P-BASIS) | 409.3abc | NPA+P (P-BASIS) | 365.1abc | NPA+P (P-BASIS) | 589.0bc |
| NPA+PHYTASE | 261.6ab | NPA+PHYTASE | 399.0abc | NPA+PHYTASE | 378.6ab | NPA+PHYTASE | 590.5bc |
| NPA | 210.2bc | NPA | 433.8ab | NPA | 310.5c | NPA | 583.3c |
| HAP | 185.2c | HAP | 343.1bc | HAP | 333.5bc | HAP | 612.0b |
| HAP+PHYTASE | 225.5bc | HAP+PHYTASE | 437.1ab | HAP+PHYTASE | 393.6ab | HAP+PHYTASE | 597.3bc |
| CONTROL | 176.3c | CONTROL | 287.6c | CONTROL | 176.3d | CONTROL | 287.6d |
| <u>Cecil soil</u> | | | | | | | |
| <u>Manure Treatment</u> | <u>mg P kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg P kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg P kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg P kg⁻¹ soil</u> |
| NPA+P (N-BASIS) | 148.1a | NPA+P (N-BASIS) | 339.3a | NPA+P (N-BASIS) | 394.1a | NPA+P (N-BASIS) | 594.5a |
| NPA+P (P-BASIS) | 100.7b | NPA+P (P-BASIS) | 220.8b | NPA+P (P-BASIS) | 184.8c | NPA+P (P-BASIS) | 386.1c |
| NPA+PHYTASE | 104.6ab | NPA+PHYTASE | 241.8b | NPA+PHYTASE | 257.0b | NPA+PHYTASE | 462.3b |
| NPA | 99.9b | NPA | 248.7b | NPA | 266.7b | NPA | 442.7bc |
| HAP | 103.9ab | HAP | 273.4ab | HAP | 295.9b | HAP | 451.8bc |
| HAP+PHYTASE | 157.7ab | HAP+PHYTASE | 284.1ab | HAP+PHYTASE | 315.5b | HAP+PHYTASE | 440.5bc |
| CONTROL | 56.5c | CONTROL | 95.3c | CONTROL | 56.5d | CONTROL | 95.3d |

*Means with same letter not statistically different by Duncan's Multiple Range Test at alpha=0.05
Statistical analyses were conducted separately on each of the twelve combinations

Fig. 2.12. Ortho-P and ICP comparison of Mehlich III-soluble P extracted from manure amended soils averaged across all three Virginia soils, manure application rates, incubation periods

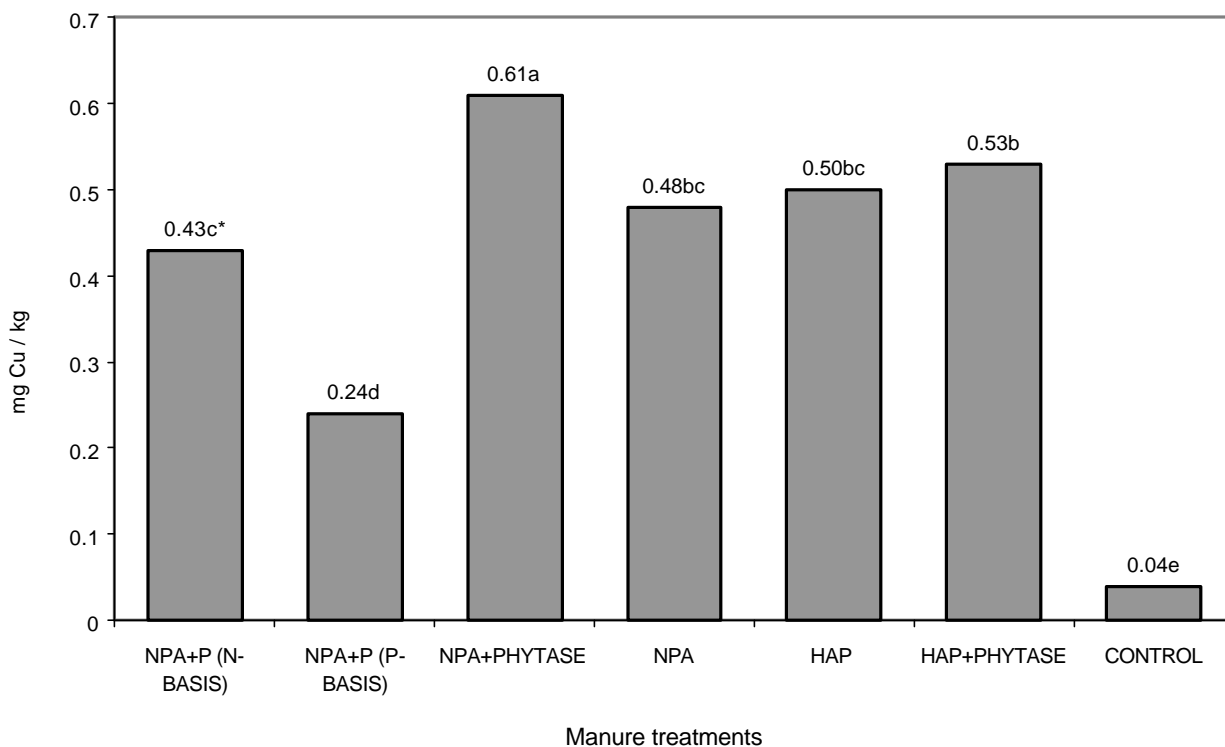


*means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
 Statistical analyses were conducted separately on each of the P fractions.

2.3.5. Water Solubility of Cu

Manure-amended soils were also analyzed for water-soluble Cu concentrations by extraction with 0.01 M CaCl₂. A total of 572 extractions were performed and are given in Fig. 2.13 for each treatment averaged across 3 soil types, 4 incubation time periods, and 2 application rates. Lawrence (2000) performed extractions on the two shortest incubation periods. The control treatments contained soil with no addition of manure.

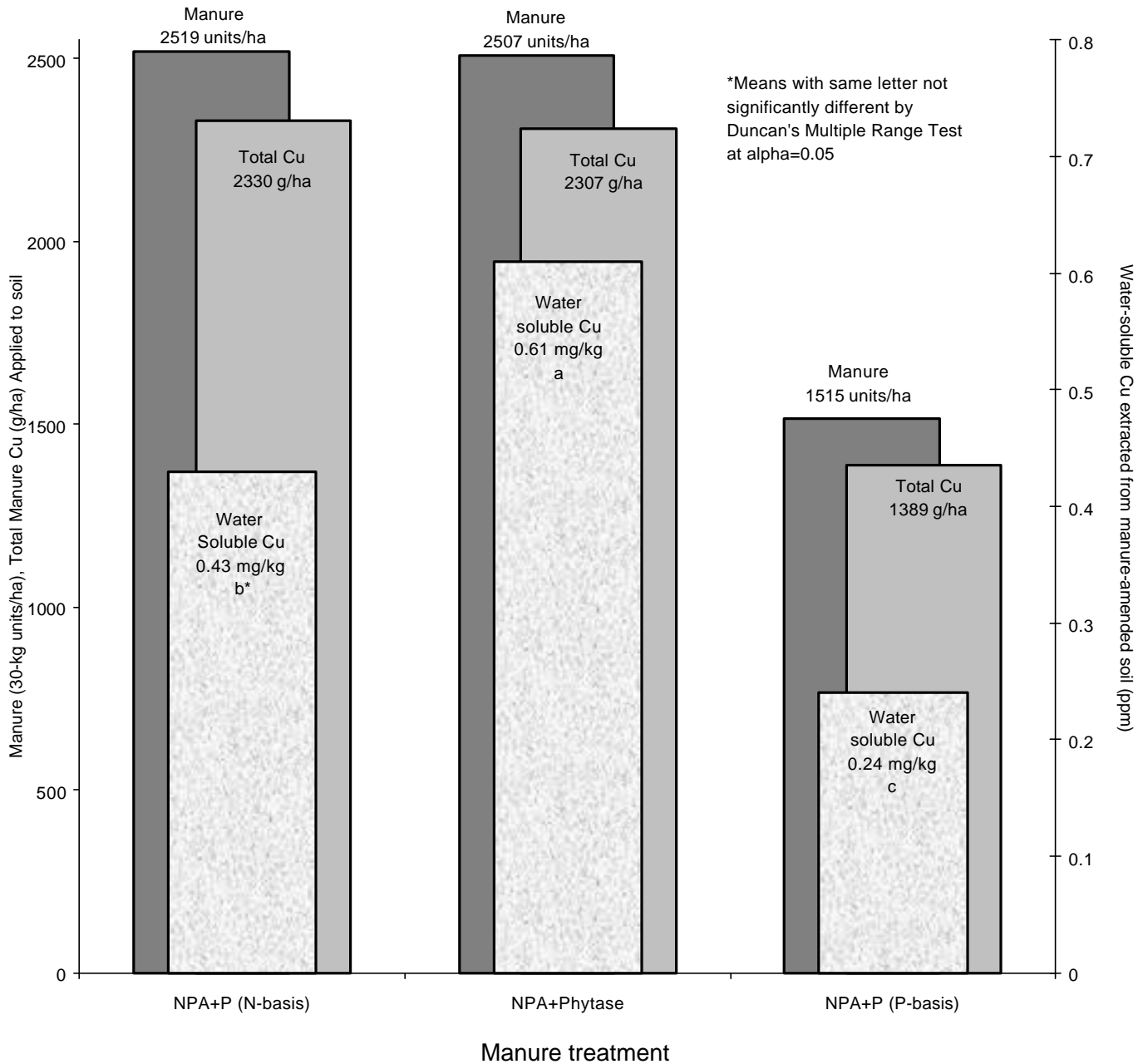
Fig. 2.13. Water-soluble Cu concentrations averaged across all three Virginia soils, incubation times (4 and 28 days, 6 and 2 months), and manure application rates (25 and 50 g/kg)



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

It is important to note that the NPA+P (N-basis) manure alone contains similar concentrations of Cu as the NPA, NPA+Phytase, HAP, and HAP+Phytase treatments. The NPA+P (P-basis) treatment should be observed when manure applications based on P are of interest. The addition of phytase to the baseline NPA treatment did produce a significant increase in water-soluble Cu concentrations as seen in Fig. 2.13. A 21% increase in Cu solubility was observed from the addition of phytase (0.61 vs 0.48 mg Cu kg⁻¹ soil for NPA+Phytase and NPA treatments, respectively), signifying that phytase did increase Cu solubility.

Figure 2.14. Comparison of Water-soluble Cu concentrations from soils amended with NPA+P and NPA+Phytase manure treatments averaged across incubation periods (4 and 28 days, 6 and 12 months), application rates (25 and 50 g/kg) and three Virginia soils



The most important issue to consider is comparing differences in water-solubility of Cu from the alternative NPA+Phytase treatment to the conventional NPA+P treatments. Data from Fig. 2.13 is presented in Fig. 2.14 for comparison of these treatments. Also given in the figure is the rate of manure and total Cu applied to soils. The same amount of total Cu is applied from both the NPA+P (N-basis) and

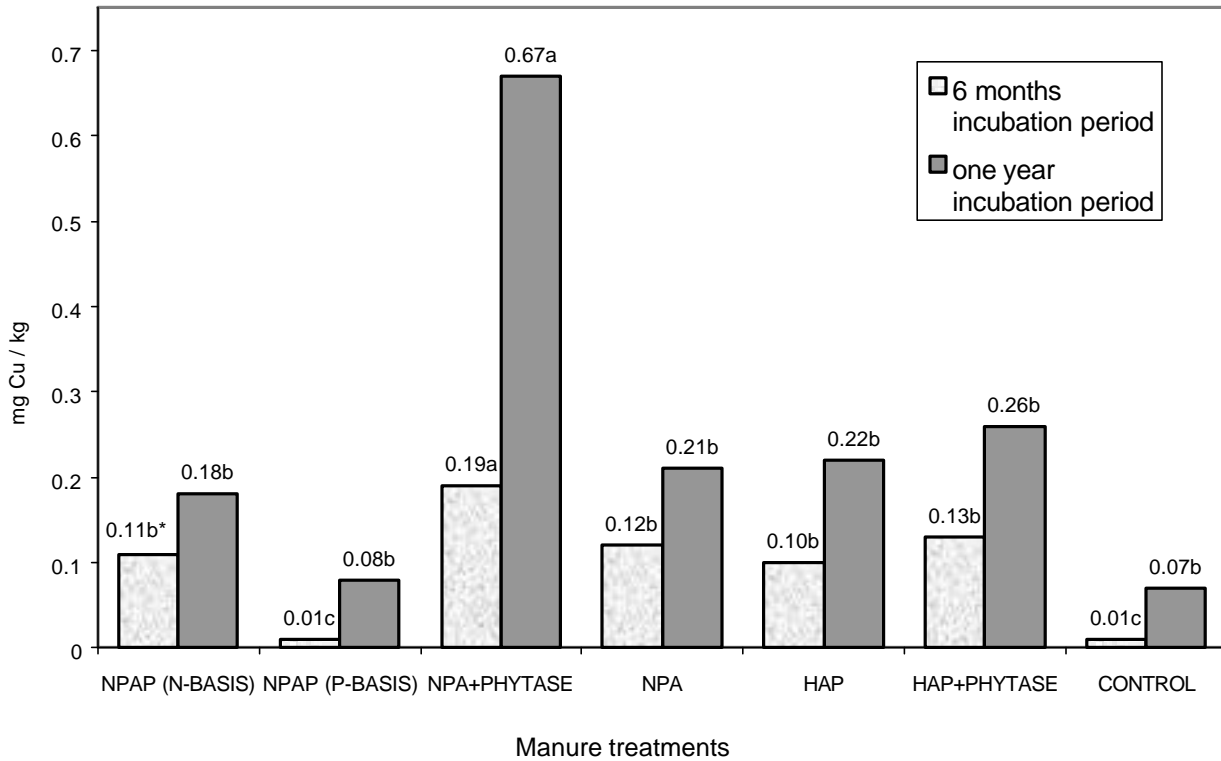
NPA+Phytase treatments, although the phytase treatment increased water-solubility of Cu. The alternative phytase treatment increased Cu solubility by a significant 30% compared to the NPA+P (N-basis) treatment (0.43 vs. 0.61 mg Cu kg⁻¹ soil for NPA+P on a N-basis and NPA+Phytase treatments, respectively). When the alternative phytase treatment is compared to the conventional NPA+P (P-basis) treatment, water solubility increased by 61%. Both the effects of increased Cu solubility by phytase and decreased Cu solubility by Ca-phosphate caused the significant difference in water-solubility of Cu between the alternative and conventional treatments. Phytase addition to the baseline NPA treatment increased Cu solubility significantly, while the addition of Ca-phosphate to the baseline NPA treatment significantly decreased Cu solubility. Substitution of HAP corn for NPA corn did not produce a significant difference in plant availability of Cu, since HAP increased Cu solubility by only 4% (0.48 vs. 0.50 mg Cu kg⁻¹ soil, for NPA and HPA treatments, respectively).

2.3.6. Effects of Incubation Time, Manure Application Rate, and Soil Type

The effects of increasing incubation time on water-solubility of Cu for each treatment averaged across soil type and manure application rate are compared in Fig. 2.15. Increasing incubation time increased extraction of water-soluble Cu over all treatments. The addition of phytase to the baseline NPA treatment produced statistical increases of 37 and 69% in Cu solubility at both 6 months and one year, respectively. Extending the incubation time thus caused increased solubility of Cu from phytase addition. Substitution of HAP for NPA corn did not produce statistical differences in either incubation time period.

The effect of increasing manure application rate to soils on Cu solubility is shown in Fig. 2.16 for each treatment averaged across all soil types and incubation periods (6 months and one year). Doubling the manure application rate increased water-solubility

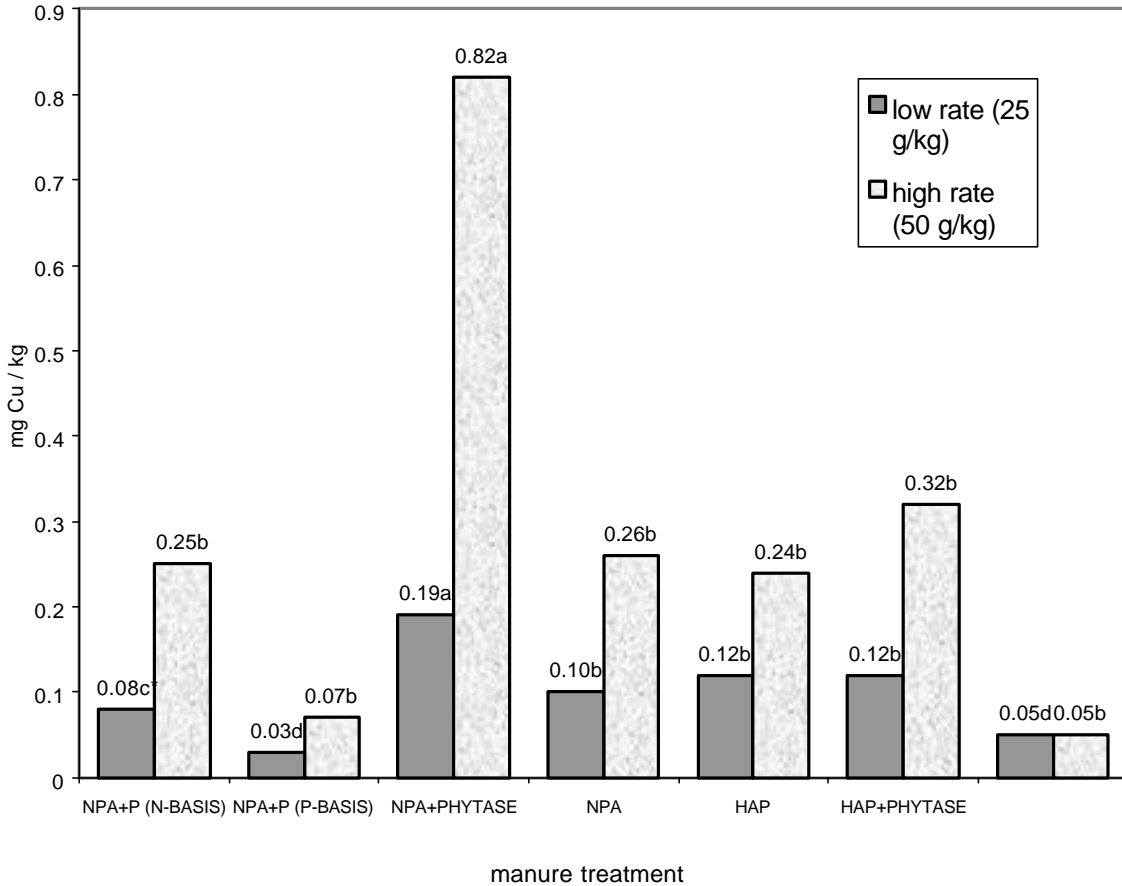
Fig. 2.15. Effects of incubation time on water-soluble Cu concentrations extracted from manure amended soils averaged across all three Virginia soils and application rates (25 and 50 g/kg)



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
 Statistical analysis were conducted separately for each incubation period.

of Cu by an average of 63% over all treatments. As seen with increasing incubation time, statistical increases in Cu solubility are observed with the addition of phytase to the baseline NPA treatment, while no significant differences exist when HAP replaces NPA corn. Note the control treatment contains higher extractable Cu levels than does the NPA+P (P-basis) treatment when soils received the low rate of manure application (0.05 vs. 0.03 mg Cu kg⁻¹ soil for control and NPA+P on P-basis treatments, respectively). This is quite possible due to the fact that P added to the NPA treatment could be suppressing the release of Cu into solution.

Fig. 2.16. Effect of manure application rate on water-solubility of Cu extracted from manure amended soils averaged across all three Virginia soils and incubation periods (6 and 12 months)

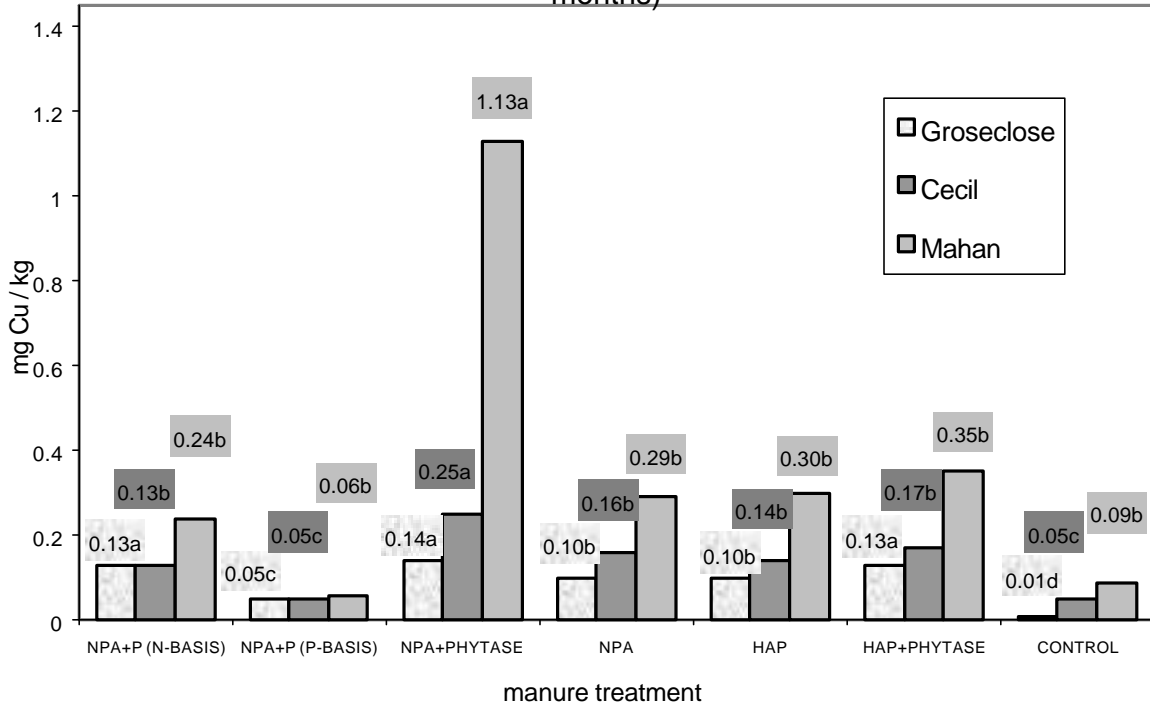


*means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

Statistical analyses were conducted separately for each application rate.

Water-soluble Cu concentrations extracted from each soil is given in Fig. 2.17 for each treatment averaged across incubation time and application rate. The Mahan soil contained the highest concentration of residual soil Cu, and also the highest concentration of water-soluble Cu. Both the Cecil and Groseclose soils contained the same concentrations of residual soil copper. Copper extracted from the Cecil soil contained higher concentrations than the Groseclose soil over all treatments except for the NPA+P treatments, which contained the same concentrations. The greatest increase in extractable

Fig. 2.17. Comparison of three Virginia soils for water-solubility of Cu extracted from manure amended soils averaged across all manure application rates (25 and 50 g/kg) and incubation periods (6 and 12 months)



*means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05. Statistical analyses were conducted separately on each soil type.

Cu between the alternative phytase treatment and conventional NPA+P (N-basis) treatment is observed in the Mahan soil, though all soils contained statistically significant differences in Cu between these treatments. The NPA+Phytase treatment increased Cu solubility by 79% compared to the NPA+P (N-basis) treatment in the Mahan soil, with increases of 7% and 48% observed in the Groseclose and Cecil soils, respectively. These increases can be attributed to the effect of phytase increasing Cu solubility, since Cu concentrations in the NPA+P (N-basis) treatment is very similar to the baseline NPA treatment. Very little differences exist when HAP is substituted for NPA corn in any of the soils. Water-soluble Cu concentrations for treatments from each of the twelve combinations of soil type, incubation period, and application rate are given in Table 2.9.

Table 2.9. Water-soluble Cu concentrations extracted from manure amended soils for all three Virginia soils, manure application rates (25 and 50 g/kg), and 6 months and one year incubation periods.

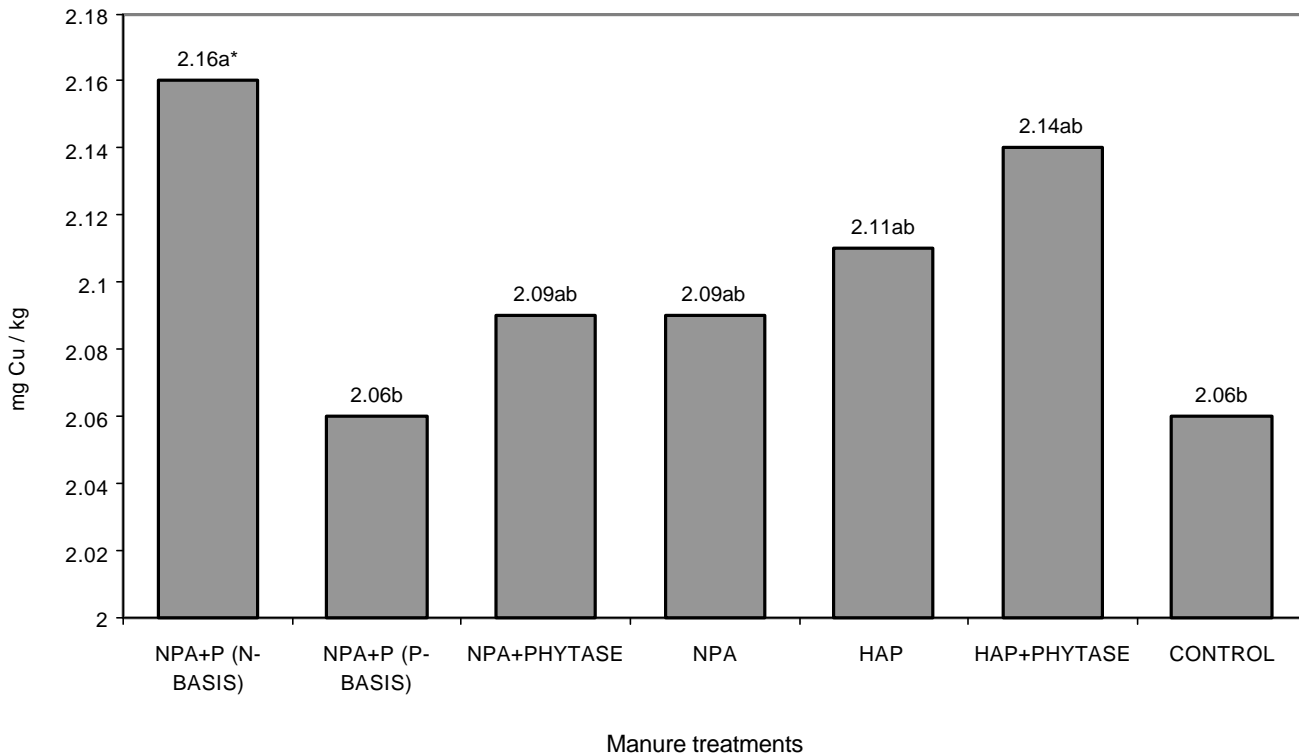
| Low application | | | | High application | | | |
|----------------------------|---|----------------------------|---|----------------------------|---|----------------------------|---|
| <u>6 months incubation</u> | | <u>One year incubation</u> | | <u>6 months incubation</u> | | <u>One year incubation</u> | |
| <u>Mahan soil</u> | | | | | | | |
| <u>Manure Treatment</u> | <u>mg Cu</u> <u>kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu</u> <u>kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu</u> <u>kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu</u> <u>kg⁻¹ soil</u> |
| NPA+P (N-BASIS) | 0.19c* | NPA+P (N-BASIS) | 0.16bc | NPA+P (N-BASIS) | 0.00a | NPA+P (N-BASIS) | 0.47ab |
| NPA+P (P-BASIS) | 0.03d | NPA+P (P-BASIS) | 0.10c | NPA+P (P-BASIS) | 0.00a | NPA+P (P-BASIS) | 0.08b |
| NPA+PHYTASE | 0.44a | NPA+PHYTASE | 0.50a | NPA+PHYTASE | 0.02a | NPA+PHYTASE | 2.66a |
| NPA | 0.31bc | NPA | 0.19bc | NPA | 0.00a | NPA | 0.52ab |
| HAP | 0.34ab | HAP | 0.24b | HAP | 0.00a | HAP | 0.49ab |
| HAP+PHYTASE | 0.38ab | HAP+PHYTASE | 0.18bc | HAP+PHYTASE | 0.00a | HAP+PHYTASE | 0.67ab |
| CONTROL | 0.00d | CONTROL | 0.13bc | CONTROL | 0.00a | CONTROL | 0.13b |
| <u>Cecil soil</u> | | | | | | | |
| <u>Manure Treatment</u> | <u>mg Cu</u> <u>kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu</u> <u>kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu</u> <u>kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu</u> <u>kg⁻¹ soil</u> |
| NPA+P (N-BASIS) | 0.00a | NPA+P (N-BASIS) | 0.09abc | NPA+P (N-BASIS) | 0.19bc | NPA+P (N-BASIS) | 0.18bc |
| NPA+P (P-BASIS) | 0.00a | NPA+P (P-BASIS) | 0.05c | NPA+P (P-BASIS) | 0.00c | NPA+P (P-BASIS) | 0.10c |
| NPA+PHYTASE | 0.00a | NPA+PHYTASE | 0.14ab | NPA+PHYTASE | 0.42a | NPA+PHYTASE | 0.40a |
| NPA | 0.00a | NPA | 0.11abc | NPA | 0.28ab | NPA | 0.22b |
| HAP | 0.00a | HAP | 0.13abc | HAP | 0.13bc | HAP | 0.23b |
| HAP+PHYTASE | 0.00a | HAP+PHYTASE | 0.16a | HAP+PHYTASE | 0.23b | HAP+PHYTASE | 0.25b |
| CONTROL | 0.01a | CONTROL | 0.07bc | CONTROL | 0.01c | CONTROL | 0.07c |
| <u>Groseclose soil</u> | | | | | | | |
| <u>Manure Treatment</u> | <u>mg Cu</u> <u>kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu</u> <u>kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu</u> <u>kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu</u> <u>kg⁻¹ soil</u> |
| NPA+P (N-BASIS) | 0.00a | NPA+P (N-BASIS) | 0.00a | NPA+P (N-BASIS) | 0.30a | NPA+P (N-BASIS) | 0.25a |
| NPA+P (P-BASIS) | 0.00a | NPA+P (P-BASIS) | 0.00a | NPA+P (P-BASIS) | 0.05de | NPA+P (P-BASIS) | 0.13b |
| NPA+PHYTASE | 0.00a | NPA+PHYTASE | 0.00a | NPA+PHYTASE | 0.22ab | NPA+PHYTASE | 0.32a |
| NPA | 0.00a | NPA | 0.00a | NPA | 0.12cd | NPA | 0.24a |
| HAP | 0.00a | HAP | 0.00a | HAP | 0.10cd | HAP | 0.26a |
| HAP+PHYTASE | 0.00a | HAP+PHYTASE | 0.00a | HAP+PHYTASE | 0.15bc | HAP+PHYTASE | 0.32a |
| CONTROL | 0.00a | CONTROL | 0.00a | CONTROL | 0.01e | CONTROL | 0.01c |

*Means with same letter not statistically different by Duncan's Multiple Range Test at alpha=0.05
Statistical analyses were conducted separately on each of the twelve combinations.

2.3.7. Mehlich III-Solubility of Cu

A total of 575 extractions were performed to determine Mehlich III-soluble Cu concentrations. An overall comparison of plant available Cu extracted from manure-amended soils for each treatment averaged across soil type, manure application rate, and incubation period is given in Fig. 2.18. Lawrence (2000) determined plant available Cu concentrations for the two shortest time periods (4 and 28 days). The NPA+Phytase and baseline NPA treatments contain equal concentrations of Mehlich III-soluble Cu, indicating that addition of phytase does not alter plant availability of Cu. (Note the addition of phytase did significantly increase Mehlich III-solubility of Cu).

Fig. 2.18. Mehlich III-soluble Cu concentrations extracted from manure amended soils averaged across all three Virginia soils, incubation times (4 and 28 days and and 12 months), and manure application rates (25 and 50 g/kg)

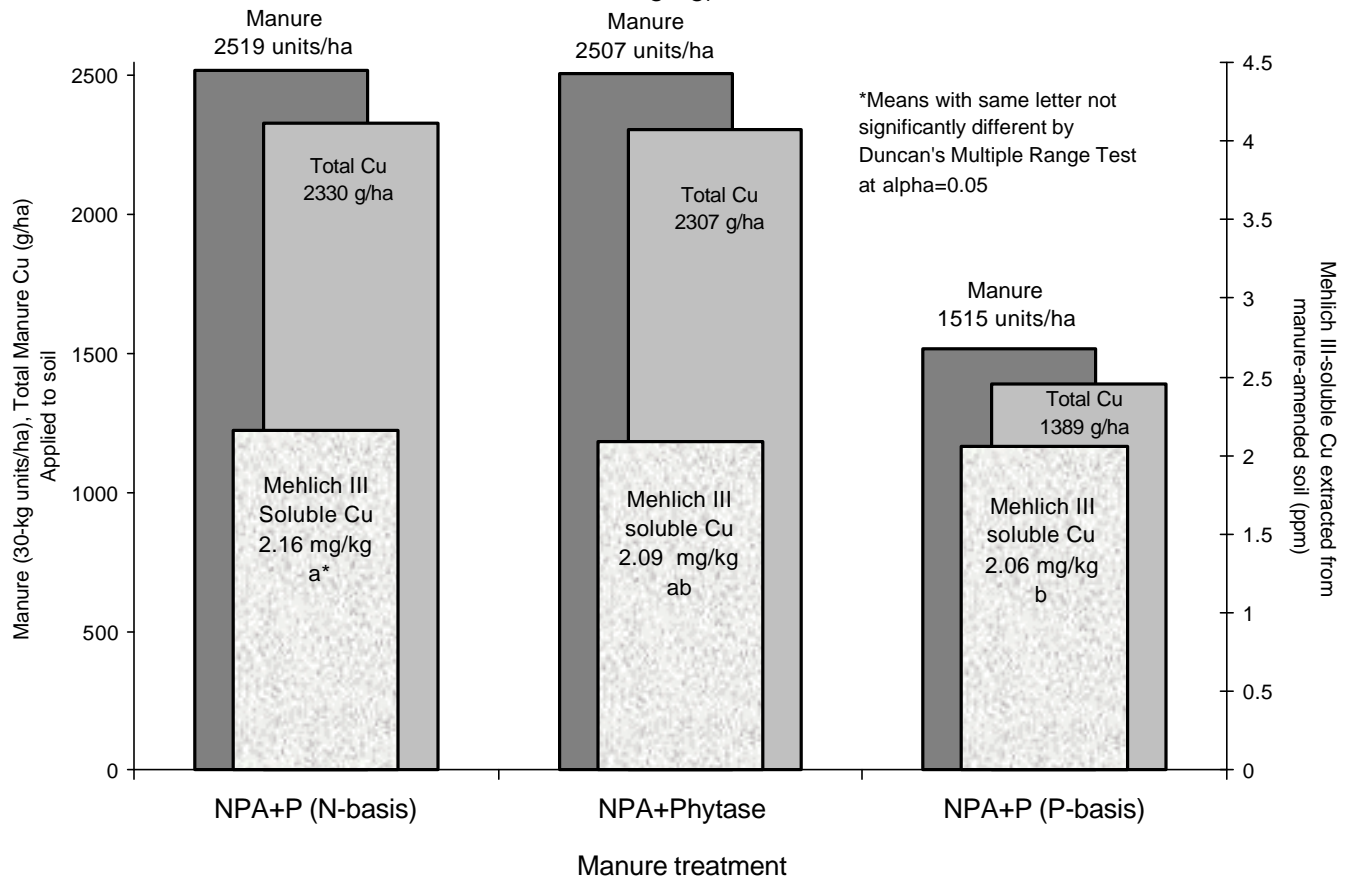


*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05.

The alternative NPA+Phytase treatment is compared to the conventional NPA+P treatments using the data obtained from Fig. 2.18 in Fig. 2.19. Comparing the treatments on an equal Cu basis causes a statistically insignificant decrease of 3% when the NPA+P (N- basis) treatment is replaced with the NPA+Phytase treatment (2.09 vs. 2.16 mg Cu kg⁻¹ soil for NPA+Phytase and NPA+P on N-basis treatments, respectively.) Conversion to a P-basis manure application regime results in a mere 1% increase in Cu by switching to the NPA+Phytase treatment. The phytase treatment is not significantly different than either of the NPA+P treatments in Cu solubility. Plant availability of Cu is therefore not affected by phytase or inorganic P addition to the baseline NPA treatment, and is reinforced by the fact that additions of either of these supplements to the NPA treatment caused very little or no differences in Cu solubility.

Substitution of either HAP corn or HAP+Phytase for NPA corn also did not create significant differences in Cu solubility, with a difference in solubility of only 1 and 2% for the HAP and HAP+Phytase treatments, respectively.

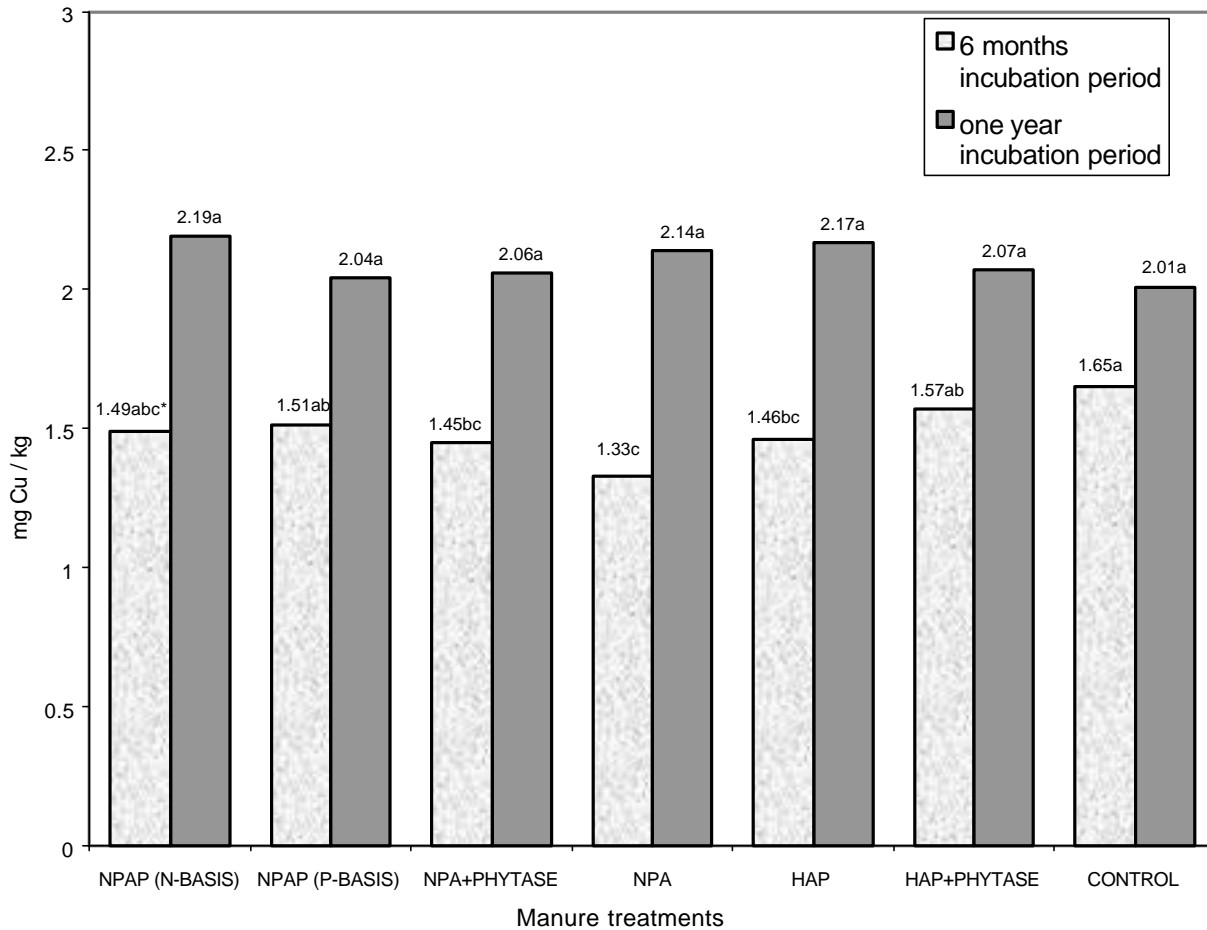
Fig. 2.19. Comparison of Mehlich III soluble Cu concentrations from soils amended with NPA+P and NPA+Phytase manure treatments averaged across all incubation periods (4 and 28 days and 6 and 12 months) and manure application rates (25 and 50 g/kg)



2.3.8. Effects of Incubation Time, Manure Application Rate, and Soil Type

This section compares the effects of incubation time, manure application rate, and soil type separately on plant availability of Cu. Once again, this section only provides comparisons using the 6 months and one year incubation periods. Extractable Cu data from treatments incubated for 6 months and one year averaged across soil type and manure application rate are presented in Fig. 2.20. Interestingly, some notable changes have taken place in Mehlich III-solubility of Cu upon increasing incubation time. For example, the highest concentration of Cu was extracted from the control treatment at six months, while it contained the lowest Cu content among the treatments at one year.

Fig. 2.20. Effects of incubation time on Mehlich III-soluble Cu extracted from manure amended soils averaged across all three Virginia soils and application rates (25 and 50 g/kg)

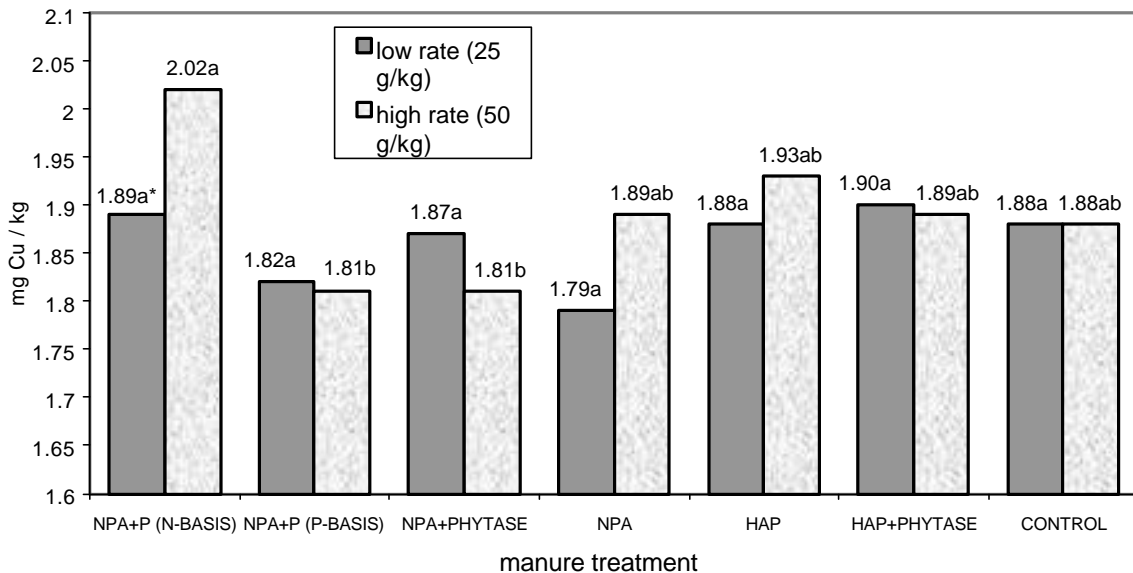


*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05. Statistical analyses were conducted separately from each incubation period

However, upon increasing incubation time the control treatment increased by 18% in Cu solubility, well below the average of 31% extracted by the manure amended soils. The increase in Cu solubility in the control soils is most likely due to mineralization of Cu upon increasing incubation time. The increase in Cu solubility in the manure-amended soils can only be partly attributed to the mineralization of native Cu in the soil, since these manured soils increased in Cu solubility at a much higher percentage than the control. Increasing incubation time did not significantly alter the additions of phytase and HAP compared to the baseline NPA treatment.

The effect of increasing application rate on plant availability of Cu is shown in Fig. 2.21. It is striking to observe that upon increasing application rate, the NPA+Phytase, NPA+P (P-basis), and HAP+Phytase treatments actually decreased in Cu solubility. Though Cu application was doubled in the high application rate, P was also doubled and may have decreased Cu solubility in these treatments, due to suppression of Cu by P.

Fig. 2.21. Effect of manure application rate on Mehlich III-solubility of Cu extracted from manure amended soils averaged across all three Virginia soils and incubation periods (6 and 12 months)

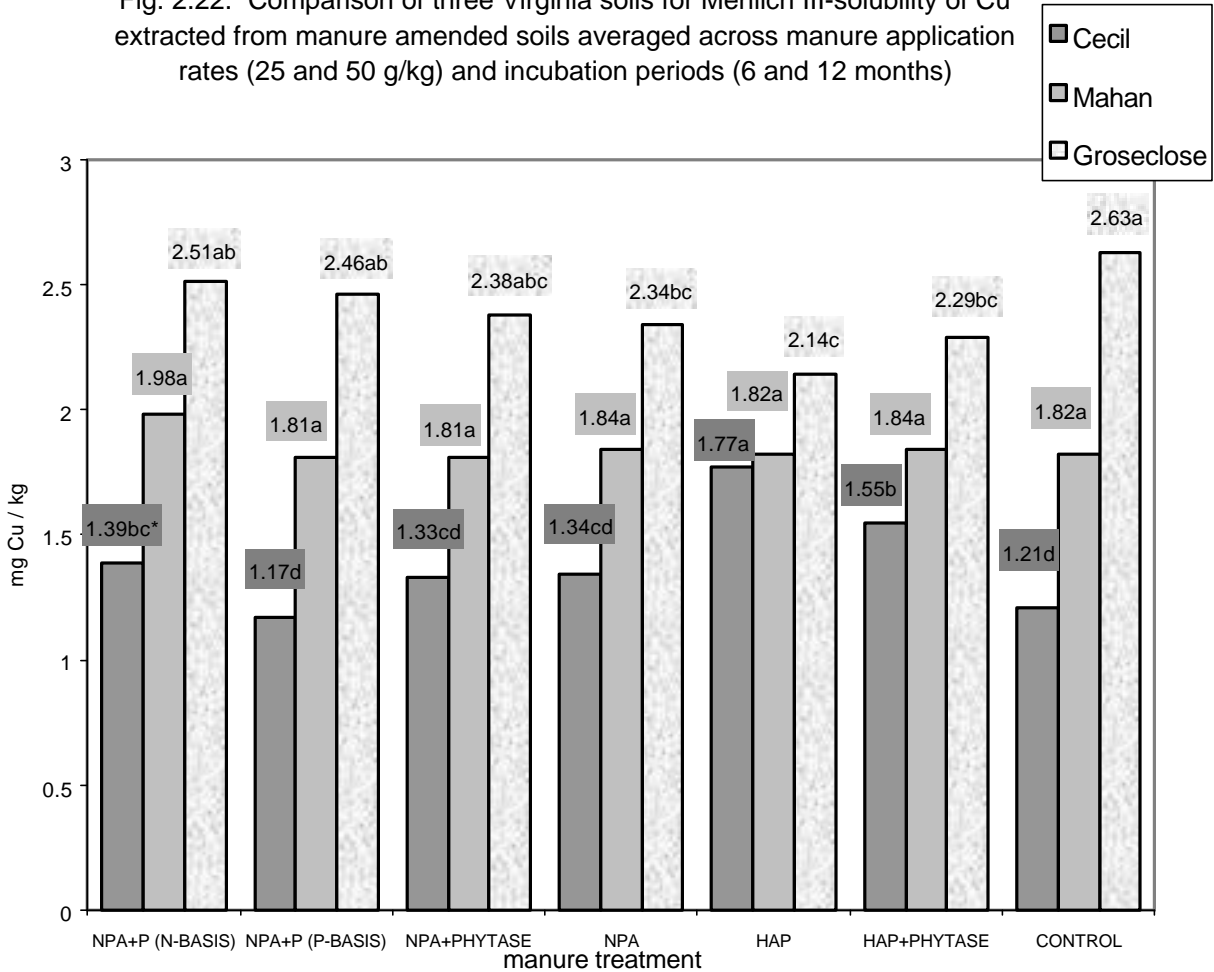


*means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05.

Statistical analyses were conducted separately from each application rate.

Treatments for each soil extracted for determination of plant available Cu are shown in Fig. 2.22 and are averaged across incubation time and application regime. The Groseclose soil extracted the highest concentration of Cu across all treatments, with the control having the highest Cu concentration. Addition of phytase to the baseline NPA treatment did not cause statistically significant differences in Cu solubility in any of the soils. Substitution of HAP corn for NPA corn created a significant difference in only the Cecil soil.

Fig. 2.22. Comparison of three Virginia soils for Mehlich III-solubility of Cu extracted from manure amended soils averaged across manure application rates (25 and 50 g/kg) and incubation periods (6 and 12 months)



*means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05.

Statistical analyses were conducted separately on each soil type.

Mehlich III-soluble Cu concentrations for treatments from all twelve combinations of soil type, manure application rate, and incubation time are given in Table 2.10. It is important to note that no significant difference exists in Cu solubility from the addition of phytase to the baseline NPA treatment in any of the twelve combinations. Conversion from the NPA to the

HAP treatment also produces no significant difference in Cu solubility in all combinations except the Cecil soil that received the low manure application incubated for one year. Copper solubility increased by 39% comparing the HAP to the NPA treatment in this case.

Table 2.10. . Mehlich III-soluble Cu concentrations extracted from manure amended soils for all soil types, application rates (25 and 50 g/kg), and 6 months and one year incubation periods

| Low application | | | | High application | | | |
|----------------------------|-----------------------------------|----------------------------|-----------------------------------|----------------------------|-----------------------------------|----------------------------|-----------------------------------|
| <u>6 months incubation</u> | | <u>One year incubation</u> | | <u>6 months incubation</u> | | <u>One year incubation</u> | |
| <u>Mahan soil</u> | | | | | | | |
| <u>Manure Treatment</u> | <u>mg Cu kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu kg⁻¹ soil</u> |
| NPA+P (N-BASIS) | 1.02bc* | NPA+P (N-BASIS) | 2.23ab | NPA+P (N-BASIS) | 1.85a | NPA+P (N-BASIS) | 2.27a |
| NPA+P (P-BASIS) | 1.23bc | NPA+P (P-BASIS) | 1.92b | NPA+P (P-BASIS) | 2.05a | NPA+P (P-BASIS) | 1.79a |
| NPA+PHYTASE | 1.04bc | NPA+PHYTASE | 2.35a | NPA+PHYTASE | 1.72a | NPA+PHYTASE | 1.74a |
| NPA | 0.81c | NPA | 2.21ab | NPA | 1.75a | NPA | 2.08a |
| HAP | 0.91bc | HAP | 2.15ab | HAP | 2.02a | HAP | 1.75a |
| HAP+PHYTASE | 1.39ab | HAP+PHYTASE | 2.12ab | HAP+PHYTASE | 2.07a | HAP+PHYTASE | 1.78a |
| CONTROL | 1.74a | CONTROL | 1.87b | CONTROL | 1.74a | CONTROL | 1.87a |
| <u>Cecil soil</u> | | | | | | | |
| <u>Manure Treatment</u> | <u>mg Cu kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu kg⁻¹ soil</u> |
| NPA+P (N-BASIS) | 0.72b | NPA+P (N-BASIS) | 1.43b | NPA+P (N-BASIS) | 1.24bc | NPA+P (N-BASIS) | 1.67ab |
| NPA+P (P-BASIS) | 0.77ab | NPA+P (P-BASIS) | 1.48b | NPA+P (P-BASIS) | 0.81d | NPA+P (P-BASIS) | 1.37b |
| NPA+PHYTASE | 0.68b | NPA+PHYTASE | 1.41b | NPA+PHYTASE | 1.02cd | NPA+PHYTASE | 1.73ab |
| NPA | 0.68b | NPA | 1.48b | NPA | 1.21bc | NPA | 1.61ab |
| HAP | 0.68b | HAP | 2.41a | HAP | 1.51ab | HAP | 1.82a |
| HAP+PHYTASE | 0.76ab | HAP+PHYTASE | 1.83ab | HAP+PHYTASE | 1.62a | HAP+PHYTASE | 1.61ab |
| CONTROL | 0.68b | CONTROL | 1.31b | CONTROL | 1.00cd | CONTROL | 1.31b |
| <u>Groseclose soil</u> | | | | | | | |
| <u>Manure Treatment</u> | <u>mg Cu kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu kg⁻¹ soil</u> | <u>Manure Treatment</u> | <u>mg Cu kg⁻¹ soil</u> |
| NPA+P (N-BASIS) | 2.50a | NPA+P (N-BASIS) | 2.60ab | NPA+P (N-BASIS) | 1.24b | NPA+P (N-BASIS) | 2.93a |
| NPA+P (P-BASIS) | 2.65a | NPA+P (P-BASIS) | 2.62ab | NPA+P (P-BASIS) | 0.99bc | NPA+P (P-BASIS) | 3.15a |
| NPA+PHYTASE | 2.74a | NPA+PHYTASE | 2.42ab | NPA+PHYTASE | 1.22b | NPA+PHYTASE | 2.75a |
| NPA | 2.21a | NPA | 2.53ab | NPA | 0.95bc | NPA | 2.91a |
| HAP | 2.29a | HAP | 1.97b | HAP | 0.81c | HAP | 2.91a |
| HAP+PHYTASE | 2.52 | HAP+PHYTASE | 2.43ab | HAP+PHYTASE | 1.01bc | HAP+PHYTASE | 2.66a |
| CONTROL | 2.17a | CONTROL | 2.85a | CONTROL | 2.17a | CONTROL | 2.85a |

*Means with same letter not statistically different by Duncan's Multiple Range Test at alpha=0.05
Statistical analyses were conducted separately on each of the twelve combinations.

2.4. SUMMARY AND CONCLUSIONS

2.4.1. Effect of Phytase Addition on Water-Solubility and Plant-Availability of P.

Addition of phytase to the baseline NPA treatment did not cause statistically significant differences in P solubility after extraction with either 0.01 M CaCl₂ or Mehlich III solution upon addition of these manure treatments to soils averaged across all soil types, incubation periods, and application rates. No statistical differences in water-solubility and Mehlich III-solubility of P were observed between these two treatments upon increasing incubation time or increasing application rate.

The most important issue to consider is the comparison of the alternative NPA+Phytase treatment to the conventional NPA+P treatments. When these treatments are compared on a N-basis, total manure P applied to soils is 41% less in the NPA+Phytase treatment. This reduction is less notable in water-soluble P extracted from soils, with a significant 20% reduction in water-soluble P concentrations when NPA+Phytase replaces the NPA+P (N-basis) treatment (15.9 vs. 12.7 mg P kg⁻¹ soil for NPA+P on N-basis and NPA+Phytase treatments, respectively). Comparing these treatments on a P-basis produces a significant increase in water-soluble P levels of 24% by replacing the NPA+P (P-basis) treatment with the NPA+Phytase treatment (12.7 vs. 9.7 mg P kg⁻¹ soil for NPA+Phytase and NPA+P on P-basis treatments, respectively). This significant difference can be attributed to the addition of inorganic Ca-phosphate to the NPA treatment, since addition of phytase did not create a statistical difference. The addition of Ca-phosphate to the NPA treatment on a P-basis decreased P solubility by a statistically significant 25%, producing the large differences in P solubility between the alternative phytase treatment and conventional NPA+P treatment. Soil type, increasing incubation time or increasing application rate did not produce statistical changes in water-soluble P concentrations between these treatments.

Comparing the alternative phytase treatment to the conventional NPA+P on a N-basis produces a significant 24% reduction in plant-available P concentrations (397.3 vs. 303.7 mg P kg⁻¹ soil for NPA+P on N-basis and NPA+Phytase treatments, respectively). When these two treatments are compared on a P-basis a significant increase of only 5% is observed by replacing the conventional NPA+P on P-basis treatment to the alternative

phytase treatment. This 5% increase is much less notable than the 24% increase in extractable P by CaCl_2 . The acidic Mehlich III extractant solubilized much more P in the NPA+P (P-basis) treatment that was not solubilized by the CaCl_2 extractant, resulting in the much smaller difference. Neither phytase addition or inorganic P supplementation to the baseline NPA treatment caused a significant difference in plant-available P concentrations. Increasing application rate or incubation time did not produce significant changes in plant-available P concentrations between the NPA+Phytase and NPA+P treatments. Soil type did produce some statistical changes in P extractability between the NPA+Phytase and NPA+P treatments only on a P-based manure application regime. The Cecil soil increased Mehlich III-soluble P concentrations by a statistically significant 15% from conversion of the NPA+P (P-basis) treatment to the NPA+Phytase treatment, though no significant differences were observed between these treatments in either the Mahan or Groseclose soils.

2.4.2. Effect of Phytase Supplementation on Water-Solubility and Plant-Availability of Cu

Supplementation of phytase to the NPA treatment caused a significant 21% increase in water-soluble Cu concentrations averaged across all soil types, incubation periods, and application regimes. The same Cu concentrations were extracted by Mehlich III from these treatments, producing no phytase effect on plant-available Cu concentrations.

Comparing the alternative phytase treatment to the conventional NPA+P treatment on an N-basis (and thus equal Cu-basis) produced a statistically significant 30% increase in water-soluble Cu concentrations (0.43 vs. 0.61 mg Cu kg^{-1} soil for NPA+P on N-basis and NPA+Phytase treatments, respectively). Comparing the phytase treatment to the NPA+P treatment on a P-basis produces an even more statistically significant increase of 61%. Note that 40% less total Cu is applied from the NPA+P (P-basis) treatment compared to the NPA+Phytase treatment. This large difference in water-soluble Cu is due both to the addition of phytase and the addition of inorganic Ca-phosphate to the baseline NPA treatment, since phytase caused a significant increase in Cu solubility and inorganic calcium phosphate caused a significant reduction in soluble Cu concentrations. Neither an increase in incubation time or application rate caused significant differences

between the alternative and conventional treatments. Both Mahan and Cecil soils did not produce changes in trends between these treatments. The only comparison which produced no statistical differences between these treatments was in the Groseclose soil when the NPA+Phytase treatment was compared to the NPA+P (N-basis) treatment.

When these treatments are compared after extraction with Mehlich III on an N-basis (and thus equal Cu-basis), a statistically insignificant difference in soluble Cu is observed (2.16 vs. 2.09 mg Cu kg⁻¹ soil for NPA+P on N-basis and NPA+Phytase treatments, respectively). Conversion to a P-based manure management situation still does not produce statistical differences between these treatments (2.09 vs. 2.06 mg Cu kg⁻¹ soil for NPA+Phytase and NPA+P treatment on P-basis, respectively). Large differences were observed between these treatments after extraction with 0.01 M CaCl₂. The insignificant differences observed here is due to the acidic Mehlich III extractant solubilizing the P bonds that had formed with copper. Soil type or increasing incubation time did not produce any significant changes between the alternative phytase treatment and conventional NPA+P treatments. However, increasing application rate did produce a significant 10% decrease in plant-available Cu concentrations by replacing the NPA+P (N-basis) treatment with the NPA+Phytase treatment (2.02 vs. 1.81 mg Cu kg⁻¹ soil for NPA+P on N-basis and NPA+Phytase treatments, respectively).

2.4.3. Effect of HAP Substitution on Water Solubility and Plant Availability of P and Cu

Substituting the NPA treatment for HAP corn did not produce statistically significant differences by extraction with either CaCl₂ or Mehlich III solution. The effects of soil type, increasing incubation time and increasing manure application rate to soils did not produce significant differences between these treatments extracted with 0.01 M CaCl₂. When Mehlich III was used to extract P, the effects of soil type and manure application rate did not produce any statistical changes between the NPA and HAP corn treatments. However, when soils are extracted at 6 months, a significant increase of 11% was observed when NPA is replaced with HAP corn.

HAP substitution for NPA corn did not produce any significant changes in water-soluble Cu concentrations averaged across all soil types, incubation times, and application rates. The same holds true when each of these factors are considered

separately. Plant available Cu concentrations are also statistically insignificant between these two treatments averaged across all soil types, incubation times, and application rates. Though no differences were observed upon increasing incubation time and application rate, the Cecil soil produced a significant 24% increase by replacement of NPA for HAP corn. Neither the Mahan or Groseclose soil produced significant differences in plant-available Cu between these treatments.

2.4.4. Comparison of Dissolved P and Dissolved Inorganic P

The dissolved inorganic P fraction analyzed colorimetrically accounted for 79% of the dissolved P fraction analyzed by ICP after extraction with 0.01 M CaCl₂, and accounted for 87% of the P fraction after Mehlich III extraction. Subtraction of the dissolved inorganic P fraction from total dissolved P yields the dissolved organic P fraction. The trends between treatments for both of these P fractions remain the same.

CHAPTER 3: GREENHOUSE STUDY

3.1. INTRODUCTION

Since phytase is becoming a popular feed additive in many poultry operations, it is important to determine if any negative impacts occur due to phytase upon addition of manure to agricultural fields. Experiments were conducted in the greenhouse to determine if any changes in corn growth or plant uptake of P, Cu, and Zn occur upon addition of phytase amended manure to soils. It was also of interest to determine if any negative impacts from phytase occur on corn growth.

3.2. MATERIALS AND METHODS

3.2.1. Corn Grown in Southeastern Soils

The objective of this study was to determine the impact on corn growth and P, Cu, and Zn uptake when manure from poultry fed a diet supplemented with phytase is applied to agricultural land, compared to those manure treatments without phytase supplementation. Refer to Chapter 2 for information on manure collection and diet formulation. The manure used in this study was recombined from selected individual pens that had no indication of Zn contamination. Therefore, Zn is being studied in this chapter. Three soils were collected from three different physiographic provinces in Virginia and are considered to be productive agricultural soils. A Groseclose loam was taken from Montgomery County located in the Ridge and Valley region and is classified as a clayey, mixed, mesic Typic Hapludult. The second soil chosen was a Cecil loam which was collected from Amelia County in the Piedmont, and is classified as a fine, kaolinitic, thermic Typic Kanhapludult. The final soil chosen was the Mahan sandy loam, taken from Isle of Wight County in the Coastal Plain. The Mahan soil has been classified as a fine, kaolinitic, thermic Typic Hapludult. It was decided to use these soils since they contain high levels of most nutrients, and are representative of many fields to which poultry manure is being applied in Virginia.

The soils were air-dried, and clods were broken up with the use of a mortar and pestle. The samples were sieved to remove coarse fragments greater than 2 mm, and were thoroughly mixed using a portable electric cement mixer. Moisture content of the air-dried soils was determined by weighing a sample before and after oven drying at

110 °C for a 24-hour period. The water holding capacity of the soils was determined by estimating the amount of water held in the soils after being freely drained by using the technique of Kuo (1996). Subsamples of the three soils were extracted with Mehlich I solution and analyzed for plant-available P, K, Ca, Mg, Cu, and Zn by ICP in the Virginia Tech Soil Testing Laboratory. (Refer to Table 2.5).

Plastic pots were double lined with plastic bags to prevent drainage, and 1500 g (dry weight basis) of air-dried soil was added to the pots. Moisture content of the manure was determined by weighing a subsample before and after oven-drying at 35 °C for 24 hours. Wet manure from each treatment was added to the pots at a rate of 4 dry tons acre⁻¹. Table 3.1 gives total elemental analyses for each manure treatment used. The NPA+P treatment was separated into two manure treatments, on an N-basis and also on a P-basis. Table 3.2 shows manure elements applied to soils at a rate of 4 tons acre⁻¹. The NPA+Phytase, NPA, and NPA+P on P basis have similar P contents, while NPA+Phytase, NPA, and NPA+P on N basis have similar N, Cu and Zn contents. The NPA+P manure treatment on a P basis allows for equal comparisons of P content, while the NPA+P treatment on an N basis allows for comparing Cu and Zn between treatments.

Table 3.1. Manure analysis for each manure treatment used in greenhouse study

| Manure treatment | Moisture Content % | <u>g kg⁻¹ manure</u> | | | | | | | | | | | |
|------------------|--------------------|---------------------------------|-------------------------------|------------------|------|------|------|------|------|------|------|------|------|
| | | N | P ₂ O ₅ | K ₂ O | S | Ca | Mg | Na | Fe | Al | Mn | Cu | Zn |
| NPA | 81.35 | 13.26 | 5.07 | 7.93 | 0.90 | 2.26 | 1.35 | 0.88 | 0.92 | 0.15 | 0.05 | 0.01 | 0.08 |
| NPA+P | 81.12 | 13.83 | 6.96 | 8.21 | 0.81 | 3.63 | 1.25 | 1.34 | 0.53 | 0.22 | 0.05 | 0.01 | 0.07 |
| NPA+PHYTASE | 80.99 | 14.79 | 4.24 | 7.51 | 0.88 | 1.83 | 1.18 | 0.91 | 0.63 | 0.13 | 0.04 | 0.01 | 0.07 |
| HAP | 82.45 | 14.82 | 4.00 | 7.76 | 0.83 | 1.67 | 1.16 | 0.90 | 0.44 | 0.17 | 0.04 | 0.01 | 0.07 |
| HAP+PHYTASE | 79.86 | 17.30 | 4.26 | 8.39 | 0.93 | 1.57 | 1.27 | 0.97 | 0.48 | 0.25 | 0.05 | 0.01 | 0.07 |

Table 3.2. Manure and manure elements applied to soils used for greenhouse study

| Manure Treatment | Manure g kg ⁻¹ (T A ⁻¹) soil | P mg kg ⁻¹ soil | N mg kg ⁻¹ soil | Cu mg kg ⁻¹ soil | Zn mg kg ⁻¹ soil |
|------------------|--|-------------------------------|-------------------------------|--------------------------------|--------------------------------|
| CONTROL | 4 | 0.00 | 0.00 | 0.00 | 0.00 |
| NPA | 4 | 47.35 | 284.13 | 0.11 | 1.72 |
| NPA+P (N-BASIS) | 4 | 64.25 | 292.53 | 0.11 | 1.48 |
| NPA+P (P-BASIS) | 2.5 | 39.92 | 181.78 | 0.07 | 0.92 |
| NPA+PHYTASE | 4 | 38.93 | 310.73 | 0.11 | 1.47 |
| HAP | 4 | 39.73 | 337.26 | 0.11 | 1.48 |
| HAP+PHYTASE | 4 | 36.92 | 343.14 | 0.10 | 1.29 |

The manure and soil was then mixed in a twin shell blender for 5 minutes and returned to the pots. The control pots contained soil without any manure addition. Fertilizer was not added due to the high fertility of the soils, as seen in Table 3.2. Water was added to 80% of field capacity and allowed to equilibrate. Five corn seeds (pioneer 33V08, 100 to 105 days to maturity) per pot were punched into the soil to a depth of 2.5 cm with the use of a glass rod fitted with a rubber stopper. Daytime greenhouse temperature was maintained at 82 °F, and 78 °F at night. Four days after plants emerged, plants were removed so that only two plants per pot remained. Pots were watered to 80% of field capacity twice daily and rotated once daily. Water was added by weighing the pots and adding the calculated amount necessary to bring pots to 80% field capacity, taking into account the weight of the pot and plant (Allen et. al., 1976). For example, the field capacity of the Cecil soil was 25%, and since 1500 g of soil was weighed into each pot, the required amount of water to reach 80% field capacity would be: $0.25 \times 1500 \text{ g} \times 0.80 = 300\text{g}$ (300 mL). The weight of the pot after water addition daily would be: $1500\text{g} + 300\text{g} + \text{container weight}$. Therefore, the difference in this calculated amount and the weight of the pot prior to water addition would be the mL of water necessary. The weight of the plants was taken into account as they became larger, and water addition was adjusted based on this

weight difference. Plants were grown for 4 weeks and harvested by clipping all plant material one inch from base of plant. Plants were placed in labeled brown paper bags and dried in a forced-air drying oven maintained at 70° C for one week. Dried corn samples were weighed and then ground using a Wiley Mill. Total concentrations of P, Cu, and Zn in the corn samples were determined by HNO₃ digestion using a Milestone Microwave Labstation. To each Teflon vessel, 0.5 g plant sample and 9 mL nitric acid was added. Select subsamples were duplicated to ensure accuracy. The vessels were sealed and digested by microwave for 35 minutes with maximum temperature reaching 220°C. The samples were filtered using No. 42 Whatman filter paper. Samples were diluted at a ratio of 6 mL deionized water: 1 mL solution and the extracts were analyzed for P, Cu, and Zn using inductively coupled plasma emission spectrometry (ICP).

3.2.2. Statistical Analysis

Each of the four manure treatments was combined with each of the three types of soil, along with controls (no manure) for each soil type. Experimental design was a completely randomized design with four replications for each soil/manure combination for a total of 60 pots. The following equation was used to calculate the quantity of P, Cu, and Zn extracted in mg per kg of manure:

$$A \times B \times C = D$$

A=mg element per L of extractant (ICP analysis)

B= ml extractant per g manure extracted (dry weight basis)

C= dilution factor

D= mg element extracted per kg plant tissue

Analysis of variance was performed using SAS (SAS Institute, 1996) to evaluate statistical differences between soils and manure treatments. Duncan's Multiple Range Test was also used in SAS to compare means at the alpha level of 0.05.

3.2.3. Soils Extractions

All large roots were removed from each pot after harvest and the soil was passed through a 10-mesh sieve to reduce the amount of small roots remaining in the soil to be extracted. The soil was returned to each pot and thoroughly mixed. Moisture content of

the soil from each pot prior to extraction was determined by weighing a subsample before and after incubation in a 110 °C oven for a 24 hour period. The calculated quantities of soil (5 g on oven-dry basis) were weighed into 100 mL plastic centrifuge tubes. In order to account for the moisture present in the soil, 25 mL double strength Mehlich III solution was added to the centrifuge tubes. The remainder of the water was added to the tubes to achieve 50 mL Mehlich III solution: 5g dry manure-amended soil (10:1 ratio), taking into account the water entrained in the soil. Plastic caps sealed the centrifuge tubes and were placed on a reciprocal shaker for 5 minutes and centrifuged at 2500 rpm for 10 minutes. The supernatant liquid from each tube was decanted into 60-cm³ syringes with Luerlok tips packed with 1 gram of non-reactive filter pulp in the bottom of the syringe. Nucleopore filter housings measuring 25-mm in diameter were attached to the Luerlok tips. The filter housings hold 0.45 µm Nucleopore filters. A syringe plunger was used to force the supernatant through the filter pulp in the syringe and then through the 0.45 µm filter in the filter housing. The liquid was collected in a plastic dilu-vial for analysis. The extracts were analyzed for P, Cu, and Zn using inductively coupled plasma emission spectrometry (ICP).

3.2.4. Statistical Analysis

One subsample was taken from each pot, which was replicated four times for each manure treatment/soil combination for a total of 60 extractions. The following equation was used to calculate the quantity of P, Cu, and Zn extracted in mg per kg of soil:

$$A \times B = C$$

A=mg element per L of extractant (ICP analysis)

B= mL extractant per g soil extracted (dry weight basis)

C= mg element extracted per kg dry soil

Analysis of variance was performed using SAS (SAS Institute, 1996) to evaluate statistical differences between soils and manure treatments. Duncan's Multiple Range Test was also used in SAS to compare means at the alpha level of 0.05.

3.2.5. Corn grown in sand

The objective of using sand as a potting medium was to ensure that P, Cu, and Zn were being compared between diet treatments with only the manure as the source of these nutrients. Any differences in corn growth would be attributed to differences in plant-availability of these nutrients between treatments. All other nutrients and water were supplied in nonlimiting quantities to ensure only the nutrients under study were the source of changes in plant growth. The water holding capacity of the sand used was determined by estimating the amount of water held in the sand after being freely drained (Kuo, 1996). Plastic pots were double lined with plastic bags to prevent drainage, and 2000 g of play sand was added to the pots. Manure treatments used in this study include the NPA, NPA+Phytase, and NPA+P on an N basis. Moisture content of the manure was determined by weighing a subsample before and after oven-drying at 35°C for 24 hours. Manure from each treatment was added to the pots at a rate of 2.24, 4.48, 8.96, and 17.92 dry Mg ha⁻¹. The manure and soil was then mixed in a twin shell blender for 5 minutes and returned to the pots. Water was added to the pots along with the first application of Ruakara fertilizer solution to 80% of field capacity. Ruakara fertilizer solution was added to the pots over a period of six days, in three applications. This solution supplied all nutrients in nonlimiting quantities and was modified to contain no P, Cu, and Zn. One set of control pots contained only sand with no fertilizer or manure addition, while another set of control pots contained all nutrients including P, Cu, and Zn in nonlimiting quantities, using the original Ruakara solution. Finally, a third set of control pots were set up to contain ample quantities of all nutrients without any P, Cu, or Zn added. Five corn seeds (pioneer 33V08, 100 to 105 days to maturity) per pot were punched into the soil to a depth of 2.5 cm with a glass rod fitted with a rubber stopper, after the first addition of the nutrient solution. Pots were watered to 80% of field capacity and rotated once daily. Pots were then watered twice daily two weeks after seeds were planted. Water was added by the same technique described in the previous greenhouse study. Plants were removed so that only two plants per pot remained four days after plants emerged. Daytime greenhouse temperature was maintained at 84 °F and 82 °F at night. Plants were grown for 6 weeks and harvested by clipping all plant material one inch from base of plant. Only the plants with the one ton acre⁻¹ application rate were sacrificed for

analysis. All other plants with 2, 4, and 8 tons acre⁻¹ application rates did not survive the six weeks growth period. The same procedures followed in the previous study were used for drying and analysis of the corn tissue.

3.2.6. Statistical Analysis

Experimental design was a completely randomized design with three replications for each of the three treatments, along with three replications for each of the three sets of control pots. Four application rates were used, for a total of 45 pots. Two subsamples per pot were analyzed. The following equation was used to calculate the quantity of P, Cu, and Zn extracted in mg per kg of manure:

$$A \times B \times C = D$$

A=mg element per L of extractant (ICP analysis)

B= ml extractant per g manure extracted (dry weight basis)

C= dilution factor

D= mg element extracted per kg plant tissue

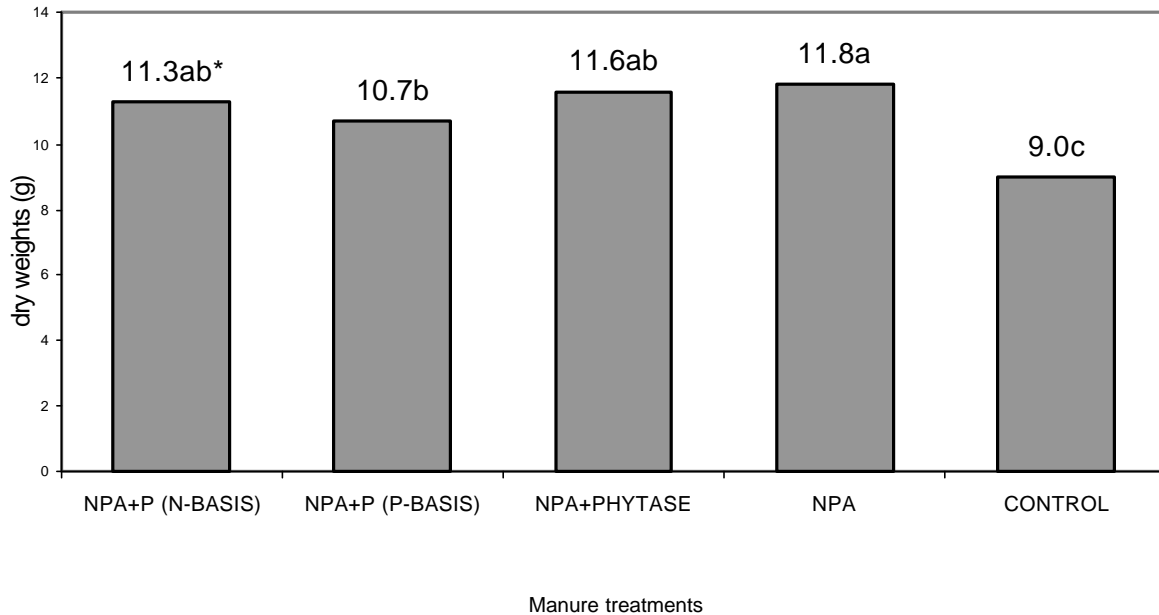
Analysis of variance was performed using SAS (SAS Institute, 1996) to evaluate statistical differences between soils and manure treatments. Duncan's Multiple Range Test was also used in SAS to compare means at the alpha level of 0.05.

3.3. RESULTS AND DISCUSSION

3.3.1. Corn grown in Groseclose, Cecil, and Mahan soils

Corn grown in Groseclose, Cecil, and Mahan soils amended with manure was dried and weighed, then digested in HNO₃ and analyzed by ICP for total P, Cu, and Zn contents. Five treatments were used in the study with 4 reps for each of the three soils for a total of 60 samples. The dry weight of corn tissue for corn grown in these three soils amended with manure are given in Fig. 3.1. Manure treatments shown in the figure give corn tissue weights averaged across all three soils. The control treatments consist of soil with no addition of manure and had the lowest yield of all treatments. The addition of phytase to the baseline NPA treatment had no effect on corn growth (Fig. 3.1). When the alternative NPA+Phytase treatment is compared to the conventional NPA+P treatment on

Fig. 3.1. Corn tissue dry weights from corn grown in all three soils amended with 8.96 Mg/ha poultry manure

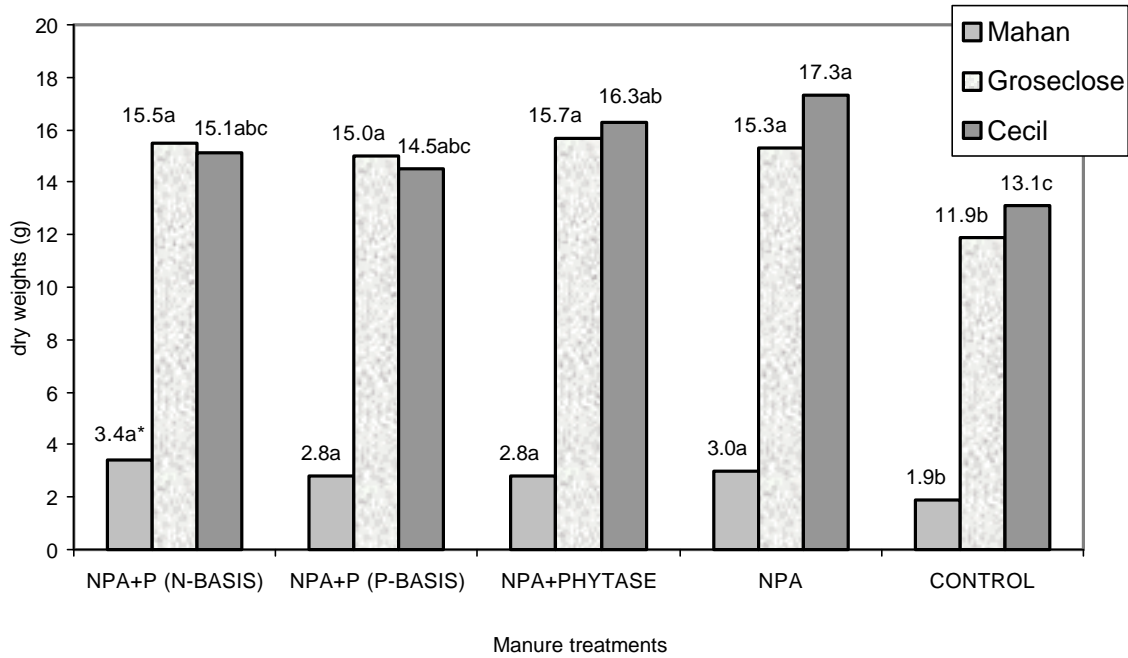


Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

an equal N basis, no significant differences in tissue growth exist. The same is true when these treatments are compared on a P basis.

Dry weights for corn tissue from corn grown in Groseclose, Cecil, and Mahan soils amended with manure are given in Fig. 3.2. Treatments for each soil type are graphed separately for soil comparison. Corn grown in the Cecil and Groseclose soils resulted in similar dry tissue quantities, while corn grown in the Mahan resulted in much less tissue growth. The Mahan soil had less plant nutrients existing in the soil compared to the Cecil and Groseclose soils (Refer to Table 2.5 for comparison of soils).

Fig. 3.2. Corn tissue dry weights from corn grown in Mahan, Groseclose, and Cecil Soils amended with 8.96 Mg/ha poultry manure.



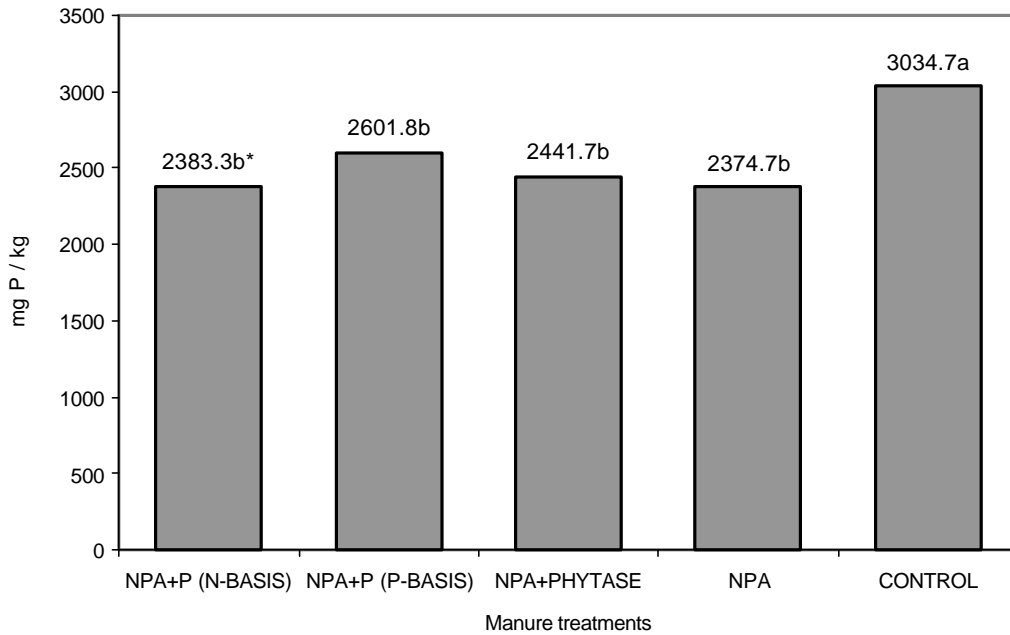
Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
 Statistical analyses were conducted separately on each soil type.

Though overall differences in dry matter exist between these soils, the trends between treatments are very similar. For example, addition of phytase to the baseline NPA treatment resulted in no statistical differences in any of the three soils. When the alternative NPA+Phytase treatment is compared to the conventional NPA+P treatments on either an N or P basis, significant differences still do not arise. Therefore, the addition of phytase did not create differences in dry matter from corn grown in any of the three soils.

3.3.2. P comparisons

An overall comparison of P in corn tissue from manure treatments averaged across all three soil types is made in Fig. 3.3. The control soils resulted in the largest amount of P in corn tissue and was significantly higher than plant matter from corn grown in any of the manure treated soils. As seen from Fig. 3.1, the control soil produced the smallest

Fig. 3.3. P in corn tissue from corn grown in three Virginia soils amended with 8.96 Mg/ha poultry manure

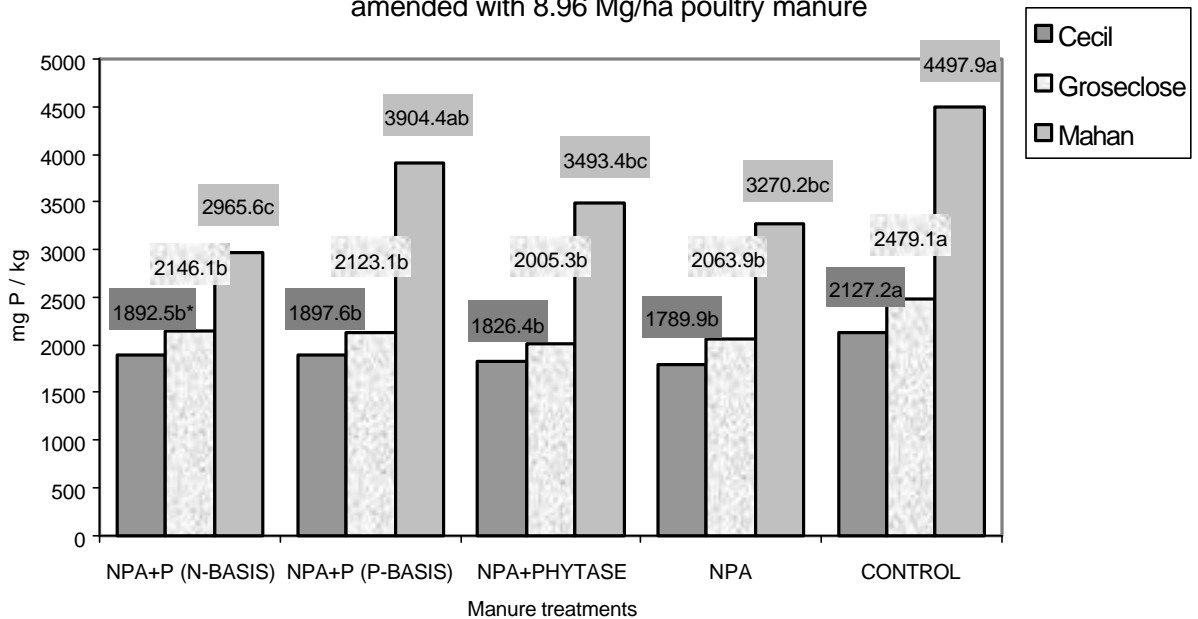


Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

amount of plant dry matter. It is common to observe a larger concentration of P accumulated in the tissue of smaller plants than larger plants, since the larger tissue tends to “dilute” P taken up by the plant. Elements found in larger concentrations also tend to accumulate in the tissue when other elements are not found at optimal levels for plant growth. Copper and Zn may be limiting plant growth in the control soils since they are found in lower concentrations in the control soils than soils treated with manure, causing an increased concentration of P in the control soils. This issue will be discussed in more detail later in this chapter. Phosphorous in corn tissue from corn grown in manure treated soils were all statistically similar, as seen from Fig. 3.3.

The three soils used in the greenhouse study are presented in Fig. 3.4 for comparison of P in corn tissue from each treatment. The Mahan soil contained much higher concentrations of P in the corn tissue than the Groseclose or Cecil soils. Once again, the Mahan soil had much lower dry matter contents than the other soils, thus allowing for more concentrated P levels in the tissue. The Groseclose and Cecil soils contained similar amounts of P in the corn tissue. Phosphorous in the plant tissue from

Figure 3.4. P in corn tissue grown in Cecil, Groseclose, and Mahan soils amended with 8.96 Mg/ha poultry manure



Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05.

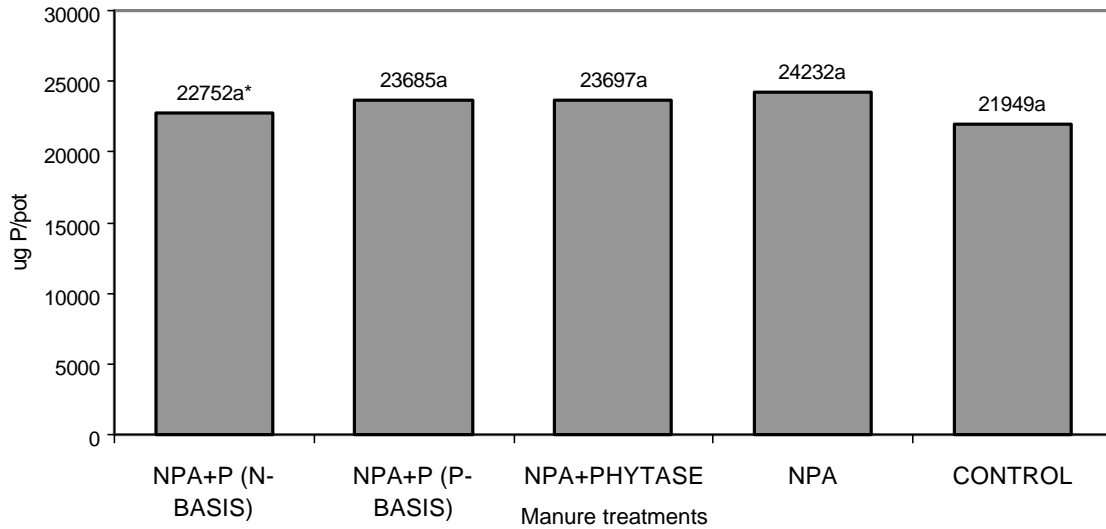
Statistical analyses were conducted separately on each soil type.

the Cecil and Groseclose soils were statistically the same across manure treatments. All manure treatments were significantly lower in P in the corn tissue compared to the control soils. The Mahan soil did show some differences between treatments, though the addition of phytase to the baseline NPA treatment showed no significant differences. The same is true when the alternative phytase treatment is compared to the conventional NPA+P treatments on either a N- or P-basis.

Plant uptake of P for each treatment averaged across all soil types is shown in Fig. 3.5. Plant uptake of P does not vary significantly among any of the treatments, including the control. The control treatment does show the lowest uptake of P but had the largest

concentration of P in the tissue. As previously explained, smaller plants take up less P in the plant, but concentrate the P that is taken up in the tissue.

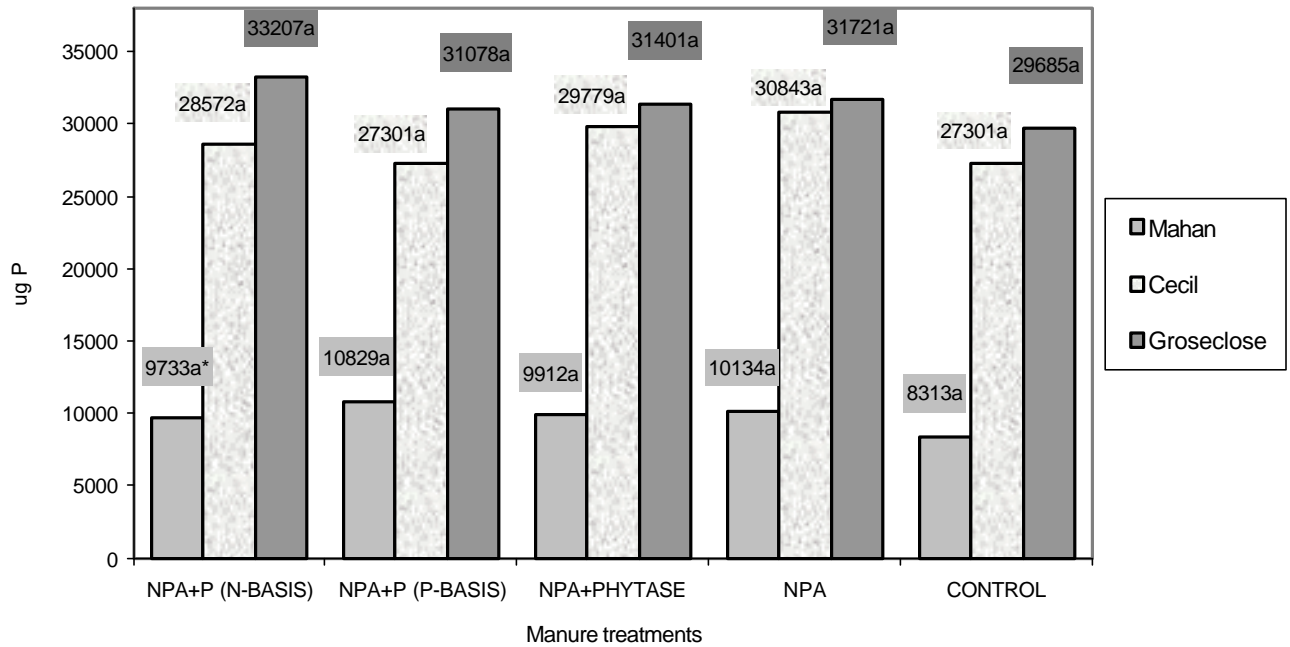
Fig. 3.5. Plant uptake of P from corn grown in three Virginia soils amended with 8.96 Mg/ha poultry manure



Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

As observed in Fig. 3.6, soil type did not affect differences in plant uptake of P between treatments. Plant uptake of P did not differ significantly between the manure treatments and control in any of the three soils. Plant uptake of P from corn grown in the Mahan soil was considerably less than the Groseclose and Cecil soils. Once again, corn grown in the Mahan soil was much smaller than corn grown in the other soils. Corn grown in the Groseclose soil had a slightly higher amount of P uptake than the Cecil soil across all treatments.

Fig. 3.6. Plant uptake of P from corn grown in Groseclose, Cecil, and Mahan Soils amended with 8.96 Mg/ha poultry manure

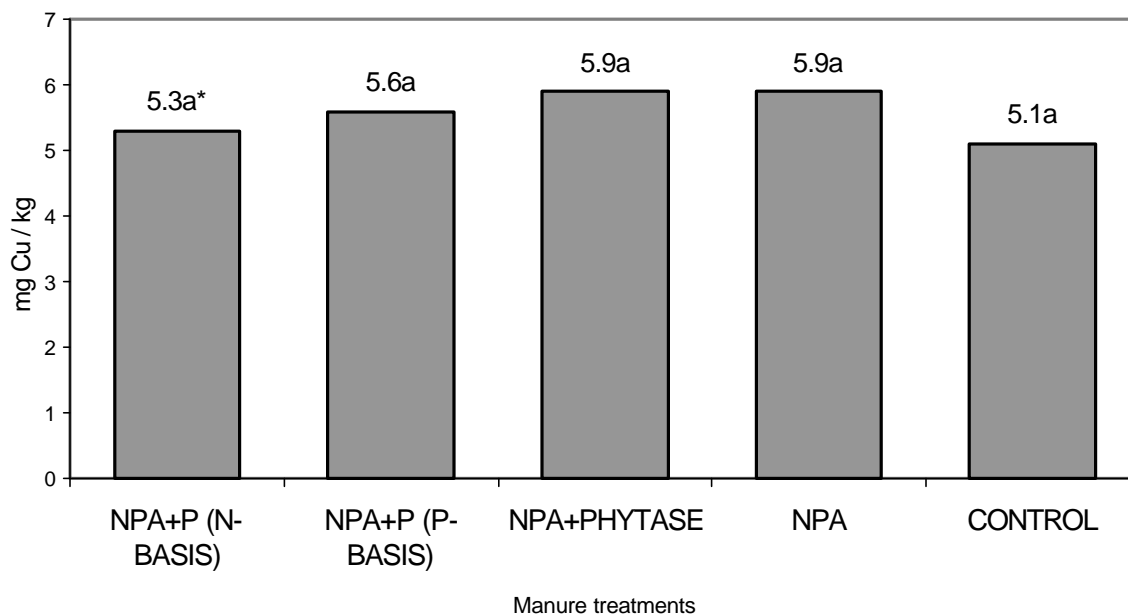


*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
 Statistical analyses were conducted separately on each soil type.

3.3.3. Cu comparisons

Copper in corn tissue for each treatment averaged across all soil types is presented in Fig. 3.7. As seen from Fig. 3.7, no statistical differences exist between any of the treatments, including the control. Therefore, addition of phytase had no impact on Cu found in plant tissue.

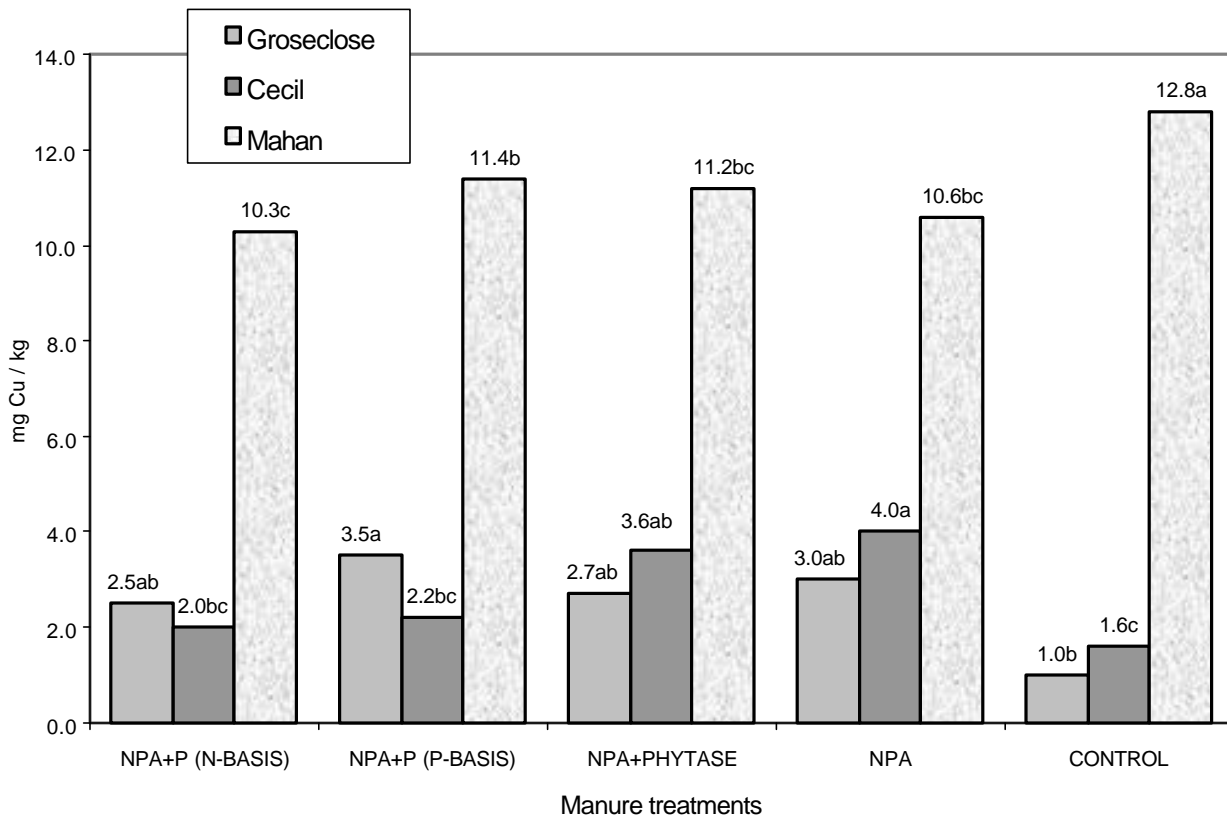
Fig. 3.7. Copper concentrations in corn tissue from corn grown in three Virginia soils amended with 8.96 Mg/ha poultry manure



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

Copper concentrations in corn tissue for corn grown in each soil type amended with manure are presented in Fig. 3.8. Corn grown in the Mahan soil contained the highest concentration of Cu, while corn grown in the Cecil and Groseclose soils had much lower Cu concentrations. When the soils are examined separately, some notable differences between treatments exist. For example, Cu in corn tissue was significantly higher in the Mahan control soil than any manure treatment, while the opposite occurs in the Cecil and Groseclose soils. The Mahan soil contains the highest concentrations of residual soil Cu, thus allowing for more Cu to accumulate in the plant tissue. It is important to note that the addition of phytase to the baseline NPA treatment did not create significant

Fig. 3.8. Copper in corn tissue from corn grown in Groseclose, Cecil, and Mahan soils amended with 8.96 Mg/ha poultry manure.

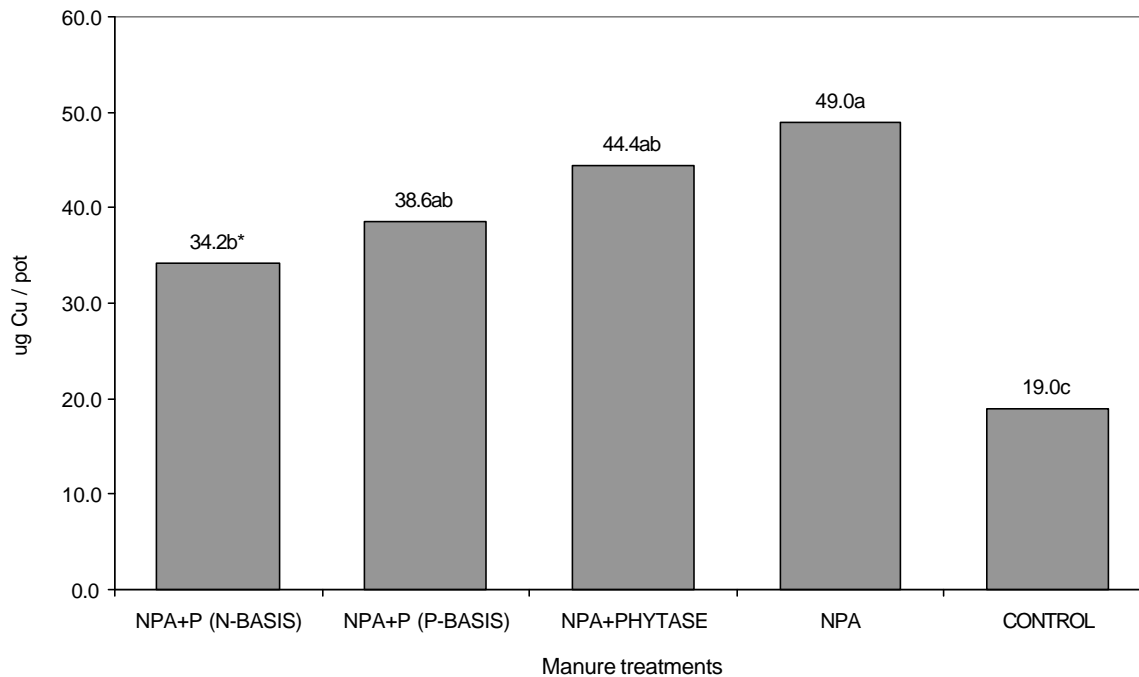


*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
 Statistical analyses were conducted separately on each soil type.

differences in plant tissue Cu in any of the three soils. The phytase treatment is statistically similar to the NPA+P treatments on both a N- and P-basis.

Plant uptake of Cu from each treatment averaged across soil type is given in Fig. 3.9. Plant uptake of Cu is consistently higher among all manure treatments compared to the control. This is the same relationship observed with Cu concentrations in corn tissue. Recall that P uptake had these same trends, although P in corn tissue was highest in the control. As stated earlier, it is common to see increased concentrations of elements in the plant tissue when found to be plentiful in the soil. This is especially true when certain elements are limiting plant growth. This may be the case with Cu for corn grown in both the Cecil and Groseclose soils, since sufficiency levels for corn are 6-20 mg Cu kg⁻¹ (Jones, 1998). Zinc could also be limiting corn growth and will be discussed later. Phosphorous is found in high concentrations in all three soils before addition of manure, resulting in high concentrations in plant tissue. Once again, no statistical differences

Fig. 3.9. Plant uptake of Cu from corn grown in three Virginia soils amended with 8.96 Mg/ha poultry manure

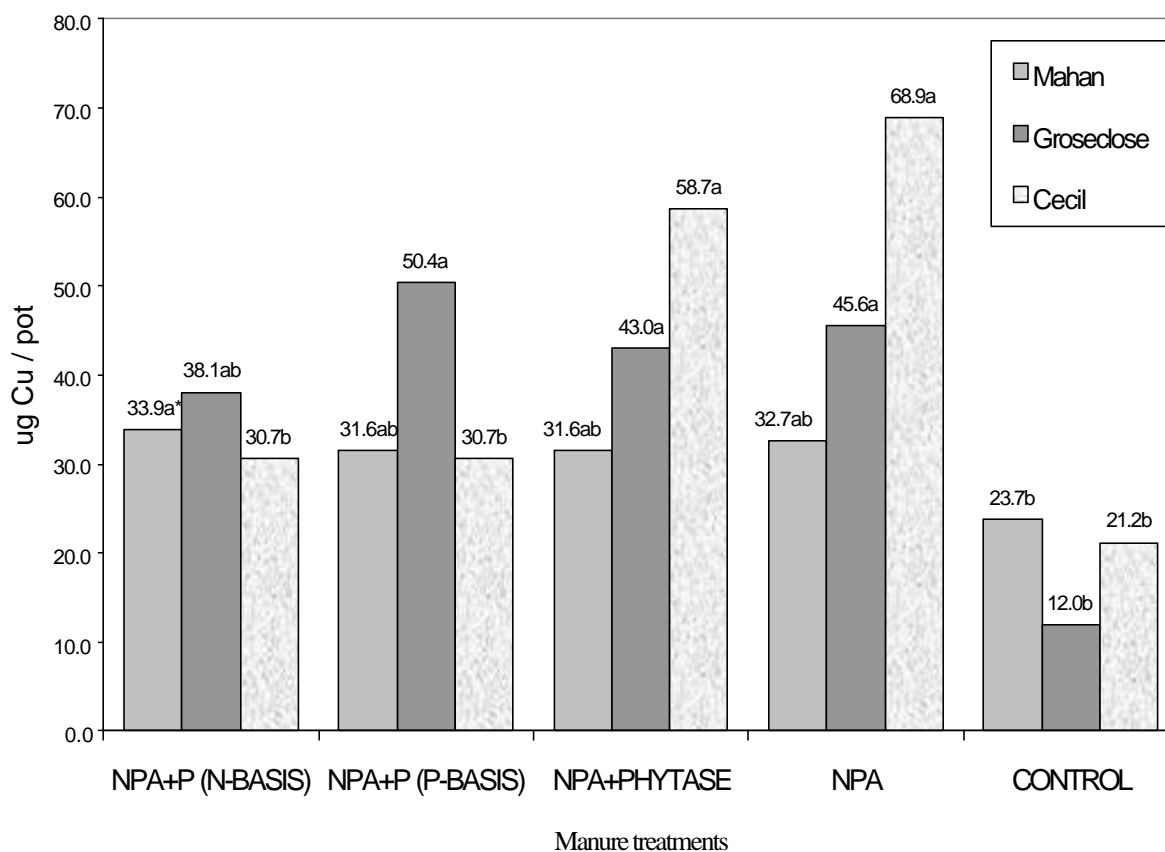


*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

exist between the NPA and NPA+Phytase treatments, as well as between NPA+Phytase and the NPA+P treatments on either a N- or P-basis. A 23% increase in plant uptake of Cu does occur when the NPA+Phytase treatment replaces the NPA+P (N-basis) treatment (44.4 vs. 34.2 ug Cu for NPA+Phytase and NPA+P treatments on equal N- and Cu-basis). A smaller 13% increase is observed in Cu uptake from corn grown in the NPA+Phytase treatment rather than the NPA+P (P-basis) treatment (44.4 vs. 38.6 ug Cu for NPA+Phytase and NPA+P treatments on P-basis, respectively).

Plant uptake of Cu graphed for each soil type amended with manure is shown in Fig. 3.10. None of the soils contain consistently higher uptake of Cu by corn among all treatments compared to the other soils. For example, the Cecil soil has higher plant uptake of Cu in the NPA and NPA+Phytase treatments, while the Groseclose soil has the highest plant uptake from the NPA+P treatments of any soil. Plant uptake of Cu is not affected significantly by the addition of phytase to the baseline NPA treatment in any of the three soils. Replacing the NPA+P treatments with the phytase treatment does not cause any significant differences to occur in either the Mahan or Groseclose soil. The

Fig. 3.10. Plant uptake of Cu from corn grown in Groseclose, Cecil, and Mahan soils amended with 8.96 Mg/ha poultry manure.



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
Statistical analyses were conducted separately on each soil type.

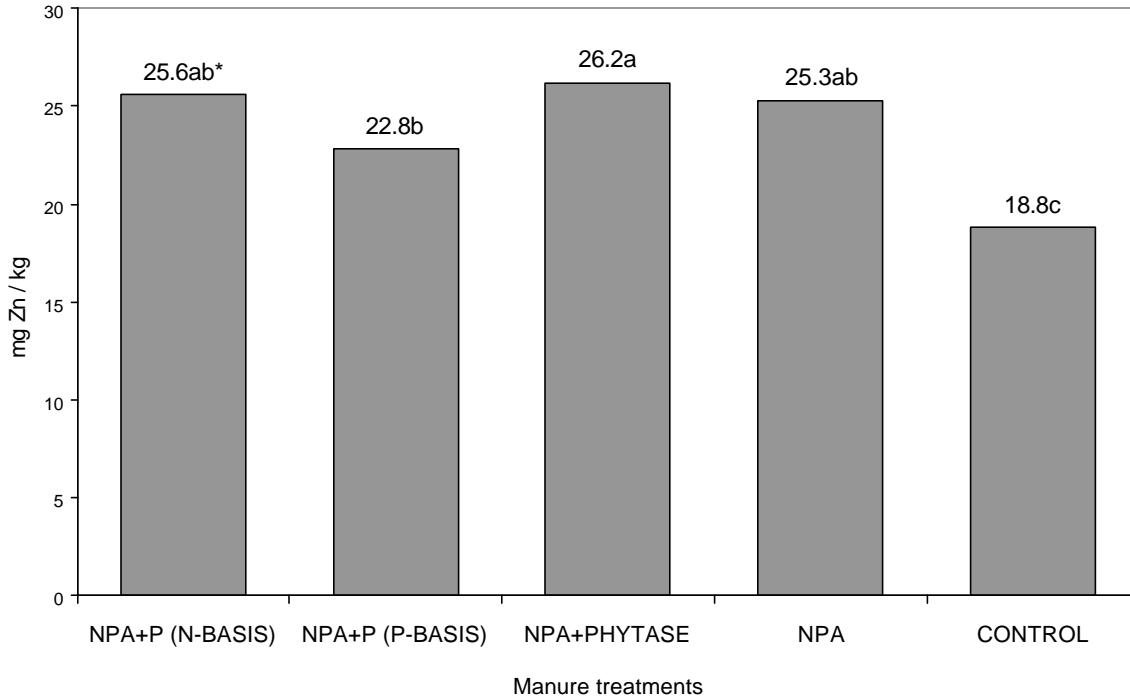
NPA+Phytase treatment is significantly higher in the Cecil soil for Cu uptake than both NPA+P treatments.

3.3.4. Zn comparisons

Zinc concentrations in corn tissue is shown in Fig. 3.11 averaged across all soil types. Like Cu, the control soil has the least amount of Zn in the corn tissue. This once again, may be attributed to Zn being a limiting nutrient in the soil. Zinc in the corn tissue is statistically lower from corn grown in the control soil than all manure treated soils. The addition of phytase to the baseline NPA treatment did not create significant differences in Zn in the corn tissue. Replacement of the conventional NPA+P (N-basis)

treatment for the alternative NPA+Phytase treatment does not result in any significant differences either. When NPA+Phytase replaces the NPA+P treatment on a P-basis, a significant 13% increase in Zn was found in the tissue results.

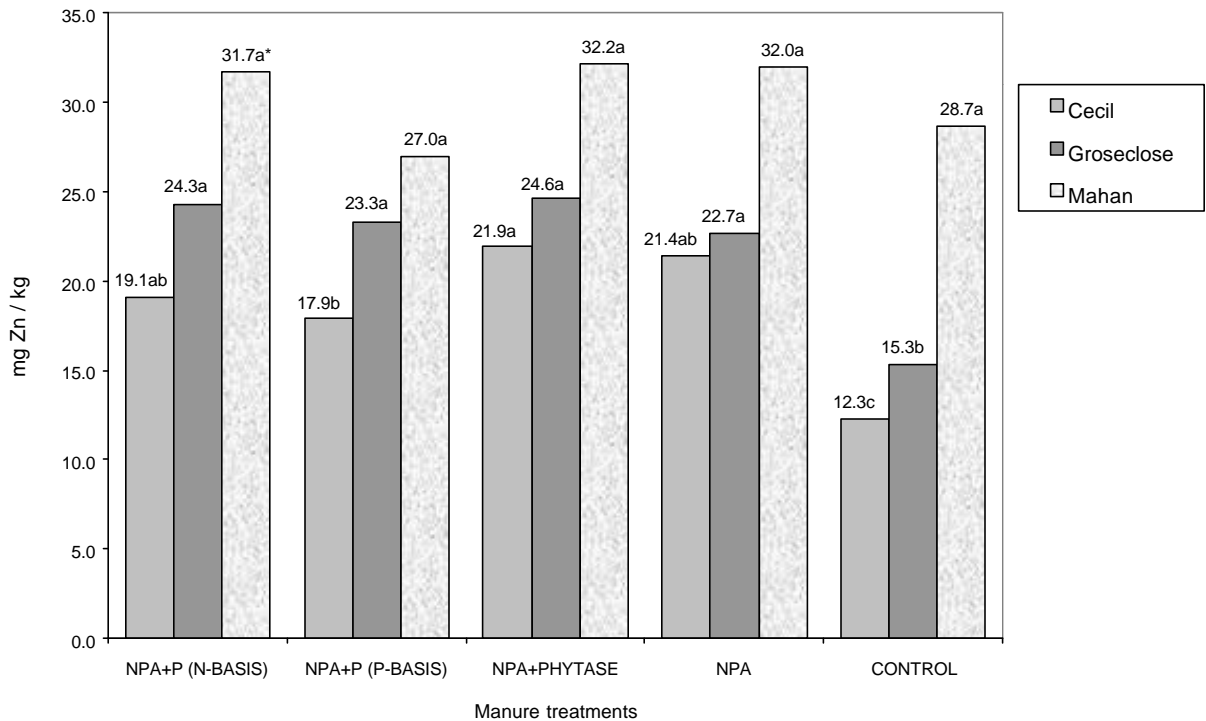
Fig. 3.11. Zinc in corn tissue from corn grown in three Virginia soils amended with 8.96 Mg/ha poultry manure



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

Zinc concentrations in corn tissue for each treatment is graphed separately for each soil type in Fig. 3.12. Corn grown in the Mahan soil contained the largest concentration of Zn in its plant tissue across all treatments. All treatments from the Mahan soil had statistically similar Zn concentrations in plant tissue. Corn grown in the Groseclose soil contained statistically similar Zn concentrations among the manure treatments, and were all significantly larger than the control. Addition of phytase to the baseline NPA treatment produced no significant differences in Zn found in plant tissue from corn grown in the Cecil soil. The same is true when the NPA+Phytase treatment is compared to the

Fig. 3.12. Zinc in corn tissue from corn grown in three Virginia soils amended with 8.96 Mg/ha poultry manure

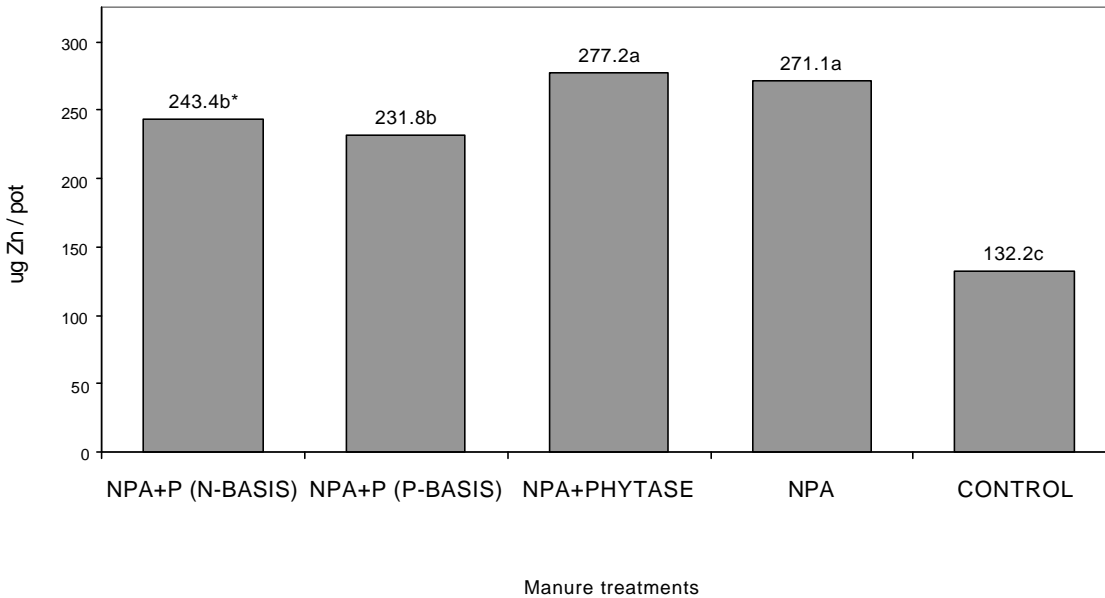


*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
 Statistical analyses were conducted separately on each soil type.

NPA+P (N-basis) treatment. A significant 18% increase in Zn is observed when the NPA+Phytase treatment replaces the NPA+P treatment on a P-basis. As previously mentioned, Zn may be limiting plant growth in the Groseclose and Cecil soils, since sufficiency levels for corn are 25-100 mg Zn kg⁻¹ (Jones, 1998).

Plant uptake of Zn for each treatment averaged across soil type is shown in Fig. 3.13. All manure treatments have significantly larger plant uptake of Zn than the control. Addition of phytase does not produce significant differences in plant uptake of Zn. Replacing the NPA+P treatment on a N-basis with the NPA+Phytase treatment does significantly increase Zn uptake by 12% (277.2 vs. 243.4 ug Zn for NPA +Phytase and NPA+P treatments on N-basis, respectively). A significant 16% increase in plant uptake of Zn is observed when NPA+Phytase replaces the NPA+P treatment on a P-basis (277.2 vs. 231.8 ug Zn for NPA+Phytase and NPA+P treatments on P-basis, respectively).

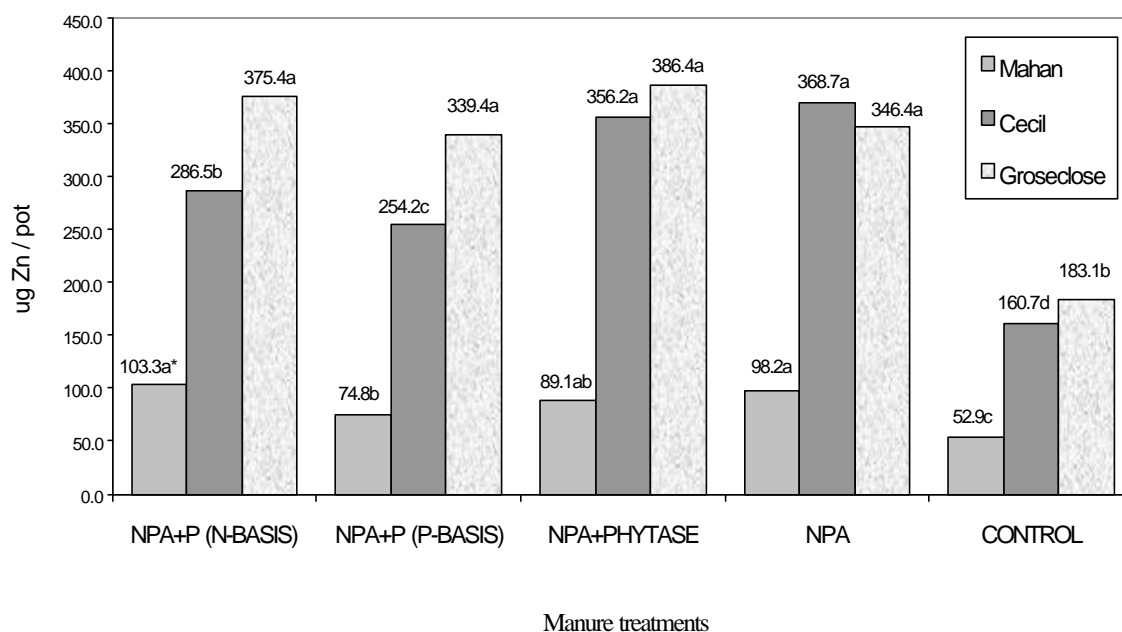
Fig. 3.13. Plant uptake of Zn from corn grown in three Virginia soils amended with 8.96 Mg/ha poultry manure



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

Plant uptake of Zn is given in Fig. 3.14 for each treatment graphed separately for each soil type. Corn grown in the Groseclose soil had the largest plant uptake of Zn for all treatments except the NPA. Plant uptake of Zn is smallest in the Mahan soil. Plant uptake of Zn is not significantly different in any of the three soils by addition of phytase to the baseline NPA treatment. Only the Cecil soil shows significant differences between the alternative NPA+Phytase treatment and the conventional NPA+P treatments. A significant 20% increase is observed in plant uptake of Zn in the Cecil soil when NPA+Phytase replaces the NPA+P (N-basis) treatment (356.2 vs. 286.5 ug Zn for NPA+Phytase and NPA+P treatments on N-basis, respectively). The increase is further heightened when the phytase treatment is compared with the NPA+P (P-basis) treatment. A 29% increase in plant uptake of Zn results when the phytase treatment replaces the NPA+P treatment on a P-basis (356.2 vs. 254.2 ug Zn for NPA+Phytase and NPA+P treatments on a P-basis, respectively).

Fig. 3.14. Plant uptake of Zn from corn grown in Groseclose, Cecil, and Mahan soils amended with 8.96 Mg/ha poultry manure



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
 Statistical analyses were conducted separately on each soil type.

3.3.5. Mehlich III extraction of soils after corn growth

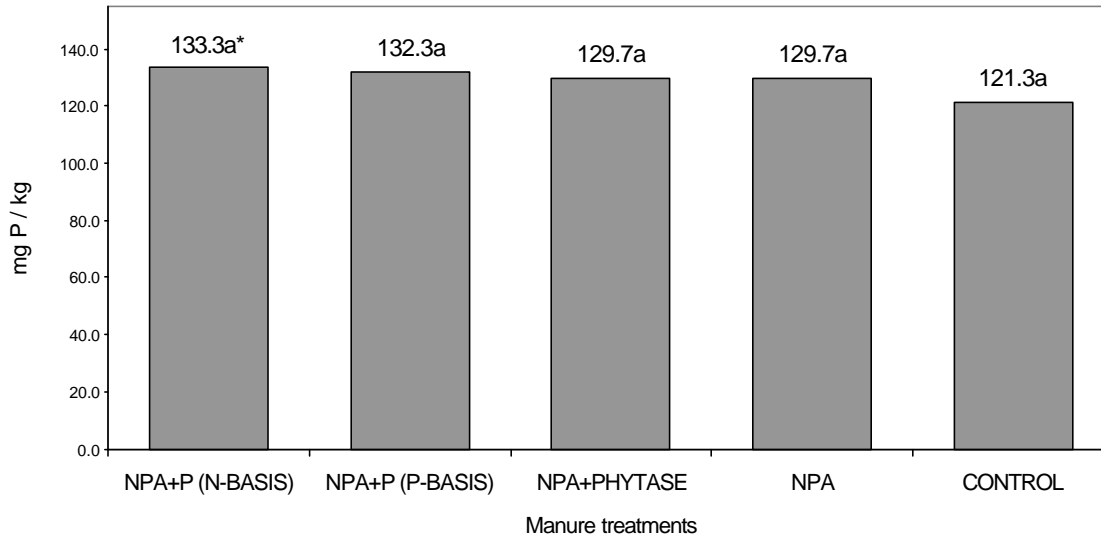
The manure-amended soils used for corn growth were extracted with Mehlich III to determine plant available P, Cu, and Zn concentrations remaining in the soil after corn growth.

3.3.6. P comparisons

The soils used in the corn study were extracted with Mehlich III and are given in Fig. 3.15 averaged across all soil types. The control soil with no manure contained the lowest concentration of plant available P, though no significant differences exist between

any treatments. Notice the addition of phytase to the baseline NPA treatment results in the same concentration of Mehlich III-extractable P.

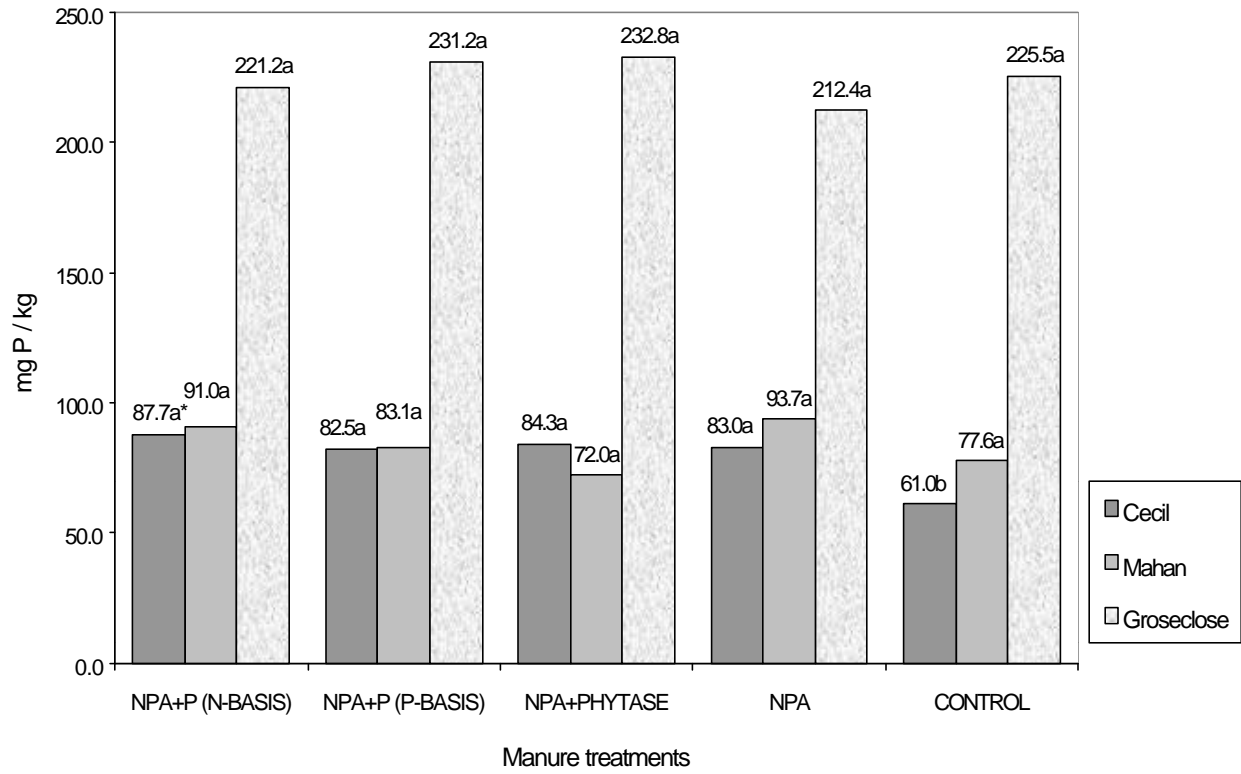
Fig. 3.15. Mehlich III extractable P extracted from three Virginia soils amended with 8.96 Mg/ha poultry manure after corn growth



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

Plant available P concentrations for all treatments are graphed separately for each soil type in Fig. 3.16. The Groseclose soil contained the highest plant available P concentrations for all treatments, and also contained the highest levels of residual soil P. The Groseclose and Mahan soils showed no statistical differences in P concentrations from each treatment. Manure amended treatments from the Cecil soil were statistically similar, with all treatments significantly higher than the control soil.

Fig. 3.16. Mehlich III extractable P extracted from Groseclose, Cecil, and Mahan soils amended with 8.96 Mg/ha poultry manure after corn growth.



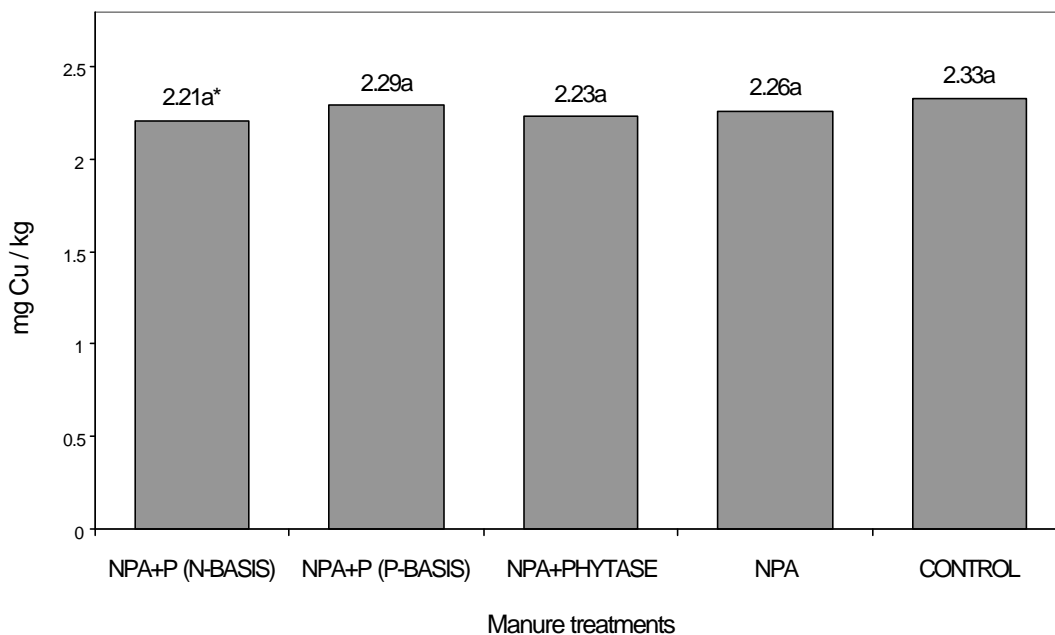
*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

Statistical analyses were conducted separately on each soil type.

3.3.7. Cu comparisons

Plant available Cu concentrations of soils after corn growth for all treatments averaged across soil type are given in Fig. 3.17. The control soil contained the highest concentration of Cu, though all treatments are statistically similar.

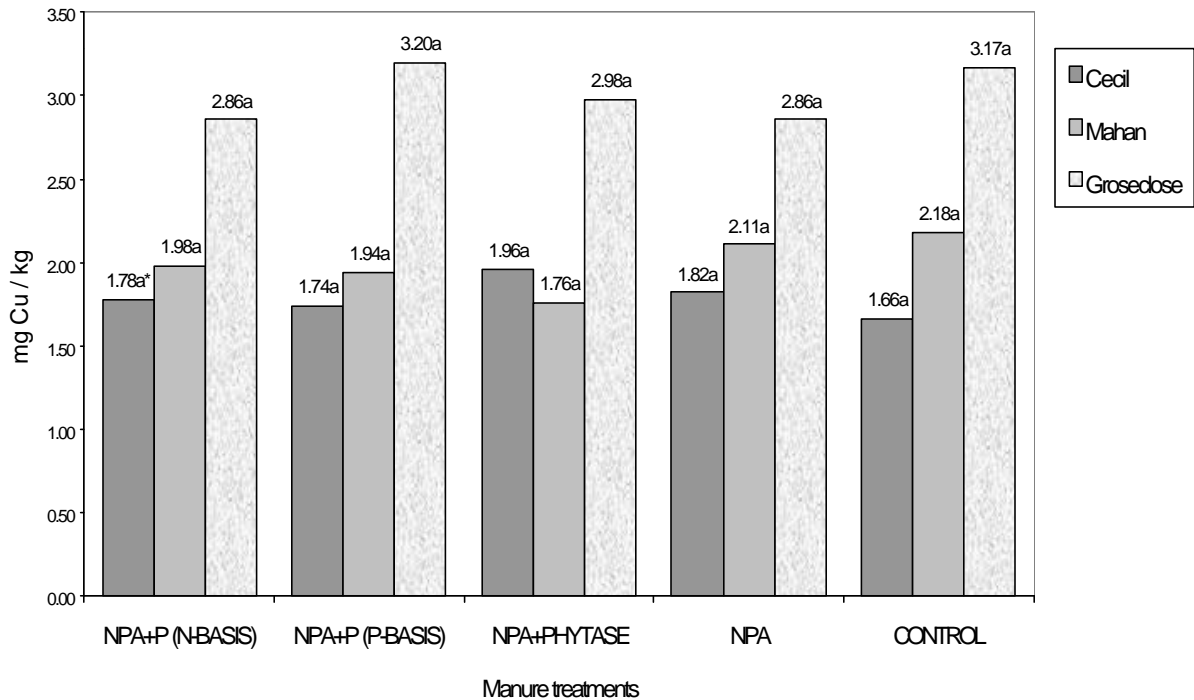
Fig. 3.17. Mehlich III extractable Cu extracted from three Virginia soils amended with 8.96 Mg/ha poultry manure after corn growth



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

Mehlich III-Cu concentrations for each treatment graphed separately for each soil type are presented in Fig. 3.18. The Groseclose soil contains the highest Cu concentrations, with more similar concentrations found in the Mahan and Cecil soils. No significant differences were observed between any treatment for any of the three soils.

Fig. 3.18. Mehlich III extractable Cu extracted from Groseclose, Cecil, and Mahan soils amended with 8.96 Mg/ha poultry manure after corn growth.



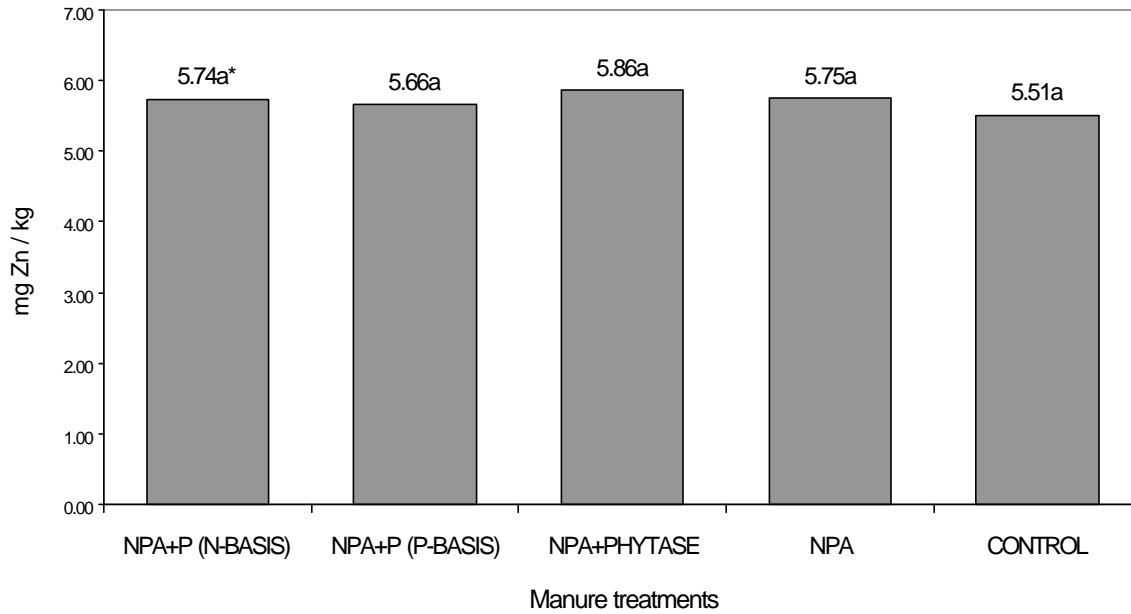
*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
 Statistical analyses were conducted separately on each soil type.

3.3.8. Zn comparisons

Zinc concentrations extracted with Mehlich III for each treatment averaged across soil type are presented in Fig. 3.19. As observed with both P and Cu, no statistical differences exist between treatments in plant extractable zinc. When these treatments are graphed separately for each soil type, differences do exist between treatments. As seen in Fig. 3.20, no significant differences exist between treatments in the Groseclose soil. Addition of phytase to the baseline NPA treatment creates no significant differences in the Cecil soil. The alternative phytase treatment also does not differ statistically from either conventional NPA+P treatment. However, addition of phytase to the baseline NPA treatment in the Mahan soil decreases Zn availability by a significant 27% (1.08 vs. 1.47

mg Zn/kg for NPA+Phytase and NPA treatments, respectively). Replacement of NPA+P (N-basis) treatment for NPA+Phytase decreases Zn solubility by a significant 21% (1.08

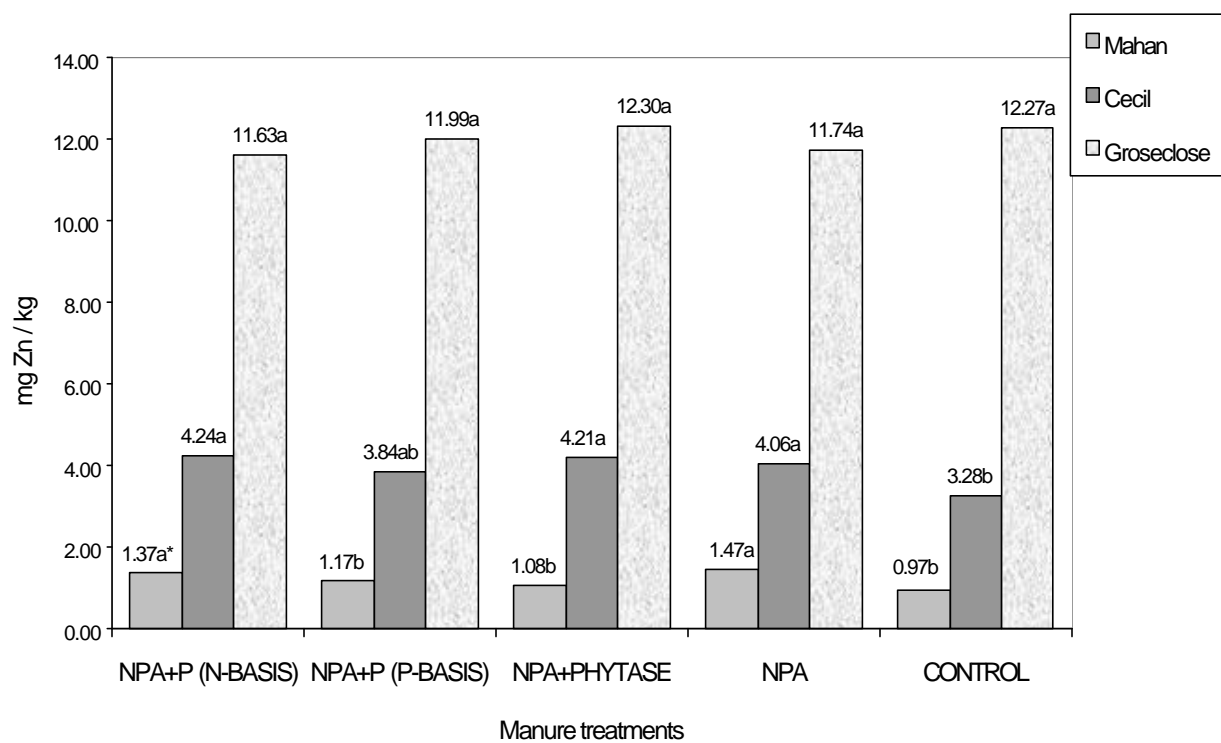
Fig. 3.19. Mehlich III extractable Zn extracted from three Virginia soils amended with 8.96 Mg/ha poultry manure after corn growth



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

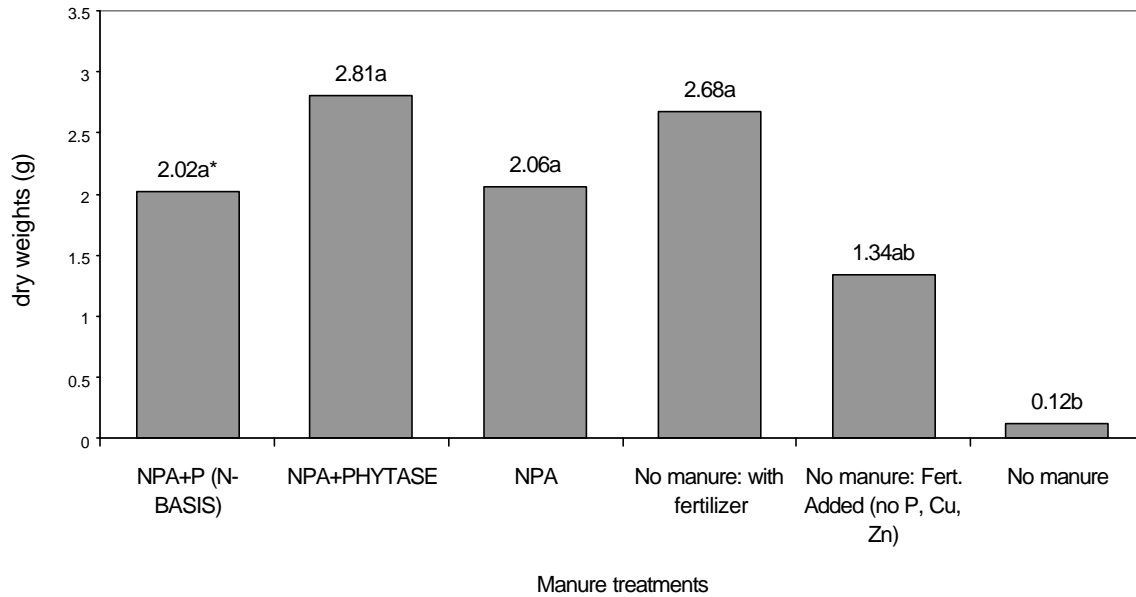
vs. 1.37 mg Zn/kg for NPA+Phytase and NPA+P treatments on N-basis, respectively), though no significant difference exists when these treatments are compared on a P-basis.

Fig. 3.20. Mehlich III extractable Zn from Groseclose, Cecil, and Mahan soils amended with 8.96 Mg/ha poultry manure after corn growth.



*Means with same letter not significantly different by Duncan's Multiple Range Test at $\alpha=0.05$
 Statistical analyses were conducted separately on each soil type.

Fig. 3.21. Corn tissue dry weights for corn grown in sand amended with 2.24 Mg/ha poultry manure



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

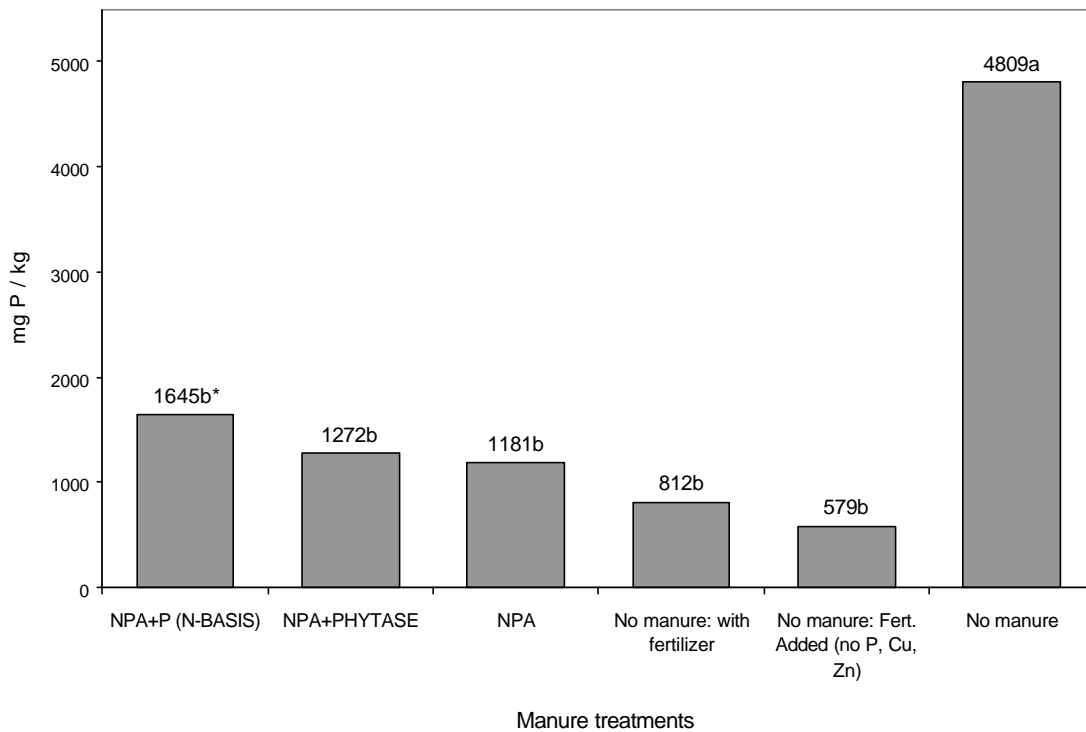
3.3.9. Corn grown in sand

Harvested corn grown in sand was dried and weighed, and digested in HNO_3 to determine P, Cu, and Zn concentrations in the plant tissue and plant uptake. The dry weight of corn tissue for each manure treatment applied to sand for corn growth is given in Fig. 3.21. It is important to note that three control treatments were used in this study. The treatment labeled “no manure” in the graphs contained only sand as a potting medium with no manure or fertilizer addition. “No manure: Fert. Added (no P, Cu, and Zn)” contains liquid fertilizer added to sand in nonlimiting quantities without addition of P, Cu, and Zn in the fertilizer. “No manure: with fertilizer” contains nonlimiting quantities of all nutrients added as a liquid fertilizer to the sand. As seen from Fig. 3.21, the manure treatments do not differ in dry tissue weight. Therefore, no effect from addition of phytase is seen in the tissue weights of the corn.

3.3.10. P Comparisons

Phosphorous concentrations in corn tissue for each of the treatments for corn grown in sand are shown in Fig. 3.22. The control treatment that contains no manure or fertilizer amendments resulted in the largest concentration of P in the corn tissue and is significantly higher than all other treatments. All other treatments contain statistically similar concentrations of P in corn tissue.

Fig. 3.22. Phosphorous in corn tissue grown in sand amended with 2.24 Mg/ha poultry manure

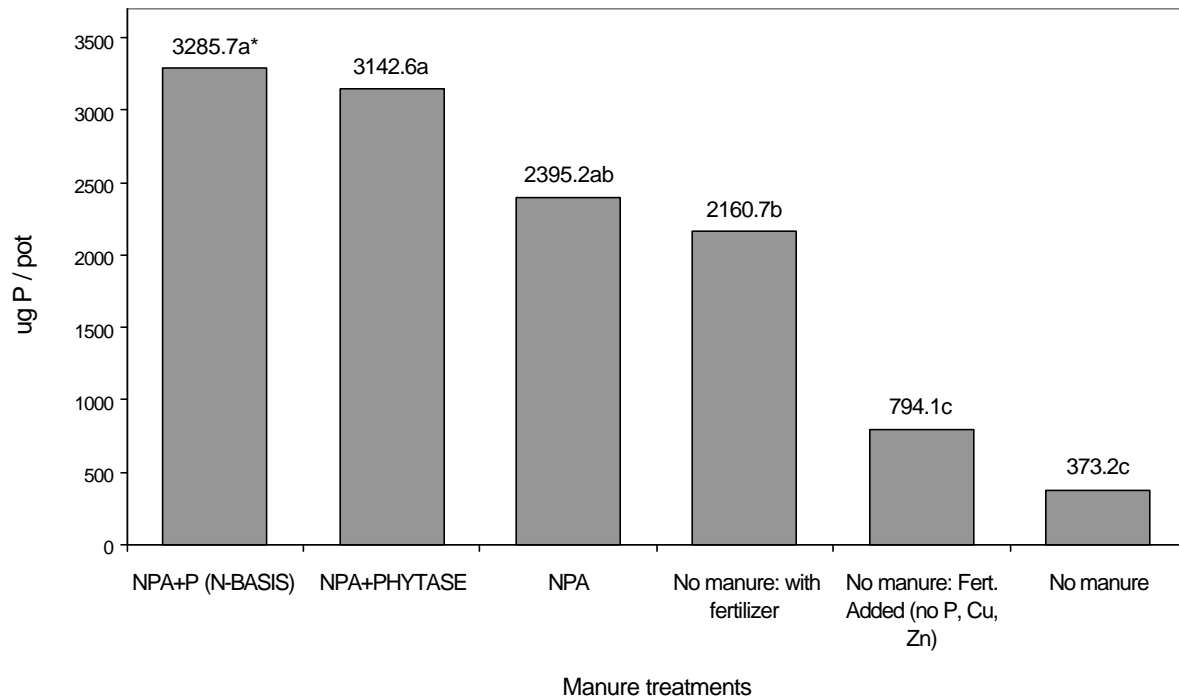


*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

Phosphorous uptake for each treatment is given in Fig. 3.23. Addition of phytase to the baseline NPA treatment does not produce significant differences in plant uptake of P. Comparison of the alternative phytase treatment with the NPA+P treatment on a N-basis does not produce significant differences either. Notice the control treatment with no manure or fertilizer amendments contains the lowest uptake of P. This is due to the

fact that nutrients tend to concentrate in the tissue of smaller plants. The control treatment with no manure addition contained the smallest dry tissue matter of any treatments, and resulted in the lowest uptake of P.

Fig. 3.23. Phosphorous uptake by corn grown in sand amended with 2.24 Mg/ha poultry manure

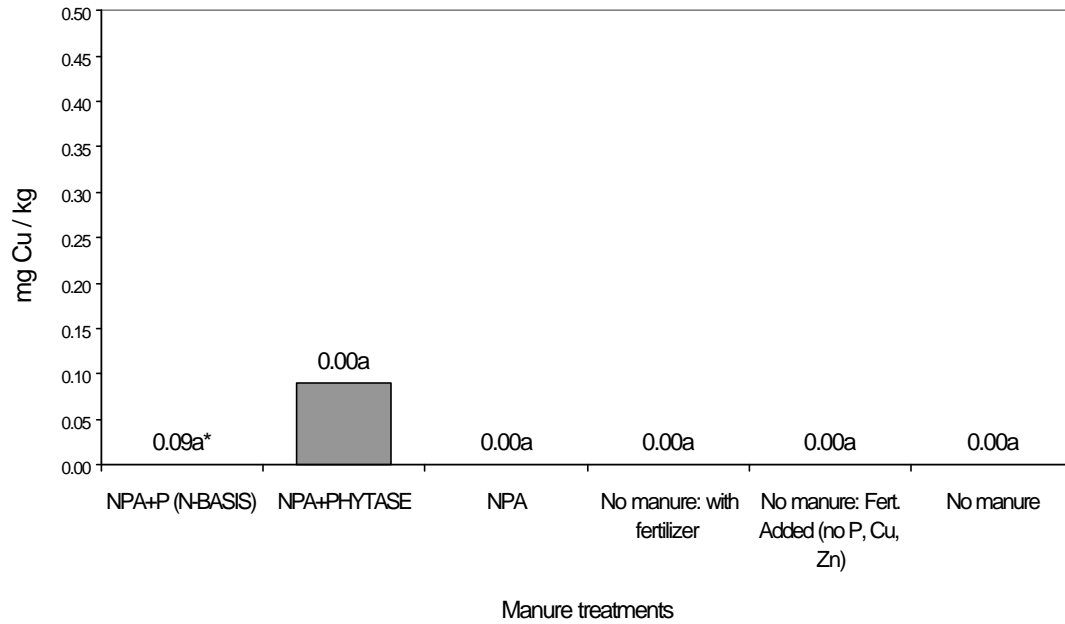


*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

3.3.11. Cu comparisons

Copper concentrations in corn tissue for each treatment grown in sand are shown in Fig. 3.24. As observed by the graph, only the NPA+Phytase treatment resulted in any detectable Cu concentration in the corn tissue, with only 0.09 ppm Cu.

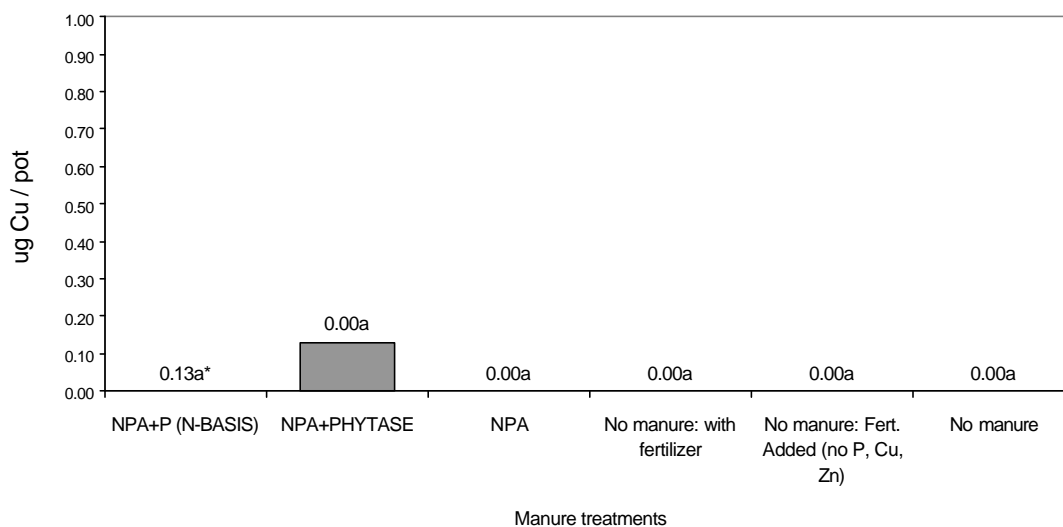
Fig. 3.24. Copper in corn tissue grown in sand amended with 2.24 Mg/ha poultry manure



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

No Cu detection in the plant tissue in any treatments except for NPA+Phytase results in no plant uptake of Cu in any treatment except for this treatment, with only 0.13 ug Cu taken up by the plant. Plant uptake of Cu is given in Fig. 3.25.

Fig. 3.25. Copper uptake by corn grown in sand amended with 2.24 Mg/ha poultry manure

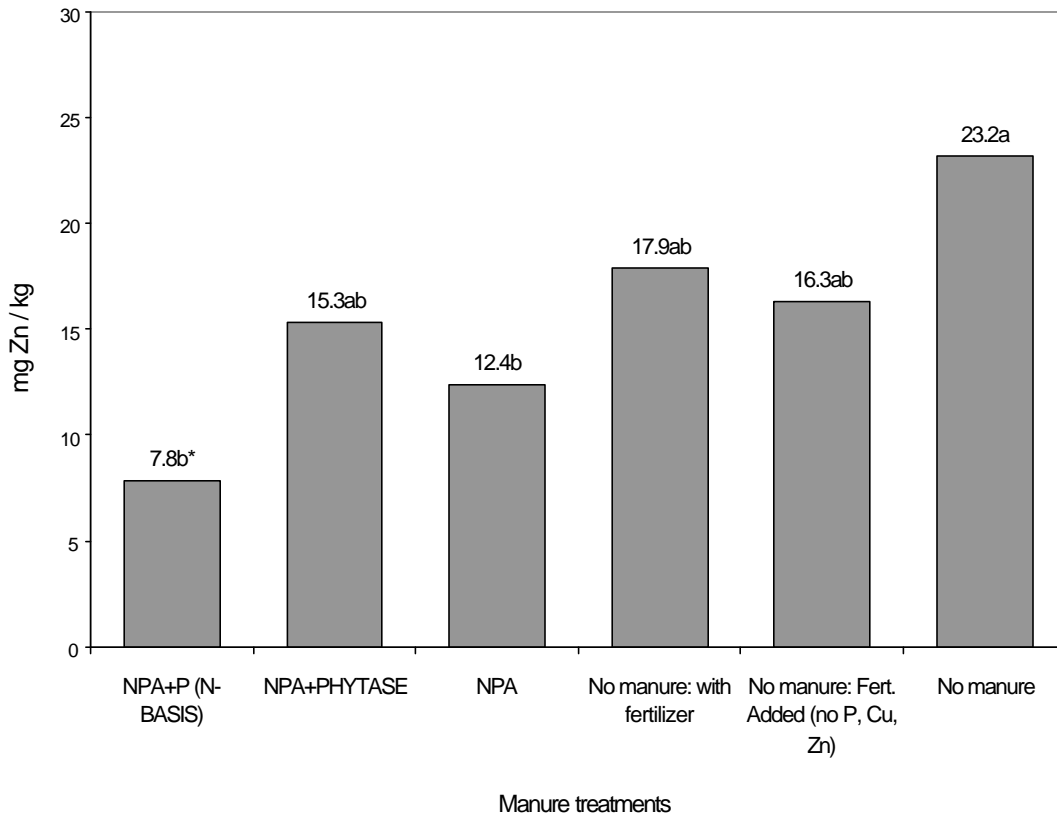


*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

3.3.12. Zn comparisons

Zinc concentrations in the corn tissue for each treatment are presented in Fig. 3.26. The control treatment with no manure or fertilizer addition contained the largest concentration of Zn in the corn tissue. Comparing the NPA+Phytase treatment to either the baseline NPA treatment or NPA+P (N-basis) treatment did not produce statistical differences.

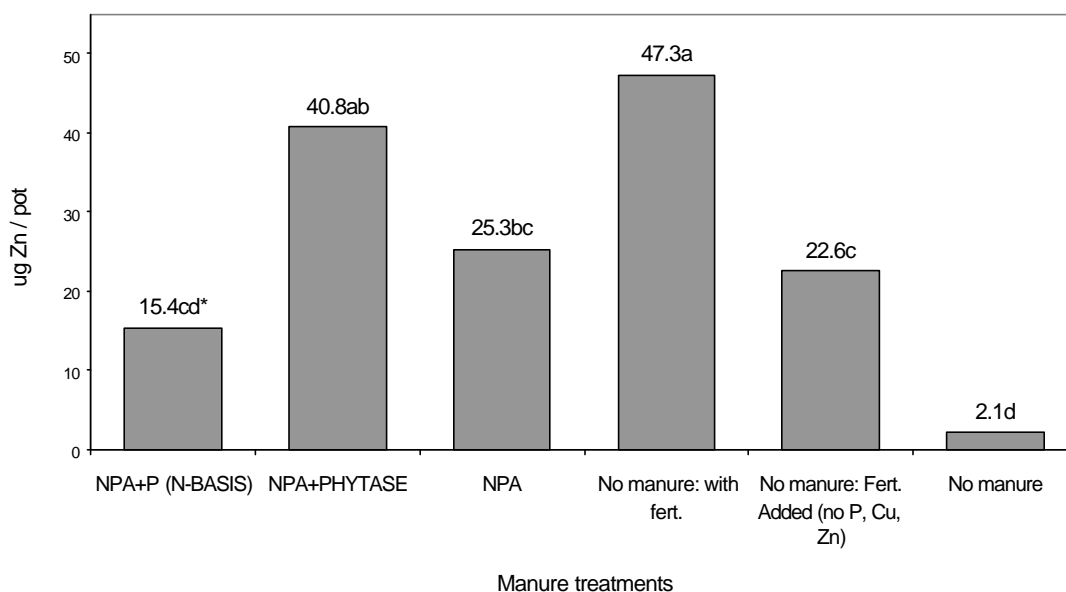
Fig. 3.26. Zinc in corn tissue grown in sand amended with 2.24 Mg/ha poultry manure



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

Treatments graphed by plant uptake of Zn are presented in Fig. 3.27. The control treatment with no manure addition has the lowest plant uptake of any treatments. This is the same scenario presented earlier with P. The control soil contained the lowest dry matter and therefore the lowest plant uptake of zinc.

Fig. 3.27. Zinc uptake by corn grown in sand.



*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05

3.4. SUMMARY AND CONCLUSIONS

3.4.1. Effect of phytase supplementation on corn grown in southeastern soils

Dry matter production by young corn seedlings was not impacted by addition of phytase to the baseline NPA treatment from corn grown in any of the three soils. The alternative phytase treatment also did not differ significantly from the conventional NPA+P treatments on either a N- or P- basis. The Mahan soil had the lowest tissue growth across all treatments, while the Cecil and Groseclose soils had similar dry tissue weights.

Corn grown in the control soils contained the highest concentration of P in corn tissue over all three soil types, and was statistically higher than all manure treatments. This may be attributed to the fact the P in the corn tissue in the control is more concentrated since much less tissue growth occurred from corn grown in the control soils. All manure treatments had statistically similar concentrations of P in the corn tissue. The Mahan soil contained the highest concentration of P in the corn tissue across all treatments, due to the low tissue weights from corn grown in this soil type. No

significant differences in P in corn tissue are observed from the addition of phytase to the baseline NPA treatment for any soil type. The same is true when the alternative NPA+Phytase treatment is compared to the NPA+P treatments on either a N- or P-basis. No significant differences occurred between treatments for P uptake for any of the three soils, though the control had the lowest P uptake of any treatment. This is due to the low tissue growth of corn. For this reason, the Mahan soil also had the lowest P uptake for all treatments.

Copper in corn tissue was highest across all treatments from corn grown in the Mahan soil. Once again, this is attributed to low tissue matter produced from corn grown in the Mahan soil. Copper concentrations did not differ significantly for any soils when phytase was added to the baseline NPA treatment. Significant differences were not observed when NPA+Phytase is compared to the NPA+P treatments on either a N- or P-basis. The control soil averaged across all soil types had the lowest concentration of Cu in corn tissue, whereas P in corn tissue was highest in the control. Nutrients found in high quantities tend to accumulate in the tissue, especially when other elements are found in quantities that may limit plant growth. Copper may be limiting plant growth since the control soil has the lowest concentration of Cu in its tissue, and has the highest concentration of P compared to the other treatments.

Plant uptake of Cu was significantly higher for all manure treatments compared to the control. Addition of phytase to the baseline NPA treatment averaged across all soil types did not produce a significant difference. A significant difference did not result when the alternative NPA+Phytase treatment is compared to the conventional NPA+P treatments on either a N- or P-basis in either the Mahan or Groseclose soil. The NPA+Phytase treatment was significantly higher than both NPA+P treatments on both a N- and P- basis in the Cecil soil.

Zinc in corn tissue was statistically lower in the control treatment compared to the manure treated soils. This once again, like Cu, may be attributed to Zn being found in limiting quantities in the control. Zinc in corn tissue averaged across all soil types resulted in no statistical differences with the addition of phytase to the baseline NPA treatment. No statistical differences resulted when NPA+Phytase was compared to the NPA+P treatment on an N-basis. When NPA+Phytase replaced the NPA+P treatment on

a P-basis, a significant 13% increase in Zn in the corn tissue resulted (26.2 vs. 22.8 mg Zn kg⁻¹ for NPA+Phytase and NPA+P treatments on a P-basis, respectively). The Mahan soil contained the highest concentration of Zn in the corn tissue results across all treatments.

Plant uptake of Zn was statistically higher for all manure treatments compared to the control. Plant uptake of Zn was not significantly different with the addition of phytase to the baseline NPA treatment for any soil type. Plant uptake of Zn was also not significantly different between the alternative NPA+Phytase and NPA+P treatments on a N- or P- basis for either the Groseclose or Mahan soil. NPA+Phytase was significantly higher than the NPA+P treatments on both a N- and P- basis for the Cecil soil.

3.4.2. Mehlich III extraction of soils after corn growth

No significant differences were observed in Mehlich III extraction of P between manure treatments for any of the three soils. Therefore, addition of phytase did not produce significant differences in extractable P from soils after corn growth. The same is true for Cu extracted by Mehlich III. Zinc extracted did not produce significant differences between manure treatments in either the Groseclose or Cecil soil. Addition of phytase to the baseline NPA treatment decreased Zn availability in the Mahan soil by a significant 27% (1.08 vs. 1.47 mg Zn kg⁻¹ for NPA+Phytase and NPA treatments, respectively). Replacement of NPA+P (P-basis) treatment for NPA+Phytase does not create statistical differences in extractable Zn. However, when these treatments are compared on an N-basis, Zn solubility decreases by a significant 21% when NPA+Phytase replaces the NPA+P treatment (1.08 vs. 1.37 mg Zn kg⁻¹ for NPA+Phytase and NPA+P treatments on N-basis, respectively).

3.4.3. Effect of phytase supplementation on corn grown in sand

Corn tissue dry weight was not affected significantly by the addition of phytase to the NPA treatment from corn grown in sand. Phosphorous in corn tissue did not statistically differ between any manure treatment. The control with no manure or fertilizer addition contained the highest concentration of P in the corn tissue, as observed in the previous corn study, due to the smaller tissue weight. Plant uptake of P was not

influenced by phytase addition to the baseline NPA treatment and thus did not affect plant uptake. Copper in corn tissue was detectable only for the NPA+Phytase treatment, with only 0.09 mg Cu kg⁻¹ observed in this treatment. Therefore, all other treatments resulted in no plant uptake of Cu. Addition of phytase to the baseline NPA treatment did not result in any statistical differences in Zn in the harvested corn tissue. The control treatment contained the highest concentration of Zn in the tissue. Plant uptake of Zn was not significantly different between the NPA and NPA+Phytase treatments, although the NPA+Phytase treatment was significantly higher than the NPA+P treatment on a N-basis, with an increase in Zn uptake by 62% (40.8 vs. 15.4 mg Cu kg⁻¹ for NPA+Phytase and NPA+P treatments on N-basis, respectively).

CHAPTER 4: FRACTIONATION STUDY

4.1. INTRODUCTION

Phytase is known to reduce total P in manure after excretion. An important question many researchers are seeking to answer is the overall effects that phytase and HAP have on P, Cu, and Zn solubility in manure. Though total P is reduced, does the chemical form of P, Cu, and Zn change with the use of phytase and HAP? This chapter focuses on the chemical forms of P, Cu, and Zn found in the manure containing phytase and HAP compared to manure with no amendments.

4.2. MATERIALS AND METHODS

4.2.1. Nonsequential Fractionation Method

Refer to chapter 2 for information on manure collection and diet formulation. The manure used in this study was recombined from selected individual pens that had no indication of Zn contamination. Therefore, Zn is being studied in this chapter. Reagents used for nonsequential extraction of P, Cu, and Zn from the five manure treatments were selected from the procedure developed by Miller et al. (1986). Moisture content was determined for each manure treatment prior to each extraction by weighing a subsample before and after oven drying at 35 °C for a 24-hour time period. Elemental analyses for each manure treatment fractionated are presented in Table 4.1. Ten extractants were used to solubilize the various forms of P, Cu, and Zn and are listed in Table 4.2 along with the extraction parameters and forms of elements solubilized. A 100:1 mL solution: g dry manure ratio was used in all extractions except for total elemental analysis. All extractants were made so that molarity was doubled to account for moisture in the manure. Therefore, a 0.5 g manure sample (dry-weight basis) was weighed into a 100 mL centrifuge tube. Afterward, 25 mL of “double strength” extracting solution was pipetted into the tube. The remaining water necessary to achieve a 100 mL solution: 1g dry matter ratio was pipetted into the centrifuge tube. The tubes were capped with plastic lids and placed on a reciprocating shaker for the time period specified in Table 4.1 for each extractant. Samples were centrifuged at 2000 rpm for 40 minutes. The supernatant liquid from each tube was decanted into 60-cm³ syringes with

Luerlok tips packed with 1 g of non-reactive filter pulp in the bottom of the syringe. Nucleopore filter housings measuring 25-mm in diameter were attached to the Luerlok tips. The filter housings hold 0.45 µm Nucleopore filters. A syringe plunger was used to force the supernatant through the filter pulp in the syringe and then through the 0.45 µm filter in the filter housing. The liquid was collected in a plastic dilu-vial for analysis. The extracts were analyzed for P, Cu, and Zn using inductively coupled plasma emission spectrometry (ICP). Total analysis was determined by microwave digestion with nitric acid. To each Teflon vessel, 0.5 g manure (dry weight basis) and 9 mL nitric acid was added. The vessels were sealed and digested for 35 minutes with maximum temperature reaching 220 °C. The samples were quantitatively transferred to a volumetric flask, diluted to 50 mL, and subsequently filtered using No. 42 Whatman filter paper. The extracts were analyzed for P, Cu, and Zn using inductively coupled plasma emission spectrometry (ICP).

4.2.2. Statistical Analysis

Three reps were used for all extractions except for total analysis, in which two reps were used, for a total of 145 extractions. The following equation was used to calculate the quantity of P, Cu, and Zn extracted in mg per kg of manure:

$$A \times B \times C = D$$

A=mg element per L of extractant (ICP analysis)

B= mL extractant per g manure extracted (dry weight basis)

C= dilution factor

D= mg element extracted per kg dry manure

Analysis of variance was performed using SAS (SAS Institute, 1996) to evaluate statistical differences between manure treatments. Duncan's Multiple Range Test was also used in SAS to compare means at the alpha level of 0.05.

Table 4.1. Manure analysis for each treatment used in fractionation study

| Manure treatment | Moisture Content % | g kg^{-1} manure | | | | | | | | | | | |
|------------------|-----------------------|---------------------------|-------------------------------|------------------|------|------|------|------|------|------|------|------|------|
| | | N | P ₂ O ₅ | K ₂ O | S | Ca | Mg | Na | Fe | Al | Mn | Cu | Zn |
| NPA | 81.35 | 13.26 | 5.07 | 7.93 | 0.90 | 2.26 | 1.35 | 0.88 | 0.92 | 0.15 | 0.05 | 0.01 | 0.08 |
| NPA+P | 81.12 | 13.83 | 6.96 | 8.21 | 0.81 | 3.63 | 1.25 | 1.34 | 0.53 | 0.22 | 0.05 | 0.01 | 0.07 |
| NPA+PHYTASE | 80.99 | 14.79 | 4.24 | 7.51 | 0.88 | 1.83 | 1.18 | 0.91 | 0.63 | 0.13 | 0.04 | 0.01 | 0.07 |
| HAP | 82.45 | 14.82 | 4.00 | 7.76 | 0.83 | 1.67 | 1.16 | 0.90 | 0.44 | 0.17 | 0.04 | 0.01 | 0.07 |
| HAP+PHYTASE | 79.86 | 17.30 | 4.26 | 8.39 | 0.93 | 1.57 | 1.27 | 0.97 | 0.48 | 0.25 | 0.05 | 0.01 | 0.07 |

Table 4.2: Reagents and extraction parameters used for nonsequential extractions

| Chemical form solubilized | Reagent | Time hours | pH |
|-------------------------------|---|---------------|-------|
| Water-soluble | H ₂ O | 16 | ----* |
| Water-soluble | 0.01 M CaCl ₂ | 16 | ---- |
| Exchangeable (neutral salt) | 0.5 M Ca(NO ₃) ₂ | 16 | ---- |
| Specifically bound | 0.05 M Pb(NO ₃) ₂ | 16 | ---- |
| Specifically bound | 0.44 M CH ₃ COOH | 16 | 2.5 |
| Acid soluble | 0.5 M HCl | 8 | ---- |
| Organically bound | 0.1 M K ₄ P ₂ O ₇ | 16 | 10.0 |
| Amorphous Fe oxide occluded | 0.2 M (NH ₄) ₂ C ₂ O ₄ (performed in dark) | 4 | 3.0 |
| Crystalline Fe oxide occluded | 0.2 M (NH ₄) ₂ C ₂ O ₄ (performed in light) | 4 | 3.0 |
| Total | Concentrated HNO ₃ | .5 | ---- |

* pH not adjusted

4.3. RESULTS AND DISCUSSION

Nonsequential extractions were conducted on each of the five manure treatments using reagents selective for partitioning elemental fractions. Eight reagents were employed for P fractionation, with 10 reagents used for partitioning both Cu and Zn. Three replicates were used for a total of 150 extractions. This chapter seeks to determine elemental P, Cu, and Zn fractions dominating each manure type, and to determine if differences exist between manure treatments for each elemental fraction.

4.3.1. P Comparisons

Data from all reagents used in the nonsequential fractionation scheme are presented for each of the five manure treatments in Table 4.3. The 0.01 M CaCl_2 reagent extracted the smallest amount of P for all NPA treatments. Both CaCl_2 and water are considered to remove the soluble P fraction from manure, though more P was extracted with water for all 3 treatments. This may be attributed to Ca suppressing P release when CaCl_2 is used. Exchangeable P was determined using 0.5 M CaNO_3 , and for all NPA treatments, extracted less P than water alone. The Ca suppressing effect of P from CaNO_3 at a higher concentration of Ca is most likely preventing greater P release. The stronger 0.5 M HCl extract removed more P than 0.44 M acetic acid for all 3 NPA treatments. Still more P was extracted from both NH_4 -oxalate in the dark and NH_4 -oxalate in the light reagents that are employed to remove crystalline and amorphous Fe, Al, and Mn. Nitric acid was used for determination of total P, and therefore extracted the highest concentration of P. The NPA+P manure treatment had the highest concentration of P from all reagents compared to either NPA or NPA+Phytase treatments. This is due to the addition of inorganic P to the ration in this manure treatment, resulting in a higher concentration of P in the manure. Compared to the NPA treatment, the NPA+Phytase treatment resulted in lower P extracted from all reagents except for the CaCl_2 extraction compared to the NPA treatment.

When HAP replaces the NPA treatment, soluble (CaCl_2 and water), exchangeable (CaNO_3), and acid soluble P (HCl and acetic) are increased. However, amorphous and crystalline Fe, Al, and Mn bound P extracted with NH_4 -oxalate both in the dark and light extracted less P from the HAP manure treatment compared to the NPA treatment. Nitric

acid also extracted less P in the HAP treatment. This can be attributed to the fact that HAP reduces total P excreted in manure due to better utilization of P by the bird. Since less P is found in the phytate form in the HAP manure, more P is soluble, as observed with both water and CaCl₂. Addition of phytase to the HAP treatment reduces total P (nitric acid) as well as soluble, exchangeable, and NH₄-oxalate extractable P. However, P extracted in acetic acid was increased by addition of phytase to the HAP treatment, while 0.5 M HCl acid extracted the same concentration of P from both treatments.

Phosphorous extracted from each manure treatment is shown in Table 4.3 for each reagent used in the nonsequential fractionation scheme. Addition of phytase to the baseline NPA treatment significantly decreases extractable P from all reagents except for the CaCl₂ extractant. No statistical differences exist between these two treatments for P extracted with CaCl₂. It is interesting to note that the conventional NPA+P treatment is significantly higher than all other treatments including the alternative NPA+Phytase treatment after any of the eight reagents are employed. Replacement of NPA for HAP significantly increases P extractability with the use of CaCl₂, water, CaNO₃, acetic acid, and HCl acid. Both NH₄-oxalate extractions along with HNO₃ result in significantly lower concentrations of P extracted when NPA was replaced by HAP. Addition of phytase to the HAP treatment results in significantly lower P concentrations in the soluble, exchangeable, and ammonium oxalate extractable fractions. Extraction with acetic acid results in significantly higher P concentrations upon addition of phytase to the HAP treatment, while the same levels of P are extracted with HCl. No statistical difference results in total P extracted between the HAP and HAP+Phytase treatments.

Table 4.3. Nonsequential extraction of P from all manure treatments

| Reagent | Manure treatment mg P kg ⁻¹ | | | | |
|---------------------------|---|-----------------|--------|--------|-----------------|
| | NPA+P | NPA+ PHYTASE | NPA | HAP | HAP+ PHYTASE |
| Calcium Chloride | 9007a* | 5520c | 5517c | 6037b | 5333d |
| Water | 9797a | 6027d | 6207c | 6460b | 5873e |
| Calcium Nitrate | 9273a | 5643d | 5950c | 6190b | 5950c |
| Acetic Acid | 9320a | 5373e | 6467d | 7020c | 7307b |
| Hydrochloric Acid | 10080a | 5957d | 7503c | 7807b | 7807b |
| Ammonium Oxalate in dark | 12627a | 7515d | 8349b | 8133c | 7651d |
| Ammonium Oxalate in light | 13494a | 8047c | 9077b | 8275c | 8059c |
| Nitric Acid | 17235a | 10376c | 11094b | 10453c | 10282c |

*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
 Statistical analyses were conducted separately for each reagent used for comparison of manure treatments

4.3.2. Cu Comparisons

Reagents used in the fractionation scheme for Cu extraction are presented in Table 4.4 for each manure treatment. Lead nitrate is used for determination of the chemically fixed fraction and extracted the lowest concentration of Cu compared with all other reagents for each treatment. Potassium pyrophosphate was included in the fractionation scheme to determine the organic fraction, and extracted 57% of total Cu averaged across all treatments. It is not surprising to observe such a high concentration in the organic fraction of manure. Hydrochloric acid extracted higher concentrations of Cu across all treatments compared to the less concentrated acetic acid. The baseline NPA treatment extracted the greatest amount of Cu across all reagents except K-pyrophosphate compared to the conventional NPA+P treatment. The NPA treatment also extracted more Cu for all reagents compared to the alternative NPA+Phytase treatment except for the NH_4 -oxalate extractions.

Replacement of NPA for HAP resulted in higher concentrations of Cu extracted across all reagents except for total Cu. Addition of phytase to the HAP treatment resulted in much lower Cu concentrations for all reagents used in the extraction process, though total Cu was decreased by only 5% (35.5 vs. 33.7 mg Cu kg^{-1} for HAP and HAP+Phytase treatments, respectively).

Addition of phytase to the baseline NPA treatment decreased solubility of Cu from all extractions except for NH_4 -oxalate (dark or light). No significant difference between these two treatments was observed for the soluble Cu fraction, though addition of phytase reduced Cu solubility (calcium chloride and water). Addition of phytase to the baseline NPA treatment significantly reduced the exchangeable (calcium nitrate), chemically-fixed (lead nitrate), acid-soluble (acetic acid and HCl), and total Cu fractions (nitric acid). The organic fraction had no significant difference upon addition of phytase to the baseline NPA treatment (K-pyrophosphate).

Substitution of the conventional NPA+P treatment for the alternative NPA+Phytase treatment significantly increased the solubility of Cu from all extractants except for the organic (K-pyrophosphate) and total Cu fractions (nitric acid). Copper extracted by K-pyrophosphate and nitric acid did not create significant differences between these two treatments. HAP was significantly higher than the NPA treatments for all reagents except

for NH_4 -oxalate in the light and nitric acid. Copper extracted with NH_4 -oxalate in the light was not statistically different between these two treatments, while total Cu in the NPA treatment was significantly higher than the HAP treatment. Addition of phytase to the HAP treatment decreased the solubility of Cu significantly from all reagents. The additional P made available from the use of both HAP and phytase may have been suppressing Cu release.

Table 4.4. Nonsequential extraction of Cu from all manure treatments

| Reagent | Manure treatment mg Cu kg ⁻¹ | | | | |
|---------------------------|--|-----------------|-------|-------|-----------------|
| | NPA+P | NPA+ PHYTASE | NPA | HAP | HAP+ PHYTASE |
| Calcium Chloride | 10.9c* | 14.3b | 15.1b | 16.8a | 8.5d |
| Water | 11.6c | 14.9b | 15.3b | 16.2a | 9.2d |
| Calcium Nitrate | 13.1d | 16.9c | 17.2b | 19.6a | 10.5e |
| Lead Nitrate | 8.2d | 10.9c | 12.3b | 15.4a | 7.9d |
| Potassium Pyrophosphate | 19.2b | 18.6b | 19.0b | 24.7a | 19.2b |
| Acetic Acid | 11.6d | 13.8c | 15.5b | 18.0a | 11.4e |
| Hydrochloric Acid | 16.5d | 18.8c | 22.4b | 24.4a | 16.4d |
| Ammonium Oxalate in dark | 15.7d | 19.7b | 18.3c | 21.1a | 10.9e |
| Ammonium Oxalate in light | 18.2c | 22.4a | 21.3b | 21.4b | 12.1d |
| Nitric Acid | 34.4c | 34.5c | 36.9a | 35.5b | 33.7c |

*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
 Statistical analyses were conducted separately for each reagent used for comparison of manure treatments

4.3.3. Zn Comparisons

Manure treatments extracted for Zn are presented in Table 4.5 for comparison of the elemental fractions solubilized by each of the 10 reagents. Water extracted the least Zn for all treatments compared with the other extractants. Larger concentrations of Zn were found in the exchangeable form (CaNO_3) compared to the soluble fraction (water and CaCl_2). More Zn was also found in the exchangeable form compared to the acid soluble fraction (acetic and HCl) from all 3 treatments. Zinc solubilized by K-pyrophosphate (used for extraction of organic fraction) comprised 76% of the total Zn averaged across all manure treatments. Both NH_4 oxalate extractions solubilized a high percentage of Zn as well.

Addition of phytase to the baseline NPA treatment significantly increased water and CaCl_2 solubility of Zn. These two treatments do not differ statistically upon extraction with nitric acid. However, addition of phytase to the NPA treatment significantly reduced Zn solubility after treatment with all other reagents. Replacement of the conventional NPA+P for the alternative NPA+Phytase treatment significantly increased the soluble (water and CaCl_2) and exchangeable (CaNO_3) Zn fractions. Using the alternative treatment significantly reduced the acid (acetic and HCl), chemically-fixed (PbNO_3), organically bound (K-pyrophosphate), and crystalline Fe, Al, and Mn bound (NH_4 oxalate in the light) fractions. NPA+P and NPA+Phytase did not statistically differ in Zn solubility with NH_4 -oxalate in the dark as well as the total Zn fraction.

Replacement of NPA for HAP also produced some interesting changes in Zn solubility for the reagents involved in the fractionation method. For example, replacing NPA with HAP significantly increased water and CaCl_2 soluble Zn, as well as acetic, HCl, and PbNO_3 soluble Zn. The use of CaNO_3 , NH_4 -oxalate in the dark and light, and nitric acid, all had significantly lower Zn solubility after replacing NPA with HAP. Potassium pyrophosphate extractable Zn created no statistical differences between the two treatments. Addition of phytase to the HAP treatment significantly lowered Zn solubility for all reagents used to fractionate the manure treatments.

Table 4.5. Nonsequential extraction of Zn from all manure treatments

| Reagent | Manure treatment mg Zn kg ⁻¹ | | | | |
|---------------------------|--|-----------------|------|-------|-----------------|
| | NPA+P | NPA+ PHYTASE | NPA | HAP | HAP+ PHYTASE |
| Calcium Chloride | 149e* | 186b | 179c | 196a | 166d |
| Water | 130e | 154b | 149c | 164a | 141d |
| Calcium Nitrate | 213e | 237c | 251a | 246b | 221d |
| Lead Nitrate | 204d | 195e | 249b | 256a | 233c |
| Potassium Pyrophosphate | 285a | 256c | 272b | 282ab | 234d |
| Acetic Acid | 172d | 166e | 206b | 210a | 188c |
| Hydrochloric Acid | 198d | 194e | 249b | 252a | 229c |
| Ammonium Oxalate in dark | 253c | 251c | 280a | 269b | 236d |
| Ammonium Oxalate in light | 275b | 266c | 295a | 270bc | 243d |
| Nitric Acid | 363ab | 366ab | 370a | 349b | 310c |

*Means with same letter not significantly different by Duncan's Multiple Range Test at alpha=0.05
Statistical analyses were conducted separately for each reagent used for comparison of manure treatments

4.4. SUMMARY AND CONCLUSIONS

4.4.1. P Comparisons

The 0.01 M CaCl_2 extractant solubilized the least amount of P across all treatments in the nonsequential fractionation scheme. Calcium suppression most likely had an effect on P solubility in the CaCl_2 extractant since water extracted higher concentrations of P across all treatments. The more concentrated HCl extracted higher concentrations of P than did acetic acid. Ammonium oxalate reacted both in the dark and light extracted rather high concentrations of P across all treatments. Nitric acid extracted the highest concentrations of P and determined total P content. NPA+P extracted the highest concentrations of P across all treatments compared to the NPA and NPA+Phytase treatments due to additional inorganic P found in the manure. The NPA+Phytase treatment contained lower P levels compared to NPA extracted from all reagents except for the CaCl_2 extractant.

The replacement of NPA with HAP significantly increases water, CaCl_2 , CaNO_3 , HCl, and acetic acid soluble P. However, both NH_4 -oxalate extractants (dark and light reactions) and nitric acid significantly decreased soluble P from the HAP treatment compared to the NPA treatment. HAP reduces total P excreted in the manure due to better utilization by poultry. Since more P is found in the nonphytate form in the HAP manure treatment, more P is soluble, as observed with both water and CaCl_2 soluble P. Addition of phytase to the HAP treatment significantly reduces all P fractions except for the acid soluble and total fraction. Acetic acid significantly increased P solubility with addition of phytase, while HCl extracted the same concentrations from both treatments. No statistical differences result upon extraction with nitric acid between these two treatments.

Addition of phytase to the baseline NPA treatment significantly decreased solubility of P from all reagents used except for the CaCl_2 extractant. NPA+P was significantly higher in P than all other treatments after extraction with any of the eight reagents.

4.4.2. Cu Comparisons

Lead nitrate extracted the lowest concentration of Cu compared with all other reagents for each treatment. CaNO_3 extracted more Cu than both water and CaCl_2 for all treatments. Potassium pyrophosphate was used to determine the organic fraction and extracted 57% of total Cu averaged across all treatments. The more concentrated HCl acid extracted more Cu than acetic acid across all treatments. The NH_4 -oxalate extractants also solubilized a high percentage of Cu. Addition of phytase to the baseline NPA treatment significantly reduced the exchangeable (Fig. 4.20), chemically-fixed (Fig. 4.21), acid-soluble (Fig. 4.24-4.25), and total Cu fraction (Fig. 4.28). Both the soluble and organic fractions were not significantly different between these two treatments (Fig. 4.19, 4.22, and 4.23). Replacement of the conventional NPA+P treatment for the alternative NPA+Phytase treatment significantly increased Cu solubility from all reagents except for the organic and total Cu fraction (Fig. 4.22 and 4.28). No significant differences were observed after extraction with these reagents between the alternative and conventional treatments. HAP was significantly higher in Cu solubility compared to the NPA treatment for all extractants except for NH_4 -oxalate in the light and nitric acid. Extraction with NH_4 -oxalate in the light did not produce statistical differences between these treatments, while Cu extracted with nitric acid was significantly higher in the NPA treatment compared to the HAP. As observed with P extraction, less total Cu was extracted with HAP due to better utilization by the bird. Addition of phytase to the HAP treatment significantly decreased Cu solubility from all reagents.

4.4.3. Zn Comparisons

Water extracted the least amount of Zn compared to all other reagents. More Zn was solubilized by CaNO_3 than both water and CaCl_2 for all treatments. Zinc solubilized by K-pyrophosphate to determine the organic fraction comprised 76% of the total fraction averaged across all manure treatments. Both NH_4 -oxalate reactions also extracted a high percentage of Zn.

Addition of phytase to the baseline NPA treatment did not significantly alter Zn solubility upon extraction with either water or CaCl_2 . However, NPA+Phytase was significantly lower in extractable Zn compared to the NPA treatment from all other reagents. Replacement of the conventional NPA+P with the alternative NPA+Phytase

treatment significantly increased the soluble (water and CaCl_2) and exchangeable (CaNO_3) fractions, while it significantly reduced the acid (acetic and HCl), chemically-fixed (PbNO_3), organically-bound (K-pyrophosphate), and crystalline Fe, Al, and Mn (NH_4 oxalate in the light) fractions. These two treatments did not statistically differ in Zn solubility upon extraction with either NH_4 -oxalate in the dark or nitric acid.

The replacement of NPA for HAP significantly increased Zn extracted with water, CaCl_2 , acetic acid, HCl , and PbNO_3 . However, extraction with CaNO_3 , NH_4 -oxalate in the dark and light, and nitric acid significantly decreased Zn solubility. Potassium pyrophosphate extractable Zn created no statistical differences between these two treatments. Addition of phytase to the HAP treatment significantly decreased Zn solubility for all reagents used in the fractionation scheme.

CHAPTER 5: SUMMARY AND CONCLUSIONS

5.1. OVERALL OBJECTIVES

The overall objectives of this study are outlined below. Results from each objective are summarized separately for discussion of P, Cu, and Zn.

- Objective 1: Determine effects of using phytase and HAP corn on water solubility of P and Cu in soils amended with manure at a period of six months and one year, which extends the 4 day and 28 day incubation study conducted by Lawrence (2000).
- Objective 2: Determine differences in water-solubility of P analyzed colorimetrically and by ICP.
- Objective 3: Determine effects of using phytase and HAP corn on plant availability of P and Cu in soils amended with manure at a period of six months and one year, which extends the 4 day and 28 day incubation study conducted by Lawrence (2000).
- Objective 4: Determine differences in plant-availability of P analyzed colorimetrically and by ICP.
- Objective 5: Evaluate the effects of phytase supplementation on P, Cu, and Zn uptake and crop yield from corn grown in a greenhouse experiment in soils amended with manure.
- Objective 6: Evaluate the effects of phytase supplementation on P, Cu, and Zn uptake and crop yield from corn grown in a greenhouse experiment using sand amended with manure.
- Objective 7: Fractionate P, Cu, and Zn in manure to determine any changes in these elements from the use of phytase and HAP corn.

5.1.1. P Comparisons

Objective 1

Replacement of the conventional NPA+P treatment on an N-basis for the alternative NPA+Phytase treatment produced a significant 20% reduction in water soluble P concentrations (15.9 vs. 12.7 mg P kg⁻¹ soil for NPA+P and NPA+Phytase treatments on N-basis, respectively) when these turkey manures were applied to three Virginia soils having diverse properties. This reduction is not as significant as the 41% reduction in total manure P applied to the soil observed by replacing the conventional treatment for the alternative phytase treatment. Comparing these two treatments on a P-basis results in a significant 24% increase in water soluble P levels after replacement of the conventional NPA+P treatment with the alternative NPA+Phytase treatment (9.7 vs. 12.7 mg P kg⁻¹ soil for NPA+P and NPA+Phytase treatments on P-basis, respectively). In order to determine the component causing the significant difference in water-soluble P levels, both the alternative phytase treatment and the conventional NPA+P treatments were compared to the baseline NPA treatment. Addition of phytase to the NPA treatment did not result in significant differences in P solubility. However, a significant 25% reduction in water-solubility of P was observed after addition of inorganic calcium phosphate to the baseline NPA treatment. Therefore, the significant differences that resulted by comparing the conventional NPA+P and NPA+Phytase treatment on a P-basis was not due to addition of phytase, but was directly related to the supplemental inorganic P. No statistical differences were observed by addition of phytase to the baseline NPA treatment upon increasing incubation time or application rate.

Replacing the NPA treatment for the HAP treatment did not produce significant differences in P solubility after extraction with CaCl₂. The effects of soil type, incubation time, and application rate did not produce statistical differences between these treatments.

Objective 2

Phosphorous extracted with CaCl₂ and analyzed colorimetrically using the molybdate blue method (dissolved inorganic P or ortho-P) comprised 79% of the P fraction analyzed by ICP (total dissolved P), averaged across all treatments. Dissolved inorganic P is the most available and most easily transported form of P. Trends between

ortho-P and ICP P are similar for the treatments under study. The major difference observed is that the addition of phytase to the baseline NPA treatment produces a significant 8% reduction in the ortho-P fraction, while these treatments have statistically similar P concentrations analyzed by ICP.

Objective 3

Replacing the NPA+P treatment for the NPA+Phytase treatment on an N-basis produced a significant 24% reduction in Mehlich III-extractable P concentrations (397 vs. 304 mg P kg⁻¹ soil for NPA+P and NPA+Phytase treatments on N-basis, respectively) when applied to three diverse Virginia soils. Comparing these two treatments on a P-basis produces a significant 5% increase in Mehlich III-extractable P concentrations by replacement with the phytase treatment (304 vs. 289 mg P kg⁻¹ soil for NPA+Phytase and NPA+P treatments on P-basis, respectively). This 5% increase is much less notable than the 24% increase observed in water-soluble P levels. The acidic Mehlich III extractant solubilized more P in the NPA+P treatment that was not solubilized with CaCl₂. This effect was apparent by comparing the NPA+P treatment to the NPA treatment on a P-basis. No statistical differences in P solubility existed between these treatments. No significant differences were noted for the upon addition of phytase to the baseline NPA treatment as well. Increasing application rate or incubation time did not produce significant changes in Mehlich III-extractable P levels.

Substituting the NPA treatment with HAP corn did not produce significant differences in Mehlich III-extractable P concentrations.

Objective 4

Ortho-P fraction comprised 87% of the total dissolved P extracted with Mehlich III averaged across all treatments. The NPA+Phytase treatment produced a significant increase in ortho-P compared to the NPA+P treatment when applied on a P-basis, while no significant difference existed between these treatments analyzed by ICP.

Objective 5

Corn tissue did not statistically differ for 4-week old corn seedlings grown in three Virginia soils amended with either the NPA+Phytase treatment or the NPA+P treatment on both a N- and P-basis. The NPA+Phytase treatment contained statistically similar concentrations of P in the corn tissue as well as plant uptake of P compared with the NPA, NPA+P (N-basis), and NPA+P (P-basis) treatments. Soils were extracted with Mehlich III after corn harvest. The alternative NPA+Phytase treatment did not statistically differ in Mehlich III-extractable P from NPA, NPA+P (N-basis), or NPA+P (P-basis). Therefore, addition of phytase did not produce significant differences in extractable P in three Virginia soils after corn growth. All three soils used in the experiment contained ample quantities of P, and was most likely responsible for the similarities in P concentrations in the corn tissue, P uptake, and P remaining in the soil after the harvest of 4-week old corn plants.

Objective 6

The NPA+Phytase treatment did not significantly differ from either the NPA or NPA+P (N-basis) treatment in the production of dry matter by 4-week old corn seedlings. Phosphorous in corn tissue as well as plant uptake of P did not statistically differ between these three treatments either. Therefore, phytase does not have an effect on corn growth, P in corn tissue, or plant uptake of P, under the conditions of this short-term greenhouse study.

Objective 7

The conventional NPA+P treatment was significantly higher in extractable P than all other treatments after extraction of turkey manure with any of the eight reagents (i.e., 0.01 M CaCl₂, etc.). This was due to the additional inorganic Ca phosphate that supplemented this diet. Addition of phytase to the baseline NPA treatment significantly reduces extractable P levels for all reagents used except for CaCl₂. For the CaCl₂, these treatments are statistically similar. Replacement of NPA with HAP significantly increased water, CaCl₂, CaNO₃, HCl, and acetic acid soluble P in the turkey manures tested. However, both NH₄-oxalate extractants (dark and light) and nitric acid had

significantly lower soluble P levels in the HAP treatment compared to the NPA treatment. HAP reduces total P in the manure due to better utilization by poultry, though more of the P was found in the nonphytate form. This nonphytate form of P was water soluble and is the reason that more P was extracted from this treatment with water and CaCl_2 as compared to the NPA treatment

5.1.2. Cu Comparisons

Objective 1

Replacing the conventional NPA+P treatment with the alternative NPA+Phytase treatment on an N-basis (and thus equal Cu-basis) produced a significant 30% increase in water-soluble Cu concentrations (0.43 vs. 0.61 mg Cu kg^{-1} soil for NPA+P on N-basis and NPA+Phytase treatments, respectively) when applied to three Virginia soils having diverse properties. Comparing these treatments on a P-basis produced an even more marked response, with a significant 61% increase in Cu-solubility (0.24 vs. 0.61 mg Cu kg^{-1} soil for NPA+P on P-basis and NPA+Phytase treatments, respectively). This large increase in Cu solubility was partly attributed to the fact that 40% more total Cu was applied from the NPA+Phytase treatment. Both the addition of phytase and inorganic P are accountable for the large difference in extractable Cu concentrations, since addition of phytase to the baseline treatment caused a significant increase in CaCl_2 -extractable Cu, while addition of inorganic Ca phosphate produced a significant reduction in extractable Cu. Neither incubation time or application rate caused a significant difference between these treatments.

Replacing the NPA treatment with the HAP treatment resulted in statistically similar Cu concentrations upon extraction of the treated soils with CaCl_2 . Increasing incubation time or application rate did not cause a statistical change between these treatments.

Objective 3

Comparing the alternative NPA+Phytase treatment to the conventional NPA+P treatments on either an N- or P- basis did not produce statistical changes in Mehlich III-extractable Cu when treated turkey manure was applied to three Virginia soils. The

acidic extractant solubilized the P bonds that had formed with Cu, producing the slight difference. The CaCl_2 extractant did not solubilize these bonds, resulting in the large differences between these treatments. Substituting the NPA treatment for HAP corn produced no statistical changes in extractable soil Cu.

Objective 5

In a greenhouse trial where the treated turkey manures were applied to three Virginia soils, no statistical differences were observed for Cu in corn tissue or plant uptake of Cu when NPA+Phytase is compared to NPA, NPA+P (N-basis), and NPA+P (P-basis) averaged across all soil types. This effect was most likely due to sufficient levels of Cu being supplied by all soils used in the experiment.

No significant differences were observed in Cu solubility upon extraction of the soils with Mehlich III after the harvest of 4-week old corn seedlings. Therefore, phytase had no effect on plant-availability of Cu in the three soils.

Objective 6

Copper in the 4-week old corn seedlings was detectable only in the NPA+Phytase treatment ($0.09 \text{ mg Cu kg}^{-1}$) from corn grown in sand culture, which resulted in no plant uptake for the other treatments. Copper extracted from the NPA+Phytase treatment was not large enough to be statistically different from the other treatments.

Objective 7

In a fractionation of the turkey manures, replacing the conventional NPA+P treatment for the alternative NPA+Phytase treatment significantly increased Cu solubility for all reagents except for the organic and total Cu fraction, in which no statistical difference existed. Addition of phytase to the baseline NPA treatment significantly reduced the exchangeable, chemically-fixed, acid-soluble, and total Cu fractions, while no statistical difference existed among treatments for the water-soluble and organic Cu fractions. NPA+Phytase treatment was significantly higher in Cu extracted from both NH_4 -oxalate extractants.

HAP manure was significantly higher in extractable Cu than NPA from all reagents used except for NH_4 -oxalate in the light and nitric acid. Extraction of manure with nitric acid produced significantly higher Cu concentrations in the NPA treatment compared to HAP. HAP reduced the total Cu extracted, but increased the soluble forms including CaCl_2 and water soluble Cu levels.

5.1.3. Zn Comparisons

Objective 5

In a greenhouse trial involving three Virginia soils, replacing the conventional NPA+P (N-basis) treatment with the alternative NPA+Phytase treatment did not produce any significant differences from Zn in 4-week old corn seedling tissue. A significant 13% increase in the Zn concentration in the corn tissue was observed when NPA+Phytase replaced the NPA+P (P-basis) treatment (26.2 vs. 22.8 mg Zn kg^{-1} for NPA+Phytase and NPA+P treatments on a P-basis, respectively). This was due to the fact that less Zn was applied to the soil from the NPA+P treatment on a P-basis, and therefore less Zn was available for uptake by the plant. Plant uptake of Zn was not significantly different between the alternative NPA+Phytase treatment and the conventional NPA+P treatments on a N- or P-basis for both the Mahan and Groseclose soils. Plant uptake of Zn by 4-week old corn seedlings from the NPA+Phytase treatment was significantly higher than both NPA+P treatments from corn grown in the Cecil soil.

Mehlich III extractable Zn measured in three Virginia soils after the harvest of the 4-week old corn seedlings did not differ statistically between treatments.

Objective 6

In the greenhouse trial involving the three Virginia soils, the NPA+Phytase treatment did not statistically differ in Zn concentrations in the corn seedling tissue from the NPA and NPA+P (N-basis) treatments. Though addition of phytase to the baseline NPA treatment did not significantly differ in plant uptake of Zn, NPA+Phytase was significantly higher in plant uptake of P than the NPA+P treatment on an N-basis.

Objective 7

In a fractionation of the treated turkey manures, replacing the NPA+P treatment for the NPA+Phytase treatment significantly increased Zn extracted by water, CaCl₂, and CaNO₃, while it significantly reduced Zn extracted by HCl, acetic acid, PbNO₃, K-pyrophosphate, and NH₄-oxalate in the light. These two treatments did not statistically differ in Zn solubility after extraction with either NH₄-oxalate in the dark or nitric acid.

When NPA was replaced with HAP, significantly higher levels of Zn were extracted by water, CaCl₂, acetic acid, HCl, and PbNO₃, while NH₄-oxalate in the dark and light, and nitric acid extracted significantly lower levels of Zn. Potassium pyrophosphate extractable Zn produced no statistical differences between these two treatments.

This research has shown that impacts on the solubility of P, Cu, and Zn in manure and manure-amended soils with the use of phytase as a feed additive and HAP grains is dependent upon many factors. For example, under N-based manure management, the use of phytase and HAP corn reduced both water-soluble and Mehlich III-extractable concentrations in the Virginia soils. The use of these alternatives under P-based manure management guidelines increased both water-soluble and Mehlich III-extractable P concentrations. This research has also shown no negative impacts on corn growth from the use of phytase as a feed additive to turkey rations. In agronomic terms, the use of phytase increases the solubility of P when poultry manure is applied on a P-basis, making the applied P more available to the plant. Farmers apply fertilizer for this same purpose. On sites that do not have high levels of residual P in the soil or a high risk of erosion, increased solubility of P would be beneficial to the farmer. However, if conditions are favorable for P movement off site, the use of phytase or HAP grains may serve to enhance P movement into surface waters. Since many different factors are involved in increasing the risk of P transport into surface waters, it is not possible at this point to give a clearcut answer as to whether the use of these amendments are environmentally beneficial or degrading. This research has shown trends with the use of these alternatives, and it demonstrates the importance of assessing not only the use of phytase and HAP grains, but also implementation of manure management guidelines appropriate to each site for controlling P movement.

CHAPTER 6: REFERENCES

- Allen, S.E., G.L. Terman, and L.B. Clements. 1976. Greenhouse Techniques for soil-plant-fertilizer research. National Fertilizer Development Center bulletin Y-104. Tennessee Valley Authority, Muscle Shoals, AL.
- Anderson, M.A., J.R. McKenna, D.C. Martens, S.J. Donohue, E.T. Kornegay, and M.D. Lindemann. 1991. Long-term effects of copper rich swine manure application on continuous corn production. *Commun. Soil Sci. Plant Anal.* 22(9&10), 993-1002.
- Baxter, C.A., B.C. Joern, and O. Adeola. 1998. Dietary P management to reduce soil P loading from pig manure. *In: Proceedings of the Twenty-eighth North Central Extension-Industry Soil Fertility Conference, Volume 14, November 11-12, 1998.* Potash and Phosphate Institute. Brookings, SD.
- Brady, N.C., and R.R. Weil. 1999. Soil Phosphorous and Potassium. P. 540-584. *In The Nature and Properties of Soils.* 12th ed. Prentice Hall, Upper Saddle River, NJ.
- Coale, F.J., and J.A. Olear. 1996. The relationship between soil test phosphorous level and the concentration of dissolved and potentially transportable phosphorous in field drainage water. CRC Cooperative Agreement #NPS-UMD95. Edgewater, Maryland.
- Coelho, M.B. 1996. Ecological nutrition: A costly or smart move? *In Coelho, M.B. and Kornegay, E.T. (eds.) Phytase in Animal Nutrition and Waste Management.* BASF Corp., Mount Olive, NJ.
- Cox, F.R. and S.E. Hendricks. 2000. Soil test phosphorous and clay content effects on runoff water quality. *J. Environ. Qual.* 29:1582-1586.

Department of Conservation and Recreation. 1993. Nutrient Management Handbook. 2nd ed. Richmond, VA.

Department of Conservation and Recreation. 1999. Means to improve the economic feasibility of transporting and selling poultry waste and pursue alternative uses for poultry waste, including the establishment of an equal matching grant program. Richmond, Va.

Donohue, S.J and S.E. Heckendorn. 1994. Laboratory procedures: Virginia Tech soil testing and plant analysis laboratory. Publication 452-881. Virginia Cooperative Extension.

Duval, M.D. 1996. Practical feed formulation and return on investment with Natuphos Phytase for broilers and turkeys. *In* Coelho, M.B. and Kornegay, E.T. (eds.) Phytase in Animal Nutrition and Waste Management. BASF Corp., Mount Olive, NJ.

Eghball, B., G.D. Binford, and D.D. Baltensperger. 1996. Phosphorous movement and adsorption in a soil receiving long-term manure and fertilizer additions. *J. Environ. Qual.* 25:1339-1343.

Galeone, D.G. 1996. Factors affecting phosphorous transport at a conventionally-farmed site in Lancaster County, Pennsylvania, 1992-95. Water Resources Investigations Report 96-4168. Lemoyne, Pennsylvania.

Gordon, R.W. and D.A. Roland, Sr. 1996. Influence of supplemental microbial phytase on calcium utilization of laying hens. *In* Coelho, M.B. and Kornegay, E.T. (eds.) Phytase in Animal Nutrition and Waste Management. BASF Corp., Mount Olive, NJ.

Harland, B.F., and D. Oberleas. 1996. Phytic acid complex in feed ingredients. *In* Coelho,

- M.B. and Kornegay, E.T. (eds.) Phytase in Animal Nutrition and Waste Management. BASF Corp., Mount Olive, NJ.
- Haygarth, P.M., and A.N. Sharpley. 2000. Terminology for phosphorous transfer. *J. Environ. Qual.* 29:10-15.
- Iragavarapu, R. 1999. Manure phosphorous-Problems, regulations, and crop genetic solutions. Part 1-summary. *Crop Insights*: vol. 9 no.6. Available at: http://www.pioneer.com/usa/nutrition/manure_phosphorous_99.htm (accessed 21 Oct 2000).
- Joern, B.C., T.L. Provin, and A.L. Sutton. 1996. Retention of swine manure phosphorous compounds in soil. Research Investment Report, National Pork Producers Council.
- Jokela, W.E. 1999. The phosphorous index: A tool for management of agricultural phosphorous in Vermont. Report to SERA-17: Minimizing P losses from agriculture. Quebec City, July, 1999. Available at <http://pss.uvm.edu/vtcrops/NutrientMgt.html#TOP> (accessed 16 Oct. 2000).
- Jones J.B., Jr. 1998. Plant Nutrition Manual. CRC Press, Boca Raton, FL.
- Kornegay, E.T. 1996. Effectiveness of Natuphos Phytase in improving the bioavailabilities of phosphorous and other nutrients for broilers and turkeys. *In* Coelho, M.B. and Kornegay, E.T. (eds.) Phytase in Animal Nutrition and Waste Management. BASF Corp., Mount Olive, NJ.
- Kornegay, E.T. and D.M. Denbow. 1999. Bioavailability of phosphorous in high available (low phytate) corn and normal phytate corn and the influence of microbial phytase for young turkeys. *S. Poultry Sci.* S35 (abstract).

- Kornegay, E.T., H. Qian, and D.M. Denbow. 1996. Influence of dietary calcium levels or calcium: phosphorous ratios on effectiveness of Natuphos Phytase for broilers and turkeys. *In* Coelho, M.B. and Kornegay, E.T. (eds.) Phytase in Animal Nutrition and Waste Management. BASF Corp., Mount Olive, NJ.
- Kuo, S. 1996. Phosphorous. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 3. SSSA Book Ser. 5. SSSA. Madison, WI.
- Lawrence, C.L. 2000. Effects of feeding phytase enzyme and HAP corn on solubility of phosphorous, copper, and zinc in turkey manure and manure-amended soils. M.S. Thesis. Virginia Polytechnic Institute and State University. Blacksburg, VA.
- Lemunyon, J.L. and R.G. Gilbert. 1993. Concept and need for a phosphorous assessment tool. *J. Prod. Agric.* 6:483-486.
- Li, Z. and L.M. Shuman. 1996a. Mobility of Zn, Cd and Pb in soils as affected by poultry litter extract-I. Leaching in soil columns. *Environ. Pollut.* 95: 219-226.
- Li, Z. and L.M. Shuman. 1996b. Mobility of Zn, Cd and Pb in soils as affected by poultry litter extract-II. Redistribution among soil fractions. *Environ. Pollut.* 95: 227-234.
- Lucas, J. 1991. Greenhouse and laboratory study for the land application of water treatment residual. M.S. Thesis. Virginia Polytechnic Institute and State University. Blacksburg, VA.
- Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of the Mehlich 2 extractant. *Comm Soil Sci Plant Anal* 15:1409-1416.
- Miller, W.P., D.C. Martens, and L.W. Zelazny. 1986a. Effect of sequence in extraction of trace metals from soils. *Soil Sci. Soc. Am. J.* 50:598-601.

- Miller, W.P., D.C. Martens, L.W. Zelazny, and E.T. Kornegay. 1986b. Forms of solid phase copper in copper-enriched swine manure. *J. Environ. Qual.* Vol. 15, no.1.
- Mitchell, C.C., S.T. Windham, D.B. Nelson, and M.N. Baltikauski. 1992. Effects of long-term litter application on coastal plain soils. *In: Proceedings 1992 National Poultry Waste Management Symposium Committee.* Auburn University Press, Auburn, AL. pp. 383-390.
- Moore, P.A., Jr. 1998. Best management practices for poultry manure utilization that enhance agricultural productivity and reduce pollution. *In* J.L. Hatfield and B.A. Stewart (ed.) *Animal waste utilization: Effective use of manure as a soil resource.* Ann Arbor press, Chelsea, Michigan.
- Moore, P.A., Jr., and D. M. Miller. 1994. Decreasing phosphorous solubility in poultry litter with aluminum, calcium, and iron amendments. *J. Environ. Qual.* 23:325-330.
- Moore, P.A., Jr., T.C. Daniel, D.R. Edwards, and D.M. Miller. 1995. Effect of chemical amendments on ammonia volatilization from poultry litter. *J. Environ. Qual.* 24:293-300.
- Moore, P.A. Jr., T.C. Daniel, J.T. Gilmour, B.R. Shreve, D.R. Edwards, and B.H. Wood. 1998a. Decreasing metal runoff from poultry litter with aluminum sulfate. *J. Environ. Qual.* 27:92-99.
- Moore, P.A. Jr., W.E. Huff, W.F. Jaynes, G.R. Huff, J.M. Balog, and N.C. Rath. 1998b. Effect of high available phosphorous corn and phytase enzyme addition to broiler diets on phosphorous runoff from fescue plots. Southern Poultry Science Society. S30 (abstract)
- Mozaffari, M., and J.T. Sims. 1996. Phosphorous transformations in poultry litter-

- amended soils of the Atlantic Coastal Plain. *J. Environ. Qual.* 25: 1357-1365.
- Mullins, G.L., and B.F. Hajek. 1997. Phosphorous accumulation and loss from Alabama soils receiving poultry litter. Bulletin No. 631. Alabama Agricultural Experiment Station, Auburn University, Alabama.
- Nys, Y., D. Frapin, and A. Pointillart. 1996. Occurrence of phytase in plants, animals and microorganisms. *In* Coelho, M.B. and Kornegay, E.T. (eds.) *Phytase in Animal Nutrition and Waste Management*. BASF Corp., Mount Olive, NJ.
- Pautler and J.T. Sims. 2000. Relationships between soil test phosphorous, soluble phosphorous, and phosphorous saturation in Delaware soils. *Soil Sci. Soc. Am. J.* 64:765-773.
- Payne, G.G., D.C. Martens, E.T. Kornegay, and M.D. Lindemann. 1988a. Availability and form of copper in three soils following eight annual applications of copper-enriched swine manure.
- Payne, G.G., D.C. Martens, C. Winarko, and N.F. Perera. 1988b. Form and availability of copper and zinc following long-term copper sulfate and zinc sulfate applications. *J. Environ. Qual.* Vol. 17, no.4.
- Pelletier, B.A. 1999. Virginia grain handling practices and corn for poultry litter exchange program. M.S. Thesis. Virginia Polytechnic Institute and State University. Blacksburg, VA.
- Peters, J.M. and N.T. Basta. 1996. Reduction of excessive bioavailable phosphorous in soils by using municipal and industrial wastes. *J. Environ. Qual.* 25:1236-1241.
- Pote, D.H., T.C. Daniel, A.N. Sharpley, P.A. Moore, Jr., D.R. Edwards, and D.J. Nichols.

- Relating extractable soil phosphorous to phosphorous losses in runoff. *Soil Sci. Soc. Am. J.* 60:855-859.
- Prasad, R. and J.F. Power. 1997. Phosphorous. *In Soil Fertility Management for Sustainable Agriculture*. Lewis Publishers, Boca Raton, FL.
- Ravindran, V. 1996. Occurrence of phytic acid in plant feed ingredients. *In Coelho, M.B. and Kornegay, E.T. (eds.) Phytase in Animal Nutrition and Waste Management*. BASF Corp., Mount Olive, NJ.
- Sallade, Y.E. and J.T. Sims. 1997. Phosphorous transformations in the sediments of Delaware's agricultural drainageways: I. Phosphorous forms and sorption. *J. Environ. Qual.* 26:1571-1579.
- SAS Institute. 1996. *SAS/STAT User's guide*. Version 6.12. SAS Inst., Cary, N.C.
- Saylor, W.W., G.W. Malone, J.T. Sims, and V. Raboy. 1999. Effects of microbial phytase, high available phosphorous corn, and dietary phosphorous level on broiler production. 1. Effects on broiler performance. *Southern Poultry Science Society*. S31 (abstract).
- Sharpley, A.N. 1996. Availability of residual phosphorous in manured soils. *Soil Sci. Soc. Am. J.* 60:1459-1466.
- Sharpley, A.N. 2000. *Agriculture and phosphorous management: The Chesapeake Bay*. Lewis Publishers, Boca Raton, FL.
- Sharpley, A.N., S.C. Chapra, R. Wedepohl, J.T. Sims, T.C. Daniel, and K.R. Reddy. 1994. Managing agricultural phosphorous for protection of surface waters: Issues and options. *J. Environ. Qual.* 23:437-451.

- Sharpley, A.N., T. Daniel, T. Sims, J. Lemunyon, R. Stevens, and R. Parry. 1999. Agricultural phosphorous and eutrophication. ARS-149. USDA-ARS.
- Sharpley, A.N., B. Foy, and P. Withers. 2000. Practical and innovative measures for the control of agricultural phosphorous losses to water: an overview. *J. Environ. Qual.* 29:1-9.
- Simpson, T.W. 1998. A citizen's guide to the Water Quality Improvement Act of 1998. Maryland Cooperative Extension.
- Sims, J.T. 1993. Environmental soil testing for phosphorous. *J.Prod. Agric.* 6:501-507.
- Sims, J.T., A.C. Edwards, O.F. Schoumans, and R.R. Simard. 2000. Integrating Soil Phosphorous Testing into Environmentally Based Agricultural Management Practices. *J. Environ. Qual.* 29:60-71.
- Sims, J.T., M.F. Lavahaun, G.W. Malone, W.W. Saylor, and V. Raboy. 1999. Effects of microbial phytase, high available phosphorous corn, and dietary phosphorous level on broiler production. 3. Effects on broiler litter composition. *S. Poultry Sci.* S33 (abstract).
- Smith, G.S., C.M. Johnston, and I.S. Cornforth. 1983. Comparison of nutrient solutions for growth of plants in sand culture. *New Phytol.* 94, 537-548.
- Stout, W.L., A.N. Sharpley, and J. Landa. 2000. Effectiveness of coal combustion by-products in controlling phosphorous export from soils. *J. Environ. Qual.* 29:1239-1244.
- Turner, B.L. and P.M. Haygarth. 2000. Phosphorous forms and concentrations in leachate under four grassland soil types. *Soil Sci. Soc. Am. J.* 64:1090-1099.

U.S. Department of Agriculture. 1997 Census of Agriculture: Virginia State and County Data: Vol. 1 Geographic Area Series, Part 46. NASS AC97-A-46, Washington, DC.

U.S. Environmental Protection Agency. 1995. National water quality inventory: 1994 Report to Congress. EPA841-R-95-005. Office of Water, USEPA, Washington, D.C.

U.S. Environmental Protection Agency. 1996. Environmental Indicators of Water Quality in the United States, EPA 841-R-96-002, U.S. Environmental Protection Agency, Office of Water (4503F), U.S. Government Printing Office, Washington, D.C.

Van der Watt, H.v.H., M.E. Sumner, and M.L. Carbrera. 1994. Bioavailability of Copper, Manganese, and Zinc in poultry litter. *J. Environ. Qual.* 23:43-49.

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