

**EFFECTS OF SYNTHETIC CHELATING AGENT APPLICATION TO SOILS ON
PHOSPHORUS AVAILABILITY**

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Cristie LeAnne Edwards

ABSTRACT

Fertilizer phosphorus (P) can become unavailable to crops due to immobilization of P in acidic soils through forming chemical bonds with iron (Fe) and aluminum (Al) amorphous oxides. Organic chelating agents form strong bonds with metals in soil and may reduce P binding with Fe and Al. Ethylenediamine tetraacetic acid (EDTA), hydroxyethyl ethylenediamine triacetic acid (HEEDTA), gluconic acid (GA), and citric acid (CA) were tested to determine their influence on water-soluble P (WSP), Mehlich-1 P and Mehlich-3 P in Loam and Sand soils fertilized with P and incubated for 49 days. Soil P sorption capacity (PSC) was estimated from an oxalate extraction of Fe and Al, and chelates were applied at rates of 90 percent of the PSC. The EDTA, HEEDTA, and CA significantly ($P < 0.05$) reduced P sorption in the Loam and Sand when measured by WSP. In soils without P fertilizer added, EDTA and HEEDTA significantly increased WSP, Mehlich-1, and Mehlich-3 P concentrations. EDTA and HEEDTA were also applied at 0, 30, 60, 90, 120, and 150 percent PSC to produce a rate response curve for WSP in a second soil incubation. With increasing chelating rate, there was a linear increase in WSP for both soils, thus indicating higher rates of chelating agents were most efficient at decreasing P sorption.

EDTA and HEEDTA were also tested in a 4-week greenhouse study for efficiency at increasing plant available P to corn (*Zea mays L.*) in two soils. Phosphorus was added with and

without the addition of chelating agents to the center of the pot, simulating a starter band of P. After 4 weeks, soils were analyzed for WSP, Mehlich-1, and Mehlich-3 P and corn above- and below-ground biomass was quantified and analyzed for total P concentration. Without the presence of chelating agents, concentrations of WSP, Mehlich-1 P, Mehlich-3 P, above- and below-ground biomass, and TKP increased linearly as P fertilizer rates increased at 0, 9.6, 19.3, 28.9, and 38.5 kg P ha⁻¹. Decreased P sorption using chelating agents was not observed in this experiment. However, with the results from the soil incubation, chelating agents do show potential for increasing plant available P, but the application and incorporation method needs to be further studied.

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DEDICATION

The author would like to dedicate this original thesis to her entire family, without their encouragement, this milestone would not have been possible. Her family has shown her that anything is possible with hard work, dedication, and a loving family.

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CHAPTER 1: LITERATURE REVIEW

PHOSPHORUS

Phosphorus fertilizer can become unavailable to crops due to immobilization of P in acidic soil. Elevated concentrations of metal ions, such as Al and Fe, in the soil solution decreases plant available P (van der Zee and van Riemdijk, 1988). Agricultural soil tests that estimate the P available for crop growth include Water Soluble P (WSP), Mehlich-1, and Mehlich-3 that are commonly used in acidic soils (Maguire et al., 2001).

Above agronomic optimum P concentrations, studies have shown an increase in P losses in surface runoff (Pote et al., 1996; Sims et al., 1998). The Mid-Atlantic Soil Test and Plant Analysis Work Group (a regional consortium of public and private soil test laboratories) stated in 1997 that for corn (*Zea mays L.*) a Mehlich-1 value of 20 mg P kg⁻¹ was the “soil test P level at 100% yield”, and 35 mg P kg⁻¹ was the “soil test P level where no fertilizer is recommended” (Sims et al. 1998). However, routine Mehlich-1 soil test values of 55 mg P kg⁻¹ is the point at which no fertilizer is recommended in Virginia (Maguire and Heckendorn, 2011). At concentrations this high, soils have a greater potential to release P into runoff waters or into surface waters. Mehlich-1 P concentrations close to optimum levels can be maintained by replacing P removed in harvest with the equivalent amount of P (McCollum, 1991). However, research has shown that P concentrations in harvest removal could not be maintained by additions of fertilizer. This result was attributed to the reversion of P into less available forms (Pierzynski and Logan, 1993).

Sources of Phosphorus

Plants absorb most of their P in the form of inorganic orthophosphate, H_2PO_4^- , therefore monoammonium phosphate (MAP) and diammonium phosphate (DAP) are both efficient P fertilizers supplying both H_2PO_4^- and HPO_4^{2-} . When MAP is applied to soil, there is an acidic zone that forms around the granule. This acidic environment temporarily inhibits ammonium volatilization. However, when DAP is applied to soil, an alkaline zone is created and the increased H^+ accelerates ammonium volatilization and the potential for ammonia toxicity to seeds if applied in band application.

Fertilizers have been categorized based on water and citrate solubility (Chien et al., 1990). Diammonium phosphate is classified as a water soluble P fertilizer and an excellent source of nutrients for its high solubility and non-hazardous handling. Diammonium phosphate has been found to form calcium (Ca) phosphates (Bennett and Adams, 1970) and magnesium (Mg) precipitates (Ensminger et al., 1965). The formation of these precipitates can lead to Ca deficiencies within the rhizosphere and therefore, a decrease in root growth (Rios and Pearson, 1974; Sorokin and Sommer, 1940), as well as, inhibiting Mg catalyzed enzymes during germination (Ensminger et al., 1965).

However, the application of ammonium based fertilizers, especially when application rates exceed crop N requirements, have been found to acidify the soil surrounding the application sites (Barak et al., 1997). Two months after the application of DAP, McGowen et al. (2001) found a decrease in pH from 7.1 to 6.5.

Plant Uptake

Phosphorus uptake and fertilizer use efficiency are becoming a major point of concern with increasing fertilizer prices. In soil solution, P is mainly acquired by plant roots in the form

of inorganic phosphate as H_2PO_4^- or HPO_4^{2-} (Raghothama, 1999). Timing of P applications can also play an important role in P uptake. Barry and Miller (1989) showed a significant increase in corn yield in response to P fertilization before V-6 growth stage on a P deficient soil.

In plants, P is required for constitution of cellular components, which include nucleic acids, cellular membrane, and adenosine triphosphate (ATP). Also, P plays a major role in enzymatic reactions and in signal transduction processes (Marschner, 1995). Phosphorus deficiency in corn will decrease the rate of plant respiration before there is a noticeable decrease in photosynthesis. Therefore, an accumulation of anthocyanin pigmentation will occur due to a build-up of sugars and the leaves and stems will turn a purple or dark-green color. Like nitrogen, P can be trans-located within the plant, so deficiency symptoms appear first in older leaves at the base of the plant. Some varieties of corn do not produce the gene responsible for the purple pigmentation; therefore stunted growth and delayed maturity are most common symptoms that can be observed. Other effects of P deficiency can include poor seed formation, root development, and straw strength in cereals (Havlin et al., 2005).

Sorption in Soils

The colloidal component of acidic soils is dominated by hydrous oxides of Al and Fe on which P is specifically sorbed by ligand exchange, thus controlling P availability (Parfitt, 1978). Parameters from sorption isotherms can be used to predict P requirements of crops and P fertilizer management (Nwoke et al., 2003).

Organic matter (OM) additions to the soil have shown to both increase or decrease P sorption by soils. The mechanism by which OM interacts with P in soils is not fully understood (Haynes and Mokolobate, 2001). However, OM can increase P sorption by increasing the

number of sorption sites of Fe and Al due to the amorphous hydroxide nature of OM (Lopez-Hernandez and Burnham, 1974).

Others found that OM decreased P sorption after applications and mineralization of organic residue in soils (Singh and Jones, 1976). This decrease in P sorption is the result of organic acids and humic acid compounds that complex Fe and Al of P sorption sites on soil minerals (Hue, 1991; Iyamuremye and Dick, 1996; Ohno and Carnell, 1996; Ohno and Enrich, 1997; Haynes and Mokolobate, 2001). Natural organic matter (NOM) interacts with Fe oxides on soil surfaces and forms a “coating” (Weng et al., 2008). Sorption of organic matter on mineral surfaces influences P sorption to goethite (Weng et al., 2008).

Iron

Iron in soil systems can be present in various crystal forms such as goethite (α -FeOOH), hydrous ferric oxide (HFO), ferrihydrite, hematite (α -Fe₂O₃), and lepidocrocite (γ -FeOOH), which have a strong affinity for phosphate (Torrent et al., 1990). Even though the crystalline structure of goethite can vary, similar binding with phosphate is maintained (Torrent et al., 1990). Phosphate ions can form surface complexes by chemically reacting with one or two singly coordinated Fe groups. The phosphate ion sorption has been described using three surface species: a monodentate, a bidentate, and a protonated bidentate. Specifically, P sorption includes two innersphere complexes as a bidentate and protonated monodentate (Rahnemaie et al., 2007).

Aluminum

A stimulatory effect of plant growth in response to low concentrations of Al has been reported (Foy et al., 1978). Chelation with organic acids such as oxalate and citric acid would prevent the precipitation of the aluminum phosphate. Additions of Al complexed with chelating

agents such as EDTA promoted the absorption of Al in mustard plants grown in a nutrient solution (DeKock and Mitchell, 1957). Plants that have been determined to be resistant to Al might do so due to the chelating mechanism (Grime and Hodgson, 1969).

DeKock and Mitchell (1957) found that trivalent cations were readily absorbed by plants and transported to leaves when chelated with nitrilotriacetic acid (NTA), diethylenetriamine pentaacetic acid (DTPA), and EDTA. However, Bartlett and Riego (1972) reported that translocation of Al to plant tops was negligible with additions of chelates. Also, increased concentrations of Al have been reported to decrease uptake of magnesium, potassium, and P (Foy and Brown 1963). However, Barlette and Riego (1972) showed no effects of P and Ca concentrations when Al was applied as a chelated source as citrate, EDTA, and soil organic matter.

Degree of P Saturation

When fertilizers are continuously applied in concentrations greater than those taken up in crop removal, a buildup of P can accumulate to concentrations considered excessive in agronomic extractions. As time progresses, soils become increasingly saturated in P (Lookman et al., 1996). Soils with high concentrations of agronomic soil test P have been reported to have higher concentrations of soluble and bioavailable P (Sims et al., 1998).

Degree of P Saturation (DPS) has been proposed as a means to predict the potential for P losses in runoff and leaching under field conditions (Lookman et al., 1996; Pote et al., 1996; Sims et al., 1998; van der Zee and van Riemsdijk, 1988). The oxalate extraction is used to determine the DPS (DPS_{ox}) and was first used on acid sandy soils, but now has proven a good predictor of P availability in acidic soils with high OM and high clay content (Maguire et al., 2001). Only a small concentration of P is weakly bound in soils below 56 percent DPS, however

concentrations of weakly bound P increases rapidly above this value (Hooda et al., 2000; Pautler and Sims, 2000; Maguire and Sims, 2002). This threshold of 56 percent is generally above the optimum recommended fertilizer P rate, therefore those agricultural soils fall below the DPS threshold. With this value, adequate crop P supply and environmental protection can both be achieved (Pautler and Sims, 2000).

CHELATING AGENTS

Within the rhizosphere, there are naturally occurring chelates that are produced by the roots to aid in the absorption of essential metals. Scientists have studied the reactions between the compounds excreted and metals within the soil in relation to synthetic chelating agents. The term chelate, for ligands like ethylenediamine, was introduced in 1920 and described the process by which two donor atoms bond to the same metal cation forming a ring structure. Metals cations are chelated when they have empty orbitals that allow the metal ion to react with a ligand that contains an atom with a pair of electrons. Depending on the chemical composition, chelating agents may require more than one molecule to bind the metal cation due to the number of bonding sites on the ligand (Clemens et al., 1990).

The most common chelating agents use nitrogen and oxygen as these elements have empty orbitals that allow them to bond metals cations. EDTA forms many complexes in which six atoms within its structure can bond one central metal cation to form a ring, making this six membered ring the most stable configuration with the highest stability constant. When EDTA bonds with Fe^{+3} , iron exhibits an octahedral environment due to EDTA using all six bonding sites. Chelating agents with less than 6 empty orbitals, like gluconic acid with only three, form weak bonds with water molecules for each empty orbital remaining (Clemens et al., 1990).

Sugar acid chelates such as citric acid and gluconic acid have the ability to form water soluble complexes with metal ions. This phenomenon is due primarily to the carboxyl and hydroxyl groups that bind cations through coordinated covalent bonding. When the soil is slightly acid, the hydroxyl groups form a loose complex with the metal ions (Clemens et al., 1990).

Salt concentrations have been found to increase the sorption of chelates, in particular Ca salts (Wallace and Lunt, 1956). With increased concentrations of Ca-salts, there is a suppression of the electrical double layer around the negatively charged surfaces, which permit anionic species to sorb to sorption sites (Bolt and Warkentin, 1958). The net surface charge of Fe and Al oxides at low pH is positive, however as pH increases, the net charge becomes negative. Sorption of EDTA and HEDTA on Fe oxides of hematite was found to decline as pH increased from 3 to 10 (Chang et al., 1983).

Natural Production

Plant production of carboxylate exudates during nutrient deficiencies has been identified for Fe (Marschner and Romheld, 1994) and P (Strom et al., 2002). Carboxylate production can be defined as either directly or indirectly effecting P availability. Direct effects on P availability generally result in immediate P release in response to the oxidized dissolution by blocking P sorption sites on mineral surfaces (Staunton and Leprince, 1996).

Less is known about the indirect effect of exudates on P availability, but there is some understanding of how exudates stimulate microbial activity and rhizosphere pH changes. Haynes and Mokolobate (2001) and Staunton and Leprince (1996) determined that citric acid is exuded in the acid form and then dissociates once outside the cell causing acidification of the soil. Another indirect effect of exudates on the P availability is the complexation of Fe and Al in

soil humic substances. These compounds are more soluble, smaller, and therefore more accessible to soil and root phosphatases. Organically bound P is then enzymatically hydrolyzed (Haynes and Mokolobate, 2001).

Synthetic Chelating Agents

Synthetic chelates used for plant nutrition are organic acids and are placed into two categories based on composition: aminopolycarboxylate and hydroxycarboxylate.

Ethylenediamine tetraacetic acid (EDTA) and Hydroxyethyl ethylenediamine triacetic acid (HEEDTA) are categorized as aminopolycarboxylate. Aminopolycarboxylates are less expensive to manufacture, however, in alkaline soil conditions, the hydroxycarboxylates were found to be more stable and not dissociate as rapidly (Clemens et al., 1990).

EDTA and HEEDTA

The most widely used Fe sources are mixtures of inorganic Fe forms and chelates (Vempati and Loeppert, 1986). Chelated forms of Fe are more effective at alleviating Fe deficiencies in plants than are inorganic forms. Chelates are rarely economically feasible for low-value crops like corn because treatments have to be applied several times during a growing season. Application of Fe chelates have been used in Fe deficient conditions as foliar applications. This method usually alleviates chlorosis. Results from foliar applications may be only temporary and actually depress the plant's Fe stress mechanisms by preventing the increase in Fe-reducing capacity of the roots that would normally occur during Fe deficiency (Romheld and Marschner, 1986). Spatial heterogeneity creates problems because Fe-deficient areas in a field are usually small and levels of plant-available soil Fe can vary within a few meters (Vempati and Loeppert, 1986).

Foliar applications of chelated Fe fertilizer sources has been inconsistent as they have been found to be successful in reducing signs of chlorosis in soybean (Goos and Johnson, 2000), increasing yields (Penas et al., 1990), but have also had no effect in soybeans yields (Lingerfelter et al., 2005) and corn yields (Godsey et al., 2003).

Of the chelating agents, EDTA has been recognized as the most effective synthetic chelating agent due to its chelating ability of cation metals such as lead, cadmium, copper, and zinc, ability to be used in different types of soils, low biodegradable that leads to recovery and reuse of EDTA, and maintained soil pH as EDTA does not acidify when applied. The low biodegradability of EDTA could be of concern for the use of EDTA.

In solutions where Ca is the only competing cation, HEDTA was found to be a more efficient chelate than EDTA (Norvell, 1984). Godsey et al. (2003) found that foliar-applied chelated Fe-HEDTA was not effective in increasing grain yield, indicating that another Fe source should be considered. However, seed-applied chelated Fe fertilizer (HEDTA) increased grain yield by approximately 55 percent for both tolerant and nontolerant soybean varieties (Liesch et al., 2011).

Citric Acid

Solubilization of Fe and Al in the presence of citrate has been observed by several other researchers (Jones et al, 1996). Ebbs et al. (1998) found that over time concentrations of Fe and Al continued to increase, indicating that solubilization of these metals was still occurring with a possibility that increased concentrations of citrate added to soil may have been physically destructive to the soil. Drouillon and Merckx (2003) concluded that the exact quantification of the effects of citrate on P solubility remains unclear because effects are not solely attributed to rhizosphere pH modification, but also to the complexation capabilities of citric acid. Chelation

of Al is restricted largely to acidic conditions in soils, where Al concentrations are relatively high. Chelates such as CIT, are best able to associate with Al due to low selectivity with Fe competition. Citric acid was less efficient at maintaining Al concentrations and this was probably due to its rapid biodegradation rather than to inherent instability of its Al chelates. Above pH 6, CIT loses its efficiency at maintaining soluble Al and Fe (Mortvedt, 1991).

As soils weather and formation of Fe and Al amorphous hydroxides increase, acidic and calcareous soils are capable of binding P due to the soils increase in capacity for P sorption. Thus far in agriculture, synthetic chelating agents have been utilized as supplemental micronutrients. Little research has been conducted to test the effects chelating agents have on decreasing P sorption. With an understanding of how synthetic chelating agents can bind sorption sites on soil colloids and block P sorption, we can test the efficiency of those chelating agents by monitoring WSP concentrations and their effects on routine soil tests. Therefore, the overall objectives are to test the efficiency of chelating agents to increase the solubility of P in soils and determine a rate response curve for the chelating agents that could increase P solubility, and evaluate chelating agents for increasing plant available P through crop uptake and biomass.

REFERENCES

- Barak, P., B. O. Jobe, A. R. Kreuger, L. A. Peterson, and D. A. Laird. 1997. Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin. *Plant Soil* 197:61-69.
- Barry, S. A., and M. H. Miller. 1989. Phosphorus nutritional requirement of maize seedlings for maximum yield. *Agron. J.* 81:95-99
- Bartlett, R. J., and D. C. Riego. 1972. Effect of chelation on the toxicity of aluminum. *Plant Soil* 37:419-423.
- Bennett, A. C., and F. Adams. 1970. Concentrations of NH_3 (aq.) required for incipient NH_3 toxicity to seedlings. *Soil Sci. Soc. Am. Proc.* 34:259-263.
- Bolt, G. H., and B. P. Warkentin. 1958. The negative adsorption of anions by clay suspensions. *Kolloid Z.* 156:41-46
- Chang, H. C., T. W. Healy, and E. Matijevic. 1983. Interactions of metal hydrous oxides with chelating agents, III. Adsorption on spherical colloidal hematite particles. *J. Colloid Interf. Sci.* 92:469-478.
- Chien, S. H., P. W. G. Sale, and D. K. Feiesen. 1990. A discussion of the methods for comparing the relative effectiveness of phosphate fertilizer varying in solubility. *Fert. Res.* 24:149-157.
- Clemens, D. F., B. M. Whitehurst, and G. B. Whitehurst. 1990. Chelates in agriculture. *Fert. Res.* 25:127-131.
- Dekock, P. C., and R. L. Mitchell. 1957. Uptake of chelated metals by plants. *Soil Sci.* 84:55-62.
- Drouillon, M., and R. Merckx. 2003. The role of citric acid as a phosphorus mobilization mechanism in highly p-fixing soils. *Gayana Bot.* 60:55-62.

- Ebbs, S. D., W. A. Norvell, and L. V. Kochian. 1998. The effect of acidification and chelating agents on the solubilization of uranium from contaminated soil. *J. Environ. Qual.* 27:1486-1494.
- Ensminger, L. E., J. T. Hood, and G. H. Willis. 1965. The mechanism of ammonium phosphate injury to seeds. *Soil Sci. Soc. Am. Proc.* 29:320-322.
- Foy, C. D., and J. C. Brown. 1963. Toxic factors in acid soils. I. Characterization of aluminum toxicity in cotton. *Soil Sci. Soc. Am. Proc.* 28:27-32.
- Foy, C. D., R. L. Chaney, and M. C. White. 1978. The physiology of metal toxicity in plants. *Ann. Rev. Plant Physiol.* 29:511-566.
- Godsey, C. B., J. P. Schmidt, A. J. Schlegel, R. K. Taylor, C. R. Thompson, and R. J. Gehl. 2003. Correcting iron deficiency in corn with seed row-applied iron sulfate. *Agron. J.* 95:160-166.
- Goos, R. J., and B. E. Johnson. 2000. A comparison of three methods for reducing Fe deficiency chlorosis in soybean. *Agron. J.* 92:1135-1139.
- Grime, J. P., and J. Sl. Hodgson. 1969. An investigation of the significance of lime chlorosis by means of large scale comparative experiments. p. 381-397. *In* I. H. Rorison (ed.). *Ecological aspects of the mineral nutrition of plants.* Blackwell, Oxford.
- Havlin, J. L., J. D. Beaton, S. L. Tisdale, and W. L. Nelson. 2005. *Soil fertility and fertilizers: An introduction to nutrient management* 7th ed.. Upper Saddle River Pearson Prentice Hall, NJ. p. 160-176
- Haynes, R.J., and M. S. Mokolobate. 2001. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved. *Nutr. Cycl. Agroecosys.* 59:47-63.

- Hooda, P. S., A. R. Rendell, A. C. Edwards, P. J. A. Withers, M. N. Aitken, and V. W. Truesdale. 2000. Relating soil phosphorus indices to potential phosphorus release to water. *J. Environ. Qual.* 29:1166-1171.
- Hue, N. V. 1991. Effects of organic acids/anions on P sorption and phytoavailability in soils with different mineralogies. *Soil Sci.* 152:463-471.
- Iyamuremye, F., and R. P. Dick. 1996. Organic amendments and phosphorus sorption by soils. *Adv. Agron.* 56:139-185.
- Jones, D. L., P. R. Darrah, and L. V. Kochian. 1996. Critical evaluation of organic acid mediated iron dissolution in the rhizosphere and its potential role in root iron uptake. *Plant Soil.* 180:57-66.
- Liesch, A. M., D. A. Diaz, K. L. Martin, B. L. Olson, D. B. Mengel, and K. L. Roozeboom. 2011. Management strategies for increasing soybean yield on soils susceptible to iron deficiency. *Agron. J.* 103:1870-1877.
- Lingerfelser, J. E., W. T. Schapaugh, Jr., J. P. Schmidt, and J. J. Higgins. 2005. Comparison of genotype and cultural practices to control Fe deficiency chlorosis in soybean. *Commun. Soil Sci. Plant Anal.* 36:1047-1062.
- Lookman, R., K. Jansen, R. Merckx, and K. Vlassak. 1996. Relationship between soil properties and phosphate saturation parameters, a transect study in northern Belgium. *Geoderma.* 69:265-274.
- Lopez-Hernandez, D., and C. P. Burnham. 1974. The effect of pH on phosphate adsorption in soils. *J. Soil Sci.* 25:207-216.
- Maguire, R. O., and S. E. Heckendorn. 2011. *Laboratory Procedures: Virginia Tech Soil Testing Laboratory, Virginia Cooperative Extension, Blacksburg, Va.*

- Maguire, R. O., and J. T. Sims. 2002. Measuring agronomic and environmental soil phosphorus saturation and predicting phosphorus leaching with Mehlich-3. *Soil Sci. Soc. Am. J.* 66:2033-2039.
- Maguire, R. O., J. T. Sims, S. K. Dentel, F. J. Coale, and J. T. Mah. 2001. Relationships between biosolids treatment process and soil phosphorus availability. *J. Environ. Qual.* 30:1023-1033.
- Marschner, H. 1995. *Mineral Nutrition of Higher Plants.*, 2nd ed Academic Press, London, England.
- Marschner, H., and V. Romheld. 1994. Strategies of plants for acquisition of iron. *Plant Soil.* 165: 261-274.
- McCollum, R. E. 1991. Buildup and decline in soil phosphorus: 30-year trends on a typical Umprabuilt. *Agron. J.* 83:77-85.
- McGowen, S. L., N. T. Basta, and G. O. Brown. 2001. Use of diammonium phosphate to reduce heavy metal solubility and transport in smelter-contaminated soil. *J. Environ. Qual.* 30:493-500.
- Mortvedt, J. J. 1991. *Micronutrients in agriculture.* Soil Sci. Soc. Am., Madison, WI. p. 187-199.
- Norvell, W. A. 1984. Comparison of chelating agents as extractants for metals in diverse soil materials. *Soil Sci. Soc. Am. J.* 48:1285-1292.
- Nwoke, O. C., B. Vanlauwe, J. Diels, N. Sanginga, O. Osonubi, and R. Merckx. 2003. Assessment of labile phosphorus fractions and adsorption characteristics in relation to soil properties of West African savanna soils. *Agr., Ecosyst., Environ.* 100:285-294.

- Ohno, T., and B. S. Carnell. 1996. Green and animal manure-derived dissolved organic matter effects on phosphorus sorption. *J. Environ. Qual.* 25:1137-1143.
- Ohno, T., and M. S. Enrich. 1997. Inhibitory effects of crop residue-derived organic ligands on phosphate adsorption kinetics. *J. Environ. Qual.* 26:889-895.
- Parfitt, R. L. 1978. Anion adsorption by soils and soil materials. *Adv. Agron.* 30:1-50.
- Paulter, M. C., and J. T. Sims. 2000. Relationships between soil test phosphorus, soluble phosphorus, and phosphorus saturation in Delaware soils. *Soil Sci. Soc. Am. J.* 64:765-773.
- Penas, E. J., R. A. Wiese, R. W. Elmore, G. W. Hergett, and R. S. Moomaw. 1990. Soybean chlorosis studies on high pH bottomland soils. *Univ. of Nebraska Inst. Agric. Nat. Res. Bull.* 312. Univ. of Nebraska, Lincoln.
- Pierzynski, G. M., and T. J. Logan. 1993. Crop, soil, and management effects on phosphorus soil test levels. *J. Prod Agric.* 6:513-520.
- Pote, D. H., T. C. Daniel, A. N. Sharpley, P. A. Moore, D. R. Edwards, and D. J. Nichols. 1996. Relating extractable soil phosphorus to losses in runoff. *Soil Sci. Soc. Am. J.* 60:855-859.
- Raghothama, K. G. 1999. Phosphate acquisition. *Annu. Rev. Plant Biol.* 50:665-693.
- Rahnemaie, R., T. Hiemstra, and W. H. Van Riemsdijk. 2007. Carbonate adsorption on goethite in competition with phosphate. *J. Colloid Interf. Sci.* 315:415-425.
- Rios, M. A., and R. W. Pearson. 1974. The effect of some chemical environmental factors on cotton root behavior. *Soil Sci. Soc. Am. Proc.* 28:232-235.
- Romheld, V. and H. Marschner. 1986. Mobilization of iron in the rhizosphere of different plant species. *Adv. Plant Nutr.* 2:155-204.

- Romheld, V., and H. Marschner. 1986. Mobilization of iron in the rhizosphere of different plant species. *Adv. Plant Nutr.* 2:155-204.
- Sims, J.T., R. R. Simard, and B. C. Joern. 1998. Phosphorus loss in agricultural drainage: Historical perspective and current research. *J. Environ. Qual.* 27:277-293.
- Singh, B.B., and J. P. Jones. 1976. Phosphorus sorption and desorption characteristics of soil as affected by organic residues. *Soil Sci. Soc. Am. J.* 40:389-394.
- Sorokin, H., and A. L. Sommer. 1940. Effect of calcium deficiency upon the roots of *Pisum sativum*. *Am. J. Bot.* 27:308-318.
- Staunton, S., and F. Leprince. 1996. Effect of pH and some organic anions on the solubility of soil phosphate: implications for P bioavailability. *Eur. J. Soil Sci.* 47: 231-239.
- Strom, L., A. O. Owen, D. L. Godbold, and D. L. Jones. 2002. Organic acid mediated P mobilization in the rhizosphere and uptake by adaptive physiology to transgenic varieties for cultivation in extreme soils. *Plant Sci.* 160:1-13.
- Torrent, J., V. Barron, and U. Schwertmann. 1990. Phosphate adsorption and desorption by goethites differing in crystal morphology. *Soil Sci. Soc. Am. J.* 54:1007-1012.
- van der Zee, S.E.A.T.M., and W.H. van Riemsdijk. 1988. Model for long-term phosphate reaction kinetics in soil. *J. Environ. Qual.* 17:35-41.
- Vempati, R.K., and R. H. Loeppert. 1986. Synthetic ferrihydrite as a potential iron amendment in calcareous soils. *J. Plant Nutr.* 9:1039-1052.
- Wallace, A., and O. R. Lunt. 1956. Reactions of some iron, zinc, and manganese chelates in various soils. *Soil Sci. Soc. Am. Proc.* 20:479-482.

Weng, L., W. H. Van Riemsdijk, L. K. Koopal, and T. Hiemstra. 2006. Adsorption of humic substances on goethite: comparison between humic acids and fluvic acids. *Environ. Sci. Technol.* 40:7494-7500.

Weng, L., W. H. Van Riemsdijk, and T. Hiemstra. 2008. Humic nanoparticles at the oxide-water interface: interactions with phosphate ion adsorption. *Environ. Sci. Technol.* 42:8747-8752.

CHAPTER 2: USING SYNTHETIC CHELATING AGENTS TO DECREASE PHOSPHORUS BINDING IN SOILS

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Abbreviations list: Fe, P, phosphorus; Fe, iron; Al, aluminum; EDTA, ethylenediamine tetraacetic acid; HEEDTA, hydroxyethyl ethylenediamine triacetic acid; GA, gluconic acid; CA, citric acid; WSP, water-soluble phosphorus; PSC, phosphorus sorption capacity; Ca, calcium; DAP, di-ammonium phosphate; OM, organic matter; Mg, magnesium; B, boron; DTPA, diethylenetriamine pentaacetic acid; CEC, cation exchange capacity; ICP-OES, inductively coupled plasma-optical emission spectroscopy; Al_{ox}, Fe_{ox}, P_{ox}, oxalate-extractable aluminum, iron, and phosphorus; 1 d, 7 d, 21 d, 35 d, 49 d, Day 1, 7, 21, 35, and 49 of the incubation study; DPS, degree of phosphorus saturation

ABSTRACT

Most acid soils have a high capacity to bind phosphorus (P) strongly on iron (Fe) and aluminum (Al) hydroxides, resulting in low P fertilizer use efficiency. Organic chelating agents form strong bonds with metals in soil and may reduce P binding with Fe and Al and increase fertilizer P use efficiency. Ethylenediamine tetraacetic acid (EDTA), hydroxyethyl ethylenediamine triacetic acid (HEEDTA), gluconic acid (GA), and citric acid (CA) were tested to determine their influence on water-soluble P (WSP), Mehlich-1 P and Mehlich-3 P in a Loam and Sand fertilized with P and incubated for 49 days. Soil P sorption capacity (PSC) was estimated from an oxalate extraction of Fe and Al, and chelates were applied at rates of 90 percent of the PSC. EDTA and HEEDTA were also applied at 0, 30, 60, 90, 120, and 150 percent PSC to produce a rate response curve for WSP in a second soil incubation. The EDTA, HEEDTA, and CA significantly ($P < 0.05$) reduced P sorption in the Loam and Sand when measured by WSP. In soils without P fertilizer added, EDTA and HEEDTA resulted in a significant increase in WSP concentrations, as well as increased concentrations of Mehlich-1 P and Mehlich-3 P. With increasing chelate rate of EDTA and HEEDTA there was a linear increase in WSP for both soils, indicating higher rates were most effective at decreasing P sorption. The application of chelating agents, with and without P fertilizer, could potentially increase plant availability of P, therefore reducing the need for P fertilizer.

INTRODUCTION

Soils adsorb P by forming chemical bonds through ligand exchange between the phosphate anion and functional groups of the soil, such as calcium (Ca) in calcareous soils and oxides and hydroxides of Fe and Al in acidic soils (van der Zee and Van Riemsdijk, 1988). Amorphous or poorly structured Al and Fe oxides and hydroxides account for most of the P sorption in acidic soils (van der Zee and van Riemsdijk, 1988). The strong binding of P by Al and Fe in acidic soils is undesirable as it can hold P in non-plant available forms. This is a concern to farmers as P fertilizer prices have been increasing in recent years. For example, from 2004 to 2012 the di-ammonium phosphate (DAP) price increased from \$304 Mg⁻¹ to \$800 Mg⁻¹ with a spike in price to \$937 Mg⁻¹ in 2008 (USDA, 2012).

Another major problem is fertilizer use efficiency. Therefore, creating an interest in products to make P more plant available and decrease P fertilizer application rates. Organic matter and organic ligands, such as EDTA, have the same carboxylic acid functional groups that could cause similar chemical reactions on soil surfaces to occur preventing P sorption. Organic ligands compete with oxyanions like phosphate in the soil for mineral surface binding sites (Campbell and Eick, 2002). This competition is pH dependent. For example, Dossa et al. (2008) showed that the additions of organic matter (OM) to soil increased the equilibrium solution P concentration and decreased P sorption. OM can decrease P sorption in soils potentially by organic acid and humic compounds that complex Fe and Al sorption sites on soil minerals (Hue, 1991; Iyamuremye and Dick, 1996; Ohno and Carnell, 1996; Ohno and Enrich, 1997; Haynes and Mokolobate, 2001). Similar results were observed by Staunton and Leprince (1996) where carboxylic acids directly affected P release by blocking P sorption sites on mineral surfaces or the mobilization of P held in humic substances.

Previous experiments have studied the foliar and soil applications of chelated metals as a source of supplemental micronutrients. Chelating agents used in agriculture can be categorized as aminopolycarboxylates or hydroxycarboxylates. EDTA and HEEDTA are aminopolycarboxylates, citric acid a tricarboxylate hydroxycarboxylate and gluconic acid a monocarboxylate hydroxycarboxylate (Clemens et al., 1990). Butler and Bray (1956) and Leonard and Stewart (1952) have shown an increase in trace metal sorption in plants with additions of EDTA to soil. Sorption of magnesium (Mg), boron (B), Ca, and P increased in wheat, corn, and okra as diethylene triamine pentaacetic acid (DTPA) treatment concentrations increased (Brown et al., 1960). Foliar applications of chelated Fe have been used to alleviate chlorosis in soybeans (Goos and Johnson, 2000) and in several situations increase soybean yields (Penas et al., 1990).

At low pH values, EDTA complexes Fe-oxide surfaces in either a binuclear or multinuclear form and at higher pH, EDTA forms a mononuclear complex (Nowack and Sigg, 1996). When binuclear and multinuclear complexes are formed more energy is required to break those bonds, therefore inhibiting dissolution (Stumm, 1992). At a low pH, the increased competition of P for Fe-oxide sites decreases the number of EDTA groups bound (Borggaard, 1991). Chang et al. (1983) found that sorption of EDTA and HEDTA on Fe oxides of hematite was found to decline as pH increased from 3 to 10. However, on sorption sites of bayerite, HEDTA sorption proved to not be pH dependent and it was suggested that the hydroxyethyl groups may have allowed for additional hydrophobic bonding (Bowers and Huang, 1985). Lunt et al. (1956) showed that losses of Fe and EDTA from applications Fe-EDTA were 1:1, thus suggesting that the chelate may have been sorbed in the Fe-EDTA complex.

With increasing cost for P fertilizer, there is an ever increasing need to study how fertilizer P can be kept plant available in soil. Using chelating agents to decrease binding of P by Al and Fe oxides is one possible mechanism to increase plant available P and decrease P fertilizer requirements. The objectives of this study were to (i) test the effectiveness of four chelating agents, EDTA, HEEDTA, CA, and GA to increase the solubility of P in soils, (ii) determine a rate response curve for the chelating agents that could increase P solubility, and (iii) evaluate the effects of chelating agents on routine plant available soil test values.

MATERIALS AND METHODS

Soil Characterization

Two acid soils were obtained for use in this study, a Shottower (Fine, kaolinitic, mesic Typic Paleudults) Loam and Conetoe (loamy, mixed, semiactive, thermic Arenic Hapludult) Sand. From here on they will be referred to as the “Loam” and “Sand”. Both soils were passed through a 1.3cm sieve during collection of the top 15cm. Following collection, the two soils were air dried in a greenhouse. Both soils were chosen based on a history of low fertilizer applications and differing textural classification.

Soil water-pH at a 1:1 volume to volume ratio of soil material to distilled water and OM by loss on ignition were measured by standard soil testing methods of the Virginia Tech Soil Testing Laboratory (Maguire and Heckendorn, 2011). Cation Exchange Capacity (CEC) was determined using the double wash method where soil was saturated with 1M CaCl₂, leached, and rinsed with deionized water. The soil was then washed with 1M KCl, displacing sorbed Ca⁺². Extract was analyzed for Ca concentration by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Field capacity was determined by completely saturating dry soil and

allowing gravitational drainage through holes in the bottom of a plastic cup for 48 hours (Bond et al., 2006). Particle size analysis was determined by pipette method (Gee and Bauder, 1986).

Mehlich-1 P was measured by standard soil testing methods of the Virginia Tech Soil Testing Laboratory (Maguire and Heckendorn, 2011). Mehlich-3 P was extracted at a 1:10 soil to solution ratio using a 0.2M CH₃COOH + 0.25M NH₄NO₃ + 0.015M NH₄F + 0.013M HNO₃ + 0.001M EDTA with a 5min reaction time and filtered through a Whatman #41 filter paper (Mehlich, 1984). Oxalate P, Al and Fe were extracted at a 1:40 soil to solution ratio using a 0.2M ammonium oxalate + 0.2M oxalic acid solution (pH 3.0) with a reaction time of 2hr in complete darkness. Samples were centrifuged at 479 g for 13min and filtered using Whatman #42 filter paper (McKeague and Day, 1966). The Mehlich-3 P and Fe and oxalate P, Fe, and Al were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES).

Concentrations of amorphous Fe and Al hydroxides extracted from an acid ammonium oxalate extraction can be used to estimate the PSC of a soil (Maguire et al., 2001; McKeague and Day, 1966). The PSC was then used to determine chelating rates based on the linear relationship between the sum of amorphous hydroxides and the maximum sorption of P (Maguire et al., 2001). PSC is expressed as a function of the sum of Fe and Al oxides:

$$PSC = \alpha (Al_{ox} + Fe_{ox}), \quad (1)$$

where PSC is P sorption capacity expressed as mmol kg⁻¹, α is a scaling factor of 0.5 (Lookman et al., 1995), and the Al_{ox} and Fe_{ox} is the sum of ammonium oxalate-extractable Al and Fe (mmole kg⁻¹) (Maguire et al., 2001). The scaling factor of 0.5 is the most commonly used value and assumes it takes 2 moles of Al and / or Fe to adsorb 1 mole of P (Lookman et al., 1995; van der Zee et al., 1987). The degree of phosphorus saturation (DPS) for each soil was calculated as follows (van der Zee and van Riemsdijk, 1988):

$$\text{DPS} = (\text{P}_{\text{ox}} / \text{PSC}), (2)$$

where P_{ox} is quantified in the acid ammonium oxalate extract in mmoles kg^{-1} and PSC calculated using eq. 1.

Chelate Efficiency Incubation

Four chelating agents were selected based on stability constants with Fe and Al (EDTA and HEEDTA) and production by plants (CA and GA). Chelating rates were based on the PSC calculated from the Al and Fe in the oxalate extraction. It is known that chelating agents are capable of forming up to 6 bonds with Fe and Al (Clemens et al., 1990). To ensure complete coverage of all sorption sites and free metal ions, EDTA and HEEDTA were applied at one mole of EDTA or HEEDTA to PSC of the soil calculated in eq. 1. Citric acid and GA were applied at twice this rate as each citric or gluconic acid molecule form only three bonds with each Fe and Al.

Two incubations of soils with chelating agents were performed, and the effects on WSP were measured. The first incubation tested the efficiency of all four chelating agents at a chelate rate based on binding, 90% PSC. The second incubation used only EDTA and HEEDTA as these proved successful in the first incubation, as described in the Results and Discussion section. This second incubation tested a range of chelating rates (0, 30, 60, 90, 120, and 150 percent PSC) to produce a response curve. The maximum chelating rate of 150 percent PSC was determined based on the solubility of chelating agents at a specific pH in deionized water ($\text{pH}=5.0$). Apart from the addition of chelating rates in the second incubation, the preparation, sampling and processing for the two incubations were identical.

Incubation cups were prepared by taking 237mL polyethylene specimen storage cups and drilling two holes at 0.32cm diameter into the top to allow gas exchange and prevent anaerobic conditions. Into each incubation cup, 0.060kg of either the air dry Loam or Sand was weighed. Each of the four chelating agents, EDTA, HEEDTA, CA, and GA, and P fertilizer were dissolved in deionized water. Solutions were applied to each air-dry soil, in triplicate, to bring 0.060kg soil per cup to 70 percent field capacity. Control cups (No Chelate and No P) were brought to 70 percent field capacity using deionized water. In addition to chelating agents, P fertilizer was added as ammonium phosphate dibasic at a rate of 49.3 kg P ha⁻¹ based on the fertilizer recommendation for corn and the Mehlich-1 P extraction (Maguire and Heckendorn, 2011). For consistency, the same P rate was applied to the Sand soil even though there were no P fertilizer recommendations based on Mehlich-1 extractable P as the Sand was categorized at “very high” for Mehlich-1 P (Maguire and Heckendorn, 2011). Before application, all treatment solutions of fertilizer and chelates were adjusted to pH 5.0 to keep concentrations of sodium added to the soil low. Solutions were mixed in soil until uniform consistency was achieved. All soils were incubated for 49-d and moisture content was maintained by weight with bi-weekly applications of deionized water.

Sub-samples were taken for WSP analysis at 1, 7, 21, 35, and 49 d intervals. Water-Soluble P was immediately extracted moist at a 1:10 soil to deionized water ratio on a dry weight basis and filtered through 0.45µm millipore filter paper after centrifuging at 479 g for 13 min (Luscombe et al., 1979). The WSP extract was analyzed colorimetrically by the molybdate blue method (Murphy and Riley, 1962). A separate subsample was taken at 2, 8, 33, and 49 d intervals to monitor water pH values. Mehlich-1 extractable P and Mehlich-3 extractable P concentrations were analyzed following the air-drying of the soil (49-d) as described above.

STATISTICAL ANALYSIS

Data from incubation and rate studies were analyzed using the GLIMMIX procedure available for SAS (SAS Institute, 2012). Treatment effects on least square means of WSP, Mehlich-1 P, and Mehlich-3 P by soil were separated using Tukeys HSD at a significant level of $P=0.05$ (SAS Institute, 2012). Regression was used to evaluate the relationship of increasing chelating rate on soil test P levels.

RESULTS AND DISCUSSION

Soil Properties

The Loam had higher OM (52 g kg^{-1}) than the Sand (10 g kg^{-1} ; Table 2. 1). The CEC of the Loam was $9.3 \text{ cmol}_c \text{ kg}^{-1}$ and $1.63 \text{ cmol}_c \text{ kg}^{-1}$ for the Sand, which are typical of soils with these textures in Virginia and North Carolina where the clay fraction in highly weathered soils is composed mainly of kaolinite. The Sand had a slightly lower pH as compared to the Loam, 6.48 and 6.04 respectively (Table 2. 1) but both are still considered to be within the range for agronomic crop productions (Maguire and Heckendorn, 2011). The two soils were in the “low” (Loam) with 6 mg kg^{-1} and “very high” (Sand) with 63 mg kg^{-1} Mehlich-1 P soil fertility categories according to current soil test P criteria of the Virginia Tech Soil Test Laboratory. Phosphorus fertilizer additions were still recommended to optimize crop productivity in the Loam, but the Sand was above the upper level of 55 mg kg^{-1} extractable P where no P fertilizer is recommended for most crops (Maguire and Heckendorn, 2011). Mehlich-3 extractable P was greater than Mehlich-1 P in both soils at 10.7 and 190 mg kg^{-1} . Mehlich-3 P concentrations have

been found to be approximately 1.5 to 2 times greater than Mehlich-1 extractable P (Daniels et al., 1998; Sims, 1989). Oxalate extractable P was 158 mg kg⁻¹ for the Loam and 265 mg kg⁻¹ for the Sand, much greater than the other measures of soil P. This is consistent with previous findings (Maguire and Sims, 2002).

The Loam had almost three times the concentration of amorphous (oxalate-extractable) Al and Fe hydroxides than the Sand, therefore almost three times the PSC, 34 and 12mmol kg⁻¹, respectively (Table 2. 1). The high value of extractable Fe and Al in the Loam may be due to its high OM and higher clay content, which increases the amorphous nature and oxalate extractability of Fe and Al (Maguire et al., 2000). Soils with a higher PSC have a greater ability to make fertilizer P less plant available, especially over a long period of time due to the slow reactions that decrease solubility of plant available P (van der Zee and van Riemsdijk 1988).

The degree of P saturation (DPS) for the Loam and Sand were calculated using eq. 2 above at 15 and 71 percent, respectively. Higher percentages of DPS are at an increased risk for potential P losses in runoff and leaching from the environment (Sims, 1989), especially a DPS above 25 percent is of environmental concern (Breeuwsma and Silva, 1992).

Efficiency of EDTA, HEEDTA, Citric Acid and Gluconic Acid at Preventing Phosphorus Sorption

Results of the 49d incubation with the four chelating agents showed that WSP decreased between 1 d and 21 d in the Loam (Fig. 2. 1a). This initial reduction in WSP can be attributed to the interaction of P with Fe and Al via sorption, which is the predominant pathway of P retention in these acidic soils (van der Zee and van Riemsdijk, 1988). The WSP also decreased in the first 21d for the Sand, but the slope of the line was not as steep, probably due to the lower PSC. Initial

decreases in WSP have been seen in other incubations, as freshly fertilized soils take time to stabilize (Maguire et al., 2001). From 21d to 49d the changes in WSP were small for both soils relative to those in the first 21d.

After 49d the WSP for the EDTA, HEEDTA, and CA treatments were all found to be significantly greater than the Control-P in both soils, indicating that they were able to block P sorption and therefore presumably maintain more plant available form (Figs. 2. 1a and 2. 1b). This reduction in P sorption by ligand exchange is consistent with studies utilizing organic residues with similar chemical reactions (Singh and Jones, 1976; Bumaya and Naylor, 1988). The mechanisms by which P sorption can decrease include complexation of Fe and Al absorption sites on mineral surface by organic acids (Iyamuremye and Dick, 1996) and competitive inhibition for sorption sites between organic acids and P (Ohno and Cranell, 1996). The WSP concentrations for the GA treatment after 49d of the soil incubation were not found to be significantly different than the Control-P for either soil. Therefore, GA was not effective at preventing P sorption by Al and Fe hydroxides. Even though GA has the highest stability constant at 10^{37} for Fe^{+3} , the increase of pH to alkaline conditions decreased the efficiency. Less is known about the effects of GA on sorption sites.

Water-soluble P is insensitive to soil type, however, researchers have found that WSP proved to have limitations in predicting plant available P as a routine soil test in soils with high P sorption capacity (Menon et al., 1988) and is only a small fraction of total P in soils (Maguire and Sims, 2002; Pote et al., 1996). No additional fertilizer P is recommended for most crops above 55mg Mehlich-1 P kg^{-1} (Maguire and Heckendorn, 2011). Mehlich-1 P is normally much greater than WSP in soils, but despite this, WSP was often well above 55 mg P kg^{-1} in this soil incubation. Indeed, EDTA, HEEDTA, and CA maintained WSP above 55 mg P kg^{-1} throughout

the 49 d of the incubation in the Loam, indicating the large scale of decreased P sorption (Fig. 2.1a). A high background P concentration in the Sand resulted in concentrations of WSP greater than 55 mg P kg⁻¹ even in the Control-P (Fig. 2. 1b).

Other studies of soil incubations where P amendments were added to soil did not observe the substantial increase in WSP, as seen with applications of chelating agents in this study. For example, WSP concentrations 1d after applications of EDTA and HEEDTA to the Loam were 178 and 192 mg kg⁻¹, respectively. However, when biosolids amendments were made to soils in an incubation study similar to this one, WSP concentrations were always numerically less than 8mg kg⁻¹ (Maguire et al., 2001). High WSP concentrations on 1d can be attributed to the additions of DAP, a highly water soluble source of P, and the sorption of chelates to soil surfaces blocking P sorption. Sorption of chelates by soil was found to occur within 1d of application (Hill-Cottingham and Lloyd-Jones, 1957).

Above a threshold of 56 percent degree phosphorus saturation, WSP concentrations increase exponentially compared to the slight increase below this level (Maguire and Sims, 2002; Hooda et al., 2000; Paulter and Sims, 2000). Therefore on 1d, additions of P fertilizer to soils with an already high DPS as in the Sand at 70.8 percent DPS (Fig. 2. 1a), should result in greater increases in WSP as compared to the Loam at 15 percent DPS (Fig. 2. 1b).

The WSP for the CA treatment was always numerically greater than both EDTA and HEEDTA, but the difference was only significant in the Loam. The WSP for the HEEDTA was significantly greater than the EDTA in the Loam, but not the Sand. Increased concentrations of WSP in both soils treated with citric acid as compared to the Control-P could be due to a pH effect as opposed to a chelate effect. Soils treated with CA averaged pH 9.6 while most treatments pH values ranged from 5.5 to 6.5. The reason for the pH of the CA treatments being

so high relative to the other treatments is not clear, as all chelate solutions were adjusted to pH 5 before addition to the soils. Results of a preliminary study (data not shown) showed that soil treated with CA that was not pH adjusted would not significantly increase WSP concentrations compared to the Control due to a decrease in pH (<4) and thus increasing P retention by Fe and Al hydroxides. Above a pH value of 6, CA efficiency at chelating Fe and Al steeply decreases (Mortvedt, 1991). Even though CA was found to significantly increase WSP concentrations, Ebbs et al. (1998) found that additions of CA to soils may have been physically destructive as concentrations of Fe and Al continually increased over time. Based on these results, the EDTA and HEEDTA were chosen for further investigation, as detailed in the following sections.

Effect of Chelates Without Added Phosphorus

In the second part of this study, soils treated with EDTA and HEEDTA without P fertilizer additions resulted in a sharp decrease in WSP concentrations between 1d and 21d in the Loam (Fig. 2. 2a) and only a slight decrease in WSP in the Sand (Fig. 2. 2b). After 49d the WSP for the EDTA and HEEDTA treatments were found to be significantly greater than the Control-No P in both soils. In the Loam (Fig. 2. 2a), HEEDTA maintained WSP at significantly higher concentrations when compared to EDTA even though EDTA has a stability constant with Fe^{+3} of 10^{25} compared to HEDTA with a stability constant of $10^{19.6}$ in an agronomic pH range (Clemens et al., 1990).

With rising fertilizer costs, increasing WSP concentrations in soils treated with chelating agents without the additions of P fertilizers could potentially lead to lower P application rates based on these results. Even in a soil with high PSC, WSP concentrations in the Loam doubled with the application of chelating agents at 90 percent of that PSC from 0.4 in the Control- No P

to between 0.8 and 1.0 mg WSP kg⁻¹ for EDTA and HEEDTA. However, in the Sand, which has a Mehlich-1 P level above 55 mg P kg⁻¹ at which no P fertilizer would be recommended (Maguire and Heckendorn, 2011) and low PSC, chelating agent application would further increase WSP concentrations, especially over the Mehlich-1 value of 55 mg P kg⁻¹ (Fig. 2.2b).

Response of WSP to Chelating Agent Rate

In the second soil incubation, increasing chelating rate of EDTA and HEEDTA from 0 to 150 percent PSC increased WSP concentrations. Sampling intervals 1, 21, and 49d were chosen to show the changes in relationship between chelating rate and WSP over the 49d soil incubation (Fig. 2. 3a, 2. 3b, and 2. 3c). As incubation time increased in the Loam, the linear relationships between increasing chelating rate and WSP improved for EDTA ($r^2=0.86, 0.98, \text{ and } 0.99$). Increasing chelating rate in the Sand maintained a positive linear relationship with increasing incubation time for EDTA ($r^2=0.95, 0.99, \text{ and } 0.99$) and HEEDTA ($r^2=0.88, 0.97, \text{ and } 0.99$). This agrees with the work of Hill-Cottingham and Lloyd-Jones (1957), who showed that sorption of Fe-EDTA and Fe-HEDTA in soils was proportional to the concentration applied. All linear regression equations were found to be significant at the $P<0.05$ level, except HEEDTA applied to the Loam on 1d after application, which had an r^2 value of 0.59 (Table 2. 2).

On 1d, based on the regression equation, the increase in chelate application by one percent PSC increased WSP concentrations by 0.38 and 0.26 mg P kg⁻¹ for EDTA and HEEDTA in the Loam (Fig. 2. 3a). The concentration of WSP was almost double, 0.71 and 0.48 mg P kg⁻¹ respectively, in the Sand. This is possibly due to the high DPS in the Sand at 71 percent compared to the Loam at 15 percent. As incubation time increased in the Sand to 49d, the regression increase in chelate application by one percent PSC increased WSP concentrations of

1.05 and 1.09mg P kg⁻¹ for EDTA and HEEDTA (Fig. 2. 3c). However, EDTA additions to the Loam increasing one percent PSC on 21d and 49d were numerically lower than 1d at 0.27 and 0.29 mg P kg⁻¹, respectively. The reason for this change is unknown, but it is beneficial to know that as incubation time increases, increased additions of EDTA and HEEDTA are capable of maintaining concentrations of WSP based on the regression lines observed here.

Figure 2. 4 shows the trends of WSP when chelates and P were applied at the greatest PSC of 150 percent over the 49d soil incubation. The Sand treated with both EDTA and HEEDTA maintained WSP at significantly higher concentrations than the Loam over the 49d soil incubation. This result is attributed to the high background P in the Sand as compared to the Loam, however, after 1d of the soil incubation, WSP concentration in both the Loam and Sand were not significantly different with the additions of chelating agents. The WSP in the Loam treated with EDTA and HEEDTA followed the same trend as application rates of 90 percent PSC as WSP concentrations steeply decreased to 21d and then leveled. As when applied at 90 percent PSC, EDTA and HEEDTA were able to significantly increase WSP concentrations when compared to the Control P. Having a higher PSC and lower DPS, the Loam was expected to decrease as incubation time increased. The higher concentrations of WSP in the Sand could be explained by the higher DPS as described above. When compared to EDTA, HEEDTA significantly increased WSP concentrations in the Loam, but not in the Sand

Effects of Chelating Agents on Soil Test Values

The addition of EDTA did not significantly increase the Mehlich-1 P concentration in the Loam, but did in the Sand at 49d of the soil incubation (Fig. 2. 5a). The HEEDTA significantly increased Mehlich-1 P concentrations when compared to the Control No P in both soils.

Mehlich-1 P is an agronomic soil test with critical levels for maximum crop production ranging from 35-55mg P kg⁻¹ (Sims et al., 1998; Maguire and Heckendorn, 2011). Increasing Mehlich-1 P in the Loam with the application of chelating agents put it closer to being in the optimum range of plant available P. Based on these results, additions of chelating agents were effective at increasing WSP concentrations when background P concentrations were low and high. However, with high background P concentrations, there would be no need for chelate additions until those high levels were reduced by crop removal without fertilization.

Additions of EDTA and HEEDTA significantly increased Mehlich-3 P in both soils when compared to the Control- No P (Fig. 2. 5b), but the scale of the increase was not as great as for Mehlich-1 P. For example, adding EDTA to the Sand increased Mehlich-1 P by 34 percent, while it only increased Mehlich-3 P by 6 percent. This is probably because the Mehlich-3 extractant includes EDTA to aid extraction of P (Sims, 1989).

With the use of chelating agents, there is potential to increase plant available P without the additions of P fertilizer based on these results and chelating rate could be adjusted according to soil characteristics. However, further research will need to be conducted. When P fertilizer was applied with the chelating agents, both EDTA and HEEDTA significantly increased Mehlich-1 P in both soils (Fig. 2. 6a). Mehlich-3 P significantly increased with the application of EDTA and HEEDTA compared to the Control P (Fig. 2. 6b) in the Loam, but significantly decreased Mehlich-3 concentrations in the Sand. The reason for this is not clear.

In the second incubation, increasing chelating rate increased Mehlich-1 and Mehlich-3 P (Fig 2. 7a and 2. 7b). On 49d, the relationship between Mehlich-1 P and increasing chelating rate could be described using a quadratic equation. Increasing the application of EDTA and HEEDTA from chelating rate 120 to 150 PSC resulted in a decrease in Mehlich-1 P. The reason

for this is unknown as we would have expected a linear response to increasing chelating rate. However, the relationship between increasing chelating agent rate and Mehlich-3 P concentrations could also be described using a quadratic equation, except chelate additions from chelating rate from 120 and 150 PSC. As chelating rate increased, Mehlich-3 P increased with r^2 values of 0.81 and 0.94 respectively, for EDTA and HEEDTA application in the Sand. Both Mehlich-1 and Mehlich-3 soil tests were developed to measure plant available P. Therefore, our results give some insight into how adding chelates to soil affect Mehlich-1 and Mehlich-3 P, but crop response trials are necessary to show whether these changes in extractable P translate into greater plant availability of P in soils amended with chelates.

CONCLUSIONS

Chelating agents have been used for over 60 years in agriculture to supplement micronutrients through foliar sprays. In this study the application of chelating agents to soils decreased P sorption. Chelating agents significantly increased WSP, Mehlich-1, and Mehlich-3 extractable P in two soils that differ in soil texture classification, PSC, OM, and DPS. Chelates were able to increase WSP irrespective of whether P fertilizer was also added. Rates of chelate were based on PSC and increasing rate of chelate caused a linear increase in WSP. These results show proof of concept that solubility of P in soils can be increased using chelating agents. It would be too expensive for farmers to treat their whole fields with chelating agents, but it may be possible to add them with P fertilizer in starter bands. However, further research is needed to conclude that increased P solubility with chelating agents translates to greater plant uptake over a growing season.

REFERENCES

- Bond, C. R., R. O. Maguire, and J. L. Havlin. 2006. Change in soluble phosphorus following fertilization is dependent on initial Mehlich-3 phosphorus. *J. Environ. Qual.* 35:1818–1824.
- Bowers, A. R., and C. P. Huang. 1985. Adsorption characteristics of polyacetic amino acids onto hydrous γ - Al_2O_3 . *J. Colloid Interf. Sci.* 105:195-215.
- Borggaard, O. K. 1991. Effects of phosphate on iron oxide dissolution in ethylenediamine- N,N,N',N' -tetraacetic acid and oxalate. *Clay Clay Miner.* 39:324-328.
- Breeuwsma, A., and S. Silva. 1992. Phosphorus fertilization and environmental effects in the Netherlands and the Po region (Italy). Rep. 57. Winand Staring Centre for Integrated Land, Soil, and Water Res., Agric. Res. Dep., Wageningen, the Netherlands.
- Brown, J. C., L. O. Tiffin, and R. S. Holmes. 1960. Competition between chelating agents and roots as factor affecting absorption of iron and other ions by plant species. *Plant Physiol.* 35:878-886.
- Bumaya, A. H., and D. V. Naylor. 1988. Phosphorus sorption and extractability in andic soil incubated with plant residues of variable P content. *Plant Soil.* 1:77-81.
- Butler, P. C., and R. H. Bray. 1956. Effect of the zinc chelate of ethylenediaminetetraacetic acid on plant available zinc and other heavy metals. *Soil Sci. Soc. Am. J.* 20:348-351
- Campbell, J. L., and M. J. Eick. 2002. Effects of oxyanions on the EDTA-promoted dissolution of goethite. *Clay Clay Miner.* 50:336-341.
- Chang, H. C., T. W. Healy, and E. Matijevic. 1983. Interactions of metal hydrous oxides with chelating agents, III. Adsorption on spherical colloidal hematite particles. *J. Colloid Interf. Sci.* 92:469-478.

- Clemens, D. F., B. M. Whitehurst, and G. B. Whitehurst. 1990. Chelates in agriculture. *Fert. Res.* 25:127-131.
- Daniels, M. T., T. Daniel, D. Carman, R. Morgan, J. Langston, and K. van Devender. 1998. Soil phosphorus levels: Concerns and recommendations. Univ. Arkansas Coop. Ext., Fayetteville, AR.
- Dossa, E. L., J. Baham, M. Khouma, M. Sene, F. Kizito, and R. P. Dick. 2008. Phosphorus sorption and desorption in semiarid soils of Senegal amended with native shrub residues.
- Ebbs, S. D., W. A. Norvell, and L. V. Kochian. 1998. The effect of acidification and chelating agents on the solubilization of uranium from contaminated soil. *J. Environ. Qual.* 27:1486-1494.
- Gee, G. W., and J. W. Bauder, 1986. Particle-size analysis. Pages 383–411 in A. Klute, ed. *Methods of soil analysis. Part 1. Physical and mineralogical methods. Agronomy Monograph 9, 2nd ed.* ASA, SSSA, Madison, WI.
- Goos, R. J., and B. E. Johnson. 2000. A comparison of three methods for reducing Fe deficiency chlorosis in soybean. *Agron. J.* 92:1135-1139.
- Haynes, R. J., and M. S. Mokolobate. 2001. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved. *Nutr. Cycl. Agroecosys.* 59:47-63.
- Hill-Cottingham, D. G., and C. P. Lloyd-Jones. 1957. Behavior of iron chelates in calcareous soils. I. Laboratory experiments with Fe-EDTA and Fe-HEEDTA. *Plant Soil* 8:263-274.
- Hooda, P. S., A. R. Rendell, A. C. Edwards, P. J. A. Withers, M. N. Aitken, and V. W. Truesdale. 2000. Relating soil phosphorus indices to potential phosphorus release to water. *J. Environ. Qual.* 29:1166-1171.

- Hue, N. V. 1991. Effects of organic acids/anions on P sorption and phytoavailability in soils with different mineralogies. *Soil Sci.* 152:463-471.
- Iyamuremye, F., and R. P. Dick. 1996. Organic amendments and phosphorus sorption by soils. *Adv in Agron.* 56:139-185.
- Leonard, C. D., and I. Stewart. 1952. Correction of iron chlorosis in citrus with chelated iron. *Proc. Florida State Hort. Soc.* 65:20-24.
- Lookman, R., N. Vandeweert, R. Merckx, and K. Vlassak. 1995. Geostatistical assessment of the regional distribution of phosphate sorption capacity parameters (Feox and Alox) in northern Belgium. *Geoderma.* 66:285-296.
- Lunt, O. R., N. Hemaidan, and A. Wallace. 1956. Reactions of some polyamine polyacetate iron chelates in various soils. *Soil Sci. Soc. Am. Proc.* 20:172-175.
- Luscombe, P. C., J. K. Syers, and P. E. H. Gregg. 1979. Water extraction as a soil testing procedure for phosphate. *Commun. Soil Sci. Plan.* 10:11, 1361-1369.
- Maguire, R. O., and S. E. Heckendorn. 2011. *Laboratory Procedures: Virginia Tech Soil Testing Laboratory*, Virginia Cooperative Extension, Blacksburg, Va.
- Maguire, R. O., and J. T. Sims. 2002. Measuring agronomic and environmental soil phosphorus saturation and predicting phosphorus leaching with Mehlich-3. *Soil Sci. Soc. Am. J.* 66:2033-2039.
- Maguire, R. O., J. T. Sims, S. K. Dentel, F. J. Coale, and J. T. Mah. 2001. Relationships between biosolids treatment process and soil phosphorus availability. *J. Environ. Qual.* 30:1023-1033.
- Maguire, R. O., J. T. Sims, and F. C. Coale. 2000. Phosphorus solubility in biosolids amended farm soils in the mid-Atlantic region of the USA. *J. Environ. Qual.* 29:1225-1233.

- McKeague, J., and J.H. Day. 1966. Dithionite and oxalate-extractable Fe and Al as aids in differentiating various classes of soils. *Can. J. Soil Sci.* 46:13–22.
- Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Commun Soil Sci. Plant Anal.* 15:1409-1416.
- Menon, R. G., L. L. Hammond, and H. A. Sissingh. 1988. Determination of plant available phosphorus by the iron-oxide impregnated filter paper (Pi) soil test. *Soil Sci. Soc. Am. J.* 53:110-115.
- Mortvedt, J. J. 1991. *Micronutrients in agriculture*. Soil Sci. Soc. Am., Madison, WI. p. 187-199.
- Murphy, J., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27:31–36.
- Nowack, B., and L. Sigg. 1996. Dissolution of EDTA and metal-EDTA complexes onto goethite. *J. Colloid Interf. Sci.* 177:106-121.
- Ohno, T., and B. S. Carnell. 1996. Green and animal manure-derived dissolved organic matter effects on phosphorus sorption. *J. Environ. Qual.* 25:1137-1143.
- Ohno, T., and M. S. Enrich. 1997. Inhibitory effects of crop residue-derived organic ligands on phosphate adsorption kinetics. *J. Environ. Qual.* 26:889-895.
- Paulter, M. C., and J. T. Sims. 2000. Relationships between soil test phosphorus, soluble phosphorus, and phosphorus saturation in Delaware soils. *Soil Sci. Soc. Am. J.* 64:765-773.
- Penas, E. J., R. A. Wiese, R. W. Elmore, G. W. Hergett, and R. S. Moomaw. 1990. Soybean chlorosis studies on high pH bottomland soils. *Univ. of Nebraska Inst. Agric. Nat. Res. Bull.* 312. Univ. of Nebraska, Lincoln.

- Pote, D. H., T. C. Daniel, A. N. Sharpley, P. A. Moore, D. R. Edwards, and D. J. Nichols. 1996. Relating extractable soil phosphorus to losses in runoff. *Soil Sci. Soc. Am. J.* 60:855-859.
- SAS Institute. 2012. The SAS system for windows. User's Guide. SAS Institute Inc., Cary, NC.
- Sims, J. T. 1989. Comparison of Mehlich-1 and Mehlich-3 extractants for P, K, Ca, Mg, Mn, Cu, and Zn in Atlantic Coastal Plain soils. *Commun. Soil Sci. Plant Anal.* 20:1707-1726.
- Sims, J. T., R. R. Simard, and B. C. Joern. 1998. Phosphorus losses in agricultural drainage: Historical perspective and current research. *J. Environ. Qual.* 27:277-293.
- Singh, B. B., and J. P. Jones. 1976. Phosphorus sorption and desorption characteristics of soil as affected by organic residues. *Soil Sci. Soc. Am. J.* 40:389-394.
- Staunton, S., and F. Leprince. 1996. Effect of pH and some organic anions on the solubility of soil phosphate: implications for P bioavailability. *Eur. J. Soil Sci.* 47: 231-239.
- Stumm, W. 1992. *Chemistry of the Solid-Water Interface*. John Wiley & Sons, Inc., New York, p. 428.
- USDA. 2012. Agricultural Prices, National Agricultural Statistics Service. Table 7- "Average U.S. farm prices of selected fertilizers, 1960-2012". Last date modified 2012, July 05. <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1002>.
- van der Zee, S.E.A.T.M., and W.H. van Riemsdijk. 1988. Model for long-term phosphate reaction kinetics in soil. *J. Environ. Qual.* 17:35-41.
- van der Zee, S. E. A. T. M., L. G. J. Fokkink, and W. H. van Riemsdijk. 1987. A new technique for assessment of reversibly adsorbed phosphate. *Soil Sci. Soc. Am. J.* 51:599-604.

Table 2. 1. Selected properties of the Loam and Sand used in the two soil incubation studies.

Soil Property	Loam	Sand
pH	6.48	6.04
CEC†, cmol _c kg ⁻¹	9.3	1.6
Organic matter, g kg ⁻¹	52	10.0
Al _{ox} ‡, mmol kg ⁻¹	44.3	18.1
Fe _{ox} ‡, mmol kg ⁻¹	23.6	6.1
PSC§, mmol kg ⁻¹	33.95	12.1
Phosphorus, mg kg ⁻¹		
Mehlich-1	6	63
Mehlich-3	10.7	190
P _{ox} ‡	158	265
DPS¶, %	15	71

† Cation exchange capacity.

‡ Oxalate-extractable Al, Fe, and P.

§ Phosphorus sorption capacity.

¶ Degree of P saturation.

Table 2. 2. Equations derived to determine the effects of chelate rate on water soluble phosphorus (WSP) of two soils, Loam and Sand, over a 49d soil incubation. Regression equations describe the relationships observed in Figure 2. 3.

Treatment	Day 1		Day 21		Day 49	
	Equation	r^2	Equation	r^2	Equation	r^2
EDTA- Loam	$y = 0.378x + 148$ *	0.86	$y = 0.268x + 37.6$ ***	0.98	$y = 0.292x + 30.6$ *	0.95
HEEDTA-Loam	$y = 0.259x + 169$	0.59	$y = 0.523x + 36.2$ ***	0.99	$y = 0.579x + 26.4$ **	0.97
EDTA- Sand	$y = 0.705x + 124$ **	0.95	$y = 0.991x + 67.8$ ***	1.00	$y = 1.05x + 54.4$ ***	1.00
HEEDTA-Sand	$y = 0.482x + 128$ *	0.88	$y = 0.913x + 75.9$ **	0.97	$y = 0.976x + 56.0$ ***	0.99

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

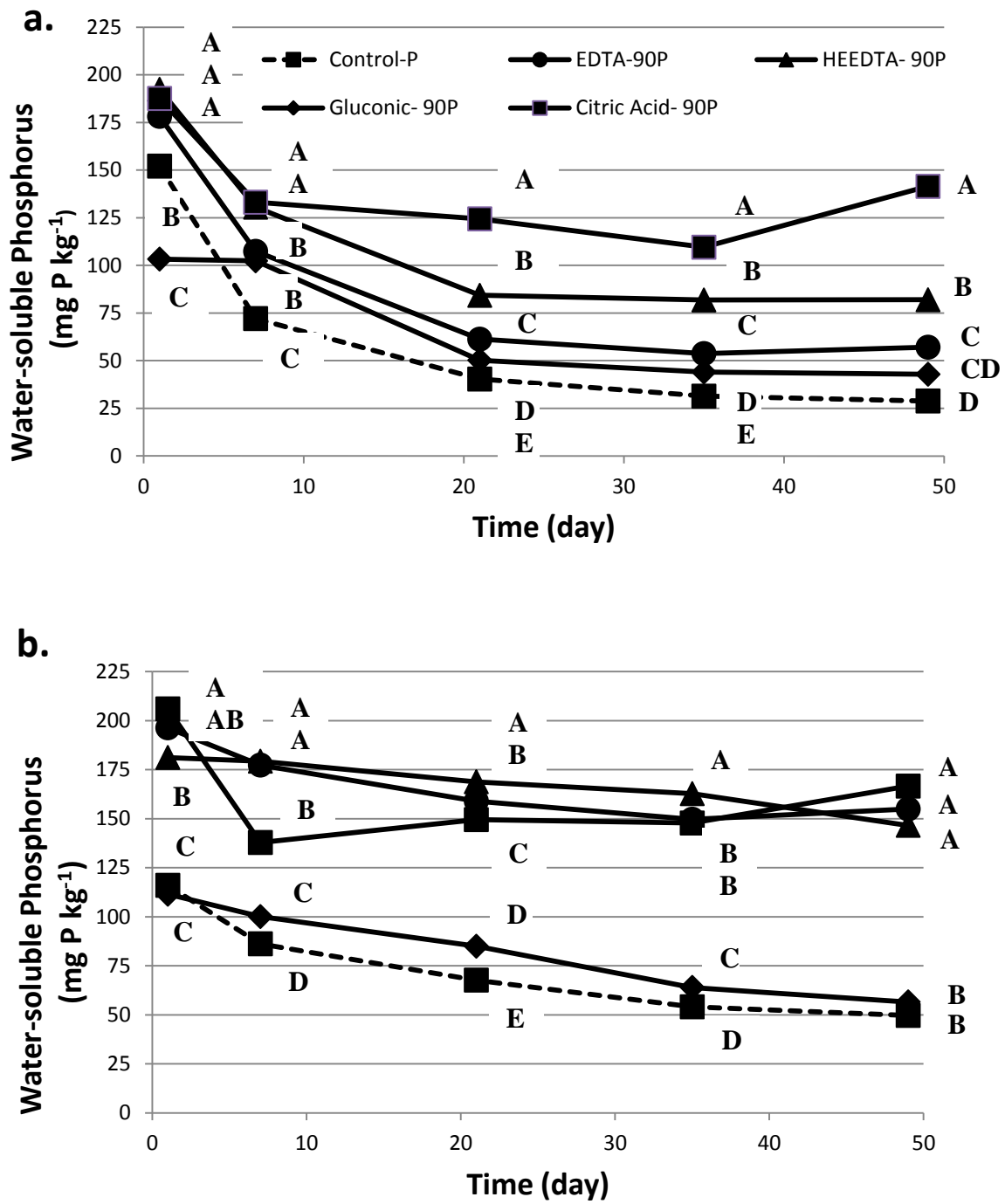


Figure 2. 1. The influence of chelating agent on water-soluble phosphorus (WSP) over a 49d soil incubation when applied at 90 percent P sorption (PSC) of the two soils (a) Loam and (b) Sand, with P fertilizer added. Letters show significant differences at each sample interval (P=0.05).

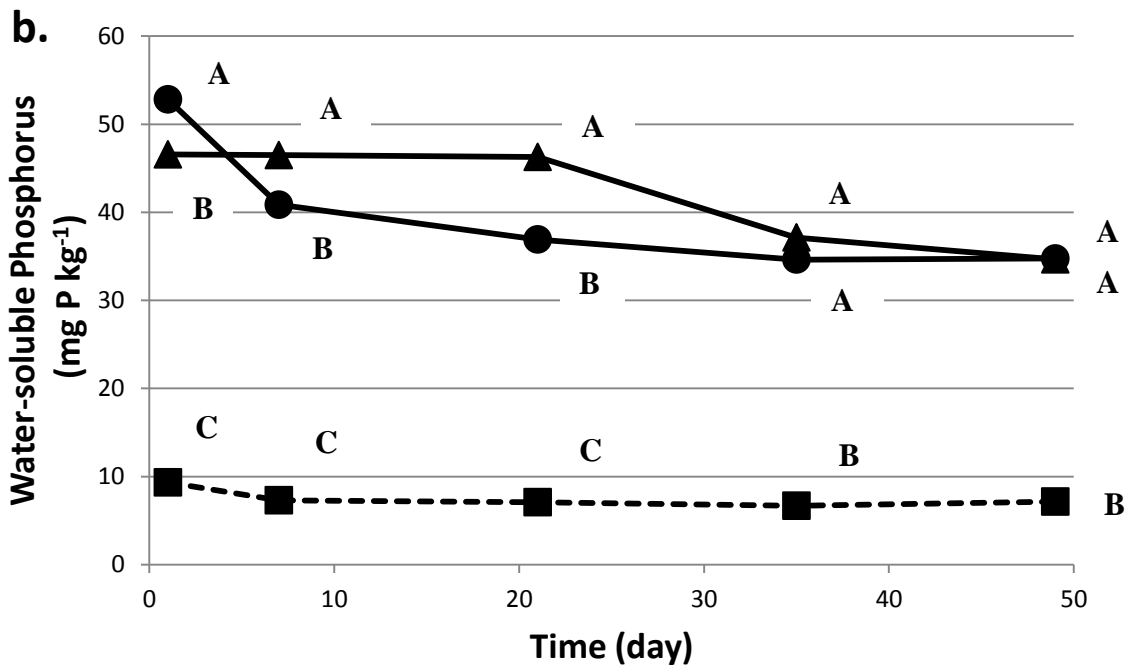
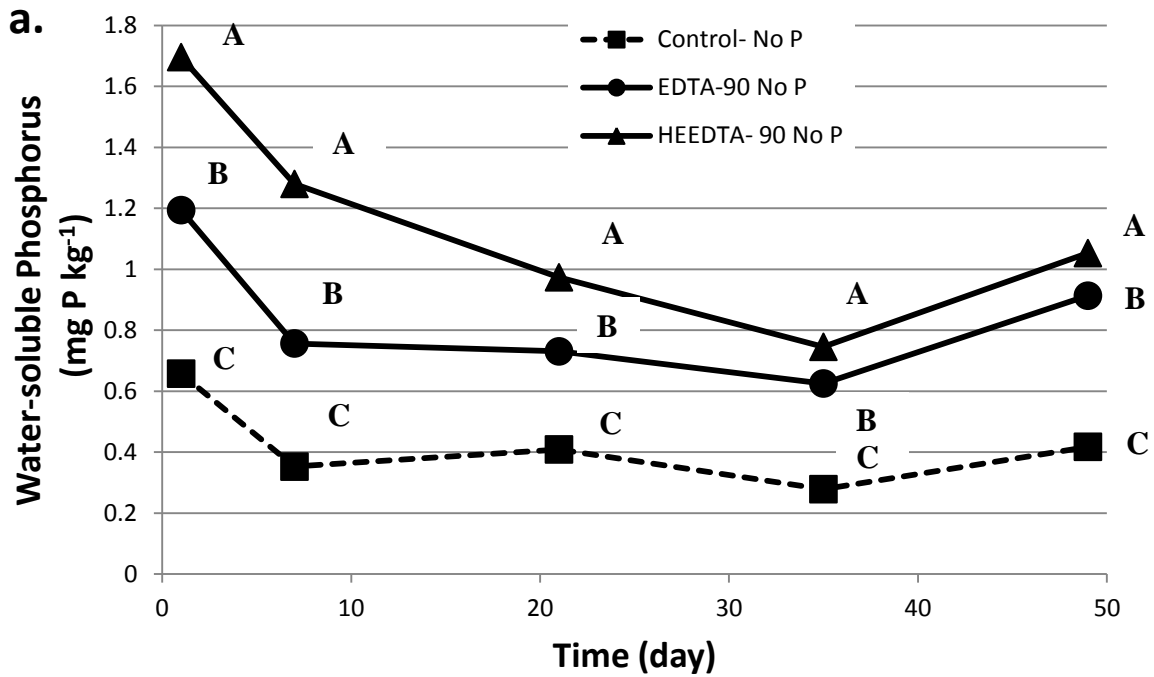
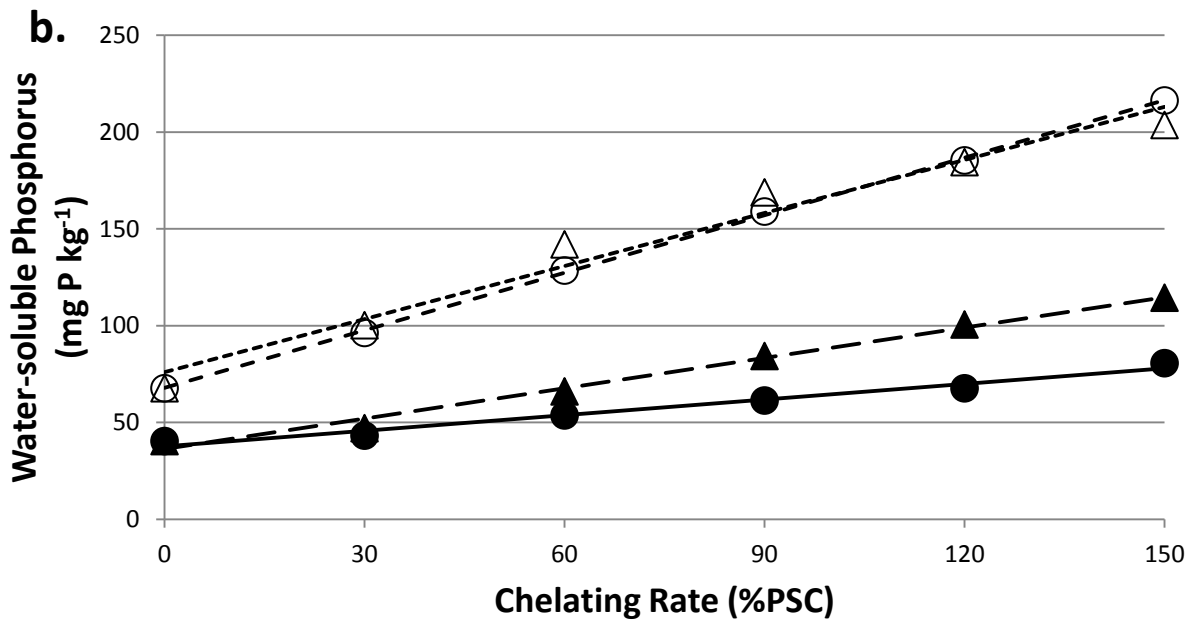
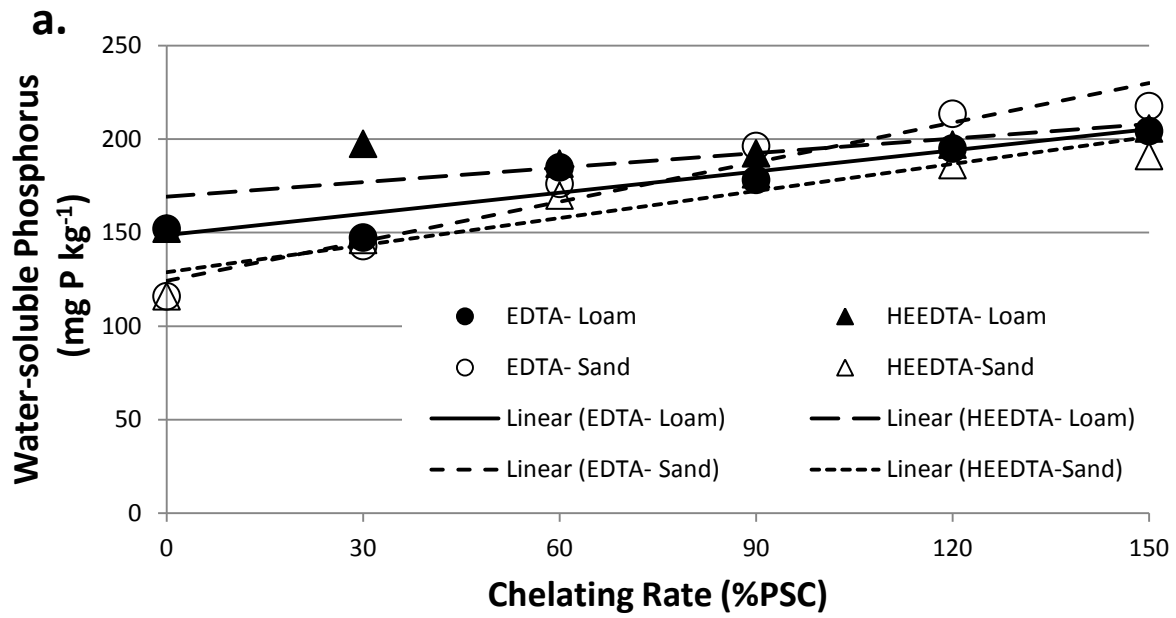


Figure 2. 2. Figure. The influence of chelating agents EDTA and HEEDTA on water-soluble phosphorus over a 49-d soil incubation when applied at 90 percent phosphorus sorption capacity of the soil (a) Loam and (b) Sand, without phosphorus fertilizer added. Letters show significant differences at each sampling interval (P=0.05).



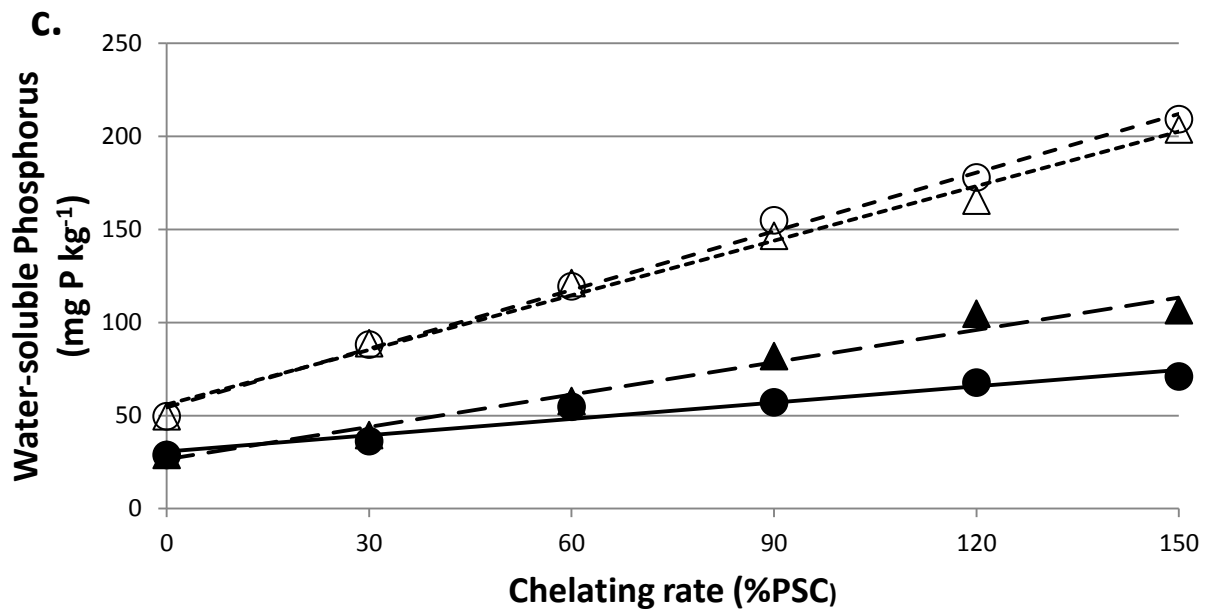


Figure 2. 3. The influence of EDTA and HEEDTA chelating rate on water-soluble phosphorus concentrations in the Loam and Sand soil with additions of phosphorus fertilizer at sampling intervals (a) Day 1, (b) Day 21, and (c) Day 49 of the 49-d soil incubation.

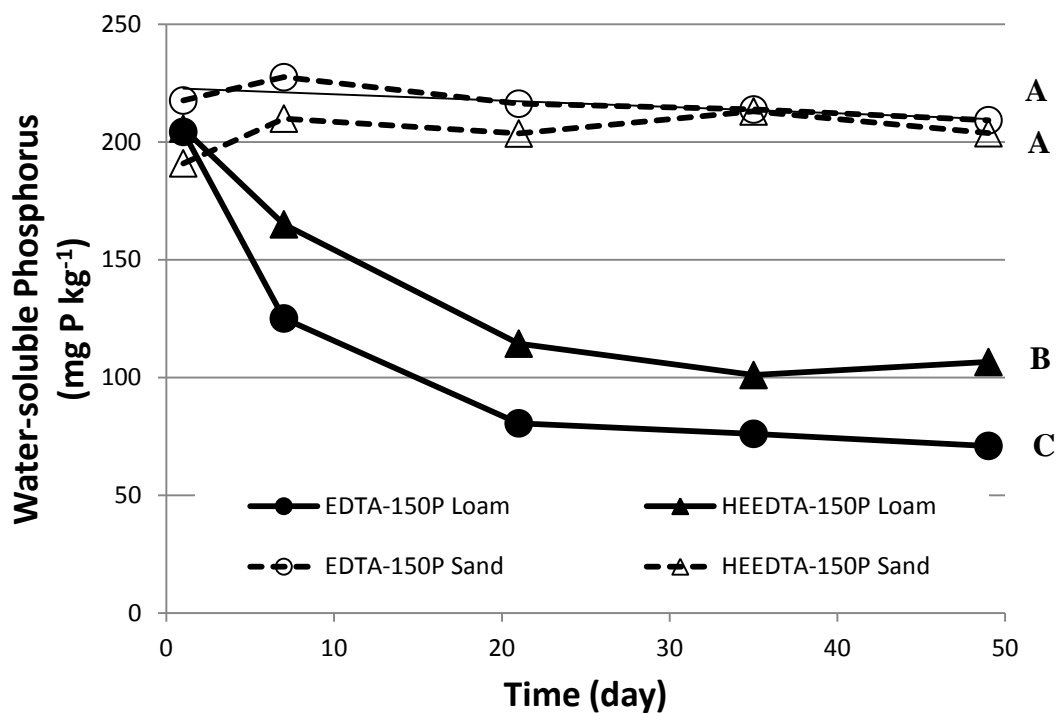


Figure 2. 4. The efficiency of EDTA and HEEDTA chelating agents when applied at 150 percent of phosphorus sorption capacity (PSC) of the soil, Loam and Sand, at maintaining water-soluble phosphorus (WSP) during a 49-d soil incubation with P fertilizer. Letters show significant differences on 49d of the soil incubation ($P=0.05$).

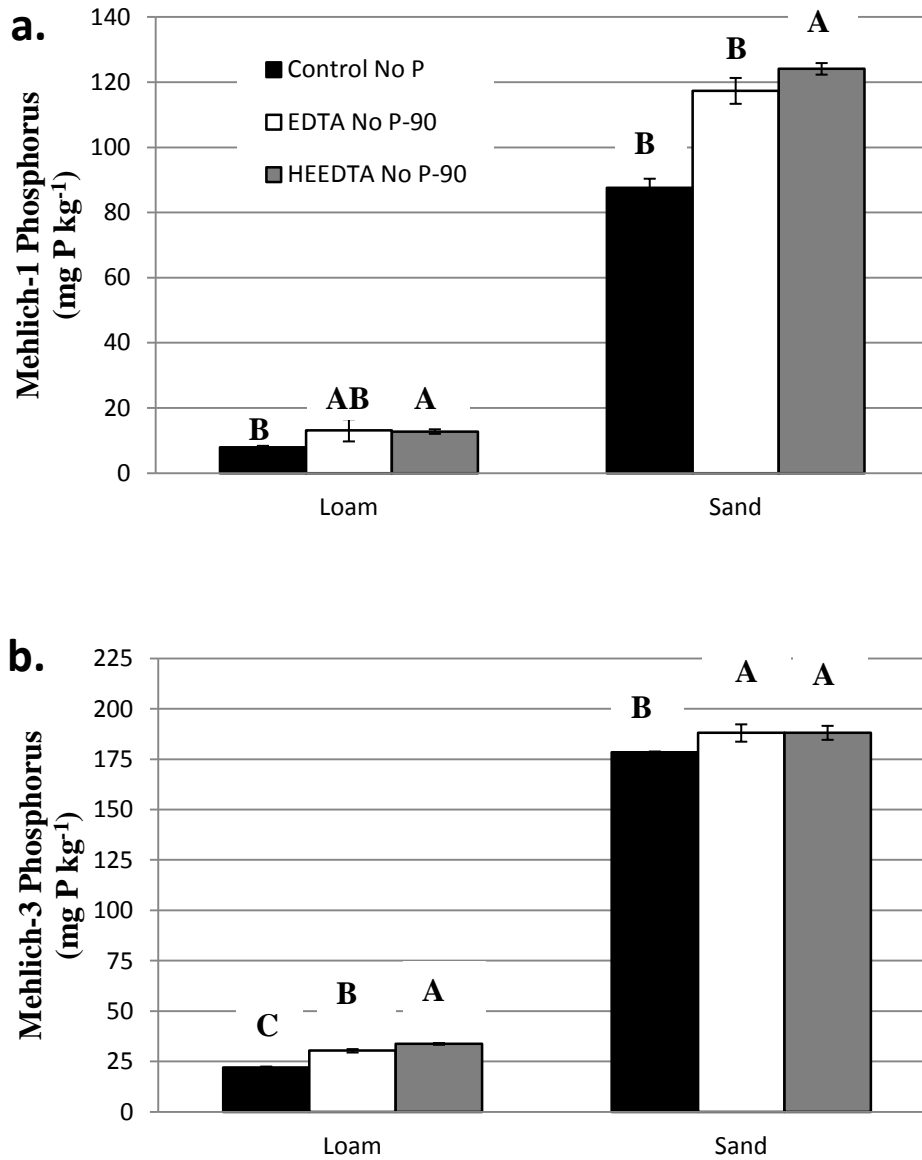


Figure 2. 5. The effects of chelating agents EDTA and HEEDTA applied at 90 percent phosphorus sorption capacity (PSC) without additions of P fertilizer on (a) Mehlich-1 and (b) Mehlich-3 P routine soil test concentrations in a Loam and Sand on 49d of the incubation. Error bars show the standard deviation. Letters show significant differences on 49d of the soil incubation (P=0.05).

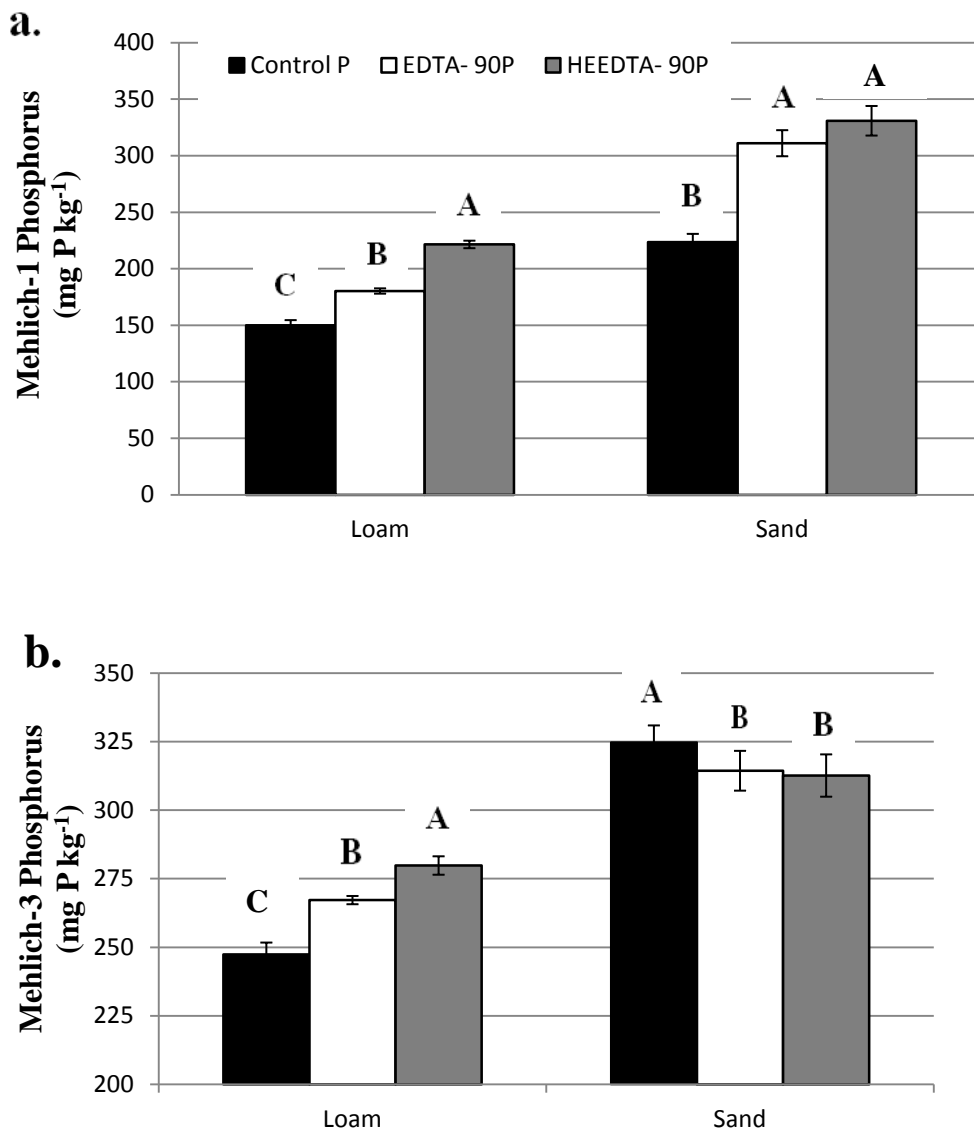


Figure 2. 6. The effects of chelating agents EDTA and HEEDTA applied at 90 percent phosphorous sorption capacity of the soil (PSC) with additions of phosphorus fertilizer on (a) Mehlich-1 and (b) Mehlich-3 phosphorus routine soil test concentrations in a Loam and Sand on 49d of the soil incubation. Error bars show the stand deviation. Letters show significant differences on 49d of the soil incubation ($P=0.05$).

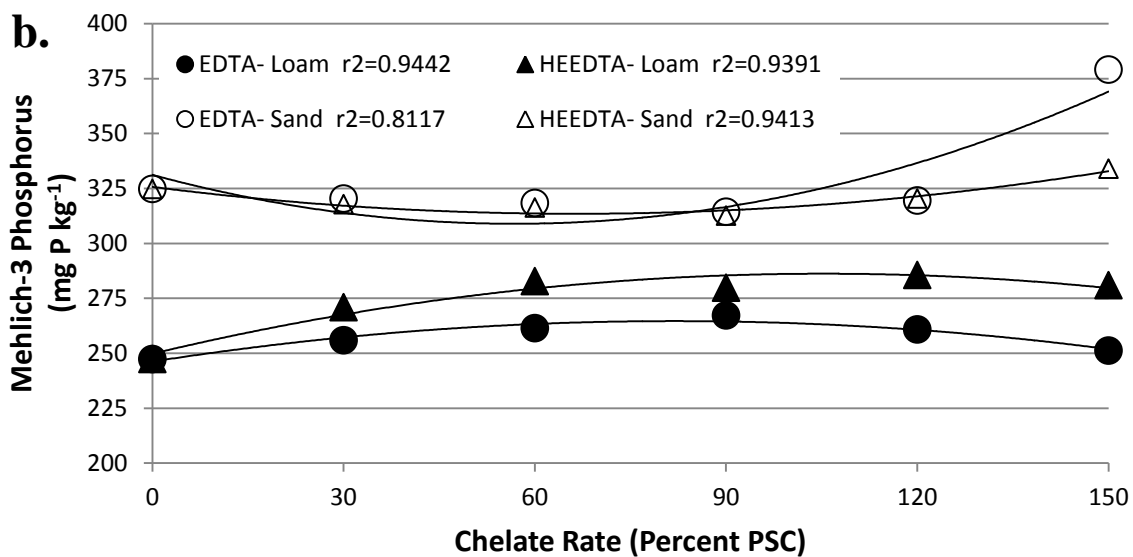
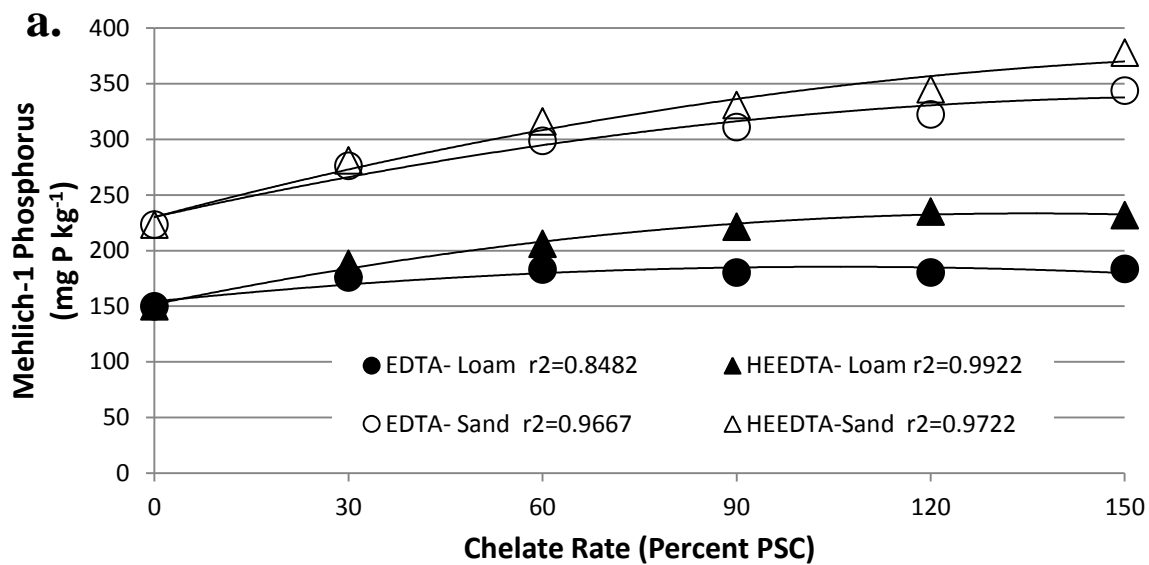


Figure 2. 7. The effects of increasing chelating rate of EDTA and HEEDTA on (a) Mehlich-1 and (b) Mehlich-3 Phosphorus concentrations on day 49 of the soil incubation in a Loam and Sand.

CHAPTER 3: PLANT AVAILABLE PHOSPHORUS AFTER APPLICATION OF SYNTHETIC CHELATINGS AGENTS TO SOIL

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Abbreviations list: P, phosphorus; Fe, iron; Al, aluminum; EDTA, ethylenediamine tetraacetic acid; HEEDTA, hydroxyethyl ethylenediamine triacetic acid; WSP, water-soluble phosphorus; PSC, phosphorus sorption capacity; OM, organic matter; CEC, cation exchange capacity; ICP-OES, inductively coupled plasma-optical emission spectroscopy; Al_{ox}, Fe_{ox}, P_{ox}, oxalate-extractable aluminum, iron, and phosphorus; DPS, degree of phosphorus saturation

ABSTRACT

Fertilizer phosphorus (P) can become unavailable to crops due to immobilization of P in acidic soils through forming chemical bonds with iron (Fe) and aluminum (Al) amorphous oxides. Two chelating agents, ethylenediamine tetraacetic acid disodium salt (EDTA) and hydroxyethyl ethylenediamine triacetic acid (HEEDTA), were tested in a 4-week greenhouse study for efficiency at increasing plant available P to corn (*Zea mays L.*) in two soils. Fertilizer P was added with or without chelate to the center of pots, to simulate a starter band of P. After 4 weeks the soils were analyzed for water-soluble P (WSP), Mehlich-1 and Mehlich-3 P and complete above and below ground biomass was sampled and analyzed for total P. Without the presence of chelates, biomass above and below ground increased linearly in both soils as P fertilizer rates increased at 0, 9.6, 19.3, 28.9, and 38.5 kg P ha⁻¹. Applications of EDTA and HEEDTA when P was added at rates 0, 9.6, and 19.3kg P ha⁻¹ did not significantly increase WSP, Mehlich-1, and Mehlich-3 P compared to soils without chelates. Applications of EDTA increased P uptake in the below-ground biomass. Despite previous research showing that chelates increased WSP in soils, a decrease in P sorption was not observed with the additions of chelating agents to soils and the sampling procedure used in this trial. Increasing chelating rate or different application method of into soil may prove effective.

INTRODUCTION

As fertilizer prices increase, fertilizer efficiency is becoming even more valuable to farmers. Phosphorus fertilizer efficiency has been reported as low as 10 percent (Mattingly and Widdowson, 1959) and as high as 85 percent throughout the world (Larsen (1952). In acid soils, P availability to plants is controlled by Fe and Al, which form chemical bonds through ligand exchange (van der Zee and Van Riemsdijk, 1988). Chelating agents compete with phosphate ions for sorption sites on amorphous oxides by complexing Fe and Al (Mortvedt, 1991).

In agriculture, inorganic forms of chelated Fe are widely utilized as foliar applications to treat Fe chlorosis (Vempati and Loeppert, 1986). EDTA and HEEDTA are commonly used chelating agents and are both aminopolycarboxylates (Clemens et al., 1990). When applied as a foliar application, chelated Fe has shown a decrease in chlorosis in soybean (Goos and Johnson, 2000), increasing soybean yields (Penas et al., 1990), but some studies have shown no effect in corn (Godsey et al., 2003) or soybean (Lingerfelser et al., 2005). The problem with foliar applications of chelated products such as Fe-EDTA for some crops is that chelating agents applications are not economically feasible as multiple foliar applications may need to be made and are not physically feasible in a crop such as corn during later growth stages.

Plant roots have developed mechanisms by which they produce chemical compounds in order to solubilize micronutrients and P for absorption. Strategy I plants, where they induce ferric chelate reductase and the Fe transporter in root systems and strategy II plants release phytosiderophores to induce a specific plasmalemma Fe (III) transporter within the root system (Hell and Stephan, 2003; Morrissey and Guerinot, 2009). Strategy II categorized plants are capable of producing several chelating compounds, such as phenolic acid, citric acid, and gluconic acid that release P bound by Fe and Al (Marschner and Romheld, 1994).

In P deficient conditions, plants have developed mechanisms by which they directly and indirectly solubilize P. Carboxylates that are produced directly affect P availability by blocking sorption sites on mineral surfaces by binding Fe and Al (Staunton and Leprince, 1996) resulting in the immediate release of P. To increase nutrient availability, some plants are capable of producing carboxylate exudates in nutrient deficient conditions of Fe (Marschner and Romheld, 1994) and P (Strom et al., 2002).

Less is known about the indirect effects, but plant exudates are capable of stimulating microbial activity and modifying rhizosphere pH to solubilize nutrients. The production of the citric acid in the conjugate base form will decrease the pH due to the production of H^+ as a counter ion to maintain electrical neutrality (Drouillon and Merckx, 2003). Microorganisms are capable of influencing soil fertility through decomposition, mineralization, storage and release of nutrients. The mechanism by which microbes can solubilize P is by lowering the soil pH in the rhizosphere with the production of organic acids and phosphatases.

A previous study showed that when chelates were applied to soil, incorporated, and incubated for 49d, EDTA and HEEDTA significantly increased WSP concentrations with and without P fertilizer (Chapter 2). The relationship of increasing chelating rate on increasing Mehlich-1 P concentrations was described by a quadratic function in soils similar to those used in the present work with additions of EDTA and HEEDTA (Chapter 2).

The majority of studies evaluating the use of chelating agents in agriculture have applied chelated micronutrients as foliar applications. Evaluating the effects of chelating agents applied to soil on corn utilization of fertilizer P is a novel approach to increasing efficiency of fertilizer P. The objectives of this study were to (i) test the efficiency of chelating agents on increasing the solubility of P in soils, and (ii) evaluate aminopolycarboxylate chelating agents at increasing

plant available P as measured through crop uptake and biomass when applied with and without phosphorus.

MATERIALS AND METHODS

Soil Characterization

Two soils were used in this study, a Shottower (Fine, kaolinitic, mesic Typic Paleudults) Loam and Bojac (Coarse-loamy, mixed, semiactive, thermic Typic Hapludults) Sandy Loam. From here on they will be referred to as “Loam” and “Sandy Loam”. Both soils were chosen based on low soil test P. The soils were collected by removing the top 15cm of soil after removing vegetation. The soil was passed through a 1.3cm sieve to remove large material prior to air-drying under greenhouse conditions.

Both soils were passed through a 2mm sieve analyzed by standard soil testing methods of the Virginia Tech Soil Testing Laboratory. Soil water pH at a 1:1 volume to volume ratio of soil material to distilled water and organic matter (OM) by loss of ignition were measured (Maguire and Heckendorn, 2011). Cation Exchange Capacity (CEC) was determined using the double wash method where soil was saturated with 1M CaCl₂, leached, and rinsed with deionized water. The soil was then washed with 1M KCl, displacing sorbed Ca⁺². Extracts were analyzed for Ca concentration by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Field capacity was determined by completely saturating dry soil and allowing gravitational drainage through holes in the bottom of a plastic cup for 48 hours (Bond et al., 2006). Particle size analysis was determined by a pipette method (Gee and Bauder, 1986).

Mehlich-1 P was measured using a 1:5 volume: volume soil to extractant ratio, and extract P concentrations determined by inductively coupled plasma-optical emission

spectroscopy (ICP-OES) (Maguire and Heckendorn, 2011). Mehlich-3 P was extracted with a 1:10 soil to solution ratio using a 0.2M CH₃COOH + 0.25M NH₄NO₃ + 0.015M NH₄F + 0.013M HNO₃ + 0.001M EDTA (Mehlich, 1984). Oxalate P, Al, and Fe were extracted using methods described by McKeague and Day (1966) at a 1:40 soil to solution ratio using a 0.2 M ammonium oxalate + 0.2 M oxalic acid solution, pH adjusted to 3.0. Mehlich 1 P, Mehlich 3 P and oxalate P, Fe, and Al, in the extracts were analyzed by ICP-OES.

Oxalate extractable Fe and Al are used as an estimate of the P sorption capacity (PSC) of both soils (Maguire et al., 2001). The PSC is expressed as a function of the sum of Fe and Al oxides:

$$\text{PSC} = \alpha (\text{Al}_{\text{ox}} + \text{Fe}_{\text{ox}}), \quad (1)$$

where PSC is P sorption capacity expressed as mmol kg⁻¹, α is a scaling factor of 0.5 (Lookman et al., 1995), and the Al_{ox} and Fe_{ox} is the sum of ammonium oxalate-extractable Al and Fe (mmole kg⁻¹) (Maguire et al., 2001). The scaling factor of 0.5 is the most commonly used value and assumes that 2 moles of Al and / or Fe are required to adsorb 1 mole of P (Lookman et al., 1995). The degree of P saturation (DPS) was calculated using PSC from eq. 1 as follows (van der Zee and van Riemdijk, 1988):

$$\text{DPS} = (\text{P}_{\text{ox}} / \text{PSC}), \quad (2)$$

where P_{ox} is extracted using an acid ammonium oxalate solution and expressed as mmoles kg⁻¹.

Greenhouse study

Two chelating agents, EDTA and HEEDTA, were selected based on their stability constants with Fe and Al and efficiency at maintaining WSP observed in previous studies in

Chapter 2. Iron and Al can form six bonds with chelating agents and both EDTA and HEEDTA are both capable of forming six bonds (Clemens et al., 1990). It was assumed that one mole EDTA or HEEDTA could bind one mole of Al or Fe as calculated in eq 1. The maximum chelating rate of 150 percent PSC was determined based on maximum solubility of EDTA in deionized water and calculated PSC using eq. 1 above.

The greenhouse study was set up in a randomized complete block with 4 replications and corn (*Zea mays L.*, Dekal B, DKC67-88) was planted at 4 seeds per pot and was grown for 4 weeks (approximately V4-V6). Phosphorus fertilizer was applied at rates equivalent to 0, 9.6, 19.3, 28.9, and 38.5 kg P ha⁻¹ as dibasic potassium phosphate to generate a P rate response curve. Fertilizer rates were based on fertilizer recommendation for silage corn and the Mehlich-1 P extraction (Maguire and Heckendorn, 2011). At P rates of 0, 9.6, and 19.3kg P ha⁻¹, applications of EDTA and HEEDTA at 150 percent PSC of each soil were added based on the volume of soil impacted by band placement as defined below.

Greenhouse pots were prepared by taking 3.79 l pots and adding 2kg of either the air dried Loam or Sandy Loam. To prevent loss of soil through the relatively large drainage holes, coffee filters were placed in the bottom of each pot. Both of the chelating agents, EDTA and HEEDTA, and P fertilizer were dissolved in deionized water with various treatments. A 2.5cm diameter PVC pipe was inserted in the center of the pot at a depth of 5 cm. This process simulating banded fertilizer applications as solutions were applied 4-5cm from the seeds planted and 5cm deep in soil. Banding fertilizer has been proven effective at increasing corn yields (Nelson, 1956; Welch et al., 1966). Air dry soil contained in the pipe for the Loam and Sandy Loam, 0.026 and 0.035 kg respectively, was determined. Solutions containing the appropriate rate of chelate and fertilizer P were applied to soil inside the pipe to bring soil to 70 percent field

capacity. Chelate concentrations were applied at 150 percent of the PSC of the volume of soil contained in the pipe, approximately 0.026 and 0.035kg. Phosphorus fertilizer was applied to soil inside the pipe at the rates specified above based on the surface area of the pot. The PVC pipe was removed, and the remainder of the dry soil was brought to 70 percent field capacity using deionized water.

All pots received the same application rates of nitrogen (N) equivalent to 45kg N ha⁻¹ as urea at planting and as a side-dress application of 45kg N ha⁻¹ urea at week 2. To keep K rates constant with varying rates of P using potassium phosphate, potash (0-0-60) was applied to assure 37kg K ha⁻¹ per pot at planting. At week 1, average height of all plants was 10.2cm. For uniformity, plants that were considerably below this point were removed and the two plants per pot closest to that average were kept. At harvest on week 4, complete above- and below-ground biomass was taken and growth stage was determined of each plant. Plant roots were carefully washed, removing all soil, with deionized water.

Following harvest, all soil in each pot was thoroughly mixed and a subsample taken, air dried, and sieved to pass a 2mm screen. Soils were extracted for water-soluble P (WSP) at a 1:10 soil:deionized water ratio and filtered (Luscombe et al., 1979) and analyzed colorimetrically by the molybdate blue method (Murphy and Riley, 1962). Mehlich-1 and Mehlich-3 extractable P were also determined using the methods stated above. Above- and below-ground wet and dry weights were taken and biomass was quantified on a dry weight basis. Corn plant tissue above- and below-ground were dried for 48hr at 80° C to reach a constant dry weight. Above- and below-ground tissue samples were ground to pass a 1mm sieve and then analyzed for total Kjeldahl P (TKP) colorimetrically following a sulfuric acid and hydrogen peroxide digestion and then analyzed colorimetrically.

STATISTICAL ANALYSIS

Data from routine soil test and plant response greenhouse study were analyzed using the GLIMMIX procedure available from SAS (SAS Institute, 2012). Treatment effects on least square means of WSP, Mehlich-1 P, and Mehlich-3 P for routine soil tests and Above- and Below-ground biomass and plant P uptake by soil were separated using Tukeys HSD at a significance level of $P=0.10$ (SAS Institute, 2012). Regression was used to evaluate the relationship of increasing phosphorus fertilizer rate on WSP, Mehlich-1 P, Mehlich-3 P, biomass, and P uptake levels.

RESULTS AND DISCUSSION

Soil Properties

Both the Loam and Sandy Loam had a water pH of approximately 6.5 which is considered within appropriate agronomic range (Maguire and Heckendorn, 2011) (Table 3. 1). The CEC of the Loam ($9.3 \text{ cmol}_c \text{ kg}^{-1}$) was twice that of the Sandy Loam ($4.5 \text{ cmol}_c \text{ kg}^{-1}$). This is within normal ranges of these soils that are highly weathered and the clay fraction is composed primarily of kaolinite. The Loam had higher OM at 52 g kg^{-1} compared to the Sandy Loam at 17 g kg^{-1} . The Loam and Sandy Loam were considered “low” in Mehlich-1 P at 8 and 3 mg kg^{-1} according to soil test P criteria of the Virginia Tech Soil Test Laboratory. Phosphorus fertilizer was recommended for crop production in both soils since they were below the cutoff value of 55 mg kg^{-1} where no P is recommended (Maguire and Heckendorn, 2011). Mehlich-3 P concentrations followed results of Daniels et al. (1998) and Sims (1989) as levels were 1.5 to 2

times greater than Mehlich-1 P at 18 and 6mg kg⁻¹ for the Loam and Sandy Loam. Previous research has shown that oxalate extractable P is greater than Mehlich-1 and Mehlich-3 (Maguire and Sims, 2002), as observed for the Loam and Sandy Loam at 163 and 32mg kg⁻¹ respectively.

The Loam had three times the PSC of the Sandy Loam at 42 and 13mmol kg⁻¹ calculated using eq. 1 above (Table 3. 1). This higher PSC of the Loam was attributed to the higher concentrations of oxalate extractable amorphous Fe and Al in finer textured soils (Maguire et al., 2000). When fertilizer is applied to acidic soils, sorption sites of Fe and Al amorphous oxides complex P making it less plant available (van der Zee and van Riemdijk, 1988). Both the Loam and Sandy Loam had low DPS at 12 and 8 percent, respectively, falling well below the value of 56 percent DPS, where soils can release excessive P and be of environmental concern (Breeuwsma and Silva, 1992).

Effects of Phosphorus and Chelate Application on Phosphorus Solubility

Regression analysis showed a positive linear relationship between WSP and applied fertilizer P in the simulated banded P application for the Loam ($r^2=0.90$) and Sandy Loam as expected ($r^2=0.81$) (Figs. 3. 1a and 3. 1b). This was similar to the results of Messiga et al. (2012) who showed a positive linear increase in WSP concentration with increasing P rate. Water-soluble P is only a small fraction of total P, but is considered the form most easily lost and is related to P losses in runoff and leaching (Maguire and Sims, 2002; Pote et al., 1996). An application of 1kg P ha⁻¹ increased WSP by 0.05 and 0.07 mg kg⁻¹, respectively in the Loam and Sandy Loam (Figs. 3. 1a and 3. 1b). This result of a greater increase in WSP concentrations in the Sandy Loam could be attributed to the lower PSC of 13.2mmol kg⁻¹ compared to the Loam at 41.9mmol kg⁻¹ (Table 3. 1).

In the Loam, applications of EDTA and HEEDTA at 150 percent PSC had no significant effect on WSP concentrations when applied in addition to 9.6 and 19.3kg P ha⁻¹ and compared to those rates without chelates (Fig. 3. 1a). However, additions of chelating agents without P fertilizer were found to significantly decrease WSP concentrations. In the Sandy Loam, increases in WSP concentrations in response to EDTA applications were only observed in addition to 19.3kg P ha⁻¹. All other additions of chelating agents in addition to P fertilizer were not found to be significant. In a soil incubation using soils similar to those used in this study, additions of EDTA and HEEDTA were found to significantly increase WSP concentrations when applied at the same chelating rate used here (Chapter 2). The reason WSP did not consistently increase in this study as in the incubation was probably because only soil in the middle of the pot was amended simulating a starter fertilizer application in a field, rather than being incorporated to the whole soil as in the incubation study. Therefore, the chelates may have decreased P sorption where they were applied, but the P could have moved out of this zone by diffusion or mass flow as the pots were watered. Similar WSP concentrations to those observed here were shown in a soil incubation with biosolids amendments where concentrations were less than 8mg kg⁻¹ (Maguire et al., 2001).

In Sandy Loam at the 19.3kg P ha⁻¹ rate EDTA increased concentrations of WSP when compared to HEEDTA at that same P rate, but failed to show any significant difference at any other rate or soil. Above a soil pH of 6, HEEDTA loses its effectiveness at complexing Al (Mortvedt, 1991), which may explain why HEEDTA was not efficient in increasing WSP concentrations as pH values were always greater than 6. However, the experimental results in Chapter 2 showed that HEEDTA increased WSP with soils having a pH similar to those of this study. As stated above, one possibility for chelating agents not having a significant effect on

WSP concentrations may be due to P leaving the application area where chelates would be able to react and P then sorbing where the chelates were not sorbed. Also, chelating agent application in this experiment was based on sorption capacity of the soil treated in center of the pot. Increased concentrations of WSP may have been observed with greater concentrations of applied chelating agents.

Effects of Phosphorus and Chelate Application on Routine Soil Tests

Similar to WSP, Mehlich-1 P increased linearly with increasing P rate with r^2 values of 0.88 and 0.92 in the Loam and Sandy Loam as expected (Figs. 3. 2a and 3. 2b). The application of each 1 kg P ha⁻¹ increased Mehlich-1 P concentrations in the Loam and Sandy Loam by 0.31 and 0.47 mg kg⁻¹, respectively (Figs. 3. 2a and 3. 2b). A greater increase in Mehlich-1 P in the Sandy Loam can be attributed to the lower PSC relative to the Loam.

The chelating agents did not significantly change Mehlich-1 P in any comparison with the same rate of P and no chelate, except EDTA and HEEDTA significantly increased Mehlich-1 P concentrations at the 19.3kg P ha⁻¹ rate in the Sandy Loam. For corn, the Mehlich-1 P concentrations at which plants should reach 100 percent yield is 20mg P kg⁻¹ (Sims et al., 1998) and the value at which no fertilizer is recommended is 55mg P kg⁻¹ (Maguire and Heckendorn, 2011). To achieve a value of 20mg P kg⁻¹ in Mehlich-1 P for maximum yield, an application rate of 38.5kg P ha⁻¹ would be required based on the results observed here for both the Loam and Sandy Loam.

Mehlich-3 P had a positive linear relationship with increasing P application rates in the Loam ($r^2=0.87$) and Sandy Loam as expected ($r^2=0.88$) (Figs. 3. 3a and 3. 3b). Messiga et al. (2012) also showed a positive linear relationship between Mehlich-3 P and increasing P

application rate. Again, the lower PSC of the Sandy Loam explained why a greater increase in Mehlich-3 P of 0.66 mg kg^{-1} occurred in the Sandy Loam as opposed to 0.52 mg kg^{-1} in the Loam for each addition of 1 kg P ha^{-1} (Table 1; Figs. 3. 3a and 3. 3b). Additions of EDTA and HEEDTA significantly increased Mehlich-3 P at the $19.3 \text{ kg P ha}^{-1}$ rate in both soils. At all other P rates, chelating agents had no significant effect on Mehlich-3 P concentrations.

Mehlich-3 P concentrations were 1.5 to 2 times greater than Mehlich-1 P concentrations at those same application rates, which have been shown in other studies and in the background soil tests here (Daniels et al., 1998; Sims 1989). In a soil incubation where chelating agents were studied for their effects on routine soil tests (Chapter 2), Mehlich-1 and Mehlich-3 extractable P were both considerably greater (above 150 mg P kg^{-1}) than those observed in this greenhouse study. Mehlich-1 and Mehlich-3 P values observed in this study with the additions of EDTA and HEEDTA were lower than 15 mg P kg^{-1} , respectively. However, in the soil incubation, chelating agents were homogeneously mixed in 0.06 kg soil and in this study; chelating agents were only applied to a small portion of 2 kg soil in the center of the pot. Mehlich-1 and Mehlich-3 P concentrations in the greenhouse study were determined after allowing the soil to dry and taking a subsample from the homogeneously mixed 2 kg soil. Taking a soil sample from the application area of the pot would likely have shown greater P concentrations as chelating agents could have blocked sorption sites. Previously, EDTA was added to several extracting solutions to increase the extractability of many nutrients, such as P (Sims, 1989). Alexander and Robertson (1972) showed that EDTA extractable P was significantly correlated with Al and Fe complexed P.

Effect of Chelating Agents on Plant Biomass

With increasing P application rate, there was a positive linear relationship with above-ground biomass in the Loam ($r^2=0.82$) and Sandy Loam ($r^2=1.0$) (Figs. 3. 4a and 3. 4b). The fact that the regression line is linear, and there is no plateau, indicates that soil P concentrations were not in excess of what the plant could uptake. Increasing the application of P fertilizer by 1 kg ha^{-1} increased above-ground biomass by 0.08 and 0.02g in the Loam and Sandy Loam, respectively. A smaller increase in biomass in response to increasing P application in the Sandy Loam can be attributed to the lower PSC of the Sandy Loam. By keeping N and K rates sufficient in this study, we were able to assure growth increases were due to increased concentrations of P. Studies have shown that N influences total P uptake and when ammonium based fertilizers are applied as a banded starter, they can promote increased P uptake (Blair et al., 1971). If chelates increased plant available P, we would have expected to see greater biomass at a given P application rate. However, applications of EDTA and HEEDTA at all P rates had no significant effect on above-ground biomass except significantly decreasing biomass with applications of EDTA at the $19.3 \text{ kg P ha}^{-1}$ rate.

There was no significant relationship between increasing P application rate and below-ground biomass in the Loam (Fig. 3. 5a). However, with increasing P application rate, there was a positive relationship ($r^2=0.40$) with below-ground biomass in the Sandy Loam (Fig. 3. 5b). Increasing the application rate of P fertilizer by 1 kg ha^{-1} increased below-ground biomass by 0.035g in the Sandy Loam. Generally, increases in grain yield in response to banded fertilizers were in soils with low P concentrations (Tarkalson and Bjorneberg, 2010).

In the Loam soil, EDTA and HEEDTA significantly decreased below-ground biomass at the 0 and $9.63 \text{ kg P ha}^{-1}$ rates, but had no significant effect at the $19.3 \text{ kg P ha}^{-1}$ application rate

(Fig. 3. 5a). In the Sandy Loam, additions of HEEDTA were found to have no significant effect when compared to the same rate without chelate application. However, EDTA applications at 9.6 and 19.3kg P ha⁻¹ only in the Sandy Loam significantly increased below-ground biomass (Fig. 3. 5b).

It has been shown that HEEDTA loses its efficiency at maintaining soluble Al above pH of 6 (Mortvedt, 1991). However, HEEDTA was shown to decrease P sorption in the soil incubation when incorporated in soil as shown in experiments reported in Chapter 2. This change in efficiency can be attributed to mixing the chelating agents into the soil as opposed to applying as banded fertilizer in this greenhouse study. Therefore, in both the Loam and Sandy Loam, as pH values were greater than 6 at harvest on 4week, this change in pH could explain why HEEDTA was inefficient at maintaining P in a plant available form. The fact that pH values were less than 7 showed that EDTA should have been able to sorbed to Fe and Al on soil colloids, therefore blocking the sorption of P.

Effects of Chelating Agents on Phosphorus Uptake

With increasing P application, there was a positive linear relationship with above-ground P uptake in the Loam ($r^2=0.83$) and Sandy Loam ($r^2=0.99$) (Figs. 3. 6a and 3. 6b). The lower PSC of the Loam could explain the increase in P uptake in response to 1 kg P ha⁻¹ application of P fertilizer compared to the Sandy Loam at 0.21 and 0.07, respectively. Applications of EDTA and HEEDTA had no significant effect on above-ground P uptake at any P rate except EDTA at the 19.3kg P ha⁻¹ rate in both soils and HEEDTA at the same rate in the Sandy Loam. Stryker et al. (1974) showed low concentrations of plant available P in soils reduced P uptake in shoots, but did not significantly affect shoot weight in corn. A reduction in plant available P resulted in a

decrease in root radius, which was attributed to the plants response to need to absorb more P by increasing root surface area.

The addition of chelating agents significantly decreased below-ground tissue P uptake at 9.6kg P ha⁻¹ rate in both soils and 19.3kg P ha⁻¹ rate in the Sandy Loam (Figs. 3. 7a and 3. 7b). With increasing P application rate there was an increase in P uptake in the below-ground portion of the corn plant based on the regression equations. The regression coefficient for this relationship was higher in the Sandy Loam with $r^2=0.67$ as opposed to the Loam at $r^2=0.38$ (Figs. 3. 7a and 3. 7b). A decrease in P uptake in the above- and below-ground portion could likely be due to the movement of P outside the application area where P could have been sorbed by Fe and Al hydroxides especially at the 9.6 and 19.3kg P ha⁻¹ rates.

CONCLUSION

A previous soil incubation study showed that applications of chelating agents to soil are capable of decreasing P binding to Fe and Al. In this greenhouse study, chelating agents applied to soils in a starter band did not decrease P binding when measured in bulk soil by three P extraction methods in two soils with differing soil texture, PSC, OM, and DPS. Phosphorus rates caused a linear increase in WSP, Mehlich-1 P, Mehlich-3 P, above- and below-ground biomass, and P uptake as shown before in similar studies. Starter banding of P and incorporation of chelating agents to soils have shown potential to increase plant available P in soil, but combining the two approaches did not produce similar results in this study. Further research on various combinations of chelating agents and P application methods are needed to clearly assess the use of soil applied chelates for increasing fertilizer P efficiency.

REFERENCES

- Alexander, T. G., and J. A. Robertson. 1972. EDTA extractable phosphorus in relation to available and inorganic phosphorus forms in soils. *Soil Sci.* 114:69-72.
- Blair, G. J., C. P. Mamaril, and M. H. Miller. 1971. Influence of nitrogen source on phosphorus uptake by corn from soils differing in pH. *Agron. J.* 63:235-238.
- Bond, C. R., R. O. Maguire, and J. L. Havlin. 2006. Change in soluble phosphorus following fertilization is dependent on initial Mehlich-3 phosphorus. *J. Environ. Qual.* 35:1818–1824.
- Breeuwsma, A., and S. Silva. 1992. Phosphorus fertilization and environmental effects in the Netherlands and the Po region (Italy). Rep. 57. Winand Staring Centre for Integrated Land, Soil, and Water Res., Agric. Res. Dep., Wageningen, the Netherlands.
- Clemens, D. F., B. M. Whitehurst, and G. B. Whitehurst. 1990. Chelates in agriculture. *Fert. Res.* 25:127-131.
- Daniels, M. T., T. Daniel, D. Carman, R. Morgan, J. Langston, and K. van Devender. 1998. Soil phosphorus levels: Concerns and recommendations. Univ. Arkansas Coop. Ext., Fayetteville, AR.
- Drouillon, M., and R. Merckx. 2003. The role of citric acid as a phosphorus mobilization mechanism in highly p-fixing soils. *Gayana Bot.* 60:55-62.
- Gee, G. W., and J.W. Bauder 1986. Particle-size analysis. Pages 383–411 *in* A. Klute, ed. *Methods of soil analysis. Part 1. Physical and mineralogical methods.* Agronomy Monograph 9, 2nd ed. ASA, SSSA, Madison, WI.

- Godsey, C. B., J. P. Schmidt, A. J. Schlegel, R. K. Taylor, C. R. Thompson, and R. J. Gehl. 2003. Correcting iron deficiency in corn with seed row-applied iron sulfate. *Agron. J.* 95:160-166.
- Goos, R. J., and B. E. Johnson. 2000. A comparison of three methods for reducing Fe deficiency chlorosis in soybean. *Agron. J.* 92:1135-1139.
- Hell, R., and U. W. Stephan. 2003. Iron uptake, trafficking and homeostasis in plants. *Planta.* 216:541-551.
- Larsen, S. 1952. The use of ^{32}P in studies on the uptake of phosphorus by plants. *Plant Soil.* 4:1-10.
- Lingerfelter, J. E., W. T. Schapaugh, Jr., J. P. Schmidt, and J. J. Higgins. 2005. Comparison of genotype and cultural practices to control Fe deficiency chlorosis in soybean. *Commun. Soil Sci. Plant Anal.* 36:1047-1062.
- Lookman, R., N. Vandeweert, R. Merckx, and K. Vlassak. 1995. Geostatistical assessment of the regional distribution of phosphate sorption capacity parameters (Feox and Alox) in northern Belgium. *Geoderma.* 66:285-296.
- Luscombe, P. C., J. K. Syers, and P. E. H. Gregg. 1979. Water extraction as a soil testing procedure for phosphate. *Commun. Soil Sci. Plan.* 10:11, 1361-1369.
- Maguire, R. O., and S. E. Heckendorn. 2011. *Laboratory Procedures: Virginia Tech Soil Testing Laboratory*, Virginia Cooperative Extension, Blacksburg, Va.
- Maguire, R. O., and J. T. Sims. 2002. Measuring agronomic and environmental soil phosphorus saturation and predicting phosphorus leaching with Mehlich-3. *Soil Sci. Soc. Am. J.* 66:2033-2039.

- Maguire, R. O., J. T. Sims, S. K. Dentel, F. J. Coale, and J. T. Mah. 2001. Relationships between biosolids treatment process and soil phosphorus availability. *J. Environ. Qual.* 30:1023-1033.
- Maguire, R. O., J. T. Sims, and F. C. Coale. 2000. Phosphorus solubility in biosolids amended farm soils in the mid-Atlantic region of the USA. *J. Environ. Qual.* 29:1225-1233.
- Marschner, H., and V. Romheld. 1994. Strategies of plants for acquisition of iron. *Plant Soil* 165: 261-274.
- Mattingly, G. E. G., and F. V. Widdowson. 1959. Uptake of phosphorus from P³² labelled superphosphate by field crops. Part II. Comparison of placed and broadcast applications to barley. *Plant Soil.* 10:161-175.
- McKeague, J., and J.H. Day. 1966. Dithionite and oxalate-extractable Fe and Al as aids in differentiating various classes of soils. *Can. J. Soil Sci.* 46:13–22.
- Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Commun Soil Sci. Plant Anal.* 15:1409-1416.
- Messiga, A. J., N. Ziadi, C. Grant, C. Morel, G. Tremblay, G. Lamarre, and L. -E. Parent. 2012. Long term impact of tillage practices and biennial P and N fertilization on maize and soybean yields and soil P status. *Field Crops Res.* 133:10-22.
- Morrissey, J., and M. L.Guerinot. 2009. Iron uptake and transport in plants: the good, the bad, and the ionome. *Chem. Rev.* 109:4553-4567.
- Mortvedt, J. J. 1991. *Micronutrients in agriculture*. Soil Sci. Soc. Am., Madison, WI. p. 187-199.
- Murphy, J., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27:31-36.

- Nelson, L. B. 1956. The mineral nutrition of corn as related to its growth and culture. *Advan. Agron.* 8:321-375.
- Penas, E. J., R. A. Wiese, R. W. Elmore, G. W. Hergett, and R. S. Moomaw. 1990. Soybean chlorosis studies on high pH bottomland soils. *Univ. of Nebraska Inst. Agric. Nat. Res. Bull.* 312. Univ. of Nebraska, Lincoln.
- Pote, D. H., T. C. Daniel, A. N. Sharpley, P. A. Moore, D. R. Edwards, and D. J. Nichols. 1996. Relating extractable soil phosphorus to losses in runoff. *Soil Sci. Soc. Am. J.* 60:855-859.
- SAS Institute. 2012. The SAS system for windows. User's Guide. SAS Institute Inc., Cary, NC.
- Sims, J. T. 1989. Comparison of Mehlich-1 and Mehlich-3 extractants for P, K, Ca, Mg, Mn, Cu, and Zn in Atlantic Coastal Plain soils. *Commun. Soil Sci. Plant Anal.* 20:1707-1726.
- Sims, J. T., R. R. Simard, and B. C. Joern. 1998. Phosphorus loss in agricultural drainage: Historical perspective and current research. *J. Environ. Qual.* 27:277-293.
- Staunton, S., and F. Leprince. 1996. Effect of pH and some organic anions on the solubility of soil phosphate: implications for P bioavailability. *Eur. J. Soil Sci.* 47: 231-239.
- Strom, L., A. O. Owen, D. L. Godbold, and D. L. Jones. 2002. Organic acid mediated P mobilization in the rhizosphere and uptake by adaptive physiology to transgenic varieties for cultivation in extreme soils. *Plant Sci.* 160:1-13.
- Stryker, R. B., J. W. Gilliam, and W. A. Jackson. 1974. Nonuniform phosphorus distribution in the root zone of corn: growth and phosphorus uptake. *Soil Sci. Soc. Am. Prob.* 38:3340-340.
- Tarkalson, D. D., and D. D. Bjorneberg. 2010. Evaluation of nitrogen and phosphorus fertilizer placement with strip tillage for irrigated Pacific Northwest corn production. Online. *Crop Management* doi:10.1094/CM-2010-0611-01-RS.

- van der Zee, S.E.A.T.M., and W.H. van Riemsdijk. 1988. Model for long-term phosphate reaction kinetics in soil. *J. Environ. Qual.* 17:35–41.
- Vempati, R. K., and R. H. Loeppert. 1986. Synthetic ferrihydrite as a potential iron amendment in calcareous soils. *J. Plant Nutr.* 9:1039-1052.
- Welch, L. F., D. L. Mulvaney, L. V. Boone, G. E. McKibben, and J. W. Pendleton. 1966. Relative efficiency of broadcast versus banded phosphorus for corn. *Agron. J.* 58:283-287.

Table 3. 1. Selected properties of the Loam and Sandy Loam used in the 4-week greenhouse study.

Soil Property	Loam	Sandy Loam
pH	6.48	6.51
CEC†, cmol _c kg ⁻¹	9.3	4.5
Organic Matter, g kg ⁻¹	52	17
Al _{ox} ‡, mmol kg ⁻¹	53.7	20.1
Fe _{ox} ‡, mmol kg ⁻¹	30.1	6.3
PSC§, mmol kg ⁻¹	41.9	13.2
Phosphorus, mg kg ⁻¹		
Mehlich-1	8	3
Mehlich-3	18	6
P _{ox} ‡	162	32
DPS¶, %	12.5	7.8

† Cation exchange capacity.

‡ Oxalate-extractable Al, Fe, and P.

§ Phosphorus sorption capacity.

¶ Degree of P saturation.

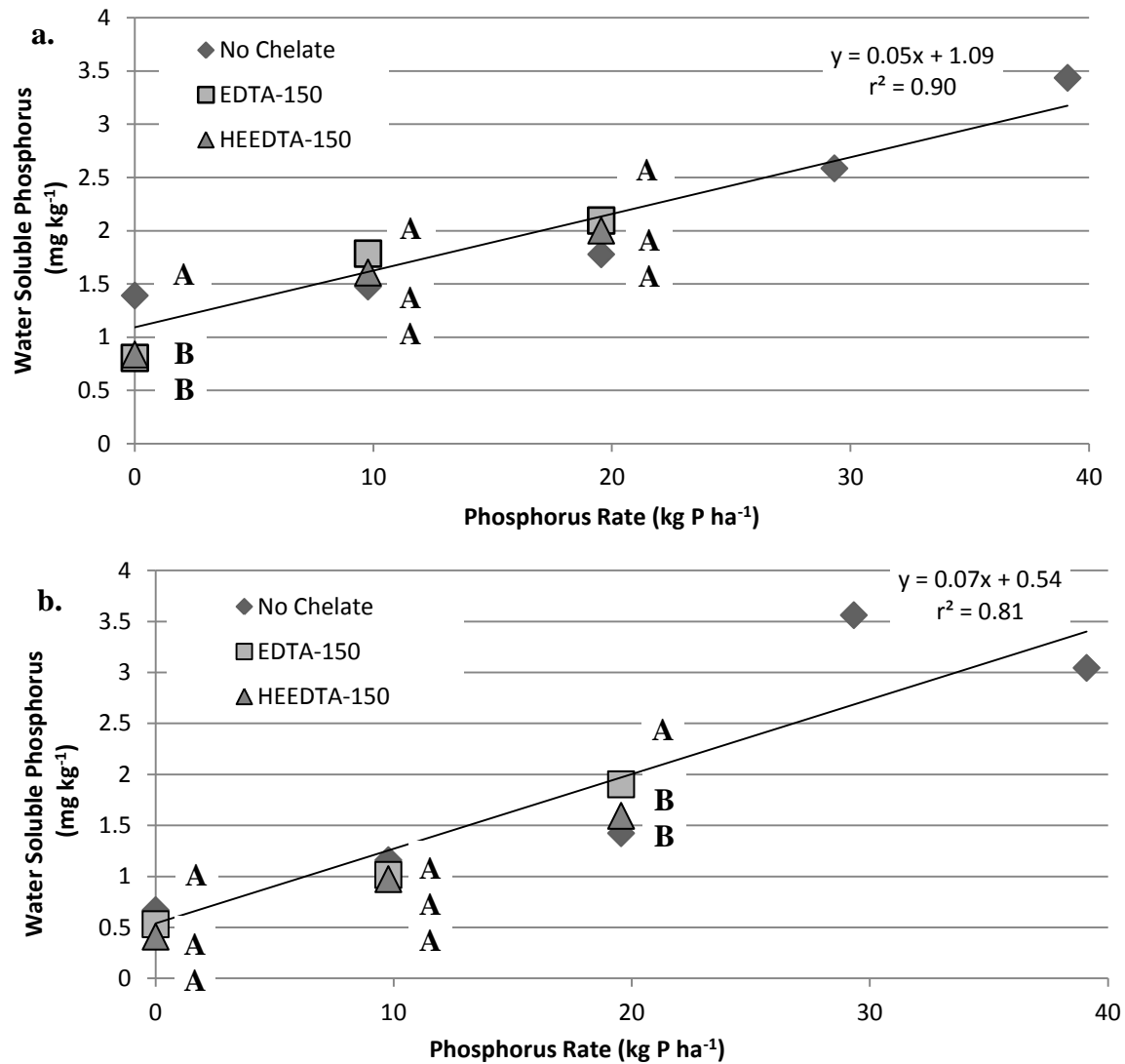


Figure 3. 1. The effects of increasing phosphorus (P) rate application on water-soluble P (WSP) in pots treated with potassium phosphate fertilizer following a 4-week greenhouse study growing corn (*Zea mays L.*) in (a) Loam and (b) Sandy Loam. Additions of EDTA and HEEDTA at 150 percent P sorption capacity (PSC) were added at P rates 0, 9.6, and 19.3kg P ha⁻¹. Letters show significant differences between treatments at each P rate (P=0.10). Regression line and equation is fit for the No Chelate treatment with increasing P rate.

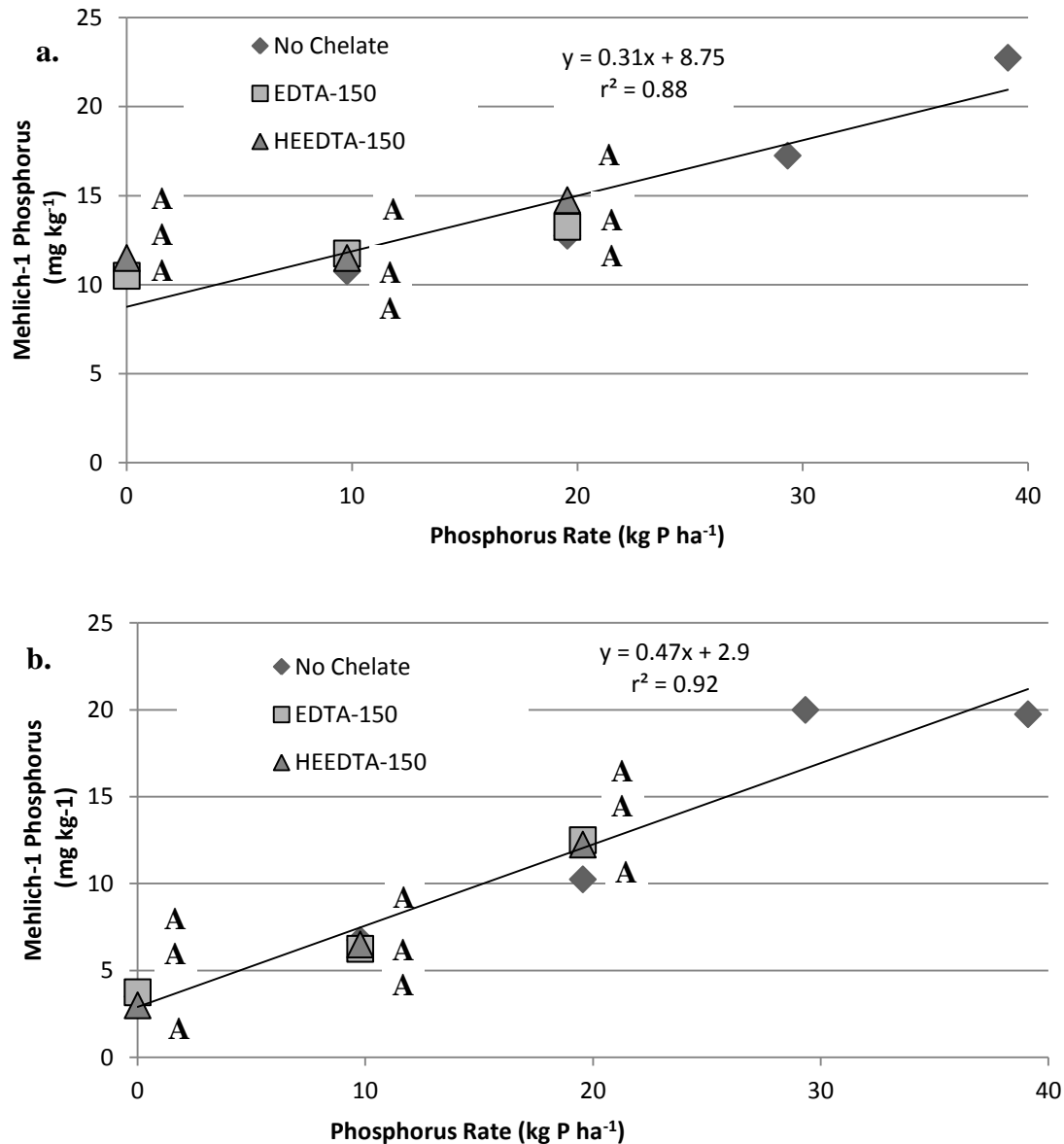


Figure 3. 2. The effects of increasing phosphorus (P) rate application on Mehlich-1P in pots treated with potassium phosphate fertilizer following a 4-week greenhouse study growing corn (*Zea mays L.*) in (a) Loam and (b) Sandy Loam. Additions of EDTA and HEEDTA at 150 percent P sorption capacity (PSC) were added at P rates 0, 9.6, and 19.3kg P ha⁻¹. Letters show significant differences between treatments at each P rate (P=0.10). Regression line and equation is fit for the No Chelate treatment with increasing P rate.

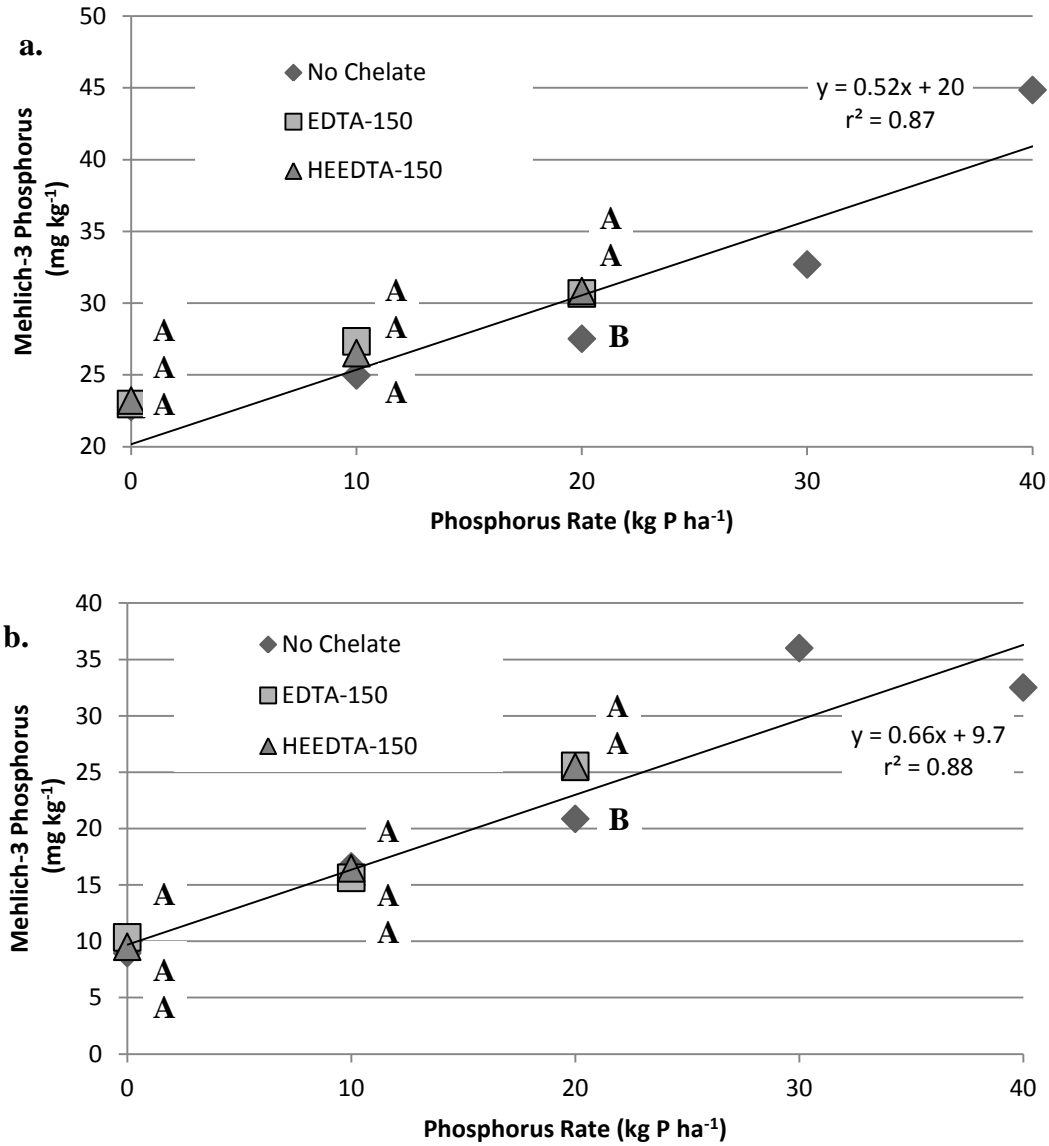


Figure 3. 3. The effects of increasing phosphorus (P) rate application on Mehlich-3 P in pots treated with potassium phosphate fertilizer following a 4-week greenhouse study growing corn (*Zea mays L.*) (a) Loam and (b) Sandy Loam. Additions of EDTA and HEEDTA at 150 percent P sorption capacity (PSC) were added at P rates 0, 9.6, and 19.3kg P ha⁻¹. Letters show significant differences between treatments at each P rate (P=0.10). Regression line and equation is fit for the No Chelate treatment with increasing P rate.

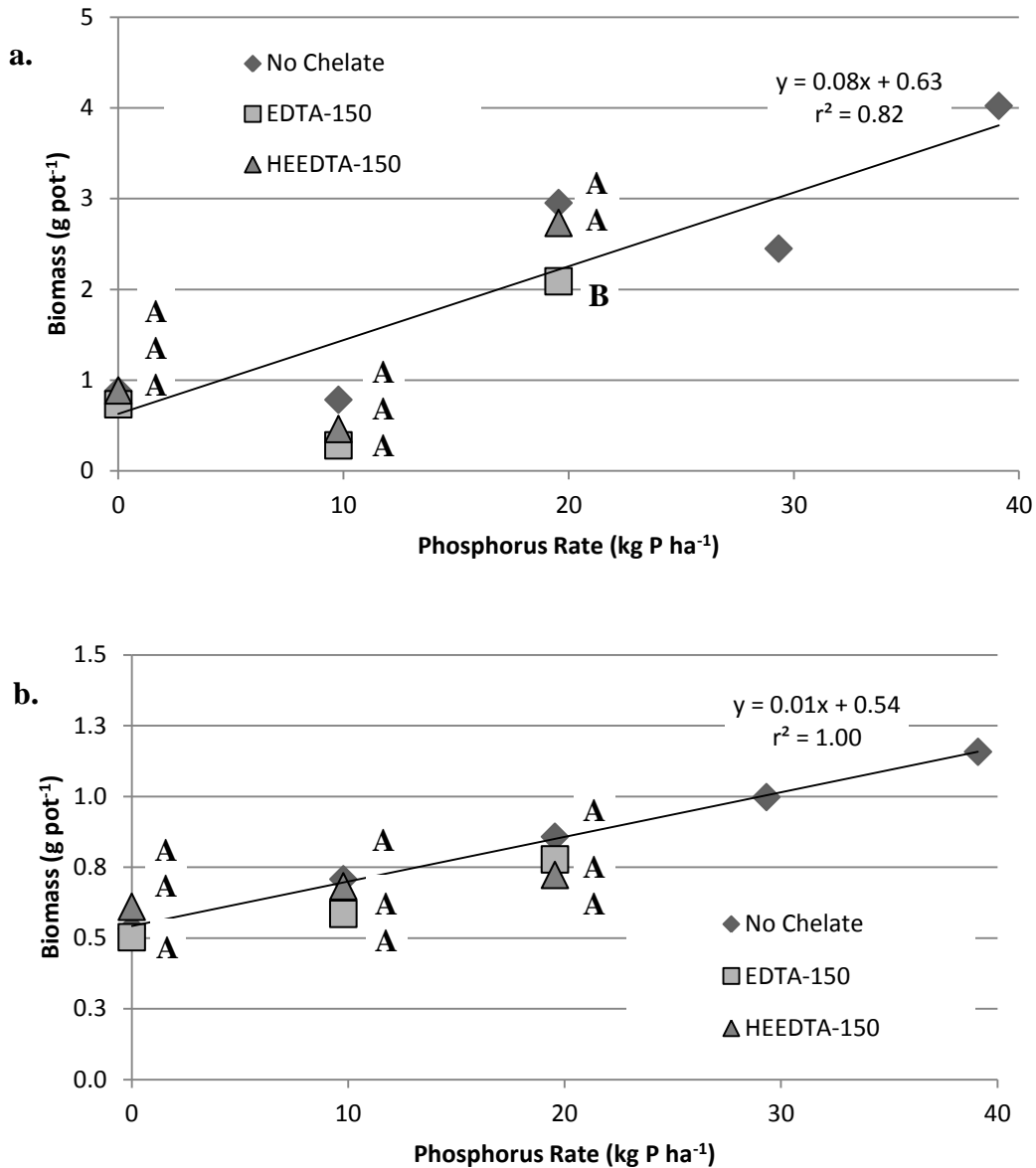


Figure 3. 4. The effects of increasing phosphorus (P) rate application on above-ground biomass of corn (*Zea mays L.*) in pots treated with potassium phosphate fertilizer following a 4-week greenhouse study in (a) Loam and (b) Sandy Loam. Additions of EDTA and HEEDTA at 150 percent P sorption capacity (PSC) were added at P rates 0, 9.6, 19.3kg P ha⁻¹. Letters show significant differences between treatments at each P rate (P=0.10). Regression line and equation is fit for the No Chelate treatment with increasing P rate.

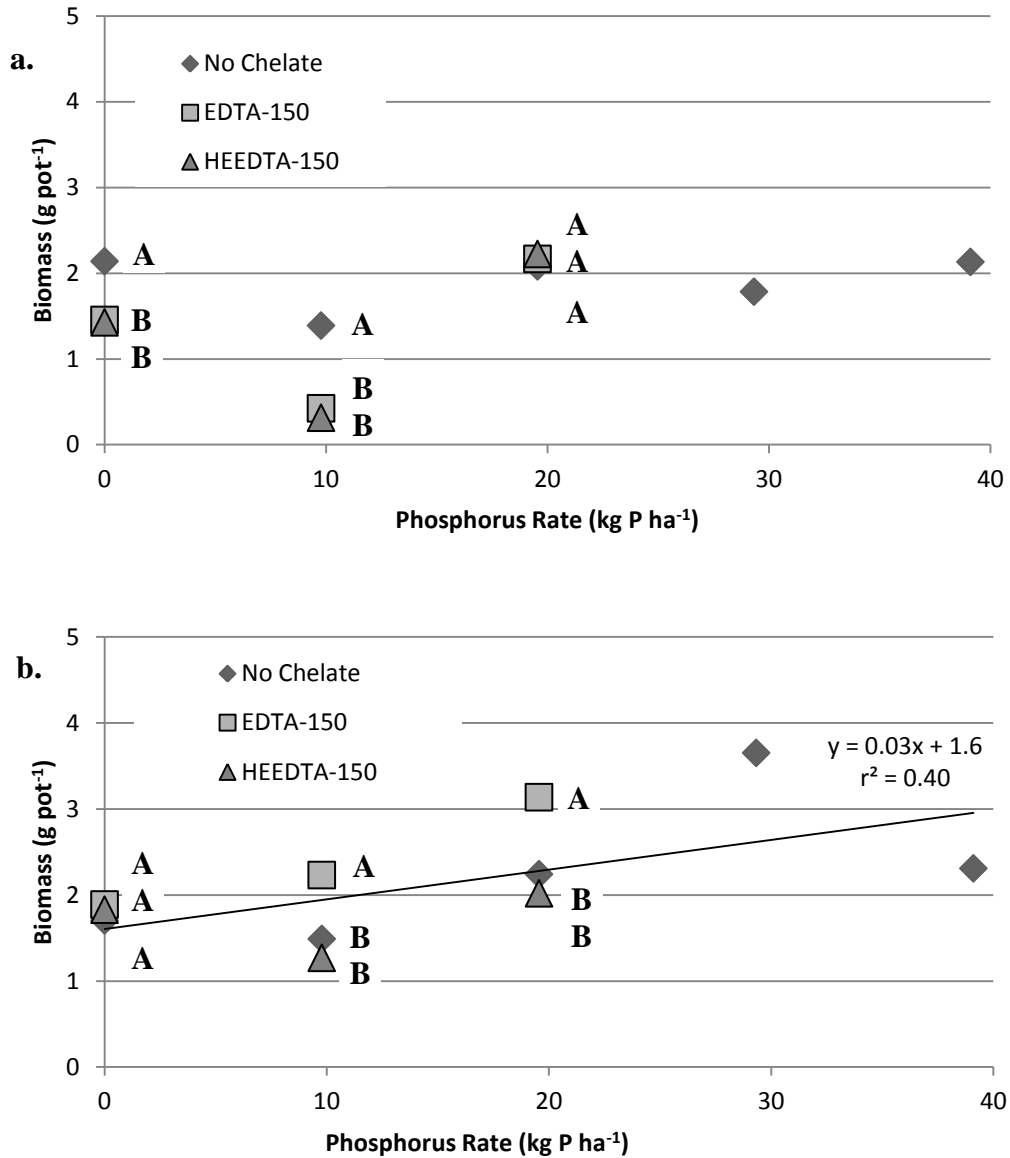


Figure 3. 5. The effects of increasing phosphorus (P) rate application on below-ground biomass of corn (*Zea mays L.*) in pots treated with potassium phosphate fertilizer following a 4-week greenhouse study in (a) Loam and (b) Sandy Loam. Additions of EDTA and HEEDTA a at 150 percent P sorption capacity (PSC) were added at P rates 0, 9.6, and 19.3 kg P ha⁻¹. Letters show significant differences between treatments at each P rate (P=0.10). Regression line and equation is fit for the No Chelate treatment with increasing P rate.

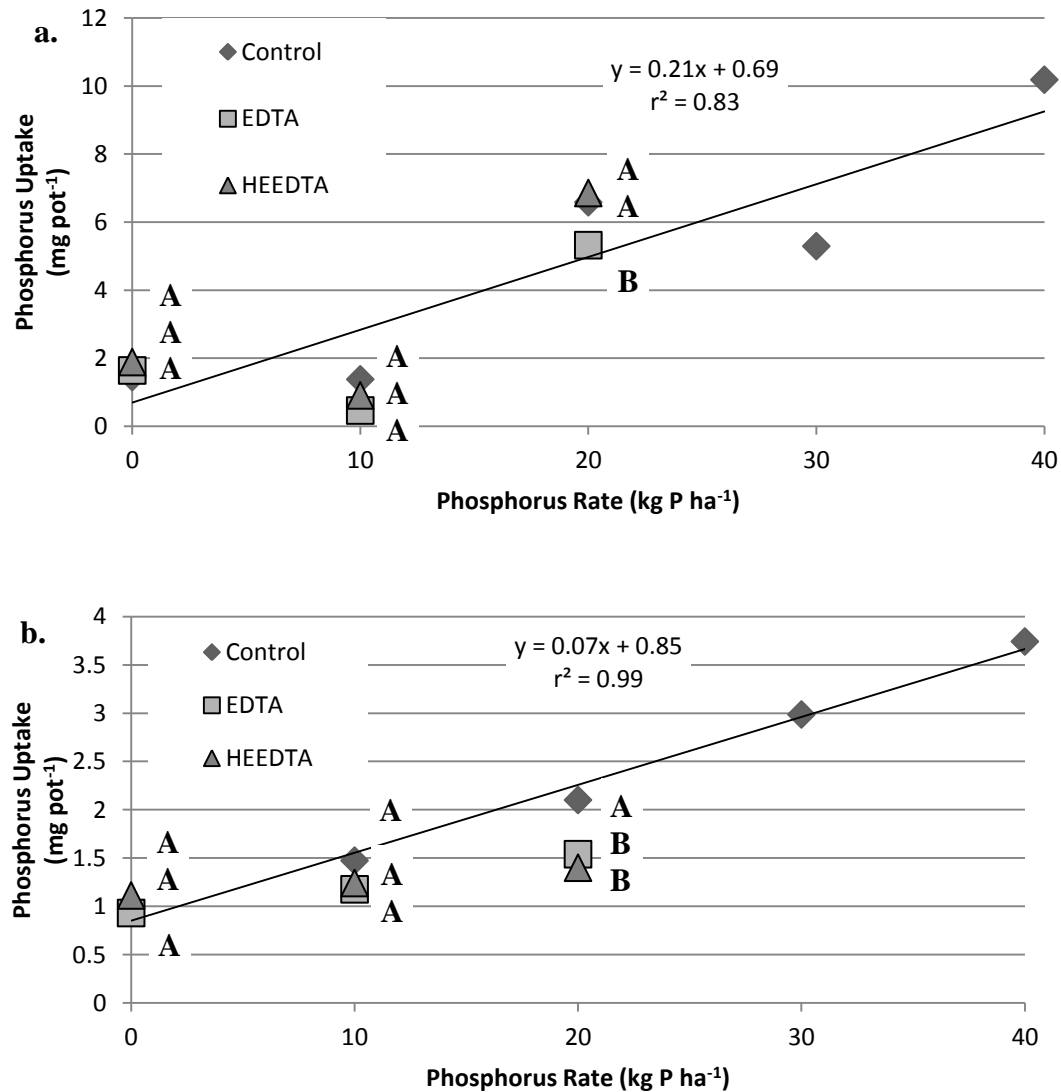


Figure 3. 6. The effects of increasing phosphorus (P) rate on above-ground P uptake by corn (*Zea mays L.*) in pots treated with potassium phosphate fertilizer following a 4-week greenhouse study in (a) Loam and (b) Sandy Loam. Additions of EDTA and HEEDTA at 150 percent P sorption capacity (PSC) were added at P rates 0, 9.6, and 19.3kg P ha⁻¹. Letters show significant differences between treatments at each P rate (P=0.10). Regression line and equation is fit for the No Chelate treatment with increasing P rate.

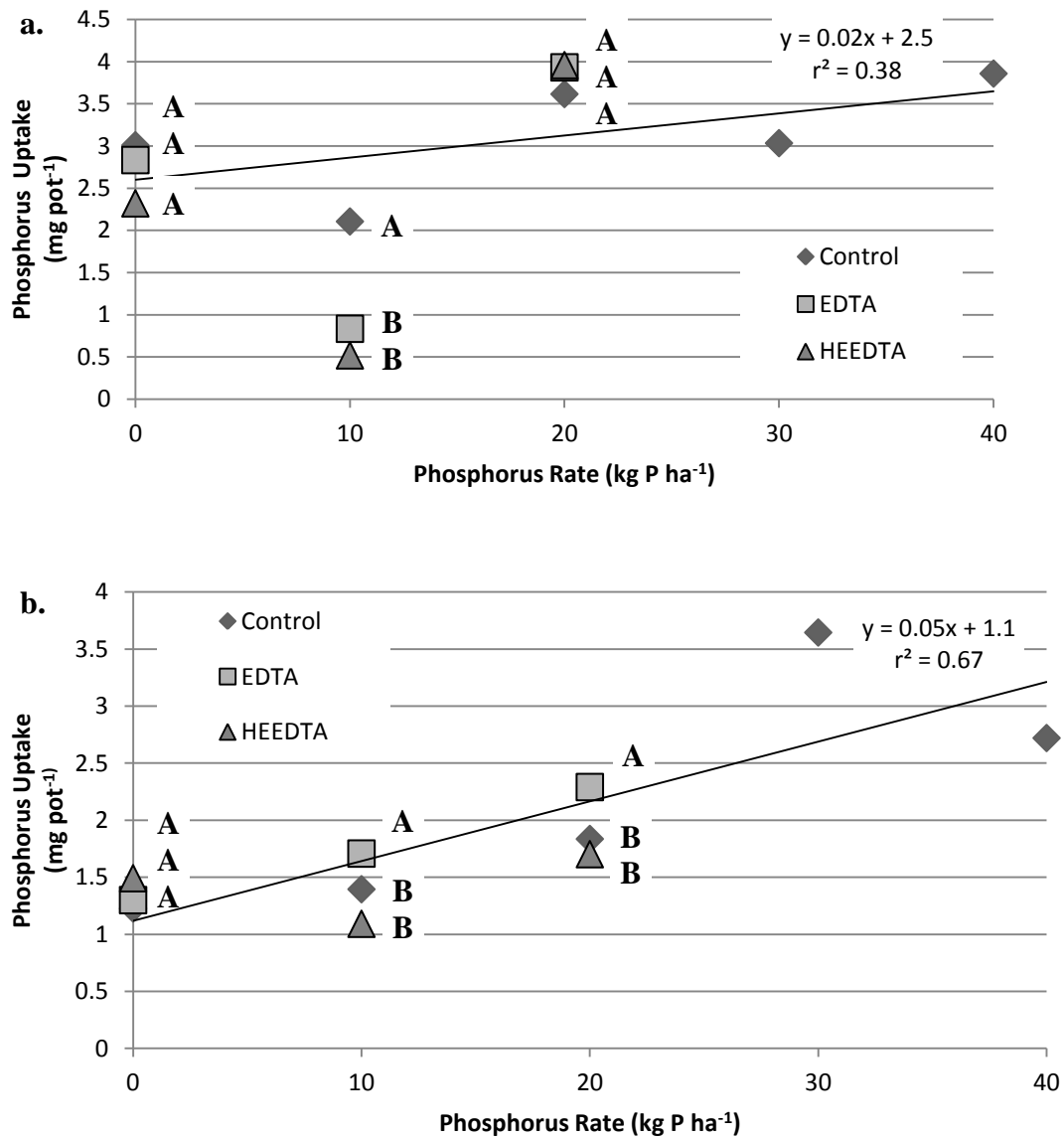


Figure 3. 7. The effects of increasing phosphorus (P) rate on below-ground P uptake by corn in pots treated with potassium phosphate fertilizer following a 4-week greenhouse study in (a) Loam and (b) Sandy Loam. Additions of EDTA and HEEDTA at 150 percent P sorption capacity (PSC) were added at P rates 0, 9.6, and 19.3kg P ha⁻¹. Letters show significant differences between treatments at each P rate (P=0.10). Regression line and equation is fit for the No Chelate treatment with increasing P rate.

APPENDIX

Appendix A. Water-soluble Phosphorus concentrations at selected sampling intervals during a 70d soil incubation comparing the efficiency of chelating agents (Incubation 1).

Cup Nu	Soil Series	Treatment	Rep	Ortho-Phosphorus†					
				1 d	7 d	21 d	35 d	49 d	70 d
				----- mg kg ⁻¹ -----					
1	Loam	Control No-P	1	0.174	0.118	0.089	0.073	0.090	0.053
2	Loam	Control No-P	2	0.184	0.119	0.085	0.086	0.074	0.055
3	Loam	Control No-P	3	0.178	0.124	0.088	0.084	0.082	0.063
4	Loam	Control P	1	80.846	54.810	32.816	29.185	24.460	20.924
5	Loam	Control P	2	99.035	57.672	30.436	27.999	22.498	18.387
6	Loam	Control P	3	92.622	53.805	30.396	26.441	23.675	18.910
7	Loam	EDTA-25	1	78.215	53.984	34.613	32.350	28.563	23.566
8	Loam	EDTA-25	2	78.695	57.384	36.587	33.243	29.854	24.837
9	Loam	EDTA-25	3	88.288	54.084	34.363	33.181	29.161	23.875
10	Loam	HEEDTA-25	1	85.897	54.366	32.897	32.260	29.723	24.471
11	Loam	HEEDTA-25	2	82.058	49.792	32.948	30.847	27.322	23.129
12	Loam	HEEDTA-25	3	85.877	51.104	35.589	31.368	29.151	23.944
13	Loam	Citric Acid-25	1	62.500	38.372	27.328	25.359	22.629	18.004
14	Loam	Citric Acid-25	2	59.315	36.273	25.961	23.484	20.358	16.244
15	Loam	Citric Acid-25	3	60.395	37.927	25.980	23.993	20.681	16.921
16	Loam	Gluconic Acid-25	1	67.921	42.869	29.431	26.483	22.265	17.797
17	Loam	Gluconic Acid-25	2	65.227	48.254	29.267	25.903	21.340	17.834
18	Loam	Gluconic Acid-25	3	71.774	45.727	28.382	26.340	21.661	18.439
25	Loam	EDTA-100	1	98.315	78.777	48.519	46.572	40.459	38.412
26	Loam	EDTA-100	2	95.503	69.155	49.833	44.615	40.074	36.767
27	Loam	EDTA-100	3	106.832	73.031	49.243	46.973	42.325	38.159

28	Loam	HEEDTA-100	1	118.344	82.994	56.413	38.383	35.733	36.204
29	Loam	HEEDTA-100	2	125.150	96.890	60.511	37.093	37.013	38.754
30	Loam	HEEDTA-100	3	109.193	90.476	63.750	42.773	40.217	37.560
31	Loam	Citric Acid-100	1	120.637	82.564	8.242	10.820	8.577	8.179
32	Loam	Citric Acid-100	2	120.669	90.287	9.015	11.135	9.010	7.888
33	Loam	Citric Acid-100	3	125.055	82.574	8.243	11.293	8.813	8.116
34	Loam	Gluconic Acid-100	1	23.217	29.716	19.249	18.524	17.015	15.246
35	Loam	Gluconic Acid-100	2	21.621	30.934	20.815	18.485	17.163	15.168
36	Loam	Gluconic Acid-100	3	20.864	33.206	20.714	19.902	18.050	15.496
37	Sand	Control No-P	1	6.480	6.193	5.623	5.935	5.406	5.455
38	Sand	Control No-P	2	6.545	6.139	5.721	5.853	5.542	5.384
39	Sand	Control No-P	3	6.619	6.309	5.545	6.020	5.467	5.424
40	Sand	Control P	1	80.945	70.041	53.210	49.468	38.853	32.529
41	Sand	Control P	2	79.112	69.453	52.602	49.300	39.706	33.125
42	Sand	Control P	3	79.097	71.836	53.255	47.290	38.453	32.537
43	Sand	EDTA-25	1	98.965	86.723	74.596	81.355	68.983	65.394
44	Sand	EDTA-25	2	96.906	84.931	77.423	77.844	72.806	64.162
45	Sand	EDTA-25	3	98.041	86.192	72.680	75.982	69.203	64.653
46	Sand	HEEDTA-25	1	100.985	92.734	70.557	68.090	61.669	57.901
47	Sand	HEEDTA-25	2	99.163	90.563	72.157	68.791	62.470	57.164
48	Sand	HEEDTA-25	3	102.649	90.304	70.897	67.902	61.155	56.626
49	Sand	Citric Acid-25	1	102.243	51.085	42.421	39.880	33.902	29.480
50	Sand	Citric Acid-25	2	97.564	47.419	41.724	39.294	33.731	29.506
51	Sand	Citric Acid-25	3	98.469	52.565	41.907	40.066	33.154	31.509
52	Sand	Gluconic Acid-25	1	70.411	62.290	51.118	45.325	36.886	30.968
53	Sand	Gluconic Acid-25	2	72.085	64.218	50.807	47.701	37.253	30.814
54	Sand	Gluconic Acid-25	3	70.659	63.790	50.081	46.884	37.954	30.759
61	Sand	EDTA-100	1	152.566	145.489	133.700	157.428	129.115	119.975
62	Sand	EDTA-100	2	160.605	143.816	132.076	144.113	126.766	119.367

63	Sand	EDTA-100	3	158.490	145.136	135.411	145.528	121.745	117.814
64	Sand	HEEDTA-100	1	156.035	150.434	136.907	105.503	103.528	101.340
65	Sand	HEEDTA-100	2	161.717	146.735	136.243	109.478	106.106	97.370
66	Sand	HEEDTA-100	3	159.866	151.183	135.294	112.019	108.748	98.793
67	Sand	Citric Acid-100	1	168.625	45.372	35.282	37.472	33.635	22.909
68	Sand	Citric Acid-100	2	167.128	46.766	36.430	37.609	37.453	35.244
69	Sand	Citric Acid-100	3	164.391	46.544	34.577	37.633	36.493	31.290
70	Sand	Gluconic Acid-100	1	64.263	41.317	41.509	44.943	38.388	34.064
71	Sand	Gluconic Acid-100	2	65.439	41.163	41.255	42.939	38.145	34.148
72	Sand	Gluconic Acid-100	3	64.705	43.694	40.927	41.894	37.544	32.382

† Ortho-Phosphorus concentrations as determined colorimetrically (Murphy and Riley, 1962).

Appendix B. Soil pH values from Incubation 1.

Treatment	Loam		Sand	
	26 d	70 d	26 d	70 d
Control No-P	6.48	6.39	5.85	5.73
Control P	5.29	4.87	4.81	4.14
EDTA-25	5.58	5.08	6.20	5.55
EDTA-100	6.34	6.22	5.96	6.08
HEEDTA-25	5.52	4.87	5.79	5.69
HEEDTA-100	5.94	5.96	4.76	4.83
Citric Acid-25	5.25	4.90	4.97	4.05
Citric Acid-100	7.11	5.21	7.23	6.60
Gluconic Acid-25	5.23	4.91	4.80	3.95
Gluconic Acid-100	5.43	5.14	6.79	4.27

Appendix C. Incubation 3 Mehlich-1 Phosphorus, Iron, and Aluminum, and Mehlich-3 Phosphorus concentrations on 49d of a soil incubation.

Cup Nu	Soil Type	Treatment	Rep	Phosphorus		Iron	Aluminum
				M-1	M-3	M-1	M-1
----- mg kg ⁻¹ -----							
1	Loam	Control No P	1	8.245	21.47	3.45	217.97
2	Loam	Control No P	2	7.36	22.54	3.34	217.55
3	Loam	Control No P	3	8.08	21.98	3.26	221.96
4	Loam	Control-P	1	144.81	243.7	5.775	268.41
5	Loam	Control-P	2	151.17	246.2	5.85	278.645
6	Loam	Control-P	3	153.77	252.1	7.755	274.16
7	Loam	EDTA No P- 90	1	17.06	30.4	215.01	233.055
8	Loam	EDTA No P- 90	2	11.125	31.2	218.91	228.07
9	Loam	EDTA No P- 90	3	11.2	29.51	225.43	237.66
10	Loam	HEEDTA No P- 90	1	11.985	33.31	253.67	224.2
11	Loam	HEEDTA No P- 90	2	12.925	33.89	262.28	225.17
12	Loam	HEEDTA No P- 90	3	13.33	34.15	270.62	235.015
13	Loam	EDTA- 30	1	165.81	246.5	277.72	284.54
14	Loam	EDTA- 30	2	181.19	262.1	275.8	296.985
15	Loam	EDTA- 30	3	180.54	259	292.38	286.2
16	Loam	HEEDTA- 30	1	185.09	263	285.67	287.23
17	Loam	HEEDTA- 30	2	188.45	273.5	281.63	285.79
18	Loam	HEEDTA- 30	3	190.95	276.2	291.27	291.075
19	Loam	EDTA- 60	1	184.57	269.9	317.29	317.095
20	Loam	EDTA- 60	2	180.53	258	326.62	310.085
21	Loam	EDTA- 60	3	183.88	256.3	312.19	316.175
22	Loam	HEEDTA- 60	1	204.52	277	320.06	295.505
23	Loam	HEEDTA- 60	2	202.67	280.9	320.45	298.505
24	Loam	HEEDTA- 60	3	211.21	290.4	332.93	302.94
26	Loam	EDTA- 90	1	179.84	268.1	345.95	304.805
27	Loam	EDTA- 90	2	178.16	265.5	353.92	309.335
28	Loam	EDTA- 90	3	182.81	268	362.22	327.555
29	Loam	HEEDTA- 90	1	219.19	275.9	379.26	334.01
30	Loam	HEEDTA- 90	2	219.95	281.6	376.81	330.955
31	Loam	HEEDTA- 90	3	225.29	281.9	386.18	342.64
32	Loam	EDTA- 120	1	178.51	262.3	438.16	353.94
33	Loam	EDTA- 120	2	180.42	256.5	420.35	362.085
34	Loam	EDTA- 120	3	182.07	263.5	439.05	359.575
35	Loam	HEEDTA- 120	1	238.2	289.5	442.11	388.995
36	Loam	HEEDTA- 120	2	233.58	288.5	419.87	374.595

37	Loam	HEEDTA- 120	3	233.5	278.8	435.34	369.01
38	Loam	EDTA- 150	1	182.88	251.1	508.15	403.935
39	Loam	EDTA- 150	2	187.84	254	504.94	410.95
40	Loam	EDTA- 150	3	179.92	248.4	527.11	414
41	Loam	HEEDTA- 150	1	237.95	285	511.54	412.375
42	Loam	HEEDTA- 150	2	215.48	268.2	433.69	371.94
43	Loam	HEEDTA- 150	3	242.18	289.6	515.47	423.61
44	Loam	Citric Acid- 90	1	157.81	190.6	41.59	244.905
45	Loam	Citric Acid- 90	2	148.88	207.6	8.81	233.235
46	Loam	Citric Acid- 90	3	151.34	197.6	5.24	225.515
47	Loam	Gluconic Acid- 90	1	149.24	203.7	4.565	242.9
48	Loam	Gluconic Acid- 90	2	148.04	209.6	4.45	246.49
49	Loam	Gluconic Acid- 90	3	146.3	210.6	4.67	243.955
51	Sand	Control No P	1	84.775	177.5	17.36	168.445
52	Sand	Control No P	2	90.29	178.3	18.66	179.73
53	Sand	Control No P	3	87.735	179.2	18.755	181.13
54	Sand	Control-P	1	217.56	331.4	22.23	228.025
55	Sand	Control-P	2	231.74	323.7	21.31	223.845
56	Sand	Control-P	3	220.91	319.2	21.855	215.69
57	Sand	EDTA No P- 90	1	121.13	192.7	138.16	255.005
58	Sand	EDTA No P- 90	2	117.64	184.1	137.68	246.295
59	Sand	EDTA No P- 90	3	113.21	187.4	127.34	243.175
60	Sand	HEEDTA No P- 90	1	122.55	191.1	151.43	239.555
61	Sand	HEEDTA No P- 90	2	126.03	189.1	165.11	245.225
62	Sand	HEEDTA No P- 90	3	123.69	184.3	152.07	233.645
63	Sand	EDTA- 30	1	271.98	319.9	126.86	248.81
64	Sand	EDTA- 30	2	278.04	329.7	124.43	246.99
65	Sand	EDTA- 30	3	278.65	311.6	126.12	253.57
66	Sand	HEEDTA- 30	1	282.32	315.4	125.62	245.79
67	Sand	HEEDTA- 30	2	278.05	322.3	120.34	235.59
68	Sand	HEEDTA- 30	3	283.04	315.7	130.26	241.49
69	Sand	EDTA- 60	1	295.2	321.3	148.65	282.355
70	Sand	EDTA- 60	2	299.36	325	156.12	294.28
71	Sand	EDTA- 60	3	301.45	308.8	152.92	290.985
72	Sand	HEEDTA- 60	1	317.66	316.5	171.6	288.74
73	Sand	HEEDTA- 60	2	313.21	302.8	166.85	277.725
74	Sand	HEEDTA- 60	3	317.53	329.5	160.85	283.43
76	Sand	EDTA- 90	1	308.8	318.7	181.93	322.7
77	Sand	EDTA- 90	2	323.54	306	192.1	337.765
78	Sand	EDTA- 90	3	300.78	318.4	168.91	311.415
79	Sand	HEEDTA- 90	1	330.15	321.5	203.55	322.475

80	Sand	HEEDTA- 90	2	344.32	307.3	216.37	340.54
81	Sand	HEEDTA- 90	3	318.18	309.1	191.82	309.17
82	Sand	EDTA- 120	1	315.17	339.8	203.09	347.705
83	Sand	EDTA- 120	2	344.35	306.1	222.1	379.785
84	Sand	EDTA- 120	3	307.73	312.5	190.6	337.635
85	Sand	HEEDTA- 120	1	351.02	301.9	229.94	355.81
86	Sand	HEEDTA- 120	2	342.66	345.8	234.39	357.635
87	Sand	HEEDTA- 120	3	341.46	313.5	225.55	346.525
88	Sand	EDTA- 150	1	356.08	349.7	258.08	414.15
89	Sand	EDTA- 150	2	341.4	414.3	243.85	399.865
90	Sand	EDTA- 150	3	334	373.2	239.15	391.405
91	Sand	HEEDTA- 150	1	417.32	350.1	277.81	455.815
92	Sand	HEEDTA- 150	2	354.98	304.1	257.01	380.3
93	Sand	HEEDTA- 150	3	361.62	347.7	264.01	382.075
94	Sand	Citric Acid- 90	1	305.16	298.7	36.52	287.36
95	Sand	Citric Acid- 90	2	298.35	295.2	25.27	276.97
96	Sand	Citric Acid- 90	3	312.91	298.2	24.77	284.75
97	Sand	Gluconic Acid- 90	1	259.59	322.6	22.205	238.66
98	Sand	Gluconic Acid- 90	2	258.63	315.8	22	236.92
99	Sand	Gluconic Acid- 90	3	263.22	322.3	22.47	241.085

Appendix D. Adjustment of chelate and diammonium phosphate fertilizer solutions before addition to soil and total concentration of sodium (Na) added to each cup for Chapter 2.

Treatment	Soil Type	Initial pH	10M NaOH	1M HCL	Adjusted pH	Applied pH	Na ion Added
			--- mL ---				--- g ---
EDTA- 150	Loam	5.40	0.00	2.00	5.07	5.32	0.148
EDTA-150	Sand	5.50	0.00	1.00	5.04	5.34	0.053
HEEDTA-150	Loam	2.44	2.00	0.00	4.98	5.20	0.134
HEEDTA-150	Sand	2.43	0.90	0.00	4.96	5.13	0.046
EDTA-120	Loam	5.51	0.00	2.40	5.03	5.35	0.119
EDTA-120	Sand	5.62	0.00	1.20	4.96	5.38	0.042
HEEDTA-120	Loam	2.46	1.60	0.00	5.08	5.30	0.107
HEEDTA-120	Sand	2.51	0.70	0.00	5.00	5.20	0.035
EDTA-90	Loam	5.65	0.00	2.60	5.03	5.44	0.089
EDTA-90	Sand	5.83	0.00	1.40	4.99	5.40	0.031
HEEDTA-90	Loam	2.54	1.10	0.00	5.05	5.33	0.073
HEEDTA-90	Sand	2.61	0.50	0.00	5.07	5.30	0.025
Citric Acid-90	Loam	2.35	4.50	0.00	5.02	5.24	0.302
Citric Acid-90	Sand	2.45	1.90	0.00	4.98	5.12	0.097
Gluconic-90	Loam	4.00	1.45	0.00	5.54	5.54	0.097
Gluconic-90	Sand	3.20	0.70	0.00	5.66	5.66	0.035
EDTA-60	Loam	5.89	0.00	3.00	5.04	5.54	0.059
EDTA-60	Sand	6.02	0.00	1.50	4.99	5.54	0.021
HEEDTA-60	Loam	3.31	0.60	0.00	5.04	5.40	0.040
HEEDTA-60	Sand	2.82	0.30	0.00	5.18	5.51	0.015
EDTA-30	Loam	6.36	0.00	3.50	5.07	5.66	0.029
EDTA-30	Sand	6.47	0.00	1.70	4.84	5.72	0.010
HEEDTA-30	Loam	4.09	0.10	0.00	5.05	5.57	0.006
HEEDTA-30	Sand	4.20	0.05	0.00	4.93	5.52	0.002

Control- P	Loam	8.04	0.00	0.00	8.04	8.04	0.000
Control- P	Sand	7.98	0.00	0.00	7.98	7.98	0.000
EDTA-90 NoP	Loam	4.03	0.10	0.00	5.29	5.35	0.066
EDTA-90 NoP	Sand	4.32	0.05	0.00	5.33	5.34	0.023
HEEDTA-90 NoP	Loam	2.12	1.00	0.00	5.13	5.18	0.067
HEEDTA-90 NoP	Sand	2.21	0.45	0.00	5.05	5.03	0.023

Appendix E. Mehlich-1 routine soil test for both soils following the soil incubation in Chapter 2.

Cup	Soil			P	K	Ca	Mg	Zn	Mn	Cu	Fe	B	Al
Nu	Type	Treatment	Rep	----- mg kg ⁻¹ -----									
Blank				0.36	2.435	36.52	7.545	0.14	0.35	0.19	0.65	0.02	1.31
1	Loam	Control No P	1	8.2	204	1781	503.6	2.35	39.9	0.32	3.45	0.95	218
2	Loam	Control No P	2	7.4	210	1842	523.6	2.46	39.9	0.31	3.34	1.00	218
3	Loam	Control No P	3	8.1	212	1838	523.4	2.62	41.5	0.34	3.26	0.98	222
4	Loam	Control-P	1	144.8	224	1832	548.2	2.63	55.6	0.40	5.78	0.65	268
5	Loam	Control-P	2	151.2	226	1811	577.2	2.79	55.6	0.41	5.85	0.65	279
6	Loam	Control-P	3	153.8	224	1843	540.4	2.60	60.2	0.42	7.76	0.60	274
7	Loam	EDTA No P- 90	1	17.1	218	1816	539.7	4.07	391.1	3.25	215.0	0.88	233
8	Loam	EDTA No P- 90	2	11.1	216	1778	519.1	3.83	378.6	3.18	218.9	0.90	228
9	Loam	EDTA No P- 90	3	11.2	215	1779	534.8	3.77	393.9	3.33	225.4	0.93	238
10	Loam	HEEDTA No P- 90	1	12.0	213	1783	520.8	3.46	449.9	2.77	253.7	0.89	224
11	Loam	HEEDTA No P- 90	2	12.9	216	1839	519.5	3.74	491.1	2.85	262.3	0.93	225
12	Loam	HEEDTA No P- 90	3	13.3	220	1859	534.1	4.03	499.8	3.01	270.6	0.93	235
13	Loam	EDTA- 30	1	165.8	229	1834	542.1	4.20	440.1	2.68	277.7	0.71	285
14	Loam	EDTA- 30	2	181.2	232	1832	553.0	4.31	427.1	2.74	275.8	0.74	297
15	Loam	EDTA- 30	3	180.5	240	1892	564.5	4.39	444.0	2.88	292.4	0.75	286
16	Loam	HEEDTA- 30	1	185.1	240	1854	555.3	4.94	457.9	2.56	285.7	0.76	287
17	Loam	HEEDTA- 30	2	188.4	238	1847	537.8	4.15	460.8	2.44	281.6	0.70	286
18	Loam	HEEDTA- 30	3	191.0	236	1873	548.2	3.98	465.6	2.52	291.3	0.73	291
19	Loam	EDTA- 60	1	184.6	235	1865	555.0	4.49	473.1	3.39	317.3	0.81	317
20	Loam	EDTA- 60	2	180.5	233	1851	551.1	4.53	484.7	3.38	326.6	0.77	310
21	Loam	EDTA- 60	3	183.9	230	1834	530.0	4.58	463.8	3.34	312.2	0.80	316
22	Loam	HEEDTA- 60	1	204.5	242	1805	527.0	4.17	501.9	2.93	320.1	0.82	296
23	Loam	HEEDTA- 60	2	202.7	236	1777	516.3	4.10	508.3	2.91	320.5	0.86	299
24	Loam	HEEDTA- 60	3	211.2	243	1840	527.2	4.47	507.4	3.04	332.9	0.91	303

26	Loam	EDTA- 90	1	179.8	230	1747	515.0	4.77	474.7	3.45	345.9	0.82	305
27	Loam	EDTA- 90	2	178.2	230	1827	518.8	4.60	488.8	3.47	353.9	0.88	309
28	Loam	EDTA- 90	3	182.8	242	1833	533.6	4.63	503.0	3.44	362.2	0.86	328
29	Loam	HEEDTA- 90	1	219.2	241	1783	500.9	4.54	540.7	3.12	379.3	0.90	334
30	Loam	HEEDTA- 90	2	220.0	242	1810	502.5	4.35	565.4	3.24	376.8	0.99	331
31	Loam	HEEDTA- 90	3	225.3	247	1766	512.7	4.58	574.2	3.25	386.2	0.94	343
32	Loam	EDTA- 120	1	178.5	241	1829	530.2	5.19	555.8	3.74	438.2	0.83	354
33	Loam	EDTA- 120	2	180.4	241	1817	543.2	4.67	525.7	3.50	420.3	0.86	362
34	Loam	EDTA- 120	3	182.1	235	1814	528.7	5.02	535.4	3.56	439.1	0.84	360
35	Loam	HEEDTA- 120	1	238.2	246	1736	512.5	4.70	592.1	3.29	442.1	0.95	389
36	Loam	HEEDTA- 120	2	233.6	237	1728	491.2	4.39	568.8	3.08	419.9	0.97	375
37	Loam	HEEDTA- 120	3	233.5	240	1755	509.9	4.52	595.4	3.20	435.3	0.98	369
38	Loam	EDTA- 150	1	182.9	236	1735	527.7	4.92	549.3	3.55	508.2	0.77	404
39	Loam	EDTA- 150	2	187.8	235	1729	526.1	4.93	564.4	3.64	504.9	0.78	411
40	Loam	EDTA- 150	3	179.9	236	1784	529.3	5.15	590.9	3.69	527.1	0.82	414
41	Loam	HEEDTA- 150	1	238.0	242	1793	529.1	4.33	650.1	3.08	511.5	0.92	412
42	Loam	HEEDTA- 150	2	215.5	236	1796	510.8	4.42	622.7	3.34	433.7	0.88	372
43	Loam	HEEDTA- 150	3	242.2	240	1783	523.1	4.50	644.3	3.23	515.5	0.94	424
44	Loam	Citric Acid- 90	1	157.8	194	1534	363.4	2.54	272.8	0.43	41.59	1.11	245
45	Loam	Citric Acid- 90	2	148.9	188	1502	350.1	2.36	246.4	0.24	8.81	1.05	233
46	Loam	Citric Acid- 90	3	151.3	186	1450	336.1	2.42	242.3	0.22	5.24	1.07	226
47	Loam	Gluconic Acid- 90	1	149.2	209	1741	474.3	2.92	73.6	0.29	4.57	0.97	243
48	Loam	Gluconic Acid- 90	2	148.0	209	1963	501.3	2.81	65.2	0.28	4.45	1.01	246
49	Loam	Gluconic Acid- 90	3	146.3	210	1767	494.5	2.76	61.9	0.29	4.67	0.96	244
51	Sand	Control No P	1	84.8	81	312	102.8	8.55	4.7	0.69	17.4	0.11	168
52	Sand	Control No P	2	90.3	85	309	106.6	8.82	4.4	0.69	18.7	0.14	180
53	Sand	Control No P	3	87.7	85	293	103.9	8.58	4.5	0.68	18.8	0.12	181
54	Sand	Control-P	1	217.6	89	316	110.5	9.93	10.2	0.67	22.2	0.05	228
55	Sand	Control-P	2	231.7	91	326	114.4	9.87	10.3	0.65	21.3	0.01	224

56	Sand	Control-P	3	220.9	85	292	102.3	9.17	9.6	0.65	21.9	0.06	216
57	Sand	EDTA No P- 90	1	121.1	95	355	110.8	12.48	20.3	1.67	138.2	0.11	255
58	Sand	EDTA No P- 90	2	117.6	95	315	105.7	11.77	21.9	1.72	137.7	0.10	246
59	Sand	EDTA No P- 90	3	113.2	94	297	103.7	10.76	17.7	1.53	127.3	0.11	243
60	Sand	HEEDTA No P- 90	1	122.5	97	312	108.3	12.17	21.1	1.68	151.4	0.15	240
61	Sand	HEEDTA No P- 90	2	126.0	100	390	120.6	14.39	23.5	1.85	165.1	0.14	245
62	Sand	HEEDTA No P- 90	3	123.7	96	334	111.1	12.81	21.3	1.76	152.1	0.10	234
63	Sand	EDTA- 30	1	272.0	99	329	112.7	12.88	18.2	1.68	126.9	0.11	249
64	Sand	EDTA- 30	2	278.0	99	325	112.2	12.98	18.1	1.70	124.4	0.12	247
65	Sand	EDTA- 30	3	278.6	100	325	111.7	12.89	17.9	1.66	126.1	0.12	254
66	Sand	HEEDTA- 30	1	282.3	100	313	109.4	12.34	19.4	1.47	125.6	0.15	246
67	Sand	HEEDTA- 30	2	278.1	97	316	108.8	12.03	18.7	1.46	120.3	0.12	236
68	Sand	HEEDTA- 30	3	283.0	102	325	114.6	13.18	19.9	1.60	130.3	0.16	241
69	Sand	EDTA- 60	1	295.2	99	325	110.1	12.28	19.7	1.94	148.7	0.10	282
70	Sand	EDTA- 60	2	299.4	103	331	114.0	13.43	20.5	2.27	156.1	0.14	294
71	Sand	EDTA- 60	3	301.4	100	329	111.8	12.74	19.7	1.88	152.9	0.13	291
72	Sand	HEEDTA- 60	1	317.7	105	337	116.6	14.31	23.3	1.84	171.6	0.12	289
73	Sand	HEEDTA- 60	2	313.2	105	363	126.3	14.11	22.5	1.86	166.9	0.15	278
74	Sand	HEEDTA- 60	3	317.5	104	355	118.5	13.59	22.0	1.72	160.8	0.13	283
76	Sand	EDTA- 90	1	308.8	98	324	109.5	12.64	20.5	1.82	181.9	0.09	323
77	Sand	EDTA- 90	2	323.5	101	343	115.0	13.37	23.6	1.91	192.1	0.13	338
78	Sand	EDTA- 90	3	300.8	96	315	104.4	11.50	19.2	1.64	168.9	0.10	311
79	Sand	HEEDTA- 90	1	330.2	102	347	112.8	13.20	27.1	1.79	203.5	0.10	322
80	Sand	HEEDTA- 90	2	344.3	106	325	114.7	13.54	23.9	1.88	216.4	0.12	341
81	Sand	HEEDTA- 90	3	318.2	100	339	112.9	12.46	21.0	1.69	191.8	0.11	309
82	Sand	EDTA- 120	1	315.2	99	325	109.5	12.26	20.3	1.80	203.1	0.12	348
83	Sand	EDTA- 120	2	344.4	102	350	116.2	13.52	21.9	2.02	222.1	0.14	380
84	Sand	EDTA- 120	3	307.7	98	325	108.4	11.39	18.5	1.66	190.6	0.07	338
85	Sand	HEEDTA- 120	1	351.0	102	341	114.0	12.88	21.6	2.18	229.9	0.15	356

86	Sand	HEEDTA- 120	2	342.7	103	335	117.2	12.85	21.8	2.08	234.4	0.11	358
87	Sand	HEEDTA- 120	3	341.5	101	326	111.9	12.68	21.9	1.79	225.6	0.12	347
88	Sand	EDTA- 150	1	356.1	100	359	112.8	12.60	20.8	1.85	258.1	0.14	414
89	Sand	EDTA- 150	2	341.4	99	326	109.8	12.03	19.3	1.79	243.8	0.11	400
90	Sand	EDTA- 150	3	334.0	97	323	106.7	11.86	19.5	1.75	239.1	0.09	391
91	Sand	HEEDTA- 150	1	417.3	105	334	114.1	13.58	26.3	1.88	277.8	0.14	456
92	Sand	HEEDTA- 150	2	355.0	97	307	103.7	11.50	19.3	1.62	257.0	0.11	380
93	Sand	HEEDTA- 150	3	361.6	97	305	105.7	11.89	19.5	1.64	264.0	0.11	382
94	Sand	Citric Acid- 90	1	305.2	88	301	94.4	9.21	14.5	0.62	36.5	0.20	287
95	Sand	Citric Acid- 90	2	298.4	87	291	91.1	8.75	14.7	0.58	25.3	0.19	277
96	Sand	Citric Acid- 90	3	312.9	88	312	96.6	9.08	14.4	0.56	24.8	0.20	285
97	Sand	Gluconic Acid- 90	1	259.6	90	315	106.4	9.74	7.5	0.62	22.2	0.09	239
98	Sand	Gluconic Acid- 90	2	258.6	90	308	105.2	9.80	7.0	0.64	22.0	0.10	237
99	Sand	Gluconic Acid- 90	3	263.2	93	325	110.3	10.19	7.1	0.65	22.5	0.13	241