The Distribution of Phosphorus Pools in Alabama Soil Regions and their Variation with Soil Depth

by

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Abstract

The poultry industry in Alabama is the state's second-largest agriculture industry generating approximately 1.25 million tons of poultry litter (PL) annually and containing 19,000 tons of phosphorus (P). The PL has a typical fertilizer grade of 3-3-2 and is applied locally to the agricultural farmlands as a source of nutrient for plants. Long-term use of PL on agricultural lands causes accumulation of P, leading to elevated soil test P levels. The excess amount of P accumulated in soil is termed as legacy P. This legacy P makes its way to the water bodies via surface runoff, leaching and erosion, and therefore is an environmental concern. Strategies for the mitigation of P pollution requires a fundamental understanding of the P dynamics and distribution of P pools in soil profile. Limited studies have documented the distribution of P pools in weathered Alabama soil regions namely Appalachian Plateau (AP), Coastal Plains (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackbelt prairie (BP). The objectives of this study were 1) to quantify the distribution of P pools within 0 to 45 cm soil depth in five Alabama soil regions and, 2) to determine how P fractions change with change in soil test P fertility rating. Soil samples were collected from Alabama farmlands from four depths: 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm. The modified Hedley fractionation procedure, which included extraction with deionized water, 0.5 M $NaHCO_3$, 0.1 N NaOH, and 1 M HCl and residue P, was used to characterize P into H_2O-P_t, $NaHCO_3$-Pt, NaOH-P_t, HCl-P_t, and residue-P_t fractions. The soil test phosphorus (STP) was determined using Mehlich-1 (M1) extraction for non-calcareous soils (AP, CP, LV, PP) and Lancaster (La) extraction for calcareous soils (BP). The samples were categorized into six Alabama STP ratings namely extremely high (EH), very high (VH), high (H), medium (M), low (L), and very low (VL) based on STP concentrations. The results from this study indicated that P fractions were highly stratified across depths in all five soil regions. The H_2O-P_t and HCl-P_t pool were found to be the least dominant P pools and NaOH-P_t and residue-P_t were the most dominant P pools in non-calcareous soil regions. In case of calcareous soils, HCl-P_t and residue-P_t were found to be the two dominant pools in the whole soil profile. In addition, with the transition of P fertility rating from VL to EH, the proportion of residue-P_t pool decreased significantly with the corresponding increase in labile P fractions (e.g., H_2O-P_t, $NaHCO_3$-P_t). Greater amounts of P in labile and moderately labile P pool (e.g., NaOH-P_t, HCl-P_t) in EH, VH, and H soils pose environmental concern. Best management practices should be targeted to manage EH, VH and H soils.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

AP- Appalachian Plateau

BP- Blackbelt Prairie

CP- Coastal Plains

La- Lancaster

LV- Limestone Valley

M1- Mehlich 1

P- Phosphorus

PP- Piedmont Plateau

PL- Poultry litter

STP- Soil test phosphorus

EH- Extremely high

VH- Very high

H- High

M- Medium

VL- Very low

L- Low

H_2O-P_t- water soluble P

$NaHCO_3$-P_t- $NaHCO_3$ extractable P

NaOH-P_t- NaOH extractable P

HCl-P_t- HCl extractable P

Residue-P_t- residue P

CHAPTER-1

Literature Review

1.1 Poultry Industry in the world and Alabama

The poultry industry is shifting towards more intensive and concentrated production during the past few decades to meet the ever-increasing demand of meat and eggs. As per the reports by FAO (2005), the growth of poultry sector is projected to double by 2050 compared to the other meat industries. The share of poultry production in the world meat production has increased from 15% to 30% in the last three decades (FAO, 2006a). Growth and development of poultry sector is accompanied by the emergence of “land-independent” farming establishments with more intensive and concentrated poultry operations (Gerber et al., 2007). This growth has resulted in a separation between animal and crop production, leading to limited access to land for manure applications.

The United States of America (USA) has the largest broiler industry in the world with the production of 20.5 million metric tons of broiler meat in 2020 (Shahbandeh, 2021). The poultry industry is flourishing with 8.9 billion birds marketed annually (USDA-NASS, 2019) and at the same time, generating around 13 million tons of poultry litter (PL) annually (approximately 1.5 kg litter is produced by broiler bird as reported by Mitchell and Tu, 2005). Georgia, Alabama, Arkansas, Mississippi, and North Carolina are the major poultry producing states in the USA.

Poultry production began in Alabama in the seventeenth and eighteenth centuries with the settlement of Europeans and European Americans and has now become a major agricultural business in the state (Conner, 2008). From a small-scale, domestic operation, the poultry industry has now evolved into a more intensive commercial farm enterprise for eggs and low cholesterol poultry meat production. In 2018, Alabama was ranked second in the USA in broiler production, processing around 1.1 billion birds annually and generating around 1.68 million tons of PL (USDA-NASS, 2019). This litter contains approximately 19,000 tons of phosphorus (P) (Chakraborty et al., 2021). Appropriate disposal of PL is a concern associated with concentrated poultry production as most of the litter produced is applied locally to agricultural fields and is not transported elsewhere due to its low bulk density which is around 500 kg m^{-3} as reported by Bernhart and Fasina (2009). Poultry litter was initially viewed as a waste product, but relatively high nutrient content of PL [3-3-2 (N-P_2O_5-K_2O)] makes it a good and an inexpensive source of nutrients for plants (Mitchell and Donald, 1995; Watts et al., 2019). Although application of PL to agricultural land seems to be a plausible management strategy, repeated application of poultry litter leads to the accumulation of P in farmlands in intensive poultry production areas. The excess P accumulated in farmlands is termed as legacy P. This legacy P in soils is lost through surface runoff or erosion and is transported to the water bodies. The enhanced P levels in water systems stimulate algal blooms accelerating eutrophication, which further leads to major ecological changes. These P enriched soils serve as a potential environmental risk to water quality problems.

1.2 Effect of PL application to soil

Poultry litter is a mixture of feathers, spilled feed, feces, and bedding materials (Chakraborty et al., 2021). The majority of PL generated is applied to agricultural lands (Bolan et al., 2010) as soil amendment and nutrient source. Land application of PL is an effective and environmentally friendly way to dispose large amount of litter produced. It is well documented that PL contains all essential nutrients as well as micronutrients for plants (Harmel et al., 2009; Chan et al., 2008). It serves as an inexpensive source of nutrients for plants and improves agricultural productivity and soil quality (He et al., 2012). Soil properties such as pH, water holding capacity, tilth, nutrient availability, cation exchange capacity and organic matter content are favorably altered by addition of PL (Bolan et al., 2010). Continuous cultivation of agricultural land results in the deterioration of soil structure which negatively impacts crop yields. Addition of PL improves soil structure by promoting aggregate stability as well as improving oxygen diffusion rate of the soil (Adeli et al., 2009). Poultry litter is reported to increase hay yield however, the yield increase was related to the number of years of PL application (He at al. 2008). Therefore, the residual effect of litter application can last for several years affecting crop production and soil conditions (Eghball et al., 2004). Phosphorus levels were impacted by PL application in two ways: number of years of PL application impacted the concentration of labile inorganic P, whereas the stable P concentrations were associated more with the cumulative quantity of PL applied (He et al., 2005).

Addition of PL by farmers is typically made to meet the N requirement of the crops (Reddy et al., 2009). Due to the disproportionate ratio of N and P in PL corresponding to the crop needs, PL applications based on N requirement of the crop leads to over-application of P to the soil (Szogi et al., 2015). This results in the build-up of P in soil over time that poses serious environmental concerns. Kingery et al. (1994) conducted an experiment in the Sand Mountain Region of Alabama, USA to discern the effect of long-term application of PL on soil and the environment and documented that the extended application of PL for years altered soil conditions and resulted in potential environmental problems. Alabama is home to five major soil regions namely Limestone Valleys and Uplands (LV), Piedmont Plateau (PP), Coastal Plain (CP), Appalachian Plateau (AP), and Blackland Prairie (BP) on which row crop and pasture production occurs. Each soil region has distinct mineralogy, chemical and physical properties. It is important to understand how P distribution and dynamic changes due to PL applications on five Alabama soil regions. The main characteristics of these soil regions are described below.

1.3 Soils of Alabama

The formation of soil is a result of an intricate weathering process involving the combined action of parent material, topography, time, biology, and climate (Jenny, 1994; Sikora and Moore, 2014). The development of soils in the Southeastern part of the USA witnessed generous amount of rainfall (more than 60 inches annually) historically. The abundant rainfall resulted in the leaching of base cations such as sodium, magnesium, calcium, and potassium leading to the acidic nature of the soils (Sikora and Moore, 2014). In addition to the rainfall, subtropical climate has resulted in a soil temperature and moisture that favors expedited mineralization of organic matter. Rapid mineralization of organic matter has caused the soils of southeastern USA to be low in organic matter content as compared to the soils in the Northern part of the USA (Sikora and Moore, 2014).

Each Alabama soil region contains soils originated from similar parent material or geological material (ACES, 2018). Within each soil region, several major series exist where each series has different properties such as color, depth to bedrock, texture etc., (Mitchell, 2008). These soil series are named based on the geographical location where similar soil was first located.

1.3.1 Appalachian Plateau

The Appalachian plateau embraces the Chandler, Lookout, Cumberland, Cuntei Brindlee, Sand and other smaller mountains. The soils of this region are derived from sandstone, shale, and limestone (ACES, 2018). The Sand Mountain located in northeastern Alabama is reported to be the largest sandstone plateau (ACES, 2018). The soil orders observed in this region are Ultisols and Inceptisols (Picconi and Swaby, 2016). These soils comprise of fine sandy loam surface layers with loamy subsoils. The majority of the slopes are less than 10 per cent and most of the regions in more rugged areas are too steeply sloping for agricultural purposes. The elevation varies from 300 to 700 feet in rugged areas of the Appalachian plateau and about 1300 feet in more level areas (Mitchell, 2008). Potatoes, tomatoes, corn and soybeans are the major crops grown in these shallow, well-drained soils and poultry is very crucial and dominant in this region (Mitchell, 2008). However, Appalachian plateau soils are acidic in nature and have low nutrient content. Additionally, these soils are reported to be environmentally sensitive soils as they are well drained soils and nutrients leach down into groundwater due to high amount of sand (ACES, 2018).

1.3.2 Coastal Plain

The Coastal Plain soil region, circumscribing most of the crops and the resulting agricultural management, is the largest physiographic region in the Southeastern USA (Sikora and Moore, 2014) that covers around southern two-thirds of Alabama (ACES, 2018). The soils in the Coastal Plain region originated from marine and fluvial sediments washed away from the Piedmont and the Appalachian plateau (Mitchell, 2008). This soil region is divided into upper and lower Coastal plains to identify the areas of different ages (ACES, 2018). The upper coastal plains have loam or sandy loam surface layers with clayey or loamy subsoils. The elevation varies from 200 to 1000 feet with level to very steep topography. Broad terraces and narrow ridgetops are cultivated, however, most of the area is under forest (Mitchell, 2008). The lower coastal plains have loamy subsoil with loamy sand or sandy loam surface layers on top. The elevations in this region of coastal plains range sea level to 500 feet with most of the slopes being less than 10 percent (Mitchell, 2008). Horticultural crops along with peanuts, soybeans and corn are grown in lower coastal plains and timber products are considered crucial here (Mitchell, 2008).

The dominant soil orders in this soil region are Ultisols, Inceptisols, and Entisols (Picconi and Swaby, 2016). The soils in the Coastal Plain region are highly weathered, acidic (soil pH < 6.0) and low in plant nutrients. These weekly buffered soils with low CEC require appropriate fertilizer, lime, and water management to ensure optimum crop yields (Sikora and Moore, 2014).

1.3.3 Limestone Valleys and Uplands

These soils originated from the weathering of limestones (carbonates of calcium and magnesium). The soils in this area are still forming and the weathering limestone, known as Karst, lead to the rolling topography which is conspicuously noticeable. Red, clayey soils of Tennessee and Coosa River valleys weathered from pure limestone and possess silt loam surface textures (Mitchell, 2008). Undulating topography with upland flats, narrow valleys and rolling hills are reported in this area (Sikora and Moore, 2014) with elevation of about 600 feet (Mitchell, 2008). Soils of upland developed from cherty limestone with gravelly silt loam surface layers and gravelly loam and gravelly clay subsoils (Mitchell, 2008). Topography in uplands range from level to steep (Sikora and Moore, 2014) with elevation up to 700 feet (Mitchell, 2008). Limestone Valleys and Uplands of Alabama represent some of the most productive agricultural lands in the state with deep and well drained soils cropped to cotton and soybeans. Ultisols, Inceptisols and Alfisols are the major soil orders found in this soil region (Sikora and Moore, 2014). The soil in Limestone Valley is less acidic than the sandstone derived upland soils and also, contain more nutrients (ACES, 2018).

1.3.4 Piedmont Plateau

Piedmont derived its name from two words “pied” means foot and “mont” means mountains, collectively as foot of the mountain (ACES, 2018). This soil region lies between Limestone valleys and Coastal plains and extends into Maryland and Pennsylvania from East Central Alabama. Piedmont plateau soils are old, highly weathered, and mainly originated from quartz, gneiss, granite, schist, and mica parent materials (ACES, 2018). The surface layers in the piedmont region are sandy loam or clay loam with red clayey subsoils. The higher amount of clay and silt in the surface layers tend to provide high cation exchange capacity (4.6 to 9.0 cmolc kg^{-1} of soil) to these soils. The topography is rolling to steep, and the elevation varies from 700 to 1000 feet. However, Talladega hills which is the highest point in Alabama (with elevation from 900 to 2407 feet) lies in this soil region (Mitchell, 2008). Cotton, tobacco, corn, and small grain crops are grown on these well drained fertile soils and the rest of the uncultivated land is used for pasture (Sikora and Moore, 2014). The soil orders found in this soil region are Inceptisols, Ultisols, Entisols, and Alfisols (Picconi and Swaby, 2016). These soils have low acidity with the dominance of low activity clays. The clays are mostly coated with iron and aluminum oxides and hydroxides imparting red orange color to the soils of Piedmont region. Additionally, these have high P fixing capacity as compared to the coastal plain soil region (Sikora and Moore, 2014).

1.3.5 Blackland Prairie

The Blackland prairie soil region extends through central Alabama into northeastern Mississippi. The name ‘Blackland prairie’ is because of the dark colored surface layers as well as prairie like vegetation in this region (ACES, 2018). The presence of humus or decomposed organic matter, coating the clay particles, imparts dark color to these soils (ACES, 2018). The soils in this region originated from acid marine clays or alkaline, Selma chalk (Mitchell, 2008). Acid and alkaline soils are intermingled in this region. These soils are clayey throughout with dark colored surface layers and red or yellowish sub-surface layers. Elevation is around 200 feet with level to undulating topography (Mitchell, 2008). This soil region has Vertisols as the dominant soil order (Picconi and Swaby, 2016). These soils are well known to possess swell shrink properties due to the dominance of smectitic clays. Additionally, due to the presence of clays, these soils are poorly drained with poor internal drainage, restricting the water movement in soil (ACES, 2018). This is the factor that makes these soils suitable for pond construction and making aquaculture as a prominent business in this region (ACES, 2018). Soybean is a dominant crop grown in this soil region with most of the soil being used for pasture and timber production (Mitchell, 2008).

1.4 Phosphorus as an element

The element phosphorus (P) derived its name from the Greek words "phôs", which means light and "phoros", which means bearer, because of its phosphorescence property (Huminicki and Hawthorne, 2002). Being a nonmetallic element with nuclear number 15 and atomic mass of 30.974 (Meija et al., 2016), P is colorless at room temperature and glows in the dark. The concentration of P in the world's landmass is about one gram for each kilogram (Ikhajiagbe et al., 2020), making P as the 12th most abundant element in the earth's crust (Britannica, 2021). Although P is widely distributed, it does not exist in free state due to its high chemical reactivity. Phosphorus is stable in pentavalent state and its compounds exist as a derivative of phosphate (PO_4^{3-}) ion, which is a tetrahedral anion (surrounded by four oxygen atoms) with high negative charge density (Tiessen, 2008). Consequently, the majority of P is dispersed in minerals in the form of phosphate (Meija et al., 2016), which is a finite, non-renewable resource. Phosphate tends to form chains and rings with P-O-P bonds and approximately 150 P minerals were reported by Cathcart (1980). However, 550 minerals (Britannica, 2021) have been recently reported to contain P, with apatite series being the principal source. Apart from phosphate ions, apatites contain calcium (Ca) ions along with variable quantities of chloride, hydroxide, or fluoride ions, with formula [$Ca_{10}(PO_4)_6$ (F, Cl, or OH)$_2$]. Other phosphate minerals include variscite ($AlPO_4 \cdot 2H_2O$), strengite ($FePO_4 \cdot 2H_2O$), vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$), monocalcium phosphate ($Ca(H_2PO_4)_2 \cdot H_2O$), brushite (dicalcium phosphate dihydrate) ($CaHPO_4 \cdot 2H_2O$), monetite (dicalcium phosphate) ($CaHPO_4$), octacalcium phosphate ($Ca_4H(PO_4)_3 \cdot 2.5H_2O$), β-tricalcium phosphate ($\beta\text{-}Ca_3(PO_4)_2(c)$) (Veith and Sposito, 1977; Lindsay, 1979; Lindsay et al., 1989). The class of phosphate minerals is large and very diverse, however, only a few minerals are relatively common.

1.5 Phosphorus as a plant nutrient

Phosphorous (P) is an important macronutrient for plants after nitrogen (Kumar et al., 2018), required for growth and development of plants. The concentration of P in plants is reported to be 0.05% to 0.5% of total plant dry weight (Malhotra et al., 2018). Phosphorus nutrition is associated with vitality needed for cell metabolism, root and seed development, crop quality, adequate yields, and straw quality in cereals (Ikhajiagbe et al., 2020), stalk and stem strength, seed and flower formation, resistance to plant diseases and N-fixation in legumes (Khan et al., 2009). Malhotra et al. (2018) also reported the positive association of P with N fixing capacity of leguminous crops. Phosphorus presence is important during root formation stage (Abel et al., 2022) and plays a significant role in root system development, both fibrous and lateral roots (Brady, 1984). Being a part of numerous cell constituents such as ATP, DNA, RNA, and phospholipids, P plays a vital role in cell division, respiration, photosynthesis, and cell broadening (Ikhajiagbe et al., 2020). Therefore, enough P must be present in soil to support growth and development of plants and prevent hindered development and diminished yields (Khan et al., 2009). However, soil solution P varies between 10^{-4} M for very high to 10^{-6} M for very low P soil and can be as low as 10^{-8} M in case of poor tropical soils (Johnston et al., 2014), whereas crops require 0.3-0.5 kg P ha^{-1} daily during rapid growth phase. Consequently, plants can get adequate supply of P only if the amount of P and rate of P availability in soil is sufficient to meet the crop needs.

Gichangi et al., (2009) and Brady and Weil (2008) reported that the adsorption and precipitation reactions of P with calcium (Ca), iron (Fe), and aluminum (Al) renders 85-90% of applied inorganic P unavailable for plant uptake. This leads to the deficiency of P in plants, resulting in stunted growth with delayed plant maturity, purple pigmentation on leaves, reduced leaf area, and upward tilting of leaves. However, these deficiency symptoms vary with the crop growth stage as well as the crop species (Peaslee, 1977). Phosphorus is needed by plants in available inorganic forms ($H_2PO_4^-$, HPO_4^{2-}). These available P forms also act as a potential water pollutant leading to eutrophication in water bodies. For that reason, it is important to determine the P concentration in soil and amount of P available in different forms to account for the crop P requirements as well as to address the environment P loss risk assessment.

1.6 Forms of phosphorus in soil

Soil total P content varies between 200–5000 ppm (an average of 600 ppm) in soils (Lindsay, 1979). Phosphorus in soil exists in both inorganic P (P_i) and organic P (P_o) forms. Inorganic forms of P are accessible to plants; however, organic P forms are inert and available to plants after mineralization. Inorganic P is reported to be 35%-70% of the total P in soil whereas 30 %-65% of total P is present as P_o (Prasad and Chakraborty, 2019). Inorganic P (P_i) forms in soil are found primarily as orthophosphates, pyrophosphates, and polyphosphates and exist in soil in bound states such as aluminum-P (Al-P), iron-P (Fe-P) and calcium-P (Ca-P) (Cho and Caldwell, 1959). The relative abundance of inorganic P forms in soil depends on the P fixing constituents (Fe, Al and Ca) present in soil. In acidic soils, Al-P and Fe-P predominate whereas in alkaline soils, Ca-P form is predominant. Phosphorus bound to Ca is stable at alkaline pH and soluble at low soil pH conditions. At neutral soil pH, all three P_i forms are equally distributed with some instances reported where Al-P and Fe-P is slightly higher than Ca-P (Chang and Jackson, 1958).

Organic P in soil marks its origin to dead plants, animal, and microbial tissues in the soil. Organic P forms include phosphonates, orthophosphate monoesters and diesters, phospholipids, and nucleic acids (Arai and Sparks, 2007; Recena et al., 2018; Stevenson, 1982). The orthophosphate monoesters possess the tendency to precipitate or adsorb on Al or Fe oxides, due to which they are relatively unavailable for enzymatic hydrolysis in soil (Recena et al., 2018). In contrast, orthophosphate diesters are fairly stable in soil (Turner and Blackwell, 2013). Inositol hexaphosphates are highly stable and contributes 50% to the total P_o in soil whereas a very little amount (0.5–7%) of phospholipids make up the total P_o (Dalai, 1977). The smallest fraction of the total P_o (less than 3%) is comprised of nucleic acids which originate from the dissolution of animal, microbial and plant remains. Microorganisms present in soil play a vital role in converting immobile P forms to mobile P forms.

1.7 Phosphorus dynamics in soil

Phosphorus dynamics in soil is very complex involving plants, soil, and microorganisms. Soils contain organic, inorganic, and microbial P indicating that P dynamics in soil are controlled by integration of biological and chemical processes and properties (Reddy et al., 2005). Other factors influencing P dynamics in soil are pH, biological activity in soil, redox potential, and ionic strength and anthropogenic activities including application of manures and fertilizers, and use of soil amendments (lime, biosolids etc.) (Pierzynski et al., 2005). Changes in P dynamics with manure application were reported by Sharpley and Kleinman (2003); Qian and Schoenau (2000); Allen and Mallarino (2008); and Brock et al. (2007). Phosphorus in soil originates from disintegration of primary apatites present in soil (Smeck, 1985). These primary apatites are transformed to secondary minerals and other inorganic forms with pedologic time. Chang and Jackson (1958) reported the use of inorganic soil P distribution to estimate level of chemical weathering in soil and found calcium phosphate, aluminum phosphate, iron phosphate, and occluded phosphate as the sequence of chemical weathering from less to highly weathered soil. Additionally, soluble P released from weathering of primary minerals can be used by plants or lost by runoff and erosion (Smeck, 1985). Secondary minerals release sesquioxides in soil that are continually occluded, and as weathering proceeds, Fe-P and Al-P is occluded by iron oxide coatings formed during weathering process (Smeck, 1985). In the initial stages of chemical weathering, when P inputs are added to the soil, more calcium and aluminum phosphate are formed as compared to iron phosphate. This is because of the high activity of calcium and aluminum ions in soil than iron ions (Chang and Jackson, 1958). Iron phosphate and some portion of aluminum phosphate and aluminum-iron phosphate is further coated by iron oxides converting these forms into occluded P. Similar findings were reported by Senwo et al. in 2003. on phosphorous distribution in five highly weathered Alabama soils. With the passage of time, all the soluble P from secondary minerals and organic pool is converted to occluded P. At this point, occluded P predominates in soil and plants are dependent on mineralization of organic P to meet their P requirements. Various chemical and biochemical processes associated with P cycling play a vital role in determining the amount of available P in soil. These processes are- sorption, desorption, oxidation, reduction, immobilization, mineralization, and dissolution, precipitation (Prasad and Chakraborty, 2019).

Majority of the orthophosphate in soil exists as HPO_4^{-2} or $H_2PO_4^{-1}$ at pH 4 to 10 (Jansson, 1977). Sorption reactions, which include both adsorption and absorption, occur in equilibrium with each other in soil solution. Adsorption reactions lead to the fixing of orthophosphates on the surfaces or edges of clay minerals, hydrous oxides and carbonates with monodentate or bidentate bonds (Pierzynski et al., 2005). Orthophosphates held with bidentate bonds are more strongly attached to the fixing sites than monodentate bonds and hence, are less labile (Pierzynski et al., 2005). Sorption processes are reversible initially, however, solid precipitates formed later transform to insoluble forms which reduces plant available P and P susceptible to runoff losses (Pierzynski et al., 2005). The reverse of sorption process is termed as desorption which describes the movement of P from solid phase to soil solution (Wiklander, 1950). Adsorption system, when becomes saturated with P, can lead to the precipitation of P in the soil. The reaction of ions (Ca^{+2}, Fe^{+3}, Al^{+3}) present in the soil with phosphate ions to form phosphate solids is termed as precipitation (Vymazal, 2007). Precipitation reactions involve a permanent change into metal phosphates and is a slow process. Release of P from metal phosphates occurs slowly and thus, precipitation reactions make P less available to plants. However, during dissolution of phosphate minerals, P is released back to the soil solution making it available for biota present in soil. Sorption, precipitation, and immobilization reactions reduce available P in soil whereas processes such as desorption, mineralization, and dissolution remove P from non-labile pool and make it available for plant uptake. Therefore, external P is added via manure or fertilizer applications to maintain adequate level of available P for plants in soil.

1.8 Availability of P in soil

Plant availability of P in soil increases with external P application. However, the applied P is also converted into insoluble forms in a very short time span making P unavailable for plants. The concentration of P in soil solution at a given time is generally low, reported as <1% of the total P (Pierzynski, 1991). Therefore, it is important to replenish P in soils over time to meet the crop P demand. Bioavailability of P is controlled by chemical, physical, and biological processes in soil. Hence the external P application should be designed in a way that limits the tie-up of P with the soil minerals and improves its bioavailability. Application of P in concentrated bands or injection closer to root system are among such strategies that can potentially improve the bioavailability of P. Addition of manure or organic matter have also been reported to reduce P fixation (Yusran, 2010). Addition of organic matter to soil masks the P fixation sites, and makes P bioavailable (Meason et al., 2009; Ahmed et al., 2019; Guppy et al., 2005). Also, organic anions compete with phosphate anions for anion exchange sites and result in chelation of Fe and Al, preventing P fixation (Scherer and Sharma, 2002; Ranatunga et al., 2013). Mineralization of organic P sources and plant residues further contributes to the plant available P pool in the soil.

Soil pH is another factor affecting the availability of P in soil (Pierzynski et al., 2005; Penn et al., 2019). Maximum bioavailability of P is observed in the pH range of 6-7 (Prasad and Chakraborty, 2019). At both acidic and alkaline pH, more P is fixed in the soil due the dominance of Al, Fe and Ca ions (Prasad and Chakraborty, 2019). Concentration of P in soil solution and P buffering capacity of the soil determines P availability in soil (Johnston et al., 2014). Soils with high P buffer capacity can replenish P at a faster rate as compared to low P buffer capacity soils.

1.9 Phosphorus fractionation

The P fractionation is the sequential extraction of soil with selective solvents of increasing extraction strength to isolate discrete P fractions of different solubility (Pierzynski et al., 2005). The fractionation procedures are operationally defined and based on the differential solubilities of the various P forms in different extractants ranging from acidic to basic pH. Soil P fractionation has been traditionally used to determine the effect of different management practices on P dynamics in soil. Several studies have been conducted to observe the effect of cultivation, manure and fertilizer application, time, aggregation etc. on the movement and transformation of P from one form to another. Kashem et al. (2004) conducted an experiment to observe the changes in P fractions with the addition of biosolids, P fertilizer, hog manure, and cattle manure and reported that water soluble P fraction varied significantly with the addition of organic and inorganic P sources. A similar study was conducted by Malik et al. (2012) to determine the changes in P pools with the addition of organic and inorganic P sources. Alabama soils have a long history of PL application and due to that, it is important to understand the effect of PL application on P fractions in soil. Ranatunga et al. (2013) conducted an experiment to determine the environmental impact of P fractions associated with different sized soil aggregates in poultry litter applied pasture fields of Appalachian Plateau soil region of Alabama. However, more research is needed to evaluate P fractions and address their agronomic and environmental importance in Alabama soil regions.

Fractionation procedure was first given by Chang and Jackson (1957) and later modified by Hedley et al., (1982). Several modifications have been done by various researchers to adapt to their research needs. However, a procedure given by Hedley et al (1982) is the most commonly used worldwide. Fractionation procedure given by Chang and Jackson (1957) includes the sequential extraction of soil with NH_4Cl, NH_4F, NaOH, H_2SO_4, and Na-citrate extract readily labile P, Al-P, Fe-P, Ca-P, and reductant soluble P. Fife (1959, 1962) modified the Chang and Jackson (1957) fractionation scheme and proposed that the use of alkaline NH_4F (pH 8.5) can lead to increased extraction of Al-P in excess of Fe-P (Pierzynski et al., 2005). Later, Williams et al. (1967) changed the Chang and Jackson (1957) fractionation scheme which included changes in shaking period of the NH_4F extraction, increased ionic strength of NaOH solution and addition of another NaOH extraction (Pierzynski et al., 2005). The most fundamental finding from the work done by Williams et al. (1967) was the P fractionation scheme is not applicable for calcareous soils as extraction with NH_4F led to the formation of CaF_2 (Syers et al., 1972; Pierzynski et al., 2005). Formation of CaF_2 results in the strong sorption of P leading to overestimation of some fractions of P and underestimation of others (Pierzynski et al., 2005). Therefore, research done by Williams et al., (1967) led to the development of P fractionation procedure for calcareous soils, which formed the basis of fractionation procedure given by Hieltjes and Lijklema (1980) that is used for evaluating P fractions in sediments (Pierzynski et al., 2005). All these schemes were widely accepted and used but they were not responsive to account for any changes in organic P fractions. Therefore, another P fractionation procedure developed by Hedley et al. (1982) and its modifications are more preferred over the other schemes. Hedley et al. (1982) fractionation includes extraction of P with resin in bicarbonate form, $NaHCO_3$, NaOH, sonication + NaOH, HCl, and residual P by digesting residue with H_2SO_4 and H_2O_2. Hedley et al. (1982) fractionation scheme and its modifications are, therefore, more efficient in the soil-plant system (Condron et al., 1990). The P fractionation schemes have advantages and disadvantages associated with them, but the selection of the P fractionation procedure mostly depends on the objective of the research study.

1.10 Fractions of P in PL

Poultry litter typically contains 3% P_2O_5 and characterizing the fractions of P in PL is important for the development of PL management strategies. Understanding the fractions of P in PL will provide an insight into the potential fate and dynamics of P in soil after long-term application of PL. Some studies inferred the runoff potential of P in PL from the amount of water-soluble P fraction in PL (Kleinman et al., 2007). However, this can potentially inform about the short-term possibility of P runoff, but the long-term impact of PL application to soil is more complicated as other P fractions in PL can potentially be converted to water soluble P fraction over time (He et al., 2012). Addition of chemical amendments (such as aluminum sulfate) to PL results in the conversion of soluble P fraction (H_2O extractable-P) to stable forms of P ($NaHCO_3$ extractable-P and NaOH extractable-P) (Duo et al., 2003). In contrary to this, He et al. (2006b) reported that the addition of chemical amendments to PL shifts stable P species in HCl extractable P fraction to relatively more soluble P forms in $NaHCO_3$-P and NaOH-P fractions. This emphasizes the importance of determining the various P fractions in PL to clearly understand the environmental implications of PL application to soil. Therefore, sequential fractionation procedures are developed and used to determine the fractions of P in PL as reported by Self-Davis and Moore (2000) and Sharpley and Moyer (2000).

In a study conducted by He et al., (2006b), P fractions in 23 PL samples were characterized using sequential fractionation into H_2O, $NaHCO_3$, NaOH and HCl extracted P as well as residual P. It was found that the majority of P in PL is present in HCl extractable P fraction followed by H_2O-P>NaOH-P>$NaHCO_3$-P (Figure 1). In 23 PL samples, the average P distribution pattern observed was 23±7% H_2O extractable-P, 8±4% $NaHCO_3$-extractable-P, 10±4% NaOH extractable-P, 36±9% acid-extractable-P and 24±12% in residues. This finding was in alignment with the results reported in other studies by Sharpley and Moyer (2000) and Duo et al. (2003). In another study, He at al. (2008) reported that the HCl-P fraction in PL was six to seven times higher than the $NaHCO_3$-P fraction and also inferred that the P fractions in PL do not just accumulate in soil but interchange between different P fractions.

1.11 Soil Test Phosphorus

The plant available P in soil is measured by various soil tests for agronomic purposes to help in fertilizer recommendation. Soil testing methods are observed to be inexpensive, widely used, and correlated with bioavailable and soluble P in soil (Sims et al., 2000). Various extractants such as Mehlich-3 (Mehlich,1984), Mehlich-1 (Mehlich,1953), Lancaster (Cox, 2001), Bray and Kurtz P1 (Bray & Kurtz,1945), and Olsen (Olsen et al.,1954) are used to measure plant available P in soil. Different states in the USA adopted different soil test methods for P, based on the differences in chemical properties of the soil to make interpretations for agronomic and environmental issues. Soils in Alabama are categorized as calcareous and non-calcareous soils based on the calcium content (Mitchell and Huluka, 2012). Mehlich-1 is the extractant used for P in non-calcareous soils and Lancaster (La) is used for calcareous soils in Alabama as a standard soil test phosphorus (STP) method for agronomic purposes (Hue and Evans, 1986; Evans and McGuire, 1990; Mitchell and Huluka, 2012). Mehlich-1 extractant is a double acid solution comprising of 0.05 M HCl and 0.0125 M H_2SO_4 (Mehlich, 1953). This extractant is commonly used in the southeastern USA and is suitable for acidic soils possessing pH<6.5, organic matter content <5%, and cation exchange capacity <10 cmol kg^{-1}.

For calcareous BP soils of Alabama, Lancaster extraction is used to analyze plant available P. Lancaster extraction is a two-step process- first, 5g of soil is treated with 0.05 M HCl and second step includes the addition of 20ml of extraction solution and samples are shaken for 10 minutes. The composition of the extraction solution is 1.58 M glacial acetic acid + 0.125 M malonic acid + 0.187 M malic acid + 0.037 M ammonium fluoride + 0.03 M aluminum chloride hexahydrate.

The P concentrations measured by soil tests are used to rate the P fertility level of the soil. Based on STP level, soils are categorized into six fertility ratings as extremely high (EH), very high (VH), high (H), medium (M), low (L), and very low (VL) (Mitchell and Huluka, 2012). These P fertility ratings are used by Auburn University soil testing lab and different soil testing labs use different P fertility ratings for soil testing. The interpretations from soil testing methods for P are aimed to determine the crop response to the added P sources. However, the P in soil serves as a potential threat to the surface water quality which is not accounted in these soil test methods. The soil test methods for P determination should serve both agronomic and environmental purposes. For example, STP is used as an input in Alabama P index. "The P index is a tool to assess the site and management practices for potential risk of phosphorus movement to water bodies as the result of additional P applications" (USDA-NRCS, 2014). The P index is used for the estimation of P loss risk and can help in the better management of resources. The extractants used for routine soil P testing are not efficient in extracting P pools that are important from environmental aspect

(Gartley and Sims, 1994). Therefore, it is important to evaluate P fractions in soil that serve both agronomic and environmental purposes.

1.12 Phosphorus transport mechanisms and association with water quality issues

The enrichment of water bodies with nutrients (N and P) is leading to increased amounts of algal blooms that contribute to eutrophication. Eutrophication leads to the creation of dead zones, turning oligotrophic water system finally to hypertrophic water system. The solution to this problem is to curtail the input of nutrients into the water systems (Carpenter, 2008). Schindler et al. (2008) conducted a study on Canadian lakes for 37 years and observed that P concentration is directly associated with enhanced algal blooms. This finding was previously observed by Schindler (1977) and Sharpley et al. (1994). Phosphorus is added to the biosphere by various anthropogenic activities as well as natural phenomenon. Cause of eutrophication can be sewage, industrial discharge, drainage water from agricultural fields and runoff from construction sites. A major concern associated with agriculture and excessive P in soil is that any factor that promotes erosion will also increase P runoff to water bodies (Daniel et al., 1994). As compared to preindustrial times when global P flux to the biosphere was 10–15 Tg P year^{-1}, it increased to 33–39 Tg P year^{-1} in 2000 (Bennett et al., 2001). This is especially true in case of Alabama state due to concentrated poultry production and application of PL to agricultural lands.

Phosphorus is transported from agricultural fields to water bodies by erosion, runoff or leaching (Haygarth et al., 1998) and can either be dissolved P or particulate P, with particulate P being the major fraction in soil runoff (75-90%) (Schuman et al., 1973; Sharpley et al., 1987). These two forms of P are distinguished based on the filtration through 0.45 µm filter paper (Toor et al., 2005). Dissolved reactive P includes water soluble orthophosphates, easily hydrolysable organic P forms (labile monoesters and diesters), whereas dissolved unreactive P includes primarily organic P compounds and some inorganic P compounds (polyphosphates) that are not detectable by acid molybdate method (Ron Vaz et al., 1993; Toor et al., 2005; Thomson-Bulldis and Karl, 1998). Particulate reactive P comprises sorbed P attached on the surfaces of clay, Ca, Al, Fe oxides and hydroxides as well as that fraction of total P which is not soluble in water (Toor et al., 2005). Relatively less information is available about the compounds present in particulate unreactive P fraction. These P forms present in water bodies are a concern for water quality because they differ in their bioavailability to freshwater algae. Dissolved P is immediately available for uptake by algal biomass in the water system, however, particulate P forms dissolve gradually with time acting as long-term P source for aquatic biota (Dorich et al., 1985; Sharpley et al., 1992).

Numerous sources contribute to the addition of P to the water systems. In some systems, particulate P can be the major fraction of total P. However, the concentration of P in sediment and amount of eroded soil can affect the loss of particulate P (Reid et al., 2018). Soil P stratification, and P enrichment conditions can complicate the estimation of particulate P loss (Reid et al., 2018) and aggravate it. During rainfall events or when snow melts, some amount of P in soil can dissolve in water and get transported with water runoff. The amount of P that desorbs from soil will depend on the mineralogy and P content of the soil (Reid et al., 2018). Addition of fertilizer or manure to the soil also adds to the easily available pool of P in soil that dissolves in water and is readily lost through runoff. However, immediate release of P from manure may be less than P released from an equivalent amount of fertilizer source applied to the soil (Reid et al., 2018). Different factors such as method, time, rate of litter application, duration and amount of precipitation following manure application can affect P loss from fields (Sharpley et al., 1998). Lamba et al. (2012) and Sistani et al. (2009) reported that applying broiler litter in the sub-surface layer leads to the reduction of nutrient transport in surface runoff as compared to the application of litter on soil surface.

Phosphorus from soil surface can be transported to water systems via both surface runoff and sub-surface flow (Lamba et al., 2013). Surface runoff occurs from soil surface when the rate of precipitation exceeds the infiltration capacity of the soil. In this situation, soil is unable to absorb any water as soil matrix is already saturated and any excess water after saturation is lost through runoff. For P loss risk assessment, it is important to calculate the amount of runoff, which depends on the total amount and intensity of rainfall, also considering the amount of rainfall that becomes runoff (Reid et al., 2018).

Sub-surface flow is important to consider in regions with extensive tile drainage (Jarvie et al., 2017). However, sub-surface flow of P is perceived to be insignificant due to the ability of the sub-surface soil to fix inorganic P (Sharpley and Syers, 1979; Burwell et al., 1977). Water movement can occur through both matrix flow and preferential flow in tile drains. However, solutes moving through the soil matrix have enough scope to interact with soil matrix through adsorption and precipitation reactions. The presence of high levels of Fe, Al, and Ca can lead to precipitation of P, retarding the movement of P through tile drains, but soils with very low sorption capacity are an exception to the rapid precipitation of P (Reid et al., 2012). Phosphorus will not precipitate in soils with low sorption capacity and hence can leach through the soil matrix. Contrary to this, macropore flow of P with water can occur through root channels, burrows made by earthworms or the fractures in soil due to shrinkage. These channels do not provide enough space for interaction between water and channel wall

(Eastman et al., 2010), providing enough conduit for water to reach tile drains leading to surface water (Eastman et al., 2010).

The biological response of the water system to P additions depends on the amount of total bioavailable P, which is calculated as sum of total dissolved P and bioavailable fraction of particulate P. This emphasizes the importance to evaluate the P fractions constituting total soil P. Phosphorus concentration of 0.01–0.03 mg dissolved P L^{-1} and 0.035–0.10 mg of total P L^{-1} is associated with eutrophic water systems (Pierzynski et al., 2005). The presence of P in water results in excessive growth of primary production and anoxic conditions due to the decomposition of the algal matter and this impairs water quality. Eutrophication can be controlled by minimizing P movement to the water bodies that can be achieved by careful nutrient management and controlling erosion and runoff from agricultural fields. Subsurface application of P sources and plowing no-till soils periodically can reduce the potential of P movement. Another P management strategy is to reduce erosion and runoff from agricultural lands, and it can be achieved by conventional tillage as runoff from no-till farms contains more P than farms with conventional tillage (Sharpley et al., 1993). Managing nutrient inputs to water systems is important to sustain agricultural systems that are both agronomically and environmentally sound. Therefore, it is important to evaluate different P fractions in soil, especially the soils with high P fertility levels, to develop better management practices that ensure minimum P loadings to water bodies.

1.13 Objectives

Alabama is home to five major soil groups (AP, CP, LV, PP, and BP), and are divided based on parent or geological material. Alabama soils are naturally deficient in P and tend to fix P in insoluble P forms by Fe and Al oxides and hydroxides present in soil. The poultry industry in Alabama has witnessed a very rapid expansion in the last decade. Poultry production has shifted from domestic, small-scale operation to more intensive and concentrated commercial production. The expansion of the poultry industry in Alabama has resulted in the generation of 1.68 million tons of poultry litter (PL) annually. To manage such large volumes of PL, land application on farmlands has been the primary strategy, as PL contains primary plant nutrients such as nitrogen, phosphorus, and potassium (3-3-2). Repeated application of PL to the farmlands has resulted in the buildup of P in soils. The P accumulated in soils is a threat to water quality as the legacy P from the farmlands is transported to water bodies via surface runoff or erosion. Significant differences exist in the quantity of P species present in soil and their dynamics within soil groups. Hence it is important to understand the distribution of P species among soil groups and how this distribution may affect the environmental P loss risks or bioavailability for plant uptake. The use of P fractionation scheme is an important tool to quantify various P pools that exist in soil. It is hypothesized that (a) different P fractions are dominant in different soil regions of Alabama, (b) different P fractions are stratified in the soil layers, (c) distribution of P fractions vary with soil P fertility rating. Therefore, the objectives of this study were (a) to investigate the distribution of P pools in Alabama soil regions namely Appalachian Plateau, Piedmont Plateau, Blackbelt Prairie, Limestone Valleys and Coastal Plains, (b) to determine the variation of P pools with soil depth from 0-45 cm, and (c) to understand how P fractions change with change in soil P fertility levels.

1.14 Figures

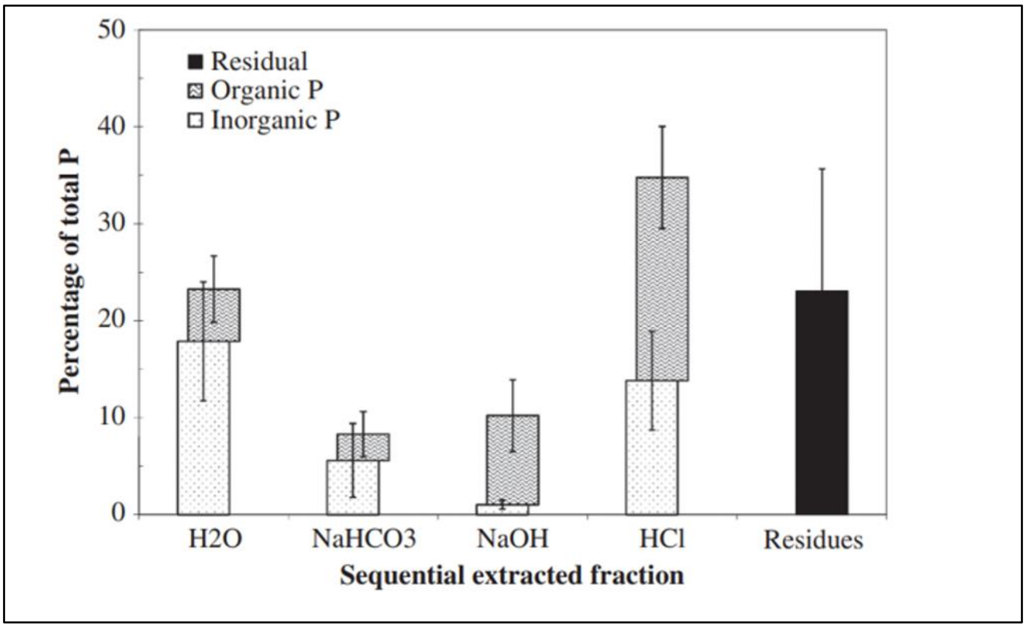

Figure 1: Distribution of P in sequentially extracted H_2O, 0.5 M $NaHCO_3$ (pH 8.5), 0.1 M NaOH, 1 M HCl, and residue P fractions in poultry litter (PL). Data presented in this figure was the average of 23 samples recalculated from He et al., (2006b) (Retrieved from He et al., 2012).

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CHAPTER 2

Distribution of Phosphorus Fractions among Soil Depths in Five Alabama Soil Regions

2.1 Abstract

The poultry industry in Alabama is the state's second-largest agriculture industry generating approximately 1.25 million tons of poultry litter (PL) and containing 19,000 tons of phosphorus (P). Long-term use of PL on agricultural lands causes accumulation of P, causing elevated soil test phosphorus levels. The P accumulated in soil makes its way into the water bodies via surface runoff and erosion, imposing a serious threat to the water quality. The objective of this study was to identify the fractions of P and their variation with soil depth between 0-45cm in five Alabama soil regions to better understand P bioavailability and environmental P loss risk. Soil samples were collected from agricultural farms in Alabama representing five major soil regions namely Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) that had a history of PL application for at least 10 years. The samples were separated into four depths: 0-5 cm, 5-15 cm, 15-30 cm, and 30-45 cm. The soil samples were sequentially extracted using modified Hedley fractionation procedure to quantify five P pools namely water extractable total P (H_2O-P_t), labile P represented by sodium bicarbonate extractable total P ($NaHCO_3$-P_t), iron-aluminum bound P represented by sodium hydroxide extractable total P (NaOH-P_t) calcium-magnesium bound P represented by hydrochloric acid extractable total P (HCl-P_t) and recalcitrant P represented by residue total P (residue-P_t). Results indicated that in CP soil region, NaOH-P_t was the dominant pool at 0-5, 5-15, and 15-30 cm soil depths

accounting for 41%, 42% and 40% of total P, while the residue-P_t accounted 47% of the total P at 30-45 cm soil depth. However, in AP soil region, NaOH- P_t, being the dominated pool at 0-5 and 5-15 cm, made 40% and 38% of total P, whereas residue-P_t was the dominant pool at 15-30 and 30-45 cm soil depth contributing 40% and 49% to the total P. In contrary, residue P_t dominated at all four soil depths from 0 to 45 cm in PP (35-64%) and LV (38-69%) soil regions. In case of calcareous BP soil region, HCl-P_t was the dominant pool at 0-5 cm soil depth accounting on an average 39% of total P and the lower profile, from 5 to 45 cm soil depth, was dominated by residue-P_t pool (46-57%). In terms of individual P fractions in all the five soil regions, the percent of H_2O-P_t was greatest in AP whereas $NaHCO_3$-P_t and NaOH-P_t percent was greatest in CP soil region at all four soil depths from 0-5 cm to 30-45 cm. Among all five soil regions, HCl-P_t was the highest in BP soil region across all four soil depths. The highest percent of residue-P_t among all soil regions at all soil depths was observed in LV soil region. This study demonstrated a significant difference in the distribution of P fractions among various soil depths across Alabama soil regions.

2.2 Introduction

With the production of 20.5 million metric tons of broiler meat in 2020, the United States was reported to be the top producer of broiler meat globally (Shahbandeh, 2021). Majority of the poultry production is witnessed in the southeastern regions of the United States (MacDonald, 2008) with Georgia, Alabama, Arkansas, Mississippi, and North Carolina being the major poultry producing states. Alabama ranks second in poultry production after Georgia, processing around 1.1 billion birds in 2018 and consequently generating 1.68 million tons of PL (USDA-NASS, 2019).

Management and disposal of PL in an environmentally sound way is a serious concern associated with concentrated poultry production. To effectively manage PL in Alabama, it is important to optimally distribute or transport PL from concentrated poultry production areas to other areas for utilization. However, PL can only be transported to a distance of 80 km cost effectively (Higgins et al., 2021) due to the low bulk density (<500 kg m^{-3}) of raw PL (Bernhart and Fasina, 2009). The key issues in PL management in Alabama are the minimal use of P index and nutrient management plan by farmers, inadequate transportation infrastructure to transfer surplus litter to deficit areas and over-application of litter in the vicinity of poultry houses (Kang et al., 2008). "The P index is a tool to assess the site and management practices for potential risk of phosphorus movement to water bodies as the result of additional P applications" (USDA-NRCS, 2014). The P index is used for the estimation of P loss risk and can help in the better management of resources.

Poultry litter is a mixture of feathers, spilled feed, feces, and bedding materials (Chakraborty et al., 2021) and serves as an inexpensive source of nutrients for plants with a fertilizer grade (N-P_2O_5-K_2O) of 3–3-2 (Mitchell and Donald, 1995; Watts et al., 2019). Therefore, the majority of the PL produced is predominantly applied to agricultural lands (Bolan et al., 2010; Sistani et al., 2009) as a part of litter management strategy. Application of PL to agricultural lands is often based on the N requirement of the crops (Reddy et al., 2009). Due to the disproportionate ratio of N and P in PL corresponding to the crop needs, long term repeated applications of PL based on N requirement of the crop leads to over-application of P to the soil (Szogi et al., 2015). Excess amount of P builds up in the surface soil which is usually termed as legacy P (Gatiboni and Condron, 2021). This legacy P in soils is lost through surface runoff or erosion and is transported to water bodies. These P enriched soils serve as a potential environmental risk to water quality. This emphasizes the importance to understand the chemical nature of legacy P in soil and its implications on the environment.

Phosphorus in soil exists in several inorganic and organic forms. Separation and determination of different P pools in soil is crucial to understand the dynamics and bioavailability of legacy P in soil (Condron and Newman, 2011). Phosphorus fractionation is the sequential extraction of soil to quantify selective P pools in the soil at each step based on the assumption that each extractant extracts an individual fraction of P. Fractionation scheme for inorganic P fractions in soil includes Chang and Jackson (1957) and Hieltjes and Lijklema (1980) whereas for organic P fractions, the methods include those proposed by Sommers et al. (1972), Bowman and Cole (1978), and Ivanoff et al., (1998). However, other fractionation procedures such as Hedley et al. (1982) and Tiessen and Moir (2008) provide detailed investigation of both inorganic and organic P fractions in soil. Li et al. (2015) revealed that the fractionation procedure given by Hedley et al. (1982) can be successfully used to quantify both available P_o and P_i instead of using fractionation schemes individually for inorganic (Chang and Jackson, 1957) and organic (Bowman and Cole, 1978) P fractions.

Several studies conducted in the past focused on understanding P dynamics in surface horizons (Gburek et al., 2005; Ahmed et al., 2019; Motavalli and Miles, 2002) and very few attempts were made to determine the accumulation and distribution of P in sub-surface horizons. This may be because of a general assumption that lower amounts of P are transferred vertically through the soil profile because of the tendency of the sub-surface horizons to fix soil P (Gburek et al., 2005). However, some recent studies have documented the movement of P vertically in the soil profile via leaching, especially in the circumstances where long term P applications in excess of the crop needs resulted in the accumulation of P in the soil profile (Tian et al., 2017; Szogi et al., 2012). The distribution and mobility of bioavailable P in soil profile determine the potential environmental risk associated with the subsurface movement of P. This emphasizes the importance to determine the forms and concentration of P in soil profile rather than P in topsoil layer of soil profile. Additionally, soil mineralogy has a major influence on P redistribution following P application. Alabama soils are broadly classified into calcareous and non-calcareous soils. Soils from Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), and Piedmont Plateau (PP) are categorized as non-calcareous soils, whereas soils from Blackland Prairie (BP) are categorized as calcareous soil (Bhatta et al, 2021; Mitchell & Huluka, 2012). It was hypothesized that 1) P forms following long term PL application varied between calcareous and non-calcareous soils of Alabama, 2) P forms were stratified in the soil profile and the amount of individual P forms changed with soil depth increment. Therefore, the objective of this study was to quantify the fractions of P and determine their variation within 0-45 cm depth in five Alabama soil regions. A better understanding of the distribution of P forms in PL impacted soils will help understand the chemical nature of legacy P and formulate strategies needed for P loss risk mitigation as well agronomic management of legacy P.

2.3 Materials and Methods

2.3.1 Soil Sampling

To conduct this study, soil samples were collected from 26 agricultural fields representing five soil regions of Alabama namely Piedmont Plateau (PP), Appalachian Plateau (AP), Limestone Valley (LV), Coastal Plain (CP), and Blackbelt Prairie (BP) located within 32 counties. These farms had a 10-year history of poultry litter (PL) application and were either in row crops or grazing systems. Samples were taken using a gas-powered soil corer (Part 360.01, AMS Equipment, American Falls, ID) and each core sample was separated into 4 different depths of 0-5, 5-15, 15-30, and 30-45 cm. At least three soil cores were collected at each sampling location and composite samples for individual depths were prepared by combining three cores collected at each sampling point corresponding to each depth. The SSURGO database (Soil Survey Staff, 2020) was used to obtain information on soil order and series. Each sample was analyzed to evaluate individual P fractions as described in the next section.

2.3.2 Chemical Analysis

Soil samples were homogenized, air-dried and passed through 2 mm sieve prior to analysis. The pH of the samples was measured using deionized water at 1:1 (w/v) soil to water ratio. Soil samples were sequentially extracted using a modified Hedley fractionation method into H_2O-P_t (water soluble P), $NaHCO_3$–P_t (considered to be labile form of P), NaOH–P_t (P bound to Fe/Al), HCl–P_t (P bound to Calcium (Ca) and magnesium (Mg)) and residue-P_t (also, considered as the recalcitrant form of P) (Tiessen and Moir, 2008). This fractionation procedure includes extraction of 0.5g of soil sample with deionized water, followed by extraction with 0.5 M $NaHCO_3$ (pH-8.5), 0.1 N NaOH and 1 M HCl (Fig. 2). The residue left at the end of sequential extraction was acid digested to determine the residue-P_t. Water extraction was carried out for 2 hours and all other extractions were carried out for 16 hours in a reciprocating shaker. The soil: extractant ratio was 0.5:30 for all the extractions. After extraction, the samples were centrifuged, and supernatant was filtrated with 0.45µm filter paper. Total P in water, 0.5M $NaHCO_3$ (pH-8.5), and 0.1N NaOH extracts was analyzed using inductively coupled argon plasma spectrometry (ICAP; Spectro Ciros, Spectro Analytical Instruments Inc., Mahwah, NH). The samples extracted by 1 M HCl were analyzed in both FIA and ICAP and the data from both the instruments was highly correlated (R^2=0.9614) (Figure appendix A (1 and 2)). The amount of P in HCl extract was determined using an autoanalyzer (FIAlab instruments Inc., Seattle, WA) by ammonium molybdate-ascorbic acid colorimetric method given by Murphy and Riley, (1962). The acid digestion of residue was carried using 5ml concentrated sulfuric acid and 5ml 30% hydrogen peroxide followed by filtration using Whatman 42 filter paper and determination of P using ICAP.

To measure available P, soil samples from non-calcareous soils were extracted with Mehlich-1 (M1) and samples from calcareous soils were extracted with Lancaster (La) solution. Extracted samples were filtered through Whatman 42 filter paper and were analyzed using inductively coupled argon plasma spectrometry (ICAP; Spectro Ciros, Spectro Analytical Instruments Inc., Mahwah, NH). Phosphorus concentration (mg kg^{-1}) obtained by M1 and La soil test was used to assign P fertility levels as extremely high (EH), very high (VH), high (H), medium (M), very low (VL), low (L) to soil samples from both non calcareous (AP, CP, LV, PP) and calcareous (BP) soil regions (Mitchell and Huluka, 2012) (Table-1).

2.3.3 Statistical analysis

The analysis of variance was conducted using Proc GLIMMIX in SAS 9.4 (SAS Institute, 2013). The mean comparisons were done using Tukey–Kramer and were considered significant at $P<0.05$. For this analysis, sample locations were considered as a random factor and soil depth and region were considered as fixed factors in the model. To estimate the differences between P fractions at soil depths and among soil types, lognormal distribution was applied to the data for normality adjustment (Cade-Menun et al., 2015; Baker et al., 2017).

2.4 Results and Discussion

The data presented in Table 1 shows the information on sample location, soil taxonomic classification, pH, STP and TP values of soil samples collected from agricultural fields representing five soil regions of Alabama (AP, CP, LV, PP, and BP). The pH of soil samples was observed to be acidic to neutral with an average pH of AP, CP, LV, PP, and BP soil regions for 0 to 45 cm of depth as 6.10, 5.97, 6.08, 5.89, and 7.15, respectively. The soil pH declined with depth across all five soil regions. The pH of BP soils was in alkaline range (pH>7). Further, the agronomic soil test P (STP) was determined using Mehlich 1 for non-calcareous soils and Lancaster for calcareous soils. The M1-P for AP, CP, LV, PP and La-P for BP soils varied with soil depth with highest concentrations found in the surface 0-5 cm and lowest at 30-45 cm depth (Table 1). Among the soil regions, the highest concentration of M1-P was found in 0-5 cm depth in AP (146.54 ±20.77 mg kg^{-1}) and PP (129.62 ±29.02 mg kg^{-1}) soils whereas CP soils had the lowest M1-P concentrations (36.96 ±9.75 mg kg^{-1}). The STP levels were found to be highly stratified with soil depth. Stratification of STP has been reported by many researchers and is a common phenomenon observed in many agricultural soils across USA (Baker et al, 2017; Bhatta et al, 2021).

The P fractions in individual soil depths were examined using modified Hedley P fractionation procedure (Hedley et el., 1982). Hedley fractionation procedure (Hedley et al., 1982) is based on the assumption that each extractant used for sequential extraction varies in their strength to extract discrete P fractions with different availability and chemical bindings (Guo et al., 2000; Maranguit et al., 2017). Accordingly, this P fractionation procedure was used to quantify P in soil into H_2O-P_t (water soluble P), $NaHCO_3$-P_t (extracts labile forms of P and easily mineralizable organic P), NaOH-P_t (extracts P bound with Fe and Al sesquioxide and more resistant organic P associated with humic and fulvic acids), HCl-P_t (extracts P bound with Ca and primary minerals such as apatites, monocalcium phosphate, brushite), and residue-

P_t fractions (recalcitrant forms of P). The distribution of P fractions in Alabama soil regions between 0 to 45 cm soil depth is presented in Table 2.

2.4.1 Distribution of P fractions in five Alabama soil regions

2.4.1.1 Water soluble P

The H_2O-P_t fraction is the most labile form of P and is readily available for plant uptake that greatly affects plant growth in agricultural farmlands (Hong et al., 2018). This P fraction is also susceptible to runoff losses as water is the primary medium for transportation of P to the waterbodies (Pote et al. 1996).

In this study, H_2O-P_t for 0-5 cm soil depth ranged between 11.74 to 63.48 mg kg^{-1} in AP, 2.88 to 14.81 mg kg^{-1} in CP, 6.67 to 102.72 mg kg^{-1} in LV, 1.81 to 156.13 mg kg^{-1} in PP, and 9.35 to 29.56 mg kg^{-1} in BP soil region with no significant difference between H_2O-P_t in all the five soil regions (Table 2). However, the percent of H_2O-P_t expressed as a percentage of total P, in AP soils ranged from 1 to 8% between 5-45 cm depth and was significantly greater ($P<0.05$) than percent of H_2O-P_t in CP, LV, PP, and BP soil regions (Figs. 5-7). Additionally, H_2O-P_t fraction was observed to be the least dominant fraction contributing between 0 to 10% to the total P between 0 to 45 cm soil depth for all the five soil regions (Fig. 8). Similar findings were reported by He et al. (2008) in an effort to characterize P fractions in PL, soils with and without PL application in Sand Mountain region of Northern Alabama. He et al. (2008) found H_2O-P_t fraction in least amounts as compared to $NaHCO_3$-P_t, NaOH-P_t, and HCl-P_t fractions in soils that had history of 20 years of PL application. Toor et al. (2020) conducted an experiment to quantify P pools in continuous and rotational pastures and observed H_2O-P_t fraction as the least dominant fraction in both the pastures between 0 to 20 cm of soil depth. The presence of small amount of P in H_2O-P_t fraction in a PL applied soils indicates lesser risk for loss as well poor availability to plants, however, P from other pools such as $NaHCO_3$ can also buffer P and make P available into H_2O pool.

Chakraborty and Prasad (2021) reported that labile P fraction contributed only 3% to the total P and the amount of $H_2O\text{-}P_t$ decreased with soil depth. The average contribution of $H_2O\text{-}P_t$ to total P for 0 to 5 cm depth was 5%, 2%, 4%, 3%, and 2% in AP, CP, LV, PP, and BP soil regions, respectively and further reduced to 3%, 2%, 1%, 1%, and 0% for 30 to 45 cm depth for respective soil regions (Fig. 8). A similar trend in reduction of P in $H_2O\text{-}P_t$ with depth was observed by He et al. (2012). He et al. (2012) conducted an experiment to evaluate the distribution of labile and stable P pools with depth in soils with history of PL applications for 0, 5, 10, 15, and 20 years. The results from He et al. (2012) study indicated that the amount of $H_2O\text{-}P_t$ at subsurface soil at 40-60 cm was 1/3 to 1/4 less than the amount of corresponding $H_2O\text{-}P_t$ levels at surface soil (0-20 cm) in soils with 15 and 20 years of PL application. Decrease in the concentration of $H_2O\text{-}P_t$ with depth increment could be because of the P fixation during the vertical downward movement of P in soil profile (Amaizah et al., 2012). Relatively lesser amount of $H_2O\text{-}P_t$ at subsurface soil than surface soil indicates poor availability of P for plant uptake as well as less risk for leaching loss (Chakraborty et al., 2021).

2.4.1.2 $NaHCO_3$ extractable P

The $NaHCO_3$ extracted P represents the labile pool and is available for uptake by plant and microbial communities as this pool readily desorbs P from the surface of soil particles through buffering action (Cross and Schlesinger, 1995; Hong et al., 2018; Mankolo et al., 2008). Johnson et al. (2003) defined labile P as the P which readily exchanges between plants and their residues, soil solution, soil microbes and loosely bound pools of P. The inorganic P in labile pool is weakly adsorbed on surfaces and found in less soluble P minerals of sesquioxides and carbonates (Tiessen and Moir, 2008; Mattingly, 1975; Chakraborty and Prasad, 2021) whereas labile organic P constitutes less stable, readily mineralizable forms of organic P (Bowman and Cole, 1978). Here we report the total labile P rather than individual inorganic and organic labile P to avoid over interpretation of data resulting from usage of two different instruments. For example, we observed that calorimetric method overestimates P concentration than ICAP resulting in zero organic P concentrations.

The labile P across 5 soil regions varied with depth where the highest concentration (expressed as percent of total P) was found at 0-5 and 5-15 cm and decreased significantly at 15-30 and 30-45 cm depth ($P<0.05$). For example, in AP soils, the labile P ranged between 20% in the 0-5 cm to 10% in the 30-45 cm depth (Fig. 8a). Similarly, in CP soils the mean concentrations of labile P ranged between 25% in the 0-5 cm and decreased gradually to 18 % in 30-45 cm depth. We also found that CP soils had the highest labile P concentrations compared to the other four soil regions. Since most CP soils are sandy to sandy loam in texture, it appears that labile P has moved vertically downwards as indicated by comparable P concentrations (Table 2, across soil depths). Downward transport of P in light textured soils have been reported by previous researchers (Nelson et al. 2005). High concentrations of labile P in lower soil profile (15 cm and below) indicates greater P availability for plant roots. However, in areas where ground water is shallow, high concentrations of labile P in bottom soil profiles can become a continuous source of P to the groundwater.

Among Alabama soil regions, the percent $NaHCO_3$-P_t in CP soil, (contributing an average of 25% to the total P) was significantly greater ($P<0.05$) than the percent $NaHCO_3$-P_t in AP, LV, and PP soil regions at 0 to 5 cm soil depth (Fig. 4). In contrary, no significant difference was observed for the percent $NaHCO_3$-P_t fraction between all the five soil regions for 5 to 30 cm of soil depth (Fig.5, 6). Further, for 30 to 45 cm soil depth, $NaHCO_3$-P_t was found to be significantly greater ($P<0.05$) in both CP and BP soil regions (Fig. 7). Besides, the results demonstrate that $NaHCO_3$-P_t fraction was also significantly greater ($P<0.05$) than H_2O-P_t between 0 to 45 cm depth for all five Alabama soil regions (Table 2). This finding is in accordance with the results documented by He et al.(2012) who reported that the amount of $NaHCO_3$-P_t was 2-4 times higher in the corresponding samples than the most labile H_2O-P_t. Similarly, Kashem et al. (2004), while determining the effect of organic and inorganic P amendments on soil P fractions, also observed that less amount of P was extracted by H_2O as compared to $NaHCO_3$. The considerable amount of P found in $NaHCO_3$-P_t pool may possibly be due to the presence of organic matter. Soil organic matter affects P sorption sites by masking the Al and Fe sorption sites that prevent the P in soil to adsorb (Meason et al., 2009; Ahmed et al., 2019; Guppy et al., 2005). Additionally, soil organic matter content alters mineral surface charges as the sorption on the surface of minerals by organic matter masks the charges of mineral colloids, reducing the sorption sites in soil that results in more labile P (Chorover and Sposito, 1995; Meason et al., 2009; Chorover et al., 2004; Beckett and Le, 1990).

In addition to this, the $NaHCO_3$-P_t labile pool in AP, CP and BP soil regions did not decrease significantly between 0 to 45 cm of soil depth (Table 2 and Fig. 9(b)). However, $NaHCO_3$-P_t decreased significantly ($P<0.05$) from 105.01 mg kg^{-1} and 177.62 mg kg^{-1} to 4.22 mg kg^{-1} and 21.92 mg kg^{-1} in LV and PP soil regions, respectively (Table 2 and Fig. 9(b)). The reduction in $NaHCO_3$-P_t concentration with depth was directly in line with previous findings (He et al. 2012; Wang et al. 2007; Hountin et al. 2000; Chakraborty and Prasad, 2021; and Toor et al. (2020). He et al. (2012) reported that the concentration of $NaHCO_3$-P_t for 0-20 cm soil layer was greater than the $NaHCO_3$-P_t fraction at 20-40 and 40-60 cm depths in soils with history of PL applications. To explain this reduction in $NaHCO_3$-P_t fraction with soil depth, Abdala et al. (2012) revealed that repeated application of PL results in the saturation of sorption sites at the surface soils. Due to the soil P saturation, any P released in soil is reapportioned into labile P fractions with high desorption potential (Chakraborty and Prasad, 2021). However, same as H_2O-P_t, greater amount of labile $NaHCO_3$-P_t fraction in surface soil is an environmental concern due to the high potential to release P to the waterbodies (Ranatunga et al., 2013; Chakraborty and Prasad, 2021).

2.4.1.3 Sodium hydroxide extractable P

The P extracted by NaOH constitutes occluded P forms, organically bound P attached to Fe and Al hydrous oxides and insoluble phosphates of Fe and Al and is considered to be moderately labile P pool (Hedley et al. 1982; Tiessen and Moir, 2008; Cross and Schlesinger 1995; He et al., 2006). The ability of Fe, Al hydroxides to bind P and other anions was due to the positive charge and the surface area of these hydroxides (Kaňa et al. 2011; Jan et al., 2015; Detenbeck and Brezonik 1991; De Vicente et al. 2008). These two metals, Fe and Al, are two most predominant metals in non-calcareous soils ($pH<7$) that are found to bind with P (Jan et al., 2015). The pH of NaOH extract is basic ($pH>11$) and the alkaline nature of NaOH extract leads to the dissolution of Al and Fe bound P substantiating the dominance of NaOH-P_t (Toor et al., 2020).

In consistence with this idea, NaOH-P_t was observed to be the most dominant pool at 0 to 5 cm depth, contributing 9 to 55% to the total P and second dominant pool, contributing 4 to 60% at 5 to 45 cm depth in non-calcareous soils. In case of calcareous BP soils, NaOH-P_t was observed to be the third dominant pool making 3 to 24% of total P, followed by HCl-P and residue P at all soil depths. The dominance of NaOH-P_t pool was observed and published in various other research findings including Toor et al. (2020), He et al. (2012), Chakraborty et al. (2021), Ranatunga et al. (2013), Hong et al. (2018), Gatiboni and Condron (2021), Hountin et al. (2000), and Chakraborty and Prasad, (2021). The build-up of P in NaOH-P_t fraction is controlled by Fe and Al oxides, owing to their high affinity for P (Wang et al. (2015). In this study, the majority of the samples (74%) belonged to the Ultisols, which is the dominant soil order in Alabama. The Ultisols are typically acidic soils and are characterized by low P availability due to the elevated amount of Al and Fe oxides imparting strong P fixing capacity to these soils (Wang et al. (2015). The greater amount of Al and Fe oxides can transform soil solution P to water-insoluble, amorphous Fe and Al P compounds (geothite, variscite, Strengite, vivianite etc), leading to reduced availability of P (Chien et al. 2011; Li, Shi, and Chen 2011; Wang, Zhang, and He 2012).

Furthermore, between 0 to 45 cm soil depth, no significant difference was observed between the percent contribution NaOH-P_t fraction to the total P among all non-calcareous soil regions (Figs. 4-7). Whereas the percent contribution NaOH-P_t fraction to the total P in calcareous BP soil region was significantly lower ($P<0.05$) than NaOH-P_t percent in all other soil regions between 0 to 45 cm depth. The amount of NaOH-P_t, alike other P pools, decreased with increase in soil depth from 0 to 45 cm (Table 2) and these results are in alignment with the findings documented by Wang et al. (2007), He et al. (2012) and Toor et al. (2020). He et al. (2012) reported that the amount of NaOH-P_t in PL applied soils at 20-40 and 40-60 cm sub-surface soil layers was less than the amount of NaOH-P_t at 0-20 cm surface soil layer.

A greater proportion of NaOH-P_t indicates that the fraction can serve as a source of plant-available P by mineralization (Randhawa et al., 2005) when the labile P sources have exhausted. However, higher amount of P in NaOH-P_t fraction has been reported to be an environment concern (Zhang et al., 2021) as the P extracted by 0.1 M NaOH-P_t in soil and sediments is associated to algal uptake (Sharpley, Troeger, and Smith 1991; Adeli et al., 2005). Under certain environment conditions, such as change in soil pH and microbial degradation, NaOH-P_t fraction can release inorganic P in the environment (Simpson et al., 2011; Chakraborty and Prasad, 2021; Wright, 2009; Richardson and Simpson, 2011). Moreover, degradation of organic matter can lead to chelation of Fe and Al by release of certain organic anions in the soil that results in reduced P adsorption, allowing more P to be lost to the environment through various transport mechanisms (Scherer and Sharma, 2002; Ranatunga et al., 2013).

2.4.1.4 Hydrochloric Acid extractable P

The HCl extracts P bound to Ca (Tiessen and Moir, 2008; Cross and Schlesinger, 1995) and is considered to represent primary minerals as in apatites (Williams et al., 1980; Tiessen and Moir, 1993). The HCl-P_t fraction is considered to be moderately labile P pool, alike NaOH-P_t (Chakraborty et al., 2021). The HCl-P_t forms are most commonly observed in soils with high pH and are found to be unstable at low soil pH (Gatiboni and Condron, 2021). Borggard et al. (1990) and Afif et al., (1993) reported negative correlation between P availability and calcium carbonate ($CaCO_3$) content of the soil. Consequently, P added in calcareous soils is sorbed on the surfaces of clay minerals and calcium carbonates, reducing the availability of P in soil (Shen et al. 2011).

In this study, HCl-P_t was found to be the dominant fraction in calcareous BP soils than non- calcareous soils. In calcareous soil, the HCl-P_t represented 39% of total P at the surface 0-5 cm depth and decreased to 33%, 28% and 27% at 5-15cm, 15-30 cm and 30-45 cm depths, respectively (Figs. 8e). The dominance of HCl-P_t in calcareous soils was also confirmed by Halajnia et al. (2009); Carreira et al. (2006);

Yang et al. (1990); Harrell and Wang (2006). Similar conclusions were drawn by Yu et al. (2006) in an effort to relate surface runoff and P fractions in Florida soils who observed that 45-60% of the total P in neutral and alkaline soils was present in HCl-P_t form.

In non-calcareous soils, HCl-P_t was the second least dominant fraction after H_2O-Pt at all soil depths (Fig. 8). At surface 0-5 cm, the HCl-Pt ranged from 4% in CP soils to 12% in LV soils. With depth increments, the HCl-Pt decreased proportionately and ranged between 1% in CP soil to 3% in LV soils (Fig. 4-7). AP soils represented an exception to this observation where the decrease was very slow from an average 9% in the 0-5 cm depth to 7% in 40-45 cm depth.

These results are in alignment with findings reported by Boitt et al. (2018); Gu et al. (2020); Gatiboni et al. (2007); Couto et al. (2017) where small amount of HCl-P_t was observed in soils with no or less amount of Ca-bound minerals.

2.4.1.5 Residue P

The residue-P_t fraction is considered to be the most stable P fraction constituting highly recalcitrant inorganic and stable organic forms of P (Tiessen and Moir 1993; Turner et al., 2005; Gatiboni and Condron, 2021; Cross and Schlesinger 1995). Tiessen, Stewart, and Moir (1984), Adeli et al. (2005) explained that residue-P_t includes occluded inorganic P covered with sesquioxides and non-extracted stable organic P. These recalcitrant forms of P were not extracted by any of the other extractants used previously in the P fractionation procedure (Kiflu et al. 2017). The residue-P_t pool is not available for plant uptake due to the low solubility and low mineralization rates (Gatiboni and Condron, 2021). In contrary, Sattari et al. (2012) and Zhang et al. (2006)

documented that the residual P accumulated in soils can serve as plant available P when external application of P sources is curtailed.

The residue-P_t ranged between 26 % to 36 % of the total P in the surface soils (0-5 cm) and increased to values ranging between 47- 69 % at the 30-45 cm depth (Figs. 4-7). The contribution of residue-P_t to the total P increased with a corresponding decrease in the contribution of labile and moderately labile P fractions from 0 to 45 cm of soil depth in all the five soil regions (Fig. 8). These results are also supported by Gatiboni and Condron (2021) who observed that the residue-P_t accounted for 65% of the total P. High amount of residue-P_t is postulated to be related to high P-sorption capacity of the soils due to high degree of soil weathering (Gatiboni and Condron (2021). Chakraborty et al. (2021) also documented the highest percent of residue-P_t in highly weathered Piedmont soils of Alabama, which suggests that the residue-P_t accumulate in soil irrespective of the land use system. In addition to this, no significant difference was observed between residue P_t fraction in all the five soil regions for 0-5 cm and 30-45 cm of depth (Figs. 4- 7).

Phosphorus applied to the soils over the span of years accumulates in residue-P_t fraction in soil that is usually unavailable for plant uptake, however, it also infers that minimum amount of P will make its way in the environment (Brunetto et al., 2013). Maranguit et al. (2017) revealed that the P in residue-P_t fraction can change to available forms depending on the soil type and land management or can undergo various processes such as desorption, mineralization and weathering, depending on the form of P.

2.4.2 Vertical Stratification of P Pools

The amount of P in all fractions and TP values were observed to decrease with depth increment from 0 to 45 cm of soil depth (Table. 1 and Figs. 9a-9e). These results suggest that P fractions in Alabama soil regions are highly stratified at the surface soil. The vertical stratification of P pools has been confirmed by Saavedra et al. (2007), Chakraborty and Prasad, (2021), Rahman et al. (2021), Firmano et al. (2021); Chakraborty et al., (2021), Yang et al. (2013), Cade-Menun et al. (2015), Nunes et al. (2020). Chakraborty et al. (2021) conducted an experiment to evaluate the impact of P in PL amended Piedmont soils on the environment and documented that the concentration of extractable P was greater in the surface than sub-surface soil layer regardless of the extractant and P fraction. Chakraborty et al. (2021) further concluded that the repeated application of PL along with conservation tillage practices may be the reason of P accumulation in surface soil. The higher amount of P in surface soil layer can be attributed to the low mobility and high reactivity of P in the soil profile (Firmano et al., 2021; Rahman et al., 2021). Maranguit et al. (2017), Farley and Kelly, (2004), and Kautz et al. (2013) explained stratification in soil by “nutrient pumping” process where nutrients absorbed by trees from sub-surface soil layer are redistributed in the topsoil by litterfall and throughfall.

Moreover, the accumulation of P in surface layer is accredited to the land management practices, sources of P added to soil and method of application of P, as these factors control the availability of P for plant uptake (Nunes et al., 2020). More accumulation of nutrients at surface layer is observed in no-till practices with minimum soil inversion as compared to conventional tillage where soil disturbance leads to the homogenization of P in the upper soil profile (Santos and Tomm, 2003, Nunes et al., 2020). Vertical stratification of P fractions observed in this study is in agreement with the prevalence of no till or reduced till practices in Alabama (Derpsch et al., 2010; Bergtold and Sailus, 2020). These findings are also confirmed by Saavedra et al. (2007) who documented that stratification of P pools as well as other nutrients is more intensive in no-till management systems. Bertol et al., (2007) reported that the nutrient accumulation in surface soil in no-till systems were three to five times greater than conventional tillage management system. Additionally, broadcast application of different P sources has been observed to intensify P stratification (Rotta et al., 2015; Nunes et al., 2020; Sims et al., 1998; Fink et al., 2016).

The nutrients present in the subsoil significantly contribute to plant uptake (Kautz et al., 2013). In case of water scarcity, plant roots tend to grow deeper in search of water and access nutrients present in subsoil. Garz et al., (2000) concluded that the P from subsoil can be potentially available for plant uptake particularly in case of dry topsoil or P depletion in surface layer. The greater concentrations of P at surface soil, especially in labile and moderately labile P pools, suggest increased availability of P for plant uptake and additionally indicates more potential of P loss to the environment (Chakraborty and Prasad, 2021). Vertical stratification of P has been observed as a potential contributor of P in runoff and leaching from agricultural farmlands (Kleinman et al., 2011). However, sub-surface application of PL or P fertilizers can help to reduce P loading in runoff water, and it will also help to manage PL in a more sustainable manner (Chakraborty and Prasad, 2021).

2.5 Conclusion

The fate of P applied and their dynamics in the soil is important for both agronomic and environmental viewpoint. The accumulation and distribution of P forms in surface and sub-surface soil depths reflect the paradigm of P transfer through the soil profile. This necessitates the development of better management practices, such as sub-surface application of PL, transforming PL into slow-release source of P or including soil amendments such as gypsum to fix P to minimize the environmental footprints of PL application to agricultural fields. This study on P fractionation in Alabama soil regions indicated occurrence of different quantities of P fractions distributed across soil depths on agricultural lands that had a history of PL application. The H_2O-P_t and HCl-P_t pool represented the two least dominant P pools and NaOH-P_t and residue P were found to be the most dominant pools in non-calcareous soil regions. In calcareous soils, HCl-P_t and residue P were found to be the two dominant pools in the whole soil profile. The contribution of H_2O-P_t, $NaHCO_3$-P_t, NaOH-P_t, and HCl-P_t to total P decreased with soil depth in all five soil regions. Whereas the percent contribution of residue-P_t to total increased with increment in soil depth. Residue-P_t P pool was the most dominant pool at all four soil depths in LV and PP soil region. In case of calcareous Blackbelt Prairie region, HCl-P_t was the most dominant pool between 0 to 5 cm and residue-P_t was the dominant pool between 5 to 45 cm soil depth. The results indicate that the excess P, above plant requirement and soil P retention capacity, accumulated in the soil profile due to the repeated applications of PL. Further, stratification of P pools at surface soils indicates a greater risk of P loss to the environment. This emphasizes the importance to develop better management practices that ensure better crop productivity while minimizing P loss to the environment.

2.6 Tables

Table 1: Sample distribution in major soil regions and ancillary properties of soil samples under study. Data is represented as mean (± standard error).

Soil Region	Soil Series	Soil Order	Depth (cm)	No. of Samples	pH	STP (mg kg^{-1})	TP (mg kg^{-1})
Appalachian Plateau	Albertville, Allen, Crossville, Hartsells, Linker, Nauvo, Tilsit	Ultisol	0-5	20	6.40 (± 0.13)	147 (± 21)	712 (± 62)
			5-15	20	6.28 (± 0.11)	95 (± 21)	514 (± 62)
			15-30	20	6.00 (± 0.12)	45 (± 12)	304 (± 40)
			30-45	20	5.74 (± 0.15)	17 (± 6)	216 (± 27)
Coastal Plain	Bowie, Dothan, Orangeburg, Red Bay, Troup	Ultisol	0-5	18	5.98 (± 0.17)	37 (± 10)	346 (± 23)
			5-15	18	5.98 (±0.17)	23 (± 4)	317 (± 23)
			15-30	18	5.85 (±0.15)	18 (± 6)	287 (± 28)
			30-45	18	5.76 (± 0.15)	128 (± 7)	235 (± 26)
Limestone			0-5	18	6.22 (± 0.11)	104 (± 25)	783 (± 120)

Valley	Capshaw, Cedarbluff, Chenneby, Colbert, Dickson, Hollywood, Holston, Linsdside, McQueen, Taft, Tupelo,Wickham	Alfisol, Inceptisol, Ultisol,	5-15	18	6.24 (± 0.12)	33 (± 10)	460 (± 57)
			15-30	18	6.07 (± 0.16)	8 (± 2)	300 (± 32)
			30-45	18	5.8 (± 0.20)	5 (± 1)	256 (± 30)
Piedmont Plateau	Lousia, Madison, Mantachie	Inceptisol, Ultisol	0-5	18	5.92 (± 0.14)	130 (± 29)	1035 (± 98)
			5-15	18	5.91 (± 0.15)	52 (± 14)	560 (± 45)
			15-30	18	6.04 (± 0.17)	12 (± 3)	350 (± 26)
			30-45	18	6.02 (± 0.15)	4 (± 0)	327 (± 29)
Blackland Prairie	Faunsdale, Sucarnoochee, Sumter, Tuscumbia, Vaiden	Inceptisol, Vertisol	0-5	11	7.09 (± 0.31)	81 (± 17)	1266 (± 155)
			5-15	11	7.25 (± 0.39)	44 (± 11)	893 (± 157)
			15-30	11	7.09 (± 0.50)	17 (± 4)	616 (± 119)
			30-45	11	7.16 (± 0.48)	13 (± 3)	586 (± 117)

Blackland Prairie soils were extracted with Mississippi/Lancaster solution and all other soils were extracted with Mehlich-1. STP is the soil test phosphorus values in mg kg^{-1} and TP is total phosphorus in mg kg^{-1}. Number of samples represents the actual number of soils analyzed for each depth within a soil region.

Table 2: The distribution of phosphorus fractions in five Alabama soil regions for 0-45 cm soil depths.

Soil region	Soil Depth (cm)	P Fractions				
		……..………..…………………………………mg kg^{-1} soil……………………………..…………………				
		H_2O-P_t	$NaHCO_3$-P_t	NaOH-P_t	HCl-P_t	Residue-P_t
Appalachian Plateau	0-5cm	36Da (3)	139Ba (11)	282Aa (29)	76Ca (14)	179ABa (15)
	5-15cm	22Da (3)	105Ba (15)	197Aa (26)	52Cab (13)	138ABab (13)
	15-30cm	13Cb (3)	63Ba (14)	105ABb (17)	22Cbc (4)	102Abc (9)
	30-45cm	6Bc (2)	29Ba (8)	75Ab (13)	14Bc (3)	92Ac (8)
Coastal Plains	0-5cm	8Ca (1)	91Ba (17)	140Aa (11)	12Ca (2)	95ABa (9)
	5-15cm	7Cab (1)	83Ba (18)	129Aa (10)	10Ca (2)	88ABa (7)
	15-30cm	6Cbc (2)	76Ba (21)	109Aa (9)	7Ca (2)	88Aa (7)
	30-45cm	4Bc (1)	64Ba (21)	71Aa (9)	4Ba (1)	93Aa (8)
Limestone Valley	0-5cm	32Ca (6)	105Ba (25)	270Aa (46)	119Ba (44)	256Aa (20)
	5-15cm	10Cab (2)	41Ba (13)	166Aa (22)	39Ba (12)	204Aa (17)
	15-30cm	3Bbc (1)	10Bb (3)	96Aa (14)	13Ba (4)	178Aa (15)
	30-45cm	2Bc (0.4)	4Bb (2)	73Aa (12)	9Ba (3)	169Aa (16)
Piedmont Plateau	0-5cm	39Ca (8)	178ABa (22)	366Aa (39)	131Ba (29)	321Aa (23)
	5-15cm	12Dab (2)	90Bab (13)	194Ab (23)	27Cab (6)	237Aab (21)
	15-30cm	5Eb (1)	34Cbc (5)	93Bc (9)	12Db (1)	199Ab (22)

	30-45cm	3Db (0.2)	22Cc (4)	70Bc (6)	10Cb (1)	221Ab (28)
Blackbelt Prairie	0-5cm	18Ca (2)	106Ba (13)	173Ba (29)	549Aa (107)	419Aa (28)
	5-15cm	8Ca (1)	65Ba (10)	107Bab (21)	356Aab (102)	357Aab (32)
	15-30cm	4Ca (0.4)	35Ba (3)	55Bbc (10)	223Ab (72)	300Aab (38)
	30-45cm	3Da (0.3)	30Ca (2)	48Cc (8)	210Bb (74)	295Ab (39)

H_2O-P_t is water extractable total phosphorus; $NaHCO_3$-P_t is $NaHCO_3$ extractable total phosphorus; NaOH-P_t is NaOH extractable total phosphorus; HCl-P_t is HCl extractable total phosphorus; Residue-P_t is recalcitrant total phosphorus. The values outside the parentheses are the mean values expressed in mg kg^{-1}, whereas the values inside the parentheses are the standard errors associated with the mean values for each soil depth in a particular soil region. The means differences were determined using Tukey–Kramer and all values are significant at $P < 0.05$. Means followed by the same uppercase letter within a row are not significantly different at $P < 0.05$ for the different P fractions (H_2O-P_t, $NaHCO_3$-P_t, NaOH-P_t, HCl-Pt, Residue-P_t) in a given soil region for that respective soil depth. Means followed by same lowercase letter within a column are not significantly different at $P < 0.05$ for a given phosphorus fraction in all five Alabama soil regions for the four depths (0-5, 5-15, 15-30, 30-45 cm).

2.7 Figures

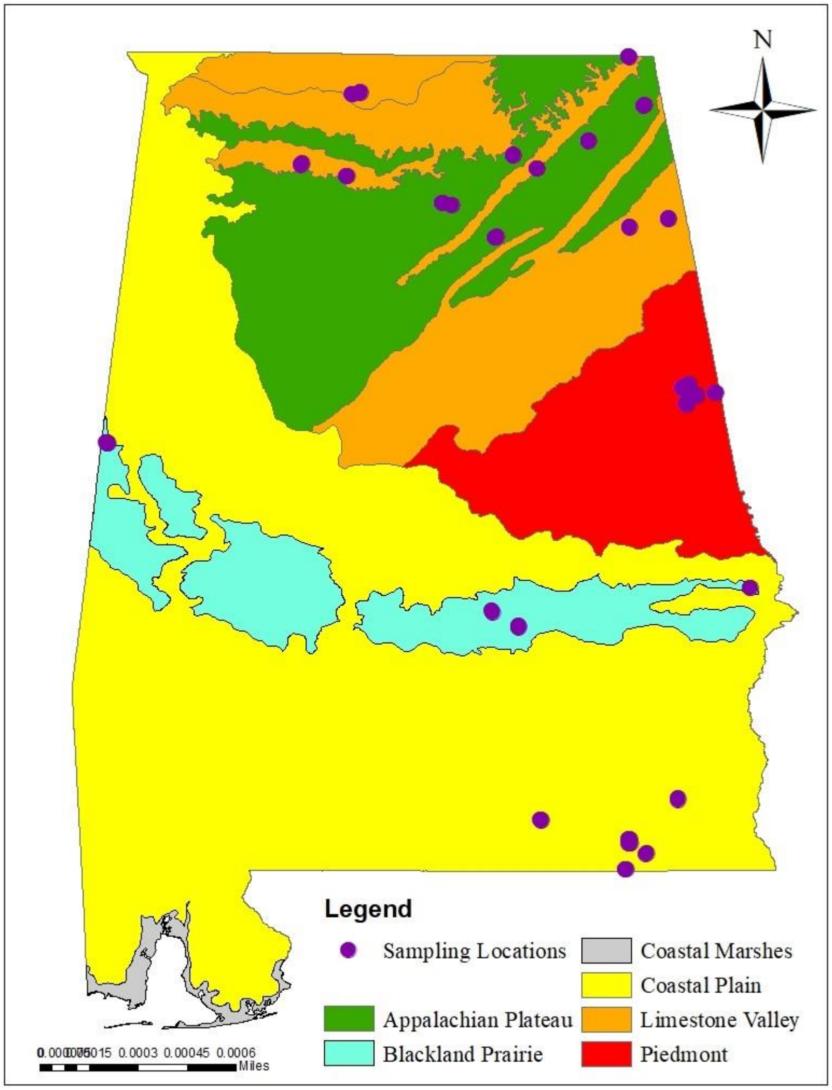

Figure 1: Major soil regions of Alabama and the dots represent the locations of sampling points for this study. Soil samples were collected from 26 farmer fields and at least three cores were collected from each sampling point.

Figure 2(a): Soil cores collected from Appalachian Plateau soil region of Alabama.

Figure 2(b): Soil cores collected from Coastal Plains soil region of Alabama.

Figure 2(c): Soil cores collected from Limestone valley soil region of Alabama.

Figure 2(d): Soil cores collected from Piedmont Plateau soil region of Alabama.

Figure 2(e): Soil cores collected from Blackbelt Prairie soil region of Alabama.

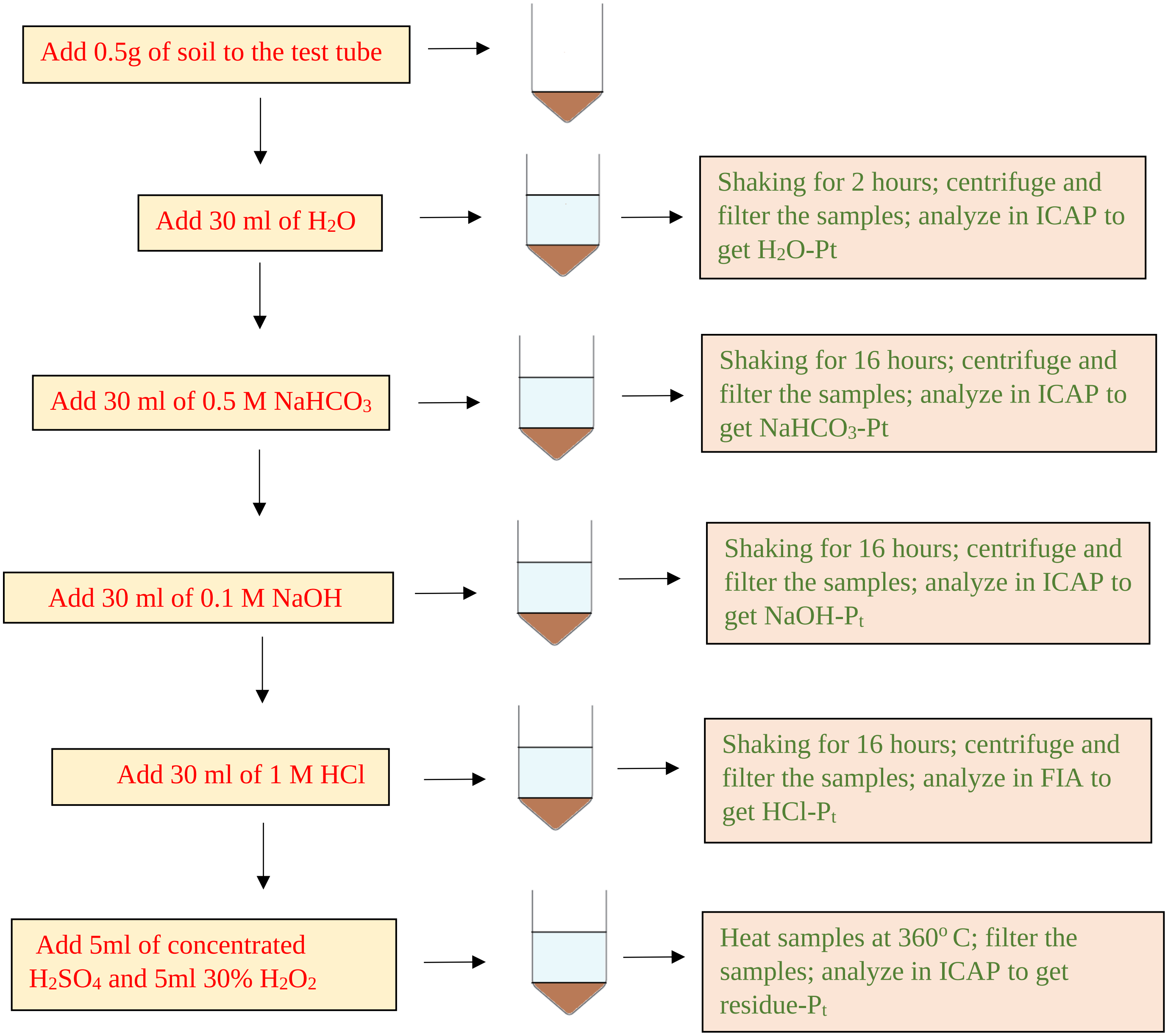

Figure 3: Phosphorus fractionation procedure (modified Hedley fractionation) used in this study.

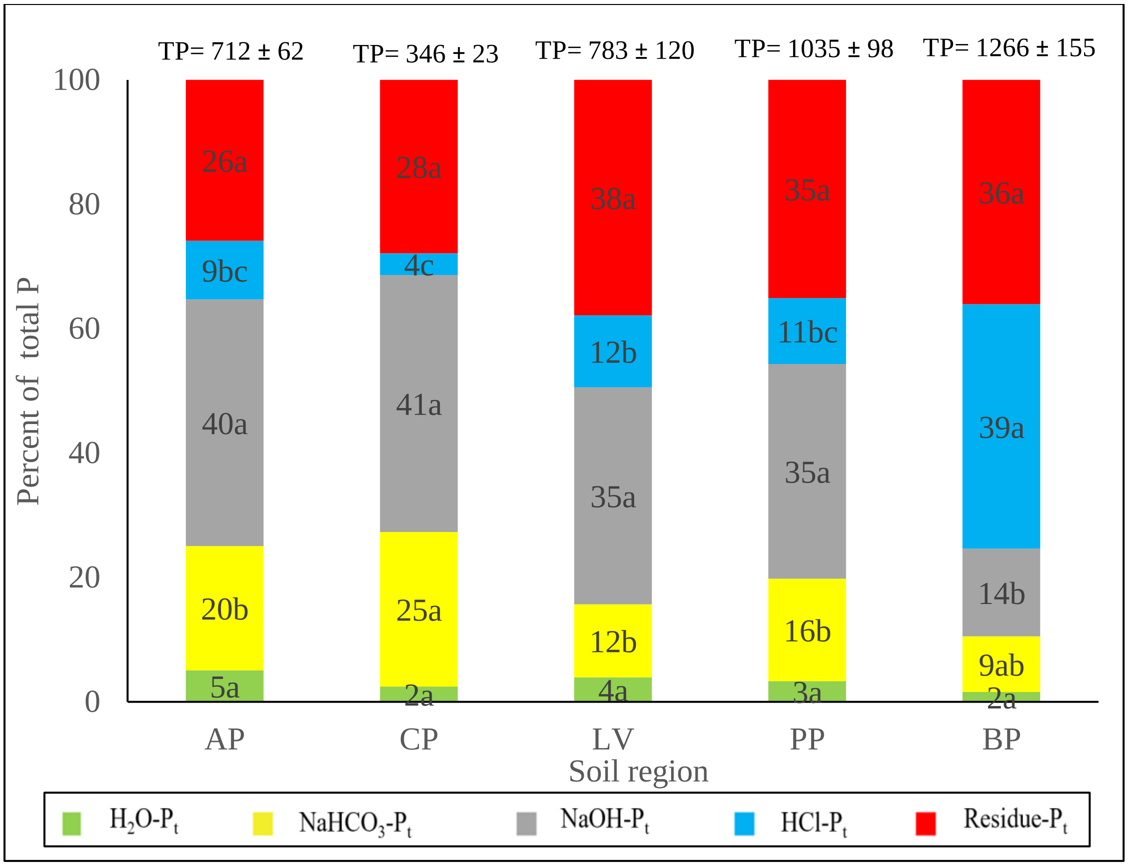

Figure 4: Stacked column graph showing the percent of P fractions in each of the five soil regions at 0-5 cm soil depth. Means followed by same letter for each P fraction are not significantly different at *P<0.05*. H_2O-P_t is water extractable total phosphorus; $NaCHO_3$-P_t is $NaHCO_3$ extractable total phosphorus; NaOH-P_t is NaOH extractable total phosphorus; HCl-P_t is HCl extractable total phosphorus; Residue-P_t is recalcitrant form of phosphorus and TP is total phosphorus in mg kg^{-1}, followed by standard error values. The TP values reported at the top of bar graphs are the average TP values and obtained by summing the individual P fractions. Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama. Means followed by same letter for each P fraction across five soil regions are not significantly different at $P<0.05$.

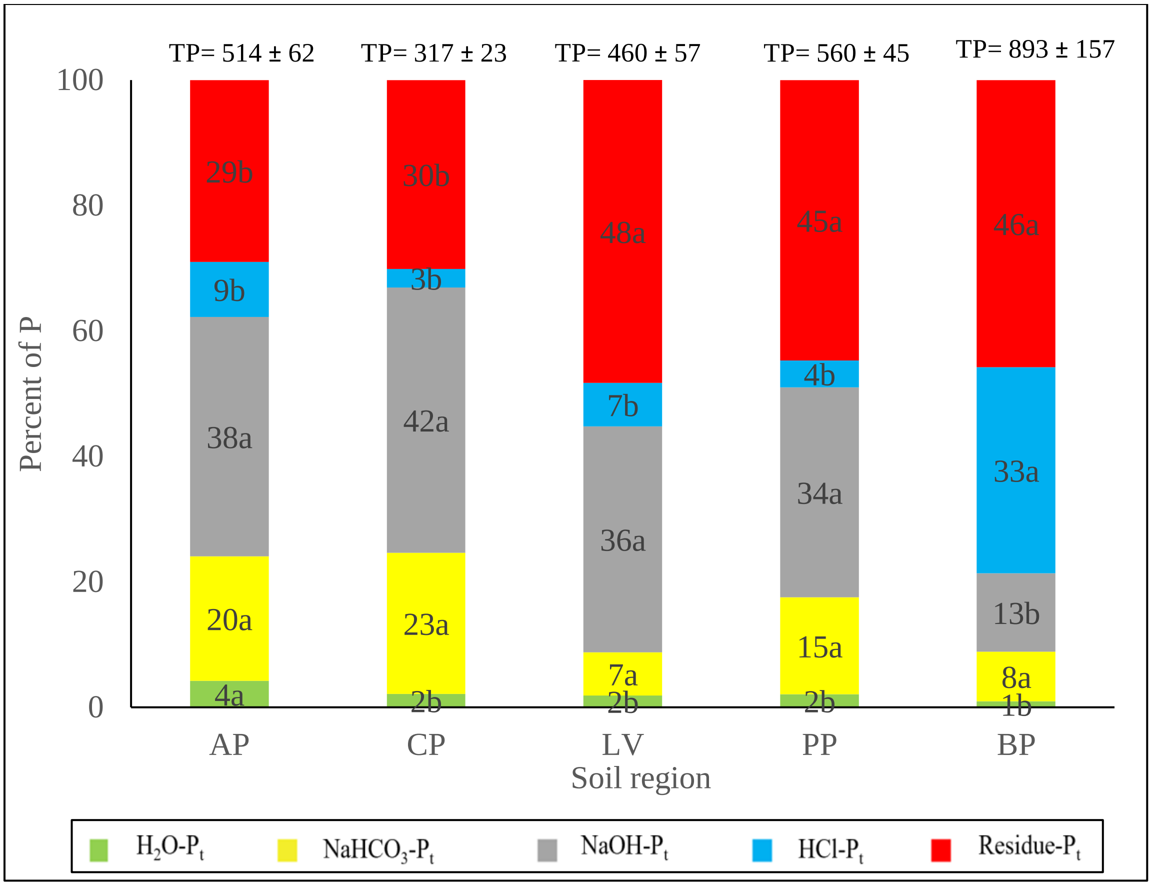

Figure 5: Stacked column graph showing the percent of P fractions in each of the five soil regions at 5-15 cm soil depth. Means followed by same letter for each P fraction are not significantly different at *P<0.05*. H_2O-P_t is water extractable total phosphorus; $NaCHO_3$-P_t is $NaHCO_3$ extractable total phosphorus; NaOH-P_t is NaOH extractable total phosphorus; HCl-P_t is HCl extractable total phosphorus; Residue-P_t is recalcitrant form of phosphorus and TP is total phosphorus in mg kg^{-1}, followed by standard error values. The TP values reported at the top of bar graphs are the average TP values and obtained by summing the individual P fractions. Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama. Means followed by same letter for each P fraction are not significantly different at *P<0.05*.

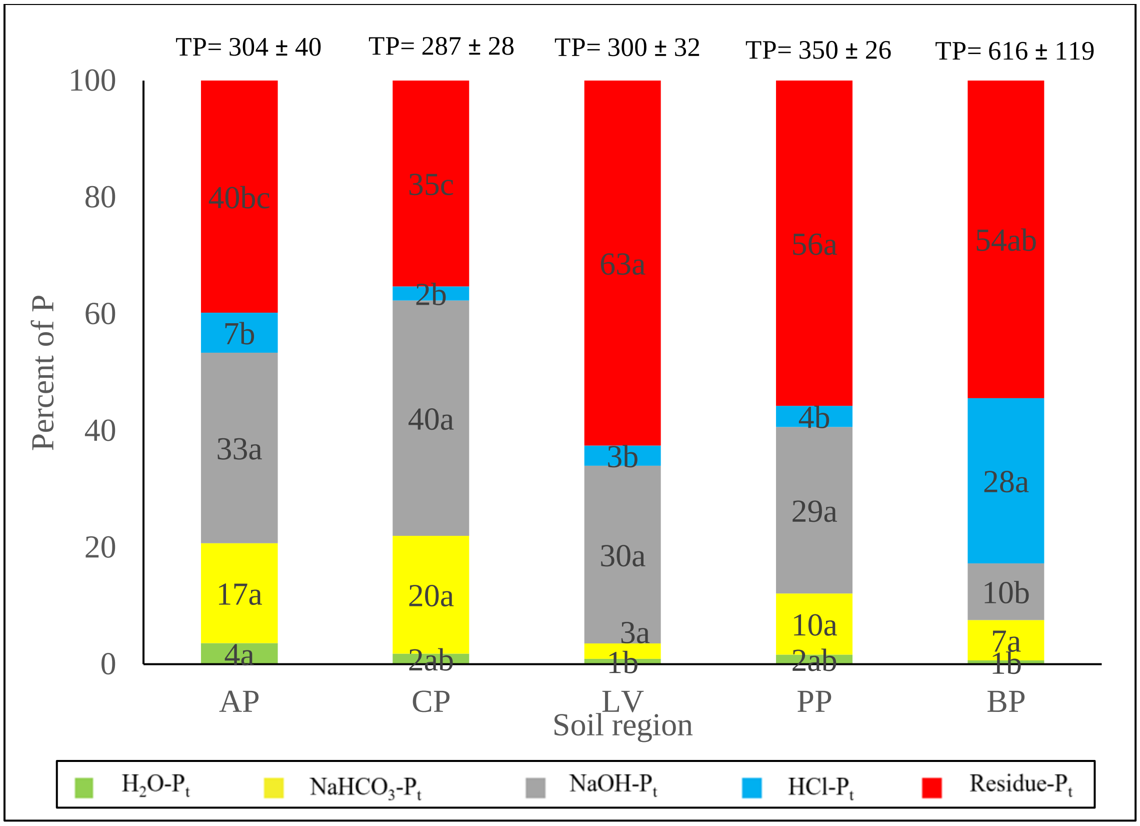

Figure 6.: Stacked column graph showing the percent of P fractions in each of the five soil regions at 15-30 cm soil depth. Means followed by same letter for each P fraction are not significantly different at $P<0.05$. H_2O-P_t is water extractable total phosphorus; $NaCHO_3$-P_t is $NaHCO_3$ extractable total phosphorus; NaOH-P_t is NaOH extractable total phosphorus; HCl-P_t is HCl extractable total phosphorus; Residue-P_t is recalcitrant form of phosphorus and TP is total phosphorus in mg kg^{-1}, followed by standard error values. The TP values reported at the top of bar graphs are the average TP values and obtained by summing the individual P fractions. Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama. Means followed by same letter for each P fraction are not significantly different at $P<0.05$.

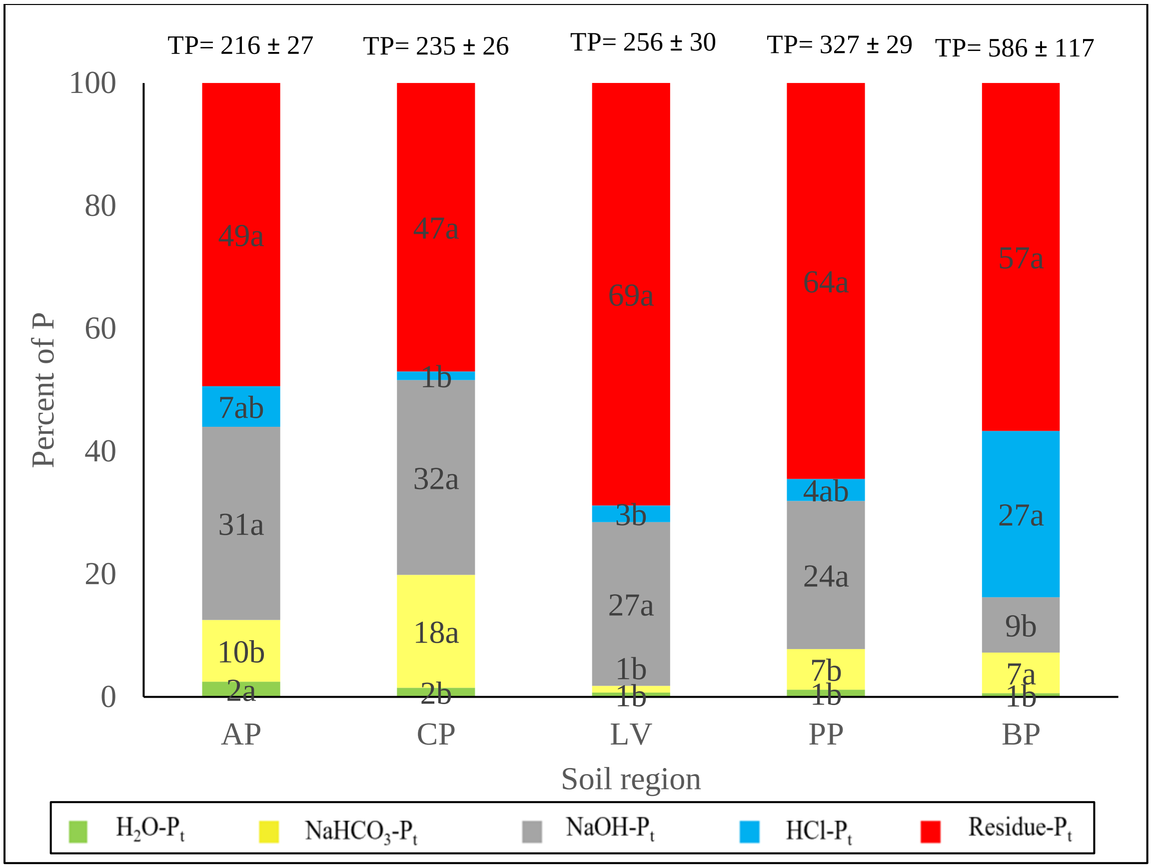

Figure 7: Stacked column graph showing the percent of P fractions in each of the five soil regions at 30-45 cm soil depth. Means followed by same letter for each P fraction are not significantly different at $P<0.05$. H_2O-P_t is water extractable total phosphorus; $NaCHO_3$-P_t is $NaHCO_3$ extractable total phosphorus; NaOH-P_t is NaOH extractable total phosphorus; HCl-P_t is HCl extractable total phosphorus; Residue-P_t is recalcitrant form of phosphorus and TP is total phosphorus in mg kg^{-1}, followed by standard error values. The TP values reported at the top of bar graphs are the average TP values and obtained by summing the individual P fractions. Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama. Means followed by same letter for each P fraction are not significantly different at $P<0.05$.

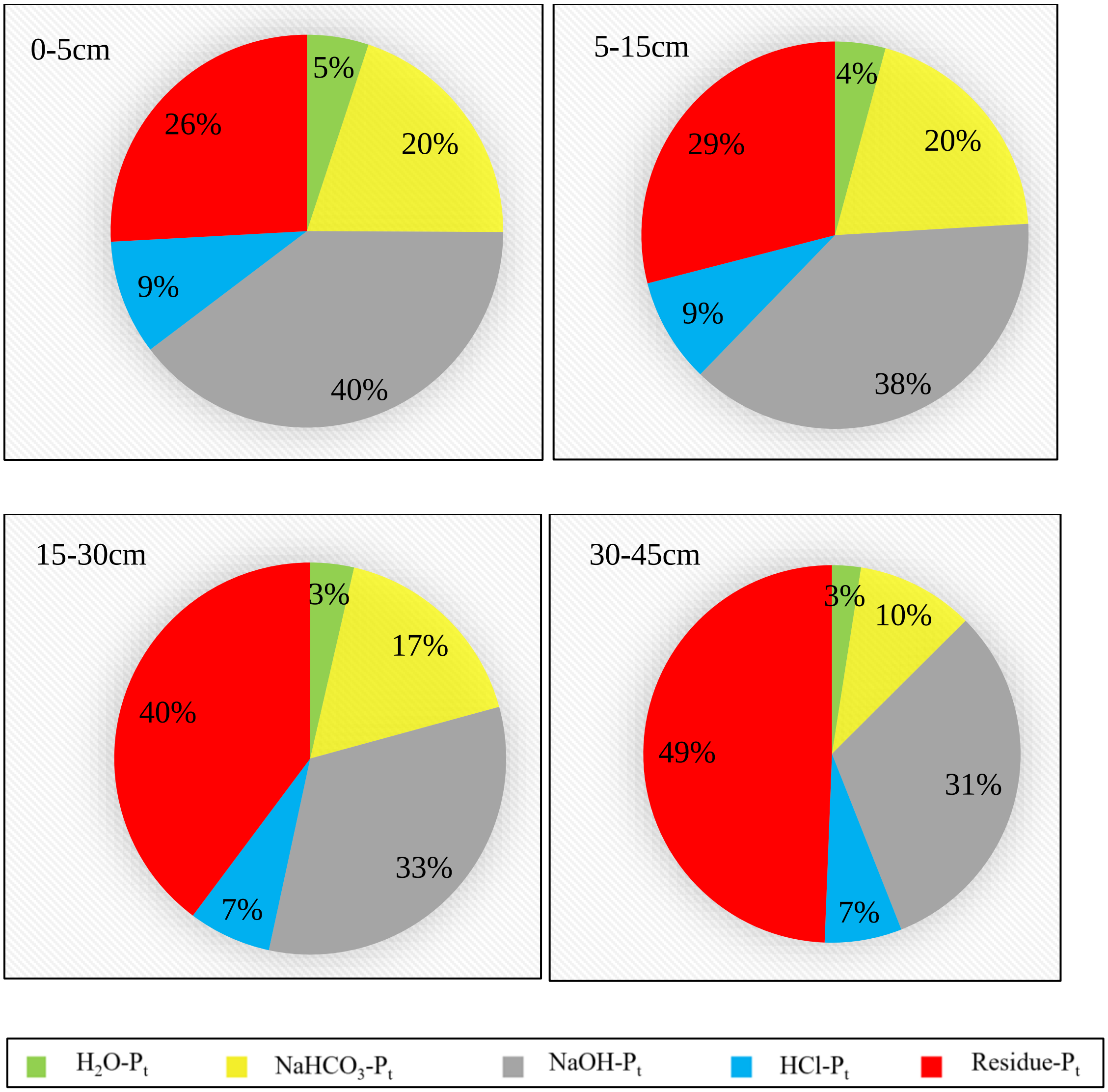

Figure 8(a): Percent distribution of P fractions in Appalachian plateau soil region between 0 to 45 cm of soil depth. H_2O-P_t is water extractable total phosphorus; $NaCHO_3$-P_t is $NaHCO_3$ extractable total phosphorus; NaOH-P_t is NaOH extractable total phosphorus; HCl-P_t is HCl extractable total phosphorus; Residue-P_t is recalcitrant form of phosphorus.

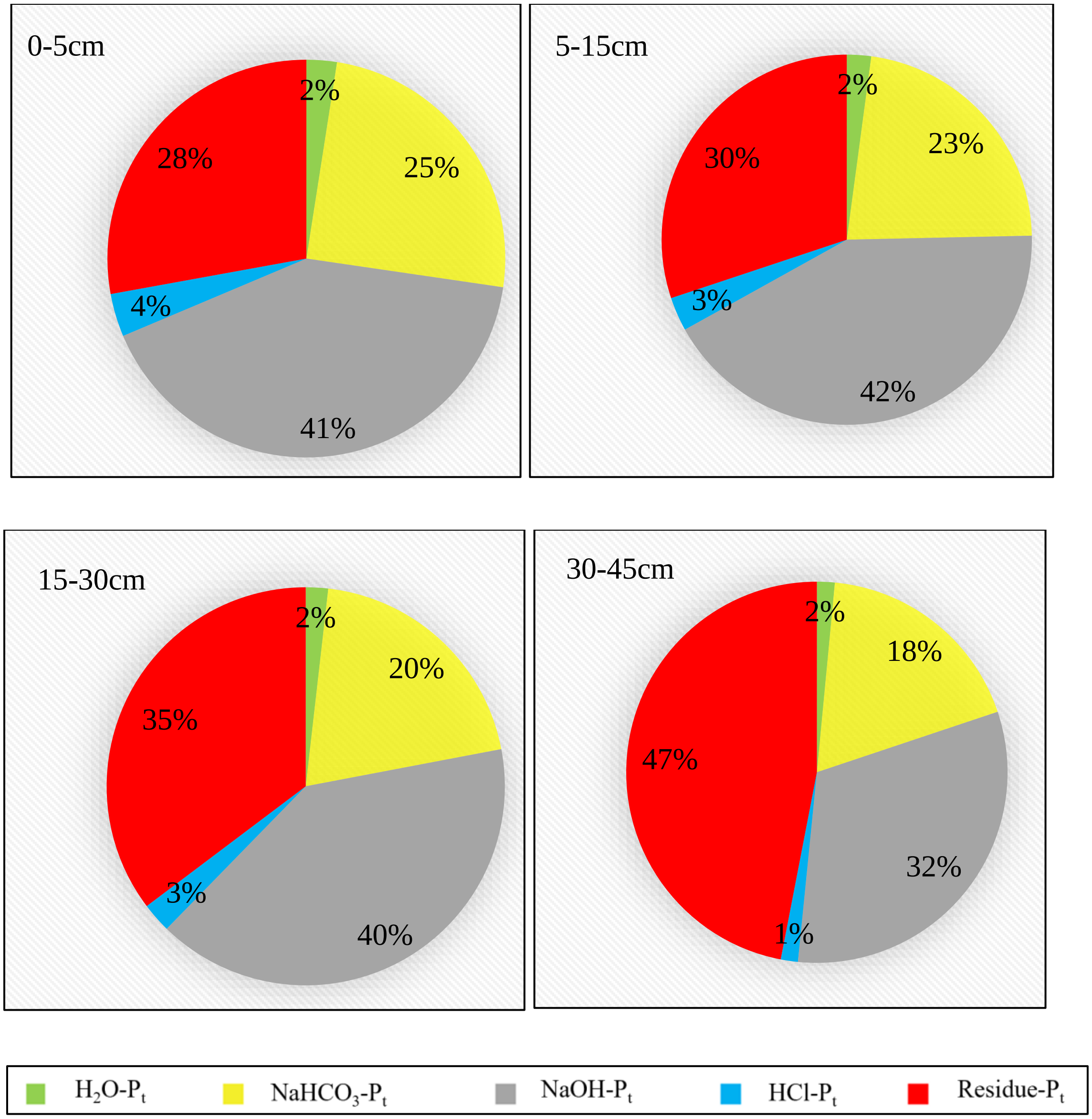

Figure 8(b): Percent distribution of P fractions in coastal plains soil region between 0 to 45 cm of soil depth. H_2O-P_t is water extractable total phosphorus; $NaCHO_3$-P_t is $NaHCO_3$ extractable total phosphorus; NaOH-P_t is NaOH extractable total phosphorus; HCl-P_t is HCl extractable total phosphorus; Residue-P_t is recalcitrant form of phosphorus.

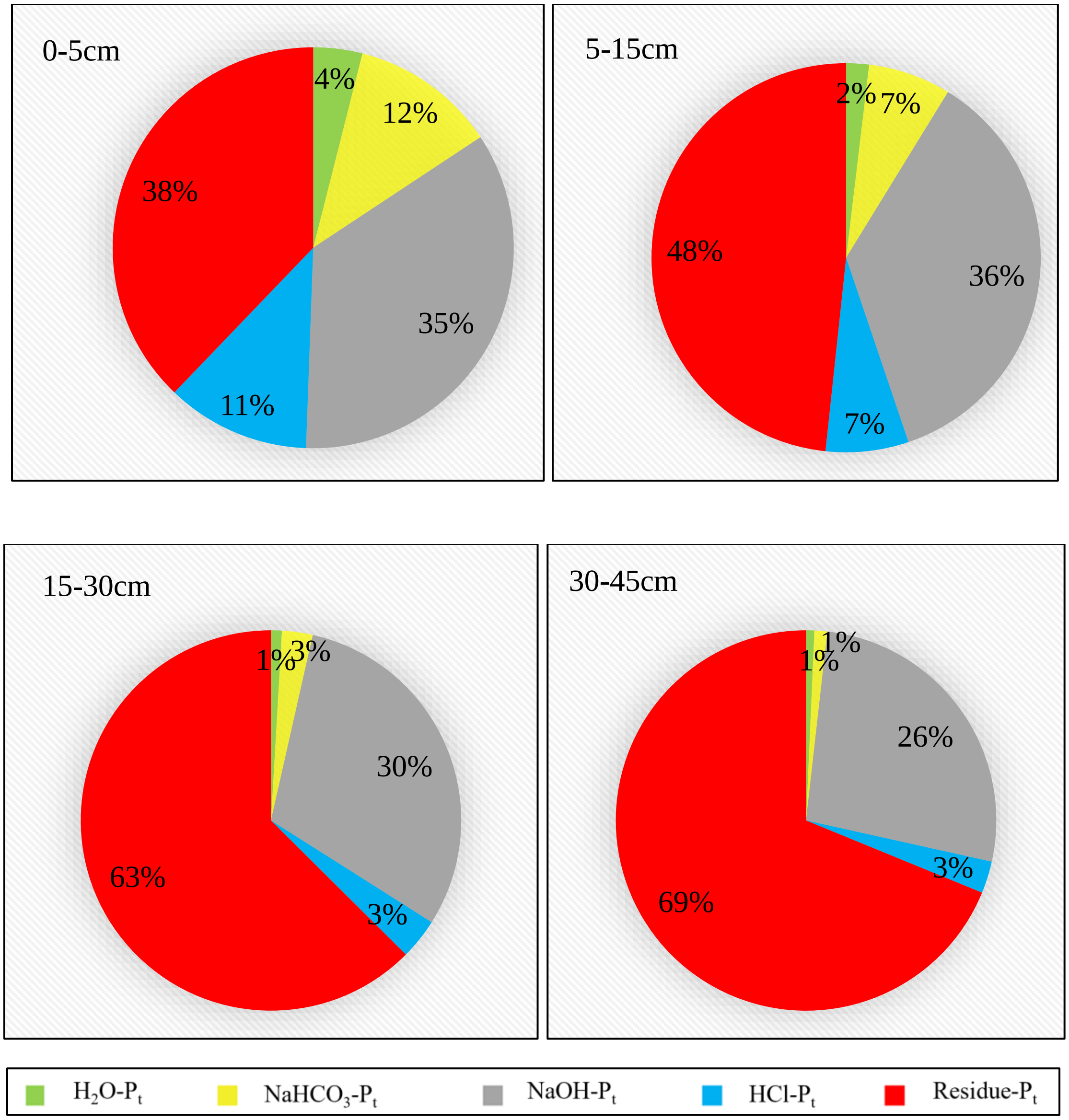

Figure 8(c): Percent distribution of P fractions in limestone valley soil region between 0 to 45 cm of soil depth. H_2O-P_t is water extractable total phosphorus; $NaCHO_3$-P_t is $NaHCO_3$ extractable total phosphorus; NaOH-P_t is NaOH extractable total phosphorus; HCl-P_t is HCl extractable total phosphorus; Residue-P_t is recalcitrant form of phosphorus.

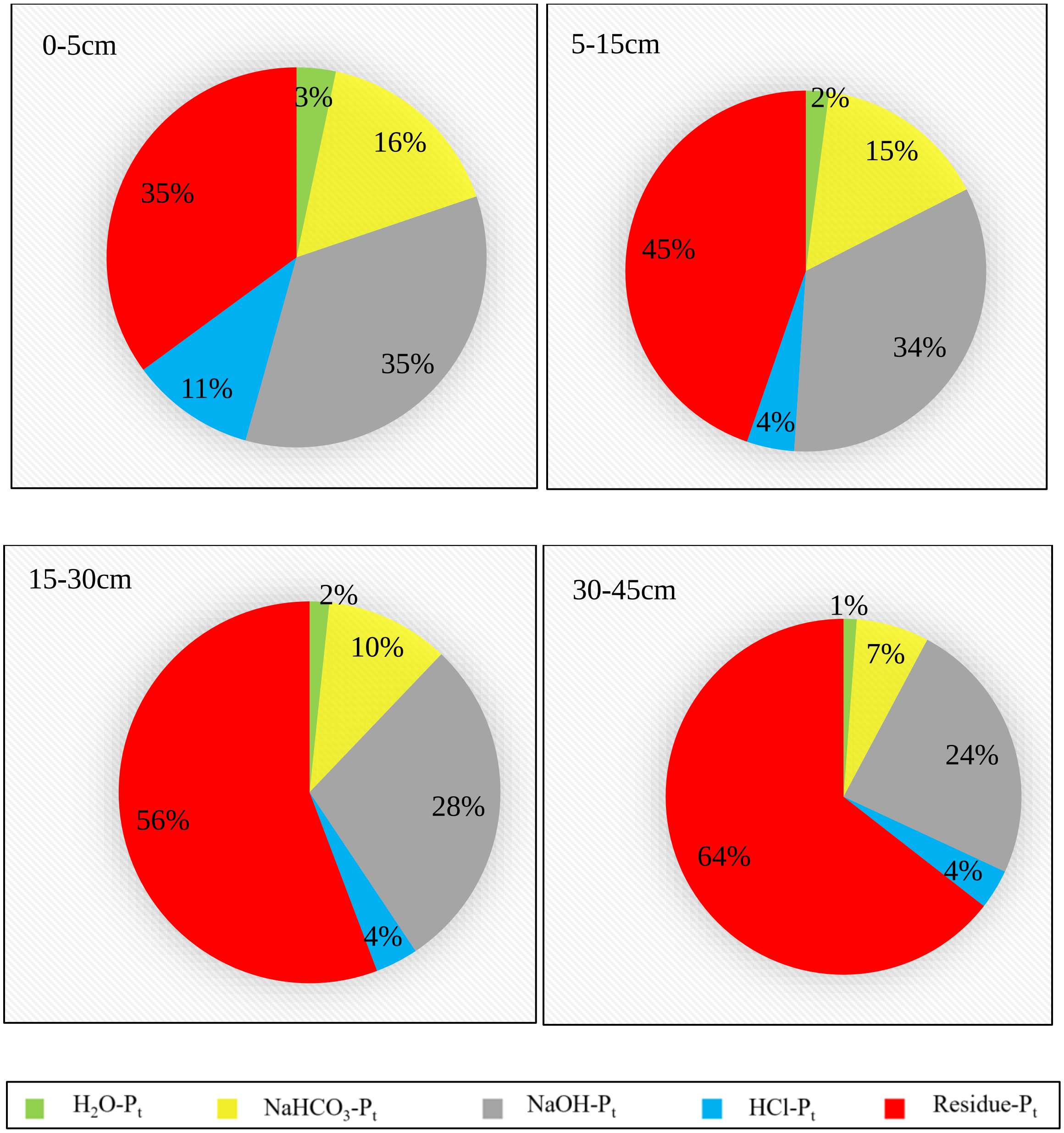

Figure 8(d): Percent distribution of P fractions in piedmont plateau soil region between 0 to 45 cm of soil depth. H_2O-P_t is water extractable total phosphorus; $NaCHO_3$-P_t is $NaHCO_3$ extractable total phosphorus; NaOH-P_t is NaOH extractable total phosphorus; HCl-P_t is HCl extractable total phosphorus; Residue-P_t is recalcitrant form of phosphorus.

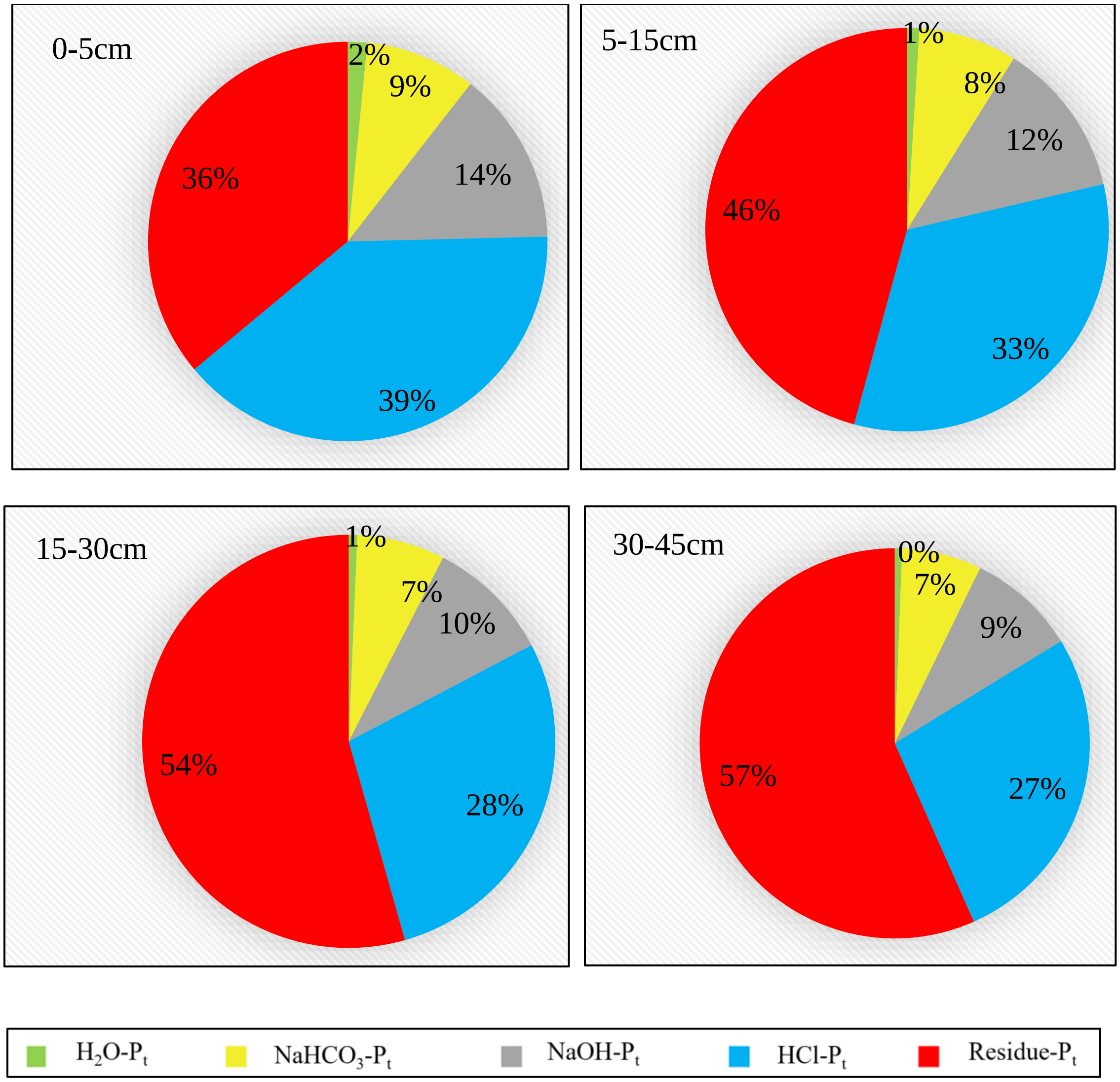

Figure 8(e): Percent distribution of P fractions in blackbelt prairie soil region between 0 to 45 cm of soil depth. H_2O-P_t is water extractable total phosphorus; $NaCHO_3$-P_t is $NaHCO_3$ extractable total phosphorus; NaOH-P_t is NaOH extractable total phosphorus; HCl-P_t is HCl extractable total phosphorus; Residue-P_t is recalcitrant form of phosphorus.

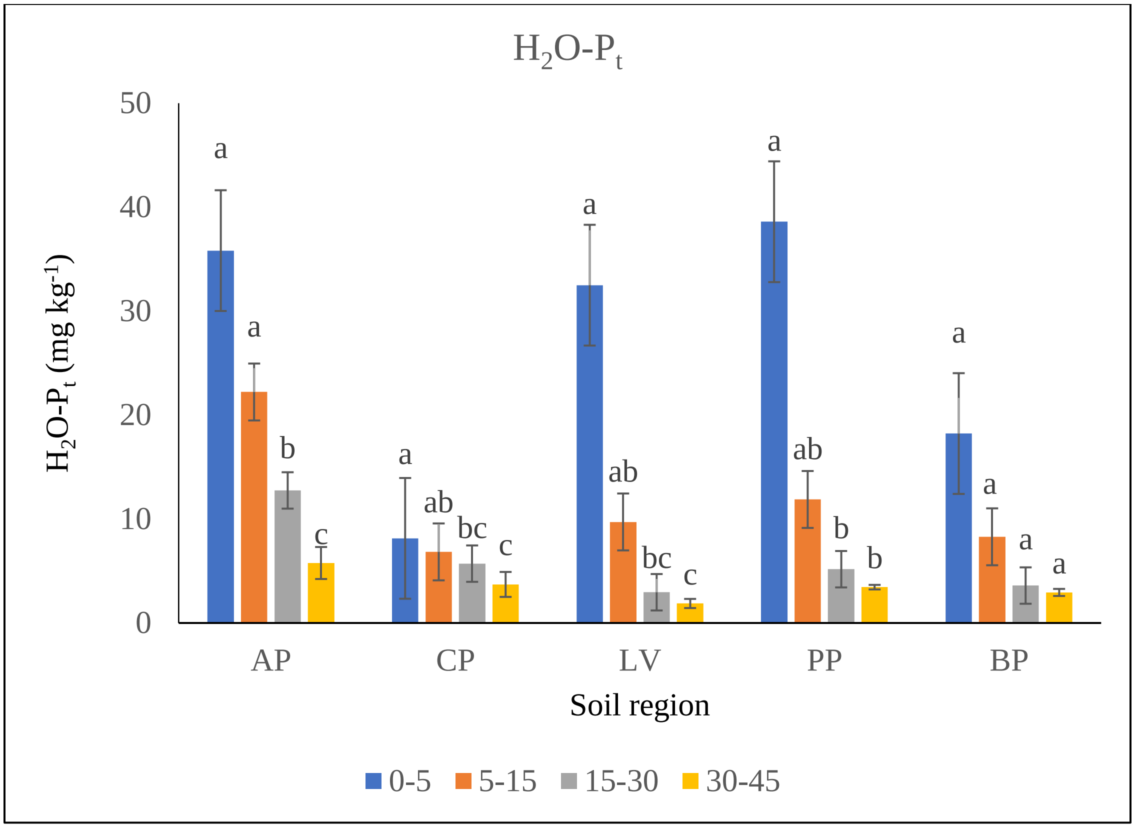

Figure 9(a): Bar plot showing mean H_2O-P_t (water soluble total phosphorus) in Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama for their respective depths (cm). Means followed by same letter for each soil region are not significantly different at *P<0.05*.

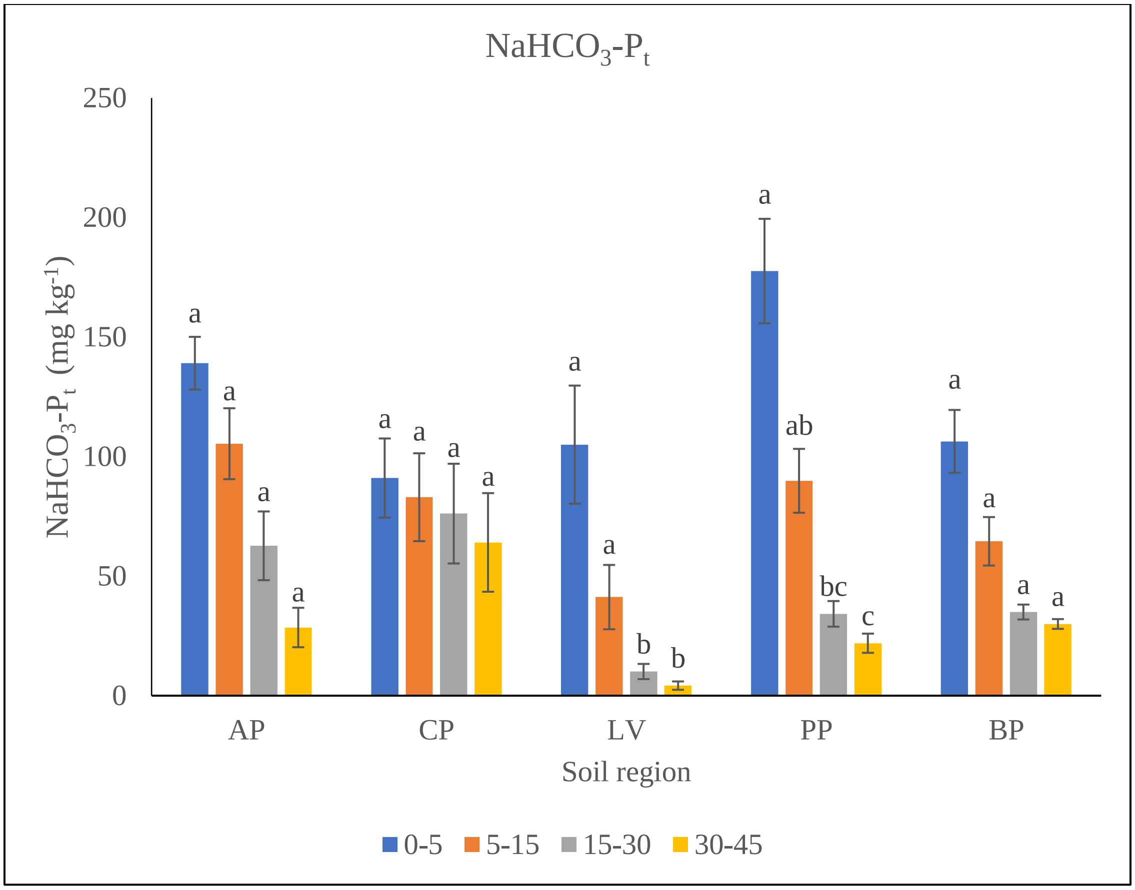

Figure 9(b): Bar plot showing mean $NaHCO_3$-P_t ($NaHCO_3$ extractable total phosphorus) in Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama for their respective depths (cm). Means followed by same letter for each soil region are not significantly different at $P<0.05$.

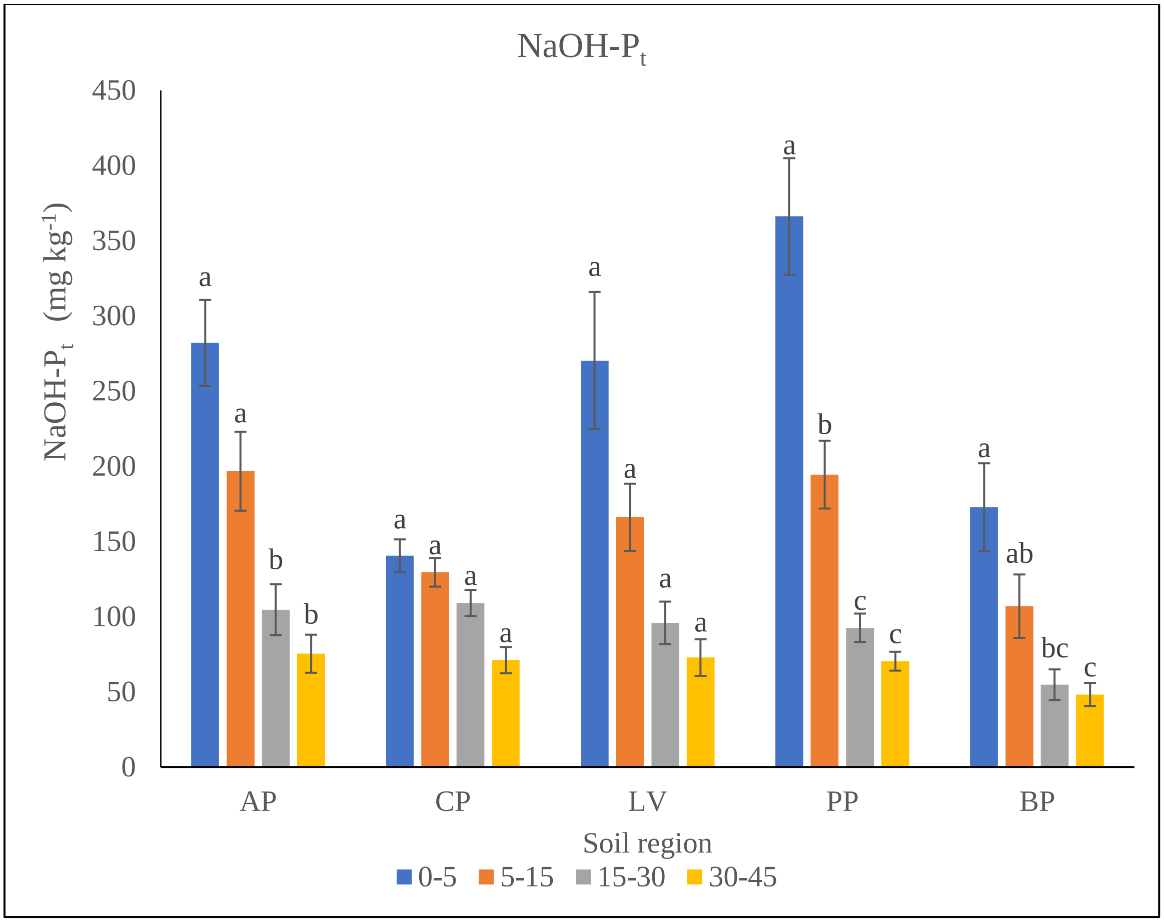

Figure 9(c): Bar plot showing mean NaOH-P_t (NaOH extractable total phosphorus) in Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama for their respective depths (cm). Means followed by same letter for each soil region are not significantly different at $P<0.05$.

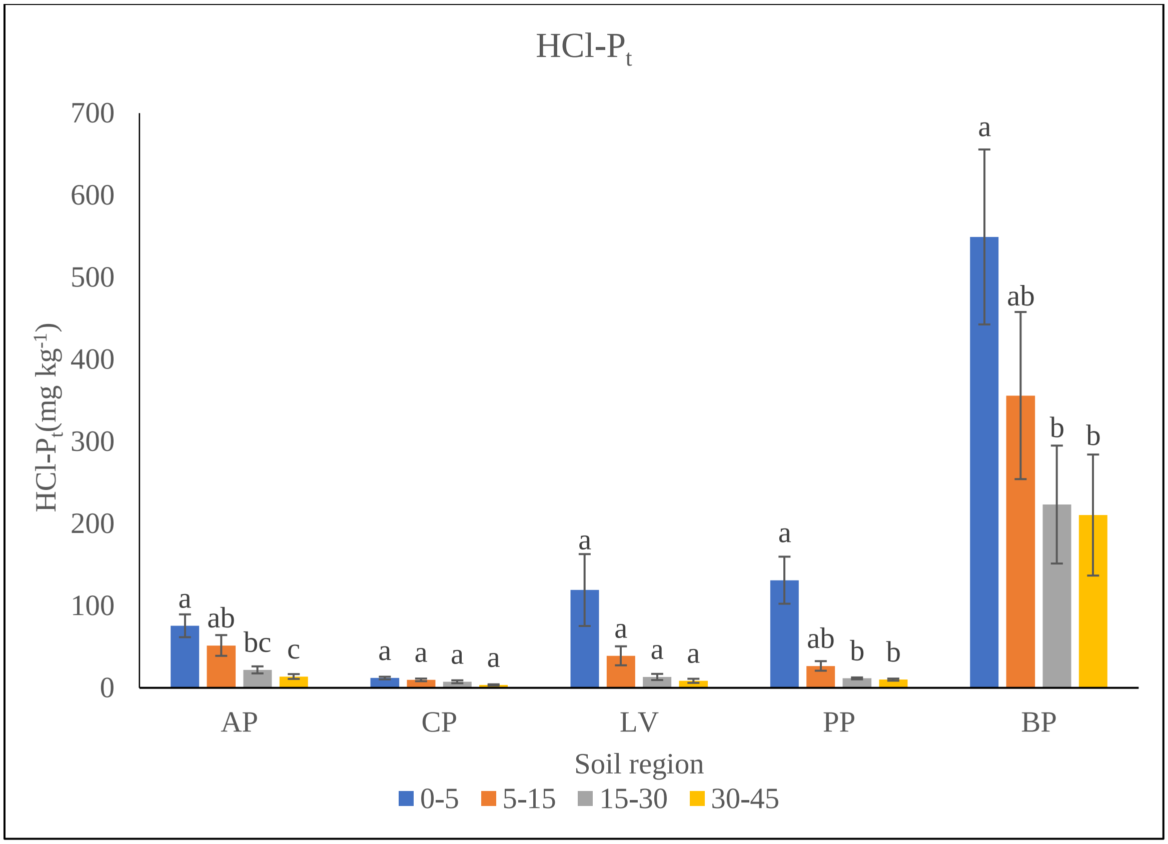

Figure 9(d): Bar plot showing mean HCl-P_t (HCl extractable total phosphorus) in Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama for their respective depths (cm). Means followed by same letter for each soil region are not significantly different at $P<0.05$.

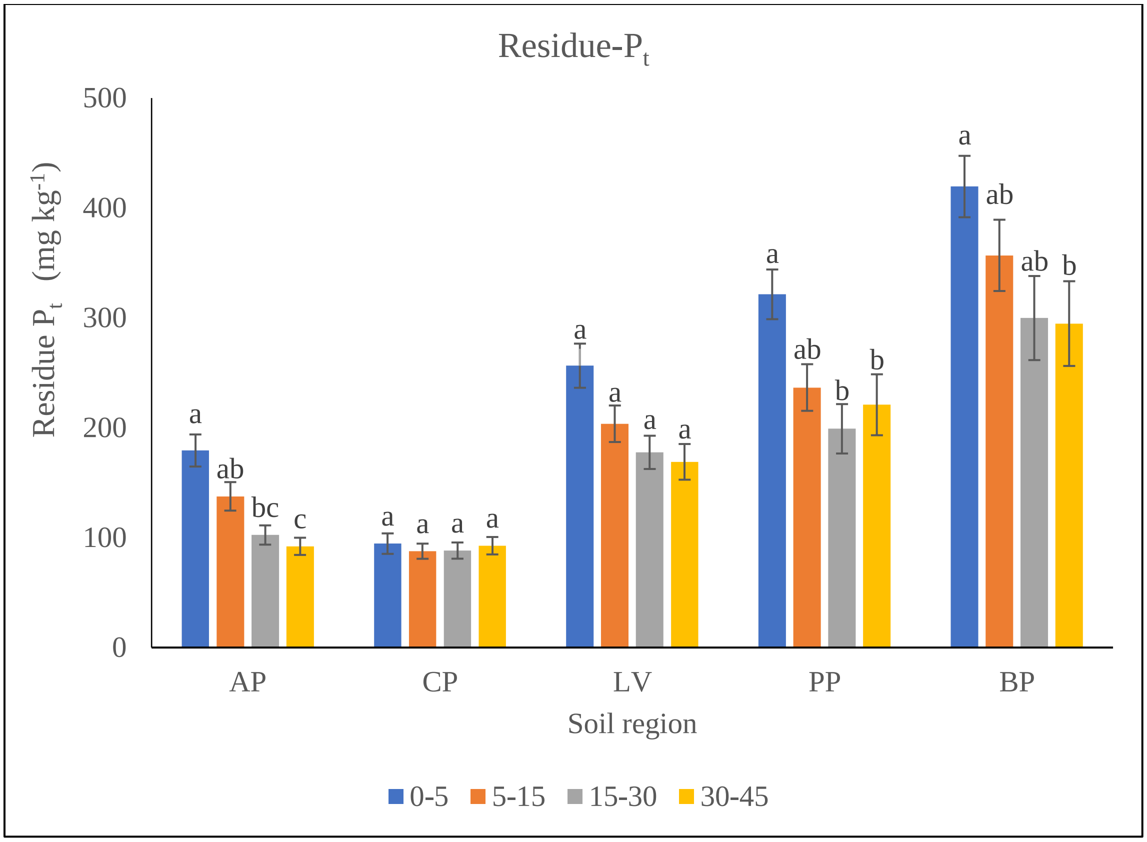

Figure 9(e): Bar plot showing mean residue-P_t (total residue phosphorus) in Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama for their respective depths (cm). Means followed by same letter for each soil region are not significantly different at $P<0.05$.

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CHAPTER-3

Distribution of Phosphorus Fractions in Soil with Different Soil Test Phosphorus Ratings in Alabama Soils Receiving Repeated Applications of Poultry Litter

3.1 Abstract

Alabama ranks second in the United States in poultry production and is one of the fastest growing agricultural industry in Alabama. Due to the expansion of the poultry industry, Alabama annually generates 1.25 million tons of poultry litter (PL) containing 19,000 tons of phosphorus (P). The safe disposal of PL in an economically and environmentally sound manner is a challenge faced by the poultry growers. The low bulk density of PL poses an economic challenge in long-distance transportation and hence, PL is applied locally to the agricultural lands in the vicinity of poultry complexes as a source of nutrient for plants. Additionally, the application of PL is based on the N requirement of the crops, which results in the over-application of P. The excess amount of P builds up in the soil over time and is an environmental concern. Soil test methods are used to determine the amount of P available for plant uptake, such as Mehlich-1 (M1) is used for non-calcareous soils and Lancaster (La) is used for calcareous soils in Alabama. However, soil test methods consider only available P that is important from agronomic viewpoint. This substantiates that P fractions measured by regular soil P tests are not the same for environmental purposes and therefore, the results and interpretations from routine soil P tests are not completely applicable for environmental purposes. We hypothesize that P pools vary between STP ratings. The objective of this study was to determine the fractions of P in Alabama soil regions for six P fertility ratings. The results indicated that when soil P fertility rating was very low (VL), majority of the P in all the Alabama soil regions was

present in the residue-P_t fraction (ranging between 18 to 90 % of the total P). However, when soil test P rating changed to extremely high (EH), most of the P was present in labile and moderately labile P forms. The presence of P in labile and moderately labile P forms for high STP values indicated a greater potential of P loss risk to the environment.

3.2 Introduction

Soils of Alabama have a long history of poultry litter (PL) application and its continued application in agricultural lands have been reported to be associated with increased level of soil extractable P (Kingery et al., 1994; Gartley and Sims, 1994; Mitchell and Tu, 2006). Kingery et al. (1994) reported an average increase of 530% in Mehlich-1 P levels at 0-60 cm depth due to the long-term application of PL in soils of Sand Mountain region of North Alabama. In the past 10 years, about 50% of the samples received by Auburn soil testing laboratory were observed to be high in soil test P (STP) as reported by Mitchell and Huluka (2012). Excess amount of P that accumulated in the surface soil is usually termed as legacy P (Gatiboni and Condron, 2021). This legacy P in soils is lost through surface runoff, leaching or sediment transport and is carried away to the water bodies, stimulating algal blooms and developing eutrophic conditions in the water bodies (Liao et al., 2019; Cordell et al., 2009). As per the reports by the United States Environmental Protection Agency (U.S. Environmental Protection Agency, 2017), agriculture is the primary source of water quality deterioration in waterbodies in the US. High STP is a potential threat to the water quality, however, low STP levels are known to diminish yields in corn and soybean as reported by Brooker et al. (2017) and Dodd & Mallarino (2005).

The amount of bioavailable P in soil, especially plant available P, is quantified using soil testing methods. Soil testing methods are observed to be inexpensive, widely used, and correlated with bioavailable and soluble P in soil (Sims et al., 2000). The most common extractants to determine P in soil are Mehlich-3 (Mehlich,1984), Mehlich-1 (Mehlich,1953), Lancaster (Cox,2001), Bray and Kurtz P1 (Bray & Kurtz,1945), and Olsen (Olsen et al.,1954). Different states in the USA have adopted different soil test methods for P to make interpretations for agronomic and environmental purposes. Soils in Alabama are categorized as calcareous and non-calcareous soils based on the calcium content. Mehlich-1 is the extractant used for P in non-calcareous soils and Lancaster (La) is used for calcareous soils in Alabama as a standard STP method for agronomic purposes (Hue and Evans, 1986; Evans and McGuire, 1990; Mitchell and Huluka, 2012). Based on STP level, soils are categorized into six fertility ratings as extremely high (EH), very high (VH), high (H), medium (M), low (L), and very low (VL). The interpretations from soil testing methods for P are aimed to determine the crop response to the added P sources. However, the P in soil serves as a potential threat to the surface water quality which is not accounted for in soil test methods. The soil test methods for P determination should serve both agronomic and environmental purposes considering the potential of a soil to serve as a source of water pollution. The extractants used for routine soil P testing are not efficient in extracting P pools that are important from environmental aspect (Gartley and Sims, 1994). It is also less known about the pools from which the extractants extract P in the STP method. This substantiates that P fractions measured by regular soil P testing extractants for environmental and agronomic uses are not the same and therefore, the results and interpretations from routine soil P tests are not completely applicable for environmental purposes (Gartley and Sims, 1994).

Phosphorus in soil exists in several inorganic and organic forms. To understand P dynamics in soil, P fractionation method has been traditionally used. Phosphorus fractionation is the sequential extraction of soil with acidic and basic extractants to quantify selective P pools. The P fractionation method is based on the assumption that each extractant extracts an individual fraction of P. Separation and determination of different P pools in soil is crucial to understand the dynamics and bioavailability of P in soil (Condron and Newman, 2011) and the method itself provides a quantitative estimate of various P pools.

The information about the P forms at different STP levels can be helpful in providing a better insight into the potential risks of P loss from agricultural farmlands. It is hypothesized that soil P fractions differ with STP fertility ratings in calcareous and non-calcareous soil regions of Alabama. Therefore, the objective of this study was to evaluate 1) the distribution of P fractions within a soil region that differ in STP fertility ratings; and 2) how P fractions changed when soil transitioned from EH to VL STP ratings.

3.3 Materials and Methods

3.3.1 Soil Sampling and STP determination

Soil sampling was done as described in chapter 2. Briefly, soil cores were collected from 26 agricultural fields representing five soil regions of Alabama namely Piedmont Plateau (PP), Appalachian Plateau (AP), Limestone Valley (LV), Coastal Plain (CP), and Blackbelt Prairie (BP) located within 32 counties. Three soil cores within a location in field were collected using a gas-powered soil corer and the samples were composited to represent 4 different depths of 0-5, 5-15, 15-30, and 30-45 cm. The SUURGO database (Soil Survey Staff, 2020) was used to obtain information on soil order and soil series information on each sampling location. Representative soil sample from individual depth was analyzed for M1 (non-calcareous soil) and La (calcareous soil) extractable P and fertility rating was assigned based on Mitchell and Huluka, (2012b). Soils in Alabama are categorized into four groups based on the estimated cation exchange capacity (ECEC) of the soils (Mitchell and Huluka, 2012b). Group 1 soils constitute sandy soils and includes Alaga, Dothan, Orangeburg, Ruston, and Troup soil series and have ECEC less than 4.6 cmolc kg^{-1} of soil. Group 2 soils constitute loamy and clayey soils and include Allen, Cecil, Hartsells, Lucedale, Madison, Pacolet, and Savannah soil series and possess an ECEC of 4.6 to 9.0 cmolc kg^{-1} of soil. Group 3 soils constitute clayey soils of Alabama (except the blackbelt soils) including Boswell, Colbert, Decatur, Dewey, Iredell and Talbott soil series, with an ECEC more than 9 cmolc kg^{-1} of soil. Group 4 soils constitute calcareous soils of blackbelt soil region. Group 4 soils include Houston, Leeper, Oktibbeha, Sumter, and Vaiden soil series. Soil samples from group 1, 2, and 3 were extracted with M-1 extracting solution whereas soil samples from group 4 were extracted using Lancaster extractant.

3.3.2 Chemical Analysis

Soil samples were homogenized, air-dried, and passed through 2 mm sieve prior to analysis. The pH of the soil samples was measured using deionized water at 1:1 (w/v) soil to water ratio. Soil samples were sequentially extracted using a modified Hedley fractionation method as described in chapter 2.

To measure available P, soil samples from non-calcareous soils were extracted with Mehlich-1 (M1) and samples from calcareous soils were extracted with Lancaster (La) solution. Mehlich-1 solution is a mixture of 0.0125 M H_2SO_4, and 0.05 M HCl. For M1 extraction, 5 g of soil sample was extracted with 20 ml of M1 solution (soil: extractant = 1: 4) and shaken for 5 minutes in a reciprocating shaker (Mehlich, 1953). Extracted samples were filtered through Whatman 42 filter paper and were analyzed using inductively coupled argon plasma spectrometry (ICAP; Spectro Ciros, Spectro Analytical Instruments Inc., Mahwah, NH).

Soil samples from BP soil region were extracted with Lancaster (La) solution. Extraction with La solution was a two-step process. First 5 g soil sample was treated with 5 ml of 0.05 M HCl solution for 10 minutes. Treatment with 0.05 M HCl was followed by the addition of 20 ml of extracting solution (1.58 M glacial acetic acid + 0.125 M malonic acid + 0.187 M malic acid + 0.037 M ammonium fluoride + 0.03 M aluminum chloride hexahydrate) and shaking for 10 minutes in a reciprocating shaker (Cox, 2001). Extracted samples were filtered through Whatman 42 filter paper and were analyzed using inductively coupled argon plasma spectrometry (ICAP; Spectro Ciros, Spectro Analytical Instruments Inc., Mahwah, NH).

Phosphorus concentration (mg kg^{-1}) obtained by M1 and La soil test was used to assign P fertility levels as extremely high (EH), very high (VH), high (H), medium (M), very low (VL), low (L) based on the STP fertility rating of Alabama (Table-1).

3.3.3 Statistical analysis

The analysis of variance was conducted using Proc GLIMMIX in SAS 9.4 (SAS Institute, 2013). The mean comparisons were done using Tukey–Kramer and the means were significant at $P<0.05$. For this analysis, sample locations were considered as a random factor and soil depth and soil region were considered as fixed factors in the model. The normality assumption was checked, and no data transformation was done prior to data analysis.

3.4 Results and Discussion

The different P fertility ratings and their respective range for STP values used for AL soil regions are presented in Table 1. The majority of the samples from 0-5 cm depth had a rating of EH, VH and H fertility. At 30 to 45 cm soil depth, a greater percent of samples had M, L and VL fertility rating (Table 2). The highest percent of samples for EH fertility rating were found in Limestone valley region totaling 50% at 0-5 cm of soil depth and least percent of samples for EH fertility rating were observed in CP soil region totaling 6% for 0 to 5 cm soil depth. None of the soils except PP soils had any sample for VL fertility rating at 0 to 5 cm soil depth. Greater percent of samples for EH, VH, and H fertility rating between 0 to 5 cm soil depth suggests that further application of P to these soils can potentially increase the P loss risk. With the depth increment from 0-5 to 30-45cm, the percent of samples for M, L, and VL fertility rating increased (Table 2).

3.4.1 Distribution of P fractions for each P fertility rating within a soil region

3.4.1.1 Non-calcareous soils

The long term, repeated application of PL to the soils resulted in the accumulation of P. This build-up of P in the soil is attributed to the saturation of soil P accumulation and adsorption capacity, indicating the potential for P loss from agricultural lands to the waterbodies (Xu et al., 2020a). The fractions of P extracted by different chemicals were categorized as labile P (H_2O-P_t, $NaHCO_3$-P_t), moderately labile P (NaOH-P_t, HCl- P_t) and non-labile/ stable P (residue-P_t). The percent contribution of P fractions to total P varied with P fertility rating in each soil region and is presented in Table 3.

The H_2O-P_t fraction is the most labile form of P and is readily available for plant uptake that greatly affects plant growth in agricultural farmlands (Hong et al., 2018). This P fraction may also help to estimate P loss to the environment by runoff as water is the medium of transportation for P loss to the waterbodies (Pote et al. 1996). The contribution of H_2O-P_t fraction to total P was observed to be greater when the concentration of P was higher in soil. The percent of H_2O-P_t fraction was significantly greater ($P<0.05$) for EH and VH in AP soil region; for VH in CP soil region and for EH in both LV and PP soil regions (Table 3). Consequently, the percent of H_2O-P_t decreased from EH to VL fertility rating in all four non-calcareous soil regions. These results demonstrate that when soil P levels are high, greater amount of H_2O-P_t fraction is available for plant uptake. However, as most of the samples with high P levels were observed in 0 to 15 cm of soil depth (Table 2), it indicates greater risk of P loss to the environment via surface runoff and erosion. Relatively greater amount of H_2O-P_t at surface soil than subsurface soil may raise concerns related to water quality as the labile H_2O-P_t fraction is highly exchangeable and has the potential to release P in the waterbodies (Ranatunga et al., 2013). The labile P movement from agricultural farmlands to water bodies via surface runoff stimulates algal growth leading to eutrophic conditions and is associated with deteriorated water quality (Hoorman et al., 2008; Rahman et al., 2021; and Correll, 1998).

The $NaHCO_3$ extracted P, along with H_2O-P_t, comprises labile pool and is readily available for uptake by plant and microbial communities as this pool readily desorbs from the surface of soil particles (Cross and Schlesinger, 1995; Hong et al., 2018; Mankolo et al., 2008). The inorganic P in labile pool is weakly adsorbed on surfaces and found in less soluble P minerals of sesquioxides and carbonates (Tiessen and Moir, 2008; Mattingly, 1975; Chakraborty and Prasad, 2021) and organic P in labile pool constitutes less stable, readily mineralizable forms of organic P (Bowman and Cole, 1978). In this study, the percent of $NaHCO_3$-P_t fraction, alike H_2O-P_t, was also observed to be greater for high P levels. Although, there was no significant difference between $NaHCO_3$-P_t fraction among six P fertility ratings in CP soil region, but the percent of $NaHCO_3$-P_t was significantly lower ($P<0.05$) for VL fertility rating in AP, LV, and PP soil regions (Table 3). Similar to the percent of H_2O-P_t fraction, $NaHCO_3$-P_t fraction percentage decreased with the transition of P fertility rating from EH to VL. The $NaHCO_3$-P_t fraction is labile form of P, therefore, in addition to plant availability, this P fraction poses similar threats to water quality as mentioned for H_2O-P_t fraction. Zhang et al. (2021) reported an increased risk of P loss to the environment due to the increase in the amount of labile and moderately labile P fractions owing to the long-term fertilization. The proportion of inorganic P in $NaHCO_3$-P_t fraction is balanced with soil solution P (Guardini et al. 2012) and organic P in $NaHCO_3$-P_t fraction is easily mineralizable that is regulated by microbial activity in the soil (Xavier et al. 2009). Hence, the amount of P in $NaHCO_3$-P_t fraction is considered to be available for plants that is agronomically desirable, but it is also associated with the possibility of environment loss risk (Ceretta et al. 2010b).

The P extracted by NaOH constitutes occluded P forms, organically bound P attached to Fe and Al hydrous oxides and insoluble phosphates of Fe and Al and is considered to be moderately labile P pool (Hedley et al. 1982; Tiessen and Moir, 2008; Cross and Schlesinger 1995; He et al., 2006). The ability of Fe, Al hydroxides to bind P and other anions was due to the positive charge and the surface area of these hydroxides (Kaňa et al. 2011; Jan et al., 2015; Detenbeck and Brezonik 1991; De Vicente et al. 2008).

These two metals, Fe and Al, are two most predominant metals in non-calcareous soils ($pH<7$) that are found to bind with P (Jan et al., 2015). The pH of NaOH extract is basic ($pH>11$) and the alkaline nature of NaOH leads to the dissolution of Al and Fe bound P substantiating the dominance of NaOH-P_t (Toor et al., 2020). The results from the present study indicated that the percent of NaOH-P_t fraction was different among all six P fertility ratings in AP, LV, and PP soil regions, however, no significant difference was observed between NaOH-P_t fraction among all the six P fertility ratings in CP soil region. In AP soil region, NaOH-P_t fraction percentage was significantly greater ($P<0.05$) for EH, VH, and M fertility rating than VL fertility rating contributing an average of 37%, 40%, 39%, and 27% to total P for EH, VH, M, and VL soil P fertility ratings (Table 3). In LV soil region, NaOH-P_t fraction was significantly greater for VH fertility rating than VL fertility rating and made 39% of total P for VH fertility rating as compared to 26% for VL fertility rating. Similarly, in PP soil region, NaOH-P_t fraction percentage was significantly greater ($P<0.05$) for VH, M, and L fertility rating than VL fertility rating (Table 3). The P bound to Fe and Al can be used by plants when the labile P fraction in soil is severely depleted following mineralization (Liang et al., 2017). Greater proportion of NaOH-P_t indicates an elevated amount of P for plant uptake and scope for crop mining (Chakraborty and Prasad, 2021). The NaOH-P_t fraction can serve as a source of plant-available P by mineralization (Randhawa et al., 2005). Under certain environment conditions, such as change in soil pH and microbial degradation, NaOH-P_t fraction can release inorganic P in the environment (Simpson et al., 2011; Chakraborty and Prasad, 2021; Wright, 2009; Richardson and Simpson, 2011). Moreover, degradation of organic matter can lead to chelation of Fe and Al by release of certain organic anions in the soil that results in reduced P adsorption, allowing more P to be lost to the environment through various transport mechanisms (Scherer and Sharma, 2002; Ranatunga et al., 2013).

Similar trend of P reduction from EH to VL fertility rating was also observed for HCl-P_t fraction (Table 3). The highest percent of HCl-P_t was observed for EH fertility rating in all the soil regions, however, there was no significant difference in HCl-P_t fraction in AP and CP soil regions among all the six P fertility ratings (Table 3). In contrary, the percent of HCl-P_t fraction in LV and PP soil regions was significantly greater ($P<0.05$) for EH fertility rating (Table 3) and the majority of the samples with high P concentrations are located in the surface layers of soil profile between 0 to 15 cm of soil depth. The HCl extracts the P bound with Ca (Tiessen and Moir, 2008; Cross and Schlesinger, 1995) and is considered to represent primary minerals as in apatite (Williams et al., 1980; Tiessen and Moir, 1993). The HCl-P_t pool is not readily available for plant uptake (Zhang et al., 2004) and is considered to be moderately labile P pool, alike NaOH-P_t (Chakraborty et al., 2021).

An important finding from this study was the significant increase in the percent of residue-P_t fraction when fertility rating changed from EH to VL. The residue-P_t pool is considered to be the most stable P pool constituting highly recalcitrant forms of P (Turner et al., 2005; Gatiboni and Condron, 2021). These recalcitrant forms of P are not extracted by any of the other extractants used in previous steps in the sequential P fractionation procedure. The residual P pool is not available for plant uptake due to the low solubility and low mineralization rates (Gatiboni and Condron, 2021). However, Sattari et al. (2012) reported that the residue-P_t accumulated in soils can serve as plant available P when external application of P sources is curtailed. The residue-P_t pool revealed a decreasing trend from VL to EH fertility rating with a corresponding increase in labile and moderately labile P fractions and the residue-P_t fraction percentage was significantly lower ($P<0.05$) for EH as compared to VL fertility rating in all the four soil regions. Similar findings were also reported by Chakraborty et al. (2021) for Piedmont soil region of Alabama. Chakraborty et al. (2021) reported a significant decrease in residue-P_t pool from VL fertility rating (82% of total P) to EH fertility rating (46% of total P). The results indicate that for VL fertility rating, the majority of the P is in residue-P_t pool, however, when M1-P increases and the soil P fertility rating transitions from VL to L, M, H, VH, and EH, more P accumulates in labile and moderately labile P pools. The increase in percent of labile and moderately labile P fractions with long term fertilization alludes to greater availability of P for plant uptake and also greater potential for P loss to the environment. These research findings are in alignment with the results reported by Zhang et al. (2020) who suggested that the long-term fertilization aggravates the proportion of labile and moderately labile P fractions that poses environmental risk due to the increased probability of P loss.

3.4.1.2 Calcareous soils

In calcareous BP soil region, none of the soil samples were observed to possess EH fertility rating. In addition to this, no significant difference was observed between the percent of H_2O-P_t, $NaHCO_3$-P_t, NaOH-P_t, and HCl-P_t fractions among VH, H, M, L, and VL fertility ratings (Table 2). However, the percent of residue-P_t fraction demonstrated a clear declining trend with transition from VL to VH fertility rating and consequently, the percent of residue-P_t fraction for VH fertility rating was significantly lower ($P<0.05$) than VL, L, M, H fertility ratings (Table 2). The similar trend of reduction in the percent of residue-P_t was also observed in non-calcareous soil regions viz. AP, CP, LV, and PP. This indicates that the repeated, long-term application of PL to the soils result in the accumulation of P in soil profile. The P accumulated in soil due to excessive P application can serve as a potential contributor of P to the aquatic environment leading to eutrophication (Sattari et al., 2012; van der Bom et al., 2019).

The decrease in residue-P_t from VL to VH was accompanied by a corresponding increase in the labile and moderately labile P fractions, although the increase was not statistically significant. The increase in the proportion of labile and moderately labile P fractions due to long term fertilization indicates increased P availability for plants, however, it is an environmental concern associated with increased potential for P loss to the environment (Zhang et al., 2021).

3.4.2 Comparison of P fractions between calcareous and non-calcareous soil regions for six P fertility ratings

The distribution of P fractions in different soil regions for EH, VH, H, M, L, and VL fertility ratings is presented in the Figures1a-f. For EH fertility rating, $NaHCO_3$-P_t was the dominant fraction contributing 44% to the total P in CP soil region (Figure 1a). In contrary, NaOH-P_t was the dominant fraction in case of LV, AP, and PP soil regions, accounting 35%, 37%, and 34% to the total P respectively (Figure 1a). Further, in case of VH fertility rating, NaOH-P_t was the dominant fraction in CP, AP, and PP soil regions and residue P_t was the dominant fraction in LV soil region. In calcareous BP soil region, none of the samples had EH fertility rating but had VH fertility rating where HCl-P_t was the dominant fraction making 43% of the total P (Figure 1). Moreover, when the P fertility rating transitioned from high P levels to VL, majority of the P was observed to be present in non-labile residue-P_t fraction. The residue-P_t fraction includes recalcitrant P forms and is not available for plant uptake (Guo et al., 2000), due to the low solubility and low mineralization rates (Gatiboni and Condron, 2021).

It was inferred from the results that when soil P levels were high (EH, VH, and H fertility ratings), majority of the P was present in labile and moderately labile P fractions. Additionally, the amount of P in moderately labile P fractions was greater than labile P fractions, demonstrating that the P added to soil is immobilized and accumulates in moderately labile P forms. Therefore, the long-term availability of P for plant uptake will depend on the mineralization of moderately labile P forms to sustain plant growth. It has been documented that more accumulation of applied P in less available P fractions serves as an extended source of P for the following crops (Pavinato et al., 2009). Conversely, majority of the samples with EH, VH, and H fertility rating were from the topsoil layer between 0 to 5 cm soil depth and greater amount of labile and moderately labile P pools in the topsoil surface suggests greater P availability for plant uptake, but it also indicates greater risk of P loss from the soil surface via surface runoff and erosion (Zhang et al., 2020). Hence, any additional input of P sources on the surface of high P fertility soils can potentially aggravate the risk of P loss from these soils (Chakraborty and Prasad, 2021).

In Alabama, around 1/6th of the surface area is covered by ponds, streams, wetlands, and rivers (Gurung et al., 2013). Around two-thirds of the water quality impairments in lakes and streams in Alabama result from non-point sources (runoff from agricultural and urban areas) (Alabama NPS, 2003). The constant loading of nutrients, both N and P, to the water results in eutrophic water bodies, stimulating algal blooms and impairing the quality of water. Although both N and P are reported to cause eutrophication, but it has been well documented that P is the main contributor of eutrophication and any efforts to reduce eutrophication should aim to reduce P loadings to the water bodies (Foy, 2005). In 2020, 443 waterbodies were listed in 303(d) list of impaired waterbodies in Alabama that included Alabama, Black Warrior, Blackwater, Cahaba, Chattahoochee, Chipola, Choctawhatchee, Coosa, Escambia, Escatawpa, Mobile, Perdido, Tallapoosa, Tennessee, Tombigbee, and Yellow river basins (ADEM 303(d) Information and Map, 2020). Out of 443 waterbodies, 35 were reported to be eutrophic due to the input of nutrients into the water systems. The waterbodies with deteriorated water quality due to the presence of nutrients are located in Elmore, Dallas, Perry, Blount, Cullman, Jefferson, Hale, Marengo, Houston, Etowah, Shelby, Mobile, Marshall, Madison, Morgan, Limestone, Lawrence, Lauderdale, Colbert, Pickens counties of Alabama. The number of impaired waterbodies for 303(d) list is projected to increase in 2022.

To combat the issue of eutrophic conditions in waterbodies, it is important that the management practices followed on the agricultural farmlands must ensure optimum P availability for plants and at the same time minimizes P release to the water bodies. The solution to this problem is to curtail the input of nutrients into the water systems (Carpenter, 2008). The STP method provides the amount of extractable P in soil that is correlated to the amount of P available for plant uptake and is used for fertilizer recommendation. The soils with same STP may indicate similar potential to release P in the environment, but the information about P pools would provide better information about the potential P loss risk to the environment. The soils with greater amount of labile P would be a bigger concern for environment as compared to another soil with similar STP but lower amount of labile P or more amount of P in non-labile P forms. The greater percent of soils in agricultural farmlands in US have high or above high P fertility levels which indicates that the STP methods have not proved helpful to prevent P buildup in soil to the levels that threaten water quality (Sharpley et al., 1994). Therefore, it is concluded that STP provides P amount that is hypothetically available for the plant, but no information is obtained regarding the forms of P in soil. So, STP along with the information regarding P fractions of different solubility in soil will help to develop better management practices to target high P soils and to mitigate the potential of environment P loss risk. In addition to this, both source and transport factors play a role to estimate P loss to the environment and the management practices followed will change with these factors. A major concern associated with agriculture and excessive P in soil is that any factor that promotes erosion will also increase P runoff to water bodies (Daniel et al., 1994). In case of CP soils, major concern would be to reduce leaching as P is transported to the groundwater via leaching. However, in case of other soil regions, P transport to water bodies can be reduced by focusing on the reduction of runoff and erosion.

To better manage soil P levels, the application of PL to the soil should be coupled with uniform and planned soil testing to prevent the build-up of excess P in soil. Phosphorus loss to the waterbody can be due to several factors and is affected by vegetative cover on the ground; method, rate and time of application; P form applied; and rainfall intensity after P application (Sharpley et al., 1998). So, these factors are to be considered for the development of effective management strategies to reduce P loss. There is need to adopt nutrient management techniques to apply nutrients to the soil at the right time, in right amount, with the right method and the right placement (Osmond and Line, 2017). Another important consideration can be to plant cover crops during Fall season that ensures minimum erosion from the soil surface (Lawrence and Benning, 2019). Phosphorus transport to water bodies can further be reduced by following reduced tillage or no-tillage practices with minimum soil disturbance that can help to minimize runoff and erosion from agricultural farmlands (Lawrence and Benning, 2019). Other edge-of-field practices such as construction of buffers, saturated buffers and blind inlets that are designed to settle the sediments and the nutrients bound to sediments will help to minimize the input of nutrients in the nearby water streams (Lawrence and Benning, 2019). It has been reported that treating PL with alum (aluminum sulfate) before application can fix the majority of the soluble P added to the soil and reduce the amount of P lost with runoff water (Kovar et al., 1999). Another effective strategy can be to convert PL into a stable nutrient source that can slowly release nutrients when needed by the plants. This will prevent instantaneous release of soluble P that typically runs off during storm events (Chakraborty et al., 2021). Additionally, sub-surface banding of PL has shown promise of reduced P loss risk. Lamba et al. (2012) reported significant reduction in nutrient loss via runoff by sub-surface banding of PL.

3.5 Conclusion

With the transition of fertility rating from VL to EH, the proportion of residue P_t fraction decreased significantly with the corresponding increase in labile and moderately labile P fractions. While STP is typically used as an index for external P application, the P fractionation provided a better understanding of the status of P in different pools. Higher amount of P in labile and moderately labile P pools increases the plant available P and also is an environmental concern due to the increased potential of P loss. Management practices should be targeted to reduce labile and moderately available pools in the surface soil. Sub surface P application might prove beneficial in soils that have clay subsurface and where groundwater is not shallow. In sandy soils, P leaching is a major concern hence P management should consider the depth of groundwater. Soil inversion may prove beneficial in regions where the surface soils have very high percentage of labile and moderately labile pool and subsurface has high amounts of recalcitrant P.

3.6 Tables

Table 1: Soil test phosphorus ratings based on soil regions of Alabama and extractable phosphorus concentration (Adopted from Mitchell and Huluka, 2012).

Soil Test P	Soil regions of Alabama				
	Appalachian Plateau	Coastal Plain	Limestone Valley	Piedmont Plateau	Blackland Prairie
	……………………………………… mg kg^{-1} ………………………………………				
Very Low	<7	<7	<4	<7	<10
Low	7-12	7-12	4-7	7-12	10-18
Medium	13-25	13-25	8-15	13-25	18-36
High	26-50	26-50	16-30	26-50	37-72
Very High	51-125	51-125	31-75	51-125	73-180
Extremely High	>125	>125	>75	>125	>180
Blackland Prairie soils were extracted with Mississippi/Lancaster solution and all other soils were extracted with Mehlich-1.					

Table 2: Percentage distribution and number of samples between 0-45cm soil depth for each soil test phosphorus (P) rating in five Alabama soil regions. Soil test P fertility ratings are based on Mehlich-1 extraction for non-calcareous soils (Appalachian Plateau, Coastal Plains, Limestone Valley, and Piedmont Plateau soil regions) and Lancaster extraction for calcareous soils (Blackbelt Prairie).

Soil Region	Soil Depth (cm)	Soil Phosphorus Fertility Rating						n
		% Distribution						
		EH	VH	H	M	L	VL	
Appalachian Plateau (AP)	0-5	40	50	5	5	0	0	20
	5-15	20	50	15	10	5	0	20
	15-30	15	10	20	20	20	15	20
	30-45	0	10	5	15	5	65	20
Coastal Plains (CP)	0-5	6	17	11	44	22	0	18
	5-15	0	11	33	17	11	28	18
	15-30	0	11	11	6	22	50	18
	30-45	6	0	6	0	6	83	18
Limestone Valley	0-5	50	17	22	11	0	0	18

(LV)	5-15	17	17	0	28	39	0	18
	15-30	0	0	17	11	22	50	18
	30-45	0	0	6	6	33	56	18
Piedmont Plateau (PP)	0-5	44	17	17	0	6	17	18
	5-15	17	22	17	11	17	17	18
	15-30	0	0	17	17	6	61	18
	30-45	0	0	0	0	11	89	18
Blackbelt Prairie (BP)	0-5	0	55	0	45	0	0	11
	5-15	0	27	18	9	36	9	11
	15-30	0	0	9	36	0	55	11
	30-45	0	0	0	18	36	45	11

EH is extremely high P fertility rating; VH is very high P fertility rating; H is high P fertility rating; M is medium P fertility rating; L is low P fertility rating; VL is very low P fertility rating.

n= number of samples for each P fertility rating at a given soil depth in five Alabama soil regions.

Table 3: Percent distribution of phosphorus (P) fractions for each soil test P fertility rating across five Alabama soil regions. The values within bracket are standard error of means.

Soil Region	Soil Test Phosphorus Fertility Rating	P Fractions				
		...………………………% of total P……………….…….………….….				
		H_2O-P_t	$NaHCO_3$-P_t	NaOH-P_t	HCl-P_t	Residue-P_t
Appalachian Plateau (AP)	EH	5a (0.42)	21ab (1.42)	37a (2.01)	13a (1.69)	24c (1.73)
	VH	5a (0.23)	22a (0.92)	40a (0.82)	7a (0.61)	26c (1.05)
	H	3b (0.38)	17bc (1.71)	35ab (1.76)	8a (1.29)	37b (2.79)
	M	3b (0.42)	16bc (1.53)	39a (1.75)	5a (1.28)	37b (1.71)
	L	2b (0.29)	13c (1.52)	32ab (3.68)	7a (2.65)	46b (3.84)
	VL	2b	6d	27b	6a	58a

		(0.18)	(0.95)	(2.54)	(2.12)	(2.88)
Coastal Plains (CP)	EH	4ab (1.50)	44a (8.50)	32a (2.50)	4a (1.50)	18ab (5.50)
	VH	3a (0.71)	29a (6.41)	43a (4.72)	3a (0.97)	21b (4.34)
	H	3ab (0.36)	34a (5.23)	39a (4.08)	4a (0.60)	20b (1.35)
	M	2ab (0.22)	19a (3.72)	41a (2.26)	3a (0.53)	35b (4.20)
	L	2b (0.22)	26a (3.72)	38a (2.26)	3a (0.53)	31ab (4.20)
	VL	1b (0.14)	13a (3.16)	38a (2.21)	2a (0.26)	47a (3.32)
Limestone Valley	EH	5a (0.60)	15a (0.96)	35ab (2.13)	16a (3.09)	29d (1.84)
	VH	3b	8b	39a	8b	42c

(LV)		(0.40)	(0.86)	(3.09)	(0.85)	(3.02)
	H	2bc (0.25)	8b (0.57)	34ab (2.25)	7b (0.78)	50bc (2.65)
	M	2c (0.22)	5b (0.92)	35ab (3.09)	4b (0.83)	55b (3.96)
	L	1c (0.15)	3c (0.33)	32ab (1.14)	4b (0.69)	60b (1.54)
	VL	1c (0.17)	0d (0.19)	26b (2.52)	2b (0.57)	71a (2.65)
Piedmont Plateau (PP)	EH	4a (0.61)	19a (1.50)	34ab (1.31)	15a (1.96)	28c (1.19)
	VH	3ab (0.55)	19a (2.21)	38a (1.31)	6b (0.80)	34bc (3.54)
	H	3ab (0.41)	18ab (1.36)	39a (2.24)	4b (0.55)	37bc (3.09)
	M	2b	15ab	35ab	4b	44bc

		(0.37)	(2.76)	(2.85)	(0.60)	(4.89)
	L	1b (0.18)	11bc (1.61)	36a (3.15)	3b (0.65)	48b (4.60)
	VL	1b (0.11)	7c (0.81)	23b (1.94)	3b (0.48)	66a (2.46)
Blackbelt Prairie (BP)	VH	1a (0.26)	9a (1.42)	16a (1.91)	43a (4.80)	31b (1.62)
	H	1a (0.20)	9a (2.79)	17a (4.98)	25a (11.69)	48ab (4.10)
	M	1a (0.15)	7a (0.77)	12a (1.38)	32a (5.47)	48a (3.80)
	L	1a (0.08)	6a (0.71)	8a (0.94)	34a (5.67)	51a (4.30)
	VL	1a (0.06)	8a (0.60)	8a (1.63)	24a (3.74)	60a (2.72)

H_2O-P_t is water soluble phosphorus; $NaHCO_3$-P_t is $NaHCO_3$ extractable phosphorus; NaOH-P_t is NaOH extractable phosphorus; HCl-P_t is HCl extractable phosphorus; Residue-P_t is recalcitrant form of phosphorus.

EH is extremely high P fertility rating; VH is very high P fertility rating; H is high P fertility rating; M is medium P fertility rating; L is low P fertility rating; VL is very low P fertility rating.

The differences between means were determined using Tukey-Kramer and all values are significant at $P < 0.05$.

The values within bracket are standard error of means.

Means followed by same lowercase letter for each phosphorus fraction for 0 to 45 cm soil depth between soil regions are not significantly different ($P < 0.05$).

3.7 Figures

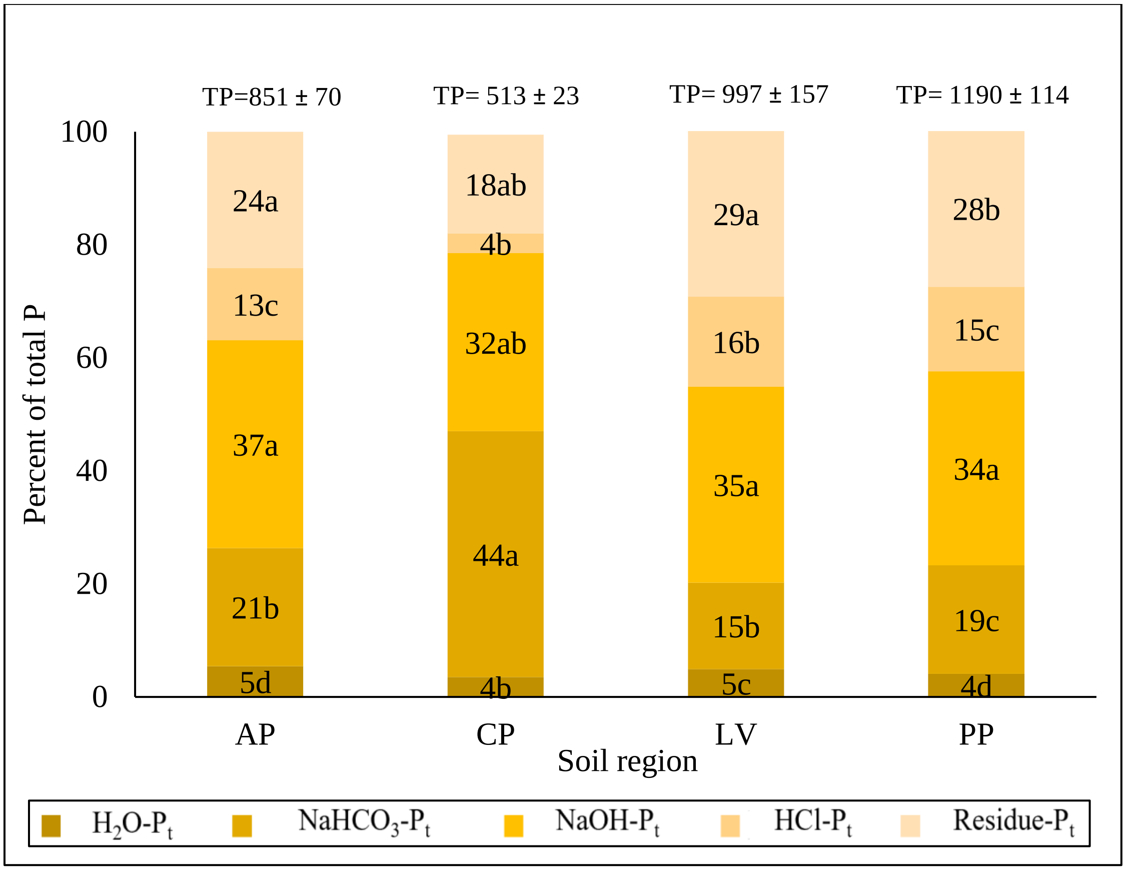

Figure 1(a): Stacked column graph showing the percent of P fractions in Alabama soil regions for extremely high (EH) soil test P. Means followed by same letter for individual P fraction between soil regions are not significantly different at $P<0.05$. TP is total phosphorus in mg kg^{-1}, followed by standard error values. The TP values reported at the top of bar graphs are the average TP values and obtained by summing the individual P fractions. Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama.

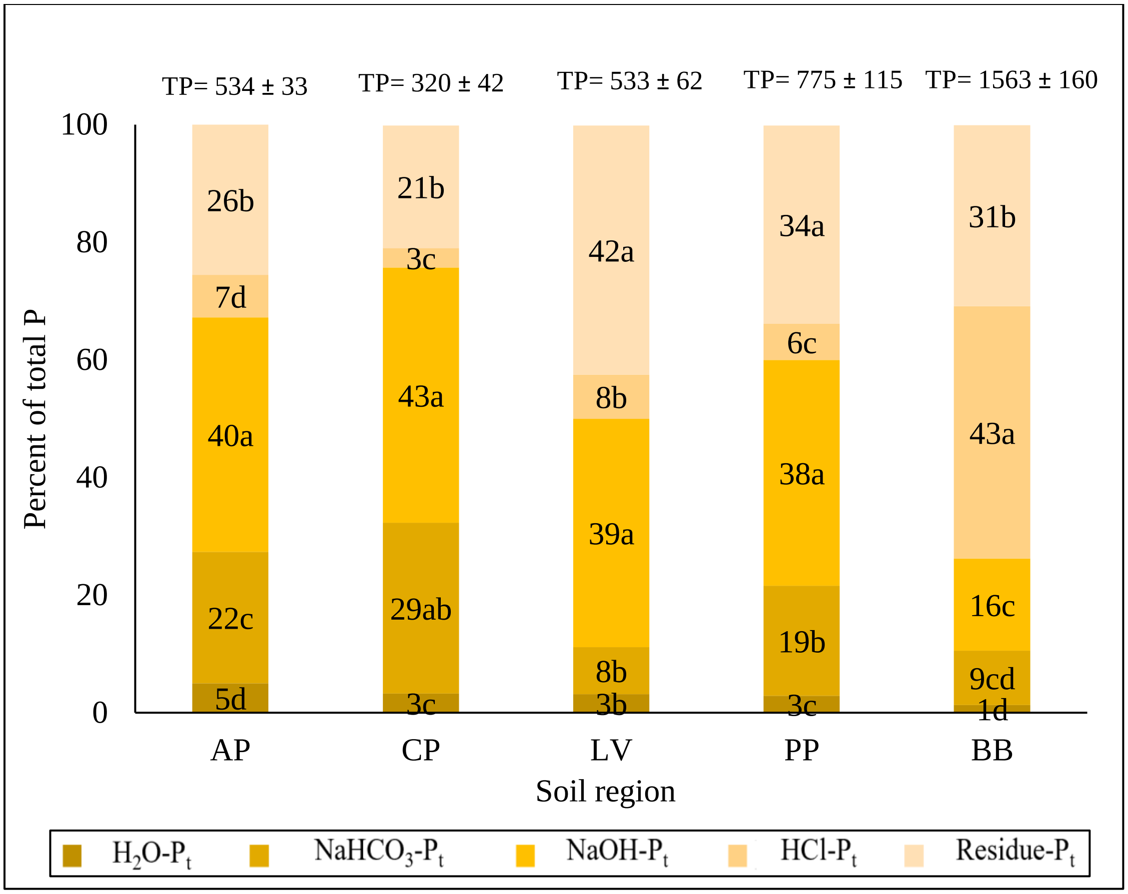

Figure 1(b): Stacked column graph showing the percent of P fractions in Alabama soil regions for very high (VH) soil test P. Means followed by same letter for individual P fraction between soil regions are not significantly different at $P<0.05$. TP is total phosphorus in mg kg^{-1}, followed by standard error values. The TP values reported at the top of bar graphs are the average TP values and obtained by summing the individual P fractions. Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama.

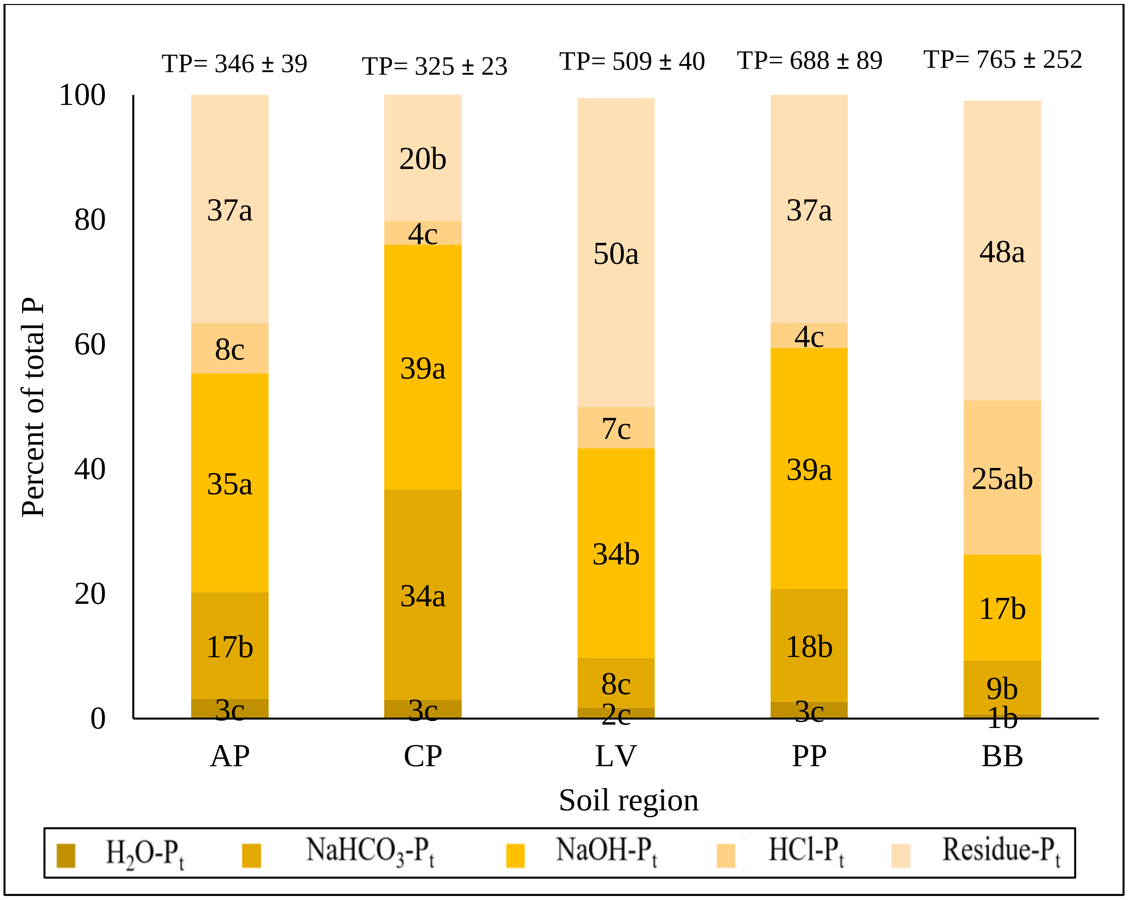

Figure 1(c): Stacked column graph showing the percent of P fractions in Alabama soil regions for high (H) soil test P. Means followed by same letter for individual P fraction between soil regions are not significantly different at $P<0.05$. TP is total phosphorus in mg kg^{-1}, followed by standard error values. The TP values reported at the top of bar graphs are the average TP values and obtained by summing the individual P fractions. Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama.

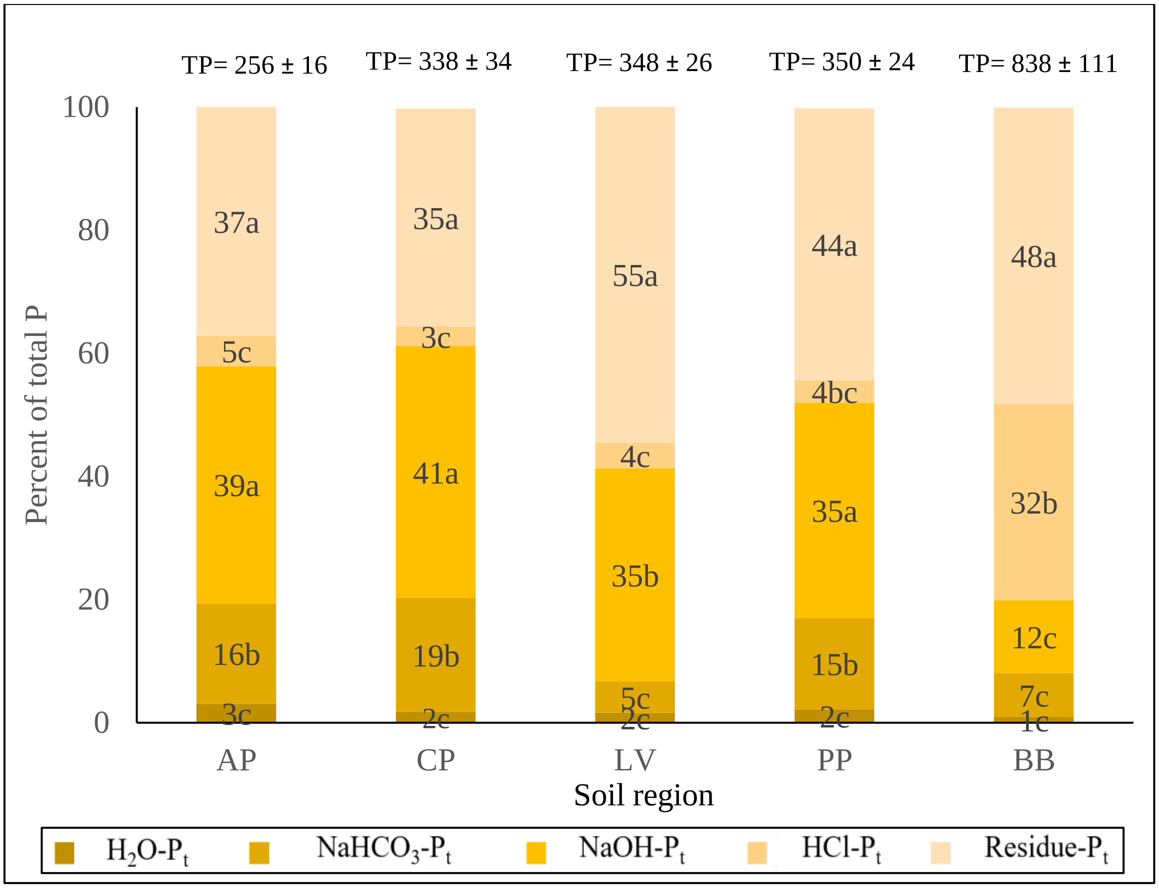

Figure 1(d): Stacked column graph showing the percent of P fractions in Alabama soil regions for medium (M) soil test P. Means followed by same letter for individual P fraction between soil regions are not significantly different at $P<0.05$. TP is total phosphorus in mg kg^{-1}, followed by standard error values. The TP values reported at the top of bar graphs are the average TP values and obtained by summing the individual P fractions. Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama.

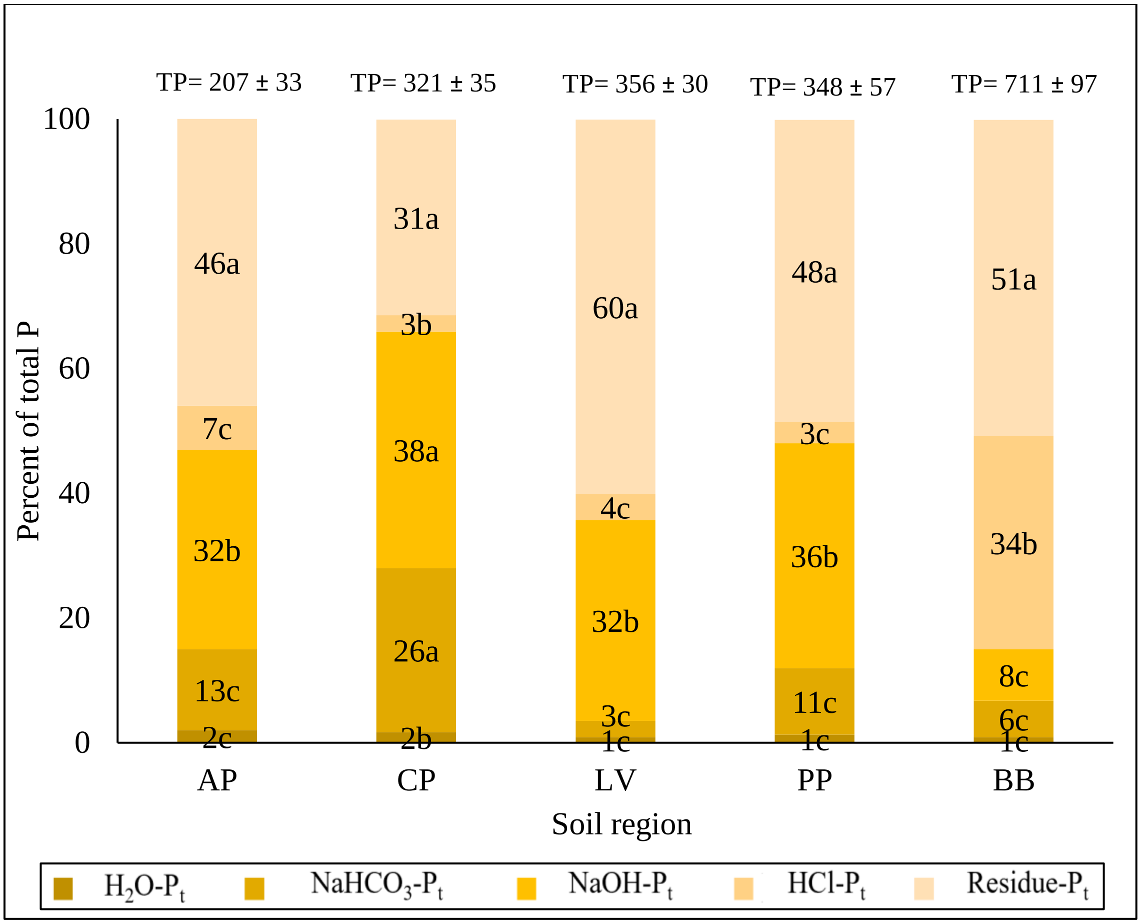

Figure 1(e): Stacked column graph showing the percent of P fractions in Alabama soil regions for low (L) soil test P. Means followed by same letter for individual P fraction between soil regions are not significantly different at $P<0.05$. TP is total phosphorus in mg kg^{-1}, followed by standard error values. The TP values reported at the top of bar graphs are the average TP values and obtained by summing the individual P fractions. Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama.

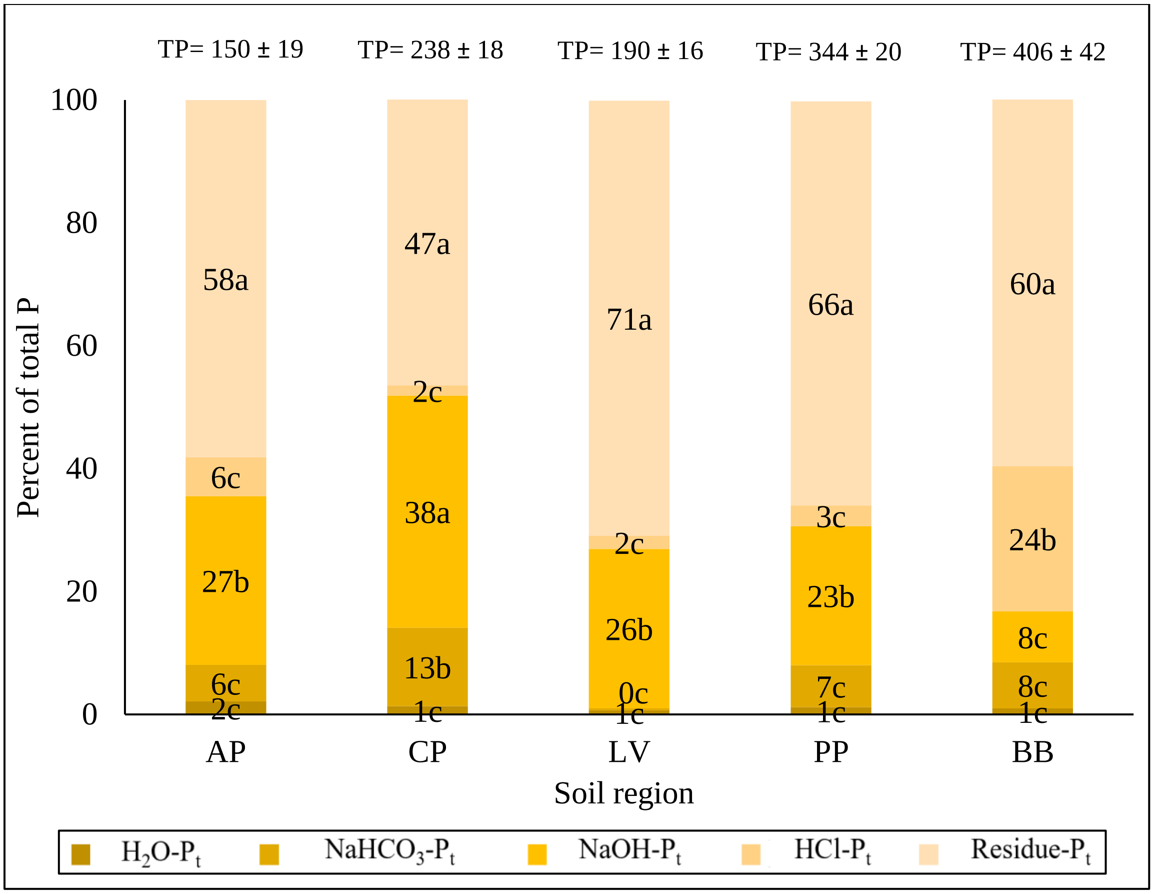

Figure 1(f): Stacked column graph showing the percent of P fractions in Alabama soil regions for very low (VL) soil test P. Means followed by same letter for individual P fraction between soil regions are not significantly different at $P<0.05$. TP is total phosphorus in mg kg^{-1}, followed by standard error values. The TP values reported at the top of bar graphs are the average TP values and obtained by summing the individual P fractions. Appalachian Plateau (AP), Coastal Plain (CP), Limestone Valley (LV), Piedmont Plateau (PP), and Blackland Prairie (BP) soil regions of Alabama.

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Appendix A

All P fractions except HCl-P_t were analyzed in ICAP. The HCl-P_t fraction of P was analyzed using FIA as no significant difference was observed between the readings obtained from ICAP and FIA instruments.

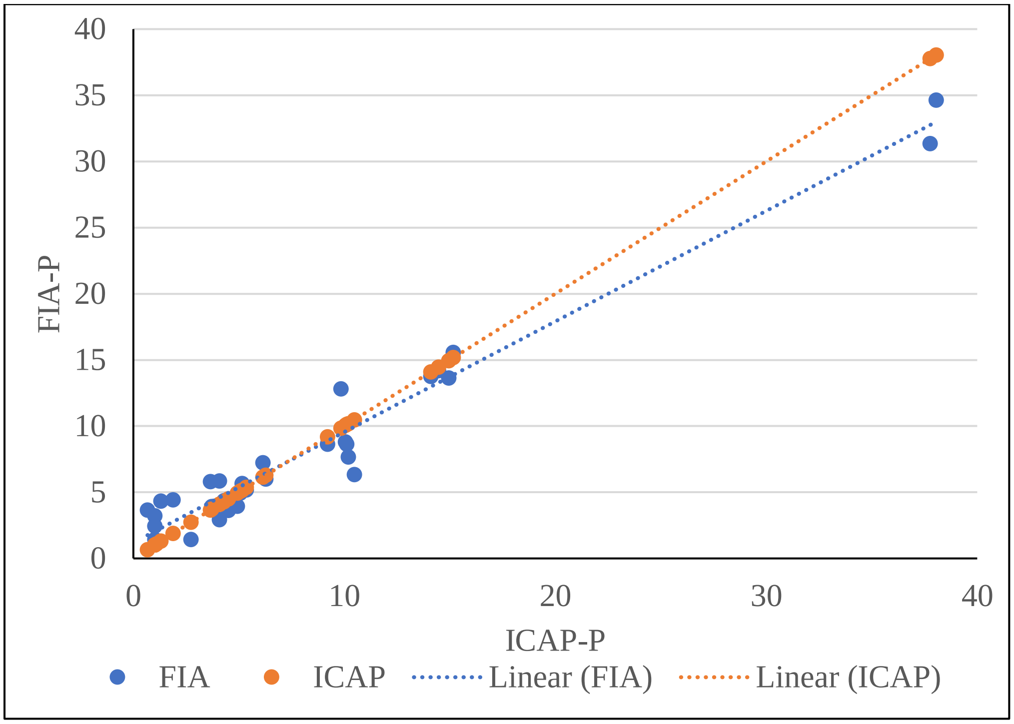

Figure appendix A (1): Relationship between ICP-P and FIA-P for HCl-P_t fraction.

Paired t-test was performed to determine the difference between the results of HCl-P_t fraction analyzed in ICAP and FIA. No significant difference was found between the HCl-P_t fraction analyzed by FIA and ICAP (P=0.69).

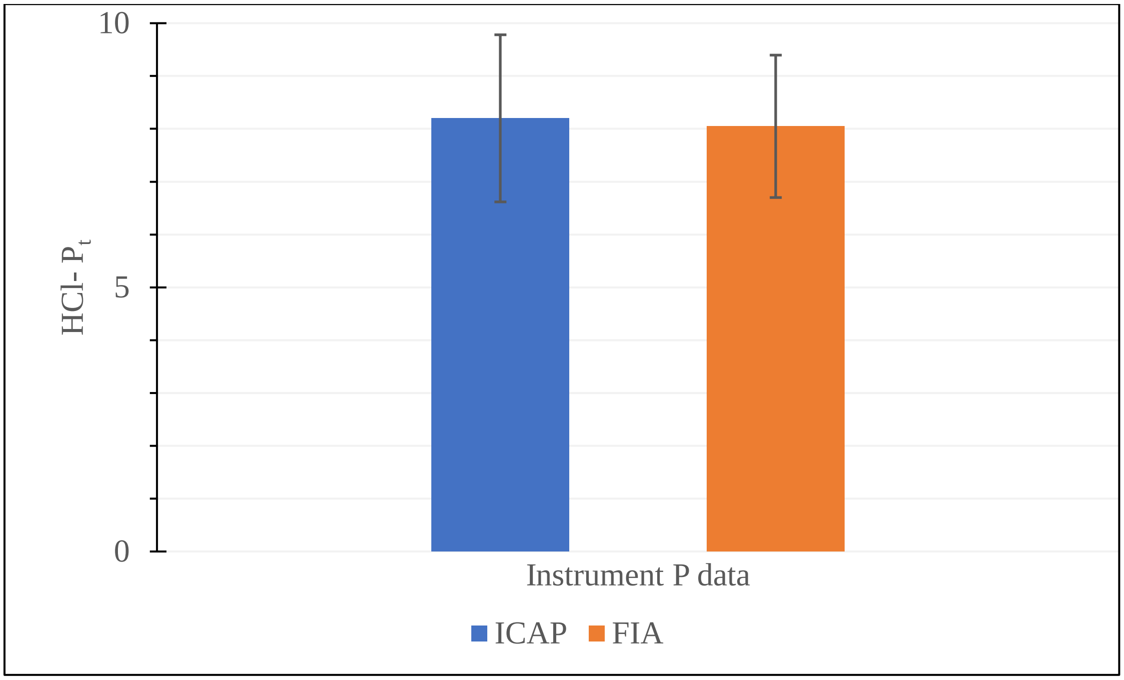

Figure appendix A(2): This bar graph represents the mean value of HCl-P_t fraction analysis obtained using ICAP and FIA instruments.