KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

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DEPARTMENT OF CHEMISTRY

COMPARISON OF EXTRACTION METHODS FOR THE DETERMINATION OF AVAILABLE PHOSPHORUS IN SOME SELECTED SOILS FROM THE EASTERN REGION OF GHANA.

A thesis submitted to the Department of Chemistry, Kwame Nkrumah University of Science and Technology in partial fulfillment of the requirements for the degree of

MPhil. Analytical Chemistry

By

Nancy Twum Barimah

(BSc. Chemistry)

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DECLARATION

I hereby declare that this submission is my own work towards the MPhil. and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

Nancy Twum Barimah	……	……
PG2849208	Signature	Date

Certified by:

Dr. Ray Bright Voegborlo	……	……
Supervisor	Signature	Date

Certified by:

Dr. Godfred Darko	……	……
Head of Department	Signature	Date

DEDICATION

This work is dedicated to the almighty God for his grace and mercy.

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ABSTRACT

Soil testing for phosphorus (P) is used agronomically to determine the amount of phosphorus needed for crop production. To characterize phosphorus in soil system and develop principles and knowledge of its nature and behaviour in soils, developing methods for soil testing and availability of soil Phosphorus to plants are essential. Soil phosphorus tests involve extraction of phosphorus from soils with chemical extractants followed by a quantification of P in the extracting solution. The concentration of available phosphorus in soil needed by plants is usually low compared to the total phosphorus in the soil. The low levels of plant available phosphorus is due to the high reactivity of soluble phosphorus with calcium, iron and aluminium that leads to its precipitation in soil and due to this it is often present in unavailable forms. As a result of the high phosphorus fixation (low soil solution phosphorus concentration) in soils, crop yields are often low. Seven soil phosphorus extraction methods were compared in this study to identify the most efficient method in extracting available phosphorus from the soil samples and to find out if the soil physicochemical properties correlate with the concentration of extracted phosphorus. The extractants include Bray 1, Bray 2, Mehlich 1, Mehlich 3, Olsen, Disodium EDTA and Distilled water. Twelve soil samples were obtained from the Eastern Region of Ghana for this study. The quantities of P removed by the extraction procedures varied for the different soil samples analyzed. The concentration of available P in the extracts was determined by the molybdenum blue method. The pH of the soils ranged from 5.08 to 7.55 that is from slightly acidic to neutral. Percentage organic matter was generally low for all the soils ranging from 0.836% to 3.078%. Texturally the soil samples varied from loamy sand to sandy clay loam. The mean concentration of available phosphorus extracted from the soils ranged from 0.100 to 9.926 µg/g soil. The highest

concentration of available P was removed from soils collected from Akwadum Cocoa farm and the least from Huhunya plantain farm. The statistical analysis on the mean concentration for all the different soil samples revealed the following order of decreasing extracting performance: Bray- 2 > Mehlich -1 > Mehlich -3 > Bray -1 > Disodium EDTA > Olsen > Distilled water extraction. Correlation analysis between the extractants indicated that there was a strong correlation between Mehlich-3 and Bray-2 P (r = 0.8059), Mehlich -3 and Mehlich - 1 (r = 0.7964), Olsen and Bray -2 P (r = 0.7190), Disodium EDTA and Bray-2 P (r = 0.713) as well as Mehlich-3 and Bray- 1 method (r = 0.7074). Similarly Olsen method showed a close correlation with that of Disodium EDTA method. There was however a poor correlation between Distilled water P and Olsen P (r = 0.2806), Distilled water P and Bray-1 P (r = 0.2828) as well as between Bray- 2 P and Distilled water P (r = 0.2417). There was also quiet significant correlation between distilled water and Mehlich-1 (r = 0.051) and also between distilled water and Mehlich-3 (r = 0.1592). Distilled water and Disodium EDTA on the other hand had a quite significant correlation. Correlation analysis between the extractants and physicochemical properties of the soil was non- significant except for the percentage organic matter which correlated with the extractants.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

1. INTRODUCTION

Phosphorus (P) is a naturally occurring element that exists in minerals, soil, living organisms and water. Phosphorus is one of the three main nutrients including Nitrogen and Potassium generally added to agricultural soils as part of fertilizers. It is classified as a macro-nutrient because of its relatively large amount required by plants. Phosphorus has many important functions in plants, the primary one being energy storage and transfer. Adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are compounds with high energy phosphate groups that drive most physiological processes such as photosynthesis, respiration, protein and nucleic acid synthesis and ion transport across cell membranes. Phosphorus is involved in controlling many enzymatic reactions and in the regulation of metabolic pathways (Theodorou and Plaxton, 1993). Phosphorus functions as a vital structural component of nucleic acids, phospho-proteins, sugar phosphates, enzymes and chloroplast. It is a major part of the nucleus of the cell and present in the cytoplasm, where it is involved in the organization of cells and transfer of hereditary characteristics. It is also well known that phosphorus is essential for seed production, enhances plant root growth, promotes early plant maturity thus decreasing time required for grain ripening and promotes stalk strength and resistance to root rot diseases (Tisdale *et al*., 1993).

Phosphorus is known to be the 11th most abundant element in the earth‟s crust. The crustal abundance of Phosphorus has been estimated as 1,180 µg/g (Mason, 1952). It is present in all soils and rocks, in water, in plant and animal remains and forms complex compounds with a wide range of elements. About 150 minerals are known to contain at least 0.44 % Phosphorus. The world‟s supply of Phosphorus comes from mineral deposits, a

nonrenewable natural resource. The Phosphorus in agricultural soils originates from primarily two sources, the parent material from which the soil developed or Phosphorus-containing amendments applied by producers (Walker and Syers 1976; Frossard *et al*., 1995). The weathering of Phosphorus from minerals such as sedimentary phosphorite and apatite can take several years and rarely releases enough Phosphorus into the soil for plant requirements (Walker and Syers 1976).Phosphorus is an essential element for plant growth and its input has long been recognized as essential to maintain economically viable levels of crop production.

Phosphorus is one of the major plant growth limiting nutrients despite being abundant in soils both in organic and inorganic forms. However many soils throughout the world are Phosphorus deficient as out of total phosphorus concentration present in soil, only 1 - 3% is available to the plants. These low levels of plant available Phosphorus are due to the high reactivity of soluble Phosphorus with calcium, iron and aluminium that leads to its precipitation in soil. Due to this it is often present in unavailable forms or in forms that are only available outside of the rhizosphere and is considered the most unavailable and inaccessible of all mineral nutrients (Holford, 1997). Phosphorus usually has a high affinity for soil, resulting in slow downward movement through the soil matrix (Eghball *et al*., 1990; Sims *et al*., 1998) or laterally through interflow. Significant amounts of Phosphorus may move by preferential flow paths (Jensen *et al*., 1998; Simard *et al*., 2000) with little adsorption to the soil matrix (Jensen *et al.,* 1998).In many agricultural systems in which the application of Phosphorus to the soil is necessary to ensure plant productivity, the recovery of applied Phosphorus by crop plants in a growing season is very low, because in the soil more than 80% of the Phosphorus becomes immobile and unavailable for plant uptake. This is normally attributed to fixation, adsorption, precipitation, or conversion of

Phosphorus to the organic form (Holford, 1997). The concentration of available-Phosphorus is always low because of continuous plant uptake and complicated by the slow replenishment of the extracted Phosphorus from the soil solution by the labile pool which is dictated by the soil Phosphorus equilibria (Holford, 1997). It must also be emphasized that concentration of available-Phosphorus pool is dictated by the prevailing soil conditions at a particular time and the ability of the crop to extract the Phosphorus from the soil solution (Raven and Hossner, 1993; Holford, 1997).

Fixation is the process by which phosphorus binds to the soil, thereby becoming unavailable for leaching or run off. The adsorption of phosphate is the process in which phosphate ions in solution react with atoms on the surface of soil particles (Abedin and Salaque, 1998). This is an important property affecting both the fate of phosphate fertilizer and the availability of phosphate to plants.

Consequently, much effort has been dedicated to the development of soil tests that determine the concentration of plant-available Phosphorus, and whether or not that concentration is sufficient for optimum crop growth. Fixation reactions in soils may allow only a small fraction (10 to 15%) of the phosphorus in fertilizers and manure to be taken up by plants in the year of application. The information on chemical forms and sorption of Phosphorus is of particular interest since it reveals not only the availability of Phosphorus but also the likely retention mechanisms when additional Phosphorus is applied to soils.

Holford, (1997) reported three important soil components controlling the supply of Phosphorus from the labile pool to replenish crop extraction. These include the amount of or concentration of Phosphorus in the soil solution; the amount of Phosphorus in the replenishment source that enters into equilibrium with the soil solution phase and

Phosphorus buffering capacity of the soil. In soil, Phosphorus is constantly cycling, mediated by the biological Phosphorus requirements of all living organisms. When soil is harnessed for agricultural production the natural cycling will be disturbed by fertilizer additions and removal of nutrients with harvesting. In soil solution, Phosphorus is available to plants in two ionic forms which include $H_2PO_4^-$ and HPO_4^{2-} known as the primary and secondary orthophosphate ions respectively (Hansen *et al*., 2004; Turner *et al*., 2007). Phosphorus solubility is restricted as the negatively charged phosphate ions can be bound to positively charged iron (Fe), aluminium (Al), Calcium (Ca) ions through reactions with aluminium and iron oxides and hydroxides as well as calcium (Ca) and magnesium (Mg) compounds at low and high pH, respectively to form relatively insoluble substances through electrostatic forces. These ionic forms of solubilized Phosphorus depend on the pH of the solution, the predominating species in

slightly acidic soils being $H_2PO_4^-$ and that in soils having a pH over 7 being HPO_4^{2-}. Solubilized Phosphorus can be leached from the soil, sorbed onto Fe and Al oxides and mineral edges of clay particles, precipitate as secondary Calcium, Iron or Aluminiumminerals or be taken up by living organisms. When this occurs, the phosphorus is considered fixed or tied up. Therefore at neutral pH the solubility of Phosphorus is the greatest.

Due to this, Phosphorus is often limiting to both aquatic and terrestrial plants because of its low solubility in water and its low mobility in the soil. Only a small proportion of soluble Phosphorus is dissolved in soil water. In this regard, phosphorus does not behave like nitrate (NO_3^-), which also has a negative charge but does not form insoluble complexes. It is then evident that the solubility of the various inorganic phosphorus compounds directly affects the availability of phosphorus for plant growth and is

influenced by the soil pH. Soil phosphorus is most available for plant use at pH values of 6 to 7. When pH is less than 6, plant available phosphorus becomes increasingly tied up in aluminum phosphates. As soils become more acidic (pH below 5), phosphorus is fixed in iron phosphates. In such soils the first products formed would be amorphous Al and Fe phosphates, as well as some Ca phosphates. The amorphous Al and Fe phosphates gradually change into compounds that resemble crystalline variscite (an Al phosphate) and strengite (an Fe phosphate).All these reactions lead to phosphorus fixation. Phosphorus is also made unavailable by fixation in calcium phosphates with pH above 7.3.Phosphorus availability is severely low in alkaline and calcareous soils due to high Calcium carbonate and clay contents in soil (Tisdale *et al*., 2002). In calcareous soils the dynamics of Phosphorus is controlled by many soil properties that strongly hold

Phosphorus and as a result maintain low Phosphorus concentration in soil solution (Delgado *et al*., 2002). The degree of Phosphorus adsorption is relatively higher in unfertilized soil and gradually decreases with increasing rate of Phosphorus in both manured and unmanured soils.

Phosphorus occurs in soil in several inorganic (mineral) and organic forms. Inorganic phosphorus (Pi) includes apatitic minerals, secondary precipitates formed with Ca, Fe and Al and free phosphate ions ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) attached to sorption surfaces or dissolved in the soil water. Organic phosphorus (Po) includes a group of organic molecules having Phosphorus as a part of their structure. It is important to emphasize that 20 to 80% of Phosphorus in soils is found in the organic form, of which phytic acid

(inositol hexaphosphate) is usually a major component (Richardson, 1994). The remainder is found in the inorganic fraction containing several mineral forms of Phosphorus (Holford, 1997).

Organic forms of Phosphorus in organic materials is released by a mineralization process involving soil organisms. The activity of these microbes is highly influenced by soil moisture, temperature and the process is most rapid in warm, well-drained soils. Research on Mississippi soils has shown that 1 % of the total soil organic phosphorus is mineralized per year during cotton and soybean production. However, since initial levels are low, and plant uptake is only one possible fate of the mineralized phosphorus, the contribution by mineralization to plant available phosphorus is small. Organic Phosphorus is also found in other organic materials example being orthophosphate

esters.

Slow Phosphorus release from minerals requires farmers to add Phosphorus containing amendments, manures or commercial fertilizers, to improve soil Phosphorus fertility for crop production. In agricultural systems Phosphorus is needed for the accumulation and release of energy associated with cellular metabolism, seed and root formation, maturation of crops (especially cereals), crop quality and strength of straw in cereals.

Phosphorus is recycled to soil in non-agricultural in plant residues and animal remains (Brogan *et al.,* 2001). Few unfertilized soils release Phosphorus fast enough to support the high growth rates of crop plant species.

The need to supplement soils with water-soluble Phosphorus fertilizers arises because the relatively small pool of native soil Phosphorus is unable to supply and maintain adequate amounts of soluble orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}) to soil solution for satisfactory crop growth.

Phosphorus is also known to exist in three pool forms in soil namely: the solution pool, the active pool and the fixed pool (Schmitt *et al*., 2009). The solution Phosphorus pool is very small and will usually contain only a fraction of a pound of Phosphorus per acre. Soil solution Phosphorus concentrations can range from 0.001mg Phosphorus L^{-1} in very infertile soils to 1 mg P L^{-1} in very fertile soils, but are 0.05 mg P L^{-1} on average (Paul and Clark 1996). It is in this pool that plants take up Phosphorus in the orthophosphate form and is the only pool that has any measurable mobility. Due to the low levels of Phosphorus in soil solution, the two inorganic forms move to plant roots primarily by diffusion, which is a slow process (approximately 0.3 to 3.3x10-9 cm^2 s^{-1} for a sandy clay loam with 20 to 40% water by volume) (Rowell *et al*., 1967, Tinker and Nye 2000). A growing crop would swiftly deplete the Phosphorus in the soluble Phosphorus pool if the pool was not being continuously reloaded. Phosphorus is primarily supplied to plants by diffusion due to its strong reactions with soil constituents (Comerford, 1998; Hinsinger, 2001). Most of the Phosphorus taken up by a crop during a growing season will probably have moved only an inch or less through the soil to the roots. A growing crop would quickly deplete the Phosphorus in the soluble Phosphorus pool if the pool was not being continuously replenished. The active Phosphorus pool is Phosphorus in the solid phase which is relatively easily released to the soil solution, the water surrounding soil particles. As plants take up phosphate, the concentration of phosphate in solution is decreased and some phosphate from the active Phosphorus pool is released. Because the solution phosphorus pool is very small, the active Phosphorus pool is the main source of available phosphorus for crops. The ability of the active Phosphorus pool to replenish the soil solution phosphorus pool in a soil is what makes a soil fertile with respect to phosphate. An acre of land may contain several pounds to a few hundred pounds of Phosphorus in the active

Phosphorus pool. The active Phosphorus pool contains inorganic phosphate that is attached (or adsorbed) to small particles in the soil, phosphate that reacted with elements such as calcium or aluminum to form quite soluble solids, and organic Phosphorus that is easily mineralized. The low availability of Phosphorus in the bulk soil limits plant uptake. More soluble minerals such as potassium move through the soil through bulk flow and diffusion, but Phosphorus is moved mainly by diffusion. Since the rate of diffusion of Phosphorus is slow (10−12 to 10−15 m^2s^{-1}), high plant uptake rates create a zone around the root that is depleted of Phosphorus (Schmitt *et al*., 2009).

Consequently, farmers who can afford to do so apply two to four times as much phosphorus as they expect to remove in the crop harvest. Repeated over many years, such practices have saturated the phosphorus-fixation capacity and built up the level of available phosphorus in many agricultural soils. Soils having such high levels of soil phosphorus no longer need to be fertilized with more than the amount of phosphorus removed in harvest.

The statistics on fertilizer use in the United States reflect the fact that farmers have recently begun to recognize that fertilizer applications can be reduced where soil phosphorus levels have been built up. Phosphorus application and low uptake of applied Phosphorus (8-33%) has resulted in significant build up of Phosphorus in the soil (Brar *et al*.,2004).

The soil can adsorb large amount of Phosphorus rapidly and firmly from solution and once adsorbed, it is difficult to release to the soil (Huang, 1998 and Varinderpal *et al*., 2006). Because of the problems associated with Phosphorus in soil, the reactions between Phosphorus and soil components have been a subject of substantial study**.** (Leytem, 2005).However, research workers agree that the reactions are complex and generally ranged from adsorption to the precipitation without clear segregation between the two

mechanisms (Mott, 1970).Partitioning of into various forms such as Iron, Aluminium, or Calcium related Phosphorus groups have been commonly done by using a sequence of chemical extractants that selectively solubilize each Phosphorus fraction (Fang, 2000).

Maintenance of plant-available Phosphorus in the soil is very imperative to avoid over exploitation of soil Phosphorus which will lead to Phosphorus deficiency and consequently, low plant yield. This maintenance is a function of the concentration of Phosphorus in the labile pool and how readily it is released into the soil solution from the solid phase (Holford, 1997).

Soil phosphorus estimation by laboratory methods is of major importance in an effective phosphate fertilizer recommendation programme. A large number of soil Phosphorus tests for plant available soil Phosphorus estimation are found in literature**.** But none of the methods can be recommended for any soil, unless such methods are evaluated and properly calibrated with respect to a particular soil - plant combination (Kurtz, 1953). Being an essential nutrient for plant growth, the estimation of plant-available Phosphorus has a long history. In later half of 19th century, papers dealing with phosphorus (P) retention in soil and extraction of Phosphorus from soils were already published (Kurtz, 1953). According to Kurtz (1953), in the beginning of the 20th century the scientific community was interested in developing a chemical extraction method for predicting the crop responses to Phosphorus fertilization. The soil Phosphorus tests that are used today provide an indication of the level of soil Phosphorus that is available to the plant. Environmentally and agronomically, the Phosphorus pool that is of the most interest in soil is the easily soluble Phosphorus that is considered to be equal to the plant available Phosphorus (labile Phosphorus). To measure the labile Phosphorus, many routine extraction tests have been developed and the results of these tests guide soil fertilization.

These tests do not determine the total concentration of Phosphorus in the soil or even the actual concentration of available Phosphorus, but provide an index measurement of the Phosphorus that can be taken up by the plant. Extraction methods used in evaluating Phosphorus status of soils include extraction with water, weak acids, bases, salts and anion exchange resin. Many authors (Morgan, 1941; Bray and Kurtz,
1945; Watanabe and Olsen, 1965; Fox and Kamprath, 1970; Barrow, 1979; Mehlich, 1984) have designed Phosphorus-testing methods using chemical extractants to determine soil-available Phosphorus. These conventional Phosphorus extractants may not give a clue on the level of available Phosphorus for plant absorption as the chemicals used for the extraction may solubilize non-labile Phosphorus. This may lead to Phosphorus fixation by Al and Fe oxides and hence unavailable for plant use (Mallarino,
1997). Moreover, these chemical extractants are not applicable over all soil types. Inadequate use of any chemical extractant over a different soil it was designed for can result to the buffering of the extractant and dissolution of non-labile Phosphorus (Myers *et al*.,2005). Bray-1 and Mehlich-3 extractants are designed to extract Phosphorus from non-calcareous soils (Bray and Kurtz, 1945; Mehlich, 1984); whereas Olsen extraction method is meant for soils characterized by calcareous nature (Watanabe and Olsen, 1965). Ion sink test has been employed by other authors (Chardon *et al*., 1996; Bache and Ireland, 1980; Raven and Hossner, 1993; Buehler *et al*., 2002) in extracting available soil-Phosphorus. These soil Phosphorus testing methods can be employed over soils with variety of physical and chemical properties (Sharpley *et al*., 1994). Ion-sink methods usually employed in Phosphorus extraction include anion and cation exchange resin membranes, resin bags, Iron oxide (FeO) coated filter papers or strip. The organic acids used are citric acid, lactic acid as well as acetic acid, and methods exploiting these are

used in the European Union at least in Austria, Belgium, Finland, France, Germany, Ireland (Vanderdeelen, 2002) and Sweden. In the United States of America, the widely used Mehlich-1 test uses 0.05 M HCl and 0.0125 M H_2SO_4. In addition to different chemical extractants, methods simulating Phosphorus uptake by plant roots have been developed. Anion exchange resins and iron-impregnated strips can act as sinks for Phosphorus, and thus, mimic plant uptake. Thus, methods and extractants intended for the same purpose, such as estimating soil plant available Phosphorus, can be expected to and do give different results for the same soil (Neyround and Lischer, 2003).

According to Nelson *et al*., (1953), the development of soil test methods is complicated by the fact that plants differ in their ability to obtain phosphorus from soil. Although the chemical forms of Phosphorus in soil largely define the potential biological availability, there are several processes that affect the quantity that can be taken up by plants. In 1965, Sauchelli reviewed limitations behind chemical soil tests and concluded that the results of soil tests reflect the prevailing soil conditions only at one brief moment of the soil's dynamic and biotic sequence of events. Therefore, the important part of soil testing is to know how to interpret the results in a practical way (Sauchelli, 1965). The efficacy of soil-Phosphorus testing method must be directed towards its ability to extract Phosphorus in a similar manner as plant roots do and at the growth stage where plants require Phosphorus most for growth and development.

Increasingly, studies of Phosphorus transport potential conducted at watershed, regional, and national scales have employed soil test Phosphorus data from several laboratories and have had to compare data from different laboratory extraction methods (Fixen, 1998; Sims *et al*., 2000). These studies point to the need for better understanding of sources of error

in soil Phosphorus analyses, either as a result of inter laboratory variance, soil specific variability, extract constraints, or a combination of these variables.

Published studies have often reported little inter laboratory error for a variety of soil Phosphorus analyses. For instance, Wolf and Baker (1985) reported strong inter laboratory correlation for Olsen, Bray-1, and Mehlich-1 soil tests conducted on 27 noncalcareous soils. Similarly, Sharpley *et al*., (1994) found a close agreement between iron-oxide strip (Fe-strip) Phosphorus measured by three different laboratories on a variety of soils (pH 5.5 to 8.0).

The suitability of specific soil Phosphorus tests for soils with various pedogenic properties is well documented. For instance, Bray-1, Mehlich-1, and, to a lesser extent, Mehlich-3, are not considered suitable for calcareous soils because soluble Phosphorus may be precipitated by Calcium fluoride (CaF_2), a product of the reaction between Ammonium fluoride(NH_4F) and Calcium carbonate ($CaCO_3$) (Smillie and Syers, 1972). Generally, acid extracts provide inconsistent measures of soil Phosphorus in calcareous soils (Fixen and Grove, 1990). Some extraction methods, however, such as Olsen, are considered suitable over a wide range of soils, from acidic to calcareous soils (Kamprath and Watson, 1980). Given the limitations of certain extraction methods with different soils, relating data from different soil tests can be problematic, since the relationship between soil tests can be soil specific. For instance, Michaelson *et al*., (1989) described variable regression equations relating Bray-1 P and Mehlich-3 P for Alaskan soils derived from two distinct parent materials. As a result, comparisons of soil Phosphorus data across large geographic areas often rely upon interpretations (example, relative agronomic Phosphorus status) to reduce variability and normalize results (Fixen, 1998). Measurement of the Phosphorus amount transferred to the solution phase during the extraction can be done colorimetrically

or by using inductively coupled plasma (ICP) spectroscopy. The most commonly used molybdenum blue method, developed by Murphy and Riley (1962) used in this study is based on the reaction of phosphate ions with molybdate to form a blue compound at low pH in reducing conditions. The intensity of the colour corresponds to the concentration of phosphate in the solution and can be measured with a spectrophotometer. The concentration of total phosphorus (TP) in the solution can be determined colorimetrically after digestion of the sample. Soil testing for Phosphorus has been formally conducted in many countries and especially in the United States, since 1940‟s and is a well established agronomic practice (Sims, 1998). However, this practice is not common in Ghana even though agriculture is Ghana's most important economic sector, employing more than half the population on a formal and informal basis and accounting for almost half of GDP and export earnings (Clark, 1994). Nye (1952) investigated the use of ammonium fluoride in extracting available phosphorus from Ghana soils. Djokoto (1964) observed that Bray's PI method of ammonium fluoride in weak hydrochloric acid solution was suitable for phosphorus determinations in all soils in Ghana. The country produces a variety of crops in various climatic zones which range from dry savanna to wet forest and which run in eastwest bands across the country. Agricultural crops form the base of Ghana's economy and these include: yams, grains, cocoa, oil palms, kola nuts, timber and many more. All require adequate fertilizer for good yield. The potential of the soils to support crop production is varied and, therefore, there is a need to formulate fertilizer recommendations to suit cocoa production in the various types of soils.

Fertilizer applications are improperly done by these farmers and the consequence is that, level of productivity is low. The agricultural sector can grow at a faster rate only if the government introduces productivity- enhancing support, which should include proper

agronomic soil test on agriculture lands. A soil test forms the basis for a fertilizer recommendation for a particular crop on a particular field. Since the community of peasant farmers in Ghana now accept the use of fertilizers, the drawing up of fertilizer recommendations based on soil tests has become necessary. Soil phosphorus (P) estimation by laboratory methods is of major importance in an effective phosphate fertilizer recommendation programme. A large number of soil Phosphorus tests for plant available soil Phosphorus estimation are found in literature. But none of the methods can be recommended for any soil, unless such methods are evaluated and properly calibrated with respect to a particular soil - plant combination.

1.2 OBJECTIVE OF THIS STUDY

The main objective of this research is:

To compare seven different extraction methods for the determination of available phosphorus in the selected soils.

The specific objectives are:

1. To identify the most efficient method of extraction of available phosphorus from the selected soils.
2. To determine the physicochemical properties of the soil samples.
3. To determine if there is any correlation between the physicochemical properties of the soil samples and the available phosphorus extracted.
4. To identify if there is any correlation between the soil physicochemical properties and the extractants and also between the various extractants.

CHAPTER TWO

2.0 LITERATURE REVIEW

Phosphate fertilizers applied to soils undergo changes in soil plant system. When Phosphorus application to soil is done, it reacts instantly with soil particles and is converted to less available form. As a result the mobility of Phosphorus is limited from the site of its application, and a complex situation is created as compared to mobile nutrients which consequently deprive plants of the required phosphate they need. These reactions between Phosphorus and soil components have been a subject of substantial studies by many researchers. To characterize phosphorus in the soil system and develop principles and knowledge of its nature and behavior in soil, developing methods for its determination and availability to plants are essential. Below is review on soil Phosphorus and research work that has been done on its various forms and methods of determining Phosphorus availability in soil.

2.1 History of Phosphorus

The name originates from the Greek words „phos“ meaning light and „phoros“ meaning bearer creating the term 'bringing light' because white phosphorus oxidizes spontaneously in air and glows in the dark. Elemental phosphorus was historically first isolated from human urine, and bone ash was an important early phosphate source. In 1669 the Hamburg merchant and alchemist Hennig Brandt heated the residue from evaporating urine with powdered charcoal, and condensed the vapor that was evolved into a waxy solid. This solid glowed in the dark, without heat, an astonishing phenomenon. He called the mysterious substance phosphorus, taken directly from the Greek "phosphoros," light-bringer. This was also the name of the planet Venus as morning star.

2.2 OCCURRENCE IN NATURE

Phosphorus is not found free in nature because of its high reactivity with air and many other oxygen-containing substances, but it is widely distributed in many minerals, mainly phosphates. Phosphate rock which is partially made of apatite (an impure tricalcium phosphate mineral) is an important commercial source of this element. About 50 % of the global phosphorus reserves are in the Arab nations.

The abundance of phosphorus in the Earth's crust is estimated to be 0.12 %, making it the 11th most common element. The only important commercial source of phosphorus is phosphate rock which is primarily calcium phosphate. The United States of America is the largest producer of phosphate rock in the world. In 1996, 13,300,000 metric tons of phosphate rock were mined in the United States. That amounted to about a third of the world's total phosphate rock. About 86 % of phosphate rock comes from North Carolina

and Florida. Smaller amounts are also mined in Idaho and Utah. Other major producers of phosphate rock are Morocco, China, Russia, Tunisia, Jordan, and Israel.

2.2.1 Mineralogy and Production of Phosphorus

The only common mineral of phosphorus is apatite, $Ca_5F(PO_4)_3$. Apatite is a family of minerals, of which this one, called fluoroapatite is the commonest. Its hardness is 5, medium hard, and its density is 3.15-3.20 g/ml. It occurs in two principal forms, crystalline apatite, and phosphorite, which is cryptocrystalline. Crystalline apatite is of inorganic origin, while phosphorite is the remains of animal bones. The largest reserve of crystalline apatite is in the Kola Peninsula, near Murmansk, Russia. The largest reserve of phosphorite is in Idaho and Wyoming in the United States. Wavellite is hydrous aluminium phosphate, a rare mineral usually formed as a secondary deposit from phosphorite. Phosphates are found in a variety of rare minerals, but never in significant quantities. Elemental phosphorus is produced in electric furnaces by heating phosphate rock, sand and coke. The silica combines with the phosphate rock to give calcium silicate, a slag, and phosphorus pentoxide. The pentoxide is then reduced by the coke to give phosphorus vapor and carbon monoxide. The phosphorus is then condensed and cast into sticks, which are kept under water. The carbon monoxide can be used as a fuel. Fertilizer, which is a soluble phosphate, is produced by treating phosphate rock with sulphuric acid. In the past, most phosphate fertilizer has been made from Florida phosphate rock, which is close to its markets, but this resource is near exhaustion. The largest reserve of phosphorite in the world is in southeastern Idaho and western

Wyoming.

2.3 PROPERTIES OF PHOSPHORUS

Phosphorus is a multivalent nonmetal chemical element that has the symbol P and atomic number15in column VA of the periodic table. Phosphorus as a mineral is almost always present in its maximally oxidized state, as inorganic phosphate rocks and exist in the oxidation states +3 +4 and +5. It has a standard atomic weight of 30.973762 and solid at 298 K. It has a density of 1.82 g/mL at 20ºC, a melting point of 44.2ºC and a boiling point of 280ºC. Its electronic configuration is [Ne] $3s^2$ $3p^3$. Ordinary phosphorus is a waxy white solid, colorless and transparent in its pure form. Phosphorus is insoluble in water, but soluble in carbon disulfide. Phosphorus burns spontaneously in air to its pentoxide. It is highly poisonous with a lethal dose of 50 mg. Elemental phosphorus exists in two major forms, white phosphorus and red phosphorus. The most important form of elemental phosphorus from the perspective of applications and chemical literature is white phosphorus. It consists of tetrahedral P_4 molecules, in which each atom is bound to the other three atoms by a single bond. This P_4 tetrahedron is also present in liquid and gaseous phosphorus up to the temperature of 800 °C when it starts decomposing to P_2 molecules (Borrmann *et al*., 1997).

2.3.1 Physical properties

Phosphorus exists in at least three allotropic forms. Allotropes are forms of an element with different physical and chemical properties. The three main allotropes are named for their colors: white phosphorus (also called yellow phosphorus), red phosphorus, and black phosphorus (also called violet phosphorus). These allotropes all have different physical and chemical properties.

2.3.1.1 White phosphorus

White phosphorus is a waxy, transparent solid. Its melting point is 44.1°C (111°F) and its boiling point is 280°C (536°F). It has a density of 1.88 gcm^3. If kept in a vacuum, it sublimes if exposed to light. Solid white phosphorus exists in two forms. At lowtemperatures, the β form is stable. At high-temperatures α form is predominant. These forms differ in terms of the relative orientations of the constituent P_4 tetrahedron. White phosphorus is the least stable, the most reactive, more volatile, less dense, and more toxic than the other allotropes. White phosphorus gradually changes to red phosphorus.

This transformation, which is accelerated by light and heat, and samples of white phosphorus almost always contain some red phosphorus and therefore appear yellow. For this reason, it is also called yellow phosphorus. It glows in the dark (when exposed to oxygen) with a very faint tint of green and blue. It is highly flammable and pyrophoric (self-igniting) upon contact with air as well as toxic (causing severe liver damage on ingestion).White phosphorus is phosphorescent. It gives off a beautiful greenish-white glow. It does not dissolve well in water, although it does dissolve in other liquids, such as benzene, chloroform, and carbon disulfide. White phosphorus sometimes appears slightly yellowish because of traces of red phosphorus.

2.3.1.2 Red phosphorus

Red phosphorus is a red powder and polymeric in structure. It can be viewed as a derivative of P_4 wherein one P-P bond is broken, and one additional bond is formed between the neighbouring tetrahedron resulting in a chain-like structure. It can be made by heating

white phosphorus with a catalyst to 240°C (464°F). Without a catalyst, red phosphorus sublimes at 416°C (781°F). Its density is 2.34 gcm^3. It does not dissolve in most liquids.

2.3.1.3 Violet phosphorus

Violet phosphorus is a form of phosphorus that can be produced by day-long annealing of red phosphorus above 550 °C. In 1865, Hittorf discovered that when phosphorus was recrystallized from molten lead, a red/purple form is obtained. Therefore this form is sometimes known as "Hittorf's phosphorus" (or violet or α-metallic phosphorus).Violet phosphorus is the least reactive allotrope and the thermodynamically stable form below 550 °C. It is also known as β-metallic phosphorus and has a structure somewhat resembling that of graphite. High pressures are usually required to produce black phosphorus, but it can also be produced at ambient conditions using metal salts as catalysts.

2.3.1.4 Black phosphorus

Black phosphorus looks like graphite powder and can be made by applying extreme pressure to white phosphorus. It has a density of 3.56 to 3.83 gcm^3. One of its interesting properties is that it conducts an electric current in spite of being a non-metal.

2.3.2 Isotopes of Phosphorus

Twenty-three isotopes of phosphorus are known including all possibilities from ^{24}P up to ^{46}P. Only ^{31}P is stable and is therefore present at 100% abundance. The half-integer nuclear spin and high abundance of ^{31}P make phosphorus-31 NMR spectroscopy a very useful

analytical tool in studies of phosphorus-containing samples. Six radioactive isotopes of phosphorus are known also. A radioactive isotope is one that breaks apart and gives off some form of radiation. Two of the known radioactive isotopes of phosphorus have half-lives that make them useful for scientific experiments. ^{32}P has a half-life of 14.262 days and ^{33}P has a half-life of 25.34 days. Biomolecules can be "tagged" with a radioisotope to allow for the study of very dilute samples. Phosphorus32, has applications in medicine, industry, and tracer studies. The isotope is injected into the system where it gives off radiation. The radiation is followed by means of detectors placed around the system. Phosphorus-32 is especially useful in medical studies, because phosphorus occurs in many parts of the body. Radioactive phosphorus can be used as a tracer to study parts of the body as well as chemical changes inside the body. It can also determine how much blood is in a person's body and can also help locate the presence of tumors in the brain, eyes, breasts, and skin.

2.3.3 Chemical properties

White phosphorus is the form that occurs most commonly at room temperatures. It is very reactive. It combines with oxygen so easily that it catches fire spontaneously. As a safety precaution, white phosphorus is stored under water in chemical laboratories. Phosphorus combines easily with the halogens. The halogens are the elements that make up Group 17 (VIIA) of the periodic table. They include fluorine, chlorine, bromine, iodine, and astatine .Phosphorus also combines with metals to form compounds known as phosphides.

2.4 APPLICATIONS

The dominant application of phosphorus is in fertilizers, which provides phosphate as required for all life and is often a limiting nutrient for crops. Phosphorus, being an essential plant nutrient, finds its major use as a constituent of fertilizers for agriculture and farm production in the form of concentrated phosphoric acids, which can consist of 70% to 75% P_2O_5. Red phosphorus, which is relatively stable, is used to make safety matches, tracer bullets, incendiary devices, pesticides, pyrotechnic devices, and many other products. There is a high demand for phosphates for use as fertilizers. Phosphorus is an essential macromineral for plants, which is studied extensively in order to understand plant uptake from soil systems. Plants appear to have severe problems in getting phosphate at a very early stage in their development. So Phosphorus deficiency symptoms most often occur in seedlings and young plants. Phosphorus is mobile within the plants; it is translocated from the older, first formed tissue to the growing points. This causes the deficiency symptoms on the lower leaves. Deficiency of phosphorus generally causes stunted growth, dark green colour associated with a purplish colour in the seedling stage. Inadequate supply of phosphorus generally causes delay in crop maturity and seed formation.

In ecological terms, phosphorus is often a limiting factor in many environments; that is why the availability of phosphorus governs the rate of growth of many organisms. In ecosystems an excess of phosphorus can be problematic, especially in aquatic systems, causing problems like eutrophication and algal bloom.

Phosphates are also used to make certain glasses (example for sodium lamps). Trisodium phosphate is used as a cleaner, water softener, and scale/corrosion inhibitor. Bones and teeth are made of apatite, a special hard version of which forms tooth enamel. If there is insufficient fluorine, then the apatite that is made is faulty, and the teeth are soft and decay

easily. Bone is made of hydroxylapatite, but there is fluorapatite in teeth. Bone ash (calcium phosphate) is used to make chinaware and to make monocalcium phosphate for baking powder. Phosphorus is used to make steels, phosphor bronze and is added to other alloys. Biologically, Living cells also use phosphate to transport cellular energy in the form of adenosine triphosphate (ATP). Nearly every cellular process that uses energy obtains it in the form of ATP. ATP is also important for phosphorylation, a key regulatory event in cells. Low-phosphate syndromes are caused by malnutrition, failure to absorb phosphate, and metabolic syndromes that draw phosphate from the blood (such as re-feeding after malnutrition) or pass too much of it into urine. All are characterized by hypophosphatemia, which is a condition of low levels of soluble phosphate in the blood serum, and therefore inside cells. Symptoms of hypophosphatemia include muscle and neurological dysfunction, and disruption of muscle and blood cells due to lack of ATP. Too much phosphate can lead to diarrhoea and calcification (hardening) of organs and soft tissue, and can interfere with the body's ability to use iron, calcium, magnesium, and zinc. Phospholipids are the main structural components of all cellular membranes. Calcium phosphate salts assist in stiffening bones

(Earnshaw, 1997).

2.4.1 Manure and fertilizer impact on soil Phosphorus

Phosphorus accumulates in the soil with long term applications of manures and fertilizers. This build up is partly due to the low Phosphorus use efficiency of most crops. On average the crop use efficiency (CUE) is less than 25% during the year of amendment application (Zhang *et al*., 2004). Making long- term environmentally sound agricultural decisions requires knowledge of the availability of soil Phosphorus forms. In order to get an

inventory of the Phosphorus in the soil most farmers will do a soil Phosphorus test. However, the results are solely based on agronomic requirements for labile inorganic phosphorus (Pi) and less on environmental impacts of the other soil Phosphorus forms.

2.4.1.1 Manure

Manure is organic matter used as organic fertilizer in agriculture. Adding manure to a soil may increase the soil test Phosphorus if Phosphorus is applied in excess of plant requirements (Hao *et al*., 2008; Zhang *et al*., 2004). The rate of change depends on the availability of the Phosphorus in the manure and the rate of application. Zhang *et al*., (2004) measured a linear increase in the Mehlich-3 Phosphorus (M3-P) with the application of manure over time. After 10 years of a normal rate of application the M3-P levels were 39 mg P kg^{-1} while at the high rate of application the M3-P levels were 157 mg P kg^{-1}. Evidently rate influences the labile Pi fractions but the M3-Phosphorus infers nothing about the other soil Phosphorus forms. The extent to which each of the other Phosphorus fractions change is dependent on several different factors including site specific differences. Research has shown that sandy soils are more vulnerable to changes in the soil Phosphorus pools while clay soils are more resistant (O"Halloran 1993; Zhang *et al*., 2004). It is equally as important to know how different manures, at the same application rate, alter pools of soil Phosphorus. A study by Sharpley *et al.,* (2004) compared dairy and poultry manures at similar rates on the same soil type. Table 2.1summarizes their results.

Table 2.1: Comparison of soil Phosphorus fractions after dairy and poultry manure applications at 120 kg per hectar per year

Manure	Dairy (mg P kg^{-1})	Poultry (mg P kg^{-1})
P Pool		
Water extractable P	53	39
Resin Pi	394	382
Bic-Pi	925	293
Bic-Po	125	139
NaOH-Pi	678	295
NaOH-Po	313	288
HCl- Pi	1780	802
Residual P	571	262

From the values obtained, it was observed that, the dairy manure typically had more Phosphorus extracted from each fraction. This could be attributed to the fact that the solid poultry manure had a higher dry matter and Carbon (C) content than the liquid dairy manure which could result in Phosphorus being used for microbial functions. Presently there are very few studies that measure the changes in Phosphorus pools when different types of manures are applied at equal rates.

2.4.1.2 Fertilizers

Understanding how fertilizers influence soil Phosphorus pools is an important tool to farmers when they have to make decisions about soil fertility. Similar to manures, soil type can alter the degree of change in the soil Phosphorus pools. O‟Halloran (1993) determined that the fertilizer treatment affected the resin Pi, NaOH-Pi, residual Phosphorus and TP on the sandy loam soil. Once fertilizers are applied they are readily available to crops due to

their high solubility. As a result the application of fertilizers leads to an increase in the readily available pools of soil Phosphorus and soil test Phosphorus (Griffin *et al*., 2003). Most manures are less soluble, therefore they are a long term source of Phosphorus (O"Halloran and Sigrist 1993). The second difference between manures and fertilizers is that fertilizers are completely Pi and lack organic matter addition. As a result, fertilizers have no direct impact on the organic pools. In a non-quantitative study of the impact of fertilizers on soil Phosphorus forms, Zhang *et al*., (2004) found the soil labile Pi fractions increased, and the Bicarbonate organic phosphorus (Bic-Po) had steady concentrations with crop removal over 6 years, then decreased when no fertilizer was applied for 4 years. All the organic fractions lessened with fertilizer application. The labile Pi increased due to the addition of fertilizers and the Po fractions decreased because no organic Phosphorus (Po) was added when

fertilizers were applied.

2.5 PHOSPHORUS IN SOIL

Phosphorus makes up about 0.12 % of the earth"s crust. It is present in all soils and rocks, in water, in plant and animal remains and it forms complex compounds with a wide range of elements, about 150 minerals are known that contain at least 0.44 %

Phosphorus. The world"s supply of Phosphorus comes from mineral deposits, a nonrenewable natural resource. The phosphate of approximately all minable deposits is one of the minerals of the apatite group. Most phosphate deposits contain silica in the form of quartz; other common diluting materials include calcite, dolomite, Fe-oxide minerals, and clay minerals. Some deposits contain diluting materials such as zeolites derivative

from the alteration of volcanic ash, glauconite, cristobalite, and pyrite. The total phosphorus content of surface soil may fluctuate from 0.02 to 0.5 % with an average value of around 0.05 %.

Phosphorus exists in soil in various mixtures, like the phosphates of iron, aluminum, calcium and so on. Solubility of these phosphate compounds is quite low and only a small portion of that phosphorus is available for plant uptake. Calcium phosphate compounds become more soluble assoil pH decreases, so, they tend to dissolve and disappear from acid soils. On the other hand, calcium phosphates are quite stable and very insoluble at higher pH, so become the prevailing forms of inorganic phosphorus present in neutral to alkaline soils. Often the relationship between total phosphorus and that available to plants is poor in the soil. Therefore, assessment of total phosphorus content of soils may be deceptive as a parameter of soil phosphorus availability to plants. The national Phosphorus contents in soils depend on the nature of parent material and extent of weathering. The source of soil Phosphorus is the primary mineral apatite. During soil development, apatite Phosphorus is weathered and gradually changed to other inorganic and organic Phosphorus forms through precipitation to other secondary minerals or through microbial uptake. Adsorption of Phosphorus can occur on the surface of soil particles (Smeck, 1985). Phosphorus can be sorbed on positive edges of kaolinite clay minerals and in calcareous soils on $CaCO_3$. The nature of clay minerals present in a soil greatly influence the extent of phosphorus retention of added phosphorus. Soils high in 1:1types minerals like kaolinite demonstrate higher phosphorus retention and thus reduced availability than those subjugated by 2:1 type minerals like montmorillonite. Kaolinite is found in great quantity in acid soils of humid and sub humid regions with high temperatures. Soils containing oxides of iron and aluminum retain enormous quantities of phosphorus through ligand

exchange. Highly weathered soils such as Oxisols are known for their high phosphorus retention due to the presence of oxides of iron and aluminium in the soils. Solution Phosphorus, labile Phosphorus and non labile Phosphorus are three forms of Phosphorus in the soil plant system. The solution Phosphorus pool is very small and usually contains only a fraction of a pound of Phosphorus per acre. The solution Phosphorus will usually be in the orthophosphate form, but small amounts of organic Phosphorus may exist as well. Plants will take up Phosphorus only in the orthophosphate form. The solution Phosphorus pool is important because it is that pool from which plants take up

Phosphorus and is the only pool that has some mobility.

Most of the Phosphorus taken up by a crop during a growing season probably have moved only an inch or less through the soil to the roots. A growing crop would swiftly deplete the Phosphorus in the soluble Phosphorus pool if the pool was not being continuously reloaded. Phosphorus is primarily supplied to plants due to its strong reactions with soil constituents (Comerford, 1998; Hinsinger, 2001). Soluble Phosphorus compounds when added to the soil react quickly with various soil components and instantly converted to slowly available forms, create one of the main problems pertinent to the maintenance and enhancement of soil fertility. The reactions between phosphate and soil are complex and have been a subject of significant study. Phosphorus fixation is a severe problem in alkaline and calcareous soils (Sharif *et al*., 2000). In calcareous soils, the dynamics of Phosphorus is controlled by many soil properties that strongly hold Phosphorus and consequently maintain low Phosphorus concentration in soil solution (Bertrand *et al*. 1999). The extent of Phosphorus sorption was relatively higher in the beginning and gradually decreased with increasing phosphorus fertilization

(Reddy

et al., 1999).

Compared with nitrogen, the atmosphere does not provide phosphorus. Instead, orthophosphates originate largely from primary and secondary minerals and/or from organic sources. However, the phosphorus cycle is by no means less complex than the nitrogen cycle, and there are many factors that affect the availability of phosphorus in the soil. Figure 2.1 is an illustration of the phosphorus cycle:

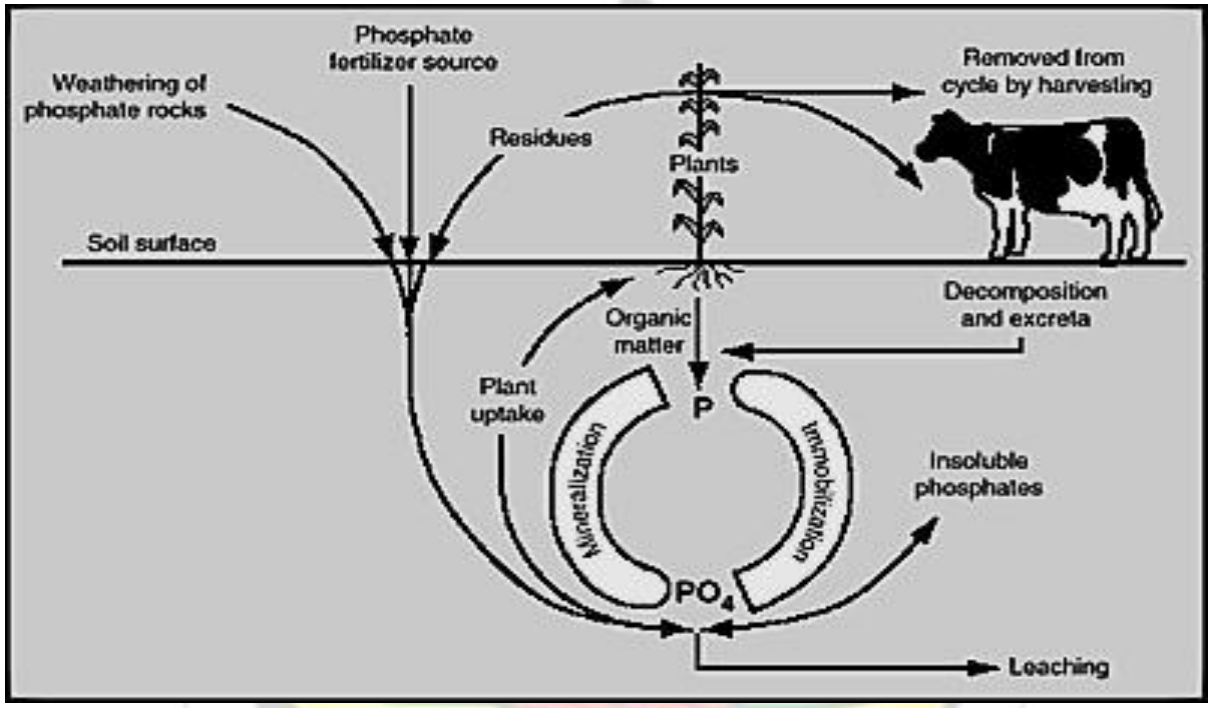


Figure 2.1: **A representation of the phosphorus cycle.**

2.6 FORMS AND REACTIONS OF SOIL PHOSPHORUS

There are several forms of Phosphorus in soils that can be categorized into two groups, organic Phosphorus (Po) and inorganic Phosphorus (Pi). Organic Phosphorus can account for 5-95% of the total phosphorus (TP) in the soil. Soil Po is derived mainly from manures, plant materials and products of microbial decomposition. The Pi fraction of TP originates from the addition of inorganic fertilizers, manures and weathering of primary minerals

such as apatite and secondary minerals such as Calcium and/or Magnesium phosphates and Iron and Aluminium phosphates (Sylvia *et al*., 2005; Morgan 1997).

Mendoza and Barrow (1987) have described four factors to determine Phosphorus status of a soil. These include: the quantity of native phosphate originally present in the soil in a form that would be available to plants; the quantity of phosphate that has been added as fertilizer or by mineralization of organic matter, the period that has elapsed since the phosphate was added and the ability of the soil to retain phosphate. In sampling and testing procedures, soils lose their physical structure, and soil particles are in close contact with the chemical used for the extraction of available phosphorus. This close contact optimizes desorption/ dissolution reactions and therefore may result in overestimate or underestimate of the amount of nutrient that can reach plant roots.

Laboratory methods focused on identifying specific Phosphorus forms in the soil can lead to misidentification and misleading results. Certain identification procedures can modify unidentified compounds from their original forms (Anderson, 1980). Other difficulties experienced when identifying specific Pi and Po forms include: the difficulty of extracting phospholipids in the presence of clays, undetectable mono phosphates in soil and the presence of complicated esters that cannot be identified (Anderson, 1980). Because of these uncertainties, researchers have used numerous methods to identify/characterize Phosphorus forms in the soil. However, methods that focus on identifying total Phosphorus and total Pi cannot infer degrees of bioavailability or the role of the Phosphorus form in the Phosphorus cycle. The forms in which phosphorus is present in soil poses problem in soil fertility. First, the total phosphorus level of soils is low, usually no more than one-tenth to one-fourth that of nitrogen, and one twentieth that of potassium. The phosphorus content of soils ranges from 200 to 2000 kg phosphorus in the upper 15

cm of 1 ha of soil, with an average of about 1000 kg Phosphorus (Bartow, 1855). Second, the phosphorus compounds commonly found in soils are mostly unavailable for plant uptake, often because they are highly insoluble. Third, when soluble sources of phosphorus, such as those in fertilizers and manure, are added to soils, they are fixed (changed to unavailable forms) and, in time, form highly insoluble compounds. Fixation reactions in soils may allow only a small fraction (10 to 15%) of the phosphorus in fertilizers and manure to be taken up by plants in the year of application. Phosphorus is primarily supplied to plants by diffusion due to its strong reactions with soil constituents (Comerford, 1998; Hinsinger, 2001).Often the relationship between total phosphorus and that available to plants is poor in the soil. Therefore, assessment of total phosphorus content of soils may be deceptive as a parameter of soil phosphorus availability to plants. The national Phosphorus contents in soils depend on the nature of parent material and extent of weathering. The critical source of soil Phosphorus is the primary mineral apatite (Smeck, 1985). During soil development, apatite Phosphorus is weathered and gradually changed to other inorganic and organic Phosphorus forms through precipitation to other secondary minerals or through microbial uptake.

2.6.1 Inorganic soil phosphorus

Inorganic solid Phosphorus compounds in soils include Aluminium (Al), Iron(Fe) and Calcium(Ca)-bound phosphates. Water in soil typically contains about 0.05 mg L^{-1} of inorganic phosphate in solution; this is equivalent to about 15 g of phosphorus in solution in one hectare of land. These very small amounts and concentrations of phosphates in soil solution compared with plant requirements, and the apparently small recovery of fertilizer phosphate in plants, have stimulated a tremendous amount of research. Studies have

concentrated on the reactions of added phosphate with soil constituents and on mechanisms controlling the amount of phosphate in solution. Although the phosphate ion can occur in three states of protonation, at pH values normally found in soils (4.5 - 6.2), $H_2PO_4^-$ and HPO_4^{2-} are the dominant species and are the forms in which phosphorus is taken up by plants. However, these ions can also absorb onto the surface (or adsorb into) solid matter in the soil. This phosphorus is then unavailable to plants. It is generally accepted that Fe and Al bound phases control the solubility and mobility of P in acidic soils while Ca bound phase is the controlling factor in calcareous environments. However, it has been debated that Fe oxides may play an equally important role in retention in these alkaline soils (Holford and Mattingly, 1975;Ryan *et al*., 1985).

Adsorption of Phosphorus can occur on the surface of soil particles. Phosphorus can be sorbed on positive edges of kaolinite clay minerals and in calcareous soils on $CaCO_3$. The nature of clay minerals present in a soil greatly influences the extent of phosphorus retention of added phosphorus. Soils high in 1:1 types minerals like kaolinite demonstrate higher phosphorus retention and thus reduced availability than those subjugated by 2:1 type minerals like montmorillonite. Kaolinite is found in great quantity in acid soils of humid and sub humid regions with high temperatures. The reactions between phosphate and soil are complex and have been a subject of significant study. Phosphorus fixation is a severe problem in alkaline and calcareous soils (Sharif *et al*., 2000). In calcareous soils the dynamics of Phosphorus is controlled by many soil properties that strongly hold Phosphorus and consequently maintain low Phosphorus concentration in soil solution (Bertrand *et al*., 1999). Investigation techniques over the past several decades have included several chemical fractionation and equilibrium techniques which have provided some insight into the question, although they have also brought further controversy over

the viability of the extraction procedures. For example, mineral solubility diagrams indicate that PO_4 solubility at equilibrium in alkaline soils is controlled by Ca-phosphate minerals while phosphate solubility in acidic soils is controlled by Fe- and Al-phosphates (Lindsay, 1979). On the other hand, dispersive xray analysis of Phosphorus-rich particles from heavily-fertilized soils indicated that Al not Ca was the predominant cation associated with Phosphorus, regardless of soil pH (Pierzynski *et al*., 1990). Physical evidence on the chemical speciation of Phosphorus in calcareous soils, can be obtained from x-ray absorption near-edge structure (XANES) spectroscopy data, it may prove invaluable in accepting or refuting the concept of Fe-P compounds contributing to Phosphorus retention in these soils. This data may also shed light on the reliability of chemical Phosphorus fractionation procedures. Recent studies have used XANES spectra to identify Phosphorus species in alum amended poultry litter
($\approx$ 20,000 mg P kg^{-1}), in extremely high saturated Phosphorus enriched soils (1,223 – 2,076 mg P kg^{-1}), and also to partition Phosphorus sorption between Fe and Al oxides (Maguire *et al.,* 2000; Beauchemin *et al*., 2003; Khare *et al*., 2004). Two types of inorganic reactions control the concentration of phosphate ions in solution, they are precipitation-dissolution and sorption-desorption processes. Precipitation dissolution reactions involve the formation and dissolving of precipitates. Sorption desorption reactions involve sorption and desorption of ions and molecules from the surfaces of mineral particles. The movement of phosphate into plants also influences soil solution concentrations and promotes dissolution and desorption reactions. Soils contain a range of crystalline and near-amorphous minerals in clay-sized particles (< 2 μm diameter). These are combined with an equally wide range of poorly characterized organic compounds which modify both

the chemical and physical properties of the clays. The major forms of inorganic soil phosphorus included are discussed below:

2.6.1.1 Iron and Aluminium Phosphates

In soil, aluminum and iron phosphate minerals have been reported to occur. The most common aluminum phosphates are wavellite ($Al_3(PO_4)$ $(OH)_3.5H_2O$) and variscite **(**$AlPO_4.2H_2O$). Studies have revealed that the occurrence of variscite has been postulated in soil of slight acidity (Lindsay and Moreno. 1960). At higher pH values, variscite dissolves incongruently, whereby a more basic solid phase of aluminum hydroxy phosphate is formed (Taylor and Gurley, 1964). This material probably controls phosphorus concentration in solution in acid soil by forming a surface complex on variscite. However, in pure systems, where the pH of the equilibrium solution is less than 3.1, the solubility product of variscite controls the phosphorus concentration in solution.

The most common iron phosphate is strengite ($FePO_4.2H_2O$). Chakravarti and Talibudeen (1962) concluded that a compound approximating to the composition of strengite may occur in temperate soils in the pH range of 3.8 to 4.2, whereas in tropical soils strengite coexists with hydrated iron oxide from pH 3.8 to 6.7. Hydrous iron and aluminium oxides and alumino silicates occur widely in soils. They will react with phosphate solutions to produce an isomorphous series of iron and aluminium phosphates

[strengite ($AlPO_4.2H_2O$) –barrandite $(Al,Fe)PO_4.2H_2O$ and variscite ($FePO_4.2H_2O$)]. While these materials have been identified by X-ray diffraction in laboratory experiments, they have not been seen in natural soils. This was assumed to be due to the very small size

of their crystals. Other studies have shown that the iron and aluminium phosphates which were assumed to occur in soils are more soluble than the

corresponding hydrous metal oxides, which definitely do occur in soils.

Variscite and strengite are the least soluble compounds at acidic pH (Tisdale *et al*., 1985). Strengite is known to crystallize more rapidly when the iron phosphate is formed. The less crystalline aluminium phosphate has greater surface area which is more favorable for release of phosphorus into the soil solution. Under very acid conditions, minerals of the variscite and strengite groups are precipitated (Wild, 1988). Soils containing oxides of iron and aluminum retain enormous quantities of phosphorus through ligand exchange. Highly weathered soils such as Oxisols are known for their high phosphorus retention due to the presence of oxides of iron and aluminium in the

soils.

Phosphate ions are strongly adsorbed by hydrous metal oxide surfaces. This suggests that it is unlikely that metal phosphates could persist for long in soils containing hydrous metal oxides. However, there is also evidence that hydrous ferric oxide coatings can form on the surface of metal phosphates and effectively slow the rate at which they dissolve.

2.6.1.2 Calcium and Magnesium Phosphates

Calcium and Magnesium Phosphate occur in soils in several forms and known compounds include the following:

- Monocalcium phosphate [Ca (H_2PO_4).H_2O],
- Hydrated and the unhydrated forms of dicalcium phosphate [$CaHPO_4.2H_2O$ and $CaHPO_4$], both are slightly soluble in water.
- Octacalcium phosphate [Ca_8H_2 (PO_4)$_6$.$5H_20$]

- Tricalcium phosphate [$Ca_3(PO4)_2$]
- Hydroxyapatite $Ca_{10}[(PO_4)_6.(OH)_2]$,
- Fluorapatite [$Ca_{10}(PO_4)_6F$]
- Struvite [$MgNH_4P0_4.6H_20$],

Dicalcium phosphate, octacalcium phosphate and hydroxyapatite are the principal crystalline phosphates that have been identified in soil (Tisdale *et al*., 1985). The native phosphorus in soils originated largely from disintegration of rocks containing the mineral apatite, $Ca_{10}(PO_4)_6(FClOH)_2$. Apatite has also been reported as a common soil mineral by Shipp and Matelski (I960). Hydroxyapatite has been reported to be a stable form over a wide range of pH (Larsen. 1967). The apatite in its primary form has little or no significance in supplying phosphate to plants, because of very low solubility and rate of solubilization (Wild, 1988).

The phosphorus concentration in calcareous soils does not correspond to any one mineral species. It may be controlled by octacalcium phosphate in some soils or by hydroxyapatite in other soils. The hydroxyapatite in soil invariably contains some carbonate ions, which makes it chemically more reactive. The presence of octacalcium phosphate has been reported in soils which have been limed and fertilized with phosphates (Webber and Mattingly, 1970).

Baifan and Yichu (1989) suggested a systematic fractionation scheme for inorganic phosphates in calcareous soils, in which they classified calcium phosphate into dicalcium phosphate, octacalcium phosphate and apatite types. They also suggested that these forms are interchangeable. Some other workers reported the fractionation of inorganic phosphorus in the calcareous soils as a series of calcium phosphates with complex

physico-chemical reactions and different availability to plant growth (Williams *et al*., 1967 and 1971, Syers *et al*., 1972 and Hooker *et al*., 1980).

The availability of Phosphorus from various inorganic compounds was compared by Tisdale *et al.,* (1985). It was revealed that there was the highest Phosphorus availability from struvite, $MgNH_4PO_4.6H_2O$for oats as compared to mono or dicalcium phosphate. In soils containing large quantities of magnesium, a number of insoluble magnesium phosphate compounds such as dimagnesium phosphate trihydrate, trimagnesium phosphate and struvite may form. However, these magnesium phosphates are more soluble than dicalcium phosphate and octacalcium phosphate.

Precipitation of calcium phosphates, including dicalcium phosphate and possibly apatite (tri-calcium phosphate) occur in soils above pH 6 and particularly those containing free calcium carbonate. Mono-calcium phosphate is soluble whilst tri-calcium phosphate is virtually insoluble. When soluble phosphate is added to a soil, its solubility first declines very rapidly. This is followed by a more gradual decrease in solution concentrations which can continue for weeks. Experiments which compare the rate at which a soil or soil component decreases solution phosphate concentrations with the rate at which adsorbed phosphate can be extracted from phosphate-treated soils are used to investigate these reactions. Calcium phosphate compounds become more soluble assoil pH decreases, so, they tend to dissolve and disappear from acid soils. On the other hand, calcium phosphates are quite stable and very insoluble at higher pH, so become the prevailing forms of inorganic phosphorus present in neutral to alkaline soils.

2.6.2 Organic Soil Phosphorus

Soil organic phosphorus occurs in chemically diverse forms. Soil microorganisms play an important role in recycling many of the organic phosphorus compounds in soils. The organic phosphorus contents of the soils throughout the world range between 7 to 1056 mg/kg of soil (Campbell and Racz, 1975).Up to one-half of the organic phosphorus in soils occurs as phytic acid (inositol hexasphosphate),which is the main compound that plants use to store phosphorus in seeds to support early seedling growth upon germination. The remaining organic phosphorus in soils occurs as mono- and diesters [that is $(RO)PO_3H_2$ and $(RO)(R‟O)PO_2H$ where R and R‟ represent aliphatic

compounds], phospholipids and nucleotides, sugar phosphates, phosphoproteins, and phosphonates (Tate, 1984).The organic phosphorus fraction is important to the phosphorus economy of these soils since it represents a very large pool of phosphates, part of which actively contributes to the phosphate nutrition of plants and hence grazing animals, while the remainder appears to be relatively stable and hence not available for plant use. Like nitrogen, organic phosphorus is converted to inorganic phosphate through the process of mineralization which is carried out by microorganisms. The immobilization of inorganic phosphate, in contrast, is the reverse reaction of mineralization. The conversion of plant available inorganic phosphates into unavailable organic forms is termed immobilization. During immobilization, microorganisms convert inorganic forms to organic phosphate, which are then incorporated into their living cells.

Mineralization and immobilization of phosphorus occur simultaneously in the soil. Ultimately, the carbon to phosphorus ratio (C:P) determines whether there is net mineralization or net immobilization. When the C:P ratio is less than 200:1, net mineralization prevails. Net mineralization indicates that there is enough phosphorus in

the soil to sustain both plants and microorganisms. On the other hand if the C:P ratio is between 200:1 and 300:1, immobilization and mineralization rates are fairly equal. In the case where the C:P ratio is greater than 300:1, net immobilization occurs. During immobilization there is not enough Phosphorus to sustain both plants and microorganisms; and so, microorganisms scavenge the soil for Phosphorus.

The composition and transformations of organic phosphorus compounds in soils have received less attention than inorganic forms. This is partly because inorganic phosphates predominate in temperate arable soils and partly because organic phosphates are even more difficult to study than the inorganic phosphates. The latter point results from difficulties in extracting all the organic phosphate components without altering them chemically. While the parent material from which a soil forms has some influence on the amount of organic phosphate in virgin soils, other soil forming factors and soil properties probably have more influence.

Organic phosphate increases as soils become more acid; cultivation decreases organic phosphate content and climatic conditions which favour the accumulation of organic matter in soils increase their organic phosphate content. Soil organic matter consists largely of carbon, oxygen, hydrogen, nitrogen, sulphur and phosphorus. When soils develop or when virgin or arable soils are put under permanent pasture, their organic matter content generally increases. While organic nitrogen and sulphur components increase to equilibrium values within a relatively short period of time (5–20years), organic phosphate compounds appear to accumulate for much longer. As a result, the phosphorus content of soil organic matter is much more variable than its carbon, nitrogen or sulphur contents. This has led to the suggestion that organic phosphates can be divided into two

fractions; one in association with carbon, nitrogen and sulphur in soil humus and the other as independent organic phosphate compounds.

However it is likely that the individual organic phosphate compounds identified in extracts are combined into complexes of high molecular weights in soils and that a continuum exists over the range of organic phosphate compounds in soils. The nature of phosphorus in defined soil humic materials is largely unknown. Of the organic phosphate compounds identified in soil extracts, inositol phosphates such as inositol hexaphosphate are the most abundant and can represent up to 50 percent of the total organic phosphates present. An average of 1 percent of the organic phosphates in soils is present as phospholipids (range0.5-7.0%). Phosphoglycerides are probably the dominant fraction but little is known about the other phospholipids in soils. They are probably derived from plant debris, animal wastes and microbial activity and may be more important to plant nutrition and phosphate cycling in soils than their relative proportion suggests. They are much more abundant in plants, suggesting that, unlike inositol phosphates, they are rapidly mineralized in the soil. While added in plant and animal remains in greater amounts than other phosphate esters, they represent less than 3 percent of the organic phosphates in soils, again implying that they are rapidly mineralized by microbes.NMR analysis of organic phosphate compounds extracted by alkaline solution shows that in native soils, the total organic Phosphorus fraction consists of 83-95% or the phosphate monoester Phosphorus, 4-9% of the phosphate diester Phosphorus and up to 12% teichoic acid Phosphorus (an orthophosphate diester form of organic Phosphorus which consists of sugar units linked by phosphate groups). The latter two forms are more easily mineralized than the monoester and were not found in soils which had a long history of cultivation. Somewhat surprisingly, there is normally more organic phosphorus in the soil solution than there is inorganic phosphorus.

In light textured soils (sands) as much as 90 percent of the total phosphorus in solution may be organic. This has considerable implications to the role of organic phosphates in the movement of phosphates in soils and to plant nutrition.

Much of the organic phosphate in the soil solution appears to be myo-inositol phosphates although a range of other soluble compounds may be released from damaged microbial cells. There is some confusion over the availability of soluble organic phosphate compounds to plants. While some appear to be as available as inorganic phosphate in aseptic culture solutions, they are of much less value in the soil. As already mentioned, the relatively simple organic phosphates in soil extracts are probably in high molecular weight complexes in the soil. In addition, inositol phosphates are strongly adsorbed on charged surfaces while the sparingly soluble Fe and Al complexes they can form may also decrease plant availability.

However, there is evidence that plant roots and rhizosphere organisms which are found around plant roots excrete phosphatase enzymes capable of hydrolyzing some organic phosphate compounds, releasing inorganic phosphate for absorption by the plants. Mycorrhizal fungi found on plant roots are known to increase the uptake of phosphates by plants in some circumstances. This may be partly due to their phosphatase activity although their role in physically extending the absorbing root surface into larger volumes of soil is probably more important. The organic phosphate fraction is much more important in pasture soils, where it may account for 50 to over 80 percent of the total phosphorus present.

2.7 Factors affecting phosphorus uptake

The soil supports plant growth, it is the physical determinant of root growth and is the main reservoir for plant-available water and nutrients. Therefore the soil controls the availability of most essential plant nutrients. It regulates availability by means of biophysiochemical processes, which are functions of soil and plant properties. There are a number of important factors affecting crop response to phosphorus and these include: soil physical, chemical, biological, crop and fertilizer factors. Chemical factors affecting Phosphorus availability in soil include the following:

2.7.1 Soil Mineralogy

Forms of mineral Phosphorus in the soil are a result of the soil‟s parent material, weathering, and, to a lesser degree, Phosphorus fertilization. Types of clay, amounts of iron and aluminum oxides, and amounts and forms of calcium affect a soil‟s ability to fix fertilizer Phosphorus.

2.7.2 Clay Mineralogy

Several research work have reported a significant correlation between clay content and Phosphorus sorption parameters. (Fox and Kamprath, 1970; Jones *et al,* 1979; Ayodele, 1981, Morais *et al,* 1996; Sharif *et al,* 2000; Chaudery *et al* 2003) .The clay content of a soil has great impact on phosphate adsorption. Soils containing large quantities of clay will adsorb more phosphate than those with less clay content.

Clays, particularly those of 1:1 lattice tend to absorb more Phosphorus in tropical soils especially at low pH ,than those of 1:2 lattice, for example soils high in kaolinite such as

those found in areas of high rainfall and high temperature, will retain larger quantities of phosphate than those containing the 2:1 types. Greater adsorption of phosphate in the former case is probably due to the higher amounts of hydrated oxides of iron, manganese and aluminum associated with the kaolinitic clays and other 1:1 type clay minerals (Hayne, 1983). In other words, the more surface area exposed with a given type of clay, the greater the tendency to retain phosphates

Additionally, kaolinite develops pH-dependent charges on its edges which can enter into adsorption reactions with phosphate. Clays such as kaolinite with a low SiO_2/R_2O_3 ratio will adsorb larger quantity of phosphorus than the clays with a higher ratio. A large number of exposed hydroxyl groups in the gibbsite layer of kaolinite are exchangeable with phosphate and cause more adsorption.

2.7.3 Iron and Aluminum oxides

Studies have revealed that oxides and hydroxides of Al and Fe play a significant role on Phosphorus availability and sorption properties. Singh and Singpuri (1986) reported that oxides of Fe and Al were correlated significantly with Phosphorus adsorption maxima. Higher value of Phosphorus adsorption maxima in case of soil containing higher content of oxides of Fe and Al might be due to formation of their respective metal phosphate (Maida, 1980).

A significant and positive relationship between Phosphorus bonding energy content and free oxides of Fe and Al was observed and suggested a mechanism of phosphate adsorption by two point attachments mainly through the colloidal surface and Al. The sorption of inorganic phosphate of soils pH less than 7.0 is closely related to the amount of reactive Fe and Al compounds (Syers *et al*., 1977).

It has been shown that the amorphous hydrous metal oxides of Fe and Al sorb relatively greater amounts of Phosphorus than their crystalline counterparts (McLaughlin *et al*., 1981). Syers *et al*., (1977) reported that phosphate sorption reduced remarkably when oxides of Al and Fe were extracted from the soil. In addition Tisdale *et al* in 1990 also showed that about 1 meq exchangeable Al per 100g soil when completely hydrolyzed can sorb up to 102mg/L Phosphorus in soil solution.

With respect to the relative importance of the two elements, Al plays a dominant role in Phosphorus retention than Fe (Bromfield, 1965, Williams *et al*., 1958). Owusu- Bennoah and Acquaye in 1989 also showed that dithionate extractable Al was a more important determinant of Phosphorus sorption maxima of some selected Ghanaian soils. Conversely Ahenkorah in 1968 observed no significant relationship between Phosphorus retention capacity and extractable Al but rather he indicated that dithionite extractable Fe was responsible for Phosphorus sorption in some cocoa growing soils of Ghana. The sorption of Phosphorus on to Al / Fe surface is usually considered to be important under acidic conditions.

2.7.4 Soil organic matter

Generally, higher soil organic matter levels are related to greater Phosphorus availability. Studies have emphasized the importance of organic Phosphorus in plant nutrition. Apparently a fairly constant portion of organic Phosphorus is converted into inorganic forms which are taken up by plants. Gradual release of organic Phosphorus provides a steady supply of Phosphorus under conditions which would otherwise result in Phosphorus fixation. Organic matter increases Phosphorus availability in the following four ways: (1) First, organic matter forms complexes with organic phosphate which

increases phosphate uptake by plants, (2) organic anions can also displace sorbed phosphate, (3) humus coats aluminum and iron oxides, which reduces Phosphorus sorption and (4) organic matter is also a source of phosphorus through mineralization reactions (Beaton, 2005). Soil biological factors such as the effects of crop residues increase microbiological action and can result in immobilization of available Phosphorus into microbial cells.

2.7.5 Soil pH

The pH of soil has an important role in Phosphorus availability and affects the efficiency of applied Phosphorus. Phosphorus fixation by Fe and Al oxides is greatest in acid soils, but declines as soils are limed. Availability in most soils is at a maximum in the pH range 6 to 7 as shown in Figure 2. As soil pH increases above 7, Calcium and Magnesium (Mg) react with Phosphorus, and the availability again declines. Trying to lower the pH of calcareous soils to improve Phosphorus availability is not practical.

Placement of Phosphorus near the seed or seedlings is much more feasible.

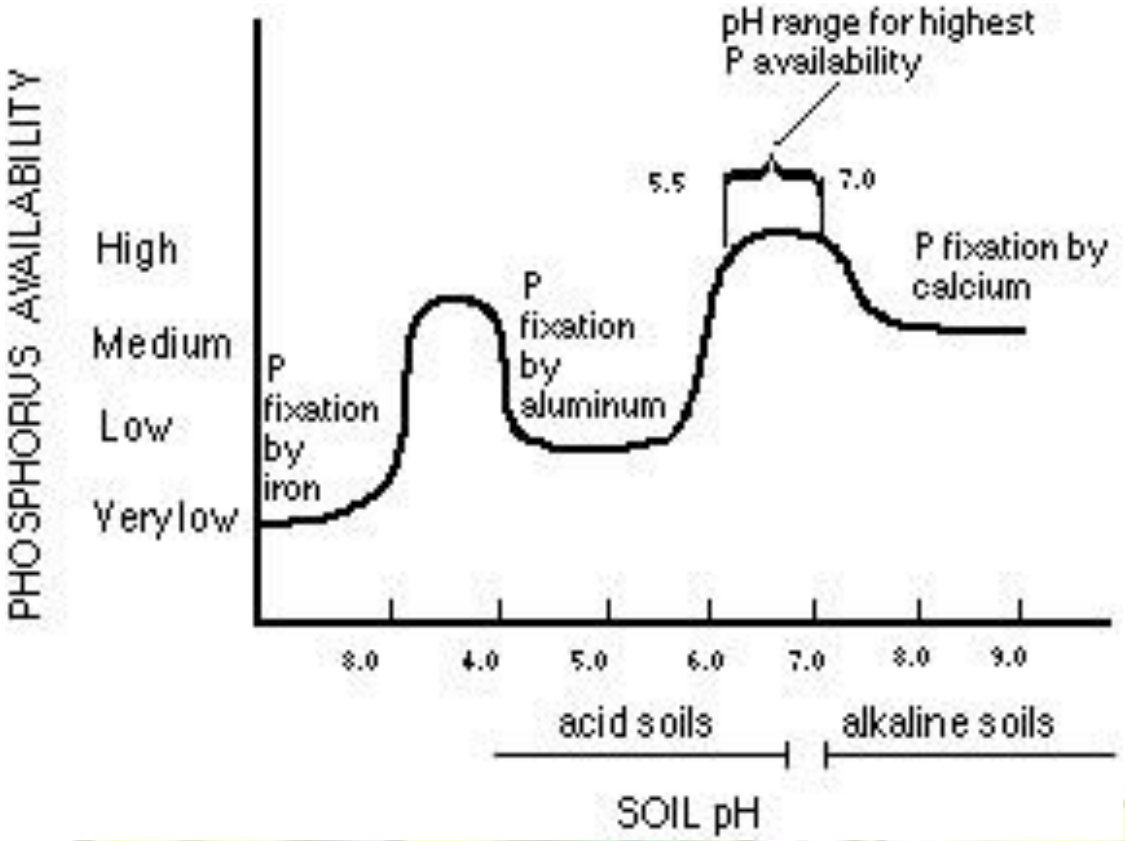


Figure 2.2: Phosphorus availability with pH

Crop responses to Phosphorus are affected by the availability of other nutrients. Interactions of Phosphorus with micronutrients, particularly zinc (Zn), usually involve lowered micronutrient availability and uptake when Phosphorus availability is high. Deficiencies of other nutrients can also limit crop response to Phosphorus. Phosphorus fertilizer absorption and use efficiency by crops is improved by the presence of ammonium-nitrogen (NH_4-N) in the soil with the Phosphorus. Ammonium-nitrogen absorption by roots lowers the pH in the vicinity of the root surface improving Phosphorus uptake. High concentration of NH_4-N can also change the soil chemistry of

Phosphorus and delay normal fixation reactions. A number of soil physical factors have also been known to affect phosphorus uptake, and these include the following: the soil texture, soil aeration and compaction, soil temperature and soil moisture.

2.7.6 Soil texture

Studies have shown that responses to fertilizer Phosphorus at a certain Phosphorus soil test level tend to be greater on sandy soils than on those containing more silt and clay. Diffusion, which occurs mainly through soil water films, is an important process in Phosphorus movement toward roots and is slower in coarse-textured soils. Higher Phosphorus soil tests or higher rates of fertilizer Phosphorus are needed on such soils. Some soil components react readily with fertilizer Phosphorus to lower its availability (Phosphorus fixation). Fixation increases as soil clay content increases. This means that larger amounts of Phosphorus must be applied to those soils in order to increase soil test values and Phosphorus availability to plants.

2.7.7 Soil aeration and Compaction

Soil aeration and compaction also account for phosphorus availability to plants in the sense that phosphorus uptake by plant roots requires energy from carbohydrates. Generating that energy requires oxygen for normal root metabolism. If soils are compacted, pore space is diminished, oxygen is limited, and Phosphorus absorption suffers. Compaction also limits Phosphorus use by decreasing the thickness of water films on soil particles through which Phosphorus moves to root surfaces. Increasing concentrations of soil Phosphorus by adequate fertilization can help offset this effect.

2.7.8 Soil Temperature

The temperature of soil also plays a role in availability of phosphorus, in that low soil temperatures depress Phosphorus availability and plant uptake. Lower temperatures reduce the rate of mineralization of soil organic Phosphorus because of lowered microbial activity. Low soil temperatures also reduce root growth rates and the rate of diffusion of Phosphorus, causing a decrease in the amount of Phosphorus accessed by roots. The metabolic release of energy, which drives Phosphorus absorption mechanisms, is also slowed by low soil temperatures. Cold soils are often associated with large Phosphorus responses, even at high test levels. Lower soil temperatures are associated with reduced tillage systems because of surface shading by residues.

2.7.9 Soil Moisture

Moisture stress also reduces Phosphorus availability and uptake. Greater crop response to Phosphorus at a given level of soil Phosphorus may be expected under moisture stress conditions. Low soil moisture has been found to decrease Phosphorus availability to wheat more than added Phosphorus fertilizer increased availability. The percentage of Phosphorus in the crop from fertilizer has been reported highest when moisture availability was lowest. Field studies indicate larger corn and soybean incremental responses to Phosphorus on a medium Phosphorus testing soil under low rainfall conditions.

2.7.10 Soil Biological processes

In addition to mineralization of organic Phosphorus, soil biological processes can influence availability and responses to applied Phosphorus. Incorporation of crop residues increase microbiological action and can result in immobilization of available Phosphorus into microbial cells. The same process affects the availability of nitrogen (N), sulfur (S), and other nutrients. Immobilized Phosphorus is gradually released for plant use as residues decompose. Soil aeration, temperature, soil moisture, pH, and supplies of other nutrients such as N have a direct effect on biological action, immobilization, and release of Phosphorus.

2.7.11 Plant roots geometry

Plant roots also have an influence on phosphorus availability in the sense that the roots affect the biology of the soil by providing energy sources for microbes and influencing soil properties such as tilth, structure, and nutrient availability. Mycorrhizae (meaning "fungus-root") are a close association of plant roots and a fungus where both partners benefit. Plants grow better when colonized by the beneficial fungi, which act as extended root surfaces. Improved nutrient absorption, especially Phosphorus, results from the plant-fungus association. Increasing the soil Phosphorus concentration to the levels needed for high yielding crops may essentially eliminate mycorrhizae as a factor in overall plant growth.

However, even with high Phosphorus testing soils, mycorrhizae may be important in early season plant growth. Mycorrhizal infection may be severely reduced by fallow periods in crop production, which increases the importance of starter Phosphorus fertilization for crops grown in a fallow rotation. Prolonged wetness and flooding can decrease the

mycorrhizal in oculum levels to the point that increased Phosphorus fertilization may be needed. Crop species, varieties, and hybrids vary in their abilities to absorb and respond to fertilizer Phosphorus. Several factors are associated with those differences and these include root development and distribution. Most available Phosphorus is present in the surface soil and helps concentrate roots in that zone.

However, if surface moisture is limiting, soil Phosphorus becomes less useable and Phosphorus use-efficiency declines. High levels of applied Phosphorus can increase Phosphorus movement into the subsoil and help overcome this problem, by allowing better root development in the subsoil and increasing the ability to extract water. Outstanding corn yields are often associated with deep distribution of nutrients, including Phosphorus. Root length and density affect response to Phosphorus since length is the major determinant of absorbing surface area. Crop varieties and hybrids differ in their requirements for Phosphorus.

Crop yield levels, an example been corn and soybean studies have indicated that the amount of Phosphorus taken up by plants per bushel or per ton of grain yield does not vary substantially. Yield effects on Phosphorus requirements are often estimated as essentially a straight line function. Increased yields with increased Phosphorus fertilization can result in higher grain Phosphorus content and a greater Phosphorus removal than what may be predicted with simple linear relationships from lower-yielding studies.

2.7.12 Chemical forms of phosphorus fertilizer

Fertilizer Factors including chemical and physical characteristics of Phosphorus fertilizers may influence crop response and management decisions on Phosphorus sources. Water

solubility of Phosphorus fertilizers is considered important in some countries, but there is little agreement on what percent of the total P should be water soluble. Research in North America has shown that water solubility is important, but it is difficult to find data that indicate superiority of one Phosphorus fertilizer source over another based on water solubility, provided water solubility is 60 % or higher. Phosphorus fertilizer materials indicate that ammonium phosphates, superphosphates, and nitric phosphates are largely equal as Phosphorus sources for plants. These classes of compounds have a high percentage of Phosphorus availability. Although research has shown some advantages to the presence of NH_4-N with Phosphorus in terms of plant Phosphorus absorption, modern crop production practices frequently involve high concentrations of Nitrogen in the soil which diminish differences among these classes of

compounds. Comparisons of monoammonium phosphate (MAP), diammonium phosphate (DAP), ammonium polyphosphate (APP), and urea-ammonium phosphate (UAP) show few consistent differences. At high rates, DAP can cause germination damage when placed in direct seed contact on alkaline soils, due to the release of some free ammonia. Limited rates of application control the problem. Formulations of UAP have an even greater probability of germination damage in direct seed contact due to ammonia release from urea hydrolysis. Application rates of UAP in seed contact should be lower than DAP. While APP provides some superior physical characteristics in liquid fertilizers, agronomic capabilities of MAP, DAP, APP, and UAP are essentially equal. Also the physical form of phosphorus fertilizers be it solid or fluid involve the same compounds mentioned earlier. Agronomic capabilities of solid and fluid Phosphorus sources are essentially equal. Handling differences, adaptability to methods of application, and abilities to co-apply

micronutrients as well as pesticides are valid management considerations when evaluating Phosphorus fertilizers.

2.8 Diffusion of Phosphorus in soil

Plant roots absorb phosphorus from the soil solution. In comparison to other macronutrients, the phosphorus concentration in the soil solution is much lower and ranges from 0.001 mg/L to 1 mg/L (Bradly and Weil, 2002). In general, roots absorb phosphorus in the form of orthophosphate, but can also absorb certain forms of organic phosphorus. Barrow (1989) suggested that phosphate mostly moves to plant roots by diffusion and it is only the phosphate in the soil solution that is free to move. The plant root reduces the soil-Phosphorus mainly by absorbing from the adjacent soil solution, which initiates the diffusion of Phosphorus in the soil solution towards the plant root and dissolution of solid phase Phosphorus, termed as the labile pool (Schofield, 1955). However, the presence of mycorrhizal fungi, which develop a symbiotic relationship with plant roots and extend threadlike hyphae into the soil, can enhance the uptake of phosphorus, as well especially in acidic soils that are low in phosphorus. The replenishment process, which involves the increase of Phosphorus concentration in soil solution, has been regarded as the primary index of available Phosphorus (Schofield, 1955: Holford, 1989).

Phosphate moves from a point of higher concentration to a point of lower concentration when a concentration gradient exists. The concentration gradient in soil across the root surfaces is an important factor influencing Phosphorus diffusion (Kamprath and Watson, 1980).

Soil texture is another factor affecting diffusion of Phosphorus (Olsen and Watanabe. 1963). As the clay content increases, the diffusion coefficients increase due to a decrease

in tortuosity and an increase in buffering capacity. The diffusion of phosphate persists until the equilibrium is established. Since the diffusion of phosphorus occurs essentially in the liquid phase and an individual phosphate ion spends a relatively short time in this phase, the diffusion coefficient of phosphorus in the soil solution will be different from that in free solution.

Diffusion coefficient of phosphate through soil is in the range of 10^{-8} to 10^{-11} cm^2S^{-1}. Diffusion through the soil phase is extremely slow and the phosphate ion, being negatively charged, would not likely diffuse along the negatively charged surfaces of soil particles. Fitter (1992) stated that a phosphate ion normally moves less than a millimeter through the soil in a day. The diffusion coefficient for phosphate ion in water

is 0.89 X $I0^{-5}cm^2S^{-1}$. Phosphorus diffusion through soil is slower than in pure water for three reasons, (i) soil water occupies only part of the soil so the cross-sectional area for diffusion is less; (ii) the diffusion path is tortuous because the water is present as films around soil particle; and (iii) most of the diffusible phosphorus is adsorbed on soil surfaces which equilibrate with and buffers the small amount of phosphorus in soil solution.

According to Sibbesen (1983) the Phosphorus uptake of a plant root over a period of time depends on: the initial concentration of Phosphorus in soil solution; the soil medium for Phosphorus-diffusion; and the Phosphorus dissolution of solid phase Phosphorus, as a function of decreasing solution Phosphorus-concentration with time, changing activity of HCO_3^- and H^+ in the rhizosphere, changing activity of exuded organic anions and changing activity of phosphate precipitating cations. All the factors that govern the rate of phosphorus diffusion to the root and the extent of root growth are important in determining the availability of phosphorus to growing plants in a soil.

2.9 Phosphorus uptake by plants

Plants obtain their phosphorus from the soil in which they grow and, if no fertilizer has been used, the phosphorus in the soil is derived from the parent material from which the soil was formed**.** Plant roots take up nearly all Phosphorus as either the primary or secondary orthophosphate anion ($H_2PO_4^-$ or HPO_4^{-2}), respectively. Primary orthophosphate is the form that is dominant in acid soils and is taken up about 10 times as readily as the secondary orthophosphate form. At a soil pH of 7.0 there is approximately equal amounts of the two Phosphorus forms and as the soil pH increases above pH 7.0, the secondary orthophosphate ion becomes the dominant form of available Phosphorus. All Phosphorus sources applied to the soil must be converted to the orthophosphate forms before a plant can utilize them. However, applying these forms of Phosphorus to the soil does not guarantee that they will remain in that form for very long. The fact is phosphorous is highly reactive, and is readily converted to other less soluble forms. The particular forms that are created depend on other soil factors such as the soil pH, temperature, moisture, other elements, and others.

This is one reason all aspects of the soil must be optimized before plants will perform at their best. On soils with higher pH, there will be some absorption of the HPO_4^{2-} ion. The absolute quantity of these ions present in the soil and available for uptake at any one time is very small. The amount that is dissolved and accessible in the soil solution is in equilibrium with solid phase phosphorus. The solid phase consists of both the organic and inorganic forms in the soil. Crops need more phosphorus than is dissolved in the soil solution to grow economically, therefore this phosphorus `pool' must be replenished many times during the growing season. It must be emphasized that the ability of a soil to

maintain adequate levels of Phosphorus in the solution phase is key to the plant available phosphorus status of a soil.

2.10 Classification of soils

Soils are grouped into three categories namely low, medium and high levels of test phosphorus for fertilizer recommendations purpose. (Fitts and Nelson 1956). Scaife (1985) and Sac (1985) classified soils into five categories on the basis of standard phosphate requirements, while Maff (1986) has ten categories of phosphorus levels for agricultural land. Sharpley (1991) divided soils into three groups based on taxonomic and chemical characteristics. Beckett and White (1964) reported that the phosphate potential of a given soil is controlled by the relative quantities of labile Phosphorus and Ca. They suggested that isotopically exchangeable Phosphorus of a field must be divided functionally into four parts: Phosphorus at surface net exchange sites; not immediately labile, requiring only to be displaced by OH^{-1} or possibly another anion to be available for uptake; Phosphorus held at occluded net exchange sites, not immediately available but capable of mobilization as a result of slow counter diffusion and exchange of H_2PO_4 and OH^{-1}, in response to depletion and consequent lowering of phosphate activity in soil solution; Phosphorus held at surface sites, crystalline phosphate not to be exchanged by OH^{-1}, but nevertheless capable of moderately rapid mobilization, this fraction will be particularly sensitive to the chelating action of root and microbial exudates; Phosphorus held within more perfect crystal lattices at sites from which Phosphorus can only be mobilized both by disposal of complementary cations and by substantial crystal arrangements, this fraction is only available to prolonged cropping.

2.11 SOIL PHOSPHORUS TESTINGPROCEDURES

Soil Phosphorus tests involve extraction of Phosphorus from soils with chemical or ionsink extractants followed by a quantification of Phosphorus in the extracting solution. Soil test is expected to determine the amount of Phosphorus that can contribute to crop growth or water contamination. From the standpoint of availability to plants, soil Phosphorus can be divided into functional pools of differing bioavailability (Tiessen *et al*., 1982). The information on soil Phosphorus transformation between those pools is useful to predict Phosphorus bioavailability as well as the risk of Phosphorus transfer from soil to surface waters. However, soil Phosphorus transformation has received less attention attributable to the difficulties associated with separation of inorganic Phosphorus (Pi) and organic Phosphorus (Po) fractions and compositional identification of soil Po pools. Such investigation is currently possible with an improved sequential fractionation procedure and adoption of advanced techniques such as nuclear magnetic resonance spectroscopy (NMR) and synchrotron-based techniques like X-ray absorption near-edge structure (XANES).

To determine the need for supplemental Phosphorus, soil tests are often used to estimate how much phosphate will be available for a crop. Different chemicals and testing methods extract different quantities of nutrient from soil. A soil test is a process by which elements are chemically removed from the soil and measured for their "plant available" content within the sample. Soil testing methods are used with varying degree of success to determine the Phosphorus status in soil and assist in making fertilizer recommendations. A major limitation of such procedures is that the results obtained are influenced by soil type requiring extensive field calibration (Luscombe *et al.,*

1979).These soil Phosphorus testing methods can be employed over soils with variety of physical and chemical properties (Sharpley *et al.,* 1994). The efficacy of soilPhosphorus testing method must be directed towards its ability to extract Phosphorus in a similar manner as plant roots does and at the growth stage where plants require Phosphorus most for growth and development. The quantities and origin of Phosphorus removed by different extraction procedures vary widely. The pH of extraction medium particularly has substantial effects on the amount and form of Phosphorus removed. A criticism against extractants is that they are either more acidic or alkaline than the soil solution and the portion of Phosphorus extracted is of low plant availability. Studies have shown that conventional methods for the determination of available Phosphorus appear to extract a portion of all chemical forms having either high solubility or high specific surface (Chang and Jackson, 1957).

Sims *et al*., (1998) also stated that: the fundamental goal of soil Phosphorus testing has always been to identify the "optimum" soil test Phosphorus concentration required for plant growth; the need for additional fertilization or manuring and the economic return on an investment in fertilizer Phosphorus could then be predicted. Other objectives of soil testing have been to "index" the Phosphorus supplying capacity of soils, thus estimating the time before fertilization would again be required, and to group soils in terms of the likelihood of an economic response to Phosphorus, based on their physical and chemical properties. Bray (1948) recognized the value of a systematic approach to soil testing and identified the following characteristics of a successful soil test extractant for phosphorus:

- The soil test should extract all or a proportionate amount of the plant available Phosphorus from soils with differing chemical and mineralogical properties.
- The Phosphorus extracted by the soil test should be well correlated with plant

Phosphorus concentration, plant growth and the response of the plant to added Phosphorus in fertilizers or manures;

- The soil test should accurately detect differences in soil p concentrations caused by previous fertilization or manuring and should be accurate and rapid.

The quantity of available nutrients in the sample determines the amount of fertilizer that is recommended hence the need for soil Phosphorus testing. Many authors have designed various soil test Phosphorus methodologies to determine soil-available Phosphorus. The available soil phosphorus originates from the breakdown of soil minerals, from soil organic matter, or from the previous addition of phosphate fertilizer and is usually only about 1 % of the total soil phosphorus (Sharpley, 2000) The main source of plant available Phosphorus is generally termed the labile pool. This provides fairly rapid exchange with soil solution, maintaining the solution concentration. The remaining fraction is the non-labile pool. This contains a large quantity of insoluble phosphate, which is very slowly released into the soil labile pool. Various organic and inorganic phosphates constitute these labile and non labile pools. There is no clear distinction by which a particular form can be assigned to labile or non labile pool. In general the labile pool can be considered as orthophosphate adsorbed onto surfaces of clay minerals, hydrous oxides and carbonates plus iron and aluminium phosphates. The relationship between the quantity of phosphorus in the labile pool and the soil solution concentration depends particularly on soil texture and pH (Archer, 1988) Adsorption of orthophosphate is stronger and is less available at low pH. Precipitation as iron and aluminium phosphate will also reduce phosphorus availability. In calcareous soils precipitation as calcium phosphate is the main factor

limiting solubility. Phosphate adsorbed on calcium carbonate will slowly convert to mineral apatite. The most common way of determining Phosphorus availability is to mix a small amount of soil with an extracting solution that contains an acid and/or complexing agent that will remove some of the phosphate from the soil particles. The extracting solution and soil are separated by filtration and the amount of Phosphorus extracted is determined. In alkaline soils, a basic solution may be used as the extractant because an acidic solution will be neutralized by the alkaline soil and be less effective in extracting Phosphorus.

In the beginning of the 20th century it was generally recognized that the total soil Phosphorus content does not define the plant available Phosphorus in soil (Kurtz, 1953). Nelson *et al*., (1953) reviewed routine soil test procedures used in estimating Phosphorus plant availability and divided them into two broad categories: (1) extraction methods and (2) biological methods. Because the biological methods involving field experiments or greenhouse experiments with higher plants or use of microbes as indicators for available Phosphorus were too time consuming to be used as routine tests, the development of extraction procedures expanded. In 1953, the chemical extraction methods used formed a long list, including water, carbon dioxide saturated water, acids, bases, salts, buffered solutions, electrodialysis and ion exchangers (Nelson *et al*., 1953). A large variety of extraction tests is used all over the world. Behind each procedure in use, there are extensive studies on the ability of the method to produce Phosphorus concentrations that correlate well with the Phosphorus uptake by plants and are thus calibrated with fertilization recommendations (Krogstad *et al*., 2008). However, the extraction tests used for bio-availability estimations of Phosphorus often serve at the same time as tests to estimate the availability of other important nutrients and might even be developed to

extract other nutrients from soil. In these cases the theory and mechanism behind the solubilization of Phosphorus is not always clear.

2.11.1 Conventional chemical extractions

Many methods exist for the determination of the various forms of soil phosphorus. Early interest in examining soil Phosphorus were primarily based on determining the quantity of supplemental phosphorus needed to adequately meet the needs of crops. Availability of Phosphorus for plant utilization is not a function of its concentration in soil, but rather on the rate of its release from soil surface into soil solution (Abdu, 2006). Available Phosphorus is composed of soil solution Phosphorus and is replenished by Phosphorus that enters the solution by desorption or dissolution of Pi associated with the soil solid phase or by mineralization of Po (Hedley *et al*.,1982). The measurement of available Phosphorus therefore needs to consider both the amount and rate of release of Phosphorus from the solid phase. Very few appropriate methods have been developed.

Isotopic dilution (^{32}P) techniques theoretically permit researchers to quantify the processes of Po mineralization, dissolution of insoluble minerals and desorption of aggregate Phosphorus, and could be likely used for this purpose. But errors involved in the measurement of change rates make it difficult to extrapolate the continuing release (or isotopic dilution) rates to a temporal scale corresponding to cropping seasons and growth cycles under field conditions(Tran *et al*., 1988; Sharpley *et al*., 1994). Thus, some limitations have to be overcome to give results that have practical application. The most widely used soil Phosphorus tests are chemical extractions that use chemical reagents to extract available Phosphorus from soils.

Soil Phosphorus extraction can be categorized into various groups on the basis of their chemical nature. In certain cases anion in the solution may have some specific effect either because of their anion replacing ability or their reaction with the cation associated with the phosphorus. The routine soil Phosphorus tests may not give insight into the level of plant available Phosphorus as the chemical reagents may solubilize non-labile Phosphorus. For instance, the acidic Bray and Mehlich I extractants can dissolve Al- and Fe-phosphates, while Olsen extractant removes dissolved and adsorbed Phosphorus on calcium carbonate and Fe-oxide surfaces (Mallarino, 1997). Moreover, these chemical extractants are not applicable over all soil types, which underscore the use of them for soil Phosphorus extractions (Myers *et al*., 2005). Bray and Kurtz (1945) used a combination of HCl and NH_4F to remove easily acid soluble Phosphorus forms, largely Al- and Fe-phosphates. In 1953, Mehlich introduced a combination of HCl and H_2SO_4 acids (Mehlich 1) to extract Phosphorus from soils in the north-central region of the U.S. In the early 1980s, Mehlich modified his initial soil test and developed a multi-element extractant (Mehlich 3) which is suitable for removing Phosphorus and other elements in acid and neutral soils (Mehlich, 1984). Olsen *et al.* (1954) introduced 0.5 M sodium bicarbonate ($NaHCO_3$) solution at a pH of 8.5 to extract Phosphorus from calcareous, alkaline, and neutral soils.

Bray andMehlich-3 extractants were designed to extract Phosphorus from noncalcareous soils, whereas the Olsen method was meant for non-acidic soils. Furthermore, those conventional soil Phosphorus tests derived from mineral soils may not necessarily be applicable for organic soils, although some routine soil Phosphorus tests are being adopted to make agronomic recommendations in muck soils (Castillo and Wright, 2008;

Wright, 2009). This, however, is an "alternative-than-never" choice, at this moment without specific test for organic soils available. A list of commonly used extractant to measure available phosphorus in soil is as follows.

2.11.2 Distilled water

Water is probably the simplest extractant used for plant available Phosphorus. A water extract removes dissolved forms of Phosphorus but very little of the adsorbed and mineral forms. It is suitable for both acid and calcareous soils. The amount of Phosphorus extracted is small for most soils, and may not reflect all forms of labile Phosphorus. Water was probably the first extractant used to measure Phosphorus in soils. The small amounts of soil Phosphorus extracted by water and difficulties related to chemical analysis limit the use of water as an extractant.

2.11.3 Sodium bicarbonate

Olsen *et al*., (1954) introduced 0.5 M sodium bicarbonate ($NaHCO_3$) solution at a pH of 8.5 to extract Phosphorus from calcareous, alkaline, and neutral soils. In calcareous soils, the main function of $NaHCO_3$ in extracting Phosphorus is to decrease the Ca^{2+} activity in solution by forming calcium carbonate ($CaCO_3$) precipitate (Olsen *et al*., 1954). For highly calcareous soils (pH greater than 7.4), the Olsen sodium bicarbonate method is used. The "Olsen Phosphorus" or sodium bicarbonate soil test is primarily used in the North Central and Western United States. The Olsen Phosphorus method is best suited for calcareous soils, particularly those with > 2% calcium carbonate, but has been shown in some research to be reasonably effective for acidic soils (Fixen and

Grove, 1990). The method also decreases the solution concentrations of soluble Al^{3+} and Fe^{+3} by formation of Al and Fe oxyhydroxides, thus increasing Phosphorus solubility. The increased surface negative charges and/or decreased number of sorption sites on Fe and Al oxide surfaces at high pH levels also enhance desorption of available Phosphorus into solution. An Olsen Phosphorus value of 10 mg P/kg is generally considered to be optimum for plant growth. This is lower than the critical values used for the Bray and Kurtz P-1, Mehlich 1 and Mehlich 3 soil tests because the Olsen extractant removes less Phosphorus from most soils than these acidic extractants. Kuo (1996) stated that proper interpretation of Olsen Phosphorus results for soils with diverse properties requires some information on soil Phosphorus sorption capacity. Similarly, Schoenau and Karamanos (1993) cautioned against use of the Olsen test to compare Phosphorus availability in soils with large differences in Phosphorus chemistry. However, in acid soils it is more likely that the solution pH, buffered to 8.5, promotes desorption of Phosphorus. In soils, containing Al- and Fe-bound Phosphorus, the Phosphorus concentration in solution increases as the pH increases because at high pH, the higher concentration of OH^- ions decreases the ability of PO_4-P to compete for sorption sites (Rajan *et al*., 1974; Hartikainen, 1981; Hartikainen and Yli-Halla, 1996). Olsen's bicarbonate method is still widely used in estimating the soil plant availability example in Denmark, England, Australia and New Zealand (Vanderdeelen, 2002). It has also been known that Sodium bicarbonate extracts more phosphorus than acetic acid because it extracts phosphorus from soil physisorbed, chemisorbed and also a small quantity of microbial phosphorus (Bowman and Cole, 1978).Barrow and Shaw (1976) pointed out that secondary reactions are minimized by the use of $NaHCO_3$ as a soil test. They enumerated that the factors which

affect their extent are: the soil solution ratio, the period of shaking with $NaHCO_3$, the kind of soil, and the level of phosphate.

2.11.4 Organic acids

Other extractants suggested for soil Phosphorus testing include organic acids. The organic acids used are citric acid, lactic acid and acetic acid, and methods exploiting these are used in the EU at least in Austria, Belgium, Finland, France, Germany, Ireland and Sweden (Vanderdeelen, 2002). Salts of weak acids have also been used as soil Phosphorus extractants. Acetic acid alone or buffered by acetate salts has been used to measure the status of plant nutrients in soil. Acetic acid solution, which generally has a pH 2-3, provides sufficient hydrogen ion activity to dissolve calcium phosphate. It also solubilizes some of aluminium and iron phosphate, Egner-Rheim‟s reagent (ammoniumlactate/acetic acid, pH 3.8) has also been used in extracting soil phosphorus.

2.11.5 Mehlich 1

The Mehlich 1 soil test for phosphorus, also known as the dilute double acid or North Carolina extractant, was developed in the early 1950s by Mehlich and his coworkers (Mehlich, 1953; Nelson *et al*. 1953). Mehlich 1 extracting solution is a combination of hydrochloric acid (HCl) and sulphuric acid (H_2SO_4) acid. Sulfate ions in this acid solution can dissolve Aland Fe phosphates in addition to Phosphorus adsorbed on colloidal surfaces in soils. The Mehlich 1 extracts Phosphorus from aluminum, iron, and calcium phosphates and is best suited to acid soils (pH < 6.5) with low cation exchange capacities (< 10 cmol/kg) and organic matter contents (< 5%). Kuo (1996) reported that the Mehlich 1 soil test was unreliable for calcareous or alkaline soils because it extracts large amounts of non

labile Phosphorus in soils with pH > 6.5, soils that have been recently amended with rock phosphate, and soils with high cation exchange capacity

(CEC) or high base saturation. In soils such as these the acidity of the Mehlich 1 solution is neutralized, reducing the capability of the dilute acid to extract Phosphorus. A Mehlich 1 Phosphorus value of 20 to 25 mg P/kg soil for the Mehlich-1 test is generally considered to be optimum for plant growth, although this may vary slightly between soil types and cropping systems. For instance, Kamprath and Watson (1980) stated a Mehlich-1 P of 20 to 25 mg P/kg soil is adequate for plants grown in sandy soils but only 10 mg P/kg soil is required for fine-textured soils, a point supported by the work of

Lins and Cox (1989).

2.11.6 Mehlich 3

In the early 1980s, Mehlich modified his initial soil test and developed a multi-element extractant (Mehlich 3) which is suitable for removing Phosphorus and other elements in acid and neutral soils. Mehlich 3 extractant (Mehlich, 1984) is a combination of acids (acetic [HOAc] and nitric [HNO_3]), salts (ammonium fluoride [NH_4F] and ammonium nitrate [NH_4NO_3]), and the chelating agent ethylenediaminetetraacetic acid (EDTA). The Mehlich 3 is similar in principle to the Bray and Kurtz P-1 test because it is an acidic solution that contains ammonium fluoride.

Acetic acid in the extractant also contributes to the release of available Phosphorus in most soils. It is more effective than the Mehlich 1 soil test at predicting crop response to Phosphorus on neutral and alkaline soils because the acidity of the extractant is neutralized less by soil carbonates (Tran and Simard, 1993). A Mehlich 3 value of 45-50 mg P/kg soil is generally considered to be optimum for plant growth and crop yields.

2.11.7 Bray 1 and Bray 2

The Bray and Kurtz P-1 soil test phosphorus method was developed by Roger H. Bray and Touby Kurtz of the Illinois Agricultural Experiment Station in 1945 and is now widely used in the Midwestern and North Central United States (Bray and Kurtz, 1945; Frank *et al*., 1998). The soil phosphorus measured is that which is extracted by a solution consisting of 0.025 N HCl and 0.03 N NH_4F, referred to as Bray-1 extractant. Phosphorus extracted by the Bray and Kurtz P-1 method has been shown to be wellcorrelated with crop yield response on most acid and neutral soils. For acid soils, the fluoride in the Bray and Kurtz extractant enhances Phosphorus release from aluminum phosphates by decreasing Al activity in solution through the formation of various Al-F complexes. Fluoride is also effective at suppressing the readsorption of solubilized Phosphorus by soil colloids. The acidic nature of the extractant also contributes to dissolution of available Phosphorus from Al, Ca, and Fe-bound forms in most soils. A Bray and Kurtz P-1 value of 25 to 30 mg P/kg soil is often considered optimum for plant growth, although Holford (1980) reported lower critical values for highly buffered soils. Bray 2 extracting solution is a strong form of the Bray 1 extracting solution and it is made up of 0.1 N HCl and 0.03 normal NH_4F. Its mode of action is similar to that of Bray 1.

Neutral ammonium fluoride is also used as an extractant, which extracts adsorbed phosphate. Strong complex formation between fluorine and aluminium makes this reagent a powerful extractant of aluminium bound Phosphorus (Williams and Knight, 1963).

Turner and Rice (1952) found that neutral ammonium fluoride can dissolve aluminium phosphate but not iron phosphate.

Chang and Jackson (1957) reported in fractionation of soil phosphorus that neutral ammonium fluoride in a single extraction dissolves aluminium phosphate completely, iron phosphate slightly and apatite negligibly, in a soil extractant ratio of 1:50 ammonium fluoride in a calcareous soil or something in acid soil reacts with the formation of CaF_2 which causes the over estimation of acid extractable $Ca_3(PO_4)_2$ and non occluded Fe and Al bound phosphate.

2.11.8 Morgan and modified Morgan

Morgan‟s reagent (3% acetic acid buffered with 10% sodium acetate) and modified Morgan‟s reagent (3% acetic acid buffered with 4% ammonium acetate) with pH 4.8 is also used as an extractant which extracts relatively small amounts of phosphorus. Acid ammonium used as an extractant breaks up the structures of aluminium oxide polymers and also extracts some of the phosphorus which is not labile.

2.11.9 Anion exchange resins and iron-impregnated strips

In addition to different chemical extractants, methods simulating Phosphorus uptake by plant roots have been developed. Example is the ion-sink method. Ion-sink methods usually employed in Phosphorus extraction include anion and cation exchange resin membranes, resin bags, iron oxide(FeO) coated filter papers or strip. Anion exchange resins and iron-impregnated strips can act as sinks for Phosphorus, and thus, mimic plant uptake (Chardon *et al*., 1996; Bache and Ireland, 1980; Raven and Hossner, 1993; Buehler

et al., 2002). These soil Phosphorus testing methods can be employed over soils with variety of physical and chemical properties (Sharpley *et al*., 1994).

Thus, methods and extractants intended for the same purpose, such as estimating soil plant available Phosphorus, can be expected to and do give different results for the same soil (Neyround and Lischer, 2003). Even though, anion exchange resins extract more Phosphorus than FeO-coated papers, the additional Phosphorus extracted may not be plant available (Robinson and Sharpley, 1994). FeO-coated papers are not so much available in the market (Myers *et al*., 2005); soil particles can contaminate the FeOcoated papers during shaking (Chardon *et al*., 1996) which can lead to error in estimating desorbable Phosphorus (Uusitalo and Yli-Halla 1999). This can however, be minimized by the use of $CaCl_2$ solution as the background electrolyte which tend to minimize soil dispersion (Myers *et al*., 2005). But this can lead to reduction in the amount of Phosphorus extracted (Koopmans *et al*., 2001). With all the mentioned disadvantages of the FeO-coated papers, Ion-sink methods especially when anion exchange membrane is used are still regarded as the best method of plant-available Phosphorus extraction technique. Its major advantage is its capability to extract Phosphorus from variety of soil types irrespective of the properties of the soil (Sharpley *et al*., 1994). Anion exchange resin membranes do not alter the chemical and physical characteristics of the soil, they quite simulate the soil aqueous solution. Furthermore, they can be re-used for several times without losing their extracting power (Schoenau and Huang, 1991). This property makes it relatively cheaper than the FeO-coated papers. The problem associated with the pH of the soil solution can be overcome by charging

the resin with either HCO_3^- or $Cl^-.HCO_3^-$ is used for charging the resin when the soil is alkaline and calcareous (Agbenin and Raij, 1999; Delgado and Torrent, 2001), while Cl^-

is used for acidic soils (Agbenin and Raij, 1999). Sibbesen (1978) observed that the use of HCO_3^-resinis more advocated than Cl^--resin because plant roots accumulate bicarbonate in the rhizosphere leading to an increase in rhizosphere pH in acid to neutral soils and a decrease in rhizosphere pH in calcareous soils. When Cl^--resin is used, the Claccumulates in solution thereby inhibiting the exchange reaction (Myers *et al*., 2005).An important aspect of resin use that needs standardization is the resin strip size and its total surface area. Different authors have used different sizes ranging from 9 x 62 mm to 25 x 62.5 mm which has led to disparity in the amount of Phosphorus extracted.

2.12 Phosphorus determination

Analysis of extracted Phosphorus is typically done by colorimetry, most notably the Murphy and Riley(1962) method which is the method used in this work. A specific ion reacts with the color developing reagents to form colorful complex (example the blue antimony phospho-molybdate), then light absorption by the formed complex is detected at a specific wavelength. Colorimetric procedures are sensitive, reproducible and amendable to automated analysis. In addition, the methods can be accommodated to water samples, digested solutions and extracts (Pierzynski *et al*., 2008).

Inductively coupled plasma (ICP) spectrophotometry is also now commonly used for Phosphorus determination, particularly in routine soil Phosphorus tests offered by public and commercial laboratories. The use of ICP has increased as the use of multi-element soil extractants becomes more popular. Results from ICP are not always directly comparable to those from colorimetric analyses (Pierzynski *et al*., 2011), as ICP estimates the total Phosphorus in a solution while the colorimetric procedures measure Phosphorus that can

react with the color developing reagents. Moreover, there are certain limitations that must be considered while evaluating data generated by ICP, such as the matrix effects, spectral interference, etc. (De Boer *et al*., 1998).

Nuclear magnetic resonance (NMR) is a physical phenomenon based upon the magnetic property of the atomic nucleus. It is observed that magnetic nuclei, like ^{1}H, ^{13}C and ^{31}P, could absorb radio frequency when placed in a magnetic field with a specific strength, described as the resonance of the nucleus. Different atoms in a molecule resonate at different frequencies at a given field strength. This is a powerful method that allows researchers to determine the structure of chemical compounds. The use of solution ^{31}P NMR spectroscopy has allowed us to identify P forms in soils and residual materials, and confirm Phosphorus forms estimated by commonly used chemical extractants, such as sequential fractionation schemes. This technique has enabled more accurate determination of organic forms of Phosphorus in soils and residual materials (Zhang *et al*., 1999; Turner and Leytem, 2008). In addition, the use of synchrotron-based techniques (example XANES) has provided insights into both Pi and Po forms in soils and residual materials. Descriptions of these approaches were detailed by Beauchemin *et al*. (2003) and Shober *et al*. (2006). These analytical advances have been critical in gaining a more detailed understanding of soil Phosphorus transformation and reaction products following land application of residual materials. This information has helped assess the fate, reactivity, behavior of specific forms of Phosphorus and the environmental implications of land application of materials such as biosolids and animal manures (Pierzynski *et al*., 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

All chemicals used were of Analar grade and commercially available. All reagents were prepared using double distilled water. Glassware were washed well with water and soaked overnight in 5% HCl. They were then rinsed with distilled water, dried and kept for use.

3.1Glass ware, Apparatus and Equipment	**3.2 Chemicals**
25 mL,50 mL ,100 mL,500 mL,1 L and 2 L volumetric flasks	Nitric Acid
25 mL, 50 mL measuring cylinders	Sulphuric Acid
50 mL Burette	Hydrochloric Acid
10 mL pipette	Disodium EDTA
Whatman No. 42 filter papers	Acetic Acid
2 mm meshsieve	Ammonium Nitrate
pH Meter	Ammonium Fluoride
Conductivity meter	Ferrous Ammonium Sulphate
UV/Visible Spectrophotometer (Shimadzu UV mini 1240 series)	Potassium dichromate
Magnetic stirrer	Sodium hexametaphosphate
Analytical balance	Potassium dihydrogen phosphate
	Ammonium paramolybdate
	Potassium antimony tartarate

3.3 PREPARATION OF REAGENTS

3.3.1 Phosphorus determination

- Sulphuric Acid (H_2SO_4)2.5 M

This was prepared by adding 141 mL of concentrated H_2SO_4 to 800 mL of distilled water. The mixture was cooled to room temperature and diluted to 1000 mL with distilled water.

- Standard phosphate solution 100 mg/L

The standard phosphate solution was prepared by weighing 0.8788 g oven dried potassium dihydrogen phosphate (KH_2PO_4) into a beaker and dissolved with about 20 mL of distilled water. The resulting solution was transferred into a 2 L volumetric flask and the solution was diluted to 2 L with distilled water. Two drops of toluene were added to inhibit microbial activity.

- Working phosphate solution 2 mg/L

This solution was prepared by diluting 2 mL standard working solution to 100 mL with distilled water

- Reagent A

Reagent A was prepared by dissolving accurately weighed 12.0 g Ammonium paramolybdate, ($(NH_4)_6Mo_7O_{24}.4H_2O$) in 250 mL distilled water. A mass of 0.2908g of potassium antimony tartarate ($KSbO.C_4H_4O_6$) was also dissolved in 100 mL distilled water. The dissolved reagents were added to the 1L solution of already prepared 2.5 M H_2SO_4 and mixed thoroughly in a 2 L volumetric flask. The resultant solution was diluted with distilled water to 2L. The solution was stored in a Winchester bottle in a dark and cool compartment.

- Reagent B

  Reagent B was prepared by dissolving 1.056g of Ascorbic acid in 200 mL of Reagent A. This solution was prepared and used on daily basis because the solution is not stable for more than 24 hours.

- Calibration curve for Phosphorus

  Calibration curve for Phosphorus was obtained by adding 4 mL of reagent B to 1, 2, 4, 6, 8, 10 and 12 mL of working phosphate solution (2 mg/L) and diluting to 25 mL with distilled water. Absorbance readings were taken at wavelength of 880 nm using spectrophotometer (Shimadzu UV mini 1240 series). A calibration curve of phosphorus concentration versus absorbance was constructed from which concentrations of unknowns were obtained.

3.3.2 Total Phosphorus

Reagents

- Perchloric acid ($HClO_4$) 60%
- Ammonium paramolybdate-vanadate. This was prepared by dissolving 25 g of ammonium paramolybdate ($(NH4)_6Mo_7O_{24}\cdot 4H_2O$) in 400 mL distilled water. Ammonium metavanadate (NH_4VO_3) was dissolved in 300 mL distilled water with boiling. The vanadate solution was cooled to room temperature and 250 mL concentrated HNO_3 was added. The NH_4VO_3-HNO_3 solution was cooled to room temperature and the $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ solution added. The mixture was then diluted to 1 L with distilled water.

- Standard phosphate solution 100 mg/L
- Sodium hydrogen sulfite ($NaHSO_3$): This was prepared by dissolving 5.2 g of $NaHSO_3$ in 100 mL of 0.5 MH_2SO_4.

3.3.3% Organic Matter Determination

- Standard potassium dichromate solution 0.0167M
- An amount of 49.13g $K_2Cr_4O_7$ was dried for 24 hours at 102 ^{0}C. The salt was dissolved in 500 mL distilled water. Exactly 167 mL concentrated H_2SO_4 was added followed by 33.3g Mercuric sulphate. The resulting solution was cooled and transferred into 1L volumetric flask and diluted to the mark.
- Standard Ferrous Ammonium Sulphate (FAS) 0.010M

Ferrous Ammonium Sulphate was prepared by dissolving 39.2 g of ferrous ammonium sulphate (FAS) in about 500 ml distilled water, 20 ml of concentrated sulphuric acid (H_2SO_4) was added , transferred into a 1 L volumetric flask and diluted to the mark with distilled water. The Fe^{2+} in this solution oxidizes slowly on exposure to air so it was standardized against the dichromate solution daily before use.

The FAS solution was standardized daily against the $K_2Cr_4O_7$ as follows:

5 mL distilled water was pipetted into a conical flask,

3 mL of dichromate solution was added followed by 7 mL concentrated H_2SO_4,

The mixture was cooled to room temperature and titrated with the FAS solution to a light green end point using two drops of N-phenylanthranilic acid indicator Molarity of FAS= (Vol. of $K_2Cr_4O_7$, mL /Vol. of FAS used) *0.1

3.3.4 Phosphorus extraction reagents

A volume of 3 L of each extraction reagent was prepared.

3.3.4.1 Bray 1

Bray 1 extracting solution consists of 0.025 M HCl in 0.03 M NH_4F. This extractant was prepared by dissolving 3.33 g ammonium fluoride in 1 L distilled water. A volume of 75 mL of previously standardized 1M HCl was added to the ammonium fluoride solution in a 2 L volumetric flask and made to volume with distilled water. The resulting solution was transferred to a clean 5 L container, followed by the addition of 1 L of distilled water. The solution was mixed thoroughly and stored for use.

3.3.4.2 Bray 2

Bray 2 extracting solution is a more concentrated solution of Bray 1. The extractant is made up of 0.1 M Hydrochloric Acid (HCl) in 0.03 M Ammonium Fluoride (NH_4F). It was prepared by dissolving 3.33g ammonium fluoride in 1 L distilled water followed by the addition of 25 mL of concentrated hydrochloric acid in a 2L volumetric flask which was topped to the mark with distilled water. The solution was transferred to a 5 L clean container, and 1 L of distilled water added. The solution was mixed thoroughly and stored for use.

3.3.4.3 Mehlich 1

This extractant was made up of 0.0125 M Sulphuric Acid (H_2SO_4) and 0.05 M Hydrochloric acid (HCl). The extractant was prepared by adding 12.5 mL of concentrated

HCl (12 M) and 2.1 mL of concentrated H_2SO_4 (18 M) to 1 L distilled water. The solution was transferred to 2 L volumetric flask and made to the mark with distilled water. The resultant solution was transferred to a 5 L clean container, followed by the addition of 1 L distilled water. The resultant solution was mixed thoroughly and stored for further use.

3.3.4.4 Mehlich 3

This extracting solution is a combination of different acids comprising: 0.2 M Acetic Acid (CH_3COOH), 0.25 M Ammonium nitrate (NH_4NO_3), 0.015 M Ammonium fluoride (NH_4F), 0.013 M Nitric Acid (HNO_3), 0.001 M Ethylenediamminetetraacetic Acid (EDTA).

Ammonium fluoride (NH_4F) and EDTA stock solution (3.75 M NH_4F:0.25 M EDTA) was prepared by dissolving 3.055 g of NH_4F and 1.607g EDTA in about 22 mL distilled water. A mass of 60 g of ammonium nitrate (NH_4NO_3) was dissolved in about 60 mL distilled water in a 2L flask and 12mL of the NH_4F-EDTA stock solution was added and mixed well. A volume of 34.5 mL glacial acetic acid (99.5%, 17.4 M) was added followed by 2.46 mL of concentrated nitric acid (HNO_3). Distilled water was added and made to the mark. The resulting solution was transferred to a 5 L clean container, 1 L of distilled water was added, mixed well and stored for use.

3.3.4.5 Disodium EDTA

This extractant is a 0.025 M disodium ETDA. It was prepared by dissolving 27.918 g Na_2EDTA in 1 litre using distilled water. The pH of the resulting solution was 5.2. The

resulting solution was transferred to a 5 L clean container, 2 L of distilled water was added, mixed well and stored for use.

3.3.4.6 Olsen' extractant

The Olsen‟s extractant is a 0.5 M Sodium bicarbonate ($NaHCO_3$) solution which was made by dissolving 126 g sodium bicarbonate in 1L using distilled water. The pH was adjusted to 8.5 with 50% sodium hydroxide. The resulting solution was transferred to a 5 L clean container, 2 L of distilled water was added, mixed well and stored for use.

3.3.4.7 Distilled water

A clean 3 L jar was used to collect distilled water. The pH of the distilled water was 6.7 and was used as the extracting solution.

3.4 Soil sampling and preparation

In this study, 12 soil samples collected from three different sampling sites were used. An uncultivated virgin land at Huhunya (HV), a cocoa farm at Akwadum (AC) and a plantain farm also at Huhunya (HP) all in the Eastern region of Ghana were the sites chosen for this study. Soils were collected from four different locations on each site. Samples from three depths comprising topsoil (0-10cm), a subsurface soil (10-20cm) and a subsoil (20-30 cm) were taken from each location in triplicate. In all a total of 36 soil samples were collected. The soil samples were air dried until their masses were constant on weighing, sieved with a 2mm mesh and kept in dry cleaned labeled containers for analysis. Table 3.1 shows the representation of the soil samples at the various soil depths.

Table 3.1: Soil samples and their code names:

Soil Depth (cm)	Akwadum Cocoa farm	Huhunya Virgin land	Huhunya Plantain farm
0-10 (Topsoil)	AC1	HV1	HP1
10-20 (Subsurface soil)	AC2	HV2	HP2
20-30 (Subsoil)	AC3	HV3	HP3

3.5 Soil analysis

3.5.1 Soil pH

Soil pH was determined using a 1:2.5 (w/v) soil: distilled water suspension as outlined by Anderson and Ingram (1993) suspension was prepared such that: for each 1 g soil sample, 2.5 mL distilled water was added, the soil solution was stirred continuously for 30 minutes with the aid of a magnetic stirrer and the pH of the suspension taken with a pH meter.

3.5.2 Soil Conductivity

The conductivity of the soil samples was measured using a soil solution which was prepared by adding 2.5 mL of distilled water to 1 g of soil. The soil solution was stirred continuously for 30 minutes with a magnetic stirrer and the suspension left to stand for 20 minutes. Conductivity meter was used to measure the conductivity of the supernatant.

3.5.3 Organic matter

Walkely- Black wet oxidation procedure (1934) was used to measure the organic carbon content in the soil samples. In this procedure, 5 mL of 0.0167M potassium dichromate

($K_2Cr_2O_7$) and 2.5 mL of concentrated sulphuric acid (H_2SO_4) were added to 0.5g of soil samples in a conical flask. The soil solution was swirled gently. Excessive swirling that could have resulted in organic particles adhering to the sides of the flask, taking them out of the solution was avoided. The mixture was then heated for 30 minutes at 150º for complete digestion. The digest was then cooled to room temperature and diluted to 50 mL with distilled water. It was then titrated against FAS (ferrous ammonium sulphate) solution to a light green end point using 2 drops of N-phenylanthranilic acid indicator.

Blanks were prepared using the same procedure but without soil samples.

Percent Organic carbon (%C) was calculated using the formula;

%C = ((B-S) x M of Fe^{2+} used x 12 x 100) / (g of soil ×4000)

Where:

B = mL of Fe^{2+} solution used to titrate blank

S = mL of Fe^{2}+ solution used to titrate sample

12/4000 = milliequivalent weight of C in g.

Percent Organic Matter was calculated using the formula

% Organic Matter = *(%* total C x 1.72) / 0.58

3.5.4 Particle size determination

The particle size of the soil samples were determined by weighing 51g of soil sample into „milkshake" mix cup. An addition of 50 mL of 10% sodium hexametaphosphate together with 100 mL distilled water was done. The mixture was shaken for 15 minutes after which the suspension was transferred from the cup into a 1 L measuring cylinder. Soil hydrometer was placed inside the suspension and distilled water was added to the 1000 mL mark. The

hydrometer was then removed. The cylinder with the soil suspension was placed on a flat surface and the time noted. The soil hydrometer was inserted immediately into the suspension and the first reading on the hydrometer taken at 40 seconds. The temperature of the suspension was also taken with a thermometer. After the first reading, the suspension was allowed to stand for 3 hours and the second hydrometer and temperature readings were taken.

Calculations:

$$\% \text{ Sand} = 100 - [H1 + 0.2\,(T1 - 20) - 2] \times 2$$

$$\% \text{ Clay} = [H2 + 0.2(T2 - 20) - 2] \times 2$$

$$\% \text{ Silt} = 100 - (\% \text{ sand} + \text{clay})$$

Where:

H = Hydrometer readings at 40 seconds

T1 = Temperature at 40 seconds

T2 = Temperature at 3 hours

H2 = Hydrometer readings at 3 hours

0.2(T – 20) = Temperature correction to be added to hydrometer reading

- 2.0 = Salt correction to be added to hydrometer reading

3.5.5 Total Phosphorus

Total phosphorus was determined by the method of Olsen and Sommers (1982). Two grams of finely ground soil (<0.5mm) was weighed into a 100 mL digestion tube and 30 mL of 60% $HClO_4$ was added to the mixture. The mixture was digested on a hot plate until the dark colour from organic matter disappeared. Heating was continued at the

boiling temperature for about 20 minutes until heavy white fumes appeared, and the insoluble material became like white sand. Two milliliters of $HClO_4$was used to wash down the black particles that stuck to the side of the digestion tube. Total digestion time was approximately 40 minutes. The digest was then cooled to room temperature, diluted to the 100 mL mark with distilled water and filtered into amber bottles for analysis.

To analyze for total P, suitable aliquots of the digest were transferred into 50 mL volumetric flasks. Ten mL of ammonium paramolybdatevanadate reagent was added and diluted to the mark with distilled water. The absorbances were read after 10 min at a wavelength of 490 nm. A calibration curve was obtained by taking 0 mL, 4 mL, 6 mL, 8 mL, 10 mL and 12 mL of 2 mg/L standard Phosphorus solution into 50 mL volumetric flasks, 10 mL of ammonium paramolybdatevanadate reagent was added to each flask and diluted to the mark with distilled water. The absorbances were read at the wavelength of 490 nm. A plot of concentration versus absorbance was constructed and the concentrations of Phosphorus in the digests were then extrapolated from the calibration curve.

3.6 AVAILABLEPHOSPHORUS EXTRACTION

Seven different procedures which are widely used were applied for extraction of soil phosphate. All soil samples were extracted in triplicate with each extractant. The procedure for the extraction process using the Bray 1, Bray 2, Mehlich 1, Mehlich 3, Olsen‟s extractant, Disodium EDTA and distilled water is as follows:

- Bray 1 Extraction

  Two grams of soil sample was weighed into 50-mL shaking container, 20 mL of Bray 1 extracting solution was added and shaken for five minutes at room

temperature. The extract was filtered through Whatman No. 42 filter paper and kept for analysis.

- Bray 2 Extraction

  A mass of 2g of soil sample was weighed into 50-mL shaking container, 20 mL of Bray 2 extracting solution was added and shaken for five minutes at room temperature. The extract was filtered through Whatman No. 42 filter paper and kept for analysis.

- Mehlich 1 Extraction

  Five grams of soil sample was weighed into 50-mL shaking container, 20 mL of Mehlich 1extracting solution was added and shaken for five minutes at room temperature. The extract was filtered through Whatman No. 42 filter paper and kept for analysis.

- Mehlich 3 Extraction

  A 2g soil sample was weighed into 50-mL shaking container, 20 mL of Mehlich 3 extracting solution was added and shaken for five minutes at room temperature. The extract was filtered through Whatman No. 42 filter paper and kept for analysis.

- Olsen Extraction

  One gram soil sample was weighed into 50-mL shaking container, 20 mL of Olsen extracting solution was added and shaken for thirty minutes at room temperature. The extract was filtered through Whatman No. 42 filter paper and kept for analysis.

- Disodium EDTA Extraction

  A 2g soil sample was weighed into 50-mL shaking container, 40 mL of Disodium

EDTA extracting solution was added and shaken for thirty minutes at room temperature. The extract was filtered through Whatman No. 42 filter paper and kept for analysis.

- Distilled water Extraction

  Two grams of soil sample was weighed into 50-mL shaking container, 20 mL of distilled water was added and shaken for five hours at room temperature. The extract was filtered through Whatman No. 42 filter paper. Activated carbon was used to remove colour from the filtrate and kept for analysis.

Phosphorus in the extracts was determined using the Molybdenum blue method modified by Murphy and Riley (1962) with ascorbic acid as the reducing agent. The blue colour was developed by adding 8 mL of reagent B to 10 mL of the extract in 50 mL volumetric flasks and diluting to the mark. The intensity of the colour was read using a spectrophotometer at a wavelength of 880 nm.

A calibration curve was obtained by taking 0 mL, 4 mL, 6 mL, 8 mL, 10 mL and 12 mL of 2 mg/L standard Phosphorus solution into 25 mL volumetric flasks; 4 mL of reagent B was added to each flask and diluted to the mark with distilled water. The absorbances were read at the wavelength of 880nm. A plot of concentration versus absorbance was done. The concentrations of Phosphorus were then extrapolated from the calibration curve.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

The pH of the soils ranged from 5.08 to 7.55 that is from slightly acidic to neutral. The least pH range of 5.08 to 5.77 was recorded for Huhunya virgin soil (HV), with the highest pH range recorded by soils from Akwadum Cocoa (AC) farm (5.44-7.55) and that of Huhunya plantain (HP) farm land recorded pH values from 6.05 to 6.92. It was observed that the pH of all the soil samples increased with depth.

The Percentage organic matter for all the soils ranged from 0.836% to 3.078%. Soil samples collected from Akwadum Cocoa farm recorded the highest percent organic matter which may be due to the clayey nature of soils and the least organic matter content was obtained for soils collected from Huhunya virgin and Huhunya plantain farm land. The soils used in this study texturally varied from loamy sand to sandy clay loam. Soils collected from Akwadum cocoa farm were sandy clay loam except soil AC
2 at depth 10–20 cm and 20–30cm which was sandy loamy. Soil samples collected from Huhunya plantain farm and the uncultivated land from Huhunya were loamy sandy in nature. The physical and chemical properties of the soil samples namely soil pH, conductivity, soil organic matter (OM), particle size distribution and the soil texture determined are presented in Table 4.1.

Table 4.1: Physicochemical properties of the soils studied

Sample Depth/cm	pH	Conductivity ms/cm	%Organic matter	Particle size %Sand	%Clay	%Silt	Remarks
AC 1 0-10	5.99	0.103	2.580	72.5	5.9	21.6	Sandy Clay Loam
AC 1 10-20	6.20	0.106	2.153	72.5	5.9	21.6	Sandy Clay loam
AC 120-30	6.60	0.074	1.779	72.5	4.0	23.5	Sandy Clay loam
AC2 0-10	7.10	0.181	3.078	68.6	7.9	23.5	Sandy Clay loam
AC2 10-20	7.00	0.129	2.762	86.3	3.9	9.8	Loamy Sand
AC2 20-30	7.55	0.147	1.762	82.4	2.0	15.6	Loamy Sand
AC3 0-10	5.44	0.145	1.833	68.6	7.9	23.5	Sandy Clay loam
AC3 10-20	5.55	0.103	1.068	66.7	5.8	27.5	Sandy Clay loam
AC3 20-30	5.80	0.075	0.836	62.7	7.9	29.4	Sandy Clay loam
AC4 0-10	5.90	0.181	1.833	66.7	11.7	21.6	Sandy Clay loam
AC4 10-20	5.94	0.099	1.566	74.5	3.9	21.6	Sandy Loam

AC4 20-30	6.42	0.082	1.619	72.5	4.0	23.5	Sandy Loam

HV1 0-10	5.62	0.115	1.548	88.2	7.9	3.9	Loamy Sand
HV1 10-20	5.70	0.102	1.833	90.2	3.9	5.9	Loamy Sand
HV1 20-30	5.77	0.059	1.512	84.3	7.9	7.8	Loamy Sand
HV2 0-10	5.23	0.109	2.189	90.2	2.0	7.8	Loamy Sand
HV2 10-20	5.34	0.055	2.384	84.3	7.9	7.8	Loamy Sand
HV2 20-30	5.63	0.054	2.171	86.3	7.8	5.9	Loamy Sand
HV3 0-10	5.46	0.116	1.334	78.4	9.8	11.8	Loamy Sand
HV3 10-20	5.81	0.065	2.117	72.5	13.8	13.7	Sandy Loam
HV3 20-30	5.08	0.057	1.334	78.4	5.9	15.7	Loamy Sand
HV4 0-10	5.37	0.072	2.082	84.3	9.8	5.9	Loamy Sand
HV4 10-20	5.44	0.041	1.317	82.4	9.8	7.8	Loamy Sand
HV4 20-30	5.39	0.044	1.673	84.3	5.9	9.8	Loamy Sand

HP1 0-10	6.92	0.125	1.726	80.4	15.7	3.9	Loamy Sand
HP1 10-20	6.83	0.084	1.566	82.4	15.6	2.0	Loamy Sand
HP1 20-30	6.78	0.083	1.157	84.3	9.8	5.9	Loamy Sand
HP2 0-10	6.05	0.024	2.028	86.3	9.8	3.9	Loamy Sand
HP2 10-20	6.76	0.075	1.495	84.3	9.8	5.9	Loamy Sand
HP2 20-30	6.70	0.100	1.299	84.3	9.8	5.9	Loamy Sand
HP3 0-10	6.75	0.059	1.334	82.4	13.7	3.9	Loamy Sand
HP3 10-20	6.76	0.496	1.317	86.3	9.8	3.9	Loamy Sand
HP3 20-30	6.80	0.045	1.299	84.3	9.8	5.9	Loamy Sand
HP4 0-10	6.78	0.107	2.260	80.4	13.7	5.9	Loamy Sand
HP4 10-20	6.82	0.864	1.495	82.4	11.7	5.9	Loamy Sand
HP4 20-30	6.87	0.060	1.477	86.3	9.8	3.9	Loamy Sand

4.1 Estimation of available soil phosphorus by extractants

The levels of phosphorus in soil system, its nature and behaviour in soil are important in soil management and its use as phosphate fertilizer. As a result, principles, knowledge of its determination and availability to plants are essential. Seven extractants were used to extract available Phosphorus from soil samples. The quantities of Phosphorus removed by the extraction procedures which varied for the soil samples are represented in Table 4.2. The pH of extracting medium had quiet substantial effect on the amount and forms of Phosphorus extracted. The mean concentration of phosphorus extracted from the soils ranged from a low of 0.100 to a high of 9.926 µg/g soil. The highest concentration of available Phosphorus which was 9.926 µg/g, was extracted from soils collected from Akwadum Cocoa farm land using Bray 2 solution, followed by Mehlich 1 and Mehlich 3, with mean values of 9.527 and 9.392 µg/g respectively. The least concentration for the extractants Bray 2, Mehlich 1 and Mehlich 3 were all recorded for subsoils collected from Huhunya plantain farm, and the values were as follows: 1.158, 1.458, 1.208 µg/g soil respectively. The use of Disodium EDTA, Olsen and Bray 1 extractants also recorded quite high concentrationof extracted Phosphorus and the values obtained were 6.210, 5.933 and 5.183 µg/g soil respectively for Akwadum cocoa farm land. The least concentration of extracted Phosphorus was also recorded for the subsoil of Huhunya plantain farm land. The Olsen test, extracted less Phosphorus as compared to the Mehlich 1 extraction for all the soil samples. The NaHCO3 test (Olsen *et al.,* 1954) generally extracts less Phosphorus compared to Mehlich- 1 or Bray –1 test (Shuman *et al.,* 1988) except in highly buffered soils (Holford, 1980) as the extractant (Olsen) undergoes neutralization before the extraction process. Distilled water extraction recorded the least concentration of extracted Phosphorus in all the soil samples with

mean concentration values from0.100 to1.733µg/g soil. While the soils from Huhunya plantain farm recorded the maximum concentration of available Phosphorus, with a value of 1.733 µg/g, the same soil sample recorded the least of 0.100 µg/g soil with distilled water.

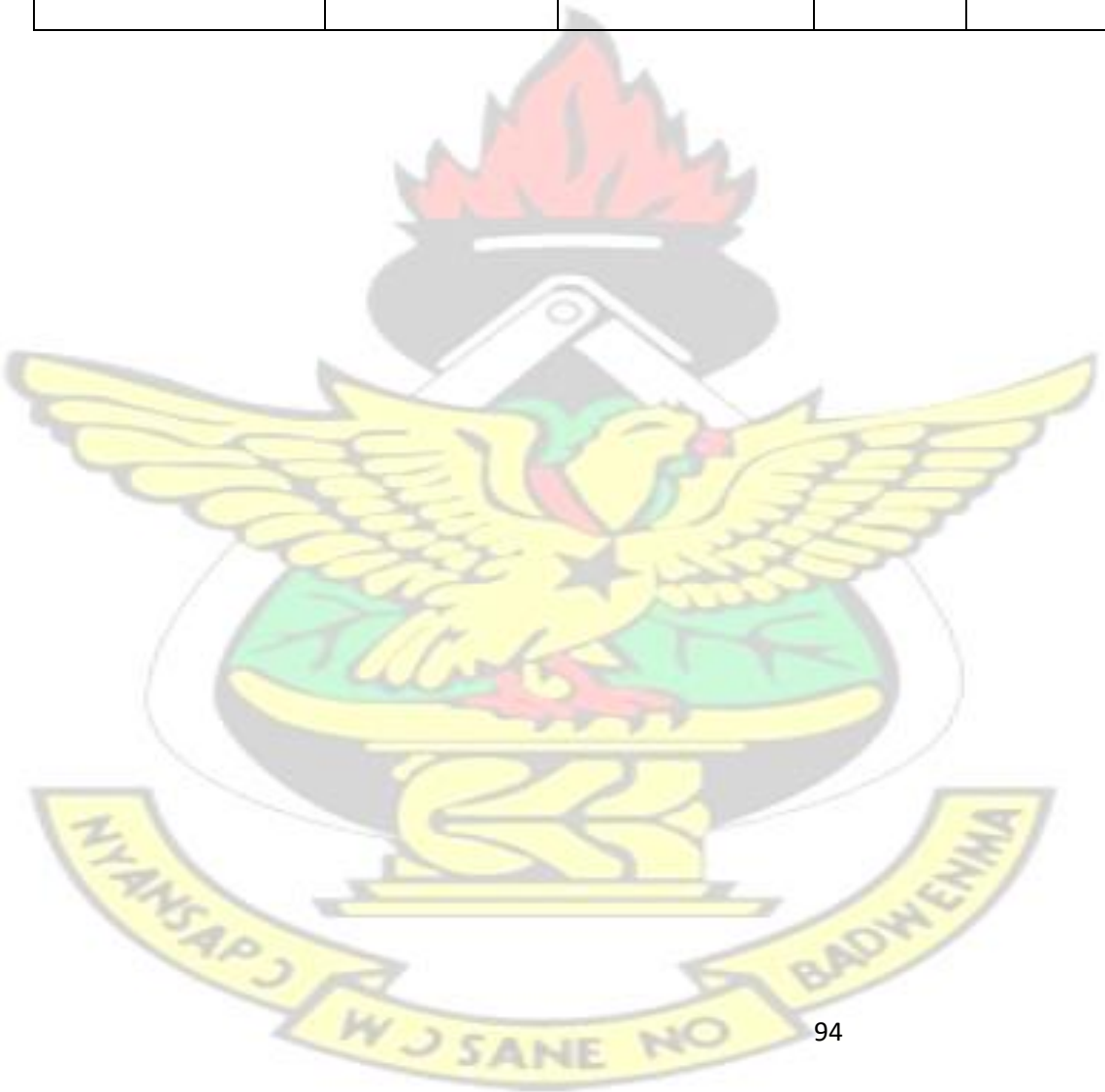
Table 4.2: Mean concentration of available phosphorus in soil samples (µg g soil)

Sample Depth/cm		Concentration of available Phosphorus						
		Distilled water	**Disodium EDTA**	**Olsen**	**Bray 1**	**Bray 2**	**Mehlich 1**	**Mehlich 3**
AC 1	0-10	0.717	3.367	5.933	3.966	6.325	6.582	5.108
AC 1	10-20	0.593	3.283	2.867	2.925	4.325	5.008	3.600
AC 1	20-30	0.517	3.250	2.267	2.700	5.258	5.150	3.108
AC2	0-10	0.933	6.210	5.300	4.325	9.146	9.527	6.113
AC2	10-20	0.217	5.672	4.717	3.233	9.926	8.167	5.613
AC2	20-30	0.217	5.490	4.383	3.575	6.787	7.084	5.534
AC3	0-10	0.250	1.914	2.267	4.850	8.858	5.900	6.258
AC3	10-20	0.217	1.791	2.817	2.967	4.333	3.742	3.533
AC3	20-30	0.250	1.336	2.600	2.175	4.992	2.867	3.183
AC4	0-10	0.620	5.983	4.650	5.183	9.525	5.915	6.525
AC4	10-20	0.617	4.450	4.633	3.417	6.892	2.908	4.275

AC4	20-30	0.600	2.550	4.617	2.425	4.359	2.367	2.450
HV1	0-10	0.600	4.470	2.833	4.767	5.825	4.867	4.942
HV1	10-20	0.647	4.233	1.500	3.900	5.775	4.265	4.440
HV1	20-30	0.623	3.283	2.033	4.400	5.303	2.692	4.108
HV2	0-10	1.000	4.467	4.500	3.017	9.333	5.475	6.725
HV2	10-20	0.633	4.067	4.550	3.092	6.883	5.883	4.892
HV2	20-30	0.967	3.633	3.833	3.208	6.275	4.275	4.517
HV3	0-10	0.350	1.417	4.700	3.175	6.925	5.683	4.715
HV3	10-20	0.250	1.330	3.417	1.783	3.008	4.925	3.067
HV3	20-30	0.200	1.200	3.350	2.767	3.925	4.458	2.492
HV4	0-10	0.850	3.053	2.833	4.775	6.133	4.558	5.350

Sample Depth/cm		Concentration of available Phosphorus						
		Distilled water	**Disodium EDTA**	**Olsen**	**Bray 1**	**Bray 2**	**Mehlich 1**	**Mehlich 3**
HV4	10-20	0.800	2.991	2.800	2.783	3.325	2.300	2.658
HV4	20-30	0.633	2.867	2.333	1.600	3.325	2.283	2.200
HP1	0-10	1.733	5.483	5.150	4.842	8.867	7.150	7.417
HP1	10-20	0.533	4.783	3.433	4.092	7.333	4.325	6.675
HP1	20-30	0.500	4.600	2.267	3.658	4.050	2.067	4.125
HP2	0-10	0.100	2.559	3.583	3.083	6.358	9.498	9.392
HP2	10-20	0.700	2.617	1.317	2.925	3.400	3.767	4.542
HP2	20-30	0.100	1.917	1.433	2.492	2.800	3.583	3.667
HP3	0-10	0.663	2.817	1.633	2.600	3.358	2.442	2.383
HP3	10-20	0.850	1.383	1.250	1.450	1.158	2.158	1.850
HP3	20-30	0.617	1.250	1.033	1.425	1.767	1.458	1.208
HP4	0-10	0.250	2.700	2.983	4.575	6.508	5.783	5.458

HP4	10-20	0.130	1.697	1.717	2.683	3.000	2.683	3.525
HP4	20-30	0.117	1.300	1.533	1.500	3.442	2.200	2.467



4.2 Comparison of the effectiveness of extractants on the soils

4.2.1 Akwadum cocoa (AC) farm land

The physical and chemical properties of soils collected from Akwadum cocoa (AC) farm and the mean concentrations of extracted P are shown in Table 4.1 and Table 4.2 respectively. Soil samples collected from Akwadum cocoa farm had pH values ranging from 5.44 to 7.55 indicating the soils are mildly acidic to slightly neutral in nature. For the different locations on the same farm land (AC1, AC2, AC3 and AC4) it was observed that soil pH increased with depth. That is the topsoils were quite acidic compared to the subsoils. Some of the soil samples did not follow the observed trend of increasing pH with depth. This irregularity in the measured pH of the same soil samples could have resulted from many factors. For example, the irregularity could result from variables such as carbon dioxide content, partial pressure, salt concentration, hydrolysis and solubility of soil constituents. Organic matter content was generally low, which ranged between 0.836% - 3.078% with the depths. The topsoils of all the soil samples recorded higher amount of organic matter than the subsoils. This could be attributed to the fact that subsoils normally lack the biological activity and warm temperatures of top soil, and have less biological and insect activity hence giving rise to less organic matter content of the soil. Akwadum soil recorded the highest percent organic matter. The concentration of extracted P from the soil samples ranged from 0.217 to 9.926 µg/g. All the extractants yielded substantial quantities of available Phosphorus with the exception of distilled water which extracted least concentrations with values ranging from 0.217 to 0.933 µg/g soil. Figure 4.1 shows the extractants used and the corresponding concentrations of extracted Phosphorus in microgram per gram soil from Akwadum cocoa farm land. From the graph, Bray 2 extracted Phosphorus had the maximum concentration value of 9.926 µg/g soil.

This indicates that the Bray 2 solution which comprises 0.1 M Hydrochloric acid in 0.03 M Ammonium fluoride solution extracted more different forms of phosphorus that may include bound forms of phosphorus from the soil. These forms of phosphorus may include Aluminium and Iron phosphate as the soil was quite acidic. A study by Djokoto (1964) showed that Bray 2 method was suitable for phosphorus determinations in all soils in Ghana. In his study six chemical methods for phosphorus soil tests were studied. The methods which include Bray1, Sodium bicarbonate method (Olsen), 0.05 N H_2SO_4 in 1% $(NH_4)_2S0_4$ (Truog), Modified

Bray's and Bray 2 were used for five areas of the study, namely Huhunya, Sunyani, Swedru and Oda. All these experimental areas were in the moist semi-deciduous forest zone. The soils at Adidwan, in the forest-savanna transition zone, were sandy loam.

Each of these extractants recorded substantial concentrations of available Phosphorus. Table 4.3 is a summary of the outcome of his research. The values of extracted Phosphorus were recorded in pounds per acre. The pattern of one extractant giving higher amounts of phosphorus with all soils was not apparent as is the case in this study.

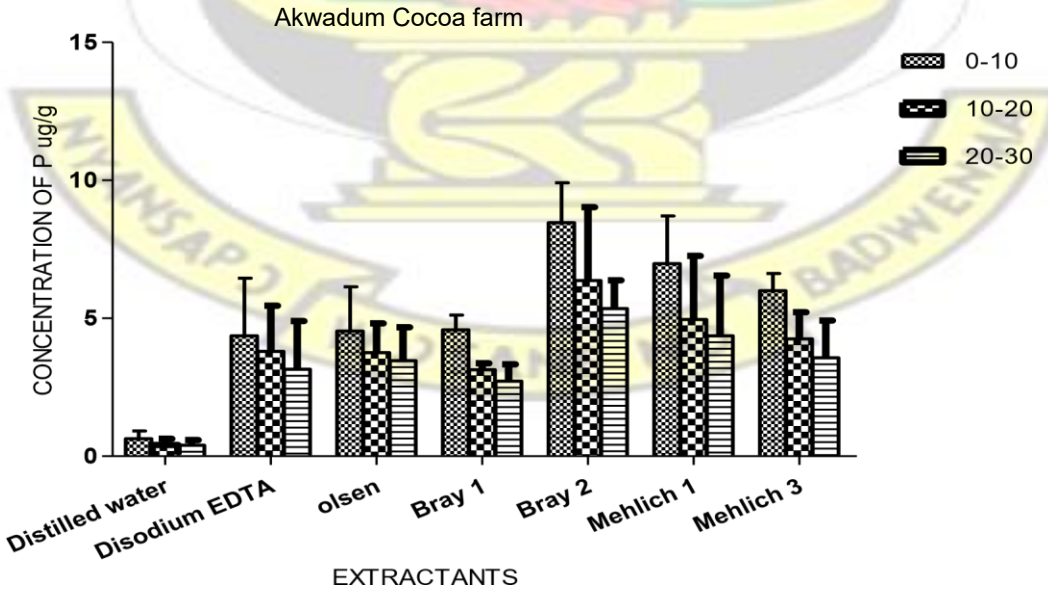


Figure 4.1: Concentration of extracted Phosphorus (µg/g) against extractants on AC soil

Table 4.3: Mean values of soil Phosphorus (P_2O_5 pound/acre) extracted by various methods from soils without added Phosphorus.

Soil location	Year	Extracted Phosphorus with extractants							
		Bray 1	Modified Bray	Bray 2	0.05 N H_2SO_4	0.002N H_2SO_4	0.5M $NaCO_3$	Average P_2O_5	Average pH
Adidwan	1964	30.1	35.6	43.3	26.6	41.9	22.4	33.3	7.2
	1964-65	24.4	40.7	55.7	60.6	52.7	23.3	42.9	7.2
	1965	44.5	48.0	44.9	62.4	53.7	22.3	45.9	6.8
Huhunya	1964	24.7	25.3	29.6	17.6	21.3	38.6	26.2	6.9
	1964-65	29.3	52.7	34.2	20.4	51.0	17.2	34.1	6.3
	1965	23.9	36.0	31.5	51.4	32.7	21.5	32.8	6.5

(Djokoto 1964, Kwame Nkrumah University of Science and Technology)

The performance of Mehlich 1 and Mehlich 3 solutions in the extraction process in the study gave an appreciable concentration of extracted Phosphorus.The values obtained from Olsen, Disodium EDTA and Bray 1 were less than Bray 2, Mehlich 1 and Mehlich 3 values. The Olsen test extracted less as compared Phosphorus to the double acid extraction. The $NaHCO_3$ test (Olsen *et al.,* 1954) generally extracts less Phosphorus compared to Mehlich 1 or Bray 1 tests (Shuman *et al.,* 1988) except in highly buffered soils (Holford 1980). A gradual decrease of available Phosphorus extracted was observed from the topsoil, subsurface to the subsoil for all the extractants used. Since a small fraction of Phosphorus applied to soil in fertilizer is utilized by plants and much is retained

in the top layer, this trend was expected as has been found in some studies. For example no additional Phosphorus was detected below 37.5 cm in plots at Rothamsted which had recieved annual application of 33 Kg P/ha for 115 years (Cooke and Williams 1963). Similarly, a research by Hingston (1959) observed that only 30% of Phosphorus from a dressing of 6900 Kg superphosphate per hectar was retained in the top 20 cm of a Coolup sand.

4.2.1.1 Effect of extractants on available phosphorus levels in soil AC at different depths

The values obtained from the concentrations of available Phosphorus obtained with the seven extractants were subjected to statistical analysis using one way Analysis of variance (ANOVA) to investigate any differences between extractants that recorded similar concentration of available Phosphorus with depth of soil. Table 4.4 represents the results from the statistical analysis on the mean concentration values from Akwadum cocoa soil samples. Same letters in the column for any extractant indicate there is no significant difference between them. Different letters indicate a significant difference between the concentrations. It was observed that the mean Phosphorus concentration values obtained using Disodium EDTA and Olsen extractant were quite similar for the topsoil, subsurface and the subsoil. For example the topsoil (AC 0-10 cm) recorded a mean value of 4.369 and 4.538, subsurface (AC10-20 cm) recorded values of 3.799 and 3.759, subsoil (AC 20-30 cm) 3.157 and 3.467 µg/g soil for Disodium EDTA and Olsen respectively. Subjecting the concentrations to statistical analysis showed that the extracting performance of Olsen

and Disodium EDTA solutions were not significantly different from each other even though the mean concentration values differed slightly from each.

Mehlich 1 and Mehlich 3 solutions extracted greater proportion of available Phosphorus compared with the values obtained from Disodium EDTA and Olsen solutions. For example, for the same soil sample AC 0-10 cm, Mehlich 1 solution gave a value of 6.981 µg/g soil, Mehlich 3 solution recorded a value of 6.001 µg/g soil compared to 4.538 and 4.369 µg/g soil values recorded for Olsen and Disodium EDTA solutions. The acidic nature of the Mehlich solutions could result to the greater proportion of the extracted Phosphorus from the same soil samples as both non labile and labile portions of Phosphorus are removed from the soil. It was observed that the mean values recorded for Mehlich 1 solution were slightly greater than those obtained from Mehlich 3 solution for all the soil depths. The mean concentration values decreased with depth of soil for both Mehlich solutions. But statistically the concentrations extracted by Mehlich solutions did not differ from each other, as indicated by the same letters (Table 4.4). Bray 2 solution ranked first as it extracted the greatest proportion of available Phosphorus from the same soil samples. A mean value of 8.464 µg/g soil was recorded for soil sample AC 0-10 cm. There was however a decrease in available Phosphorus extracted with depth of soil for Bray 2 solution. A significant difference was observed between concentrations extracted by Bray 2 solution and all the other extracting solutions. Distilled water extracted the least available Phosphorus as it only removed only water soluble phosphorus. Statistically, distilled water extraction was significantly different from any of the other extractants. The general trend for the extracting performance was Bray 2 > Mehlich 1 > Mehlich 3 > Bray 1 > Olsen > Disodium EDTA> Distilled water.

Table 4.4: Effect of extractants on available phosphorus levels in soil AC at different depths

Extractants	AC 0-10	AC 10-20	AC 20-30
Distilled water	0.630 ± 0.285[a]	0.411 ± 0.224[a]	0.396 ± 0.191[a]
Disodium EDTA	4.369 ± 2.084[b]	3.799 ± 1.656[ab]	3.157 ± 1.745[ab]
Olsen	4.538 ± 1.602[b]	3.759 ± 1.059[ab]	3.467 ± 1.205[ab]
Bray 2	8.464 ± 1.452[c]	6.369 ± 2.661[b]	5.349 ± 1.030[b]
Mehlich 1	6.981 ± 1.727[bc]	4.956 ± 2.308[b]	4.367 ± 2.179[b]
Mehlich 3	6.001 ± 0.619[bc]	4.255 ± 0.965[b]	3.569 ± 1.351[b]

Results are reported as mean±SD. Same letters in columns represents no significant difference.

4.2.2 Huhunya Virgin (HV) land

The loam sandy soil collected from the uncultivated land at Huhunya had pH values ranging between 5.08 and 5.81, indicating the moderately acidic nature of the soil.It was observed that, the pH of the soils increased with depth, (0–10, 10–20 and 20–30 cm). Some exceptions occurred with samples HV3 and HV4. The subsoils of these samples gave less pH values. For example, for sample HV3, the top soil had pH of 5.46, the subsurface 5.81 and the subsoil 5.08. The percent organic matter of the soil also varied with the depth but did not follow any particular pattern. The percent organic matter ranged from 0.041% to 2.384%. Considering the effectiveness of the seven extractants, Bray 2 solution gave the maximum value of extracted Phosphorus (9.333 µg/g soil), followed by Mehlich 1, Mehlich 3, Bray 1, Olsen and Disodium EDTA. Distilled water extract had the least concentrations of available Phosphorus. The concentration ranged between 0.200 and 0.967 µg/g soil. The concentration of available Phosphorus in the soil samples decreased

with depth for all the methods with some exception where the subsurface showed a slightly larger value than the top soil. Example is the extracted Phosphorus values obtained with sample HV1. Top soil of HV 1 recorded a concentration of 0.600µg/g, subsurface recorded a value of 0.647µg/g and the subsoil had a value of 0.623 µg/g. Even though the land has not been cultivated, amount of available Phosphorus was appreciable. This could be as a result of accumulation from the past. Figure 4.2 is a representation of how the different extractancts performed on the soil samples with depth.

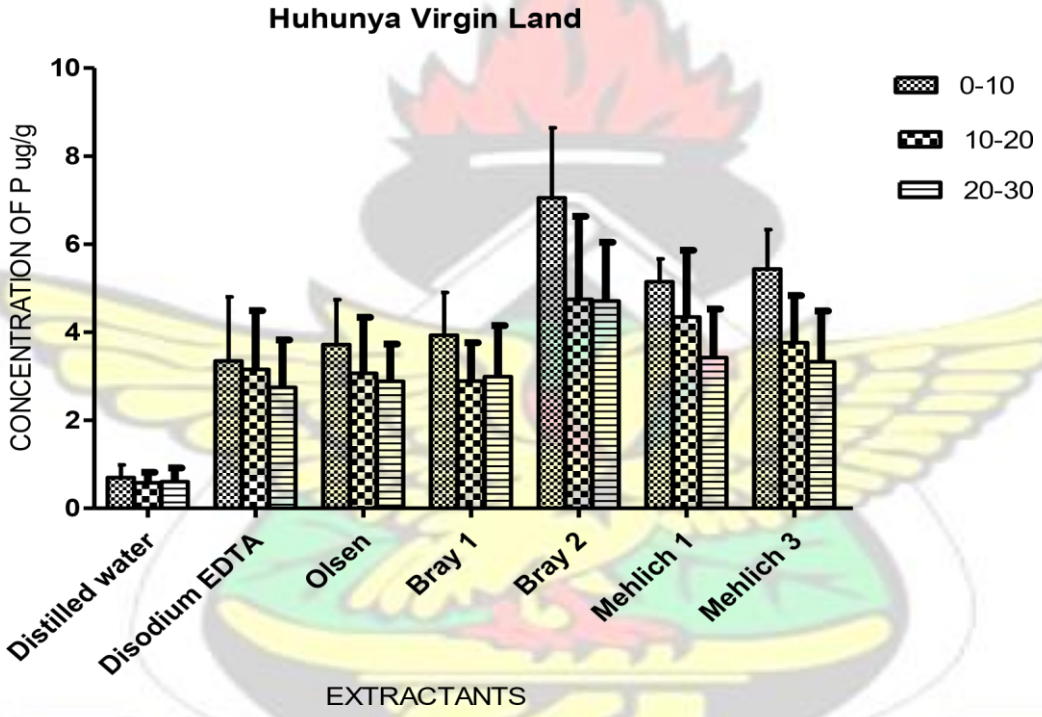


Figure 4.2: Concentration of extracted Phosphorus (µg/g) against extractants on HV soil

4.2.2.1 Effect of extractant on available phosphorus levels in soil HV at different depths

Analysis of variance (ANOVA) revealed that the use of distilled water showed a significant difference between the amount of Phosphorus removed from the soil samples and the other six extractants. Distilled water recorded the least of 0.700 µg/g for the top soil (0-10 cm), which reduced to0.583µg/g for the subsurface (10-20 cm) soil. But the regular pattern observed with a decrease in Phosphorus concentration with depth was not observed as the subsoil (20-30 cm) concentration got higher (0.606µg/g).The concentration of available Phosphorus removed by distilled water was relatively small as it removed only a small fraction (only water soluble Phosphorus) of available Phosphorus from the soil. Bray 1, Olsen and Disodium EDTA extractants recorded mean concentrations of available Phosphorus between 3.300 and 3.950 µg/g for the three soil depths. Each of the extracting solution indicated a decrease in amount of extracted Phosphorus with depth. The slight difference between these values showed no significant difference for the three methods (Bray 1, Olsen and Disodium EDTA solutions) acording to ANOVA (Table 4.5). Thus any of the three methods could be used to extract available Phosphorus from soils with similar characteristics.

Mehlich 1 and Mehlich 3 extractants on the other hand recorded mean concentration values of 5.146, 5.433 for the topsoil, 4.343,3.764 for subsurface soil and 3.427, 3.329 µg/g for subsoil respectively. The differences in these mean values were not significant according to ANOVA. On the average Mehlich 1 or Mehlich 3 extractants could be used for the extraction of available Phosphorus from soil with physicochemical properties similar to those of Huhunya Virgin soil. Bray 2 solution recorded the highest concentration

value of 7.054 µg/g for the top soil, which reduced with depth to 4.748 (10-20 cm) and to 4.707 (20-30 cm) µg/g soil. The general trend of extracting ability was Bray 2 > Mehlich 3 > Mehlich 1 > Bray 1 > Olsen > Disodium EDTA > Distilled water.

Table 4.5: Effect of extractant on available phosphorus levels in soil HV at different depths

Extractants	HV 0-10	HV 10-20	HV 20-30
Distilled water	0.700 ± 0.286^{a}	0.583 ± 0.234^{a}	0.606 ± 0.314^{ab}
Bray 1	3.934 ± 0.969^{b}	2.890 ± 0.875^{ab}	2.994 ± 1.157^{ab}
Olsen	3.717 ± 1.023^{b}	3.067 ± 1.271^{ab}	2.887 ± 0.846^{ab}
Disodium EDTA	3.352 ± 1.452^{b}	3.155 ± 1.336^{ab}	2.746 ± 1.077^{ab}
Mehlich 1	5.146 ± 0.523^{bc}	4.343 ± 1.516^{b}	3.427 ± 1.100^{b}
Mehlich 3	5.433 ± 0.901^{bc}	3.764 ± 1.071^{b}	3.329 ± 1.154^{b}
Bray 2	7.054 ± 1.588^{c}	4.748 ± 1.886^{b}	4.707 ± 1.334^{b}

Results are reported as mean±SD. Same letters in columns represents non significant difference

4.3.3 Huhunya plantain (HP) farm land

The soil samples collected from Huhunya plantain farm had pH value ranging from 6.05 to 6.87, indicating the soil is moderately acidic in nature. A gradual increase of pH of the soil was observed from topsoil to subsoil. An exception was however seen with soil sample HP 1 as a decrease in pH values from the topsoil to the subsoil was observed. This could be due to agricultural practices and the topography of the land. Percent organic matter content of the Huhunya plantain farm varied from 1.157% to 2.260% and decreased with depth. The gradual decrease in the percent organic matter could be attributed to the fact that subsoils normally lack the biological activity and warm temperatures of top soil, and

have less biological and insect activity hence giving rise to less organic matter content of the soil. The extracted Phosphorus obtained by the

Mehlich 1 method was the maximum (9.498 µg/g soil). Holford (1980) found that Mehlich 1 extracts large amounts of non-labile Phosphorusaside the soluble P it extracts in soils with pH greater than 6.0. This was expected as soil samples from Huhunya plantain farm recorded pH values above 6.

The least available P was extracted by distilled water with a value of 0.100 µg/g. Mehlich 3 and Bray 2 also extracted a significant amount of P (9.392 and 8.867 µg/g) respectively. Bray 2 solution ranked first in extracting available P from the soil samples. Halm (1965) carried out a research on correlation of soil tests for available phosphorus and his findings was that amongst the various methods used for soils collected from Huhunya Bray 2 solution extracted P was the highest.

Olsen and Disodium EDTA methods also extracted 5.150 and 5.483 µg/g respectively from the soil. For this soil, on the whole, three of the methods namely Mehlich 1, Mehlich 3 and Bray 2 showed similar extraction performance. Disodium EDTA and Olsen method also produced similar values. Graphical representation of the extractants used and concentration of available Phosphorus extracted from Huhunya plantain farm land with depth is shown in figure 4.3. A similar trend seen from the graph of Akwadum cocoa and Huhunya virgin land in the concentration of available Phosphorus extracted decreasing with depth of soil was observed for Huhunya plantain farm soil as well. It has been known from other studies that subsoil generally contains much less Phosphorus, as

Phosphorus content of soil decreases with soil depth (Memon, 1986).

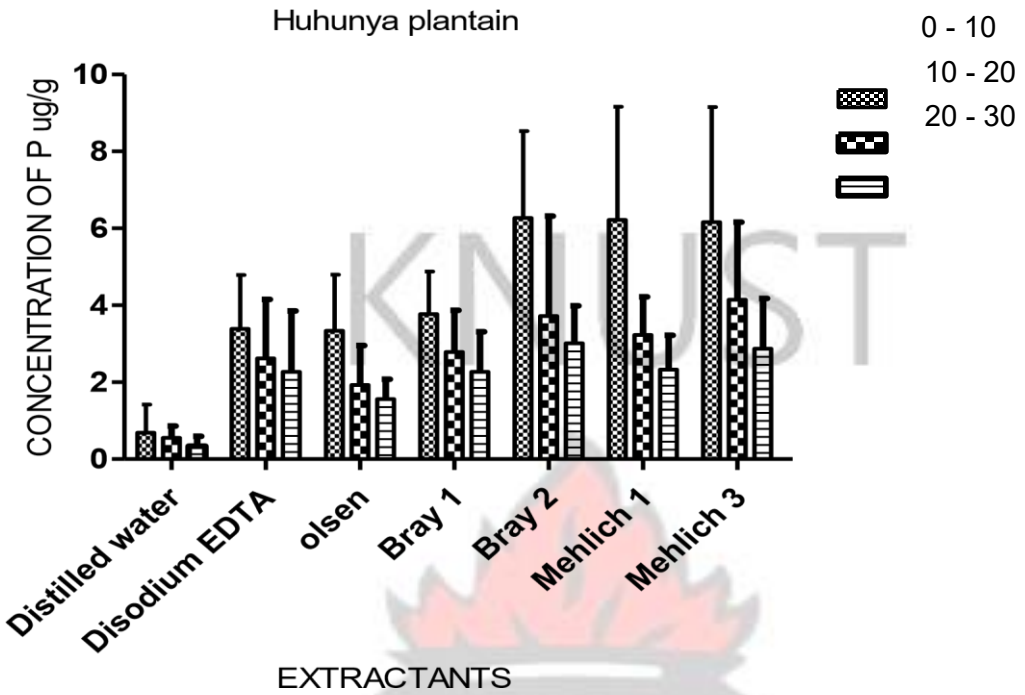


Figure 4.3: Concentration of extracted Phosphorus (µg/g) against extractantsfor HP soil

4.3.3.1 Effect of extractant on available phosphorus levels in soil HP at different depths

Data from statistical analysis indicates that distilled water extracted the least concentration of available Phosphorus, the extraction effect distilled water had on soils HP was different from all the other six extractants. It was observed that the extractant performance (distilled water) was similar for the topsoil (0-10 cm) and the subsurface soil (10-20 cm).Bray 2, Mehlich 1 and Mehlich 3 methods showed statistically no difference in the amount of extracted Phosphorus for the topsoil, but for the subsurface, Bray 2 and Mehlich 1 methods extracted equal concentrations with mean values between

3.233 and 3.723 µg/g soil. Mehlich 3 extracted the highest value of 4.148 µg/g which was recorded for the subsurface soil. A different pattern was however observed for the subsoil

(20-30 cm) as Bray 2 and Mehlich 3 solutions extracted similar amounts of available Phosphorus, which were higher than that removed by Mehlich 1. The mean concentration values obtained with the use of Bray 1, Disodium EDTA and Olsen methods statistically were not different from each other. The extraction ability of these three methods is similar for the extraction from the topsoil, subsurface and subsoil. The mean concentration values obtained are 3.337µg/g for Olsen, 3.390 µg/g and 3.775 µg/g for Bray 1. Overall the performance of Bray 2 was the highest, recording a mean concentration of 6.273 µg/g for Huhunya plantain farm soil. Table 4.6presents the results obtained from the statistical analysis of the effect the extractants had on phosphorus levels on HP soil at different depths.

Table 4.6: Effect of extractant on available phosphorus levels at HP at varying depths

Extractants	HP 0-10	HP 10-20	HP 20-30
Distilled water	0.687 ± 0.737^{a}	0.553 ± 0.310^{a}	0.334 ± 0.264^{ab}
Bray 2	6.273 ± 2.257^{b}	3.723 ± 2.597^{ab}	3.015 ± 0.976^{b}
Mehlich 1	6.218 ± 2.948^{b}	3.233 ± 0.989^{ab}	2.327 ± 0.897^{ab}
Mehlich 3	6.163 ± 2.988^{b}	4.148 ± 2.017^{b}	2.867 ± 1.308^{b}
Bray 1	3.775 ± 1.101^{ab}	2.788 ± 1.083^{ab}	2.269 ± 1.046^{ab}
Disodium EDTA	3.390 ± 1.399^{ab}	2.620 ± 1.534^{ab}	2.267 ± 1.585^{ab}
Olsen	3.337 ± 1.458^{ab}	1.929 ± 1.023^{ab}	1.567 ± 0.515^{ab}

Results are reported as mean±SD. Same letters in columns represents non significant difference

4.4 cORRELATION ANALYSIS

4.4.1 Correlation (r) between extractants and soil physicochemical properties To ascertain if the available Phosphorus extracted by the various methods used in this study

is related to any of the soil properties, correlation between the extractants and the soil physical and chemical properties were carried out. The correlation between extractants and the soil physicochemical properties is presented in Table 4.7 as correlation coefficient (r). There was a strong dependence of all the extractantsapart from distilled water on percent organic matter content of the soil ($p < 0.05$). The simple linear correlations between the extractants used and the physicochemical properties however showed no significant correlation between the soil pH, conductivity, percent clay, sand and silt. This was indicated by the pearson (p) values being greater than 0.05

($p > 0.05$). Soil pH has been known to be a major factor in soil test for available Phosphorus. The investigation carried out however indicated no significant correlation between soil pH and the extractants. Maghanga (2012) made similar observation as there was no correlation between soil pH and Mehlich 1extractant ($r = 0.42$), when Olsen and Mehlich 1 methods of extraction of available P from soil was performed. The implication of his findings was that one cannot predict soil available Phosphorus using pH data alone. He also observed no correlation between soil pH and Olsen Phosphorus ($r=0.49$), hence Olsen Phosphorus cannot be estimated using soil pH values alone. Mehlich 1 ($p = 0.0000^*$) and Bray 2 ($p = 0.0001^*$), as well as Olsen ($p = 0.0002^*$) showed a high correlation with the % organic matter. It was observed that, as the % organic matter (OM) content of the soil increased, the available Phosphorus nutrient level also increased. Soils with greater values of organic matter yielded corresponding higher concentrations of extracted Phosphorus with the different extractants. For example the % Organic matter for soil samples AC1 (0-10), AC2 (0-10) and HV2 (0-10) were 2.580, 3.078 and 2.189 respectively and the extracted Phosphorus from each of these samples were 6.325, 9.146 and 9.333 µg/g soil respectively for Bray 2 method. Similarly, Olsen method recorded

high values of extracted Phosphorus for the same soil samples, for AC1 (0-10) a value of 5.933, AC2 (0-10) recorded 5.300 and HV2 (0-10) recorded 4.500 µg/g soil. For soil samples with low % Organic matter for example,

1.495 was recorded for HP2 (10-20) and the extracted P with Olsen was 1.317 µg/g soil. Sample HP3 (20-30) also gave a low % Organic matter (1.299) and a corresponding low value of extracted Phosphorus with Bray 2 (1.767 µg/g soil). There was a significant correlation between Disodium EDTA and % organic matter (p = 0.0013*), Mehlich 3 and % organic matter (p = 0.0023*) and Bray 1 and % organic matter (p = 0.0355*).

These observations were seen with soils from Akwadum Cocoa, followed by soils from Huhunya virgin land and Huhunya plantain farm soils. There was a gradual decrease in % organic matter in the soil samples with depth. This trend was not observed in some of the soil samples from Huhunya Virgin land. This may be a result of past accumulation of unused organic matter for longer periods.

Table 4.7: A correlation matrix between extractants and soil physicochemical properties

		Distilled water	Disodium EDTA	Olsen	Bray 1	Bray 2	Mehlich 1	Mehlich 3
pH	r	-0.0396	0.2328	-0.1050	-0.0805	-0.0791	0.0851	0.0120
	p	0.8185	0.1719	0.5424	0.6407	0.6467	0.6219	0.9444
Conductivity	r	-0.0867	-0.1070	-0.1593	-0.0673	-0.1545	-0.1003	-0.0811
	p	0.6149	0.5345	0.3533	0.6967	0.3681	0.5604	0.6381
	r	0.2107	0.5159	0.5786	0.3515	0.5915	0.7309	0.4914

%Organic matter	p	0.2173	0.0013*	0.0002*	0.0355*	0.0001*	0.0000*	0.0023*
%sand	r	0.1412	0.0819	-0.2986	-0.1108	-0.1591	-0.1361	0.0413
	p	0.4116	0.6350	0.0769	0.5201	0.3541	0.4285	0.8111
%clay	r	0.1131	-0.1541	-0.1957	0.1178	-0.1524	-0.0828	0.1033
	p	0.5113	0.3696	0.2528	0.4939	0.3748	0.6314	0.5490
%silt	r	-0.1746	-0.0060	0.3503	0.0474	0.2076	0.1569	-0.0815
	p	0.3086	0.9723	0.0362*	0.7835	0.2245	0.3606	0.6367

*indicates $p < 0.05$

4.4.2 Correlation matrix between the various extractants

High linear correlations were found between six of the soil test methods. This was observed between Bray 2, Bray 1, Mehlich 1, Mehlich 3, Olsen and Disodium EDTA.The highest correlation (r = 0.8059) was found between Bray 2 and Mehlich 3 extraction methods. Bray 1 and Mehlich 3 solutions are all acidic in nature and their strong positive correlation suggests both extractants have a similar extracting ability of Phosphorus in the soils. A similar correlation has been observed between Mehlich-3 and Bray 1 soil test results, and have been found to be highly correlated in neutral to acid soils with Mehlich-3 extracting slightly more Phosphorus than Bray 1 Phosphorus in most soils because Mehlich-3 uses a more acidic extracting solution (Tran *et al*., 1990, Beegle and Oravec, 1990, Lucero *et al*., 1998; Mallarino, 2003).Correlation coefficient between Bray 1 and Bray 2 (r = 0.7236), between Bray 1 and Mehlich 3 (r = 0.7074), Bray 2 and Olsen (r = 0.7190) as well as that between Disodium EDTA and Bray 2 (r = 0.7130) were also significant. Even though these extractants differ in their chemical compositions and is expected that different forms of Phosphorus will be removed from the soil samples, the

significant correlation between these extractants suggests that the concentrations of available Phosphorus extracted were similar. Mallarino (1997) found a similar pattern as observed here when Mehlich 3, Olsen, and Bray 1 Phosphorus extractions were compared and the outcome was that Mehlich 3, Olsen, and Bray 1 Phosphorus correlated well with each other. Similarly, Wolf and Baker (1985) reported strong interlaboratory correlation for Olsen, Bray-1, and Mehlich-1 soil tests conducted on 27non-calcareous soils. Overall Bray 2 solution showed a superior performance in extracting available Phosphorus from all the soil samples. The weakest correlations were found between the following extraction methods; Distilled water Phosphorus and Olsen Phosphorus ($r = 0.2806$), Distilled water Phosphorus and Bray 1 Phosphorus ($r = 0.2828$) as well as between Bray 2 Phosphorus and Distilled water Phosphorus ($r = 0.2417$). There was insignificant correlation between distilled water and Mehlich 1 ($r = 0.051$) and also between distilled water and Mehlich 3 ($r = 0.1592$). Distilled water and disodium EDTA however revealed a significant correlation, although this is not comparable to the correlation with the other extractants ($r = 0.4567$) $P < 0.05$. Table 4.8 presents the correlation coefficients (r) between the extractants used in this study.

Table 4.8: A correlation matrix between the various extractants

		Distilled water	Disodium EDTA	Olsen	Bray 1	Bray 2	Mehlich 1	Mehlich 3
Distilled water	r		0.4567	0.2806	0.2828	0.2417	0.0501	0.1592
	p		0.0051	0.0973	0.0946	0.1555	0.7715	0.3537
Disodium EDTA	r	0.4567		0.5565	0.6246	0.7130	0.5058	0.5775
	p	0.0051		0.0004	0.0000	0.0000	0.0016	0.0002
Olsen	r	0.2806	0.5565		0.4070	0.7190	0.6544	0.5370
	p	0.0973	0.0004		0.0138	0.0000	0.0000	0.0007
Bray 1	r	0.2828	0.6246	0.4070		0.7236	0.5073	0.7074
	p	0.0946	0.0000	0.0138		0.0000	0.0016	0.0000
Bray 2	r	0.2417	0.7130	0.7190	0.7236		0.7438	0.8059
	p	0.1555	0.0000	0.0000	0.0000		0.0000	0.0000
Mehlich 1	r	0.0501	0.5058	0.6544	0.5073	0.7438		0.7964
	p	0.7715	0.0016	0.0000	0.0016	0.0000		0.0000
Mehlich 3	r	0.1592	0.5775	0.5370	0.7074	0.8059	0.7964	
	p	0.3537	0.0002	0.0007	0.0000	0.0000	0.0000	

Data from analysis of variance (ANOVA) indicatesthat, statistically there is a significant difference between some of the extractants and others too were similar. Subsequently Multiple range test was carried out to identifythe differences between the extractants that were statistically similar and those that were different. Tukey"s test performed on the data indicates four differenthomogenous groups of extractants. On the table the various extractants have been arranged from the least to the highest interms of their ability to remove available Phosphorus. These groups were identified using columns of X's presented in Table 4.9. The levels containing X's form a group of means within which there are no statistically significant differences between those extractants and those on different columns indicates a statistical difference between the extractants. The four different groups identified include: Distilled water extractant in one different column; Olsen, Disodium EDTA and Bray 1 are in the same column; Mehlich 3 and Mehlich 1 in the same column and Bray 2 extractant in a different column**.** Figure 4.4 is a Box plot graphically depicting the four different groups of extractants. The plot indicates distilled water has a different extracting range from all the other extractants located at the lower range, disodium EDTA, Olsen and Bray 1 are in one different group all presented in the mid range, the Mehlich solutions are in one group as seen lying at upper mid range and lastly Bray 2 is also in a different group with the uppermost position. Distilled water extraction which is known to remove small fractions of available P recorded the least concentration values for all the soil samples. The values obtained ranged from a low of 0.1 recorded from HV3 (20-30 cm) to a high of 1.733 µg/g soil for HP 2 (0-10 and 20-30 cm). The values obtained were so low compared to the values obtained from the other extractants. The two salts Olsen ($NaHCO_3$) and disodium EDTA as well as Bray 1 extractants also recorded similar concentration values ranging between 1.2 to 5.93 µg/g soil. Mehlich 1 and Mehlich 3 all

being acidic solutions with Mehlich 3 as a modified form of Mehlich 1 extracted similar values between 1.208 for HP3 (20-30 cm) to 9.527 µg/g soil for sample AC2 (0-10 cm). Bray 2 solution showed a different extracting ability from the other solutions as it removed the highest available Phosphorus from all the soil samples with values ranging from 1.158 for HP3 (10-20 cm) to 9.926 µg/g soil for AC2 (10-20 cm).

Table 4.9: Multiple Range Test: 95.0 % Tukey HSD

Extractant	*Count*	*Mean*	*Homogeneous Groups*
Distilled water	108	0.544275	X
Olsen	108	3.14069	X
Disodium EDTA	108	3.20592	X
Bray 1	108	3.23147	X
Mehlich 3	108	4.3921	X
Mehlich 1	108	4.55542	X
Bray 2	108	5.52228	X

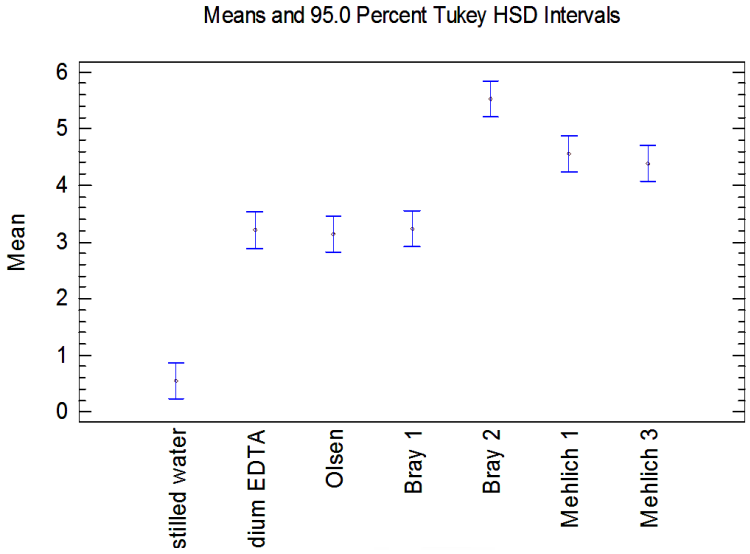


Figure 4.4: Box plot showing the different groups of extractants

The position of each vertical line inside the box shows the range of the mean values of extracted available Phosphorus for the different groups of extractants. Each of the vertical lines drawn inside the box indicates variability outside the upper and lower quartiles. The groups identified include Bray 2 in a different group, disodium EDTA, Olsen and Bray 1 in same group, Mehlich 1 and Mehlich 3 in same group and distilled water in another group.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

From the investigation carried out the following conclusions were drawn. The soils used were moderately acidic to neutral in nature as the pH of the soils ranged from 5.08 to 7.55. The Percentage organic matter for all the soils was generally low ranging from 0.836% to 3.078%. Texturally, the soil samples varied from loamy sand to sandy clay loam. The concentration of Phosphorus extracted by the different methods varied. The mean concentration of Phosphorus extracted from the soils ranged from 0.100 to 9.926 µg/g soil. The results revealed that the most effective extractant in extracting available Phosphorus from all the soil samples was Bray 2 solution which produced the highest mean values for the soils under study, namely Akwadum Cocoa farm (9.926 µg/g), Huhunya Virgin land (9.333 µg/g) and Huhunya plantain farm land (8.867 µg/g).The least mean values were obtained with the use of distilled water. Akwadum Cocoa farm soil yeilded 0.933 µg/g soil, Huhunya Virgin land recorded 1.00 µg/g soil and Huhunya plantain farm land recorded 1.733 µg/g soil for distilled water.

Statistical analysis of the results revealed the following order of decreasing extracting performance: Bray 2 > Mehlich 1 > Mehlich 3 > Bray 1 > Disodium EDTA > Olsen > Distilled water. ANOVA with Multiple Range Tests using Tukey‟s HSD method indicated that the seven extracting solutions could be grouped into four. Bray 1, Olsen and Disodium EDTA methods were in the same group, meaning there is no significant difference between them. This means any of the methods can be used to extract available Phosphorus from soils with similar properties. Mehlich 1 and Mehlich 3 methods were also in another group, inferring no significant difference between them statistically hence any of these two extractant can be used to extract available Phosphorus from soils with similar properties. Bray 2 extractant belonged to a different group, indicating a significant

difference between all the other extractants. Distilled water was also in a different group indicating a significant difference between the other extractants. The extraction performances of Bray 2 (highest extractant) as well as distilled water (least extracting ability) were significantly different from all the other extractants.

Correlation analysis performed on the results revealed there was a strong correlation between Mehlich-3 and Bray-2 (r= 0.8059), Mehlich 3 and Mehlich 1(r = 0.7964), Olsen and Bray 2 (r = 0.7190), Disodium EDTA and Bray 2 (r = 0.713) as well as Mehlich 3 and Bray 1methods (r = 0.7074). Similarly Olsen method showed some correlation with that of Disodium EDTA solution. Distilled water on the other hand indicated a poor correlation with Bray 2, Bray 1, Mehlich 1, Mehlich 3 and Olsen extracted Phosphorus. There was however a significant correlation between Distilled water and Disodium EDTA extracted Phosphorus. Correlation between the various extractants and the soil physicochemical properties was poor apart from soil organic matter content which showed a statistically significant correlation with the seven extractants apart from distilled water.

Although a rigid, maximum value of available Phosphorus concentration has not been set by soil scientists or the Natural Resources Conservation Service, one suggested limit that has been debated is 136.076 Kg Phosphorus per acre (by the Mehlich 3 extraction testing method). Also an Olsen Phosphorus of 18 to 22 mg/kg is recommended for large gains in crop production. Thomas and Peaslee (1973) also found that for Olsen

($NaHCO_3$) test, levels of 10 mg/kg soil are adequate for optimum plant growth.

Kamprath and Watson (1980) indicated that for Mehlich –1 test, Phosphorus levels of 20-25mg/kg soil are adequate for plant growth in sandy soils, but 10ppm is required for fine textured soils. From these deductions it can be concluded that the Phosphorus status of the studied soils were generally low.

5.2 RECOMMENDATION

- Soil test should be a well established agronomic practise in Ghana. When a soil test indicates that available phosphorus is low, application of phosphate fertilizer is needed, the rate recommended is intended to satisfy immediate crop needs and begin to build soil phosphorus levels to optimum range which will increase crop productivity. Wasting of fertilizers will be minimized if there exist knowledge about the phosphorus status of soils in agriculture.
- The physicochemical properties of soils should be determined in other to predict the best soil test for available phosphorus determination.
- Further work should be carried out to cover other lands country wide.

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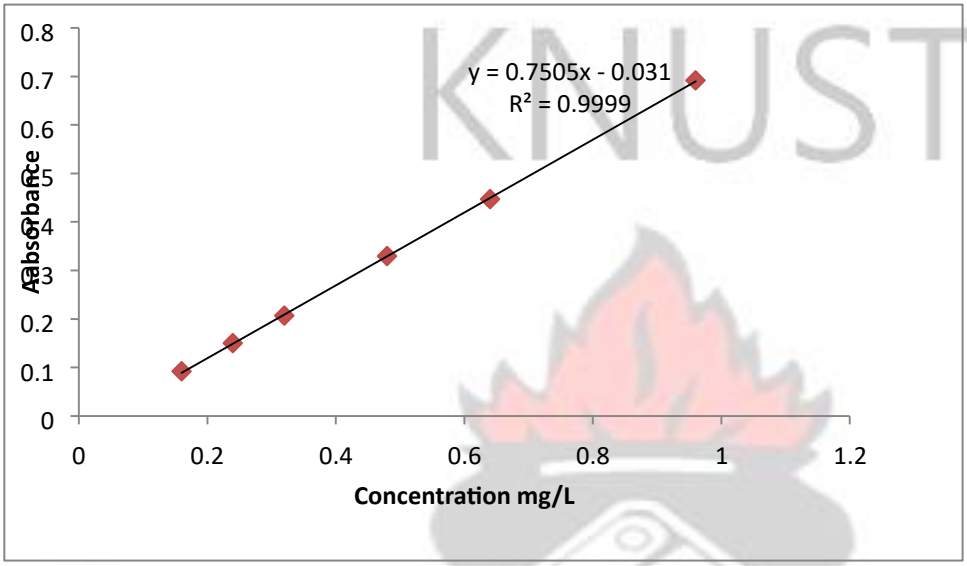
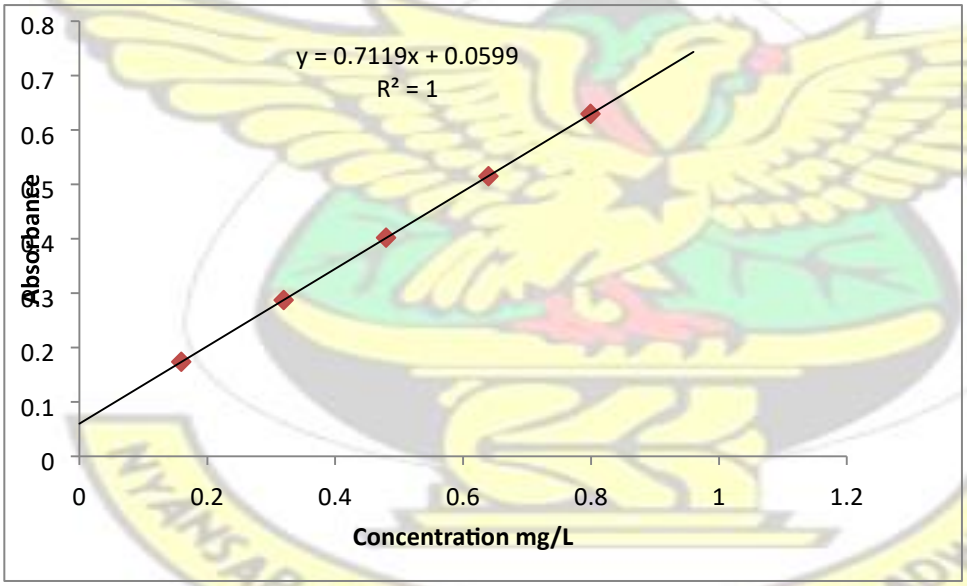
APPENDICES

APPENDIX A

CALIBRATION CURVES USED





APPENDIX B

Comparison of extraction methods.

Summary Statistics

	Count	*Average*	*Standard deviation*	*Coeff. of variation*	*Minimum*	*Maximum*	*Range*
Distilled water	108	0.544275	0.330868	60.7905%	0.0941	1.73601	1.64191
Disodium EDTA	108	3.20592	1.4866	46.3705%	1.19966	6.2103	5.01064
Olsen	108	3.14069	1.33076	42.3715%	1.03252	5.93353	4.90101
Bray 1	108	3.23147	1.03823	32.1289%	1.4201	5.1885	3.7684
Bray 2	108	5.52228	2.27809	41.2527%	1.15732	9.92625	8.76893
Mehlich 1	108	4.55542	2.05651	45.1443%	1.45716	9.52753	8.07037
Mehlich 3	108	4.3921	1.74871	39.8148%	1.20714	9.39229	8.18515
Total	756	3.51316	2.15629	61.3773%	0.0941	9.92625	9.83215

ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1622.39	6	270.399	107.27	0.0000
Within groups	1888.04	749	2.52074		
Total (Corr.)	3510.43	755			

APPENDIX C

Multiple Range Tests

Method: 95.0 percent Tukey HSD

	Count	*Mean*	*Homogeneous Groups*
Distilled water	108	0.544275	X
Olsen	108	3.14069	X
Disodium EDTA	108	3.20592	X
Bray 1	108	3.23147	X
Mehlich 3	108	4.3921	X
Mehlich 1	108	4.55542	X
Bray 2	108	5.52228	X

Contrast	*Sig.*	*Difference*	*+/- Limits*
Distilled water - Disodium EDTA	*	-2.66164	0.638675
Distilled water - Olsen	*	-2.59642	0.638675
Distilled water - Bray 1	*	-2.68719	0.638675
Distilled water - Bray 2	*	-4.978	0.638675
Distilled water - Mehlich 1	*	-4.01114	0.638675
Distilled water - Mehlich 3	*	-3.84782	0.638675
Disodium EDTA - Olsen		0.0652222	0.638675
Disodium EDTA - Bray 1		-0.0255528	0.638675
Disodium EDTA - Bray 2	*	-2.31636	0.638675
Disodium EDTA - Mehlich 1	*	-1.3495	0.638675
Disodium EDTA - Mehlich 3	*	-1.18618	0.638675
Olsen - Bray 1		-0.090775	0.638675
Olsen - Bray 2	*	-2.38158	0.638675
Olsen - Mehlich 1	*	-1.41472	0.638675
Olsen - Mehlich 3	*	-1.2514	0.638675

Bray 1 - Bray 2	*	-2.29081	0.638675
Bray 1 - Mehlich 1	*	-1.32395	0.638675
Bray 1 - Mehlich 3	*	-1.16063	0.638675
Bray 2 - Mehlich 1	*	0.966861	0.638675
Bray 2 - Mehlich 3	*	1.13018	0.638675
Mehlich 1 - Mehlich 3		0.163318	0.638675

* denotes a statistically significant difference.

APPENDIX D

Comparing the effect of Location and depth on phosphorus levels using each extraction method. (Does the sample location or depth of sample have any effect on the phosphorus levels?)

Statistical Method used: Two-way ANOVA (multifactor ANOVA)

Summary of statistics

	Factor	
Extraction Method	**Location**	**Depth**
Distilled Water	ND	SD
Disodium EDTA	SD	SD
Olsen	SD	SD
Bray 1	SD	SD
Bray 2	SD	SD
Mehlich 1	SD	SD
Mehlich 3	ND	SD

ND=Non significant difference SD=Significant difference

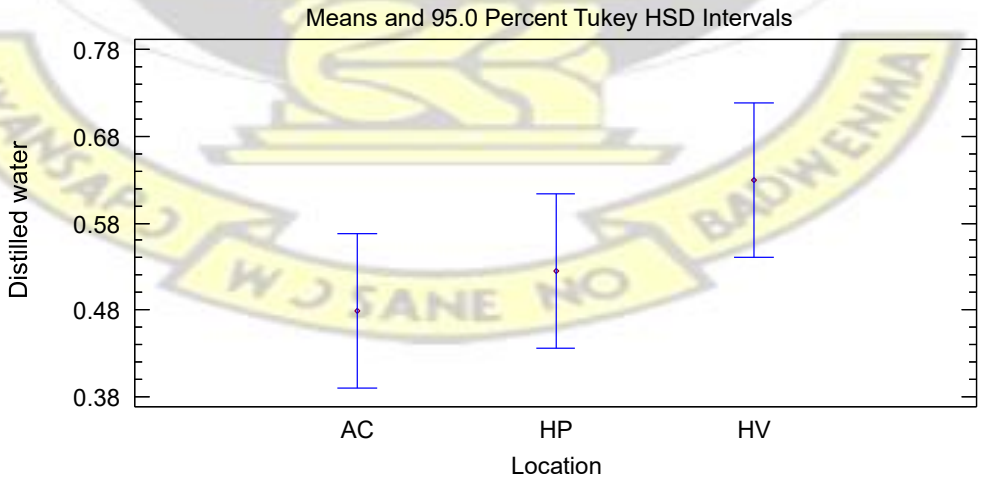
Distilled Water

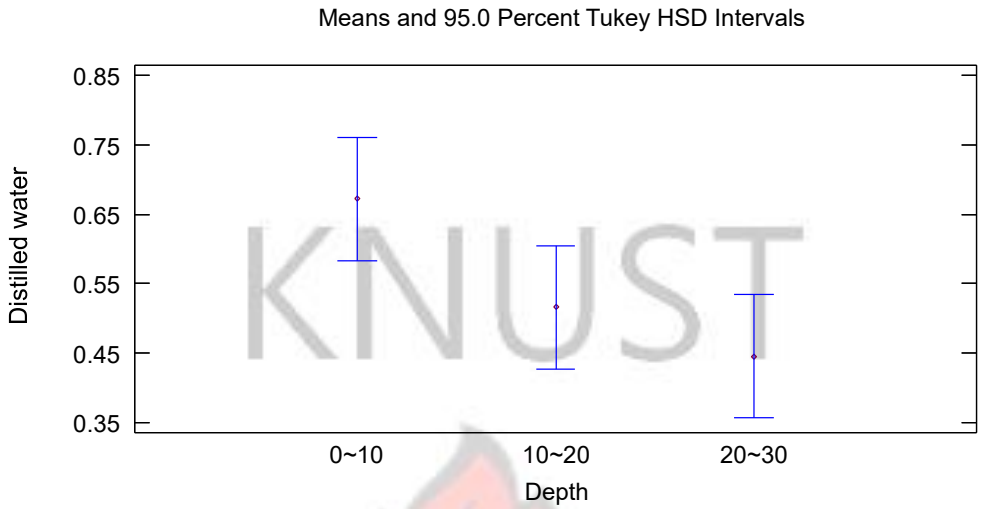
Analysis of Variance for Distilled water - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Location	0.428593	2	0.214297	2.12	0.1257
B:Depth	0.972588	2	0.486294	4.81	0.0102
INTERACTIONS					
AB	0.294627	4	0.0736567	0.73	0.5750
RESIDUAL	10.0178	99	0.10119		
TOTAL (CORRECTED)	11.7137	107			

All F-ratios are based on the residual mean square error.

The ANOVA table decomposes the variability of Distilled water into contributions due to various factors. Since Type III sums of squares (the default) have been chosen, the contribution of each factor is measured having removed the effects of all other factors. The P-values test the statistical significance of each of the factors. Since one P-value is less than 0.05, this factor has a statistically significant effect on Distilled water at the 95.0% confidence level.





Multiple Range Tests for Distilled water by Location

Method: 95.0 percent Tukey HSD

Location	*Count*	*LS Mean*	*LS Sigma*	*Homogeneous Groups*
AC	36	0.478992	0.0530174	X
HP	36	0.524417	0.0530174	X
HV	36	0.629417	0.0530174	X

Contrast	*Sig.*	*Difference*	*+/- Limits*
AC – HP		-0.045425	0.178411
AC – HV		-0.150425	0.178411
HP - HV		-0.105	0.178411

* denotes a statistically significant difference.

This table applies a multiple comparison procedure to determine which means are significantly different from each other. The bottom half of the output shows the estimated difference between each pair of means. There are no statistically significant differences between any pair of means at the 95.0% confidence level. At the top of the page, one homogenous group is identified by a column of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences.

Multiple Range Tests for Distilled water by Depth

Method: 95.0 percent Tukey HSD

Depth	*Count*	*LS Mean*	*LS Sigma*	*Homogeneous Groups*
20~30	36	0.445083	0.0530174	X
10~20	36	0.515583	0.0530174	XX
0~10	36	0.672158	0.0530174	X

Contrast	*Sig.*	*Difference*	*+/- Limits*
0~10 - 10~20		0.156575	0.178411
0~10 - 20~30	*	0.227075	0.178411
10~20 - 20~30		0.0705	0.178411

* denotes a statistically significant difference.

This table applies a multiple comparison procedure to determine which means are significantly different from each other. The bottom half of the output shows the estimated difference between each pair of means. An asterisk has been placed next to 1 pair, indicating that this pair shows a statistically significant difference at the 95.0% confidence level. At the top of the page, 2 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences.

Disodium EDTA

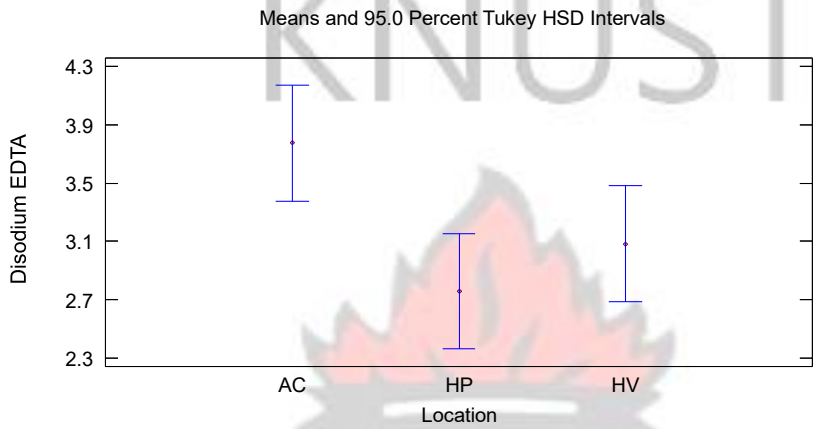
Analysis of Variance for Disodium EDTA - Type III Sums of Squares

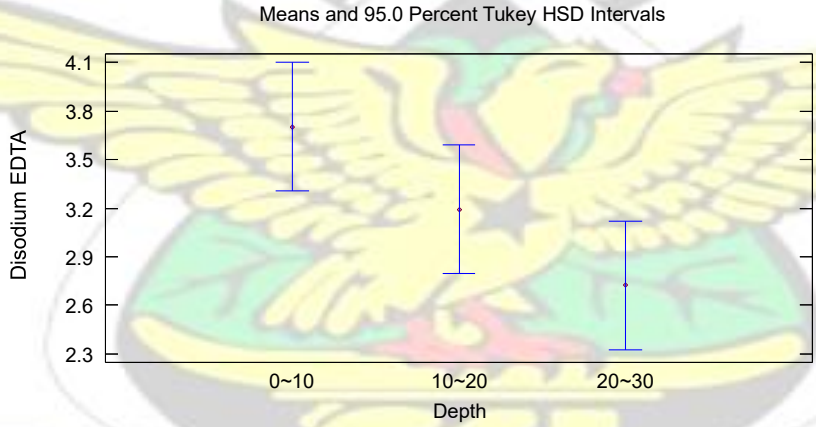
Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Location	19.3739	2	9.68693	4.84	0.0099
B:Depth	17.3103	2	8.65516	4.33	0.0158
INTERACTIONS					
AB	1.72188	4	0.43047	0.22	0.9294
RESIDUAL	198.062	99	2.00063		
TOTAL (CORRECTED)	236.468	107			

All F-ratios are based on the residual mean square error.

The ANOVA table decomposes the variability of Disodium EDTA into contributions due to various factors. Since Type III sums of squares (the default) have been chosen, the contribution of each factor is measured having removed the effects of all other factors. The

P-values test the statistical significance of each of the factors. Since 2 Pvalues are less than 0.05, these factors have a statistically significant effect on Disodium EDTA at the 95.0% confidence level.





Multiple Range Tests for Disodium EDTA by Location

Method: 95.0 percent Tukey HSD

Location	*Count*	*LS Mean*	*LS Sigma*	*Homogeneous Groups*
HP	36	2.75883	0.235739	X
HV	36	3.08425	0.235739	XX
AC	36	3.77467	0.235739	X

Contrast	*Sig.*	*Difference*	*+/- Limits*
AC - HP	*	1.01583	0.793296
AC - HV		0.690417	0.793296
HP - HV		-0.325417	0.793296

* denotes a statistically significant difference.

This table applies a multiple comparison procedure to determine which means are significantly different from each other. The bottom half of the output shows the estimated difference between each pair of means. An asterisk has been placed next to 1 pair, indicating that this pair shows a statistically significant difference at the 95.0% confidence level. At the top of the page, 2 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences.

Multiple Range Tests for Disodium EDTA by Depth

Method: 95.0 percent Tukey HSD

Depth	*Count*	*LS Mean*	*LS Sigma*	*Homogeneous Groups*
20~30	36	2.723	0.235739	X
10~20	36	3.19142	0.235739	XX
0~10	36	3.70333	0.235739	X

Contrast	*Sig.*	*Difference*	*+/- Limits*
0~10 - 10~20		0.511917	0.793296
0~10 - 20~30	*	0.980333	0.793296
10~20 - 20~30		0.468417	0.793296

* denotes a statistically significant difference.

This table applies a multiple comparison procedure to determine which means are significantly different from each other. The bottom half of the output shows the estimated difference between each pair of means. An asterisk has been placed next to 1 pair, indicating that this pair shows a statistically significant difference at the 95.0% confidence level. At the top of the page, 2 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences.

Olsen

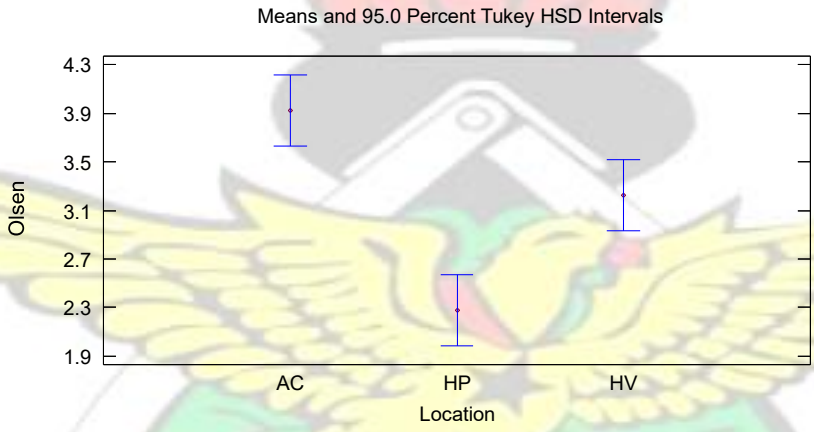
Analysis of Variance for Olsen - Type III Sums of Squares

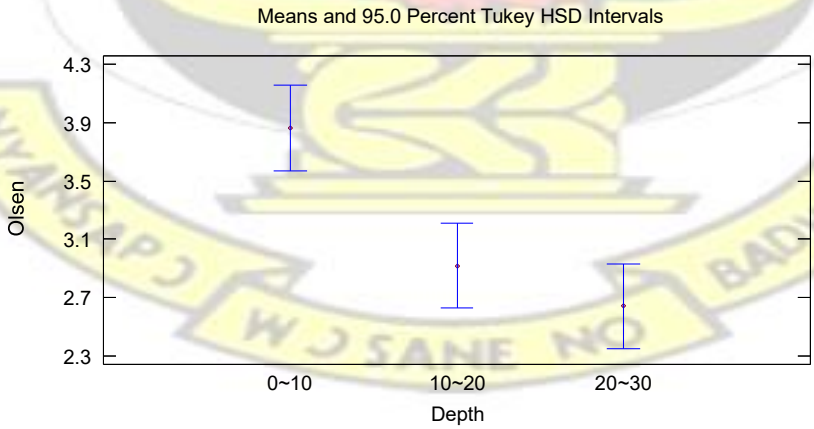
Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Location	48.9751	2	24.4876	22.53	0.0000
B:Depth	29.6228	2	14.8114	13.63	0.0000
INTERACTIONS					
AB	3.29767	4	0.824419	0.76	0.5547
RESIDUAL	107.593	99	1.08679		

TOTAL (CORRECTED)	189.488	107			

All F-ratios are based on the residual mean square error.

The ANOVA table decomposes the variability of Olsen into contributions due to various factors. Since Type III sums of squares (the default) have been chosen, the contribution of each factor is measured having removed the effects of all other factors. The P-values test the statistical significance of each of the factors. Since 2 P-values are less than 0.05, these factors have a statistically significant effect on Olsen at the 95.0% confidence level.





Multiple Range Tests for Olsen by Location

Method: 95.0 percent Tukey HSD

Location	*Count*	*LS Mean*	*LS Sigma*	*Homogeneous Groups*
HP	36	2.27767	0.173749	X
HV	36	3.2235	0.173749	X
AC	36	3.92092	0.173749	X

Contrast	*Sig.*	*Difference*	*+/- Limits*
AC - HP	*	1.64325	0.58469
AC - HV	*	0.697417	0.58469
HP - HV	*	-0.945833	0.58469

* denotes a statistically significant difference.

This table applies a multiple comparison procedure to determine which means are significantly different from each other. The bottom half of the output shows the estimated difference between each pair of means. An asterisk has been placed next to 3 pairs, indicating that these pairs show statistically significant differences at the 95.0% confidence level. At the top of the page, 3 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences.

Multiple Range Tests for Olsen by Depth

Method: 95.0 percent Tukey HSD

Depth	*Count*	*LS Mean*	*LS Sigma*	*Homogeneous Groups*
20~30	36	2.64017	0.173749	X
10~20	36	2.91817	0.173749	X
0~10	36	3.86375	0.173749	X

Contrast	*Sig.*	*Difference*	*+/- Limits*
0~10 - 10~20	*	0.945583	0.58469
0~10 - 20~30	*	1.22358	0.58469
10~20 - 20~30		0.278	0.58469

* denotes a statistically significant difference.

This table applies a multiple comparison procedure to determine which means are significantly different from each other. The bottom half of the output shows the estimated difference between each pair of means. An asterisk has been placed next to 2 pairs, indicating that these pairs show statistically significant differences at the 95.0% confidence level. At the top of the page, 2 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences.