Behaviour of monoammonium phosphate in alkaline and calcareous sandy soils

by

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DECLARATION

I declare that the dissertation hereby handed in for the qualification at the University of the Free State, is my own independent work and that I have not previously submitted the same work for a qualification at/ in another University/ faculty. I furthermore cede copyright of the dissertation in favour of the University of the Free State.

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ABSTRACT

Behaviour of monoammonium phosphate in alkaline and calcareous sandy soils

Soluble phosphorus (P) in soil is subject to fixation in either low or high pH soils. A variety of other soil processes contributes to this process, for example clay mineralogy, organic matter, sesquioxides and carbonates. Arid and semi-arid areas which are high in carbonates require proper P fertilisation to ensure sustainable crop production. Proper P fertilisation is hampered by the choice of which agronomic soil P test is best employed for fertiliser recommendations. The main objectives of the study were, firstly, to establish the amount of monoammonium phosphate needed to increase extractable P in the upper Orange River catchment soils with different calcium and phosphorus contents. Moreover, relationships between the application of P and the P extracted by the Olsen, Bray 1, Mehlich 3 and the Ambic 1 methods were investigated in the study soils which are used for irrigation. Lastly, the study compared extractable P contents in these catchment soils, which were analysed by three analytical laboratories.

Soil samples from the orthic A horizon were collected at six sampling sites in the upper Orange River water management area below the Vanderkloof dam in the southwestern parts of the Free State, and eastern parts of the Northern Cape. These samples were dried and sieved before conducting a two-month laboratory incubation study at room temperature, where they were treated with seven levels of monoammonium phosphate. During incubation, the samples were exposed to several wetting and drying cycles. The P in the soil samples was extracted with the Olsen, Bray 1, Mehlich 3 and Ambic 1 methods for colorimetric determination by the UFS laboratory. These samples were also analysed for extractable P by two commercial laboratories with the Olsen, Bray 1 and Mehlich 3 methods. Analyses of variance were conducted with IBM SPSS Statistics 25 at a 95% confidence level. The P content means of extraction methods and application levels were then compared with Tukey’s procedure, also at a 95% confidence level. Simple regression analyses were also done to meet the objectives of the study.

The six sites can be catagorised as low calcareous (<0.7% calcium carbonate) with low to high P contents (8.9 to 24 mg kg^{-1} Olsen P) and as high calcareous (>3.3% calcium carbonate) with low to high P contents (2.4 to 42 mg kg^{-1} Olsen P).

Thus phosphorus requirement factors (PRF) estimated from regression equations varied significantly between the four extraction methods. The Bray 1 method showed significantly unrealistic PRFs of 1.8 to 384.6 kg P ha^{-1}. By contrast, the variation of the PRFs for the Mehlich 3 method was very slight (0.9 to 2.1 kg P ha^{-1}). The PRFs of the Olsen method (4.6 to 6.1 kg P ha^{-1}) and Ambic 1 method (1.7 to 4.3 kg P ha^{-1}) were more in line with other studies. Relationships between applied P and extracted P showed that various regression equations fitted the data with different methods. Although almost linear, polynomial equations best described the relationship with R^2-values exceeding 0.98 for the Olsen method. Poor relationships (R^2- values less than 0.57) were regressed with Bray 1 method data at calcareous sites. A variety of equations fitted the data best when using the Ambic 1 method. Good relationships between the P extracted with these methods may have a positive influence on fertiliser recommendations when conversion of P contents is required. Some significant differences between extractable P for a particular method were observed by the three analytical laboratories. These differences can result in fertiliser recommendations bring offered with limited confidence.

This study proved that the mineralogical, physical and chemical properties of a soil ultimately prescribed which method is the most suitable to extract P for reliable P recommendations. The Olsen method proved to be the most reliable on both the non-calcareous as well as the calcareous soils. The PRFs estimated with Olsen data ranged from 4.6 to 6.1 kg P ha^{-1}. These values can serve as a basis for increasing extractable Olsen P to the required optimal levels. However, field studies are warranted to establish threshold values for fertiliser recommendations for each extraction method.

Key words: phosphorus extraction methods, phosphorus fertiliser recommendations, phosphorus reactions and availability, phosphorus requirement factors

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CHAPTER 1

INTRODUCTION

1.1 Motivation

Phosphorus (P) is one of the most essential macronutrients that are required in relatively large quantities (~0.2 to 0.8%) for plant growth and crop productivity (Mengel and Kirby 1987; Stutter *et al*., 2012; Daly *et al*., 2015). Only potassium (K) and nitrogen (N) are required in amounts larger than P. Phosphorus plays an important role in key functions of the plant, for example it forms part of structural components of numerous macromolecules. Generally P is widely spread in nature and occurs together with the other two important macronutrients, K and N (Sanchez and Uehara, 1980). Phosphorus in soil occurs in extremely insoluble forms such as organic complexes, salts, crystals, or is attached to the base-exchange complex (Smith, 1976). Only a very small part is available for crop uptake (Larsen, 1976). The insolubility of P in the soil leads to less leaching of this nutrient, but also leads to disadvantages where plants cannot always absorb a sufficient amount for P from the soil solution (Smith, 1976).

The term soil fertility refers to the ability of soil to supply the essential nutrients for crop development and maturation for good yields. Thus soil fertility is connected to the capability of soils to supply essential nutrients at rates and in amounts that are needed to produce high-quality and high-yielding crops, on a sustained basis (Stewart, 1990). Soil fertility is declining over the world, particularly in developing countries (Ayoub, 1999), because nutrient mining by crop removal without adequate replenishment occurs (Food and Agricultural Organisation [FAO], 1998; Ayoub, 1999.) This problem combined with unbalanced plant nutrition practices cause a serious problem to agricultural production. Therefore the efficient use of fertilisers is crucial. The improvement of fertiliser use is a major challenge. It is very important that farmers know which plant nutrients are required, and how much they should apply to provide the optimum economic increase in yield without causing any pollution risks.

The availability of P is strongly correlated to the soil pH. In acidic soils the formation of iron (Fe) and aluminium (Al) phosphates leads to a reduction in solubility, while free excess lime (calcium or magnesium) in calcareous or alkaline soils leads to the formation of calcium (Ca) or magnesium (Mg) phosphates. Soils containing free lime are common in arid and semi-arid regions with little rainfall (Hopkins and Ellsworth, 2005), such as in the Northern Cape and South Western parts of the Free State in South Africa. The effect of low P solubility and availability in alkaline or calcareous or acidic soils causes moderately poor fertiliser P efficiency, which leads to P deficiency in different crops (Hopkins and Ellsworth, 2005). Phosphorus availability is usually evaluated by soil exposure to different solutions that aim to remove an amount of the plant-available P. Numerous extraction methods with different extractants have been developed for the valuation of available P for plant uptake. However, no chemical extraction which is well related with plant uptake under all conditions exists (Power and Prasad, 1997). Chemical measures generally used for extraction of P are created on chemical principles that relate mostly to P minerals found in soils. These different minerals can dissolve, or P that is adsorbed can be released and may resupply the soil solution P, when this P is taken up by different plant species. The different chemical extractants simulate this process by reducing the Al, Fe and Ca in the soil solution through precipitation or complexation. During extraction, the iron phosphate (Fe-P), aluminium phosphate (Al-P) and calcium phosphate (Ca-P) compounds dissolve to resupply Fe, Al and Ca to the soil solution. The P in the solution increases, which provides a measure of the soil's ability to supply P to plant species (Havlin *et al*., 2014). To reduce the environmental impact of agricultural used P, the appropriate management strategy, which includes the agronomic analyses methods may be intensified to increase the quality of P fertiliser corrections (Do Carmo Horta and Torrent, 2006).

Due to the various complex reactions of P in soil, P has a very low availability as previously discussed. More or less 15 million tons of P fertiliser is applied annually worldwide (Wang *et al*., 2012), but only 5 to 30% is absorbed by plants (Price, 2006). Free Ca is an important factor established by calculating the P correction factor in calcareous or alkaline soils in arid areas. The relationship between free Ca content and P requirement is very important. Free Ca content in soil must be taken in consideration by the P requirement factor (PRF). It is very important to calculate this factor correctly to establish successful requirements for a specific soil with a specific P source, which is economically viable, and helps to avoid eutrophication risks. The normal approach to correcting P deficiencies involves the following: determine test value on specific soil from the known optimum level of the specific crop, establish the deficit in terms of the test value and calculate mass of nutrient needed per unit area. Deficit is multiplied by the factor, therefore: P requirement (kg ha^{-1}) = (Optimum soil P-measured soil P) x PRF. The requirement factor must bring into account the depth of fertiliser incorporation as well as different sorption effects related to the specific soil (Johnston *et al*., 1991). As mentioned earlier, soil P correction calculations are challenging for specific soils which differ in PRF values. Therefore it is important to calculate the PRF's of soils with their P adsorption factors in order to avoid excess P and hence eutrophication.

Eutrophication is a process where P accumulates in water bodies and leads to pollution risks. In certain areas there are severe environmental concerns about P losses from either point or non-point sources. Point sources refer to waste waters which contain manures and slurry from intensive livestock production farms where leakages from manure storage facilities occur, while non-point sources include losses from individual fields through erosion and leaching. In Europe, the average P pollution is estimated as follows: 50 to 75% from point sources, 20 to 40% non-point sources, and 5 to 15% from natural loadings (Crouzet *et al*., 1999). Fertiliser management practices are therefore very important when attempting to formulate safe P fertiliser recommendations to achieve a sufficient crop yield, while protecting the environment against eutrophication.

Food security is suppressed by the shortage of P. Throughout the world the P-content in soil is low; therefore P management in soil-plant systems is very important (Faucon *et al*., 2015). According to Batjies (1997) 5700 million hectares of soil worldwide does not have sufficient available P which is needed for optimum crop growth. Phosphorus is one of the most important macronutrients in agronomy due to the large amounts that are necessary for production.

The world population is projected to near eight billion by 2020. Sustainable food security in many developing countries will face major challenges due to the projections on population pressure and the availability of water and land resources globally. Considering their availability per capita per land area, socio-economic conditions and the severe scarcity of fresh water resources and certain infrastructure, food security is facing increasing risks. A further problem scenario involves global soil degradation, especially sub-Saharan Africa and South Asia and an increasing risk of soil erosion, in particular desertification. Soil degradation is increasing currently at a rate of 5 to 7 million hectares each year, while the currently estimated rate is 1.9 billion hectares (Lal, 2000; Zapata and Roy, 2004).

Modern agriculture on a global scale, is mostly dependent on the adequate supply of P inputs via different P sources, which mostly derive from rock phosphates. The known rock phosphate reserves of geological origin are non-renewable and are estimated to be exhausted before the end of this century (White and Cordell, 2010). Therefore major challenges relating to P management exist, namely: supplying satisfactory P inputs for improving soil P status to achieve sustainable growth of agricultural production and guarantee food security in the long term, and minimising the disadvantages of excess P on the environment, like fresh water bodies. To achieve these goals and reach a proper sustainable agriculture system it would be necessary to review the P cycle carefully in terrestrial ecosystems and describe combined strategies to improve management of P resources for food production over the world (White and Cordell, 2010).

The purpose of this study is therefore to provide information on the importance of soil fertility and P availability in alkaline and calcareous soils for sustainable crop production in arid areas. The importance of different agronomic soil tests measuring the concentration of available P is also highlighted for the purpose of examining the effectivity of these tests in different soils for proper regulation and management of different available P sources for precision agronomic purposes.

The study focused on the Upper Orange Water Management Area (UOWMA). The area consists of the main branch of the Orange River, from where it exists in Lesotho to the confluence with the Vaal River. The UOWMA has been divided into four distinct sub areas. Agricultural crop production in the area is predominantly by irrigation (Department of Water Affairs and Forestry, 2004). The Vanderkloof Water Association forms part of the UOWMA, of which 16 726.65 ha is irrigated from the Vanderkloof dam to the Douglas weir, as well as 5 457 ha of the main canal, Ramah 1, 2 and 3 from the dam (Personal communication: Ms. M. Peters., 2018. Vanderkloof Water Accosiation. Vanderkloof).

1.2 Objectives

- To review the behaviour of P in the soil-plant system.
- To establish the amount of monoammonium phosphate (MAP) needed to increase extractable soil P in Upper Orange River catchment soils with different Ca and P contents, used for irrigation.
- To establish relationships between the application effect of MAP and extractable P by different extractable methods in Upper Orange River catchment soils with different Ca and P contents, used for irrigation.
- To compare extractable P contents in Upper Orange River catchment soils, analysed by three analytical laboratories.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Phosphorus is an essential nutrient for crop growth and next to N, the second most important macronutrient for plant nutrition (Ragothama, 1999). The nutrient is involved in many plant processes such as respiration, photosynthesis, energy generation, nucleic acid biosynthesis and is an integral component in several plant structures. Due to the low availability of P in soils it is the least accessible macro-nutrient in most agricultural soils and is therefore the most frequently deficient nutrient (Vance *et al*., 2003).

The total P in soil ranges from 0.035 g kg^{-1} to 5.3 g kg^{-1}, with a mean content in the earth's crust of 1 g kg^{-1} (Sparks, 2003). Due to the process of soil degradation, the P availability is declining in many agroecosystems, which affected up to 75% of agricultural land in Africa. There is a global demand for an increase in agricultural production. This refers to a strict and sustainable P management strategy involving manipulation of soil and rhizosphere processes, improve P recycling efficiency and the development of P efficient crops in the future (Shen *et al*., 2011). Approximately 15 to 80% of total P are in the organic P form that includes a range of compounds such as phospholipids (1 to 5% of organic P), nucleic acid (0.2 to 2.5%), inositol-phosphates(2 to 50%), metabolic phosphates (trace), sugar phosphates and other unknown compounds (>50%) (Hiradate *et al*., 2007).

Phosphorus forms insoluble complexes with cations such as Al and Fe in soils which are acidic, and this reaction restricts P availability. Under alkaline conditions P forms complexes with Ca and Mg. A lack of P limits crop yield on 30 to 40 % of the world's arable land. This characteristic of P results in poor fertiliser recovery, because P applied through fertilisers is mainly adsorbed by the soil, and becomes unavailable for plant uptake (Vance *et al*., 2003; Cordell *et al*., 2011). One option to enrich P availability in soil is the addition of different P fertilisers. Supplementing phosphorus shortage by means of fertiliser applications is not practicably for the ordinarily resource-poor farmers in the tropics and subtropics, especially in soils (e.g. Ultisols, Oxisols) with high P-fixing capacity (Sanchez and Uehara, 1980).

The uptake of P by plant roots from the soil occurs in the form of inorganic orthophosphate ($H_2PO_4^-$ or HPO_4^{2-}) ions present in the soil solution, depending on the soil pH. At any stage the soil solution contains only 1% of the P taken up by plant roots. The remaining 99% of P for plant uptake is released by the soil solid phase in the soil solution during the growing season (Grant *et al*., 2005). Desorption and dissolution reactions are responsible for the transfer of P from the solid to the solution phase (Frossard *et al*., 2000).

Eutrophication in reservoirs and rivers can happen if there is an accumulation of soil P in the water as a result of run-off, affecting growth of fauna and flora in water. The challenge is to increase the P fraction in soil and to decrease the P input by reducing manufactured P inputs (Faucon *et al*., 2015). Applications of P through fertilisers or manure are the reason for enhancing the loss of P through water runoff. This can be affected by time, method and rate of application, form of P fertiliser, and the placement method of P in the soil. Other factors that also play a role are the runoff volume, temperature, soil type, tillage method of soil and soil cover (Faucon *et al*., 2015; Jalali, 2016a; b).

Soil tests are the main method to make P recommendations and to determine whether there are environmental risks. According to Jalali (2016a; b) soil tests, as well as P sorption foretell the mobility and the availability of P in the soils. As indicated by Pierzynski (2000) soil testing for P has been formally in use in the United States of America since the late 1940s. Today it is a general agronomic practice. The aim of soil analyses is to identify the optimum P level that plants need for optimal growth (Pierzynski, 2000). When the soil P content is determined, predictions can be made to gauge the correct level of economic investment in P fertilisers to obtain the optimum yield. Jalali (2016a; b) indicated that the extractability and the sorption of P fluctuate with the formation of soil, such as the contents of either organic matter or clay in soil.

The purpose of this literature review is to provide comprehensive and up-to-date information on several topics of P behaviour in soil-plant systems. This review will also focus on the cycle of P and the management of P in soil with regard to crop production. Lastly this review highlights the use of different extraction methods, especially those in use in South Africa.

2.2 Phosphorus cycle

Soil, plants and micro-organisms are involved in the P cycle. The form of P in soils depends on several processes in the soil. Micro-organisms in the soil are essential for maintaining these processes. Bacteria are the main micro-organisms that control all the P cycle processes in soil (Hu *et al*., 2009). The P cycle in soils is a very complicated phenomenon and is influenced by several factors. These factors included the nature of inorganic and organic phases present, forms and extent of biological activity occurring, chemistry of the soil solution like pH, ionic strength and redox potential and other environmental factors such as soil-water content and temperature of soil. The optimum P concentration in the soil solution must be maintained for optimum plant uptake. This is necessary to manage the P cycle and all the chemical and biochemical processes in this cycle (Lanyon and Simard, 2005). Mineralisation, immobilisation, adsorption, desorption, precipitation and dissolution are principal processes involved in the P cycle. Figure 2.1 refers to all these processes involved in the conversion of P in the soil and atmosphere.

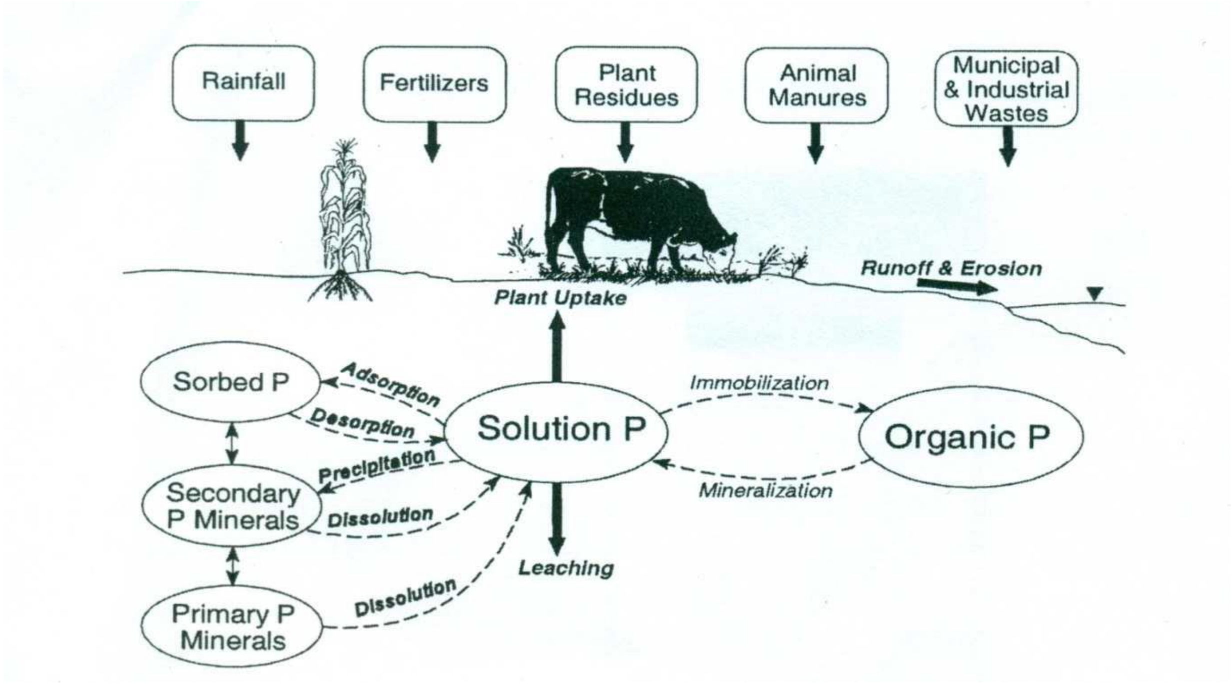


Figure 2.1 Phosphorus cycle in soil-plant systems (Campbell and Edwards, 2001).

2.2.1 Mineralisation and Immobilisation

Mineralisation and immobilisation are two processes that occur concurrently in soil although the one may dominate the other depending on carbon: phosphorus (C:P) ratios. Mineralisation is the process where organic P is converted to inorganic P which is available for plant uptake and immobilisation is the process where inorganic P is converted to organic P which is unavailable for plant uptake (Van der Laan *et al*., 2009). Inorganic P reacts very quickly with various other mineral surfaces and clay; it leads to the formation of Fe-P, Al-P or Ca-P minerals that precipitate and become unavailable to plants. The amount of P that is mineralised during the growing season fluctuates across different kind of soils (Havlin *et al*., 2014).

On average, total soil P consists of 30 to 65% organic P (Blair, 1993). Either the mineralisation or immobilisation processes to which organic P is subject are controlled by combined activities of micro-organisms, free enzymes, phosphatases and intracellular enzymes. These enzymes catalyse the hydrolysis of both ester and anhydrides of phosphoric acid. Organic soil P is mostly present as inositol P esters, which is prone to adsorption resulting in less available P in soils that have a higher adsorption capacity. A wide range of micro-organisms are proficient to mineralise organic P through their phosphatases dexterity (Havlin *et al*., 2014).

Soil factors that are responsible for microbial activities determine the process of mineralisation and immobilisation of organic and inorganic matter in soil. The following factors have an influence on the rate of mineralisation; organic P content and rate of organic matter breakdown, which depend on the ratio between organic C and P, soil pH, liming, soil temperature (optimum temperature for growth of most bacteria is between 30 and 45°C), soil water content, P retention capacity of the specific soil and alternate wetting and drying of the soil, phosphatase activity, fertiliser application, cultivation intensity and the type of microbes (Havlin *et al*., 2014). The chemical form of P in the soil is dependent on soil pH, parent material, vegetation cover, time and the degree of pedogenesis. The organic P content in the soil increased with soil development, but the organic P content decreased in highly weathered soils (Spohn *et al*., 2013).

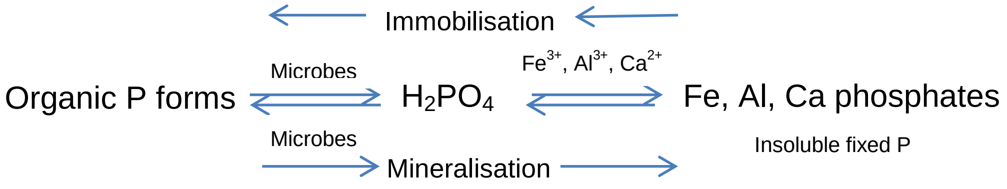
Amino acids or monosaccharides, especially glucose, increase organic C mineralisation. The glucose serves as an energy source for the micro-organisms

(Spohn *et al*., 2013). Spohn *et al*., (2013) indicated that micro-organisms located in moderate forest soils incorporated only a small proportion of P, while the organic moiety of phosphorylated organic compound as a source of carbon (C) was used. The P undergoes mineralisation without incorporation of P. Phosphorus mineralisation is driven by microbial demand for C in the soil and is a highly beneficial process for plants. Spohn and Kuzyakov (2013) also indicated that microbial demand is not only driven by the process of N and sulphur (S) mineralisation, but also by P mineralisation. Mineralisation of C and N is opposite to the mineralisation of organic P. The latter increases with a rise in the soil pH, where organic C and N do not (Shreeja, 2016).

Moist and warm conditions are regarded as optimum for P mineralisation. This process determines the form of P in the soil and whether it is available for plant uptake, run-off or leaching. Mineralisation of P, through bacteria that occurs intracellular as well as extracellular, maintains soil fertility. Inorganic plant available P increases while organic P decreases due to mineralisation, especially in ecosystems where inorganic plant available P is low and soil organic matter is high in soil (Bünemann *et al*., 2016).

The C:P ratio in soil determines the mineralisation and immobilisation rates. If the ratio is less than 200:1, mineralisation is favoured and if the ratio is greater than 300:1, immobilisation is favoured. A balanced ratio would be in the range of 200:1 to 300:1. Mineralisation and immobilisation rates depend therefore on the addition of organic P or inorganic P. Moreover, the C:N ratios in soil will increase by adding N sources which can promote N mineralisation and P mineralisation, because the two plant nutrients are used concurrently by mineralising microbes (Campbell and Edwards, 2001). Conversely, immobilisation can be favoured by having a high C content in the soil. This will lead to a higher organic P content. A C:N:P ratio of 100:10:1 for soil organic matter is recommended, but for mineralisation of organic phosphatic substances, the ratio ranges from 229: 10 : 0.39 to 71: 10: 3.05, depending on nature and type of soils. The C:N:P:S ratio in soils varies, with an average of 140:10:1.3:1.3. On 10 calcareous soils in Scotland an average ratio of 113:10:1.3:1.3 was found, while on 40 non-calcareous soils an average of 147:10:2.5:1.4 was found (Havlin *et al*., 2014). Continuous fertiliser application of P can lead to an increase in organic P content which will afterwards lead to an increase in mineralisation. Annual increases of 3.4 to 11.2 kg ha^{-1} in organic P are possible with P fertilisation. The maximum immobilisation rate occurs at a temperature of 30 °C, although it can proceed at temperatures as low as 5 to 7 °C and even lower. For net mineralisation to take place, a concentration of about 0.2% of P is critical, while a concentration of less than 0.2% will lead to immobilisation (Shreeja, 2016).

Organic substances which contain P can be mineralised and immobilised by the same general processes that release N and S form soil organic matter:



Studies in the midwest of the USA showed that mineralisation reduces the organic P by 24% in the surface soil when cultivated for 25 years, which was less than the loss of organic C and N. Organic P losses in the southern plains of the USA are greater because of higher soil temperature (Havlin *et al*., 2014).

2.2.2 Adsorption and desorption

According to Campbell and Edwards (2001) adsorption and desorption refers to the level in which P is held by chemical bonds on the reactive components of soil. Desorption refers to the release of adsorb P into the solution, which is available for plant uptake. In spite of the fact that desorption does not occur as easily as adsorption, a fraction of P is available for plant uptake, run-off transport and leaching.

Several factors have an influence on P adsorption in soils: organic matter, clay, Fe, Al, and Ca content, surface charge, surface area, dominant cation and anion on exchange complex, P sorption value, pH, concentration of P in the soil solution, parent material, repeated fertiliser additions, flooding, oxygen supply, cultivation, liming practices, time, temperature and electrolyte concentration (Sibbesen, 1981; Mattingly, 1985; Mengel and Kirby, 1987; Blair *et al*., 1990; Syers and Ru-Kun, 1990; Wada *et al*., 1990; Morgan, 1997; Addiscott and Thomas, 2000; Campbell and Edwards, 2001; Havlin *et al*., 2014). Metal hydrous oxides are highly effective in adsorbing $H_2P0_4^-$ ions that may exist in the soil solution (Morgan, 1997). Kaolinite (1:1) and montmorillonite (2:1) clays are the primary types of crystalline Al silicates, which are responsible for P adsorption on the edge face of these crystals. Calcium carbonate ($CaCO_3$) is the dominant molecule in calcareous soils (Kissel *et al*., 1985). The sorption capacity of these molecules lies somewhere between that of crystalline clay minerals and hydrous oxides. In acidic soils adsorption is favoured due to the fact of less competition on the adsorption sites. Adsorption in weathered soils is high, because of the high clay, Al and Fe contents. In sandy soils the reaction sites are less and the P content is higher in the solution. Organic matter favours P adsorption, but only in limited cases (Campbell and Edwards, 2001).

2.2.3 Precipitation and dissolution

Precipitation is a process in soil where P is fixed and forms solid materials. If P precipitates it is less precipitive to transport by runoff than P that is associated with fine soil particles (Campbell and Edwards, 2001).

According to Campbell and Edwards (2001) precipitation reactions are highly pH dependent. If soil P reacts with $CaCO_3$ it forms apatite. At a lower pH the P would rather reacts with the Al and Fe in the soil. The Ca or Fe and Al content in the soil determines the precipitation rate.

Dissolution is the opposite process of precipitation. Dissolution is also pH dependent and the maximum dissolution happens in the pH range from 6 to 6.5. In agriculture a pH which is slightly acidic would be preferred, due to P availability (Campbell and Edwards, 2001).

2.2.4 Losses and additions

2.2.4.1 Losses

Orthophosphates in the soil can get lost by various processes which include erosion and leaching. They can also be lost in an aqueous solution via runoff (Zhang *et al*., 2016). There are two transport pathways in which P can be lost via runoff, namely overland in surface runoff on eroded soils, and secondly, vertical leaching with drainage. The dominant pathway is controlled by a few factors. The factors include weather conditions, topography and soil properties. Surface runoff and erosion are responsible for the largest P losses. Dissolved P for plant uptake is mostly available, but its concentration in runoff water varies (Sharpley and Rekolainen, 1997). In Booneville, United States of America (USA) a field study on a silty loam soil with a clay content of 8% resulted in a P content of 0.57 mg^{-1} in runoff water during May and during August the P content was 1.05 mg^{-1} in runoff water (Pote *et al*., 1999). Due to the adsorption property of P for example to Fe and Al-oxides in the soil, leaching of P in soil is not a major form of P loss. When the land has a slope and runoff is high, P can be lost in particle bound form. Another way in which P can be lost is if there is saturation-excess runoff in a flat land, especially in rice fields. Phosphorus losses in soil can be affected by different crop covers (Jian *et al*., 2016). All these losses refer to the worldwide consternation about eutrophication and the resulting hypoxia. Anthropogenic P inputs on the wrong growth stage of the crop contribute to P losses and eutrophication (Chen *et al*., 2016). Erosion in New Zealand from small catchment areas can cause up to 0.1 to 6.3 kg P ha^{-1} year^{-1} P losses for pastures (Ward *et al*., 1990).

The amount of P that is lost and carried away by water can be controlled through two main approaches: firstly, more efficient use of P is necessary. This can be effected by limiting P inputs according to the requirement of the specific crop's growth stage, and secondly P losses must be reduced through more economically viable management techniques (Campbell and Edwards, 2001). For example, the runoff of orthophosphates can be reduced by subsurface application of P (Randall *et al*., 1985). An increase in the amount of water, concentration of P in the soil solution, P saturation of the soil and a decrease of P buffer capacity will cause higher P losses from soil (Holford and Mattingly, 1976; Soon, 1985). In Western Australia, areas receiving more than 450 mm rain year^{-1} experience P leaching in sandy soils with a low P adsorbing capacity, for example low clay content and low Fe and Al-oxide contents (Bolland, 1998).

2.2.4.2 Additions

In agricultural land-chemical fertilisers, recycled manure, atmospheric deposition as well as seeds-input are the primary inputs of P in the soil. Residential lands receive P from human and animal wastes (Chen *et al*., 2016). Plants primarily use inorganic P that depends on a few factors (soil temperature, water, pH and the availability of other nutrients) (Campbell and Edwards, 2001). Dust deposits can also affect the P concentration in soil. Microbial P also plays a role in the addition of P in soil. Application of phosphobacteria increased P uptake, resulting in an average yield increase of 10%, although the results varied. *Penicillium bilaii* increase the P uptake in high P, calcareous soils (Havlin *et al*., 2014).

2.3 Reaction of phosphorus in soil and availability

When soluble phosphatic sources are added to the soils, several processes can occur. Initially they dissolve and cause a rise in the soil solution P, which leads to adsorption and precipitation processes (Power and Prasad, 1997). Phosphorus fixation is the reaction that occurs among the P ions which are in the soil solution. The constituents and the non-phosphatic components in the fertilisers, mainly remove the P from the soil solution phase and the P become less soluble over time (Sample *et al*., 1980). Phosphorus in soil thus plays an important role in determining the definitive availability of fertiliser P to different crops and the mobility of P in soils. An understanding of the different reactions underlying P fixation in acid and alkaline/ calcareous soils is a first step towards obtaining optimum P nutrition in a specific soil with a specific P source to achieve efficient management for soil P corrections. Soils throughout the world have a shortage of P and the result is a yield which is not economically viable. Slow P diffusion and P fixation are held accountable for this. Due to fixation, all the P in the soil is not available for plant uptake and this causes deficiencies (Zhang *et al*., 2016). As mentioned in section 2.2.2, the presence of Al-P and Fe-P minerals prevail in acid soils, while Ca-P dominate in neutral and calcareous soils. In acid soils, inorganic P can precipitate as Al-P or Fe-P, or can be adsorbed to Fe/Al oxide surfaces and different clay minerals. Inorganic P in neutral and calcareous soils can precipitate either as Ca-P or Mg-P or can also be adsorbed to clay mineral surfaces and $CaCO_3$ (Havlin *et al*., 2014). Adsorption on the edges of kaolinite or on Fe-oxide coating on kaolinite clays at moderate soil pH values are possible (Brady and Weil, 2017). Organic P such as phytins form Fe and Al phytases in acid soils while they are able to precipitate under alkaline conditions as Ca phytases. Clay minerals, especially montmorillonite are adsorbed nucleic acids that cause a decrease in their rate of decomposition as well as P availability for plant uptake. Phytins can be absorbed by plants directly, but nucleic acids must be fragmented to inorganic P by enzymes at the root surfaces of the plant (Brady and Weil, 2017). The optimum pH value where P is available for plant uptake is in the range of 6.5. Figure 2.2 indicates the pH ranges where P is fixed, due to the presence of certain elements at different soil pH values.

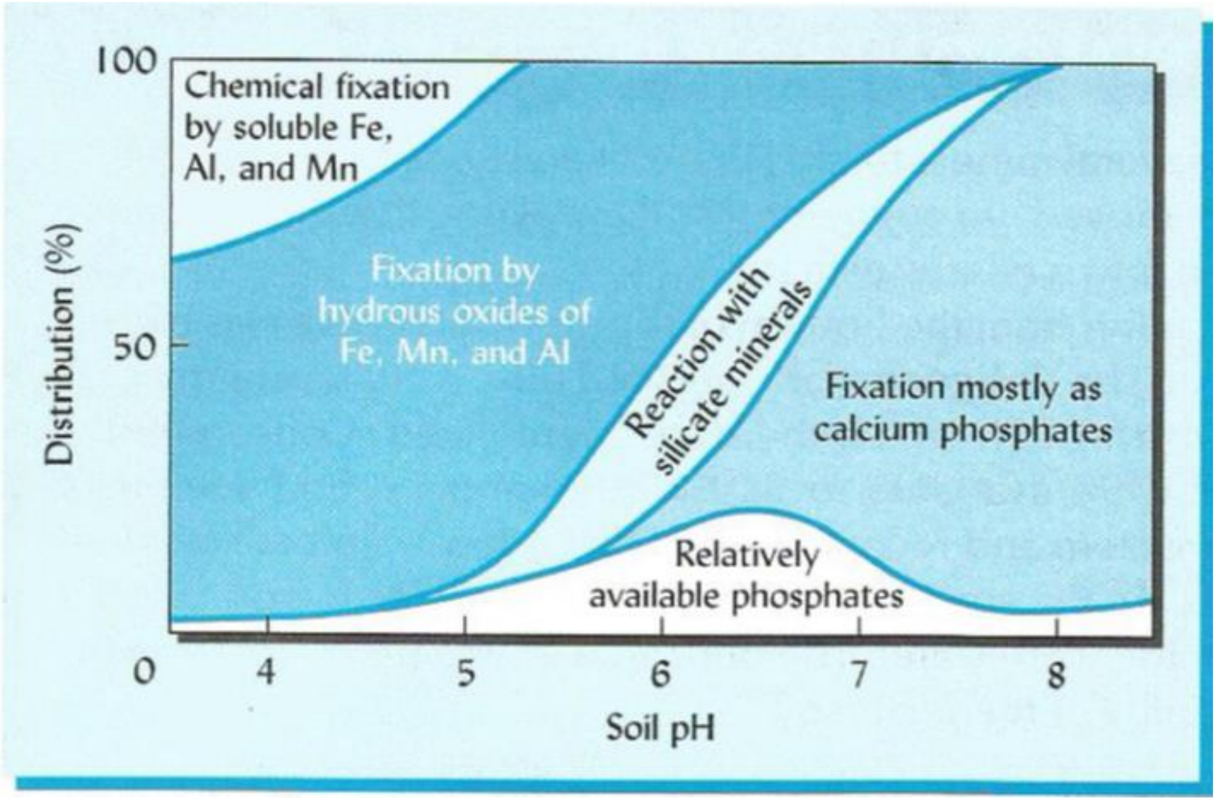


Figure 2.2 Inorganic P fixation of P added at various soil pH values (Brady and Weil, 2017).

South African soils can be divided into highly weathered, slightly weathered or calcareous soils (Van der Laan *et al*., 2009). Highly weathered soils usually contain more Fe and Al than slightly weathered soils. Fluorapatite is believed to be the original P mineral present in the soil and is even found in the lower horizons of the most weathered soils. This mineral is an indication of the extreme insolubility and resulting unavailability of the P contained therein (Brady and Weil, 2017). Table 2.1 indicated different inorganic compounds of Al-P, Fe-P and Ca-P commonly found in acid, alkaline and calcareous soils.

Table 2.1 Inorganic P containing compounds commonly found in acid, alkaline and calcareous soils (Brady and Weil, 2017)

The compounds is listed in order of increasing in solubility in each group	
Compound	**Formula**
Iron and aluminium compounds	
Strengite	$FePO_4 . 2H_2O$
Variscite	$AlPO_4 . 2H_20$
Calcium compounds	
Fluorapatite	$[3Ca_3(PO_4)_2] . CaF_2$
Carbonate apatite	$[3Ca_3(PO_4)_2] . CaCO_3$
Hydroxy apatite	$[3Ca_3(PO_4)_2] . Ca(OH)_2$
Oxyapatite	$[3Ca_3(PO_4)_2] . CaO$
Tricalcium phosphate	$Ca_3(PO_4)_2$
Octacalcium phosphate	$Ca_8H_2(PO_4)_6 . 5H_2O$
Dicalcium phosphate	$CaHPO_4 . 2H_2O$
Monocalcium phosphate	$Ca(H_2PO_4)_2 . H_2O$

2.3.1 Acid soils

Several factors like vegetation, soil solution and soil minerals are responsible for the H^+ transfer processes of acidification and alkalinisation in soil. The flux of H^+ can be caused by direct proton addition or depletion (Van Breemen *et al*., 1983). In areas across the world with a high rainfall, a low soil pH is common as a result of all the basic cations like Ca^{+2} that are removed. The basic cations such as Ca^{+2} are being replaced with hydrogen ions (Hopkins and Ellsworth, 2005).

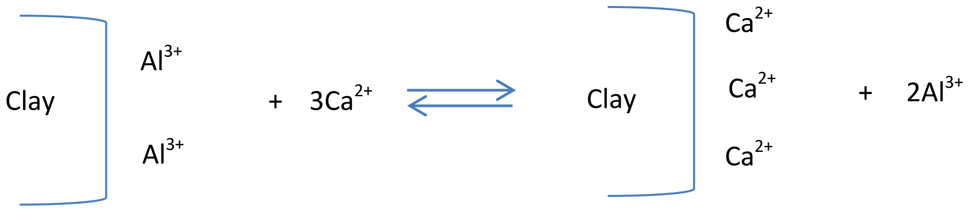
Soil acidity is one of the major problems that limit agricultural production as well as the availability of P in the soil. Acid soils have high levels of Al in the soil which restrict the uptake of plant nutrients such as P. The presence of phytotoxic levels of exchangeable Al, complicate P availability. In highly weathered soils hydrated oxides of Al and Fe play a major role in the adsorption of P (Parfitt, 1978; Sanchez and Uehara, 1980). Such hydrous oxides can occur in soil as coatings on soil particles and as interlayer precipitates in silicate clays (Brady and Weil, 2017). The P adsorption capacities of pure oxide systems such as goethite normally decrease as the pH levels rise from 4 to 12. Adsorption of P by goethite decrease when there is a rise in the pH from 2 to 12. The divalent HPO_4^{2-} ion increases 10-fold for each unit increase in pH from 2 to 7 (Hingston *et al*., 1972; Breeuwsma and Lyklema, 1973; Chen *et al*., 1973; Bowden *et al*., 1980).

The most common P fixation reaction is that between $H_2PO_4^-$ ions with dissolved Al^{3+}, Mn^{3+} and Fe^{3+}, resulting in insoluble hydroxyl P which precipitates (Brady and Weil, 2017):

$$Al^{3+} + H_2PO_4^- + 2H_2O \rightleftharpoons 2H^+ + Al(OH)_2H_2PO_4$$

The surfaces of insoluble oxide of Fe, Al and manganese (Mn), such as goethite ($Fe_2O_3 \cdot 3H_2O$), gibbsite ($Al_2O_3 \cdot 3H_2O$) and 1:1 type silicate clays adsorb P (Brady and Weil, 2017). The 1:1 type of silicate clay minerals adsorbed P to a larger extent than the 2:1 type of clay minerals since they contain larger amounts of Fe and Al oxides. More hydroxide (OH) groups are exposed on these Al layers and are able to exchange with P. The charge of kaolinite is pH dependant and this characteristic also contributes to P adsorption (Brady and Weil, 2017). The following reactions illustrate how hydrolysed Al^{3+} can adsorb soluble P (Havlin *et al*., 2014).

Step 1 Cation Exchange



Step 2 Hydrolysis

$$Al^{3+} + 2H_2O \rightleftharpoons Al(OH)^{2+} + 2H^+$$

Step 3 Precipitation and/or Adsorption

$$Al(OH)_2^+ + H_2PO_4^- \rightleftharpoons Al(OH)_2H_2PO_4$$

Liming can often increase P uptake by plants by decreasing Al toxicity in the soil. Through this management practice plants are able to use available P in the soil more efficiently (Haynes, 1982). Lime materials such as $CaCO_3$ or $Ca(OH)_2$ are being used to raise soil pH. The Ca^{2+} addition reduces the capacity of the original surfaces of P adsorption due to removal of hydroxyl-Al or Fe species, through neutralisation (Fried and Dean, 1955; Coleman *et al*., 1960). Phosphorus adsorption increases impressively when Al and Fe hydroxyl species are present on mica surfaces (Fried and Dean, 1955; Perrott *et al*., 1974). When comparing limed acid soils and calcareous soils, it is interesting to note where the content of metal phosphates are high in both soils, due to pedogenesis or fertiliser application in excess of the plant's requirement. It was found that the calcareous soils contain less surface P than in the case of acid lime soil. In limed acid soils the P availability is greater than in calcareous soils (Von Wandruszka, 2006).

A study in Malaysia was conducted to improve P availability in acid soils by adding biochar, compost produced from chicken litter and pineapple leaves. The organic amendments increased the soil pH and reduced the exchangeable acidity and exchangeable Al and Fe fractions in the soil. This particular study proved that P becomes more available with all three organic amendments, because Al and Fe have a high affinity to organic components. The result is the chelation of Al and Fe by biochar and compost instead of P. Inorganic P in the bio-available labile P pool will last for a longer period compared to the addition of inorganic fertilisers such as triple superphosphate. Thus biochar in the trial improves soil fertility, crop productivity, soil-water retention as well as C sequestration. Organic amendments in the soil also increase the mineralisation rate in the soil (Pizzeghello *et al*., 2011).

In a study by Robarge and Corey (1979) a resin as a model of an acid soil was used, to determine the effect of pH on the adsorption of P. The pH was raised from 3 to 7 and the amount of Al^{3+} was decreased by the neutralisation of Al^{3+} and the formation of positively charged hydroxyl-Al polymers. In the pH zone from 4.5 to 5.5 the most P was actively adsorbed. At higher pH zones the adsorption of P was repressed due to both competition with hydroxyl ions and because of the hydroxyl-Al species that tend to become negatively charged in more alkaline conditions.

The positive charges of Al and Fe oxides/ hydroxides in acid soils attract the negative orthophosphates ions ($H_2PO_4^-$ and HPO_4^{2-}). Figure 2.3 represents the mechanism of P adsorption on Al and Fe oxide surfaces. The OH is replaced by P groups, allowing for the bonding of orthophosphate ions through aluminium-oxygen-phosphorus (Al-O-P) bonds. The $H_2PO_4^-$ ion is considered as labile, because it can be desorbed from the mineral surface to the soil solution and is available for plant uptake. When two Al-O bonds form, a stable six-membered ring forms, the $H_2PO_4^-$ is non-labile and hence unavailable for plant uptake (Havlin *et al*., 2014).

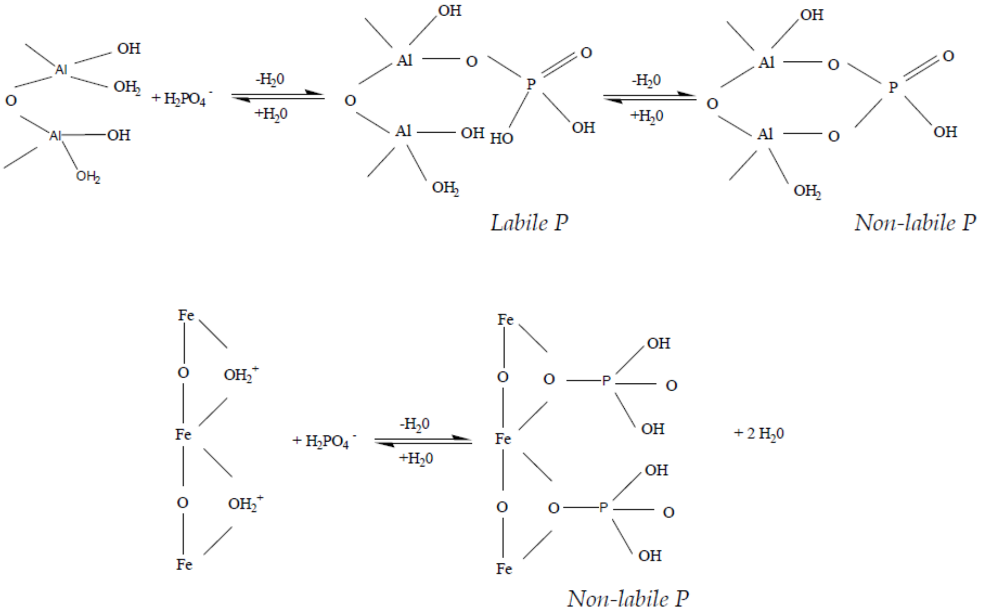
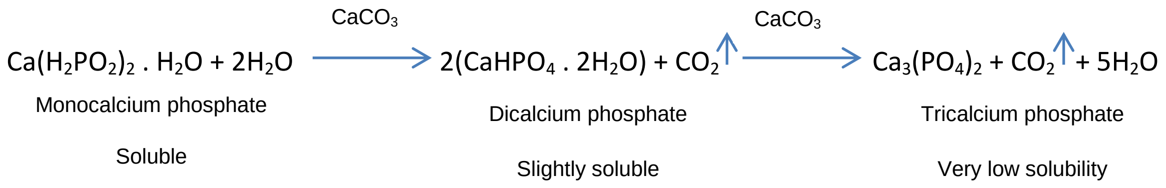


Figure 2.3 Mechanism of P adsorption to Al/Fe oxide surface (Havlin *et al*., 2014).

2.3.2 Alkaline and calcareous soils

Calcareous soils are soils which have a pH (H_2O) value in the range of 7.6 to 8.3 and contain free $CaCO_3$ or $MgCO_3$. The origin of these two compounds is generally the parent material. According to the South African taxonomic soil classification system, calcareous soils which contain $CaCO_3$ or $MgCO_3$ effervesce visibly when 10% cold hydrochloric acid (HCl) is added (Soil Classification Working Group, 1991). The pH of the soils can evenly increase till 9 if the soil contains sodium carbonate (Na_2CO_3). Calcareous soils are generally found in arid or semi-arid regions. The available P levels decrease as a result of the precipitation of Ca and Mg insoluble phosphates. Adsorption and precipitation take place in soils with a high pH, although it is difficult to distinguish between these two reactions. Adsorption and precipitation determine the availability of P after fertiliser application. If the P content of a specific soil is low, the non-carbonate clays provides the surface for P adsorption in calcareous soils (Von Wandruszka, 2006).

In soils with a high pH (e.g pH= 8) the soluble $H_2PO_4^-$ will quickly react with Ca to form products in a sequence of solubility. The reaction below illustrated an example of products formed in calcareous soils that vary in solubility. Tricalcium phosphate can undergo further reactions in $CaCO_3$ rich soils to form even more insoluble P molecules. Hydroxy-, oxy-, carbonate-, and fluorapatite compounds (apatites) are examples of less soluble molecules (Brady and Weil, 2017).



$$Ca(H_2PO_2)_2 \,.\, H_2O + 2H_2O \xrightarrow{CaCO_3} 2(CaHPO_4 \,.\, 2H_2O) + CO_2\uparrow \xrightarrow{CaCO_3} Ca_3(PO_4)_2 + CO_2\uparrow + 5H_2O$$

For example, with time the dicalcium or tricalcium phosphate will dissolve if the concentration of soluble P decreases and octacalcium phosphate ($Ca_8(HPO_4)_2(OH)_2$) will form. Octacalcium phosphate will control the amount of P in the solution when all the dicalcium phosphate has dissolved. When the P in the solution can no longer support octacalcium phosphate, it will dissolve and hydroxyapatite will form. If hydroxyapatite is the stable form in a particular soil, it will be the last Ca-P compound formed and hence will represent the lower limit of P in the soil solution and the lower limit of P available to plants (Syers and Ru-Kun, 1990; Addiscott and Thomas, 2000).

Soil carbonates ($CaCO_3$) can also be responsible for P adsorption in calcareous soils. The interaction of P with $CaCO_3$ involves two reactions: the first reaction occurs at low P concentration and consists of the adsorption of P on $CaCO_3$ surfaces, and the second reaction is a nucleation process to form phosphate crystals (Power and Prasad, 1997).

Calcareous soils can be productive as long as the producer manages the soil properly. Cultivated land can easily be modified through the use of fertilisers or lime applications (Van der Laan *et al*., 2009). By making use of this practice the reactions in the soil change dramatically.

2.4 Phosphorus uptake by plants

The largest fraction of P is absorbed during the vegetative growth period of the plant. During the reproductive growth stages most of the absorbed P is re-translocated into fruits and seeds (Marschner, 2000). Plant species and cultivars differ in their ability to take up P. Hanway and Olson (1980) researched the P nutrition of maize, sorghum and soybeans and indicated that the total amount of P taken up was from 7 to 15 kg P ha^{-1} with 2 to 8 kg P ha^{-1} being returned to the soil via the crop residues left on the land. Studies on P nutrition of maize, rice and wheat showed that for each tonne of grain produced, the total crop contained about 4.2 kg P, the range of P is given as 2.7 to 3.3 kg P in the grain, and 0.83 to 1.6 kg P in the stover. The P concentration in soil is usually low due to fixation or retention reactions. Therefore it is very important that crops such as maize cover a large soil volume through extensive root systems enabling maize to absorb large amounts of P. Root growth and morphology of the specific crop are very important for the uptake process of P by plants (Mengel and Kirby, 1987). Mengel and Kirby (1987) stated that lettuce absorb P only from the upper 180 mm soil layer. Carrots on the other hand are able to utilize a significant percentage P from the upper 400 mm soil layer, while total P uptake by carrots from the soil layer deeper than 1 m can amount to 10%. Soil properties (depth, pH, cation exchange capacity and water content), crop characteristics (type, growth stage and root system), climatic factors (rainfall, solar radiation and temperature), and P added through fertilisation and removed by the crop are factors that influenced P uptake by the plant (Nye, 1969; Jones, 1982; Parnes, 1990; Simpson, 1991; Wolf, 1999).

For the application of control measures to ensure P availability in soil it is necessary to have knowledge of the different P forms in soil by determining their availability. Figure 2.4 refers to inorganic P that occurs in three main pools: labile, active and stable. Plants can take up P from the soil solution also known as the active pool, in the orthophosphate ion ($H_2PO_4^-$ and HPO_4^{2-}) form. The amount of these orthophosphate ions present in the soil solution is pH dependent. In a pH range from 4 to 5.5 (strongly acidic), the monovalent ion, $H_2PO_4^-$ will dominate, while the divalent ion, HPO_4^{2-} dominates in the more alkaline range. In soils with a neutral pH, both ions play an important role. However, of the two ions, $H_2PO_4^-$ is slightly more available to plants, but effects of pH on P reactions with other soil components are more important than the specific anion present (Brady and Weil, 2017). Shen *et al*. (2011) stated that at a neutral pH (6 to 7) each anion represents 50% of the total P in the soil solution, and an acidic pH value of 4 to 6, represents $H_2PO_4^-$ 100% of P in the soil solution, while a pH value of 8 represents $H_2PO_4^-$ 80% and HPO_4^{2-} 20% of the total P in the soil solution. Soluble, low molecular-weight organic P compounds such as nucleic acid and phytin which are products of soil organic matter decomposition can also be absorbed by plants (Havlin *et al*., 2014). Phosphorus uptake mainly relies on the process of diffusion, because of its immobility in the soil. According to Lambers *et al*. (2006) mass flow only contributes a small portion (1 to 5%) of P supply to roots, while Havlin *et al*. (2014) stated that mass flow can contribute up to 20% of the total P requirement. The concentration of P in the soil solution is usually less than 10 µmole P m^{-3}, in some cases the concentration can even drop to 0.01 µmole P m^{-3}. In most soils the P concentration in the soil solution is roughly 1% of the total P in the soil, therefore P uptake occurs against a massive P concentration due to a higher P concentration of about 10 mmole m^{-3} which is incorporated in the plant tissue. The young tissue near the root tips is the absorbing tissue which is responsible for the active uptake of P by the plant (Blair, 1982).

The three P pools previously mentioned consists of soluble and weakly sorbed P on surfaces (soluble Ca-P and adsorbed P), and this labile P is subject to run-off (Van der Laan *et al*., 2009). A flux of P can occur between the labile and active P pools as well as between the active and stable P pools in soil. The direction and magnitude of these fluxes between the labile, active and stable pools are determined by the Phosphorus Availability Index (PAI). Phosphorus which is strongly adsorbed through the active sites of colloids is not immediately available for plants. This P represents the active P pool, which is followed by the stable P pool. When the labile P in soil is exhausted, some of the non-labile P becomes available for plant uptake; the rate of this process is so slow that most of this fraction can be classified as unavailable for crops. The P in the soil solution can also be replenished by a mechanism whereby the soil solution is recharged through dissolution and desorption reactions of P, causing the P uptake to remain sustainable (Nye, 1969; Godwin and Wilson, 1976; Shapiro and Fried, 1985; Morgan, 1997). Blair *et al*. (1976) proved in a study in Australia that soil with a bicarbonate extractable P value of 35 mg kg^{-1} and higher was non-responsive to P applications, while soils with an extractable P value of less than 35 mg kg^{-1} were responsive to P applications, regardless of parent material and the past history of that specific soil. According to Sharpley *et al*. (1984; 1989) the most accurate estimation of labile P was achieved when soils were divided into calcareous, slightly weathered or highly weathered groups, based on the presence of $CaCO_3$ and the degree of weathering. The following equilibrium equation demonstrates the relationship between these three forms of P in soil (Beaton and Nelson, 2005).

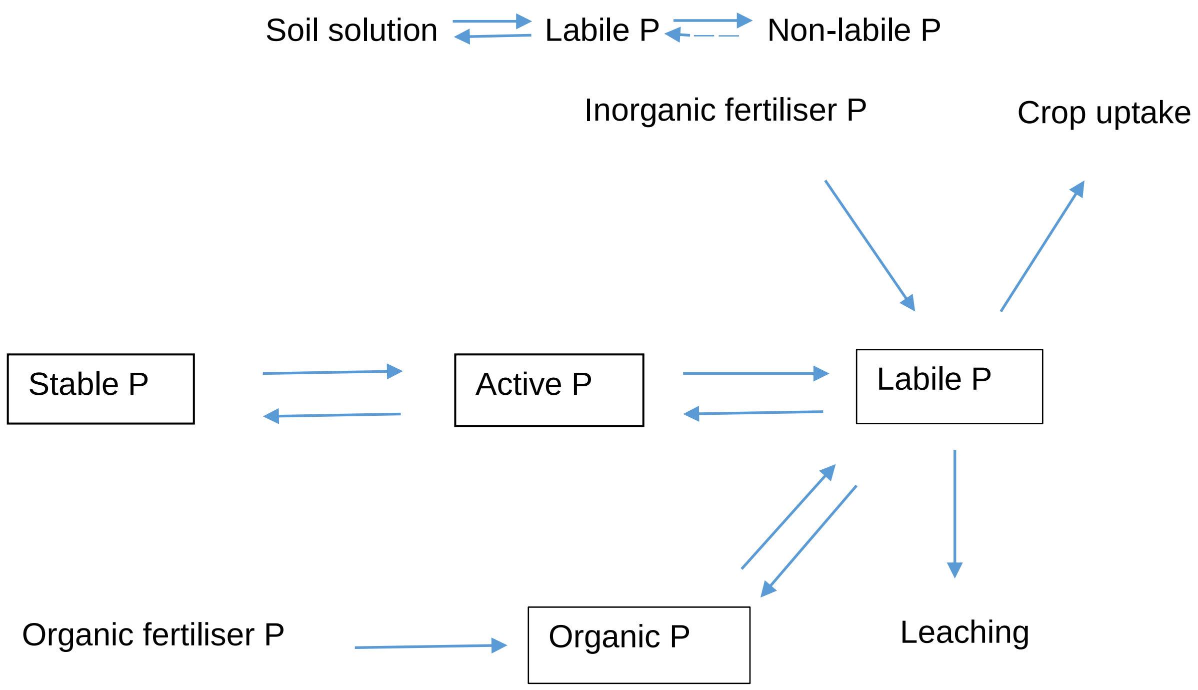


Figure 2.4 Different P forms in the soil (Van der Laan *et al*., 2009).

Plant species differ in P uptake, as indicated in Table 2.2. Maize is an important crop produced in South Africa, since it serves as a food source for humans and animals, an input provider to other sectors and a source of job creation. Therefore it is very important to have sufficient production levels of maize, where P uptake has a positive effect on the yield. Uptake of P by maize amounted to 50 kg ha^{-1} for a yield of 11.3 ton ha^{-1} (Table 2.2).

Table 2.2 The typical uptake of P by crops (Campbell and Edwards, 2001)

Crop	**Yield (ton ha^{-1})**	**P uptake (kg ha^{-1})**
Maize	11.3	50
Soybeans	3.46	26
Grain sorghum	8.4	39
Wheat	9.5	25
Oats	3.6	20
Barley	6.5	32
Tall fescue	13.5	55
Clover	13.5	44
Bermudagrass	18.0	47
Alfalfa	18.0	59

Plant roots have a significant influence on P availability since it induces pH changes in the rhizosphere. The changes in pH are caused by the uptake of cations and anions that are associated with H^+ and OH^- effluxes, respectively. The form of N nutrition plays also an important role in pH changes. When nitrate (NO_3^-) is used for N nutrition, anion uptake exceeds cation uptake and OH^-, or bicarbonate (HCO_3^-), is released from the roots. This caused a more alkaline rhizosphere than in the bulk soil. On the other hand, when ammonium (NH_4^+) is used for N nutrition, it will cause a more acidic rhizosphere. These changes in soil pH will have a great influence on P availability, hence on uptake. An increase in the rhizosphere pH should lead to desorption of soil P and will therefore increase P availability. In alkaline and calcareous soils, where Ca-P is the dominant molecule, a decrease in soluble P will be the result, due to an increase in pH (Mengel and Kirkby, 1987).

2.5 Phosphorus function in plants

The macronutrients, N, P and K are essential for optimal plant growth and reproduction. An adequate supply of these decreases plant stress improves physiological resistance and lowers disease risk of the plant. The P concentration in plant species varies between 0.1 and 0.5% and is therefore considerably lower than N and K (Havlin *et al*., 2014). Phosphate, in comparison with sulphate and nitrate, is not reduced in plants but remains in the highest oxidised form. Phosphorus plays an important role in energy metabolism and in the production of cellular structures of the plants (Wyngaard *et al*., 2016). Moreover P is an important building block for nucleic acids, phospholipids, sugar phosphates, nucleotides and coenzymes. Orthophosphates ($H_2PO_4^-$ and HPO_4^{2-}) which are taken up by the plant, are incorporated in adenosine mono-phosphate (AMP), adenosine di-phosphate (ADP) and adenosine tri-phosphate (ATP). These molecules store energy that is produced during photosynthesis and metabolism of carbohydrates. Enzyme reactions also depend on sufficient P in the soil. An example is the complex processes of photosynthesis where water and carbon dioxide (CO_2) are converted to sugars and starches. When the P ion is split from either ADP or ATP molecules, a large amount of energy (12 000 cal/mol) is released which is available for plant growth and reproductive systems. The ATP transfer energy rich orthophosphate molecules to energy requiring ADP in the plant, a process known as phosphorylation (Havlin *et al*., 2014). The energy rich molecule, nicotinamide adenosine dinucleotide phosphate

(NADP) can be reduced to ATP through the combination of two photoreactions, where light energy is absorbed by the chlorophyll, a large amount of energy is also being released. Phosphorus is also responsible for regulating the partitioning of photosynthates between the sink reproductive organs and the source aerial organs. This effect is important for N-fixing grain legumes (Marschner, 2000). Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) contain the genetic code of the plant and are involved in protein syntheses which are important P-containing molecules (Havlin *et al*., 2014). Root growth and development of plant harvest parts also require a sufficient amount of P in the plants. Sufficient P in forage fruit, vegetable and grain crops lead to better quality. For example wheat will have stronger straw and greater straw strength, particularly the larger grains that graded better with higher tolerance to fungal diseases. Phosphorus also decreases cold damage in grain crops (Young *et al*., 1985).

Phosphorus is important for electron transport in redox reactions as well as in the production and translocation of sugars and starches. Other processes in which P is involved include maturation, seed formation and symbiotic N fixation in legume crops (Prabhu *et al*., 2007). Phosphorus is stored as phytic acid, phytin (Ca or Mg salts) and the hexa-phosphate ester of myo-inositol in seeds (Sanchez, 2007). In a study done by Chrysargyris *et al*. (2016) it was reported that N, P and K contribute to growth and essential oil synthesis in medical plants. Lavender plants specifically benefited in their anti-oxidant status by applying 60 mg L^{-1} of P. Enough P in cereal crops ensure optimum number of tillers, optimum number of panicles and grain yield.

2.5.1 Deficiency symptoms

Phosphorus is probably one of the most difficult nutrients to identify visually as deficient. Vance *et al*. (2003) and Zhang *et al*. (2016) indicated that roughly 40% of the soils all over the world are suffer a P deficiency, especially in high rainfall areas, with acidic weathered soils in tropical and subtropical regions, and because of P fixation. Due to this deficiency of P in soil, one of the first symptoms is restricted early growth in the plant and slow developing plants. In the seed of cereal crops, lupines and pasture legume species, concentrations in surplus of 0.3% P in the seed adequate for early growth stages are found, while concentrations smaller than 0.2% P in the seed is able to reduce crop yields by up to 70% during early growth stages.

Phosphorus is translocated to seeds and fruit. Thus, P deficiencies late in the growing season affect both seed development and crop maturity (Havlin *et al*., 2014).

Since P is mobile in plants and moves from older leaves to younger leaves, this results in deficiency symptoms on the older leaves. Stunted growth, reduced yields, purple or reddish discoloration, especially along the veins, due to the accumulation of sugars which favour anthocyanin (a purple pigment) synthesis and poor root systems are visual symptoms of P deficiencies. Grayish-green to a bluish-green metallic lustre colour is the result of increasing P deficiency. Poor pollination of maize and apples sometimes results in a bronze to purple discoloration of the young leaves and the growing points of the shoots**.** Phosphorus is not a building block or component of chlorophyll, and therefore the chlorophyll is able to increase in the younger leaves, causing the leaves to turn dark green. The deficiency symptoms include chlorosis and necrosis. Insufficient P supply results in slow cell division and the plant becomes dwarfed. Good tillering as well as maturity of crops such as rice is negatively affected (McCauley *et al*., 2009). Seed numbers, their size and viability can be reduced by P deficiency (Ozanne, 1980).

In cool, wet soil conditions P can become deficient in P-sensitive crops with small root systems due to reduced P diffusion. The P deficiency can usually be corrected by increasing soil temperature and expanding root growth (Havlin *et al*., 2014).

2.5.2 Toxicity symptoms

An excess of any nutrient can also cause direct visible symptoms in plants. In most cases macronutrients such as N, P and K are toxic due to the over application of fertilisers or manure, especially when Al is low. A high concentration of P in the plants affects plant growth negatively. Uptake of Fe, Mn and zinc (Zn) are reduced due to toxic levels of P and nutrient deficiency symptoms can occur. Another possibility is that these micronutrients undergo immobilisation by P in the root zone and also within the conducting tissue. Precipitation of these elements by P can also be a result of deficiencies in copper (Cu) and Zn (Wolf, 1999). The deficiency of Zn and Fe under these conditions is the most common. Deficiency of Ca can also occur due to excess available P (McCauley *et al*., 2009).

2.6 Phosphorus fertility management

The total P content in soil consists of organic and inorganic compounds (Havlin *et al*., 2014). These compounds are subject to transformation processes between P pools in soil (section 2.4) and affect the availability of P for plants. Chen *et al*. (2015) stated that nearly 80% of the total P is unavailable for plant uptake, thus causing problems with soil-fertility management. This confirms the necessity for efficient management of this nutrient as well as the importance of understanding the factors that influence the availability of P in soil.

The low availability of P can be explained by the physical adsorption of P to Al- and Fe-oxides/ hydroxides, as well as to Ca and Mg carbonate surfaces. This adsorbed P ultimately precipitates as Al-P (variscite), Fe-P (strengite) in more acidic soils and Ca-P (apatite) in alkaline soils. The inorganic P from fertiliser sources can be immobilised to organic P, which is not available for plant uptake (Holford, 1997). Reserves of P are exhausted worldwide and this could result in a lack of P reserves by the year 2050 (Vance *et al*., 2003; Cordell *et al*., 2011).

According to Havlin *et al*. (2014) several factors are of importance in determining the efficiency of P fertilisers used for crop production. These factors include time, placement, rate and frequency of fertiliser P application. After application, P undergoes several chemical reactions that reduce its solubility and hence availability in soil (Lowell *et al*., 2009). The best application time for P is just before planting or at planting. The use of starter P fertilisers is known to promote early growth and development, therefore P restrictions in the early growing season are more vital for plant growth than P restrictions which occur later during the crop season (Bundy *et al*., 2005). According to Klatt *et al*. (2003), P is immobile in soil and moves mainly by diffusion, which is very slow (Lambers *et al*., 2006).

Proper P fertiliser application can improve crop recovery of this nutrient. This can be achieved when P is placed sufficiently close to the root surface. Thus, for effective plant nutrition, P fertilisers must be band-placed to concentrate P in a small soil volume that saturates the P binding sites and lowers the fixation buffering capacity of the soil. The result is a higher mobility of P ions in the soil, with diffusion towards the roots becoming more efficient. Band-placement reduces the contact between the fertiliser source and the soil, which results in less fixation. Phosphorus will thus be in an available form for a longer period (Havlin *et al*., 2014). Band-placement is especially important under dry land conditions in South Africa (Schmidt *et al*., 2004). This management practice ensures a better fertiliser-root contact early in the season. Grant *et al*. (2001) stated that a high efficiency was obtained by the band placement of the fertiliser below the seed-row to avoid damage to seedlings, rather than placing the fertiliser in the seed-row, especially when MAP or di-ammonium phosphate (DAP) is utilised. Both fertilisers improved root proliferation due to the synergetic effect of N and P (Balemi and Negisho, 2012). Band-placement and broadcast application methods of these P sources may result in different optimum extractable soil P values for the same grain yield. Ammonia volatilization from either MAP or DAP in water-deficit conditions is also lower with deep application of P in the soil (Klatt *et al*., 2003). A larger granule size or band placement of MAP and DAP decreases soil-fertiliser contact and the time of P in the soil solution will last longer compared with a smaller granule size or broadcast of these fertilisers. Soil water is also important for P uptake by plants. At 50 to 80% of field capacity, soluble P diffuses from the fertiliser within 24 hours, resulting in better P uptake by plants and hence higher yields since interference of cations such as Al, Fe, Ca and Mg decreased. Residual P availability has a heightened influence in acid soils than in basic soils. The availability of residual P availability was lower in weathered, acid soils than in slightly weathered and calcareous soils (Havlin *et al*., 2014). In a wide range of soils less than 50% of fertiliser P was plant available after 6 months.

In high pH soils there is no economic benefit to be gained by reducing the soil pH. According to Hopkins and Ellsworth (2005), there are various P management strategies to improve P fertility in alkaline and calcareous soils. The following strategies may improve P nutrition in plants:

- high rates of P applications
- phosphorus fertiliser bands which are concentrated
- complexed P fertilisers
- phosphorus fertilisers with a slow release
- cation complex P fertiliser
- application of P fertilisers in the season
- good balance of P in relation to the other nutrients.

Frequent applications may be very efficient on high P fixation soils, but is not always economically attainable or environmentally friendly. Organic materials such as farmyard manure along with P fertilisers can improve the efficiency of applied fertilisers through microbial activities on calcareous soils. Excretion of organic anions and carbonic acid (H_2CO_3) formed due to respiration of microbes. Organic materials assure a slow release of P and other nutrients for plants (Hopkins and Ellsworth, 2005). The uptake of N in the form of NH_4^+ will induce the release of the hydrogen cation (H^+) by plant roots, resulting in acidification of the rhizosphere. The organic anions and H_2CO_3 will also play a role in desorption of P ions that are fixed to Ca and Mg carbonates (Balemi and Negisho, 2012). In calcareous soils the performance of liquid fertilisers is better than that of granular fertilisers.
Rock phosphate can be used to saturate Al and Fe ions in strongly weathered acidic soils to maintain P availability. Dissolution of rock phosphate, fineness of grinding and soil pH plays an enormous role in its efficiency (Yusdar *et al*., 2007). Rock phosphate will react most effectively when it is broadcast on a field and incorporated into soil. The dissolution processes will be intensified when contact is enhanced with soil H^+ (Bolland and Gilkes, 1990). It is not always economically viable to add fertilisers such as rock phosphate, and the practice is also not environmentally friendly, because an excess of P can cause eutrophication and ammonia volatilization. Alternatively, green wastes and animal manure can be applied, which may increase soil fertility in acid soils. Soil-chemical properties causing P fixation can be improved by biochar and compost. The fixation of P in acid soils can also be reduced by this practice since biochar and compost have a high affinity for Fe and Al. Moreover the addition of biochar increases soil pH. Other additional advantages of this practice include a higher porosity and specific surface with more surface functional groups which can adsorb Fe and Al and will decrease the potential of P fixation. Therefore P and basic cations will become more available for plant uptake (Ch'ng, 2014).

Sustainable crop production with yields under P-deficient conditions can be maintained by planting cultivars that are more efficient in uptake. These cultivars make use of various adaption mechanisms to gain sufficient nutrients. The mechanisms include altered root morphology, synergism association of roots with mycorrhizal bacteria and exudation of chemical compounds, that enable plants to exploit a greater soil volume (Balemi and Negisho, 2012). Through the larger root system, with longer root hairs, the plant is able to take up more P. These cultivars are also able to mineralise organic P sources by freeing acid phosphates, phytases or ribonuclease (RNase). Gahoonia and Nielsen (2004) found that barley genotypes that are able to form root hairs of 1 mm length, took up more P than barley genotypes with hairs half the length. These researchers reported that root hairs of plants can contribute to about 63% of the P uptake by plants under P deficient conditions.

Conservation tillage, crop residue management, buffer strips, terracing, contour tillage, riparian strips, cover crops and small reservoirs can reduce P loss via erosion and runoff (Daniel *et al*., 1998). In comparison with conventional tillage, conservation tillage may reduce erosion through runoff, but, conversely conservation tillage is able to enhance nutrient losses via leaching. Efficient irrigation practices, especially furrow irrigation, may decrease the P losses by minimising runoff and erosion (Sharpley and Withers, 1994).

The interaction between N and P are beneficial for P absorption by plants. Nitrogen stimulates P uptake by increasing top and root growth, altering plant metabolism and increasing P solubility and availability. Larger P uptake by crops is dependent on increased root mass. Nitrogen fertilisers containing NH_4^+ are more effective in stimulating the process of P uptake than those containing NO_3^-, for example the yield of winter wheat increased from 0.35 to 0.4 t ha^{-1} by concurrently injecting anhydrous NH_3 and ammonium polyphosphates into the soil (Havlin *et al*., 2014).

Recent studies on the response of P forms in soil to fertilisation P rates have shown that the application of P fertiliser at higher rates increased the sodium bicarbonate ($NaHCO_3$) extractable labile fraction of P significantly. The labile organic P content increased two-fold, while the labile inorganic fraction increased five-fold. Phosphorus fertilisation had no significant effect on microbial biomass. However immobilisation was favoured at significantly high P fertiliser rates (Picone *et al*., 2003).

2.6.1 Phosphorus requirements and corrections

Phosphorus availability to plants is determined by many interacting factors such as the environment which includes water, temperature and soil management practices

(Havlin *et al*., 2014). The P need to increase crop yield further depends on the P requirement of the crop and on the P supplying capacity of the soil. In South Africa diagnostic and fertiliser recommendations systems for P nutrition of crops rely heavily on the use of different P extraction procedures. The common approach to correcting P deficiencies include the following: determine test value on specific soil from the known optimum level of the specific crop; establish the deficit in terms of the test value and calculate mass of nutrient needed per unit area. Thus the P (kg ha^{-1}) required is equal to: (Optimum soil P – measured soil P) x PRF. The PRF must bring into account the depth of fertiliser incorporation as well as different sorption effects related to the specific soil (Johnston *et al*., 1991). The PRF value is a P test characteristic which is important in calculating the P required per ha to raise the level of P test by one unit and, then allowing for P fixation with respect to the recovery of added P by the test. This PRF value differs between soils because each soil has its own characteristics. It is essential to calculate a PRF for each soil to prevent over or under fertilisation. As discussed earlier, the calculation to correct soil is challenging because soils differ in their PRF.

2.6.2 Phosphorus fertilisation

Commercial mining of P deposits began in the mid 1800s with only 5 000 tons per annum. It increased globally to more than 100 Mt in the 1970s. In 2000 the world production of rock phosphate was 133 Mt. The United States of America (28%) is currently the largest producer of rock phosphate, followed by China (21%), Morocco and Western Sahara (15%), Russia (8%) and Tunisia (6%). Up to 80% of the mined rock phosphate globally is used for the manufacturing of fertiliser, 12% for detergents and 5% for animal food production and speciality applications (Stewart *et al*., 2005).

Phosphorus sources for plant production include rock phosphate products, inorganic manufactured P fertilisers and organic P fertilisers such as sewage sludge, crop residues, manure and composts. Only a fraction of the total P content of fertilisers is plant available, namely, P that is soluble in 2% citric acid ($C_6H_8O_7$). A wide range of inorganic P fertilisers that vary in solubility are commercially available (Havlin *et al*., 2014). For crops with short growth cycles that require a quick start, applied P should be readily available for plant uptake. In this instance, water soluble P fertilisers that are band placed next to the seeds are the most suitable. However, salt damage to seeds and seedlings must be avoided. Fertilisers with citric-soluble P can be considered for slow-growing crops before planting (Havlin *et al*., 2014; International Atomic Energy Agency [IAEA], 2016).

Holford (1997) and Syers *et al*. (2008) stated that only 10 to 30% of the applied P fertiliser can be absorbed by the plant, due to complex physicochemical adsorption, precipitation and the conversion of P to unavailable organic forms. As a result, only a small proportion of the P ions in the soil solution are available for plant uptake. This explained why P fertilisation alone is not cost effective regarding the increase crop production in the most P limiting soils (Tilman *et al*., 2002). The appropriate amount and source of P fertiliser for a crop, combined with the most effective management strategy are important for sustainable crop production. Soil and crop scientists must develop fertilisation strategies that enhance P acquisition by plants. The focus of these strategies must be the improvement of P utilization by plants for the sustainable management of P in agriculture (Balemi and Negisho 2012)**.**

In the tropical and subtropical regions, where most of the world’s population is concentrated, there is a scarcity of chemical fertilisers. In addition to the scarcity of P, most of the P in fertilisers is rapidly transformed into organic and inorganic forms, which are limited in terms of plant availability. In rural regions constraints are encountered regarding accessibility of fertiliser P, due to lack of fertiliser infrastructures, poor transportation facilities and financial systems (Balemi and Negisho 2012).

2.6.3 Phosphorus sources

Ammonium phosphates, which are commercially available as MAP and DAP, were known from the 1900s, but became very popular from the 1960s (Leikam and Achorn, 2005). A wet process is used to produce ammonium phosphates by reacting H_3PO_4 with NH_3 (Havlin *et al*., 2014). Both MAP (11:22:0, citric acid soluble P and water soluble) and DAP (18:20:0, citric acid soluble P and water soluble) are also excellent N sources. The ammonium they contain will gradually be converted to nitrate by soil bacteria, resulting in a subsequent drop in pH. However, DAP due to its alkaline nature will temporarily increase soil pH near the fertiliser granules. This rise in soil can influence the micro-site reactions of the applied P. On the other hand MAP is by nature acidic (Kamel *et al*., 2010). Both MAP and DAP are highly water soluble and dissolve easily to release NH_4^+ and either $H_2PO_4^-$ or HPO_4^{2-}. The N bearing cation, NH_4^+ has a positive effect on P absorption by the roots of the different crops. The two fertilisers can be used for direct application as starting fertilisers, but the application of DAP in the row or near the seed can cause seedling injury and prevent root growth through the production of ammonia (NH_3). This problem is more common in calcareous or alkaline soils (Havlin *et al*., 2014).

The reaction between pyrophosphoric acid ($H_4P_2O_7$) and NH_3 produces **ammonium polyphosphate**. Polyphosphates usually combine to some extent with orthophosphates, through the loss of one water molecule per two $H_2PO_4^-$ molecules. A property of ammonium polyphosphates is that it can bind with metal cations, which retains higher possible concentrations of micronutrients with $H_2PO_4^-$ solution. For example the concentration of Zn can be maintained at 2% in a $H_4P_2O_7$ solution compared to 0.05% in a $H_2PO_4^-$ solution (Havlin *et al*., 2014). Polyphosphate fertilisers which are added to soil for P nutrition of crops also undergo adsorption and precipitation reactions. This is because the orthophosphates available in these fertilisers and those which form via hydrolyses of polyphosphates react with soil components. Hydrolysis of polyphosphates result in different short chain polyphosphate fragments, which are subject to further hydrolysis. The most important factor that controls the rate of hydrolysis is enzymatic activity. Soil pH, water, temperature and organic carbon are factors that inter alia effect the transformation of polyphosphates in soil (Shreeja, 2016).

Phosphoric acid contains 17 to 24% P. This acid (H_3PO_4) is produced by the reaction between rock phosphate and sulphuric acid (H_2SO_4). Gypsum is a by-product and can be used as a fertiliser of Ca and S, for industrial purposes, and as an amendment for sodic soils. Phosphoric acid is beneficial to acidulate rock phosphate for the manufacture of Ca and NH_4 phosphates. Moreover H_3PO_4 is also beneficial as an additive either to irrigation water or soils which are alkaline (Havlin *et al*., 2014).

Single superphosphate (SSP) and **triple superphosphate** (TSP) are important calcium P fertilisers. SSP is derived from rock phosphate when reacting with H_2SO_4 and is commonly recommended for correcting soil P deficiencies. The treating rock phosphate with H_3PO_4 results in TSP (Havlin *et al*., 2014). The latter qualifies for the most highly concentrated commercial P source. The P quality in SSP is 10.5% water soluble P, and 11% citric acid soluble P, while the P quality in TSP is 19.6% water soluble P and 20.4% citric acid soluble P. Ordinary superphosphate or SSP supplied over 60% of the world's P in 1955 (Leikam and Achorn, 2005). Neither SSP nor TSP has any appreciable effect on soil pH (Havlin *et al*., 2014).

Gilliam *et al*. (1985) found that superphosphate, when applied at 600 to 2000 kg P ha^{-1} can be leached to a depth of 2 m in a sandy soil, with maximum build-up in the 150 to 450 mm zone. In this soil, superphosphate can move to 4 m depth when applied at 13 000 kg P ha^{-1}, and only 22% was retained in the upper 150 mm layer.

Rock phosphate is a P-bearing mineral found in geological deposits all over the world, with the exception of Antarctica. Major rock phosphate deposits are found in the United States of America, Morocco, China and Russia which provide almost 70% of the total world production (Havlin *et al*., 2014). Mined rock phosphate serves as the raw material in manufactured P products. The manufactured products also contain, in addition to P, also uranium, thorium, potassium, cadmium, zinc, iron, manganese radionuclides and other trace materials. The most reactive rock phosphate is that which contains the mineral francolite, a carbonate-fluorapatite (CO_3-F-apatite). In developing countries, the use of rock phosphate is becoming increasingly important, particularly on acid soils. Rock phosphate is very effective for plantation crops such as rubber, oil palm and cacao on acidic soils (Havlin *et al*., 2014). The use of rock phosphate on high pH soils and for crops with short growth periods is not recommended. Direct applications of rock phosphate will not always deliver beneficial results, since the agronomic effectiveness of this fertiliser is affected by a large number of factors such as the rate of rock phosphate dissolution in a given soil. Other factors that influenced the agronomic effectiveness of rock phosphate are climate conditions, plant characteristics and management strategies (Bolan *et al*., 1990; Rajan *et al*., 1996; Rajan *et al*., 2004).

The effectiveness of rock phosphate is, for example, better in moist soils with warm climates and long growing seasons. Applications of 0.2 $t.ha^{-1}$ that are repeated every 5 to 10 years can restore low P levels of soils (Havlin *et al*., 2014).

Davies (1984) found that the use of Gafsa rock phosphate in England and Wales was only 50% as effective as control SSP in the first year after application. However, Gafsa rock phosphate compared well with SSP in the next two years on unlimed soil. In New Zealand, Cullen (1958) and Kavlovsky (1958) showed that Gafsa rock phosphate was comparable with SSP with regard to the absence of lime at a number of sites. These results differed from results obtained in other New Zealand trials where Gafsa rock phosphate was applied (Elliot and Lynch, 1942; Grigg and Crouchley, 1980). Different organic and inorganic P sources as well as their different P fraction in the source are indicated in Table 2.3.

Table 2.3 Chemical composition of rock phosphate, commercial fertilisers and organic fertilisers used as phosphorus sources for crop production (Pierzynski *et al*., 2000; FERTASA, 2016)

P source and chemical composition		**P (%)**	**Other nutrients**
Rock phosphates	$Ca_{10}F_2 (PO_4)_6 . XCaCO_3$ (varies between mineral deposits)	14-17	Major impurities: Al, Fe, Si, F, CO_3^{2-}
Commercial fertilisers			
SSP	$Ca(H_2PO_4)_2 + CaSO_4$	7-10	Ca, S (8-10%)
TSP	$Ca(H_2PO_4)_2$	19-23	Ca
MAP	$NH_4H_2PO_4$	22	N (11%)
DAP	$(NH_4)_2HPO_4$	20	N (18%)
Ammonium polyphosphates (liquids)	$(NH_4)_3HP_2O_7$	15	N (11%)
Organic P sources			
Cattle manure		0.9	N, P, K, S, Ca, Mg and other trace elements
Dairy manure		0.6	
Poultry manure		1.8	
Swine manure		1.5	
Composted sludge		1.3	

Soils react positively to application of reactive rock phosphate when they have inter alia a low pH, little plant available P, high Cation Exchange Capacity (CEC) and low exchangeable Ca (Randhawa *et al*., 2006). It is therefore understandable that Bolan and Hedley (1998) found that reactive rock phosphate can be as effective as soluble P fertilisers on pastures since most soils had a pH lower than 6, and received more than 800 mm rain annually. These findings emerged during several trials conducted in New Zealand. Long term studies in developed countries also proved that residual soil P values from rock phosphate can exceed those from soluble P fertilisers (Rajan *et al*., 1996; Hu *et al*., 2009). De Sousa *et al*. (2015) supported this observation and proved that rock phosphate efficiency increased over time due to a steady supply of P.

In summary, most agriculture systems are dependent on the supply of P from commercial fertilisers, which derive from rock phosphates. Rock phosphate reserves are a non-renewable resource and the estimation is that it will be depleted by the end of this century. Therefore sufficient maintenance of soil P is vital.

2.7 Extraction methods of phosphorus

Different extraction methods were developed to determine the P status in soils (Haygarth *et al*., 1999). This is necessary for the identification of a need of P fertilisation (Wuenscher *et al*., 2015). With soil tests, economic returns can be predicted, and the economic benefits of investment in P fertilisers may be determined. As mentioned above, several P tests are used to establish the plant availability of P in soils. Soil tests do not reflect P losses in the soil, although they determine the risk of P losses (Wang *et al*., 2015). No single chemical extraction method for P that is suitable and workable for all soils and fertilisers exists at present.

Wuenscher *et al*. (2015) indicated that the different extraction methods extract different pools of soil P, resulting in different values. The extractability of a certain pool may be influenced by various soil properties at different degrees. Further examination is required to investigate if and how these relationships translate to plant P uptake (Wuenscher *et al*., 2015).

Particle size distribution, mineralogical composition, total surface area, organic matter, pH, Fe/Al-oxides and Ca/Mg carbonates are soil properties that influence P extractability in soils as well as the effectiveness of extraction methods (Nafiu, 2006). Various forms of P in soil can be estimated, depending on the different extraction method used (Schmidt *et al*., 2004). Extractants in different methods, which are commonly in use over the world, are indicated in Table 2.4. The solubility of P, and therefore its extractability, are dependent on a large combination of factors, including the properties of soils and the chemical composition of extractants.

During the extraction of P from soil, the Al, Fe and Ca in the soil solution is reduced through complexation or precipitation. This results in the dissolution of Al-P, Fe-P and Ca-P and the release of P in the soil solution. Thus the P content in the soil solution increases during extraction (Schmidt *et al*., 2004).

Wuenscher *et al*. (2015) found in a study done in Austria and Germany that the P extracted with methods such as calcium chloride ($CaCl_2$), Olsen, Bray II and Mehlich 3 correlated negatively with the pH and carbonate content of soils. This observation seems reasonable, because $CaCO_3$ can bind with readily available P and thereby fix P in the Ca-P form.

Soil texture has a strong influence on the P fractions in soils (Zheng and Zhang, 2012). In contradiction Fernandes *et al*. (1999) stated that P which was extracted by Olsen, Fe-oxide Pi and CAEM was less dependent on soil texture. However, Wuenscher *et al*. (2015) found that soil texture has a great influence on P extractability since the P extracted with $CaCl_2$, LiCl, Olsen, Bray II, Mehlich 3, CAL and Fe-oxide Pi had a negative correlation with clay and/or silt content and a positive correlation with sand content. According to Kumar *et al*. (1994) the composition of P fertilisers also played a major role in P extractability in addition to extraction methods and soil properties.

Jalali (2016a;b) showed in a study that the P extracted with $CaCl_2$, Citrate, HCl, Olsen and Mehlich 3 was higher in soils with sandy, sandy clay loam and sandy loam textures than in soils with clay and mixed loam textures. Wang *et al*. (2015) reported that if the P content of a soil in Ontario is 30 mg P kg^{-1} soil according to the Olsen method, there is no need for P fertilisation. Moreover, this study also proved that the Bray 1 and Mehlich 3 methods performed best in soils with a low pH and the Olsen method best in soils high in pH, and calcareous soils.

Schmidt *et al*. (2004) compared analysis results of the Bray 1 and Ambic extractions methods using linear regression. Soil samples from maize fertilisation trials carried out at Heidelberg, Lichtenburg, Wolmaransstad, Koppies, Ventersdorp, Viljoenskroon, Stilfontein, Athole, Belfast, Vrede and Dirkiesdorp were analysed. A wide range of extractable P was found with either the Bray 1 or Ambic extractions for the soils ranging in clay content from 8.4 to 47%. Significant linear relationship with a R^2- value of 0.91 was obtained between soil P test values of Bray 1 and Ambic. Buys and Venter (1980) studied soil P test values of Bray 1 and Bray 2 and observed a greater correlation for acid soils than for soils with a high pH as well as for soils which were treated with rock phosphate. These values were measured in soil samples from maize fertilisation trials done by the Fertiliser Society of South Africa. A R^2- value of 0.95 was obtained for 36 South African soils where the relationship between Bray 1 and ISFEI was tested (Buys and Venter, 1980). Phosphorus requirement factors of 5, 7 and 9 kg P ha^{-1} for soils were established by Schmidt *et al*. (2004) with clay contents of 1 to 10%, 1 to 20% and 21 to 35%, respectively. These amounts of P must be applied when determined to increase soil P by 1 mg P kg^{-1} when determined with the Bray 1 method.

Dryland maize is commonly produced on the Highveld of South Africa. Soil samples from nine P fertilisation trials were analysed with the Ambic 1 and Bray 1 methods for extractable P. The trials were subjected to control traffic and therefore band placement of P. Most of the variation in extractable P was explained by related soil properties such as degree of leaching and silt-and-clay content. The relationship between silt-and-clay content and extractable P only was explored. The extractable P concentration according to Bray 1 for the top 150 mm soil layer to obtain 90% relative yield varied from 33.5 mg kg^{-1} at 13% silt-and-clay to 14.6 mg kg^{-1} at 60% silt-and-clay. Fertiliser P which is needed to obtain 90% relative yield was much higher on sandy soils than on clayey soils (Schmidt *et al*., 2004).

The ability of four P extraction methods (Ambic, Bray 1, Mehlich 3 and a modified Truog) to extract P from a sandy loam Avalon soil type and from a Balmoral soil clay type was tested in Langebaan, Western Cape. Both soils were fertilised with either superphosphate or langfos for three levels of acidity. Regardless of soil type, application of superphosphate resulted in larger amounts of extractable P than langfos for all the extractants except, Truog. According the study of Thibaud *et al*. (1994), the efficiency of the four extractants when Langfos was used for both soil types followed the order Truog> Mehlich 3> Bray 1> Ambic. However, the order for superphosphate on the Avalon soil type was Bray 1> Mehlich 3> Truog> Ambic.

Table 2.4 Different extraction methods and their extraction composition (Van der Merwe *et al*., 1984; Fixen and Grove, 1990, Fageria *et al*., 1997)

Extractant name	Soil/reagent ratio	Extraction composition
AB-DTPA		1 M NH_4HCO_3 + 0.005 M DTPA – pH 7.5
Bray 1	1:10	0.03 M NH_4F + 0.025 M HCl
Bray 2	1:17	0.03 M NH_4F + 0.1 M HCl
Citric acid	1:10	1 % citric acid
Egner	1:20	0.01 M Ca lactate + 0.02 M HCl
ISFEI		0.25 M $NaHCO_3$ + 0.01 M NH_4F + 0.01 M EDTA – pH 8.5
Mehlich 1	1:4	0.05 M HCl + 0.0125 M H_2SO_4
Mehlich 2	1:10	0.015 M NH_4F + 0.2 M CH_3COOH + 0.2 M NH_4Cl +0.012 M HCl
Mehlich 3	1:10	0.015 M NH_4 + 0.2 M CH_3COOH + 0.25 M NH_4NO_3 + 0.013 M HNO_3 + 0.001 M EDTA
Morgan	1:10	0.54 M CH_3COOH + 0.7 M $NaC_2H_3O_2$ – pH 4.8
Olsen	1:20	0.5 M $NaHCO_3$ – pH 8.5
Truog	1:100	0.001 M H_2SO_4 + $(NH_4)_2SO_4$ – pH 3
Ambic 1		(0.25 mol L^{-1} NH_4HC0_3 + 0.01 mol L^{-1} $(NH_4)_2$ EDTA + 0.01 mol L^{-1} NH_4F + 0.05 g L^{-1} Superfloc N100
Egner	1:20	0.02N Ca-lactate + 0.02NHCl

The extractants given in Table 2.4 are commonly employed to extract soil P over a huge range of climate and soil conditions (Fageria *et al*., 1997). Diluted solutions of strong acids like HCl, HNO_3 and H_2SO_4 are used as extractants in the Bray and Mehlich methods. All P soil tests are not reliable for the different soil types in South Africa since different extractants may dissolve non-labile P that is tightly bound to Al, Fe and Ca complexes at varying degrees (Myers *et al*., 2005). Each extraction method has therefore its own optimum soil P value indicating the best P availability for plants. The best extraction method for a specific set of conditions is normally the one which gives the best correlation between the soil P extracted and the measured crop growth. A reliable reference method for P determination in South African soils is a modification of the ISFEI extractant renamed to the Ambic extractant (Van der Merwe, 1984). The six most common extraction methods employed in South Africa are: Olsen, Bray 1, Bray 2, Mehlich 1, Truog (Schmidt *et al*., 2004) and Ambic 1 (Van der Laan *et al*., 2009). These methods are briefly discussed below, as well as the citric acid extraction method, which is used in the Western Cape.

2.7.1 Olsen

The Olsen soil test, also called the sodium bicarbonate soil test for P is based on an extraction with bicarbonate. This 0.5 M $NaHCO_3$ solution with a pH of 8.5 is very effective for agronomic purposes where soils are neutral or calcareous and in soils which are moderately weathered. This method may also be useful in some acid soils, although more research is warranted. However, the Olsen method is based on the principle that at pH 8.5 anions like OH^-, HCO_3^- and CO_3^{2-} reduced $Al^{3+,}$ Fe^{3+} and Ca^{2+} in solution resulting in a P increase (Do Carmo Horta and Torrent, 2007). An Olsen P value of less than 15 mg kg^{-1} is regarded as too low for optimum crop growth. The Olsen method is more useful as an agronomic P test than as an environmental P test in acid soils. According to Dodd *et al*. (2012) the Olsen method does not predict the potential P loss in soil. The Free State, Department of Agriculture and The University of the Free State are major institutions in South Africa employing the Olsen extraction method, especially on the irrigated soils (Van der Laan *et al.,* 2009).

2.7.2 Bray

The Bray 1 and Bray 2 methods make use of a diluted strong acid for P extraction (Sims, 1998). In South Africa the Bray 1 soil P extraction method is a very common tool in the fertiliser industry used to make fertiliser recommendations (Schmidt *et al*., 2004). Sometimes this industry also makes use of the Bray 2 method (Van der Laan *et al.,* 2009). In most acid and neutral soils the extracted P of Bray 1 is well correlated with crop growth. The Bray 1 extraction method is well-correlated in most acid and neutral soils. This extractant, which consists of 0.03 M NH_4F and 0.025 M HCl, is the main force behind the solubilisation of Al-P, Fe-P and Ca-P in the protonation of PO_4^{-3}, complexation of fluoride with Al^{3+} and Fe^{3+} and the precipitation of CaF_2 (Olsen and Sommers, 1982). Fluoride therefore decreases the activity of Al, Fe and Ca in the soil (Schmidt *et al*., 2004). The concentration of HCl in the Bray 2 extractant has been raised to 0.1 M to give an increased capacity for the extraction of the less soluble Ca-P (Fageria *et al*., 1997).

Values of 25 to 30 mg P kg^{-1} are considered as optimum for plant growth when the Bray extraction methods are used for soil tests. The Bray extraction method can’t be used for soils with high clay content as well as a high degree of base saturation. This also applies to calcareous soils with a pH of more than 6.8, which have a fine texture, soils of which the base saturation has more than 7% $CaCO_3$, and soils that contain more than 2% lime (Pierzynski, 2000).

2.7.3 Mehlich

This method is also known as the dilute double acid or North Carolina extractant. Mehlich and his co-workers developed the extraction method in the early 1950s. Several essential plant nutrients such as P, K, Ca, Mg, Cu, Fe, Mn and Zn can be extracted from soil through the method of Mehlich. In soils with a pH less than 6.5 the Mehlich 1 method is very suitable since it extracts P bound to Al, Fe and Ca. Moreover the method is also very suitable for soils with low CEC (<10 $_{c}$mol kg^{-1}) and organic matter (<5%) values (Pierzynski, 2000). The method is, however, not recommended for use on alkaline and calcareous soils. This is because the method extracts a large amount of non-labile P in soils with a pH of more than 6.5, soils which were fertilised with rock phosphate, soils with a high CEC and a high base saturation. With the Mehlich 1 method an amount of 20 to 25 mg P kg^{-1} soil is the optimum content for plant growth (Pierzynski, 2000). The Mehlich 3 method contains weaker acids than either the Mehlich 1 or Bray methods and therefore can be used for soils with a wider range of pH values (Latrou *et al*., 2014). The strong binding of P in soil usually leads to a slow decline in concentration. For example McCollum (1991) reported that it can took more than 17 years for Mehlich 1 extractable P in a fine sandy loam soil in the United States to decline from 99 g P m^{-3} to the agronomic optimum of 20 to 25 g P m^{-3}.

2.7.4 Truog

This is a dilute acid extraction method consisting with of a 0.004 M H_2SO_4 solution. The reaction of H^+, which increases the solubility of the different forms of P is responsible for the P extraction (Kamprath and Watson, 1980). The Fertiliser Advisory Service (FAS) at the South African Sugarcane Research Institute (SASRI) make use of the Truog P extraction method. The main form of P which is extracted by this method is Ca-P, while Al-P and Fe-P are extracted to a lesser extent. This method is very effective on acidic soils, especially in soils with a pH ($CaCl_2$) lower than 5. A disadvantage of the Truog method is that it over estimates the P availability on neutral to alkaline soils. As a result of this, the method does not reflect a deficiency on leaf samples and insufficient amounts of fertiliser that are applied. Factors like soil pH, Ca content of soil, and empathy of sesquioxides for P in soil determines the efficiency of this extraction method (Miles *et al*., 2013). In Kwazulu-Natal the Truog method is a very popular P extraction method, due to low soil pH values (Van der Laan *et al*., 2009).

2.7.5 Ambic 1

Institutions in South Africa like the Agricultural Research Council (ARC) Grain Crops Institute and the North West Department of Agriculture use the Ambic 1 extraction method for P calibration research (Schmidt *et al*., 2004; Van der Laan *et al*., 2009). This method was developed by Van der Merwe *et al*. (1984) who modified the ISFEI extraction method of Hunter (1975). For the modification $NaHCO_3$ and sodium ethylenediaminetetraacetic acid (Na-EDTA) were replaced by ammonium bicarbonate (NH_4HCO_3) and ammonium ethylenediaminetetraacetic acid (NH_4-EDTA) and the pH of the extractant is adjusted, using NH_4OH and not NaOH. Similar forms and amounts of P should therefore be extracted by these two different P extraction methods (Farina and Channon, 1979). Due to high pH values (pH 8.3) which is created by NH_4HCO_3 in the extractant, protonation of PO_4^{3-} will be less. The main solubilizing factor in this instance is the complexation of Al^{3+}, Fe^{3+} and Ca^{2+} by EDTA (Thomas and Peaslee, 1973; Olsen and Sommers, 1982; Hahne *et al*., 1988). This method is suitable for the determination of P on a wide range of soils, which differs in pH and texture. Through this method the concentration of K, Ca, Mg, Cu, Zn, Fe and Mn can also be determined (Van der Merwe *et al*., 1984).

2.7.6 Citric acid

In the Western Cape, the citric acid method is the most common extraction method for soil P (Van der Laan *et al*., 2009). The weak acid in the extractant solubilises the insoluble P in soil. Moreover the organic citrate anion forms complexes with metal cations on which P is adsorbed. Thus it competes with P for adsorption sites on the soil surface, which increase the release of P into the soil solution by substituting adsorbed P and also decreasing its re-adsorption (Kamprath and Watson, 1980).

Soil tests such as Bray 1, Mehlich 1 and Mehlich 3 are the most effective means to extract P from non-calcareous soils, and the Olsen test is effective in calcareous soils. A low R^2 value of 0.35 for the Bray 1 method in the study of Van der Laan *et al.* (2009) in calcareous soils proved the non-effectiveness of the Bray 1 method in such soils. An R^2 value of 0.90 was found with the Olsen method in these calcareous soils (Van der Laan *et al*., 2009).

2.8 Conclusion

The contribution of P to sustainable agricultural crop production is very important worldwide. It is challenging, however that the P concentration which is available for plant uptake is very low, due to the high level of P fixation in different soils. By understanding the chemical reactions that P can undergo in soil, plant deficiencies can be prevented and necessary management strategies may be introduced.

Various strategies exist to increase P availability, all of them are not economically or environmentally friendly. By applying P sources, the P in the soil solution can increase as well as the plant uptake. Different P sources exist on the market, which react differently in calcareous/alkaline and acid soils. Some contribute more to the soil P solution and others are fixed quickly, depending on the soil content.

The P status of the soil and the optimum P level required for crop production are determined through different extraction methods. None of them are suitable for all the different soil conditions. The most widely spread extraction method used in South Africa for alkaline and calcareous soils is the Olsen method. However, this method is also used on acidic soils although to a lesser extent.

CHAPTER 3
MATERIALS AND METHODS

3.1 Study area

The soils for this study were collected in sub area four of the Upper Orange Water Management Area (UOWMA). Sub area four occurs under the Vanderkloof dam and lies predominantly within the Free State, but also occupies portions of the Eastern and Northern Cape provinces. The main towns that rely on the water resources for domestic use are Colesberg, Hopetown, Vanderkloof, Orania, Koffiefontein, Jacobsdal and Luckhoff. However, most of the water is used for irrigation. The main crops produced are maize, wheat, lucern, pecan nuts, soyabeans and some vegetables (Department of Water Affairs, 2009). Moderate to deep soils varying in texture from sandy to clayey occur in the UOWMA. These soils support the following vegetation types under prevailing climate; pure grassveld, false karoo, false bushveld, karoo and karroid (Acocks, 1988).

The climate in the area is cool to temperate and ranges from semi-arid to arid (Basson and Rossouw, 2003). In the study area the mean annual maximum temperature varies around 25 °C, and the mean annual minimum temperature around 9 °C. However, the average annual temperature for the six sampling sites varies from 16.02 to 17.55 °C, with the highest annual mean rainfall of 377.52 mm at site 5 and lowest of 318.24 mm at site 1 (Table 3.1). The rainfall occurs mainly as summer thunder showers. Due to high temperatures the result is a high evapotranspiration rate, which means that sustainable crop production in this low rainfall area is only possible under irrigation, which creates a micro-climate.

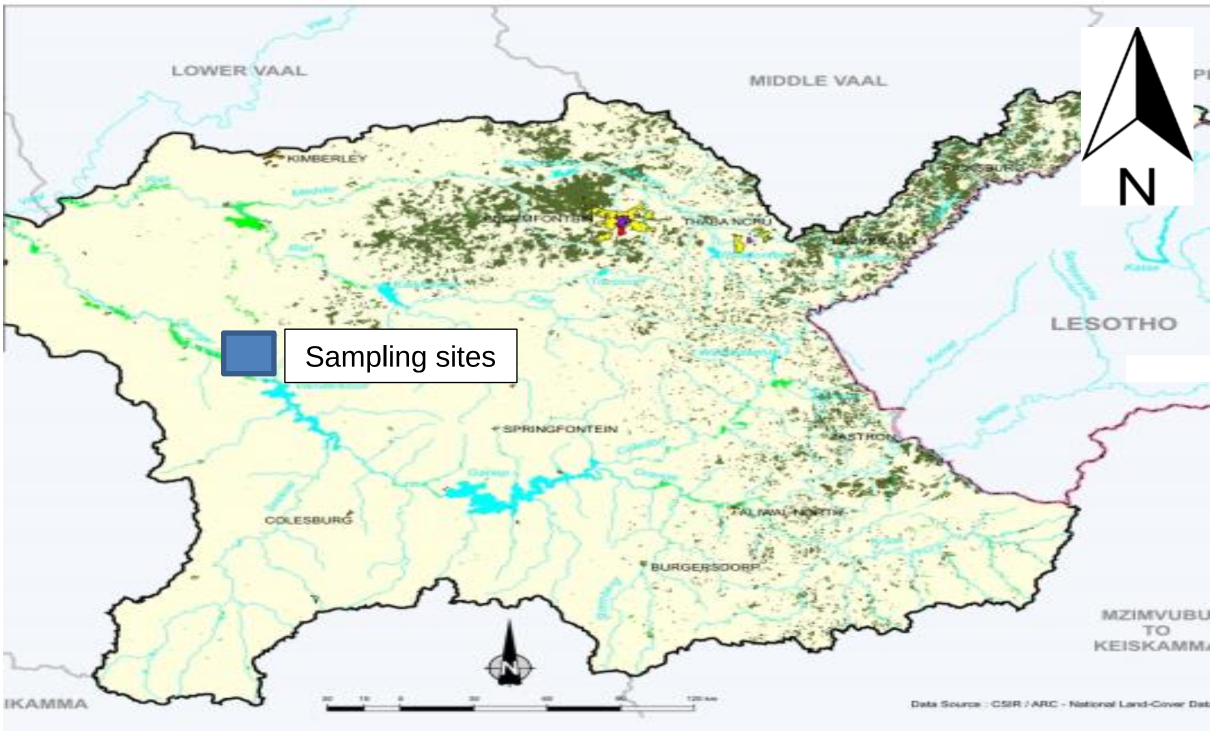


Figure 3.1 Location of the six sampling sites in the UOWMA, South Africa (Basson and Rossouw, 2003).

Some climate parameters for the six sampling sites were estimated by the New LocClim freeware tool. New LocClim is a software tool to estimate local climatic conditions for a certain location on earth. The software makes use of different popular interpolation techniques for example kriging and inverse weight technique. Altitude regression as well as local or regional gradients can be taken into account. New LocClim use the FAO database with observations from nearly 30 000 stations worldwide. The program runs in three modes, namely; single point, workbench and lastly the automatic mode. For this study, single point mode was preferred. Rainfall was estimated through the kriging interpolation mode and temperature through distance inverse technique (Grieser *et al*., 2006).

Table 3.1 Monthly average daily climate parameters estimated by a local climate estimator, New LocClim freeware tool (Grieser *et al*., 2006)

	Site 1				**Site 2**				**Site 3**			
Month	**Tx**	**Tn**	**T**	**Rain**	**Tx**	**Tn**	**T**	**Rain**	**Tx**	**Tn**	**T**	**Rain**
January	32.53	17.02	24.68	43.62	32.4	16.83	24.19	47.96	32.7	17.8	25	50.43
Febuary	31.3	16.54	23.86	52.34	31.15	16.36	23.32	57.66	31	17.2	24.2	61.68
March	28.42	14.31	21.29	54.62	28.28	14.14	20.81	59.74	28.7	15.1	21.6	60.99
April	24.59	9.8	17.21	40.61	24.46	9.61	16.67	40.79	24.7	10.8	17.6	41.4
May	20.78	5.26	12.8	18.14	20.67	5.1	12.34	19.51	21.3	6.5	13.1	19.4
June	17.95	1.7	8.48	7.08	17.84	1.53	7.86	7.89	18.2	3.2	8.8	7.92
July	17.73	1.2	8.01	5.5	17.63	1.06	7.4	7	18.7	2.7	8.3	6.85
August	20.74	3.47	11.22	7.1	20.63	3.33	10.65	8.54	21.2	4.9	11.6	8.44
September	23.96	6.93	15.26	8.66	23.87	6.77	14.79	12.46	25.5	8.8	15.6	12.54
October	27.36	10.66	18.92	22.88	27.22	10.49	18.4	28.21	27.7	11.8	19.3	30.3
November	29.36	13.21	21.32	28.13	29.22	13.03	20.8	32.13	30.2	14.6	21.7	34.2
December	31.57	15.56	23.48	29.5	31.43	15.38	23	34.32	32	16.6	23.8	36.87
Mean	25.53	9.64	17.21	26.52	25.4	9.47	16.69	29.68	25.99	10.83	17.55	30.92

	Site 4				**Site 5**				**Site 6**			
Month	**Tx**	**Tn**	**T**	**Rain**	**Tx**	**Tn**	**T**	**Rain**	**Tx**	**Tn**	**T**	**Rain**
January	32.4	16.85	24.06	49.52	31.83	16.31	23.56	49.75	32.39	16.85	24.06	49.73
Febuary	31.14	16.38	23.2	59.68	30.57	15.97	22.69	59.06	31.13	16.37	23.2	59.95
March	28.28	14.16	20.7	60.28	27.81	13.79	20.21	63.94	28.28	14.15	20.7	60.41
April	24.47	9.63	16.59	40.84	24.07	9.49	16.1	40.67	24.46	9.63	16.59	40.89
May	20.69	5.13	12.27	19.51	20.34	5.21	11.8	21.49	20.69	5.13	12.27	19.53
June	17.85	1.56	7.61	7.87	17.43	1.65	6.92	8.53	17.85	1.56	7.61	7.9
July	17.65	1.09	7.14	7.03	17.36	1.32	6.43	7.93	17.65	1.09	7.14	7.05
August	20.65	3.35	10.46	8.51	20.22	3.52	9.86	9.8	20.65	3.35	10.45	8.54
September	23.9	6.81	14.75	12.57	23.66	6.83	14.27	15.24	23.9	6.81	14.75	12.64
October	27.24	10.52	18.32	29.25	26.65	10.23	17.8	29.77	27.24	10.52	18.32	29.41
November	29.24	13.06	20.71	32.96	28.64	12.7	20.2	34.25	29.24	13.06	20.71	33.11
December	31.44	15.4	22.89	35.33	30.91	14.96	22.38	37.07	31.44	15.4	22.89	35.51
Mean	25.41	9.49	16.56	30.28	24.96	9.33	16.02	31.46	25.41	9.49	16.56	30.39

Tx: Maximum Temperature (°C)
Tn: Minimum Temperature (°C)
T: Average Temperature (°C)
Rain: Rainfall (mm)

3.2 Site selection

Sampling sites for this study were selected with a view to represent the spectrum of irrigated soils from the UOWMA below the Vanderkloof dam. Six sites were sampled in sub area four covering irrigated portions of the Free State and Northern Cape provinces. Four sites were cultivated, and two uncultivated, as illustrated in Figure 3.2. The four cultivated sites have high calcareous topsoils with high (site 1) and low (site 2) P contents, and low calcareous topsoils with high (site 3) and low (site 4) P contents. The topsoils of the two non-cultivated sites are high (site 5) and low (site 6) calcareous. At all six sites the topsoils were classified as diagnostic orthic A horizons (Table 3.2).

Table 3.2 Selected sampling sites, locations, land types (Land Type Survey Staff, 2002) and diagnostic horizons of soils sampled (Soil Classification Working Group, 1991)

Site	Site description	Latitude	Longitude	Altitude	Land type	Horizon of sample
1	Cultivated (irrigated), calcareous soil with high P content.	-29.62638	24.131507	1080	Da 78	Orthic A
2	Cultivated (irrigated), calcareous soil with low P content.	-29.735047	24.391312	1080	Da 103	Orthic A
3	Cultivated (irrigated), non-calcareous soil with high P content.	-29.690681	24.42007	1100	Ae 278	Orthic A
4	Cultivated (irrigated), non-calcareous soil with low P content.	-29.692083	24.41361	1100	Ae 278	Orthic A
5	Uncultivated (virgin), calcareous soil.	-29.83895	24.61362	1100	Ae 278	Orthic A
6	Uncultivated (virgin), non-calcareous soil.	-29.68958	24.42008	1100	Ae 278	Orthic A

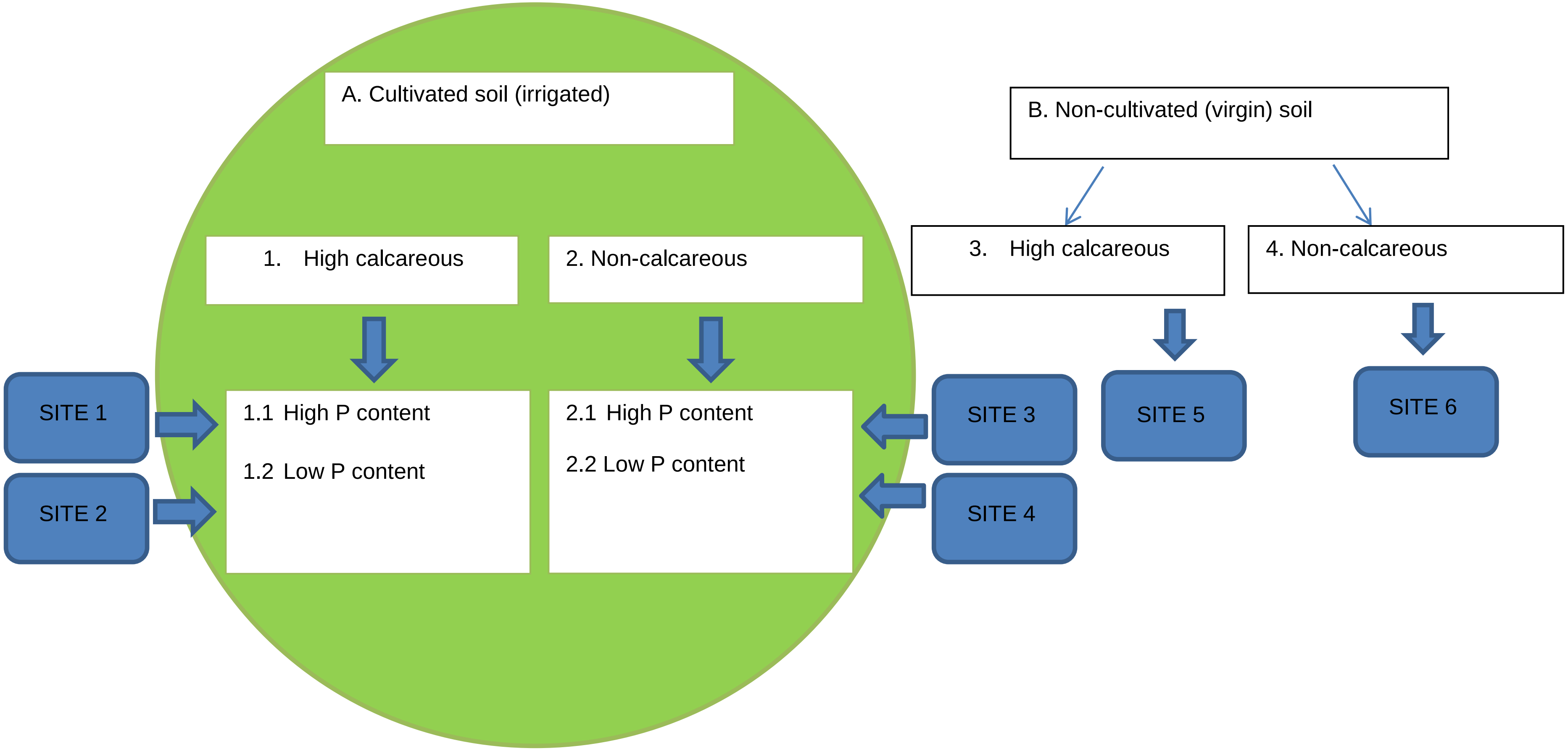


Figure 3.2 Schematic illustration of site selection.

3.3 Soil sampling

Soil samples were taken from the orthic A horizon at each of the selected six sites. A spade was used to collect the samples to a depth of 200 mm. A simple random sample technique was applied to composite representative samples from each site of not less than 20 kg. Crop residues were removed before sampling and all the samples were taken from between wheat rows to avoid direct sampling of fertiliser bands. Soil sampling was done in July 2017 after wheat planting. These samples were transported to the University of the Free State, where they were spread out to be air-dried in glasshouses. Samples were turned at least five times, to ensure effective drying of the soils. The soil samples were sieved through a mechanical 2 mm sieve in preparation for laboratory analysis and incubation treatments.

3.4 Incubation experiment

The average N, P and K application for this irrigation area is 350 kg ha^{-1} N, 70 kg ha^{-1} P and 100 kg ha^{-1} K. About 80% of the P is applied at plant. MAP ($NH_4H_2PO_4$) is the most dominant P source used for P corrections. The composition of MAP is 11:22:0 (N:P:K) and is soluble in water and citric acid. Usually MAP with a pH of 4.5 is more efficient in reducing ammonia volatilisation of urea than DAP with a pH of 8.2 (Smalberger, 2012).

A bulk density of 1 400 kg m^{-3} was used to calculate the amount of MAP required for raising the P levels of the sampled soils with 0, 6, 12, 18, 24, 30 and 36 mg kg^{-1} over 0.2 m depth (Table 3.3). The incubation experiment was therefore set up with six soils each having seven P levels that were replicated five times.

Table 3.3 Amount of MAP required to raise sampled soils' P contents with predetermined levels

Phosphorus (mg kg^{-1})		Required amount (kg ha^{-1})		Incubation (g MAP 400 g^{-1} soil)
Level	**Content**	**P**	**MAP**	
1	0	0	0	0
2	6	16.8	62.4	0.00891
3	12	33.6	124.8	0.01783
4	18	50.4	187.2	0.02674
5	24	67.2	249.6	0.03565
6	30	84	311.9	0.04456
7	36	100.8	374.3	0.05348

Plastic containers of 500 ml each were filled with 400 g of the applicable soil. Each soil was spread evenly on an uncontaminated plastic sheet, where it was treated with an appropriate MAP solution. The solution was sprayed on the soil till it was wet to 70% field capacity. This was done with a sprayer connected to a measure cylinder to ensure precise application. After this process the soil was mixed thoroughly and returned to the plastic containers. The lid was removed 6 days before the next rewetting process, which allowed drying during incubation at a temperature of 28 °C. The soil was rewetted several times to 70% field capacity with distilled water during the two months of incubation and was mixed as described above. The soil was air-dried after incubation and sieved through a 2 mm mechanical sieve to prepare it for analysis by the different P extraction methods.

3.5 Soil analyses

The bulk soils' mineralogical composition and total elemental composition were determined by the Department of Geology at the University of the Free State, using X-ray diffraction (Anonymous, 2014a) and X-ray fluorescence (Anonymous, 2014b) respectively. Both methods have the advantage of being non-destructive. Patterns of XRD were obtained with a Panalytical Empyrean theta-theta diffractometer equipped with a Ca anode X-ray tube operating at 45 kV and 40 mV. The measurements were carried out in Bragg-Brentano mode. Phase identification and semi-quantitative analyses were done using the Highscore software. For XRF a Panalytical Axios that contains a Rh end window tube was employed. This sequential wavelength dispersive XRF has a 4 kW Rh anode and a W cathode. Only one element’s X-ray wavelength is measured at a time, instead of its energy. The mineralogical composition of the six bulk soils is displayed in Table 3.4 and Table 3.5, respectively.

The $CaCO_3$ and $MgCO_3$ in the soil was dissolved in a 1:3 soil to HCl suspensions. These suspensions were boiled, filtered and washed with distilled H_2O. The Ca and Mg contents of the filtrates were determined with atomic absorption to calculate the percentage of $CaCO_3$ and $MgCO_3$ in the soils (Personal communication: Ms Y.M Dessels., 2017. Department of Soil, Crop and Climate Sciences, University of the Free State, Bloemfontein). Standard methods of The Non-affiliated Soil Analysis Working Committee (1990) were used to determine the following: particle size distribution (pipette and sieve method), total carbon and nitrogen (Leco combustion), organic carbon (Walkley-Black method), pH (1:2.5 soil to water/KCl suspension), soluble and exchangeable cations as well as cation exchange capacity (1 mol dm^{-3} NH_4OAc at pH 7) and extractable micronutrient cations (diethylene triamine pentaacetic acid [DTPA]) of the six bulk soil samples. These samples were analysed in triplicate, for the above mentioned parameters, which are displayed in Table 3.6.

Table 3.4 Mineralogical composition of the soils from the sampling sites

Mineral (%)	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Quartz	76	44	63	59	66	69
Mica	12	13	9	-	9	-
Clinochlore	-	10	-	-	7	-
Kaolinite	-	-	8	10	-	10
Smectite	-	-	-	13	-	-
Calcite	4	9	-	-	2	-
Plagioclase	8	12	10	10	7	10
K-Feldspar/ Rutile	-	-	-	-	6	-
Pyroxene	-	12	9	8	5	11

Table 3.5 Total elemental composition of the soils from the sampling sites

Element (%)	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
SiO_2	76.56	58.55	74.47	75.74	78.94	78.16
TiO_2	0.60	0.77	0.83	0.81	0.89	0.80
Al_2O_3	5.92	9.45	7.25	7.16	6.92	7.16
Fe_2O_3	4.18	5.64	5.62	5.31	3.71	5.43
MgO	1.19	3.39	2.43	2.13	1.25	2.43
MnO	0.05	0.10	0.09	0.09	0.06	0.09
CaO	3.68	8.63	2.97	2.65	2.28	3.18
K_2O	0.87	1.40	0.99	0.93	1.18	0.95
P_2O_5	0.16	0.13	0.09	0.07	0.06	0.07
Na_2O	0.46	0.80	1.13	1.06	0.60	1.19
LOI	7.13	10.08	3.44	4.10	4.88	2.95

Table 3.6 Physical and chemical properties of the soils selected for the study

Soil property	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Particle size distribution (%)						
Coarse sand (2 to 0.5 mm)	2.4	5.2	0.6	1.2	1.2	0.4
Medium sand	18.4	10.7	26.3	29.2	14.7	26.0
Fine sand	46.6	38.6	52.9	50.0	46.8	56.6
Very fine sand	12.6	19.3	13.8	12.1	23.5	11.4
Coarse silt	2.4	9.5	3.6	3.6	3.6	3.0
Fine silt	6.0	6.0	1.2	1.0	4.8	1.3
Clay (<0.002 mm)	8.3	8.3	1.2	2.4	3.6	2.4
Total N (%)	0.097	0.087	0.076	0.074	0.080	0.055
Total C (%)	1.583	1.997	0.530	0.599	1.020	0.277
Organic C (%)	0.604	0.432	0.402	0.198	0.368	0.178
Inorganic C (%)	0.980	1.565	0.128	0.401	0.652	0.099
pH (H_2O)	7.71	7.83	7.12	7.31	8.10	8.18
pH (KCl)	8.47	8.44	8.09	8.27	8.68	8.78
Olsen P (mg kg^{-1})	41.82	13.94	24.07	19.25	2.35	8.85
Extractable macronutrients cations (mg kg^{-1})						
Soluble Ca	110	68	17	10	28	18
Exchangeable Ca	6058	8125	1208	1398	5825	1235
Soluble Mg	7	4	2	1	2	2
Exchangeable Mg	254	253	255	302	164	185
Soluble K	2	1	1	0.28	0.42	1
Exchangeable K	287	186	257	175	159	221
Soluble Na	7	5	5	5	3	4
Exchangeable Na	23	28	25	36	13	23
CEC (cmol$_c$ kg^{-1})	10	14	9	10	10	10
ECEC bases (cmol$_c$ kg^{-1})	33	43	9	10	31	8
$CaCO_3$ (%)	6.47	12.40	0.62	0.65	3.28	0.73

$MgCO_3$ (%)	0.77	1.14	0.58	0.62	0.72	0.69
Extractable micronutrient cations (mg kg^{-1})						
Cu	0.8	0.7	1.2	1.2	0.6	0.6
Fe	5.4	4.4	6.6	6.3	5.2	2.5
Mn	3.1	3.2	12.7	10.3	2.8	3.9
Zn	7.6	3.0	2.4	1.4	0.5	0.8

After termination of incubation, the P treated soil samples were analysed twice in the laboratories of the Department of Soil, Crop and Climate Sciences at the University of the Free State for extractable P according to the Olsen, Mehlich 3, Bray 1 and Ambic 1 methods (UFS data). These samples were also analysed once by two commercial laboratories for extractable P with Olsen, Mehlich 3 and Bray 1 methods (Lab A data), and with Olsen, Mehlich 3, Bray 1 and Bray 2 methods (Lab B data). All the above mentioned methods are described concisely below, although their details are published elsewhere (The Non-affiliated Soil Analysis Working committee [1990]).

3.5.1. Olsen

To determine the Olsen P content, 2.5 g of soil was weighed in a 250 ml extraction bottle, 50 ml of the extractant (0.5N $NaHCO_3$) with a pH of 8.5 was added and the content was shaken for 30 minutes. One to two drops of a flocculate substance were added. The content was filtered through a Whatman nr. 40 filtrate paper. Ten ml of the extract were pipette, 8 ml of distilled water and 2 ml colour reagent. The reading was taken 30 minutes after the aforementioned and the P content in the soil was determined through a calibration curve (Olsen, 1954).

3.5.2 Mehlich 3

For the extraction of P with the Mehlich 3 solution, 2.5 g of soil was weighed in a 50 ml Erlenmeyer flask. Then 25 ml of the extracting solution (0.015 *M* NH_4F + 0.2 *M* CH_3COOH + 0.25 *M* NH_4NO_3 + 0.013 *M* HNO_3 + 0.001 *M* ethylenediaminetetraacetic acid [EDTA]) was added. This suspension was shaken at 200 or more rpm for 5 minutes at a room temperature which fluctuated between 24 and 27°C. One to two drops of a flocculate substance were added. The content was filtered through a Whatman nr. 40 filter paper. The extract was diluted by adding 8 ml of the extracting solution, 8 ml of distilled water and 2 ml colour reagent to 2 ml of the extract. Absorption readings were taken after 30 minutes. Both blank and standards were prepared in the Mehlich 3 extracting solution to determine the P concentration of the soil with a calibration curve (Mehlich, 1984).

3.5.3 Bray 1

This method extracts more soluble P and is most valuable when analysing cultivated soil. To determine Bray 1 P content, 6.67 g of soil were weighed in a 250 ml extraction bottle. The extractant was prepared as follows: 1.11 g ammonium fluorite (NH_4F) was mixed with 700 ml of distilled water. Two and a half ml of concentrated HCl was added and the solution was diluted to 1 litre. Fifty ml of the prepared extraction agent was added to the soil and shaken for 60 seconds. One to two drops of a flocculate substance were added and filtered through a Whatman nr. 40 filtrate paper. Ten ml of the extract were pipette, 8 ml of distilled water and 2 ml colour reagent. The readings were taken after 30 minutes and the P content in the soil was determined through a calibration curve (Bray and Kurtz, 1945).

3.5.4 Ambic 1

The Ambic 1 method is a modification of the Hunter (1975) method, by Van der Merwe. *et al* (1984). To determine the Ambic 1 extractable P, 5 g of fine grinded soil was weighed in a 250 ml extraction bottle and then 50 ml of the extraction agent was added to the soil and this suspension was shaken for 30 minutes. One to two drops of a flocculate substance were added and the content was filtered through a Whatman nr 40 filtrate paper. A dilution was prepared by pipetting 2 ml of the extract and 8 ml of the extraction solution in a glass container with 8 ml distilled water and 2 ml colour reagent. Absorption readings were taken after 30 minutes. A blank and standard was prepared in the Ambic 1 extracting solution to determine the P concentration of the soil with a calibration curve.

3.6 Data processing

Two-way analyses of variance for P extraction methods and P application levels were calculated with the measured values at a 95% confidence level. The treatment means were compared with Tukey’s procedure, also at a 95% confidence level. Furthermore, either simple regression analyses were done to establish the amount of MAP required to raise the extractable soil P by one unit for each soil and extraction combination. In both instances IBM SPSS Statistics 25 software designed for windows was used.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Characteristics of study soils

From this point onward, the numbers of the sites are appended to the soil samples drawn from them, and vice versa. Based on the mineralogical analysis of the study soils, quartz is the dominant mineral, ranging from 44% in soil 2 to 76% in soil 1 (Table 3.4). All six soils contain plagioclase (7 to 12%), five of which contain pyroxene (5 to 12%). Mica occurs in soils 1, 2, 3 and 5, kaolinite in soils 3, 4 and 6, and smectite in soil 4. Only soils 1, 2 and 5 have calcite. These minerals are typical of the soils that are irrigated in the UOWMA below the Vanderkloof dam (Figure 3.1).

The dominance of quartz in the study soils caused a high content of SiO_2 that varied between 59% in soil 2 and 79% in soil 5 (Table 3.5). Elements that influence the plant availability of P, namely Al_2O_3 (5.9 to 9.5%) and Fe_2O_3 (3.7 to 5.6%) in acidic soils, and CaO (2.3 to 8.6%) in alkaline and calcareous soils, are quite abundant (Figure 2.2). The P_2O_5 content of the six soils ranged from 0.06% in soil 5 to 0.16% in soil 1. However, the P_2O_5 content of soils is not an indication of the amount of P available for plant uptake as discussed in section 2.7.

All six soils' physical and chemical properties were determined in triplicate (Appendix 1). Except for soils 1 and 2 with a clay content of 8.3%, the clay content of the other four soils is less than 3.6% (Table 3.6). This small variation in clay content implies little difference of P adsorption on this particle size fraction. The minerals, namely mica, kaolinite and smectite are presumably present in the clay fraction of the soils (Table 3.4) and may have a larger influence on the adsorption of P than the clay content (Campbell and Edwards, 2001). However, some other soil attributes, such as organic matter and either Ca or Mg carbonates, could result in a larger variation of P adsorption in these alkaline and calcareous soils.

Organic C, an indication of organic matter, varies, for example from 0.18% in soil 6 to 0.60% in soil 1 (Table 3.6). A high organic matter content can lead to larger adsorption of P and can therefore increase the efficiency of applied P through microbial activities in alkaline and calcareous soils. The organic matter assured a slow release of P for plant nutrition, which will increase the availability of P for plant uptake (Hopkins and Ellisworth, 2005).

Calcium carbonate, which contributes to the amount of either soluble or exchangeable Ca, ranges from 0.62% in soil 3 to 12.40% in soil 2. In comparison with $CaCO_3$ content of $MgCO_3$ is far smaller, namely from 0.58% in soil 3 to 1.14% in soil 2 which manifested largely in the amount of soluble and exchangeable Mg in the soils. Despite the large range of Ca and Mg in the study soils, their pH (H_2O) values fall into a relative narrow range of 7.2 in soil 3 to 8.18 in soil 6. Von Wandruszka (2006) mentioned that soils which have a pH (H_2O) value between 7.6 and 8.3, and contain free $CaCO_3$ and $MgCO_3$ are calcareous. Soils 1, 2 and 5 comply with this criteria as displayed in Table 3.6.

It can be assumed that due to the dominance of Ca in the study soils, applied P will react with this cation to form Ca phosphate which will precipitate with ageing. In this precipitated form P is not available for plant uptake as explained in detail in section 2.3.2. The study soils have a large variation in soluble Ca which should influence the fate of applied P.

The adsorption of P and its ultimate fractionation usually resulted from a combination of soil attributes which are addressed thoroughly in section 2.3. Based on this existing knowledge of P reactions in alkaline and calcareous soils and the discussed characteristics of the soils selected for this study, it can be stated that they comply with the criteria set for achieving the objectives (Table 3.2). The four cultivated soils comprise two soils that are high calcareous with high (soil 1) and low (soil 2) P contents, and two soils that are low calcareous with high (soil 3) and low (soil 4) P contents. The two non-cultivated soils are high (soil 5) and low (soil 6) calcareous, both having a low P content.

The focus this far was on soil characteristics that could influence the reaction of applied P. However, it is also essential to address soil characteristics that influence their fertility status. The accessibility of certain plant nutrients decline in soils with pH values lower than 5.5 and higher than 7.5 (FERTASA, 2016).

Pierzynski (2005) indicated that the reactions of P in soil is primarily a function of pH, and added that soils with a pH value of 4.1 to 7.3, are more likely to produce fixation of P with hydrous oxides than with carbonates. In high pH soils, less P is extracted, especially by methods which are not suitable for soils with high pH values. Less P is therefore available for crop uptake, which leads to shortages of P in plants. The highest pH (H_2O) values were measured in both the uncultivated soils (Table 3.6). Lower pH (H_2O) values of 7.12 (soil 3) and 7.31 (soil 4) occurred in soils with lower Ca and Mg carbonate contents.

A high degree of variance of extractable Olsen P was evident between the six soils, meeting the study's objectives. Soil of site 1 has the highest Olsen P content of 41.8 mg kg^{-1}. This value thus exceeded the optimum Olsen P soil content of 15 mg kg^{-1} (FERTASA, 2016). Soil of sites 5 and 6 which are uncultivated have a low extractable Olsen P value of 2.4 mg kg^{-1} for soil of site 5, and 8.9 mg kg^{-1} for soil of site 6. The Olsen P content of soil 6 is unexpectedly high for an uncultivated soil (Table 3.6).

High pH values in soils caused high Ca cation activities. The soluble Ca ranged from 10 to 110 mg kg^{-1} and the exchangeable Ca ranged between 1 208 and 8 125 mg kg^{-1} (Table 3.6). For grain crops, an acceptable value is 300 to 2 000 mg kg^{-1} and for vegetables and potatoes 400 to 2 500 mg kg^{-1} (FERTASA, 2016). According to above, all the sites have sufficient Ca to support plant growth. However, the high Ca content at site 1, 2 and 5 may inhibit the availability of other plant nutrients and hence restrict plant growth in these highly alkaline and calcareous soils. Except for P, the high Ca contents may also cause deficiencies of K, Mg and some micronutrients. For most crops an acceptable Ca: Mg ratio is 4:1 (FERTASA, 2016). However, analysis of the study soils showed high exchangeable Mg and K levels (Table 3.6). The values range from 164 to 302 mg kg^{-1} for Mg and 159 to 287 mg kg^{-1} for K. A recommended level of Mg for optimum crop growth is 80 to 300 mg kg^{-1} for grain crops and for vegetables and potatoes 100 to 400 mg kg^{-1}. For K, values ranged from 80 to 160 mg kg^{-1} for grain crops and from 120 to 240 mg kg^{-1} for vegetables and potatoes (FERTASA, 2016). A Ca: Mg ratio of 36 at site 5 is extremely high when compared to the recommended ratio of 4:1. The high K content also leads to a low Mg: K ratio for all six sites. Due to the high Ca content at site 1, 2 and 5, a high (Ca+Mg): K ratio is evident. Besides having sufficient quantities of Ca, Mg and K in the soil, it is very important that the nutrients are balanced, because an excess of one can supress the uptake of another. Thus all six sites have enough Ca, Mg and K for crop nutrition, except for the excess Ca at site 1, 2 and 5. According to FERTASA (2016) the optimum Ca: Mg: K: Na ratio is 65: 25: 8: 2 for the growth of most crops. The most favourable soil for crop growth according to this ratio is site 4 with a Ca: Mg: K: Na ratio of 78: 17: 10: 2. The other soils seem to be more unbalanced with respect to exchangeable cations. The excess amount of K at site 1 and 3 may cause deficiencies of boron (B) and Mg (FERTASA, 2016).

The critical levels for micronutrient cations extracted with the DTPA method, are reported by Lindsay and Novell (1978) as 0.8 mg kg^{-1} for Zn, 4.5 mg kg^{-1} for Fe, 1 mg kg^{-1} for Mn and 0.2 mg kg^{-1} for Cu when maize is produced. The Cu ranges from 0.6 mg kg^{-1} for both uncultivated soils to 1.2 mg kg^{-1} for soil 3 and 4 (Table 3.6). Soil 3 (12.7 mg kg^{-1}) and 4 (10.3 mg kg^{-1}) have a high Mn content. This high Mn content can lead to an insufficient amount of Fe in soil for crop growth. The Zn content fluctuates from a very high amount of 7.6 mg kg^{-1} in soil 1 to a very low amount of 0.5 mg kg^{-1} in soil 5 and 0.8 mg kg^{-1} in soil 6. The Fe content of only soil 6, namely 2.5 mg kg^{-1} is less than the recommended value of 4.5 mg kg^{-1}.

The cation exchange capacity of the soils ranged from 9 to 14 cmol$_c$ kg^{-1}. A soil with a high CEC is considered as fertile since it is able to retain more plant nutrients than a soil with a low CEC. Nutrients in soils with a low CEC are subject to loss through leaching and therefore require higher fertiliser applications. The effective cation exchange capacities (ECEC) of the base cations in the non-acidic soils coincide with the amount of exchangeable cations they contain if not buffered. An extremely high ECEC of the base cations occurred at site 1, 2 and 5 (Table 3.6). For the individual sites, the ECECs were ordered from highest to lowest as follows: site 2 (43 cmol$_c$ kg^{-1}), site 1 (33 cmol$_c$ kg^{-1}), site 5 (31 cmol$_c$ kg^{-1}), site 4 (10 cmol$_c$ kg^{-1}), site 3 (9 cmol$_c$ kg^{-1}) and site 6 (8 cmol$_c$ kg^{-1}).

These wide-ranging fertility indicators presented in Table 3.6 are considered as typical in this irrigation area and are managed by farmers depending on the optimum yield potential and the production potential for a specific crop.

4.2 Extractable P contents of soils

The complete dataset on the analyses of extractable P in the incubated soil samples is presented in Appendix 2. For convenience the average extractable P values per treatment combination are summarized in Table 4.1 (site 1), 4.2 (site 2), 4.3 (site 3), 4.4 (site 4), 4.5 (site 5) and 4.6 (site 6). The two-way ANOVAs were conducted with IBM SPSS Statistics 25, which examined the effect of seven P application levels and four extraction methods on extractable P analysis. There were statistically significant interactions between these two effects for all six sites. The least significant difference test of Tukey (LSD_T) was conducted to compare the means for P application levels and extraction methods as well as the interaction between these two main effects.

Table 4.1 Summary of average extractable P and Tukey's LSD values for site 1

P levels	**kg P ha^{-1}**	**Olsen**	**Bray 1**	**Mehlich 3**	**Ambic 1**
		Extractable P (mg kg^{-1})			
1 (control)	0	41.82	1.39	241.26	62.43
2	16.8	45.77	1.69	250.42	68.95
3	33.6	46.85	1.95	291.49	74.32
4	50.4	52.71	2.31	327.42	77.20
5	67.2	54.32	2.36	286.73	79.73
6	84	57.62	1.92	318.68	85.22
7	100.8	59.56	1.50	311.39	86.11

LSD_T (0.05) for methods: 3.39
LSD_T (0.05) for levels: 4.48
*LSD_T (0.05) for levels*methods: 7.19*

Table 4.2 Summary of average extractable P and Tukey's LSD values for site 2

	kg P ha^{-1}	Olsen	Bray	Mehlich 3	Ambic 1
P levels		**Extractable P (mg kg^{-1})**			
1 (control)	0	13.94	0.44	78.02	36.11
2	16.8	16.87	0.24	82.69	39.59
3	33.6	20.74	0.45	88.88	42.93
4	50.4	23.40	1.60	98.87	49.25
5	67.2	25.17	0.55	129.92	58.08
6	84	27.92	1.12	137.69	63.19
7	100.8	31.04	2.10	174.09	64.34

LSD_T (0.05) for methods: 1.93
LSD_T (0.05) for levels: 2.56
*LSD_T (0.05) for levels*methods: 4.1*

Table 4.3 Summary of average extractable P and Tukey's LSD values for site 3

	kg P ha^{-1}	Olsen	Bray 1	Mehlich 3	Ambic 1
P levels		**Extractable P (mg kg^{-1})**			
1 (control)	0	24.07	82.53	75.79	80.79
2	16.8	26.48	87.64	79.36	87.06
3	33.6	30.97	95.97	112.82	92.88
4	50.4	34.87	98.05	129.51	99.70
5	67.2	35.82	105.42	147.09	108.47
6	84	39.54	107.89	168.05	120.43
7	100.8	44.64	113.07	180.25	129.52

LSD_T (0.05) for methods: 2.00
LSD_T (0.05) for levels: 2.65
*LSD_T (0.05) for levels*methods: 4.26*

Table 4.4 Summary of average extractable P and Tukey's LSD values for site 4

	kg P ha^{-1}	Olsen	Bray 1	Mehlich 3	Ambic 1
P levels		**Extractable P (mg kg^{-1})**			
1 (control)	0	19.25	66.72	62.49	63.56
2	16.8	23.50	75.59	65.29	71.58
3	33.6	27.43	83.34	72.19	80.32
4	50.4	30.70	89.16	75.01	85.91
5	67.2	31.76	90.17	78.61	98.11
6	84	36.21	96.89	119.07	104.53
7	100.8	39.68	104.45	138.40	117.46

LSD_T (0.05) for methods: 1.76
LSD_T (0.05) for levels: 2.33
*LSD_T (0.05) for levels*methods: 3.74*

Table 4.5 Summary of average extractable P and Tukey's LSD values for site 5

P levels	kg P ha^{-1}	Olsen	Bray 1	Mehlich 3	Ambic 1
		Extractable P (mg kg^{-1})			
1 (control)	0	2.35	15.13	23.88	23.53
2	16.8	5.14	7.32	32.21	28.33
3	33.6	8.71	11.83	41.11	30.57
4	50.4	11.96	6.94	49.05	36.17
5	67.2	15.57	22.68	61.78	38.17
6	84	18.52	14.49	59.64	44.14
7	100.8	21.86	12.43	73.91	46.79

LSD_T (0.05) for methods: 2.94
LSD_T (0.05) for levels: 3.89
*LSD_T (0.05) for levels*methods: 6.24*

Table 4.6 Summary of average extractable P and Tukey's LSD values for site 6

P levels	kg P ha^{-1}	Olsen	Bray 1	Mehlich 3	Ambic 1
		Extractable P (mg kg^{-1})			
1 (control)	0	8.85	45.14	48.69	48.12
2	16.8	12.76	53.95	53.18	54.81
3	33.6	15.51	62.22	54.58	61.64
4	50.4	19.58	71.34	54.91	71.31
5	67.2	25.39	81.53	58.03	83.83
6	84	26.84	91.94	82.33	97.43
7	100.8	30.08	100.28	103.03	106.38

LSD_T (0.05) for methods: 1.73
LSD_T (0.05) for levels: 2.29
*LSD_T (0.05) for levels*methods: 3.68*

Soils of sites 1 and 2 which are rich in $CaCO_3$ and $MgCO_3$ displayed similar increases of extractable P upon P application of the Olsen method. There was however, a difference in the initial P values of the two soils. For comparative purposes, larger increases in extractable P with the Mehlich 3 and Ambic 1 methods were measured for soils with lower initial P values. The Ambic 1 method showed an almost similar increase of extractable P for both cultivated soils which are highly calcareous. With Bray 1, relatively constant extractable P values were measured, indicating no significant differences between P application levels at either site 1 or site 2. Despite the increase in extractable P due to higher P application levels for site

1 and 2 according to the Olsen and Ambic 1 methods, the differences between levels are not always clearly signified. However, a high content of $CaCO_3$ and $MgCO_3$ in soils of site 1 and 2 leads to clear significant differences between the four extraction methods. On account of this, in most instances, the differences between P values of the interactions were significant.

Some interesting trends were observed in soils of sites 3 and 4. Both sites showed initial P contents of the Bray 1, Mehlich 3 and Ambic 1 methods to be less than than a 10% difference. Compared to these three methods the P contents of the Olsen method were much lower and therefore resulted in larger differences. The largest increase of extractable P due to P application at site 3 and 4 was measured with the Mehlich 3 method. The low LSD_T value for levels at site 3 (LSD_T= 2.65) and site 4 (LSD_T= 2.33) indicated significant differences, except between 50.4 kg P ha^{-1} and 67 kg P ha^{-1} for the Olsen method at site 3, and for the Olsen and Bray 1 methods at site 4. Significant differences of extractable P between P application levels occurred at site 3 except for some instances with either the Bray 1 or Ambic 1 methods. At site 4 higher application of P in all instances resulted that the Mehlich 3 method extracted significantly more P. However, the difference of extractable P with the Ambic 1 method was not significant at the two highest levels of P application. Based on the LSD_T value of 4.26 at site 3 and 3.74 at site 4, significant differences between some application levels were measured with the Olsen method and the Bray 1 method for site 3.

Remarkably high initial P contents were analysed by the Bray 1, Mehlich 3 and Ambic 1 methods for uncultivated soils at site 5 and 6. The Mehlich 3 and Ambic 1 methods extracted almost the same amount of P from soils of site 5 and 6. These initial extractable P contents were therefore not significantly different. However, a clear significant difference between Olsen and the other methods (Bray 1, mehlich 3 and Ambic 1) as well as Bray 1 and the other methods (Olsen, Mehlich 3 and Bray 1) are evident. However, applied P caused very similar increases in extractable P when determined by the Olsen and Mehlich 3 methods for both uncultivated soils. Soil of site 5 contained an exchangeable Ca content of 5 825 mg kg^{-1} and has a pH (H_2O) value of 8.1. This confirms that the Bray 1 method did not reflect any significant increase of extractable P in the soil of site 5 when P is applied. Olsen is apparently a suitable method for the extraction of P from calcareous soils that have high P contents (Do Carmo Horta and Torrent, 2007). An LSD_T value of 6.24 (site 5) and 3.68 (site 6) indicate significant differences of extractable P between the treatment combinations of four methods and seven levels used for this study.

As pointed out earlier, the four methods extracted different pools of soil P, which may influence fertiliser P recommendations. The extractability of a certain pool is determined by a batch of various soil properties. In this study the exchangeable Ca content has a great influence on the amount of P which a soil test extracted and mainly determines whether there will be a significant difference between the main effects of four P extraction methods and seven P application levels as well as the interactions between them. In section 2.7 the soil properties that could influence P extractability in soils as well as the effectiveness of extraction methods are discussed in some detail. They are inter alia; particle size distribution, mineralogical composition, total surface area, organic matter, pH, Fe/Al-oxides and Ca/Mg carbonates (Nafiu, 2006).

Thus it can be concluded that the Mehlich 3 method reflected the largest increase of extractable P on account of P application. However, for comparable levels of applied P extracted, the Olsen method reflected the lowest P contents. At all six sites, increased higher levels of P application the extractable P significantly, irrespective of the extraction method. The exception was for the Bray 1 method on the calcareous soils. Significant differences of P extracted with the Olsen and the other three methods were noteworthy.

4.3 Relationships between applied P and extractable P

The relationships between applied P as MAP and P extracted with the Olsen (Figure 4.1), Bray 1 (Figure 4.2), Mehlich 3 (Figure 4.3) and Ambic 1 (Figure 4.4) methods showed interesting trends. A general trend in all four P extractable methods suggested that fixation of P is not yet saturated at the highest P application level of 100.8 kg P ha^{-1}. In the case of the Olsen method, the relationship for each of the six sites is almost linear and best described by polynomial equations with R^2 values ranging from 0.9826 for site 1 to 0.9997 for site 5.

By using the Bray 1 method the relationship between applied P and extractable P showed trends similar to that of the Olsen method for soils of sites 3, 4 and 6 which are non-calcareous. Similar to the Olsen method, described polynomial equations the relationship between applied P and Bray 1 extractable P most effectively for the 3 sites (R^2= 0.9784 to 0.9992). However, for the soil of site 5, the relationship was exponential and very poor (R^2= 0.0698), better for soil of site 2 (R^2= 0.6122) and best for soil of site 1 (R^2= 0.9026). These three sites were all alkaline and calcareous (Table 3.6). It is noteworthy that for soil of site 1 the extractable P increased to a level of 50 kg ha^{-1} and then decreased, probably due to precipitation of Ca-phosphate.

The P extracted with the Mehlich 3 method increased in almost linear fashion with higher levels of P application for soils of site 3 and 5, and polynomial for soils of sites 2, 4 and 6. Soil of site 1 was the exception since extractable P increased with higher P application until a level of 60 kg ha^{-1} and then decreased, as was observed with the Bray 1 method. However, despite the differences between the sites' soils, the relationships between applied P and extractable P was best described by polynomial equations with R^2 values ranging from 0.7720 for site 1 to higher than 0.9480 for the soils of the other five sites.

For all soils of the six sites increased higher P application levels the Ambic 1 extractable P contents. This relationship was linear for the soil of site 5 (R^2= 0.990), polynomial for the soils of sites 1 (R^2= 0.9887), 3 (R^2= 0.9980) and 4 (R^2= 0.9951) and exponential for soil of site 2 (R^2= 0.9738) and 6 (R^2= 0.9963). The increase of Ambic 1 extractable P due to higher levels of applied P for the soil of site 1 seems to show a trend similar to that observed with either the Bray 1 or Mehlich 3 extraction methods, although not as clear. In all three instances the extractable P began declining from a certain P application level. The soil of sites 1, 2, and 5 is regarded as alkaline and calcareous (Table 3.6).

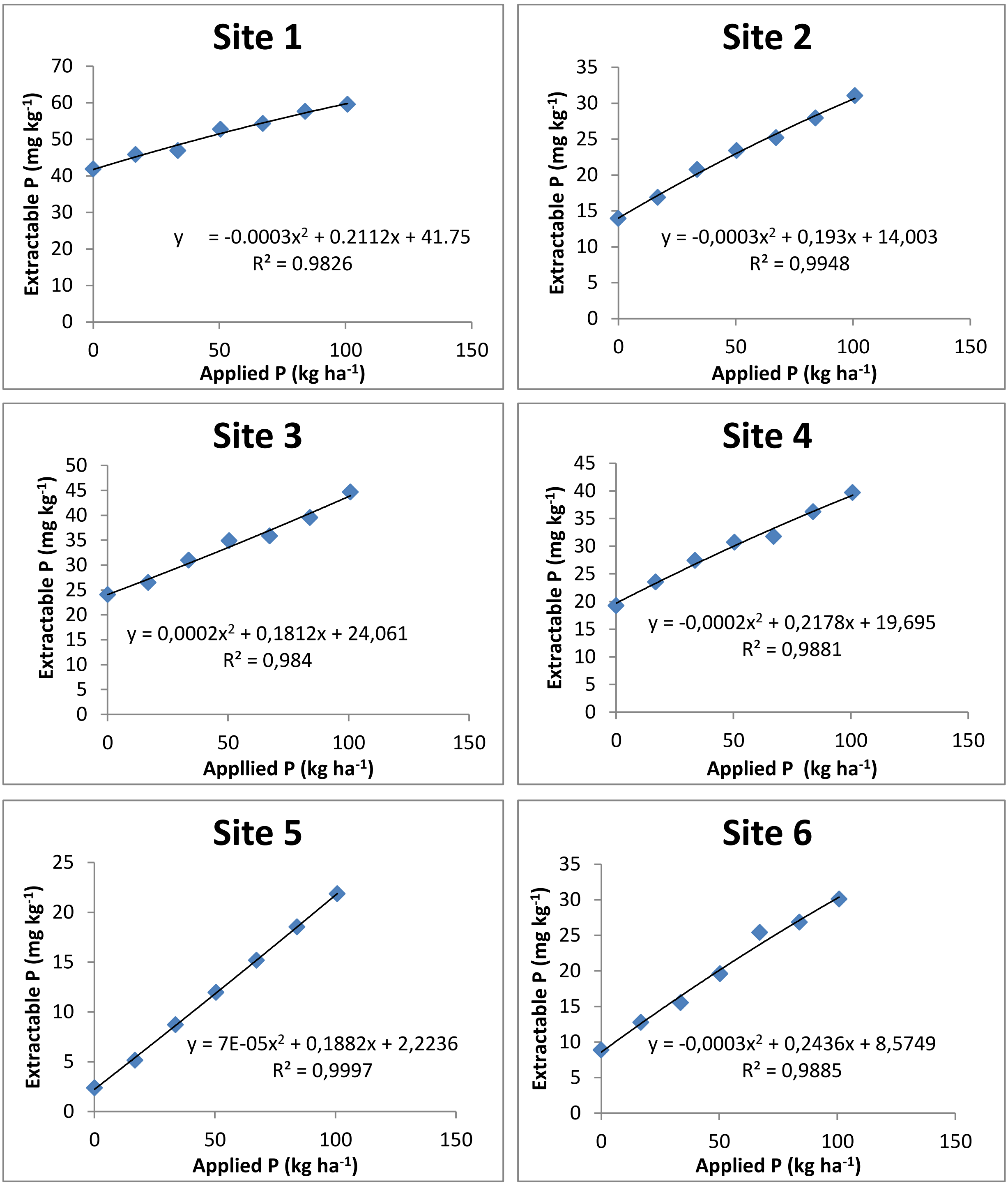


Figure 4.1 Relationships between applied P levels and Olsen extractable P contents for soils of the six sites, using equations fitted the data best.

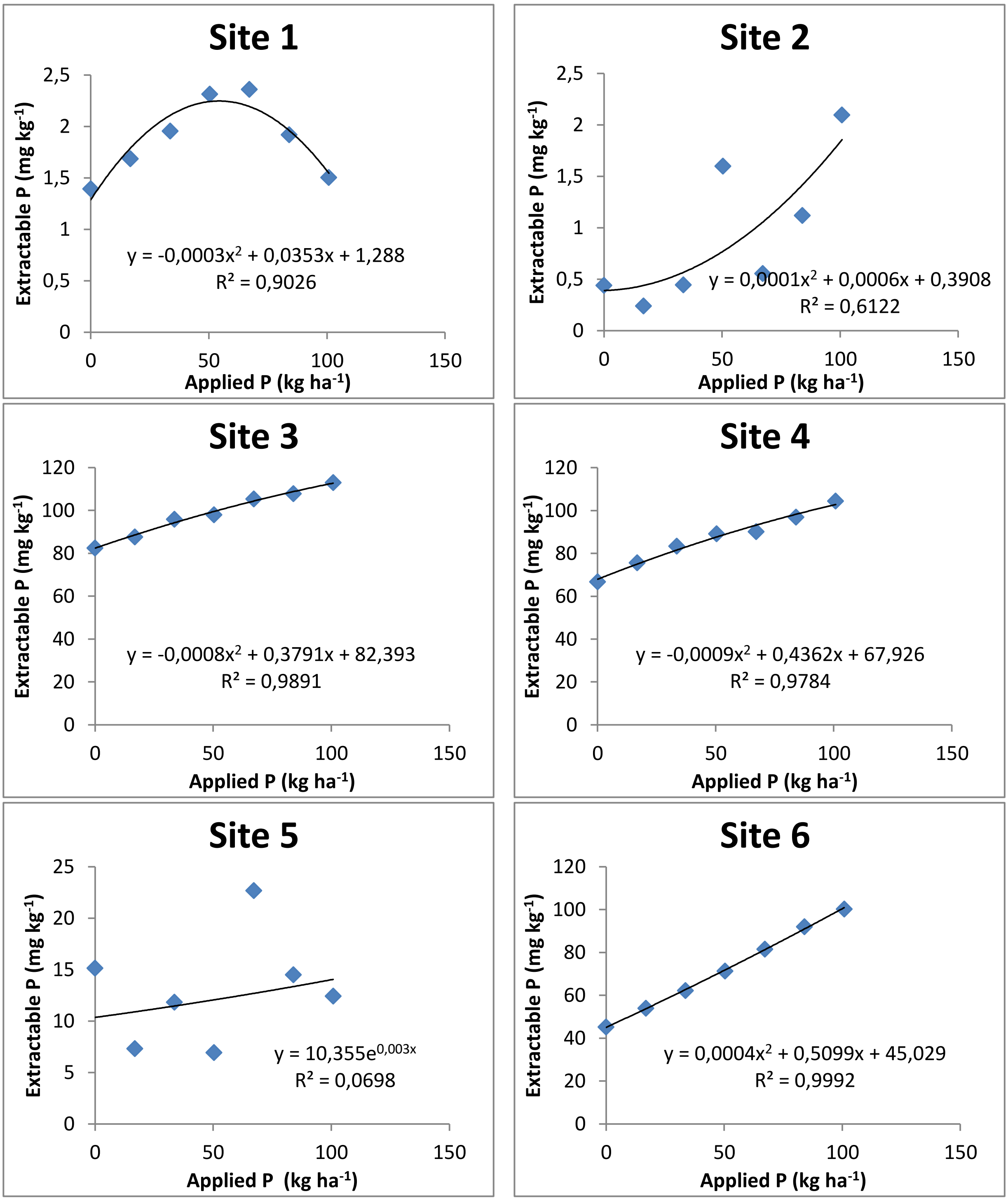


Figure 4.2 Relationships between applied P levels and Bray 1 extractable P contents for soils of the six sites, using equations fitted the data best.

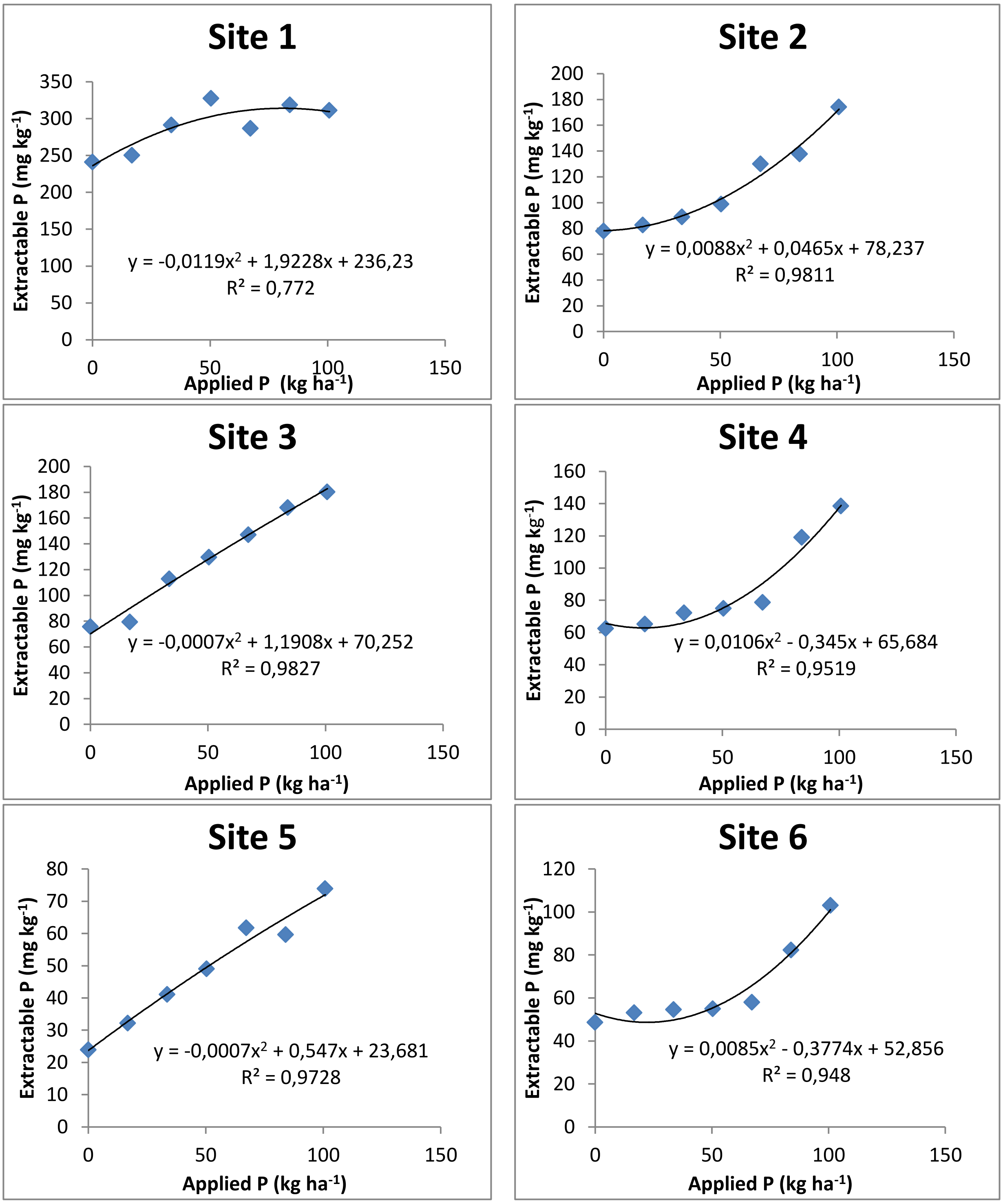


Figure 4.3 Relationships between applied P levels and Mehlich 3 extractable P contents for soils of the six sites, using equations fitted the data best.

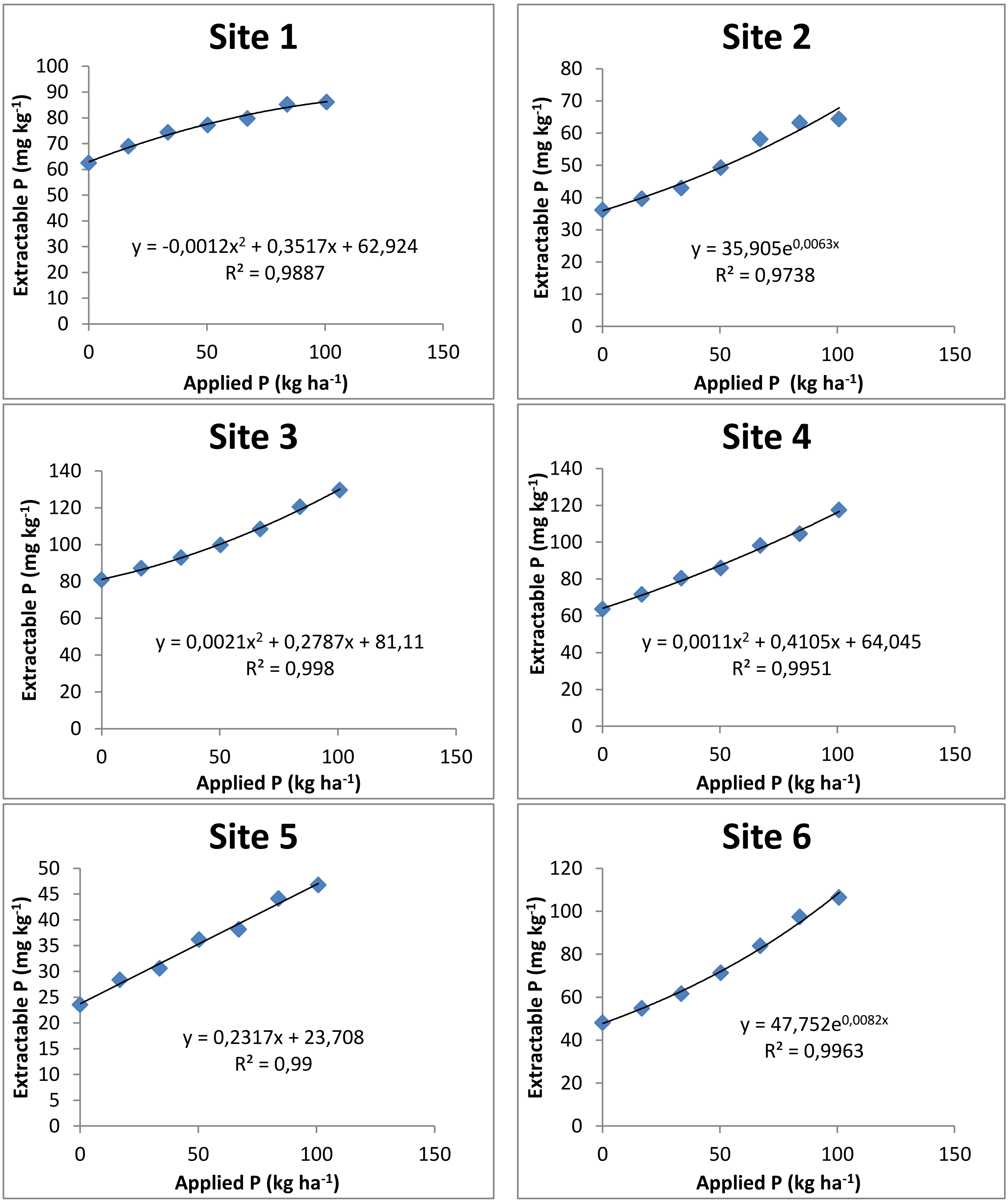


Figure 4.4 Relationships between applied P levels and Ambic 1 extractable P contents for soils of the six sites, using equations fitted the data best.

4.4 Phosphorus requirement factors

The relationships between applied P and extractable P could be used to estimate the amount of P required for raising the extractable P by unity if they were linear, viz. the Phosphorus Requirement Factor (PRF). Unfortunately, the relationships in most instances were not linear and therefore two other approaches were employed to estimate PRF. For the first approach, a linear equation was fitted between a soil's initial extractable P content and the extractable P content induced by the first P application level of 16.8 kg ha^{-1}. Based on the assumption of Molete (2000), the slope of this linear equation is an indication of the kg P ha^{-1} needed to increase the extractable P with 1 mg kg^{-1} when soils have low P contents. All of these equations have R^2 values of 1. In the second approach, a linear equation was fitted between the entire range of P application levels and the resulting extractable P contents. Like above, the slope of this linear equation gives the kg P ha^{-1} required to raise the extractable P with 1 mg kg^{-1} when soils have a wide range of P contents. These equations have variable R^2 values. The results of the two approaches are listed below.

The PRFs estimated with the first approach are displayed in Table 4.7. Based on this data it is clear that the PRFs of the Bray 1 method should be neglected for P recommendations due to inconsistency. However, the PRFs of the Olsen, Mehlich 3 and Ambic 1 methods could be useful. In the case of Olsen, the PRFs varied from 3.95 kg ha^{-1} for soil 4 to 6.98 kg ha^{-1} for soil 3. The estimated PRFs of the Mehlich 3 method ranged from 1.89 kg ha^{-1} for soil 1 to 6.01 kg ha^{-1} for soil 4. For the Ambic 1 method, the PRFs varied between 2.10 kg ha^{-1} for soil 4 and 4.84 kg ha^{-1} for soil 2. These PRF data showed no consistency with respect to either the selected soils or the extraction methods. It is therefore suggested to rather use the average PRFs for P recommendations: 5.20, 3.65 and 3.04 kg ha^{-1} for the Olsen, Mehlich 3 and Ambic 1 methods, respectively.

In the second approach linear equations were fitted to the applied versus extractable P data and these outcomes are presented in Figure 4.5 to 4.8. For convenience, however, the slopes and R^2 values of the equations are given in Table 4.8. The slopes as mentioned earlier can be regarded as the PRFs of the soils. Only the PRFs estimated with data of the Olsen, Mehlich 3 and Ambic 1 methods seem useful for P recommendations. This is because the PRFs estimated with Bray 1 data are extremely high for the soils of site 1 (385 kg ha^{-1}), 2 (69 kg ha^{-1}) and 5 (28 kg ha^{-1}). Soils of sites 3, 4 and 6 have acceptable PRFs of less than 3.3 kg ha^{-1}. The estimated PRFs with the Olsen (4.62 to 6.05 kg ha^{-1}), Mehlich 3 (0.90 to 2.09 kg ha^{-1}) and Ambic 1 (1.67 to 4.32 kg ha^{-1}) methods had narrow ranges with averages of 5.27, 1.48 and 2.90 kg ha^{-1}, respectively. These average PRFs for the Olsen, Mehlich 3 and Ambic 1 methods can be employed for P recommendations on soils with characteristics similar to the study soils.

It is noteworthy that the two approaches yielded almost similar PRFs with the Olsen (5.20 versus 5.27 kg ha^{-1}) and Ambic 1 (3.04 versus 2.90 kg ha^{-1}) data. The estimated PRFs based on the Mehlich 3 data were, however, 2.5 times higher with the first approach (3.65 kg ha^{-1}) than with the second approach (1.48 kg ha^{-1}). This may be an indication that it would be better to use the Olsen or Ambic 1 rather than the Mehlich 3 method for P recommendations on alkaline and calcareous soils.

Table 4.7 Phosphorus requirement factors estimated between initial extractable P and extractable P induced by the first level of P application, using linear regression

	PRF (kg ha^{-1})			
Site	**Olsen**	**Bray 1**	**Mehlich 3**	**Ambic 1**
1	4.25	57.80	1.83	2.58
2	5.73	-84.03	3.60	4.84
3	6.98	3.29	4.71	2.68
4	3.95	1.89	6.01	2.10
5	6.01	-2.15	2.02	3.50
6	4.29	1.91	3.74	2.51

Table 4.8 Phosphorus requirement factors estimated between extractable P contents induced by the different levels of P application, using linear regression

	PRF ($kg\ ha^{-1}$)							
Site	**Olsen**		**Bray 1**		**Mehlich 3**		**Ambic 1**	
	PRF	**R^2**	**PRF**	**R^2**	**PRF**	**R^2**	**PRF**	**R^2**
1	5.57	0.9801**	384.62	0.0611	1.37	0.6302*	4.32	0.9671**
2	6.05	0.9925**	68.97	0.5685	1.07	0.9125**	3.20	0.9713**
3	5.08	0.9834**	3.32	0.9835**	0.90	0.9824**	2.06	0.9831**
4	5.17	0.9868**	2.89	0.9728**	1.38	0.8058**	1.92	0.9914**
5	5.13	0.9996**	27.55	0.0608	2.09	0.9712**	4.22	0.9900**
6	4.62	0.9872**	1.80	0.9987**	2.09	0.7482*	1.67	0.9850**

***** *significant*
*******highly significant*

The discussion will now focus primarily on the PRFs yielded by the second approach because of the linear relationships fitted between applied P and P extracted with the Olsen (Figure 4.5), Bray 1 (Figure 4.6), Mehlich 3 (Figure 4.7) and Ambic 1 (Figure 4.8) methods. The PRF range in magnitude was extremely wide, probably on account of differences in P adsorption (Table 4.8). If no adsorption of any kind occurred, then the PRF should be theoretical 2.8 kg P ha^{-1} (section 3.4). The estimated PRFs concurred strongly with the alkaline and calcareous characteristics of the soils (section 4.1). Some of the other soil properties may also have an effect on the PRF values, but severe differences were largely eliminated with the selection of the study soils.

Figure 4.5 indicated a good correlation between the two variables, since R^2 values of more than 0.9801 were regressed between applied P and extractable Olsen P. This implied that the P requirement calculated from the slopes of the regression analyses is applicable for sandy soils having more or less similar physical and chemical properties. For example, soils with higher exchangeable Ca (site 1, 2 and 5) showed a less pronounced gradient than soils with lower exchangeable Ca (site 3, 4 and 5). Thus, based on the PRF values displayed in Table 4.8, the soil from site 2, with an exchangeable Ca content of 8 125 mg kg^{-1} needs 6.05 kg P ha^{-1} to increase extractable Olsen P by 1 mg kg^{-1}. while soil from site 4 with an exchangeable Ca content of 1 398 mg kg^{-1} requires 5.17 kg P ha^{-1} to raise extractable Olsen P by 1 mg

kg^{-1}. For example, this implies a difference of 8.8 kg ha^{-1} P application when an increase of 10 mg kg^{-1} Olsen extractable P is essential for the two soils. Thus the fixation of P by exchangeable Ca can have a big influence on the soils' sustainability and economic viability.

Generally, Bray 1 P contents showed larger variation between high and low calcareous soils (Figure 4.6) Due to low R^2 values for the relationships between applied P and Bray 1 P contents, it is not possible to make P recommendations with respect to soils with high $CaCO_3$ and $MgCO_3$ contents (Figure 4.6). Compared to soils of sites 1, 2 and 5, soils of sites 3, 4 and 6 have lower exchangeable Ca contents of 1 208, 1 398 and 1 235 mg kg^{-1} which manifested in PRFs of 3.32, 2.89 and 1.8 kg ha^{-1}, respectively. For soils of sites 3, 4 and 6 the estimated PRFs with Bray 1 data are generally lower than those with Olsen data. This phenomenon can cause substantial differences in the amount of P required to raise the three soils' P contents to an optimum level. Compared to the calculated value given in section 3.4, the estimated PRF for soil at site 6 is much lower since fixation of applied P was not considered. Extremely low Bray 1 extractable P values were analysed in soils containing high exchangeable Ca, while soils with lower exchangeable Ca reflected much higher Bray 1 extractable P values.

Of the two methods, the Mehlich 3 method (Figure 4.7) seems more suitable than the Bray 1 method (Figure 4.6) to determine PRFs of highly calcareous soils. Based on the Mehlich 3 method, the soils of all six sites have PRF values lower than those used in section 3.4 to calculate the range of P application levels for the incubation experiment. This difference can be attributed to the fact that potential fixation of applied P to the study soils was not taken into account. Plants rely largely on the P in the labile pool and not on the P in the stable pool (Van der Laan *et al*., 2009). Much higher PRFs were expected for soils of sites 1, 2 and 5 than for soils of sites 3, 4 and 6 due to differences in exchangeable Ca between them. The low R^2 values for relationships between applied and extractable P in soils of sites 1, 4 and 6 imply that the slopes of these linear equations cannot be assumed as reliable PRF values. Thus the Mehlich 3 method is not recommend for use on alkaline and calcareous soils, because the method extracts a large amount of non-labile P which is not available for crop uptake, especially in soils with a pH of more than 6.5 (Pierzynski, 2000).

By using the Ambic 1 data, much higher PRFs (Table 4.8) were estimated for soils with high contents of either $CaCO_3$ or $MgCO_3$ (Table 3.6). The high contents of either $CaCO_3$ or $MgCO_3$ resulted in high pH values and Ca contents which increase the potential of P fixation. For example the difference in PRF for soil of the calcareous site 2 and the non-calcareous site 4 was 1.28 kg ha^{-1}. The difference in the PRF between the soils of these two sites was in the case of the Olsen method only 0.88 kg ha^{-1}. However, significant linear relationships were obtained between applied and extractable P with the methods indicated by the R^2 values. Interesting to note that soil of site 2 required less P to increase Ambic 1 extractable P with 1 mg kg^{-1} than soil of site 1, although soil of site 2 contained more exchangeable Ca. This explained that not only the amount of $CaCO_3$ and $MgCO_3$ played a role in the fixation process of applied P, but also the physical and chemical properties of $CaCO_3$ and $MgCO_{3.}$

Based on literature, the Olsen method is most suitable for soils which are alkaline and calcareous by nature. In this study the linear regressions with Olsen data has the highest R^2 values, followed by that of the Ambic 1 data. The R^2 values of the Bray 1 method indicate that it is less suitable for high pH soils, especially soils containing extreme amounts of $CaCO_3$ and $MgCO_3$, like those of the UOWMA. On the other hand, the Olsen method is highly recommended for use of P recommendations on such soils due to the high R^2 values obtained between applied and extractable P relationships. This concurs with the findings of Eloff (1971) on the soils of Vaalharts Irrigation Scheme, and those of Nthejane (2012) on the soils of Orange Riet Irrigation Scheme. The Olsen extraction method for P was specifically developed for soils which are alkaline and calcareous in nature.

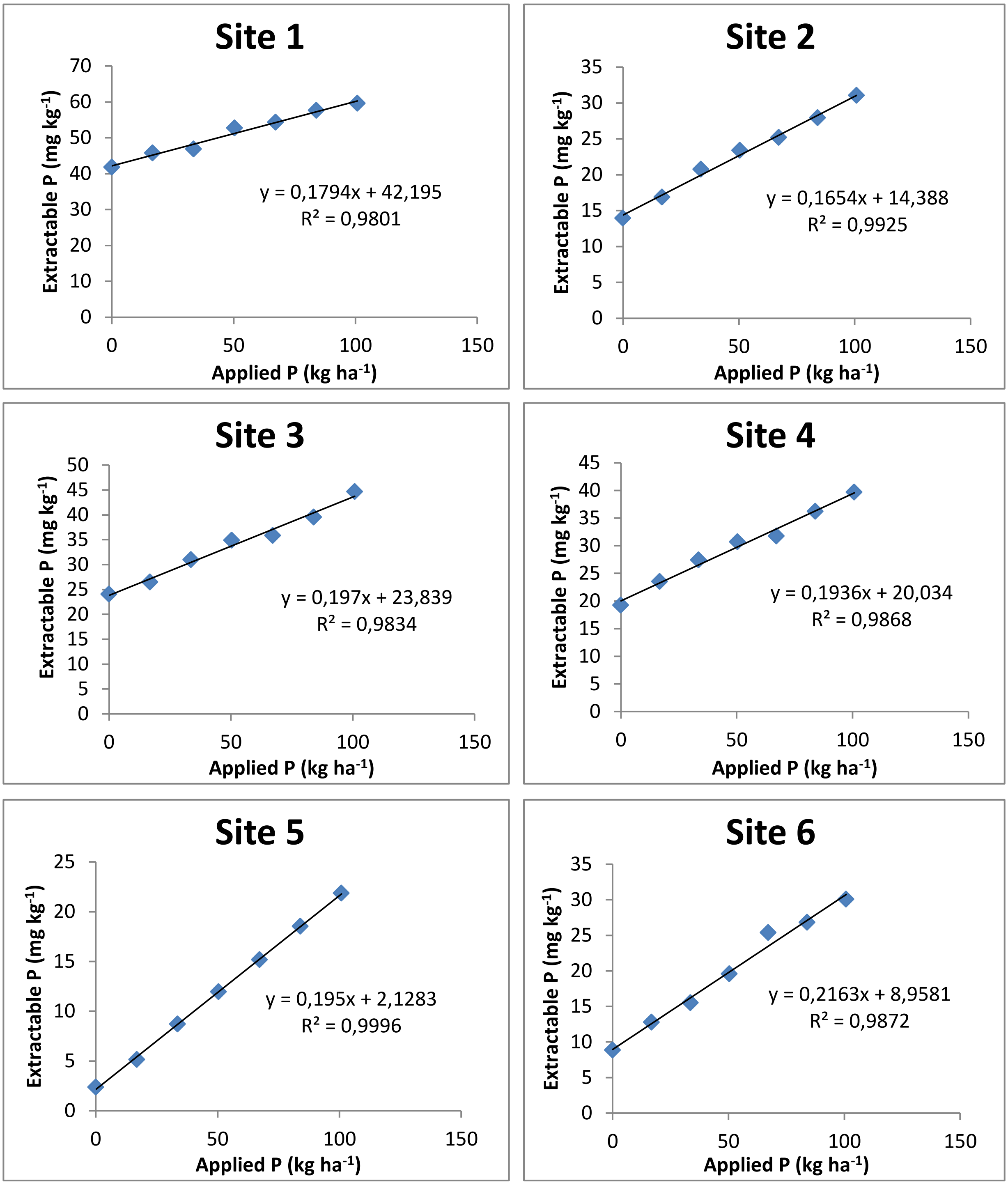


Figure 4.5 Relationships between applied P levels and Olsen extractable P contents for soils of the six sites, fitted linear equations only.

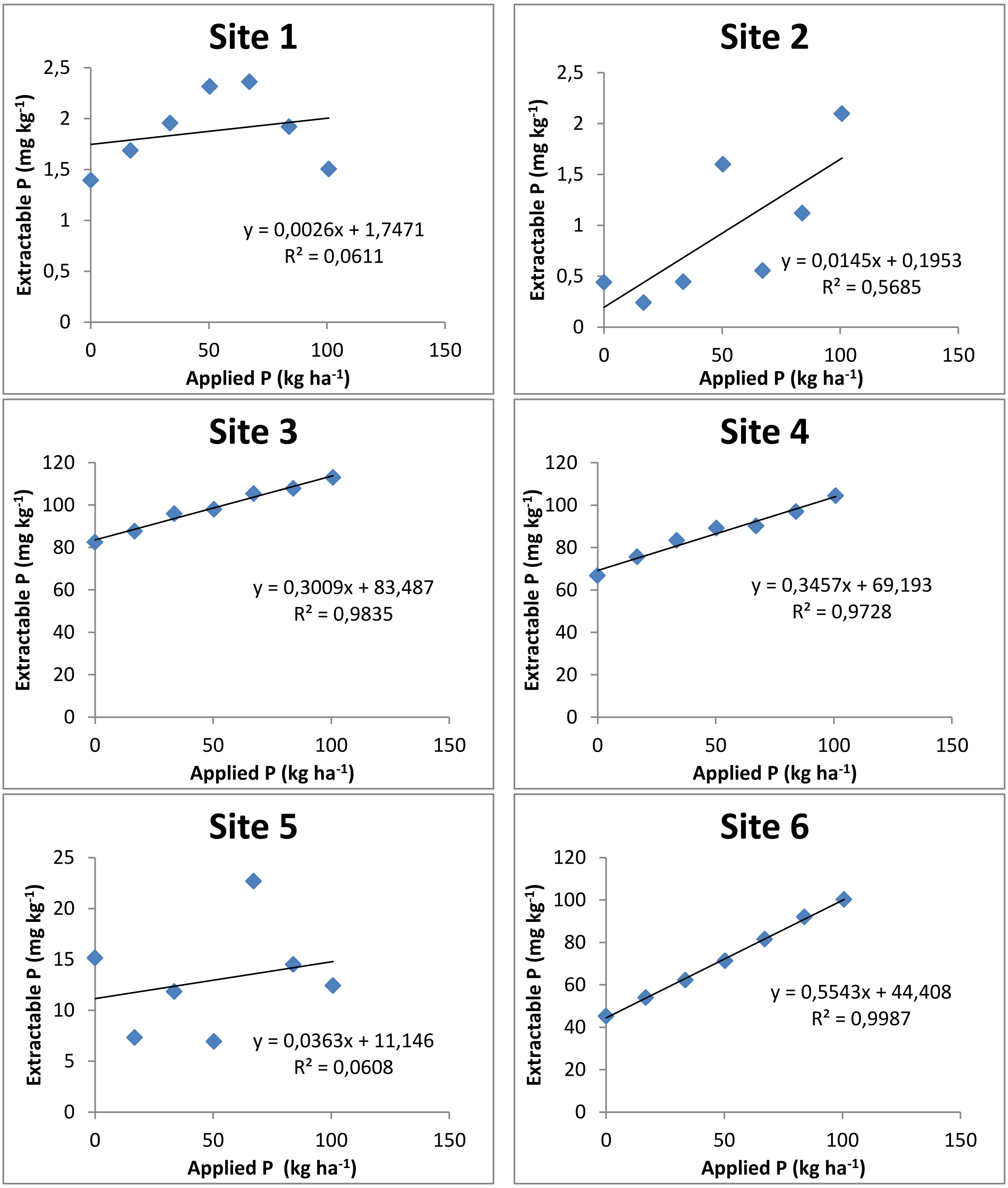


Figure 4.6 Relationships between applied P levels and Bray 1 extractable P contents for soils of the six sites, fitted linear equations only.

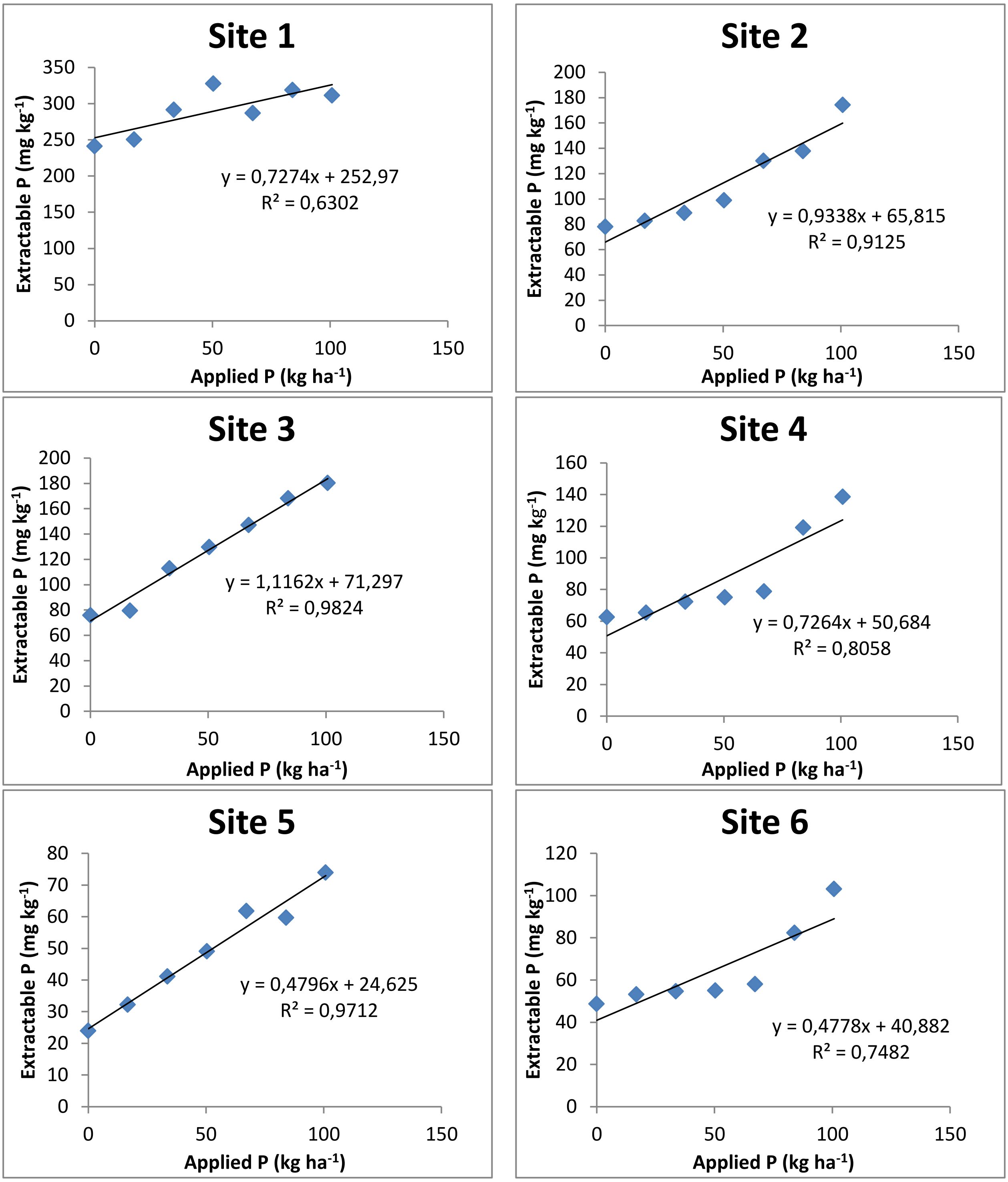


Figure 4.7 Relationships between applied P levels and Mehlich 3 extractable P contents for soils of the six sites, fitted linear equations only.

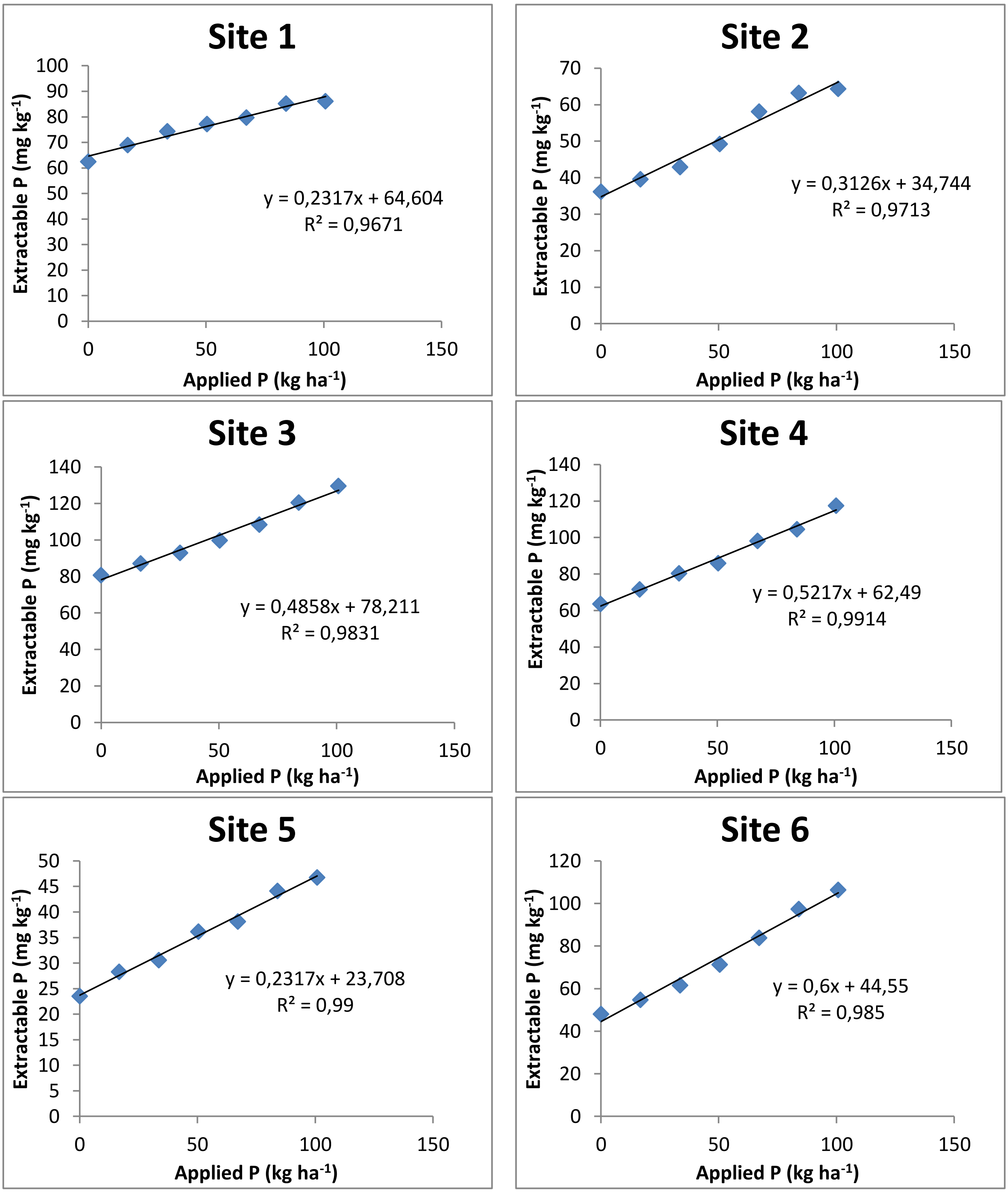


Figure 4.8 Relationships between applied P levels and Ambic 1extractable P contents for soils of the six sites, fitted linear equations only.

4.5 Relationships between extractable P determined with different methods

Different methods like Olsen, Bray 1, Mehlich 3 and Ambic 1 are used to determine extractable P in alkaline and calcareous soils of UOWMA. The interpretation of these extractable P values for fertiliser recommendations is sometimes hampered. This is because no thorough study has yet been done in the UOWMA to establish critical thresholds between extractable P contents and crop yields, except perhaps for the Olsen method. If good relationships between extractable P contents of the above mentioned methods exist, this may be useful for fertiliser recommendations. Thus the P extracted by the different methods were related, as shown in Figures 4.9 to 4.14.

Figure 4.9 displayed good relationships between Olsen and Bray 1 extractable P contents across soils of sites 3, 4 and 6 that have lower exchangeable Ca. The relationships for soils of sites 3, 4, 5 and 6 were linear, and that for soils of sites 1 and 2 polynomial. Soils of sites 1, 2 and 5 contain high amounts of exchangeable Ca and hence lower values of Bray 1 extractable P than Olsen extractable P due to precipitation of Ca-phosphate, thus causing low R^2 values which range from 0.0175 (site 5) to 0.4330 (site 1). The Olsen method extracted more P than the Bray 1 method in soils 3, 4 and 6 which have lower exchangeable Ca. The data presented in Figure 4.9 showed that Olsen and Bray 1 methods have different abilities to extract P in calcareous soils and would yield into inaccurate conversions from one to the other. A high pH (H_2O) value of 8.10 at site 5 caused no relationship between the P extracted with the Olsen and Bray 1 methods. Despite high pH values in soils at all sites, a clear difference in the relationship between Olsen and Bray 1, extractable P was found due to large differences in exchangeable Ca at the sites. This concurred with the work of Sawyer and Mallarino (1999) who reported a good correlation between the P extracted with Olsen and Bray 1 in soils with a pH of less than 7.05. For these relationships improved the R^2 values when the pH values decline. However, Sawyer and Mallarino (1999) found no relationship between Olsen and Bray 1 extractable P in soils with a pH ranging from 7.45 to 8.2.

The relationships between Olsen and Mehlich 3 extractable P contents were independent of exchangeable Ca in soils of the six sites (Figure 4.10). These results were not expected because of the common belief that the Olsen and Mehlich 3 methods would not extract similar amounts of P, due to the weaker acids used in Mehlich 3 to make this method more suitable for P analyses over a wider pH range (Latrou *et al*., 2014). The Olsen P values of soils at the six sites correlated well with the Mehlich 3 P values with the poorest relationship in soil of site 1 (R^2= 0.6454). A low R^2 value for the relationship between applied P and extractable Mehlich 3 P supported the poorer correlation between the two methods at site 1. Soils of all six sites showed polynomial relationships between the Olsen and Mehlich 3 methods.

Generally, Olsen extractable P correlated well with Ambic 1 extractable P for each soil of the six sites (Figure 4.11). These relationships' R^2 values ranged from 0.8322 for soil of site 1 to 0.9764 for soil of site 6. Thus conversion of Olsen P to Ambic 1 P and vice versa seems a strong possibility for fertiliser recommendation for alkaline and calcareous soils of the UOWMA. This possibility justifies a thorough investigation since the ideal should be the development of a single equation for conversion.

The relationships in Figure 4.12 suggested that extractable P contents of the Mehlich 3 method cannot be converted to those of the Bray 1 method or vice versa. This is especially true for the calcareous soils of sites 1, 2 and 5. Even for the non-calcareous soils of site 4 (R^2= 0.9151) and site 6 (R^2= 0.8794) the relationships of extractable P between the two methods seems due to their polynomial nature which is not useful for conversion. It seems, however, that the conversion of Bray 1 to Mehlich 3 P contents and vice versa could be a viable option for soil at site 3.

The relationships of contents by the Bray 1 and Ambic 1 methods (Figure 4.13) resulted in R^2 values of 0.1184 (site 1), 0.1846 (site 2), 0.9309 (site 3), 0.9489 (site 4), 0.0604 (site 5) and 0.9876 (site 6). These R^2 values indicated that the conversion of the P contents of the two methods is an option for the non-calcareous soils of sites 3, 4 and 6, but not for the calcareous soils of sites 1, 2 and 5 (Table 3.6).

The relationships of P contents yielded by the Mehlich 3 and Ambic 1 methods were not ideal for conversion for either the non-calcareous soils of sites 3, 4 and 6, or for calcareous soils of sites 1, 2 and 5. The R^2 values in Figure 4.14 ranged between 0.7052 for soil of site 1 which has a higher exchangeable Ca content to 0.9663 for soil of site 3 which has a lower exchangeable Ca content. Most of the relationships between P content of the Mehlich 3 and Ambic 1 methods are polynomial. The only exception was the exponential relationship that fitted the data for soil of site 5 best.

Results from the study indicated that in most instances the P contents of one extraction method cannot be converted to reflect the P contents of another method. This observation is especially true for the calcareous soils and to a lesser extent for the non-calcareous soils. The conversion of P contents between the Olsen and Ambic 1 methods is the most promising and therefore justifies a thorough investigation.

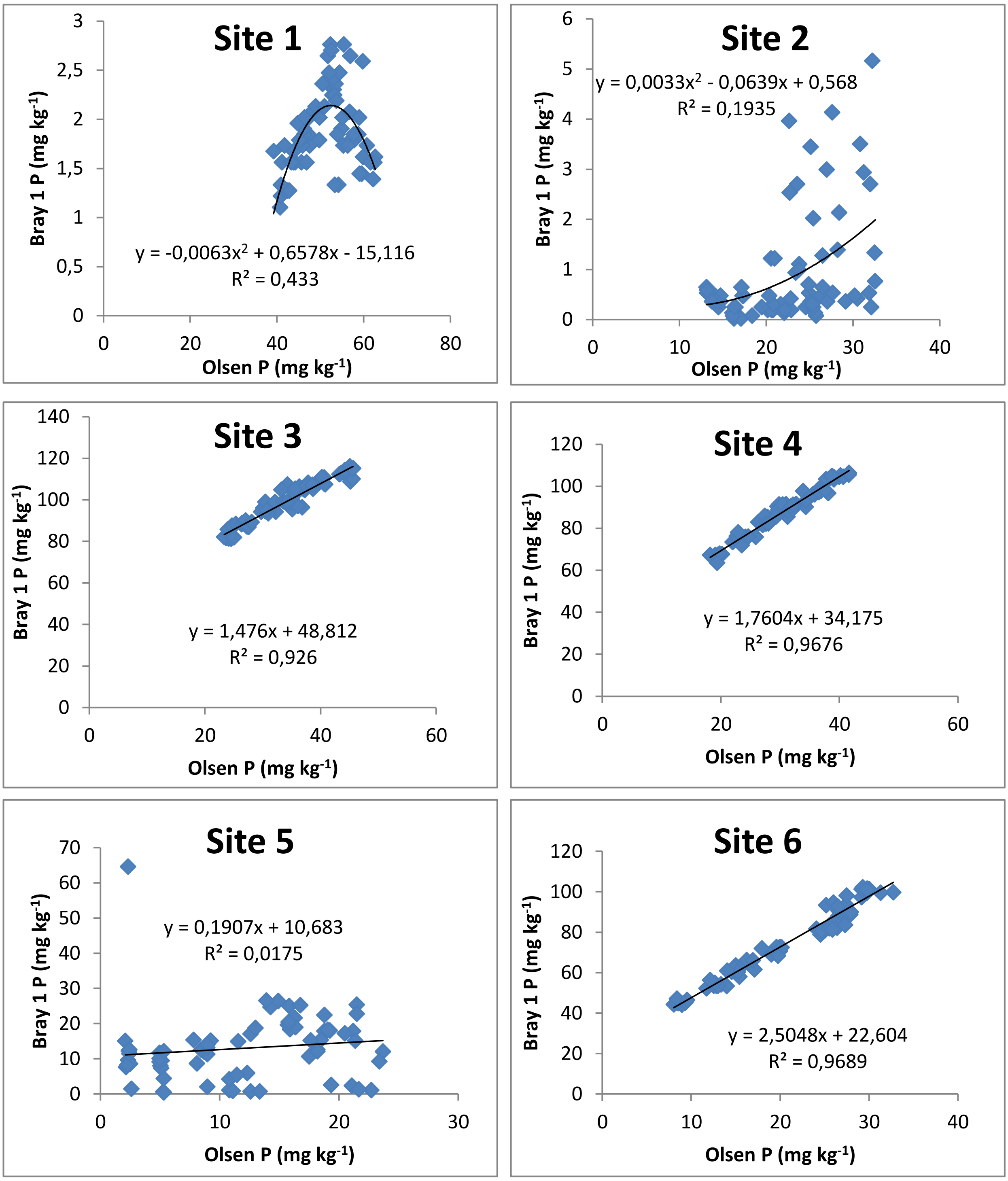


Figure 4.9 Relationship between Olsen and Bray 1 extractable P contents for soils of the six sites, using equations fitted the data best.

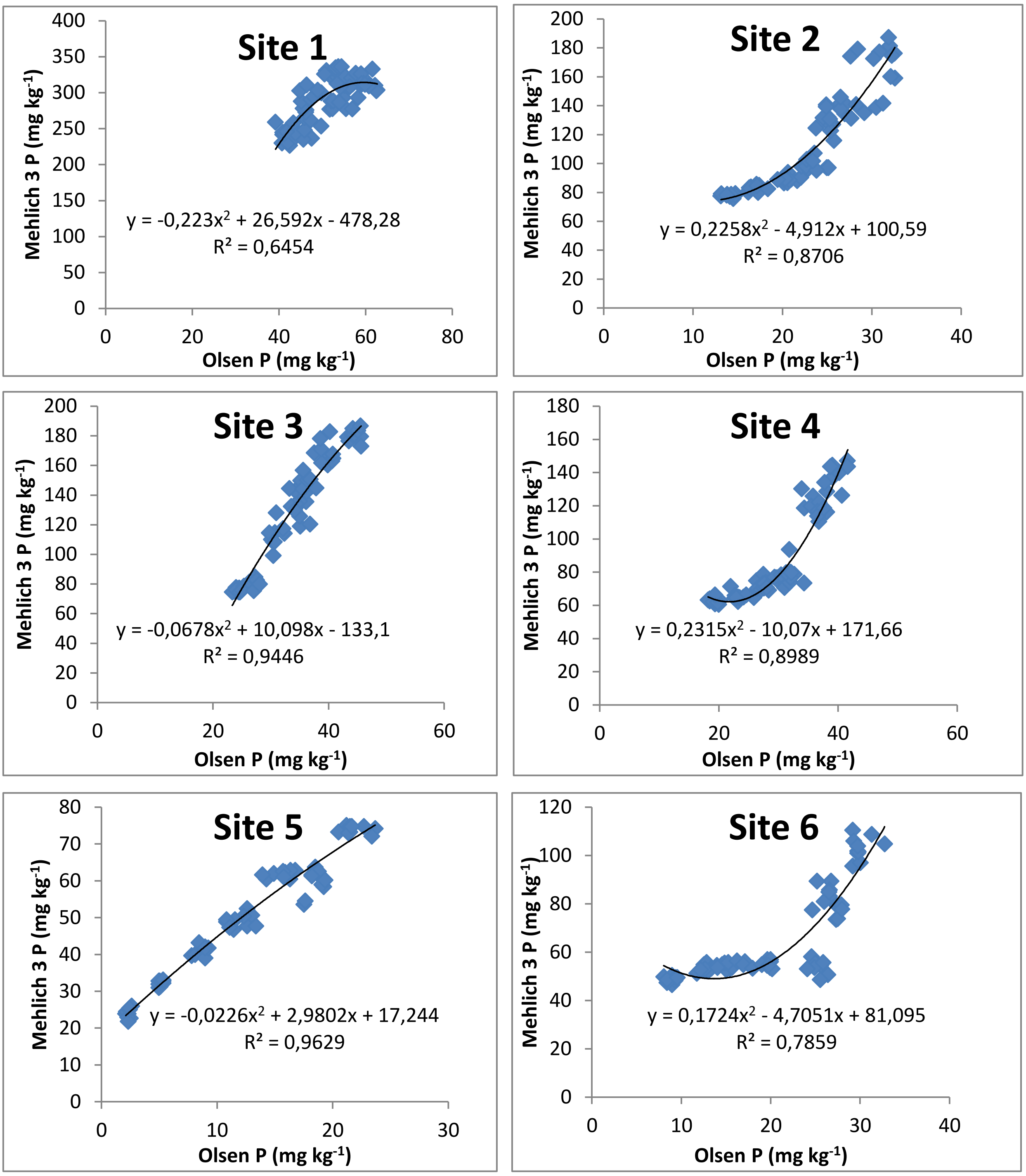


Figure 4.10 Relationship between Olsen and Mehlich 3 extractable P contents for soils of the six sites, using equations fitted the data best.

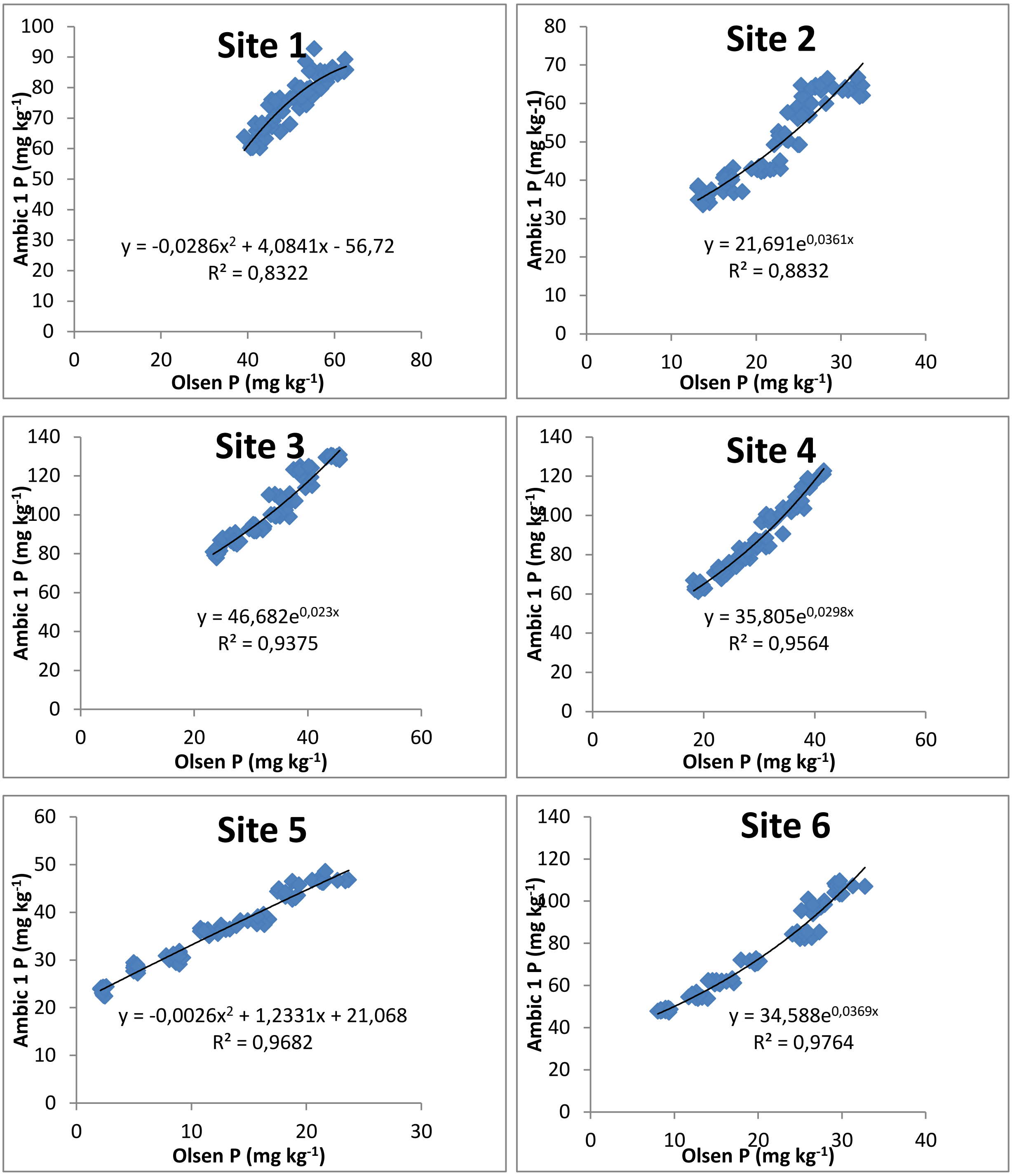


Figure 4.11 Relationship between Olsen and Ambic 1 extractable P contents for soils of the six sites, using equations fitted the data best.

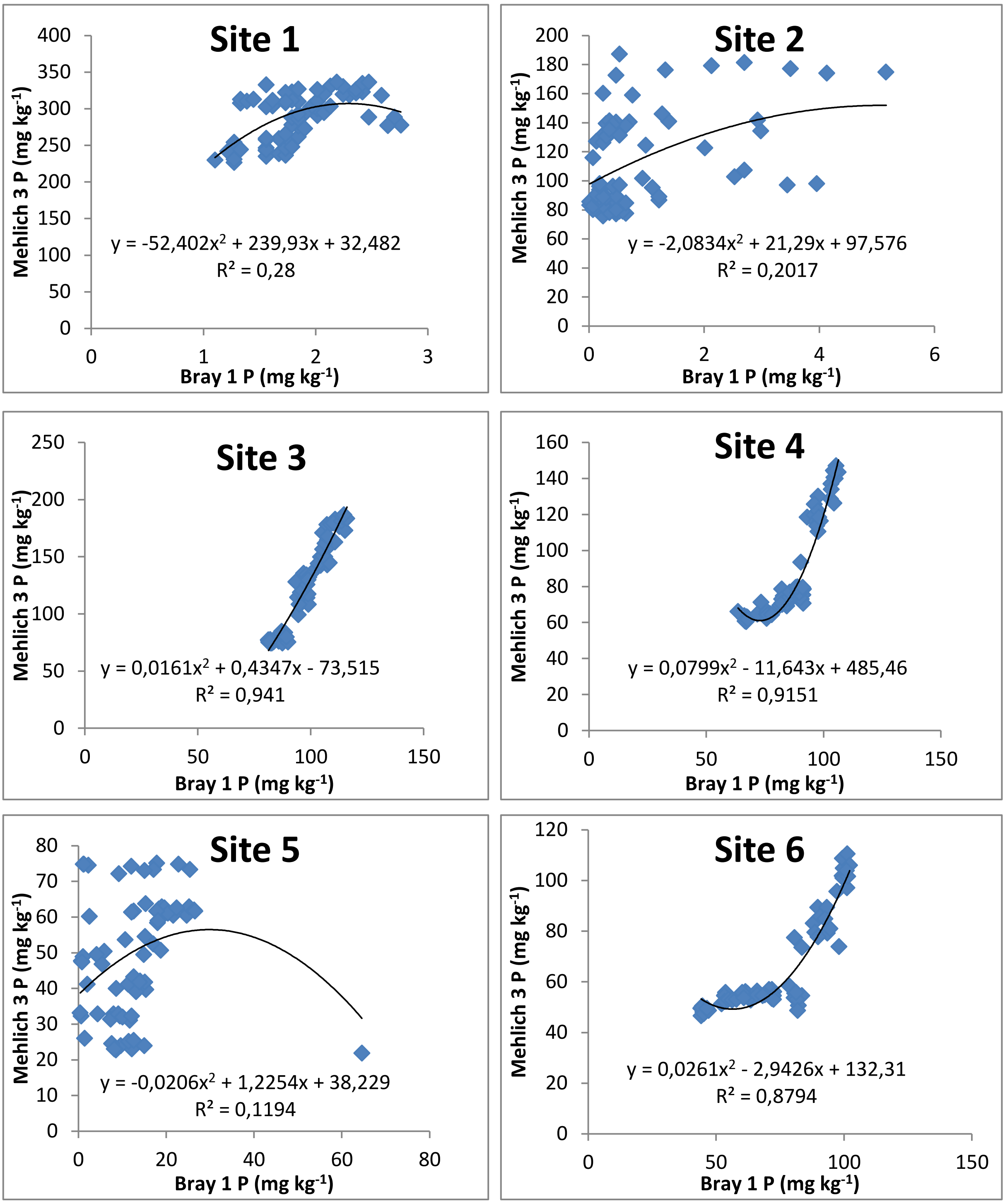


Figure 4.12 Relationship between Bray 1 and Mehlich 3 extractable P contents for soils of the six sites, using equations fitted the data best.

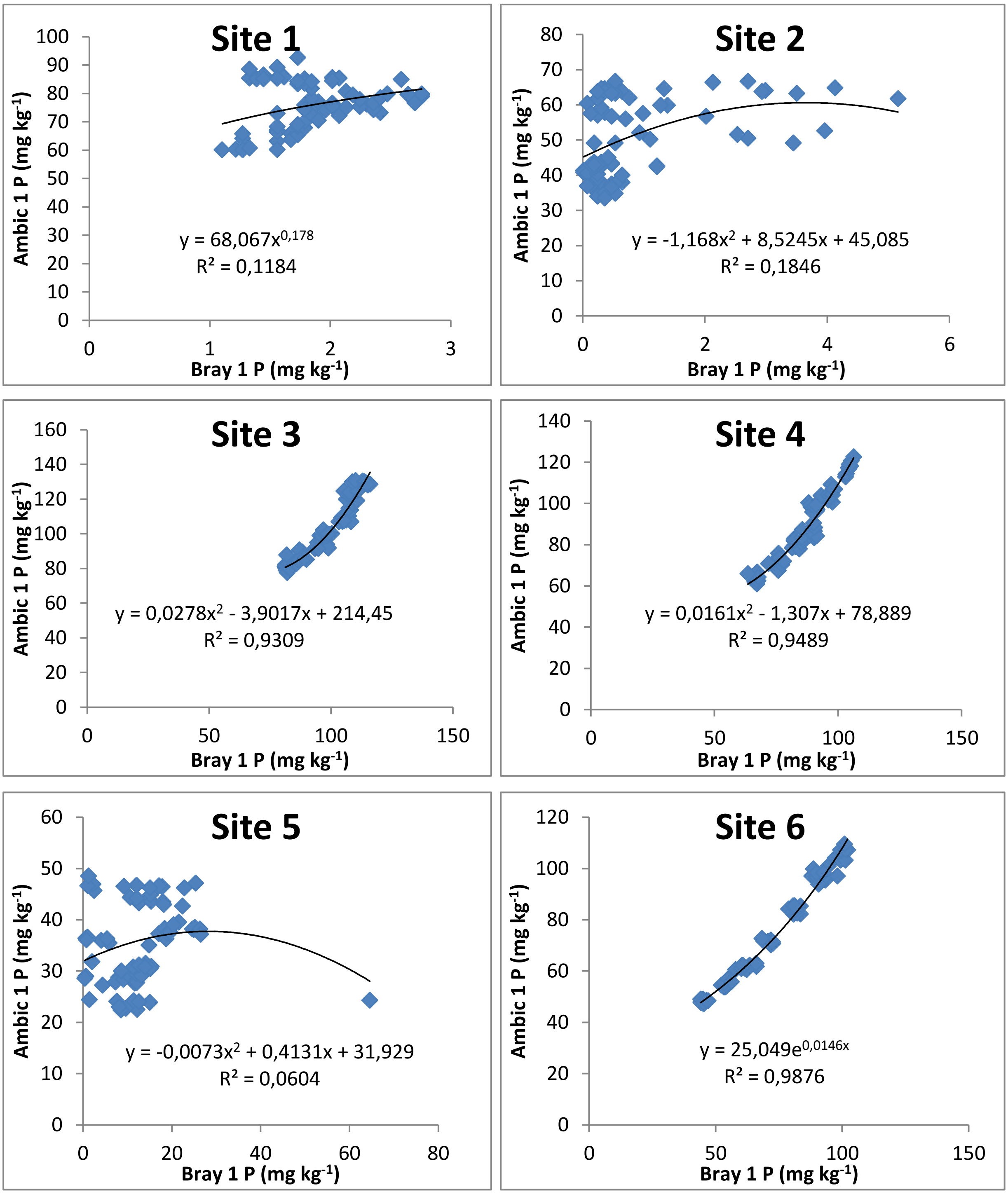


Figure 4.13 Relationship between Bray 1 and Ambic 1 extractable P contents for soils of the six sites, using equations fitted the data best.

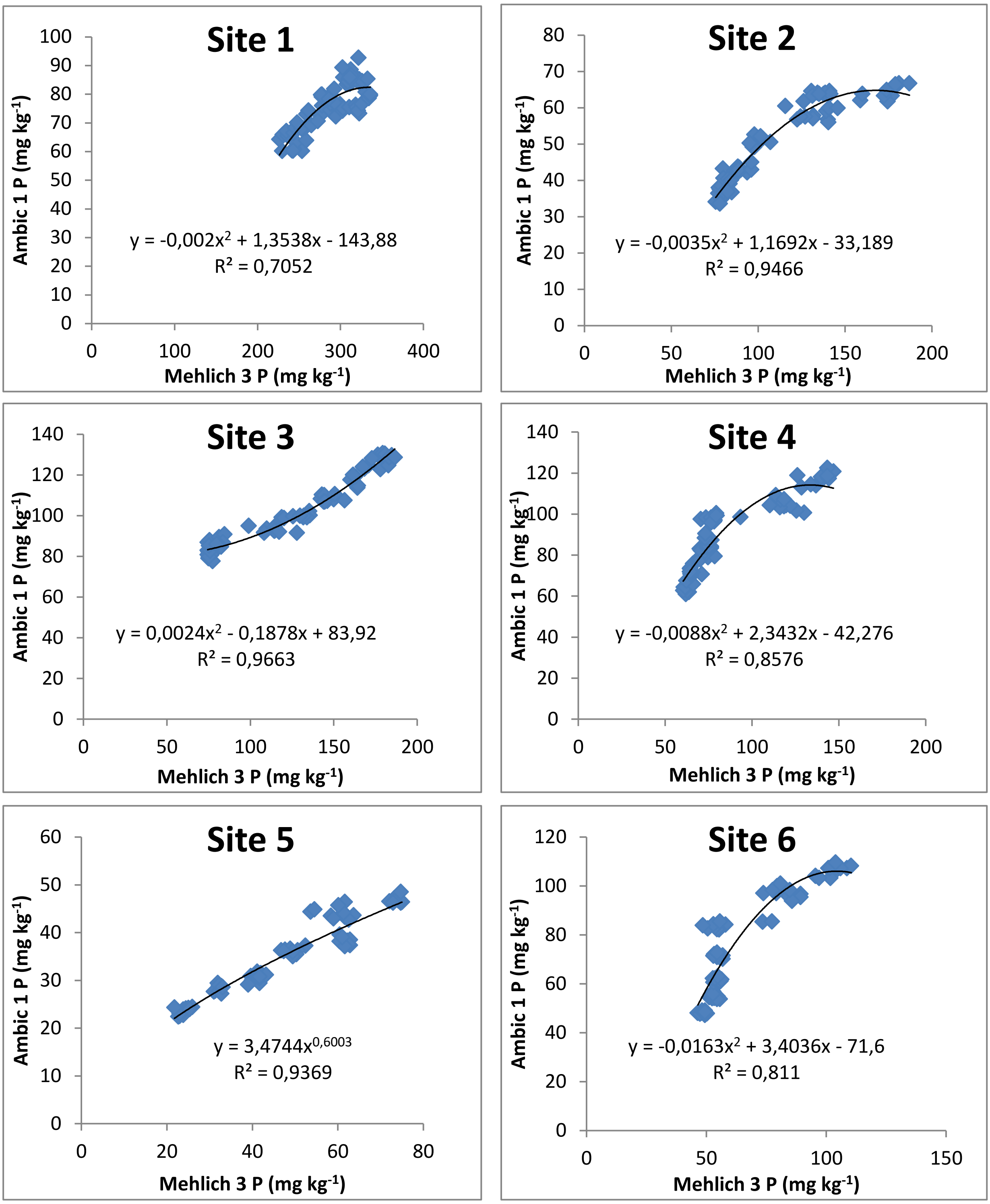


Figure 4.14 Relationship between Mehlich 3 and Ambic 1 extractable P contents for soils of the six sites, using equations fitted the data best.

4.6 Comparison of extractable P contents determined by three analytical laboratories

The soil samples were each separated into three portions for analysis of extractable P at the UFS laboratory and two commercial laboratories with the Olsen, Bray 1 and Mehlich 3 methods, after incubation. These data are presented and discussed below.

4.6.1 Olsen method

The P extracted by the Olsen method from the incubated soil samples in the three laboratories is illustrated in Figure 4.15. Irrespective of the P level, laboratory A reported the highest extractable P contents, followed by those of laboratory UFS and laboratory B. For example an application of 50 kg P ha^{-1} to soil of the non-calcareous site 3 resulted in extractable P contents of 5, 35 and 75 mg kg^{-1} as determined by laboratories B, UFS and A, respectively. The extractable P contents at an application of 50 kg P ha^{-1} to soil of the calcareous site 5 were determined as 5, 10 and 27 mg kg^{-1} by laboratory B, UFS and A, respectively. Generally, the most consistent increase of extractable P in the P treated soils was observed with data of the laboratory UFS, as indicated by the lowest standard deviations and highest R^2 values. Conversely, the extractable P data of laboratory A had the highest standard deviations and lowest R^2 values. The standard deviations and R^2 values for extractable P data from laboratory B were intermediate.

4.6.2 Bray 1 method

Comparisons of Bray 1 extractable P contents reported by the three laboratories were very poor, especially for sites 1, 2 and 5 which have calcareous soils (Figure 4.16). No clear trends evolved from the three laboratories' extractable P data. However, the extractable P data of sites 3, 4 and 6 with non-calcareous soils showed clear trends with respect to the three laboratories. The P contents reported by laboratories A and B were very similar and those of laboratory UFS much higher. At an application level of 50 kg P ha^{-1} the P contents of soils from sites 3, 4 and 6 were twice as high in laboratory UFS than in either laboratory A or B. Nevertheless, the extractable P contents of the non-calcareous soils increased with higher P application levels.

4.6.3 Mehlich 3 method

The data in Figure 4.17 suggested that the Mehlich 3 extraction method resulted in highly variable extractable P contents for the soils of the six sites as confirmed by high standard deviation values. Laboratory UFS reported much higher P contents in soils of sites 1, 2, 3 and 6 than either laboratory A or B whose P contents were almost similar. For site 4 higher P contents were determined by laboratory A than by laboratories UFS and B. The P contents reported for site 5 by laboratory A were initially much higher than those of laboratories UFS and B, but at application levels higher than 16.8 kg P ha^{-1}, the extractable P contents of the three laboratories were very similar.

4.7 Comparison of Bray 1 and Bray 2 extractable P contents determined by laboratory B

Laboratory B determined Bray 2 extractable P in addition to the requested Olsen, Bray 1 and Mehlich 3 extractable P. This allows for the comparison of Bray 1 and Bray 2 extractable P contents in soils of the six sites (Figure 4.18). The ratio of soil to extractant is 1:10 for Bray 1 and 1:17 for Bray 2. These extractants also differ with respect to the HCl concentration used: 0.025 M HCl for Bray 1 and 0.1 M for Bray 2. Despite the diluted soil-to-extractant ratio, the Bray 2 method, due to its higher HCl concentration has a larger capacity to extract the less soluble Ca phosphate than the Bray 1 method (Fageria *et al*., 1997).

Thus it is not surprising that a higher amount P was extracted by the Bray 2 method than by the Bray 1 method. The difference of extractable P contents between the two methods is larger in the calcareous soils of sites 1, 2 and 5 than in the non-calcareous soils of sites 3, 4 and 6. Generally, the Bray 1 method had smaller standard deviation values than the Bray 2 method. The standard deviation values for the calcareous soil of site 2 are extremely large, following the application of the Bray 2 method to extract P. Thus the Bray 2 method like the Bray 1 method seems for similar reasons not to be suitable for use in the extraction of P from alkaline and calcareous soils.

4.8 Comparison of Olsen and Ambic 1 extractable P contents determined by laboratory UFS

Based on the foregoing results, it can be stated that the Olsen extraction method for P is the most suitable for use on alkaline and calcareous soils. In comparison with Olsen, the performance of Ambic 1 was also of such a nature that it could be considered for use on alkaline and calcareous soils. However, neither the Mehlich 3 nor the Bray 1 methods are regarded as fit to extract P from alkaline and calcareous soils.

Only the Olsen and Ambic 1 extractable P contents at different levels of applied P, as determined by laboratory UFS are therefore displayed in Figure 4.19. The standard deviation values were small throughout. The Ambic 1 method extracted more P than the Olsen method without exception. This is probably due to the chemical composition of the two methods’ extractants that is given in Table 2.4. The Olsen extractant consists of $NaHCO_3$ only, while that of Ambic 1 of NH_4HCO_3, $(NH_4)_2EDTA$ and NH_4F. Thus it may be worthwhile to establish P fertilisation guidelines for cropping in UOWMA with either the Olsen or Ambic 1 extraction methods.

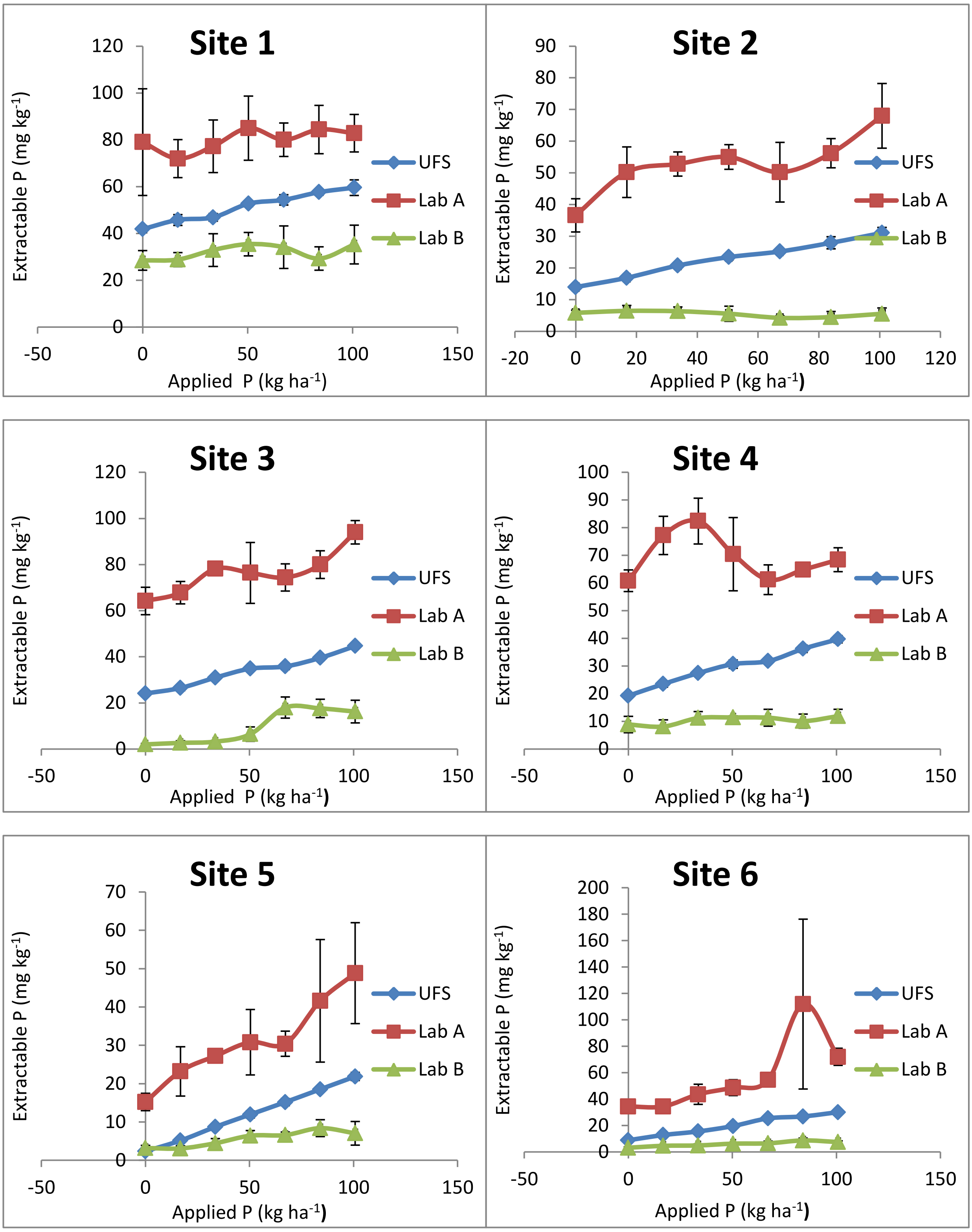


Figure 4.15 Olsen extractable P contents at different levels of applied P as determined by three laboratories. Standard deviation is indicated by error bars.

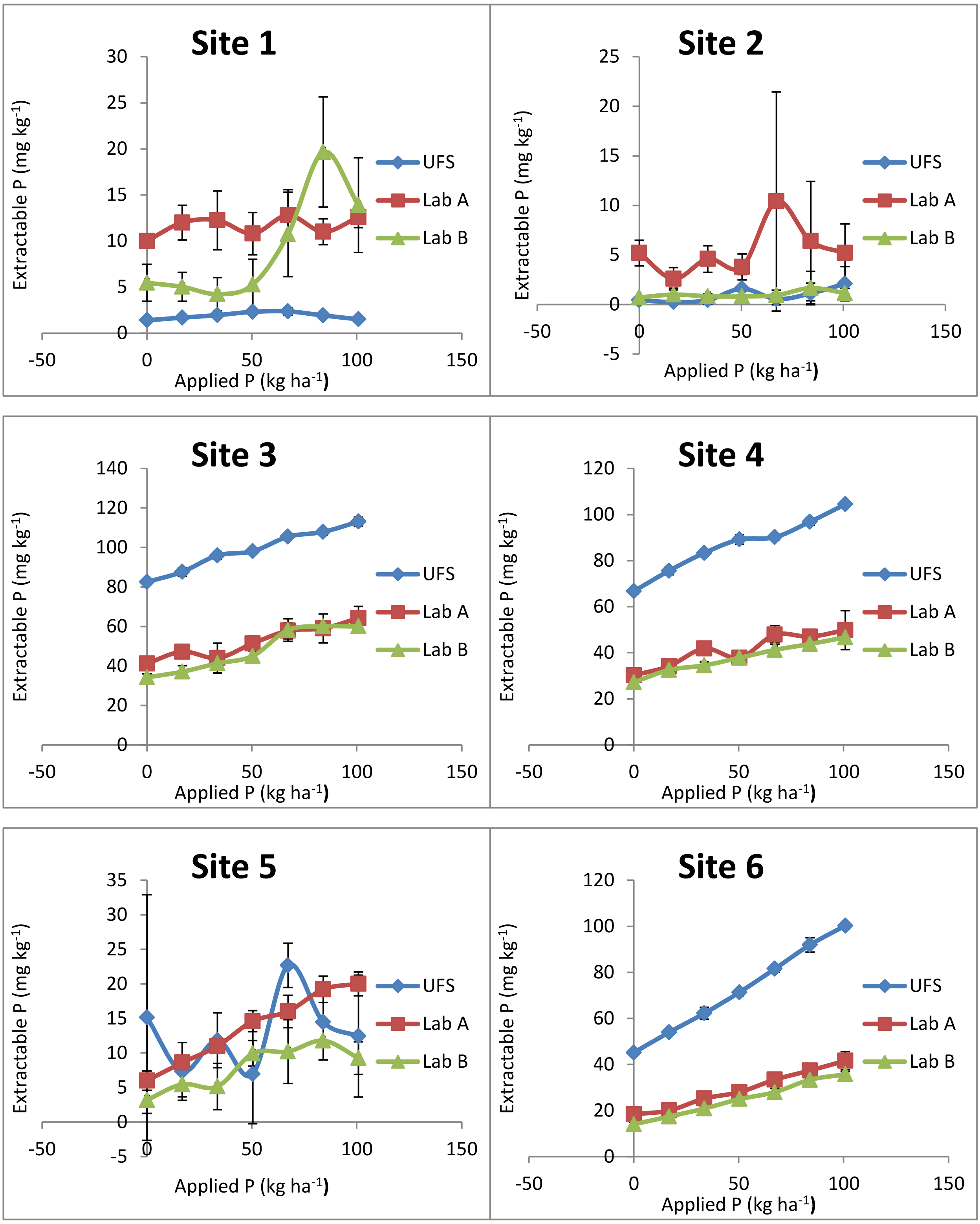


Figure 4.16 Bray 1 extractable P contents at different levels of applied P as determined by three laboratories. Standard deviation is indicated by error bars.

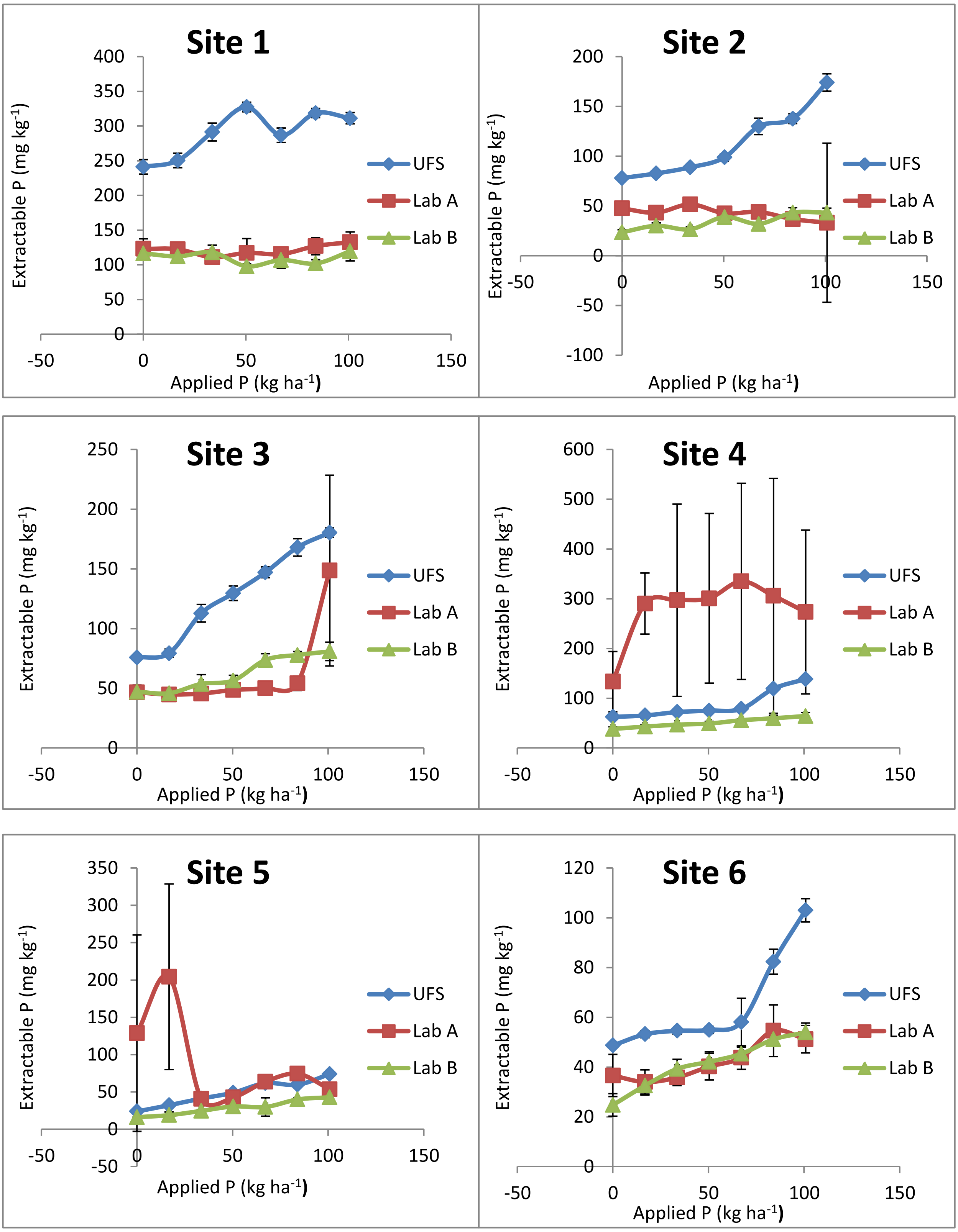


Figure 4.17 Mehlich 3 extractable P contents at different levels of applied P as determined by three laboratories. Standard deviation is indicated by error bars.

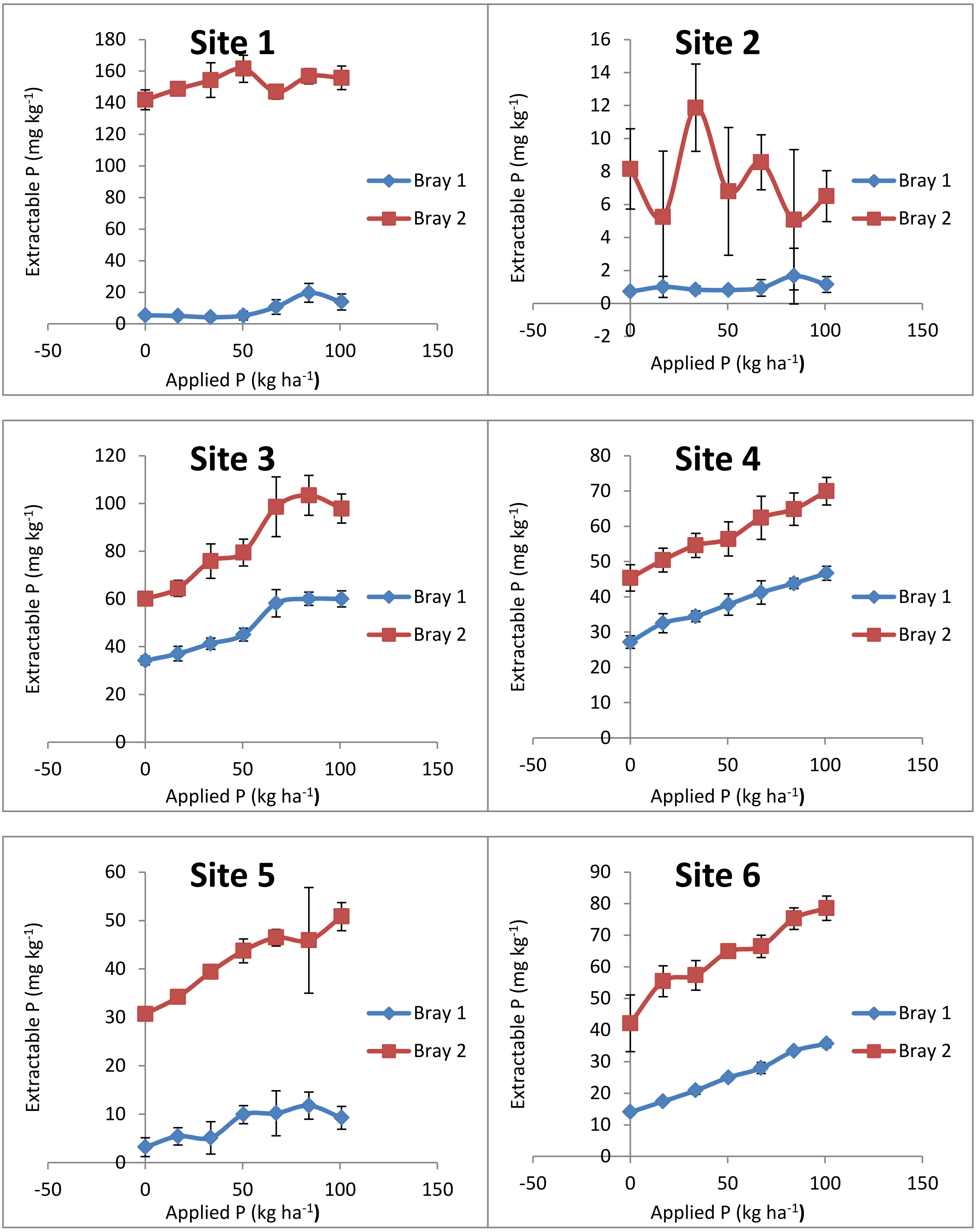


Figure 4.18 Comparison between Bray 1 and Bray 2 extractable P facing applied P by seven elevated levels done by laboratory B. Standard deviation is indicated by error bars.

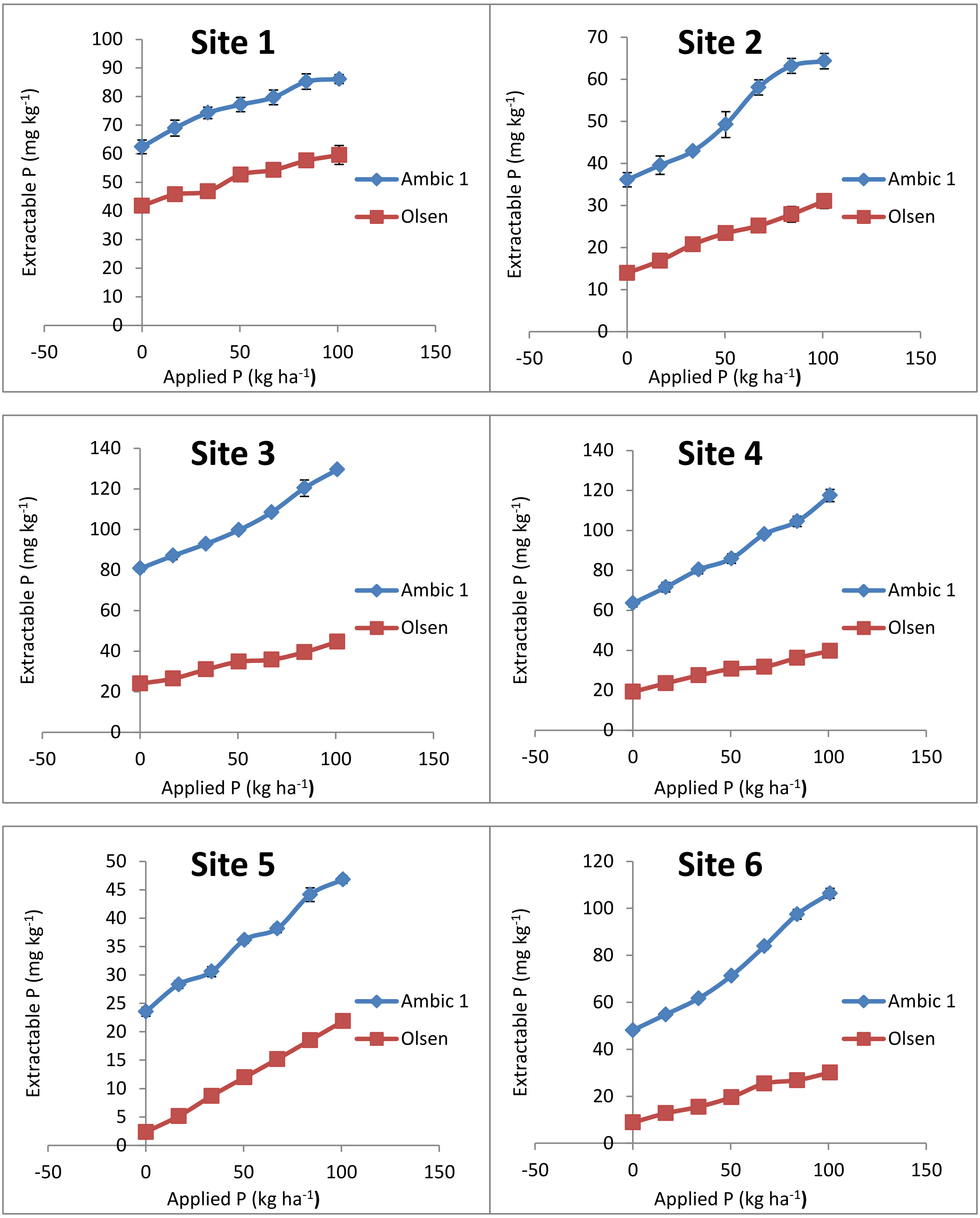


Figure 4.19 Comparison between Ambic 1 and Olsen extractable P facing applied P by seven elevated levels done by laboratory B. Standard deviation is indicated by error bars.

CHAPTER 5
SUMMARY AND RECOMMENDATIONS

5.1 Summary

The availability of P for plants is strongly related to the pH of soil. This is because pH influences the solubility of P. Moreover, it is general knowledge that soluble P is subject to fixation in either low or high pH soils. In addition, pH contributes, a variety of other soil properties to the process, for example clay mineralogy, organic matter, sesquioxides and carbonates.

A lack of knowledge on the behaviour of applied P in alkaline and calcareous soils exists in South Africa. This hampered the choice involving which agronomic soil P test is best to employ for fertiliser recommendation. An incorrect P recommendation may lead ultimately to unsustainable crop production on the one hand, and pollution of freshwater bodies on the other.

Carbonates of Ca and Mg occur commonly in soils of arid to semi-arid irrigation areas. Such areas are located in the southwestern Free State and Northern Cape of South Africa. Proper fertilisation of P is essential on these irrigated soils to ensure sustainable crop production with optimal yields.

The objectives of the study on soils from the Upper Orange River catchment were therefore to establish the amount of MAP required to increase the P extracted, using the Olsen, Bray 1, Mehlich 3 and Ambic 1 methods. Furthermore, the samples were analysed to obtain an indication of which of these four methods are potentially the most suitable for the determination of plant available P in the study soils. Lastly, the study involved the comparison of three laboratories with respect to extractable P contents measured by them, using the four methods. This kind of information could serve as a basis for the ultimate development of proper P fertiliser guidelines when cropping is practised under irrigation on alkaline and calcareous soils.

Soil samples from the orthic A horizon were collected at six sampling sites in the UOWMA below the Vanderkloof dam in the southwestern parts of the Free State and eastern parts of the Northern Cape. These samples were dried and sieved before

MAP was applied at different levels. Application levels of MAP were equivalent to 0, 16.8, 33.6, 50.4, 67.2, 84.0 and 100.8 kg P ha^{-1}. The fertilised samples were incubated at room temperature for two months. During this period the samples were subjected to several wetting and drying cycles to ensure equilibration of the applied P. After incubation the P in the soil samples was extracted with the Olsen, Bray 1, Mehlich 3 and Ambic 1 methods for colorimetric determination by the UFS laboratory. The samples were also analysed for extratable P by commercial laboratory A and B with the Olsen, Bray 1 and Mehlich 3 methods.

The six study soils have diverse mineralogical, physical and chemical characteristics. Based on these characteristics the soils may be categorised as low calcareous (<0.7% $CaCO_3$) with low to high P contents (9 to 24 mg kg^{-1} Olsen P), and high calcareous (>3.3% $CaCO_3$) with low to high P contents (2 to 42 mg kg^{-1} Olsen P). On account of this diversity the soils were deemed to be suitable for an investigation into the behaviour of applied P in alkaline and calcareous soils.

1. Estimated phosphorus requirement factors of study

Two way analyses of variance were done of the extractable P contents of each soil. This showed that the P contents of a soil varied significantly between extraction methods and application levels, as well as in their interactions. This is confirmed with regression analyses between applied P and extractable P. A variety of equations fit the data best, resulting in a wide range of R^2 values. The R^2 values ranged from 0.07 to 0.99 for calcareous sites with Bray 1 and Olsen, respectively. However, the R^2 values of these relationships were generally better for the Olsen (0.98 to 0.99) and Ambic 1 (0.97 to 0.99) than for the Bray 1 (0.07 to 0.97) and Mehlich 3 (0.77 to 0.98) extraction methods. The Mehlich 3 especially Bray 1 methods were not suitable for the extraction of P from the high calcareous soils as manifested in the PRF values estimated via linear regression analyses. The PRF values for the Bray 1 method showed unrealistically large variation of 1.8 to 384.6 kg P ha^{-1}. By contrast, the variation of PRF values for the Mehlich 3 method was very small, namely from 0.9 to 2.1 kg P ha^{-1}. For the Ambic 1 method (1.7 to 4.3 kg P ha^{-1}) and especially the Olsen method (4.6 to 6.1 kg P ha^{-1}) the PRF values were more in line with the values of other studies.

2. Relationships of extracted phosphorus with different methods

It is important to note that the soils' fixation capacity was not yet saturated at the highest P application of 100.8 kg ha^{-1} for all four extraction methods. The relationship between applied P and extractable P for the Olsen method was almost linear although best described by polynomial equations with R^2 values exceeding 0.98. Non-calcareous soils showed similar trends with the Bray 1 method than with the Olsen method. Poor relationships (R^2 values less than 0.57) were regressed with calcareous soils for the Bray 1 method. However, differences occurred between the sites, using the Mehlich 3 method. For this method the relationship (R^2 values of 0.63 to 0.98) between applied P and extractable P was best described by polynomial equations. By using data of the Ambic 1 method the following equations were regressed as optimal for soils at site 1: polynomial, site 2: exponential, site 3: polynomial, site 4: polynomial, site 5: linear and site 6: exponential. The R^2 values of these equations exceeded 0.97. Good relationships between P extracted with the four methods may have a positive influence on fertiliser recommendations when conversion of P contents is required. All four methods are employed for fertiliser recommendation on the study soils without proper thresholds, except for Olsen. Due to the different capacity of these methods to extract P, especially in calcareous soils, it is clear in most instances that the P contents of one extraction method cannot be converted to reflect the P contents of another method. This observation is to a lesser extent true for non-calcareous soils. However, the conversion of P contents between the Olsen and Ambic 1 methods for all six study soils was promising since R^2 values of 0.83 to 0.98 were realised.

3. Comparison of extractable P determined by three laboratories

The incubated soil samples were analysed by two commercial laboratories and the UFS laboratory for extractable P with the Olsen, Bray 1 and Mehlich 3 methods. In some instances, significant differences of extractable P with a specific method were observed between laboratories. These differences were of such a nature that fertiliser recommendations could not be made with confidence.

In the case of the Olsen method, the extractable P contents and coinciding standard deviations were highest for laboratory A, followed by laboratory B and laboratory UFS. Comparisons between the three laboratories for the Bray 1 method, especially for alkaline and calcareous soils were very poor since no clear trends evolved. However, some clear trends occurred between the laboratories when non-calcareous soils were analysed. Laboratory A and B reported more or less similar P contents for non-calcareous soils, while those of the UFS were much higher. The three laboratories suggested variable extractable P contents for the soils of the six sites, using the Mehlich 3 method. High standard deviations were estimated at laboratory A.

5.2 Conclusions

Results in the study proved that it is essential to use the P extraction method that fits the properties of a soil best. The mineralogical, physical and chemical properties of a soil ultimately prescribed which method is the most suitable to extract P. In agreement with other research, Olsen was evidently the most reliable method for extraction of P from the study soils, followed by the Ambic 1 method. The extraction of P by the Mehlich 3, especially Bray 1 method was very unreliable on the calcareous soils. These two methods performed better on the non-calcareous soils but not as effectively as both the Olsen and Ambic 1 methods. The phosphorus requirement factors estimated with data of Olsen ranged from 4.6 to 6.1 kg P ha^{-1} and those estimated with data of Ambic 1 varied from 1.7 to 4.3 kg P ha^{-1}. These values can serve as a basis for increasing the extractable P contents of the study soils with 1 mg kg^{-1}. An increase of the soils extractable P contents to optimal levels is essential to ensure sustainable cropping, and to reduce water pollution. However, field studies are warranted to establish threshold values for fertiliser recommendations for each extraction method. The data of this study showed that the conversion of extractable P contents between the methods is at all not reliable, except between Olsen and Ambic 1. Moreover, extractable P contents reported by the three laboratories differ significantly in some instances and this can jeopardise fertiliser recommendations.

5.3 Shortcomings of study

For a similar future study, higher levels of P application are necessary to ensure that the saturation point of P fixation in study soils is exceeded.

Soils with a more representative range of $CaCO_3$ and $MgCO_3$ are necessary to investigate the processes involved after the application of P, until the point of extraction of P by the different methods.

If possible, a greater number of extraction methods regarded as suitable for use on alkaline and calcareous soils should be included in a study of this nature.

5.4 Further research required

When analysed with the Mehlich 3 method, the extractable P contents of the incubated soils showed no constant pattern over the six different sites. This is not supportive of other research where the method gave acceptable results on calcareous soils, although more suitable for soils with a lower pH. Further research is therefore required to establish how the Mehlich 3 method performs on more soils with a wide range of $CaCO_3$ and $MgCO_3$ contents.

A study to determine the amount of $CaCO_3$ and $MgCO_3$ in soils below which P can be extracted reliably with the Bray 1 method, is required.

An investigation into different P pools and their relation to plant available P as determined by agronomic tests could enhance the understanding of P fixation and its release in alkaline and calcareous soils.

It is recommended that the relationships between different P pools and plant available P during crops' growing season be determined in soils high in $CaCO_3$ and $MgCO_3$.

A proper survey of soil analysis laboratories' performance with respect to determination of extractable P with different methods should be conducted.

Studies including different crops and P sources are essential for proper corrections of P in soils with various levels of $CaCO_3$ and $MgCO_3$.

Lastly, the challenge is not only to reduce the addition of P fertilisers, but also to maintain sustainable yields and decrease water eutrophication by adding more organic P fertilisers, or developing P fertilisers which are less subject to fixation.

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Appendix 1 Chemical analyses of bulk soils used in study

							Lime content (mg kg-^{1})				Extractable macronutrients cations (mg kg^{-1})									Extractable micronutrient cations (mg kg^{-1})			
Laboratory nr	**Total N (%)**	**Total C (%)**	**Organic C (%)**	**Inorganic C (%)**	**pH (H20)**	**pH (KCl)**	**Ca**	**Mg**	**$CaCO_3$**	**$MgCO_3$**	**Soluble Ca**	**Exhangeable Ca**	**Soluble Mg**	**Exchangeable Mg**	**Soluble K**	**Exchangeable K**	**Soluble Na**	**Exchangeable Na**	**CEC ($_c$molc kg^{-1})**	**Cu**	**Mn**	**Fe**	**Zn**
Site 1																							
1	0.09	1.60	0.60	1	7.71	8.46	25600	2100	64000	7350	104.90	5950	7.14	257.50	2.39	280	7.52	22.50	2324.75	0.81	5.45	3.19	7.82
2	0.10	1.57	0.61	0.96	7.77	8.50	26100	2200	65250	7700	112.80	6225	7.35	257.50	2.48	285	7.44	22.50	2319	0.79	5.32	3.01	7.52
3	0.10	1.58	0.60	0.98	7.65	8.45	25900	2300	64750	8050	111.80	6000	7.15	247.50	2.27	295	7.01	22.50	2332.75	0.76	5.41	3.02	7.38
Average	**0.10**	**1.58**	**0.60**	**0.98**	**7.71**	**8.47**	**25866.67**	**2200**	**64666.67**	**7700**	**109.83**	**6058.33**	**7.22**	**254.17**	**2.38**	**286.67**	**7.32**	**22.50**	**2325.50**	**0.79**	**5.39**	**3.07**	**7.57**
Site 2																							
1	0.09	2.03	0.41	1.62	7.87	8.30	49200	3200	123000	11200	67.28	7975	3.73	252.50	0.56	187.50	5.29	30	3156.25	0.70	4.33	3.22	3
2	0.08	1.96	0.45	1.51	7.86	8.46	50100	3400	125250	11900	68.44	8275	3.74	257.50	0.51	187.50	5.57	27.50	3295.75	0.69	4.38	3.14	3.03
3	0.08	2	0.43	1.57	7.77	8.55	49500	3200	123750	11200	68.88	8125	3.86	250	0.52	182.50	5.55	27.50	3308.50	0.73	4.64	3.25	3.09
Average	**0.09**	**2**	**0.43**	**1.57**	**7.83**	**8.44**	**49600**	**3266.67**	**124000**	**11433.33**	**68.20**	**8125**	**3.78**	**253.33**	**0.53**	**185.83**	**5.47**	**28.33**	**3253.50**	**0.71**	**4.45**	**3.21**	**3.04**
Site 3																							
1	0.07	0.54	0.40	0.14	7.21	8.08	2570	1700	6425	5950	17.80	1217.50	2.08	255	0.87	255	4.75	25	1939	1.21	6.58	12.77	2.40
2	0.08	0.51	0.40	0.11	7.10	8.07	2430	1700	6075	5950	17.22	1197.50	2.24	252.50	0.87	255	4.75	25	2070	1.12	6.62	12.47	2.38
3	0.08	0.54	0.41	0.13	7.04	8.12	2440	1600	6100	5600	16.88	1207.50	2.13	257.50	0.82	260	4.49	25.50	2108.25	1.16	6.48	12.92	2.34
Average	**0.08**	**0.53**	**0.40**	**0.13**	**7.12**	**8.09**	**2480**	**1666.67**	**6200**	**5833.33**	**17.30**	**1207.50**	**2.15**	**255**	**0.85**	**256.67**	**4.66**	**25.17**	**2039.08**	**1.17**	**6.56**	**12.72**	**2.38**

Site 4																							
1	0.07	0.60	0.18	0.42	7.30	8.31	2680	1800	6700	6300	10	1352.50	1.30	302.50	0.28	172.50	4.65	35	2372	1.25	6.23	10.24	1.45
2	0.07	0.61	0.21	0.40	7.32	8.24	2560	1700	6400	5950	10.22	1425	1.31	302.50	0.29	180	4.67	37.50	2314.50	1.26	6.50	10.54	1.46
3	0.07	0.59	0.20	0.39	7.30	8.26	2590	1800	6475	6300	10.18	1417.50	1.33	300	0.27	172.50	4.88	35	2446.25	1.21	6.09	10.18	1.41
Average	**0.07**	**0.60**	**0.20**	**0.40**	**7.31**	**8.27**	**2610**	**1766.67**	**6525**	**6183.33**	**10.13**	**1398.33**	**1.31**	**301.67**	**0.28**	**175**	**4.74**	**35.83**	**2377.58**	**1.24**	**6.27**	**10.32**	**1.44**
Site 5																							
1	0.08	1.01	0.36	0.65	8.00	8.73	13000	2000	5000	7000	28.16	5675	1.91	170	0.41	162.50	2.82	12.50	2295.75	0.63	5.11	2.66	0.49
2	0.08	1.03	0.39	0.64	8.16	8.68	13100	2100	5250	7350	27.92	5850	1.86	162.50	0.41	157.50	2.87	12.50	2516.75	0.61	5.13	2.91	0.52
3	0.08	1.02	0.36	0.66	8.13	8.64	13200	2100	5250	7350	27.06	5950	1.83	160	0.45	157.50	2.79	12.50	2307	0.62	5.46	2.87	0.52
Average	**0.08**	**1.02**	**0.37**	**0.65**	**8.10**	**8.68**	**13100**	**2066.67**	**5166.67**	**7233.33**	**27.71**	**5825**	**1.87**	**164.17**	**0.42**	**159.17**	**2.83**	**12.50**	**2373.17**	**0.62**	**5.23**	**2.81**	**0.51**
Site 6																							
1	0.06	0.27	0.17	0.10	8.19	8.89	2900	2000	7250	7000	18.30	1217.50	1.95	185	0.94	222.50	4.35	23	2145.75	0.53	2.39	4.09	0.78
2	0.06	0.27	0.18	0.09	8.15	8.60	2780	1900	6950	6650	17.86	1227.50	1.82	182.50	0.88	220	4.03	22.50	2289.75	0.58	2.39	3.73	0.76
3	0.05	0.29	0.18	0.11	8.19	8.85	3080	2000	7700	7000	17.68	1260	1.94	187.50	0.92	220	4.35	22.50	2208	0.58	2.57	3.86	0.84
Average	**0.06**	**0.28**	**0.18**	**0.10**	**8.18**	**8.78**	**2920**	**1966.67**	**7300**	**6883.33**	**17.95**	**1235**	**1.90**	**185**	**0.92**	**220.83**	**4.24**	**22.67**	**2214.50**	**0.56**	**2.45**	**3.89**	**0.79**

Appendix 2 Extractable P contents (mg kg^{-1}) analysed after the treatment of MAP

Site	P level (kg ha^{-1})	Sample number	Phosphorus extraction methods (mg kg^{-1})			
			Olsen P	Bray 1	P Mehlich 3	Ambic 1
1	0	1	40.72	1.10	229.68	60.13
		2	42.45	1.27	226.41	64.15
		3	40.89	1.22	241.58	60.29
		4	42.04	1.27	230.58	65.77
		5	42.79	1.27	253.78	60.13
		6	40.89	1.33	244.26	60.76
		7	43.83	1.67	238.31	65.88
		8	44.18	1.56	246.34	63.16
		9	41.18	1.56	242.77	60.24
		10	39.21	1.67	258.83	63.79
		Average	**41.82**	**1.39**	**241.26**	**62.43**
		STDev	**1.53**	**0.20**	**10.43**	**2.38**
		SE	**0.49**	**0.06**	**3.30**	**0.75**
1	16.8	11	47.53	1.73	236.23	65.41
		12	45.62	1.56	234.74	67.08
		13	43.83	1.56	243.37	66.40
		14	41.76	1.73	245.45	68.18
		15	43.31	1.56	257.64	68.28
		16	46.84	1.56	259.13	72.98
		17	49.72	1.79	253.18	67.91
		18	45.91	1.79	247.83	70.00
		19	45.91	1.73	265.08	69.06
		20	47.30	1.85	261.51	74.23
		Average	**45.77**	**1.69**	**250.42**	**68.95**
		STDev	**2.31**	**0.11**	**10.55**	**2.79**
		SE	**0.73**	**0.04**	**3.34**	**0.88**
1	33.6	21	46.26	1.85	275.79	72.51
		22	46.20	1.90	272.81	70.58
		23	45.57	1.79	277.87	75.96
		24	45.16	1.79	287.69	74.39
		25	47.36	2.02	290.36	76.53
		26	49.78	2.02	299.58	76.43
		27	48.05	2.07	294.53	72.18
		28	44.82	1.96	302.56	74.13
		29	48.91	2.13	303.15	75.07
		30	46.37	2.02	310.59	75.44
		Average	**46.85**	**1.95**	**291.49**	**74.32**
		STDev	**1.64**	**0.12**	**12.89**	**1.99**
		SE	**0.52**	**0.04**	**4.08**	**0.63**

1	50.4	31	51.92	2.42	322.78	73.24
		32	53.07	2.30	318.32	76.22
		33	52.78	2.25	319.51	75.38
		34	53.53	2.36	321.30	74.23
		35	53.19	2.25	329.92	77.84
		36	50.94	2.13	330.52	80.66
		37	50.53	2.36	325.76	76.53
		38	53.07	2.42	334.38	78.52
		39	53.71	2.19	335.87	79.46
		40	54.40	2.47	335.87	79.87
		Average	**52.71**	**2.31**	**327.42**	**77.20**
		STDev	**1.23**	**0.11**	**6.78**	**2.48**
		SE	**0.39**	**0.03**	**2.15**	**0.79**
1	67.2	41	51.74	2.65	276.68	79.67
		42	55.44	2.76	277.57	78.99
		43	52.32	2.76	277.57	79.82
		44	52.03	2.47	288.28	79.82
		45	56.94	2.65	276.98	79.61
		46	52.55	2.70	288.28	76.69
		47	54.86	1.90	293.04	77.00
		48	53.94	1.85	286.20	78.31
		49	58.27	1.85	292.74	81.81
		50	55.15	2.02	309.99	85.57
		Average	**54.32**	**2.36**	**286.73**	**79.73**
		STDev	**2.21**	**0.40**	**10.47**	**2.53**
		SE	**0.70**	**0.13**	**3.31**	**0.80**
1	84	51	57.75	1.79	322.78	85.10
		52	57.81	1.79	322.19	84.21
		53	58.79	1.85	309.70	84.16
		54	57.00	1.79	311.48	83.58
		55	55.32	1.73	321.89	92.67
		56	56.77	2.07	320.11	85.41
		57	56.30	1.73	307.91	83.37
		58	57.69	1.85	326.65	84.21
		59	59.77	2.59	318.02	84.99
		60	58.96	2.02	326.06	84.47
		Average	**57.62**	**1.92**	**318.68**	**85.22**
		STDev	**1.33**	**0.26**	**6.74**	**2.70**
		SE	**0.42**	**0.08**	**2.13**	**0.85**

1	100.8	61	54.17	1.33	307.61	85.41
		62	53.36	1.33	312.37	88.54
		63	62.66	1.62	303.45	85.78
		64	62.19	1.39	309.99	85.15
		65	59.54	1.45	312.67	86.51
		66	62.48	1.56	302.56	89.22
		67	60.75	1.73	309.10	84.26
		68	59.08	1.45	312.37	85.15
		69	59.83	1.62	311.18	85.78
		70	61.56	1.56	332.60	85.31
		Average	**59.56**	**1.50**	**311.39**	**86.11**
		STDev	**3.30**	**0.13**	**8.27**	**1.58**
		SE	**1.05**	**0.04**	**2.62**	**0.50**
2	0	71	14.50	0.25	75.61	34.05
		72	13.81	0.36	78.58	36.40
		73	14.73	0.36	79.18	36.92
		74	14.10	0.47	77.10	36.40
		75	14.73	0.47	77.99	37.45
		76	13.18	0.53	78.88	34.83
		77	13.12	0.65	77.39	37.97
		78	14.27	0.36	78.58	35.10
		79	13.18	0.59	78.88	38.49
		80	13.75	0.36	77.99	33.53
		Average	**13.94**	**0.44**	**78.02**	**36.11**
		STDev	**0.63**	**0.12**	**1.08**	**1.68**
		SE	**0.20**	**0.04**	**0.34**	**0.53**
2	16.8	81	16.12	0.13	80.37	36.92
		82	17.39	0.47	84.83	36.66
		83	16.29	0.02	83.05	41.36
		84	17.27	0.47	79.77	43.19
		85	16.47	0.25	83.64	39.01
		86	17.10	0.02	85.43	40.84
		87	16.18	0.07	80.07	40.58
		88	16.35	0.25	82.75	40.32
		89	17.16	0.65	84.53	40.06
		90	18.37	0.07	82.45	36.92
		Average	**16.87**	**0.24**	**82.69**	**39.59**
		STDev	**0.72**	**0.22**	**2.04**	**2.17**
		SE	**0.23**	**0.07**	**0.64**	**0.69**

2	33.6	91	20.16	0.19	86.32	42.67
		92	20.57	1.22	86.62	42.67
		93	20.97	1.22	88.99	42.41
		94	20.80	0.19	88.40	43.71
		95	21.66	0.30	88.10	42.67
		96	20.62	0.19	93.75	42.15
		97	22.07	0.13	90.18	42.93
		98	20.80	0.30	88.40	43.71
		99	20.33	0.47	89.29	43.45
		100	19.47	0.25	88.70	42.93
		Average	**20.74**	**0.45**	**88.88**	**42.93**
		STDev	**0.73**	**0.42**	**2.07**	**0.54**
		SE	**0.23**	**0.13**	**0.65**	**0.17**
2	50.4	101	22.88	0.19	96.13	42.93
		102	22.82	0.42	96.13	45.02
		103	23.57	2.70	107.14	50.50
		104	22.12	0.19	97.92	49.20
		105	24.95	0.53	97.03	49.20
		106	22.64	3.96	97.92	52.59
		107	25.13	3.45	97.03	49.20
		108	23.80	1.10	95.24	50.24
		109	22.70	2.53	102.68	51.55
		110	23.39	0.93	101.49	52.07
		Average	**23.40**	**1.60**	**98.87**	**49.25**
		STDev	**0.99**	**1.42**	**3.75**	**3.07**
		SE	**0.31**	**0.45**	**1.19**	**0.97**
2	67.2	111	25.13	0.36	131.83	57.81
		112	25.76	0.07	115.76	60.43
		113	26.28	0.47	140.15	56.77
		114	24.55	0.25	131.53	57.03
		115	24.90	0.70	140.45	55.99
		116	24.90	0.30	139.26	59.12
		117	25.65	0.13	127.07	57.55
		118	25.42	0.25	126.17	61.73
		119	25.42	2.02	122.61	56.77
		120	23.74	0.99	124.39	57.55
		Average	**25.17**	**0.55**	**129.92**	**58.08**
		STDev	**0.71**	**0.58**	**8.27**	**1.81**
		SE	**0.22**	**0.18**	**2.62**	**0.57**

2	84	121	27.03	0.36	141.05	64.60
		122	26.97	2.99	134.21	64.08
		123	25.30	0.30	130.64	64.60
		124	26.51	0.65	137.78	63.82
		125	30.50	0.42	138.67	64.08
		126	31.25	2.93	141.64	63.82
		127	27.67	0.53	131.23	63.30
		128	29.17	0.36	135.10	63.82
		129	28.24	1.39	140.75	59.90
		130	26.51	1.27	145.81	59.90
		Average	**27.92**	**1.12**	**137.69**	**63.19**
		STDev	**1.88**	**1.04**	**4.87**	**1.78**
		SE	**0.60**	**0.33**	**1.54**	**0.56**
2	100.8	131	32.58	0.76	158.89	61.99
		132	32.11	0.25	160.08	63.82
		133	32.52	1.33	176.15	64.60
		134	30.84	3.50	177.04	63.30
		135	32.00	2.70	181.20	66.69
		136	32.23	5.16	174.66	61.73
		137	31.88	0.53	187.15	66.69
		138	30.21	0.47	172.58	63.30
		139	27.61	4.13	174.06	64.86
		140	28.42	2.13	179.12	66.43
		Average	**31.04**	**2.10**	**174.09**	**64.34**
		STDev	**1.77**	**1.72**	**8.75**	**1.84**
		SE	**0.56**	**0.55**	**2.77**	**0.58**
3	0	141	24.55	81.26	77.10	81.32
		142	24.20	81.43	75.01	82.36
		143	24.09	81.38	76.50	80.53
		144	23.63	81.66	75.01	78.97
		145	23.97	82.12	77.39	77.66
		146	23.34	82.18	74.42	80.79
		147	24.26	83.32	76.80	81.05
		148	24.49	82.75	74.42	82.88
		149	24.20	83.43	75.31	79.75
		150	23.97	85.78	75.91	82.62
		Average	**24.07**	**82.53**	**75.79**	**80.79**
		STDev	**0.37**	**1.38**	**1.11**	**1.66**
		SE	**0.12**	**0.44**	**0.35**	**0.53**

3	16.8	151	24.67	87.43	74.42	86.80
		152	25.36	88.40	78.29	86.54
		153	26.45	88.80	78.29	86.54
		154	25.01	81.83	75.61	87.84
		155	26.34	88.29	81.26	89.41
		156	27.26	87.09	84.53	90.72
		157	27.03	88.52	83.64	86.80
		158	27.03	89.95	75.31	85.23
		159	27.55	86.86	82.45	84.71
		160	28.07	89.26	79.77	86.02
		Average	**26.48**	**87.64**	**79.36**	**87.06**
		STDev	**1.14**	**2.25**	**3.58**	**1.83**
		SE	**0.36**	**0.71**	**1.13**	**0.58**
3	33.6	161	30.73	96.12	114.57	94.63
		162	30.44	94.52	99.11	94.89
		163	30.09	96.12	109.82	93.59
		164	29.75	94.23	114.28	92.54
		165	32.34	98.35	113.98	93.85
		166	32.23	94.23	114.57	92.54
		167	30.96	93.49	127.96	91.50
		168	30.44	98.92	108.33	91.76
		169	32.11	98.86	117.25	92.02
		170	30.61	94.86	108.33	91.50
		Average	**30.97**	**95.97**	**112.82**	**92.88**
		STDev	**0.93**	**2.06**	**7.42**	**1.27**
		SE	**0.29**	**0.65**	**2.35**	**0.40**
3	50.4	171	35.12	95.49	118.74	99.07
		172	36.79	96.29	120.23	98.81
		173	35.00	98.40	125.58	99.60
		174	36.10	96.92	135.40	102.21
		175	34.42	98.69	131.83	99.07
		176	34.19	98.12	131.83	99.33
		177	35.00	100.35	135.69	100.12
		178	34.36	98.17	133.91	99.07
		179	33.56	99.20	132.12	99.86
		180	34.13	98.86	129.74	99.86
		Average	**34.87**	**98.05**	**129.51**	**99.70**
		STDev	**0.97**	**1.44**	**6.03**	**0.98**
		SE	**0.31**	**0.46**	**1.91**	**0.31**

3	67.2	181	36.21	104.40	142.24	108.21
		182	37.25	104.75	146.10	107.17
		183	35.17	104.40	149.67	109.26
		184	34.25	107.26	142.83	110.30
		185	33.21	104.80	144.32	110.04
		186	35.52	103.32	144.02	106.91
		187	36.33	106.29	149.97	107.95
		188	37.83	108.23	144.62	106.91
		189	36.85	105.49	150.57	110.56
		190	35.58	105.32	156.51	107.43
		Average	**35.82**	**105.42**	**147.09**	**108.47**
		STDev	**1.39**	**1.47**	**4.49**	**1.45**
		SE	**0.44**	**0.46**	**1.42**	**0.46**
3	84	191	39.62	108.17	164.25	113.70
		192	40.77	107.37	164.54	114.74
		193	39.85	108.57	160.08	117.61
		194	40.60	110.69	162.76	119.18
		195	38.81	106.34	161.57	119.96
		196	37.54	106.74	168.41	123.10
		197	38.70	105.37	170.79	124.66
		198	38.58	107.14	177.93	122.84
		199	40.72	107.60	167.52	123.88
		200	40.20	110.86	182.69	124.66
		Average	**39.54**	**107.89**	**168.05**	**120.43**
		STDev	**1.09**	**1.77**	**7.30**	**4.06**
		SE	**0.35**	**0.56**	**2.31**	**1.28**
3	100.8	201	43.26	112.29	179.12	129.36
		202	43.49	112.69	176.44	129.89
		203	45.16	108.80	178.52	129.89
		204	44.12	113.03	180.61	130.41
		205	45.57	110.06	179.42	130.67
		206	45.62	115.14	172.87	128.06
		207	44.47	113.66	180.90	130.15
		208	44.18	114.23	184.77	129.63
		209	45.05	116.06	183.28	128.58
		210	45.51	114.74	186.56	128.58
		Average	**44.64**	**113.07**	**180.25**	**129.52**
		STDev	**0.87**	**2.26**	**4.00**	**0.86**
		SE	**0.27**	**0.71**	**1.26**	**0.27**

4	0	211	18.49	66.35	63.71	61.99
		212	18.49	66.35	62.52	63.30
		213	19.35	66.64	60.74	64.34
		214	18.20	67.26	63.12	66.69
		215	19.53	67.32	61.93	62.78
		216	19.06	67.21	61.93	60.95
		217	20.22	67.61	62.82	62.51
		218	19.99	67.15	60.44	62.78
		219	19.82	67.78	61.63	64.34
		220	19.35	63.55	66.09	65.91
		Average	**19.25**	**66.72**	**62.49**	**63.56**
		STDev	**0.68**	**1.22**	**1.62**	**1.77**
		SE	**0.22**	**0.38**	**0.51**	**0.56**
4	16.8	221	22.01	73.44	71.15	70.61
		222	22.76	75.61	65.79	71.65
		223	24.15	75.89	64.90	69.83
		224	23.22	75.83	62.22	67.48
		225	23.22	77.26	64.90	70.35
		226	23.51	71.89	64.60	70.87
		227	25.88	75.83	64.60	73.74
		228	24.61	76.01	66.09	75.83
		229	22.70	76.18	64.31	73.48
		230	22.93	77.95	64.31	71.92
		Average	**23.50**	**75.59**	**65.29**	**71.58**
		STDev	**1.11**	**1.75**	**2.30**	**2.34**
		SE	**0.35**	**0.55**	**0.73**	**0.74**
4	33.6	231	26.34	82.86	74.72	78.97
		232	27.49	82.23	78.58	79.49
		233	26.45	82.46	69.96	83.14
		234	27.09	81.49	69.36	78.44
		235	28.36	84.35	69.07	77.92
		236	27.55	84.29	70.26	77.92
		237	27.90	85.20	73.53	80.79
		238	27.44	85.89	72.93	82.36
		239	27.67	82.29	72.93	81.84
		240	28.01	82.29	70.55	82.36
		Average	**27.43**	**83.34**	**72.19**	**80.32**
		STDev	**0.65**	**1.48**	**2.97**	**2.01**
		SE	**0.20**	**0.47**	**0.94**	**0.64**

4	50.4	241	29.57	90.40	76.50	83.41
		242	29.80	88.29	76.50	84.19
		243	30.15	90.52	74.72	86.54
		244	31.25	85.49	74.12	83.67
		245	31.82	90.40	74.42	84.19
		246	29.80	91.32	75.31	84.19
		247	29.69	88.80	75.61	86.80
		248	29.34	85.60	76.80	87.32
		249	31.25	90.58	72.93	88.37
		250	34.31	90.18	73.23	90.46
		Average	**30.70**	**89.16**	**75.01**	**85.91**
		STDev	**1.52**	**2.10**	**1.37**	**2.36**
		SE	**0.48**	**0.66**	**0.43**	**0.75**
4	67.2	251	31.02	91.38	70.55	97.51
		252	32.69	91.55	78.58	96.98
		253	31.88	90.12	73.53	98.29
		254	32.11	89.03	76.50	98.29
		255	30.44	91.32	78.29	96.46
		256	32.06	89.72	76.50	95.94
		257	31.30	88.18	79.48	100.38
		258	32.06	88.92	79.77	99.33
		259	31.82	90.35	93.46	98.55
		260	32.17	91.15	79.48	99.33
		Average	**31.76**	**90.17**	**78.61**	**98.11**
		STDev	**0.65**	**1.19**	**5.99**	**1.39**
		SE	**0.21**	**0.38**	**1.89**	**0.44**
4	84	261	33.90	97.72	130.04	100.64
		262	35.81	96.12	125.58	101.68
		263	34.36	93.09	118.44	103.77
		264	35.52	96.06	119.63	104.30
		265	36.85	97.72	122.01	104.03
		266	38.12	96.69	116.06	103.25
		267	36.79	97.66	110.41	104.30
		268	36.56	98.69	116.36	106.91
		269	36.56	97.15	113.68	109.26
		270	37.66	98.00	118.44	107.17
		Average	**36.21**	**96.89**	**119.07**	**104.53**
		STDev	**1.34**	**1.57**	**5.70**	**2.60**
		SE	**0.42**	**0.50**	**1.80**	**0.82**

4	100.8	271	38.12	103.03	128.55	112.91
		272	39.10	103.20	136.88	113.96
		273	37.77	103.37	133.91	114.48
		274	39.97	104.46	140.45	116.83
		275	40.20	105.09	139.86	118.14
		276	40.66	104.52	126.17	118.92
		277	41.58	105.43	147.00	120.75
		278	41.64	106.34	143.43	122.57
		279	39.04	104.29	144.32	117.35
		280	38.75	104.80	143.43	118.66
		Average	**39.68**	**104.45**	**138.40**	**117.46**
		STDev	**1.35**	**1.05**	**6.95**	**3.04**
		SE	**0.43**	**0.33**	**2.20**	**0.96**
5	0	281	2.61	1.45	25.94	24.38
		282	2.38	12.19	22.96	22.50
		283	2.32	9.62	23.85	22.71
		284	2.49	8.59	22.67	22.39
		285	2.09	15.04	23.85	23.86
		286	2.15	7.62	24.45	24.07
		287	2.26	8.19	22.96	23.02
		288	2.32	64.58	21.77	24.27
		289	2.44	11.44	25.04	24.17
		290	2.44	12.59	25.34	23.96
		Average	**2.35**	**15.13**	**23.88**	**23.53**
		STDev	**0.16**	**17.76**	**1.32**	**0.79**
		SE	**0.05**	**5.62**	**0.42**	**0.25**
5	16.8	291	5.15	9.44	32.18	28.87
		292	5.03	8.02	32.78	28.77
		293	5.09	7.33	31.29	27.83
		294	4.98	11.67	30.99	27.62
		295	4.98	9.10	32.78	28.35
		296	4.98	10.07	31.89	29.39
		297	5.32	12.13	32.18	27.72
		298	5.27	0.65	32.18	28.98
		299	5.32	4.42	32.78	27.20
		300	5.32	0.42	33.08	28.56
		Average	**5.14**	**7.32**	**32.21**	**28.33**
		STDev	**0.15**	**4.20**	**0.68**	**0.71**
		SE	**0.05**	**1.33**	**0.21**	**0.22**

5	33.6	301	7.81	15.39	39.62	30.86
		302	8.09	8.64	39.92	30.02
		303	8.67	12.02	41.70	29.39
		304	8.96	2.02	41.11	31.80
		305	8.96	11.33	41.11	30.75
		306	8.96	14.07	42.00	31.48
		307	8.96	13.16	39.02	29.08
		308	9.25	15.16	41.70	30.44
		309	8.44	12.59	43.19	31.17
		310	8.96	13.96	41.70	30.75
		Average	**8.71**	**11.83**	**41.11**	**30.57**
		STDev	**0.46**	**3.98**	**1.25**	**0.87**
		SE	**0.14**	**1.26**	**0.40**	**0.27**
5	50.4	311	12.60	17.04	52.41	37.23
		312	13.00	18.76	50.62	36.29
		313	12.60	0.70	47.65	36.39
		314	13.35	0.70	47.65	36.39
		315	12.31	5.90	50.33	35.45
		316	11.56	14.87	49.43	35.03
		317	10.81	1.05	48.84	36.60
		318	10.81	4.13	49.43	35.97
		319	11.44	5.44	46.76	36.29
		320	11.10	0.82	47.35	36.08
		Average	**11.96**	**6.94**	**49.05**	**36.17**
		STDev	**0.93**	**7.19**	**1.76**	**0.60**
		SE	**0.29**	**2.28**	**0.56**	**0.19**
5	67.2	321	15.89	18.36	62.22	38.27
		322	15.83	24.99	61.93	38.06
		323	14.27	24.70	60.44	38.17
		324	16.76	25.21	62.82	38.48
		325	14.91	26.36	61.93	38.17
		326	16.29	21.61	60.44	39.52
		327	16.35	18.99	62.82	37.33
		328	15.72	19.61	62.52	37.54
		329	13.93	26.53	61.63	37.12
		330	15.77	20.41	61.04	39.00
		Average	**15.57**	**22.68**	**61.78**	**38.17**
		STDev	**0.92**	**3.20**	**0.89**	**0.73**
		SE	**0.29**	**1.01**	**0.28**	**0.23**

5	84	331	18.78	17.84	61.63	46.42
		332	18.20	12.59	61.63	43.29
		333	18.49	15.33	63.71	43.60
		334	18.78	22.41	62.52	42.66
		335	17.51	10.64	53.60	44.33
		336	17.62	15.21	54.49	44.85
		337	19.24	18.01	58.36	43.49
		338	19.06	18.19	58.95	42.97
		339	18.20	12.13	61.33	44.12
		340	19.35	2.53	60.14	45.69
		Average	**18.52**	**14.49**	**59.64**	**44.14**
		STDev	**0.64**	**5.46**	**3.35**	**1.21**
		SE	**0.20**	**1.73**	**1.06**	**0.38**
5	100.8	341	21.49	25.38	73.23	47.15
		342	21.09	2.30	74.42	46.94
		343	23.68	12.07	74.12	46.73
		344	23.39	9.22	72.04	46.52
		345	21.20	17.84	75.01	46.42
		346	21.49	22.81	74.72	46.21
		347	22.70	1.10	74.72	46.63
		348	20.51	17.16	73.23	46.63
		349	21.37	15.10	72.93	46.21
		350	21.66	1.27	74.72	48.51
		Average	**21.86**	**12.43**	**73.91**	**46.79**
		STDev	**1.04**	**8.82**	**0.99**	**0.67**
		SE	**0.33**	**2.79**	**0.31**	**0.21**
6	0	351	8.96	44.24	46.46	47.99
		352	9.31	45.04	48.25	49.03
		353	8.56	44.93	47.95	47.99
		354	8.96	44.13	49.43	49.03
		355	8.96	44.70	50.33	47.78
		356	9.36	45.27	49.43	47.15
		357	8.04	44.30	49.73	47.67
		358	8.38	45.21	47.35	47.67
		359	8.38	47.10	48.54	48.40
		360	9.54	46.47	49.43	48.51
		Average	**8.85**	**45.14**	**48.69**	**48.12**
		STDev	**0.49**	**0.97**	**1.20**	**0.61**
		SE	**0.16**	**0.31**	**0.38**	**0.19**

6	16.8	361	11.73	52.24	51.22	54.46
		362	12.54	53.55	54.79	54.77
		363	12.83	53.72	55.68	53.73
		364	13.35	54.18	53.30	54.15
		365	12.54	54.30	52.41	54.04
		366	12.83	53.50	52.11	55.30
		367	13.98	53.32	54.49	53.73
		368	13.00	53.44	52.11	55.30
		369	12.66	54.92	53.00	56.76
		370	12.14	56.29	52.71	55.82
		Average	**12.76**	**53.95**	**53.18**	**54.81**
		STDev	**0.62**	**1.09**	**1.40**	**0.99**
		SE	**0.20**	**0.34**	**0.44**	**0.31**
6	33.6	371	15.43	57.95	53.00	60.52
		372	14.56	60.41	54.79	62.19
		373	17.10	61.55	55.98	61.04
		374	15.02	63.55	52.71	62.19
		375	15.66	63.15	53.60	61.98
		376	14.85	62.29	55.38	60.52
		377	15.25	60.24	55.68	60.94
		378	14.04	60.87	53.60	62.19
		379	16.24	66.12	56.28	61.77
		380	16.93	66.06	54.79	63.03
		Average	**15.51**	**62.22**	**54.58**	**61.64**
		STDev	**0.99**	**2.59**	**1.28**	**0.84**
		SE	**0.31**	**0.82**	**0.40**	**0.27**
6	50.4	381	19.64	71.89	56.87	70.13
		382	19.82	72.18	55.68	70.65
		383	17.97	71.89	53.30	72.01
		384	19.93	71.89	53.60	71.70
		385	19.99	72.58	55.98	70.65
		386	19.99	70.69	56.87	71.49
		387	19.58	72.52	54.49	70.86
		388	19.01	69.04	54.79	71.59
		389	20.16	72.46	53.00	71.38
		390	19.76	68.29	54.49	72.64
		Average	**19.58**	**71.34**	**54.91**	**71.31**
		STDev	**0.65**	**1.52**	**1.40**	**0.74**
		SE	**0.21**	**0.48**	**0.44**	**0.24**

6	67.2	391	24.55	78.86	58.06	84.13
		392	25.01	80.81	55.38	82.14
		393	25.59	83.61	54.49	82.25
		394	24.90	80.63	53.60	83.19
		395	26.40	82.23	50.62	82.56
		396	24.09	81.66	53.00	84.23
		397	27.32	83.55	73.53	85.27
		398	24.67	80.69	77.39	85.38
		399	25.53	81.95	48.54	83.81
		400	25.88	81.32	55.68	85.38
		Average	**25.39**	**81.53**	**58.03**	**83.83**
		STDev	**0.96**	**1.42**	**9.60**	**1.27**
		SE	**0.30**	**0.45**	**3.04**	**0.40**
6	84	401	26.57	88.00	83.05	96.98
		402	26.74	89.78	89.29	96.72
		403	25.18	93.32	89.29	95.42
		404	26.57	90.75	85.72	93.85
		405	27.44	93.55	79.18	96.98
		406	27.90	88.69	79.48	99.86
		407	27.49	98.12	73.83	96.98
		408	27.96	89.95	77.69	98.29
		409	25.99	94.63	80.96	100.90
		410	26.51	92.63	84.83	98.29
		Average	**26.84**	**91.94**	**82.33**	**97.43**
		STDev	**0.87**	**3.10**	**5.04**	**2.04**
		SE	**0.28**	**0.98**	**1.60**	**0.64**
6	100.8	411	29.17	97.26	95.54	104.03
		412	29.75	99.37	101.78	103.25
		413	29.75	99.66	100.89	107.17
		414	30.03	101.26	97.03	103.25
		415	32.75	99.72	104.76	106.91
		416	31.30	99.43	108.63	107.17
		417	29.17	101.32	110.41	108.21
		418	29.28	102.23	105.95	107.17
		419	29.80	101.55	101.49	107.17
		420	29.75	100.97	103.87	109.52
		Average	**30.08**	**100.28**	**103.03**	**106.38**
		STDev	**1.12**	**1.46**	**4.70**	**2.14**
		SE	**0.35**	**0.46**	**1.49**	**0.68**

STDev: Standard deviation
SE: Standard error