Improving phosphate fertilizer recommendations using soil phosphorus buffer capacity and evaluation of various P extraction tests on a variety of South-African soils

By

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Summary:

Phosphorus (P) fertiliser recommendations are based on extractable P levels as measured by a variety of extraction tests and should incorporate factors such as depth, bulk density and the soils phosphorus buffering capacity. Internationally and in South-Africa a variety of extraction test are used with contrasting norms and crop norms are not available for all of these extraction tests. Therefore the first aim of this study was to examine the relative aggressiveness and relationships between the various P extraction tests using 49 soils with widely varying physicochemical properties. The relative efficiency as compared to Total P (acid digestion) of the extraction tests were as follows; 1:2 H_2O < Olsen < Colwell < Bray I < Ambic 1 < Bray II < Mehlich III ≈ Citric acid. Bray II and Mehlich III were the only extractions test that correlated significantly (R^2 = 0.78 & 0.76) with total P. Strong linear relationships were observed between Bray II, Olsen and Mehlich III extractions tests and based on the weighted standard error of measurement, direct conversions between these tests were most reliable. It is thus possible to convert with confidence from Olsen to Bray II and Bray I by using a factor of 5.20 and 3.88 respectively. For the conversion from Bray II to Mehlich III a factor of 1.10 can be used. Phosphorous buffering capacity (PBC) can be determined by a multiple-point sorption isotherm and through fitting the Freundlich equation or through a single-point isotherm method by making various assumptions. A subsample set of 10 soils varying in P sorbing capabilities was used, a large variance in parameter *b* was observed and ultimately two distinct groups with both a different value for *b* were determined. By equilibrating the samples with 1000 mg P kg^{-1} and by using the untransformed variation of the Freundlich equation and using the two distinct manually selected values for *b* (one for the low PBC soils and one for the high PBC soils) the best results were obtained. The single point estimate of PBC correlated significantly with PBC (Ozanne and Shaw, 1968) (Eq. 1 & 2). However, a large RMSE was observed and this predicted estimate of sorption is not reliable. These estimations utilise unrealistically high P levels and a 1:10 soil: solution, completely saturating the soil and allowing for most of the added P to react with the soil. This would be highly unlikely when incorporating P fertilizer into the soil thus a simple laboratory incubation method was investigated. A strong linear relationship existed between applied P and percentage extractable P by the three extraction tests used (Bray II, Mehlich III and Olsen) using the incubation method. It was found that at an application rate exceeding 150 mg P kg^{-1} the regression lines plateaued and that and the percentage extractable P at rates (100 – 150 mg kg^{-1}) correlated highly significantly (R^2 = 0.92 and 0.99 respectively) with the percentage extractable P derived from the slope of the regression line of applied P against extractable P. The influence of time also needed to be investigated and it was found that Bray II extractable P only significantly decreased after 1

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month and that after 24 hours of equilibration the percentage extractable P remains fairly constant up to one month. Therefore it is possible to get an estimate of the amount of applied P that will be plant available with a single-application incubation method allowing for 24 – 72 h of equilibration. Lastly the Bray II and Olsen extractable P was correlated to plant response in high pH soils of the Northern cape planted to grapevine and citrus, no significant response was observed to the applied P fertilizer due to soil P already being at significant levels. Bray II was most sensitive in detecting applied P and excessive soil P levels, where Olsen was the least effective in detecting applied P. Therefore these soils do run the risk of reaching excessively high P levels which can in turn lead to P loss, micronutrient deficiencies and groundwater contamination.

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Opsomming:

Fosfaat (P) bemestings aanbeveelings word gebasseer op ekstraheerbare P vlakke soos gemeet deur een van ‘n verskeindenheid fosfaat ekstraksie toetse en moet faktore soos grond diepte, bulk digthied en die grond se fosfaat buffer kapasiteit insluit. Internationaal en in Suid – Afrika word ‘n verskeindenheid P ekstraksie toetse gebruik met varieerende gewas norms, norms is ook nie beskikbaar vir al die ekstraksie toetse nie. Dus, is die eerste doel van hierdie studie was om die relatiewe agresiwiteit van die onderskeie ekstraksies en die verhouding tussen die ekstraksies te ondersoek op 49 verskillende gronde met varieerde grond fisiese en chemise eienskappe. Die relatiewe doeltreffendheid soos vergelyk met totale P van die ekstraksie toetse was soos volg; 1:2 H_2O < Olsen < Colwell < Bray I < Ambic 1 < Bray II < Mehlich III ≈ Citroen suur. Bray II en Mehlich III was die enigste ekstraksies wat statisties bedeuidend met totale P gekorroleer het (R^2= 0.78 & 0.76). Sterk liniêre verwantskappe was gevind tussen Bray II. Olsen en Mehlich III ekstraksies en gebasseer op die standard foout van meting sal direkte omskakelings tussen hierdie P ekstraksie toetse die mees betroubaarste wees. Daar kan dus met sekerheid van Olsen na Bray I en Bray II omgeskakel word met ‘n factor van 5.20 en 3.88 respektiewelik. Vir die omskakeling van Bray II na Mehlich III kan ‘n factor van 1.10 gebruik word. Fosfaat buffer kapasiteit (FBK) kan bepaal word meervoudige-punt isotermes en deur gebruik te maak van die Freundlich vergelyking of deur ‘n enkel-punt isterme deur om verskeie aannames te maak. ‘n Stel van 10 verskillende grond-monsters was gebruik met varieërende P bindings bindings vermoëns. ‘n Groot variasie in konstante *b* was was op gemerk en uiteindellik was daar twee unieke groepe geïdentifiseer met statisties verskillende *b* waardes. Die beste resultate was verkry deur die grond-monsters met 1000 mg P kg^{-1} te ekwilibreer en gebruik te maak van die standard Freundlich vergelyking met die twee geselekteerde waardes vir konstante *b* (een vir die hoë FBK gronde en een vir die lae FBK gronde). Hierdie enkel-punt FBK waarde het statisties beduidend gekorroleer met FBK (Ozanne & Shaw, 1968). Ongeag hierdie sterk korrolasie was ‘n groot RMSE waarde bepaal en hierdie geskatte enkel-punt indikasie van FBK is nie betroubaar nie. Hierdie sorpsie-isotemre skattings van FBK gebruik egter onrealistiese hoë vlakke van P en ‘n 1:10 grond : oplossing, dit lei tot totale water versadiging van die grond end at meeste van die toegediende P met die grond reageer. Dit is onwaarskynlik vir veld toestande en gevolglik was ‘n eenvoudige inkubasie metode ondersoek. ‘n Sterk liniêre verhouding was waargeneem tussen toegediende P en die persentasie ekstraheerbare P vir die drie ekstraksies wat gebruik was (Bray II, Mehlich III en Olsen). Dit was vas gestel dat by toedienings hoeveelhede groter as 150 mg P kg^{-1} het die regressive lyne begin af plat. Die persentasie ekstraheerbare P by toedienigs (100 – 150 mg kg^{-1}) het sterk gekoroleer (R^2 = 0.92 and 0.99 onderskeidellik) met

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die persentasie ekstraheerbare P soos verky van die liniëre regressive lyn van toegediende P teenoor ekstraheerbare P. Die Inloed van tyd was ook ondersoek en daar was bevind dat Bray II ekstraheerbare beduidend afgeneem het na 1 maand van inkubasie end at na 24 uur van ekwilibrasie die persentasie ekstraheerbare P relatief constant bly tot en met 1 maand. Dit is dus moontlik om ‘n indikasie van die hoeveelheid toegediende P wat plant beskikbaar sal wees te kry van ‘n enkele P toediening met ‘n inkubasie periode van 24 – 72 uur. Daar was ook besluit om Bray II en Olsen ekstraheerbare P te korroleer met gewas-reaksie van tafel druiwe en sitrus op hoë pH gronde van die Noordkaap. Geen beduidende effek was waar geneem nie, dit kan toegeskryf word aan die reeds voldoende P vlakke van hierdie gronde. Daar was wel vas gestel dat Bray II meer sensitief was om toegediende P en oormatige P vlakke te meet. Daar is dus ‘n risiko dat P vlakke in hierdie gronde kan op bou tot onreëlmatige vlakke wat tot P verliese, mikro-nutiriënt tekorte en grond-water kontaminasie kan lei.

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Chapter 1 **Introduction**

Phosphorus (P) being a finite resource, the effective application and management thereof becomes significant in modern agriculture. The ineffective management of P can have negative affects both on yield and through having a polluting effect of ground water. Consequently, it is important to have an estimate of a specific soils ability to bind P in order to make accurate management decisions and to prevent adverse environmental affects due to over application of P. Internationally a wide range of P extraction tests has been developed to determine plant available P. Currently in South Africa the Bray II, Bray I, Olsen, Ambic 1 and citric acid extraction test are widely used. The Mehlich III extraction test as well as Colwell extraction test are not used for routine P determination in South-Africa. Different tests are used due to the variety of soil properties affecting P extractability. Considering the large variation in extraction mechanisms of these extraction test and large amount of soil physical and chemical properties affecting the extractability of P, various tests extract varying amounts of P. Norms for P extraction tests vary and can lead to confusion, Bray II (25-30 mg kg^{-1}), Olsen (10-15 mg kg^{-1}) and Mehlich III (45-50 mg kg^{-1}) (Pierzynski, 2000). Phosphorous norms for all of the above-mentioned P extractions test do not exist for all crops in South-Africa and considering that various tests are used across the country it would be useful to understand the correlation between the various tests to make it possible to convert between the various norms. Therefore this study aims to develop conversion equations based on regression models to make it possible to convert between the various extraction test norms. On soils of the Northern-Cape of South-Africa the contrasting P levels as indicated by Bray II and Olsen highlights the variation within the various extraction tests. It thus becomes uncertain which extraction test is accurately reflecting soil P levels. It is common practice to apply P annually due to the expected immobilisation of P through the formation of tricalcium phosphate ($Ca_3(PO_4)_2$). The effect of applying P fertilizer on these soils with sufficient or excessive P levels will be investigate and soil P levels and fertilizer rates will be correlated to crop response of grapevine and citrus. Due to negative affect an excess of P can have on ground water through eutrophication and the availability of micro-nutrients it is important to understand which extraction test gives the most realistic estimation of plant available P and is most effective in detecting fresh applied P. It then also becomes clear that accurate fertilizer recommendations are also of great significance. Currently in South-Africa fertilizer recommendations are made by determining the P deficit of the soil and then multiplying that by a factor of 4.5, which is the amount of P (kg ha^{-1}) required to raise soil P levels by 1 mg kg^{-1}. This method however does not consider the effect P sorption has on available P. Traditionally P sorption is determined

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with multiple point sorption isotherms and by fitting the Freundlich equation to sorption data, researchers such as Burkitt el at. (2002) and Barrow (2000) has however proposed the use of a single point method. This has also been investigated by Henry & Smith (2004) on South-African soils. These methods of sorption utilise unrealistically high levels of P do not represent field conditions. Thus, this study will also aim to compare the multiple point isotherm and sing-point isotherm estimates of sorption for South-African soils. An effort will also be made towards developing an incubation method to accurately and realistically describe P sorption and to use when making P fertilizer recommendations.

This thesis will be divided into five chapters additional chapters. Chapter 2 a literature study will include relevant literature that is critical to understanding P reactions in the soils and work that has been done regarding P sorption and fertilizer recommendations. Chapter 3 will include a characterisation of the soils being studied and a comparison of soil P extraction tests. Chapter 4 will investigate the use soil P buffering capacity when making fertilizer recommendations, determined by multiple- and single point sorption isotherms and through a proposed incubation method. Chapter 5 will describe the field trial done on grapevine and Nadorcott manderins on high pH soils of the Northern-Cape attempting to correlate soil P to crop response and to investigate the effect of P application on soils with already high levels of P and to determine which extraction test most accurately reflects soil P levels of these neutral pH soils. Chapter 6 will summarise all the main conclusions of this study and make recommendations for future research regarding soil P extracting tests and P fertilizer recommendations.

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Chapter 2 **Literature review**

2.1 Introduction

Phosphorus in soil solution as orthophosphate ions, $H_2PO_4^-$ (<pH 7.2) and to a lesser extent $H_2PO_4^{2-}$ (>pH 7.2) is very reactive by nature (Johnston *et al.,* 2014). Therefore the extractability of P by soil P extraction tests and the availability of P to crops is influenced by a large amount of soil physical and chemical properties. Generally, P fertilizer recommendations are made based on soil P levels as determined by a soil P extraction test. Work has been done by various other workers to investigate the correlation between these soil P extraction tests and soil physiochemical properties (Wuenscher *et al.,* 2015). Due to this reactive nature of P and the soils ability to sorb P it becomes necessary to incorporate a measure of sorption into fertilizer recommendations. P sorption describes the partitioning of soil P between sorbed and solution phases and is commonly referred to as the soils phosphorus buffering capacity (PBC) (Burkitt *et al.,* 2002). Work has been done by Burkitt *et al.,* (2015) to develop a single-point method to determine P sorption on Australian soils. Similarly, Johnston *et al.,* (1991) did work on P sorption on South-African soils working toward a more practical approach suggesting using an incubation method. A need thus exists to improve P fertilizer practices due to (i) the limited P resource, (ii) reactions with soil colloids affecting availability and (iii) the potential for pollution and micro-nutrient deficiencies if P is over applied.

In this literature review consisting of three sections an attempt will be made to provide all the necessary information to understand phosphorus reactions in the soil and how P availability is affected by the solid soil and how this influences P fertilizer recommendations. The first section aims to describe P reactions in the soil, the second section aims to describe soil P extraction tests and work done to correlate and compare these tests. The third section aims to explain P sorption and work done to determine various sorption parameters and how sorption can be used to accurately predict P fertilizer requirements.

2.2 Phosphorus reactions in soils

Phosphorus and the efficient use and application thereof is essential to productive agriculture as well as a contributing factor to precision farming. Phosphorus is essential to life with a key role in major metabolic processes such as energy transfer in the form of adenosine triphosphate (ATP). Consequently, plants rely on phosphate for energy production during photosynthesis (Wuenscher *et al.,* 2015). Critical to achieving maximum yields in agricultural

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production through phosphate application is an understanding of the soil P cycle as well as the chemical and biochemical processes that control the forms and availability of P in the soil (Tabatabai & Sparks, 2005). The major components of the soil P cycle include: dissolution-precipitation (mineral equilibria), sorption-desorption (interaction between solution P and soil solid phase) and mineralization-immobilization (Tabatabai & Sparks, 2005). Soil P can be in solution where it is easily taken up by plant roots, it can be adsorbed to the surfaces of clay minerals and Fe/Al oxides, it can be precipitated in mineral form or found in organic substances and living organisms (Wuenscher *et al.,* 2015). Phosphorus availability is reliant on factors such as: nature and amount of soil components, soil pH, other ions, kinetics and saturation of the sorption complexes (Tisdale *et al.,* 1990).

In general, organic forms of phosphorus occur as esters orthophosphoric acids. These P esters can be grouped as follows: inositol phosphates, phospholipids, nucleic acids, nucleotides and sugar phosphates (Tisdale *et al.,* 1990). However most of the P in soils is inorganic of nature and is mostly associated with Al and Fe in acidic soils and Ca in alkaline, calcareous soils (Tabatabai & Sparks, 2005). Inorganic P can occur as primary minerals such as apatite, as secondary minerals formed through the precipitation of P with Ca, Al and Fe, as P absorbed to onto the surface of clay minerals or Fe and Al oxyhydroxides or carbonates and as P physically occluded in secondary minerals (Tabatabai & Sparks, 2005). Secondary minerals generally refer to minerals to which P has chemisorbed and these include Fe and Al oxides, carbonates and minerals with a low temperature crystallization e.g. variscite (Smeck, 1985). Phosphorus generally becomes occluded in the iron oxides, hematite and goethite, and can only be released by a reducing agent such as citrate-bicarbonate-dithionite (Smeck, 1985). Phosphorus occluded by gibbsite can be released by NaOH, whereas, P encapsulated by silicate minerals can only be released by a very strong treatment such as HF or Na_2CO_3. It can thus be assumed that P occluded by these minerals won't be easily taken up by plant roots.

The solubility products of P are generally controlled by pH, concentration of P and divalent- and trivalent cations. Phosphorus interacts or binds to the mineral fraction of soil through sorption reactions and thus binds strongly through covalent bonds. In the case of the interactions between phosphorus and the mineral fraction, adsorption is believed to occur via the formation of an inner-sphere complex between the orthophosphate anion and a metal cation that is a constituent of a soil solid (Tabatabai & Sparks, 2005). Generally during P adsorption in acidic conditions, the phosphate ion undergoes an exchange reaction with either a hydroxyl ion or water. The result of this is the formation of a covalent bond and the lowering of the metal cations point of zero charge due to an increase in negative charge density. Point of zero charge (PZC) is the pH at which there is an equal distribution of positive and negative

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charges, a positive charge would be expected at a pH below the PZC and vice versa. These covalent bonds formed are very strong, and adsorptive reactions by this mechanism are not readily reversible. Also, important to note that ligand exchange can result in the formation of monodentate, bidentate, or binuclear forms of adsorbed P (Tabatabai & Sparks, 2005). The monodentate form of adsorbed P is considerably more reversible than other forms and can still be labile. Phosphorus sorption is also considered to be kinetically biphasic, which entail that the first part of sorption is rapid (one day or less) and the latter part is typically much slower (weeks or months). The initial reaction involves non-specific ligand exchange reactions on mineral edges. After weeks or longer phosphorus sorption slows down and involves surface precipitation or polymerization on mineral surfaces and the diffusion of adsorbed P into the interior of the solid phase (Tabatabai & Sparks, 2005).

Phosphorus can react via sorption reactions with mainly two kinds of surfaces, one's with surface constant charges and ones with variable surface charges. Minerals with a surface constant charge refer to the crystalline clay minerals, these minerals react with phosphorus principally through the cations bound to their plate like surfaces (Tisdale *et al.,* 1990). With regards to sorption via surface constant charges, the change in surface charge properties of the crystalline clay mineral is mainly due to the interaction of polyvalent cations such as Ca^{2+} and Al^{3+}, or Fe and Al polymers close to the surface thereof (Tisdale *et al.,* 1990). Bivalent cations such calcium will generally bind P via cation bridging. Phosphorus can also interact with minerals with a variable surface charge, including Fe(III) and Al oxides and organic matter. Important to note is that H^+ and OH^- are largely responsible for the surface charge of these minerals and relating to this, the effect of pH on sorption reactions and these minerals should be clear. At a lower pH values the Fe or Al oxides would be protonated resulting in a net positive charge favouring phosphate binding. The PZC for these oxides are 8.5 and 9 respectively, thus as previously mentioned at a lower pH than the PZC a positive charge would be expected (Tisdale *et al.,* 1990). Also, an important factor in phosphorus adsorption is calcium carbonate occurring as calcite in alkaline soils. Calcite develops a negative charge due to the greater tendency of calcium to go into solution via carbonate (Tisdale *et al.,* 1990). Phosphorus chemisorption to calcite involves adsorption of small amounts of P followed by the precipitation of Ca-P at higher concentrations. Only a small fraction of the calcite mineral interacts with phosphorus, thus calcite can still control soil pH while reacting with phosphorus. Besides the precipitation of calcium-phosphate, calcite can retain phosphorus electrostatically through the exchange of HCO_3^- for $H_2PO_4^{2-}$ (Antonaidis *et al.,* 2016). This effect of calcite becomes more prominent with increase in effective surface area, thus with increase in clay content. Other minerals such as kaolinite also have variable charged binding sites on their broken edges. Thus, to summarize, Fe/Al oxides bind phosphorus at low pH where calcite

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interacts with phosphorus at higher pH values, the crystallinity of the metal oxides also greatly influence its interaction with phosphorus. In general oxide content increases with weathering, older more weathered soils would contain more metal oxides, on the other hand crystallinity also increases which in turn decrease the oxides reactivity (Antonaidis *et al.,* 2016). Weakly crystalline metal oxides are much more reactive, these oxides seek to be in a more stable state and binding phosphorus increases its stability.

Desorption refers to the release of phosphorus, the sorbate from a sorbent, the solid soil. In general, the sorption of phosphorus is poorly reversible, the reversibility of sorption is of importance because it effects the availability of added or inherent P for uptake by plant roots and the potential loss op P in surface and subsurface runoff (Tabatabia & Sparks, 2005). The reversibility of sorption would also shed light on whether P tightly bound or in non-labile forms can eventually become plant available. Solubility diagrams have been used to identify phosphorus minerals in the soil and predict whether it will precipitate and be unavailable or dissolve and be available. It has been proposed that the concentration of solution P in neutral and acid soils is controlled by the solubility equilibria of the crystalline phosphorus compound (Tisdale *et al.,* 1990). If the soluble P concentrations of a compound are above the compound's isotherm line, this signifies supersaturation indicating that precipitation is prominent. Levels lower than the isotherm line indicates under-saturation and that the compound would be expected to dissolve (Tisdale *et al.,* 1990). Variscite ($AlPO_4.2H_2O$) and strengite ($FePO_4.2H_20$) are believed to be the most stable and least soluble at low pH values, thus they play a key role in phosphorus retention in acid soils. Strengite crystallizes more rapidly and consequently P availability will decline at a more rapid rate when the Fe-oxide is formed opposed to the formation of the aluminium oxide. In alkaline soils with high concentrations of Mg and Ca, P availability will also decline due the formation dimagnesium phosphate trihydrate, trimagnesium trihydrate, dicalcium phosphate and octacalcium phosphate. However, Mg-P compounds are said to be more soluble than Ca-P compounds (Tisdale *et al.,* 1990).

Referring to the mechanism of P sorption, factors such as pH and clay content as well as crystallinity should become apparent as influential in affecting phosphorus retention in soils. The primary factors controlling soil P availability, is the quantity of labile P, the concentration of P in solution as well as the buffering capacity that controls the distribution between P in solution and P in the solid phase (Sparks, 1996). As previously mentioned, factors that influence P retention include: nature and amount of soil components, pH, other ions, kinetics and saturation of the sorption complexes. Sorption-desorption reactions are largely affected by the type of surface the P encounter (Tisdale *et al.,* 1990). Hydrous metal oxides of iron and aluminium absorb large amounts of P and are abundant in weathered soils. These

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hydrous oxides can occur as discrete particles or as coatings on other soil particles. They can also exist as amorphous aluminium hydroxyl compounds between the layers of expandable aluminium silicates (Tisdale *et al.,* 1990). Consequently, soil with large amounts of hydrous iron/aluminium oxides that are weakly crystalline can retain large amounts of P, however crystalline metal oxides still retain more P than layer silicates. Also, important in affecting P retention is the clay mineralogy, 1:1 clay minerals such as kaolinite, because of its variable charged broken edges retain P stronger that 2:1 clays. The amount and not just primarily the type of clay also greatly affects P retention. An increase in clay will lead to an increase in specific surface area that can react with P, and consequently lead to greater P retention (Tisdale *et al.,* 1990). Calcium carbonates in high quantity and specific surface area will retain large amounts of phosphorus, thus to maintain a high activity of P in the soil it would be necessary to add higher quantities of phosphate fertilizer to such soils (Tisdale *et al.,* 1990). The effect pH on the variable charged edges of certain clays and metal oxides is prominent in affecting P retention in soils. The PZC of certain compounds will determine the dominant charge that compound will possess at certain pH values. Gibbsite adsorbs most efficiently at an ambient pH of between 4 and 5, where goethite adsorbs best at pH 3. It has been found that raising the pH of clays above mentioned will increase P adsorption due to the increase in Al hydrolysis (Tisdale *et al.,* 1990). This increase in pH will also decrease P retention of vermiculites due to the presence of interlayer hydroxy-aluminium polymers (Tisdale *et al.,* 1990). In general P availability is at a maximum between pH 6.0 and 6.5. The presence of certain cations and anions in solution also effect to a varying degree the retention of P in soils. Divalent cations enhance P retention relative to monovalent cations, where anions reduce the retention of P through competition for binding sites (Tisdale *et al.,* 1990).

Alongside soil factors that affect P retention and consequently P uptake by plant roots, factors that affect plant growth also affect P uptake and the amount of applied P used by the plant. These factors include soil moisture and the extent to which weeds and pest have been controlled (Johnston *et al.,* 2014). Also important to consider is root size and root distribution through the soil matrix and the effectiveness of the roots to extract phosphorus from the soil (Johnston *et al.,* 2014). Johnston *et al.,* (2014) reported a positive correlation between P uptake and the volume of the root hair cylinder. Plants have developed many mechanisms for extracting phosphorus from the soil, one is the development of a symbiotic relationship with arbuscular mycorrhizal fungi and the other is through the excretion of organic acids (Johnston *et al.,* 2014).

2.3 Plant available soil P extraction tests

It is critical that a P extraction test adhere to certain specification as put out by Bray (1948). These specifications include; 1) the P test should extract all or an appropriate amount of plant

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available P from soils with differing chemical and mineralogical properties, 2) the test should be accurate and rapid, 3) the extracted P should be correlated to plant P content and plant response and 4) the test should be able to detect differences in P content caused by previous fertilization and manuring (Pierzynski, 2000). Various methods exist for the quantitative analysis of phosphorus in the soil, these methods all differ in their extracting power and conditions under which they perform optimally. These tests extract varying amounts of P, depending on the type of extractants used and can be categorised as follows: 1) Water or unbuffered salt solutions, 2) dilute concentrations of weak acids with or without complexing agents, 3) dilute concentrations of strong acids with or without complexing agents, 4) buffered alkaline solutions, 5) anion exchange resin or iron oxide-impregnated filter paper strips and 6) isotopic exchange with P isotopes (Sparks, 1996). Important to note that different characteristics of the solid soil such as, pH, clay content, organic matter content and metal oxide content will influence which method should be used to determine extractable P.

The determination of total P via acid digestion will ultimately release all phosphorus, labile and non-labile. All methods used aim to convert organic P to inorganic P to facilitate total P determination. The four main methods that have been implemented to indicate total P include, sodium carbonate fusion, perchloric acid digestion, sulfuric acid and hydrogen fluoride digestion and sodium hypobromite oxidation followed by dissolution in dilute sulfuric acid (Sparks, 1996). It is believed that total P determination via sodium bicarbonate fusion is more reliable than acid digestion (Pierzynski, 2000). Due to the inability of acid digestion methods to extract P from apatite inclusions, these methods generally underestimate total P. Total P determination via sodium carbonate extraction as described by Sparks *et al.,* (1996) is a very time-consuming procedure and requires very specialized laboratory equipment, on the other hand digestion with perchloric acid only requires perchloric acid and nitric acid and is simple to execute. After completion of digestion with perchloric acid, P concentration should be determined by using ascorbic acid method as outlined by Riley and Murphy (1962). Values of total P determined by this method would serve as control when comparing the effectiveness of the various other extraction methods to each other and to different soil characteristics.

For the determination of P availability indices, dilute acid solutions dissolve Ca-P, Al-P and to a lesser extent Fe-P complexes, fluoride is included in the Bray II extraction test to complex Al and to prevent reabsorption to Fe oxides (Sparks *et al.,* 1996). EDTA is added to the dilute acid and fluoride solution in the Mehlich 3 test to create an multielement test (Sparks, 1996). The Bray I and Bray II extraction test proposed by Bray and Kurtz (1945) are not suitable for soils with a high degree of base saturation, silty clay-loam or finer textured soils with a pH>6.8, soils with a calcium carbonate equivalent of >7 % or soils with large amounts (>2 %) of lime (Pierzynski, 2000). In alkaline soils, such as these described above the acidity of the

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extracting solution may be neutralised, unless the soil:solution ratio is raised considerably. The fluoride used in the solution may also complex with the free Ca^{2+} in the soil and form Ca-F complexes which in turn can react with and immobilize P (Pierzynski, 2000). Also important to note that the Bray and Kurtz extractant can dissolve rock phosphate, this may lead to an over estimation in soils recently amended with this (Pierzynski, 2000). The Bray I extraction test is based on the principle that F^- promotes P desorption by decreasing Al activity and that F^- is also effective in suppressing the reabsorption of solubilized P by soil colloids (Sparks *et al.,* 1996). The main driving force for the solubilization of Fe-, Al- and Ca-phosphates is the protonation of PO_4^{3-} and the complexation of fluoride with Al^{3+} and Fe^{3+} and the precipitation of CaF_2 (Schmidt, 2004). The Bray I extracting solution contains 15 ml of 0.03 M NH_4F and 25 ml of 0.025 M HCL and P concentration in the aliquot can be determined with the ascorbic acid method (Sparks *et al.,* 1996).

The Bray II extraction test is like the Bray I extraction test, with a higher concentration of 0.1M HCl used and is suitable for moderately to highly weathered soils of low to medium CEC (AgriLasa, 2004). Phosphorus-free charcoal is added in this procedure to remove interfering organic acids and to decolourise the extract. This extraction test extracts acid soluble and adsorbed or available and reserve phosphates present in the soil (AgriLasa, 2004). The critical value for the Bray and Kurtz extraction test ranges from between 25-30 mg/kg.

Extraction with a buffered alkaline solution, the Olsen method was developed by Olsen *et al* (1954) and is used to determine phosphorus content in calcareous soils (Pierzynski, 2000). The extracting solution has a pH of 8.5 and contains $NaHCO_3$ and is consequently suitable for alkaline soils. The OH^- and CO_3^- is believed to decrease the concentration and activity of Ca^{2+} and Al^{3+}, resulting in increased P solubility (Sparks *et al.,* 1996). The increased number of surface negative charges and decreased number of sorption site on Al/Fe oxides at high pH also enhance the desorption of available P into solution (Southern Cooperative Bulletin 2000). In general, the Olsen test extracts less P than other test, with a critical value of 10 mg kg^{-1} (Sparks *et al.,* 1996). Cowell (1963) modified the Olsen method by increasing the soil:solution ratio from 1:20 to 1:100 and the reaction time was increased from 30 min to 16 h (Sparks, 1996). By increasing the soil:solution and reaction time the influence of soil P buffering on P extractability was decreased, the Cowell method in general extracts more P than the Olsen method. Also, important to note is the dependence of the $NaHCO_3$ extraction of clay content and citrate-dithionite-bicarbonate extractable Fe and P buffering capacity (Sparks *et al.,* 1996).

Extraction via the Mehlich III extraction test is well suited for acidic and basic soils and functions similarly to the Bray II extraction test seeing that it is an acidic solution containing ammonium fluoride (Pierzynski, 2000). In addition to containing ammonium fluoride it also

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contains EDTA and can also be used to detect other macro-and micro-elements. The acetic acid in the extractant also contributes to release P from most soils by promoting the dissolution of Ca-P (Wuenscher *et al.,* 2015). The Mehlich III test is more effective than the Mehlich I test because the acidity of the extracting solution is neutralized less by soil carbonates in alkaline soils. The Mehlich III test extracts more P than other test with a critical value ranging from 40-45 mg kg^{-1} (Pierzynski, 2000). Mehlich (1984) investigated the role of ammonium fluoride in die dissociation of Ca-P, Fe-P and Al-P compounds, he noted that it was critical to maintain a pH below 2.9 for extraction with fluoride. Acetic acid was found to digest apatite to a lesser extent than other mineral acids, this led to the development of the Mehlich II extraction test and also reduced the possibility of an over estimation of P content (Mehlich, 1984). In the Mehlich III extraction test, chlorides where substituted with nitrates and EDTA and DPTA was used as chelating agents, used to detect micro-nutrients (Mehlich, 1984).

In South-African soil science analytical laboratories generally use Bray I, Bray II, Ambic 1, Truog and Olsen extraction test. The fertilizer industry prefers to use the Bray I extraction for fertilizer recommendations, Ambic 1 is used by the ARC-Grain crop institute for calibration research (Schmidt *et al.,* 2004). For this reason, the correlation between Ambic 1 and other extraction tests needs to be determined, especially between Ambic 1 and Bray I. The Ambic 1 extraction test is a modification of the ISFEI method of Hunter (1975) and was developed by Van der Merwe *et al*., (1984). The Ambic 1 test uses NH_4HCO_3 and NH_4-EDTA instead of $NaHCO_3$ and Na-EDTA as proposed by Hunter (1975). Contrary to the mechanism by which the Bray I extraction extracts P, the effect of protonation of PO_4^{3-} is less due to the higher pH of 8.3 and the Ambic 1 relies on the complexation of Al^{3+}, Fe^{3+} and Ca^{2+} by EDTA as the main driver of solubilization with the effect of the fluoride being minimal (Schmidt, 2004). These methods would thus extract different amounts and forms of P and consequently their correlation two one and other is of great importance. Schmidt *et al.,* (2004) compared Bray I- and Ambic 1 P, they found a strong linear relationship between these two methods for most of their experimental sites.

Unique to South-African soil science is the determination of extractable phosphorus with 1 % citric acid, the method was originally developed by Dyer (1894). Citric acid increases the solubility of calcium phosphates, aluminium phosphates and iron phosphates while exchangeable cations are displaced from the exchange complexes of the solid soil (Non-Affiliated Soil analysis work committee, 1990). The reabsorption of phosphate is prevented by the formation of citric-acid complexes, 1 % citric acid, hydrochloric acid and nitric acid are the main active reagents for this method.

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In a comparative study conducted by Wuenscher *et al.,* (2015), 14 P extraction tests were compared to each other, samples were collected from the ploughed layer (0-30 cm) from agricultural fields in Germany and Austria . The sites were specifically chosen to include soils that vary in soil properties and inherent P content and all the soils used were exposed to diverse tillage, fertilizer applications and crop rotations prior to sampling. Wuenscher *et al.,* (2015) found that the Bray II extraction test extracted more phosphorus than the Mehlich III test which extracted more phosphorus than the Olsen test. This could be due to the more aggressive nature of the Bray II extractant with its low pH of 1. In this study, it was found that most of the extraction methods correlated well with each other, with total P being an exception and only correlating positively to the Olsen method. The highest correlation was found between the Bray II extraction and the Mehlich III extraction, this is mainly due to the similarity in their mechanism of extraction. As mentioned previously the solubility and extractability of phosphorus is dependent on a variety factors, The Bray II extraction, Olsen and Mehlich III all correlated negatively with pH and carbonate content. A positive correlation was found between soil carbon and P extracted by oxalate, organic P and total P test, showing that only these methods can extract phosphorus from the organic pool. Wuenscher *et al.,* (2015) also found a strong negative correlation between clay content and P extracted by Mehlich III, Olsen and Bray II and a positive correlation was found between these methods and sand content. Finer soil texture relating to higher specific surface area, will consequently lead to stronger P binding and thus reducing the extractability by weaker extraction tests. Wuenscher *et al.,* (2015) found that finer texture corresponded with a higher organic carbon content and thus also a higher organic P content. Oxalate- and dithionite- extractable iron correlated positively with only the stronger extractions e.g. oxalate extraction. The Fe_0/Fe_d ratio, where a higher ration indicates lower iron oxide crystallinity, correlated strongly with the Olsen, Bray II -and Mehlich III method. It can then be assumed that a higher P extractability can be expected from soils containing more amorphous iron oxides. Important to notice is that P extractability via one of these methods is not only determined by inherent soil properties but also by the composition of the fertilizer used.

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Table 2.1 Summary of soil P extractions tests

Method	Extracting solution	Solution pH	Soil: Solution ratio	Time of extraction
Bray I	0.03M NH_4F 0.025M HCl	2.0	1:7	60 s
Bray II	0.03M NH_4F 0.1M HCl	1.0	1:7	40 s
Olsen	0.5M $NaHCO_3$	8.5	1:20	30 min
Mehlich III	0.015M NH_4F 0.013M HNO_3 0.001M EDTA	2.5	1:10	5 min
Colwell	0.5M $NaHCO_3$ 0.2N H_2SO_4 2.5% acetic acid	8.5	1:100	16 h
Citric acid	1% citric acid	1.0	1:10	1 h
Ambic P	0.25M NH_4HCO_3 0.01M $(NH_4)_2EDTA$ 0.01 M NH_4F 0.05 $g.L^{-1}$ Superfloc N100	8.3	1:10	30 min
Total P	70% perchloric acid 15.8M HNO_3 5M NaOH	<1.0	1:100	16 h

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Bolland *et al.,* (2003) compared the Mehlich III for soils in South Western Australia. In their study they applied two different P fertilizers in the form of superphosphate and rock phosphate, they found that the Mehlich III extraction with a pH of 2 could have extracted unreacted rock phosphate leading to an over estimation in plant available phosphate when rock phosphate was applied (Bolland *et al.,* 2003). They also found that the Colwell extraction, extracted far less P, it is believed that bicarbonate extractions such as the Olsen and Cowell extract less phosphate from soils treated with apatite rock phosphate. It has been reported that Cowell extracts P that is not isotopically extractable from calcareous soils (Speirs *et al.,* 2013). Zbiral, (2000) compared different soils test including the Mehlich III test on calcareous soils and found that the Mehlich III extraction was less influenced by the presence of free carbonates than for example the Bray I test. In their work then measured the pH of the extractants after the addition of calcium to the soil and measured the influence on extractable P. They observed a gradual increase in pH with increase of lime content for the Mehlich III test (Figure 2.2) compared to a sharp increase for other methods of extraction, extractable P also decreased gradually with increased carbonate content. Consequently, the extraction of phosphorus by acids extractants strongly depends on the increase in pH of the extractant (Zbíral, 2000).

2.4 Soil P Buffering capacity indexes and P fertilizer requirements

Soil P as indicated by the various extraction methods show excellent correlation with concentration of P in plants and relative yield for a number of soils with varying characteristics (Quintero *et al.,* 2003). However, estimating P availability strongly relies on a variety of factors such as; P-intensity, which refers to phosphorus in solution, P-Quantity, which refers to labile P and lastly phosphorus buffer capacity, which serves as an indication of the soils sorption capacity (Shirvani *et al.,* 2005). Consequently, these chemical indices of P availability correlate to plant P-uptake but they do not effectively predict fertilizer requirement considering that phosphorus buffering capacity (PBC) of the soil isn't brought into consideration (Quintero *et al.,* 2003). When making a fertilizer recommendation the extracted P by a specific test should be known as well as the known optimum level for that test, the nutrient deficit can then be calculated and multiplied by the requirement factor to ultimately determine fertilizer application rate in kg ha^{-1} (Johnston *et al.,* 1999). The requirement factor should include factors such as; depth of sampling, bulk density of the soil and sorption effects on phosphorus (Johnston *et al.,* 1999). Phosphorus sorption is key in affecting P availability, it expresses the partitioning of soil P between the solid and solution phase and is commonly described as the soils phosphorus buffer capacity (PBC) (Burkitt *et al.,* 2002). Phosphorus sorption and desorption are both influenced by a variety of factors; pH, concentration of phosphate, concentration of other electrolytes, temperature and period of reaction (Barrow, 2015). Consequently, making it hard to describe P sorption by the soil. P sorption is believed to

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increase with a decrease in soil pH, the effects only becoming notable at low pH levels, it has however also been shown that P sorption can increase at higher pH levels. Due to the effect P sorption has on the charge of the soil and that the soil only has a finite amount of sorption sites, P sorption also decreases with an increase in applied P through previous fertilization (Barrow, 2002). Considering all the various factors influencing P sorption, Barrow (2015) constructed a model aimed to describe P sorption. The model makes three main assumptions, the first being that the reaction of P is with a variable-charged surface. The second, a range of sorption sites exists, and their summed behaviour can be modelled by assuming a normal distribution of the parameters of the variable-charge model. Third assumption is that the initial reaction with a soil particle induces a diffusion gradient towards the interior of the particle and begins a solid-state diffusion process. This model suggests that P that has reacted with soil over a long period of time is not fixed but can be recovered if enough surface activity is induced.

PBC can be described as the soils ability to moderate P solution concentration when P is added or removed from the soil (Burkitt *et al.,* 2002). PBC largely influences the extent of sorption and precipitation reactions and thus influences the amount of fertilizer needed to raise the soil P level by a desired amount. It is thus important to have estimate of the soils PBC to adjust the critical value for some soil tests, consequently it would be expected that as the PBC of a soil increases so also does the critical value to achieve 90 % of maximum yield. Generally, PBC can be determined from the slope of a P sorption curve when a range of P concentrations are added and P sorbed is measured after a period of equilibration (Burkitt *et al.,* 2002). Conditions in solution during this period of equilibration or adsorption have a big influence on the final result. Factors such as; growth of micro-organism, presence of other ions, temperature, soil: solution ratio, initial P and agitation intensity, all greatly influence the accuracy of the amount of P obtained in the equilibrated solution.

Commonly three equations are fitted to P sorption data to estimate sorption characteristics of the soil, these include the Freundlich, Langmuir and Tempkin equations (Holford, 1997). In general, the Freundlich equation best describes P sorption and should be used when estimating PBC. The Freundlich equation considers the fact that P sorption energy decreases as sorption increases (Tisdale *et al.,* 1990). Based on a review by Barrow (2008) and use thereof by Burkitt *et al.,* (2002) and Malaysiana *et al.,* (2018) the Freundlich equation (1) most accurately describes P sorption in soils. From equation (1) q is the amount P sorbed (mg kg^{-1}) , Kd and b are both constants and C is the equilibrium P concentration in mg L^{-1}. Kd has the units of q/C^b and b is dimensionless (Barrow, 2008). Parameters b and Kd are said to be constants and are correlated to each other. Thus, an increase in b will lead to a smaller estimate of Kd, highlighting the significance of the size of b on sorption. This parameter

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indicating the curvature of the sorption curve is also indicative on how well the Freundlich equation describes the sorption data, values ranging from 0.1 -1.0 are indicators of a good fit (Burkitt *et al.,* 2002). Alternatively, the Freundlich equation can be written as $S= ac^b – q$ (equation 3), where q in this case refers to the amount of P that can be desorbed. By fitting the linear variation of the Freundlich equation (2) *b* can be derived from the gradient of the regression line and *Kd* can be derived as the y-intercept.

$$\mathbf{q = Kd \times C^b} \quad \mathbf{(1)}$$

$$\mathbf{Log\ q = b\ log\ C + log\ Kd} \quad \mathbf{(2)}$$

$$\mathbf{S = ac^b – q} \quad \mathbf{(3)}$$

The Langmuir equation assumes that apart from occupying all sorption sites, reaction with the surface does not affect the surface. This is not true for the reaction of ions with the solid-soil, the electrical potential of the surface will be affected both directly and indirectly. Directly through the effect on the charge and indirectly through an effect on pH. It is known that the specific sorption of anions changes the charge of the reacting particle, in this case the soil making it more negative. With time the reacting ion penetrates towards the centre of the reacting particle and consequently leading to a semi-permanent change in charge (Barrow 2002). Due this effect the sorption of P has on the soil, the Langmuir equation (4) has seldomly given realistic estimates of sorption (Barrow, 2015).

$$\mathbf{q = (K_{lang} \times C \times qmax)/ (1+ K_{lang} \times C)} \quad \mathbf{(4)}$$

$$\mathbf{1/q= 1/K_{lang} \times qmax \times 1/C + 1/qmax} \quad \mathbf{(5)}$$

Determining sorption indices are of large importance but commercial laboratories are reluctant to measure sorption curves, referring to the cost and time requirement of these procedures (Allen *et al*, 2001). Thus these laboratories use simple measures of buffer indexes, these can be divided into two groups; i) indirect measures such as iron dissolved by ammonium oxalate or ii) measures derived from a single measurement of sorption (Barrow, 2002). Then the resulting concentrations and values for sorption can used to determine different sorption indices such as; phosphorus retention index (PRI), phosphorus saturation index (PSI) and phosphorus buffering index (PBI). PRI can be obtained by dividing sorption by concentration and PSI by Bache & Williams (1971), can be determined by dividing sorption by the log of concentration (Allen *et al.,* 2001).

In a study conducted by Burkitt *et al.,* (2002), PBC was determined from a P sorption curve, they equilibrated 4 g of soil in a 40 ml P solution with a 1:10 soil: solution, containing potassium dihydrogen phosphate at concentrations ranging from 5 – 1000 mg P kg^{-1} in 0.01M $CaCl_2$.

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They also added 50 μg of chloroform to prevent microbial growth. McGee (1972) also found that microbial growth during equilibration decreased the P concentration in the soil-P solution. In theory the quantity of P adsorption for a constant equilibrium P concentration should be independent of soil:solution ratio and initial P content (McGee, 1972). McGee (1972) studied the effect of soil:solution ratio on P supernatant concentration and concluded that the effect was negligible, this has been found to be incorrect and the soil:solution greatly affects P supernatant concentrations. The soil-solution mixtures were shaken at 14 rpm for 17 h at a temperature of 25℃ (Burkitt *et al.,* 2002). The shaking time and vigour at which the samples are shaken should also be considered. If the samples are shaken to vigorously the increased abrasion on the soil might expose more surfaces to interact with the sorbate and might lead to an over estimation of sorption (Barrow, 2008). McGee (1972) also conducted a study on the influence of equilibrating time on supernatant P concentrations. McGee (1972) used two soils from the Natal region, a Griffen and Balmoral soil was collected. Two grams of each soils could equilibrate with 50 ml of a P solution, the equilibration periods varied from 1 h to 144 h. The conclusion was that most adsorption occurs within the first hour and adsorption after 24 h is negligible. Also important is the temperature during equilibration, small changes in P adsorption has been noticed with increase in temperature. Since P adsorption is entropic by nature an increase in temperature could lead to an increase in adsorption sites. In the study conducted by Burkitt *et al.,* (2002) $CaCl_2$ was used as a background electrolyte and was added to the solution at a much higher concentration than the sorbate, in this case being KH_2PO_4. The use of a background electrolyte is deemed necessary due to the fact that naturally P is adsorbed in the presence of other ions, and at the low concentration at which $CaCl_2$ is added and because Cl does not bind strongly it does not really affect P adsorption. A possible disadvantage of using $CaCl_2$ as the background electrolyte is that it might react with P to form calcium phosphate (Barrow, 2008). It was later found by Allen *et al.,* (2001) as cited by Barrow (2008) that the formation of Ca-P had not biased their results at high pH. The amount of phosphorus sorbed by the soil was calculated as the difference between the amount added (5 - 1000 mg P kg^{-1}) and the amount in the equilibrated solution, they also suggested the use of single-point sorption indices. In the study conducted by Burkitt *et al.,* (2002) they evaluated the efficiency of a single-point sorption test by comparing it to the standard method of described by Ozanne & Shaw (1968) using equation (6).

$$\mathbf{PBC_{O\&S} = Kd\ (0.35^b - 0.25^b)} \qquad \mathbf{(6)}$$

The Ozanne & Shaw PBC value was used as benchmark to which single point estimates of sorption was compared and was determined by measuring the amount of P sorbed between equilibrium concentrations of 0.25 and 0.35 mg P L^{-1}. The selection of an external solution P concentration is a necessary requirement for making fertilizer recommendations, values

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higher or lower than that required for optimum yield will lead to an over- or under estimation of the P requirement of a specific soil (Henry and Smith, 2003). However, Ozanne and Shaw (1967) found that P requirement interpolated at one solution P concentration related well to that found at another concentration, thus selecting a specific range of solution concentrations matters less in theoretical studies where the objective is to obtain a range of requirement factors of a variety of soils. The Ozanne & Shaw (1967) PBC value was determined by shaking four to five soil samples in 0.01M $CaCl_2$ and adding mono-ammonium-phosphate at concentrations ranging from 0-25 ppm (Ozanne & Shaw, 1967). It was found that at equilibrium solution concentration of P of 0.3ppm crops generally do not respond to P fertilizer application, this being the motive for measuring P sorbed between solution concentrations of 0.25 and 0.35 mg P L^{-1} (Ozanne & Shaw, 1967). Sorption data from 290 soils across Australia were collected , sorption was determined as put out by Rayment and Higginson (1992) and through fitting the Freundlich equation to the data (Burkitt *et al.,* 2002). Due to all soils containing some P there will be some desorption and hence sorption equations should have a negative intercept with the vertical axis. Thus, with equation (7) by using initial P as determined by the Colwell P extraction test as a direct measure of P was used as a surrogate for the *q* parameter in equation (3). This was a necessary adjustment to make for the sorption curve too pass through the origin. Considering that P availability is dependent on PBC which in turn is influenced by both P-intensity and P-quantity it is important to use an estimate of P that will accurately represent both these parameters. Where P-intensity is simply the solution P concentration measured at a defined soil: solution ratio, however the measurement of P-quantity is a bit more complicated. P-quantity can be estimated with isotopically exchangeable P, this method is however time dependant and has been ineffective with high P soils (Moody, 2007). An approach to this problem is to assume that an extractant comprising of an anion capable of displacing P from sorption sites at a wide range of soil: solution ratio and a long extraction time is correlated to the quantity of P that may be released into solution after intensive plant uptake (Williams, 1962 as cited by Moody, 2007). The Colwell extraction test has been found to meet this criteria although PBC still has an effect on P extractability by this method Colwell P has however been correlated to P that is isotopically exchangeable within 24h (Moody, 2007) For most soils especially, natural unfertilised soils the amount of desorption is too small to be measured by standard techniques but with high P soils it has been proposed to modify the Freundlich equation. The modified variation of the Freundlich equation for low P soils has been found to yield too large *b* values, underestimating sorption. Alternatively, they determined sorption indices such PBI (phosphorus buffering index) from (S + Colwell P)/C^b through the single-point method by adding 1000 mg kg^{-1} and using the parameters solved by the multiple sorption isotherm. Where *S* is sorption (mg kg^{-1}) , *b* is a constant and *C* is the equilibrated solution concentration. The best results were obtained by

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using a constant value of 0.41 for parameter *b,* they found that PBI correlated more strongly to PBC determined by Ozanne and Shaw (1968) (R^2=0.99). Thus, for Australian soils a single point method for determining P sorption has been developed and correlates significantly to multiple point estimates of sorption (Burkitt *et al.,* 2002).

(q + Colwell P) = Kd × C^b **(7)**

Barrow (2000) also reviewed the use of a single-point method to estimate P sorption in Australian soils. Again, a sing-point estimate of sorption was compared to $PBC_{O\&S}$, and it was found that the best results were obtained when Colwell P was used as a direct estimate for the *q* parameter by using the transformed Freundlich equation ($S = ac^b - q$). A single point estimate of *Kd* in this case *a* was determined from $(S – q)/ C^b$, where S is the amount of P sorbed and q is the amount of be that can be desorbed thus the initial amount of Colwell P and *C* is again the equilibrated solution P concentration (mg L^{-1}). Consequently, a single point estimate of $PBC_{O\&S}$ can be determined from $(S + q) (0.35^b – 0.25^b)/C^b$. Barrow (2000) argued that due to *b* being a denominator and numerator the estimate of PBC was not very sensitive to the value of *b* provided C was in the range of 2 – 5 mg P L^{-1}. Barrow (2000) found that for moderate P sorbing soils Colwell P was a useful estimate of *q* and single point estimates was close to values found from the fitted curves. However, exceptions to this were observed for low P sorbing soils that has received a single high application of P fertilizer as well as for very high P sorbing soils. Barrow (2000) also pointed out that over a large range of log concentrations *b* is not constant and varies a large amount especially for low P sorbing soils. The most reliable values for *b* was found to be between 0.2 and 0.5 , and between solution concentrations of 2-5 mg P L^{-1} the estimate of sorption was less sensitive to the value of *b.* Thus, it was found that the most accurate single point estimate was determined by using Colwell P as a direct estimate for *q* and to use value for *b* as 0.35 and 0.41 (Barrow, 2000). To conclude it is important to aim for a specific solution concentration in order to get accurate estimates of *b* , similarly Burkitt *et al.,* (2002) and Moody (2007) found that single point estimates using *b as 0.41* for Australians soils correlated well to $PBC_{O\&S}$. It was also found that fertilizer recommendations made by using this single point estimate correlated well to crop response (Moody, 2007).

Henry & Smith (2003) also suggest the use of single-point sorption indices, but this can only be done once a sorption isotherm of a specific soil already exist or if the correlation between different soil properties that are routinely measured, and the sorption parameters are known. Henry & Smith (2003) constructed P adsorption isotherms and derived the indices of P-fixation; Lm-Langmuir adsorption maximum and Bt- the slope of the Temkin isotherm, which in turn is the buffer capacity of the soil. Consequently, they constructed regressions equations

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for each of the isotherms, making it possible to estimate the amount of P adsorbed (Y mg kg^{-1}) when the amount of P in solution is known. Two single point sorption test were included in this study by adding rates of 70 mg kg^{-1} and 100 mg kg^{-1} to the soil and allowing it the equilibrate for 24 h (Henry & Smith 2003). They could derive two sets of sorption indices including the amount of P in solution and the amount P adsorbed expressed as a percentage of the amount added. They interpolated the P requirement from the isotherms as a concentration of P in solution.

Table 2.2 Calculations used by Burkitt *et al.,*(2002) for various single-point sorption indices and their correlation to PBC determined by Ozanne & Shaw (1968).

Index	Method of calculation	R^2
PBI_{colP}	$P_s + \theta ColwellP/C^b$	0.99
PRI_{colP}	$P_s + \theta ColwellP/C$	0.74
PBI_{olsP}	$P_s + \theta OlsenP/C^b$	0.99
PRI_{OlsP}	$P_s + \theta OlsenlP/C$	0.80

C = the concentration remaining in solution (mg/L), b and θ are parameters, Ps = amount of P sorbed (mg kg^{-1}) and subscripts ColP and OlsP represent the addition of Olsen or Colwell extractable P

Quintero *et al.,* (2003) studied the effect of PBC on P fertilizer requirement. In this study 13 soils varying in physiochemical characteristics were selected and six rates of P were added ranging from 0 – 300 mg dm^{-3}. The extractable P was determined by four methods including the Bray II and Olsen methods of extraction. From this a linear equation was created to obtain the slope of the relation between added P and extracted P for each soil and extraction method. Phosphorus sorption indices such as PBC and PRI (phosphorus retention index, L kg^{-1}) were also determined for each of the soils. From the linear models the relationship between applied P and P extracted by the extractants could be estimated, and it was found that the proportion of extracted P was inversely related to capacity factor of the soil, referring to the sorption capacity of the soil. Like the findings of Wuenscher *et al.,* (2015), Quintero *et al.,* (2003) also found that Bray II extracted the most P and that the amount of clay and amorphous Al was negatively related to recovered P. It was also observed that the Bray II extractant had the greatest extraction power, this could be due to the fact that this extractant, extracts non-labile forms of P (Quintero *et al.,* 2003). Sorption capacity indices also correlated with the required rate to obtain maximum yield; PBC (R^2=0.81) and PRI (R^2=0.77). Quintero *et al.,* (2003)

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concluded that the P extraction tests are capable to evaluate P availability but are not satisfactory for making fertilizer recommendations, to determine the required rate of P application the slope of the recovered soil P has to be considered (Quintero *et al.,* 2003). With incorporating depth and bulk density of the soil these values can be transformed into the amount of fertilizer (kg ha^{-1}) needed to raise the P availability by 1 mg kg^{-1}. The required rate of application can be calculated by multiplying the equivalent rate (ER) with the difference between the critical value for each test and the amount extracted by the test. In conclusion it was necessary to get an estimate of a capacity factor such as PRI to make accurate fertilizer recommendations, by doing this it is possible to consider the slope of P recovery and to determine the amount needed to reach a certain level of availability (Quintero *et al.,* 2003). The method proposed by Quintero *et al.,* (2003) consisted of shaking 1 g of soil with 10 ml of 0.01M $CaCl_2$ solution with 8 levels of P as KH_2PO_4 for one hour. A linear equation was fitted between the mono-calcic phosphate potential of the equilibrium solution and the phosphate sorbed per kg of soil (Quintero *et al.,* 1999). PRI could also easily be determined following the method proposed by Bache & Williams (1997). This involves 1 g of soil being placed in a centrifuge tube and 20 ml of a 75mg P L^{-1} solution and toluene being added (Gutierrez Boem *et al.,* 2008). The P concentration in solution was determined calorimetrically, the PSI($L.kg^{-1}$) was then determined as the ration between sorbed P and Log_{10} of the final P concentration in solution. The sorption capacity of the soil or the maximum sorption capacity of the soil (MPSC, mg kg^{-1}), estimated with sorption isotherms showed a strong correlation with the quick and easy determination of PRI (Quintero *et al.,* 1999). Phosphorus buffer capacity correlated significantly with PRI with R^2=0.99, a strong positive correlation was also found between Al, Fe and clay content, indicating that these variables are good indicators of the sorption capacity of the soil (Quintero *et al.,* 1999).

In a similar study McGee (1972) correlated P adsorption to plant response, he explained that for P adsorption to be of any practical use it needs to correlate to plant response. McGee (1972) conducted a pot trial with five soils amended with lime to correct pH and added various rates of P fertilizer as a fraction of the P max. P max can be defined as the maximum amount of phosphorus adsorbed to the solid soil and was determined by adding know amounts of P to a soil, measuring the P content after a period of equilibration and then applying the Langmuir equation to the data. The Langmuir equation was preferred by McGee (1972) since it implies that only a monolayer of adsorbate is formed on the adsorbent, the other advantage it has it that P_{max} can be calculated. Alternatively, other researchers such as Burkitt *et al* (2002) have found the Freundlich equation to be empirically sounder for describing P adsorption to the solid soil. After the five soils were amended with lime and the six rates of P in the form superphosphate the soil was allowed equilibrate for 10 days, haygrazer seeds were planted

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into each pot. The plants were allowed a growing period of 30 days and were then harvested and dried. In a similar study conducted by Barber *et al.,* (1963) they reported that a maximum response in the yield of millet was related to the degree of saturation of the phosphorus adsorption maximum (McGee, 1972). Important to note that after the equilibration period Pmax and P requirement values were determined and ratios of 0, 1/20, 1/10, 1/5, ½ and 1/1 of the Pmax was added as P fertilizer. After the trial the soil was air dried again and equilibrium P concentrations were determined and PBC could be determined from the first derivative of the Langmuir equation. McGee (1972) found a that as the ratio of P added increased the yield of the haygrazer also increased. P concentrations calculated from the Langmuir equation correlated significantly with mean yield with R^2=0,68. When McGee (1972) included PBC in the correlation the correlation coefficient rose to 0,94. It was noted that the yield curves tailed of between ½ and 1/1 of Pmax levels, indicating that that maximum response to phosphate for these soils was attained at full saturation of the phosphate adsorption maximum (McGee, 1972). The work done by McGee (1972) thus also revealed that an estimation of PBC is need for accurate fertilizer recommendations.

Johnston *et al.,* (1991) conducted a study on 54 soils from South-Africa, focussing on soils from the Natal region. In this study, they compared the Ambic, Bray I and Truog methods and considered the use of routinely measured soil factors in determining the P requirement factor. Topsoil samples from a depth of 0-200 mm were takin that vary in pH, organic carbon content and CEC (Johnston *et al.,* 1999). Subsamples of each soil were incubated with four levels of P fertilizer (0, 35, 70 and 140 mg P L^{-1}) in the form of potassium dihydrogen phosphate (KH_2PO_4). Each of these samples were maintained under 25℃ and wetted to field capacity, there after the samples were exposed to three wetting and drying cycles, after six weeks the samples were dried and extractable P was measured via the three extraction tests (Johnston *et al.,* 1999). The phosphorus desorption index (PDI) was determined, where the fraction of P not sorbed by the soil reflects the sorption properties of the soil. Various other soil properties including pH in KCl, clay content and CEC cmolc kg^{-1} were measured. The relationship between the P measured in mg.L^{-1} and the P added in kg ha^{-1} was found to be near linear, thus a linear regression function could be fitted to each soil. From the slope of this function the requirement factor could be estimated (Johnston *et al.,* 1999). The PRF (phosphorus requirement factor) values for the three extractants evaluated statistically against certain soil properties and functions of best fit were established. They concluded that highest correlation was found against PDI and thus justifies the use of this test for evaluating phosphorus sorption (Johnston *et al.,* 1999).

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2.5 Gaps in Knowledge

Considering the variety of soil P extraction tests being used in South-Africa and internationally it would be beneficial to correlate these tests with each other on a range of South-African soils and then investigating the possibility of using a single conversion factor to convert between the norms of the various tests. Also due to the contrasting P levels given by certain soil P extraction tests on high pH soils from the northern-cape, as determined by industry, soil extractable P needs to be correlated to plant response for these areas. Consequently, due to the confusion that arises from the contrasting Bray II and Olsen P levels farmers have continued to apply P fertilizer year after year. The effect applying P annually on already P sufficient soils also needs to be investigated due to the environmental effect over fertilisation can have.

Currently in South-Africa fertilizer recommendations are made without incorporating soil P buffering capacity into the calculation. Previous work suggests that single point sorption isotherm methods correlate well to multiple point sorption isotherm methods, this however has not been done for South-African soils using extraction test used locally. Henry & Smith (2004) did investigate the use of a single point estimate of sorption on South-African soils but they used the Langmuir equation to describe sorption where most other researchers believe the Freundlich equation to be superior when describing P sorption. Work done by Burkitt *et al* (2002) showed that using initial P as a surrogate for the *q* parameter (explaining desorption) and by assuming *b* to be constant, single-point estimates of sorption correlated well to multiple point estimates. Barrow (2008) however pointed out that *b* can have a wide range, thus this needs to be investigated for South-African soils. Also, work needs to be done towards a more realistic estimate of sorption, a method simulating field conditions should be investigated and also correlated to sorption-isotherms estimates of sorption. An Incubation method would not just give a realistic estimate of the amount of P fertilizer available for plant uptake but also of the efficiency of the specific P extraction tests used to determine the amount of available P.

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Chapter 3 **Soil characterisation and comparison of commonly used soil P extraction tests**

3.1 Introduction

Various tests exist and use different mechanism to extract phosphorus and are consequently affected to a greater or lesser extent by the soil's physiochemical properties (Sparks *et al.,* 1996). In South- Africa, Bray I, Bray II, Olsen, Ambic 1 extraction tests are predominantly used. Increasingly, Mehlich III and Colwell tests are being used as local soil samples are being sent to soil labs in the USA and Australia, respectively. Bray I is the preferred test of the fertilizer industry (FSSA 2007), while the ARC-Grain crops institute uses the Ambic 1 for research purposes (Schmidt *et al.,* 2016). Soil analytical labs in the Western- Cape predominantly use the 1 % Citric acid, Olsen and Bray II extraction tests, whereas, the Ambic 1 test is preferred in KZN-province. A few Western Cape consultants make use of the 1:2 water extract to assess plant P availability. Internationally, bicarbonate extractions (Olsen, Colwell) are used on the more alkaline soils of western United States, western Europe and Australia. Bray I test is used in Central United states and Western Europe while the Mehlich III test is used in southern United States and the Czech Republic (Csathó & Magyar, 2005). Extractable P by these tests has been correlated to various crop responses and yields, but soil critical norms for all the P tests are not available for all crops in South Africa or internationally. Due to the varied mechanisms of extraction these tests use it often becomes difficult to interpret P levels (mg kg^{-1}) reported by these tests. In the table grape producing area of Upington in the Northern-cape of South- Africa contrasting Bray II P and Olsen P levels have been reported. These soils are sandy and alkaline by nature and show deficits in P when extracted by Olsen, but sufficient P levels are reported by Bray II extraction tests. It thus becomes important to understand the relationship between these tests and the affect various soil physiochemical properties have on extractable P.

The main aim of this study was to examine the correlation between soil P extraction tests commonly used in South Africa and Internationally (Bray II, Bray I, Mehlich III, Citric acid, Olsen, Ambic 1 and 1:2 water extraction) with each other and with total soil P (acid digestion), in order to assess the relative efficiency of the tests on South African soils, and also to derive linear regression equations to make conversion between test soil P norms possible. A further aim was to examine the relationship between P extraction efficiency of the soil P tests and selected soil chemical and physical properties, to see which soil properties were affecting the tests.

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3.2 Materials and methods

3.2.1 Soil sampling

After consultation with a statistical expert at Stellenbosch University, Prof Martin Kidd, a soil sampling strategy was devised that would select soil samples that vary with regards to soil pH, C content, inherent P content, Fe and Al content and soil texture. The majority of the 49 soils were selected from a commercial soil analytical laboratory's soil sample database (Bemlab Pty Ltd., Somerset West). Farmers were contacted and composite samples were collected at defined depths of 0 mm - 300 mm and/or 300 mm – 600 mm of 49 soils from the Western Cape, Northern Cape, KZN and Limpopo provinces were collected. Soils were also sampled from natural areas (sample J1, 41, S1, S2 and 40A - *See list of soils in Appendix A*). Soil samples were air-dried and then passed through a 2 mm sieve prior to analysis.

3.2.2 Soil characterisation

Soil pH (1M KCl), EC (1:2 extract), exchangeable basic cations (ammonium acetated pH 7) and titratable acidity (K_2SO_4), cation exchange capacity (pH 7), organic C (Walkely-Black), total C (LECO analyser) and particle size analysis were determined by a commercial laboratory (Bemlab Pty Ltd., Somerset West). Soil CBD and oxalate extractable Fe and Al was determined by the MSc candidate at Department of Soil Science, Stellenbosch University.

Soil pH was measured in a 1 *M* KCl suspension with a glass electrode using a soil to solution ratio of 1:2.5. The electrical conductivity (EC) of the soils were determined of a water extract made by using 50 g of soil and 100cm^3 of deionised water, the mixture was centrifuged, and the EC of the clear solution was determined with a *Jennway conductimeter*. Cation exchange capacity (CEC) was determined using 0.2 *M* ammonium acetate (pH 7) method as described by the non-affiliated Soil Analyses Work Committee (1990), soils were then leached with 0.2 *M* K_2SO_4.The total NH_4^+ was then extracted with 1 *M* KCl and determined colormetrically in a SEAL Autoanalyzer 3 with a 15 mm flow cell and 520 nm filter. Organic C was determined with the oxidative titration method of Walkley-Black described by Walkley (1946). Total C was determined by dry combustion with a LECO instrument (USA). Clay, sand and fine silt was determined through calgon dispersion and the pipette method (Gee & Bauder, 1986).

Total Fe and partially crystalline AL oxides was determined with the citrate-bicarbonate-dithionite method as described by Jackson *et al.,* (1986). Finally, the analyte was analysed with an atomic adsorption spectrophotometer (AAS). Amorphous Fe and Al oxides were determined as put out by the acid ammonium oxalate in darkness method. The procedure followed was a modification of the procedure of Schwertmann (1964) and McKeague and Day (1966) described in Methods of Soil Analysis by Sparks *et al.,* (1996).

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3.2.3 Soil extractable P

Soil extractable P was determined with eight different extraction tests, namely, Bray II, Bray I, Mehlich III, 1 % Citric acid, Ambic 1, Olsen, Colwell and 1:2 water extraction with total acid digestible P being used as the reference method. Extractable Bray II, Bray I, Mehlich III, Olsen, 1:2 water P and total P was also determined by Bemlab (Pty) Ltd., (Somerset West, Western Cape Province). Citric acid extractable P was determined by Elsenburg analytical laboratory, Dept. Agriculture Western Cape. Ambic 1 P was determined by Labserve (Pty) Ltd., (Nelspruit, Mpumalanga Province). Colwell P was determined by the MSc candidate at the Department of Soil Science, Stellenbosch University.

Total soil P was determined using digestion with perchloric acid as described by Jackson (1958). Samples that contained high amounts of organic material were also treated with 15.8 *M* HNO_3. Bray I extractable P was measured as described by Bray and Kurtz (1945), shortly 45 ml of an extracting solution containing 0.03 *M* NH_4F and 0.025 *M* HCl was shaken for 40 seconds at 250 rpm on an automatic shaker with 6 g of soil sample. The suspension was passed through whatman.40 filter paper and the clear filtrate P concentration was determined with ICP-OES. Bray II extractable P was determined similarly to Bray I P, however an extracting solution of 0.03 *M* NH_4F and 0.1 *M* HCl was used. Mehlich III extractable P was determined as described by Frank *et al.,* (1998) and filtrate P concentration was determined with ICP-OES. An extracting solution consisting of 0.2 N acetic acid (CH_3COOH); 0.25 N ammonium nitrate (NH_4NO_3); 0.015 N ammonium fluoride (NH_4F); 0.013 N nitric acid (HNO_3) and 0.001 M EDTA was used and suspensions were shaken for 5 minutes. Citric acid P was determined as set out in the Handbook of standard soil testing methods for advisory purposes (Non-Affiliated Soil Analysis Work Committee 1990). Briefly, a 1 % citric acid solution is heated to 80°C. Twenty grams of soil is allowed to react with 200 cm^3 of 1 % citric acid solution. The soil-citric acid solution was then filtered and a 50 cm^3 aliquot was then heated to dryness for two hours to remove organic material. The residue was allowed to cool and 5 cm^3 concentrated HCl and 5 cm^3 concentrated HNO_3 were added and evaporated to dryness, this last step was repeated. Lastly 5 cm^3 and 20 cm^3 of de-ionised water was added to the residue and heated until dissolution. The aliquot was filtered, and P solution concentration was then determined by ICP-OES.

Olsen extractable P was determined as described by Olsen *et al.,* (1954) as set out by Sparks *et al.,* (1996). Briefly, a 0.5 *M* $NaHCO_3$ solution adjusted to pH 8.5 with NaOH was used and the suspensions were shaken with an automatic shaker at 250 rpm for 30 min. Colwell P was determined according to the method of Colwell (1963). Briefly, 1 g of soil was shaken for 16 h at room temperature (±23 °C) with 100 ml (1:100 soil-solution ratio) of $NaHCO_3$ at pH 8.5. The suspensions were then passed through a Whatman No.42 filter paper and the clear filtrate P

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concentration was determined by Bemlab using ICP-OES. Ambic 1 extractable was determined using the method described by the non-affiliated soil analyses work committee, 1990. Briefly an extracting solution containing 0.25 M NH_4HCO_3, 0.01M $(NH_4)_2EDTA$, 0.01M NH_4F and Superfloc N100 at pH 8.3 was used. P solution concentration was determined with ICP-OES. Water extractable P was determined with 1:2 soil: solution ratio. Briefly 10 g of soil was used and 20ml of deionised water was used. P solution concentration was determined with ICP-OES.

3.2.4 Statistical analysis

Statistical analyses of the data were done using STATISTICA 13.3 software. Multifactorial analyses were done on soil physiochemical properties and soil P test extractable P to get an estimate of the correlation between soil properties and extractable P. Pearson correlation coefficients (r) were determined between soil P extraction test and soil properties as well as between the various soil P extraction tests. Linear regression models were created comparing the various extraction test with each other and to generate conversion equations to convert between the norms of the various extraction tests. Standard error of measurement (SEM) for the correlation between the various extraction tests were also determined. Statistical analyses were done under the guidance and after consultation of Proff. Micheal Kidd from the Centre for Statistical Consultation (Department of Statistics and Actuarial Sciences University of Stellenbosch Private Bag X1, Matieland 7602, South Africa).

3.3 Results and Discussions

3.3.1 General soil properties

As designed by the initial sampling strategy, soils used in this study displayed a large range of soil chemical and physical properties (Table 3.1). With soil pH being a critical determining factor in influencing P reactions in the soil, a large range in the sample set pH was necessary. The pH (in KCl) ranged from 4.00 – 7.90 with a mean pH of the sample set being 5.83. The electrical conductivity (EC) showed a large variation ranging from 7 - 961 mS m^{-1} with a mean value of 80.7 mS cm^{-1}. Organic C ranged from 0.18 % - 2.24 % with a mean C content of 0.74 %, whereas, total C ranged between 0.17 % - 4.08 % with a mean of 0.72 %.

Central to P reactions in the soil is texture, influencing the availability of reactive functional groups available to bind applied P. Clay content expressed ranged from 6 % - 45 % with a mean of 17.7 %. Sand content also varied widely with a minimum of 10 % a maximum of 91 % and a mean of 73 %. The cation exchange capacity (CEC at pH 7) of the studied soil ranged from 2.19 $cmol_c$ kg^{-1} – 17.78 $cmol_c$ kg^{-1} and had an average value of 5.64 $cmol_c$ kg^{-1}. The dithionite- and oxalate- extractable iron (Fe_d and Fe_o) respectively ranged from 447 mg kg^{-1} –

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33801 mg kg^{-1} and 68 mg kg^{-1} – 18548 mg kg^{-1} . The Fe_O/Fe_d ratio is indicative of the iron oxide crystallinity (Wuenscher *et al.,* 2015). Within our sample set the Fe_O/Fe_d ratio varied from 0.01 – 6.2 with a mean value of 1.1. Theses ratio's show that our sample set featured many soils containing poorly crystalline/amorphous iron oxides (Fe_O/Fe_d > 0.5) but also featured soils containing primarily crystalline iron oxides (Fe_O/Fe_d <0.5) (Wuenscher *et al.,* 2015). In general, metal oxide content would be expected to increase in highly weathered soils, but oxide crystallinity is also said to increase with weathering (Antoniadis *et al.,* 2016). The CBD- and oxalate extractable Al of our sample set ranged between 69 mg.kg^{-1} – 7738 mg kg^{-1} and 41 mg kg^{-1} – 88389 mg kg^{-1} , respectively.

Table 3.1 Summary of physiochemical properties of soils used in this study (n=49)

	pH	EC	Carbon %		Clay	Sand	CEC	Fe_{CDB}	Fe_{ox}	Al_{CBD}	Al_{ox}	Fe_o/Fe_d
	KCl	$mS.m^{-1}$	Org C	Total C	%		$cmol_C\ kg^{-1}$	$mg\ kg^{-1}$		$mg\ kg^{-1}$		
Mean	5.83	80.6	0.74	0.72	17.7	73.0	5.64	8259	4100	1709	6056	1.10
Median	5.70	32.55	0.59	0.53	13.0	81.0	5.32	3828	2723	836	3442	0.49
Standard dev.	1.14	165.38	0.51	0.70	10.5	19.2	2.65	9225	4063	1919	12782	1.43
Min	4.00	7.00	0.18	0.17	6.0	10.0	2.19	447	68	69	41	0.02
Max	7.90	961.00	2.24	4.08	45.0	91.0	17.78	33801	18548	7738	88389	6.16

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3.3.2 Comparison of P extraction tests

3.3.2.1 Comparison of ICP-OES (Bemlab) and Colorimetric P data (Stellenbosch University)

Generally soil testing for plant available P is done by extracting P with a suitable extractor followed by a colorimetric assay to measure the concentration of P In solution (Hart & Cornish 2009). Currently in South-Africa commercial laboratories use inductively coupled plasma (ICP) spectroscopy to determine solution P concentrations, it is therefore important to understand the correlation between soil P determined with ICP-OES and P measured colorimetrically with the Ascorbic Acid method of Murphy and Riley (1962). There is a need to understand the relationship between the two methods, the shift could lead to the potential over or under fertilization which will have adverse environmental and economic implications (Hart & Cornish, 2009). It is widely recognised that ICP measures organic forms of P that may not by measured by the colormetric methods which predominantly measures orthophosphate (Hart & Cornish, 2009). A slight over estimation of solution P as measured by ICP-OES would thus be expected. Ten soils were selected and Bray II, Mehlich III and Olsen P were determined by Bemlab and by the MSc candidate. Bemlab used ICP-OES to quantify P in the extracts while the MSc candidate used the colorimetric ascorbic acid (Murphy & Riley, 1962), method to quantify P. For the selected soils, Bemlab's ICP-OES correlated significantly with the MSc candidate's colorimetric method for all the extraction tests ($p < 0.05$) (Figure 3.1). The most significant correlation was obtained between ICP-OES and colorimetric P as extracted by Bray II with a Pearson correlation coefficient ($r = 0.99$, $p < 0.05$) (Figure 3.1a). Mehlich III ($r = 0.94$, $p < 0.05$) and Olsen ($r = 0.96$, $p < 0.05$) also showed strong linear relationships between the two methods. These Pearson correlation coefficients with p-values smaller than 0.05 are indicative of highly significant relationships between the two methods and laboratories. Bemlab's ICP-OES tended to over-estimated solution P concentrations compared to colorimetrically determined concentration of Bray II (11 %) (Figure 3.1a) and Olsen (27 %) (Figure 3.1c) tests, whereas, it was underestimated for the Mehlich III test (-30 %) (Figure 3.1b). Adesanwo *et al.,*(2013) reported significantly higher P amounts determined by ICP-AES for Mehlich III, they however also reported a linear relationship for the two methods. It is however important to note that not only is this a comparison of P determination by ICP-OES and the Ascorbic acid method, P extraction tests performed by two different laboratories are also being compared. From the data collected it becomes apparent that there isn't a large difference between the two methods of determination and the two laboratories.

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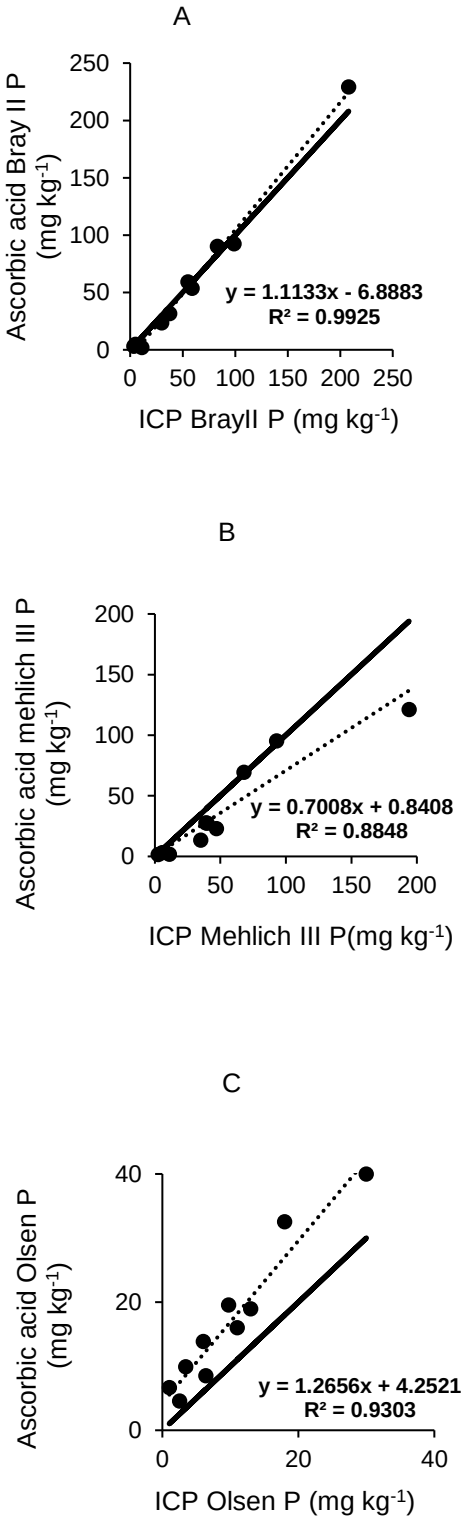

Figure 3.1 Relationship between solution P concentrations determined by ICP- OES and colorimetrically for a) Bray II b) Mehlich III and c) Olsen extraction tests (n=10). Black solid line represents 1:1 relationship.

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3.3.2.2 Relative effectiveness

For our sample set the measure of relative effectiveness of each extraction test was expressed as the percentage of P each extraction extracted of total P. Assuming that total P represents all the P in both P_i (inorganic) and P_o (organic) pools, the percentage each extraction test extracts of the determined total P will be indicative of how aggressive the tests under consideration are and how effective they are in predicting available P in the soil. However, it is also important to consider that not all of the P determined by acid digestion will be plant available. The mean percentage P each test extracted of total P increased in the following order: 1:2 H_2O < Olsen < Colwell < Bray I <Ambic 1 <Bray II < Mehlich III < Citric acid (Figure 3.2). This sequence is similar to that found by (Wuenscher *et al.,* 2015). Citric acid (38.9 %), Mehlich III (38.8 %) and Bray II (36.7 %) extracted the highest amount of soil P, significantly more than the other tests ($p < 0.001$) (Figure 3.1). Olsen (7.2 %) and 1:2 water extract (2.9 %) tests extracted the least amount of total P and did not differ significantly in terms of effectiveness ($p < 0.001$). Wuenscher *et al.,* (2015) however reported a significantly higher amount of P extracted by the Bray II extraction than for Mehlich III on European soils. However, in this study the difference of P extracted by Bray II and Mehlich III was not statistically significant ($p < 0.001$). Ambic 1 was the most aggressive of the alkaline extractors, extracting roughly 29 % of total P. The more aggressive nature of the Ambic 1 extraction could be due to the use of NH_4F in the extracting solution assisting in the desorption of P from Fe and Al oxides. In the case of the Ambic 1 extraction complexation of Al^{3+}, Fe^{3+} and Ca^{2+} by EDTA is the main solubilizing factor (Thomas and Peaslee, 1973) as cited by Schmidt *et al.,* (2004).

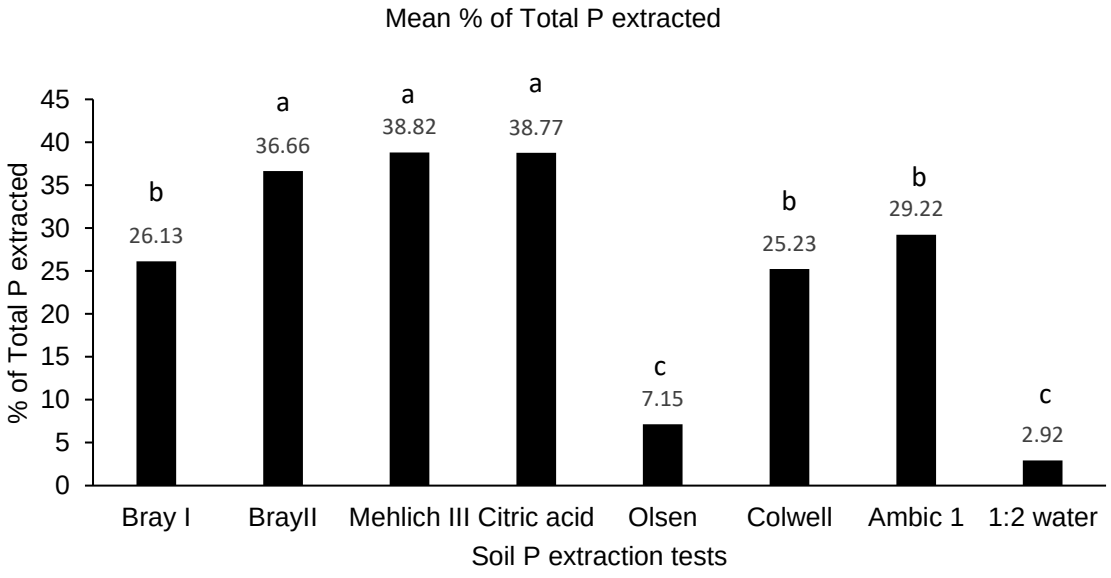

Figure 3.2 Mean % of total soil P extracted by the various soil P extraction tests ($p < 0.05$)

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The mean amount (n=49) of P (mg kg^{-1}) each test extracted can be grouped as follows; Olsen and 1:2 water extraction extracting 13.56 mg kg^{-1} and 5.45 mg kg^{-1} respectively, Bray I, Ambic 1 and Colwell extracting mean P amounts of 55 mg kg^{-1} and lower and Bray II, Citric and Mehlich III extracting mean P amounts of between 75 - 80 mg kg^{-1}. For our sample set the mean amount of P extracted via acid digestion (total P) was 190.88 mg kg^{-1}. Cross & Schlesinger (1995), reported a total P of 64 - 908 mg kg^{-1} in 88 soils globally, similarly in this study total P to varied largely (45 - 556 mg kg^{-1}), reflecting a large degree a variation in terms of P status within our sample set. Our findings compared to that of Wuenscher *et al.,* (2015), however they reported lower mean P levels for the Olsen extraction. As expected and reported by other researchers the acidic extraction was the most aggressive, with the alkaline extractions (Olsen, Colwell and Ambic) and 1:2 water extraction extracting the least amount of P. Acid extractions can however also dissolve unreacted rock phosphate P fertilizer which can lead to an over estimation of available P and artificially inflate their aggressiveness (Bolland *et al.,* 2003). In general, the acid extractions were more aggressive and showed a stronger correlation with total P. Bray II ($r = 0.78$; $p < 0.01$) and Mehlich III ($r = 0.76$; $p < 0.01$) both correlating significantly with total P for this study. It is however important to consider that most of the soils under consideration are acidic in nature (n = 34) and a better correlation between the acidic extraction tests and total P in acidic soil conditions are expected. Our soil sample set is thus favouring the acidic extraction tests but, is however representative of South-African and more specifically Western-cape soil conditions. These correlations are thus representative of the efficiency of these extractions for South- African soils.

As expected and reported by Colwell (1963), the Colwell test extracted more P than the Olsen test, this could be due to the increased reaction time and soil: solution ratio of the Colwell test. For our sample set the highest variation between minimum and maximum amount of P extracted was observed for Bray II, Mehlich III and citric acid extractions with a 704-fold, 515-fold and 336- fold increase from minimum P extracted to maximum P extracted respectively. The lowest variation between minimum and maximum amount of P extracted was observed for Olsen and 1:2 water extraction with a 114-fold increase between minimum and maximum. Wuenscher *et al.,* (2015) also reported the largest range of P for Bray II, however for this sample set the increase from minimum to maximum amount of P extracted was much greater. This could be due to some of the sampled soils not being used under intensive agriculture and have not received any P fertilizer. Saggar *et al.,* (1999), suggested that a wider range of P extracted would assist in the categorisation in P status groups. This range could be indicative of both the aggressiveness of the test and the sensitivity thereof. When expressing the relative effectiveness of these extraction tests as a correlation to total P it would be useful to split the

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sample set into two pH groups i) pH < 6.5 and ii) pH > 6.5. The correlation between total P and most of the acidic extractions (Bray II, Bray I and Mehlich III) showed higher significance at pH < 6.5 ($p < 0.01$). Most noteworthy is the increase in fit from r =0.45 at pH > 6.5 to r =0.64 at pH < 6.5 observed for the Mehlich III extraction. Ambic 1 P correlated more significantly with total P at alkaline pH levels as expected from alkaline extraction tests. Contrary to what was expected Olsen P showed a stronger linear relationship with total P at acid pH levels (r = 0.54) in comparison with a r = 0.41 at pH > 6.5. Regarding this, the Olsen extraction is relatively effective on larger pH range than initially thought. Citric acid although acid in nature is designed to increase the solubility of Ca-P explaining the significant relationship with total P at pH > 6.5 ($r = 0.60$; $p < 0.01$)

Table 3.2 Pearson coefficients between extraction tests and Total P at pH > 6.5 (n=14) and pH < 6.5 (n=33) (*P < 0.05 **P < 0.01)

Extraction test	**pH > 6.5**	**pH < 6.5**
	r	**r**
Bray I	0.20	0.54**
Bray II	0.61**	0.67**
Mehlich III	0.45**	0.64**
Citric acid	0.60**	0.45**
Olsen	0.41*	0.53**
Colwell	0.10	0.31**
Ambic 1	0.54**	0.42*
1:2 water	0.03	0.24*

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3.3.2.3 Correlation and conversion factors between soil P extraction tests

The Pearson correlation coefficients and standard error of measurement between the amount of P extracted (mg kg^{-1}) by the various soil P tests are shown in Table 3.2. As expected, the strongest linear relationship was observed between acid soil extractors Bray I, Bray II, Citric acid and Mehlich III (r = 0.92 - 0.99) (Figure 3.2a). Similarly, Kleinman *et al.,* (2001) reported a strong relationship between Bray II and Mehlich III (r = 0.94). Olsen P also correlated significantly with Bray II (r = 0.86) (Figure 3.2b) and Mehlich III (r = 0.85), this was also observed by Kleinman *et al.,* (2001) and *et al.,* (2015). Both Olsen and Colwell are alkaline and use $NaHCO_3$ as the key extracting reagent, a strong correlation between the two methods would thus be expected. This was not the case for the soils of our sample set, Olsen and Colwell did not correlate highly significantly with each other (r = 0.59; $p < 0.01$) (Figure 3.2c). This poor fit could be due to the increased reaction time and soil: solution ratio used by the Colwell test consequently resulting in larger amounts of P being extracted. In general, the Olsen P test is considered to be a measure of P intensity (amount of solution P), the Colwell being a longer extractor is considered to be a measure of P quantity (amount of labile P) (Rayment and Higginson, 1992). Citric acid P extraction being unique to South-Africa correlated significantly with Bray II, Mehlich III and Ambic 1 but not with Olsen. A strong correlation between Ambic 1 and other alkaline extractors would be expected. For our sample set Ambic 1 correlated most significantly with Citric acid extractable P (r = 0.96; $p < 0.01$). Ambic 1 also correlated significantly with Bray II and Mehlich III P, Schmidt *et al.,* (2004) reported a strong relationship between Bray I and Ambic 1 (r = 0.92-0.98). a significant relationship between Ambic 1 and Bray I (r = 0.72; $p<0.01$) was observed. Schmidt *et al.,* (2004) also found a highly significant relationship between Bray I P and Ambic P (r = 0.91).

Only Bray II P and Mehlich III P correlated highly significantly with total P demonstrating that these are the most aggressive extractants. Colwell P showed the weakest relationship with total P; this could be due to the fact that this soil set was predominantly acid soils, thus the alkaline extractors would not be as effective.

Through fitting linear regression equations to the data, it was possible to get an estimate of the correlation between the various methods and also to generate conversion equations to converts between test values (Table 3.3). It was important to have an estimate of the accuracy of these conversion equations, this was done by using the SEM (standard error of measurement in mg kg^{-1}) (Table 3.2) for the relationship between the various tests. It is important to consider that the SEM represents the standard error between the two tests and would consequently be larger when comparing an acidic extractor with and alkaline extractor than when comparing two acidic extractors, due to differences in efficiency of P extraction

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(Figure 3.1). Due to this the SEM needed to be weighted for relative efficiency of the tests, this was done by using the gradient of the regression line (conversion factor) fitted between two test and ultimately a weighted standard error of measurement (WSEM) (Table 3.4) could be generated as a true measure of the accuracy of the conversion equations. The WSEM (mg kg^{-1}) for the acidic extractors are larger than that for the alkaline extractors, this is due to these extractors being more aggressive and extracting a larger range of P (Table 3.3). Bray II and Mehlich III correlated highly significantly and has WSEM of 12.67 mg kg^{-1}, consequently when make a conversion using the linear regression equation will be subjected to a variation of 12.67 mg kg^{-1}. On the contrary, Olsen P and Bray II did not correlate as significantly but a WSEM of 9.35 mg kg^{-1} was determined and consequently a conversion from a Bray II P value to an Olsen P value would be subjected to less variation. It would also be possible to directly convert between P values obtained by Ambic 1 and Citric acid extraction, due to a highly significant correlation between these tests and a low WSEM of 17.28 mg kg^{-1}. The largest WSEM values were reported for conversions between Citric acid P and Mehlich III P and Citric acid P and Colwell P. It is thus of great importance to consider the WSEM when converting between the various test values using the regression equations. It is however important to mention that these conversions are based on extractions done by Bemlab using ICP-OES to determine solution P content and the conversion would thus be more accurate using data collected by Bemlab.

These regression equations were created by plotting a certain test (X) against another (Y) and then using the same equation for converting both ways. It is thus important to understand the relative aggressiveness of the two tests being converted in order to know which tests was used as dependent and independent variables in the linear regression model. Referring to Table 3.5, the gradient of the regression line fitted for the relationship between Bray II P and Olsen P is 4.82, keeping in mind that Bray II is the stronger extractor any Olsen P (mg kg^{-1}) value will need to be substituted for x and multiplied by a factor of 4.82, this will yield a corresponding Bray II P (mg kg^{-1}) value. For the conversion between Citric acid P and Bray II P, the regression equation is given as $y = 0.79x + 13.79$, consequently any Citric acid P value will be used as the independent variable (y) and this will yield a corresponding Bray II P value. Using these regression equations with an intercept will yield a more direct and accurate conversion between the various methods. However as suggested by Sonmez *et al.,* (2008) using regression equations with no intercept gives us a simple conversion factor and makes the conversion simpler. From there work comparing different soil :water ratios in soil salinity studies they didn't observe a significant change in fit for regression equations with an intercept and regression equations passed through the origin. When comparing the regressing equations given in Table 3.5 and 3.6 a similar trend for the relationship between highly

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significantly correlated tests was observed. For the relationship between Olsen and Bray I, Bray II and Mehlich III the gradient of the regression equation without an intercept was not significantly different from regression done with an intercept. The same was observed for other highly correlated tests such as Bray II and Mehlich III of which the correlation changed from r = 0.99 -0.97 when passed though the origin and the gradient changes from 1.13 – 1.10. These single factor conversions can be used with confidence when converting between the highly correlated extraction tests. Alternatively, for weakly correlated tests such as Olsen and Citric acid the gradient varied from 2.95 – 4.14 when passed through the origin, converting with a single factor from Olsen P to Citric acid P will most likely lead to an overestimation of Olsen P.

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Table 3.3 Pearson correlation coefficients between various extraction test and SEM (standard error of measurement) values expressed in mg kg^{-1}

P<0.01, ** highly significant correlations, SEM = standard error of the mean. Coloured blocks indicating significant correlations.

Extraction test	Bray I		Bray II		Mehlich III		Citric acid		Olsen		Colwell		Ambic 1		1:2 water		Total P	
	r	SEM	r	SEM	r	SEM	r	SEM	r	SEM	r	SEM	r	SEM	r	SEM	r	SEM
Bray I			0.92**	23.34	0.95**	26.57	0.68	39.23	0.89**	35.76	0.62	35.30	0.73**	31.57	0.68	41.37	0.68	55.75
Bray II	0.92**	23.34			0.99**	13.46	0.85**	29.83	0.86**	47.96	0.66	45.03	0.85**	30.80	0.63	53.41	0.78**	47.49
Mehlich III	0.95**	26.57	0.99**	13.46			0.82**	37.03	0.85**	56.48	0.66	52.91	0.84**	38.01	0.64	61.86	0.76**	49.75
Citric acid	0.68	39.23	0.85**	29.83	0.82**	37.03			0.57	46.99	0.74**	39.66	0.96**	17.28	0.33	50.56	0.60	61.00
Olsen	0.89**	35.76	0.86**	47.96	0.85**	56.48	0.57	46.99			0.59	17.75	0.56	36.35	0.75**	7.17	0.69	69.18
Colwell	0.62	35.30	0.66	45.03	0.66	52.91	0.74**	39.66	0.59	17.75			0.72**	29.42	0.35	20.61	0.58	73.14
Ambic 1	0.73**	31.57	0.85**	30.80	0.84**	38.01	0.96**	17.276	0.56	36.35	0.72**	29.422			0.34	39.71	0.60	62.00
1:2 water	0.68	41.37	0.63	53.41	0.64	61.86	0.33	50.56	0.75**	7.17	0.35	20.61	0.34	39.71			0.58	73.14
Total P	0.68	55.75	0.78**	47.49	0.76**	49.75	0.60	61.00	0.69	69.18	0.58	73.14	0.60		0.58	73.14		

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Table 3.4 SEM (standard error of measurement) and WSEM (weighted SEM) in units of measurement (mg kg^{-1}) of P extraction methods that showed significant correlations

Extraction Test	Olsen		Bray I		Bray II		Mehlich III		Citric acid		Colwell		1:2 H_2O		Ambic 1	
	SEM	WSEM	SEM	WSEM	SEM	WSEM	SEM	WSEM	SEM	WSEM	SEM	WSEM	SEM	WSEM	SEM	WSEM
Olsen			35.76	9.79	47.96	9.94	56.48	10.40					7.17	2.93		
Bray I	35.76	9.79			23.34	16.64	26.57	17.89							31.57	39.49
Bray II	47.96	9.35	23.34	16.64			13.46	12.76	29.83	28.22					30.80	26.28
Mehlich III	56.48	10.40	26.57	17.89	13.46	12.76			37.03	36.99					38.01	28.40
Citric acid					29.83	28.22	37.03	36.99			39.66	25.81			17.28	13.41
Ambic 1			31.57	39.49	30.80	26.28	38.01	28.40	17.28	13.41	29.42	76.22				

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Table 3.5 Linear regression functions with intercept describing the relationship between the various P extraction tests. Converting from (x) to (y).

Extraction method (x) / (y)	Olsen	Bray I	Bray II	Mehlich III	Citric acid	Colwell	1:2 water	Ambic 1	Total P
Olsen		-2.17+ 3.96X	10.18+4.82X	5.59 +5.52X	33.37 +2.95X	24.40 +1.29.X	0.75 +0.35.X	23.63 + 2.29X	119.65+5.25.X
Bray I			15.55+1.17X	9.07+1.39X	32.44+0.79X	26.29+0.30X	1.83+0.07X	19.92 + 0.67X	130.70+1.17X
Bray II				-5.25+1.13X	13.97+0.79X	22.74+0.25X	1.54+0.05X	7.88 + 0.61X	111.48+1.05X
Mehlich III					20.40+0.66X	24.05+0.22X	1.79+0.05X	0.53X + 11.95	119.33+0.89X
Citric acid						12.03+0.45X	3.31+0.03X	-0.292 + 0.75X	129.84+0.87X
Colwell							2.40+0.07X	-1.72 + 1.35X	128.18+1.50X
1:2 water								38.406 + 2.98X	138.89+9.52X
Ambic 1									129.72+1.12X
Total P									

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Table 3.6 Simplified conversion factors (regression equations without an intercept) between extraction tests that are significantly correlated. P < 0.01, ** highly significant correlations.

Extraction test (y) / (x)	Olsen		Bray I		Bray II		Mehlich III		Citric acid		Ambic	
	Y =	R^2	Y =	R^2	Y =	R^2	Y =	R^2	Y =	R^2	Y =	R^2
Olsen			3.88x	0.80**	5.20x	0.72**	5.72x	0.72**	4.14x	0.21	3.12x	0.23
Bray I					1.29x	0.82**	1.46x	0.90**	1.05x	0.34	0.83x	0.47
Bray II							1.10x	0.97**	0.87x	0.70**	0.66x	0.71**
Mehlich III									0.77x	0.63	0.59x	0.68
Citric acid											0.75x	0.92**
Ambic												

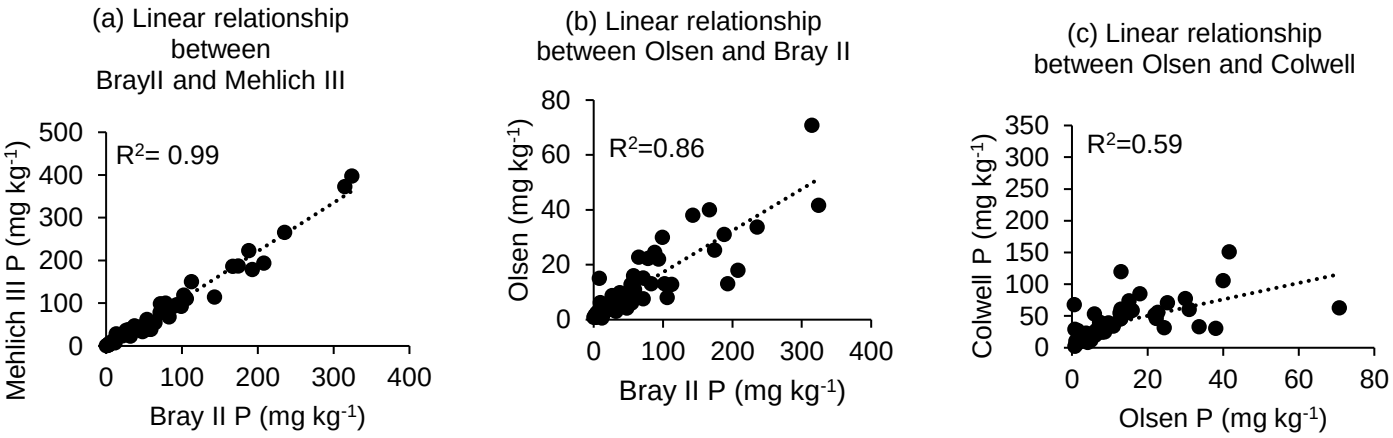

Figure 3.3 Linear relationship between (a) Bray II P and Mehlich III P, (b) Bray II P and Olsen P and (c) Olsen and Colwell P, correlation coefficient is shown (R^2) (n= 49, p <0.01)

3.3.3 P test efficiency in relation to soil physiochemical properties

Available P can be regarded as the amount of P that can be extracted by plant roots and used by the plant, due to the reactive nature of phosphorus in soils available P is influenced by the prevailing soil conditions and the plants ability to extract the available P (Zheng & Zhang

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2012). On the other hand, P availability is a parameter that does not reflect P solution concentration but is rather a function of the rate of its release from the solid soil into solution. The availability, solubility and extractability are largely influenced by soil pH, $CaCO_3$ content, texture, Fe/Al oxide content and organic matter content. In this study extractable P was correlated to pH, CEC, CBD- and oxalate Fe and Al, Clay and sand content as well as organic material content. The correlation between $CaCO_3$ and extractable P was not investigated in this study, Wuenscher *et al.,* (2015) reported a negative relationship between Bray II P and Olsen P with $CaCO_3$ content with Pearson correlation coefficients of -0.380 and -0.363 respectively. In this study it was decided upon to correlate a measure of the relative effectiveness of each soil P extraction test to selected soil properties. The measure of effectiveness was determined as a % of P extracted by each P test of total soil P (acid digestion).

The sorption of orthophosphate by Fe- and Al oxides is well known; particularly in acid soils these oxides have a net positive charge which favours P sorption to the metal oxide surface. In this study, CBD extractable Fe (Fe_d) was significantly negatively correlated with all of the extraction tests (Table 3.2). Bray II, Mehlich III, Citric acid and Ambic 1 showed the strongest negative correlations with CBD extractable Fe ($p < 0.01$), possibly indicating that they were more likely extracting P fractions more associated with Fe oxides. Dithionite extractable Al-oxides was only significantly negatively correlated with Bray II P ($r = -0.43$ $p < 0.01$), Mehlich III ($r = -0.43$; $p < 0.01$) and Citric acid P ($r = -0.54$; $p < 0.01$). This strong negative correlation with Bray II, Ambic 1 and Mehlich III P levels would be expected considering that these methods employ similar mechanism to extract P, targeting the release of P from Fe and Al oxides. Similarly, the Citric acid method, contains a high concentration of citrate which would complex Al and Fe. Olsen, Colwell and 1:2 water extractable P correlated weakly, and less significantly to CBD extractable Fe ($r = -0.32$, -0.43 and -0.41 respectively). Water extractable P only represents P in solution and not bound to Fe/Al oxides also explaining why no significant correlation with dithionite extractable Fe was observed.

In contrast, no significant correlations were observed between the any of the P tests and oxalate extractable Fe and Al (Fe_o and Al_o) (Table 3.2). However, the Fe_o/Fe_d (a higher value indicating lower iron oxide crystallinity) was significantly positively correlated with mainly the acid P tests, i.e., Bray I ($r = 0.58$, $P<0.01$), Mehlich III ($r = 0.55$; $p<0.01$) and Bray II ($r = 0.45$; $p<0.01$) (Table 3.2). This positive correlation between Fe_o/Fe_d extractable P indicates higher P extractability from soils with dominantly amorphous iron oxides (Wuenscher *et al.,* 2015). Wuenscher *et al.,* (2015) also reported negative correlations between Fe_o and Fe_d and extractable P, with water extractable P being least affected by oxide content. It was found that for the soils in our sample set extractable P was more affected by total oxide content than by

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amorphous oxide content. Similarly, to our findings a significant correlation between Fe_o/Fe_d and Bray II P (r = 0.405) and Mehlich III P (r = 0.427) was reported by Wuenscher *et al* (2015). Our results are however consistent with those of Torrent (1987), who found that Fe_d was a more effective P sink in some Mediterranean soils. It is believed that dithionite extractable Al can be substituted into crystalline Fe oxides such as goethite and hematite, resulting in the structural distortion of crystalline Fe oxides with implications on anion retention and surface area (Agbenin, 2003). Some studies reported higher P sorption for Al-substituted goethites than for pure goethites. This effect of Al substitution could also explain why a more significant correlation between CBD Fe and extractable P was observed than for oxalate extractable Fe. Also important to note is the relationship of soil pH and Fe oxide content in affecting P availability, maximum P fixation by Fe and Al oxides is said to occur at a pH(KCl) of 5 (Mabilde *et al.,* 2017). There is thus an interaction between the various soil properties in affecting P sorption.

Referring to Figure 3.1 (correlation circle), it is clear that there is a negative relationship between % sand and Fe/Al oxide content and that a positive correlation exists between oxide content and clay content. Due to this interaction a similar relationship between extractable P and clay and sand content would be expected. Menon *et al.,* (1989b) (as cited by Wuenscher *et al.,* 2015) also reported an increase in P extractability with an increase in % sand. Extractable P as determined by all the extraction tests showed a significant negative correlation with % clay (Table 3.2). The Pearson coefficients observed for Olsen P and % clay (r = -0.30) and Olsen P and % sand (r = 0.31) suggest that Olsen P was less dependent on texture than some of the other P extraction tests. Fernades *et al.,* (1999) (as cited by Wuenscher *et al.,* 2015) also reported that P extracted by Olsen was less dependent on soil texture. This is due to the Olsen test extracting P from Ca-P precipitates and not from P sorbed to Fe and Al oxides, which is strongly correlated to clay content. Wuenscher *et al.,* (2015) contrary to our findings reported that water extractable P did not correlate significantly with % sand, in our study however, % sand correlated more significantly (positively) with water extractable P, due to the fact that sandy soils have less fixed P which water is unable to extract.

Soil pH greatly affects P sorption and consequently is known to affect the amount of extractable P, due to its effect on the precipitation and adsorption of P. In general it is expected that Bray II, Bray I, Mehlich III and citric acid P tests will extract higher amounts of P on acidic soils, and Olsen and Colwell tests will extract more P at alkaline pH levels. However, for this soil sample set, there were no significant correlations between soil pH and extractable P by the various tests (Table 3.2). Zheng & Zang (2012) reported that not only does soil physiochemical properties affect the extractability of P but that the composition of fertilizer

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used may also influence the fractionation and solubility of P. Similar to the findings of Wuenscher *et al* (2015), a poor (negative) correlation between CEC and extractable P, with Bray II (r = -0.30) and Mehlich III (r = -0.31) was observed, showing a negative relationship with regards to CEC. A weak relationship between CEC and extractable P would be expected considering that CEC refers to the cation exchange capacity of the soil. CEC can be related to clay type; however, XRD analyses was not done to determine clay type. Knowing that the majority of the soils under consideration are from the western-cape the assumption can be made that kaolinite is the dominating clay mineral of our soils. Kaolinite has a low CEC but can bind P by the pH dependent variable charged broken edges of these clay minerals, this could explain the negative relationship observed between CEC and extractable P. Carbon content did not correlate highly significantly to any of the extraction tests. The strongest significant correlation with C was observed with Citric acid P and Ambic 1 P, which were also highly correlated to each other.

In addition to correlating the relative effectiveness of each extraction to soil physiochemical properties a multifactorial analysis was done on all the properties under investigation. Figure 3.1 is a correlation circle showing the correlation between the various physiochemical properties and soil P extraction tests. Factors within the outer circle apposing each other are highly significantly (negatively) correlated, factors that are perpendicular show no relationship to each other. From this the strong negative correlation between CBD Fe and Al, oxalate Fe and Al and clay content becomes apparent. Also, the negative correlation between % sand and CBD Fe and Al and oxalate Fe and Al explains the positive correlation between sand content and extractable P.

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Table 3.7 Pearson correlation coefficients between percentage each extraction extracted of total P (Relative effectiveness) and soil properties (n= 49, *P <0.05, **P<0.01)

	pH (KCl)	CBD Fe	Oxalate Fe	CBD Al	Oxalate Al	Fe_o/Fe_d	CEC ($cmol_c$ kg^{-1} soil)	C_{total} (Leco)	$C_{organic}$ (Walkley Black)	Clay %	Silt %	Sand %
Olsen	-0.11	-0.43*	-0.08	-0.35*	-0.21	0.32*	-0.24*	-0.08	-0.13	-0.30*	-0.17	0.32*
Bray I	-0.13	-0.54**	-0.03	-0.35*	-0.12	0.58**	-0.37**	-0.16	-0.02	-0.42**	-0.31*	0.43**
Bray II	0.19	-0.61**	-0.04	-0.43**	-0.17	0.45**	-0.30*	-0.18	-0.11	-0.50**	-0.06	0.32*
Mehlich III	0.08	-0.61**	-0.03	-0.43**	-0.18	0.55**	-0.31*	-0.15	-0.00	-0.49**	-0.17	0.35*
Citric acid	0.25	-0.60**	-0.15	-0.54**	-0.19	0.41*	-0.31*	-0.31*	-0.29*	-0.56**	0.05	0.22
Colwell	-0.12	-0.32*	-0.28	-0.31*	-0.24	0.19	-0.18	-0.21	-0.25	-0.31*	-0.11	0.23
1:2 H_2O	-0.03	-0.41**	-0.21	-0.37**	-0.27	0.07	-0.38**	-0.22	-0.27	-0.28*	-0.18	0.37**
Ambic 1	0.02	-0.57**	-0.21	-0.48**	-0.27	0.43**	-0.32*	-0.30*	-0.23	-0.52**	-0.01	0.20

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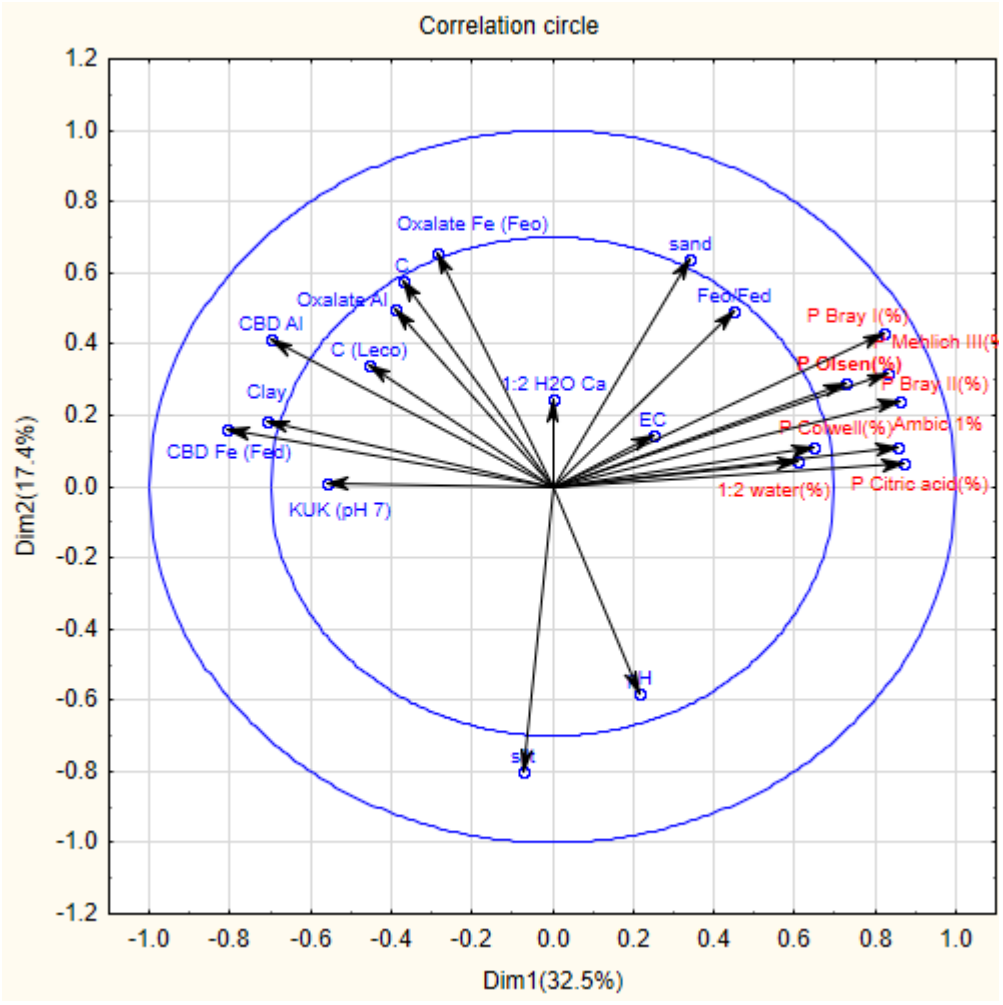

Figure 3.4 MFA Correlation circle showing the relationship between soil properties and extractable P

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3.4 Conclusions

The relative efficiency of the P tests in extracting total soil P was found to be as follows: 1:2 H_2O < Olsen < Colwell < Bray I < Ambic 1 < Bray II < Mehlich III ≈ Citric acid. Mehlich III and Bray II were the only two tests to correlate highly significantly with total P. The strongest linear correlations were observed between Mehlich III, Bray I and Bray II ($r = 0.92$-0.99, $p<0.01$), Ambic 1 and Citric acid ($r = 0.96$, $p<0.01$), and Olsen and Bray I, Bray II and Mehlich III ($r = 0.85$-0.89, $p<0.01$). Therefore, it is possible to directly convert between the soil P norms of these tests with more confidence. However, the WSEM (mg kg^{-1}) should be considered when making the conversion. It can recommended that field validation is required to determined what WSEM value can be considered as critical. Regression equations generated without an intercept have been used to generate single factor conversion equations. These conversions are however only effective for extraction tests that correlated highly significantly. Conversions from Olsen to Bray II and Bray I by using a factor of 5.20 and 3.88 respectively is thus possible. For the conversion from Bray II to Mehlich III a factor of 1.10 can be used. Converting from Citric acid to Ambic 1 can also be done accurately with a factor of 0.75. These conversion factors also comment on the relative aggressiveness of the tests, a larger conversion factor being indicative of a conversion from a weak extractor to a strong extractor. Considering that crop norms aren't available for all soil P extraction tests; these conversions will be a useful tool in converting between the various tests. These regression equations are based on soil P extraction data performed by various commercial labs (Bemlab, Elsenburg and Labserve) and should preferably be used to interpret soil P data produced by these labs. Further field validations of these conversions are necessary. These conversion equations are also only currently valid for typical Western and Northern Cape soils used predominantly in our study.

The multifactorial analysis (MFA) done on the extractable P and soil physiochemical properties revealed the strongest negative correlations between CBD extractable Fe and Al and clay content with regards to extractable P. As expected, the acidic P tests (Bray I, Bray II, Mehlich III, Citric acid, Ambic I) designed to extract labile P from Al and Fe minerals, were most significantly (negatively) correlated to metal oxide content. A weaker, yet significant, negative relationship was observed between CBD Fe and Olsen, Colwell and 1:2 water extract. For our soils, no significant correlations were observed between pH and extractable P, this could be due to the effect of the variation in fertilizer regimes affecting soil P fractionation and possibly masking the effect of some soil properties on soil P. Ultimately the mechanism by which the various extractors extract P will largely influence the effect soil properties will have on it.

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Chapter 4 **Making fertilizer recommendation based on soil sorption capacity and soil P test efficiency: Comparison of Phosphate Buffering Capacity, single-point sorption test and incubation methods**

4.1 Introduction

Phosphorous extraction test are effective in predicting amounts of plant available P but when making fertilizer recommendations the P sorption capacity of the soil also needs to be incorporated (Quintero *et al*, 2003). Phosphate sorption can be described by various sorption equations, most commonly the Freundlich equation has been used to describe P sorption. Sorption isotherms need to be created by equilibrating a known amount of P (mg kg^{-1}) with the soil and then measuring the resulting P concentration left in solution after the equilibration period. Multiple rates of P need to be added to the soil, this however is time consuming and expensive and thus a need exists for a single point measure of sorption. Burkitt *et al.,* (2002) compared various single point measures of sorption to a benchmark measure of phosphorus buffering capacity (PBC) as described by Ozanne and Shaw (1968) and found the strongest relationship between Phosphorus Buffering Index (PBI) and $PBC_{O\&S}$. However, this single point estimate of P assumes that some of the Freundlich parameters are constants. For it to be possible to determine single point estimates of PBC without assuming the parameters of the sorption equations to be constant it would be necessary to determine the correlations between sorption parameters and various soil physical and chemical properties (Henry and Smith, 2003). Consequently, it will require an intensive sample set to able to make accurate predictions for the various sorption parameters and their correlations to soil properties. Due to an excessive amount of P being equilibrated with the soil at a soil:solution ratio of 1:10 all of the available sorption sites react with the added P which would not be the case with P applied under field conditions (Johnston *et al.,* 1991). This method for estimating sorption may yield an unrealistic estimate of sorption, therefore a quick and simple incubation method simulating field conditions would be a more realistic approach. This estimate of sorption can then be used to adjust the amount of P fertilizer required (kg P ha^{-1}) to raise soil P levels with 1 mg kg^{-1}. Work has been done by Johnston *et al.,* (1991) to express P sorption as a phosphorus requirement factor (PRF) determined through an incubation study. This study was done on soils from the KZN-province with the use of the Truog, Bray I and Ambic extraction tests. However soils were only incubated for 6 weeks, a need exists to evaluate P sorption with an incubation method for a longer time period and with different soil P extraction tests.

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The objective of this study is to compare a multiple point estimate of sorption with a single-point estimate, and a simple incubation method that was developed in this study.

4.2 Materials and Methods

For this experimental unit, ten soils were selected based on expected P sorption capabilities. Five acid, clayey soils with low to moderate P contents from the Western-Cape and Limpopo provinces, and five alkaline, sandy soils with high P contents from the Northern-Cape were used (Table 4.1). Soils were sampled at a depth of 0 – 300mm and selected based on inherent P content and potential P sorbing capabilities.

Table 4.1. Physical and chemical properties of soils selected for the incubation study. Sample location given as province.

Sample	Location (province)	pH (KCl)	Clay %	Sand %	Bray II P (mg kg^{-1})	Mehlich III P (mg kg^{-1})	Olsen P (mg kg^{-1})
J1	Western-Cape	4.4	6	87	5	3	6
S1	Western-Cape	4.6	20	10	2	2	9
S2	Western-Cape	4.1	27	14	23	13	10
41	Western-Cape	4.2	37	53	3	2	7
48	Limpopo	6.7	13	75	31	23	20
53	Northern-Cape	7.5	11	81	92	95	40
54	Northern-Cape	7.9	11	83	54	28	16
56	Northern-Cape	7.2	7	89	90	69	19
57	Northern-Cape	7.5	9	85	229	121	33
58	Northern-Cape	7.9	11	87	59	27	14

4.2.1 Sorption isotherms

Similarly, to the work done by Burkitt *et al* (2002), sorption isotherms were created as described by Rayment and Higginson (1992). Shortly, 4 g of soil was equilibrated with 40 ml of a solution containing phosphate from KH_2PO_4 in 0.01M $CaCl_2$ (P concentrations ranged from 5 – 1000 mg P kg^{-1}). Calcium chloride was used as the background electrolyte and it has been found that the use thereof does not lead to Ca-P forming in the short time of the sorption equilibration reaction (Barrow, 2008). To reduce microbial activity 50 µL of chloroform was added to the equilibrating solutions (Rayment and Higginson 1992). Samples were shaken end-over-end for 17 h at room temperature (22 °C) and then passed through a Whatman no. 5 filter paper. The P concentration in the clear filtrate was determined colorimetrically as described by Murphy and Riley (1962). All treatments were performed in triplicate. The

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amount of P sorbed (q) by the soil (mg P kg^{-1}) was calculated as the difference between the amount of P added in mg P kg^{-1} and the concentration of P in the equilibrated solution (C) in mg P L^{-1}. Estimates of sorption were derived by fitting the Tempkin, Freundlich and Langmuir equations.

Phosphorus buffering capacity (PBC) was determined from the sorption curve as described by Rayment and Higginson (1992). The amount of P sorbed (mg P kg^{-1}) was plotted against solution P concentration (Log_{10} µ P L^{-1}) and the slope of what should be a linear line was derived to obtain PBC. By plotting sorption against the log of the equilibrated solution P concentration is was possible to derive the Tempkin PBC (mg P kg^{-1} / Log_{10}µ P L^{-1}) from the linear regression equation fitted to the data.

The Freundlich equation (Eq. 1) was selected based on a review by Barrow (2008) and use the thereof by Burkitt *et al* (2002) in estimating single-point sorption indices.

$$\mathbf{q = Kd \times C^b} \qquad \mathbf{(1)}$$

From equation 1, *q* refers to the amount of P sorbed, *Kd* (Freundlich constant) and *b* are constants and *C* refers to the P concentration in the equilibrating solution. The units of *q* and *C* are mg P kg^{-1} and mg P L^{-1} respectively. Parameter *b* is dimensionles and the dimensions of *Kd* is those of sorption devided by C^b. The linear form of the freundlich equation (Eq. 2) was fitted to the data, kd and b were derived from the linear regression equation.

$$\mathbf{Log\ q = b\ log\ C + log\ Kd} \qquad \mathbf{(2)}$$

Alternatively the freundlich equation can be written as $S = aC^b - q$, where in this case q refers to the amount of P that can be desorbed. Burkitt *et al* (2002) suggested using initial P content (BrayII and Olsen P) as a surrogate for this *q* parameter in the freundlich equation in order to make the curve pass through the origin. This transformed form of the Freundlich equation (Eq. 3) was fitted to the raw sorption data by using intitial P content (Bray II P and Olsen P). Due to all soils containing some P, sorption equations should have a negative intercept with the y-axis thus, there should be some desorption. For low P or virgin soils the amount of desorption is too small to measure, however this is not the case for fertilized soils.

$$\mathbf{(q + Bray\ II\ P/\ Olsen\ P) = Kd \times C^b} \qquad \mathbf{(3)}$$

For this study the $PBC_{o\&s}$ (Ozanne & Shaw 1968) was used as a benchmark measure of PBC to which single-point PBC indices were compared. This was done based on work done by Burkitt *et al* (2002). Parameters *Kd* and *b* derived from the Freundlich equation was used to calculate $PBC_{o\&s}$ (Eq. 4)

$$\mathbf{PBC_{o\&s} = Kd\ (0.35^b - 0.25^b)} \qquad \mathbf{(4)}$$

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Based on the work done by Burkitt *et al* (2002) and a review written by Barrow (2008), a measure of P sorption was determined with a single point method. This was done by using an estimate of parameter *b* for our sample set and the amount of P sorbed (q) and P in the equilibrated solution at a 1000 mg.kg^{-1} application rate. By calculating PBI (Eq. 5) a measure of P sorption with a single application of P at a rate of 1000 mg.kg^{-1} could be determined.

$$\mathbf{PBI = (q_{1000\ mg.kg^{-1}} + \theta\ Brayll\ Por\ Olsen\ P) / C^{b}} \quad \mathbf{(5)}$$

The Langmuir equation (Eq. 6) was also fitted to the raw data by plotting the inverse of solution P concetration against the inverse of P sorbed. Although it has been identified that the Langmuir equation is not suitable in describing the sorption of anions to a non-uniform surface such as the soil, It was still deemed it nessassary to investigate whether it explained the sorption of P to our soils (Barrow, 2008). By fitting the linear form of the Langmuir eqaution (Eq. 7) to the sorption data, *qmax* could be derrived, which gave us an estimate of the maximum amount of P sorbed by each soil.

$$\mathbf{q = (K_{lang} \times C \times qmax)/ (1+ K_{lang} \times C)} \quad \mathbf{(6)}$$

$$\mathbf{1/q= 1/K_{lang} \times qmax \times 1/C + 1/qmax} \quad \mathbf{(7)}$$

4.2.2 Incubation study

For the incubation study the aim was to i) add P at more realistic agricultural rates to the soil ii) to incorporate the added P in a manner simulating field conditions, consequently not allowing excessive mixing iii) to maintain the moisture at field water capacity (FWC). Samples were incubated with five rates of P (0, 50, 100, 150 and 200 mg P kg^{-1}), replicated three times in the form of KH_2PO_4 for 6 months (Table 4.2). This entailed adding 200 g of each soil to 250 ml console glass jars pre-cleaned with 1 % HCl. A P stock solution of 2000 mg L^{-1} was made using KH_2PO_4 pre-dried at 105 °C for 48 h and deionised water. The P treatments (0, 10, 20, 30 and 40 mg P) were added dropwise over the surface of each sample to ensure homogeneity of P application (Table 4.2). Approximately 30 - 45 ml (15.0 - 22.5 % of soil mass) of deionised was added to each soil including the KH_2PO_4 solutions to achieve FWC, the jars were covered with paper to prevent excessive water loss but to also allow for air exchange. The soil moisture content was readjusted monthly, this was done to allow the soils to dry out and consequently exposing the samples to wetting and drying cycles.

Representative soil samples of approximately 40 g were taken from each of the jars at 7 days, 30 days and 6 months. These samples were air dried and passed through a 2 mm sieve prior to determination of extractable P with Bray II, Mehlich III and Olsen extraction tests. Bray II P was determined as described by Bray and Kurtz (1945), however 0.1 *M* HCl was used and not

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0.025 *M* HCl. Mehlich III P was determined as described by Frank *et al.,* (1998), an adjustment was made that 4 g of soil was reacted with 40 ml of extracting solution, still maintaining a 1:10 soil to solution ratio. Olsen P was determined as described by Olsen *et al.,* (1954) as described in Sparks *et al.,* (1996). All glass (volumetric flasks and beakers) and plastic (centrifuge tubes and bottles) containers were rinsed with 1 % HCl prior to use. The P concentration in the filtrates was determined using the ascorbic acid method as described by Murphy and Riley (1962). Standard curves were created using all the reagents used in extraction tests and blanks were created similarly without P.

Table 4.2. Phosphorous Incubation treatments

Treatment	Rate (mg kg^{-1})	mg P 200 g^{-1}	Volume added (ml)
T1	0	0	0
T2	50	10	5
T3	100	20	10
T4	150	30	15
T5	200	40	20

Absorbance measurements were converted to available P (mg kg^{-1}) and plotted against P added (mg kg^{-1}) to obtain an estimate of P sorption, the gradient of the linear line was taken as the amount (percentage) of added P that is extractable. From the percentage of added P that was extractable the amount of P kg ha^{-1} required to raise soil P by 1 mg kg^{-1} can be adjusted to make a more accurate fertilizer recommendation. From the data collected it was decided upon to do a two-week incubation with two contrasting soils (high sorbing and low sorbing) applying one rate of P (100 mg P kg^{-1}) in the form of $KH_2PO_4^-$, $Ca(H_2PO_4)_2$ and Maxiphos®. Laboratory grade sources of P (KH_2PO_4 and $Ca(H_2PO_4)_2$) were oven dried at 105 °C for 48h and applied the same way as for the incubation study. Maxiphos® was ground to pass through a 1 mm sieve and applied directly to a 500 g dry sample, water was added, and the sample and fertilizer were thoroughly mixed, the beaker was then covered with parafilm to prevent excessive moisture loss. Due to the difficulty of ensuring a homogenous distribution of the fertilizer, 500 g of soil was used instead of 200 g consequently increasing the mass of fertilizer used to obtain the same end concentration. This larger mass of Maxiphos® made it easier to thoroughly mix it with the soil. Soil was sampled at 24 hours, 72 hours, 7 days and 14 days, dried and sieved to pass through a 2 mm sieve. Bray II extractable P was determined.

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4.2.3 Statistical analyses

All PBC indices were determined by using the mean of the three replicates done on each soil for each treatment. Sorption equations was fitted with MS Excel 2016 software and all correlation matrices were done with STATISTICA® 13.2 software. A 95 % confidence interval was used and one-way ANOVA analyses with post-hoc Fisher LSD test was used to investigate the significance of time on extractable P.

4.3 Results and Discussions

4.3.1 Sorption study: towards a single point PBC

The Freundlich, Langmuir and Tempkin equations all aim to make an estimate of the soil's ability to sorb a specific sorbate, in this case P, from batch equilibrium sorption data. All of the selected sorption equations succeeded in predicting the soils ability to sorb P. Both Freundlich and Langmuir described sorption equally well with R^2 - values ranging from (0.93 – 0.99) and (0.91 - 0.99), respectively (Table 4.3). For our data set, the Tempkin equation also gave a good measure of sorption but did not show the same goodness of fit (for sample 41 and 48) as the other equations. The soils under investigation showed a large range in sorption capabilities as indicated by the various sorption indices derived from the three sorption equations. Freundlich parameter *Kd* had a range of (5.35 – 22.86) and *b* (0.29 – 0.72), Langmuir *qmax* of (31.95 – 666.67 mg P kg^{-1}) and Tempkin a PBC of (60.82 – 208.76 mg P kg^{-1}/ Log µg P L^{-1}). Similarly, Burkitt *et al.,* (2002) also observed a *b* (0.11 – 0.98). Burkitt *et al.,* (2002) however observed much smaller values for *Kd* (0.0025 – 4) for the soils of their study. The *qmax* as derived from the Langmuir equation gives an indication of the maximum amount of P that can be sorbed by the soil, the soils of this sample set exhibited lower *qmax* values compared to Malaysiana *et al.,* (2018) but corresponded with *qmax* values obtained by Henry and Smith (2003) who investigated P sorption of soils from the KZN province of South-Africa.

The Freundlich equation showed a good fit and explained sorption for all of the soils of our samples set relatively well with *b* within the range of 0.1 -1 (Burkitt *et al*, 2002). The mean values for the replications of *Kd* and *b* were used to get an estimate of the Ozanne and Shaw (1968) PBC (mg kg^{-1}) for each soil of the sample set (Table 4.4). This measure of PBC determines the amount of P sorbed by the soil at solution concentrations ranging from 0.25 – 0.35 mg $P.L^{-1}$ and was used as the benchmark measures of PBC to which the single-point estimate PBI was compared.

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Table 4.3. Freundlich, Langmuir and Tempkin sorption indices. R^2 given as estimate of goodness of fit for each isotherm.

Sample	Freundlich		Langmuir		Tempkin	
	R^2	*Kd*	R^2	*qmax*	R^2	PBC
53	0.97	9.7	0.99	117.7	0.98	65.6
54	0.99	12.1	0.99	73.0	0.94	109.9
56	0.98	9.6	0.99	55.9	0.99	78.8
57	0.90	5.4	0.99	32.0	0.97	70.6
58	0.98	13.0	0.99	51.6	0.93	60.8
J1	0.93	22.9	0.91	57.8	0.98	174.4
S1	0.99	21.4	0.95	41.0	0.91	140.0
S2	0.99	22.2	0.95	270.3	0.90	130.2
41	0.99	26.2	0.99	666.7	0.87	204.2
48	0.98	15.9	0.99	90.9	0.73	208.

Kd – Freundlich constant

qmax – maximum amount of P sorbed (mg kg^{-1})

PBC – Phosphorus buffering capacity (mg kg^{-1}/ Log µg P L^{-1})

$PBC_{O\&S}$ varied significantly across the sample set (0.54 – 1.99 mg kg^{-1}) (Table 4.4). On the contrary, Burkitt *et al.,* (2002) determined larger values for $PBC_{O\&S}$ for their sample set. For our sample set the Freundlich equation was fitted by using initial P as a surrogate for the *q* parameter and without using initial P. The mean value for the *b* parameter excluding initial P (0.52), including Bray II P *b* (0.23) and Olsen P *b* (0.32) (Table 4.4). At lower solution concentrations (low P soils) the *b* parameter for our soils was not significantly affected by adding initial P, this however was not the case for higher solution concentrations. For the high P soils when excluding initial Bray II P from the Freundlich equation and using Equation 1, *b* = 0.66 and when using Equation 3 and including Bray II initial P *b* = 0.14. This decrease in *b* causes an increase in the curvature of the sorption curve and consequently leads to a higher estimate of *Kd*. From this is becomes clear that *b* parameter with a standard deviation of 0.18 is not a constant and is affected by various other soil properties and that *Kd* is sensitive to the size of *b*. Similarly, Barrow (2000) also observed a wide range of *b*, this was attributed due to error of measurement due to PBC being measured between a small concentration range and error induced by subsampling. The deviation was especially large with low P sorbing soils. Barrow (2008) also pointed out the limitations of the transformed Freundlich equation when

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working with low solution P concentrations, using this form of the Freundlich might lead to higher *b* values and consequently to an underestimation of sorption. However, Bach & Williams (1971) as sited by (Samadi, 2017) reported that initial P content has no effect on PBC as it gives a value of the isotherm slope and that the isotherm slope at the same equilibrium concentration will be the same. Barrow (2008) later determined that the slope of the isotherm could change with P addition highlighting the effect of initial P status on P sorption. However, using the transformed Freundlich equation was not significantly beneficial especially for the low P soils (Table 4.5). However, Burkitt *et al.,* (2002) found that for their sample set of 290 soils the transformed Freundlich equation using Colwell P and Olsen P showed a better fit (R^2 = 0.99) compared to the untransformed Freundlich equation (R^2 = 0.97). Barrow (2000) however reported that there were cases where using Colwell P as correction term was unsatisfactory, one such a case was when the amount of extractable P exceeded *q*. This was the case for the high P soils of our sample set with the Freundlich equation showing a poorer fit when initial P was included, but values for *b* still fell within the range of 0.1 -1. (Table 4.5).

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Table 4.4. List of Freundlich (Kd and b) parameters as derived from the transformed and untransformed Freundlich equations and PBCO&S (mg P kg^{-1}) values

Sample	*Kd*	*Kd* (Bray II)	*Kd* (Olsen)	*b*	*b* (Bray II)	*b* (Olsen)	PBC O&S (Bray II)	PBC O&S (Olsen)	PBC O&S
53	9.68	20.60	17.77	0.72	0.17	0.27	0.96	1.16	0.98
54	12.14	19.33	16.20	0.58	0.21	0.34	1.05	1.23	1.17
56	9.60	20.20	15.35	0.64	0.14	0.31	0.82	1.11	0.95
57	5.35	23.70	16.43	0.93	0.08	0.29	0.59	1.13	0.54
58	13.04	11.81	23.43	0.41	0.12	0.23	0.41	1.39	1.09
J1	22.86	23.02	22.74	0.34	0.33	0.30	1.70	1.59	1.72
S1	21.40	21.50	21.80	0.44	0.44	0.42	1.86	1.84	1.86
S2	22.21	23.01	22.58	0.29	0.25	0.27	1.43	1.49	1.54
41	26.22	26.27	26.31	0.34	0.34	0.34	1.98	1.97	1.99
48	15.92	19.86	18.99	0.52	0.27	0.31	1.30	1.37	1.48
Mean	15.84	20.93	20.16	0.52	0.23	0.31	1.21	1.43	1.33
Standard deviation	6.97	3.84	3.70	0.20	0.11	0.05	0.53	0.30	0.46

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Table 4.5. Investigating the effect of including initial P on calculated sorption indices, standard deviation and R^2. ***b*** and ***Kd*** are mean parameters derived from the Freundlich equation. Standard deviations given for estimate of sorption (***Kd***).

Index	Parameter estimates		Standard deviation	R^2
	b	***Kd***		
Freundlich	0.52	15.84	6.97	0.97
		Inclusion of initial extractable P		
Freundlich $_{+ \text{Bray II}}$	0.23	20.93	3.84	0.93
Freundlich $_{+ \text{Olsen}}$	0.31	20.16	3.70	0.95

From the multiple point sorption data Freundlich parameters *Kd* and *b* for each sample was used in Equation 4 to calculate $PBC_{O\&S}$ which was used as benchmark to which single-point estimates of sorption were compared. From the multiple point sorption data, sorption (q) and equilibrium solution P concentration (C) at an application rate of 1000 mg P kg^{-1} were used to calculate PBI (phosphorus buffering index) using Equation 5. This is simply a variation of the Freundlich equation and essentially a single point estimate of *Kd* is determined from this. $PBC_{O\&S}$ had a range of 0.55 – 2.01 mg kg^{-1} with a mean of 1.33 mg kg^{-1}, PBC as determined with initial Bray II P and Olsen P had means of 1.21 and 1.43, respectively (Table 4.6). $PBC_{O\&S}$ was also lower for the high P fertilised soils of the sample set and sorption was higher for the low P soils. Various estimates of *b* were used and investigated in order to get as close as possible to the measured values for *Kd*, *b* was manually adjusted for each sample and the mean of these respective *b* values were used to calculate PBI and later PBC. For our sample set two distinct groups ($P < 0.001$) were identified with each having a different estimate of *b*. For the high P soils, *b* was lower than for the low P soils, these estimates of *b* were used accordingly to predict *Kd* using single-point sorption data (Table 4.6). When using these selected *b* values (Table 4.4) PBC could be predicted more accurately than when using the mean for b (0.52). When using the mean values for the sorption data RMSE (root mean square error) for PBC decreased from 0.53 to 0.43 $mg.kg^{-1}$. Consequently, is was better to determine sorption using the mean q and C values for each sample, this could be due to the error introduced by subsampling (Barrow, 2000). This RMSE is still however high considering that the mean PBC measured was 1.33 mg kg^{-1}. When using initial Bray II P and Olsen P the RMSE increased significantly to 1.20 and 0.53 respectively, again showing that for our sample

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set using the transformed Freundlich equation was not beneficial in predicting sorption indices. When splitting the sample set into two groups using *b* values (Table 4.6), the RMSE was lower for the high P soils (0.12) than for the low P soils (0.65). Consequently, the single-point estimate of sorption was more effective for the high P, low P sorption soils. For the high P sorption soils it is thus much harder to get an accurate estimate of *b* and *b* varies largely due to the effect of other soil properties. The same trend was observed when using the transformed Freundlich equation for both Bray II P and Olsen P (Table 4.6). However no significant correlations between soil properties such as texture and the value of *b* was found. Thus, the biggest pitfall of this single point estimate is that it assumes *b* to be constant (Barrow, 2008).

Table 4.6. Comparison of single-point estimate of PBC and PBC O&S (mg P kg^{-1}). Kd is a single point Freundlich parameter, b is a freundlich parameter and was manually selected. Subscripts + Bray II and + Olsen indicate PBI calculated with initial P. Root mean square error (RMSE) in mg P kg^{-1} gives the mean amount the predicted PBC differed from the measured PBC $_{O\&S}$. Pearson correlation coefficients given as R^2, marked (**) correlations are significant at $p < 0.05$.

Single-point sorption indices	*b*	Kd (mean)	RMSE (mg P kg^{-1})	R^2
PBI^A general	0.63	9.59	0.12	0.85**
PBI^A + Bray II	0.57	19.62	1.19	0.49
PBI^A + Olsen	0.52	17.71	0.53	0.51
PBI^B general	0.84	20.68	0.65	0.85**
PBI^B + Bray II	0.82	22.69	1.19	0.49
PBI^B + Olsen	0.84	21.49	1.13	0.51

PBI^A = Phosphorus buffering capacity without initial P for high P fertilized soils

PBI^B = Phosphorus buffering capacity without initial P for low P unfertilized soils

Figure 4.1 also shows that without initial P, the single-point prediction of PBC was much closer to the actual measured $PBC_{O\&S}$, accordingly predicted PBC only correlated significantly with PBC without using initial P as a surrogate for *q* with a Pearson correlation coefficient of 0.85; P <0.05. Burkitt *et al.,* (2002) however used a constant value for *b* (0.41) and used Colwell P as a measure of initial P and found a strong relationship between PBI and $PBC_{O\&S}$ ($R^2 = 0.99$). Barrow (2008) also suggested selecting specific values for *b*, from his work it was best to assume *b* as 0.35 or 0.41 and to use Colwell P as direct estimate for *q*. However, for these studies a much larger sample set was used possibly making it possible to assume a specific value for *b* for the entire sample set. The Colwell extraction test using a larger soil:solution ratio reflects P quantity rather than P intensity and is less affected by the soils P buffering

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capacity. Whereas, the Olsen and Bray II extractions are both considered P buffering capacity sensitive extractants, however it is not certain whether they accurately reflect the affect P buffering has on plant available P (Moody 2007).

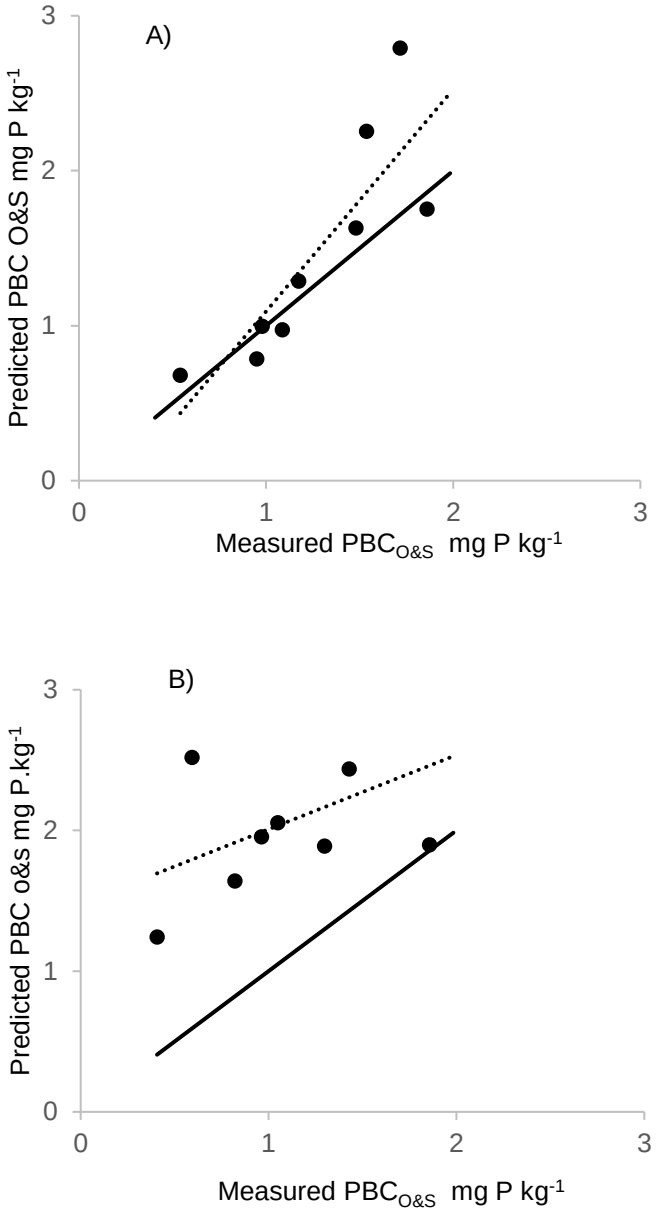

Figure 4.1 Plots of measured $PBC_{O\&S}$ values against predicted single-point estimates of PBC. The sample set was split into two groups using values of *b* as indicated. Figure a) is calculated without adjustment (*b = 0.63 & 0.84)*, Figure b) was calculated using Bray II P as a measure of initial P (*b = 0.57 & 0.82)*. In both case the solid lines are 1:1.

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4.3.2 Incubation study: towards a practical approach

It is hypothesised that estimates of sorption determined by sorption equations utilize unrealistically high levels of P and that sorption sites would be saturated to an artificial degree (Johnston *et al.,* 1991). These estimates of sorption also use soil:solution ratios that allow for complete saturation of the soil and all of the applied P to react with the entire sample, this however would not be the case with field conditions. Adding known amounts of P to a known amount of soil and incubating the sample at field water capacity and allowing for wetting and drying cycles would be a more field-realistic approach to estimate P sorption and could make accurate fertilizer recommendations to achieve a specific soil P concentration. The relationship between P measured (mg P kg^{-1}) and P applied (mg P kg^{-1}) was found to be linear (Table 4.7 and Appendix C), this agreed with observations of Johnston *et al.,* (1991). From the slope of the linear regression equations the amount of P extracted expressed as a percentage of applied P could be derived (Table 4.7). Using the percentage of P extracted with Bray II, Mehlich III and Olsen extraction tests a measure of the efficiency of the extraction tests and of sorption could be determined. A strong fit was observed between applied P (mg kg^{-1}) and extracted P (mg kg^{-1}) however, at an application rate of 200 mg kg^{-1} the slope of the regression line plateaued especially for the Olsen extraction test. Cole *et al.,* (1959) observed a similar trend for the Olsen extraction test on sandy soils, this could be due to the interferences caused by P precipitation with the Olsen reagents. For this reason, it was decided upon to only use a maximum application rate of 150 mg P kg^{-1} which lead to a better fit of the incubation data (Table 4.8). On the sandy, high pH soils from Upington, Mehlich III was the most aggressive test and extracted all of the added P, whereas, Bray II extracted 91 % of applied P followed by the Olsen (83 %). However, on the acidic clay soils, Bray II was more effective (64 %) followed by Mehlich (51 %) and Olsen (49 %) (Table 4.7). From the percentage extractable P the percentage of applied P sorbed by soil could also be determined. Ultimately from this the percentage of applied P sorbed and not extracted can be used to adjust fertilizer recommendations. Due to the strong linear relationship that exists between applied P and extracted P it was deemed necessary to investigate at which application rate the percentage extractable P best represents the soils ability to sorb P. At low application rates not all the sorption sites will be saturated, and sorption will be underestimated thus the applied rate should be sufficiently high.

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Table 4.7. Percentage of applied P extracted by Bray II, Olsen and Mehlich III extraction tests after one week and R^2 value of linear regression equation fitted to data of applied P (mg kg^{-1}) versus extractable P (mg kg^{-1}) (Max P treatment = 150 mg kg^{-1}).

Sample	Bray II		Olsen		Mehlich III	
	% P extracted	R^2	% P extracted	R^2	% P extracted	R^2
53	91	0.998	98	0.997	89	0.952
54	92	0.999	99	0.995	100	0.999
56	87	0.999	89	0.999	100	0.973
57	89	0.942	90	0.997	100	0.999
58	97	0.999	87	0.997	86	0.991
J1	72	0.999	47	0.993	61	0.988
S1	49	0.959	43	0.951	43	0.966
S2	58	0.994	41	0.990	43	0.998
41	45	0.980	32	0.976	33	0.987
48	92	0.987	77	0.999	73	0.954

Table 4.8. Comparison of goodness of fit of linear model describing Olsen P extractability using 5 rates (max. 200 mg kg^{-1}) or 4 rates (max. 150 mg kg^{-1}) in the incubation study.

Sample	% P extracted (max 150 mg kg^{-1})	R^2	% P extracted (max 200 mg kg^{-1})	R^2
53	96	0.997	90	0.993
54	96	0.995	85	0.979
56	86	0.995	79	0.991
57	87	0.997	71	0.945
58	85	0.997	92	0.993
J1	46	0.993	45	0.996
S1	41	0.951	43	0.975
S2	40	0.99	42	0.992
41	32	0.976	36	0.974
48	77	0.999	80	0.998
Mean	69		66	

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For The T3 (100 mg kg^{-1}) and T4 (150 mg kg^{-1}) treatments the percentage of applied P extracted correlated highly significantly with the percentage extractable P derived from the slope of the linear regression equations applied to each sample (R^2 = 0.92 and 0.99; $p < 0.05$). Therefore using a single application of 100 mg kg^{-1} – 150 mg kg^{-1} would give an accurate estimate of the amount of applied fertilizer that is extractable after a week.

Over the incubation period of six months the percentage P extractable with Bray II and Olsen extraction tests would be expected to decrease considering that the sorption of P is biphasic. The initial stages involving non-specific ligand exchange on broken edges and the latter involving precipitation reactions and the diffusion into the interior of the solid soil which would be less extractable (Tabatabai & Sparks, 2005). For our sample set percentage extractable Bray II P didn't decrease significantly over time for the light textured soils (Figure 4.2). However, an increase in extractable P was observed for two samples (57 and J1), this could be due to a sampling error or organic matter mineralisation. Both these samples are predominantly sandy textured and have a carbon content of 1.6 and 0.41 % respectively. Therefore this increase in extractable P cannot be explained by organic matter mineralisation and is most likely due to subsampling error. Discordantly, for the clayey soils the percentage extractable Bray II P did decrease significantly ($p<0.05$) after one month of incubation (Figure 4.2). In general, a mean decrease of 14 % in percentage extractable Bray II P was observed after 6 months. When sorbed ions react with the soils they alter the charge of the soil making it more negative and after a period of time this affect can became semi-permanent altering the sorbing capabilities of the soil (Barrow, 2002). This could explain why no significant change in percentage extractable Bray II P was observed especially for the high P soils and why most of the applied P could be extracted. These sandy soils having less Fe and Al oxides as well as a lower clay content, would also be expected to have less P binding sites. It is also important to considerer the effect pH has on P sorption, consequently the high P sorbing capabilities of these clayey soils cannot just be attributed to clay content. At pH values approaching 3 sorption is believed to increase for Fe-oxides whereas for clays the pH of maximum sorption is shifted to neutral pH values (Gerard, 2016). For this sample set these clayey soils were predominantly acidic in nature with pH < 5 thus Fe and Al – oxides are believed to be responsible for the decline in extractable P over time. Similar trends were observed for percentage Mehlich III extractable P this would be expected due to this test's strong relationship with the Bray II extraction test. On the contrary the percentage extractable Olsen P decreased highly significantly over 6 months for all of the soils ($p<0.05$) (Figure 4.3). The largest decrease in extractable P was however observed for the high pH sandy soils from the Upington region (49 %). For the clayey soils the decline in percentage extractable P over 6 months (18 %) was similar to that of Bray II. This large decrease in extractable P and

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increase in P sorption over time can be attributed to the slower nature of Ca-P precipitation (Barrow, 2008) and the less aggressive nature of the Olsen extraction.

The effect of texture and initial P content became apparent when 92 % of applied P could be extracted by Bray II from a high P sandy soil only 72 % could be extracted from a low P clayey soil. Considering that the soil has a set amount of sorption sites it is clear that the amount of sorption sites available to bind P and consequently the P level of the soil, will have an effect on P sorption. The sample set used for this incubation study was relatively small and thus no conclusive statements can be made about the effect of texture and initial P content but significant correlations between the percentage P extracted, and the initial P levels and texture was observed for our sample set. Bray II, Mehlich III and Olsen initial P levels correlated significantly with percentage P extracted (R^2= 0.78, 0.73 and 0.66; $P < 0.05$). As expected, clay content had a negative correlation with extractable P (R^2= 0.73, 0.76 and 0.66; $P<0.05$) with percentage extractable Mehlich III P correlating most significantly to clay content. Similarly, Johnston *et al.,* (1991) also observed a strong relationship between clay content and PRF (phosphorus requirement factor). The percentage P extracted increased with an increase in sand content for all three extraction tests (R^2 = 0.74, 0.68 and 0.69; $p < 0.05$).

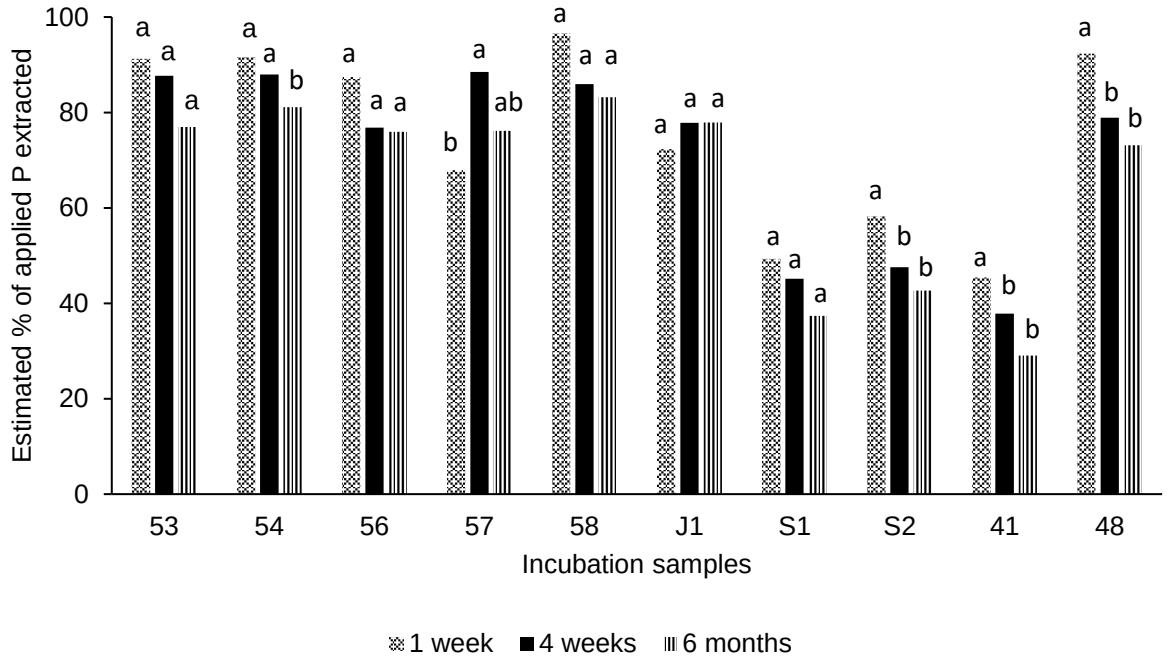

Figure 4.2 Percentage of applied P extractable by Bray II test after 1 and 4 weeks and 6 months. Significant letters applicable to each sample, $p < 0.05$.

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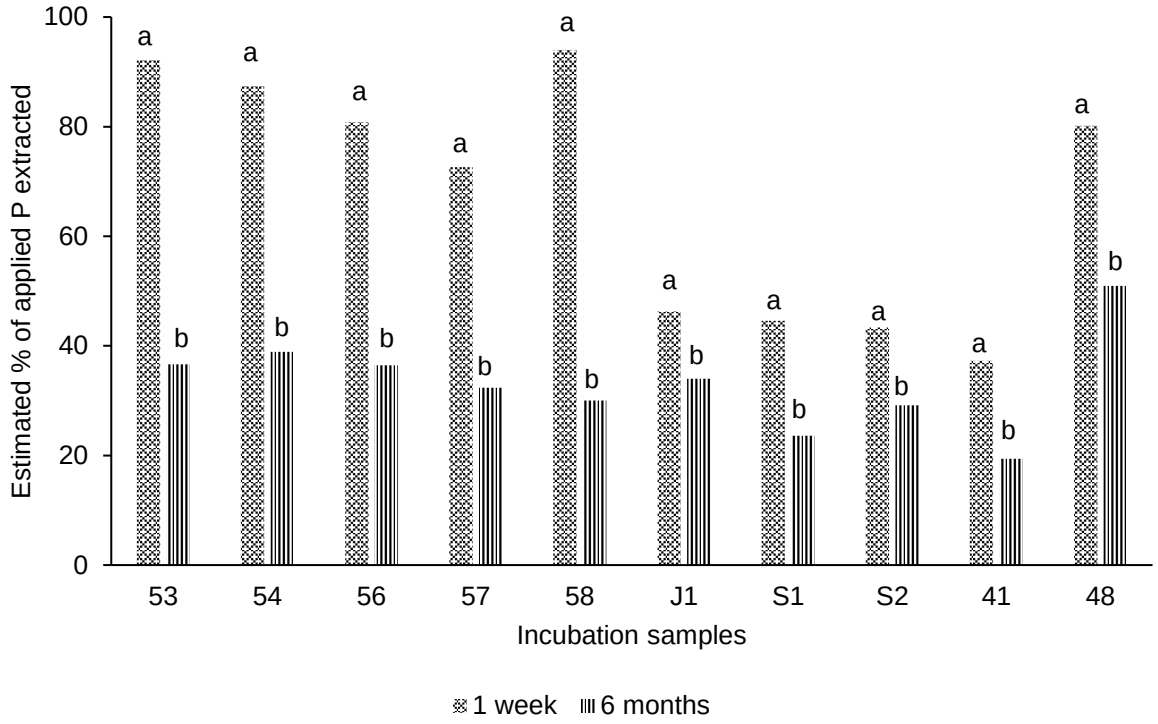

Figure 4.3 Percentage of applied P extractable by Olsen test after 1 week and 6 months. Significant letters applicable to each sample, $p < 0.05$.

The Bray II extractability of three different forms of P (KH_2PO_4, Ca $(H_2PO_4)_4$ and Maxiphos® was compared after an incubation period of one month (Figure 4.4). For the sandy high P sample (Figure 4.4 a) the amount of P extractable did not decrease significantly for each of the applied forms of P up to two weeks of incubation. Only for P in the form of KH_2PO_4 did extractable P significantly differ after one month of incubation, however extractable increased after a month of incubation. This could be due to a sampling error or mineralisation of organic P. For the other forms of P no statistically significant change in extractable P was observed over the one-month incubation period. For the $Ca(H_2PO_4)_4$ treatment however extractable P did significantly decrease after 72 hours as compared to extractable P measured after 24 hours. For both Ca $(H_2PO_4)_4$ and Maxiphos® treatments extractable P after 24 hours of incubation exceeded the applied amount, this could be indicative of an application error or insufficient incorporation of the applied P. For the moderate P, clayey soil (figure 4.4 b) extractable P did decrease significantly after one week of incubation for P applied as KH_2PO_4 . No significant difference between KH_2PO_4 and $Ca(H_2PO_4)_4$ was observed, P applied as Maxiphos® did however differ significantly for sample 48 but not for sample 53. This difference in extractable P applied as Maxiphos opposed to the other two forms can be due to the inaccuracy of the labelling of this commercial P fertilizer. The possibility exists that Maxiphos®

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contained more than 18 % P as indicated by the manufactures, leading to the higher observed extractable P when compared to the other forms of applied P, this was especially true for the high P sorbing soil (Figure 4.4 b). However extractable P decreased significantly after 24 hours of incubation with Maxiphos® where after extractable P then increased, no significant change was observed between one week and two weeks of incubation.

Therefore, when estimating sorption with this incubation method either KH_2PO_4 or Ca $(H_2PO_4)_4$ should be used. It also becomes apparent that for low sorbing soils the amount of applied P extractable does not significantly decrease over a one-month incubation period, this is however not the case for soils with higher P sorbing capabilities. For these soils extractable P significantly decreases after one week of incubation, there after soil P remains constant. Therefore for low P sorbing soils an incubation period of 24 hours can be recommended. However, for the high P sorbing soils due to the nature of P sorption an incubation period exceeding 72 hours (three days) can be recommended.

For this sample set the incubation Bray II sorption percentage as determined with the incubation method correlated significantly with the multiple point isotherm estimate of $PBC_{O\&S}$ (R^2= 0.80; $p<0.05$). This correlation although not highly significant is indicative that this incubation method succeeded in describing P sorption for the soils of this sample set. Similarly, is linear relationship was observed between PBC determined as described by Rayment & Higginson (1992) and percentage of applied Bray II P sorbed as determined by the incubation method (R^2=0.78) (Figure 4.5a). Sorption as described by the incubation method with percentage Olsen P sorbed was least effective in describing sorption when compared to sorption isotherm estimates of sorption. Due to the unrealistically high P concentrations used with sorption isotherms some non-linear responses between sorbed P and solution P concentration was observed, therefore with the lower levels of P utilised in the incubation method stronger linear relationships were observed giving a more realistic estimate of sorption.

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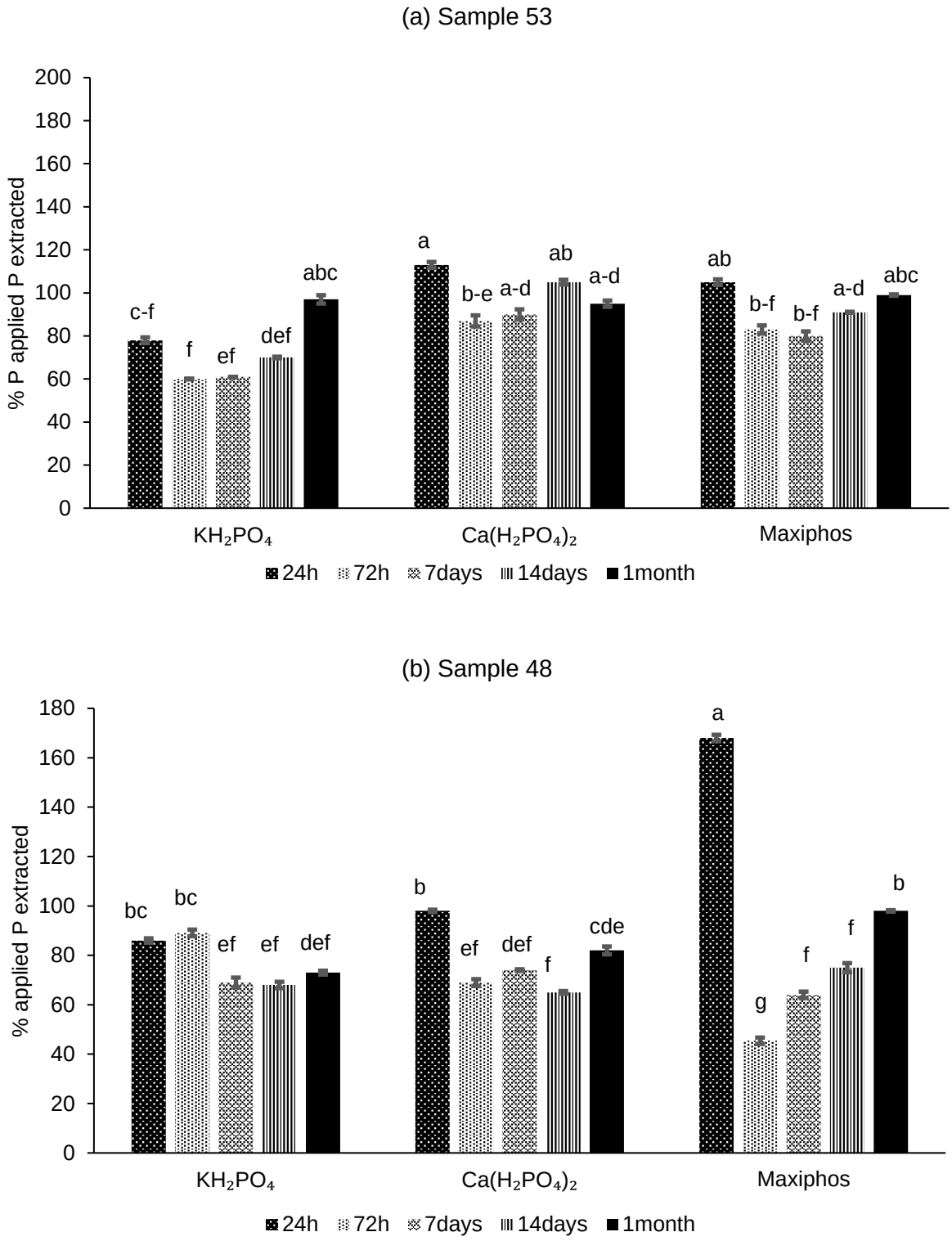

Figure 4.4 Effect of P source on percentage of applied P extractable by Bray II test for (a) an alkaline, high P sandy soil (sample 53) and (b) acid, moderate P clayey soil (sample 48) over an incubation period of one month. Error bars show standard error for the various treatments. P was applied at a rate of 100 mg kg^{-1}. Significant letters are applicable to each form of P and each sampling time ($p < 0.05$).

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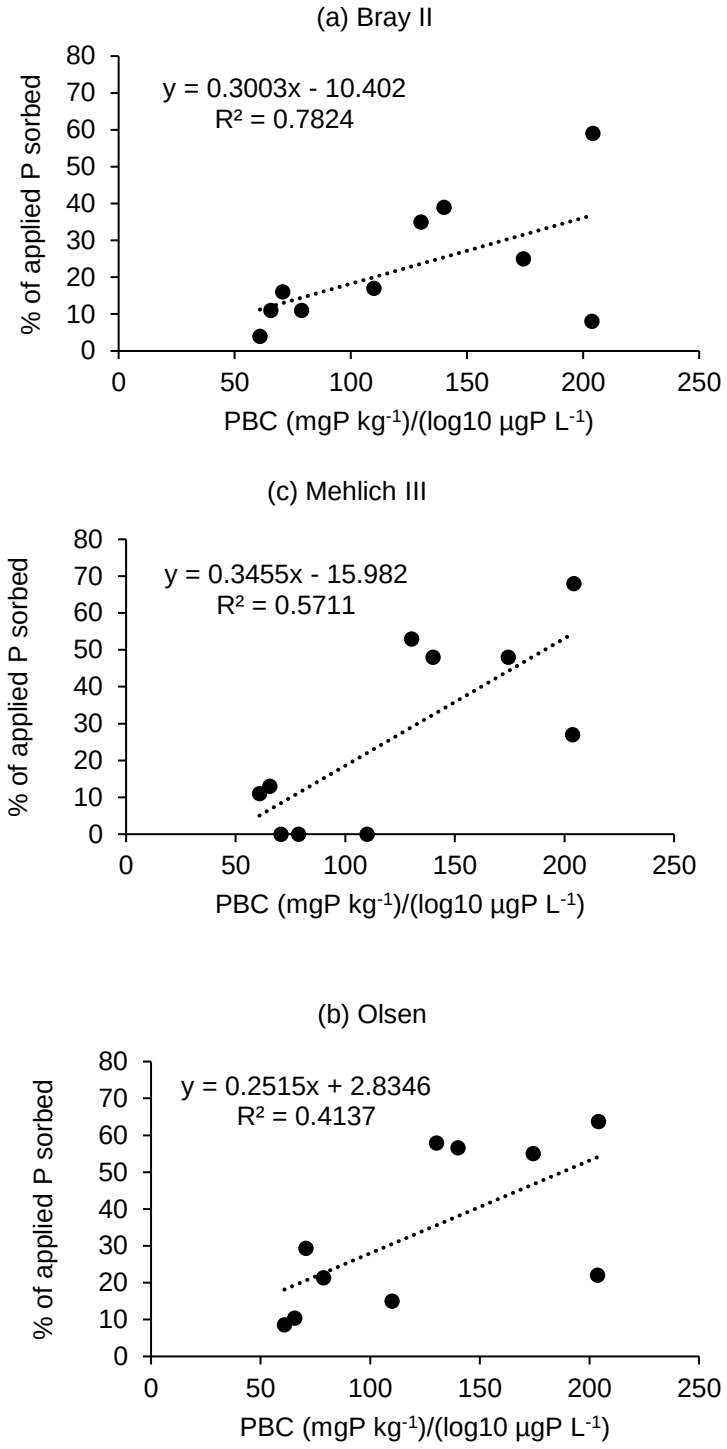

Figure 4.5 Relationship between PBC as determined through a sorption isotherm and as determined with an incubation method. Percentage sorption determined with (a) Bray II (b) Mehlich III and (c) Olsen extraction tests.

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4.4 Conclusions

Strong linear relationships were observed for the three sorption equations used to describe P sorption for the soils of our sample set. However, based on previous studies it was decided upon to use the Freundlich equation as well as a transformed variation thereof. The Freundlich equation without using initial P (Bray II P or Olsen P) described sorption for our sample set better (R^2 = 0.97) than the transformed variation using Bray II P (R^2=0.93) and Olsen P (R^2 = 0.95) (Table 4.3) . For our sample set the values for parameter *b* for both variations was within the range of 0.1 - 1 confirming that sorption was adequately described by these equations. It was found that using the transformed variation of the Freundlich equation had no beneficial effect on the estimate of sorption as compared to the general Freundlich equation for the low P, high sorbing soils of our sample set. This however was not the case for the high P sandy soils, being indicative of the affect initial P has on sorption. For these high P soils the size of *b* decreased when using the transformed Freundlich equation yielding a higher estimate for *Kd*. From the sorption data $PBC_{O\&S}$ (Ozanne and Shaw, 1968) could be determined. Sorption as shown by $PBC_{O\&S}$ was lower for the high P sandy soils, this could be due to both initial P content and texture. From the sorption data it became clear that parameter *b* varied across the sample set with the largest standard deviation (0.18) being observed for the high P clayey soils. Consequently, Freundlich parameter *b* is not a constant and is affected by various soils properties. Thus, a need exists to investigate the variation of *b* over a larger sample set and its correlation to various soil properties. When using the single point estimate of sorption (PBI) to predict $PBC_{O\&S}$ the strongest linear relationship was observed between PBI without initial P being used and $PBC_{O\&S}$ (R^2 = 0.85;$p < 0.05$). Although the single point estimate of sorption and $PBC_{O\&S}$ did correlate significantly it did not succeed in predicting PBC very accurately with an RMSE of 0.33, this deviation between the predicted single point measure of sorption and the measured $PBC_{O\&S}$ was however greater for the high P sorbing soils, this being indicative of the unpredictability of parameter *b* especially in high P sorbing soils. The most accurate measures of single point sorption were obtained by manually selecting for specific *b* values, from this two distinct groups with significantly different *b* values were obtained. One being that of the High P, low sorbing soils and the other being of the low P, high sorbing soils. Again, this confirms that a single estimate or constant value for *b* cannot be used to accurately predict PBC, without the original multiple point sorption data. Therefore an accurate estimate of a single point PBC could not be determined for the soils of this sample set. Therefore, it's clear that making an accurate prediction of sorption using a single point sorption isotherm is subject to a large amount factors and requires more knowledge regarding the effect of soil properties on sorption parameters and a larger sample set to understand how and to what degree parameter *b* varies across South-African soils. Therefore making fertilizer recommendations requires a more realistic and practical approach.

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For the incubation study a strong linear relationship between applied P (mg kg^{-1}) and extractable P (Bray II, Mehlich III and Olsen) was observed and it was possible to derive the percentage of applied P extractable from the regression equation fitted. At high application rates the regression line, especially for the Olsen extraction, plateaued and a better fit was obtained by using a maximum application rate of 150 mg kg^{-1}. The sorption explained by this incubation method also correlated significantly with sorption determined by the multiple point isotherms (R^2=0.80; $p<0.05$) indicating that this method did succeed in describing the P sorption for the soils of this sample set. Over the incubation time of 6 months the percentage extractable Bray II P did not significantly decrease for the high pH, sandy soils. However, a significant decrease in percentage extractable P was however observed for the low pH clayey soils. This being indicative of the biphasic nature of P sorption and that P will become less available over time. The decrease in percentage Olsen extractable P was much greater after 6 months, especially for the high pH soils of our sample set. The percentage Bray II extractable P also correlated more significantly (R^2=0.78) to PBC by sorption isotherm. Therefore using the Bray II extraction test in the incubation method would more accurately describe sorption. Extractable P at application rates of 100 mg kg^{-1} and 150 mg.kg^{-1} correlated significantly with percentage extractable P obtained from the slope of the regression equations fitted to the multiple point incubation data, therefore using a single application of 100-150 mg kg^{-1} would accurately predict the amount of applied P that is extractable and can be used to adjust fertilizer recommendations. Incubation with KH_2PO_4 and Ca $(H_2PO_4)_2$ did not yield significantly different estimates of percentage extractable Bray II P however, incubation with Maxiphos® varied significantly. For low P sorbing soils percentage extractable P remains constant up to a month and for high P sorbing soils percentage extractable P remained constant after 72 hours of incubation. Therefore it can be recommended to use either KH_2PO_4 or $Ca(H_2PO_4)_2$ and incubate rate of 100 mg kg^{-1} for 72 hours at field water capacity and room temperature (22 °C). This estimate of sorption expressed as percentage of applied P extractable can then be used to adjust fertilizer recommendations and accurately increase soil P by the desired amount.

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Chapter 5 **Evaluating P extraction tests based on plant response of grapevine and citrus in the Northern-cape**

5.1 Introduction

Phosphorus (P) is an essential macronutrient and considering that P is a finite resource, the effective management thereof is of critical importance. The over application of P can lead to its build-up in the soil, which in turn can lead to P loss, groundwater contamination and micronutrient deficiencies (Bai *et al.,* 2013). Thus, the possibility of P-build up and its effect on crop response needs to be investigated. Generally P deficiency is known to inhibit the initiation and maintenance of cluster primordia and consequently fruit yield of grapevine (Grant & Matthews, 1996). Phosphorous is also essential for citrus root growth and colour development of citrus fruit. The most significant effect a deficiency in P has on citrus is a decrease in fruit quality, with an increase in acidity, a decrease in juice content and fruit appearing coarse and over mature. (Coetzee, 2007). It is accepted that P concentration in grapevine leaves will increase with P fertilization and that low leaf concentrations are indicators of poor P supply (Conradie & Saayman, 1989). It is also understood that leaf and petiole P concentrations, for both grapevine and citrus aid in identifying soils with inadequate P levels however, soils where over fertilization has occurred it may be difficult to identify from leaf analysis (Conradie & Saayman, 1989).

For the neutral to alkaline soils of the Northern Cape region of South Africa, contrasting soil P concentrations, as determined by Bray II and Olsen extraction tests have been reported. Typically, the Olsen P test indicates sub-optimal soil P, while Bray II P test shows sufficient or excessive soil P. Annual P fertilizer application is common practice due to expected immobilisation of P through the formation of tricalcium phosphate [$Ca_3 (PO_4)_2$]. A need to establish which of these soil P extraction tests correlate best with leaf and petiole P concentrations in grapevine (Prime and Sultana) and leaf P concentrations in citrus (Nadorcott) thus exists. Furthermore, it is also not clear whether P application on these high P soils has a significant effect on soil P content and crop P nutritional status. It was hypothesised that for high pH soils with high P concentrations there will be a minimal response to applied P with regards to soil P content and leaf P concentration, and that this could eventually lead to undesirable soil P build-up and run-off or leaching losses. Therefore, the objective of this study was to examine the relationship between Olsen, Bray II and Mehlich III soil P levels and P uptake by grapevines and soft citrus on alkaline soils in the Northern Cape region. The Mehlich III extraction test will be included as it is known to be less affected by soil pH and functions at a wider pH range. Highly significant correlations between Mehlich III and

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total P has been observed and the use of this extraction, if correlated to crop response, could be beneficial for the industry.

5.2 Materials and Methods

5.2.1 Experimental Design

The trial was conducted on high pH sandy loam soils located in the lower Orange River region. Sites were selected according to their inherent Bray II and Olsen P concentration from data collected by Bemlab (Table 5.1). Extractable Olsen and Bray II P concentrations of 12 mg kg^{-1} and 20 mg kg^{-1}, respectively, are considered soil critical values for grapevine production (Oberholzer, 2016). Thus, it can be seen that the according to the Olsen test, the New Grow site was deficient in soil P, whereas, according to the Bray II P test, that site contained excessive soil P (Table 5.1). According to general P guidelines for crop production (Pierzynski, 2000), the Mosplaas site contained sufficient Bray II P (above 25 - 30 mg kg^{-1}) but insufficient Olsen P (less than 10 - 15 mg kg^{-1}).

Table 5.1 Initial soil pH (KCl), Bray II and Olsen P concentrations at experimental sites used in the study.

Experimental site	Soil pH (KCl)	Bray II P (mg kg^{-1})	Olsen P (mg kg^{-1})
Yarona	7.3	121	18
New Grow	7.5	59	11
Mosplaas	7.3	45	8

It was assumed that grape vines remove about 0.7 kg P ha^{-1} for every ton of production, consequently the standard yearly fertilizer application amounts to 30 kg P ha^{-1}. This rate of 30 kg P ha^{-1} was used as the standard rate to which the treatments were adjusted. Similarly, for the site planted to Nadorcott manderins adjustments were made according to an industry standard rate of 30 kg P ha^{-1}. Karsten farming uses fertigation systems and for the duration of this trial the rows under investigation (where possible) were taken off this system and P was added manually. However, this could not be done for experimental site two (New-grow) and three (Mosplaas) thus, it was not possible to have a control treatment of 0 kg P ha^{-1}. Maxiphos® (supplied by Omnia, 18 % P content) was used as source of P and applied as a split application with two instalments before harvest and one directly after harvest. After the second instalment soil and leaf samples were taken from each of the sites. Bray II, Olsen and Mehlich III extractable P and foliar P were determined for each treatment determined by Bemlab Pty (Ltd), Somerset West.

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5.2.2 Experimental Site 1 – Yarona

Experimental site 1 was located on Yarona farm (28°39'42.74"S, 21° 7'36.04"E) and planted to Thompson seedless/Ramsey grapevines, spaced 1.8 m × 3.3 m. Each experimental plot consisted of a row of four vines with a barrier vine at each side, a six-vine barrier was included on both sides of the rows to separate the site from the access roads. Four treatments (Table 5.2) were replicated six times in a randomised block design, the granular P fertilizer Maxiphos® was applied evenly on the vine row. The treatments were split in three instalments and two were applied before soil and leaf samples were taken at veraison. Soil samples were taken of the 0 - 300 mm layer with a manual auger in the application zone. Leaf samples were taken as described by Oberholzer (2016), e.g. leaves were selected from bearing shoots between node three and five. Leaf blades and petioles were separated in the field and placed in brown paper bags for processing. Extractable Bray II (Bray and Kurtz, 1945), Mehlich III (Frank *et al.,* 1998) and Olsen P (Olsen *et al.,* 1945 as cited by Sparks *et al*, 1996). Leaf blade and petiole P concentrations were determined as described by Clesceri *et al.,* (1998) by Bemlab.

5.2.2 Experimental Site 2 – New-grow

Experimental site 2 was located on New-grow farm (28°40'8.48"S, 21° 9'3.25"E) and planted to prime grapevines spaced at 2 m × 3.3 m. Each experimental plot consisted of four vines with a barrier vine on each side, a six-vine barrier was included on both sides of the rows to separate the site from the access roads. Three treatments (Table 5.3) was replicated seven times in a randomised block design. Fourteen kg P ha^{-1} was applied through fertigation with potassium fertilizer, while the remainder of each treatment (T1 = 16 kg P ha^{-1} , T2 = 31 kg P ha^{-1}, T3=46 kg P ha^{-1}) was granular P fertilizer Maxiphos®, applied by hand to the vine rows. The granular P were split in three instalments and two were applied before soil and leaf sampling at veraison. Leaf and soil sampling was done similarly to the Yarona site.

5.2.3 Experimental Site 3 - Mosplaas

Experimental site 3 was located on Mosplaas farm (28°47'49.44"S, 20°36'31.12"E) and planted to Nadorcott citrus spaced at 3 m × 6 m. Each experimental plot consisted of three trees, a six-tree break was included as a barrier between access roads at each end of the row. Three treatments (Table 5.4) were replicated seven times in a randomised block design. Fourteen kg P ha^{-1} was applied through fertigation together with potassium fertilizer, while the remainder of each treatment was granular P fertilizer Maxiphos®, applied by hand to the trees (T1 = 16 kg P ha^{-1} , T2 = 31 kg P ha^{-1}, T3 = 46 kg P ha^{-1}). The treatments were split in three instalments and two were applied before soil and leaf sampling at veraison. Leaves were sampled at fruit set and selected from bearing and non-bearing shoots. The third to fifth leaves from the apical fruit and from the shoot apical was sampled. Soil samples were taken according the same method used at the Yarona and New Grow sites.

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5.2.4 Statistical Analyses

Statistical analyses of the data was done using STATISTICA® 13.3 software. The trial was set up as a randomised block design, a one-way Anova analyses was done of the various treatments and their effect on soil extractable P and leaf P concentration. LSD-fisher post hoc tests was used to report the significance of the treatments. Olsen soil extractable P data was determined as not homogenastic with the Levene test for homogeneity and the Games-Howell post hoc test was done for these data points. A 95 % confidence interval was used for all statistical procedures. All statistical analyses were done under the guidance of and after consultation with Prof Micheal Kidd from the Centre for Statistical Consultation (Department of Statistics and Actuarial Sciences University of Stellenbosch Private Bag X1, Matieland 7602, South Africa).

Table 5.2 Phosphorous fertilizer application rates at Yarona experimental site

Treatment code	Rate (kg P ha^{-1})	% of standard rate	Maxiphos® Per application (g $vine^{-1}$)	Total Maxiphos® (g $vine^{-1}$)
T1	0	0	0	0
T2	15	50	17	51
T3	30	100	34	102
T4	60	200	67	201

Table 5.3 Phosphorous fertilizer application rates at New Grow experimental site

Treatment code	Rate (kg P ha^{-1})	% of standard rate	Maxiphos® Per application (g $vine^{-1}$)	Total Maxiphos® (g $vine^{-1}$)
T1	30	100	20	60
T2	45	150	38	114
T3	60	200	57	171

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Table 5.4 Phosphorous fertilizer application rates at Mosplaas experimental site

Treatment code	Rate (kg P ha^{-1})	% of standard rate	Maxiphos® /instalment (g $vine^{-1}$)	Total Maxiphos® (g $vine^{-1}$)
T1	30	100	53	159
T2	45	150	103	309
T3	60	200	153	459

5.3 Results and Discussions

5.3.1 Effect of P fertilizer application on extractable soil P

Figures 5.1 to 5.3 show the effect of the different P application rates on soil test extractable P at the three experimental sites. When examining the initial P (control treatment) of the Yarona site (Figure 5.1), a large degree of variation was observed in all the tests. Olsen extractable P with a mean of 18.12 mg kg^{-1} in the control had a standard deviation (SD) of 9.56 mg kg^{-1}, this amounts to a 52 % variance for background P. This large variation in initial P would make it hard to detect any changes that might be brought about by P application. Similarly, the Bray II and Mehlich III, with SDs of 29.56 mg kg^{-1} and 47.22 mg kg^{-1}, respectively, varied 24 % and 35 % from the mean, respectively. This large amount of variation in background P can be accounted for by natural soil variation and the routine method of P application. Due to the immobility of P in the soil, P fertilizer build-up from previous seasons accumulated in specific zones around the vines, consequently sampling in or outside these zones could lead to a large variation the observed initial P level. Thus, because the large background variation of soil P at the high soil P sites, Yarona and New Grow (Table 5.1), the effect of the fertilizer treatments was not statically significant, using the more aggressive Bray II and Mehlich III P tests (Figures 5.1 and 5.2). Whereas, Olsen P did produce some statically significant differences at the Yarona and New Grow sites (Figures 5.1 and 5.2). At the Mosplaas site, with lowest inherent soil P (Table 5.1), some statistically significant differences were observed between treatments using all three tests (Figure 5.3).

At the Yarona site, extractable P seemed to decrease at highest P application rate (Figure 5.1). This was possibly due experimental error in the field and to the large P background variation at the site. The potential of P leaching due to excessive P concentrations can be ruled out as, an Olsen-P value of ≥ 40 mg kg^{-1} has been considered a critical level for having

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a risk of P loss through leaching and surface runoff (Zhong *et al.,* 2004). When performing linear regression on the relationship between applied P (mg kg^{-1}) and extracted P (mg kg^{-1}) the correlation coefficient (R^2) increased from 0.69 to 0.96 for Olsen, from 0.24 to 0.99 for Bray II and from 0.29 to 0.97 for Mehlich III P when removing the data points of the highest P treatment. This also suggests that last treatment was applied incorrectly.

Figure 5.4 a-c shows the strong linear relationship between extractable P and applied P (excluding the highest P treatment) at the Yarona site (R^2 = 0.96-0.99). When comparing the efficiency of the various tests (as indicated by the magnitude of the gradient (m) of the linear regression equations), it is clear that the more aggressive P tests, Mehlich III (m = 0.85) and Bray II (m = 0.68), were considerably more effective at detecting applied P than the Olsen test (m = 0.30). Similarly, for the Mosplaas experimental site (Figure 5.4 d-f), Mehlich III (m = 0.90) and Bray II (m = 0.78) were more effective in detecting applied P than Olsen (m = 0.20). However, at the New grow site (Figure 5.4 g-i), Bray II was more effective (m = 1.14) than Mehlich III P correlating poorly to applied P (m = 0.59, R^2= 0.38), whereas Olsen remained weak (m = 0.17). This illustrates that the more aggressive soil P tests are likely better at picking up soil P excesses compared to Olsen test, even on neutral to alkaline soils.

Zhong *et al.,* (2004), as cited by Bai *et al.,* (2013), reported that an Olsen-P value of ≥ 40 $mg.kg^{-1}$ has been considered a critical level for having a risk of P loss through leaching and surface runoff. Mabilde *et al.,* (2017) also found that excessive P application in North West - Europe to sandy soils has resulted in saturation of sorption sites and eventually leaching of P from the soil to the groundwater. Using the Olsen to Bray II conversion factor as calculated in ***Chapter 3,*** a Bray II P of approximately 208 mg kg^{-1} would be considered as a critical level for risk of P loss. The highest Olsen P and Bray II P levels achieved in this trial was 32 mg kg^{-1} and 153 mg kg^{-1}, respectively, at the 20 kg ha^{-1} treatment at the Yarona site (Figure 5.1). These levels were reached at an application rate which is lower than the annual amount of P applied year after year. Thus, both the Yarona and New-grow sites run the greatest risk of reaching sufficiently high P levels to risk P loss due to run-off and leaching. The Mosplaas site hasn't reached the same critical P level but should still be managed to prevent the build-up of P and consequent loss of P.

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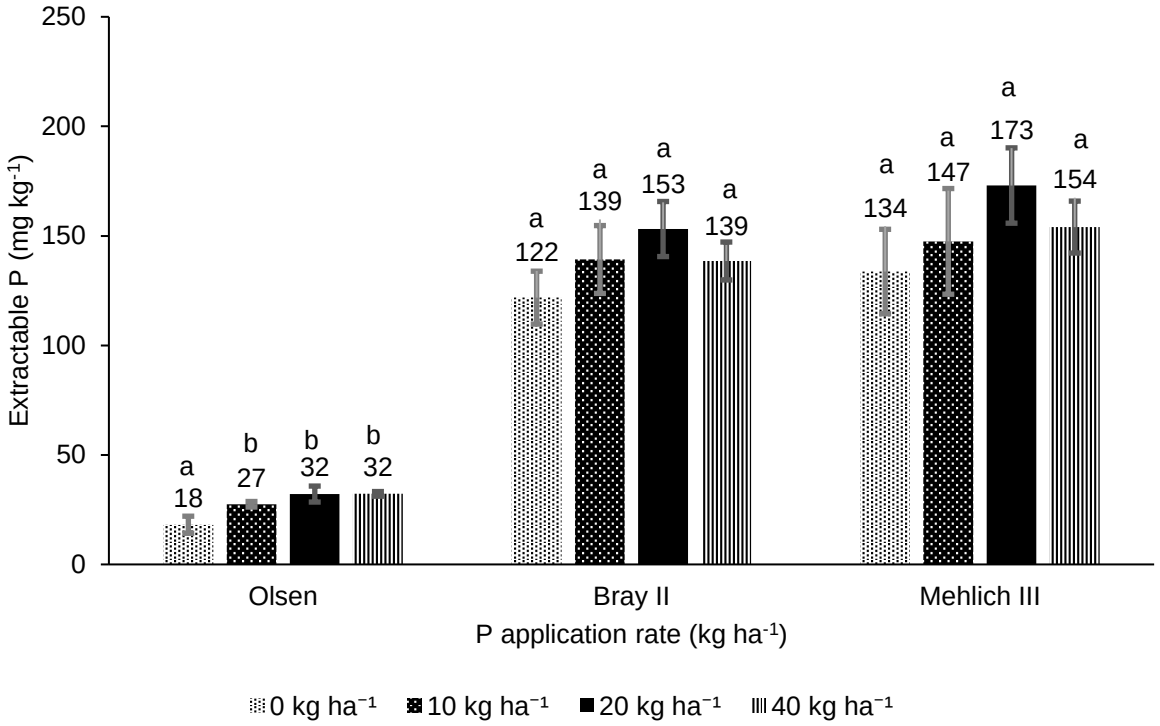

Figure 5.1 Mean extractable Olsen, Bray II and Mehlich III P (mg kg^{-1}) at the Yarona experimental site. Significant letters are applicable to each extraction test ($p<0.05$).

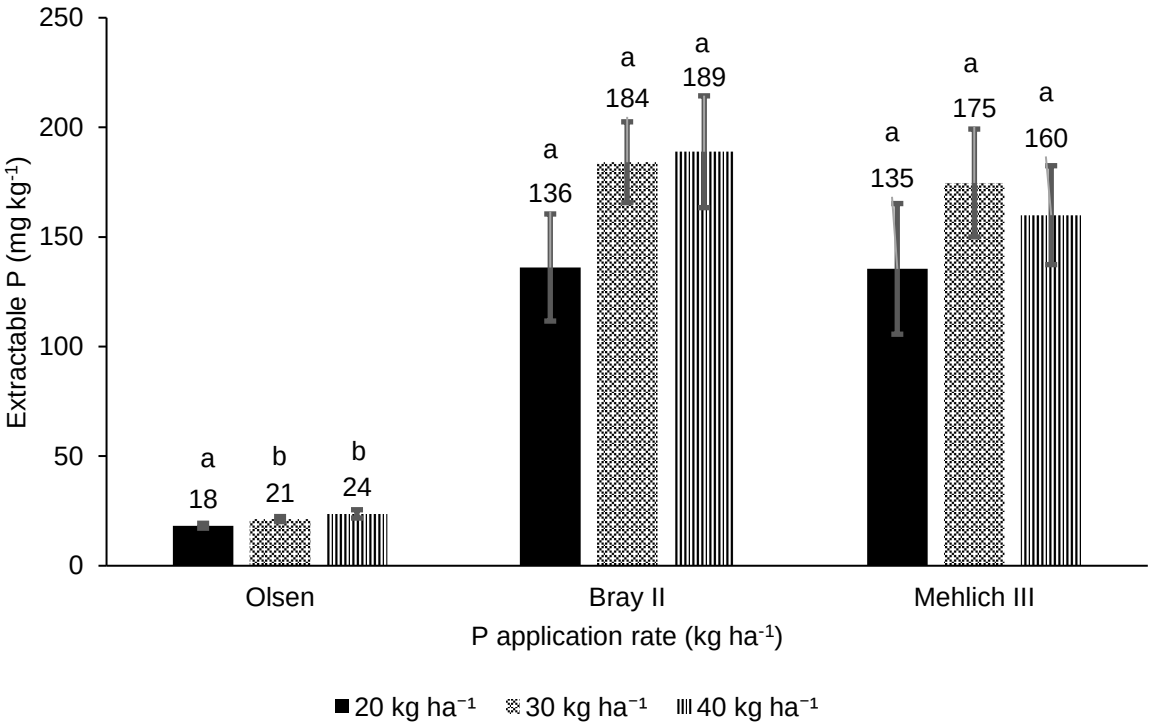

Figure 5.2 Mean extractable Olsen, Bray II and Mehlich III P (mg kg^{-1}) at New Grow experimental site. Significant letters are applicable to each extraction test ($p<0.05$).

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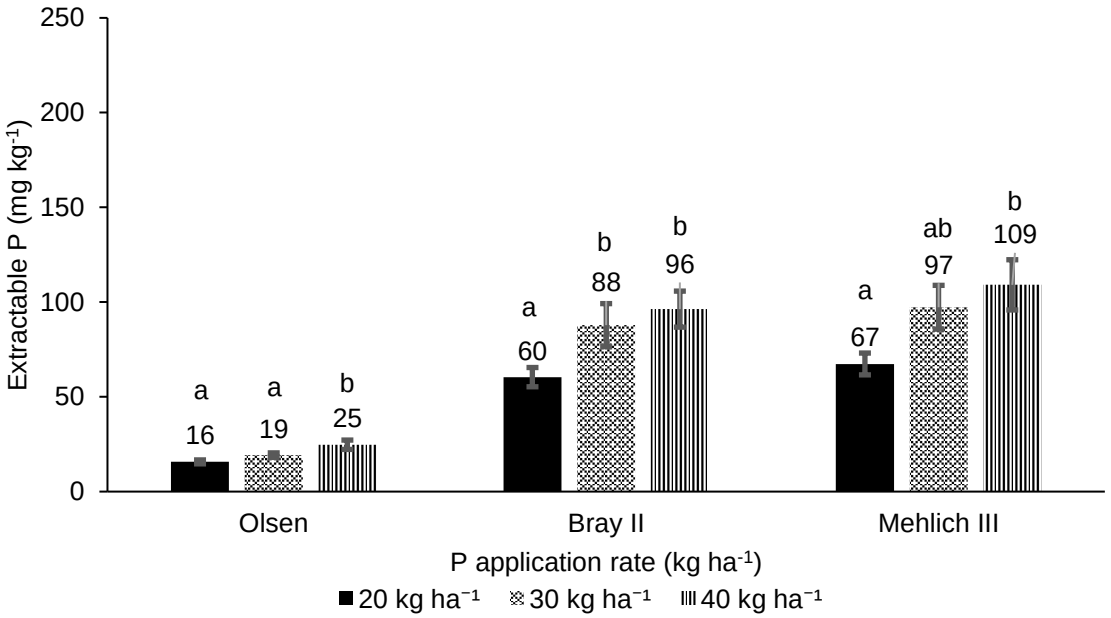

Figure 5.3 Mean extractable Olsen, Bray II and Mehlich III P (mg kg^{-1}) at Mosplaas experimental site. Significant letters are applicable to each extraction test ($p<0.05$).

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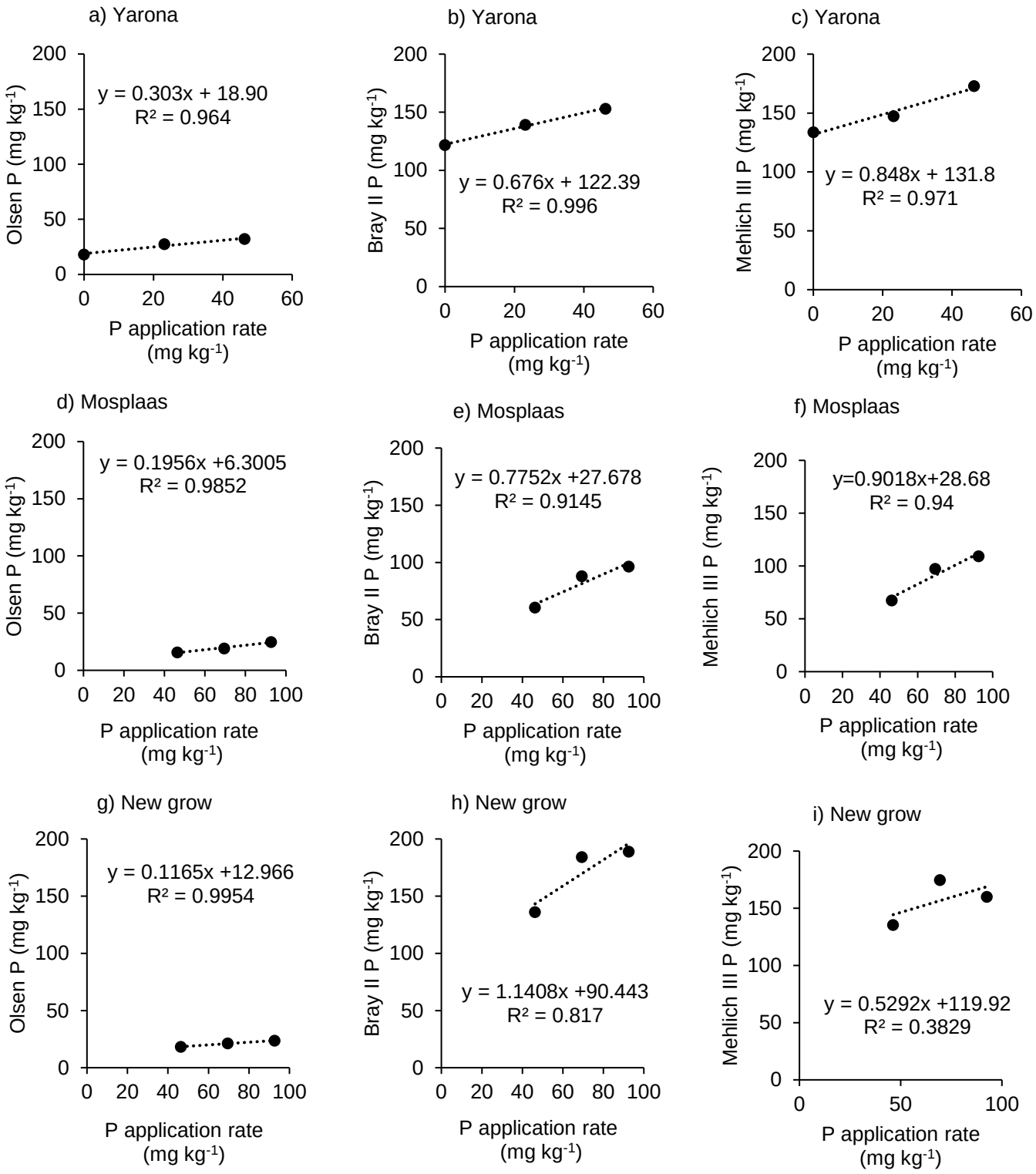

Figure 5.4 Linear relationship between applied P and a) Olsen P, b) Bray II P and c) Mehlich III P at the Yarona experimental site, d) Olsen P, e) Bray II P and f) Mehlich III P at the Mosplaas site and g) Olsen P, h) Bray II P and i) Mehlich III P at the New grow site.

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5.3.2 Effect of phosphorus fertilizer application on leaf P content and the relationship between soil P tests and leaf P content

A soil test critical value can be considered as a soil P status above which crop yield does not respond to P application (Bai *et al.,* 2013). Extractable Olsen, Bray II and Mehlich III P concentrations of 12 mg kg^{-1}, 18 mg kg^{-1} and 21 mg kg^{-1}, respectively, are considered sufficient for grapevine production (Oberholzer, 2016). In general, Olsen (10-15 mg kg^{-1}), Bray II P (25-30 mg kg^{-1}), and Mehlich III P (40 - 45 mg.kg^{-1}) are deemed as sufficient soil concentrations for crop production (Pierzynski, 2000). As previously mentioned, according to Bray II test all sites initially had sufficient P, whereas, according to Olsen tests, the New Grow and Mosplaas sites initially had insufficient P for grapevine and citrus production, respectively (Table 5.1). According to all the soil P test results (Figures 5.1 - 5.3), all P fertilizer treated sites had sufficient soil P for grapevine and citrus production. As reported by Conradie (1989), P concentrations of between 0.13-0.45 and 0.12-0.64 % in grapevine leaf blades and petioles, respectively, at veraison are considered sufficient. Thus, all of the grapevines had sufficient leaf P (0.27 - 0.37 %) and petiole P (0.46 - 0.60 %) (Figures 5.4 and 5.5). It is widely accepted that grapevines do respond to P fertilization and that P concentrations in blades and petioles are significantly increased with increased P application (Conradie & Saayman 1989). Petioles are also extensively used as indicators of vine P status (Stanley *et al.,* 1996). This was only observed at the Yarona site (Figure 5.4), where a small, significant increase in grapevine leaf P content was observed in the 10 and 40 kg P ha^{-1} treatments compared to the control. There were no significant changes in leaf petiole concentrations (Figure 5.4 and 5.5).

For citrus, leaf P concentrations of 0.09 - 0.15 are considered sufficient (Srivastava & Singh, 2008). Therefore, the Nardorcott citrus foliar P concentrations (0.13 - 0.17 %) were also sufficient at all application rates (Figure 5.6). Interestingly, the 40 kg ha^{-1} P application rate resulted in a significant decline in foliar P in the fruit-bearing leaves compared to the 20 kg ha^{-1} treatment (Figure 5.6).

Due to the critical level of P already being reached, no strong correlations were observed between soil P extraction test and leaf P concentrations. Weak, yet significant, relationships were only observed between Olsen and Bray II P and petiole P concentration of Thompson seedless grapevine (Table 5.5). The general lack of plant P response to P-fertilisation on these high pH soils can be attributed to both the soil and foliar P being at a sufficient level prior to P application. It is also clear that Olsen test gave the least accurate results regarding plant P availability, which is surprising considering the alkaline nature of these soils.

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Table 5.5 Pearson correlation coefficients (R^2) for the relationship between soil test P and P content in grapevine leaf blades and petioles.

Extraction test	% P (leaf blade)		% P (Petiole)	
	Thompson seedless grapes (Yarona)			
	R^2	p	R^2	p
Olsen	0.13	0.07	0.33	0.01
Bray II	0.29	0.01	0.25	0.02
Mehlich III	0.24	0.04	0.11	0.10
	Prime seedless grapes (New Grow)			
	R^2	p	R^2	p
Olsen	0.14	0.06	0.18	0.052
Bray II	0.11	0.14	0.04	0.40
Mehlich III	0.02	0.50	0.0001	0.97

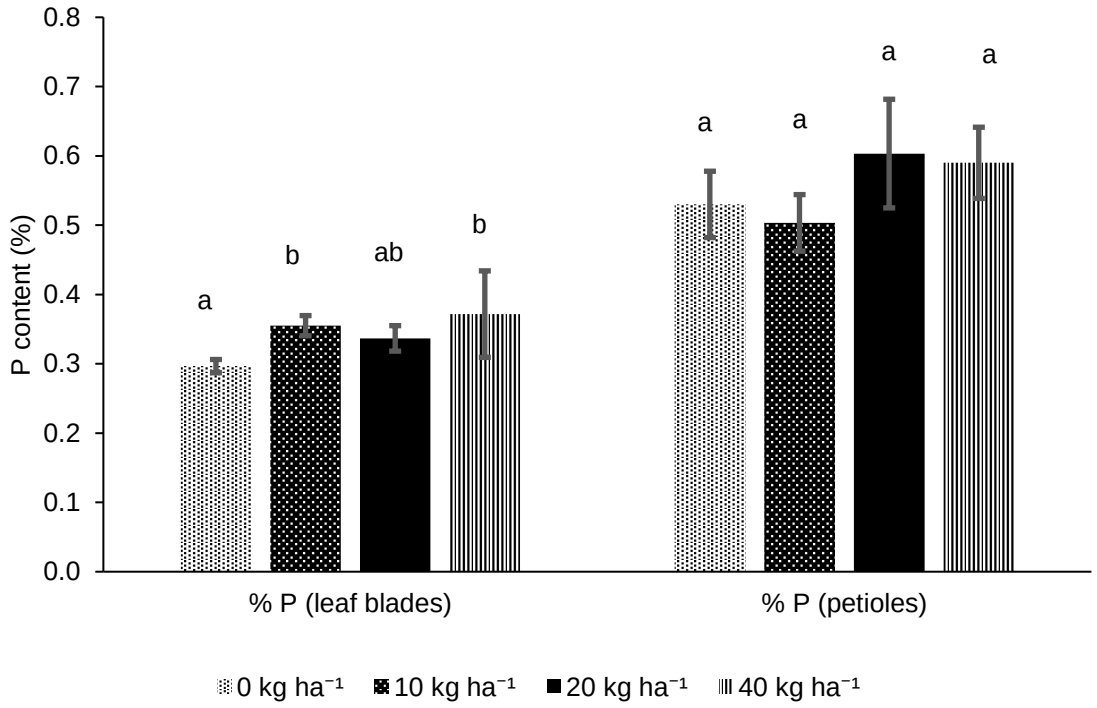

Figure 5.5 Concentration of P in leaf blades and petioles of Thompson seedless grapevine in response to P application at Yarona site. Significant letters are applicable to % P in leaf blades and % P in petioles respectively. Significance indicated at; $p < 0.05$

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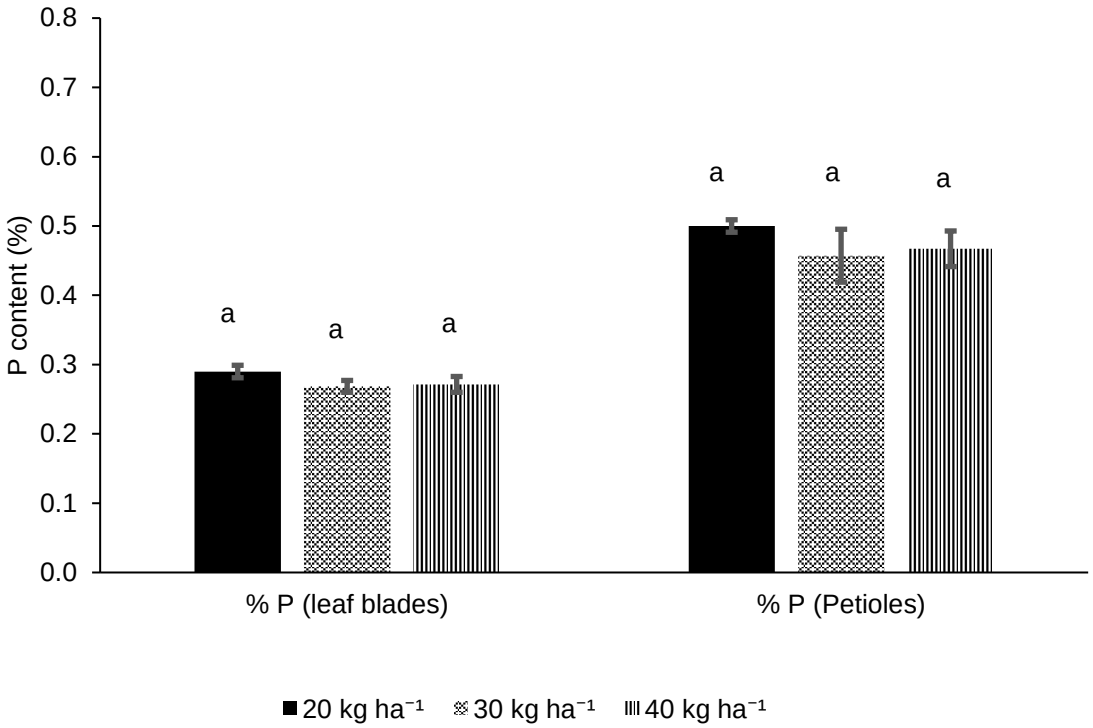

Figure *5.6* Concentration of P in leaf blades and petioles of Prime seedless grapevine in response to P application at New Grow site. Significant letters are applicable to % P in leaf blades and % P in petioles respectively. Significance indicated at; $p < 0.05$

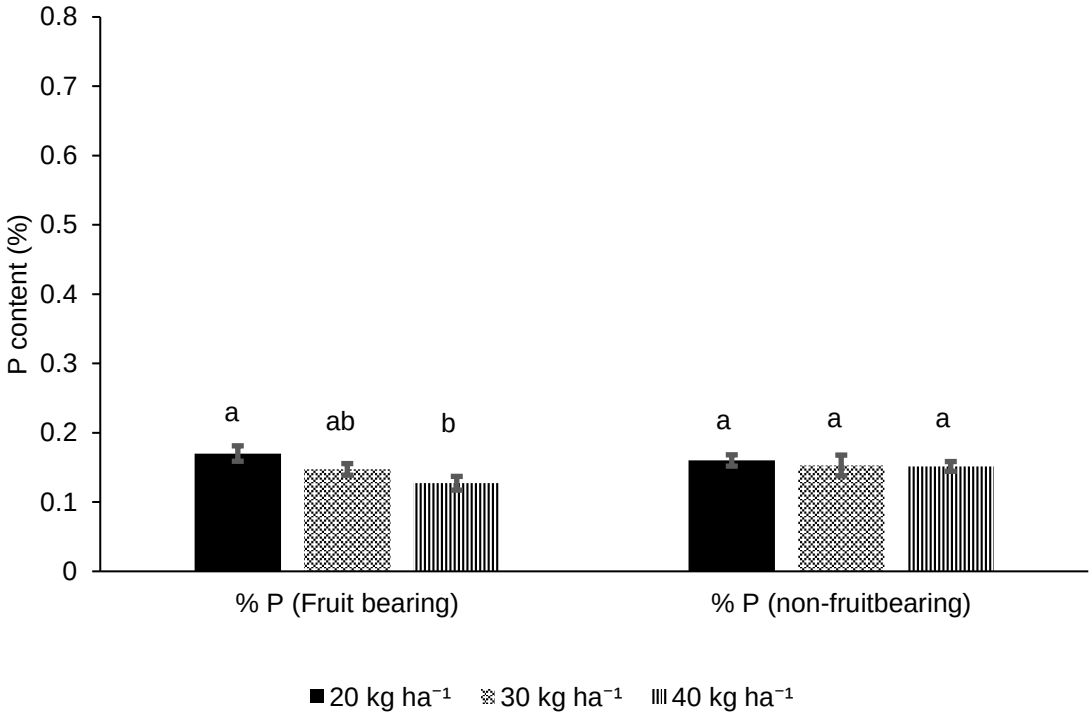

Figure 5.7 Concentration of P in leaves of Nadorcott manderins in response to P application at Mosplaas Site. Significant letters are applicable to % P in leaves from fruit bearing shoots and non-fruit bearing shoots respectively. Significance at, $p<0.05$

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5.4 Conclusions

Olsen, Bray II and Mehlich III extractable P showed no significant increase with increase in P application rate. This is ascribed to the large variation in initial soil P making it difficult to detect changes brought about by P fertilizer application. Initial soil P status will greatly influence plant response to applying P fertilizer. Leaf and petiole P concentration did not respond to or show any significant differences for the various P application rates, for both grapevines and manderins. This can be explained by the already sufficient soil P levels. Olsen, Bray II and Mehlich III extractable P levels were determined as adequate or excessive for the highest P application rate as well as for the standard rate of P application which is 30 kg P ha^{-1} per year for all three experimental sites. Consequently, applying P fertilizer year after year at these rates will lead to build up of P to levels at which there is a risk of P loss through leaching and surface runoff as well as the possible pollution of groundwater and micro nutrient deficiencies. It then becomes clear that when adding P to soils with adequate soil P, crop P concentration and consequently crop response will not be significantly affected. Bray II and Mehlich III were the most sensitive and correlated most significantly to applied P, whereas, Olsen extractable P was the least sensitive to applied P. Therefore, it is advisable to use Bray II and Mehlich III P tests for the alkaline Northern Cape soils levels give the most accurate representation of available P and P build-up.

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Chapter 6 **General conclusions and recommendations**

The aggressiveness and effectiveness as compared to total P (acid digestion) of eight P extraction test were compared on forty-nine top and sub-soil samples varying in physiochemical properties from high and low rainfall regions across South-Africa. These P extraction tests (Olsen, Colwell, Bray I, Bray II, Mehlich III, Ambic 1, Citric acid and 1:2 water extractable P) were evaluated based on their correlation to total P and the fraction of total P extractable by each method. A Multifactorial-analyses was done correlating extractable P by each method to certain soil properties, linear regression equations were used to investigate the relationship between the various test and to ultimately obtain a conversion factor to make conversion between the norms of the tests possible. Considering the contrasting P levels indicated by soil P extraction test especially for Olsen and Bray II on high pH soils it was necessary to correlate extractable P to plant response and investigate the effect of annual P application on crop P content as well as on soil P content. The effect of P sorption on the availability of P is well understood and should be included into fertilizer recommendations, however a quick and realistic approach needs to be developed. Work has been done on single-point sorption isotherms and strong correlations to multiple point sorption estimates has been reported, however uncertainty arose regarding the variability of some of the parameters used and what influences this variability. Therefore, work was done to develop an incubation method that simulates field conditions and that provides an estimate of the percentage of applied P that can be extracted by a specific test and then also giving a measure of the effectiveness of the P extraction test being used.

Chapter Three reported on the general characterisation of the soils being studied as well as the correlation between the soil P extraction tests being investigated. The relative efficiency as compared to Total P of the extraction tests were as follows; 1:2 H_2O < Olsen < Colwell < Bray I < Ambic 1 < Bray II < Mehlich III ≈ Citric acid. Bray II and Mehlich III were the only extractions test that correlated significantly (R^2 = 0.78 & 0.76 respectively) with total P for the entire sample set. By fitting linear regression equations explaining the relationship between the various tests conversion factors have been reported for tests that correlated significantly. Due to the variance in relative aggressiveness of the tests and the standard error of measurement that exists between the tests the WSEM (weighted standard error of measurement) was determined describing the accuracy of the conversions between the highly significant tests. From this conversions between Mehlich III, Olsen and Bray II extraction tests are the most accurate and these tests correlated most significantly with each other. The possibility of using single factor conversions was also investigated, the regression equations were transformed without crossing the vertical axis, and this did not significantly affect the fit

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of the regression equations. Therefore is was possible to report single factor conversion equations. Conversions from Olsen to Bray II and Bray I by using a factor of 5.20 and 3.88 respectively can be done with confidence. For the conversion from Bray II to Mehlich III a factor of 1.10 can be used. Converting from Citric acid to Ambic 1 can also be done accurately with a factor of 0.75. Through the multifactorial analyses done, the affect CBD extractable Fe and Al as well as clay content has on P extractability should be highlighted, respectively correlating significantly (negatively) with extractable P.

Chapter Four aimed to incorporate the soils phosphorus buffering capacity into fertilizer recommendations and to evaluate multiple-point, single-point and incubation estimates of sorption. The Freundlich equation was found to describe sorption the best for this sample set of 10 soils. From the Freundlich equation parameters *Kd* and *b* were used to determine $PBC_{O\&S.}$ The effect of using initial P in a transformed variation of the Freundlich was deemed not be beneficial for the soils under investigation. Parameters *Kd* and *b* varied throughout the sample set and using the mean value for *b* was not effective in predicting PBI with a single application of 1000 mg P kg^{-1}. The best results were obtained when values for *b* were manually selected, from this two distinct groups with significantly different *b* values were identified. When using the selected *b* values (0.63 and 0.84) with the untransformed Freundlich equation a strong correlation between measured multiple-point $PBC_{O\&S}$ and predicted single-point $PBC_{O\$S}$ (R^2 = 0.85) was observed. This however does not accurately reflect the accuracy of the singly point estimate and therefore the RMSE was also determined as 0.43 mg kg^{-1} which considering the mean $PBC_{O\&S}$ is large. Ultimately for this sample set it was not possibly to predict sorption with a single-point method accurately due to the variation of *b.* Evidence of the variability of certain sorption parameters has been provided and it is clear that for the sample set used in this study *b* is not a constant. Therefore a need exists for a method that realistically predicts P sorption and that can reflect the effectiveness of the extraction test used. From the incubation study a strong linear relationship was found between applied and extracted P. Incubation estimates of sorption also correlated significantly to $PBC_{O\&S}$ (R^2 = 0.80). Percentage Bray II extractable P (R^2 = 0.78) showed the strongest relationship with PBC as described by Rayment and Higginson (1992), therefore using the Bray II extraction test most accurately described P sorption for this sample set. Percentage extractable P could be derived from the slope of the regression equation and correlated significantly with extractable P at 100 mg kg^{-1} and 150 mg kg^{-1} application rates. Therefore, it is possible to predict the fraction of applied P that will be plant available from a single application and incubation of P at a rate of 100 -150 mg kg^{-1}. It was also found that there was no significant difference between the extractability of KH_2PO_4 and $Ca(H_2PO_4)_2$, extractable Maxiphos® however, varied significantly from the other forms of applied P. It was also found

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that extractable Bray II P did not decrease significantly after one month of incubation for especially the low P sorbing soils, percentage extractable P did however decrease significantly after one month for the high P sorbing soils. Olsen extractable P decreased significantly and to a larger extent after 6 month of incubation for the high pH, high P soils. It was also found that for the low P sorbing soils percentage extractable P did not vary significantly after 24 hours of incubation but only remained constant after 72 hours for the high P sorbing soils.

Chapter Five reported the findings on the field trial conducted on high pH soils from the Northern cape planted to grapevine (Prime and Thomson seedless) and Citrus (Nadorcott manderins). For these soils with already sufficient and in some cases excessive Bray II and Olsen P, no significant plant response was observed when adding various rates of P. When correlating applied P (mg kg^{-1}) to extracted P Bray II and Mehlich III extraction test were the most sensitive in detecting applied P, whereas Olsen was the least sensitive in detecting fresh P. Therefore, for theses alkaline soils, Bray II gave the most realistic estimate of available P and the annual application of P on these soils can lead to P build up and the eventual loss of P and contamination of ground water.

The significance of the research findings presented in this thesis include the development of useful conversion factors to accurately convert between the most commonly used soil P test norms in South Africa. An easy and relatively quick incubation method has also been developed to improve accuracy of field fertilizer recommendations using specific soil P tests and taking soil P fixation capacity into account.

Based on the results of this study, a further understanding of the variability of parameter *b* and its effect on estimates of sorption is required. It can be recommended that the influence of specific soil properties such as pH, clay content, carbonates and Fe and Al-oxide content on the value of *b* should be investigated, an understanding of the correlation between various soil properties and *b* would make it possible to predict its size and therefore accurately use an estimate of *b* when determining single-point measures of sorption. The effectiveness of the fertilizer recommendations generated from the incubation method needs to be validated with crop response and it would also be useful to compare fertilizer recommendations of both the sorption isotherm method and the incubation method as used in this study. For estimating sorption and making fertilizer recommendations using the incubation method both KH_2PO_4 and $Ca(H_2PO_4)_2$ are suitable forms of P and P applied at a rate of 100 – 150 mg P kg^{-1} should be incubated with soil samples for 24 hours for low sorbing P soils, and for 72 hours for high P sorbing soils. Phosphorous sorption should be revaluated after one month for high P sorbing, clayey soils and after 6 months for low P sorbing, sandy soils. Further it can be recommended that Bray II P should be used as measure of percentage extractable P.

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Conversions between the various extraction test will be possible with the most accurate conversions being between Bray II, Bray I, Mehlich III and Olsen, it is important to note that these conversions are suitable for South-African soils and for P extractions done by Bemlab. Further validation of these conversion factors would be necessary using other laboratories. From the field trial results, the need arises to correlate Bray II, Mehlich III and especially Olsen P to crop response on low P, low – moderate pH soils, to understand when the Olsen test would be most useful.

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Appendix A-1

sample number	sample ID	Depth cm	pH KCl	EC mS m^{-1}	C %	C (Leco) %	CBD Fe (Fed) mg kg^{-1}	CBD Al mg kg^{-1}	Oxalate Fe (Feo) mg kg^{-1}	Oxalate Al mg kg^{-1}	Feo/Fed	1:2 H2O Ca mg kg^{-1}	Clay %	Silt %	Sand %	KUK (pH 7) cmol(+) kg^{-1}
1	J1.1	30	4.4	30.0	1.6	1.2	3574.8	3491.0	8196.0	17926.0	2.3	7.4	9.0	4.0	87.0	4.2
2	F1.1	30	4.3	10.0	0.8	0.4	446.5	1300.3	2650.2	9261.9	5.9	3.2	9.0	2.0	89.0	3.4
3	3.1	45	6.2	227.0	0.8	1.1	11352.9	1458.5	8440.6	1322.3	0.7	168.4	25.0	14.0	61.0	5.3
4	6.1	30	4.4	659.0	0.3	0.3	736.4	632.0	1284.5	41.3	1.7	38.1	9.0	2.0	89.0	3.0
5	7.1	30	5.5	31.0	0.4	0.4	1344.3	725.0	3899.1	165.7	2.9	23.4	9.0	2.0	89.0	3.8
6	8.1	30	5.9	21.0	1.2	0.9	2564.0	1188.3	7522.5	4215.1	2.9	31.8	15.0	10.0	75.0	5.7
7	9.1	100	4.6	25.0	0.3	0.2	3218.6	770.0	2329.3	2467.8	0.7	23.9	11.0	8.0	81.0	3.0
8	11.1	30	4.9	18.0	1.4	1.7	2395.2	1460.5	9373.1	8218.1	3.9	29.6	11.0	10.0	79.0	6.9
9	12.1	30	7.2	157.0	0.3	0.7	4825.4	723.0	1823.8	2897.0	0.4	69.8	29.0	8.0	63.0	9.1
10	14.1	80	5.2	38.0	0.4	0.6	10467.2	1768.5	4088.5	1935.9	0.4	50.2	23.0	6.0	71.0	5.6
11	16.1	30	5.5	41.0	0.8	0.5	1271.4	901.3	1299.7	883.0	1.0	64.2	17.0	4.0	79.0	4.5
12	17.1	30	5.0	13.0	0.4	0.2	1816.6	641.0	833.1	1367.1	0.5	26.2	9.0	2.0	89.0	2.9
13	18.1	30	5.7	167.0	0.9	0.6	617.4	562.3	1065.7	576.2	1.7	355.3	11.0	2.0	87.0	4.1

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sample number	sample ID	Depth cm	pH KCl	EC mS m^{-1}	C %	C (Leco) %	CBD Fe (Fed) mg kg^{-1}	CBD Al mg.kg^{-1}	Oxalate Fe (Feo) mg kg^{-1}	Oxalate Al mg kg^{-1}	Feo/Fed	1:2 H2O Ca mg kg^{-1}	Clay %	silt %	sand %	KUK (pH 7) cmol(+) kg^{-1}
14	19.1	30	6.3	135.0	0.8	0.9	3556.2	858.0	5175.0	3337.1	1.5	688.2	19.0	4.0	77.0	7.0
15	20.1	30	6.1	41.0	0.3	0.2	3828.0	641.0	1697.7	1022.4	0.4	93.9	9.0	0.0	91.0	3.5
16	21.1	30	5.7	8.0	0.2	0.2	1398.5	652.3	751.1	1142.4	0.5	8.0	7.0	4.0	89.0	2.2
17	22.1	90	6.6	31.0	0.4	0.2	1484.7	832.3	3139.1	1451.8	2.1	34.9	11.0	2.0	87.0	3.3
18	25.1	60	4.7	7.0	0.5	0.5	14243.9	2488.5	11695.3	8123.1	0.8	6.6	27.0	10.0	63.0	6.2
19	26.1	30	5.5	7.0	1.0	0.6	1117.6	1418.0	6882.5	8300.6	6.2	16.6	9.0	4.0	87.0	5.0
20	27.1	30	5.4	15.0	0.3	0.3	1523.4	789.0	4115.0	2071.9	2.7	14.4	11.0	8.0	81.0	3.3
21	30.1	120	4.0	9.0	0.3	0.3	29488.6	4067.3	2102.2	5488.3	0.1	24.8	29.0	12.0	59.0	7.6
22	31.1	30	4.9	29.0	0.8	0.2	1718.9	839.3	1552.8	3442.4	0.9	68.0	15.0	4.0	81.0	4.0
23	32.1	30	5.4	53.0	1.6	1.7	6913.0	2830.8	2723.1	6988.0	0.4	87.4	11.0	4.0	85.0	5.9
24	33.1	30	7.0	961.0	0.6	0.3	2800.0	427.8	1820.2	4183.5	0.7	522.7	17.0	2.0	81.0	5.3
25	34.1	30	6.4	106.0	0.6	0.3	4731.5	578.5	18547.9	4289.5	3.9	58.5	13.0	4.0	83.0	4.5
26	35.1	30	6.8	33.0	0.4	0.2	2608.2	723.3	2619.6	4220.7	1.0	51.7	9.0	0.0	91.0	3.4
27	38A.1	60	6.6	24.0	0.8	1.0	8325.6	7738.0	4987.9	11583.6	0.6	135.9	37.0	14.0	49.0	5.8
28	38B.1	30	5.8	94.0	1.6	1.7	20504.6	5870.3	17617.3	88389.2	0.9	396.5	15.0	8.0	77.0	6.9
29	39.1	30	6.3	48.0	1.4	1.2	7612.1		7144.0	6653.1	0.9	86.2	17.0	8.0	75.0	5.3
30	40A.1	30	5.0	18.0	1.3	1.2	25412.7	1614.0	8392.3	7656.8	0.3	16.9	33.0	22.0	45.0	6.9

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sample number	sample ID	Depth cm	pH KCl	EC mS m^{-1}	C %	C (Leco) %	CBD Fe (Fed) mg kg^{-1}	CBD Al mg kg^{-1}	Oxalate Fe (Feo) mg kg^{-1}	Oxalate Al mg kg^{-1}	Feo/Fed	1:2 H2O Ca mg kg^{-1}	Clay %	silt %	sand %	KUK (pH 7) cmol(+) kg^{-1}
31	40B.1	30	5.6	58.0	2.2	2.6	13617.6	5128.0	6666.3	9495.2	0.5	134.8	31.0	22.0	47.0	8.4
32	T41.1	60	4.2	38.0	0.8	0.7	27172.3	3392.5	6836.6	18557.7	0.3	39.0	37.0	10.0	53.0	4.9
33	43A.1	120	6.1	11.0	2.1	0.3	27384.7	5280.0	2178.7	9307.8	0.1	25.2	33.0	4.0	63.0	5.3
34	43B.1	40	4.7	76.0	0.4	0.6	33801.4	7221.5	6943.5	11486.3	0.2	242.7	39.0	8.0	53.0	6.5
35	47.1	30	5.9	33.0	1.4	1.0	2098.1	1004.8	2468.5	3880.7	1.2	58.8	11.0	6.0	83.0	5.7
36	L48.1	30	6.7	25.0	0.6	0.7	8957.2	578.3	3840.0	4509.9	0.4	54.4	13.0	12.0	75.0	6.4
37	49.1	30	7.7	236.0	0.7	0.8	10376.9	522.8	2845.4	3690.2	0.3	40.0	19.0	6.0	75.0	6.4
38	50.1	30	5.0	55.0	0.5	0.4	5094.6	436.3	1642.2	960.8	0.3	113.9	37.0	10.0	53.0	3.2
39	stellen1.1	60	4.6	41.0	0.8	0.7	20589.9	3655.0	3096.9	6605.2	0.2	37.4	20.0	37.0	10.0	4.9
40	stellen2.1	60	4.1	34.0	1.0	0.7	31146.2	4223.8	4015.8	4902.8	0.1	49.0	27.0	51.0	14.0	13.8
41	V51	30	4.7	24.4	0.8	4.1	14149.8	1230.7	2927.4	1946.7	0.2	59.1	45.0	18.0	37.0	17.8
43	K53	30	7.5	10.2	0.4	0.5	4008.8	191.1	215.7	235.0	0.1	44.9	11.0	8.0	81.0	6.2
44	K54	30	7.9	30.6	0.3	0.7	3954.2	162.4	68.2	131.5	0.0	72.8	11.0	6.0	83.0	4.7
45	K55	30	7.5	27.3	0.3	0.4	3146.5	141.6	919.4	65.4	0.3	92.2	9.0	2.0	89.0	5.0
46	K56	30	7.2	35.6	0.2	0.2	2895.8	274.2	345.2	149.4	0.1	64.0	7.0	4.0	89.0	5.7
47	K57	30	7.5	36.7	0.4	0.4	3009.7	248.3	259.9	178.7	0.1	50.9	9.0	6.0	85.0	7.3
48	K58	30	7.9	32.1	0.3	0.3	2961.4	104.0	85.5	100.5	0.0	58.2	11.0	2.0	87.0	5.6
49	K59	30	7.8	13.2	0.2	0.3	3348.8	69.0	103.1	99.1	0.0	94.5	9.0	2.0	89.0	5.7

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Appendix A-2

sample number	sample ID	Olsen	Bray I	Bray II	Mehlich III	Citric acid	Colwell	1:2 water	Ambic 1	Total P
		←				mg kg^{-1}	→			
1	J1.1	2.55	4.79	5.04	5.63	11.00	14.36	0.48	24	93.00
2	F1.1	1.87	22.66	13.57	28.51	18.00	12.35	0.20	16	45.00
3	3.1	38.05	95.64	143.10	114.31	32.00	30.43	7.40	20	302.00
4	6.1	22.77	63.54	64.74	54.51	77.00	55.72	8.48	68	70.00
5	7.1	15.95	29.74	57.53	40.06	66.00	58.74	1.78	40	74.00
6	8.1	25.26	105.98	174.46	187.45	148.00	70.89	9.60	83	273.00
7	9.1	1.64	2.06	2.36	2.35	6.00	7.89	0.12	8	45.00
8	11.1	41.60	256.97	324.12	397.45	318.00	150.85	10.40	296	430.00
9	12.1	2.97	10.70	32.23	23.42	44.00	12.99	1.54	17	117.00
10	14.1	1.73	6.08	9.53	9.44	6.00	10.82	20.00	7	181.00
11	16.1	7.34	29.63	29.63	38.48	52.00	40.15	2.18	50	73.00
12	17.1	22.02	96.14	93.35	97.00	78.00	51.83	12.98	61	172.00
13	18.1	7.60	40.25	71.56	98.65	88.50	39.06	4.50	70	139.00
14	19.1	40.02	113.20	167.11	186.50	169.00	105.86	6.14	99	215.00
15	20.1	3.74	18.83	21.91	23.78	22.00	22.89	7.46	13	146.00
16	21.1	1.13	3.98	4.47	6.72	7.00	5.95	2.08	7	51.00
17	22.1	3.41	24.19	31.08	33.25	46.00	19.81	4.58	25	71.00
18	25.1	1.57	2.15	3.20	3.52	20.00	26.98	1.76	25	210.00
19	26.1	31.06	212.03	188.05	223.31	169.00	60.31	6.66	146	300.00
20	27.1	24.48	73.86	88.14	92.32	89.00	31.69	9.32	66	99.00
21	30.1	0.61	5.32	11.98	7.23	41.00	67.70	0.14	49	146.00
22	31.1	15.12	63.01	71.43	78.56	44.00	73.69	2.56	49	137.00
23	32.1	70.74	280.83	314.76	373.22	95.00	62.86	37.30	89	556.00
24	33.1	8.02	66.76	106.00	111.00	111.00	24.67	2.28	93	228.00

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sample number	sample ID	Olsen	Bray I	Bray II	Mehlich III	Citric acid	Colwell	1:2 water	Ambic 1	Total P
		←				mg kg^{-1}				→
25	34.1	33.66	133.90	235.62	266.17	228.00	33.15	10.68	135	361.00
26	35.1	12.99	81.26	102.38	119.22	112.00	119.63	8.94	82	127.00
27	38A.1	4.19	17.54	47.94	33.64	7.00	8.47	1.42	8	163.00
28	38B.1	5.02	29.78	49.20	35.73	54.50	12.08	0.38	8	165.00
29	39.1	8.72	18.41	26.75	36.49	32.00	24.95	4.70	23	177.00
30	40A.1	6.22	7.13	9.73	12.62	10.00	19.94	4.62	13	164.00
31	40B.1	12.83	35.24	54.03	62.55	39.00	44.92	2.90	39	319.00
32	T41.1	1.03	2.37	3.57	2.52	3.00	10.18	0.12	7	277.00
33	43A.1	0.85	1.08	0.46	0.77	7.00	2.11	0.18	1	79.00
34	43B.1	0.82	2.37	3.52	2.90	1.00	28.94	0.12	7	196.00
35	47.1	12.76	82.68	112.67	150.37	76.50	54.86	4.68	66	173.00
36	L48.1	9.73	31.07	37.64	47.05	93.00	39.06	2.66		167.00
37	49.1	5.05	15.65	46.84	43.99	49.00	19.02	2.46	25	223.00
38	50.1	22.29	88.08	78.31	100.64	63.00	46.31	11.80	61	155.00
39	stellen1.1	6.39	20.88	30.13	35.23	21.00	27.14	0.94	16	159.00
40	stellen2.1	3.42	5.38	11.62	11.46	5.00	20.37	0.26	13	74.00
41	V51	15.00	6.00	8.00	10.00	18.00	55.80	1.26	11	260.00
43	K53	30.00	45.00	99.00	93.00	111.00	77.46	14.38	60	293.00
44	K54	11.00	19.00	59.00	39.00	51.00	33.95	4.72	28	194.00
45	K55	13.00	69.00	193.00	179.00	275.00	46.36	4.44	210	382.00
46	K56	13.00	35.00	83.00	68.00	133.00	60.75	6.24	72	193.00
47	K57	18.00	69.00	208.00	194.00	232.00	85.17	10.56	168	334.00
48	K58	6.00	14.00	55.00	40.00	94.00	53.13	0.90	67	165.00
49	K59	8.00	18.00	45.00	44.00	50.00	30.57	2.76	36	189.00

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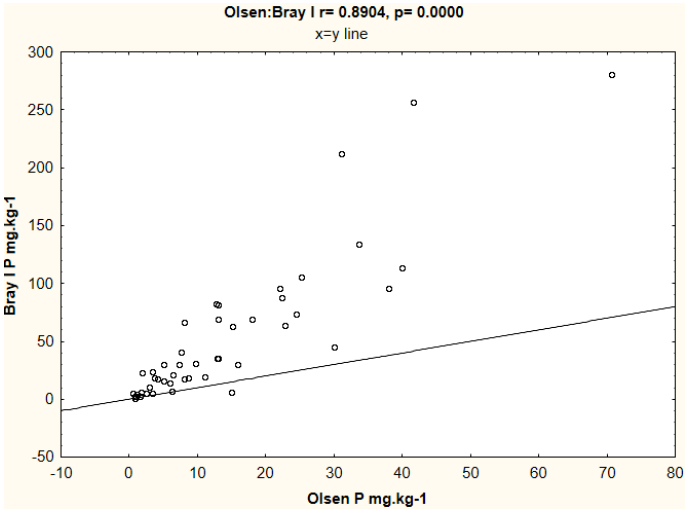
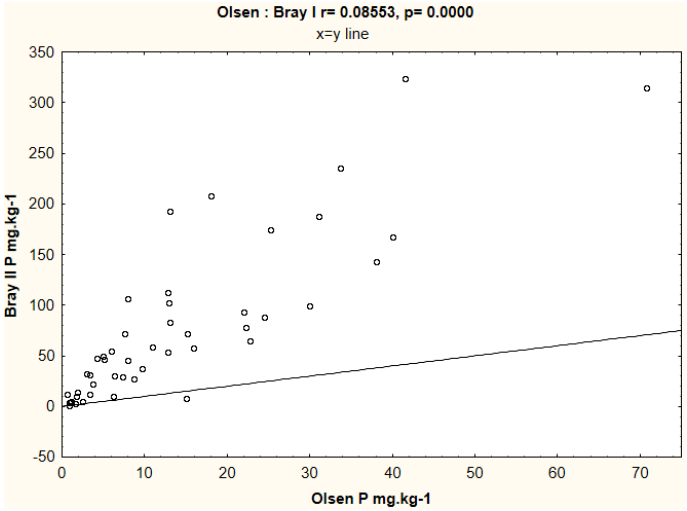
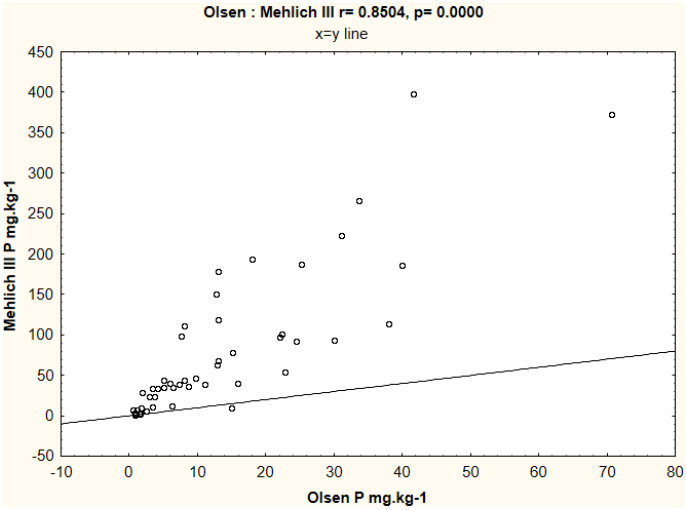
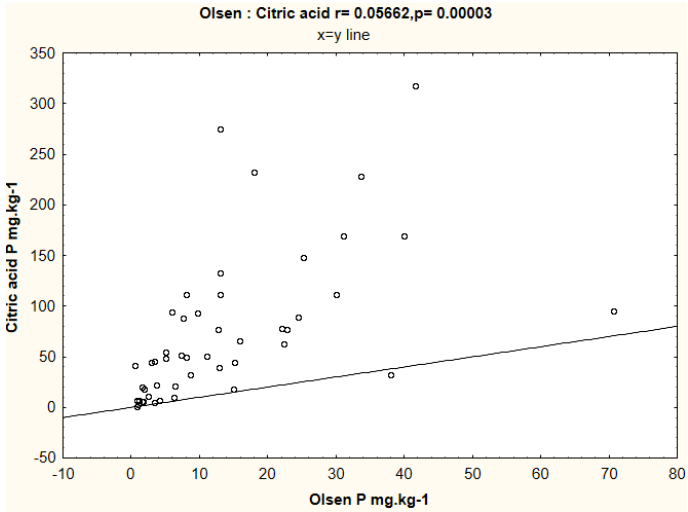
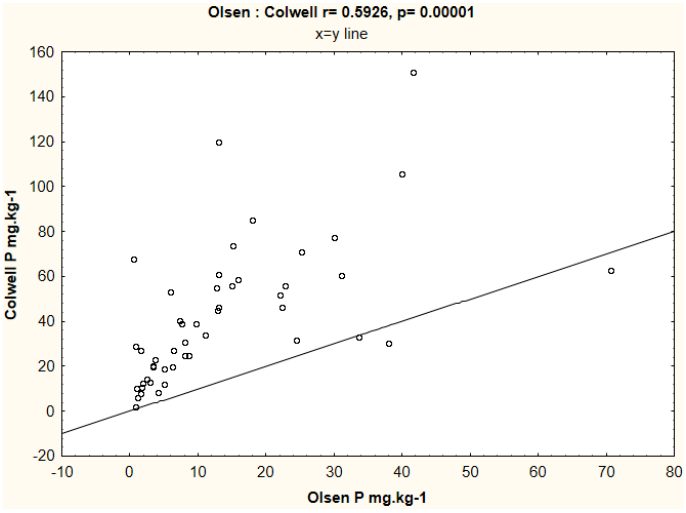
Appendix B

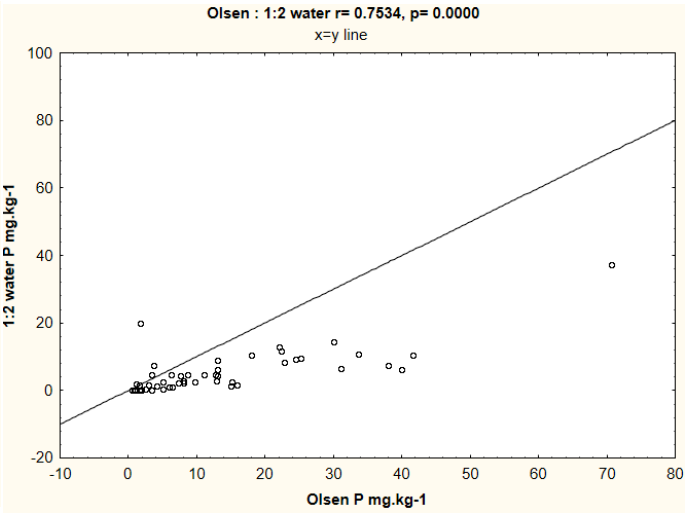












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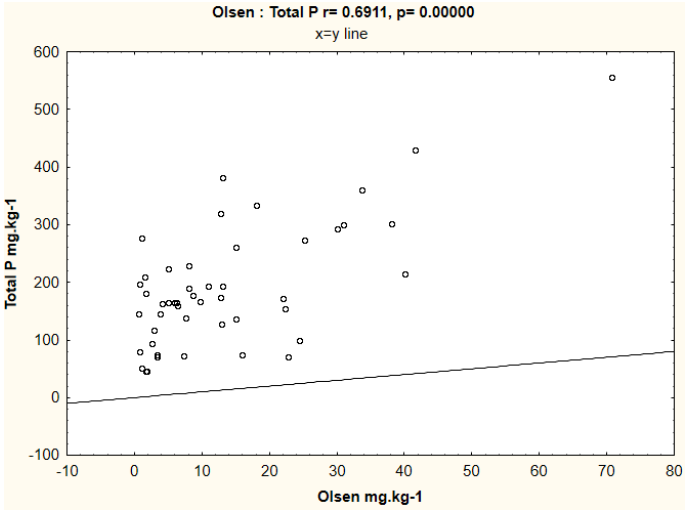

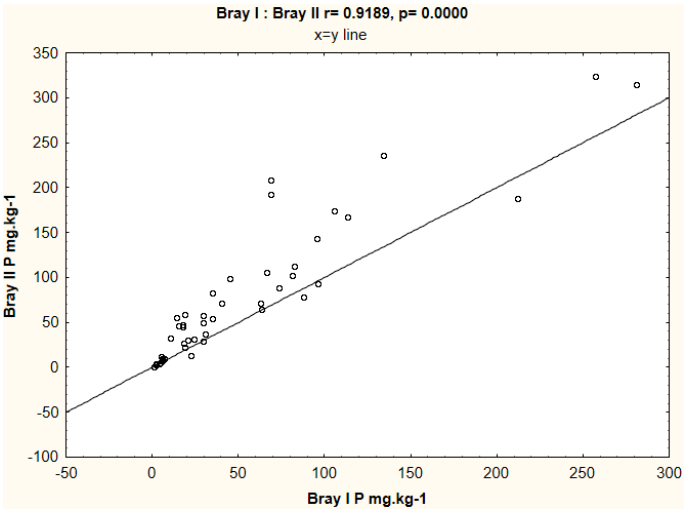

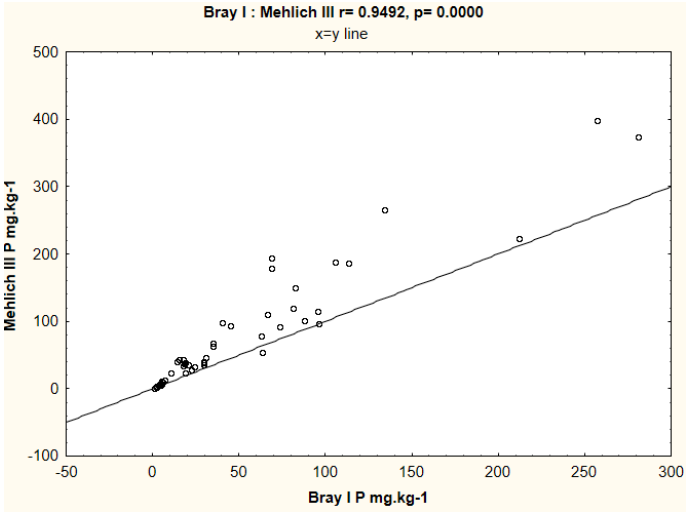

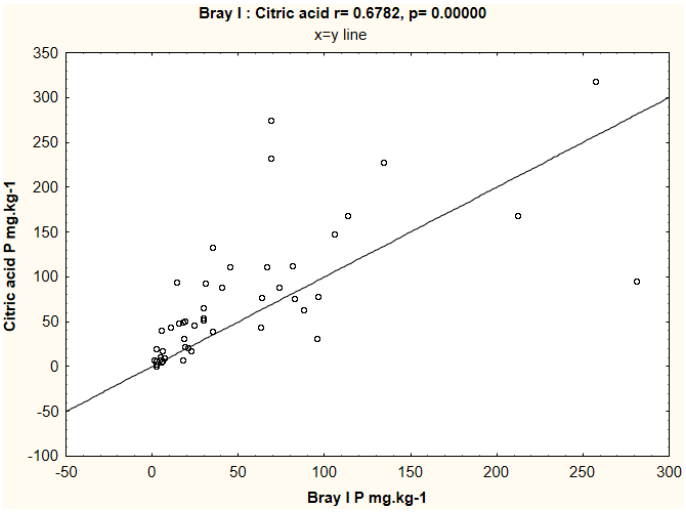

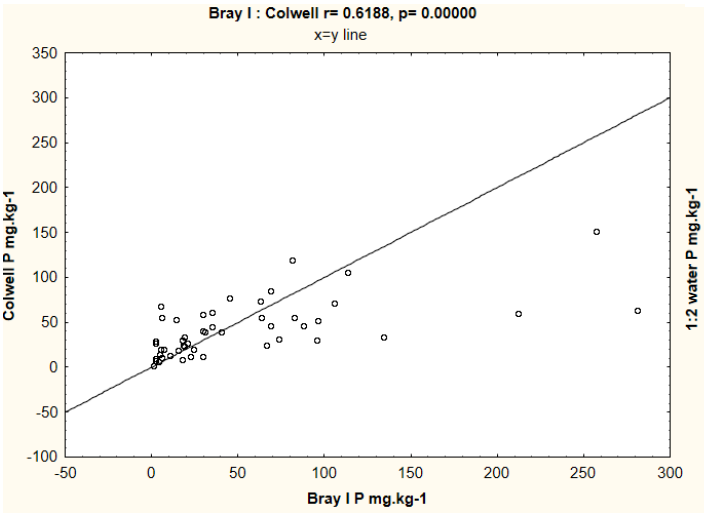

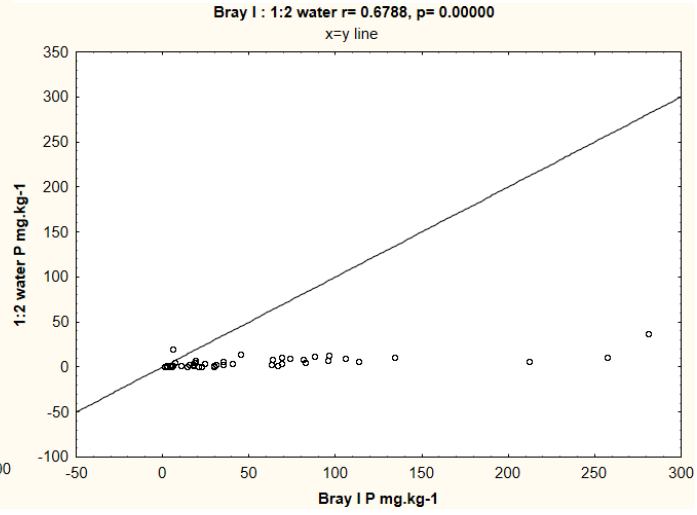

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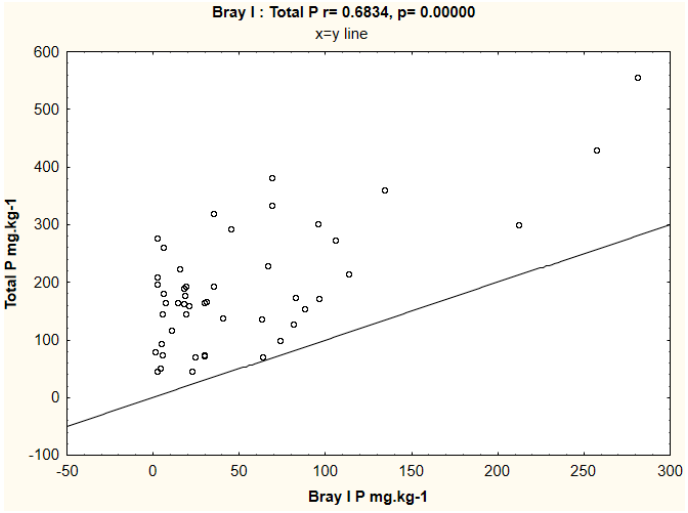

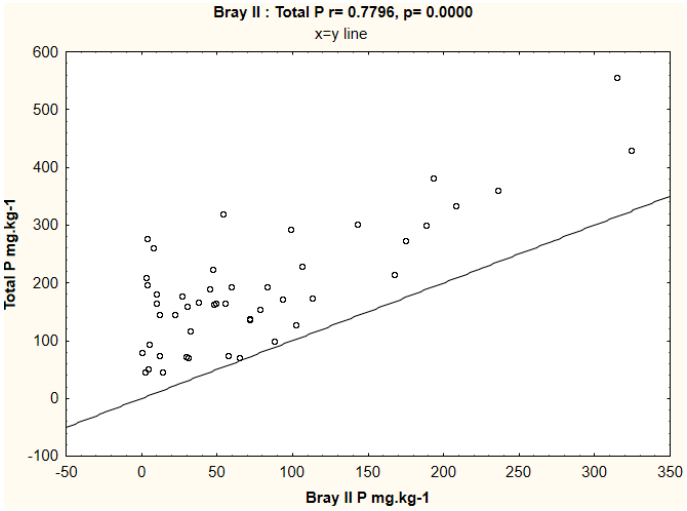

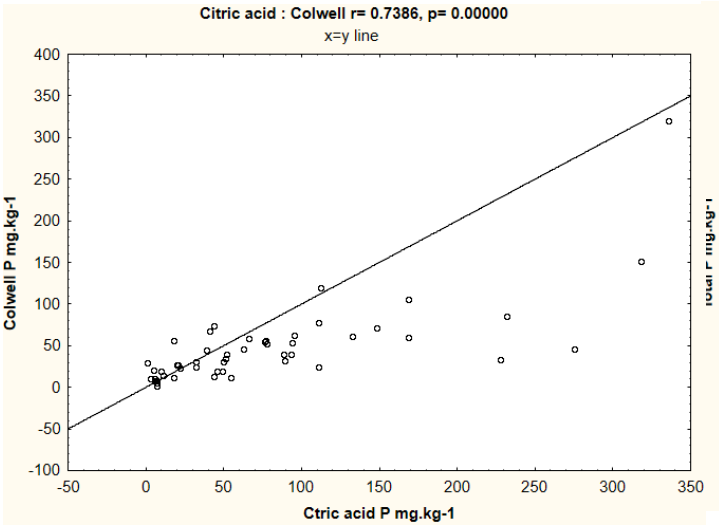

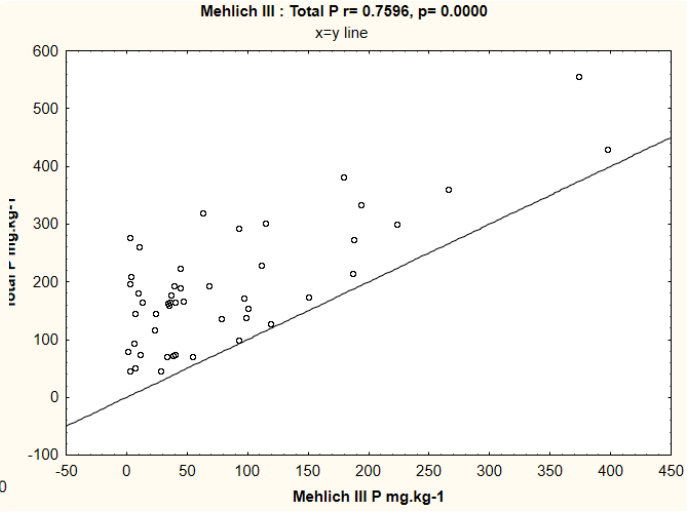

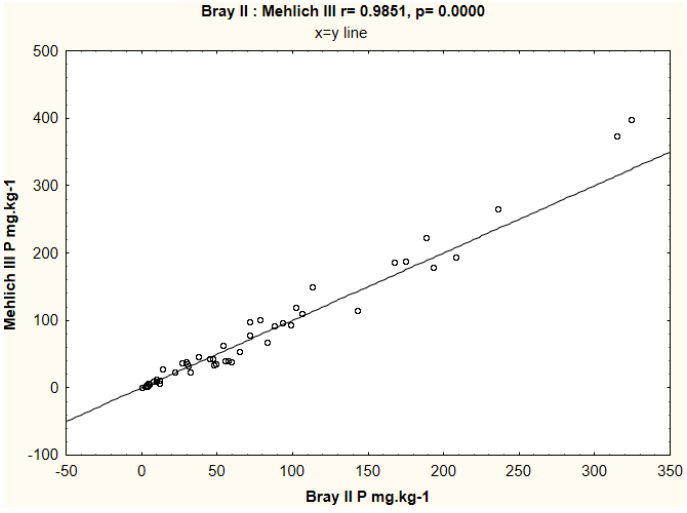

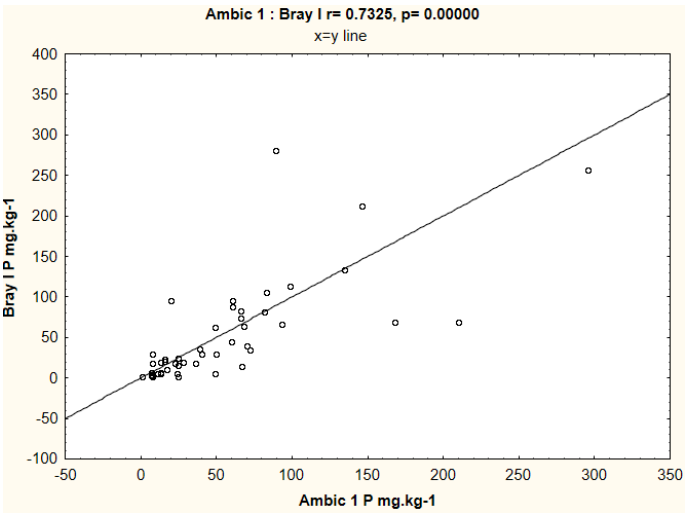

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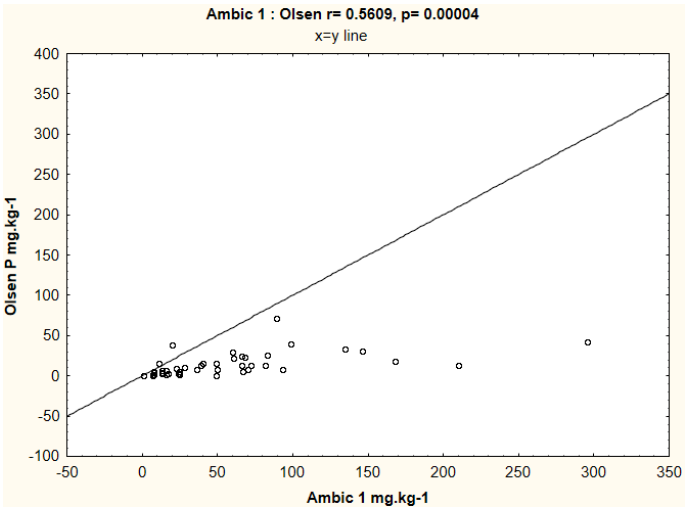

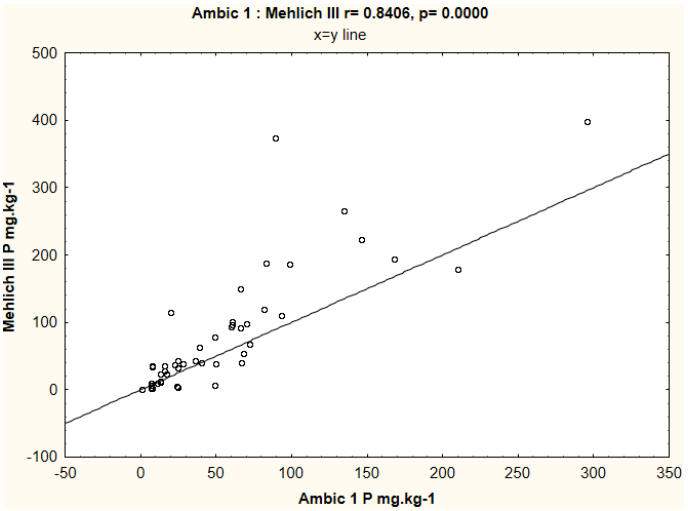

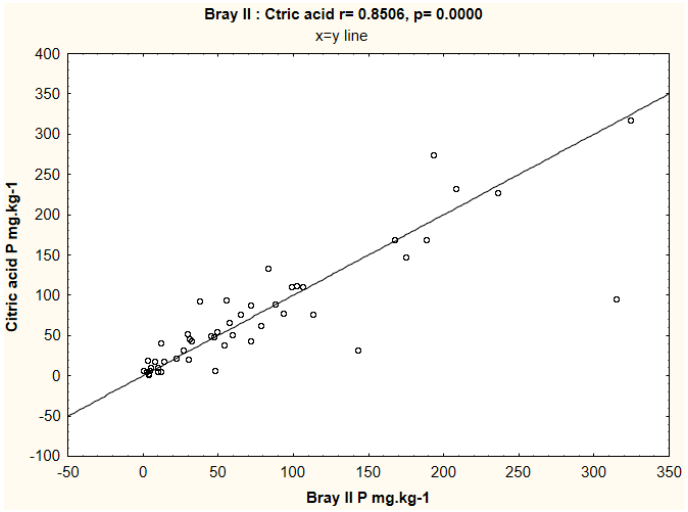

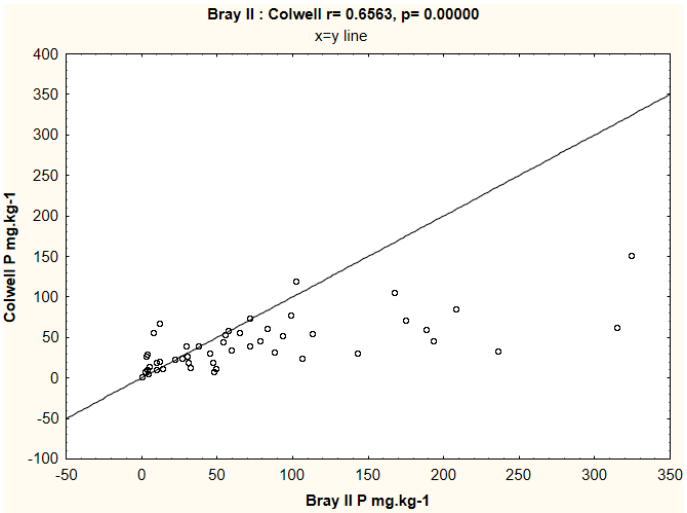

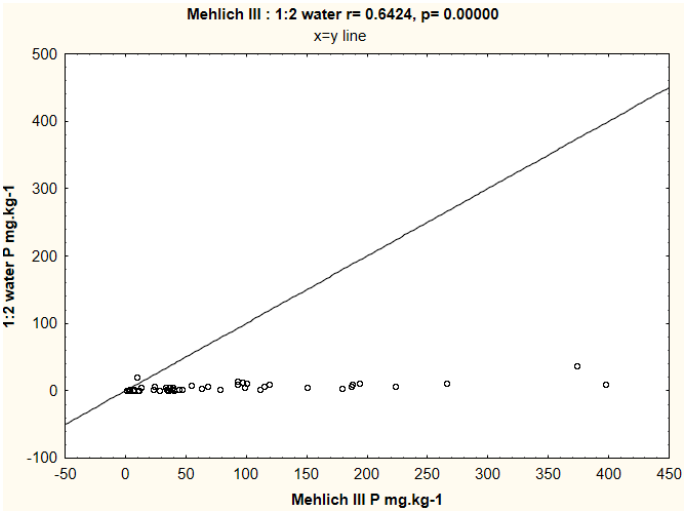

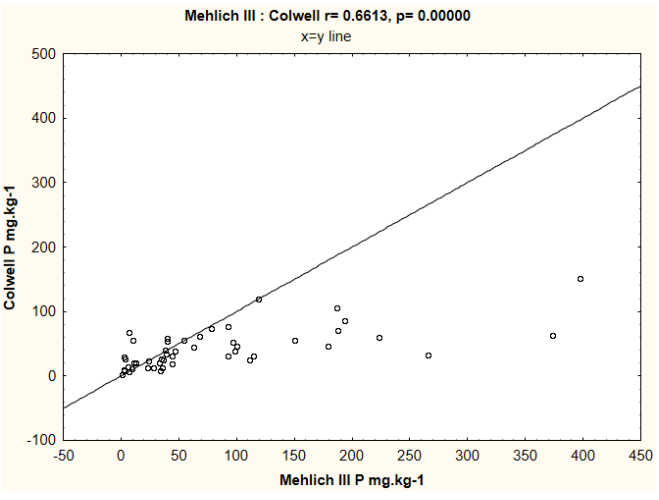

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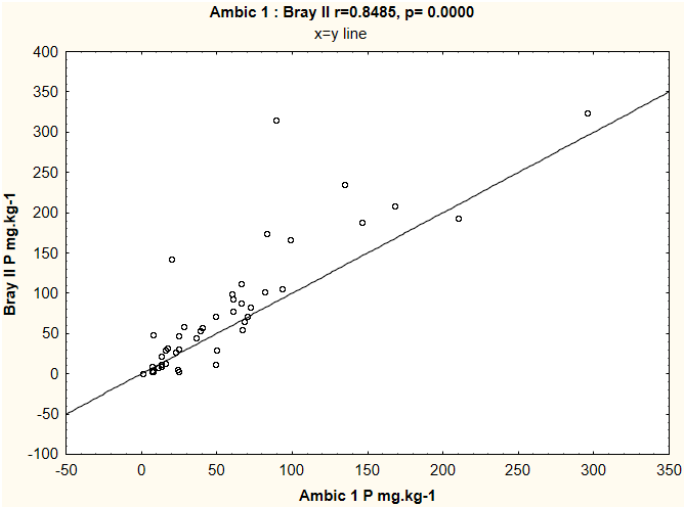

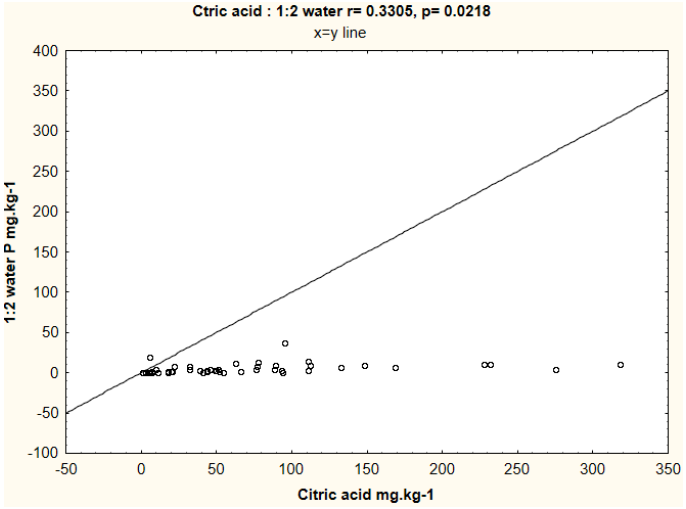

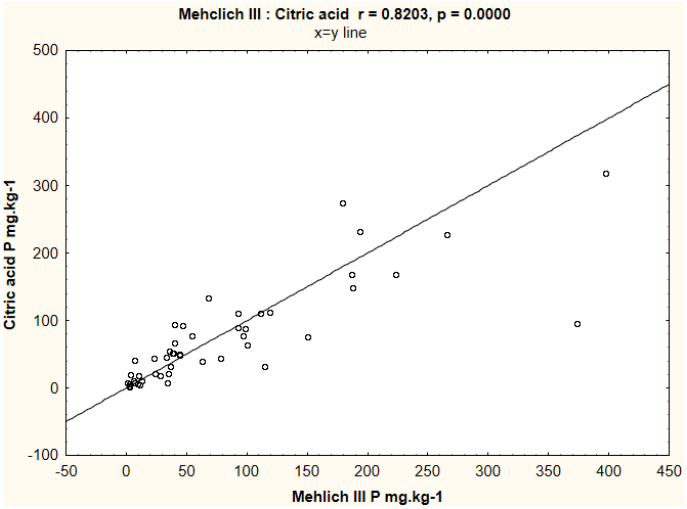

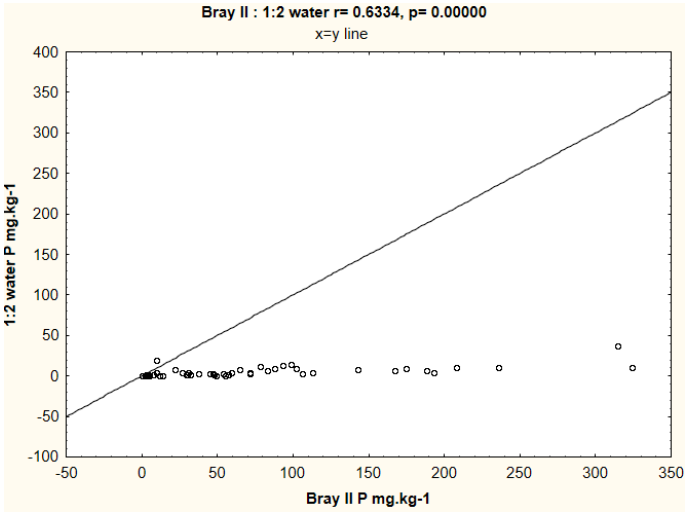

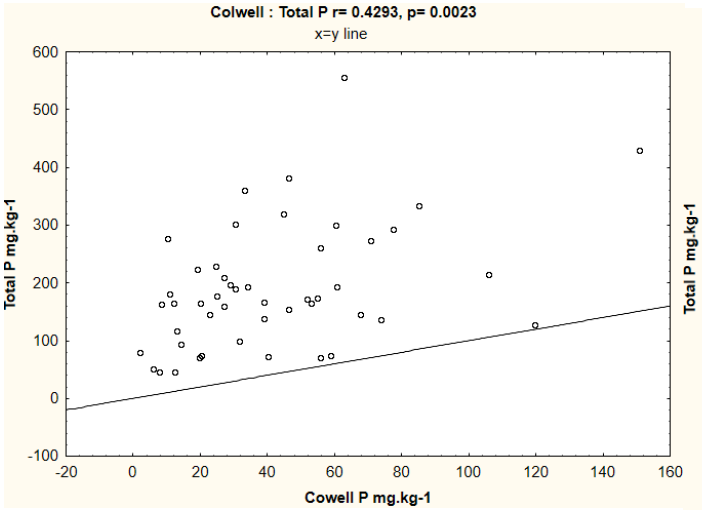

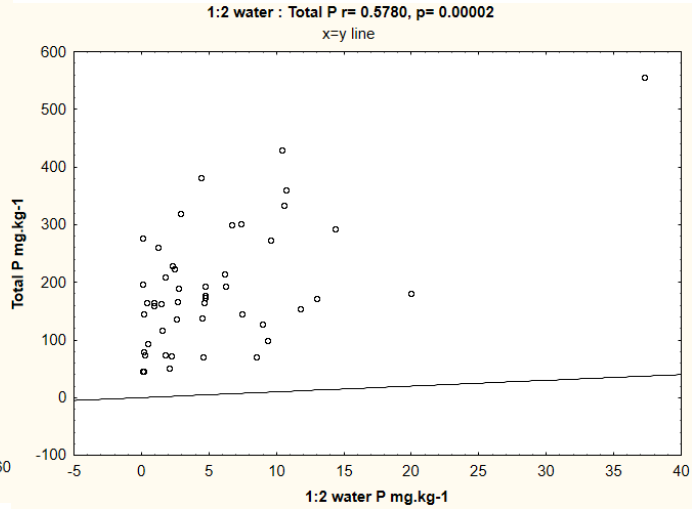

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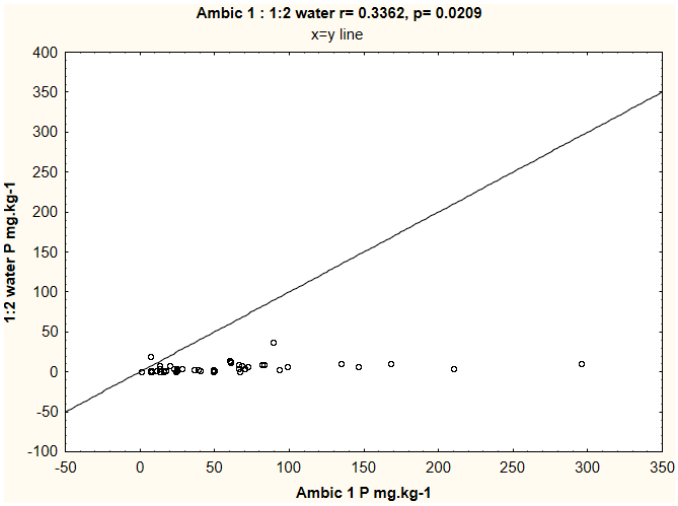

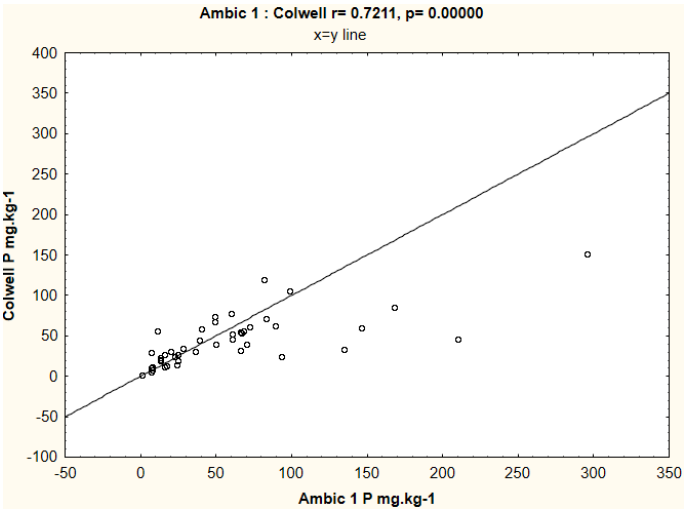

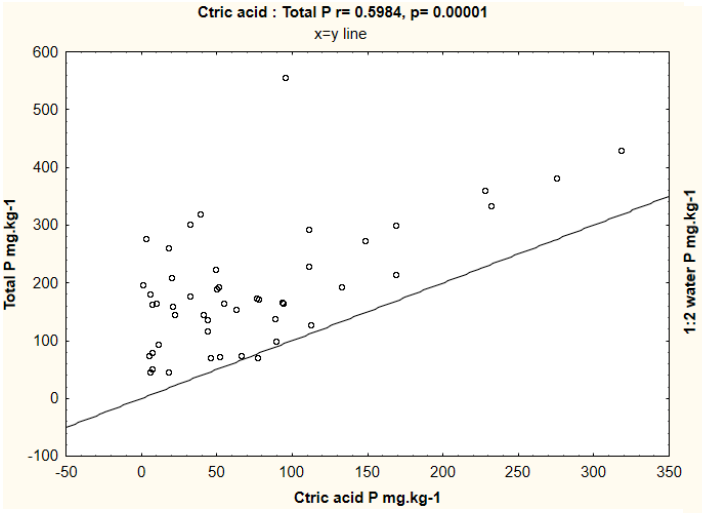

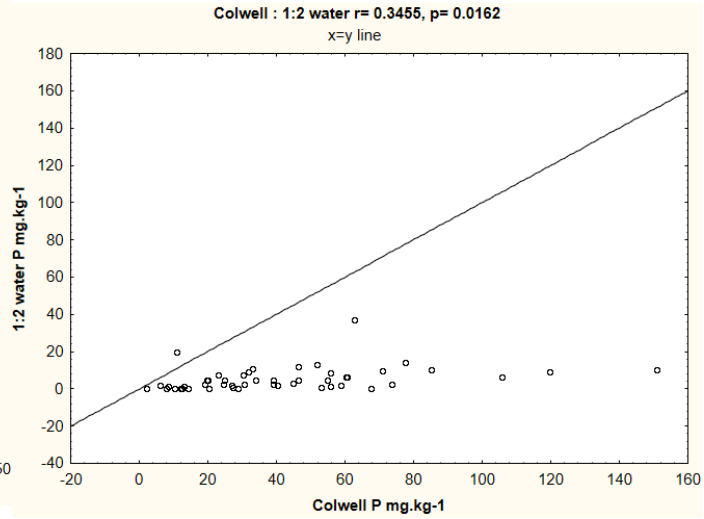

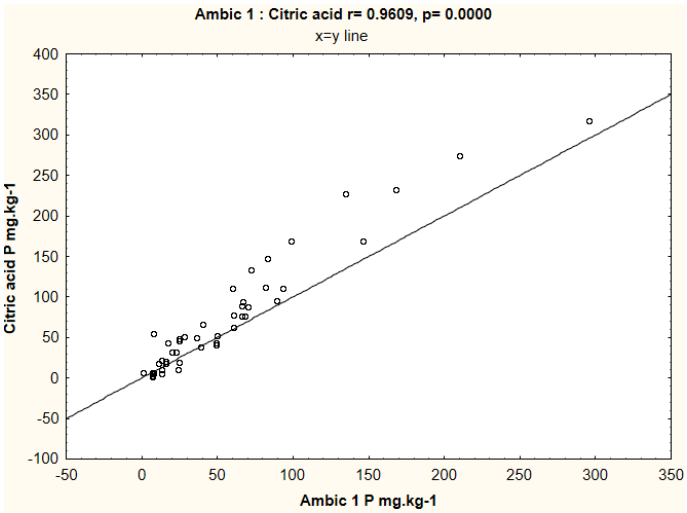

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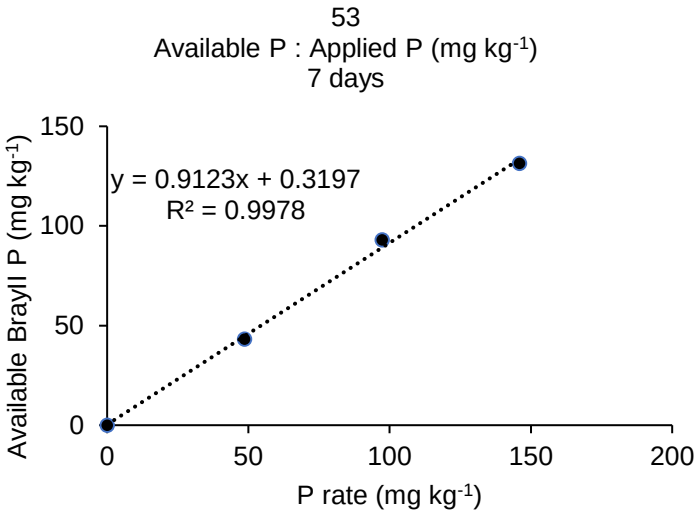
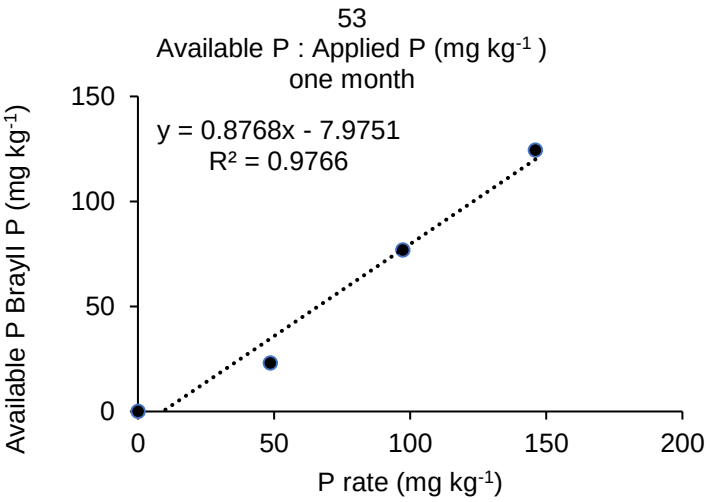
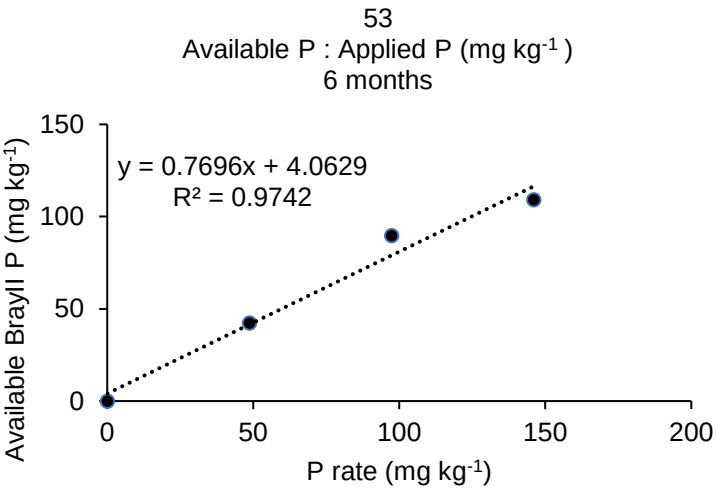
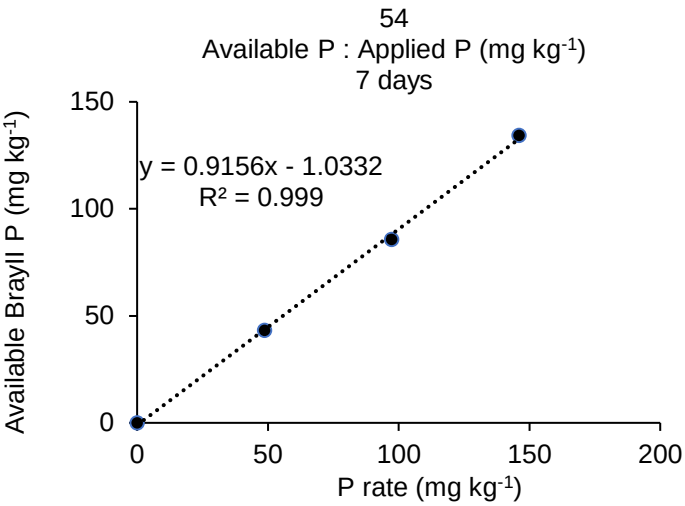
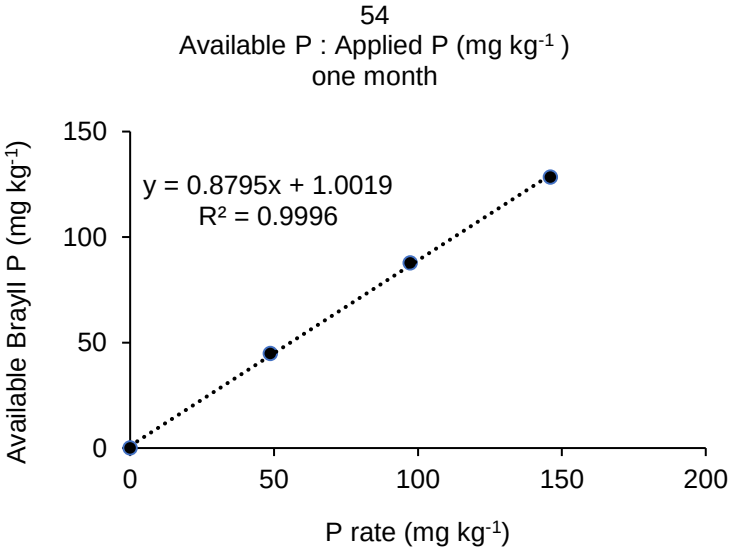
Appendix C

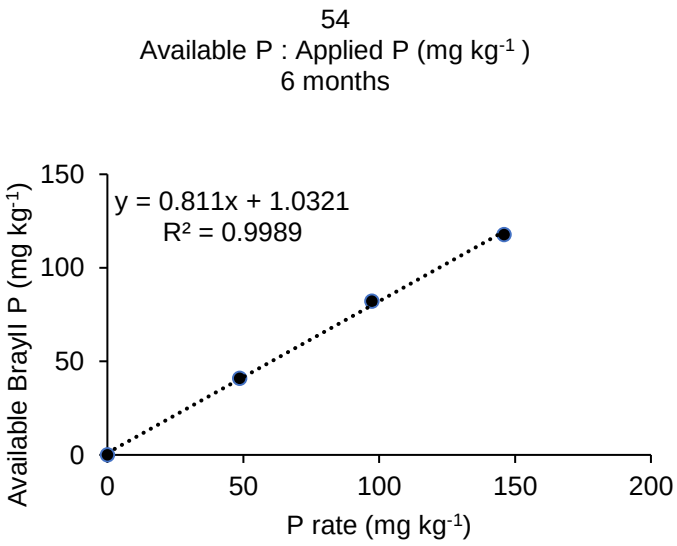












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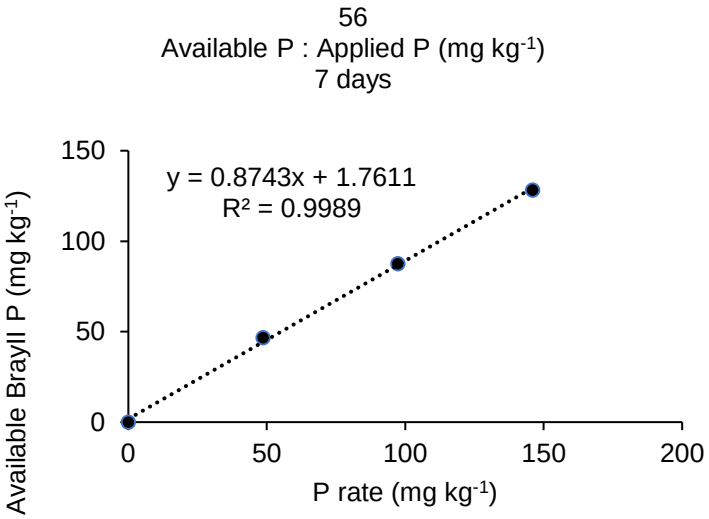

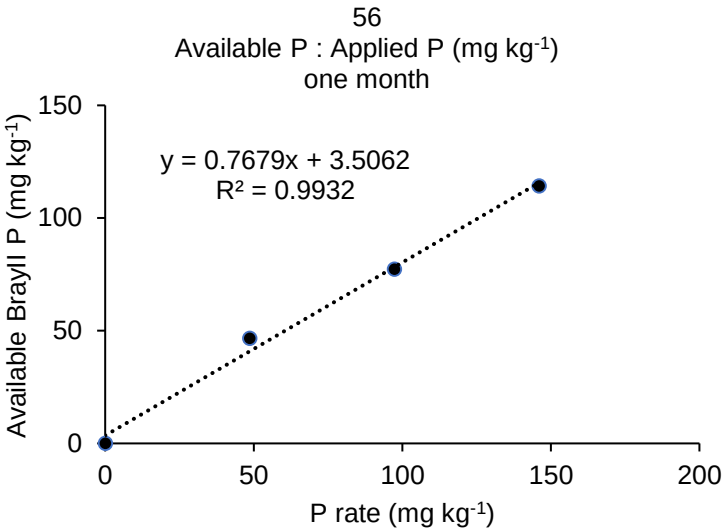

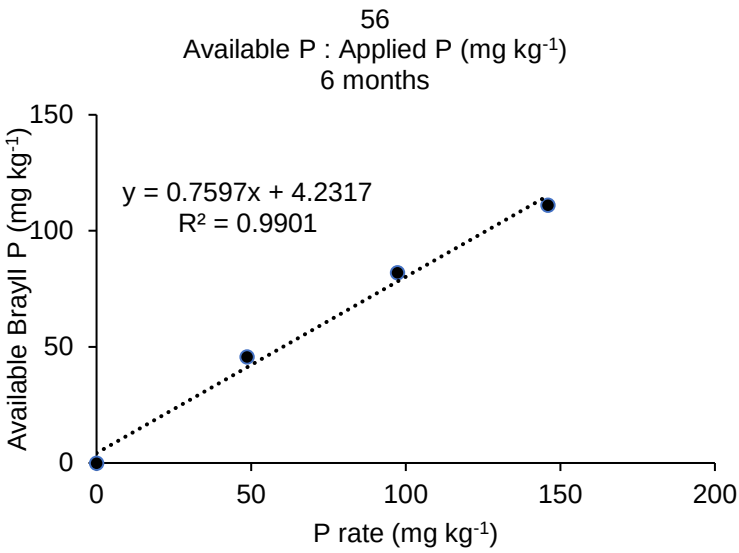

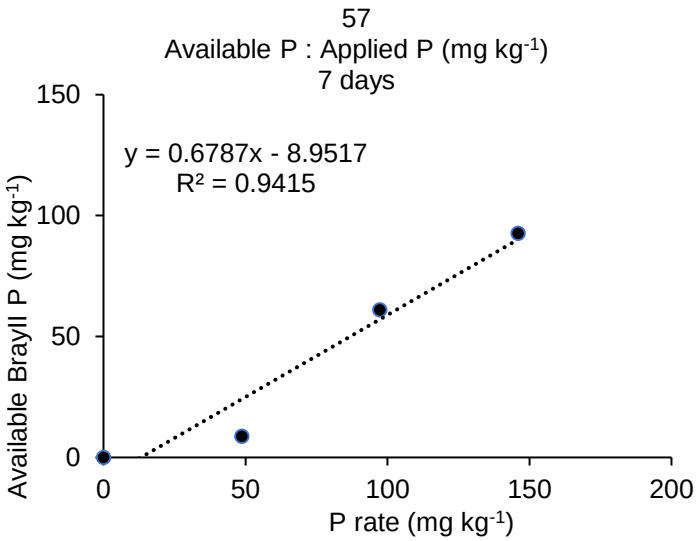

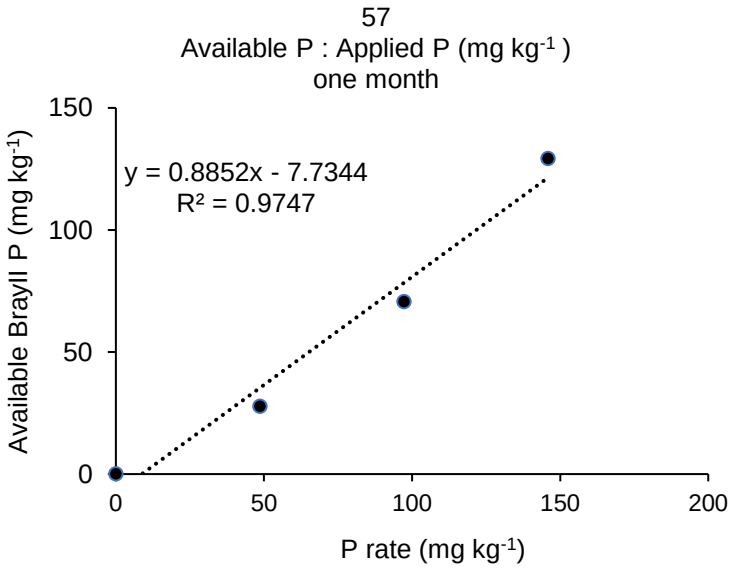

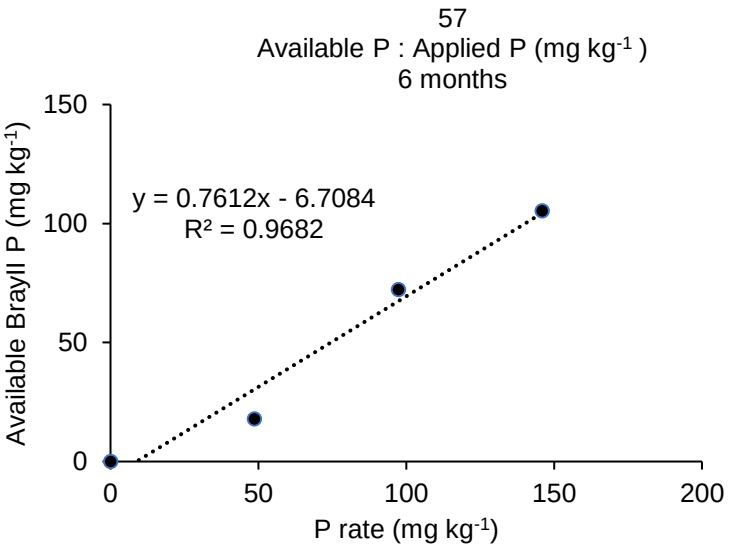

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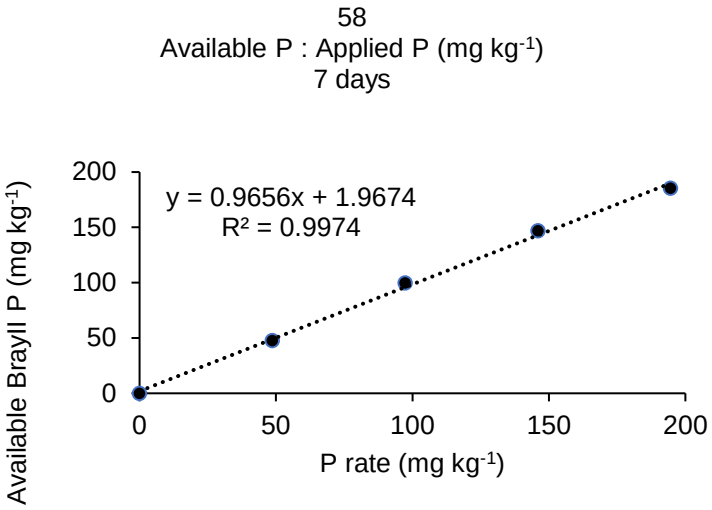

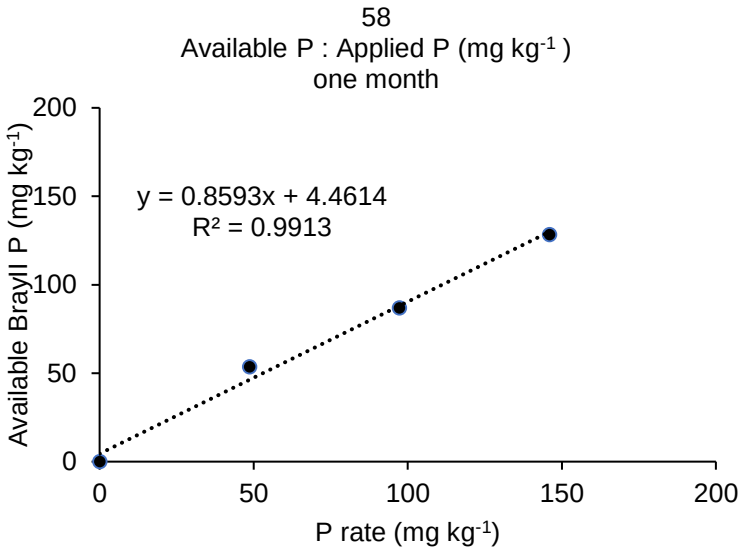

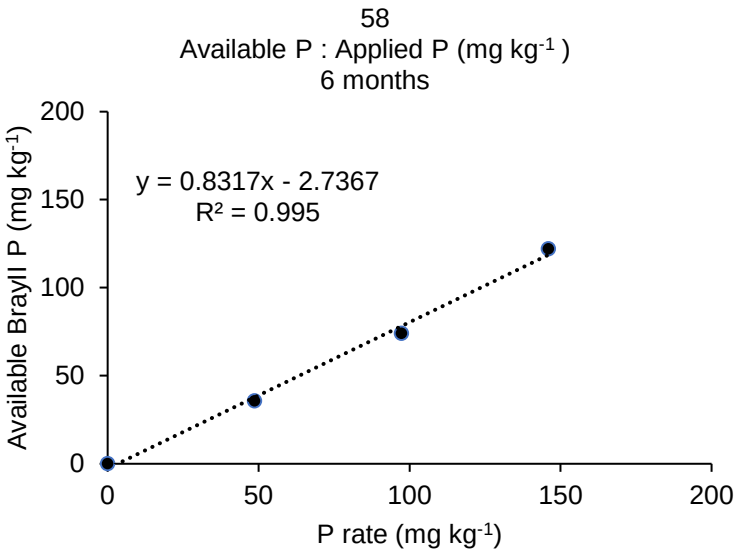

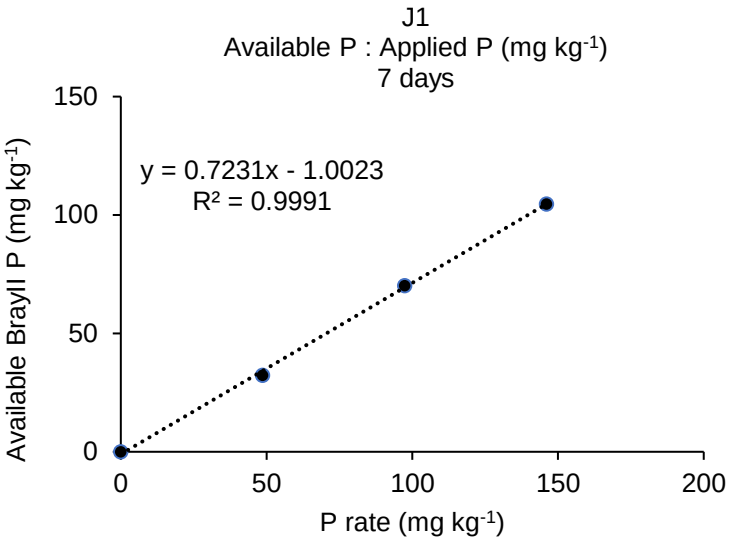

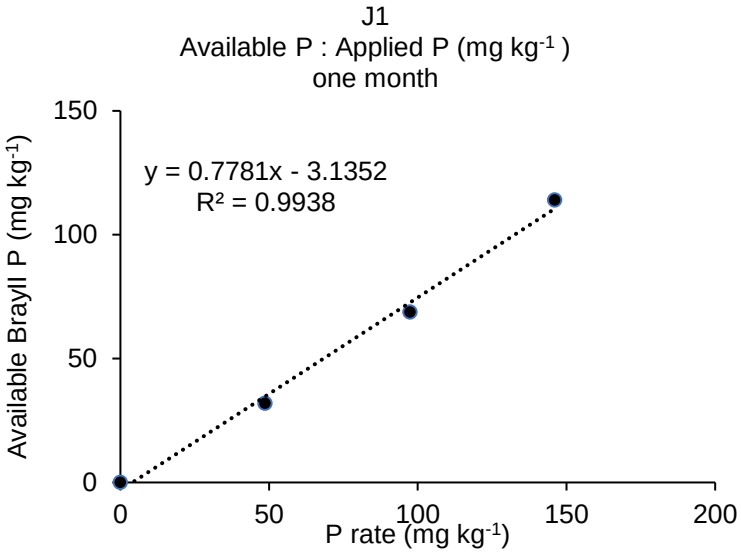

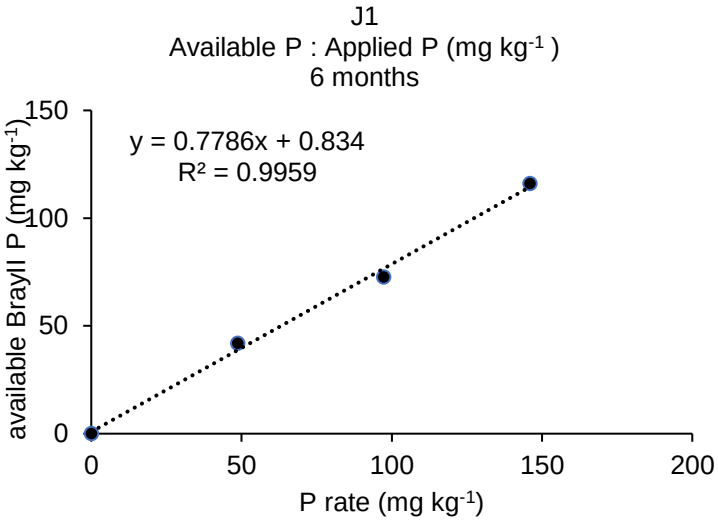

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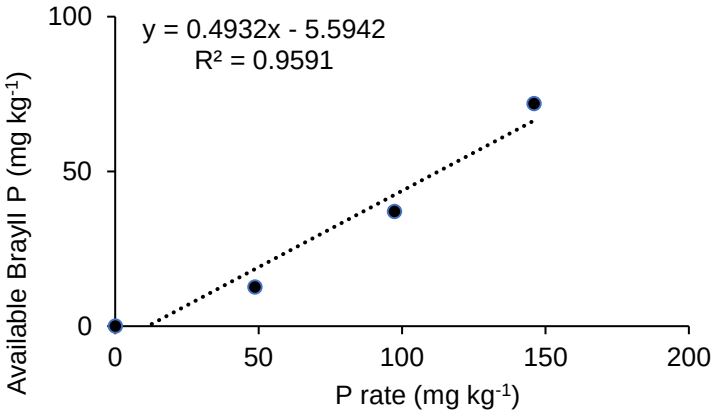
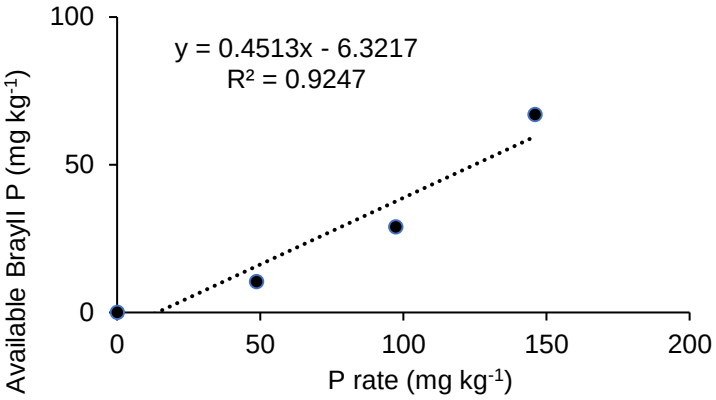
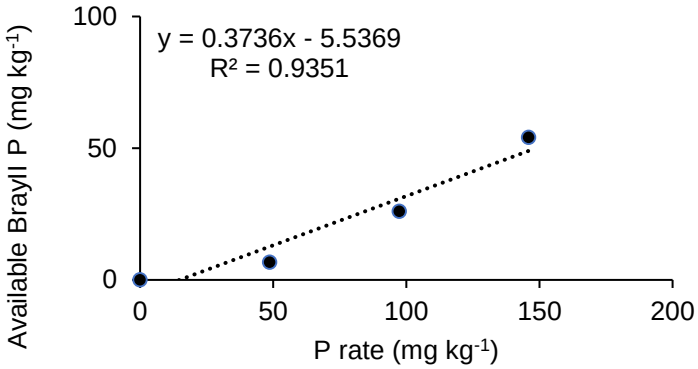
S1
Available P : Applied P (mg kg^{-1})
7 days



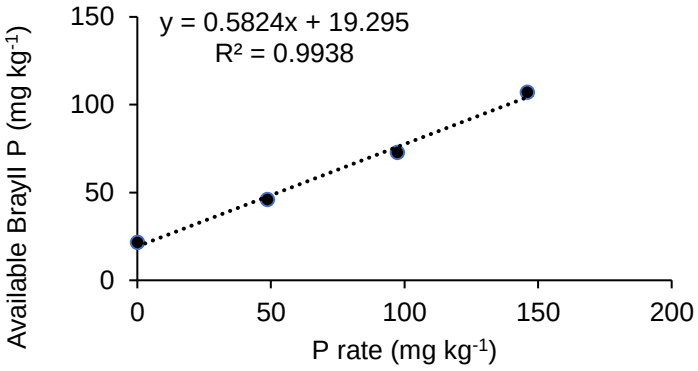
S1
Available P : Applied P (mg kg^{-1})
one month



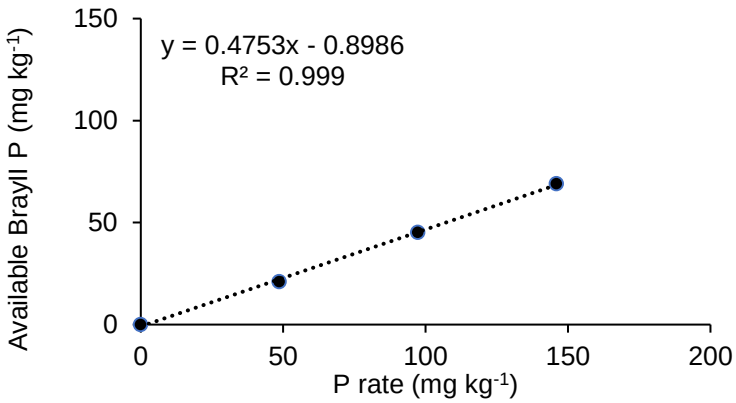
S1
Available P : Applied P (mg kg^{-1})
6 months



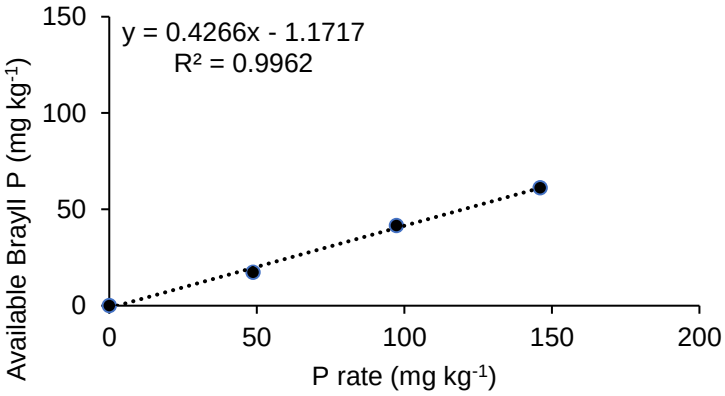
S2
Available P : Applied P (mg kg^{-1})
7 days



S2
Available P : Applied P (mg kg^{-1})
one month



S2
Available P : Applied P (mg kg^{-1})
6 months



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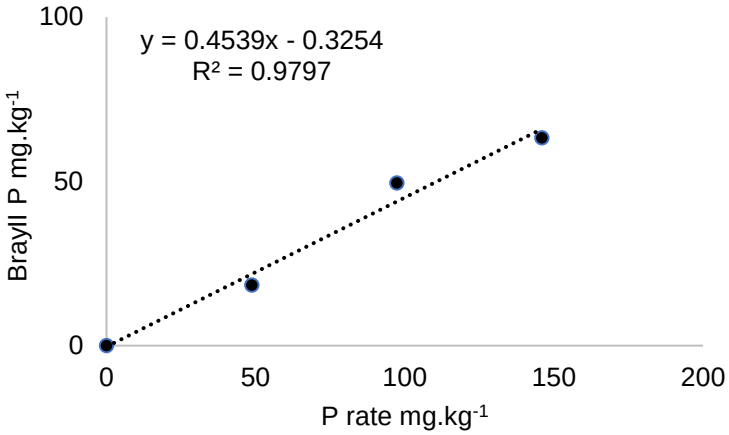
41
Available P : Applied P (mg.kg^{-1})
7 days



41
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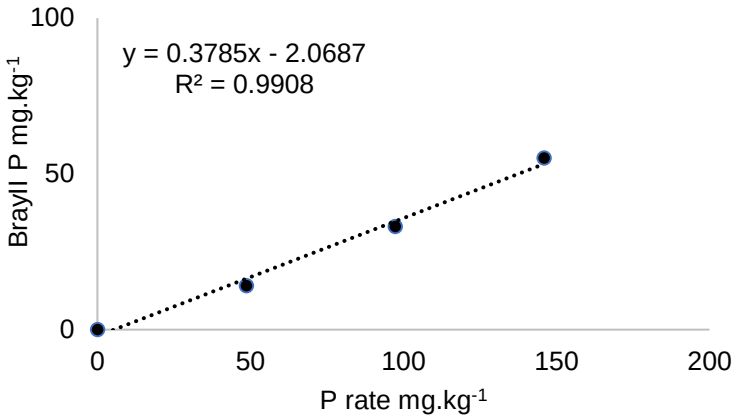
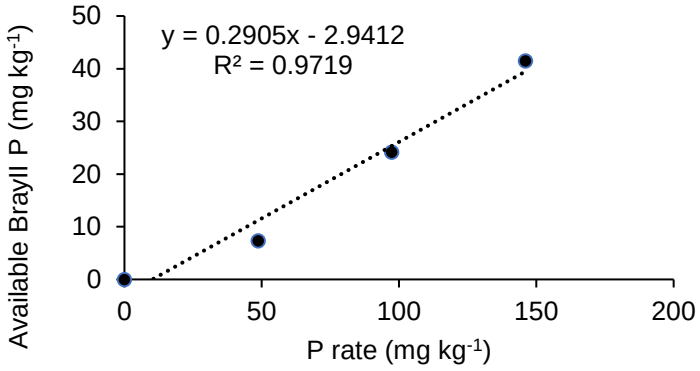
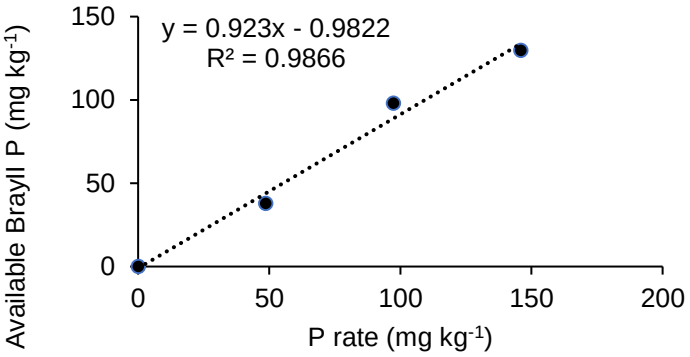
one month



41
Available P : Applied P (mg kg^{-1})
6 months



48
Available P : Applied P (mg kg^{-1})
7 days



48
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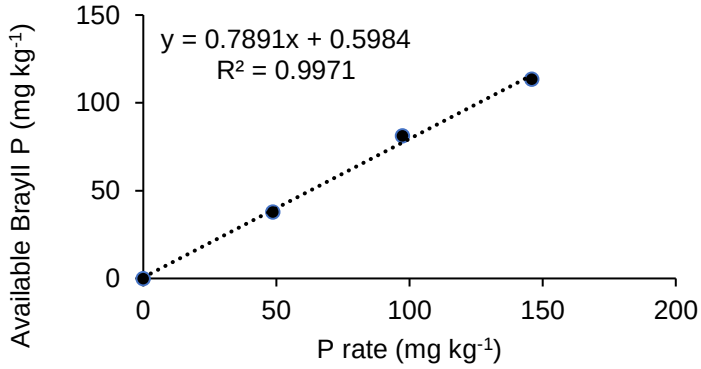
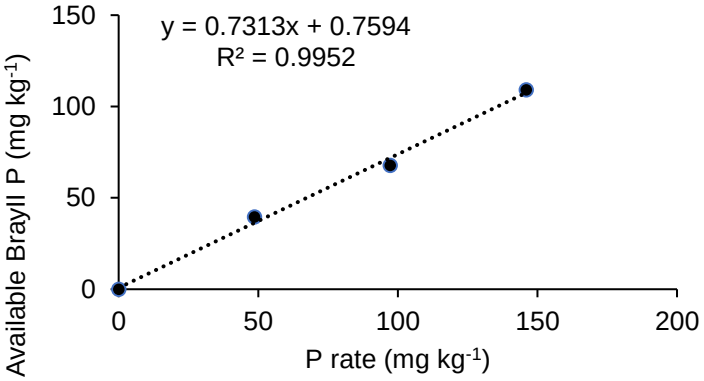
one month



48
Available P : Applied P (mg kg^{-1})
6 months



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