



Monash University

Doctor of Philosophy

**Investigation into the effects of K-humate-blended
phosphate fertilisers on soil-available phosphorus
and plant growth**

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Abstract

The world's population now stands at billion people. Although the current application of fertilisers including nitrogen, phosphorus, potassium (NPK), and other essential elements enables the industry to obtain adequate crop yields, it has raised serious concerns over environmental damage caused by surplus NPK, including its deleterious effects on waterways, soil, and soil microorganisms, high levels of fertiliser wastage and its associated negative economic consequences. Different strategies are employed to increase the use-efficiency of chemical fertilisers and to reduce their destructive impact on the environment, such as improvement in the accuracy of application, the development of slow-release fertilisers, and the organic coating and blending of fertilisers. Some agricultural manufacturers produce fertiliser engineered as a combination of organic and chemical materials. Although organic-blended fertilisers vary substantially in nutrient constitution and percentage, insufficient research has been undertaken regarding their relative efficacy in making necessary nutrients available to crop plants throughout their growth stages.

Every year, an enormous amount of phosphorus (P) is removed from agricultural soils in the process of plant growth for cropping, horticulture, and pastoralism, including the production of vegetables and fruit, meat and dairy goods for consumption. In order to replenish P losses from the soil, fertilisers are applied in varying rates. As phosphate is a highly water-soluble element, phosphate fertilisers are regarded as particularly damaging to highly weathered soils such as those common to Australia. Depending on the type of industry and the systems of plant growth involved, not all P introduced to the soil in fertiliser is recoverable in terms of being available to crop plants for growth. In Australia, the P recovery from harvested products is 48% for grains production, 29% for dairy cattle, and only 19% for beef cattle. Most of the applied P accumulates in soils via a fixing process and remains out of reach of plants. For this reason, P fertilisers are applied at levels far in excess of the amount necessary for optimal production. In

Western Australia, 87% of cropping soils exceed the required level of P sufficiency.

In this thesis, combined organic and phosphorus fertilizer application has been investigated in regard to its usage efficiency and availability in Victorian soils for uptake by plants.

Chapter 2 of this thesis describes the range of methods used in the production of the granular humate-blended phosphate (HBP) fertiliser that is the subject of this research investigation. In addition, the chapter outlines physiochemical properties of the HBP that have been determined by wet chemistry methods including solubility testing, product pH, elemental analysis, and structural information. The reported results indicate an improvement of soil P availability after 2 and 7 days of adding the HBP fertiliser to acidic soils, while in calcareous soils it takes further time before any beneficial effect on soil P availability is evident.

Chapter 3 details the process and results of a soil slurry incubation study into the effect of HBP fertilisers on soil phosphorus availability. Two different acidic soils and one alkaline soil were selected. The HBP fertilisers were compared with triple superphosphate (TSP) as a matter of P availability in these soils during a specified time duration. The results demonstrate greater diffusion of P on addition of HBP2 in an acidic soil with high-P fixing capacity. The visualisation technique is in agreement with the diffusion results.

Chapter 4 describes research conducted for the thesis project in which HBP fertilisers were compared with TSP in a comparative soil experiment. A specific quantity of P was added to soil in a series of Petri dishes. The phosphorus diffusion rate was monitored over a set duration, with the results confirmed by application of a visualisation method.

Three different glasshouse studies were also undertaken. These took place a range of Victorian soils and differing crops in order to evaluate the HBP fertilisers in regard to soil phosphorus availability and plant growth improvement. The HBP fertilisers were compared with TSP in relation to the plants' growth indexes, as well as for levels of post-harvest soil- available P. The effect of HBP fertiliser on plant growth was not consistent across the three glasshouse trials, with the results demonstrating that soil characteristics and the demands of plant nutrition play crucial roles in the effects of HBP. A finding from the glasshouse studies was that in acidic soils HBP appear to improve the level of post-harvest P available in the soil.

In the final chapter discussion concentrates on further chemical investigation into the possible positive effect of organic acids on soil phosphorus availability – this was conducted for the purpose of adducing additional results that would either confirm or falsify the glasshouse study results regarding acidic soil effects on P availability in relation to HBP. An incubation study was run in soil with a high P-fixing capacity. Two carboxylic acids were introduced into the soil slurry solutions in two different concentrations. Results indicated that the addition of organic acids to soil solution did not improve P availability in acidic soil.

Declaration

In accordance with Monash University Doctorate Regulation 17.2 Doctor of Philosophy and Research Master's regulations, the following declarations are made: I hereby declare that this thesis contains no material that has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis. Firstly, I would like to express my sincere gratitude to my great supervisor Prof. Tony Patti for the continuous support of my Ph.D study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better supervisor and mentor for my Ph.D study.

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Arsenic	As
Aluminium	Al
Carbon nitrogen and hydrogen analyser	CHN
Cadmium	Cd
Calcium phosphate	Ca-P
Dalton	Da
Department of Economic Development, Jobs, Transport and Resources	DEDJTR
Diammonium phosphate	DAP
Exchange capacity	EC
Fulvic acid	FA
Honest significant difference (Tukey's range test)	HSD
Humate-phosphate blended	HPB
Humic acid	HA
Humic substances	HS
Inductively coupled plasma	ICP
Iron	Fe
Kilo Dalton	kDa
Lead	Pb
Monoammonium phosphate	MAP
Nitrogen	N
Organic complexed Superphosphate	CSP
Phosphorus	P
Phosphorus buffering capacity	PBC
Potassium	K
Potassium humate	K-humate
Single superphosphate	SSP
Soil organic matter	SOM
Triple superphosphate	TSP
Total phosphorus	TP
Total iron	TFe
Total aluminium	TAI
Victorian brown coal	VBC
Victorian Centre for Sustainable Chemical Manufacturing	VCSCM
Water-extract phosphorus	WEP
X-ray fluorescence	XRF

Chapter 1

Literature Review and Background

1.1 Introduction

1.1.1 Background

The world's population has increased to 7.7 billion people. There is a consequent need for worldwide changes from ongoing ecologically damaging land-use practices to reliance on land and agricultural resources in a sustainable manner (Foley et al., 2005). Among necessary inorganic nutrients for plant growth, the elements nitrogen (N), phosphorus (P) and potassium (K) play crucial roles during the stages of growth for all plants. These elements are applied by a variety of means and in disparate forms throughout the world. The increasing need for global food security has led to an increase in the quantities of fertiliser applied to farmland. Although the current excessive application of fertilisers including nitrogen, phosphorus, potassium (NPK), and other essential elements enables the industry to replenish nutrient deficiencies in the soil and obtain adequate crop yields, it has raised serious concerns over environmental damage caused by surplus NPK, including its deleterious effects on waterways, soil, and soil microorganisms, high levels of fertiliser wastage, and its associated negative economic consequences (Nkoa, 2014, Geisseler and Scow, 2014, Chen et al., 2018). The list of ecological damage is extensive, including soil degradation, air pollution, groundwater pollution, changing ecosystems (Tilman et al., 2002, Khan et al., 2008, Wen et al., 2016) accumulation of salt and nitrate concentrations (Ju et al., 2007), and increasing concentrations of heavy metals such as cadmium (Cd), lead (Pb) and arsenic (As) (Atafar et al., 2010, Moe et al., 2017). However, increasing the quantities of NPK has not always improved the efficiency of nutrient delivery to plants. For instance, phosphorus (P) deficiency is a major problem worldwide, especially in highly weathered soils like Ferrosol in Australia, in which a strong chemical reaction occurs between P and soil compounds such as iron (Fe) and aluminium (Al). This reaction varies from surface adsorption to precipitation depending on reaction time and soil pH (Koopmans et al., 2003, Kruse et al., 2015). For instance, administering excessive quantities of NPK in

glasshouse vegetable production has caused unsustainable levels of accumulated salt and nitrate concentrations (Ju et al., 2007). In another study, results indicated the concentration of some heavy metals (Cd, Pb and As) increased due to the over-application of fertilisers (Atafar et al., 2010). Results such as these demonstrate there is a pressing need to identify alternative solutions in providing sufficient nutrients to plants; one plausible alternative involves increasing the efficiency of fertilisers in delivering those nutrients.

Different strategies exist in seeking to increase the use-efficiency of chemical fertilisers and reduce their harmful impacts on the environment, such as improving the specificity of fertiliser application, and the use of slow-release fertilisers and organic coated fertilisers (Chen et al., 2018, Lü et al., 2014). The most common and commercially available technology is coating chemical fertiliser with environmentally friendly materials that degrade in soil and convert to inorganic compounds (Naz and Sulaiman, 2016). Various materials have been used for this purpose, usually naturally derived (Schneider Teixeira et al., 2016). The most commonly recognised feature of coated fertilisers is their controlled release of the elements fulfilling the crop nutrient requirement at a single application (Azeem et al., 2014). In addition to the abovementioned new coating technologies directed at nutrient use efficiency, some manufacturers have developed organic-chemical fertilisers, which combine the organic material with chemical fertiliser (Summerhays et al., 2015).

Although these organic-blended fertilisers vary in nutrient percentages, only very few studies have investigated their efficacy in regard to nutrient availability and plant growth.

In this thesis, combined organic and phosphorus fertilisers have been investigated as a matter of usage efficiency and availability in soils for uptake by plants. Detail concerning the functions and interrelations of phosphorus in soils, the organic matter that has been used in combined

fertilisers, and how the new combined fertilisers affect phosphorus availability are set out in the following sections:

1.1.2 Phosphorus in soils and chemistry of phosphorous

Typically there are two forms of phosphorus in the soils, organic and inorganic form. Usually the organic form of phosphorus consists of 5% to 95% of total phosphorus (Harrison, 1987). The conversion of inorganic P to organic P phosphorus happens when the plants utilize the orthophosphate (George et al., 2017). Both organic and inorganic forms of phosphorus accumulate in the soil after the application of P fertilisers (McLaren et al., 2015a). The chemical nature and reaction of organic phosphorus still remains unclear (McLaughlin et al., 2011).

Regarding to the chemical behavior of P, phosphorus occurs in soil both in the solid phase and in the soil solution phase, based on its reaction with other soil constituents. In solid phase P can react with minerals such as Al, Fe in low-pH soils (<5), and Ca in soils with higher pH ranges (>6). These reactions involve P adsorption onto amorphous or crystalline Al- and Fe-(hydro)oxide, the formation of complexes of P-Al (hydro) oxide or Fe- (hydro)oxide in acidic soils, and P-calcium carbonate in alkaline soils (Schoumans, 1995). According to Sposito (1985) the mechanism of P adsorption by mineral surfaces includes two aspects of phosphate-hydroxyl reaction and the adsorbed phosphate ion configuration. Studies results showed that orthophosphate adsorbed onto hydroxyl minerals by ligand exchange mechanism (Beek and van Riemsdijk, 1977; Parfitt, 1978; Mott, 1981; Hingston, 1981; Sposito, 1984). However, if soil P content is high, reversible adsorption of P plunges, and adsorbed P persists in the soil, to be mobilized again when precipitation occurs. This mechanism was accurately described by Lookman (Lookman et al., 1995). In view of the abovementioned reactions, it is apparent that P can be found in different forms in its soil solid phase. There are three main groups of P reserve that occur in soil: first is the soluble form of P that occurs in soil solution. Secondly, gradually available P (labile) forms; and finally, the very gradually available P (non-labile) form, which

often forms over a considerably long period of time (McKenzie et al. 2004; Syers et al. 2008).

In agriculture, the most important instantiation of P is its soluble form. By definition, this encompasses all reactions that lead to the release of P from solid phase to the soil. For the purposes of this research, the function of – and capacity for – P to form a solution is termed solubilisation. The soluble form of P is important for agricultural purposes as plants can only absorb P in soluble form (Kuo, 1996). However, it should be considered that this is a necessary but insufficient condition: not all soluble forms of P can be assimilated by plants, and only a small fraction of soluble P can be used by plants, which is referred to as “plant-available P”. Based on this definition, plant-available phosphorus is the part of total P in soil solution that can be absorbed into a plant’s root system and be made available for its use (Dhillon et al., 2017). It is accepted that plant-available P – And this form is only a small portion of P in soil solution – mostly presents in orthophosphate forms (H_2PO_4^- and HPO_4^{2-}) (Nyle C. Brady & Weil, Ray R., 2002).

1.1.3 Phosphorus fertiliser efficiency

Every year, a significant amount of P is removed from agricultural soils in cropping, horticulture, pastoralism, the production of vegetables, dairy, meat and fruit. In order to replenish soil P losses, fertilisers are applied, but in varying amounts and at differing rates. The most commonly used P fertilisers are acidulated phosphates such as single superphosphate (SSP), monoammonium phosphate (MAP), diammonium phosphate (DAP), and triple superphosphate (TSP). These forms of phosphate are highly soluble, with greater P concentration than non-acidulated fertilisers. However, their high solubility is regarded as a drawback in highly weathered soils like those in Australia (Herrera et al., 2016). By increasing the soluble P concentration, P adsorption and precipitation also increase, which reduces plant-available P over the time. On the other hand, not all the applied P is recovered by fertiliser

application, depending on the industry and the targeted plant growth systems (Syers et al. 2008). In Australia, the P recovery from harvested products is 48% for grains production, 29% for dairy cattle, and only 19% in beef cattle (Wong et al. 2012). Most of the applied P accumulates in the soil, remaining out of reach to plants. For this reason, P fertilisers are added to the soil at excessive levels considered necessary in order to maximise production (Simpson et al. 2010). For example, in Western Australia, 87% of cropping soils tested exceed naturally occurring and conventionally accepted levels of P (Weaver and Wong 2011).

The abovementioned issues, as well as the limited rock phosphate reserves and the increasing demand for P to maintain high yields, have given rise to new approaches to improve P use efficiency via different methods and techniques.

1.1.4 Soil organic matter and its effect on phosphorus availability and plant growth

Soil organic matter (SOM) is a complex substance that contributes to a variety of biological, chemical and physical properties of soil and is essential for soil health. By definition, soil organic matter is composed of live organisms and their undecomposed remains and is a heterogeneous mixture of different products which are resulted from microbial and chemical transformations of organic debris (Hayes and Swift, 1978). The original source of soil organic matter is plant tissue. Animals are a secondary source of organic matter. When plant and animal debris is added to soil, it is broken down by macro- and micro-organisms, initially into particulate organic matter, and finally into humus. Some of the major characteristics of SOM are: cation exchange improvement, increased water-holding capacity, formation and stabilization of soil aggregates, and its functions as an energy supplier for microbial activities and as a source of slow-release nutrients.

SOM consists of subsets including humic substances. Humic substances are natural organic substances which are present in soil, water and sediments and has a beneficial effect on soil

physiochemical properties (Piccolo, 2002).

Humic substances include humic acid (HA), fulvic acid (FA) and humins. These are the generalized terms for the HS components. Humic Acid and FA differ in their solubility. HA are insoluble at low pH, and they are precipitated by adding strong acid while FA remain soluble under all pH range (Piccolo, 2002).

Chemically, HAs comprise a mixture of weak aliphatic (carbon chains) and aromatic (carbon rings) organic acids, which are not soluble in water under acid conditions, but are soluble in water under alkaline conditions. On average, 35% of humic acid (HA) molecules are aromatic (carbon rings), while the remaining components exist in the form of aliphatic (carbon chains) molecules. Landmark papers by Piccolo (2001, 2002) proposed a new view of humic substances. Piccolo proposed that humic substances should be regarded as supramolecular associations of self-assembling heterogeneous and relatively smaller molecules derived from the transformation (both biotic and abiotic) dead biological material. These supramolecular structure were proposed to be predominantly stabilized by weak dispersive forces instead of covalent linkages, including hydrophobic (van der Waals, π - π , CH- π) and hydrogen bonds, leading to the “apparent” large molecular size of humic substances

Piccolo and Spiteller (2003) definitely put a stone on top of the "molecular size" problem of substances using electrospray ionization mass spectrometry. “Unexpectedly, their average molecular mass was only slightly less than for the bulk sample and, despite different nominal molecular size, did not substantially vary among size-fractions. The values increased significantly (up to around 1200 Da) after on-line analytical HPSEC for the H A bulk sample, at both pH 8 and 4, and for the HA size-fractions when pH was reduced from 8 to 4. Humic acid bind clay minerals to form stable organic clay complexes. Also, HAs readily form salts with inorganic trace mineral elements. Analysis of extracts of naturally occurring humic acids will reveal the presence of over 60 different mineral elements. These trace elements are bound

to humic acid molecules in a form that can be readily used by various living organisms. As a result, HAs function as important ion exchange and metal complexing (chelating) systems (Pettit, 2006)

It has been well-established that the composition, extent, and relative proportions of soil humic substances, bioavailable mineral nutrients, and soil microbiota are strongly related to soil fertility, and thereby to crop yield and quality (Erro et al., 2016). The positive roles of HS on soil and plant systems are typically divided into two main categories: direct effects and indirect effects (Chen and Aviad, 1990, Mora et al., 2014). The ability of HS to form stable natural complexes with soil cations leads to improved soil texture and provision of an available nutrient reserve, which are regarded as indirect effects (Stevenson, 1994); direct effects of HS are those that operate upon the cell membranes of root and leaf surfaces. These direct effects take place by means of a complex network of signaling pathways that are regulated by plants' hormones (Mora et al., 2014). The direct role of humic substances in plant nutrients and growth was emphasized in the early 19th century (Chen, 1990). In a number of studies Bottomley (1914) demonstrated that HS increased plant growth in a variety of species.. Results from other studies have shown the benefit of humic substances in improving the solubility of some soil minerals, especially iron (Olsen, 1930, Burk et al., 1932).

Many studies have been conducted to investigate the effect of organic matter on phosphorus efficiency. Some of these have shown that the application of organic matter to soil can decrease P adsorption onto soil minerals (Scheffe et al., 2008, Wang et al., 1995, Diez et al., 1992). It has also been widely reported that naturally derived organic matter enhances P availability in soils (Iyamuremye and Dick, 1996, Haynes and Mokolobate, 2001). The effect of humic substances on P use efficiency has also been investigated. Wang and colleagues (1995) demonstrated that the addition of humic acids to soil in conjunction with P fertiliser significantly increased the amount of water-soluble phosphate, strongly retarded the formation of occluded phosphate, and

increased P uptake and yield by 25%; Wang also indicated that coating phosphorus fertilisers with humic-derived alkaline extracts such as potassium humates (K-humate) leads to increased P efficiency. Research by Diez and colleagues (1991) suggested the use of diammonium Phosphate (DAP) coated with 22% rosin based humates enabled phosphorus fixation to be controlled in calcareous soils. Another study revealed that a low rate of HA (1.7kg/ha) applied with MAP caused no significant difference in P uptake compared to MAP without HA (Clain et al.2007). Phosphorus availability increased in calcareous soils when superphosphate fertiliser was coated with lignin (Garcia et al., 1997). Quan-Xian and co- researchers (2008) have run an investigation on the effect of humic substances on enhancement of P solubility in acidic soils. They demonstrated that in soils treated with humic substances the water-soluble P concentration was significantly higher than in untreated soils.

In another study it has been shown that the application of organic matter derived from clover residue reduced P retention on hydrous ammonia and amorphous aluminosilicate (Perrott, 1978). A separate study investigated the effect of humic acid-coated monoammonium phosphate (MAP) on P solubility, micronutrient uptake and spring wheat yield was investigated. The results did not show any significant difference of P solubility and P uptake by wheat between HA coated MAP and MAP fertilisers (Jones et al., 2007). Despite the latest study results that did not show a significant difference between HA coated P fertilisers and uncoated one, Mesut and his research team has shown that the application of humic acid to lettuce improved P availability (Cimrin and Yilmaz, 2005).

One fundamental body of research over recent decades has been the use of humic substances (HS) to improve crop growth. There have been many studies over different influence of humic substances on plant growth. A study demonstrated the direct availability of soil nutrients from humic substances (Stevenson and Xin-Tao, 1990), while (Stevenson, 1991) and Varanini and Pinton (1995) showed the effects of HS on chelation of nutrients. Other studies indicated the

effect of HS on more complex physiological reactions (Chen and Aviad, 1990, Vaughan et al., 1985). Most of the demonstrated positive effects of humic substances on plant growth have derived from hydroponic or sand culture experiments (Chen et al., 2004).

Little (2014) has shown that the effect of lignite-derived humic substances varied in different soils and pastures (Little et al., 2014). The results of a glass house study on spring wheat in U.S on 2006, demonstrated that the humic substances did not have a significant effect on nutrient uptake, shoot biomass and grain (Jones et al., 2007). Sharif applied HA at the rates of 50–300 mg kg⁻¹ into corn pots and no significant difference was observed in root and shoot biomass (Sharif et al., 2002). The most positive effects of the application of humic acids on crop yield have been proven in a controlled condition like glasshouse or growth chamber according to Olk et al., (2018). However, in recent years, more studies reported the positive effects of humic substances on plant growth indexes in the filed condition. Verlinden compared the yield and nutrient uptake of grass, maize, potato and spinach under application of a leonardite based humic product in six filed experiments. He observed a general positive effect on dry matter yield and nutrient uptake of the crops (Verlinden et al., 2009). In another filed study, there was an increase in the potato yield under application of commercial humic substances at four different sites (Seyedbagheri, 2010). Yellow corn yield under application of the humic substances mixed with micronutrients has been studied in two following season in the field (Mesker et al., 2014). The results indicated some positive effects on corn yield under application of humc substances combined with the micronutrients. Olk applied the liquid lignite based humic product on corn and evaluated the growth response for 3 years in the filed condition. He found a significant increase in corn grain weight under product treatment (Olk et al., 2012). Previous explications of potential avenues for the improvement of plant growth response to HS application were studied in the literature. These primarily concern increasing water-holding capacity, nutrient availability, hormonal activity, and microbial activity

improvement (Sharif et al., 2002, Ayuso et al., 1996). Potential alternative mechanisms for enabling HS improvement have been studied for years. The most notable mechanisms which lead to plant growth improvement by HS are: increasing the P availability to be taken up by inhibiting calcium phosphate (Ca-P) precipitation rates (Grossl and Inskeep, 1991, Inskeep and Silvertooth, 1988); and competing for adsorption site or /and decreasing the adsorption site by chelation of metals (Guppy et al., 2005). The effect of the humic substances on soil and plant yield was studied. The post – harvested soil microbial activity has been increased significantly in HA-treated soils as well as a significant increase of potato yield and potato quality (Sharif et al., 2002).

In this thesis a potassium humate extraction of Victorian brown coal has been considered as humic substances resources to produce an organic-phosphate blended fertilisers. The definition of brown coal and its characterisation occur in the following section.

1.1.5 Brown Coal as a source of humic substances

Victoria has 430 billion tonnes of brown coal that represents a significant proportion of the world's brown coal reserves. About 80% of this is located in the Gippsland basin Victorian Brown coal or lignite is a low rank coal with very low impurities such as ash, sulphur and heavy metals. It typically contains 50–60% carbon (on a dry basis), very low in nitrogen (typically <2% db), and has a very high in moisture content (48–70% water) (Department of Economic

Development, Jobs, Transport and Resources: <http://agriculture.vic.gov.au/agriculture>, access November 2017).

It has been shown that alkaline extraction of humic materials, humate salts, from brown coal have positive effects on plant nutrient uptake as well as stimulation of crop growth (Fong et al., 2006). Imbufe and colleagues (2004) have showed that humic substances from brown coal increase soil exchange capacity (EC) by providing reactive sites for cation exchange as well as assisting nutrient transport to plants and improvement in soil pH buffering (Imbufe et al., 2004). Organic matter which is derived from brown coal can also reduce bioavailable heavy metals in soils (Sklodowski et al., 2006).

Interestingly, a number of studies have shown Lignite (or brown coal) and the alkaline extracted humates of lignite, are being widely used to promote nutrient uptake and plant growth (Cao et al., 2016, Fong et al., 2006, Schindler et al., 2016). For example, it has been demonstrated that humic acid derived by alkaline extraction from brown coal has positive effects on soil nutrient efficiency and plant uptake as well as stimulation of crop growth (Fong et al., 2006).

In this thesis the humate alkaline extracts of brown coal were blended with triple superphosphate (TSP) fertilisers to evaluate the P efficiency in soils and plants. More detailed description of the blended humate-phosphate fertilisers used in this study is available in chapter 2.

1.1.6 Organic-phosphate fertiliser blends

There are different methods and techniques that are used to increase fertiliser efficiency without compromising the environment (Chen et al., 2018). Use of humic substances/ humics in association with chemical fertilisers is one the most effective methods to improve nutrient efficiency in soils. Usually these kinds of fertilisers are formulated in a way of coating or mixing with humic or organic substances that are soluble in soil solution. The combination of phosphate fertilisers with organic matter as complexed or blended fertilisers is a demonstrably efficient way of increasing P fertiliser use efficiency (Erro et al., 2012) (Gerke, 2010, Baigorri et al., 2013). A study mixing of urea with lignin increased CO₂ and N₂O emission from soil and yield per plant (Fernández et al., 2016a). The results of one study indicated that soil-available phosphorus increased when phosphorus was applied in lignin-coated P fertiliser form (García et al., 1997), while a similar study at the University of Utah found that a carbon-blended P fertiliser (unknown C source) could enhance fertiliser efficiency, crop yields and quality, and P uptake (Hill et al., 2015b). In a study, the application of coated urea fertilizer (unknown source) delayed urea release from the coted fertilisers and reduced ammonia gas loses (Guimarães et al., 2015). The recovery of plant phosphorus from P fertilisers at the first year of application is usually less than 20% due to immobility and P fixation by other soil particles. A study results have demonstrated that coating the monoammonium phosphate fertiliser with organic polymer increased the P release rate in the soil (Zhang et al., 2000). Bolan (1994) combined a low-molecular organic acid with P fertiliser into the soil and observed a longer presence of P in soil when it was applied with organic substrates in comparison with the P fertiliser without organic substances (Bolan et al., 1994). A new organic complexed superphosphate (CSP) has been developed to evaluate the P solubilisation. The organic substances have been extracted from peat and the complexed organic phosphate fertilisers has been provided in pellet form. The study results have shown that HS can promote P solubilisation and decrease P retention through

chemical and physical stabilisation mechanisms, thus resulting in more P availability in the soil (Erro et al., 2012).

Previous studies demonstrated the beneficial effect of a new type of organic-complexed mineral fertilisers mostly stem from the formation of phosphate – stable metal – humic acid bridge (Guardado et al., 2007,; Guardado et al., 2008,; Erro et al., 2011,; Erro et al.,2009). The NMR characterization study of a humic-metal-phosphate complex revealed the formation of metal – phosphate – humics occurs through chelating group of humic acids (Erro et al., 20110).

In this thesis a series of new organic phosphate complexed fertiliser with the name of humate-phosphate blended (HPB) fertilisers were investigated in laboratory and glasshouse conditions for phosphorus solubility, availability for plant uptake and plant growth indexes. The characteristics of humate-blended phosphate fertilisers which have been used in this study can be found at chapter 4.

1.2 Research Hypothesis, Objectives and overview

1.2.1 The research hypothesis

Does the application of triple superphosphate (TSP) fertiliser in combination with Victorian lignite coal-derived humate improve soil phosphorus availability and plant growth?

1.2.2 Research objectives

The research hypothesis will be tested through addressing the following objectives:

Sourcing commercial TSP and a lignite-derived humate for a preparation which will be used to prepare a series of humate-blended phosphate fertilisers with different proportions of carbon and phosphorus

Examination of the humate-blended fertilisers using X-ray fluorescence (XRF), carbon nitrogen and hydrogen (CHN) analyser, and inductively coupled plasma (ICP); conducting chemical evaluation of the blended humate-phosphate fertilisers through measurement of a range of parameters including: solubility and availability; evaluation of the effect of selected blended humate-phosphate fertilisers on plant growth in glasshouse conditions; and understanding the chemistry mechanism of the blended fertilisers in a laboratory study.

1.2.3 Thesis overview

This thesis prepares and investigates a new series of granular organic blended phosphate fertilisers both in laboratory and glasshouse conditions. The blended fertilisers have been manufactured for different total P content at a commercial manufacturer (Feeco International, Inc.).

The effect of the humate-blended fertilisers on phosphorus solubility, availability, diffusion and also on plant growth and P bioavailability both in laboratory and glasshouse was investigated in different Victorian soils.

The characteristics of soils and blended fertilisers are presented in Chapter 2. The blended fertilisers were examined for total P by XRF (X-Ray Fluorescence) and ICP (Inductively Coupled Plasma) and total C by CHN analysis.

In chapter 3 the humate-blended fertilisers are compared with triple superphosphate (TSP) on P solubility and availability in soil solution matrix over the time in three different soils. The highly weathered Ferrosol with highest response to the blended fertiliser was picked to conduct further studies.

Chapter 4 describes a diffusion study of different phosphate fertilisers conducted in different soils. In this section, the P diffusion distance from the fertiliser in soil is investigated for both humate-blended and TSP fertilisers.

Chapter 5 presents a glasshouse study. In this study, all humate-blended phosphate fertilisers as well as TSP were applied in two different Victorian soils with different P reserves under the controlled environment. The focus for this study was pasture growth (annual Ryegrass) indexes as well as pasture end of season yield.

Chapter 6 presents sequential glasshouse studies based on the results of the first glasshouse study in chapter 5. This study was conducted with one soil and two different plants. The first glasshouse outcome indicated that the rye grass was not the best plant to see the effect of the different P fertilisers. Radish and corn were chosen for the second round of the glasshouse research as these plants are considered to be sensitive to the lack of phosphate. Again, the plant growth indexes as well as plant yields were measured at

the end of the growth season. Soil with the highest response to the humate-blended fertilisers was used for this round.

Chapter 7 investigates how humate and phosphate fertilisers interact to improve the phosphorus availability in a soil solution incubation study involving the effect of several simple organic acids on P availability.

The key outcomes of all previous chapters are summarised in Chapter 8 and some directions for future research areas are suggested.

1.3 References

ATAFAR, Z., MESDAGHINIA, A., NOURI, J., HOMAEE, M., YUNESIAN, M.

AHMADIMOGHADDAM, M. & MAHVI, A. H. 2010. Effect of fertiliser application on soil heavy metal concentration. *Environmental monitoring and assessment*, 160, 83–89.

AZEEM, B., KUSHAARI, K., MAN, Z. B., BASIT, A. & THANH, T. H. 2014. Review on materials & methods to produce controlled release coated urea fertiliser. *Journal of Controlled Release*, 181, 11–21.

BAIGORRI, R., URRUTIA, O., ERRO, J., MANDADO, M., PÉREZ-JUSTE, I. & GARCIA-MINA, J. M. 2013. Structural Characterization of Anion–Calcium–Humate Complexes in Phosphate-based Fertilisers. *ChemSusChem*, 6, 1245–1251.

BEEK, J., and W. H. van RIEMSDIJK. 1977. Interaction of orthophosphate ions with soil. p. 259-284. In G. H. Bolt (ed.) *Soil chemistry. B. Physico-chemical models*. Elsevier, Amsterdam

BOLAN, N. S., NAIDU, R., MAHIMAIRAJA, S. & BASKARAN, S. 1994. Influence of low- Molecular-weight organic acids on the solubilization of phosphates. *Biology and Fertility of Soils*, 18, 311–319.

CHEN, Y. AND AVIAD, T., 1990. Effects of humic substances on plant growth 1. Humic substances in soil and crop sciences: Selected readings, (humic substances), 161-186.

CHEN, J., LÜ, S., ZHANG, Z., ZHAO, X., LI, X., NING, P. & LIU, M. 2018. Environmentally Friendly fertilisers: A review of materials used and their effects on the environment. *Science of the Total Environment*, 613, 829–839.

DHILLON, J., TORRES, G., DRIVER, E., FIGUEIREDO, B. & RAUN, W. R. 2017. World Phosphorus Use Efficiency in Cereal Crops. *Agronomy Journal*, 109, 1670–1677.

EI-MESKER HKA, MOHAMED ZEOM, ALI MAM (2014) Influence of humic acid and some micronutrients on yellow corn yield and quality. *World Appl Sci J* 32(1):1–11

ERRO, J., URRUTIA, O., BAIGORRI, R., APARICIO-TEJO, P., IRIGOYEN, I., STORINO, F., MANDADO, M., YVIN, J. C. & GARCIA-MINA, J. M. 2012. Organic Complexed Superphosphates (CSP): physicochemical characterization and agronomical properties. *J. Agric. Food Chem*, 60, 2008–2017.

ERRO, J., ZAMARRENO, A.M., GARCIA-MINA, J.M., YVIN, J.C. 2009. Comparison of different phosphorus-fertiliser matrices to induce the recovery of phosphorus-deficient maize plants. *Journal of*

Science of Food and Agriculture, 89, 927-934.

ERRO, J., BAIGORRI, R., YVIN, J.C., GARCIA-MINA, J.M. 2011. ³¹P NMR characterization and efficiency of new types of water-insoluble phosphate fertilizers to supply plant-available phosphorus in diverse soil types. *J Agric Food Chem.* 59, 1900-1908.

ERRO, J., O. URRUTIA, R. BAIGORRI, P. APARICIO-TEJO, I. IRIGOYEN, F.STORINO, M. MANDADO, J. C. YVIN, J.M. GARCIA-MINA. 2012. Organic Complexed Superphosphates (CSP): Physicochemical Characterization and Agronomical Properties. *J Agr. Food. Chem.*, 60, 2008–2017.

FERNÁNDEZ, L., BAIGORRI, R., URRUTIA, O., ERRO, J., APARICIO-TEJO, P., YVIN, J. & GARCÍA-MINA, J. 2016. Improving the short-term efficiency of rock phosphate-based fertilisers in pastures by using edaphic biostimulants. *Chemical and Biological Technologies in Agriculture*, 3, 1–9.

FOLEY, J. A., DEFRIES, R., ASNER, G. P., BARFORD, C., BONAN, G., CARPENTER, S. R., CHAPIN, F. S., COE, M. T., DAILY, G. C. & GIBBS, H. K. 2005. Global consequences of land use *Science*, 309, 570–574.

GARCÍA, M. C., VALLEJO, A., GARCIA, L. & CARTAGENA, M. C. 1997. Manufacture and evaluation of coated triple superphosphate fertilisers. *Industrial & engineering chemistry research*, 36, 869–873.

GERKE, J. 2010. Humic (organic matter)-Al (Fe)-phosphate complexes: an underestimated phosphate form in soils and source of plant-available phosphate. *Soil Science*, 175, 417–425.

GIOVANNINI, C., J. M. GARCIA-MINA, C. CIAVATTA, C. MARZADORI. 2013. Effect of organic-complexed superphosphates on microbial biomass and microbial activity of soil. *Biol Fertil Soils.*, 49:395–401.

GUARDADO, I., URRUTIA, O. and Garcia-Mina, J.M. (2007). Size distribution, complexing capacity and stability of phosphate-metal-humic complexes. *Journal of Agricultural and Food Chemistry*, 55, 408-413.

GUARDADO, I., URRUTIA, O. and GARCIA-MINA, J.M. (2008). Some structural and electronic features of the interaction of phosphate with metal-humic complexes. *Journal of Agricultural and Food Chemistry*, 56, 1035-1042.

GUIMARAES, G. F., PAIVA, D. M., CANTARUTTI, R. B., MATTIELLO, E. M., & REIS, E. L. (2015).

Volatilization of Ammonia Originating from Urea Treated with Oxidized Charcoal. *Journal of the Brazilian Chemical Society*, 26(9), 1928-1935.

HARRISON, A.F. 1978. Phosphorus cycles of forest and upland grassland ecosystems and some effects of land management practices. In: *Ciba Foundation Symposium 57. Phosphorus in the Environment: its Chemistry and Biochemistry* (eds R. Porter & D.W. Fitzsimons), 175–193. Elsevier, Amsterdam.

HAYES, M.H. and SWIFT, R.S., 1978. The chemistry of soil organic colloids. *The chemistry of soil constituents*, 179-320.

HERRERA, B., FERNEY, W., RODRIGUES, M., BETTONI TELES, A. P., BARTH, G. & PAVINATO, P. S. 2016. Crop Yields and Soil Phosphorus Lability under Soluble and Humic- Complexed Phosphate Fertilisers. *Agronomy Journal*, 108, 1692–1702.

HILL, M. W., HOPKINS, B. G., JOLLEY, V. D. & WEBB, B. L. 2015. Phosphorus Mobility Through Soil Increased with Organic Acid-Bonded Phosphorus Fertiliser (Carbond® P). *Journal of Plant Nutrition*, 38, 1416–1426.

HINGSTON, F. J. 1981. A review of anion adsorption. p. 51-90. In M. A. Anderson, and A. J. Rubin (ed.) *Adsorption of inorganics at solid-liquid interfaces*. Ann Arbor Science, Ann Arbor

JU, X. T., KOU, C. L., CHRISTIE, P., DOU, Z. & ZHANG, F. 2007. Changes in the soil environment from excessive application of fertilisers and manures to two contrasting intensive cropping systems on the North China Plain. *Environmental Pollution*, 145, 497–506.

LOOKMAN, R., FREESE, D., MERCKX, R., VLASSAK, K. & VAN RIEMSDIJK, W. H. 1995. Long-Term Kinetics of Phosphate Release from Soil. *Environmental Science & Technology*, 29, 1569–1575.

LÜ, S., GAO, C., WANG, X., XU, X., BAI, X., GAO, N., FENG, C., WEI, Y., WU, L. & LIU, M. 2014. Synthesis of a starch derivative and its application in fertiliser for slow nutrient release and water-holding. *RSC Advances*, 4, 51208–51214.

MCLAUGHLIN, M. J., McBeath, T. M., Smernik, R. J., Stacey, S. P., Ajiboye, B., Guppy, C. N. (2011): The chemical nature of P accumulation in agricultural soils—implications for fertiliser management and design: an Australian perspective. *Plant Soil* 349, 69–87.

MCLAREN, T. I., SIMPSON, R. J., MCLAUGHLIN, M. J., SMERNIK, R. J., MCBEATH, T. M., GUPPY,

C. N. and RICHARDSON, A. E. (2015a): An assessment of various measures of soil P and the net accumulation of P in fertilized soils under pasture. *J. Plant Nutr. Soil Sci.* 178, 543–554

MOTT, C. J. B. 1981. Anion and ligand exchange, p.179-219. In D. J. Greenland, and M. H. B. Hayes (ed.) *Chemistry of soil processes*. John Wiley and Sons, Inc., New York

NAZ, M. Y. & SULAIMAN, S. A. 2016. Slow release coating remedy for nitrogen loss from conventional urea: a review. *Journal of Controlled Release*, 225, 109–120.

OLK, D.C., DINNES, D.L., SCORESBY, J.R., CALLAWAY, C.R. and DARLINGTON, J.W., 2018. Humic products in agriculture: potential benefits and research challenges—a review. *Journal of Soils and Sediments*, 1-11.

OLK DC, DINNES DL, CALLAWAY C, RASKE M (2013) On-farm evaluation of a humic product in Iowa (US) maize production. In: Xu J et al (eds) *Functions of natural organic matter in changing Environment*. Zhejiang University Press and Springer Science+Business Media, Dordrecht, 1047–1050.

PARFITT, R. L. 1978. Anion adsorption by soils and soil materials. *Adv. Agron.* 30:1-50

PERROTT, K.W., 1978. The influence of organic matter extracted from humified clover on the properties of amorphous aluminosilicates. II. Phosphate retention. *Soil Research*, 16(3), 341-346.

PETTIT, R. E. 2004. *Organic matter, humus, humate, humic acid, fulvic acid and humin: Their importance in soil fertility and plant health*. CTI Research.

PETTIT, R. E. 2006. *Organic matter, humus, humate, humic acid, fulvic acid, and humin. The Wonderful World of Humus and Carbon*.

PICCOLO, A., 2001. The supramolecular structure of humic substances. 2001. *Soil Science*: 166(11), 810-832

PICCOLO, A., 2002. The supramolecular structure of humic substances: a novel understanding of humus chemistry and implications in soil science. *Advances in Agronomy*, 75, 57-13

PICCOLO, A. and SPITELLER, M., 2003. Electrospray ionization mass spectrometry of terrestrial humic substances and their size fractions. *Analytical and bioanalytical chemistry*, 377(6), 1047-1059.

SCHNEIDER T. A., DELADINO, L. & ZARITZKY, N. 2016. Yerba mate (*Ilex paraguariensis*) waste and alginate as a matrix for the encapsulation of N fertiliser. *ACS Sustainable Chemistry & Engineering*, 4, 2449–2458.

SCHOUMANS, O. 1995. Description and validation of the process formulation of abiotic phosphate reactions in acid sandy soils. (Dutch.) Rep.

SEYEDBAGHERI M.M (2010) Influence of humic products on soil health and potato production. *Potato Res* 53(4):341–349.

SPOSITO, G. 1984. *The surface chemistry of soils*. Oxford University Press, New York. STEELINK, C., AIKEN, G.R., MCKNIGHT, D.M., WERSHAW, R.L., MACCATHY P. *Elemental Characteristics of Humic Substances, Humic Substances in Soil, Sediment, and Water*, Wiley (1985), 457-476

SUMMERHAYS, J. S., HOPKINS, B. G., JOLLEY, V. D., HILL, M. W., RANSOM, C. J. & BROWN, T. R. 2015. Enhanced phosphorus fertiliser (Carbond P®) supplied to maize in moderate and high organic matter soils. *Journal of Plant Nutrition*, 38, 1359–1371.

URRUTIA, O., GUARDADO, I., ERRO, J.M., MANDADO, J.M. GARCIA-MINA. 2013. Theoretical chemical Characterization of phospho-metal–humic complexes and relationships with their effects on both phosphorus soil fixation and phosphorus availability for plants. *J Sci Food Agr*. 93: 293–303.

URRUTIA, O., ERRO, J.M., GUARDADO, I., SAN FRANCISCO, S., Mandado, M., BAIGORRI, R., Yvin, J.C. and Garcia-Mina, J.M. (2013/2014) Physico-chemical characterization of humic-metal-phosphate complexes and their potential application to the manufacture of new types of phosphate-based fertilizers. *Journal of Plant Nutrition and Soil Science*.

VERLINDEN, G., PYCKE, B., MERTENS, J., DEBERSAQUES, F., VERHEYEN, K., BAERT, G., BRIES, J. and HAESAERT, G. (2009) Application of humic substances results in consistent increases in crop yield and nutrient uptake. *J Plant Nutr* 32(9):1407–1426.

ZHANG, M., NYBORG, M., MALHI, S., MCKENZIE, R. & SOLBERG, E. 2000. Phosphorus release from coated monoammonium phosphate: effect of coating thickness, temperature, elution medium, soil moisture and placement method. *Canadian Journal of Soil Science*, 80, 127–134.

Chapter 2

Production and Characterization of granular humate-phosphate fertilisers

2.1 Introduction

It has been widely reported that the commercial lignite-derived materials promote plant nutrient availability and uptake. As previously described, brown coal or lignite derived products have been introduced to farmers as the plant growth promoter for years. There are some other lignite related materials which are manufactured based on lignite extraction. Most of them are alkaline extraction of lignite and are highly soluble in water. The product which has been used in this thesis is the potassium (K) salt of humic (HA) acid derived from lignite coal which from here on in, for the purpose of clarity, it will be referred K-humate. The process of extraction, usually involves increasing the temperature of an alkaline digest that causes some breakdown in the chemical structure of lignite, as well as some oxidation of the lignite structure itself (Durie, 2013). Highly oxidised lignite have also been utilised and one such common material sourced in the USA is leonardite, which is a mixture humic and fulvic acids (Harrell Jr and Saeed, 1977). The humate products are supplied in many different forms, and are often mixtures involving other organic materials (eg sea-weed extract and fortified with essential plant nutrients).

The humate mixtures also consist of a wide range of peptides, phenols, aldehydes, and nucleic acids combined with other cations (Weber, 2008). Their beneficial effects on plant growth and efficiency in the delivery of plant have been widely reported (Albayrak and Camas, 2005, Danyaei et al., 2017, Haider et al., 2017). The application of K-humate was also shown to significantly improve mean weight-diameter of soil aggregates (Imbufe et al., 2005).

Usually, the humate products are available in markets in the form of liquid, powder or pallet. Although the forms are different, the main ingredient is humic substances. The incorporation of humic materials with conventional fertilisers has been gaining attention. This approach provides “controlled release “fertilisers that are prepared from granular fertilisers coated with different organic materials such as humates and synthetic polymers (Han et al., 2009)

Granular fertilisers combining conventional P fertilisers with humified organic matter represent another new generation of fertilisers. Although many studies have shown the beneficial effects of the use of organic, or the organic matter derived materials on plant growth, fewer studies have been done on BC- derived material blended with mineral fertilisers. The interaction between organic matter and mineral fertilisers (in this study triple superphosphate) can be useful in providing more efficient fertilisers.

Among phosphorus (P) fertilisers, triple superphosphate (TSP), is a highly concentrated P fertiliser with 46% soluble P which is chemically expressed as diphosphorus pentoxide (P_2O_5). It can be used in a wide range of soil with different pH. Because of its high water-solubility character it generally satisfies the plants' P needs. TSP can accelerate the growth of young plants root system, florescence and ripening of the fruits. TSP has been chosen to be combined with K-humate in the manufacture humate blended phosphate granules as it is the simplest form of P fertilisers. In addition, the higher P content of TSP enabled the production of blended granules with a range of P contents while still containing a substantial quantity of P overall.

Conditioner produced from Victorian brown coal, (commercially known as "K-humate") was used as the organic matter source in this study. K-humate contains about 60% humic acid to provide both short term and sustained benefits (<http://www.australianhumates.com.au/>, accessed April 2018).

Making the granular humate- blended phosphate fertiliser and its characterization have been described in this chapter..

2.2 Methods and material

The initial approach for the fertilizer manufacture was to coat the superphosphate fertilizers with an alkaline extract of Victorian brown coal. Different coating methods have been employed to make the products. The granular K-humate was supplied from Omnia Company (<http://www.australianhumates.com.au/>, accessed April 2018). The granular TSP in this study was supplied by Elders Agribusiness Company, Australia (elders.com.au).

Basic characteristics of commercial K-humate and TSP are shown in Table 1.

Table 2.1 Basic measurements of K-humate

Product name (Manufacturer)	Original C source	Water solubility	%C	%H	%N	%P	%K	%Al	pH
K-humate	Victorian brown coal	>95%	47.4	4.19	1.32	0.02	8.5	1	9.4
Triple superphosphate	Phosphate rocks	>90%	0		0	17%	0	0	1-3

2.2.1 Laboratory scale - Coating fertilisers by drum

With the aim of obtaining a humate coated phosphate fertiliser, coating the granular superphosphate fertilisers has been done in laboratory scale. In this method granular TSP were sprayed with diluted K-humate in a drum at 2:1 ratio at the chemical engineering department laboratory. Diluted K-humate was sprayed on fertiliser in a rotating drum. However, the results were not favourable – the coated fertilisers were shattered in the process of separation, the breaks exposing fertiliser; in addition wholly uncoated surfaces were also apparent (Fig 2.1).



Figure 2.1 Rotating Drum Coating granules

2.2.2 Coating fertilisers by spraying

In this method, diluted K-humate was sprayed on TSP at ratio of 1:2 and then left to dry. The same problem occurred, involving strong adhesion of the humate particles to each other. As a result particles shattered when separating from the plate (Fig 2.2).

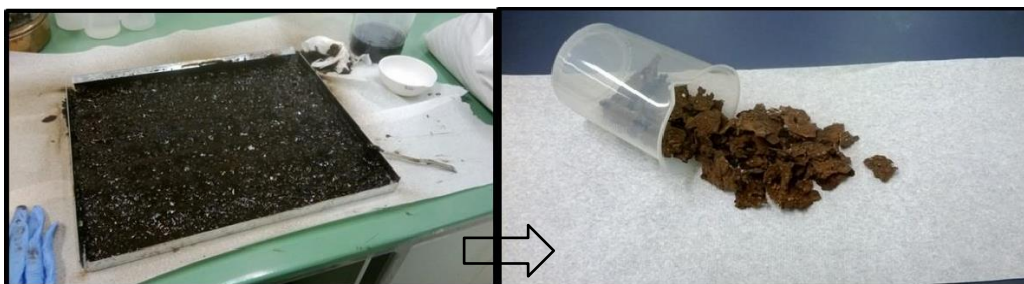


Figure 2.2 Unsuccessful coated fertilisers by spraying

2.3 Results

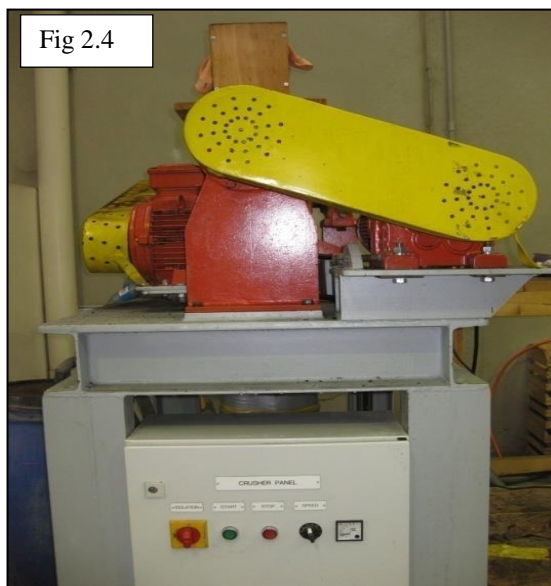
As a result of unsuccessful coating procedure that were based on available facilities, the procedure of preparation of new products changed to blended fertilisers and these humate-blended fertilisers have been made commercially. In brief, the process of manufacturing includes:

First, the granular TSP and K-humate have been ground and crushed with the crusher to reach a homogenised fine texture for both. They mixed in special blender while molasses 3% was sprayed on their surface. The blender shot the new blended granule in a right size. At the end the new granules were dried at 180 to 200 °C in a rotary drum evaporator. The final products have enough stability and are in same size range as phosphate granule fertilisers (Figures 2.4 to 2.8). To obtain similar control product, the common granule TSP as control fertilisers grinded and re- granulate again. The Humate-blended fertilises have been analysed for %P with X-ray fractionation technique (by the Victorian centre for sustainable chemical manufacturing laboratory, Monash University, <http://www.vcscm.org/>, accessed December 2017). They also have been analysed for and carbon (C), hydrogen (H) and nitrogen (N) element with CHN analyser (by the school of chemistry laboratory, Monash University, <https://www.monash.edu/science/schools/chemistry>, accessed December 2017). (Table 2.2).

Table 2.2 Phosphate and carbon percentage of blended fertilisers

Fertiliser	ingredients	%P ^a	%C ^b	%H ^b	%N ^b
TSP	Triple superphosphate	17.47	2.74	2.58	0.60
HBP 1	TSP+ K-humate	15.57	7.02	2.62	0.91
HBP 2	TSP+ K-humate	14.59	12.7	2.61	0.85
HBP 3	TSP+ K-humate	12.94	16.6	2.79	0.83
HBP 4	TSP+ K-humate	7.12	30.26	3.20	1.22

a) Total elemental phosphorus by X-Ray fractionation, b) Total C, H and N by CHN analyser



Figures 2.4 to 2.8 the granular humate- blended phosphate (HBP) fertilisers

2.4 References

ALBAYRAK, S. & CAMAS, N. 2005. Effects of different levels and application times of humic acid on root and leaf yield and yield components of forage turnip (*Brassica rapa* L.). *Journal of Agronomy*.

DANYAEI, A., HASSANPOUR, S., BAGHAEE, M. A., DABBAGH, M. & BABARABIE, M. 2017. The Effect of Sulfur-Containing Humic Acid on Yield and Nutrient Uptake in Olive Fruit. *Journal of Ecology*, 7, 279–288.

DURIE, R. 2013. *The science of Victorian brown coal: structure, properties and consequences for utilization*, Butterworth-Heinemann.

EL-HAMADY, M. M., BADDOUR, A. G., SOBH, M. M., ASHOUR, H. M. & MANAF, H. H. 2017. Influence of Mineral Fertilisation in Combination with K-humate, Amino Acids and Sodium Selenite on Growth, Chemical Composition, Yield and Fruit Quality of Sweet Pepper Plant. *Middle East J*, 6, 433–447.

ERRO, J., URRUTIA, O., BAIGORRI, R., FUENTES, M., ZAMARREÑO, A. & GARCIA-MINA, J. 2016. Incorporation of humic-derived active molecules into compound NPK granulated fertilisers: main technical difficulties and potential solutions. *Chemical and Biological Technologies in Agriculture*, 3, 1–15.

HAIDER, N., ALAM, M., MUHAMMAD, H., GUL, I., HAQ, S. U., HUSSAIN, S. & RAB, A. 2017. Effect of humic acid on growth and productivity of okra (*Abelmoschus esculentus*) cultivars. *Pure and Applied Biology*, 6, 932–941.

HAN, X., CHEN, S. & HU, X. 2009. Controlled-release fertiliser encapsulated by starch/polyvinyl alcohol coating. *Desalination*, 240, 21–26.

IHESHIULO, E. M.-A., ABBEY, L. & ASIYEDU, S. K. 2017. Response of Kale to Single-Dose Application of K Humate, Dry Vermicasts, and Volcanic Minerals. *International Journal of Vegetable Science*, 23, 135–144.

IMBUFE, A. U., PATTI, A. F., BURROW, D., SURAPANENI, A., JACKSON, W. R. & MILNER, A. D. 2005. Effects of potassium humate on aggregate stability of two soils from Victoria, Australia. *Geoderma*, 125, 321–330.

JONES, C. A., JACOBSEN, J. S. & MUGAAS, A. 2007. Effect of Low-Rate Commercial Humic Acid on Phosphorus Availability, Micronutrient Uptake, and Spring Wheat Yield. *Communications in soil science and plant analysis*, 38, 921–933.

KOVÁČIK, P., ŽOFAJOVÁ, A., ŠIMANSKÝ, V. & HALÁSZOVÁ, K. 2016. Spring Barley Yield Parameters after Lignite, Sodium Humate and Nitrogen Utilization. *Agriculture (Polnohospodárstvo)*, 62, 80–89.

LITTLE, K. R., ROSE, M. T., JACKSON, W. R., CAVAGNARO, T. R. & PATTI, A. F. 2014. Do lignite-derived organic amendments improve early-stage pasture growth and key soil biological and physicochemical properties? *Crop and Pasture Science*, 65, 899–910.

LODHI, A., TAHIR, S., IQBAL, Z., MAHMOOD, A., AKHTAR, M., QURESHI, T. M., YAQUB, M. & NAEEM, A. 2013. Characterization of commercial humic acid samples and their impact on growth of fungi and plants. *Pakistan journal of soil science*, 32, 63–70.

MICIC, V., SCHMID, D., BOSSA, N., GONDIKAS, A., VELIMIROVIC, M., VON DER KAMMER, F., WIESNER, M. R. & HOFMANN, T. 2017. Impact of Sodium Humate Coating on Collector Surfaces on Deposition of Polymer-Coated Nanoiron Particles. *Environmental science & technology*, 51, 9202–9209.

Chapter 3

Evaluation of a new generation of organic blended phosphate fertilisers: phosphorus availability in a batch equilibrium study

Abstract

Although the effects of humic substances on soil nutrient availability have been widely studied, most investigations have focused on them as soil amendments in order to improve soil properties. As a means of innovative application of humic substances, different quantities of phosphorus (P) supplied as triple superphosphate (TSP) were blended with a lignite-derived humate to prepare blended fertilisers with different carbon (C) and phosphorus contents in this study. The solubility (availability) of the P in the humate-blended phosphate (HBP) fertilisers was compared with standard triple superphosphate (TSP) in two acidic soils: a highly-P fixing Ferrosol and a Podosol. Humate-blended fertiliser with 7.0% organic C content (HBP1) maintained higher concentrations of soluble P than TSP in the Ferrosol, while no significant differences in s P were observed between HBP and TSP fertilisers in the Podosol. At the end of the experiment (15 days), the post-incubated Colwell (available) P was also significantly higher in HBP1 in the Ferrosol. The results indicated that addition of humate into phosphate fertiliser could improve the plant-available P in high P-fixing acidic soils.

Keywords: Humic substances, brown coal, phosphorus, availability, humate-blended

3.1 Introduction

Chemical phosphate fertilisers are often used to make up for the lack of plant-available P in the soil (Herrera et al., 2016). Superphosphates (SP) are the most commonly used phosphate fertiliser in agriculture, particularly single superphosphate (SSP), while triple superphosphate (TSP), mono-ammonium phosphate (MAP) and di-ammonium phosphate (DAP) are also common. However, the P- use efficiency of applied fertilisers in agriculture is low (Dawson and Hilton, 2011). Their efficacy depends strongly on the phosphorus buffering capacity (PBC) of the soil, which is a measure of the soil's capacity to bind applied phosphorus and reduce the P concentration in the soil solution (Sui and Thompson, 2000). Managing phosphorus fertilisers is particularly challenging in highly-weathered soils with high "P-fixing" capacity, such as those high in aluminium and iron (hydr) oxides (Hinsinger 2001; Raghothama and Karthikeyan 2005). Usually, these types of soils need a higher application of phosphate fertilisers to optimize the plants needs of phosphate.

In order to solve these issues much research has focused on increasing P-use efficiency and reducing P losses to waterways (Dawson and Hilton, 2011, Rowe et al., 2016). Of particular interest is the use of organic matter to improve P-use efficiency. A number of studies have reported that naturally derived organic matter enhances P availability in soils (Iyamuremye and Dick, 1996) (Haynes and Mokolobate, 2001) and furthermore, that the application of organic matter to soil can decrease P sorption (Scheffe et al., 2008, Wang et al., 1995, Diez et al., 1992). Because the availability of organic amendments is low, and transport costs are high, the practicality of using high rates of bulk organic wastes is logistically and economically challenging.

In order to overcome these limitations, research has focused on the ‘active’ fractions of organic matter responsible for improving P-availability in soil. This includes the alkaline extract of organic matter, commonly known as humates, which are being widely used to promote nutrients uptake and plant growth (Cao et al., 2016, Fong et al., 2006, Schindler et al., 2016). Three main mechanisms by which humic substances can promote P-availability have been proposed: 1) they can provide binding sites for Al/Fe and Ca in acidic and alkaline soils respectively, 2) by chelating Al, Fe and Ca via complex formation, and 3) increasing the solubility of Al, Fe and Ca bonded to the phosphorus (Bolan et al., 1994). (Giovannini et al., 2013) demonstrated that peat-extracted humate complexed with superphosphate fertiliser could maintain the available phosphorus about 73% more than superphosphate itself in an incubation study. In another study, the concentration of soil water extracted-P increased with increasing amounts of humic substances in the soil slurries (Quan-Xian et al., 2008). Sharif et al. (2002) showed that the concentration of available phosphorus increased significantly by adding 200 mg/kg humic acid into silty clay soil. Addition of humic substances to soil along with triple superphosphate significantly increased the available phosphorus as well as crop P uptake (Summerhays et al., 2015), (Wang et al., 1995), (Hill et al., 2015a, Hill et al., 2015b). Another study showed that the available phosphorus as well as potato yield was increased by adding humates into the soil. The results showed that complexing ions into stable compounds which allows phosphorus to remain exchangeable for plant uptake from 11.4% to 22.3% (Seyedbagheri, 2010).

In Victoria, Australia, a number of soil types used for agriculture are highly P-fixing. These include the ferrosols and the Vertosol (Isbell, 2002). The first Ferrosol is high in Fe and often require high P-fertiliser inputs (Gérard, 2016, Harris, 2016) for the productive potato, dairy and horticultural industries that are predominant and Vertosol is high in Ca ions and usually fix the soil phosphorus by making Ca-P compounds (Grossl and Inskeep, 1991) (Department of

Economic Development, Jobs, Transport and Resources, 2017). Victoria also contains large reserves of lignite (brown coal) which are used as a commercial source of humic substances for application in agriculture (Chen et al., 2015). In this study, we have hypothesized that plant-available P can be maintained at a higher concentration and longer duration in highly weathered soil by blending conventional triple superphosphate (TSP) with lignite-derived humic substances. The aim of this study was to investigate the effect of lignite derived humate on different soil phosphorus availability via soil slurry experiment laboratory conditions.

3.2 Methods and Materials

3.2.1 Soils

The soils used in this study were classified as a Ferrosol (Nitisol based on FAO classification), Podosol (Podozols based on FAO classification) and Calcarosol (Calcareous based on FAO classification) based on the Australian Soil Classification (Isbell, 2002). All soils were collected from experimental fields of Department of Economic Development, Jobs, Transport and Resources (DEDJTR), Victoria, Australia. The Ferrosol was collected from dairy pasture at Ellinbank in West Gippsland, Victoria (38° 14'06''S lat. 145°55'26''E long). The Podosol was sourced from a vegetable farm in Cranbourne, Victoria (38° 11'6''S lat. 145°18'50''E long) and Calcarosol was collected from a wheat field at the Mallee Research Institute, Victoria (34° 58'0.16''S lat. 142°20'45.85''E long). All the topsoils (0-15 cm) collected were air dried and sieved to < 2 mm. All soils were analyzed for a range of key physicochemical properties (Table) (Environmental Analysis Laboratory, Southern Cross University, Lismore, NSW: <http://scu.edu.au/eal/>; accessed March 2017).

Table 3.1 Soils characterization

Soil type	pH (1:5) water	Location	OM w/w	Colwell P mg/kg	Al mg/kg	Fe mg/kg	Ammonium mg/kg	Nitrate mg/kg	texture
Ferrosol	5.3	Ellinbank	13.3%	73	61	191	62	28.3	Loam
Podosol	5.4	Cranbourne	2.1%	31	19	122	3.6	2.7	Sandy
Calcarosol	6.8	Ouyen	0.2%	22	1	7.8	1.5	2.2	Sandy

3.2.2 Humate blended phosphate (HBP) fertilisers

The humic substance used in this study is a commercially available solid granular potassium humate product derived from Victorian brown coal via alkaline extraction (Table 2). Humate blended phosphate (HBP) fertiliser granules were synthesized with different P and C percentages (Table 3) with the assistance of Feeco International (Pakenham, Australia). In order to characterize the HBP fertilisers, P was measured by X-Ray fluorescence at the Victorian Centre for Sustainable Chemical Manufacturing (VCSCM) and total C, H and N were measured by combustion using a high-frequency induction furnace CHN analyser. (Vario Micro Cube).

Table 3.2 Lignite-derived humate characterization

	C (%)	H (%)	N (%)	S (%)	P (%)	pH (1:5)water
Lignite- derived humate (K-humate)	47.4	4.19	1.32	0.33	0.02	10

Table 3.3 Blended fertiliser's characteristics

Fertiliser	Components	%P ^a	%C ^b
TSP	Triple superphosphate	17.4	2.7
HBP 1	TSP+Humate	15.5	7.0
HBP 2	TSP+Humate	14.5	12.7
HBP 3	TSP+Humate	12.9	16.6
HBP 4	TSP+Humate	7.1	30.2

^{a)} Total elemental P by X-ray fluorescence, ^{b)} Total C by dry combustion 1

3.2.3 Solubility experiment

To evaluate the P solubility of the granular HBP fertilisers, amounts equivalent to 10 mg P from different granular fertilisers were weighed into 50 ml polypropylene centrifuge tubes (Falcon brand, VWR International Pty Ltd) before addition of 50 mL of deionised water with five replications for each time. The samples were incubated for 20 days and shaken manually every day. A set of sample set were prepared for each time. The aqueous samples were taken on days 1, 3, 8, 15 and 20 days. Samples then filtered (Whatman No. 42) followed by Minisart® syringe filtered (0.45 µm) (Sartorius Stedim Australia Pty Ltd.) to recover fertiliser pellets and then kept in a freezer before analysis. A set of samples were prepared for each time of sampling to avoid sample destruction. Soluble P_i was measured by the ammonium molybdate method (Murphy and Riley, 1962) and the solution pH was measured directly using a TPS WP81 meter and probe (TPS Pty Ltd, Springwood, QLD).

3.2.4 Soil slurry experimental set up

A batch equilibration study was undertaken to evaluate the phosphorus availability in soil solution in three different soils of Ferrosol, Podosol and Calcarosol. Sieved soil (5 g) was

incubated with deionised water (25 mL) in a 50 ml centrifuge tube and left overnight to equilibrate at room temperature.

At the beginning of the experiment fertiliser equivalent to 10 mg P per 5 gr soil was added to the soil slurry solution from each HBP fertiliser, replicated four times. The study lasted 15 days and non-destructive samples were taken at 2, 7 and 15 days after adding the fertiliser to the soil. The mixtures were shaken at 125 rpm for 1h before sampling on a rotary shaker at room temperature and allowed to settle before sampling. Then, the mixture was filtered in two steps: firstly through a 42 Whatman filter paper followed by a Minis art[®] syringe filter (0.45 μ m) (Sartorius Stedim Australia Pty Ltd.) to recover soil and fertiliser pellets. The supernatants were analysed for water-extractable P (Murphy and Riley, 1962). Also the post incubated soil of Ferrosol treatments were air-dried and analysed for Colwell P (Rayment and Lyons, 2011) and pH (Rayment and Lyons, 2011).

3.2.5 Statistical Analysis

All data was analysed using JMP statistical software (JMP[®], Version 14; SAS Institute Inc., Cary, NC, USA). A Two-way ANOVA was used to investigate the effect of HBP fertilisers and time on soils water-extracted P and post-harvest available P. Tukey's honestly significant difference (HSD) was used where significant differences were found between different treatments.

3.3 Results

3.3.1 Blended fertilisers solubility

The solubility of phosphorus from humate-blended fertilisers and TSP followed a similar pattern over time (Figure 1). There was a decrease in P solubility at three days after addition of fertilisers to the solution followed by an increase of soluble P at the end of the study. There were no significant difference ($P < 0.05$) in soluble P between TSP and HBPs fertilisers.

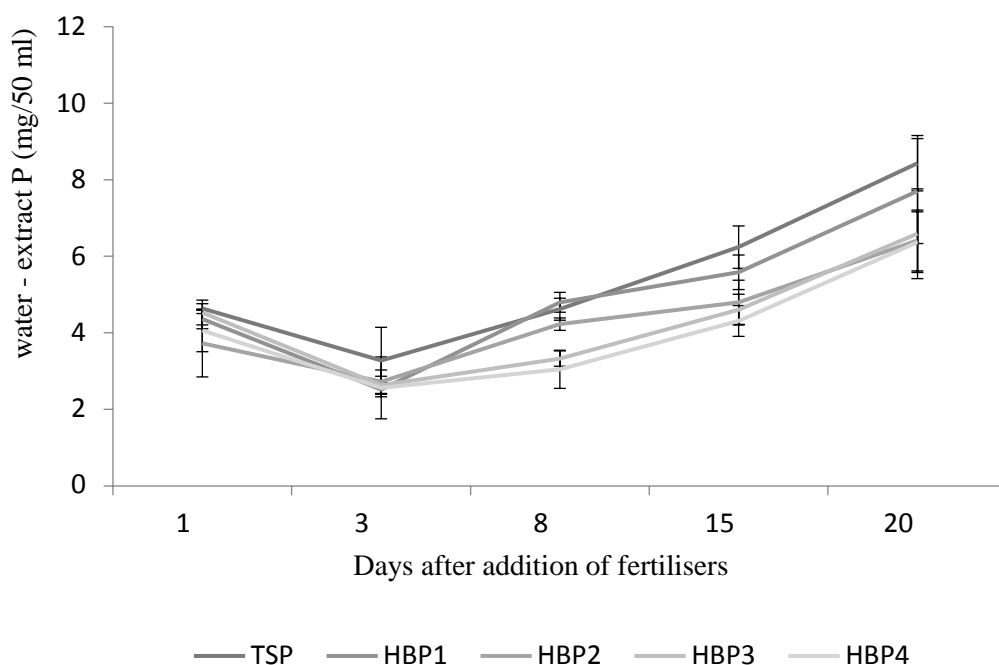


Figure 3.1 Water Soluble Phosphorus of TSP and humate-blended fertilisers

The pH of all fertiliser solutions was consistent over time (Figure 2). The solution pH of fertilisers HBP3 and HBP4, containing the highest humate content were higher, but did not change over the time course of this experiment.

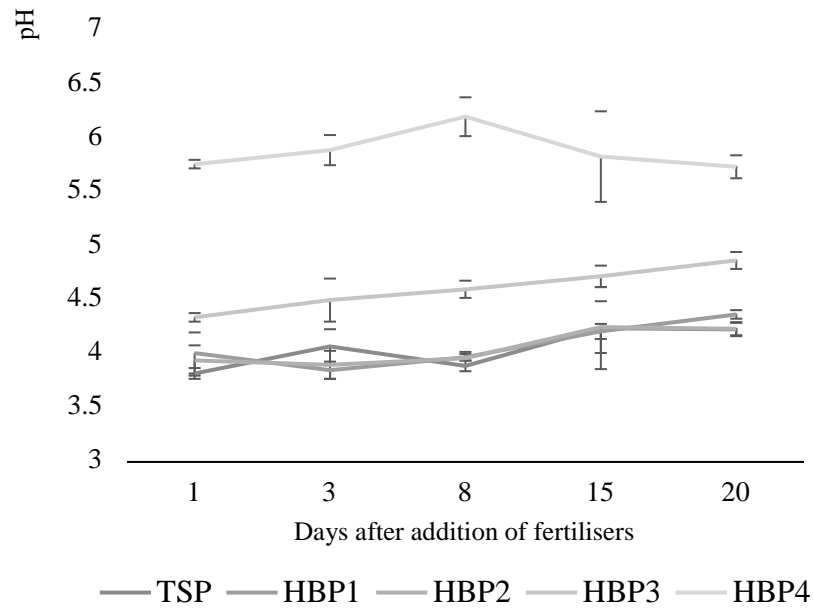


Figure 3.2 The pH of the solutions over the time

3.3.2 Water-extracted P (WEP) in soils slurries

3.3.2.1 Ferrosol

Data analysis by ANOVA demonstrated a significant effect ($p < 0.05$) of HBPs fertilisers as well as significant interaction effect between time and fertilisers on WEP in Ferrosol. However, time did not show a significant effect on WEP. Application of HBP1 significantly increased WEP with 21.1 mg/L and HBP4 with 7.5 mg/L showed the lowest available P in soil solutions (Figure 3.3)

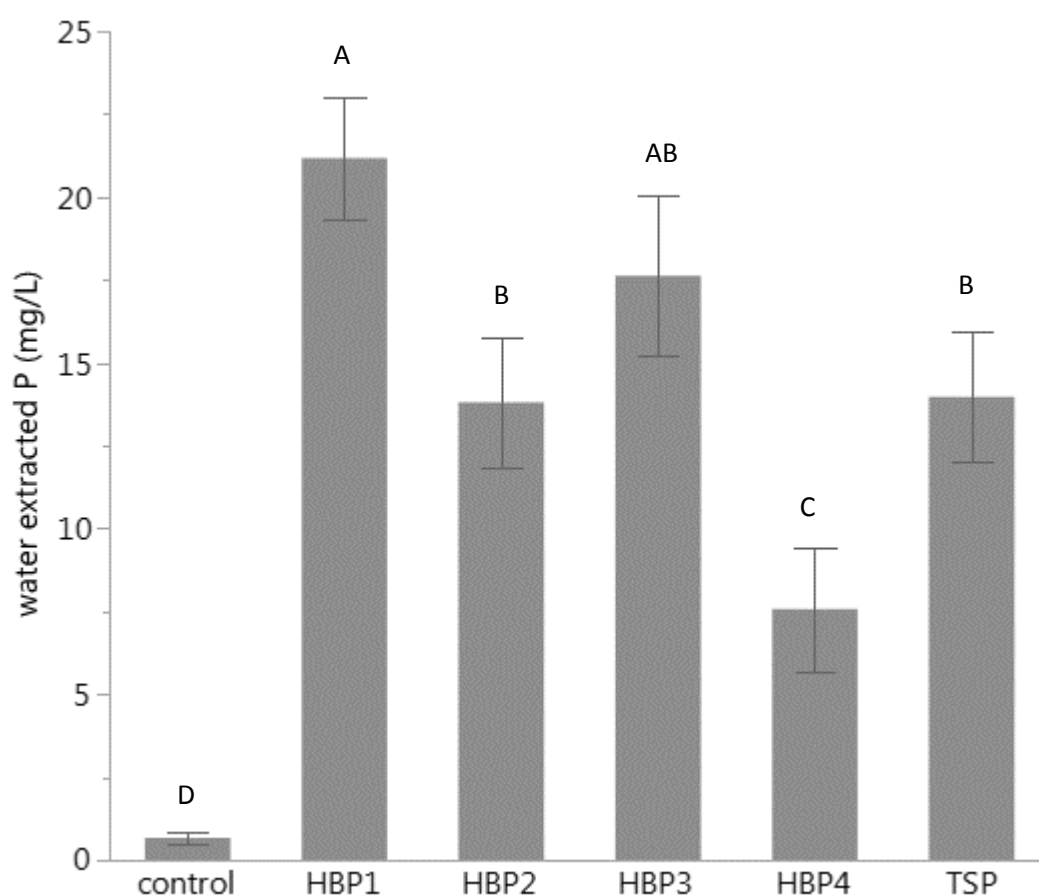


Figure 3.3 Effect of different fertilisers on water-extracted P in Ferrosol. Mean values are presented ($n=4$) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

. ANOVA showed the interaction of time and fertiliser is significant ($p < 0.05$). The highest water-extractable P was observed in HBP1 at 7 days after addition of fertilisers with 27.9 mg/L and the lowest water-extractable P was observed in control treatments at 2, 7 and 15 days after addition of the fertilisers into the solution with 0.8, 0.7 and 0.4 mg/L, respectively (Table 3.4).

Table 3.4 The effect of the interaction between time and different fertilisers on water-extract P. Mean values are presented (n=4). Numbers in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

Fertilisers	Time	Mean
HBP1	7	27.9 (2.3) A
HBP3	15	26.7 (2.3) AB
HBP1	2	20.7 (2.3) ABC
HBP2	7	16.6 (2.3) ABC
HBP3	2	16.3 (2.3) ABC
TSP	2	15.7 (2.3) ABCD
HBP2	2	15.6 (2.3) ABCDE
HBP4	2	15.5 (2.3) BCDE
HBP1	15	14.8 (2.3) BCDE
TSP	15	14.5 (2.3) BCDE
TSP	7	11.6 (2.3) CDEF
HBP3	7	9.7 (2.3) CDEF
HBP2	15	9.1 (2.3) CDEF
HBP4	15	3.7 (2.3) DEF
HBP4	7	3.3 (2.3) EF
Control	2	0.8 (2.3) F
Control	7	0.7 (2.3) F
Control	15	0.4 (2.3) F

3.3.2.2 Podosol

Although application of HBP1 and HBP3 resulted in higher WEP with 94.6 and 93.4 mg P/L, in comparison with TSP with 90.3 mg P/L, however, ANOVA did not show this higher result to be significant (Figure 3.4). Application of HBP4 showed the lowest WEP with 84.3 mg P/L.

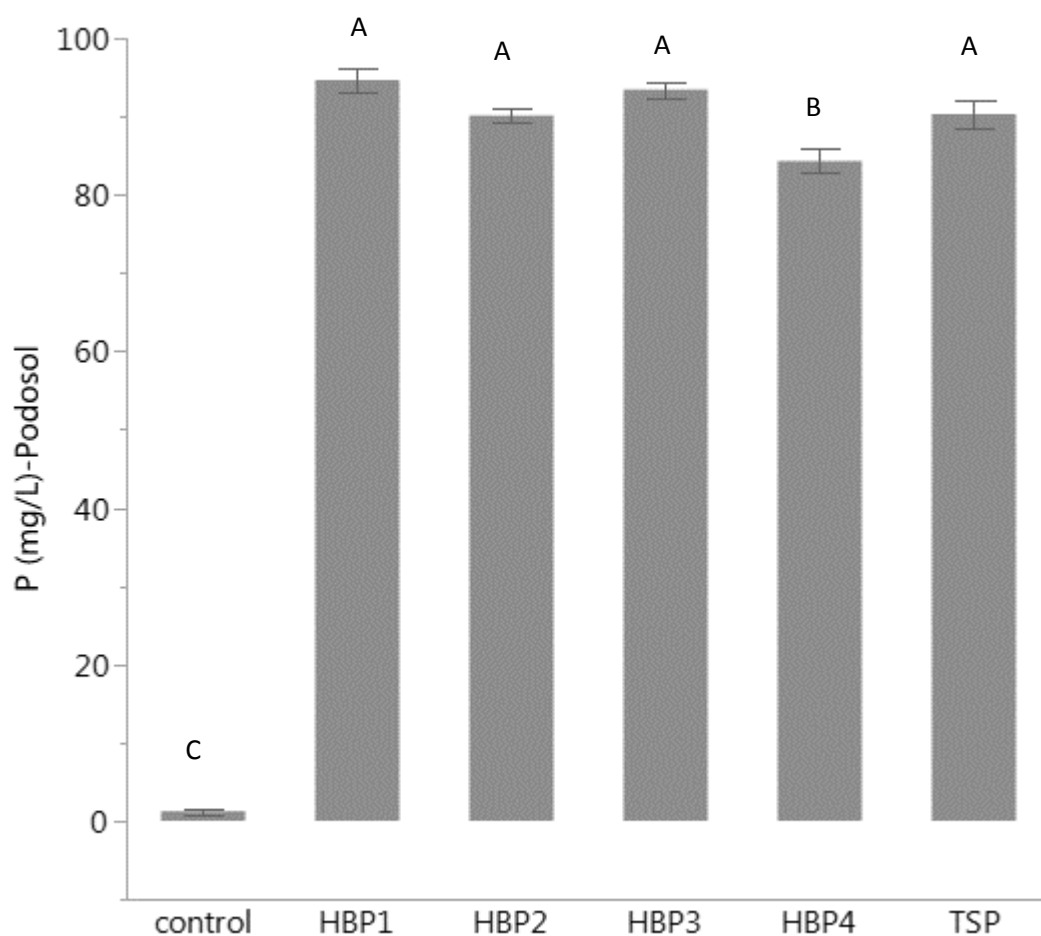


Figure 3.4 Effect of different fertilisers on water-extracted P in Podosol. Mean values are presented (n=4) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

Data analysis by ANOVA showed no significant effect ($p < 0.05$) of time, while interaction effect between time and fertilisers was significant (Table 3.5). The application of HBP1 released a significantly higher amount of WEP into the soil solution at 7 days after fertiliser addition with 99.9 mg P /L, but this difference was no longer apparent after 15 days. At 7 d, HBP4 with 81.8 mg P /L of WEP showed the lowest P concentration. Treatments containing TSP, HBP2 and HBP3 were not different from any of the other fertilisers at any time points. The range of P available in the Podosol (~2.0-2.5 mg/ 25 ml) was approximately 5 times greater than the P available in the Ferrosol.

Table 3.5 Interaction effect of time and different fertilisers on water-extract P. Mean values are presented (n=4). Numbers in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

Fertilisers	Time	Mean
HBP1	7	99.9 (1.9)A
HBP1	2	95.9 (1.9) AB
HBP3	15	95.9 (1.9) AB
TSP	7	93.8 (1.9) ABC
HBP3	2	92.2 (1.9) ABC
HBP3	7	92.1 (1.9)ABC
HBP2	7	91.3 (1.9)ABCD
HBP2	2	90.9 (1.9)ABCD
TSP	2	90 (1.9)ABCD
HBP2	15	87.9 (1.9) BCD
HBP1	15	87.9 (1.9) BCD
TSP	15	87.1 (1.9) BCD
HBP4	15	86.8 (1.9) BCD
HBP4	2	84.3 (1.9) CD
HBP4	7	81.8 (1.9) D
Control	7	2.3 (1.9) E
Control	2	0.9 (1.9) E
Control	15	0.3 (1.9) E

3.3.2.2 Calcarosol

ANOVA demonstrated a significant effect ($p < 0.05$) of HBPs fertilisers have on WEP in comparison with TSP, in Calcarosol. Application of HBP1 released higher water-extractable P in the soil solution with 217 mg P/L (Figure 3.5) and HBP4 with 200.5 mg P /L showed the lowest P concentration.

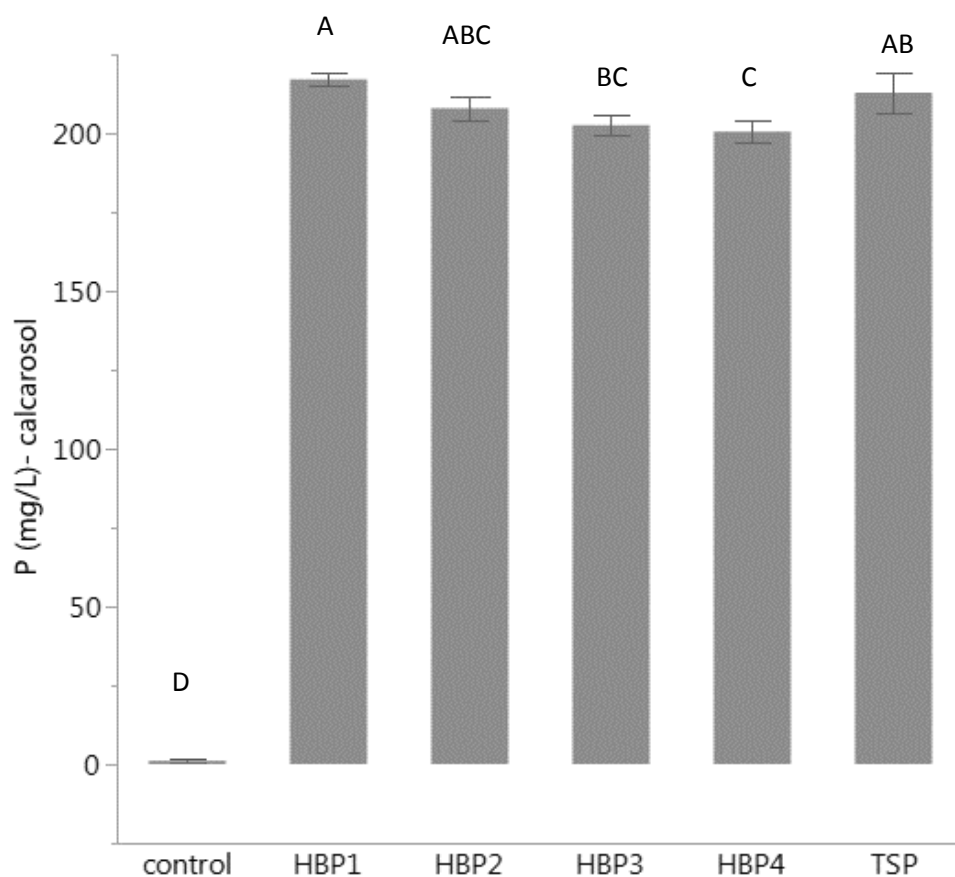


Figure 3.5 Effect of different fertilisers on water-extracted P in Calcarosol. Mean values are presented ($n=4$) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

Data analysis by ANOVA showed the higher WEP were released into the soil solutions at 2 days after addition of the fertilisers while at 7 days there was a significant reduction on WEP in soil solutions for all treatments (Table 3.6).

Table 3.6 Effect of time on water-extract P in Calcarosol. Mean values are presented (n=4). Numbers in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

Time (days)	Mean
2	177.8 (1.9) A
15	173.2 (1.9) AB
7	169.8 (1.9) B

The interaction effect between time and different fertilisers was significant ($p < 0.05$) (Table 3.7). While application of TSP at 2 days showed the highest WEP concentration with 231.5 mg P /L, HBP4 at 7 days resulted in the lowest WEP in soil solutions.

Table 3.7 Interaction effect on water-extract P in Calcarosol. Mean values are presented (n=4). Numbers in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

Fertilisers	Time	Mean
TSP	2	231.5 (4.6) A
HBP1	2	221.3 (4.6) AB
HBP1	15	218.1 (4.6) AB
HBP2	15	217.5 (4.6) ABC
TSP	7	214.4 (4.6) ABCD
HBP2	7	212.4 (4.6) ABCDE
HBP1	7	211.7 (4.6) ABCDE
HBP3	2	210.2 (4.6) ABCDE
HBP4	2	210 (4.6) ABCDE
HBP3	15	205.8 (4.6) BCDE
HBP4	15	203 (4.6) BCDE
HBP2	2	193.7 (4.6) CDE
TSP	15	192.4 (4.6) DE
HBP3	7	191.4 (4.6) DE
HBP4	7	188.6 (4.6) E
Control	15	2.5 (4.6) F
Control	7	0.6 (4.6) F
Control	2	0.2 (4.6) F

3.3.3 Colwell P of post – incubated soils- Ferrosol

Although data analysis by ANOVA showed a significant positive effect ($p < 0.05$) of HBP1 and HBP2 fertilisers in post-incubated soils available P with 520 mg P /kg, however the effect was not very significant with TSP with 500 mg P /kg soil (Figure 3.6). The application of HBP4 significantly reduced available P into 448 mg P /kg soil.

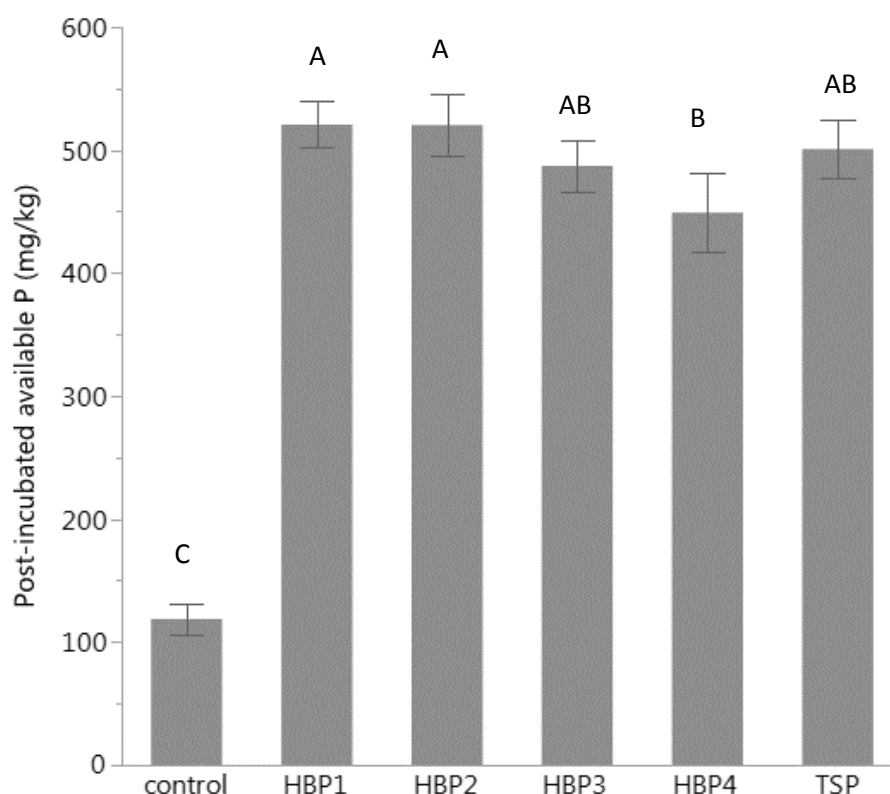


Figure 3.6 Post-incubated available (Colwell) P under different treatments in Ferrosol. Mean values are presented (n=4) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

3.4 Discussion

The humate blended phosphate (HBP) fertilisers showed the same pattern of solubility as triple superphosphate (TSP) in water, with a gradual increase in dissolved P over time.

The humate blended phosphate (HBP) fertilisers have shown similar results in keeping phosphorus available over the time in all three types of soils with exception of HBP1 which could keep more water-extract P over the time. Across treatments, the Ferrosol maintained the lowest range of water-extractable P, with only 0.1-0.5 mg from the initial added P (10 mg) remaining in solution at the end of the incubation, compared with 5.0-5.6 mg P in the Calcarosol. This different response to P fertilisers in Ferrosol stems from the difference in phosphorus buffering capacity (PBC) of the soils. The Australian Ferrosol PBC range is 27-104 mg/kg while the Australian Calcarosol is about 0-4 mg/kg (Burkitt et al., 2002b). The Ferrosol has the highest PBC and Calcarosol has the lowest PBC among the soils examined in this study (Vu et al., 2010, Burkitt et al., 2002a). The high PBC of Ferrosol is attributed to the high concentration of Fe-mineral adsorption sites (Bolan et al., 1994, Sharpley and Moyer, 2000b). Of all the soils, the Ferrosol was also the most responsive to the different HBP fertilisers, with HBP1 providing almost double the water-extractable P than the TSP in this soils and HBP4 providing less than half that of TSP. (Quan-Xian et al., 2008) previously found that P and solubility was enhanced by the addition of humic substances to a Ferrosol, and (Ch'ng et al., 2014) showed that in a soil with high Fe and Al concentration, organic matter could keep the inorganic P in available form for a longer period of time in comparison with TSP by reducing soluble Fe and Al ions which would otherwise contribute to P-fixation. Other studies have also shown that there is competition for adsorption sites between humate and phosphate on Fe and Al (hydr) oxide minerals (Violante et al., 1991, Antelo et al., 2007). Thus, in this study, the increase of water-extractable P concentration by addition of HBP1 in Ferrosol,

was probably due to the competition of humate and P for adsorption sites on soil mineral surfaces or dissolved Fe/Al.

The HBP4, containing a higher proportion of humate material, showed a lower P availability, particularly with respect to HBP1. This result is in contrast to a number of other studies that have shown that increasing the concentration of humic substances in the presence of Fe and Al (hydr)oxides generally results in decreased P adsorption and a higher equilibrium solution concentration of P (Borggaard et al., 2005, Antelo et al., 2007). The reason for this is not definitively known, but the addition of carbon into the soil is known to stimulate soil microbial populations, which can subsequently cause microbial immobilisation of inorganic P pools (Bünemann et al., 2011) (Ehlers et al., 2010). A localised increase in pH and C content around HBP4 may have provided more conducive conditions for microbial growth and P immobilisation at 7 d, with subsequent microbial turnover releasing P possibly diminishing this effect by 15 d.

Similar to the Ferrosol, the water-extractable P in the Calcarosol was higher in HBP1 treatment during the incubation time. Because the Calcarosol is a soil with very low Fe and Al concentrations, beneficial effects of HBP1 fertiliser is probably attributed to the less amount of available P in this soil but this positive effect was relatively less significant than Ferrosol due to the higher overall soluble P concentration in the Ferrosol.

Not significant positive effect of HBPs was observed in Podosol. The water-extract P was similar in TSP and HBPs treatments, likely due to the high concentration of background soil available P and that prevented P fertilization from having any meaningful impact.

3.5 Conclusion

The results show that blending the TSP fertilisers with K-humate has the potential to improve available phosphorus in highly weathered soils solution such as Ferrosol with high contents of Fe and Al ions, but that it is dependent of the ratio of humate:phosphate in the blended fertiliser. Improvements in P availability are likely due to competitive binding of humate for sites on Fe and Al oxides. Nevertheless, the post-incubated available P was not affected by the presence of the humate substances and it is possible the high concentration of soil available P and organic matter prevented a significant response from HBPs fertilisers (Summerhays et al., 2015, Hill et al., 2015b) Further investigation is needed into the mechanism of the positive role of HBP fertilisers in different soils with wide range of P: C ratios.

3.6 References

ANTELO, J., ARCE, F., AVENA, M., FIOL, S., LÓPEZ, R. & MACÍAS, F. 2007. Adsorption of a soil humic acid at the surface of goethite and its competitive interaction with phosphate. *Geoderma*, 138, 12-19.

BOLAN, N. S., NAIDU, R., MAHIMAIRAJA, S. & BASKARAN, S. 1994. Influence of low-molecular-weight organic acids on the solubilization of phosphates. *Biology and Fertility of Soils*, 18, 311-319.

BORGGGAARD, O. K., RABEN-LANGE, B., GIMSING, A. L. & STROBEL, B. W. 2005. Influence of humic substances on phosphate adsorption by aluminium and iron oxides. *Geoderma*, 127, 270-279.

BURKITT, L., MOODY, P., GOURLEY, C. & HANNAH, M. 2002. A simple phosphorus buffering index for Australian soils. *Australian Journal of Soil Research*, 40, 497-514.

CAO, X., DROSOS, M., LEENHEER, J. A. & MAO, J. 2016. Secondary Structures in a Freeze-Dried Lignite Humic Acid Fraction Caused by Hydrogen-Bonding of Acidic Protons with Aromatic Rings. *Environmental science & technology*, 50, 1663-1669.

CH'NG, H. Y., AHMED, O. H. & MAJID, N. M. A. 2014. Improving phosphorus availability in an acid soil using organic amendments produced from agroindustrial wastes. *The Scientific World Journal*, 2014.

CHEN, D., SUN, J., BAI, M., DASSANAYAKE, K. B., DENMEAD, O. T. & HILL, J. 2015. A new cost-effective method to mitigate ammonia loss from intensive cattle feedlots: application of lignite. *Scientific reports*, 5, 16689.

DAWSON, C. J. & HILTON, J. 2011. Fertiliser availability in a resource-limited world: Production and recycling of nitrogen and phosphorus. *Food Policy*, 36, S14-S22.

DIEZ, J., CARTAGENA, M. & VALLEJO, A. 1992. Controlling phosphorus fixation in

calcareous soils by using coated diammonium phosphate. *Fertilizer research*, 31, 269-274.

FONG, S. S., SENG, L., CHONG, W. N. & ASING, J. 2006. Characterization of the coal derived humic acids from Mukah, Sarawak as soil conditioner. *Journal of the Brazilian Chemical Society*, 17, 582-587.

GÉRARD, F. 2016. Clay minerals, iron/aluminum oxides, and their contribution to phosphate sorption in soils—A myth revisited. *Geoderma*, 262, 213-226.

GIOVANNINI, C., GARCIA-MINA, J., CIAVATTA, C. & MARZADORI, C. 2013. Effect of organic-complexed superphosphates on microbial biomass and microbial activity of soil. *Biology and fertility of Soils*, 49, 395-401.

GUEDES, R. S., MELO, L. C. A., VERGÜTZ, L., RODRÍGUEZ-VILA, A., COVELO, E. F. & FERNANDES, A. R. 2016. Adsorption and desorption kinetics and phosphorus hysteresis in highly weathered soil by stirred flow chamber experiments. *Soil and Tillage Research*, 162, 46-54.

HARRIS, M. A. 2016. Quenching of Phosphorus-Fixation in a Disturbed Caribbean Bauxite Mine Overburden Using Root Exudates: Implications for Acidic Tropical Soils. *Geobiotechnological Solutions to Anthropogenic Disturbances*. Springer.

HAYNES, R. & MOKOLOBATE, M. 2001. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved. *Nutrient cycling in agroecosystems*, 59, 47-63.

HERRERA, B., FERNEY, W., RODRIGUES, M., BETTONI TELES, A. P., BARTH, G. & PAVINATO, P. S. 2016. Crop Yields and Soil Phosphorus Lability under Soluble and Humic-Complexed Phosphate Fertilizers. *Agronomy Journal*, 108, 1692-1702.

HILL, M. W., HOPKINS, B. G. & JOLLEY, V. D. 2015a. Maize in-Season Growth Response to Organic Acid-Bonded Phosphorus Fertilizer (Carbond P®). *Journal of Plant Nutrition*, 38, 1398-1415.

HILL, M. W., HOPKINS, B. G., JOLLEY, V. D. & WEBB, B. L. 2015b. Phosphorus Mobility Through Soil Increased with Organic Acid-Bonded Phosphorus Fertilizer (Carbond® P). *Journal of Plant Nutrition*, 38, 1416-1426.

ISBELL, R. 2002. *The Australian soil classification*, CSIRO publishing.

IYAMUREMYE, F. & DICK, R. 1996. Organic amendments and phosphorus sorption by soils. *Advances in agronomy (USA)*.

KRUSE, J., ABRAHAM, M., AMELUNG, W., BAUM, C., BOL, R., KÜHN, O., LEWANDOWSKI, H., NIEDERBERGER, J., OELMANN, Y. & RÜGER, C. 2015. Innovative methods in soil phosphorus research: A review. *Journal of plant nutrition and soil science*, 178, 43-88.

LEHMANN, J. & KLEBER, M. 2015. The contentious nature of soil organic matter. *Nature*, 528, 60-68.

MURPHY, J. & RILEY, J. P. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica chimica acta*, 27, 31-36.

PICCOLO, A. 2001. The supramolecular structure of humic substances. *Soil science*, 166, 810-832.

QUAN-XIAN, H., JIAN-YUN, L., JIAN-MIN, Z., HUO-YAN, W., CHANG-WEN, D. & XIAO-QIN, C. 2008. Enhancement of phosphorus solubility by humic substances in ferrosols. *Pedosphere*, 18, 533-538.

RAYMENT, G. E. & LYONS, D. J. 2011. *Soil chemical methods: Australasia*, CSIRO publishing.

ROWE, H., WITHERS, P. J., BAAS, P., CHAN, N. I., DOODY, D., HOLIMAN, J., JACOBS, B., LI, H., MACDONALD, G. K. & MCDOWELL, R. 2016. Integrating legacy soil phosphorus into sustainable nutrient management strategies for future food, bioenergy and water security.

Nutrient Cycling in Agroecosystems, 104, 393-412.

SCHEFE, C. R., PATTI, A. F., CLUNE, T. S. & JACKSON, W. R. 2008. Interactions between organic amendments and phosphate fertilizers modify phosphate sorption processes in an acid soil. *Soil Science*, 173, 433-443.

SCHINDLER, D. W., CARPENTER, S. R., CHAPRA, S. C., HECKY, R. E. & ORIHIEL, D. M. 2016. Reducing phosphorus to curb lake eutrophication is a success. ACS Publications.

SEYEDBAGHERI, M.-M. 2010. Influence of humic products on soil health and potato production. *Potato research*, 53, 341-349.

SHARPLEY, A. & MOYER, B. 2000. Phosphorus forms in manure and compost and their release during simulated rainfall. *Journal of environmental quality*.

SUMMERHAYS, J. S., HOPKINS, B. G., JOLLEY, V. D., HILL, M. W., RANSOM, C. J. & BROWN, T. R. 2015. Enhanced phosphorus fertilizer (Carbond P®) supplied to maize in moderate and high organic matter soils. *Journal of Plant Nutrition*, 38, 1359-1371.

VIOLANTE, A., COLOMBO, C. & BUONDONNO, A. 1991. Competitive adsorption of phosphate and oxalate by aluminum oxides. *Soil Science Society of America Journal*, 55, 65- 70.

VU, D., TANG, C. & ARMSTRONG, R. 2010. Transformations and availability of phosphorus in three contrasting soil types from native and farming systems: A study using fractionation and isotopic labeling techniques. *Journal of Soils and Sediments*, 10, 18-29.

WANG, X., WANG, Z. & LI, S. 1995. The effect of humic acids on the availability of phosphorus fertilizers in alkaline soils. *Soil Use and Management*, 11, 99-102.

WITHERS, P. J., SYLVESTER-BRADLEY, R., JONES, D. L., HEALEY, J. R. & TALBOYS, P. J. 2014. Feed the crop not the soil: rethinking phosphorus management in the food chain. ACS Publications.

ZWETSLOOT, M. J., LEHMANN, J., BAUERLE, T., VANEK, S., HESTRIN, R.

&NIGUSSIE, A. 2016. Phosphorus availability from bone char in a P-fixing soil influenced by root-mycorrhizae-biochar interactions. *Plant and Soil*, 408, 95-105.

Chapter 4

Evaluation of humate-blended phosphate fertiliser on soil phosphorus availability: diffusion and Visualisation

Abstract

Petri Dish experiments were done to evaluate the diffusion rate of soil available phosphorus from triple superphosphate (TSP) and K-humate-blended phosphate (HBP) fertilisers in two different soils. In the first part of the study, a resin membrane technique was used to measure available phosphorus in Ferrosol. The results indicated not significant difference between TSP and HBPs fertilisers in soil available phosphorus over the time. All treatments showed the highest available P in 2 weeks after addition of fertilisers.

Based on the first part of the study results, it was hypothesized that the humate-blended fertiliser did not show a beneficial effect on soil available P because of the high concentration of available P in soils. Therefore, the second part of the study was conducted using a Ferrosol with lower P concentration than the Ferrosol used in part (I). TSP and humate-blended TSP were applied into the soil. The results showed that among HBPs, only HBP2 could keep phosphorus more available than TSP.

Also, a visualisation technique was applied to visualise P diffusion zones in soil and fertiliser addition treatments. The results were in agreement with chemical extraction of soil phosphorus as the scanned filter papers showed a bigger area of diffused phosphorus in HBP2 treatment.

The results indicated that the beneficial effect of HBPs fertilisers depends on soil characteristics and soil phosphorus content. In addition only humate-phosphate blended fertiliser with the proper carbon to phosphorus ratio may have positive effect on soil phosphorus availability.

4.1 Introduction

In soil solution, P moves mainly through diffusion flux which allows plants to absorb it. The diffusion of P usually is assessed by sampling different sections of soils around the application point (Lawton and Vomocil, 1954) and phosphate extraction method depends on soil type. The efficiency of P in soil for crop production depends on sufficient concentration of the P in soil solution. The diffusion flux of P in the soil solution and its availability are affected by numerous factors including soil moisture, soil bulk density, mineralogy, and P concentration in soil solution (Liu et al., 2014, Hinsinger, 2001). The mobility and availability of the phosphorus will be reduced by sorption and precipitation reactions in soils (Degryse and McLaughlin, 2014). The reactions between P and soil compounds which lead to change available form of phosphorus to unavailable form is called “P-fixation” (Hue, 1991, Chand and Tomar, 1993) have shown that the physiochemical properties of the soil play the most important role on P availability in P-fixing soils.

In weathering acidic soils with high Fe and Al concentration the reaction of P with Al and Fe can happen as fast adsorption at soil surfaces sites in short term (<1 day) to slow diffusion through the solid phase followed by precipitation (Sharpley et al., 1984). After application of phosphate into the soil, the free Al and Fe ions as well as amorphous Al and Fe oxides react with P. These reactions are described as a ligand exchange reactions between phosphate ions and OH⁻ or H₂O⁻ groups at the surface of soil particles (Sharpley and Moyer, 2000). It was shown that in acidic soils P adsorption is highly positively correlated with Al and Fe content (Cuttle and Bourne, 1993). In longer periods of time, P slowly diffuses through soil aggregates to form P-Al or P-Fe precipitates.

The strong affinity of P for soil particles surfaces and reactions with Ca and Fe/Al in calcareous and acidic soils may be influenced by the application of humic substances into the soil through

interaction with soil P and reduce the P fixation then increase P availability and mobility (Quan-Xian et al., 2008). The beneficial effect of humic substances on soil chemical and biological process specifically phosphorus enhancement have been well established (Iyamuremye and Dick, 1996, Haynes and Mokolobate, 2001) and have been described in detailed in chapter 1 of this thesis.

Various studies investigated the role of humic substances on P diffusion behaviour in the soil. For example, the results of the studies revealed a significant increase of P fertiliser's mobility and efficiency when humic acids and fulvic acids were added to the soil (Fixen et al., 1983, Havlin and Westfall, 1984). The results of a study have shown that the P availability in soils and then plant P absorption has been increased with lignin-coated P fertilizers (Garcia et al., 1997). Coating diammonium phosphate fertilizers with resin could control the P-fixing issue in arid regions soils (Diez et al., 1992). In another study, it was shown the granular phosphorus fertilisers release P into the soil via diffusion movement (Degryse et al., 2015). Coating monoammonium phosphate with a natural organic acid resulted in higher phosphate availability in highly weathered soil as well as greater agronomic efficiency in maize plant growth with 13% increase in yield (Teixeira et al., 2016). However, some studies results showed no significant effect of application of humic substances on soil P availability. For instance, there were not a significant difference between mono ammonium phosphate fertilisers and phosphate fertilisers that with coated with humic acids in P uptake in spring wheat (Clain et al., 2007).

Australian soils are known as low P efficiency soils despite a long-term history of phosphorus fertilizer application (Iqbal, 2009). Although farmers have applied large quantities of phosphorus fertilizers over years, just a small fraction of it has been used by plants, particularly in high P-fixing soils (Lombi et al., 2004). So, in order to investigate the effect of humic

substances -which were blended with TSP- on phosphorus fertiliser efficiency a series of diffusion and visualization studies have been conducted.

This chapter describes two separate laboratory studies involving triple superphosphate (TSP) and the humate-blended TSP fertilisers previously described. In one study the P diffusion rate in soil compared in humate blended phosphate fertiliser and TSP at Ferrosol. The incubation time was 15 weeks with soil sampling at 2, 6 and 15 weeks after addition of the fertilisers. A set of Petri dishes were filled with soil and granular fertilizer was placed in the centre of a Petri dish in a certain depth. At the end of the incubation time, the sealed dishes were opened and soil sample were collected from different distance from the application point with concentric plastic rings. The available P then was measured by resin membrane technique.

The results of the first experiment were used to inform the experimental design of the second part of the study. In the second experiment, soil available phosphorus has been evaluated in a Ferrosol with lower phosphorus concentration under different P treatments in 30 days.

4.2 Methods and Materials

4.2.1 Soil and fertilizers – Part (I)

Ferrosol was used for the first part of the study (Table 4.1). Ferrosol (Australian Soil Order), collected from an experimental field of Department of Primary Industries (DPI), Ellinbank, Research Institute, West Gippsland, Victoria (38° 14' 00") Australia from an experimental field of Department of Economic Development, Jobs, Transport and Resources (DEDJTR), The soil was collected in 2013, air-dried and sieved to <2 mm. A subsample was then analysed for a range of key physiochemical properties (Environmental analysis laboratory, Southern Cross University, Lismore, NSW). The key physiochemical properties of the soil with the extraction methods and the fertilisers that were used in this part of the study presented in Tables 4.1 and 4.2, respectively.

Table 4.1 Some of the key physiochemical properties of the soils

Soil type	pH (1:5) water	Location	OM %	Colwell P mg/kg	KCl extracted Al mg/kg	DTPA* Fe mg/kg	KCl Ammonium mg/kg	KCl Nitrate mg/kg	Morgan Ca	texture
Ferrosol	5.3	Ellinbank	13.3	73	61	191	62	28.3	793	Loam

- DTPA : Diethylene Triamine Pentaacetic Acid

Table 4.2 Fertilisers which were used in part (I)

	TSP	HBP2	HBP4
P%	17.5%	14.6%	7.12%
C%	2.7%	12.7%	30.2%

4.2.2 Soil and fertilizers –Part (II)

A Ferrosol soil with low available P was utilised in the second part of this study. This was collected from a roadside in Ellinbank, in West Gippsland, Victoria (38° lat. 146° long.) Australia. This roadside soil had not received fertilizers so it was low in N and P concentration. The most important characteristics of this soil are shown in Table 4.3. The soil was air-dried and sieved to <2 mm. A subsample was then analysed for a range of key physiochemical properties (Environmental analysis laboratory, Southern Cross University, Lismore, NSW). The key physiochemical properties of the soil with the extraction methods and fertilisers were used in part (II) presented in Tables 4.3 and 4.4, respectively.

Table 4.3 Some of the key physiochemical properties of the soil

Soil type	pH (1:5 water)	Location	%OM	Colwell P mg/kg	KCl extractad Al mg/kg	DTPA* Fe mg/kg	KCl Ammonium mg/kg	KCl Nitrate mg/kg	texture
Ferrosol-road side	5.8	Ellinbank	12	31	4	236	7.8	8	Loam

- DTPA : Diethylene Triamine Pentaacetic Acid

Table 4.4 Fertilisers which were used in part (II)

	TSP	HBP1	HBP2	HBP3
%P(w/w)	17.	15.5	14.6	12.9
%C (w/w)	2.7	7.0	12.7	16.6

4.2.3 Experimental set up and sampling – Part (I)

Individual 9 cm diameter Petri dishes were filled with 56.80 g of the Ferrosol to simulate the soil bulk density of the field at four replications (Lombi et al., 2004). The soils were wetted to 60% field capacity moisture. The field capacity moisture has been calculated based on soil water content and drainage test. The surface of the soil then flattened using a spatula (Figure 4.1).



Figure 4.1 Addition of distilled water to the soil

The dishes were then capped tight and sealed with Para film and left overnight at room temperature (25°C). A day after preparation of Petri dishes, 10 mg P (~ 176mg/kg soil) in the form of the granular fertilizers (TSP and HBPs) were placed at 2 mm depth in the soil at the centre of the Petri dishes by using a sculpture. To avoid a direct contact of granules and soil the fertilisers were covered in a fine texture that could pass the water (Figure 4.2).



Figure 4.2 Placing the granular fertiliser into the 2mm depth of the soil

Then Petri dishes capped and sealed with Para film and incubated at room temperature (Lombi et al., 2004). Four different treatments (Table 4.3) with four replication in three sampling times giving a total of 48 Petri dishes. A set of sixteen Petri Dishes made for each sampling time (Figure 4.3). The incubation time was twelve weeks.

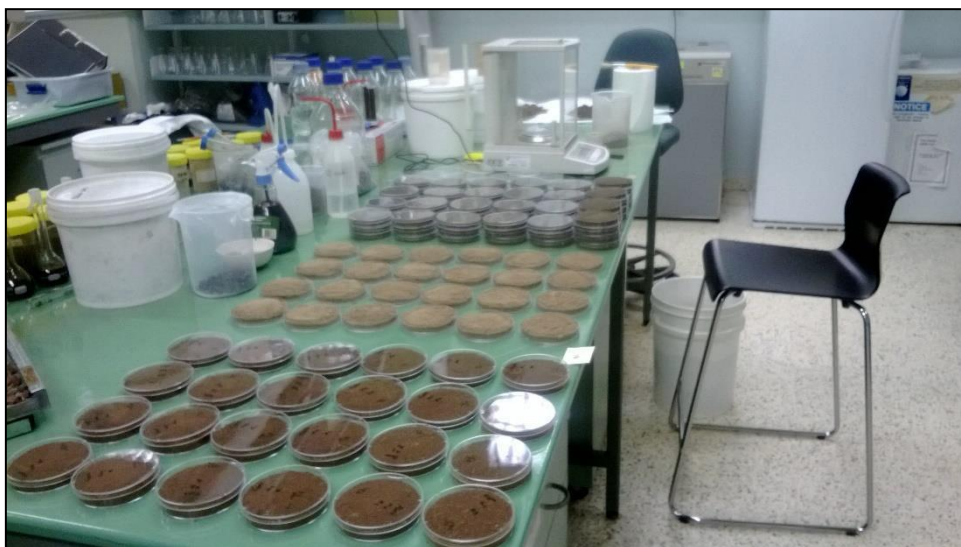


Figure 4.3 Preparation of Petri Dishes

The Petri Dishes opened at two, six and twelve weeks after placing the fertilizers into the soils to do soil sampling around the fertiliser's application point. Plastic concentric circles placed around the granules at two different radius of 0-1, 1-4.5 cm for soil sampling (Figure 4.4). The available phosphorus was measured by the resin membrane technique (Amer et al., 1955). All data were analysed using JMP statistical software (JMP®, Version 14; SAS Institute Inc., Cary, NC, USA). Three-way ANOVA was used to investigate the effect of different fertilisers and sampling zone as well as sampling time on soil available phosphorus. Tukey's honestly significant difference (HSD) was used where significant differences were found between different treatments

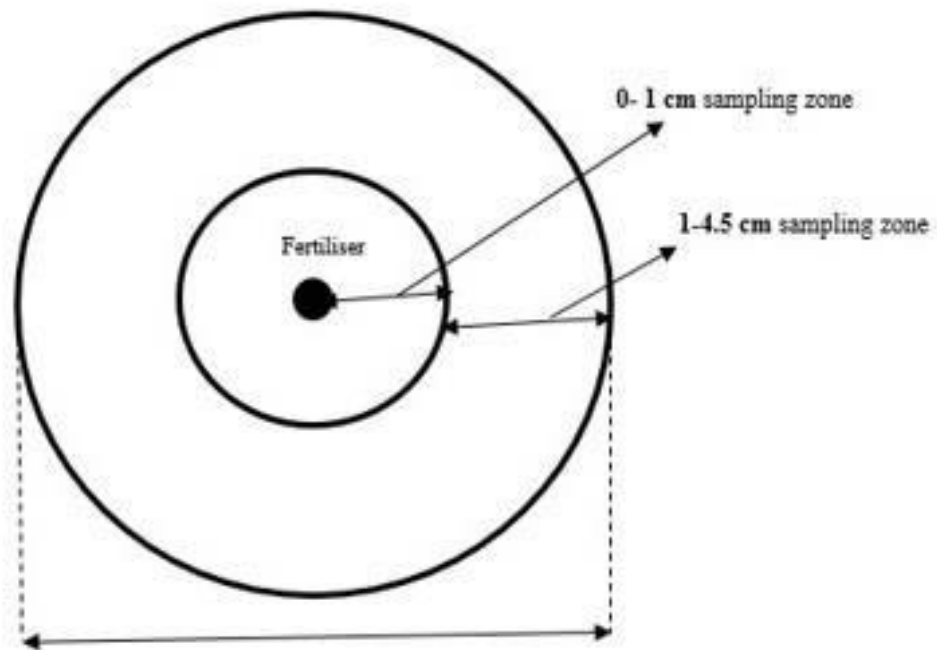


Figure 4.4 Schematic drawing of soil sampling in 2 zones of 0-1 and 1-4.5 cm in a Petri Dish

4.2.4 Experimental set up and sampling- Part (II)

In the second part of this study with a lower P Ferrosol soil, the same experimental design as described above was used and an incubation period, of 30 days after addition of granules to the Petri dishes was used with five replicates. In addition, the available phosphorus in the different soil zones was measured using Colwell method (Rayment and Lyons, 2011) following ammonium molybdate procedure to read the P concentration. The P measurement technique has been changed from Resin membrane P in part (I) to Colwell P in part (II) because the access to the resin membrane was limited.

All data was analysed using JMP statistical software (JMP®, Version 14; SAS Institute Inc., Cary, NC, USA). Two-way ANOVA was used to investigate the effect of different fertilisers and different sampling zone on soil available phosphorus after 30 days. Tukey's honesty significant difference (HSD) was used where significant differences were found between different treatments

4.2.5 Visualization experimental set up and sampling

In this part of the study, the extent of diffusion of the different P fertilisers was monitored by visualisation. The technique has been adopted from the original work (Degryse and McLaughlin, 2014) and briefly, a filter paper (9 cm diameter, which were the same size as the Petri Dishes), which has been impregnated with iron oxide acting as P sink at soil surface area. The captured P on filter papers is visualized with malachite green reagent. A mirror image of P diffused zone will be created at the surface of filter papers. After drying the papers, the scanned papers will analysis with free software (GNU Image Manipulation programme v.2.6.11).



A) Opening the Petri Dishes contain soil



B) Impregnation of filter papers with iron oxide by soaking the filter papers in iron oxide solution at least for 15 minutes



C) Deployment of phosphorus by a good contact of soil surface and wetted filter papers



D) Development of filter papers



E) Staining the filter papers by soaking in Malachite green in HCl solution

Figure 4.5 A-E steps of Visualisation technique

4.3 Results and Discussion

4.3.1 Part (I)

Data analysis by ANOVA demonstrated not significant effect of different fertilisers on soil available phosphorus while sampling zone and sampling time significantly were different (Table 4.5).

Table 4.5 ANOVA summary table for available phosphorus. Main effects and their interaction terms are presented. *P<0.05; **P<0.01; n.s., not significant at P<0.05

Source	Available P
Fertiliser	n.s
Sampling zone	**
Time	**
Fertiliser * sampling zone	n.s
Fertiliser*time	n.s

For the whole incubation period, the mean comparison by Tukey’s revealed that the availability of P was significantly ($p<0.05$) higher in zone 1(0-1cm from the application point) than zone 2 (91-4.5 cm from the application point), for all treatments (Figure 4.6).

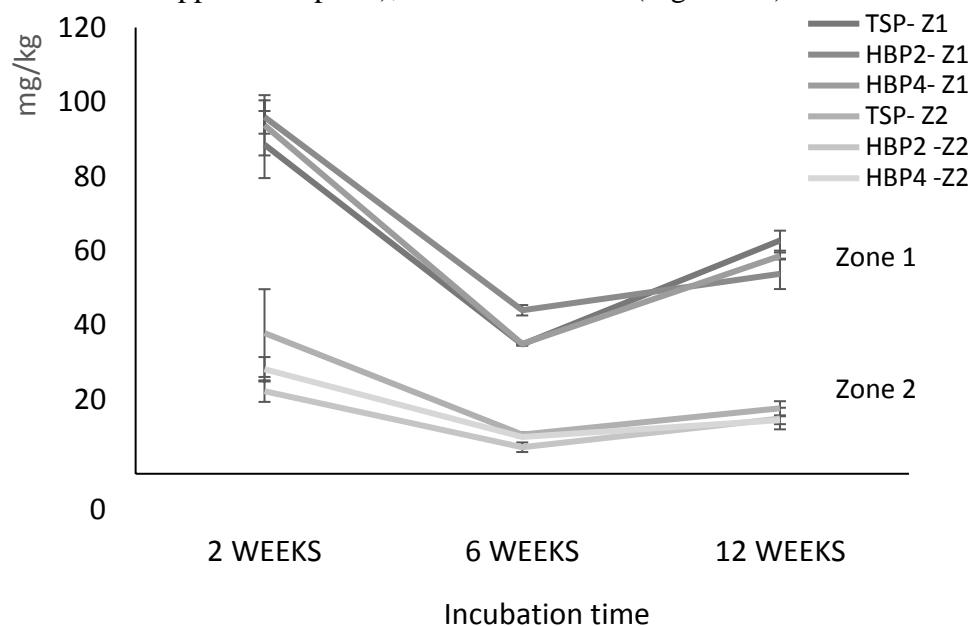


Figure 4.6 Soil available P under different fertilisers at two different sampling zones during 12 weeks

The most applied P recovered in the 0-1 cm zone from the application point. This is likely because of the slow diffusion rate of phosphorus in the soil. P Concentrations around a fertilizer granule are initially very elevated (Bolland and Gilkes, 1998).

Data analysis showed all treatments had a same trend of available P during 12 weeks. Further analysis by Tukey's demonstrated at 2 weeks after application of fertilisers into the soil, TSP, HBP2 and HBP4 released 63, 59 and 606 mg/kg available P, respectively. There was a significant decrease of soil available P for the same treatments order with 22, 25 and 22 mg/kg, respectively. A significant increase in soil available P happened between 6 and 12 weeks for the same treatments order with 40, 34 and 36 mg/kg of available P. Phosphorus is a dynamic element in the soil. After application of phosphate fertilisers to the soil, a small fraction of it will easily dissolve into the soil solution and become available for plant uptake. It has been reported that most of the movement of P away from the fertilizer granule occurs in the first week after application (Lawton and Vomocil, 1954; Williams, 1971). The P adsorption reaction is completely reversible. Adsorbed P may become plant available again in the soil due to the fast desorption reaction (Schoumans 1999, 1995). In a long-term situation, in acidic soils, soluble P usually moves by diffusion through the soil's solid phase or microspores of Al- and Fe-(hydr) oxides. This is followed by precipitation in form of Al-P and Fe-P, inside soil aggregates (Van Riemsdijk et al., 1984a, b; Bolan et al., 1985; Madrid and Armbarri, 1985). Also, P can be immobilized in organic matter due to chemical and biological procedures (Sharpley and Moyer, 2000a). However, the adsorbed P can be released by desorption of adsorbed P or dissolution of precipitated P, followed by diffusion to the soil solution.

The application of HBPs fertilisers did not have a positive effect on soil available P in comparison with TSP over the time. While the application of TSP at both sampling zones totally resulted in 126.3 mg/kg available P in 2 weeks, HBP2 and HBP4 released 118.1 and

121.8 mg/kg available P, respectively (Table 4.6). Application of TSP released 45.3 mg/kg available P in 6 weeks while HBP2 and HBP4 released 51 and 44.7 mg/kg of available P, respectively. At 17 weeks, TSP could release 80.2 mg/kg available P and HBP2 and HBP4 showed 68.5 and 73 mg/kg of available P which were not significant difference from TSP.

Table 4.6. Effect of different fertilisers on soil available P in 2, 6 and 17 weeks. Mean values are presented (n=4) and values in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $P < 0.05$ level as assessed by Tukey’s HSD within a column

Fertiliser	Soil available P in both zones (Z1+Z2) (mg/kg)		
	2 weeks	6 weeks	12 weeks
TSP	126.3 (20.7)	45.31(0.9)	71.3 (4.5)
HBP2	118.1 (7.2)	51 (2.6)	68.5 (6.9)
HBP4	121.8 (11.3)	44.7 (0.2)	72.9 (1.9)

These results likely contributed to the high concentration of organic matter in background soil. It contained a high level of organic matter. Many studies indicated the high organic matter soil may mask the effects of humic substances (Patti et al., 2013, Lee and Bartlett, 1976, Duplessis and Mackenzie, 1983, Hartz and Bottoms, 2010). Furthermore, another study also showed the effect of “Carbond P” fertiliser (unknown carbon source) had no effect compared with ammonium polyphosphate fertiliser on maize growth in a high organic matter soil (Summerhays et al., 2015). The soil background organic matter can behave in the same way as the K-humate in HBPs fertilisers(Chen et al., 2004), which may then minimize or negate any beneficial effect of HBPs applications.

4.3.2 Part (II)

4.3.2.1 Diffusion

Data analysis by ANOVA indicated a significant effect of fertilisers as well as sampling zone on available P at the end of the study (Table 4.7).

Table 4.7 ANOVA summary table for available phosphorus after 30 days. Both main effects and their interaction terms are presented. *P<0.05; **P<0.01; n.s., not significant at P<0.05

Source	Available P
Fertiliser	**
Sampling zone	**
Fertiliser * sampling zone	**

Further analysis by Tukey’s test, demonstrated a significant positive effect of HBP2 on soil available P after 30 days with 160.3 mg P/kg (Figure 4.7). TSP and HBP3 with 139 mg P/kg of available P showed the lowest P concentration in soil after 30 days.

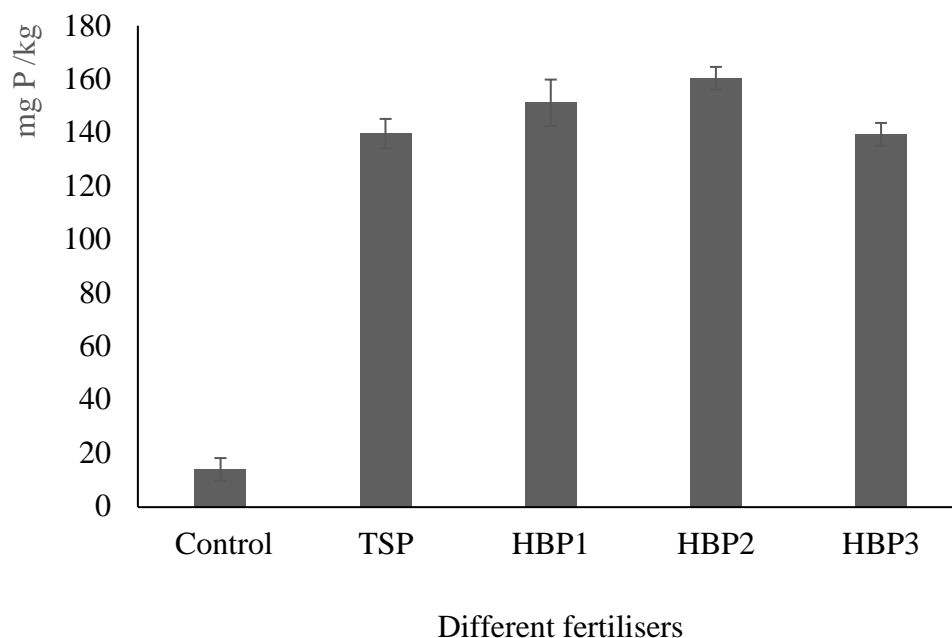


Figure 4.7 Effect of different fertilisers on Colwell P in Ferrosol-road side. Mean values are presented (n=5) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p<0.05$ level as assessed by Tukey’s HSD

Ferrosol has been selected for this study because of its high concentration of Fe and Al and it represents a typical Victorian pasture soils. The application of HBP2 increased soil available P at the end of the study. The concentration of Fe and Al were too high in Ferrosol (Table 4.1). The positive effect of humate that associated TSP in HBP2 may be attributed to the competition between humate and P for binding sites (Hue, 1991, Violante et al., 1991). In this case, humate interact with Al and Fe and decrease the soil available P sorption sites. Another possibility of the potential beneficial properties of humate utilised in HBP2 is P mobilization and sequestration like Al and Fe (Hill et al., 2015). Looking at all HBPs fertilisers effect, reveal that increasing the amount of humic substances in humate-blended fertilisers not necessary increase the soil available P. HBP2 contains 14% P and 12% C. The P: C ratio in this blend is about 1.3 that it is likely is the best P: C ratio of humate-phosphate fertilisers for soil P availability.

Analysis of variance of data demonstrated higher amount of the added P remained within 1 cm from the application point (Figure 4.8). Other studies results have shown that when P fertilizers are added to the soil in a granular triple superphosphate form, most of the P remained in the closest zone around the granules (Lombi et al., 2004) (Lawton and Vomocil, 1954).

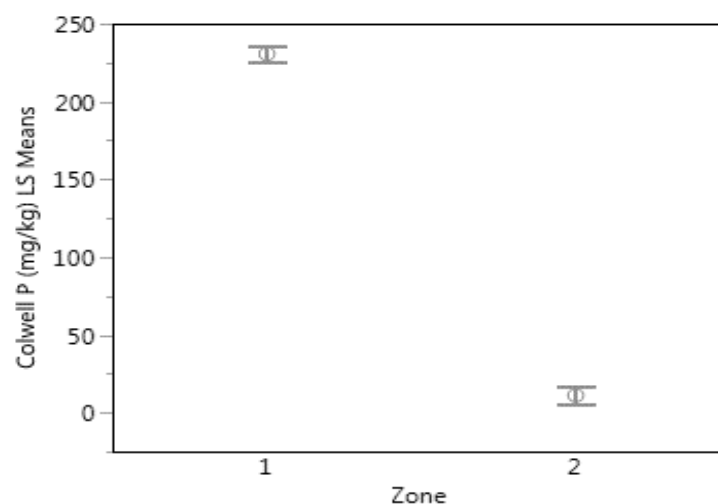


Figure 4.8 The comparison of P concentration of fertilisers at zone 1 (0-1cm) and zone 2 (1-4.5cm)

ANOVA indicated a significant ($p < 0.05$) interaction effect on soil available P. further analysis by Tukey's demonstrated application of HBP2 at zone 1 (0-1cm) released the most available P in the soil with 311.6 mg P /kg (Table 4.8). After HBP2, HBP1 and TSP at zone 1 released 290 and 270 mg P /kg available P, respectively. The lowest available P was observed in all treatments in zone 2 (1-4.5cm).

Table 4.8 Interaction effect between different fertilisers and sampling zone on soil available P. Mean values are presented (n=5). Numbers in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

Fertilisers	Zone	Mean
HBP2	1	311.6 (6) A
HBP1	1	290 (6) AB
TSP	1	270.7 (6) B
HBP3	1	262.3 (6) B
Control	1	18.7 (6) C
HBP3	2	16.5 (6) C
HBP1	2	12.2 (6) C
Control	2	9.4 (6) C
HBP2	2	9 (6) C
TSP	2	8.5 (6) C

4.3.2.2 Visualisation

The visualised diffusion zones of P in the different treatments after 30 days are shown in figure 4.9. The high intense black areas indicate the higher value for available phosphorus. The available P results from direct soil analysis in previous section showed that most of the available P from the granular fertilisers was present in the 0–1 cm zone from the application point and at 1–4.5 cm distance from the application point, the intensity of the stain on the filter was reduced (Figure 4.9). The analysis of the scanned filter papers also showed a higher P concentrated zone at HBP2 treatment in comparison with other treatments. The analysis-scanned filter paper demonstrated no P diffusion at both control (soil background P) and K-humate treatments. The visualisation technique is sensitive to zones of high P concentration, so there were no dark points in control and K-humate treatments. According to diffusion results of the previous part of the study, there were no significant differences between TSP and HBP3 treatment as a matter of available P. These results also are obvious in scanned filter papers of visualisation technique.

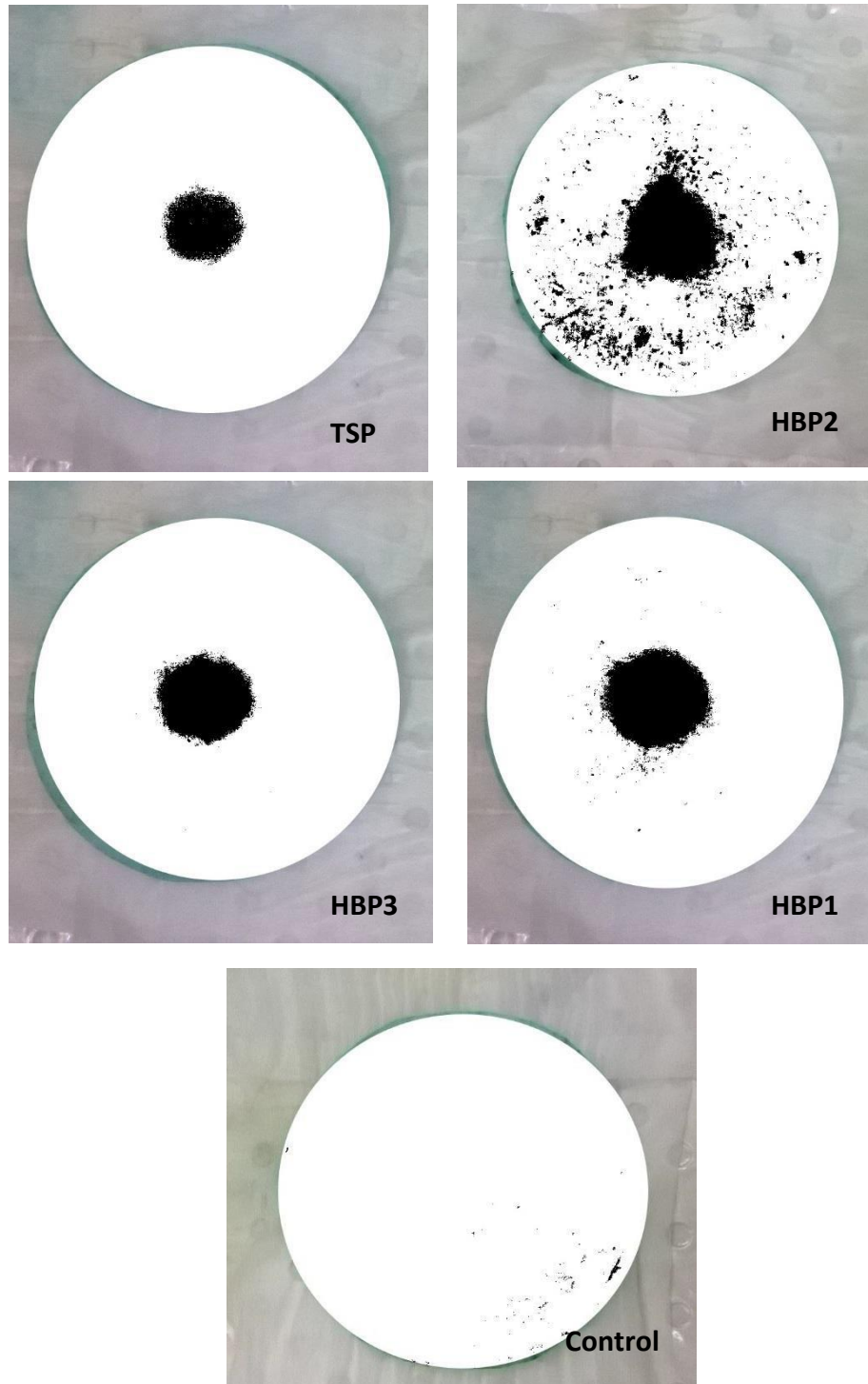


Figure 4.9 Visualized P diffusion zones at Ferrosol in which granular P-humate fertilizers have been applied to the centre of the Petri Dishes.

4.4 Conclusion

Available phosphorus mostly moves in soil solution via diffusion. After application of granular fertilisers, the concentration of diffused phosphorus from the application point was higher in closer distance to the application point.

HBP2 blended fertiliser demonstrated a positive effect in retaining more available phosphorus in Ferrosol with high Al and Fe concentrations. It is likely that HBP2 is more effective in soils with a high capacity of P-fixing. In addition, the results suggested that increasing humate in blended fertilisers is not necessarily more effective in increasing soil P availability.

The results of the Visualisation technique in measurement of soil phosphorus concentration were in agreement with the results of the diffusion study: the HBP2 fertiliser stain was more concentrated in comparison with other treatments.

4.5 References

- DEGRYSE, F. & MCLAUGHLIN, M. J. 2014. Phosphorus diffusion from fertiliser: visualisation, chemical measurements, and modelling. *Soil Science Society of America Journal*, 78, 832–842.
- DIEZ, J., CARTAGENA, M. & VALLEJO, A. 1992. Controlling phosphorus fixation in calcareous soils by using coated diammonium phosphate. *Fertiliser research*, 31, 269–274.
- EGHBALL, B., BINFORD, G. & BALTENSPERGER, D. D. 1996. Phosphorus movement and adsorption in a soil receiving long-term manure and fertiliser application. *Journal of environmental quality*, 25, 1339–1343.
- FAGBENRO, J. & AGBOOLA, A. 1993. Effect of different levels of humic acid on the growth and nutrient uptake of teak seedlings. *Journal of Plant Nutrition*, 16, 1465–1483.
- FIXEN, P., LUDWICK, A. & OLSEN, S. 1983. Phosphorus and potassium fertilisation of irrigated alfalfa on calcareous soils: II. Soil phosphorus solubility relationships. *Soil Science Society of America Journal*, 47, 112–117.
- FORTUN, C., FORTUN, A. & ALMENDROS, G. 1989. The effect of organic materials and their humified fractions on the formation and stabilisation of soil aggregates. *Science of the Total Environment*, 81, 561–568.
- GARCIA, M., DIEZ, J., VALLEJO, A., GARCIA, L. & CARTAGENA, M. C. 1997. Effect of applying soluble and coated phosphate fertilisers on phosphate availability in calcareous soils and on P absorption by a rye-grass crop. *Journal of Agricultural and Food Chemistry*, 45, 1931–1936.
- GARLAND, G., BÜNEMANN, E., OBERSON, A., FROSSARD, E., SNAPP, S., CHIKOWO, R. & SIX, J. 2018. Phosphorus cycling within soil aggregate fractions of a highly weathered tropical soil: A conceptual model. *Soil Biology and Biochemistry*, 116, 91–98.
- GROSSL, P. R. & INSKEEP, W. P. 1991. Precipitation of dicalcium phosphate dihydrate in the presence of organic acids. *Soil Science Society of America Journal*, 55, 670–675.
- GUPPY, C., MENZIES, N., MOODY, P. & BLAMEY, F. 2005. Competitive sorption reactions between

phosphorus and organic matter in soil: a review. *Soil Research*, 43, 189–202.

HALAJNIA, A., HAGHNIA, G. H., FOTOVAT, A. & KHORASANI, R. 2007. Effect of organic matter on phosphorus availability in calcareous soils. *JWSS-Isfahan University of Technology*, 10, 121–133.

HARTZ, T. K. & BOTTOMS, T. G. 2010. Humic substances generally ineffective in improving vegetable crop nutrient uptake or productivity. *HortScience*, 45, 906–910.

HAVLIN, J. & WESTFALL, D. 1984. Soil test phosphorus and solubility relationships in calcareous soils. *Soil Science Society of America Journal*, 48, 327–330.

HAYNES, R. & MOKOLOBATE, M. 2001. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved. *Nutrient Cycling in Agroecosystems*, 59, 47–63.

HILL, M. W., HOPKINS, B. G., JOLLEY, V. D. & WEBB, B. L. 2015. Phosphorus Mobility Through Soil Increased with Organic Acid-Bonded Phosphorus Fertiliser (Carbond® P). *Journal of Plant Nutrition*, 38, 1416–1426.

HINSINGER, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil*, 237, 173–195.

HOOKE, M., PETERSON, G., SANDER, D. & DAIGGER, L. 1980. Phosphate fractions in calcareous soils as altered by time and amounts of added phosphate. *Soil Science Society of America Journal*, 44, 269–277.

HUE, N. 1991. Effects of organic acids/anions on P sorption and phytoavailability in soils with different mineralogies. *Soil Science*, 152, 463–471.

IBRAHIM, H. & PRATT, P. 1982. Effects of rate of application and time on phosphorus sorption by soils. *Soil Science Society of America Journal*, 46, 926–928.

INSKEEP, W. P. & SILVERTOOTH, J. C. 1988. Inhibition of hydroxyapatite precipitation in the presence of fulvic, humic, and tannic acids. *Soil Science Society of America Journal*, 52, 941–946.

IYAMUREMYE, F. & DICK, R. 1996. Organic amendments and phosphorus sorption by soils.

Advances in Agronomy (USA).

JARDINE, P., MCCARTHY, J. & WEBER, N. 1989. Mechanisms of dissolved organic carbon adsorption on soil. *Soil Science Society of America Journal*, 53, 1378–1385.

KONONOVA, M. A. M. 1966. *Soil organic matter; its nature, its role in soil formation and in soil fertility*. Pergamon Press.

KUNKEL, R. & HOLSTAD, N. 1968. Effects of adding humates to the fertiliser on the yield and quality of Russet Burbank potatoes. *American Potato Journal*, 45, 449–457.

LAWTON, K. & VOMOCIL, J. 1954. The dissolution and migration of phosphorus from granular superphosphate in some Michigan soils. *Soil Science Society of America Journal*, 18, 26–32.

LEE, Y. S. & BARTLETT, R. J. 1976. Stimulation of plant growth by humic substances. *Soil Science Society of America Journal*, 40, 876–879.

LIPTZIN, D. & SILVER, W. L. 2009. Effects of carbon additions on iron reduction and phosphorus availability in a humid tropical forest soil. *Soil Biology and Biochemistry*, 41, 1696–1702.

LIU, F.-P., LIU, H.-Q., ZHOU, H.-L., DONG, Z.-G., BAI, X.-H., BAI, P. & QIAO, J.-J. 2014. Isolation and characterisation of phosphate-solubilizing bacteria from betel nut (*Areca catechu*) and their effects on plant growth and phosphorus mobilisation in tropical soils. *Biology and Fertility of Soils*, 50, 927–937.

LOMBI, E., MCLAUGHLIN, M. J., JOHNSTON, C., ARMSTRONG, R. & HOLLOWAY, R. 2004.

Mobility and lability of phosphorus from granular and fluid monoammonium phosphate differs in a calcareous soil. *Soil Science Society of America Journal*, 68, 682–689.

MONTALVO, D., DEGRYSE, F., AJIBOYE, B. & MCLAUGHLIN, M. J. Visualisation of phosphorus diffusion from granular and fluid fertilisers in non-calcareous highly phosphorus-fixing soils.

QUAN-XIAN, H., JIAN-YUN, L., JIAN-MIN, Z., HUO-YAN, W., CHANG-WEN, D. & XIAO-QIN, C. 2008. Enhancement of phosphorus solubility by humic substances in Ferrosols. *Pedosphere*, 18, 533–538.

RAYMENT, G. E. & LYONS, D. J. 2011. *Soil chemical methods: Australasia*, CSIRO publishing.

RYAN, J., HASAN, H., BAASIRI, M. & TABBARA, H. 1985. Availability and transformation of applied phosphorus in calcareous Lebanese soils. *Soil Science Society of America Journal*, 49, 1215–1220.

SAMPLE, E., SOPER, R. & RACZ, G. 1980. Reactions of phosphate fertilisers in soils. The role of phosphorus in agriculture, 263–310. Published by: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America

SHARPLEY, A. & MOYER, B. 2000. Phosphorus forms in manure, compost, and their release during simulated rainfall. *Journal of environmental quality*, 29, 1462–1469.

SIBANDA, H. & YOUNG, S. 1986. Competitive adsorption of humus acids and phosphate on goethite, gibbsite and two tropical soils. *European Journal of Soil Science*, 37, 197–204.

SINSABAUGH, R. L. & SHAH, J. J. F. 2012. Ecoenzymatic Stoichiometry and Ecological Theory. *Annual Review of Ecology, Evolution, and Systematics*, 43, 313–343.

SUMMERHAYS, J. S., HOPKINS, B. G., JOLLEY, V. D., HILL, M. W., RANSOM, C. J. & BROWN, T. R. 2015. Enhanced phosphorus fertiliser (Carbond P®) supplied to maize in moderate and high organic matter soils. *Journal of Plant Nutrition*, 38, 1359–1371.

TEIXEIRA, R. D. S., RIBEIRO DA SILVA, I., NOGUEIRA DE SOUSA, R., MÁRCIO MATTIELLO, E. & BARROS SOARES, E. M. 2016. Organic acid coated-slow-release phosphorus fertilisers improve P availability and maize growth in a tropical soil. *Journal of Soil Science and Plant Nutrition*, 16, 1097–1112.

CHAND, T. & TOMAR, N. 1993. Effect of soil properties on the transformation of phosphorus in alkaline calcareous soils. *Journal of the Indian Society of Soil Science*, 41, 56–61.

COLE, C., OLSEN, S. R. & SCOTT, C. 1953. The nature of phosphate sorption by calcium carbonate. *Soil Science Society of America Journal*, 17, 352–356.

CUTTLE, S. & BOURNE, P. 1993. Uptake and leaching of nitrogen from artificial urine applied to grassland on different dates during the growing season. *Plant and Soil*, 150, 77–86.

DEGRYSE, F. & MCLAUGHLIN, M. J. 2014. Phosphorus diffusion from fertiliser: visualisation, chemical measurements, and modeling. *Soil Science Society of America Journal*, 78, 832–842.

FREEMAN, J. & ROWELL, D. 1981. The adsorption and precipitation of phosphate onto calcite. *European Journal of Soil Science*, 32, 75–84.

GRIFFIN, R. & JURINAK, J. 1973. The Interaction of Phosphate with Calcite 1. *Soil Science Society of America Journal*, 37, 847–850.

GUPPY, C., MENZIES, N., MOODY, P. & BLAMEY, F. 2005. Competitive sorption reactions between phosphorus and organic matter in soil: a review. *Soil Research*, 43, 189–202.

HILL, M. W., HOPKINS, B. G., JOLLEY, V. D. & WEBB, B. L. 2015. Phosphorus Mobility Through Soil Increased with Organic Acid-Bonded Phosphorus Fertiliser (Carbond® P). *Journal of Plant Nutrition*, 38, 1416–1426.

HUE, N. 1991. Effects of organic acids/anions on P sorption and phytoavailability in soils with different mineralogies. *Soil Science*, 152, 463–471.

JARDINE, P., MCCARTHY, J. & WEBER, N. 1989. Mechanisms of dissolved organic carbon adsorption on soil. *Soil Science Society of America Journal*, 53, 1378–1385.

LOMBI, E., MCLAUGHLIN, M. J., JOHNSTON, C., ARMSTRONG, R. & HOLLOWAY, R. 2004. Mobility and lability of phosphorus from granular and fluid monoammonium phosphate differs in a calcareous soil. *Soil Science Society of America Journal*, 68, 682–689.

MONTALVO, D., DEGRYSE, F., AJIBOYE, B. & MCLAUGHLIN, M. J. Visualisation of phosphorus diffusion from granular and fluid fertilisers in non-calcareous highly phosphorus-fixing soils.

SAMPLE, E., SOPER, R. & RACZ, G. 1980. Reactions of phosphate fertilisers in soils. The role of phosphorus in agriculture, 263–310.

SCHOUMANS, O. 1995. Description and validation of the process formulation of abiotic phosphate reactions in acid sandy soils. Dutch.) Rep.

SHARPLEY, A. & MOYER, B. 2000. Phosphorus forms in manure and compost and their release during simulated rainfall. *Journal of environmental quality*, 29, 1462–1469.

SHARPLEY, A., SMITH, S., STEWART, B. & MATHERS, A. 1984. Forms of phosphorus in soil receiving cattle feedlot waste. *Journal of Environmental Quality*, 13, 211–215.

SIBANDA, H. & YOUNG, S. 1986. Competitive adsorption of humus acids and phosphate on goethite, gibbsite and two tropical soils. *European Journal of Soil Science*, 37, 197–204.

VIOLANTE, A., COLOMBO, C. & BUONDONNO, A. 1991. Competitive adsorption of phosphate and oxalate by aluminium oxides. *Soil Science Society of America Journal*, 55, 65–70.

Chapter 5

The Effect of Humate- blended phosphate fertilizers on pasture growth in two different acidic soils

Abstract

Dairy production constitutes one of the most important industrial sectors in Victoria. The success of this industry relies on growing high quality pasture. Although dairy farmers apply nitrogen, potassium and phosphorus (NPK) fertilisers to overcome pre-existing soil nutrient deficiencies and to replace the nutrients lost or removed from soil in grassland farming systems, phosphorus (P) persists in large quantities in the soil due to its relative soil immobility in regard to a number of relevant chemical reactions. The importance of phosphorus fertilisers and the effect of phosphate fertiliser on pasture yields have been widely studied. Rather than acceding to the practice of applying large quantities of phosphate fertiliser, which usually is considered as surplus P, many studies have been directed at increasing soil phosphorus efficiency in diverse soil types.

In this study an innovative organic blended phosphate fertiliser was applied to two different soils to investigate the P efficiency related to annual Ryegrass (*Lolium multiflorum* Lam.) growth improvement and nutrient uptake. The organic matter was lignite-derived, and both selected soils were Victorian dairy typical soils. The results indicated that the humate-blended phosphate would be likely to improve plant yields and P uptake in soils with low available P. The results also exhibited beneficial effects of humate in plants requiring high levels of phosphate.

5.1. Introduction

The dairy sector is one of the most important industrial sectors in Victoria. There are more than 4200 dairy farms in Victoria which accounts for about 80% of Australia dairy products exports. The success of this industry relies on growing high quality pasture. The pasture species yield depends on some main factors including soil type, soil fertility and water availability. The capability of different plants to use nutrients efficiently is highly dependent on species and variety. A 25 year-study on the effect of phosphorus on Victorian pasture productivity has shown that the phosphate fertilisers should be applied at an optimum level where economic return and environmental sustainability can be achieved (Department of Economic Development, Jobs, Transport and Resources, 2017).

The dairy farmers apply nitrogen, potassium and phosphorus (NPK) fertilisers to overcome the pastures' soil nutrient deficiencies and to replace the nutrients lost or removal from soil in grassland farming systems but, phosphorus is relatively immobile in soils due to the 'fixing' capacity of the soils (Ho et al., 2015). Fixing refers to any reaction between phosphorus and other soil particles and nutrients which reduce the phosphorus availability for plant uptake (Gérard, 2016, Harris, 2016). The importance of phosphorus fertilisers and the effect of phosphate fertiliser on pasture yields have been widely studied (Simpson et al., 2014, Jorquera et al., 2014, Mendoza et al., 2016, Lagos et al., 2016, Fernández et al., 2016b). To improve phosphate efficiency with regard to plant growth, it has been showed that the application of humic acid based substances could improve the short-term efficiency of rock phosphate as the phosphate fertiliser on pasture growth (Fernández et al., 2016a).

In a different study, the application of coal combustion by-product with phosphate rock could increase Ryegrass yields on fertile acidic soils (Baligar et al., 1997). The effects of coating TSP with organic matter (lignin) on phosphorus availability and Ryegrass P uptake were compared

with TSP. The results indicated that phosphate availability and plant P uptake increased in lignin-coated TSP (Garcia et al., 1997). Study results have shown that phosphorus balance efficiency (P output/P input) for Australian farming systems in dairy industry is about 29% which is considered as surplus P (Weaver and Wong, 2011). The surplus phosphorus indicates the P fertiliser's inefficiency.

Other study results have indicated that the application of humic substances did not have a significant effect on pastures plant growth (Nikbakht et al., 2014). In another study, humic acid applied foliar on perennial Ryegrass with different concentration. The results did not show any improvement of plant dried biomass as well as P uptake (Daneshvar Hakimi Maibodi et al., 2015). Interestingly, investigation of the effect of humic substances on nutrient uptake, availability and plant growth showed variable results in different species and soil types. The application of six commercial lignite-derived humic substances on the early stage of two pasture species in two different soils resulted in variable responses of plant species and soil types (Little et al., 2014).

In this context, humic substances which have been derived from Victorian lignite, were blended with commercial phosphate fertiliser to evaluate the effect of humic substances on phosphorus availability and pasture growth in two different Victorian acidic soils with high “P-fixing” capacity.

5.2. Methods and Materials

5.2.1 Soil collection and characterization

The soils used for this study were Ferrosol and Podosol, collected from a pasture farm in Ellinbank, Victoria (38° 14'06''S lat. 145°55'26''E long) and a vegetable farm recently converted from a pasture in Cranbourne, Victoria (38° 11'6''S lat. 145°18'50''E long) respectively. The soils were air-dried and sieved to <2 mm. A 200 g subsample was then analysed for a range of key physicochemical properties by Environmental Analysis Laboratory (EAL), Southern Cross University, Lismore, NSW: <http://scu.edu.au/eal/>; accessed March 2017). Key properties of the soils are shown in Table (5.1).

Table 5.1 Soil physicochemical properties before the addition of fertilisers

Soil	Texture	pH _{water} (1:5)	OM%	C%	N%	P _{Colwell} (mg/Kg)	K (mg/Kg)	Ca (mg/Kg)	Fe (mg/Kg)	Al (mg/Kg)
Ferrosol	Clay- Loam	5.3	13.3	7.59	0.65	73	191	793	191	61
Podosol	Sandy	5.4	2.1	1.19	0.07	31	28	152	122	19

Based on the soil analysis, nitrogen (urea), phosphate (triple superphosphate and blended fertilisers) and potassium (potash) were added at 30, 25 and 100 kg/ha for Ferrosol and 0, 25 and 10 kg/ha for Podosol respectively.

5.2.2 Characterization of fertilisers

The granular blended fertilisers (HBP1, HBP2 and HBP3) and TSP have been used in this study. The characterization of different fertilisers have been described in detail Chapter 2, table 2.2.

5.2.3 Glass house study set up: Annual Ryegrass (*Lolium multiflorum* Lam.) growth

A glasshouse study was conducted from March to June, 2015 in the glasshouse complex at Monash University Clayton Campus. Light and temperature conditions in the glasshouse were controlled. The light was maintained at a high level with supplemental lighting (16 hr/d) and averaged $220 \pm 17 \mu\text{mol m}^{-2} \text{s}^{-1}$. The temperature was $25.2 \pm 1.8^{\circ}\text{C}$ during the day and $23.3 \pm 1.4^{\circ}\text{C}$ at night.

Plastic pots (20 cm height x 20 cm diameter) were filled with 1kg soil to obtain the field condition soil bulk density. Urea and potash were uniformly mixed thoroughly to the soil. Each phosphate/carbon blended fertiliser was applied as a concentrated band at a soil depth of 1 cm at a rate of 25kg/h (Figure 5.1). Each treatment was replicated 4 times. The pots were left for to equilibrate for 2 days. After equilibration, six annual Ryegrass (*Lolium multiflorum* Lam.) seeds were sown approximately 2 mm below the soil surface. Seeds were irrigated to field-capacity moisture (Asghari and Cavagnaro, 2012), following with tap water, usually every second day to maintain the soil moisture content at 50% field capacity. After seven days post-seeding, plants were thinned to two per pot. Pots were arranged in a completely randomised design, with their positions rotated every second day.

5.2.4 Plants harvesting (plants tissue and post-harvest soil) analysis

After 12 weeks (84 days) (Figure 5.2), the plants were removed from the soil. The roots were gently shaken to remove the soil and then washed with deionised water. Plants, shoots, and roots were dried in an oven for three days at 55C⁰ to determine the dried biomass weight. The oven-dried shoot and root weight (SDW and RDW) was then ascertained. Also, the macro- and micro-nutrient concentration of dried shoots and roots were microwaved, digested with nitric acid, and analysed on the ICP-MS at Environmental Analysis Laboratory, Southern Cross University, Lismore, NSW: <http://scu.edu.au/eal/>; accessed March 2017.



Figure 5.1 Different P fertilisers, sown at 1cm soil depth, Podosol

Post-harvested soils were transferred to the laboratory and analysed for pH by suspension of an air-dried soil sub-sample (5g) in deionised water (1:5 w/v) using a TPS WP81 meter and probe (TPS Pty Ltd, Springwood, QLD), as well as soil-available P (Colwell)(Rayment and Lyons, 2011) and total P by X-Ray Fractionation (XRF) method.

5.2.5 Calculation and data analysis

All data was analysed using JMP statistical software (JMP®, Version 14; SAS Institute Inc., Cary, NC, USA). One-way ANOVA was used to investigate the effect of HBP fertilisers and time on soils water-extracted P and post-harvest available P. Tukey's honestly significant difference (HSD) was used where significant differences were found between different treatments.



Figure 5.2 Twelve weeks Ryegrass (*Lolium multiflorum* Lam.) plants in Podosol

5.3 Results

5.3.1 Ferrosol

Data analysis by ANOVA showed no significant beneficial effect ($p < 0.05$) of HBPs fertiliser on ryegrass dried biomass (Table 5.2) neither on post-harvest available P. ANOVA indicated no significant ($P < 0.05$) difference between TSP and HBPs fertilisers in shoot nutrients uptake (Table 5.2).

Table 5.2 ANOVA summary table for ryegrass dried biomass weight. Fertiliser's effects is presented.

* $P < 0.05$; ** $P < 0.01$; n.s., not significant at $P < 0.05$

Variabales	Fertilisers
Shoot dried weight (g/pot)	n.s
Root dried weight (g/pot)	n.s
Post-harvest Colwell P (mg/kg)	n.s
N in shoot (mg/g)	n.s
P in shoot (mg/g)	n.s
K in shoot (mg/g)	n.s
Ca in shoot (mg/g)	n.s
Mg in shoot (mg/g)	n.s
Na in shoot (mg/g)	n.s
Cu mg/kg	n.s
Zn mg/kg	n.s
Mn mg/kg	n.s
Fe mg/kg	n.s

The application of HBP2 showed 1.9 g/pot of ryegrass shoot dried weight (SDW) and HBP1 with 1.2 g/pot showed the lowest SDW.

No significant difference was observed between TSP and HBPs on root dried weight (RDW).

While application of TSP resulted in 0.5 mg/pot of RDW, HBP1 with 0.3 mg/pot showed the lowest RDW.

Regarding to ryegrass nutrients uptake, the values are shown in Table 5.3.

Table 5.3 Effect of different P fertilisers on shoot nutrients concentrations in Ferrosol. Mean values are presented (n=5).

	P	N	K	S	Ca	Mg	Na	Cu	Zn	Mn	Fe
	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	mg/kg	mg/kg	mg/kg	mg/kg
TSP	3.1	64.1	76.4	4.8	11.9	5.7	3.2	13.6	72.8	359.1	1152.2
s.e	0.5	8.7	9.8	0.8	1.8	0.9	0.8	0.5	4.7	10.4	203.1
HBP1	3.4	60.4	78.8	4.7	9.6	5.6	2.1	13.0	69.9	377.5	1068.4
s.e	0.3	6.5	7.9	0.6	1.0	0.8	0.3	0.5	2.2	12.3	128.8
HBP2	3.4	66.1	74.1	5.1	10.8	5.9	3.0	12.6	73.3	362.8	1165.2
s.e	0.3	11.2	16.5	0.9	0.7	1.4	0.6	0.7	4.4	21.7	116.7
HBP3	3.5	65.9	76.7	5.3	10.4	6.0	3.3	12.7	72.9	372.6	1120.0
s.e	0.5	8.3	12.5	0.5	0.7	0.9	0.3	0.3	11.3	84.6	80.1

Analysis by ANOVA indicated that there was no significant difference ($p < 0.05$) in post-harvest soils available (Colwell P) between TSP and humate and HBPs fertilisers (Figure 5.3). However, there was a significant effect of fertilisation between control and treated soils. The lowest and post-harvest phosphorus belonged to the soil with no P fertiliser with 49.94 mg/kg.

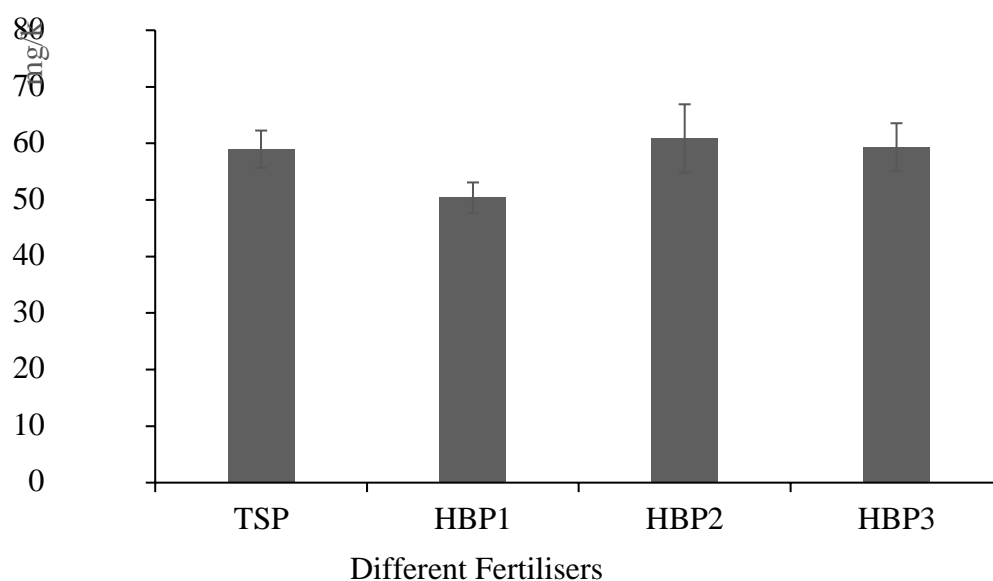


Figure 5.3 Post harvest Colwell P of Ferrosol under different P fertilisers. Data is expressed as mg phosphorus per kg soil. Means value are presented ($n=5$) and error bars present \pm s.e.

5.3.2 Podosol

Data analysis by ANOVA indicated that the fertilisers effect was not significant ($p < 0.05$) on ryegrass SDW and RDW in Podosol (Table 5.4). Although dried root biomass showed

Differences in response to the TSP and HBPs fertilisers, these differences were not significant statistically ($p < 0.05$) (Figure 5.4). The highest root biomass were observed in HBP2 with 0.61 gr/pot then HBP3 treatment with 0.51 gr/pot. The lowest dried root biomass belonged to TSP treatments with 0.42 gr/pot (Figure 5.5).

Table 5.4 ANOVA summary table for ryegrass dried biomass weight. Fertiliser's effects is presented. * $P < 0.05$; ** $P < 0.01$; n.s., not significant at $P < 0.05$

Variabales	Fertilisers
Shoot dried weight (g/pot)	n.s
Root dried weight (g/pot)	n.s
Post-harvest Colwell P (mg/kg)	n.s
N in shoot (mg/g)	n.s
P in shoot (mg/g)	n.s
K in shoot (mg/g)	n.s
Ca in shoot (mg/g)	n.s
Mg in shoot (mg/g)	n.s
Na in shoot (mg/g)	n.s
Cu mg/kg	n.s
Zn mg/kg	n.s
Mn mg/kg	n.s
Fe mg/kg	n.s

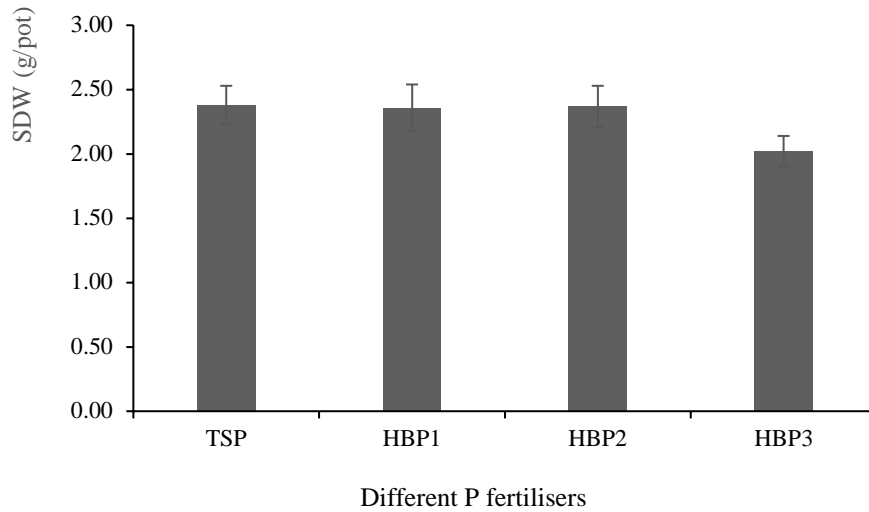


Figure 5.4 Ryegrass dried shoot weight (SDW) under different P fertilisers in Podosol. Data is expressed as g/pot. Mean values are presented (n=5) and error bars represent \pm s.e.

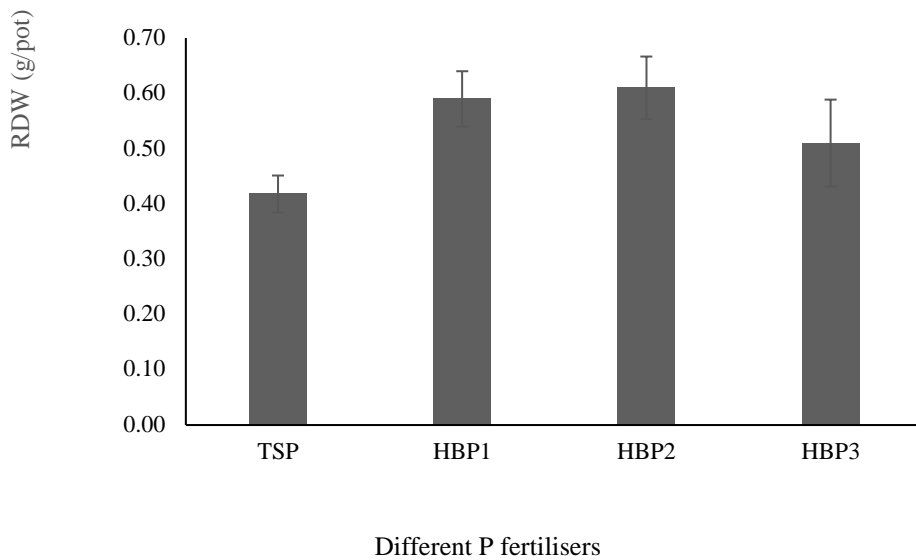


Figure 5.5 Ryegrass dried root weight (RDW) under different P fertilisers in Podosol. Data is expressed as g/pot. Mean values are presented (n=5) and error bars represent \pm s.e.

The ryegrass shoot nutrients concentration have not been affected by HBPs fertilisers (Table 5.5).

Table 5.5 Effect of different P fertilisers on shoot nutrients concentrations in Pododsol. Mean values are presented (n=5).

	N	P	K	S	Ca	Mg	Na	Cu	Zn	Mn	Fe
	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	mg/kg	mg/kg	mg/kg	mg/kg
TSP	75.8	4.5	66.8	6.6	13.8	11.0	25.4	10.6	70.5	209.2	146.9
Ste	3.5	0.3	3.3	0.6	0.6	0.8	1.9	0.1	5.6	7.9	6.8
HBP1	76.2	5.0	61.3	6.6	13.2	10.1	25.0	10.0	62.2	197.9	158.0
Ste	7.5	0.5	4.1	0.5	1.2	0.8	1.8	0.3	5.0	9.4	8.3
HBP2	77.2	4.6	65.6	7.3	13.3	10.7	22.7	10.9	63.4	223.0	155.3
Ste	5.2	0.5	1.6	0.5	1.1	0.6	3.9	0.5	5.0	13.0	10.3
HBP3	72.7	4.9	64.2	6.6	9.7	8.1	19.4	9.8	63.1	217.6	125.2
s.e	4.5	0.3	3.4	0.5	0.4	0.5	1.3	0.5	9.6	10.7	5.7

Analysis by ANOVA indicated that there was not a significant difference ($p < 0.05$) in post-harvest soils available (Colwell P) between TSP and HBPs fertilisers (Figure 5.6).

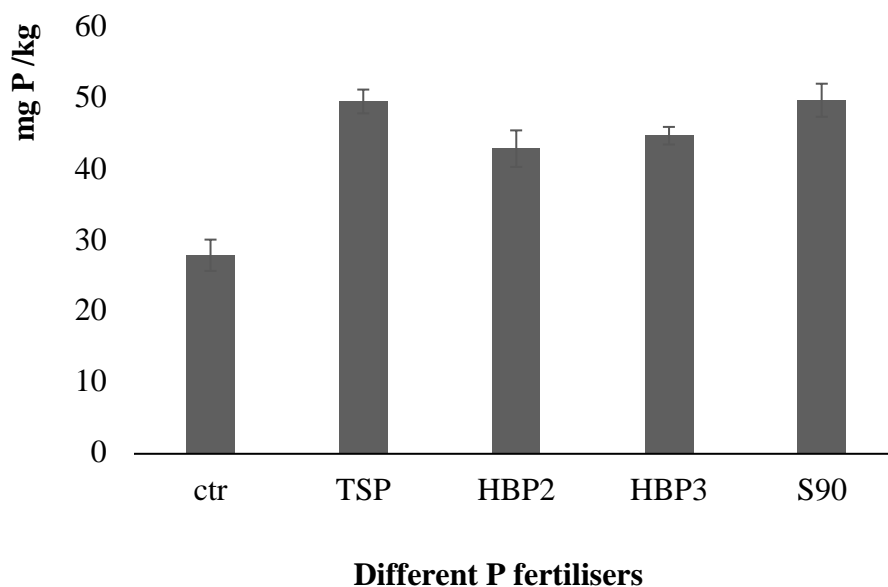


Figure 5.6 Post harvest Colwell P of Podosol under different P fertilisers. Data is expressed as mg phosphorus per kg soil. Means value are presented (n=5) and error bars present + s.e.

5.4 Discussion

5.4.1 Ryegrass dried biomass and nutrients uptake in Ferrosol

Ferrosol was selected for this study because of its high P-fixing capability. It was needed to select a Victorian soil with the most P availability limitation. In Victoria most Ferrosol is located in pasture or field crops that have been regularly fertilised with NPK. For this reason it was extremely difficult to find a soil site exhibiting both high P availability and low fertilisation and organic matter content. The high organic matter and available P concentration of Victorian soil might affect biomass weight and P uptake.

The blended phosphate fertilisers did not improve P and other nutrient uptake and ryegrass shoot and root biomass. This finding suggests that if application of humate has not increased the soil-available P, plant growth response would not have been observed. It was hypothesised that the addition of humate substances (K-humate in this study) would improve the P uptake and plant growth and plant nutrient concentration (Kumar and Singh, 2017a) (Tripura et al., 2017) in Ferrosol by inhibiting Fe-phosphate (Fe-P) precipitation rates (Grossl and Inskeep 1991, 1992; Inskeep and Silvertooth 1988), competing for adsorption sites (Sibanda and Young 1986), and/or promoting dissolution of metal solid compounds via chelation (Guppy et al. 2005).

There was a slight increase of dried shoot biomass of ryegrass in HBP2 treatment but the statistic's mean comparison did not show this difference significantly ($p < 0.05$). Also, Ryegrass dried root biomass did not affected by HBP fertilisers. One of the possible reasons of not increasing the dried shoot and root biomass of HBP treatment is the high concentration of soil organic matter. Similar reason was identified in a glasshouse study with application of unknown carbond P fertilisers on an industrial crop (Summerhays et al., 2015) and an

incubation study in which HA derived from Leonardite shale applied with P fertilisers to the soil with high organic matter (Hartz and Bottoms, 2010). The Ferrosol of this study was collected from a dairy pasture which had a high soil organic matter. Application of different HBP fertilisers at the same P application rate (25k/h), resulted in application of a high range humate carbon (8 to 39 kg/h). Some studies have indicated root and shoot inhibition by higher humate application rate (Sladký and Tichý, 1959). (Little et al., 2013) reported the highest pasture growth was obtained at 4 and 20 kg/h humate and by increasing the application rate to 50 and 100 kg/ha no benefit was observed in term of plant growth.

Previous studies indicate variable shoot and root growth benefit from humic substances in soils with different organic matter content with more growth effects in soil with low organic matter (Kunkel and Holstad, 1968, Lee and Bartlett, 1976, Fagbenro and Agboola, 1993)

Another possibility in which no significant effect was observed from HBP fertiliser may be the plant species. (Akinremi et al., 2000, Lodhi et al., 2013). A similar result was obtained in a glasshouse study in which the similar humate products have been applied in a ryegrass study (Little et al., 2014).

The lack of uptake response in the ryegrass shoot nutrients is in total agreement with a study in which no change were observed in ryegrass nutrient concentration after application of humate substances in different application rates and partial agreement with a study in which no beneficial effect was observed in tomato nitrogen, potassium and iron concentration after application of humate substances (Adani et al., 1998).

5.4.2 Ryegrass dried biomass and nutrient uptake in Podsol

Podsol as a low organic matter soil was selected for this study because published studies indicated that humate effects can be masked in high organic matter soils (Summerhays et al., 2015, Hill et al., 2015b). Uptake of P was about similar in TSP and HBPs likely due to the high

concentration of background soil-available P and organic matter that prevented fertilisation from being statistically different between treatments.

Although higher dried biomass range was observed in Podosol compared with Ferrosol, phosphate fertiliser did not show beneficial effects on Ryegrass shoot biomass. The higher dried biomass range in Podosol is likely due to lower Fe and Al contents in comparison with Ferrosol. One possibility that Ryegrass dried shoot biomass did not improve is ryegrass is less responsive to P fertilisers (Schindler et al., 2016). Forage grass dried matter yield does not always respond to P fertilisers (Bélanger et al., 2017, Valkama et al., 2014, Valkama et al., 2016). A study results indicated that the grass P requirement is less than clover (Barrow, 1975, Simpson et al., 2014). So, it is likely that Ryegrass needed higher P application rate to respond to different P fertilisers.

Another reason that probably contributed to not dried shoot biomass respond to P fertilisers, is ryegrass harvesting time. Ryegrass were harvested at early growth stage (12 weeks after planting). A study results have indicated that the ryegrass highest P demand usually happens at late growth stage (Ozanne et al., 1969). In another study ryegrass responded well to application of the same humic substances after 112 days after starting the study (Little, 2017).

5.5 Conclusion

The beneficial effects of K-humate substances on soil phosphorus availability for pasture uptake are likely more at Ferrosol with high Fe and Al concentration comparing with Podosol. In order to see the real benefit of use of humate-blended phosphate fertilisers, it is needed to investigate them on soils with low available P content as well as low organic matter on different crops.

5.6 References

- ADANI, F., GENEVINI, P., ZACCHEO, P. & ZOCCHI, G. 1998. The effect of commercial humic acid on tomato plant growth and mineral nutrition. *Journal of plant nutrition*, 21, 561–575.
- AKINREMI, O., JANZEN, H., LEMKE, R. & LARNEY, F. 2000. Response of canola, wheat and green beans to Leonardite additions. *Canadian Journal of Soil Science*, 80, 437–443.
- ASGHARI, H. R. & CAVAGNARO, T. R. 2012. Arbuscular mycorrhizas reduce nitrogen loss via leaching. *PLoS one*, 7, e29825.
- BALIGAR, V., HE, Z., MARTENS, D., RITCHEY, K. & KEMPER, W. 1997. Effect of phosphate rock, coal combustion by-product, lime, and cellulose on ryegrass in an acidic soil. *Plant and soil*, 195, 129–136.
- BARROW, N. 1975. The response to phosphate of two annual pasture species. I. Effect of the soil's ability to adsorb phosphate on comparative phosphate requirement. *Crop and Pasture Science*, 26, 137–143.
- BÉLANGER, G., ZIADI, N., LAJEUNESSE, J., JOUANY, C., VIRKAJARVI, P., SINAJ, S. & NYIRANEZA, J. 2017. Shoot growth and phosphorus–nitrogen relationship of grassland swards in response to mineral phosphorus fertilisation. *Field Crops Research*, 204, 31–41.
- DANESHVAR HAKIMI MAIBODI, N., KAFI, M., NIKBAKHT, A. & REJALI, F. 2015. Effect of Foliar Applications of Humic Acid on Growth, Visual Quality, Nutrients Content and Root Parameters of Perennial Ryegrass (*Lolium Perenne* L.). *Journal of Plant Nutrition*, 38, 224–236.
- FAGBENRO, J. & AGBOOLA, A. 1993. Effect of different levels of humic acid on the growth and nutrient uptake of teak seedlings. *Journal of Plant Nutrition*, 16, 1465–1483.
- FERNÁNDEZ, L., BAIGORRI, R., URRUTIA, O., ERRO, J., APARICIO-TEJO, P., YVIN, J. & GARCÍA-MINA, J. 2016a. Improving the short-term efficiency of rock phosphate-based fertilisers in pastures by using edaphic biostimulants. *Chemical and Biological Technologies in Agriculture*, 3, 1–9.

FERNÁNDEZ, L., BAIGORRI, R., URRUTIA, O., ERRO, J., APARICIO-TEJO, P. M., YVIN, J. C. & GARCÍA-MINA, J. M. 2016b. Improving the short-term efficiency of rock phosphate-based fertilisers in pastures by using edaphic biostimulants. *Chemical and Biological Technologies in Agriculture*, 3, 5.

GARCIA, M., DIEZ, J., VALLEJO, A., GARCIA, L. & CARTAGENA, M. C. 1997. Effect of applying soluble and coated phosphate fertilisers on phosphate availability in calcareous soils and on P absorption by a rye-grass crop. *Journal of Agricultural and Food Chemistry*, 45, 1931–1936.

HARTZ, T. K. & BOTTOMS, T. G. 2010. Humic substances generally ineffective in improving vegetable crop nutrient uptake or productivity. *HortScience*, 45, 906–910.

HILL, M. W., HOPKINS, B. G., JOLLEY, V. D. & WEBB, B. L. 2015. Phosphorus Mobility Through Soil Increased with Organic Acid-Bonded Phosphorus Fertiliser (Carbond® P). *Journal of Plant Nutrition*, 38, 1416–1426.

JORQUERA, M. A., MARTÍNEZ, O. A., MARILEO, L. G., ACUÑA, J. J., SAGGAR, S. & MORA, M. L. 2014. Effect of nitrogen and phosphorus fertilisation on the composition of rhizobacterial communities of two Chilean Andisol pastures. *World Journal of Microbiology and Biotechnology*, 30, 99–107.

KUMAR, D. & SINGH, A. 2017. Efficacy of Potassium Humate and Chemical Fertilisers on Yield and Nutrient Availability Pattern in Soil at Different Growth Stages of Rice. *Communications in Soil Science and Plant Analysis*.

KUNKEL, R. & HOLSTAD, N. 1968. Effects of adding humates to the fertiliser on the yield and quality of Russet Burbank potatoes. *American Potato Journal*, 45, 449–457.

LAGOS, L. M., ACUÑA, J. J., MARUYAMA, F., OGRAM, A., DE LA LUZ MORA, M. & JORQUERA, M. A. 2016. Effect of phosphorus addition on total and alkaline phosphomonoesterase-harboring bacterial populations in ryegrass rhizosphere microsites. *Biology and Fertility of Soils*, 52, 1007–1019.

LEE, Y. S. & BARTLETT, R. J. 1976. Stimulation of plant growth by humic substances 1. *Soil Science Society of America Journal*, 40, 876–879.

LITTLE, K., ROSE, M., PATTI, A., CAVAGNARO, T. & JACKSON, R. 2013. Effect of application rate of commercial lignite coal-derived amendments on early-stage growth of *Medicago sativa* and soil health, in acidic soil conditions. *Functions of Natural Organic Matter in Changing Environment*. Springer.

LITTLE, K. R., ROSE, M. T., JACKSON, W. R., CAVAGNARO, T. R. & PATTI, A. F. 2014. Do lignite-derived organic amendments improve early-stage pasture growth and key soil biological and physicochemical properties? *Crop and Pasture Science*, 65, 899–910.

LODHI, A., TAHIR, S., IQBAL, Z., MAHMOOD, A., AKHTAR, M., QURESHI, T. M., YAQUB, M. & NAEEM, A. 2013. Characterization of commercial humic acid samples and their impact on growth of fungi and plants. *Pakistan journal of soil science*, 32, 63–70.

MENDOZA, R., GARCÍA, I., DEPLAMA, D. & LÓPEZ, C. F. 2016. Competition and growth of a grass–legume mixture fertilised with nitrogen and phosphorus: effect on nutrient acquisition, root morphology and symbiosis with soil microorganisms. *Crop and Pasture Science*, 67, 629–640.

NIKBAKHT, A., PESSARAKLI, M., DANESHVAR-HAKIMI-MAIBODI, N. & KAFI, M. 2014. Perennial ryegrass growth responses to mycorrhizal infection and humic acid treatments. *Agronomy Journal*, 106, 585–595.

OZANNE, P., KEAY, J. & BIDDISCOMBE, E. 1969. The comparative applied phosphate requirements of eight annual pasture species. *Australian Journal of Agricultural Research*, 20, 809–818.

RAYMENT, G. E. & LYONS, D. J. 2011. *Soil chemical methods: Australasia*, CSIRO publishing.

M. 2016. Reducing phosphorus to curb lake eutrophication is a success. ACS Publications.

SIMPSON, R. J., RICHARDSON, A. E., NICHOLS, S. N. & CRUSH, J. R. 2014. Pastureplants and soil fertility management to improve the efficiency of phosphorus fertiliser use in temperate grassland systems. *Crop and Pasture Science*, 65, 556–575.

SLADKÝ, Z. & TICHÝ, V. 1959. Application of humus substances to overground organs of plants. *Biologia Plantarum*, 1, 9–15.

SUMMERHAYS, J. S., HOPKINS, B. G., JOLLEY, V. D., HILL, M. W., RANSOM, C. J. & BROWN, T. R. 2015. Enhanced phosphorus fertiliser (Carbond P®) supplied to maize in moderate and high organic matter soils. *Journal of Plant Nutrition*, 38, 1359–1371.

TRIPURA, P., KUMAR, S. & VERMA, R. 2017. Effect of Potassium Humate and Bio-inoculants on Nutrient Content, Uptake and Quality of Cowpea (*Vigna unguiculata* (L.) Walp). *Int. J. Curr. Microbiol. App. Sci*, 6, 1735–1741.

VALKAMA, E., VIRKAJÄRVI, P., UUSITALO, R., YLIVAINIO, K. & TURTOLA, E. 2014. Phosphorus fertilisation and herbage production in Finland. *Maataloustieteen Päivät 2014*, 8.-9.1. 2014 Viikki, Helsinki: esitelmät-ja posteritiivistelmät/Toim. Risto Kuisma, Nina Schulman, Hanna-Riitta Kymäläinen ja Laura Alakukku.

VALKAMA, E., VIRKAJÄRVI, P., UUSITALO, R., YLIVAINIO, K. & TURTOLA, E. 2016. Meta-analysis of grass ley response to phosphorus fertilisation in Finland. *Grass and forage science*, 71, 36–53.

WEAVER, D. M. & WONG, M. T. F. 2011. Scope to improve phosphorus (P) management and balance efficiency of crop and pasture soils with contrasting P status and buffering indices. *Plant and Soil*, 349, 37–54.

Chapter 6

Effect of humate-blended phosphate on plant growth

6.1 Introduction

Significant attention has been given to innovate and improve technologies for brown coal (BC) or/and BC derived substance utilization in fertilization systems in the agricultural sector. It is well established that humic substances, which are derived from BC, can improve nutrients uptake as well as plant growth under some conditions (Tahir et al., 2011, Kumar and Singh, 2017, Little et al., 2014). Potassium humate (K-humate) that is the alkaline salt of humic acid of BC is sold in Australia as a soil amendment to improve nutrient availability in soils. K-humate has the potential to improve soil properties and nutrients dynamics (Turgay et al., 2011). The natural accumulation of K-humate in the soil reduces the need for commercial fertilizers because of proposed positive effects on chemical fertilizers efficiency (Kumar et al., 2013). However, a comparison of different studies indicated that the BC derived humate varied in effects depending on the plant, soil type and application rate. The majority of these studies investigated humate substances as soil amendments. Very few studies have been conducted with manufactured humate-blended fertilizers to enhance P fertilizer efficiency. This is likely due to the limited knowledge of manufacturing technology of the combined fertilizers, and their agronomical and economical effects. (Erro et al., 2016). Although the investigation of incorporated humate-chemical fertilizers is scarce, some studies have been conducted on this matter. A humate containing product (Carbond P[®]) (unknown carbon source) was investigated to evaluate P uptake and plant growth (Hill et al., 2015a, Hill et al., 2015b, Hopkins and Stark, 2003, Hopkins et al., 2008, Summerhays et al., 2015) and the results indicated that utilization of blended/mixed P fertilizers with organic matter did not always result in significant plant yield increases. In another study, the effect of coating diammonium phosphate (DAP) pellets with a resin on P-fixing in calcareous soil was investigated, resulting in P-fixing decrease with 22% resin coated DAP (Diez et al., 1992, Garcia et al., 1997). Integrated plant nutrient systems

(IPNS) (organic-inorganic fertilizer) were applied in a cabbage field study and the results revealed that the marketable yield of cabbage was increased by IPNS fertilizers (Islam et al., 2017). In another study, bio-fertilizers (BF) combined with chemical fertilizers (CF) were tested on cotton yield and the results showed a significant increase in soil total nitrogen, available phosphorus, available potassium and soil organic carbon when plants received 60% CF+ BF compared with plants which received just CF (Li et al., 2017). Sesame (*Sesamum indicum L.*) yield was significantly improved when N and P fertilisers were applied in combination with farmyard manure (Ketema, Dadi Waka and Nigussie, Dechassa, 2016). Some other studies have indicated that humic-micronutrients complexed fertilizers can be used to improve the micronutrients uptake, although their efficiency was limited (Chen and Aviad, 1990, Garcia-Mina, 2006).

Here, in this study humate-blended phosphate fertilisers (HBP) were investigated, first on Radish (*Raphanus sativus.*) and then Sweet Corn (*Zea Mays L.*) Growth and nutrient uptake in two different acidic soils in two separate glasshouse trials were conducted. Radish (*Raphanus sativus*) was chosen for this study because it is a root vegetable and phosphorus is vital for root development (Sanchez et al., 1991). A second glasshouse study was conducted as an extension based on the radish growth experiment. The HBPs fertiliser were investigated on corn (*Zea Maize L.*) growth and nutrients uptake. Glasshouses studies will be presented in two parts separately.

The aim of the studies was to see whether blending the common chemical phosphate fertiliser (TSP in this study) with brown coal derived humate would increase the P use efficiency and uptake in two different plant species in comparison with TSP alone. In addition, the effect of the humate-blended phosphate fertilisers on plant growth was investigated.

Glasshouse study 1: Radish (*Raphanus sativus.*) growth

6.2 Methods and Materials

6.2.1 Fertilisers

Two granular humate blended phosphate fertiliser were selected for this study (Table 6.1). The characterisation of all humate blended fertiliser was presented in chapter 2 of this thesis.

Table 6.1 Fertilisers characteristics

Fertiliser	Ingredients	%P ^a	%C ^b
TSP	Triple superphosphate	17.1	0
HBP 2	TSP+Humate	14.6	12.7
HBP 3	TSP+Humate	12.9	16.6

a) Total elemental phosphorus by X-Ray Fluorescence, b) Total Carbon by CHN analyser

6.2.2 Soils characterisation

A Ferrosol (Isbell, 2002) soil was chosen for this study, the same soil as previously used (See chapter 3, Table 3.1). Some of the characteristics of the Ferrosol are also shown in Table 3.1.

6.2.3 Experimental design and treatments

Radish (*Raphanus sativus.*) seeds were planted at the surface depth of pots, which had been packed with about 1.5 kg sieved soil to reach to the bulk density of the field (1 g/cm³). Pots were arranged in a complete randomized design with their position rotated every second day (Figure 6.1). The plants had been thinned to one plant per pot after germination (Figure 6.2).

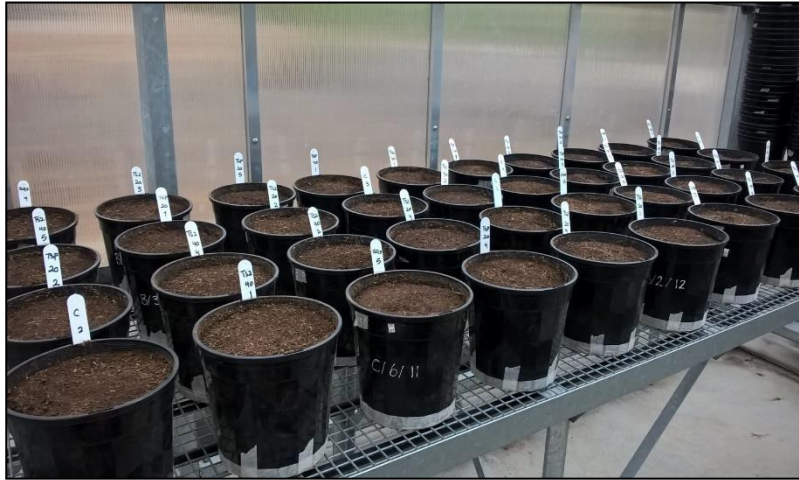


Figure 6.1 Plastic pots that were packed with 1.5 kg Ferrosol.



Figure 6.2 Plants thinned to one plant per pot.

Nitrogen (N) and potassium (K) were mixed through with the top 5 cm soil as urea and potash at 100 and 20 kg/ha, respectively. The pots were irrigated every other day to maintain the soil water at 60% field capacity moisture. The determination of field capacity moisture has been explained in chapter 5, section 4.2.3. Granular phosphate fertilisers were applied in a band just under the radish seeds at depth of 2 mm. TSP and HBPs were applied in three P rates of 0, 20 and 40 kg/ha.. There were five replicates of each treatment (Figure 6.3).



Figure 6.3 Pots were arranged in a complete randomized design.

The study was conducted in the glasshouse complex at Monash University Clayton Campus. Light and temperature conditions in the glasshouse were controlled. The light was maintained at a high level with supplemental lighting (16 h/d) and averaged $220+17 \mu\text{mol m}^{-2} \text{s}^{-1}$. The temperature was $25.2+1.8 ^\circ\text{C}$ during the day and $23.3+1.4 ^\circ\text{C}$ at night. Radishes were harvested after 5 weeks (Figure 6.4) and analyzed for fresh and dried root and shoot biomass, number of leaves, radish diameters (the widest part of radish), root depth (distance from soil surface to the end of the root). The samples have been sent to a laboratory to analysis macro and micro nutrients in radish bulbs based on plant dried weight. (Environmental Analysis Laboratory, Southern Cross University, Lismore, NSW; (<http://scu.edu.au/eal/>) (Accessed March 2017). Post-harvest soil pH was determined by suspending an air-dried soil sub-sample (5g) in deionized water (1:5 w/v) using TSP WP81 meter and probe (TSP Pty Ltd, Springwood, QLD).



Figure 6.4 Radish plant after five weeks

6.2.4 Calculation and data analysis

All data was analyzed using JMP statistical software (JMP®, Version 14; SAS Institute Inc., Cary, NC, USA). Two-way ANOVA was used to investigate the effect of HBP fertilisers with two application rates on plant biomass and shoot nutrient content as well as soil nutrient uptake, post harvested available and total P and soil pH. Both main effects and their interaction have been analyzed. Tukey's honest significant difference (HSD) was used where significant differences were found between different treatments.

6.3 Results

6.3.1 Radish growth parameters

The ANOVA table for all radish growth indexes are presented in Table 6.2. The table indicated that while the main factor of application rate has a significant effect on all radish growth parameters, the main factor of different P fertiliser had a significant effect just on radish shoot dried weight (SDW) and root depth. The ANOVA also showed a significant interaction of main factors on radish SDW and root depth.

Table 6.2 ANOVA summary table for all variables. Both main effects and their interaction terms are presented.

*P<0.05; **P<0.01; n.s., not significant at P<0.05

Source	Root dried weig	Shoot dried weight	Number of leaves	Root depth	Radish bulb diameter
Fertiliser	n.s	*	n.s	*	n.s
Application rate	**	**	**	**	*
Fertiliser * application rate	n.s	*	n.s	*	n.s

6.3.1.1 Radish root dried weight

The ANOVA indicated a significant difference ($p < 0.05$) between control treatments which had not received P fertilisers and fertilised treatments which received P fertilisers at rates of 20 and 40 kg/ha (Figure 6.5). While the control treatment showed the lowest RDW with 1.4 g/pot, application rates of 20 and 40 kg/ha resulted in the higher RDW with 2.9 and 2.8 g/pot, respectively. There was no significant difference ($p < 0.05$) between TSP and HBPs in RDW. In addition, ANOVA indicated there was not a significant interaction effect of different treatments and different application rates ($p < 0.05$) on RDW.

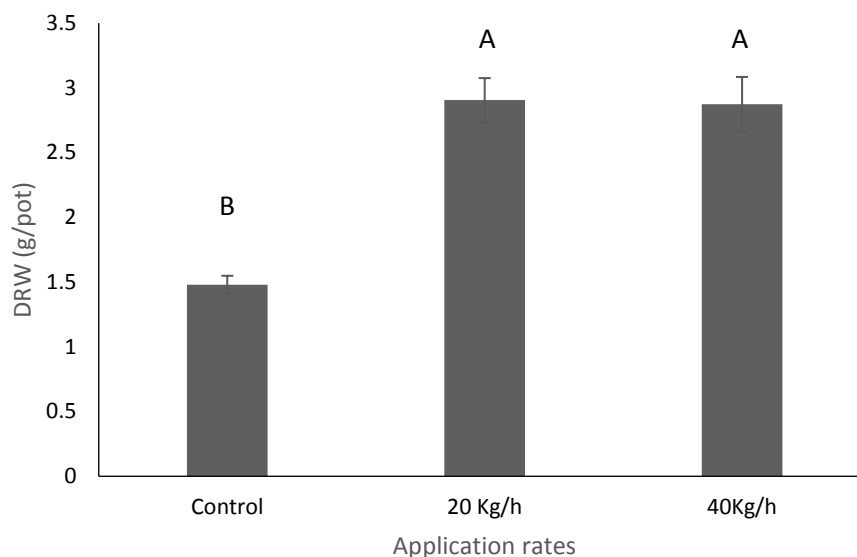


Figure 6.5 Radish shoot dry weight (SDW) in response to the application rates. Mean values are presented (n=15). Error bars are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

6.3.1.2 Radish shoot dried weight

Data analysis by ANOVA indicated that the different P fertilisers had a significant effect on SDW (Figure 6.6). TSP showed the highest SDW with 2.8 g/pot and control with 1g/pot showed the lowest SDW.

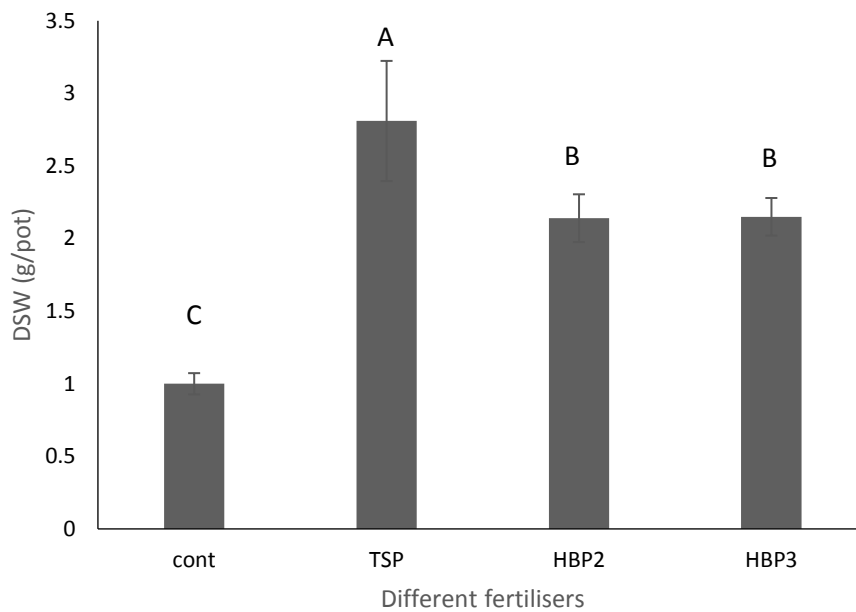


Figure 6.6 Shoot dried weight (SDW) under different P fertilisers. Mean values are presented (n=10) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

In addition, data analysis by ANOVA indicated a significant effect of application rate on SDW. In the presence of different P fertilisers, an application rate of 40 kg/ha resulted in the highest SDW with 2.6 g/pot (Figure 6.7).

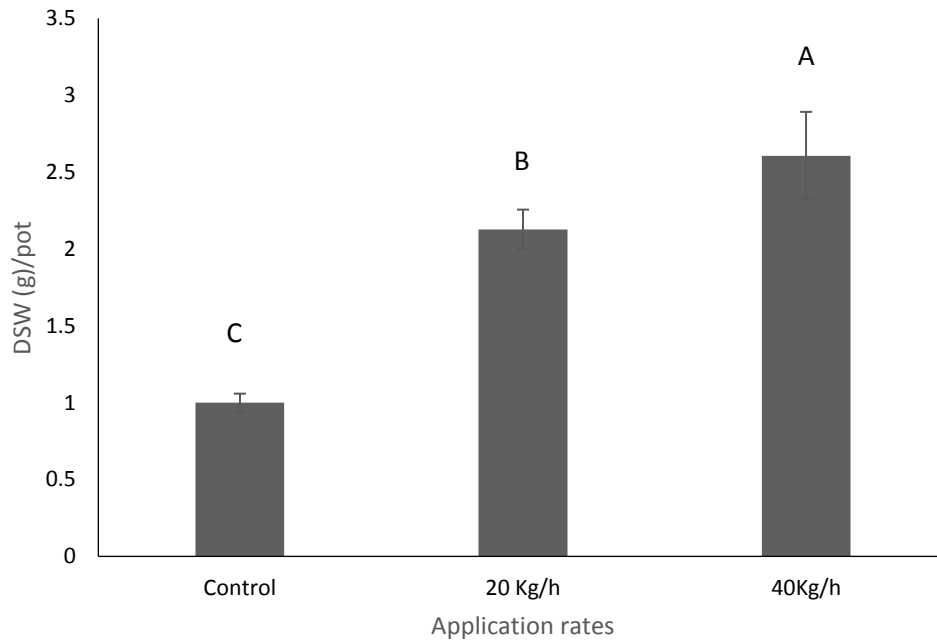


Figure 6.7 Shoot dried weight (SDW) under different application rates. Mean values are presented (n=15) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

ANOVA indicated a significant interaction ($p < 0.05$) between P fertilisers and application rates on SDW, with fertiliser TSP with application rate of 40 kg/ha showed the highest SDW with 3.3 g/pot and all P fertilisers with 0 kg/ha application rate indicated the lowest SDW with 1.4 g/pot (Figure 6.8).

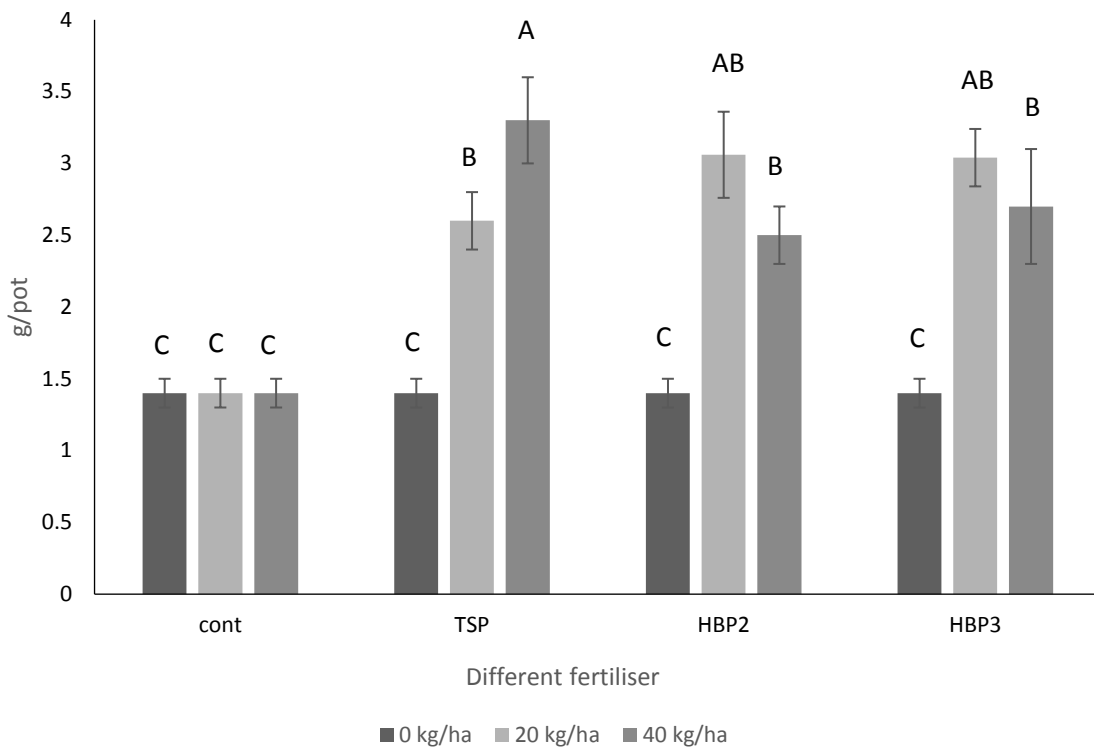


Figure 6.8 Interaction effect of different fertilisers and application rates on SDW. Mean values are presented ($n=5$) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey’s HSD

6.3.1.3 Radish number of leaves

Data analysis by ANOVA indicated that while different fertilisers did not have any significant effect on number of leaves, the application rate had a significant effect on the number of Radish leaves (Figure 6.9). The control treatment showed 8 leaves per plant while 20 and 40 k/h rate showed higher number of leaves per plant with 11.8 and 11, respectively.

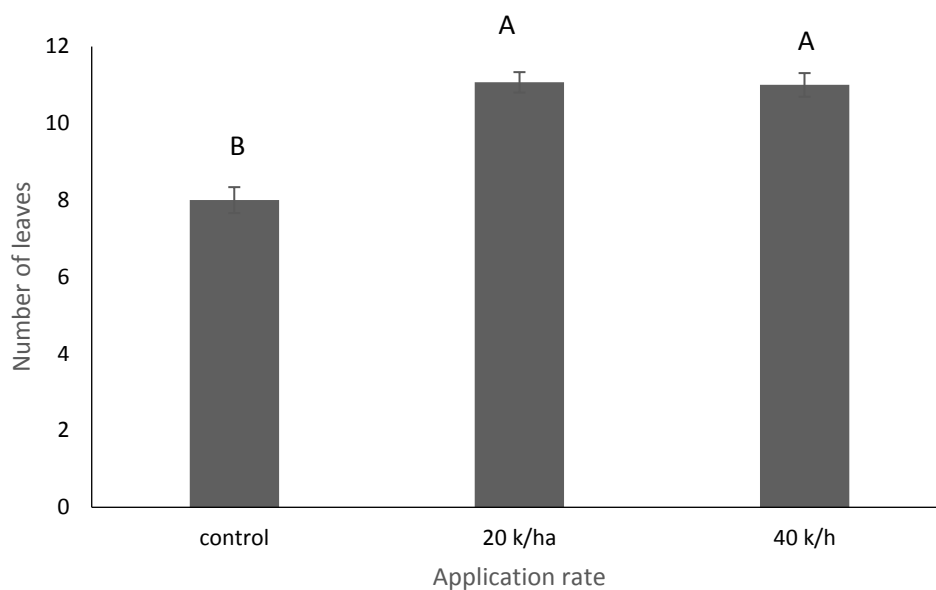


Figure 6.9 Radish number of leaves under different application rates. Mean values are presented (n=15) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

6.3.1.4 Radish bulb diameter

Data analysis by ANOVA showed there was just a significant difference between application rates for bulb diameter (6.10). Application of fertilisers at the rates of 20 and 40 k/ha resulted in 4.9 and 4.7 cm of radish bulb diameter while the control treatments resulted in bulb diameter at 3.8 cm.

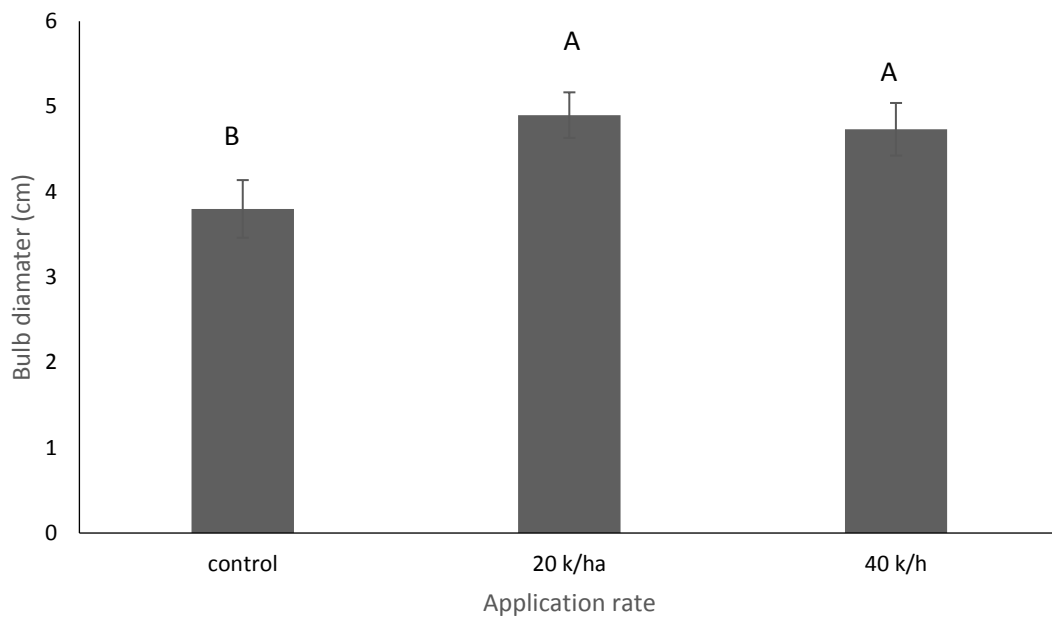


Figure 6.10 Radish bulb diameter under different application rates. Mean values are presented (n=15) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

6.3.1.4 Radish root depth

ANOVA indicated a significant effect of both main factors application rate and P fertilisers on radish root depth as well as a significant interaction term. With Regard to the effect of different P fertilisers, the application of TSP significantly increase the root depth to 12.05 cm and the control treatment showed the lowest root depth, 7.6 cm (Figure 6.11).

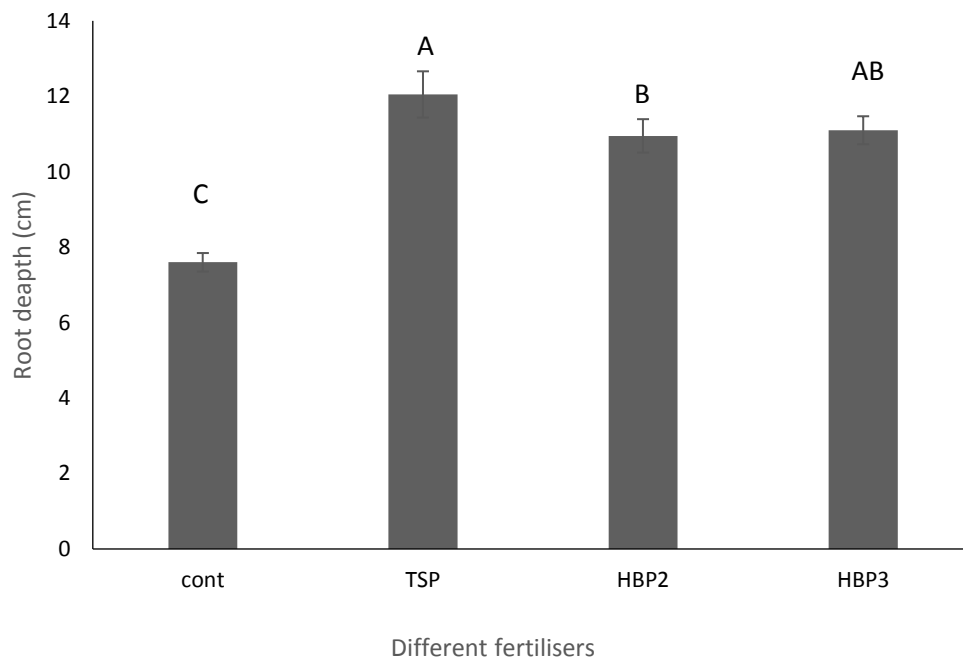


Figure 6.11 Radish root depth under different fertilisers. Mean values are presented (n=10) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

Application of the fertilisers at both rates of 20 and 40 k/h resulted in a significant higher root depth with 11 and 11.7 leaves/pot in comparison with the control treatment with 7.6 leaves /pot (Figure 6.12).

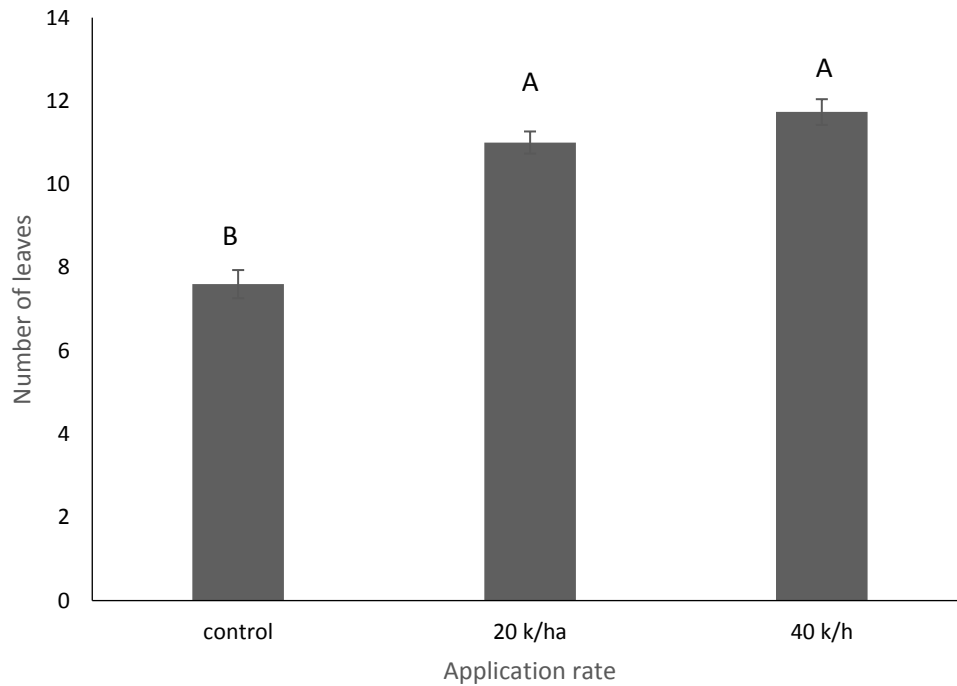


Figure 6.12 Radish root depth under different application rates. Mean values are presented (n=15) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

In addition, the interaction between application rates and different P fertilisers was significant on radish root length. The longest root depth was observed in TSP at the application rate of 40 kg/ha with 13.6 cm and the shortest root depth was observed in control treatment with 7.6 cm (Figure 6.13).

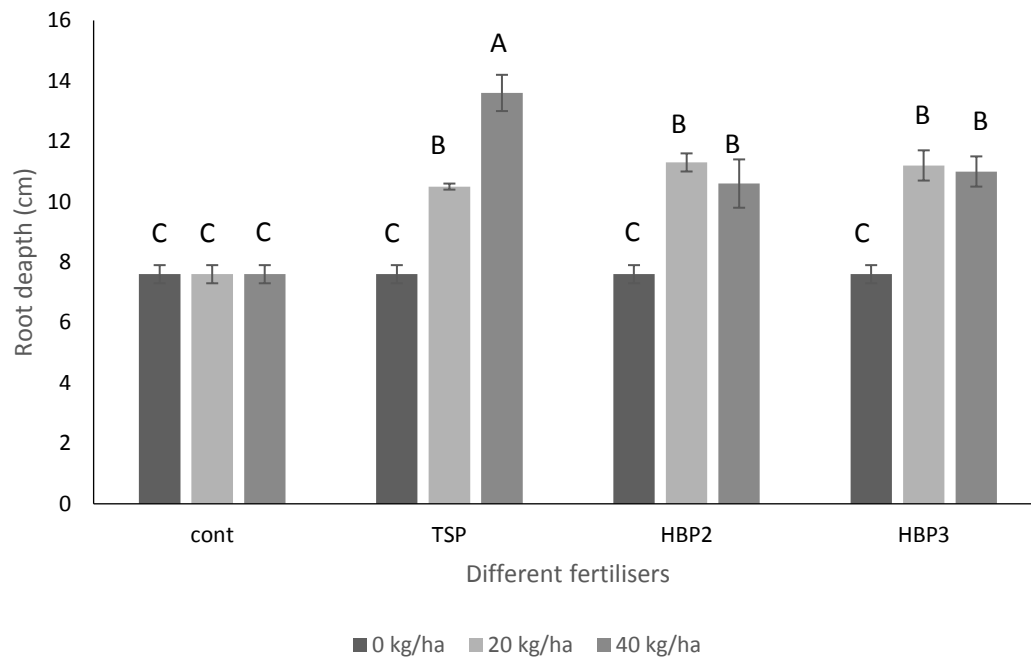


Figure 6.13 Interaction effect of application rate and P fertilisers on root depth. Mean values are presented (n=5) and error bars represent \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey’s HSD

6.3.2 Radish bulbs nutrients uptake

Data analysis by ANOVA demonstrated that the different P fertilisers did not have a significant effect on nutrients concentration in radish bulbs (Table 6.3). Application rates of 20 and 40 kg/ha significantly increased the nutrients concentration in radish bulbs in comparison with control treatments which have not been received any P fertilisers. In addition, the interaction effect was not significant on bulb nutrient concentration.

Table 6.3 ANOVA summary table for all nutrients concentration in radish bulbs (dried weight).

Both main effects and their interaction terms are presented.*P<0.05; **P<0.01; n.s., not significant at P<0.05

Elements	N	P	K	S	C	Ca	Mg	Na	Cu	Zn	Mn	Fe
	mg/ g	mg /g	mg/ g	mg/ g	mg/ g	mg/ g	mg/ g	mg/ g	mg/ k	mg/ k	mg/ k	mg/ k
P fertilisres	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Application rate	**	**	**	**	**	**	**	**	n.s	**	**	n.s
P fertilizer*application rate	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

With respect to application rate effect, data analysis by ANOVA showed the application of the phosphate fertilisers (all types) at both 20 and 40 kg/ha rates, significantly increased the radish bulb nutrients concentration compared with control (0 kg/ha) except for zinc (Zn) which significantly decreased from 50 mg/g in control treatment to 36.5 and 36.5 mg/g, respectively. (Table 6.4).

Table 6.4 Effect of different application rates on radish bulb nutrients concentration. Mean values are presented (n=5) and values in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $P < 0.05$ level as assessed by Tukey's HSD

Application rate	N mg/g	P mg/g	K mg/g	S mg/g	C mg/g	Ca mg/g	Mg mg/g	Na mg/g	Zn mg/kg	Mn mg/kg
0 kg/ha	51.4 ^b (2.6)	2.5 ^b (0.1)	94.6 ^b (4.7)	12.9 ^b (0.8)	539 ^b (25.5)	9 ^b (0.7)	4.1 ^b (0.3)	2.9 ^b (0.1)	50 ^a (0.9)	16.7 ^b (0.4)
20 kg/ha	92.3 ^a (5.7)	9.1 ^a (0.4)	183.3 ^a (9.7)	19.4 ^a (0.9)	1083 ^a (64.1)	16.1 ^a (1.1)	6.9 ^a (0.4)	9.8 ^a (0.7)	36.5 ^b (1.4)	21.4 ^a (1.5)
40 kg/ha	86.5 ^a (6.4)	10.9 ^a (1.2)	171 ^a (12.5)	19.2 ^a (1.6)	1075 ^a (81.1)	15.8 ^a (1.2)	7 ^a (0.5)	10.2 ^a (0.8)	36.6 ^b (1.7)	26.8 ^a (3.5)

6.3.3 Radish shoot nutrients uptake

Analysis by ANOVA indicated a significant effect of different fertilisers on radish shoot N, P, C and Ca concentration. There was also a significant effect of application are on all shoot nutrients concentration except for zinc (Cu) and iron (Fe). The interaction of fertilisers and application rates were significant on shoot P, C, Ca and Mg concentration (Table 6.5).

Table 6.5 ANOVA summary table for all nutrients concentration in radish shoots (dried weight). Both main effects and their interaction terms are presented.*P<0.05; **P<0.01; n.s., not significant at P<0.05

Elements	N	P	K	S	C	Ca	Mg	Na	Cu	Zn	Mn	Fe
	mg/ g	mg /g	mg/ g	mg/ g	mg/ g	mg/ g	mg/ g	mg/ g	mg/ k	mg/ k	mg/ k	mg/ k
P fertilisres	*	*	n.s	n.s	*	*	n.s	n.s	n.s	n.s	n.s	n.s
Application rate	**	**	**	**	**	**	**	**	n.s	**	**	n.s
P fertilizer*application rate	n.s	**	n.s	n.s	**	*	*	n.s	n.s	n.s	n.s	n.s

The ANOVA analysis indicated that the application of TSP significantly increased the shoot N, P, C and Ca concentration with 115.4, 6, 1075 and 110.8 mg/g, respectively (Table 6.6).

Table 6.6 Effect of different P fertilisers on shoot N, P, C and Ca concentrations. Mean values are presented (n=10) and values in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $P < 0.05$ level as assessed by Tukey's HSD

P fertiliser	N mg/g	P mg/g	C mg/g	Ca mg/g
TSP	115.4 ^a (8.9)	6 ^a (0.8)	1075 ^a (137)	100.8 ^a (9.0)
HBP2	90.9 ^b (7)	4.4 ^b (0.2)	791 ^b (49)	76.2 ^b (4.6)
HBP3	98.8 ^b (6.7)	4.5 ^b (0.3)	798.8 ^b (46)	84.7 ^b (5.3)

With respect to the application rate effect, data analysis by ANOVA showed the application of the phosphate fertilisers (all types) at both 20 and 40 kg/ha rates, significantly increased the shoot nutrients concentration compared with control (0 kg/ha) except for copper (Cu) and iron (Fe) concentration which no significant effect was observed between 0 and 20/40 kg/ha application rates (Table 6.7).

Table 6.7 Effect of different application rates on shoot nutrients concentration. Mean values are presented (n=5) and values in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $P < 0.05$ level as assessed by Tukey's HSD

Application rate	N mg/g	P mg/g	K mg/g	S mg/g	C mg/g	Ca mg/g	Mg mg/g	Na mg/g	Zn mg/kg	Mn mg/kg
0 kg/ha	46.5 ^b (3.2)	1.1 ^b (0.04)	43.8 ^b (3.2)	7.9 ^b (0.5)	353 ^b (20.8)	35.1 ^b (1.9)	5.5 ^b (0.3)	1.6 ^b (0.09)	103.2 ^a (11.7)	91.5 ^b (3.5)
20 kg/ha	95.2 ^a (5.7)	4 ^a (0.2)	78.3 ^a (7.5)	13.1 ^a (0.7)	775 ^a (50.1)	79.4 ^a (4.3)	13.6 ^a (0.8)	6.89 ^a (0.5)	38.6 ^b (1.2)	109.7 ^a (4.9)
40 kg/ha	108.2. ^a (9.2)	5.9 ^a (0.7)	98.7 ^a (13.9)	14.7 ^a (1.3)	987 ^a (115)	95 ^a (8.5)	16.6 ^a (1.6)	7.9 ^a (0.7)	37 ^b (1.2)	115.3 ^a (3.50)

The significant interaction between different P fertilisers and application rates on some of the shoot nutrients concentration are shown in Table 6.8. The application of TSP at the rate of 40 kg/ha showed the highest P, C, Ca and Mg concentration in radish shoot with 8.2, 1441.4, 123.6 and 22.7 mg/g, respectively.

Table 6.8 Inorganic nutrient composition of radish shoot tissue. Mean values are presented (n=5) and values in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $P < 0.05$ level as assessed by Tukey's HSD

	Application rate	P mg/g	C mg/g	Ca mg/g	Mg mg/g
TSP	0	1.1 ^c (0.1)	353.4 ^c (39)	35 ^c (3.7)	5.5 ^c (0.7)
	20	3.8 ^b (0.6)	710.2 ^b (99.7)	78 ^b (10)	13.9 ^b (1.9)
	40	8.2 ^a (0.6)	1441.4 ^a (99.7)	123.6 ^a (14)	22.7 ^a (2.8)
HBP2	0	1.1 ^c (0.1)	353.4 ^c (39)	35 ^c (3.7)	5.5 ^c (0.7)
	20	4.3 ^b (0.2)	857.9 ^b (97.7)	75.1 ^b (2.8)	13.4 ^b (1)
	40	4.5 ^b (0.6)	724.4 ^b (99.7)	77.2 ^b (11.8)	13 ^b (1.9)
HBP3	0	1.1 ^c (0.1)	353.4 ^c (39)	35 ^c (3.7)	5.5 ^c (0.7)
	20	4 ^b (0.3)	759.4 ^b (75.9)	85.1 ^b (8.6)	13.4 ^b (1.6)
	40	5 ^b (0.7)	795.7 ^b (66.1)	84.2 ^b (10.9)	14 ^b (1.6)

6.3.3 Radish post-harvest soil available phosphorus

Analysis by ANOVA did not show any significant difference between TSP and HBPs on post- harvest soil Colwell P. However, a higher post-harvest P was observed for HBP2 and HBP3 treatments, but ANOVA didn't indicate this higher P concentration as significant (Table 6.9). There was no significant interaction effect between P fertilisers and application rate on post- harvest soil Colwell P.

Table 6.9 Effect of different fertilisers on post-harvest Colwell P. Mean values are presented (n=5) and values in parentheses are \pm s.e.

Fertilisers	Post-harvest Colwell P (mg/kg)
TSP	173 (9.4)
HBP2	193 (9.4)
HBP3	195 (9.4)

Glasshouse study 2: Sweet Corn (*Zea Maize L.*) growth

6.4 Methods and Materials

6.4.1 Fertilisers

The same fertilisers of radish study have been used for a sweet corn study (Table 6.1).

6.4.2 Soil characterisation

A Ferrosol (Isbell, 2002) was chosen for this study, the same soil; previously used (See chapter 3, Table 3.1 of this thesis). Some of the most characteristics of the Ferrosol are also shown in Table 3.1.

6.4.3 Experimental design and treatments

About two kg Ferrosol-road side was packed in plastic soil columns (40×9 cm) to reach to field condition bulk density. Pots were arranged in a complete randomized design with their position rotated every second day in five replications. Three Sweet corn (*Zea mays L.*) seeds were planted at the narrow surface of the top soil. Plants were thinned to one plant/pot at two- leaf stage (Figure 6.10).



Figure 6.10 Plants thinned to two plants per pot after germination

Nitrogen (N) and potassium (K) were mixed through with the top 5 cm s soil as urea and potash at 100 and 50 kg/ha, respectively. The pots were irrigated every other day to maintain the soil water at 60% field capacity moisture. Phosphate fertilisers were applied in a band just under the corn seeds at depth of 2mm. TSP and HBPs were applied in three rates of 0, 20 and 40 kg/ha (table 4). Corn was harvested after 63 days just before flowering (Figure 6.11) and analysed for fresh and dried root and shoot biomass, number of leaves, stem diameter (the widest part of the root), and height (from soil surface to end of furthest leaf) and shoot and root P content and post-harvest soil pH, measured as previously described for the radish study.

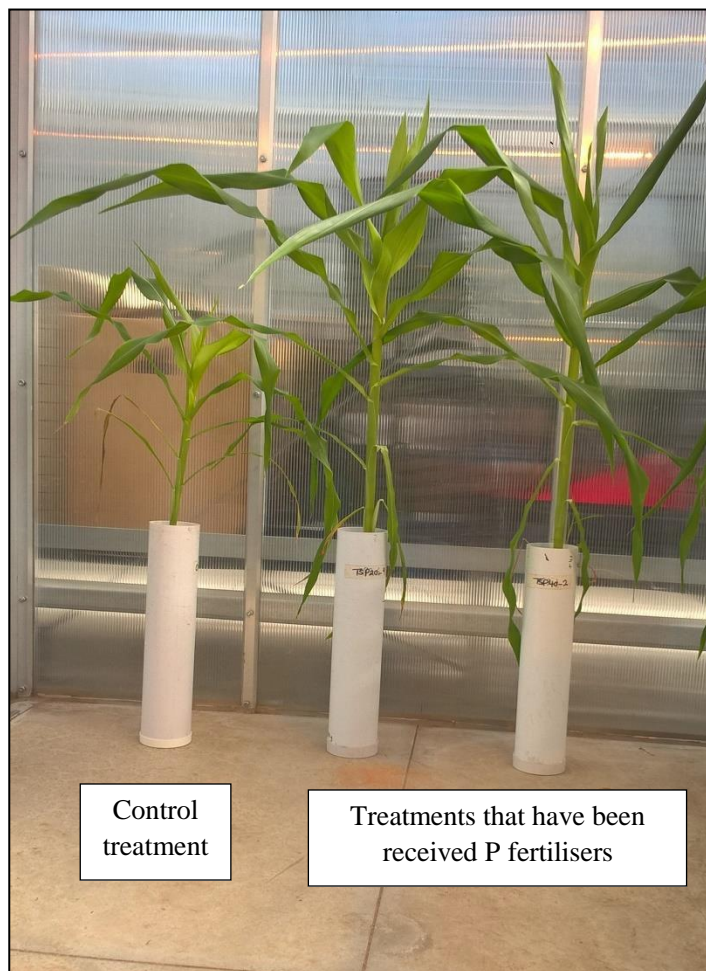


Figure 6.11 Sweet corn plants before harvesting

6.4.4 Calculation and data analysis

All data were analysed using JMP statistical software (JMP®, Version 14; SAS Institute Inc., Cary, NC, USA). Two-way ANOVA was used to investigate the effect of HBP fertilisers with two application rates on plant biomass and shoot nutrient content as well as soil nutrient uptake, post-harvest available and total P and soil pH. Tukey's honestly significant difference (HSD) was used where significant differences were found between different treatments.

6.5 Results

6.5.1 Sweet corn growth parameters

The ANOVA table for all corn growth parameters are presented in Table 6.9. The data indicated that while the main factor of application rate has a significant effect ($p < 0.05$) on all corn growth parameters, with the exception of stem diameter, the different P fertilisers did not have any significant effect on growth parameters. The ANOVA did not show a significant interaction effect ($p < 0.05$) on corn growth parameters as well (Table 6.10).

Table 6.10 ANOVA summary table for all variables. Both main effects and their interaction terms are presented. * $P < 0.05$; ** $P < 0.01$; n.s., not significant at $P < 0.05$

Source	Root dried weight	Shoot dried weight	Number of leaves	Stem height	Stem diameter
Fertiliser	n.s	n.s	n.s	n.s	n.s
Application rate	**	**	**	**	n.s
Fertiliser *application rate	n.s	n.s	n.s	n.s	n.s

6.5.1.1 Corn root dried weight

The ANOVA indicated a significant difference ($p < 0.05$) between control treatments which had not received P fertilisers and fertilised treatments which received P fertilisers at rates of 20 and 40 kg/ha (Figure 6.12). While the control treatment showed the lowest RDW with 1.6 g/pot, application rates of 20 and 40 kg/ha resulted in the higher RDW with 2 and 3.3 g/pot, respectively.

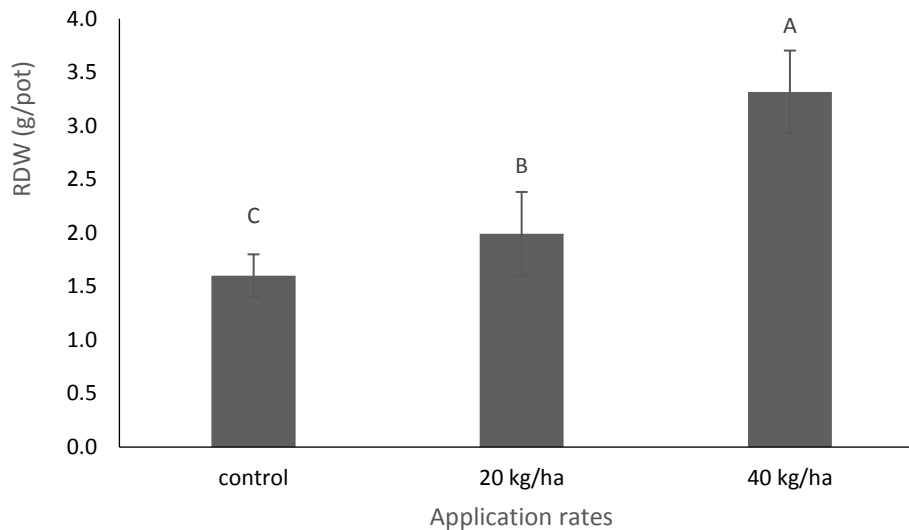


Figure 6.12 Corn root dry weight (RDW) in response to the application rates. Mean values are presented ($n=15$). Error bars are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

6.5.1.2 Corn shoot dried weight

The application of fertilisers at the rates of 40 kg/ha, significantly increase the corn shoot dried weight with 10.1 g/pot and the control treatment resulted in 5.5 g/pot of SDW (Figure 6.13).

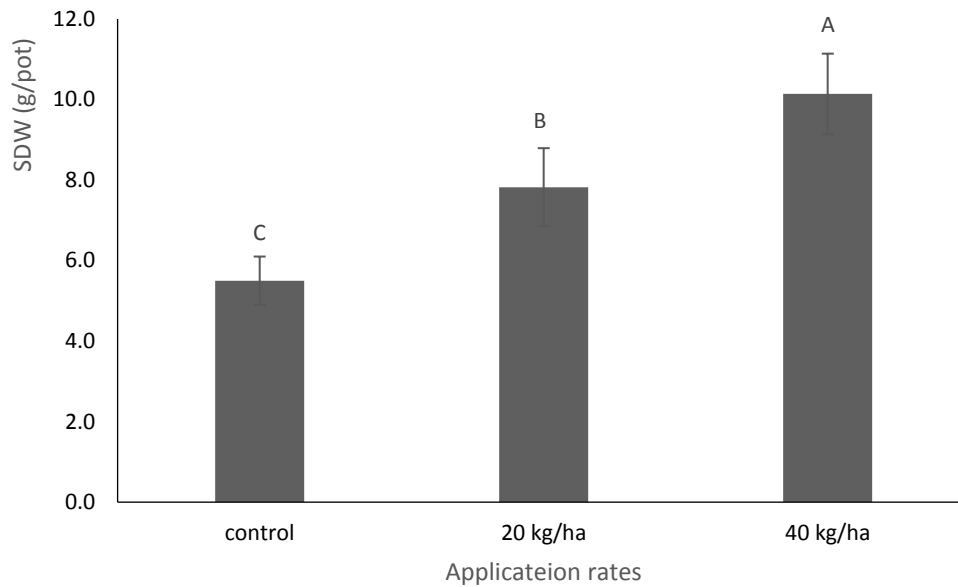


Figure 6.13 Corn shoot dry weight (SDW) in response to the application rates. Mean values are presented (n=15). Error bars are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

6.5.1.3 Corn number of leaves

The application of fertilisers at the rates of 20 and 40 kg/ha, significantly increase the corn number of leaves with 14.5 and 14.4, respectively. The control treatment resulted in 12.8 of number of leaves/pot (Figure 6.14).

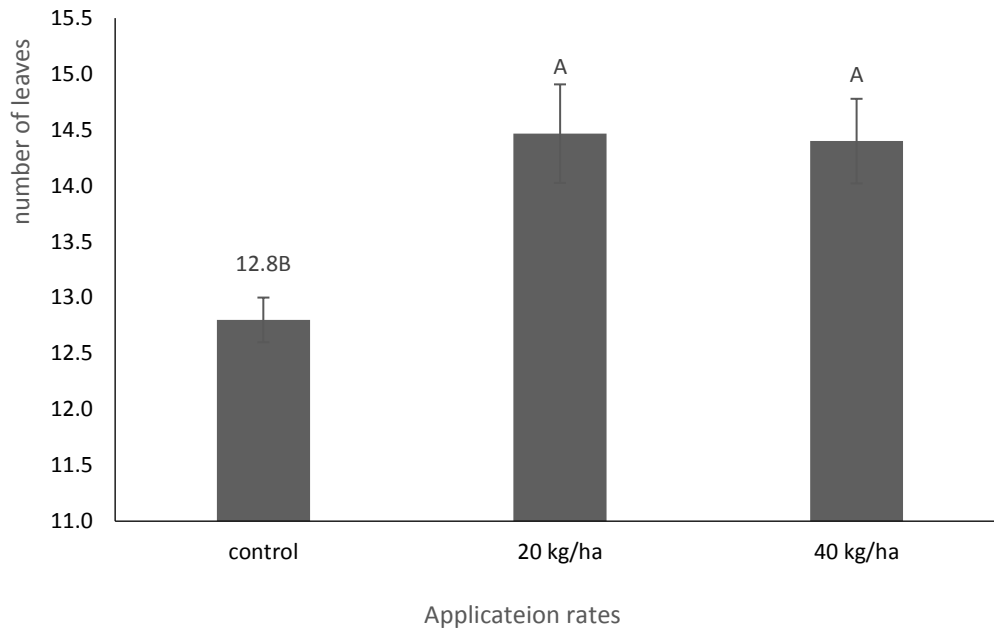


Figure 6.14 Corn number of leaves in response to the application rates. Mean values are presented (n=15). Error bars are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

6.5.1.4 Corn stem height

The application of fertilisers at the rates of 20 and 40 kg/ha, significantly increase the corn number of leaves with 59.6 and 62.5 cm, respectively. The control treatment resulted in 54.2 cm of number of leaves/pot (Figure 6.15).

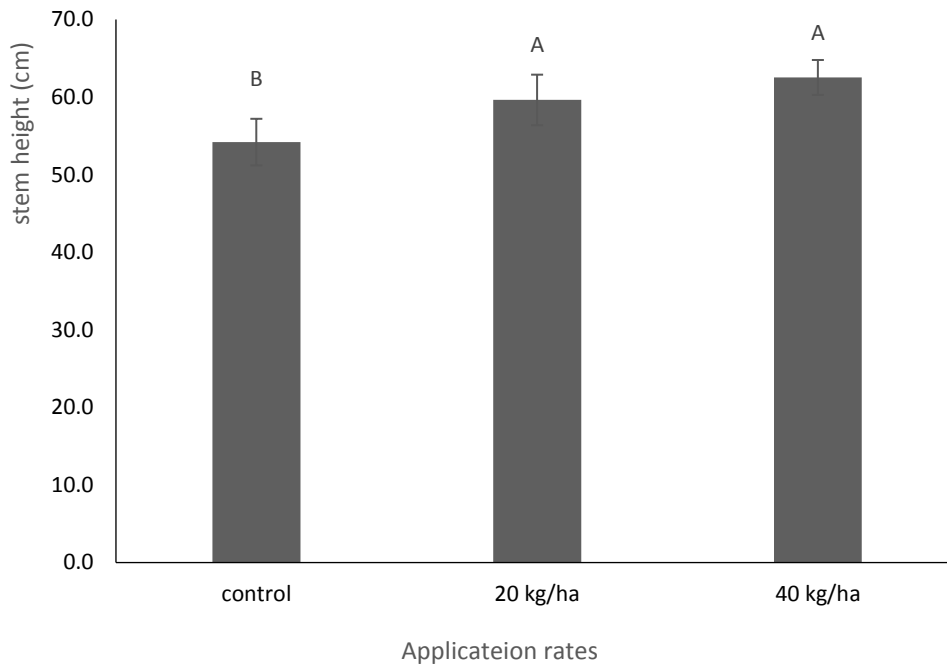


Figure 6.15 Corn stem height (cm) in response to the application rates. Mean values are presented (n=15). Error bars are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

6.5.2 Corn shoot nutrients

Data analysis by ANOVA demonstrated that the different P fertilisers did not have a significant effect on nutrients concentration in corn shoot (Table 6.11). Only the application of P fertilisers at both rates significantly increased the shoot nutrients concentration except for Cu and Fe, in comparison with control treatments which have not been received any P fertilisers. In addition, ANOVA demonstrated no significant interaction effect on corn shoot nutrients concentration for all variables (Table 6.11).

Table 6.11 ANOVA summary table for all nutrients concentration in corn shoot. Both main effects and their interaction terms are presented. *P<0.05; **P<0.01; n.s., not significant at P<0.05

Elements*	P fertiliser	Application rate	P fertiliser *Application rate
N mg/g	n.s	**	n.s
P mg/g	n.s	**	n.s
K mg/g	n.s	**	n.s
S mg/g	n.s	**	n.s
C mg/g	n.s	**	n.s
Ca mg/g	n.s	**	n.s
Mg mg/g	n.s	**	n.s
Na mg/g	n.s	**	n.s
Cu mg/kg	n.s	n.s	n.s
Zn mg/kg	n.s	**	n.s
Mn mg/kg	n.s	**	n.s
Fe mg/kg	n.s	n.s	n.s

*Nitrogen (N), phosphorus (P), potassium (K), sulphur (S), carbon (C), calcium (Ca), magnesium (Mg), sodium (Na), copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe)

With respect to the application rate effect, data analysis by ANOVA showed the application of the phosphate fertilisers (all types) at both 20 and 40 kg/ha rates, significantly increased the corn nutrients concentration compared with control (0 kg/ha) except for zinc (Zn) which the application of P fertilisers resulted in Zn concentration decrease in corn tissue. (Table 6.12).

Table 6.7 Effect of different application rates on shoot nutrients concentration. Mean values are presented (n=5) and values in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $P < 0.05$ level as assessed by Tukey's HSD

Application rate	N mg/g	P mg/g	K mg/g	S mg/g	C mg/g	Ca mg/g	Mg mg/g	Zn mg/g	Mn mg/g
0 kg/ha	159.3b	9.7 b	232.4 b	10.5 b	2436.0 a	20.1 b	18.3 b	60.4 a	55.2 b
	20.7	1.3	27.5	1.1	279.9	1.6	1.8	4.7	1.4
20 kg/ha	184.8 a	11.3 a	287.1 a	13.4 a	3479.5 a	25.2 a	21.1 a	47.3 b	46.5 a
	18.2	0.8	29.7	1.4	440.4	2.6	2.1	2.5	3.1
40 kg/ha	201.8 a	13.6 a	308.1 a	15.1 a	4563.7 a	29.4 a	25.1 a	40.5 b	46.8 a
	17.0	1.0	27.3	1.3	458.6	2.9	2.2	2.5	2.0

6.5.3 Corn post-harvest soil available phosphorus

Analysis by ANOVA did not show any significant difference between TSP and HBPs on post-harvest soil Colwell P. However, higher values for post-harvest P was observed at HBP2 and HBP3 treatments, but ANOVA did not indicate that this higher P concentration was significant (Table 6.13). There was no significant interaction effect between P fertiliser and application rate as a matter of post-harvest soil Colwell P.

Table 6.13 Effect of different fertilisers on post-harvest Colwell P. Mean values are presented (n=5) and values in parentheses are \pm s.e.

Fertilisers	Post-harvest Colwell P (mg/kg)
TSP	45 (1.2)
HBP2	46 (1.2)
HBP3	47 (1.2)

6.6 Discussion

The application of TSP significantly increased the radish shoot dried weight and root depth as well as some of shoot nutrients uptake at both application rates. Also, the higher application rate (40kg/ha) increased the all radish growth parameters and shoot and bulb nutrients uptake. Generally, radish growth parameters were greater at the higher application rate of P. (Pezzarossa et al., 1993) reported radish dried root biomass and radish root increased with higher P application rates.

Radish responds well to phosphate fertiliser and phosphorus shortage restricts growth of radish and its maturity (Baloch et al., 2014); hence it was selected for this study. On the other hand, its short life cycle is a benefit for ease of investigation of the blended fertilisers. In this study, the HBP fertilizer did not show any beneficial effect on radish growth parameters. There are several possible explanations for these results. One of the possible mechanism could be the high organic matter concentration in the soil which did not allow HBPs to show their positive effect on plant growth. Native soil organic matter can perform some of the humic substances functions. Chen and Avid (1990) showed the highest effect of humic substance occurs in in soil with less than 100mg/L dissolved organic matter. Some other studies explained the lack of positive effect of humic substances in the higher organic matter soils in the incubation and field studies (Fagbenro and Agboola, 1993; Kunkel and Holstad, 1968; Lee and Bartlett, 1976).

Despite fertiliser HBP3 having the higher humate content (carbon \approx 16.6%), a lack of growth benefit even at the higher application rates could attributed to a tighter bound between P and K-humate hence P couldn't being released easily to meet the radish demands.

A significant decrease of Zn content in the radish root and shoot by application of phosphate fertilisers in all forms was observed. There is an adverse relation between soil Zn content and phosphate application. Similar results were obtained previously in several studies (Cimrin and Yilmaz, 2005, Marschner and Rimmington, 1988). The decrease in soluble Zn cations can be

explained by the formation of insoluble zinc-phosphate – zinc compounds (Marschner, 1995).

Zinc ions may also complex with cation exchange sites on the humate molecules, thus decreasing free zinc ion concentrations.

The HBPs fertilisers showed no significant differences from TSP in corn growth parameters. These results are in agreement with another literature report (Cimrin and Yilmaz, 2005) with some exceptions. They found no significant effect of humic acids on lettuce yield and nutrients uptake when it was applied along with phosphate fertilisers. The higher application rate of both TSP and HBPs fertilisers (40 kg/ha) had greater biomass production than the control treatment but there were no differences between different P-fertilisers applied.

Corn was selected for this study because previous studies demonstrated it can be an indicator plant for humate product effects (Hill et al., 2015a, Summerhays et al., 2015, Tan and Nopamornbodi, 1979, Duplessis and Mackenzie, 1983, Fink et al., 2016a, Teixeira et al., 2016, Sharif et al., 2002). The results of radish study suggested that one of the main reasons for inconsistent effects of HBPs could be the Ferrosol already had sufficient plant available P. So for the corn plant trial study another Ferrosol was chosen with lower available P concentration. The importance of selection of a Ferrosol soil was the high concentration of Fe and Al in these soils. The Ferrosol for the corn study was selected from a roadside section of a pasture. It contained a high level of organic matter. Many studies indicated the high organic matter soil may mask the effects of humic substances (Patti et al., 2013, Lee and Bartlett, 1976, Duplessis and Mackenzie, 1983, Hartz and Bottoms, 2010). Furthermore, another study also showed the effect of “Carbond P” fertiliser (unknown carbon source) had no effect compared with ammonium polyphosphate fertiliser on maize growth in a high organic matter soil (Summerhays et al., 2015). The soil background organic matter can behave in the same way as the K-humate in HBPs fertilisers (Chen et al., 2004), which may then minimize or negate any beneficial effect of HBPs applications.

Duplessis and Mackenzie, 1983 found some similar results to the study described in this chapter. They observed no interaction benefit between leonardite and phosphate fertiliser in a corn plant trial. In their study, leonardite humate was applied with a range of phosphate fertiliser in two different soils in glasshouse conditions. They observed that the leonardite humate had no effect on corn growth in a clay soil with a high organic matter content.

The content of zinc (Zn) and iron (Fe) in tropical soils was significantly decreased by application of phosphate fertilisers (Saeed and Fox, 1979, Pérez-Novo et al., 2011, Pardo, 1999). (Bingham and Garber, 1960) studied the availability and uptake of micronutrients with phosphate fertilization. They found that the application of excess P led to decreased copper and zinc uptake in acid soils. The possible mechanisms in which application of P decreased the micronutrients efficiency may related to formation of complexes on adsorbing surfaces (Agbenin, 1998) or/and Zn bridging between phosphorus and organic matter (Ahumada T et al., 1997).

In this study at both radish and corn experiments, although the post-harvest available phosphorus in HBPs treatments were higher than TSP treatments but ANOVA did not show this difference to be significant. A similar trend was observed in other (Cimrin and Yilmaz, 2005), where it was found that the application of phosphate fertilisers along with humic acids does not increase soil available P.

6.7 Conclusion

Both radish and corn plant growth trial findings suggested that the effect of K-humate-blended phosphate fertilisers greatly depended on crop and soil properties and soil phosphorus content. While K-humate blended phosphate showed some beneficial effect in radish shoot dried weight and root depth, no significant beneficial effect was observed in corn growth parameter. The interaction between fertilisers and application rates was significant in radish root and shoot dried weight as well as radish root depth. The application of TSP at rate of 40 kg/ha resulted in higher radish root and shoot dried weight and root depth. Application of TSP alone and in interaction with applicate rate of 40 kg/ha, significantly increased the radish N and P uptake which is in agreement with significant increase of radish shoot dried weight.

In contrast, for the corn study, HBPs fertilisers showed the beneficial effect only on the corn number of leaves and no positive effect was observed with regard to corn nutrients uptake.

The different response of radish and corn to the same fertilisers likely stems from the different Ferrosols which were used in studies. Ferrosol with a higher concentration of P and Fe/Al than Ferrosols used in the corn study. The higher concentration of Fe and Al ions in the radish study soil resulted in higher P absorption sides. While the presence of humate along with TSP in HBPs fertiliser was supposed to attribute to see the positive effect of HBPs on some of radish growth parameters and nutrients uptake, no significant effect was observed by HBPs application.

On the other hand, harvesting corn plants before flowering likely did not allow enough time for the humate to show the positive effects on corn growth parameters. In some studies the beneficial effect of humic acid was shown at the end of the corn growing season.

6.8 References

AGBENIN, J. 1998. Phosphate-induced zinc retention in a tropical semi-arid soil. *European Journal of Soil Science*, 49, 693-700.

AHUMADA T, I., BUSTAMANTE, A. & SCHALSCHA, E. 1997. Zinc speciation in Phosphate-affected soils. *Communications in soil science and plant analysis*, 28, 989-995.

BALOGH, P. A., UDDIN, R., NIZAMANI, F. K., SOLANGI, A. H. & SIDDIQUI, A. A. 2014. Effect of nitrogen, phosphorus and potassium fertilizers on growth and yield characteristics of radish (*Raphanus sativus* L.). *American-Eurasian Journal of Agricultural & Environmental Sciences*, 14, 565-569.

BINGHAM, F. T. & GARBER, M. 1960. Solubility and Availability of Micronutrients in Relation to Phosphorus Fertilization 1. *Soil Science Society of America Journal*, 24, 209-213.

CHEN, Y. & AVIAD, T. 1990. Effects of humic substances on plant growth. *Humic substances in soil and crop sciences: Selected readings*, 161-186.

CHEN, Y., DE NOBILI, M. & AVIAD, T. 2004. Stimulatory effects of humic substances on plant growth. *Soil organic matter in sustainable agriculture*. CRC Press.

CIMRIN, K. M. & YILMAZ, I. 2005. Humic acid applications to lettuce do not improve yield but do improve phosphorus availability. *Acta Agriculturae Scandinavica, Section B- Soil & Plant Science*, 55, 58-63.

DIEZ, J., CARTAGENA, M. & VALLEJO, A. 1992. Controlling phosphorus fixation in calcareous soils by using coated diammonium phosphate. *Fertilizer research*, 31, 269-274.

DUPLESSIS, G. & MACKENZIE, A. 1983. Effects of leonardite applications on phosphorus availability and corn growth. *Canadian journal of soil science*, 63, 749-751.

ERRO, J., URRUTIA, O., BAIGORRI, R., FUENTES, M., ZAMARREÑO, A. & GARCIA-MINA, J. 2016. Incorporation of humic-derived active molecules into compound NPK granulated fertilizers: main technical difficulties and potential solutions. *Chemical and Biological Technologies in Agriculture*, 3, 1-15.

FAGBENRO, J.A. and A.A. AGBOOLA. 1993. Effect of different levels of humic acids on growth and nutrient uptake of teak seedlings. *J. Plant Nutr.* 16:1465–1483.

FINK, J. R., INDA, A. V., BAVARESCO, J., SÁNCHEZ-RODRÍGUEZ, A. R., BARRÓN, V., TORRENT, J. & BAYER, C. 2016a. Diffusion and uptake of phosphorus, and root development of corn seedlings, in three contrasting subtropical soils under conventional tillage or no-tillage. *Biology and fertility of soils*, 52, 203-210.

FINK, J. R., INDA, A. V., TIECHER, T. & BARRÓN, V. 2016b. Iron oxides and organic matter on soil phosphorus availability. *Ciência e Agrotecnologia*, 40, 369-379.

GARCIA-MINA, J. M. 2006. Stability, solubility and maximum metal binding capacity in metal–humic complexes involving humic substances extracted from peat and organic compost. *Organic Geochemistry*, 37, 1960-1972.

GARCIA, M., DIEZ, J., VALLEJO, A., GARCIA, L. & CARTAGENA, M. C. 1997. Effect of applying soluble and coated phosphate fertilizers on phosphate availability in calcareous soils and on P absorption by a rye-grass crop. *Journal of Agricultural and Food Chemistry*, 45, 1931-1936.

HARTZ, T. K. & BOTTOMS, T. G. 2010. Humic substances generally ineffective in improving vegetable crop nutrient uptake or productivity. *HortScience*, 45, 906- 910.

HILL, M. W., HOPKINS, B. G. & JOLLEY, V. D. 2015a. Maize in-Season Growth Response to Organic Acid-Bonded Phosphorus Fertilizer (Carbond P®). *Journal of Plant Nutrition*, 38, 1398-1415.

HILL, M. W., HOPKINS, B. G., JOLLEY, V. D. & WEBB, B. L. 2015b. Phosphorus Mobility Through Soil Increased with Organic Acid-Bonded Phosphorus Fertilizer (Carbond® P). *Journal of Plant Nutrition*, 38, 1416-1426.

HOPKINS, B. & STARK, J. H. HUMIC ACID EFFECTS ON POTATO RESPONSE TO PHOSPHORUS. Presented at the Idaho Potato Conference January, 2003. 23.

HOPKINS, B. G., ROSEN, C. J., SHIFFLER, A. K. & TAYSOM, T. W. 2008. Enhanced Efficiency fertilizers for improved nutrient management: potato (*Solanum tuberosum*). *Crop management*.

HUE, N. 1991. Effects of organic acids/anions on P sorption and phytoavailability in soils with different mineralogies. *Soil Science*, 152, 463-471.

ISBELL, R. 2002. *The Australian soil classification*, CSIRO publishing.

ISLAM, M. A., ISLAM, S., AKTER, A., RAHMAN, M. H. & NANDWANI, D. 2017. Effect of organic and inorganic fertilizers on soil properties and the growth, yield and quality of tomato in Mymensingh, Bangladesh. *Agriculture*, 7, 18.

KETMA, DADI WAKA., NIGUSSIE, DECHASSA. 2016. Response of Sesame (*Sesamum indicum* L.) to Organic and Inorganic Fertilizers under Irrigation at Gode, Somali Region, South Eastern Ethiopia

KUMAR, A. & GUPTA, R. 2018. The effects of vermicompost on growth and yield parameters of vegetable crop radish (*Raphanus sativus*). *Journal of Pharmacognosy and Phytochemistry*, 7, 589-592.

KUMAR, D. & SINGH, A. 2017. Efficacy of Potassium Humate and Chemical Fertilizers on

Yield and Nutrient Availability Patterns in Soil at Different Growth Stages of Rice. *Communications in Soil Science and Plant Analysis*, 48, 245-261.

KUMAR, D., SINGH, A., RAHA, P., RAKSHIT, A., SINGH, C. & KISHOR, P. 2013. Potassium Humate: A Potential Soil Conditioner and Plant Growth Promoter. *International Journal of Agriculture, Environment and Biotechnology*, 6, 441-446.

KUNKEL, R. and N. HOLSTAD. 1968. Effect of adding humates to the fertilizer on the yield and quality of russet burbank potatoes. *Potato J.* 45:449– 457.

LEE, Y. S. & BARTLETT, R. J. 1976. Stimulation of plant growth by humic substances 1. *Soil Science Society of America Journal*, 40, 876-879.

LI, R., TAO, R., LING, N. & CHU, G. 2017. Chemical, organic and bio-fertilizer management practices effect on soil physicochemical property and antagonistic bacteria abundance of a cotton field: Implications for soil biological quality. *Soil and Tillage Research*, 167, 30-38.

LITTLE, K. R., ROSE, M. T., JACKSON, W. R., CAVAGNARO, T. R. & PATTI, A. F. 2014. Do lignite-derived organic amendments improve early-stage pasture growth and key soil biological and physicochemical properties? *Crop and Pasture Science*, 65, 899-910. MARSCHNER, H. 1995. *Mineral nutrition of higher plants*. ed. 2.

MARSCHNER, H. & RIMMINGTON, G. 1988. Mineral nutrition of higher plants. *Plant Cell Environ*, 11, 147-148.

PARDO, M. 1999. Influence of phosphate on zinc reaction in variable charge soils. *Communications in soil science and plant analysis*, 30, 725-737.

PATTI, A., ROSE, M., LITTLE, K., JACKSON, R. & CAVAGNARO, and T. A meta-analysis of Plant-growth response to humic substance applications. *EGU General Assembly Conference Abstracts*, 2013. 12892.

PÉREZ-NOVO, C., BERMÚDEZ-COUSO, A., LÓPEZ-PERIAGO, E., FERNÁNDEZ-CALVIÑO, D. & ARIAS-ESTÉVEZ, M. 2011. Zinc adsorption in acid soils: influence of phosphate. *Geoderma*, 162, 358-364.

PETRUS, A. C., AHMED, O. H., MUHAMAD, A. M. N., NASIR, H. M. & JIWAN, M. 2010. Effect of KN-humates on dry matter production and nutrient use efficiency of maize in Sarawak, Malaysia. *The Scientific World Journal*, 10, 1282-1292.

PEZZAROSSA, B., PETRUZZELLI, G., MALORGIO, F. & TOGNONI, F. 1993. Effect of repeated phosphate fertilization on the heavy metal accumulation in soil and plants under protected cultivation. *Communications in Soil Science and Plant Analysis*, 24, 2307-2319.

SAEED, M. & FOX, R. L. 1979. Influence of Phosphate Fertilization on Zinc Adsorption by Tropical Soils. *Soil Science Society of America Journal*, 43, 683-686.

SANCHEZ, C., LOCKHART, M. & PORTER, P. 1991. Response of radish to phosphorus and potassium fertilization on Histosols. *HortScience*, 26, 30-32.

SHARIF, M., KHATTAK, R. A. & SARIR, M. 2002. Effect of different levels of lignitic coal derived humic acid on growth of maize plants. *Communications in Soil Science and Plant Analysis*, 33, 3567-3580.

SUMMERHAYS, J. S., HOPKINS, B. G., JOLLEY, V. D., HILL, M. W., RANSOM, C. J. & BROWN, T. R. 2015. Enhanced phosphorus fertilizer (Carbond P®) supplied to maize in moderate and high organic matter soils. *Journal of Plant Nutrition*, 38, 1359-1371.

TAHIR, M., KHURSHID, M., KHAN, M., ABBASI, M. & KAZMI, M. 2011. Lignite-derived humic acid effect on growth of wheat plants in different soils. *Pedosphere*, 21, 124- 131.

TAN, K. & NOPAMORN BODI, V. 1979. Effect of different levels of humic acids on nutrient content and growth of corn (*Zea mays* L.). *Plant and soil*, 51, 283-287.

TEIXEIRA, R. D. S., RIBEIRO DA SILVA, I., NOGUEIRA DE SOUSA, R., MÁRCIO MATTIELLO, E. & BARROS SOARES, E. M. 2016. Organic acid coated-slow-release phosphorus fertilizers improve P availability and maize growth in a tropical soil. *Journal of soil*

science and plant nutrition, 16, 1097-1112.

TURGAY, O., KARACA, A., UNVER, S. & TAMER, N. 2011. Effects of coal-derived humic substance on some soil properties and bread wheat yield. Communications in soil science and plant analysis, 42, 1050-1070.

Chapter 7

The effect of organic acids on soil phosphorus availability

Abstract

The phosphorus availability in acidic soil decreased due to phosphate fixation with Fe (oxide) and Al (oxide).. It has been shown that humic acid can improve soil P availability (Wang, Wang et al. 1995, Iyamuremye and Dick 1996, Haynes and Mokolobate 2001) through a number of mechanisms, previously discussed, including: chelation, competition with minerals for P binding and forming a soluble metal-P complexes. In this study, the solubilisation of P was studied under different organic acid treatments. Oxalic acid, salicylic acid and K-humate solutions were applied to air-dried soils at two different concentrations. In contrast to the initial hypothesis, the effects of organic acids and K-humate on total P solubility were opposite to what was expected in the Ferrosol soil. Previous studies demonstrated that the application of organic acids into soil solutions increased soluble P (Hu et al., 2002, Jones, 1998, Scheffe et al., 2011, Scheffe and Tymms, 2013, Scheffe et al., 2008), so it was expected that the addition of organic acids would release more P into the soil solution compared with the untreated control. As the effects of organic amendments were not consistent among treatments and time, further experiments will be needed in different types of soils and with different organic amendments.

Introduction

The effects of lignite-derived-humate blended with triple superphosphate on soil available phosphorus and plant growth were investigated in Chapters 2-6, and has given variable results. In my previous experiments, P availability and plant growth has been driven primarily by soil type (including mineralogy), existing soil P reserves and soil organic matter, with only a minor influence from the fertiliser formulation. The P requirement of different plants is also an important factor. (Dalal and Hallsworth 1976; Mkhabela and Warman 2005). The availability of P especially in soils with lower pH, usually decreases due to phosphate fixation with Fe^{3+} and Al^{3+} and other solid binding sites (Barrow, 1989; Haynes and Mokolobate, 2001; Guedes, Melo et al. 2016; Zwetsloot, Lehmann et al. 2016). P is considered to be immobilised predominantly by the formation of a surface complex through ligand exchange between H_2O and / or OH^- coordinated with phosphate and Fe^{3+} and Al^{3+} ions at soil surface (Hingston et al., 1967, Muljadi et al., 1966).

Results of previous studies have indicated that there are several mechanisms by which organic acids can influence P availability in the soil solution. It has been shown that humic acid can improve soil P availability (Wang, Wang et al. 1995, Iyamuremye and Dick 1996, Haynes and Mokolobate 2001) through a number of mechanisms, including:

- D) Competition with P for adsorption sites on soil particles surface. This happens when organic acids block adsorption sites on soil materials and reduce P adsorption (Nagarajah et al., 1970, Nagarajah et al., 1968, Hue, 1991, Violante et al., 1991, Zamuner et al., 2008, Fink et al., 2016). Organic acid competition efficiency decreases in the order of tricarboxylic acids > dicarboxylic acids > monocarboxylic acids (Earl et al., 1979, Bolan et al., 1994). Model, low-molecular weight organic

acids are frequently used in studies on competition efficiency with P, including oxalic, salicylic, malic and citric acids. These compounds have a low dissociation constant, so their competitive inhibition of P sorption generally increases as soil pH decreases (Nagarajah et al., 1970, Bolan et al., 1994, Lopez-hernandez et al., 1979).

- II) Organic acids form humic-metal-P complexes which are soluble in the soil solution (Chen et al., 1973, Earl et al., 1979, Stevenson and Fitch, 1986). This mechanism involves the formation of a metal bridge between P and organic ligands (Guardado et al., 2008). The schematic figure of the mechanism is shown in Figure 7.1. Factors which influence the stability of the metal-organic complexes include pH, the presence of cations, dissociation of functional groups and the saturation of binding sites (Tan, 2010).

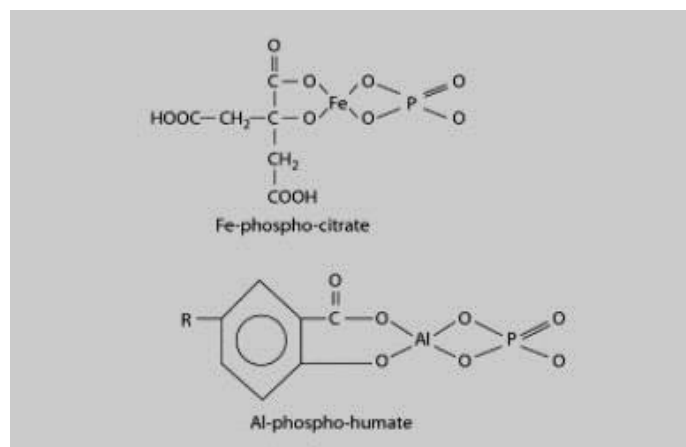


Figure 7.1 Forming humic-metal-phosphate complexes in soils (Tan, 2010).

III) Organic acids capable of chelating free Fe^{3+} and Al^{3+} cations in acidic and Ca^{2+} in alkaline soils act as chelating agent for dissolved cations like Fe and Al (Hue et al., 1986, Nagarajah et al., 1970, Hu et al., 2005). Numerous low molecular weight organic acids including oxalic, salicylic and citric acids are effective in the chelation of metal ions (McCull and Pohlman, 1986, Stevenson and Ardakani, 1972). The structure of the organic acid and the position of the functional groups are important factors in determining the stability constants, with stable organo-metal complexes occurring when an organic acid can form five- or six-membered chelate rings (Martell, 1952) (Figure 7.2). For example, Bolan et al. (1994) showed that the addition of organic acids increased the availability of P in soils and ryegrass dry matter through forming soluble complexes with soil cations.

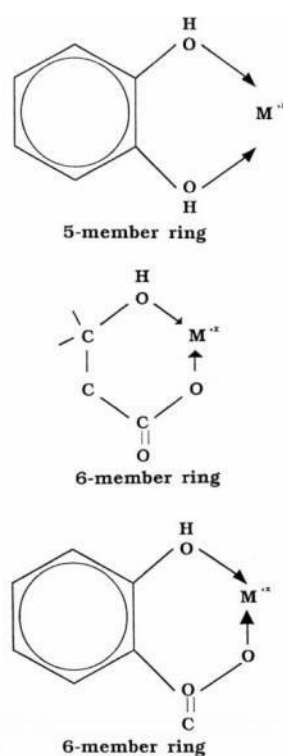


Figure 7.2 Forming stable organo-metal complexes with very stable five or six membered chelate rings (Harrold and Tabatabai, 2006)

Previous studies which examined the effect of a wide range of organic acids on P availability have indicated that di- and tricarboxylic acids are generally more effective than mono carboxylic or phenolic acids (Hue, 1991, Hu et al., 2005, Scheffe et al., 2011, Harrold and Tabatabai, 2006). The increase in soluble P concentration is partly the result of dissociation of the organic acids in the soil solution, releasing H⁺ and resulting in acidification and partly via the formation of stable organo-metal complexes preventing the precipitation/sorption of P.

Complex mixtures of humic materials contain organic acids with different chemical structures. ¹³C NMR spectroscopy of the K-humate indicated that the chemical structure of K-humate was dominated by aromatic carbon (>60%) (Little et al., 2013). To better understand the mechanism of the observed increase in P solubility and diffusion in Chapters 2 and 3, the effect of K-humate versus different organic acids on P availability in the soil was investigated. Oxalic acid, a non-aromatic low molecular weight organic acid (LMWOA), and salicylic acid, a phenolic LMWOA (acid) were applied as model LMWOAs as previous research indicated their efficacy in metal cation chelation (Harrold and Tabatabai, 2006).

7.2 Methods and Materials

7.2.1 Soil sampling

The soil used in this study was a Ferrosol (Isbell, 2002) collected from 0-15 cm from road side of a pasture, at Ellinbank, Victoria (Department of Economic Development, Jobs, and Transport & Resources). The main soil characteristics can be found in Chapter 4, Table 4.3.

7.2.2 Treatments

Oxalic acid and salicylic acid were of analytical grade and purchased from Sigma Aldrich Pty Ltd (Castle Hill, NSW) and K-humate was purchased from Omnia specialties (Morwell, Vic). Each acid solution was prepared at two concentrations of 0.1 mM and 1 mM (Table 7.1). K-humate solutions were prepared at two carbon concentrations which were equal to the highest and lowest carbon percentage of the organic acid solutions.

Table 7.1 Treatments

Treatments
Oxalic acid - 1 mM
Oxalic acid - 0.1 mM
Salicylic acid - 1 mM
Salicylic acid - 0.1 mM
K-humate – High concentration (0.06% C)
K-humate – Low concentration (0.02% C)
Control (distilled water)

7.2.3 Methodology

Air dried soil (5 g, sieved to <2mm) was mixed with 50 ml of oxalic acid, salicylic acid or K-humate solution in 50 ml centrifuge tubes (1:10 soil: solution) with 4 replications for each treatment and the same soil was mixed with 50ml deionised water as the control. Two to three drops of chloroform was added to the mixture to prevent microbial activity. The soil mixture was incubated at room temperature using continuous shaking on a rotating shaker at 120 rpm. Samples were taken at 2, 5 and 17 hours after the start of incubation. For each time of sampling, a separate sample set was prepared to avoid sampling destruction. Samples then were centrifuged at 3000 rpm for 10 min. The solution pH was measured using a TPS WP81 meter and probe (TPS Pty Ltd, Springwood, Qld). The solution was filtered (Whatman 42 filter paper), followed by a 0.45µm syringe filter (Sartorius Stedim Australia PTY Ltd, Dandenong South, VIC). The filtrates were then analysed for total P (TMP), Al and Fe by ICP-OES (Perkin Elmer) (Schefe and Tymms, 2013).

7.2.4 Calculation and data analysis

All data was analysed using JMP statistical software (JMP®, Version 14; SAS Institute Inc., Cary, NC, USA). Two-way ANOVA was used to investigate the effect of organic acids and time on soil total phosphorus (TP), total iron (TFe) and total aluminium (TAI). Tukey's honestly significant difference (HSD) was used where significant differences were found between different treatments.

7.3 Results

Data analysis by ANOVA showed the main effects and interaction effect were significant ($p < 0.05$) on total P, total Fe and total Al (Table 7.2).

Table 7.2 ANOVA summary table for total phosphorus, total iron and total aluminium. Both main effects and their interaction terms are presented. * $P < 0.05$; ** $P < 0.01$; n.s., not significant at $P < 0.05$

Source	Total P	Total Fe	Total Al
organic acid	**	**	**
Time	**	**	**
organic acid*Time	*	**	**

7.3.1 Total phosphorus (TP) concentration

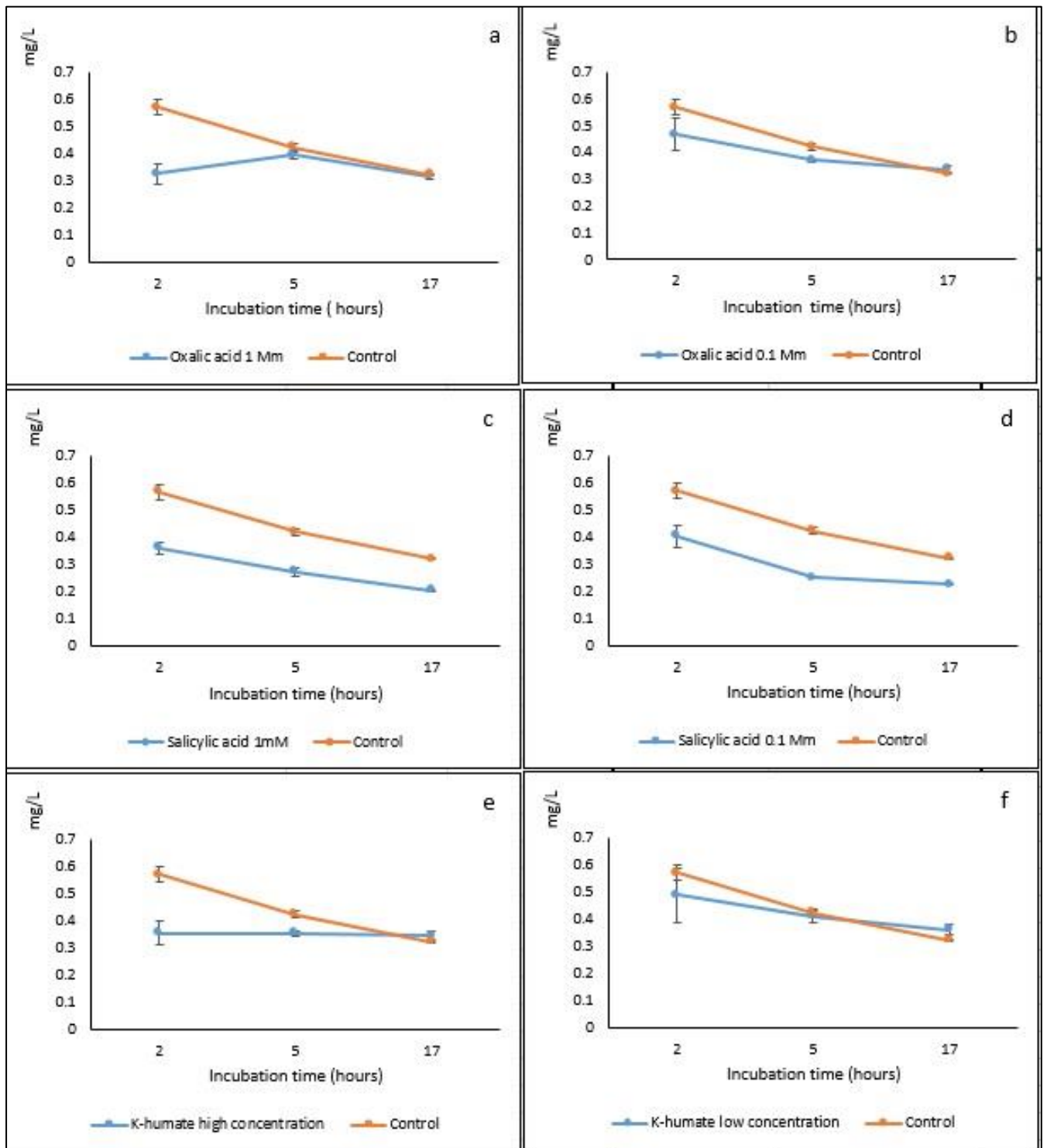
The variation of TP during the incubation time for all treatments compared to the control is shown in Figures 7.3 a-f. All treatments showed a significant decrease ($p < 0.05$) of TP with time except for K-humate at the higher concentration. Data analysis by ANOVA indicated that all treatments showed the highest TP at 2 hours of sampling and the lowest TP concentration at 17 hours of sampling. All the treatments showed a lower level of total P in solution than control except for K-humate which showed a higher TP at 17 hours of sampling. However, this difference was not significant at the $p = 0.05$ level.

At 2 hours after start of the incubation, the control treatment with 0.57 mg/L significantly ($p < 0.05$) showed the highest TP value while oxalic acid-1 mM showed the lowest TP concentration with 0.32 mg/L.

At 5 hours, while the control and K-humate low concentration showed the highest TP with 0.42 and 0.41 mg/L, respectively, notably, a significant decrease in TP was observed in the salicylic

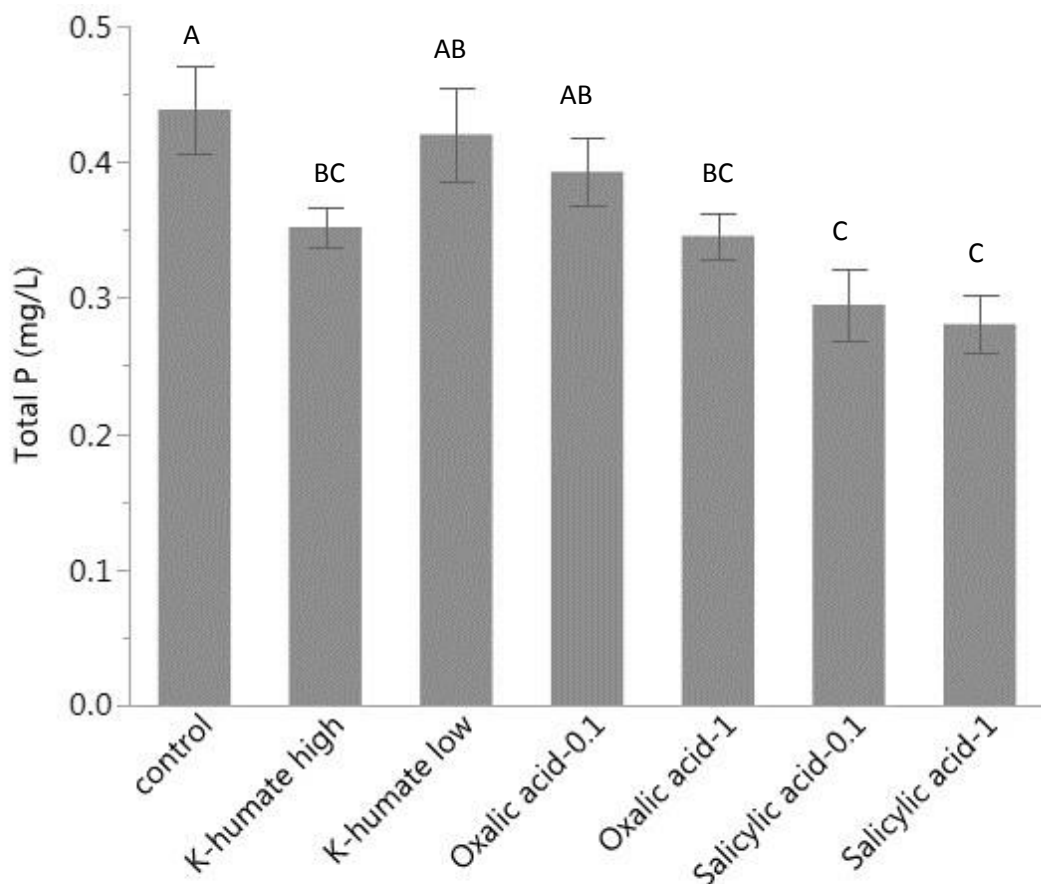
acid treatments with 0.27 mg/L for salicylic acid-1 mM and 0.25 mg/L for salicylic acid-0.1 mM. Between 5 and 17 hours of incubation time, there was a slight decrease in all treatments except again for K-humate treatment where no change was observed in the K-humate at the higher concentration (Figure 7.3, e).

At 17 hours, the K-humate treatments and oxalic acid-0.1 mM both gave TP concentrations with 0.36 and 0.33 mg/L, respectively, and these values were not significantly different from the control (0.32 mg/L of TP). Application of salicylic acid showed the lowest TP concentration with 0.20 mg/L.



Figures 7.3 (a-f) Effect of time on total phosphorus (mg/L) in different treatments. Mean values are presented (n=4) \pm s.e

Data analysis by ANOVA showed a significant effect of different treatments on TP in 17 hours. Figure 7.4 shows that while control treatment showed the highest concentration of TP with 0.43 mg/L, application of salicylic acid significantly decreased the TP concentration to 0.2 mg/L. Comparison of means by Tukey's HSD demonstrated that there was not any significant difference ($p < 0.05$) among oxalic acid-0.1 mM, and K-humate in low concentration as they all showed TP concentrations 0.42 and 0.39 mg/L, respectively. In addition, there was not any significant difference between oxalic acid -1 mM and K-humate high concentration as both showed 0.34 mg/L TP.



Figures 7.4 Effect of different treatments on total phosphorus (TP) in different treatments. Mean values are presented (n=4). Error bars represent standard error. Comment about what the letter represent.

ANOVA indicated a significant ($p < 0.05$) interaction of time and organic acid on soil TP. Further analysis by Tukey's demonstrated control treatment at 2 hours showed the highest TP concentration with 0.57 mg P/L of TP. However, it was not very different (0.48 and 0.46 mg P g/L, respectively) from K-humate in low concentration and oxalic acid (0.1 mM) at 2 hours (Table 7.3). Application of salicylic acid 0.1 showed the lowest TP concentration at 2 hours.

Table 7.3 Interaction effect between organic acids and time on soil TP. Mean values are presented ($n=4$). Numbers in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

Level	Time								Mean of TP (mg /L)
Control	2	A							0.579 (0.03)
K-humate low	2	A	B						0.48 (0.03)
Oxalic acid-0.1	2	A	B	C					0.46 (0.03)
Control	5	A	B	C	D				0.42 (0.03)
K-humate low	5	A	B	C	D	E			0.41 (0.03)
Salicylic acid-0.1	2	A	B	C	D	E			0.40 (0.03)
Oxalic acid-1	5		B	C	D	E	F		0.39(0.03)
Oxalic acid-0.1	5		B	C	D	E	F	G	0.37 (0.03)
K-humate low	17		B	C	D	E	F	G	0.36 (0.03)
Salicylic acid-1	2		B	C	D	E	F	G	0.36 (0.03)
K-humate high	2		B	C	D	E	F	G	0.35 (0.03)
K-humate high	5		B	C	D	E	F	G	0.35 (0.03)
K-humate high	17		B	C	D	E	F	G	0.34 (0.03)
Oxalic acid-0.1	17		B	C	D	E	F	G	0.33 (0.03)
Oxalic acid-1	2		B	C	D	E	F	G	0.32 (0.03)
control	17		B	C	D	E	F	G	0.32 (0.03)
Oxalic acid-1	17			C	D	E	F	G	0.31(0.03)
Salicylic acid-1	5				D	E	F	G	0.27(0.03)
Salicylic acid-0.1	5					E	F	G	0.25(0.03)
Salicylic acid-0.1	17						F	G	0.22(0.03)
Salicylic acid-1	17							G	0.20(0.03)

7.3.2 Total iron (TFe) concentration

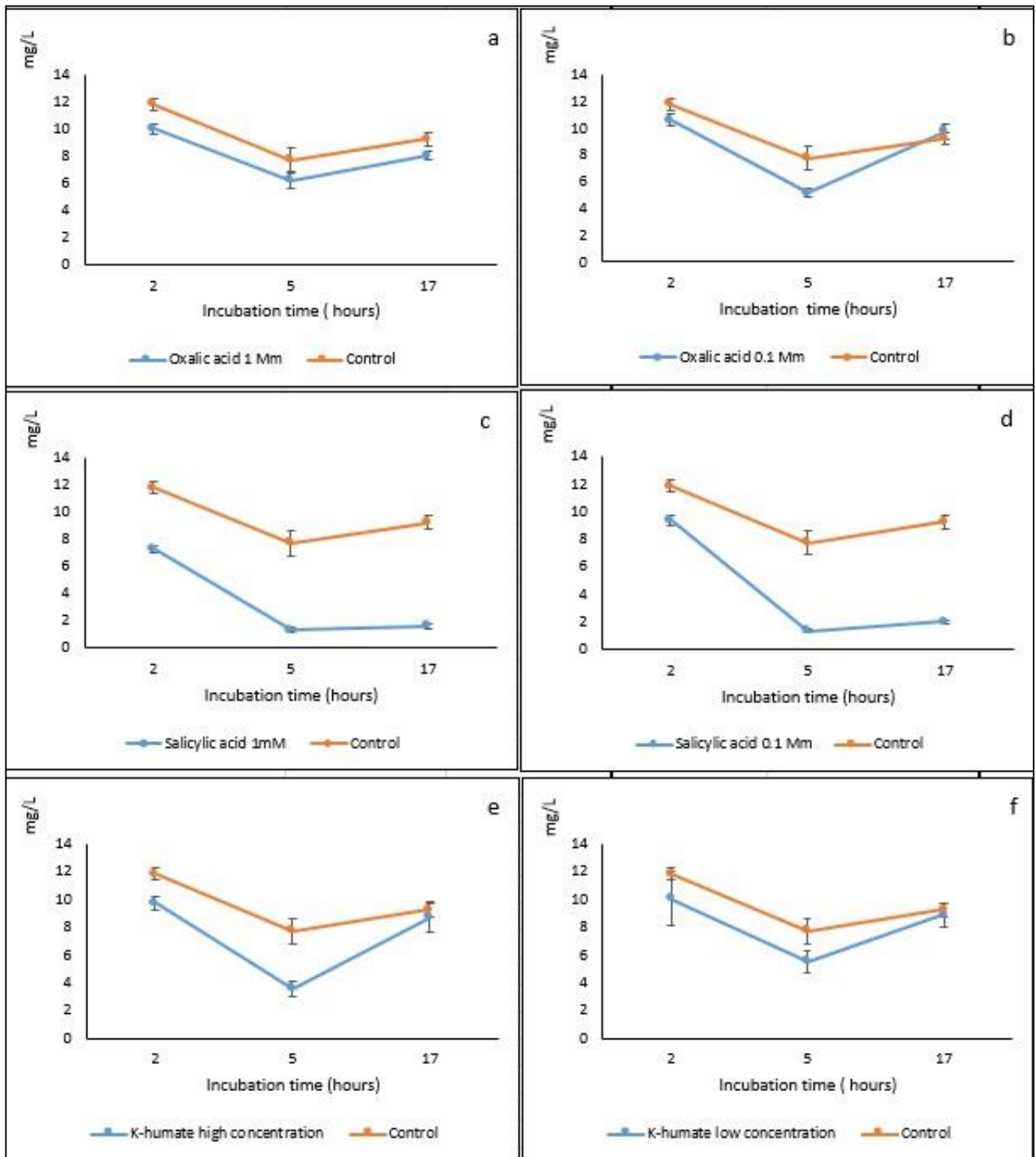
Data analysis by ANOVA showed that both different treatments and sampling time had a significant effect ($p < 0.05$) on total iron (TFe) concentration.

All treatments showed a significant higher concentration of TFe at 2 hours and significantly lower TFe concentration at 5 hours after the start of incubation (Figures 7.5 a-f). There was a significant reduction of TFe concentration between 2 and 5 hours and a significant increase of TFe concentration was observed between 5 and 17 hours. This trend was similar in all treatment during 17 hours with a significant higher values for the control.

At 2 hours after the start of incubation, the control treatment with 11.8 mg/L of TFe concentration showed the highest TFe and salicylic acid – 1mM showed the lowest TFe with 7.3 mg/L.

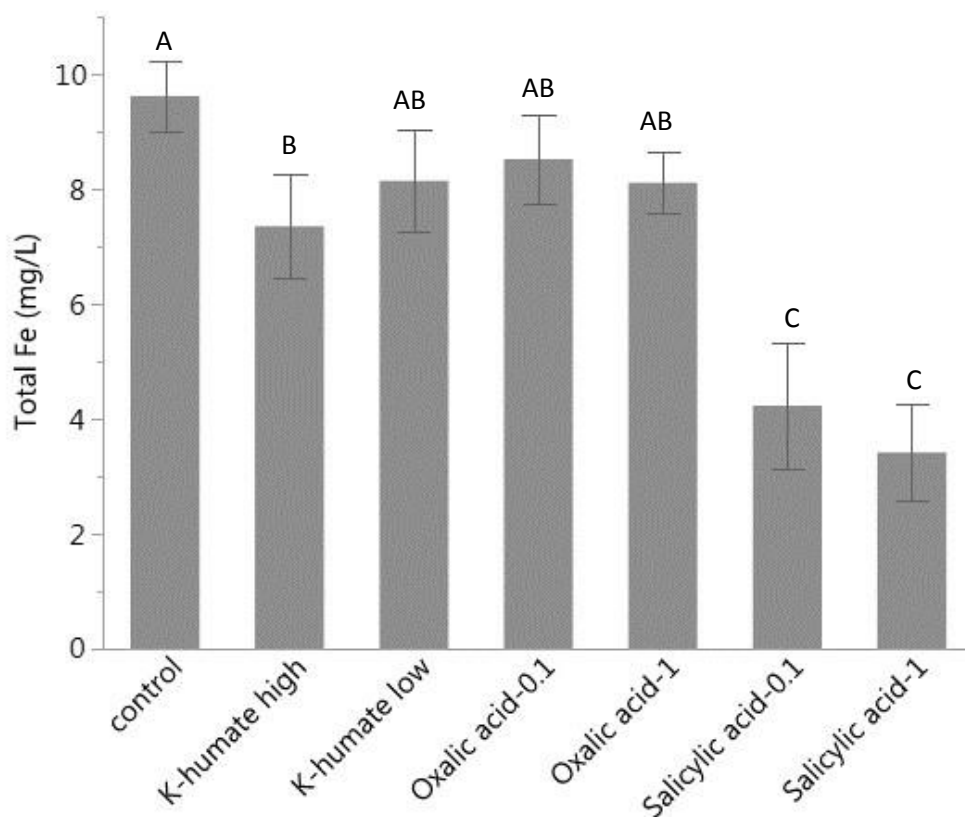
At 5 hours, although the highest TFe was observed in control with 7.7 mg/L however it was much lower than control value at 2 hours. Application of salicylic acid-1mM resulted in the lowest TFe at 5 hours with 1.3 mg/L.

At 17 hours, application of oxalic acid -0.1 mM showed the highest TFe with 9.7 mg/L and salicylic acid -1 mM with 1.6 mg/L resulted in the lowest TFe concentration.



Figures 7.5 (a-f) Effect of time on total iron (TFe) in different treatments. Mean values are presented ($n=4$) \pm s.e

The effect of different treatments on TFe concentration within 17 hours is shown in Figure 7.6. While control treatment showed the highest concentration of TFe with 9.6 mg/L, application of salicylic acid at lower and higher concentration (0.1 and 1 mM), significantly decreased the TFe concentration to 4.2 and 3.4 mg/L, respectively. Comparison of means by Tukey's HSD demonstrated that there was not any significant difference ($p < 0.05$) among oxalic acid-1 mM, oxalic acid-0.1 mM and K-humate at low concentration as they all showed 8 mg/L of TFe.



Figures 7.6 Effect of different treatments on total iron (TFe) in different treatments. Mean values are presented (n=4). Error bars represent \pm standard error. Columns with the same letter are not statistically different as assessed by Tukey's HSD.

ANOVA indicated a significant ($p < 0.05$) interaction of time and organic acid on soil TFe. Further analysis by Tukey's demonstrated that the control treatment at 2 hours showed the highest TFe concentration with 11.8 mg Fe/L; however it was not very different from the other treatments at 2 and 17 hours (Table 7.4). Application of salicylic acid at 5 hours showed the lowest TFe concentration with 1.3 mg/L.

Table 7.4 Interaction effect between organic acids and time on soil TFe. Mean values are presented ($n=4$). Numbers in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

Treatments	Time					Mean of TFe (mg/L)					
control	2	A				11.8(0.6)					
Oxalic acid-0.1	2	A	B			10.6(0.6)					
Oxalic acid-1	2	A	B			10(0.6)					
K-humate low	2	A	B			10(0.6)					
Oxalic acid-0.1	17	A	B	C		9.7(0.6)					
K-humate high	2	A	B	C		9.7(0.6)					
Salicylic acid-0.1	2	A	B	C		9.3(0.6)					
control	17	A	B	C		9.2(0.6)					
K-humate low	17	A	B	C	D	8.8(0.6)					
K-humate high	17	A	B	C	D	8.7(0.6)					
Oxalic acid-1	17		B	C	D	E	8(0.6)				
control	5		B	C	D	E	7.7(0.6)				
Salicylic acid-1	2		B	C	D	E	7.3(0.6)				
Oxalic acid-1	5			C	D	E	F	6.2(0.6)			
K-humate low	5				D	E	F	G	5.5(0.6)		
Oxalic acid-0.1	5					E	F	G	H	5.1(0.6)	
K-humate high	5						F	G	H	I	3.5(0.6)
Salicylic acid-0.1	17							G	H	I	1.9(0.6)
Salicylic acid-1	17								H	I	1.6(0.6)
Salicylic acid-0.1	5									I	1.3(0.6)
Salicylic acid-1	5									I	1.3(0.6)

7.3.3 Total aluminium (TAI) concentration

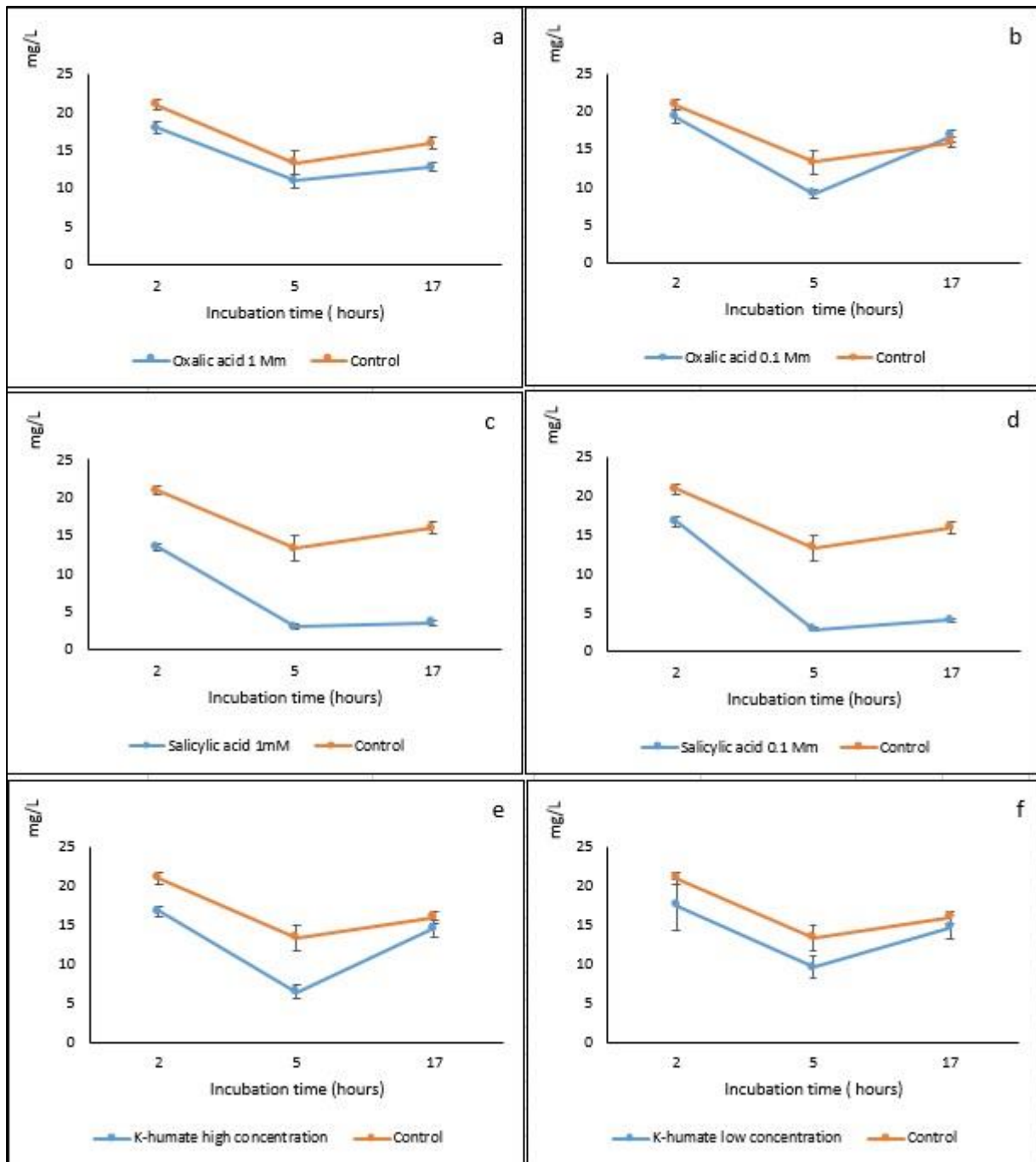
Data analysis by ANOVA showed that both treatment and sampling time had a significant effect ($p < 0.05$) on total iron (TAI) concentration.

All treatments showed a significant higher concentration of TAI at 2 hours after start of the incubation and a significant lowest TAI concentration at 5 hours after start of incubation (Figures 7.7 a-f). There was a significant reduction of TAI concentration between 2 and 5 hours and a significant increase of TAI concentration was observed between 5 and 17 hours. This trend was similar in all treatment during 17 hours with a significant higher values for control.

At 2 hours after the start of incubation, the control treatment with 20.9 mg/L showed the highest TAI and salicylic acid – 1mM showed the lowest TAI with 13.5 mg/L.

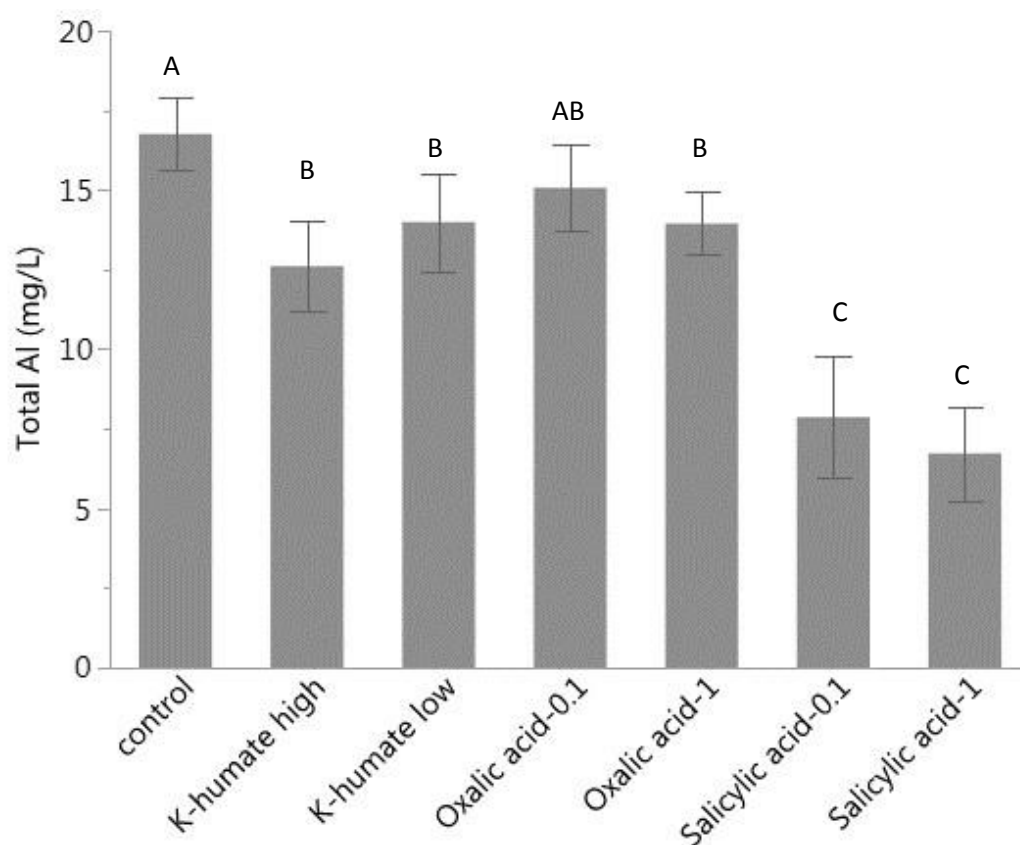
At 5 hours, although the highest TAI was observed in the control with 13.3 mg/L. It was much lower than the control value at 2 hours. Application of salicylic acid-0.1mM resulted in the lowest TAI at 5 hours with 2.8 mg/L.

At 17 hours, application of oxalic acid -0.1 mM showed the highest TAI with 16.8 mg/L and salicylic acid -1 mM with 3.5 mg/L resulted in the lowest TAI concentration



Figures 7.7 (a-f) Effect of time on total aluminium (TAI) in different treatments. Mean values are presented ($n=4$) \pm s.e

The effect of different treatments on TAl concentration within 17 hours is shown in Figure 7.8. While the control treatment showed the highest concentration of TFe with 16.7 mg/L, application of salicylic acid at both the lower and higher concentrations (0.1 and 1 mM), significantly decreased the TAl concentration to 7.8 and 6.7 mg/L, respectively. Comparison of means by Tukey's HSD demonstrated that there was not any significant difference ($p < 0.05$) among oxalic acid-1 mM, K-humate in high concentration and K-humate in low concentration with 13.9, 13.9 and 12.6 mg/L, respectively.



Figures 7.8 Effect of different treatments on total aluminium (TAl) in different treatments. Mean values are presented (n=4).

ANOVA indicated a significant ($p < 0.05$) interaction of time and organic acid on soil TAl. Further analysis by Tukey's demonstrated control treatment at 2 hours showed the highest TAl concentration with 20.9 mg Al /L, however it was not very different from the other treatments at 2 and 17 hours (Table 7.5). Application of salicylic acid at 5 hours showed the lowest TAl concentration with 3 mg Al /L.

Table 7.5 Interaction effect between organic acids and time on soil TAl. Mean values are presented ($n=4$). Numbers in parentheses are \pm s.e. Values allocated the same letter were not significantly different at the $p < 0.05$ level as assessed by Tukey's HSD

Treatments	Time					Mean of Tal (mg/L)						
control	2	A				20.9(1.1)						
Oxalic acid-0.1	2	A	B			19.3(1.1)						
Oxalic acid-1	2	A	B			17.9(1.1)						
K-humate low	2	A	B			17.5(1.1)						
Oxalic acid-0.1	17	A	B	C		16.8(1.1)						
Salicylic acid-0.1	2	A	B	C		16.7(1.1)						
K-humate high	2	A	B	C		16.7(1.1)						
control	17	A	B	C		15.9(1.1)						
K-humate low	17	A	B	C	D	14.7(1.1)						
K-humate high	17	A	B	C	D	14.5(1.1)						
Salicylic acid-	2		B	C	D	E	13.5(1.1)					
control	5		B	C	D	E	13.3(1.1)					
Oxalic acid-1	17		B	C	D	E	12.8(1.1)					
Oxalic acid-1	5			C	D	E	F	11(1.1)				
K-humate low	5				D	E	F	G	9.6(1.1)			
Oxalic acid-0.1	5					E	F	G	H	9.1(1.1)		
K-humate high	5						F	G	H	I	6.4(1.1)	
Salicylic acid-0.1	17							G	H	I	4(1.1)	
Salicylic acid-1	17								H	I	3.5(1.1)	
Salicylic acid-1	5									I	3(1.1)	
Salicylic acid-0.1	5										I	2.8(1.1)

7.3.4 The pH of solution

Table 7.2 shows the pH of the solutions measured during the 17 h incubation time. Application of oxalic and salicylic acids resulted in lower pH of the solution containing those acids. K-humate treatments showed higher pH level as K-humate is an alkaline salt with high pH value. Data analysis by ANOVA did not show any significant effect ($p < 0.05$) of time on the pH of the solutions.

Table 7.6 The pH of the solutions over 17 hours. Mean values are presented (n=4). Standard error is shown in brackets.

Treatments	2 hours	5 hours	17 hours
control	5.2 (0.1)	5.2 (0.1)	5.3 (0.1)
Oxalic acid -0.1mM	4.2 (0.02)	4.5 (0.02)	4.7 (0.2)
Oxalic acid -1mM	4.6 (0.02)	4.8 (0.02)	4.7 (0.02)
salicylic acid -0.1mM	4.2 (0.02)	4.7 (0.02)	4.8 (0.1)
salicylic acid -1mM	4 (0.02)	4.5 (0.02)	4.9 (0.1)
K-humate low concentration	5.5 (0.1)	5.5 (0.1)	5.4 (0.1)
K-humate high concentration	5.4 (0.1)	5.6 (0.1)	5.6 (0.1)

7.4 Discussion

The results showed a reduction of total P over 17 h in all treatments including the control. In this study, total soluble P was measured via 0.45 μm syringe filtration following by TCP reading. Therefore, the phosphorus values represented all soluble forms of P in solution including organic and inorganic forms. The decrease in total P concentration with time may be attributed to shaking the solutions over 17 hours. It is possible that shaking resulted in the disruption of soil aggregates, resulting in the release of Fe and Al into solution and/or exposing more surfaces that could adsorb P or lead to the formation of more insoluble Fe and Al phosphate complexes. In addition, shaking could liberate organic P and polyphosphates already present that might then be hydrolysing to release orthophosphate. This then may have reacted with the Al and Fe and become insoluble precipitates or adsorbed onto mineral surfaces. This would lower the overall concentration of measured total soluble P (Miller and Arai, 2017).

In contrast to the initial hypothesis, the effects of organic acids and K-humate on total P solubility were opposite to what was expected in the Ferrosol soil. Previous studies demonstrated that the application of organic acids into soil solutions increased soluble P (Hu et al., 2002, Jones, 1998, Schefe et al., 2011, Schefe and Tymms, 2013, Schefe et al., 2008), so it was expected that the addition of organic acids would release more P into the soil solution compared with the untreated control. Aliphatic acids, like oxalic acid, in particular are generally more effective than phenolic acids in mobilising soil P reserves (Schefe et al., 2011, Hue, 1991), however, the soil used in this study contained a relatively high available P content (Table 4.3) and this may have reduced the ability to detect an effect of organic ligand competition for cation (Fe/Al) adsorption sites. Furthermore, the high level of soil organic matter may have masked the effectiveness of additional organic matter, including the LMWOAs and the K-humate in mobilising soil P (Patti et al., 2013, Lee and Bartlett, 1976, Duplessis and Mackenzie, 1983, Hartz and Bottoms, 2010). To illustrate this, the application of LMWOAs significantly

increased the available P when the organic matter content of the soil was approximately 1% (Mihoub et al., 2017), while the Ferrosol used in this study contained approximately 12% organic matter (Table 7.1). The observation that oxalic acid and K-humate had limited effects on P mobilisation was mirrored by a lack of effect on Fe/Al solubility with respect to the untreated controls. This confirms a limited interaction with metal cations involved in regulating P availability. In addition, a high organic matter soil is likely to possess a higher level of enzyme activity due to a larger soil microbial community. For example, phosphatase enzymes can promote the hydrolysis of naturally occurring phospholipids in the soil (Turner et al., 2002), thus releasing orthophosphate which could then be “fixed” by the various mechanisms previously discussed.

The observation that the addition of the organic acids further reduced the total soluble P measured, particularly at 2 hours and further decreased by 5 hours, suggested additional interactions are involved. The pH of the solutions involved in this study could be a factor (Table 7.2). The salicylic and oxalic acid solution pH may have contributed to further hydrolysis of naturally occurring organic phosphate esters, polyphosphates and phospholipids, associated with the high soil organic matter. A lowering in pH could contribute to release of additional Al^{3+} and Fe^{3+} cations into the soil solution, which could then interact with the orthophosphate, as well as the organic acids themselves. Insoluble complexes may then have formed.

Interestingly, salicylic acid actually reduced, rather than increased, P availability in the soil solution compared to controls, whilst simultaneously reducing soluble Fe and Al concentrations. A number of authors have found that salicylic acid displays anomalous behaviour in soil solutions, as it has a high stability constant for exchangeable Al ($\log K_{\text{Al}} = 12.9$ cf. oxalic acid $\log K_{\text{Al}} = 6.2$), yet it apparently forms less stable complexes with mineral oxide surfaces (Fox

et al., 1990). Although several studies have shown that salicylic acid can mobilise Al and/or P from soil (Fox et al., 1990; Li et al., 2006; Khorassani et al., 2011), most of the soils studied had an organic C content of less than 1%. It is possible that the high organic C content of this soil (>4%) may have increased salicylate adsorption via phenolic interactions with hydrophobic organic coatings on clay particles, leaving the acidic functional group free to chelate soluble Al/Fe from the soil solution. Because these metal ions are multivalent, the excess positive charge may have then stimulated additional complexation and sorption of dissolved phosphate, forming metal-organo-phosphate complexes.

In the case of K-humate, the intermediate drop in total soluble P might be due to the capacity of the humate to complex Al and Fe, similarly to salicylic acid, but due to its complex structure and being largely insoluble, further interactions on its surface could involve organo-metal-phosphate complexes. This is consistent with observations by (Guardado et al., 2008, Arp and Meyer, 1985) who showed that the phosphorus in these complexes can then be released over time. Indeed after 17 hours of equilibration in this study, the K-humate treatment showed an increase in total soluble P.

As the effects of organic amendments were not consistent among treatments and time, further experiments will be needed in different types of soils and organic amendments.

7.5 References

- ARP, P. A. & MEYER, W. L. 1985. Formation constants for selected organo—metal (Al^{3+} , Fe^{3+})—phosphate complexes. *Canadian journal of chemistry*, 63, 3357-3366.
- BOLAN, N. S., NAIDU, R., MAHIMAIRAJA, S. & BASKARAN, S. 1994. Influence of low-molecular-weight organic acids on the solubilization of phosphates. *Biology and Fertility of Soils*, 18, 311-319.
- CHEN, Y.-S. R., BUTLER, J. N. & STUMM, W. 1973. Adsorption of phosphate on alumina and kaolinite from dilute aqueous solutions. *Journal of Colloid and Interface Science*, 43, 421-436.
- DUPLESSIS, G. & MACKENZIE, A. 1983. Effects of leonardite applications on phosphorus availability and corn growth. *Canadian journal of soil science*, 63, 749-751.
- EARL, K., SYERS, J. & MCLAUGHLIN, J. 1979. Origin of the effects of citrate, tartrate, and acetate on phosphate sorption by soils and synthetic gels. *Soil Science Society of America Journal*, 43, 674-678.
- FINK, J. R., INDA, A. V., TIECHER, T. & BARRÓN, V. 2016. Iron oxides and organic matter on soil phosphorus availability. *Ciência e Agrotecnologia*, 40, 369-379.
- GUARDADO, I., URRUTIA, O. & GARCIA-MINA, J. M. 2008. Some structural and electronic features of the interaction of phosphate with metal–humic complexes. *Journal of agricultural and food chemistry*, 56, 1035-1042.
- HARROLD, S. & TABATABAI, M. 2006. Release of Inorganic Phosphorus from Soils by Low-Molecular-Weight Organic Acids. *Communications in soil science and plant analysis*, 37, 1233-1245.

HARTZ, T. K. & BOTTOMS, T. G. 2010. Humic substances generally ineffective in improving vegetable crop nutrient uptake or productivity. *HortScience*, 45, 906-910.

HU, H., LIAO, L. & WANG, X. 2002. Effect of low molecular weight organic acids on inorganic phosphorus transformation in red soil and its acidity. *Ying yong sheng tai xue bao= The journal of applied ecology*, 13, 867-870.

HU, H., TANG, C. & RENGEL, Z. 2005. Role of phenolics and organic acids in phosphorus mobilization in calcareous and acidic soils. *Journal of Plant Nutrition*, 28, 1427-1439.

HUE, N. 1991. Effects of organic acids/anions on P sorption and phytoavailability in soils with different mineralogies. *Soil Science*, 152, 463-471.

HUE, N., CRADDOCK, G. & ADAMS, F. 1986. Effect of organic acids on aluminum toxicity in subsoils. *Soil Science Society of America Journal*, 50, 28-34.

ISBELL, R. 2002. *The Australian soil classification*, CSIRO publishing.

JONES, D. L. 1998. Organic acids in the rhizosphere—a critical review. *Plant and soil*, 205, 25-44.

LEE, Y. S. & BARTLETT, R. J. 1976. Stimulation of plant growth by humic substances 1. *Soil Science Society of America Journal*, 40, 876-879.

LITTLE, K., ROSE, M., PATTI, A., CAVAGNARO, T. & JACKSON, R. 2013. Effect of application rate of commercial lignite coal-derived amendments on early-stage growth of *Medicago sativa* and soil health, in acidic soil conditions. *Functions of Natural Organic Matter in Changing Environment*. Springer.

LOPEZ-HERNANDEZ, D., FLORES, D., SIEGERT, G. & RODRIGUEZ, J. 1979. The effect of some organic anions on phosphate removal from acid and calcareous soils. *Soil Science*, 128, 312-326.

MARTELL, E. 1952. *Chemistry of the metal chelate compounds*.

MCCOLL, J. & POHLMAN, A. 1986. *Soluble organic acids and their chelating influence on Al and other metal dissolution from forest soils. Acidic Precipitation*. Springer.

MIHOUB, A., DADDI BOUHOUN, M., NAEEM, A. & SAKER, M. L. 2017. Low-molecular weight organic acids improve plant availability of phosphorus in different textured calcareous soils. *Archives of Agronomy and Soil Science*, 63, 1023-1034.

MILLER, A. P. & ARAI, Y. 2017. Investigation of acid hydrolysis reactions of polyphosphates and phytic acid in Bray and Mehlich III extracting solutions. *Biology and Fertility of Soils*, 53, 737-742.

NAGARAJAH, S., POSNER, A. & QUIRK, J. 1968. Desorption of phosphate from kaolinite by citrate and bicarbonate. *Soil Science Society of America Journal*, 32, 507-510.

NAGARAJAH, S., POSNER, A. & QUIRK, J. 1970. Competitive adsorption of phosphate with polygalacturonate and other organic anions on kaolinite and oxide surfaces. *Nature*, 228, 83-85.

PATTI, A., ROSE, M., LITTLE, K., JACKSON, R. & CAVAGNARO, T. A meta-analysis of plant-growth response to humic substance applications. *EGU General Assembly Conference Abstracts*, 2013. 12892.

SCHEFE, C., KAPPEN, P. & PIGRAM, P. 2011. Carboxylic acids affect sorption and micro-scale distribution of phosphorus in an acidic soil. *Soil Science Society of America Journal*, 75, 35-44.

SCHEFE, C. & TYMMS, K. 2013. Phased addition of organic and phenolic acids with phosphate fertiliser increases P availability in an acid soil. *Soil Research*, 51, 437-446.

SCHEFE, C. R., PATTI, A. F., CLUNE, T. S. & JACKSON, W. R. 2008. Interactions between organic amendments and phosphate fertilizers modify phosphate sorption processes in an acid soil. *Soil Science*, 173, 433-443.

STEVENSON, F. & ARDAKANI, M. 1972. Organic matter reactions involving micronutrients in soils. *Micronutrients in agriculture*.

STEVENSON, F. & FITCH, A. 1986. Chemistry of complexation of metal ions with soil solution organics. *Interactions of soil minerals with natural organics and microbes*, 29-58.

TAN, K. H. 2010. *Principles of soil chemistry*, CRC press.

TURNER, B., MCKELVIE, I. & HAYGARTH, P. 2002. Main content area Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil biology & biochemistry*, 34, 27-35.

VIOLANTE, A., COLOMBO, C. & BUONDONNO, A. 1991. Competitive adsorption of phosphate and oxalate by aluminum oxides. *Soil Science Society of America Journal*, 55, 65-70.

ZAMUNER, E., PICONE, L. & ECHEVERRIA, H. 2008. Organic and inorganic phosphorus in Mollisol soil under different tillage practices. *Soil and Tillage Research*, 99, 131- 138.

Chapter 8

Conclusion and Future Developments

8.1 Conclusion

The phosphorus efficiency of soil and plants was compared between humate-blended phosphate (HBP) fertilisers and triple superphosphate (TSP). The blended fertilisers were manufactured as granules by a commercial company (Chapter 2). There is limited information regarding the effectiveness of such humate-blended phosphate fertilisers in the literature. Consequently, the research reported in this dissertation seeks to address this knowledge gap with a program that investigates HBP granules effectiveness in a series of laboratory and glasshouse studies.

In the first phase of the work, the blended fertilisers were evaluated in laboratory conditions in different soil solution matrices. Although the HBP granules demonstrated a beneficial effect on the level of available P in acidic soil solutions, the effect was consistent and not different to with the release of P by TSP. The solubility study, or soluble P released over time, which was outlined in Chapter 3, revealed that the solubility trend of HBP during a certain time is similar to the TSP solubility trend. The study into the addition of HBP to the soil solution in slurry demonstrated that HBP was effective within 15 days in three different soils. While the HBP1 fertiliser with 15.5% phosphorus revealed a beneficial effect on soil-available P in Ferrosol and Calcarosol, no significant benefit was observed in a Podosol. This difference may be attributed to the different soil characteristics of Ferrosol and Podosol. Although both soils were acidic, the higher concentration of free Fe and Al cations in the Ferrosol caused better response to HBP1.

When selected HBPs were added into the soil in Petri dishes, HBP2 (p= 14.5%) released more available phosphorus in comparison with TSP only in a Ferrosol-road side which contained a lower concentration of available P in comparison with Ferrosol which was collected from a pasture.

The HBP2 fertiliser in the Petri dish experiment retained more available P than TSP in a post-incubated Ferrosol soil after 30 days post fertilization. The visualisation technique that was used in this study (Chapter 4) demonstrated the higher phosphate diffusion rate from HBP2 granules compared with TSP. Using the visualisation technique in parallel with soil P chemical extraction confirmed the advantage of HBP2 fertiliser (p=14.5%) in the Petri dish study.

In Chapter 5 detailed glasshouse plant growth studies were carried out with selected HBP fertilisers. The glasshouse studies assessed the effect on pasture growth indexes in two different acidic soils. Although the application of HBP2 slightly increased ryegrass dried shoot biomass in the Ferrosol, the results did not confirm any significant distinction, compared with TSP. Generally, pasture growth indexes were similar for both TSP and HBP applications in Ferrosol and Podosol soils. It is likely that the concentration of P was too high in both soils, thereby preventing HBP from eliciting any potential benefits of K-humate associated with HBP fertilisers. Non-significant differences between TSP and HBPs fertilisers in pasture biomass were evident in the Ferrosol, which exhibited high P-fixing capacity, and in Podosol which had a lower P concentration.

A second glasshouse study was conducted in a different Ferrosol with lower available P. The effect of selected HBP fertilisers were studied on two further plant varieties, Radish (*Raphanus sativus.*) and Sweet Corn (*Zea Maize L.*). The application of HBP at two rates, 20 and 40 kg/ha resulted in no significant radish growth effects. The phosphate application rate was shown to be important for radish growth, with a maximum growth benefit observed at 40 kg/ha, however the TSP fertilisers only showed benefit in radish dried shoot biomass and root depth. TSP also indicated a beneficial effect on radish shoot N, P, C and Ca concentrations.

In the corn study, none of the corn growth parameters have been affected by the HBPs fertilisers. However, further analysis indicated an interaction between HBP2 and both application rates (20 and 40 kg/h). It showed a greater number of leaves than TSP for both application rates (20 and 40 kg/h). No significant difference was observed on corn shoot nutrients uptake between TSP and HBPs fertilisers. Although a Ferrosol- road side was selected for the corn study, it is likely that the soil still had a high organic matter to evaluate the HBPs fertiliser. The high organic matter content of these soils was likely to have masked any beneficial effects of the K-humate, which were used in HBPs. A similar effect was observed in a previous study (Duplessis and Mackenzie, 1983).

The final step of this thesis research involved a mechanistic study in order to explain the role of humic substances in the inhibition of phosphate absorption in the soil. Previous studies have described the possible mechanisms whereby organic acids may increase the nutrient efficiency in soil and plant uptake (Wang et al., 1995, Iyamuremye and Dick, 1996, Haynes and Mokolobate, 2001, Nagarajah et al., 1970, Nagarajah et al., 1968, Violante et al., 1991, Zamuner et al., 2008, Fink et al., 2016a, Fink et al., 2016b, Hue, 1991). Oxalic acid and salicylic acids were chosen because it was demonstrated that the low-molecular weight organic acids are more effective in affecting soil phosphorus availability. The application of oxalic acid and K-humate did not improve total soluble P in the soil solution; however, the application of salicylic acid significantly reduced the total soluble P in the soil solution over the time of the study. It is likely that the high concentration of soil organic matter masked the beneficial effect of oxalic acid and K-humate with respect to soil P availability. The application of salicylic acid significantly decreased the total soluble P, Fe and Al concentration in soil solution. This P

concentration reduction, was attributed to the formation of less soluble metal-phosphate complexes by salicylic acid at the soil surface (Fox et al., 1990, Fox and Comerford, 1990).

8.2 Limitation

A collection of soils from all around Victoria was used in initial pre-test studies to choose the most responsive soil to use in further investigations. The soils were collected from pasture farms of the Department of Economic Development, Jobs, Transport and Resources (<http://agriculture.vic.gov.au/agriculture>, accessed November 2017). The high organic matter content of these soils was likely to have masked any beneficial effects of the K-humate, which were used in this study. A similar effect was observed in a previous study (Duplessis and Mackenzie, 1983). To avoid the effects of high levels of organic matter, soil sampling was undertaken from a pasture roadside region for the second and third glasshouse studies. Although the results did not indicate a similar trend to the first glasshouse study, no beneficial effect was observed from changing the soil-sampling region. The native soil organic matter which can perform some of the same functions of humate (Chen and Aviad, 1990) may nonetheless exist in sufficient concentration to minimise or negate the effects of applied K-humate in the form of blended fertilisers.

Previously reported glasshouse studies results are in agreement with our results. They demonstrated that the application of humic substances and lignite-derived humate at the typical agricultural use rates are generally ineffective in improving plant growth, with some exceptions (Hartz and Bottoms, 2010, Chen et al., 2004, Duval et al., 1998, Feibert et al., 2003). The inconsistent results of different studies with respect to plant growth probably relate to the complexity of the soil environment, and as such, issues of representative sampling arise. There are some sources of bias and errors in soil and plant studies in relation to the inconsistency in reactions of humic substances introduced to soil.

8.3 Future work

This thesis research has established valuable new knowledge, particularly in regard to: the effect of lignite-derived humate-blended phosphate fertilisers on phosphorus availability; plant growth indexes in different soils; and the mechanism of reaction of organic acid with soil minerals. This work indicated that blends of traditional superphosphate fertilisers, TSP in the case of this study, with humic materials, are not always effective in increasing P availability and plant growth. However, there remain opportunities to expand and strengthen our understanding of Victorian brown coal (VBC) applications for agricultural applications. Furthermore, the knowledge gained in the present work can be employed in to produce more efficient blended fertilisers from VBC. This could open up a significant new area of study to optimise the semi-organic fertiliser's quality and develop a broad scope of application. This section illustrates some of these directions, which – with the research outcomes of this thesis – can be taken as a starting point.

1. Investigating the potential of lignite-phosphate blended fertilisers and studying the effect of brown coal rather than K-humate on soil phosphorus availability in the form of blended fertilisers.
2. Studying the effect of the application of blended humic rich materials-phosphate fertilisers on different forms of phosphorus in soil. This would be approached by application of the phosphate fractionation method (Hedley and Stewart, 1982).
3. Studying the effect of blended-phosphate fertilisers in another range of soils with different properties.
4. Investigation of blended fertilisers with innovative methods and techniques that measure the phosphate quantity in soils, such as the Fourier-transform infrared spectroscopy (FTIR) technique.

8.4 References

- BOLAN, N. S., NAIDU, R., MAHIMAIRAJA, S. & BASKARAN, S. 1994. Influence of Low-molecular-weight organic acids on the solubilization of phosphates. *Biology and Fertility of Soils*, 18, 311–319.
- CHEN, Y. & AVIAD, T. 1990. Effects of humic substances on plant growth. *Humic substances in soil and crop sciences: Selected readings*, 161–186.
- CHEN, Y., DE NOBILI, M. & AVIAD, T. 2004. Stimulatory effects of humic substances on plant growth. *Soil organic matter in sustainable agriculture*. CRC Press.
- DUPLESSIS, G. & MACKENZIE, A. 1983. Effects of leonardite applications on phosphorus availability and corn growth. *Canadian journal of soil science*, 63, 749–751.
- DUVAL, J. R., DAINELLO, F. J., HABY, V. A. & EARHART, D. R. 1998. Evaluating leonardite as a crop growth enhancer for turnip and mustard greens. *HortTechnology*, 8, 564–567.
- FEIBERT, E. B., SHOCK, C. C. & SAUNDERS, L. D. 2003. Nonconventional additives leave onion yield and quality unchanged. *HortScience*, 38, 381–386.
- FINK, J. R., INDA, A. V., BAVARESCO, J., SÁNCHEZ-RODRÍGUEZ, A. R., BARRÓN, V., TORRENT, J. & BAYER, C. 2016a. Diffusion and uptake of phosphorus, and root development of corn seedlings, in three contrasting subtropical soils under conventional tillage or no-tillage. *Biology and fertility of soils*, 52, 203–210.
- FINK, J. R., INDA, A. V., TIECHER, T. & BARRÓN, V. 2016b. Iron oxides and organic matter on soil phosphorus availability. *Ciência e Agrotecnologia*, 40, 369–379.
- FOX, T. & COMERFORD, N. 1990. Low-molecular-weight organic acids in selected forest soils of the southeastern USA. *Soil Science Society of America Journal*, 54, 1139– 1144.

FOX, T., COMERFORD, N. & MCFEE, W. 1990. Phosphorus and aluminum release from a spodic horizon mediated by organic acids. *Soil Science Society of America Journal*, 54, 1763–1767.

HALAJNIA, A., HAGHNIA, G. H., FOTOVAT, A. & KHORASANI, R. 2007. Effect of Organic matter on phosphorus availability in calcareous soils. *JWSS-Isfahan University of Technology*, 10, 121–133.

HARTZ, T. K. & BOTTOMS, T. G. 2010. Humic substances generally ineffective in improving vegetable crop nutrient uptake or productivity. *HortScience*, 45, 906–910.

HAYNES, R. & MOKOLOBATE, M. 2001. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved. *Nutrient cycling in agroecosystems*, 59, 47–63.

HEDLEY, M. & STEWART, J. 1982. Method to measure microbial phosphate in soils. *Soil Biology and Biochemistry*, 14, 377–385.

HU, H., TANG, C. & RENGEL, Z. 2005. Role of phenolics and organic acids in phosphorus mobilization in calcareous and acidic soils. *Journal of Plant Nutrition*, 28, 1427–1439.

HUE, N. 1991. Effects of organic acids/anions on P sorption and phytoavailability in soils with different mineralogies. *Soil Science*, 152, 463–471.

HUE, N., CRADDOCK, G. & ADAMS, F. 1986. Effect of organic acids on aluminum toxicity in subsoils. *Soil Science Society of America Journal*, 50, 28–34.

IYAMUREMYE, F. & DICK, R. 1996. Organic amendments and phosphorus sorption by soils. *Advances in agronomy (USA)*.

LOPEZ-HERNANDEZ, D., FLORES, D., SIEGERT, G. & RODRIGUEZ, J. 1979. The effect of some organic anions on phosphate removal from acid and calcareous soils. *Soil Science*, 128, 312–326.

MCCOLL, J. & POHLMAN, A. 1986. Soluble organic acids and their chelating influence on Al and other metal dissolution from forest soils. *Acidic Precipitation*. Springer.

NAGARAJAH, S., POSNER, A. & QUIRK, J. 1968. Desorption of phosphate from kaolinite by citrate and bicarbonate. *Soil Science Society of America Journal*, 32, 507–510.

NAGARAJAH, S., POSNER, A. & QUIRK, J. 1970. Competitive adsorption of phosphate with polygalacturonate and other organic anions on kaolinite and oxide surfaces. *Nature*, 228, 83–85.

SCHOUMANS, O. & GROENENDIJK, P. 2000. Modeling soil phosphorus levels and phosphorus leaching from agricultural land in the Netherlands. *Journal of Environmental Quality*, 29, 111–116.

STEVENSON, F. & ARDAKANI, M. 1972. Organic matter reactions involving micronutrients in soils. *Micronutrients in agriculture*.

VIOLANTE, A., COLOMBO, C. & BUONDONNO, A. 1991. Competitive adsorption of phosphate and oxalate by aluminum oxides. *Soil Science Society of America Journal*, 55, 65–70.

WANG, X., WANG, Z. & LI, S. 1995. The effect of humic acids on the availability of phosphorus fertilisers in alkaline soils. *Soil Use and Management*, 11, 99–102.

ZAMUNER, E., PICONE, L. & ECHEVERRIA, H. 2008. Organic and inorganic phosphorus in Mollisol soil under different tillage practices. *Soil and Tillage Research*, 99, 131–138.

ZHEN-YU, D., QING-HUA, W., FANG-CHUN, L., HAI-LIN, M., BING-YAO, M. & MALHI, S. 2013. Movement of phosphorus in a calcareous soil as affected by humic acid. *Pedosphere*, 23, 229–235.

Summary of projects output

Papers

Submitted to Journal

A new generation of organic blended phosphate fertilizers: phosphorus availability in a batch equilibrium study. Azita. Kargosha ^{A, D}, Michael T. Rose ^B, William R. Jackson ^A, Timothy R. Cavagnaro ^C, and Antonio F. Patti ^A

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Ready to be submitted to Journal

Effect of humate-blended phosphate on plant growth- Glass house studies. Azita. Kargosha, Michael T. Rose, William R. Jackson, Timothy R. Cavagnaro and Antonio F. Patti

Conference posters and presentations

Kargosha, A., M. Rose., T. Cavagnaro. And A. F. Patti. The comparison of granular blended humic-phosphate fertiliser and mono ammonium phosphate on solubility of phosphorus and on phosphorus availability. 2014. 17th International conference of Humic Substances Society.

Kargosha, A., M. Rose., T. Cavagnaro. and A. F. Patti. 2014. The effect of humate- phosphate fertilisers on phosphorus solubility and P efficiency. National soil science conference.