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Nitrophosphate as an alternative phosphate fertiliser for acidic sandy soils

By

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DECLARATION

I hereby certify that this thesis is my own work, except where duly acknowledged. I also certify that no plagiarism was committed in writing this thesis.

Signed……

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AN	Ammonium nitrate
BBCH	Biologische Bundesanstalt, Bundessortenamt and Chemical Industry
cm	Centimetre
cmol(+) kg^{-1}	Centimol positive charge per kilogram of soil
CV	Coefficient of variance (%)
DAP	Diammonium phosphate
DCP	Dicalcium phosphate
ECEC	Effective cation exchange capacity ($cmol_c$ kg^{-1})
FC	Field capacity (%)
FSSA	Fertiliser Society of South Africa
FAO	Food and Agriculture Organisation
g	Gram
g kg^{-1}	Grams per kilogram
IFDC	International Fertiliser Development Centre
kg	Kilogram
kg ha^{-1}	Kilogram per hectare
kg m^{-3}	Kilogram per cubic metre
LAI	Leaf area index (m^2 m^{-2})
LSD	Least significant difference
ℓ	Litre
MAP	Monoammonium phosphate
mg	Milligram
mg kg^{-1}	Milligrams per kilogram
mS m^{-1}	Milli Siemens per metre
^oC	Degrees Celsius
PR	Phosphate rock
PAW	Plant available water (mm)
PWP	Permanent wilting point (mm)
ppm	Parts per million
R^2	Regression coefficient
WAE	Weeks after emergence

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ABSTRACT

It is a common practice to use large quantities of reduced nitrogen (N) such as urea, ammonia (NH_3) and ammonium (NH_4^+) as pre-plant (before planting) fertilisers in the Free State, Mpumalanga and North West where farmers apply monoammonium phosphate (MAP(33)) together with urea before planting. However, MAP(33) a source of phosphorous (P), which is pre-applied resulted in soil acidification, the accumulation of NH_4^+ and loss of cations. Nitrophosphate was therefore suggested as an alternative phosphate fertiliser to remedy low P use efficiency, soil acidity and to replace lost calcium (Ca) and magnesium (Mg). A study was conducted with the aim to compare nitrophosphate with MAP(33) when used as pre-plant fertilisers in acidic sandy soils commonly found in commercial agriculture. To meet this aim, two greenhouse experiments were conducted at the research facilities of Omnia (Pty) Ltd in Sasolburg, South Africa in 2013. The biomass, residual soil nutrient status and nutrient uptake of potted wheat plants were compared when fertilised with nitrophosphate and MAP(33). The experiments consisted of a completely randomised design (CRD) with two P fertiliser sources (nitrophosphate and MAP(33)), applied at four different rates (0, 15, 30 and 45 kg P ha^{-1}) replicated five times. Urea was added to all treatments, except the controls, to ensure that all treatments received the same amount (106 kg N ha^{-1}) of nitrogen (N). Micronutrients were supplied by topdressing with HIDROSPOOR™ at a rate of 2 kg ha^{-1}. For the experiments wheat was planted in an acidic sandy soil (pH_{KCl} of 4.1) collected from a commercial farm in Bothaville with a low Bray-1 P (15 mg kg^{-1}), S (13 mg kg^{-1}), ammonium acetate extractable Ca (79 mg kg^{-1}) content and 5% clay.

Results from the study indicated that the pH of soils treated with MAP(33) was higher than the pH of soils treated with nitrophosphate. The leaves of MAP(33) fertilised wheat had a consistently higher chlorophyll content than the leaves of nitrophosphate fertilised wheat, except for the period 2-3 weeks after emergence (WAE). MAP(33) fertilised wheat had 108% (first trial) and 105% (second trial) more root growth and 96% (first trial) and 167% (second trial) more leaf growth compared to nitrophosphate fertilised wheat. MAP(33) also resulted in higher sulphur (S), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), copper (Cu), manganese (Mn), zinc (Zn) and iron (Fe) uptake. There was a strong correlation (R^2 = 0.81) between soil pH_{KCl} and root growth where the lower soil pH resulted in impaired

root development which adversely affected wheat growth and nutrient uptake. The secondary nutrients associated with nitrophosphate therefore did not improve wheat growth.

Results from the experiments indicated that the MAP(33) treatments resulted in lower concentrations of S, N, K, Cu, Mn, Zn, Fe and Mo in the dry leaf matter than nitrophosphate treatments. The results of this study suggests nitrophosphate is not the preferable fertiliser to substitute MAP(33) as a pre-plant fertiliser in soils with a low pH. However, under less acidic soil conditions (pH 6.5) nitrophosphate resulted in better growth than MAP(33).

Keywords: acidic soils, monoammonium phosphate, nitrophosphate, wheat

CHAPTER 1

INTRODUCTION

It has become common practice to use large quantities of fertilisers containing reduced nitrogen (N) such as ammonium (NH_4^+) and ammonia (NH_3), and urea that can hydrolyse to NH_4^+, as pre-plant fertilisers or part of a pre-plant blend for maize production in the Free State, North West and parts of Mpumalanga (Bornman 2013). Pre-plant fertilisers in this context refers to the application of granular or liquid fertilisers before planting (DeGroot et al. 1982). Reduced N fertilisers are N that occur in the NH_4^+ form with the potential of causing soil acidity. In this adopted practice farmers mix monoammonium phosphate (MAP(33)) and urea as a pre-plant blend in the top 20-30 cm of the soil to reduce N losses due to volatilisation and denitrification. An added advantage of this practice is that the need to topdress is reduced, which result in less labour requirements during the festive season, when workers break for holidays.

The N in MAP(33) ($(NH_4)_3PO_4$) is in NH_4^+ form, while urea (CH_4N_2O) is hydrolysed to NH_4^+, NH_3, water (H_2O) and carbon dioxide (CO_2). With repeated use of these fertilisers, NH_4^+ accumulate and may cause the acidification of the subsoil (the soil directly below the root zone) when nitrified with the subsequent loss in macro cations such as calcium (Ca), potassium (K) and magnesium (Mg). This was confirmed by Bornman (2013) who analysed data from 5 200 subsoil samples from the Omnia Chemtech laboratory soil analysis database for the period 2002-2011. The results indicated that in the Bothaville, Viljoenskroon and Hoopstad regions of the Free State the soil pH_{KCl} had decreased by more than 0.4 units, from 2002 to 2011, to a critical level of 4.7, where crop growth is adversely influenced by the low pH. The extractable soil acidity had significantly increased from zero to more than 10% (highest value recorded is 53%) for more than 20% of the samples and in 40% of the samples the pH_{KCl} was less than 4.5 (lowest value recorded is 3.5). Cation deficiencies were also detected and 50% of the soil samples had Ca concentrations lower than 330 mg kg^{-1}, Mg concentrations lower than 60 mg kg^{-1} and K concentrations lower than 70 mg kg^{-1}. As such, crops cultivated in these soils produce lower yields and farmers suffer

financial loses. Generally the minimum threshold values, in South Africa, for ammonium acetate extractable Ca is 300 mg kg^{-1} and for Mg it is 50 mg kg^{-1} in the topsoil (Miles 2012). The minimum threshold value for K for soils with a low clay content is 125 mg kg^{-1} and 225 mg kg^{-1} for soils with a high clay content and high base saturation (> 95) (Meyer and Wood 1985). In soils with a low base saturation (< 70), K recommendations range between 100 and 200 kg ha^{-1}, with a maximum of 200 kg ha^{-1} for soils with severe K deficiency and up to 300 kg ha^{-1} for soils with a high base saturation (Miles 2012).

It is well known that soil acidity adversely affect crop growth and yield (Foy 1992; Conyers et al. 1996; Mengel and Kirkby 2001; FSSA 2007). When MAP(33) and urea are applied they result in NH_4^+ accumulation, especially on fairly cold, acidic sandy soils low in organic matter, because these conditions inhibit the nitrification of NH_4^+ (Bornman 2013). This situation is exacerbated in soils that received MAP(33) and urea as pre-plant fertilisers and that is prone to waterlogging particularly during the early planting season after the first rains. Many plants develop symptoms of toxicity when subjected to excessive concentrations of NH_4^+ (Britto and Kronzucker 2002) such as: leaf chlorosis, decrease in net photosynthesis, inhibited root growth, decrease in yield and leaf nutrient concentration and changes in several metabolites, such as amino or organic acids concentrations (Britto and Kronzucker 2002; Bornman 2013). When N is exclusively provided as NH_4^+, germination and cation uptake are suppressed which results in a decrease in the Ca and Mg content of plant tissues (Jones 1973; Borgognone et al. 2013).

Failure of plant roots to intercept reduced N from sources such as ammonium sulphate ($(NH_4)_2SO_4$), ammonium nitrate (NH_4NO_3), anhydrous ammonia (NH_3) and urea facilitate the conversion of NH_4^+ to NO_3^- through nitrification. This causes severe soil acidification (FSSA 2007; Bornman 2013), which worsens when N management is poor. Increased acidity results in elevated concentrations of H^+ in the soil that subsequently displace cations adsorbed onto soil colloids (Figure 1.1), resulting in the possible leaching of Ca, Mg and K from the root zone (Chan et al. 1992; Chan and Heenan 1993a; Haynes and Mokolobate 2001; Mengel and Kirkby 2001; Bornman 2013).

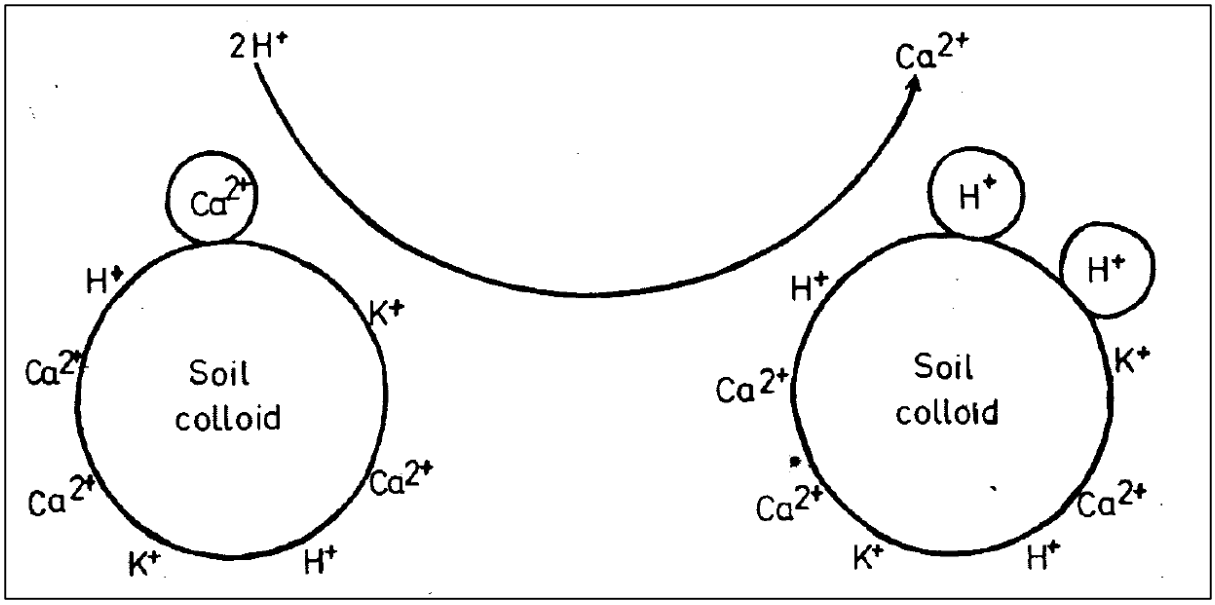


Figure 1.1 Schematic illustration of H^+ replacing other cation species on soil colloids (Mengel and Kirkby 2001)

The challenge in these soils, with excessive NH_4^+ in the subsoil, low pH (< 4.7) and deficient in basic cations (K, Ca and Mg), will be to find solutions for farmers to continue producing high yields. The first suggestion is to apply base cations to the acidified subsoil by means of fertilisers containing high concentrations of base cations combined with lime or gypsum (Bornman 2013). This will increase the pH of the soil and the cation content, especially Ca, which will enhance nitrification even under low pH conditions. The second option is to replace fertilisers containing NH_4^+, or that can be converted to NH_4^+, with a NO_3^- containing fertiliser (Bornman 2013). An added advantage to this practice will be that a more favourable ratio between NO_3^- and NH_4^+ will be achieved. The optimum NH_4^+:NO_3^- ratio is reportedly close to 3:1 (Adriaanse and Human 1986; Adriaanse and Human 1988a,b; Kant et al. 2007) and several studies have proved that plant growth is favoured more when a combination of NH_4^+ and NO_3^- is supplied to crops than when N is supplied solely as NH_4^+ (Adriaanse and Human 1986, Adriaanse and Human 1988a,b; Adriaanse and Human 1993; Gerendas et al. 1997; Britto and Kronzucker 2002; Siddiqi et al. 2002; Kant and Kafkafi 2003; Kant et al. 2007; Adriaanse 2012; Borgognone et al. 2013).

Nitrophosphate is a phosphate fertiliser that contains N and P as primary nutrients and Ca, Mg and S as secondary nutrients. The N fraction in nitrophosphate comprises of both NO_3^- and NH_4^+ at a ratio of approximately 2:1 (NH_4^+:NO_3^-), making it an ideal

option to replace MAP(33). It should be clarified that only nitrophosphate produced through the double acid process has secondary nutrients, while nitrophosphate produced through the Odda process (Steen et al. 1986), does not contain secondary nutrients. The Odda process is a Norwegian method that predominantly uses sulphuric acid (H_2SO_4). The double acid process is a method used in South Africa which is similar to the Odda process (Mizane 2012), except that it predominantly uses nitric acid (HNO_3). The final nitrophosphate is 97% water soluble and 100% citric acid soluble with 18% N, 8% P, 6% Ca, 0.3% Mg and 6% S (Bornman 2012).

MAP(33) blended with urea, as in the adopted practice of applying a pre-plant blend (Bornman 2012), is also a source of N (11%) and P (22%), but does not contain base cations to alleviate K, Ca and Mg soil deficiencies. MAP(33) can be found in granular, crystalline or powder forms (Mullins and Sikora 1990). It has good physical properties and good agronomic performance under a wide variety of conditions (IFDC 1991). The production of MAP(33) uses the phosphate concentrate ($Ca_{10}(PO_4)_6F_2$) as the starting material (Saueia and Mazzilli 2006) which goes through a series of chemical reactions to form phosphoric acid. The latter is then ammoniated in 1:1 mole ratio of ammonia (NH_3) to phosphoric acid to form MAP(33) (Bornman 2012). The main disadvantage of this production process is that it generates a large volume of phosphogypsum that create disposal problems (Khurana et al. 2004; Saueia and Mazzilli 2006).

It is generally assumed that nitrophosphate gives a similar agronomic efficiency as most other ammoniated phosphates (Bornman 2012). Khurana et al. (2004) and Lewis (1955) also shared the same sentiments that nitrophosphate was equally effective as diammonium phosphate (DAP), another ammoniated fertiliser. However, it is not known if the researchers were using the double acid or Odda processed nitrophosphate. Therefore, a study was conducted with the aim to compare nitrophosphate with MAP(33) when used as pre-plant fertilisers in acidic sandy soils commonly found in commercial agriculture. To validate if nitrophosphate could substitute MAP(33) as a pre-plant fertiliser in soils with a low pH. It was hypothesised that,

1. MAP(33) applied as a pre-plant fertiliser in an acidic sandy soil could result in a further decrease in soil pH due to the occurrence of NH_4^+-N in MAP(33).

2. The application of nitrophosphate, as a pre-plant fertiliser in an acidic sandy soil, will result in more wheat biomass produced when compared with MAP(33), due to the additional supply of Ca, Mg, and S.
3. The nutrient uptake of wheat will be higher with nitrophosphate application than MAP(33) application due to reduced soil acidity and better root growth that will facilitate efficient nutrient absorption.

The objectives of this study were to:

1. Compare post-harvest soil pH, soil residual P, Ca, Mg and S of potted wheat grown in an acidic sandy soil fertilised with nitrophosphate and MAP(33) as pre-plant fertilisers.
2. Determine root and above ground growth of potted wheat grown in an acidic sandy soil fertilised with nitrophosphate and MAP(33) as pre-plant fertilisers.
3. Compare nutrient uptake of potted wheat grown in an acidic sandy soil fertilised with nitrophosphate and MAP(33) as pre-plant fertilisers.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Comprehensive literature has already been published on the functions and cycle of P in plants and soils (Mengel and Kirkby 2001; FSSA 2007; Hilton et al. 2010; Mundus et al. 2013; Wall et al. 2013), therefore only a short summary on these aspects will be presented. The focus will be on fertilisers and the factors affecting fertiliser use efficiency. Although nitrophosphate and MAP(33) contain N, the study aims at validating nitrophosphate as an alternative P fertiliser and therefore the literature will focus on P nutrition.

2.2 Role of P in plants

Nitrophosphate and MAP(33) are fertilisers that supply P and significant amounts of N to crops, especially at planting. Phosphorus is one of the macro-elements (C, O, N, P, K, Ca, Mg, S) that is supplied by the soil and is required in abundance for normal plant growth (Mengel and Kirkby 2001). It is the second most widely used fertiliser nutrient after N (Grain SA 2011) and is the least available global resource among the macro-elements needed for plant growth (Hilton et al. 2010). Phosphorus scarcity is due to the fact that only a few selected areas worldwide have deposits of phosphate rock (PR), which is the raw material for the production of phosphate fertilisers. Mundus et al. (2013) described it as a finite natural resource that must be used economically in order to sustain the world for many years.

Critical functions of P in plants include its involvement in cell division, root growth, flowering and fruit ripening (FSSA 2007). It also plays a role in photosynthesis, respiration, reproduction and in the maintenance of genetic identity (Vance et al. 2003; FSSA 2007). Orthophosphates are built into organic compounds such as phytine, adenosine triphosphate (ATP), adenosine diphosphate (ADP) and phospholipids (Mundus et al. 2013; Wall et al. 2013). Numerous studies in Africa have shown that P fertilisers such as ground PR, modified PR products and other water soluble P fertilisers can significantly increase crop yield (Buresh et al. 1997; Amanullah et al. 2010) by increasing root growth, which leads to the absorption of nutrients and water

from larger soil volumes. Plants grown in P deficient soils have a reduced adventitious root system which also result in a lower leaf area index (LAI) with less interception of photosynthetic active radiation (PAR) (Amanullah et al. 2010). Plants with limited P supply have stunted growth and the leaves and stems turn red-blue or dark green. This colouration is the result of anthocyanin that accumulates in plants (anthocyanosis) to protect the photosynthetic areas from oxidative damage by sunlight (Mundus et al. 2013) or due to the accumulation of carbohydrates once the respiration shuts down (Wall et al. 2013). However, when P supply is restored these symptoms disappear and the crop grows normally. Studies have shown that adequate supply of P improve yields and therefore contribute to food and energy security (Hilton et al. 2010).

Plants have response mechanisms to cope with moderate P scarcity. Under low P supply plants are capable of secreting low molecular weight organic anions and phosphatase exo-enzymes to solubilise and mobilise organic P (Mundus et al. 2013; Wall et al. 2013). Plants can also increase P availability by modifying the chemistry of the rhizosphere through the exudation of protons (H^+) from the roots to acidify the rhizosphere, organic anions (e.g. the ligands malate, citrate) to increase P desorption through ligand-exchange and exo-enzymes to increase the solubility of sparingly soluble P minerals (George et al. 2011).

Plants assimilate P as an orthophosphate ion (Figure 2.1), which could be absorbed as mono-hydrogen phosphate (HPO_4^{-2}) or di-hydrogen phosphate ($H_2PO_4^-$) (Mengel and Kirkby 2007). These forms coexist and their ratio depends on the soil pH. At a pH below 7, the $H_2PO_4^-$ form dominates, while in soils with a pH above 7 the HPO_4^{-2} form dominates (FSSA 2007; Noack et al. 2010), which is also the dominant ion absorbed by plants (Hilton et al. 2010).

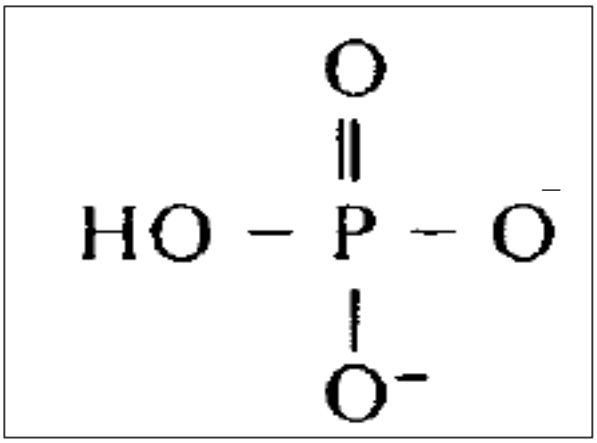


Figure 2.1 The structure of a mono-hydrogen phosphate ion

Phosphorus also plays an important role in humans and animals which they acquire through the consumption of plants (Wall et al. 2013). Humans require a minimum of 0.6-0.7 g P day^{-1} (van Rossum et al. 2011), but the average daily intake could be up to 2-3 g P day^{-1} (Flynn et al. 2009). A typical 70 kg adult is assumed to contain about 780 g P and it is recommended that the daily P intake must be 1 mg P $capita^{-1}$ day^{-1} (Hilton et al. 2010). To meet or maintain these figures agriculture must keep producing quality food that contains substantial amounts of P for human consumption.

2.3 Forms and cycling of P in the soil

The total amount of P in the form of orthophosphate in soil is estimated to range between 0.02-0.15% P (Mengel and Kirkby 2001). In agricultural land, the general concentration varies from 1 500 to 3 000 kg P ha^{-1}. However only 15 g P in solution is bioavailable in the top-soil of one hectare (Mundus et al. 2013). Most of the applied P is fixed by aluminium (Al), iron (Fe) hydrous oxides or Ca (Haynes and Mokolobate 2001), which reduces the bioavailability of P. Soils with a high Al and Fe content are typically found in the highly weathered soils of the tropical and subtropical regions of the world (Mundus et al. 2013). The small proportion of P in solution that is bioavailable cannot meet the crop requirements, hence re-supply is imperative. Vance et al. (2003) estimated that on a global scale more than 30% of arable land is P deficient and these deficiencies are mostly located in the developing world (Mundus et al. 2013). This is common with resource poor farmers who cannot afford to supplement P with inorganic fertilisers. The same sentiments were shared by Mundus et al. (2013) that most P

limited soils are often found in regions where farmers have limited economic resources and investment in P fertilisers is challenging or impossible.

2.3.1 Fractions of P

Phosphorus in soils is associated with the rhizosphere and soil minerals and four different P fractions can be identified: i) Non-labile P that form part of the crystal structure of soil minerals, such as apatite, aluminium and iron compounds. This fraction is not bioavailable. ii) Labile P such as phosphate precipitates and P adsorbed on clay particles. This fraction is unavailable but could slowly over time become bioavailable. iii) Phosphorus in living micro-organisms and in soil organic forms that constitutes 10–60% of total P in topsoil. This fraction is immobilised and temporarily not bioavailable. iv) Phosphorus in solution fraction, in both organic and inorganic forms (FSSA 2007).

Phosphorus ($H_2PO_4^-$ or HPO_4^{2-}) in solution is the only fraction that is readily available for plant uptake. This is also the only fraction that has any measurable mobility (Mundus et al. 2013). When P fertiliser is applied to the soil, it dissolves and a large part of the P is adsorbed on soil colloids and precipitates with Al, Fe or Ca (labile fraction). However, the latter depends on soil pH. Applied P that becomes labile has cost implications for farmers, because more fertiliser must be applied to compensate for the fraction that becomes unavailable to the crop. However, fertilisers react differently in soils, and the conversion of the available P to labile fraction partially explains why certain fertilisers have low P use efficiency (Shen et al. 2011).

Non-labile P is the insoluble P which is released very slowly into the labile form (Mengel and Kirkby 2001). Phosphorus in this form is not available to plants due to the fact that P is strongly bound to Ca (apatites - $Ca_5(PO_4)_3(OH,F,Cl)$) in soils with a pH higher than 5.8 (Figure 2.2). In soils with a pH lower than 4.2 P is strongly bound with the crystal structure of Fe to form strengite ($FeH_2PO_4(OH)_3$) and Al to form variscite ($AlH_2PO_4(OH)_2$) (Mundus et al. 2013). In acidic soils, pH lower than 4.2, P reacts with Al and Fe hydrous oxides to form stable insoluble oxides (Figure 2.2) which cannot be absorbed by the plant (Haynes and Makolobate 2001).

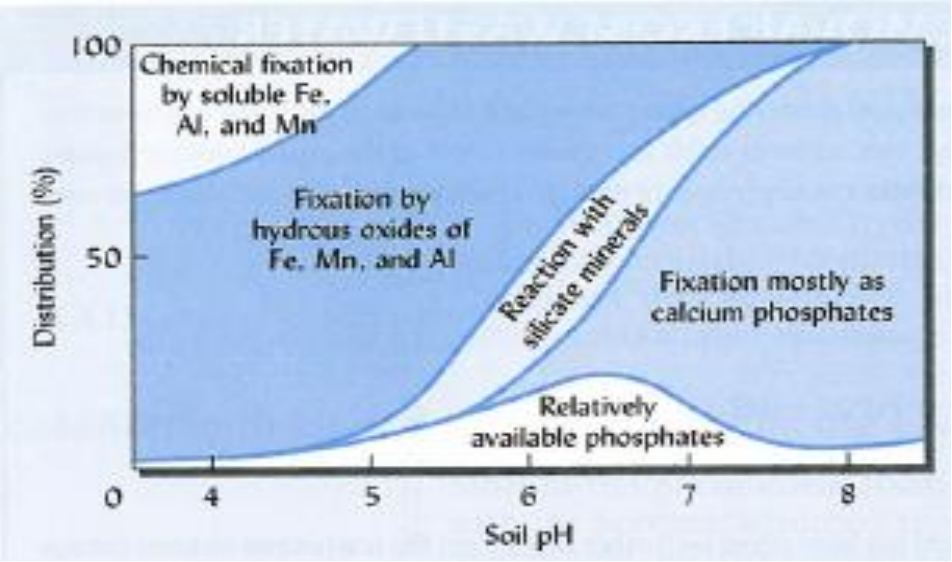


Figure 2.2 Fractions of soil P in the soil as influenced by pH (Brady 1990)

The labile P fraction consists of phosphate that is not strongly bound to the surfaces of cations and is in rapid equilibrium with soil solution phosphate (Mengel and Kirkby 2001). When P becomes depleted from the rhizosphere the adsorbed P, which is covalent or electrostatically bound to Al and Fe oxides, Ca carbonates and various clay minerals is displaced by anions such as silicates (SiO_4^{-4}), sulphates (SO_4^{2-}), arsenate (AsO_4^{-3}), molybdate (MoO_4^{-2}) and carbonates (CO_3^{-2}) that compete with ortho-phosphates for the anion exchange sites and transferred to the soil solution as plant available P (Mundus et al. 2013). Highly weathered soils with high clay content usually retain more P than more course sandy textured soils (Brady 1990).

Phosphorus associated with the organic fraction in soils consists mainly of phosphate derived from plant residues, for example, phytin, phospholipids and inositol phosphates. The quantities, forms and dynamics of organic P are determined by a combination of biological, chemical and physical factors (Condron et al. 2005). For instance, a soil containing high amounts of microorganisms has the capacity to breakdown organic matter and release P. However, P can also be immobilised by microorganisms by converting P from an inorganic to an organic form. This can be favourable to reduce the mineral P fixation because P is locked within the living organisms until they die and therefore acts as storage (Wallace and Knausenberger 1997). Abiotic factors such as soil texture, water content and temperature influence the formation of organic P (Skopp et al. 1990). For plants to utilise organic P, it must be converted into an inorganic form through the mineralisation process which is

facilitated by microorganisms, plant root exudate exo-enzymes and phosphatase enzymes that catalyses the mineralisation process (Mundus et al. 2013; Wallace and Knausenberger 1997). The C:P ratio is another factor affecting the mineralisation process. Low C:P ratio favours mineralisation while a high ratio of C:P of more than 300:1 will favour P immobilisation (Havlin et al. 1999; Marschner 2008).

2.3.2 P cycle in the soil

The P cycle starts with mineral P in rocks that weathers physically and/or chemically to yield inorganic P. The weathered P could be found either dissolved in solution, adsorbed on clay particles or as secondary precipitates (labile and non-labile fractions). Phosphorus in solution is then taken up by plants or consumed by soil living organisms (Schroder et al. 2010). The P in solution that is not utilised can either be completely lost from the cultivated land or become temporarily unavailable through adsorption and precipitation reactions (labile fraction) or being absorbed by plants (Figure 2.3). The adsorbed P could become recycled into the solution phase when favourable soil conditions prevail. Immobilised P re-enters the solution phase when mineralisation occur or the microbes die and decompose. A loss from the cultivated land includes erosion or run-off which removes P from the cropping area making the loss permanent (Figure 2.3).

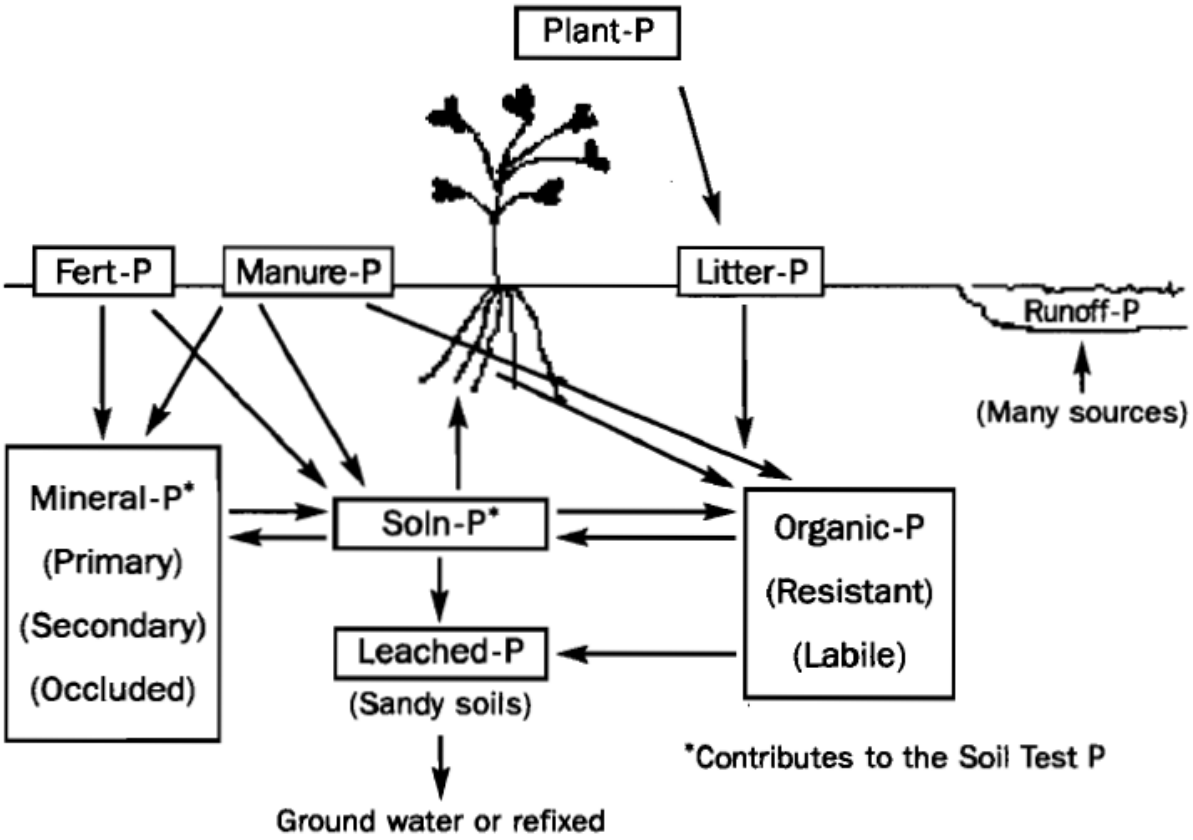


Figure 2.3 Phosphorus cycle in the soil (Bowman and Vigil 2002)

A substantial amount of P is absorbed by crops and subsequently recycled back into the soil when crop residues decompose (Figure 2.3). However, not all of the P is recycled back into the soil. During harvest, plant parts are removed from the field (Figure 2.3) that indirectly contributes to P losses. The proportion of P cycled back into the soil in grain crops, assuming complete crop residue return, is approximately 40% compared to 50–70% for N and 90% for K (SSSA 1997). This suggests a farmer need to supplement at least 60% of the P after every harvest to maintain high soil P content. Phosphorus is removed from agro-ecosystems through the milk, meat or wool from livestock (Wall et al. 2013) and by small animals such as deer, rats or locusts as they defecate outside the field (Schroder et al. 2010). Lost P in agrosystems is replenished by different methods which include the use of organic and inorganic fertilisers (Figure 2.3).

2.4 Phosphate rock as basis for P fertilisers

Phosphate rock (PR) forms a basis for all inorganic P fertilisers which include sedimentary phosphate concentrate (e.g. Langfos), single superphosphate (SSP), enriched superphosphate, double superphosphate, triple superphosphate (TSS), urea ammonium phosphate (urea-P), nitrophosphate, MAP(33) and DAP. It is used as raw material for the production of nitrophosphate, MAP(33), DAP, SSP and TSP (Saueia and Mazzilli 2006; FSSA 2007). Phosphate rock (PR) is also used as a P fertiliser (Buresh et al. 1997), but its P content is low. Large amounts are therefore needed to satisfy the crop requirements and this make it economically unattractive because of the high transport costs involved for delivery onto the farm. The utilisation and suitability of PR also depends on the reactivity of the rock (Buresh et al. 1997). Only PR's with medium to high reactivity are potentially suitable for direct application. Chemical extracting solutions such as neutral ammonium citrate (NAC), 2% citric acid and 2% formic acid can estimate the solubility of PR and its potential for direct application (IFA 2013). Using 2% citric acid, PR solubility (% P_2O_5) below 6% is regarded low, 6.7-8.4% medium and above 9.4% high (Diamond 1979). Table 2.1 shows classification of PR by solubility using 2% citric acid, 2% formic acid solutions and potential responses.

Table 2.1 Classification of PR for direct application by solubility in aqueous media (extraction solutions) and potential response (Diamond 1979)

	Potential response	Solubility (% P_2O_5)	
	NAC	2% citric acid	2% formic acid
High	>5.4	>9.4	>13.0
Medium	3.2-4.5	6.7-8.4	7.0-10.8
Low	<2.7	<6.0	<5.8

*The phosphorus content expressed as a percentage (by weight) measured as P2O5, the anhydride of phosphoric acid.

It is estimated that 130–150 million tonnes PR are annually mined, with the United States and Morocco dominating the world production while developed countries such as Europe, North America and the Pacific Rim (countries such as Australia, Cambodia, Malaysia, South Korea, Vietnam) are major users (Cook et al. 1989). Significant amounts are also produced in China and South Africa (Jasinski 2011).

The main mining site for PR in South Africa is Phalaborwa, where approximately 1.5 x 10^6 t per annum are mined, mainly for domestic use (FSSA 2007). Phosphate rock (PR) production in South Africa represents 1.5% of the total PR produced worldwide, while China is the leading producer (35%), with the USA (17%) in second place followed by Morocco and Western Sahara (15%) (Figure 2.4). In South Africa almost 90% of the annual phosphate rock mined is used in fertilisers, while 6% is used in the industrial sectors and 4% as animal feed additives (Grain SA 2011).

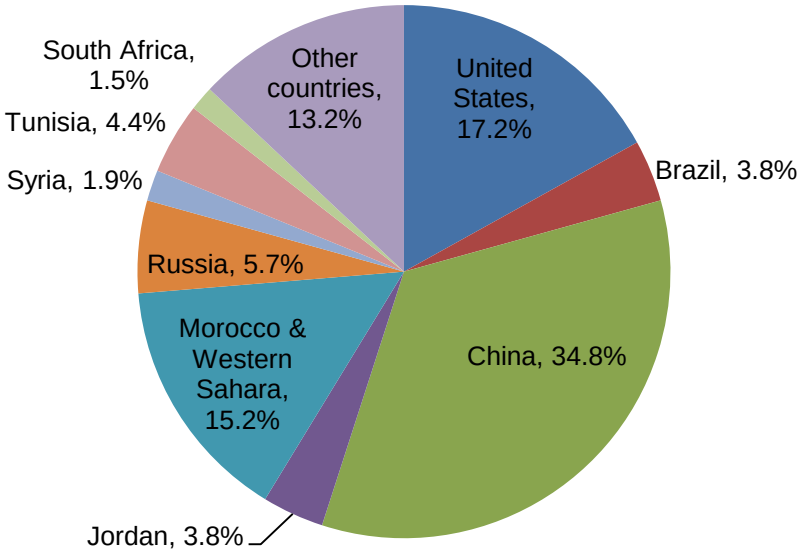


Figure 2.4 Leading PR producing countries in the world for 2009 (Grain SA 2011)

Fertiliser producers prefer PRs with a high P and low heavy metal content. Phosphate rocks (PR) with at least 33.4% P_2O_5 are regarded as adequate for nitrophosphate production and those with less not suitable, because they increase the hydraulic load of the plant (Hussain 2012). The chemical and mineralogical compositions of an ideal PR mined in Ayetoro, Ogun State, Nigeria is presented in Table 2.2.

Table 2.2 Chemical and mineralogical composition of phosphorite mined in Ayetoro, Ogun State, Nigeria (Olanipekun 2003)

Chemical composition		Mineralogical composition	
Constituent	Wt.%	Constituent	Wt.%
P_2O_5	33.81	Fluorapatite	80.3
CaO	52.35	Calcite	13.5
MgO	0.16	Silica	6.2
Fe_2O_3	0.30		
Al_2O_3	0.35		
F-	2.16		
SiO_2	6.83		

2.5 Monoammonium phosphate (MAP(33))

MAP(33) is a source of N (11%) and P (22%) and is widely used by farmers in South Africa especially when blended with urea (Bornman 2012). It does not contain secondary nutrients but has good physical properties and good agronomic performance under a wide variety of conditions (IFDC 1991). MAP(33) is produced in granular, crystalline or powder forms (Mullins and Sikora 1990), with the crystalline form being less desired, because of the uneven distribution of NH_4^+ (Kearns 1965). MAP(33) has a pH of 4-5 and when applied in the soil, the pH decrease further to approximately 3.5-4.5 close to the granule (Rehm 2002; Bornman 2012). On high pH soils this tends to be an advantage because the acid produced may offset the calcareous soils. However, in low pH soils over-application of MAP(33) could have depressing effects on plant growth due to NH_4^+ toxicity on germinating seed, osmotic effect and/or inhibition of the uptake of cations (Bennet and Adams 1970; Dowling 2001; Rehm 2002).

Production of MAP(33) began around the 1920s (Ivell 2012) with phosphate concentrate ($Ca_{10}(PO_4)_6F_2$) from PR as the starting material (Saueia and Mazzilli 2006). Unlike with the nitrophosphate process, phosphoric acid (H_3PO_4) and NH_3 is used during the neutralisation stage in a mole ratio of 1:1 to form MAP(33) (Bornman 2012). The final product contains 11% N in the NH_4^+ form and 22% P in the $H_2PO_4^-$

form and is citric acid soluble (Saueia and Mazzilli 2006). The chemical reaction of phosphoric acid (H_3PO_4) with NH_3 is represented by Saueia and Mazzilli (2006):

$$H_3PO_4 + NH_3 \longrightarrow NH_4H_2PO_4 \qquad (2.1)$$

The main disadvantage with the production of MAP(33) is the large volumes of phosphogypsum that are produced, which create disposal problems (Khurana et al. 2004; Saueia and Mazzilli 2006). This is typical of most phosphoric acid based P-fertilisers including SSP and DAP where up to 5 tonnes of phosphogypsum could be produced per tonne of P_2O_5 (Mizane 2012). In Europe, a decision was once taken by the main European phosphate fertiliser producer to close its phosphoric acid (H_3PO_4) plant at Antwerp in Belgium (IFDC 1991) following difficulties in disposing phosphogypsum. Large volumes of phosphogypsum pollute the environment (Olanipekun 2003; Khurana et al. 2004; Mizane 2012) and pose the risk of contaminating underground and surface water. Furthermore, disposing phosphogypsum is a costly exercise. During the MAP(33) production process gaseous pollutants are released such as hydrogen fluoride (HF), SO_2 and sulphur trioxide (SO_3) (Mizane 2012) which are also detrimental to the environment.

Examples of MAP(33) producers are Omnia Fertiliser (Pty) Ltd and Foskor (Pty) Ltd, (Sasol stopped production of MAP(33) in 2010/2011) in South Africa, and Mosaic (Pty) Ltd in the USA (Grain SA 2011).

2.6 Nitrophosphate

Nitrophosphate is a phosphate fertiliser that contains N and P as primary nutrients with N in the NH_4^+ and NO_3^- forms at a ratio of approximately 2:1 and Ca, Mg and S as secondary nutrients. However, only nitrophosphate produced with the double acid process has secondary nutrients, while those produced with the Odda process does not contain secondary nutrients (Bornman 2013).

Physically, nitrophosphate is found as smooth round prills, free flowing, with low dust and evenly sorted particles of approximately the same size (Hussain 2012). It is also available in liquid and powder forms (Al-Shawi and Dahl 1995). The N, P and Ca nutrients in nitrophosphate are present as ammonium phosphates, calcium phosphates and ammonium nitrate (Abdel-aal and Amer 1995). Chien (2010) also

revealed that nitrophosphate contains N and P as DAP, ammonium nitrate (AN) and dicalcium phosphate (DCP) compounds. In South Africa, nitrophosphate produced through the double acid process contains 18% N (12% NH_4^+ and 6% NO_3^-), 8% P, 6% Ca, 0.3% Mg and 6% S and is 97% water soluble and 100% citric acid soluble (Bornman 2012). There are also advantages associated with the production of nitrophosphate when compared to MAP(33): (i) The production process does not generate sulphur dioxide and large volumes of solid waste and wastewater which may pollute the environment (Olanipekun 2003; Khurana et al. 2004; Mizane 2012), (ii) the production cost is 20% lower than phosphoric and sulphuric acid based fertilisers and this is attributed to lower energy requirements (Olanipekun 2003; Khurana et al. 2004; Mizane 2012).

2.6.1 Production of nitrophosphate

The largest producer and exporter of nitrophosphate worldwide is Russia, which accounts for approximately 20% of the world's production and 35% of the export (Grain SA 2011), while Omnia Fertiliser (Pty) Ltd is one of the companies that produce nitrophosphate in South Africa.

In the first production step of nitrophosphate, PR is chemically digested with nitric acid (HNO_3) (Olanipekun 2003; Hussain 2012) to yield a phosphate concentrate that is supplied mostly by Foskor (Pty) Ltd in South Africa (Grain SA 2011). The phosphate concentrate is further mixed with nitric acid (HNO_3) (Olanipekun 2003) and ammoniated to yield nitrophosphate that consists of dicalcium phosphate (DCP), monocalcium phosphate (MCP) and AN (FSSA 2007). The use of nitric acid (HNO_3) to digest the phosphate ore into nitrophosphate furnishes the N into the product (Abdel-Aal and Amer 1995; Olanipekun 2003). Using nitric acid (HNO_3) is also an economically viable reagent for producing phosphates without the concomitant production of large volumes of solid wastes, waste by-products and sulphur dioxide (SO_2) (Olanipekun 2003; Khurana et al. 2004; Mizane 2012). There are two production methods of nitrophosphate, namely the Odda (Hussain 2012) and double acid processes (Bornman 2012).

2.6.2 Odda process

The Odda process was invented in 1927 by Erling Johnson in the city of Odda, Norway, hence the name Odda process which was named after the city (Steen et al. 1986). A schematic illustration of the Odda process in the production of nitrophoshate is presented in Figure 2.5 (Mizane 2012).

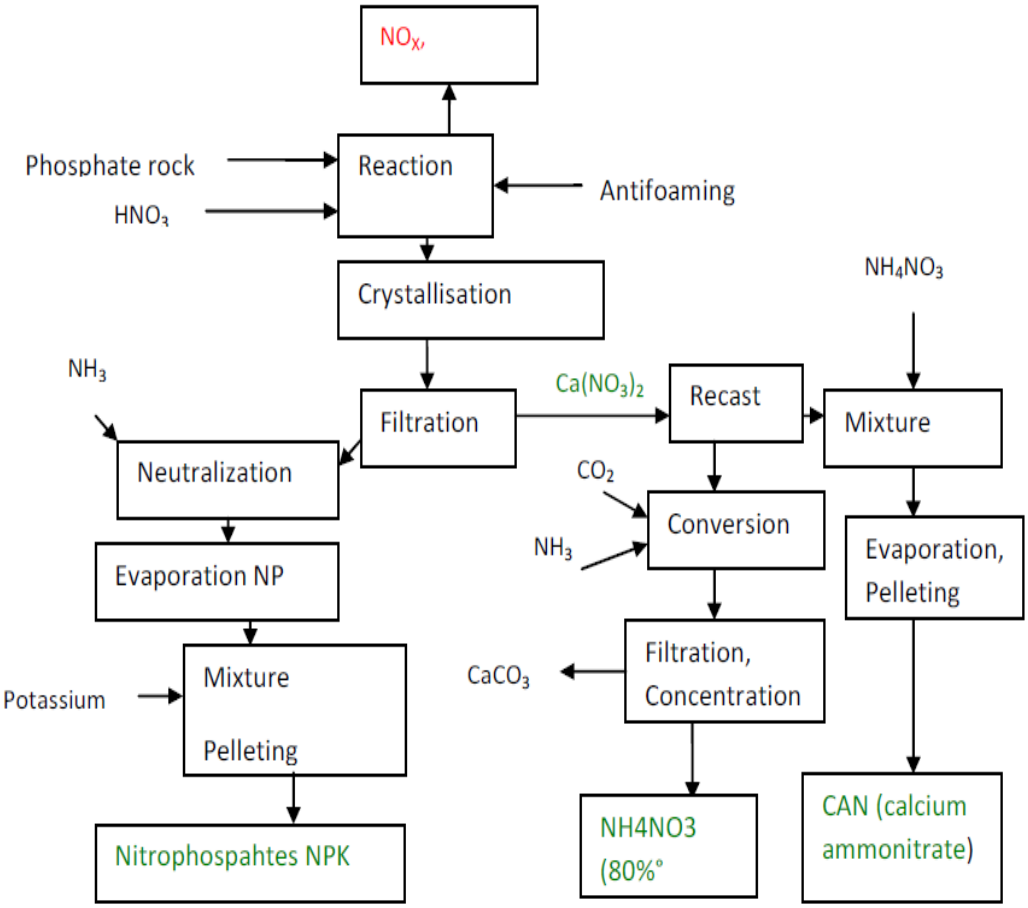


Figure 2.5 Odda process flow diagram for the production of nitrophoshate (Mizane 2012)

The Odda process begins with the dissolution of rock phosphate with nitric acid (HNO_3). Thereafter $Ca(NO_3)_2$ (Abdel-Aal and Amer 1995) is removed from the slurry (Bornman 2012; Hussain 2012; Mizane 2012) before neutralisation with ammonia (NH_3) (Abdel-Aal and Amer 1995). Calcium nitrate ($Ca(NO_3)_2$) that is removed as a by-product can be used for the production of calcium ammonium nitrate ($5Ca(NO_3)_2.NH_4.NO_3.10H_2O$) fertiliser (Hussain 2012; Mizane 2012). The purpose of

removing $Ca(NO_3)_2$ is to improve the solubility of nitrophosphate (Bornman, 2012) and there are three possible methods of removing $Ca(NO_3)_2$:

- Removing $Ca(NO_3)_2$ by cooling and crystallisation (The Odda process).
- Re-addition of phosphoric acid and/or sulphuric acid to precipitate most of the calcium.
- Addition of sulphate salts to precipitate calcium as gypsum (Bornman, 2012).

Up to 25% of the P in nitrophosphate, produced with the Odda process, is present as polyphosphate which is 60–65% water soluble (Bornman 2012). The N content is approximately 20% (Hussain 2012) with no secondary nutrients. Most of the nitrophosphate imported into South Africa is manufactured through this process (Bornman 2012). The only disadvantage of the Odda process is that it results in N oxide (NO_x) emissions into the environment and produces large amounts of nitrate-N (IFDC 1991). However, the process is still more ecological suitable than the production of sulphur-based fertilisers (SSP, DAP, MAP(33)) (Khurana et al. 2004). The IFDC (1991) once expressed that the production of nitrophosphate had the least impact on the environment because phosphogypsum with its associated process water are not produced.

2.6.3 Double acid process

The double acid process is another method for producing nitrophosphate and is mainly used in South Africa (Bornman 2012). The production method is similar to the Odda process (Mizane 2012), except that the double acid process does not remove all the $Ca(NO_3)_2$, which subsequently becomes available as Ca in the final product. It also differs in that sulphuric acid (H_2SO_4) and NH_3 are reintroduced via a pipe reactor during the production process. The final nitrophosphate is 97% water soluble and 100% citric acid soluble with more than 6% S and Ca with P in the form of orthophosphate and not as polyphosphate as produced through the Odda process (Bornman 2012). It also contains some Mg which comes from the PR. Advantages of the double acid process are similar to those of the Odda process (IFDC 1991; Olanipekun 2003; Kharana et al. 2004; Mizane 2012).

2.7 Hydrolyses reactions of nitrophosphate, MAP(33) and urea in soils

Nitrophosphate contains N and P as diammonium phosphate (DAP), ammonium nitrate (AN) and dicalcium phosphate (DCP). During the hydrolysis reactions of nitrophosphate, DAP form orthophosphates and NH_4^+, and AN form NH_4^+ and NO_3^-, while calcium phosphate (DCP), under acidic soil (pH_{KCl} of approximately 4.1), releases phosphoric acid (Lindsay 1979) that has the potential to decrease soil pH (Bornman 2012).

$$Ca(H_2PO_4)_2.H_2O + H_2O \rightleftharpoons CaHPO_4.H_2O + H_3PO_4 \qquad (2.2)$$

MAP(33) also split into orthophosphates and NH_4^+ which are readily absorbed by plants while urea ($(NH_2)_2CO$) hydrolysed to NH_4^+ prior plant absorption (FSSA 2007; Mengel and Kirkby 2001; Bornman 2013). In the first step urease enzyme catalyses the reaction of $(NH_2)_2CO$ and water (H_2O) to form carbamic acid (H_2NCO_2H) that simultaneously decomposes into NH_3 and CO_2 (equation 2.3) (Tisdale et al. 1985). The NH_3 that is formed is further hydrolysed (react with H_2O) to form NH_4^+ and OH^- (equation 2.4).

$$(NH_2)_2CO + H_2O \xrightarrow{\text{Urease}} NH_3 + H_2NCOOH \longrightarrow 2NH_3 + CO_2 \qquad (2.3)$$

$$NH_3 + H_2O \longrightarrow NH_4^+ + OH^- \qquad (2.4)$$

The NH_4^+ released from nitrophosphate, MAP(33) and urea have the potential to decrease the soil pH when it is nitrified to NO_3^- (FSSA 2007; Mengel and Kirkby 2001; Bornman 2013):

$$2NH_4^+ + 3O_3 \longrightarrow 2NO_2^- + 4H^+ \qquad (2.5)$$

$$2NO_2^- + O_2 \longrightarrow 2NO_3^- \qquad (2.6)$$

In the first step of nitrification NH_4^+ is converted to nitrite (NO_2^-) (equation 2.5) and this reaction releases protons (H^+) which are responsible for decreasing soil pH. In the second stage NO_2^- is oxidised to NO_3^- (equation 2.6).

2.8 Factors affecting P use efficiency

3.8.1 P fertiliser and solubility

Fertiliser solubility is an important factor that determines how efficiently crops utilise nutrients (Govil 1972; Meelu et al. 1977; Hundal et al. 1979) and the solubility is determined by measuring the concentration of the nutrient in a citrate or water solution (Meelu et al. 1977). The physical state in which the fertiliser exists, that is, granular or powder influences its solubility and therefore the efficiency of P uptake (Govil 1972; Meelu et al. 1977; Hundal et al. 1979). A fertiliser that has a large specific surface area will have an increased solubility and release of nutrients. However, at times the rapid release of nutrients could be a disadvantage due to the fact that nutrients are susceptible to leaching and the fixation of P is eminent. However, this depends on the prevailing conditions such as the soil pH and the type of crop.

A study by Meelu et al. (1977) showed that wheat response to the P fertilisers DAP, urea ammonium phosphate, suphala (30% WSP), nitrophosphate (50% WSP) and SSP, was directly related to the water-soluble P content. High water-soluble P sources gave significantly higher yields. Similarly Hundal et al. (1979) reported findings where the effectiveness of various sources of P fertilisers was directly proportional to the amount of water–soluble P. It was established that nitrophosphate with 30% water–solubility P was the least effective source of P when compared to the nitrophosphate and SSP whose solubility were 50% and 70% respectively. A study, at the Indian Agricultural Research Institute revealed that the response of sorghum to P application was considerably reduced when the water solubility of triple superphosphate/dicalcium phosphate mixtures were below 50% and below 75% for triple superphosphate/rock phosphate mixtures (Govil and Prasad 1972).

3.8.2 Soil pH

The adsorption and precipitation of P in the soil is influenced by the pH of the soil. Under acidic conditions (pH<5) the solubility of Al, Fe and Mn increases resulting in elevated concentrations of Al^{3+}, Fe^{2+} and Mn^{2+} (Haynes and Mokolobate 2001) that form metal-phosphate complexes with a low solubility (Wall et al. 2013) rendering P unavailable for plants (SSSA 1997; Haynes and Mokolobate 2001; Wall et al. 2013). Increased Al^{3+} concentration also poses toxicity effects on plants by inhibiting root

growth through impedance of both cell division and elongation (Wall et al. 2013). This consequently restricts the ability of plant roots to explore maximum soil volume to absorb nutrients and water (Haynes and Makolobate 2001). As a mitigation measure, farmers often lime the soil by applying calcium carbonate ($CaCO_3$) to raise the pH and precipitate exchangeable Al (Haynes and Mokolobate 2001). At high pH (alkaline) conditions, P reacts with Ca and precipitate as insoluble calcium phosphates that are not available for plant uptake (Niazi et al. 1991). Phosphorus is therefore most available at slightly acidic soil with the pH between 6 and 7 (Figure 2.2) being generally optimum for most field crops (Brady 1990, Mengel and Kirkby 2001; Wall et al. 2013).

3.8.3 Organic matter

Organic matter has been shown to increase P availability in the soil (Palm et al. 1997; Haynes and Mokolobate 2001) when P is released from organic material into the soil during the mineralisation of the organic material (Earl et al. 1979). The released P as orthophosphate becomes involved in equilibrium reactions between the free P in the soil solution and the adsorbed P ions (Mengel and Kirkby 2001) on oxide surfaces (Haynes and Mokolobate 2001). During the mineralisation of organic matter an increase in CO_2 production occurs, which may increase the solubility of soil phosphates (Mengel and Kirkby 2001). Organic acids formed during the decomposition of organic matter may also increase P availability by:

- Forming complexes (or chelate) with Fe and Al in the soil solution and thus preventing the precipitation of phosphates with these elements. Al and Fe toxicity is also reduced.
- Competing with P for sorption sites.
- Solubilising P from the insoluble Ca, Fe and Al phosphates (Palm et al. 1997).

Organic acids commonly found in plant leaves include malic, citric acids and to a lesser extent succinic, fumaric and oxalic acids (Haynes and Mokolobate 2001; Sepehr et al. 2012). Most of these organic acids are present in the soil solution when leaves decompose, but only for short periods of time, because they are highly susceptible to microbial degradation (Wong et al. 1995). Humic and fulvic acids have also been reported to prevent P fixation in soils, where the addition of humic and fulvic acids at

100–350 kg ha^{-1} can improve plant growth and ameliorate the negative effects of high Al concentrations (Suthipradit et al. 1990; Haynes and Mokolobate 2001).

3.8.4 Crop type

Plants vary widely in their ability to grow in soils with low P content. This can be explained by two root attributes, namely i) the ability to explore the soil volume and ii) their geometry and morphology to absorb P from the soil solutions (Schachtman et al. 1998). For example, grass species are more effective in absorbing P from soils with a low P content than legumes, because the total fine root length of grasses, which is considerably longer than the total root length of legumes, plays a bigger role in P absorption than the rate of P absorption per unit root length (Hedley et al. 1989).

Some plants exudate high amounts of organic acids (up to 23% of net photosynthesis) to mobilise P through acidifying the soil and chelating metal ions around the roots (Schachtman et al. 1998). Wheat seedlings can according to Barber and Martin (1976) release up to 20% of its photosynthetic products that contain a significant amount of chelating acids exudate such as citric acid, malic acid and succinic acid (Christiansen-Weniger et al. 1992; Haynes and Mokolobase 2001). Roots exudate these acids to increase the P availability in soils for e.g. in high pH soils the organic acids decrease the soil pH and favour calcium phosphate (Ca-P) precipitates to release orthophosphates (Lajtha and Harrison 1995). In low pH conditions organic acids form strong bonds with Al^{3+} and other polyvalent cations that prevent these cations to bind with P (Haynes and Mokolobate 2001).

3.8.5 Soil temperature

The effect of soil temperature on P availability is largely linked to the biological and chemical reactions of the soil (Wall et al. 2013). Lower quantities of P may be available for crop uptake, due to the decrease in biological activity, in soil temperatures less than 5^{o}C (Wall et al. 2013). Lower soil temperatures reduce the mineralisation of organic P because of lower microbial activity. It also reduces root growth rates and the rate of diffusion of P resulting in reduced amounts of P absorbed by roots (IPNI 1999). Sorption and desorption rates of P are also decreased with lower soil temperature

which adversely affect P use efficiency (Barrow 1979b). For these reasons, more P fertiliser is generally required at lower soil temperatures to ensure sufficient P uptake (Singh and Jones 1977).

2.9 Effect of P source on chlorophyll content, dry matter yield and root biomass of crops

The impact of fertiliser source on the chlorophyll content, yield or root biomass is based on the solubility of the fertiliser. A highly soluble P source is capable of releasing nutrients quicker for plant absorption. To be agronomically feasible it is agreed by scientist that nitrophosphates should be at least 60% water soluble but the European Union requires at least 75% solubility in 2% citric acid (Bornman 2012). MAP(33) contains 22% citric acid soluble P (FSSA 2007). A readily available P source offers good P supply to plants and accelerate root growth (Mengel and Kirkby 2001) that explores larger soil volume. Subsequently other essential nutrients such as C, H, O, N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Ni, B, Mo and Cl (Mengel and Kirkby 2001; FSSA 2007; Pilon-Smiths et al. 2009) are absorbed. Some of these improve chlorophyll synthesis (N, Mg, Zn) and growth. High P increases chlorophyll concentration in plants and this was confirmed by Van Nieuwenhuyse and Jones (1996) who found a strong curvilinear relationship (R^2 = 0.67) between total P concentration and chlorophyll concentration. The effect of P on chlorophyll was also investigated by Schertz (1919) and revealed that the lack of P reduces the daily variation of the chlorophyll components and narrows the absorption bands.

2.10 Effect of P source on crop nutrient uptake

Phosphate fertiliser solubility influences the P uptake by plants. The inherent P content in different sources also influence the nutrient uptake by crops where higher P content increases the P uptake compared to low P content sources. For example P uptake of PR is lower (Bolland et al. 1997) than inorganic fertilisers such as MAP(33) and nitrophosphate. The chemical form of P contained in fertiliser also affect P uptake, that is, orthophosphates and polyphosphates (Ottman et al. 2005). Orthophosphates are single ions (HPO_4^{-2} and $H_2PO_4^-$) that readily dissolve and become available for plant uptake (Noack et al., 2010). Polyphosphates are polymers of orthophosphates that

are formed when orthophosphate units join together. Polyphosphates need to be hydrolysed into simple orthophosphate units before plant uptake (Robertson 2004) and the hydrolysis is dependent on the soil temperature (Anonymous 2008). Nitrophosphate and MAP(33) contain orthophosphate and ammonium polyphosphate (APP) polyphosphates (Bornman 2012).

2.11 Effect of P source on residual soil pH and nutrient composition

Phosphate sources that contain NH_4^+ pose a threat of acidifying the soil when the NH_4^+ undergoes nitrification. MAP(33) and DAP contain N in the NH_4^+ form. Nitrophosphate has fractions of the NH_4^+ and NO_3^- forms. With residual nutrient composition, P sources that slowly release P takes longer to be depleted hence these could potentially have residual soil P after the crop is harvested.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

The purpose of the study was to compare nitrophosphate with MAP(33), in terms of wheat growth and nutrient uptake, when used as pre-plant fertilisers in acidic sandy soils. Therefore, a greenhouse experiment was conducted where biomass produced, residual soil nutrient content, soil pH and nutrient uptake of potted wheat plants fertilised with nitrophosphate and MAP(33) was compared. Biomass production of a crop is a good indicator of plant growth and potential grain yield (Wu and Bao 2011) that can be used to evaluate fertiliser efficiency. The residual soil pH and P, K, Ca, Mg and S content of the soil were determined to evaluate if the soil pH was changed and if the residual Ca and Mg decreased with the application of MAP(33) and nitrophosphate. Two trials were conducted and the first trial will be referred to as Trial 1 and the validation trial as Trial 2.

3.2 Soil analysis

An acidic subsoil sandy soil, with pH_{KCl} 4.1, was collected from a commercial farm in the Bothaville region. The soil was thoroughly mixed and a representative sample was taken for analysis at the Omnia Chemtech analytical laboratory. The Omnia Chemtech analytical laboratory is a member and participates in the Agri Laboratory Association of Southern Africa (AgriLASA) quality scheme and is ISO/IEC 17025: 2005 and ISO 9001: 2000 accredited (South African National Accreditation System, SANAS). The soil was analysed for macro- and micronutrients, pH and texture and these results are presented in Table 3.1.

Table 3.1 Soil characteristics and analyses from the subsoil used in the trials

Soil property	Units	Value
Colour		Yellow brown
Sand	%	90.00
Clay	%	5.00
Silt	%	5.00
pH_{KCl}		4.10
Exchangeable acidity	cmol(+) kg^{-1}	0.17
*ECEC	cmol(+) kg^{-1}	1.00
S	mg kg^{-1}	13.00
P (Bray-1)	mg kg^{-1}	15.00
K (ammonium acetate)	mg kg^{-1}	60.00
Ca	mg kg^{-1}	79.00
Mg	mg kg^{-1}	28.00
Na	mg kg^{-1}	19.00
B	mg kg^{-1}	0.04
Cu	mg kg^{-1}	0.90
Fe	mg kg^{-1}	4.10
Mn	mg kg^{-1}	2.90
Ni	mg kg^{-1}	0.01
Zn	mg kg^{-1}	0.90

*ECEC calculated from the sum of the cations (K^+, Na^+, Mg^{2+} and Ca^{2+} $cmol_c$ kg^{-1}).

The cation content of the soil was determined with the ammonium acetate (NH_AOA_C) extraction method (Kistopoulus 1999) and the P content with the Bray-1 method (Sims 2000). Sulphur was determined using the calcium phosphate ($Ca(H_2PO_4)_2$) extraction method (Wrenshall and McKibbin 1935) and the Effective Cation Exchange Capacity (ECEC) was calculated based on summation of the extracted cations, K^+, Na^+, Mg^{2+} and Ca^{2+} and extractable acid determined close to the inherent pH of the soil. The analysis of Fe, Mn, Zn, Cu and Ni was done with the diethylenetriaminepentaacetic acid (DTPA) extraction method (Carter and Gregorich 2007) and then B was analysed using the hot water extraction method described by Jones (1999).

3.3 Experimental conditions

To meet the objective of the study, two experiments were conducted in a greenhouse, in Sasolburg (26º 49' 0" S and 27º 49' 0" E). The first experiment started on 3 July 2013 and the second (confirmation experiment) on 9 July 2013. The purpose of the confirmation experiment was to corroborate the findings of the first experiment. The study was conducted in winter with the greenhouse temperature set to 25ºC during the day and 18ºC at night. Greenhouse temperature and relative humidity (RH) were managed with a misting and ventilation system to ensure that temperatures were kept below 30ºC, while electrical heaters were used to maintain temperatures above 18ºC. Relative humidity was maintained at 50%. Three weeks after emergence (WAE) heaters were switched off and wheat plants were exposed to chilling temperatures (below 5ºC) to induce tillering.

3.4 Experimental design and treatments

Ten litre plastic pots were filled with 16 kg of soil collected from the Bothaville region. Soil characteristics and chemical analyses information is given in section 3.2 (Table 3.1). Wheat was used as test crop and the experiment was laid out in a completely randomised design (CRD) with two P fertiliser sources (nitrophosphate & MAP(33)), applied at four different rates (0, 15, 30 & 45 kg P ha^{-1}) and replicated five times. A treatment was included where MAP(33) was applied with secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1}) equal to those in nitrophosphate (T9) therefore the entire experiment consisted of nine treatments. The experiment was repeated as a confirmation experiment, so that a total of 90 pots were used that were divided into two batches of 45 pots that were placed on a rotating table, which were adjacent to each other in the same greenhouse, to minimise the influence of variable climatic conditions in the greenhouse (Figure 3.1).

Figure 3.1 Completely randomised design on the rotating tables in the greenhouse

The nitrophosphate and MAP(33) used in the pot trials were also analysed at the Omnia Chemtech analytical laboratory and the results are presented in Table 3.2.

Table 3.2 Chemical analysis of nitrophosphate and MAP(33) used in pot trials

	Nitrophosphate	MAP(33)
Citric P	7.64%	21.2%
Total N	18.9%	12.04%
Ammonium-N	12.4%	12.04%
Nitrate-N	6.5%	LD*
Calcium	5.71%	LD
Magnesium	0.39%	LD
Sulphur	4.97%	LD
pH	3.6	4.4

*Lower than the detection limit

In Table 3.3 a summary of the experimental treatments is presented. Nitrogen was added to all treatments as urea to mimic farmer's practice and to ensure a standardised N content of 106 kg N ha^{-1}. Potassium was not supplemented because, according to the soil analysis, the soil contained sufficient K (60 mg kg^{-1}) for wheat production. Phosphorus was applied as treatments while the controls received no N, K and P.

Table 3.3 Fertiliser treatments

Treatment description	Phosphorus applied (kg P ha^{-1})	N applied with treatment (kg N ha^{-1})	Urea applied (kg N ha^{-1})	Total N applied (kg N ha^{-1})
Control	0	0	0	0
Nitrophosphate + Urea	15	35	71	106
Nitrophosphate + Urea	30	71	35	106
Nitrophosphate + Urea	45	106	0	106
MAP(33) + Urea	15	8	98	106
MAP(33) + Urea	30	17	89	106
MAP(33) + Urea	45	25	81	106
MAP(33) + Urea + Ca, Mg & S	30	17	89	106

T9: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1}) where S, Ca and Mg were applied as gypsum ($CaSO_4$), $MgSO_4$ and $Mg(NO_3)_2$.

To mimic farmers' practices fertilisers were applied as pre-plant by mixing it with the top 20 cm of the soil in the pot (Figure 3.2). The pots were then tapped lightly to compact the soil and ensure that they had the same bulk density.

Figure 3.2 Mixing fertiliser treatments with the top 20 cm of the soil in the pot

Ten wheat seeds were planted per pot at 10 mm depth. Ten days after emergence the seedlings were thinned to five per pot. Four weeks after emergence (WAE)

micronutrients were applied to all treatments using HIDROSPOOR™, except the controls. HIDROSPOOR™ (Table 3.4) is a multi-micronutrient fertiliser and 0.01 g was dissolved in 500 ml of water and applied to each pot, which is equivalent to an application rate of 2 kg ha^{-1}. The micronutrient composition of HIDROSPOOR™ is presented in Table 3.4.

Table 3.4 Nutritional composition of HIDROSPOOR™

Fe	Mn	Zn	Cu	B	Mo
		g kg^{-1}			
87	18	11	1.2	22	2

3.5 Irrigation

For irrigation purposes the permanent wilting point (PWP) was taken as the mass of the pot filled with oven dried soil (60 ^{o}C) and field capacity (FC) as the mass of the pot with soil after it was saturated with water and left to drain freely for two days. The mass of water (kg) at field capacity (plant available water (PAW)) was calculated from equation 3.1:

$$\text{Plant Available Water (PAW)} = \text{FC} - \text{PWP (kg)} \qquad (3.1)$$

The mass of the pot containing 70% PAW was calculated as shown on equation 3.2:

$$70\%\ \text{PAW} = (\text{PAW} \times 0.7) + \text{Mass of pot filled with oven dried soil (kg)} \qquad (3.2)$$

Each pot was weighed twice a week to monitor the soil moisture content and pots were irrigated to 70% PAW if they went below 50% PAW. At later growth stages pots were weighed thrice a week to meet the crop water demands.

3.6 Plant measurements and analyses

Chlorophyll measurements were taken weekly, starting 2 WAE until harvest, using a SPAD meter (Minolta chlorophyll meter SPAD 502 Plus). Thirty readings were taken per pot on the last fully developed leaves and the average was recorded. Wheat was harvested when at least 50% of the plants reached the flag leaf stage (growth Stage 37-38, BBCH 37-38) as described by Lancashire et al. (1991).

At harvest plants were cut at the base of the stem and the following growth parameters were measured and recorded: fresh root volume, fresh root mass, fresh leaf mass, dry root mass and dry leaf mass (leaves + stems). Roots were washed by placing them on a sieve and then washing with running water to remove soil particles. The fresh biomasses and root volume were measured on the day of harvest. Fresh root volume was determined by placing fresh roots in a measuring cylinder with water and recording the displacement. Dry mass was measured with an electronic scale and was determined by first oven drying the fresh biomass at 60 ^{o}C until constant mass. After drying, leaf samples were sent to NviroTek Labs, in Brits for analyses. NviroTek Labs is a member of and participate in the AgriLASA quality scheme that promotes the use of standard analytical methods, competency and reliable results. Nitrogen in samples was analysed according to the Dumas method (Shea and Watts 1939) by combustion of the milled samples in a Leco TruSpec® CN analyser. The concentrations of S, P, K, Ca, Mg, Na, Cu, Mn, Zn, Fe, B and Mo were determined according to the chemical procedures described in the Handbook of Standard Soil Testing Methods for Advisory Purposes (Kalra 1990). Oven dried plant samples were finely milled mechanically before there were incinerated at 500 °C for at least 3 hours, allowed to cool and then wetted with concentrated nitric acid and incinerated for another hour. After cooling, 10 ml of a 1:2 distilled water to nitric acid solution was added to the silica crucibles, heated on a sand bath and when warm enough washed over into a 100 ml volumetric flask with distilled water. The determination of P in solution was done colorimetrically and other nutrients were determined with atomic absorption spectrometry (Palic et al. 2000).

Nutrient uptake indices were also calculated by multiplying the specific nutrient concentration with the dry leaf mass produced per pot, and the units are given as milligrams per pot.

$$NU\ (mg\ pot^{-1}) = NC\ (mg\ g^{-1}) \times DM\ (g\ pot^{-1}) \quad (3.3)$$

Where: NU-specific nutrient uptake (mg pot^{-1})

NC-specific nutrient concentration (mg g^{-1})

DM-dry matter (dry leaf mass) (g pot^{-1})

At the end of the trial soil samples were taken from the top 20 cm in the pots and sent for macronutrients, micronutrients and pH analysis to Omnia Chemtech analytical laboratory as explained in section 3.2. In Trial 1 a composite sample was taken per treatment by pooling replicates together due to logistics and budget constraints. For the validation trial (Trial 2), soil samples were taken and analysed separately for each replicate.

3.7 Statistical analysis

Data were analysed using the software STATISTICA (Version 12) 2013 from StatSoft. Three different statistical analyses were performed:

In the first analyses, the independent t-test was used to compare the two trials with each other at a 5% significance level to confirm that the results were repeatable. For the top soil analyses, a single t-test was used to compare the results of Trial 1 with the average for Trial 2, because the first experiment only had composite data.

In the second analyses, different treatments were analysed using the analyses of variance tests to indicate the significant effects and Fisher's least significant difference test (LSD) at the 5% significance level, was used to determine statistically significant differences between means.

In the third analyses, the analyses of variance (factorial) tests were used to indicate the significant effects and Fisher's least significant difference test (LSD) at the 5% significance to determine statistically significant differences between the means. The analysis of variance consisted of three factors namely, two treatments (Nitrophosphate and MAP(33)), four P application rates (0, 15, 30 and 45) and the interaction between treatments and P application rate. This was to establish if these factors in combination

had an effect on the growth of wheat. Please note that for the third analyses, treatment nine was excluded from the analyses.

CHAPTER 4

SOIL PH, POTASSIUM, PHOSPHORUS, MAGNESIUM, CALCIUM AND SULPHUR CHANGES AS AFFECTED BY NITROPHOSPHATE AND MAP(33) APPLICATION

4.1 Introduction

For decades soil acidity has been a major constraint for crop production throughout the world (Sumner and Noble 2003). The total area covered by acid topsoils is estimated to be between 3.777 X 10^9 and 3.950 X 10^9 ha (von Uexkull and Mutert 1995; Eswaran et al. 1997) and are found mostly in South and North America, Asia and Africa, which represent 40% of the total arable land area in the world (Haug 1984). In South Africa, Fey (2001) estimated that approximately 5 x10^5 ha of production soils are acidic and a further 11 x 10^6 ha are ofmoderate acidic. Almost 40% of the production soils, to the west of the Drakensberg, and the western and southern Cape are acidic (Fey 2001). The same author revealed that in KwaZulu Natal approximately 85% of soil analyses have pH_{KCl} values lower than five, of which half had potentially dangerous exchangeable acid (Al) levels. It is essential that proper agricultural management practices which minimise acidification, such as frequent liming or the use of fertilisers that has a minimum effect on soil acidity be adopted to mitigate the problem (Conyers 1996).

Poor crop growth on acid soils is usually a direct result of Al toxicity, where the Al becomes more soluble and severely inhibits root development when soil pH drops below 5 (Sumner and Noble 2003). Subsequently water and nutrient uptake is inhibited. In acidic soils the Mn concentrations can increase to toxic levels (Haynes and Mokolobate 2001; Mengel and Kirkby 2001) while P, Mo and B decreases (FSSA 2007).

Mechanisms include natural acidification (Lesturgez et al. 2006), acid deposition and the hydrolysis of NH_4^+ containing fertilisers. However, in intensive agriculture fertilisation with NH_4^+ containing compounds such as urea (hydrolyses to NH_4^+), ammonium sulphate and MAP(33) acidifies the soil if not properly managed (Malhi et al. 1998; FSSA 2007; Zhao and Xing 2009; Bornman 2013). MAP(33) contains N in the NH_4^+ form and it is a stronger acidifying fertiliser than anhydrous ammonia, urea

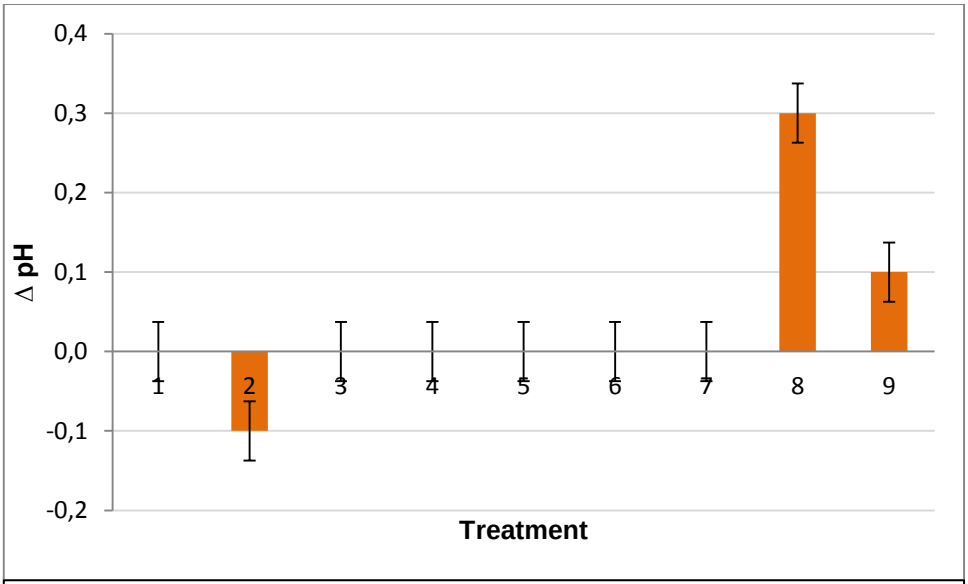
and ammonium sulphate (FSSA 2007). This is due to the fact that the dihydrogen phosphate releases a proton when forming hydrogen phosphate. It can produce an acidity of 2 moles of H^+ per mole of N which is double than that of anhydrous ammonium and urea (FSSA 2007). On the other hand, nitrophosphate contains a combination of the NH_4^+ and NO_3^- and the objective of this chapter was to compare residual soil pH and residual K, P, Ca, Mg and S of soils treated with nitrophosphate and MAP(33).

4.2 Results and discussion

The pH and P, K, S, Mg and Ca concentration in the soil showed significant differences ($P<0.05$) for the fertiliser treatments (nitrophosphate and MAP(33)) and application rates (0, 15, 30 and 45 kg P ha^{-1}). There was also an interaction effect between the fertiliser and the application rate meaning the two factors influenced each other.

Two trials were conducted and the first trial will be referred to as Trial 1 and the validation trial as Trial 2. In Trial 1 a composite sample was taken per treatment (by pooling replicates together) and the change in pH (ΔpH, pH at end of trial – pH at start of trial) over the trial period are presented in Figure 4.1. An increase in pH is denoted by positive ΔpH values and a decrease with negative ΔpH values.

The pH of the soil treated with nitrophosphate remained unchanged (ΔpH = 0) except when nitrophosphate was applied at 15 kg P ha^{-1} a decrease of 0.10 pH units occurred, because of the acidifying effects of urea, which will be explained in detail when discussing the validation trial (Trial 2). MAP(33) applied at lower rates (15 and 30 kg P ha^{-1}) also had no effect on soil pH, but pH increased when MAP(33) was applied at 45 kg P ha^{-1} (0.30 pH units) and MAP(33) combined with secondary nutrients (MAP(33)+) applied at 30 kg P ha^{-1} (0.10 pH units). There were no clear trends exhibited from this data, most probably due to the fact that this data was compiled from composite samples. More focus was therefore on the data of Trial 2 that was sampled per replicate (Figure 4.2).



1. Nitrophosphate 0 kg P ha^{-1}
2. Nitrophosphate 15 kg P ha^{-1}
3. Nitrophosphate 30 kg P ha^{-1}
4. Nitrophosphate 45 kg P ha^{-1}
5. MAP 0 kg P ha^{-1}
6. MAP 15 kg P ha^{-1}
7. MAP 30 kg P ha^{-1}
8. MAP 45 kg P ha^{-1}
9. MAP(33)+

*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 4.1 Trial 1: Change in soil pH over the trial period (ΔpH) in response to nitrophosphate, MAP(33) and MAP(33)+ application

In Table 4.1 the amount of urea, as kg N ha^{-1}, for the different treatments is given. More urea was added to the MAP(33) treatments to ensure an equivalent N content than nitrophosphate (Table 4.1).

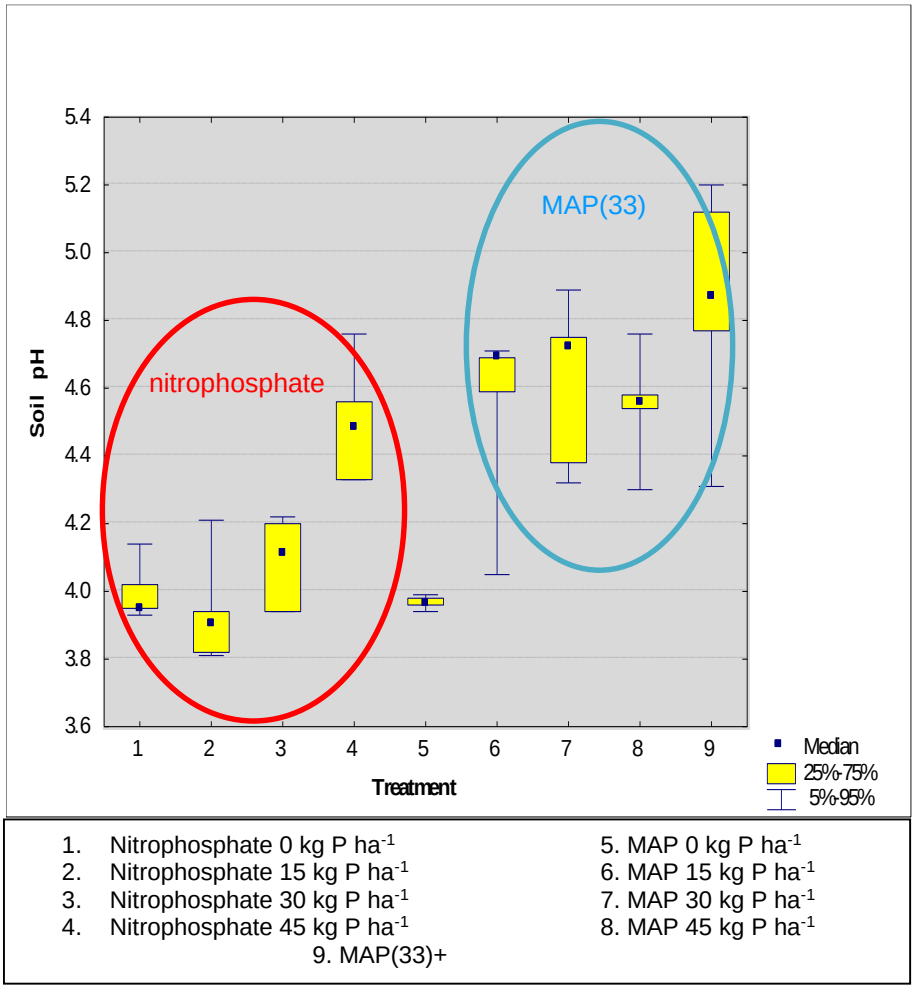
Table 4.1 Nitrogen applied as urea at planting to the nitrophosphate and MAP(33) treatments to ensure an equivalent N content

	Treatments		
	Nitrophosphate	MAP(33)	MAP(33)+
P applied (kg ha^{-1})	Urea-N (kg ha^{-1})	Urea-N (kg ha^{-1})	Urea-N (kg ha^{-1})
0	0	0	-
15	71	98	-
30	35	89	89
45	0	81	-

*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

For the validation trial (Trial 2), soil samples were taken and analysed separately for each replicate and the results are presented in Figure 4.2. The soil pH for the MAP(33) treatments, at the end of the trial, were significantly higher ($P<0.05$) than for the nitrophosphate treatments. The lower pH in the nitrophosphate treatment is most probably due to a combination of three factors, namely i) more N was taken up by wheat applied with MAP(33) as discussed in more detail after Figure 4.2 ii) the nitrification of urea that acidifies the soil and iii) the release of phosphoric acid from nitrophosphate. Nitrophosphate contains mono-calcium phosphate that hydrolyses and releases phosphoric acid (Lindsay 1979) that can potentially decrease soil pH (Bornman 2012).

However, the prime cause of the decrease in pH for the nitrophosphate treatments was postulated to be the release of phosphoric acid. The reason is that the nitrophosphate treatments received less urea than MAP(33) (Table 4.1) and still resulted in more acidification. Therefore, less nitrification related acidification should have occurred for the nitrophosphate treatments.



1. Nitrophosphate 0 kg P ha^{-1}
2. Nitrophosphate 15 kg P ha^{-1}
3. Nitrophosphate 30 kg P ha^{-1}
4. Nitrophosphate 45 kg P ha^{-1}
5. MAP 0 kg P ha^{-1}
6. MAP 15 kg P ha^{-1}
7. MAP 30 kg P ha^{-1}
8. MAP 45 kg P ha^{-1}
9. MAP(33)+

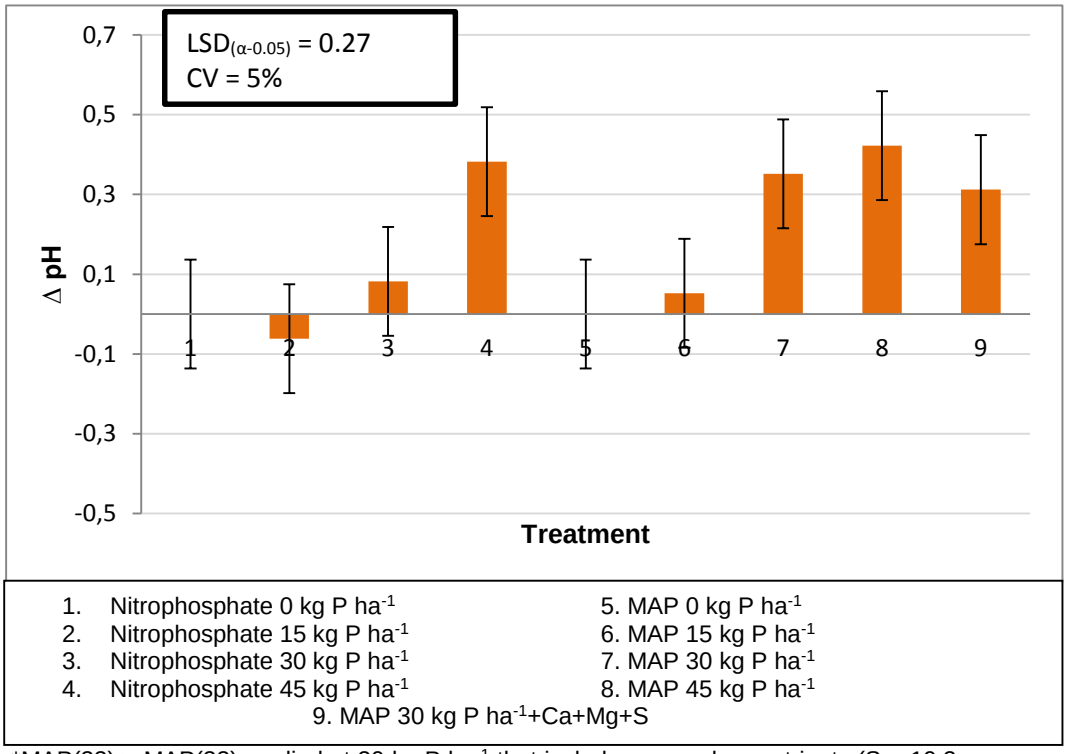
*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 4.2 Trial 2: Soil pH measured after harvest for different nitrophosphate, MAP(33) and MAP(33)+ application rates

For MAP(33) treatments the soil pH remained high for all application rates (Figure 4.2) despite the fact that it received more urea (Table 4.1) that releases more NH_4^+ when hydrolysed. MAP(33) is regarded as an acidifying fertiliser due to the NH_4^+ that decreases soil pH when it is nitrified (FSSA 2007). However, it is only the fraction of NH_4^+ that is not taken up by the plant that is nitrified and can cause a decrease in pH. Hence, the more NH_4^+ that is not absorbed by the plant roots the more is available (left over) to be oxidised to NO_3^- through nitrification, which is a highly acidifying process. In this study MAP(33) did not decrease the soil pH, because wheat treated with MAP(33) grew more vigorously with higher biomass production (Chapter 5) that resulted in the absorption of most N (Chapter 6). Therefore, it is postulated that nitrification of NH_4^+ did not occur. The pH increase for MAP(33) treatments and nitrophosphate applied at 45 kg P ha^{-1} treatment could be explained by the exudation

of hydroxyl (OH^-) or hydrogen carbonate (HCO_3^-) ions from the roots when anions, such as HPO_4^- are taken up by the plants (Mengel and Kirkby 2001). These anions (OH^-, HCO_3^-) could raise the pH of the rhizosphere by up to 1 unit (Whitehead 2000; Rengel 2015). This conclusion is further supported by the results discussed in Chapter 6, where it is shown that MAP(33) and nitrophosphate applied at 45 kg P ha^{-1} resulted in more P absorption (Chapter 6).

Changes in soil pH (ΔpH) over the trial period were observed for different nitrophosphate treatments in Trial 2 (Figure 4.3). The soil pH decreased (as indicated by a negative ΔpH) when nitrophosphate was applied at 15 kg P ha^{-1} (Figure 4.3), due to the nitrification of urea. More urea (71 kg N ha^{-1}), that causes soil acidification during the nitrification process (Mengel and Kirkby 2001; FSSA 2007; Bornman 2012), was added in this treatment to ensure that the same amount of N was applied to all treatments (Table 4.1). For the 30 kg P ha^{-1} and 45 kg P ha^{-1} nitrophosphate treatments, soil pH increased (as indicated by a positive ΔpH) (Figure 4.3) because less urea was added (35 kg N ha^{-1} and 0 kg N ha^{-1}) (Table 4.1) and thus less acidification due to nitrification occurred.



1. Nitrophosphate 0 kg P ha^{-1}
2. Nitrophosphate 15 kg P ha^{-1}
3. Nitrophosphate 30 kg P ha^{-1}
4. Nitrophosphate 45 kg P ha^{-1}
5. MAP 0 kg P ha^{-1}
6. MAP 15 kg P ha^{-1}
7. MAP 30 kg P ha^{-1}
8. MAP 45 kg P ha^{-1}
9. MAP 30 kg P ha^{-1}+Ca+Mg+S

*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 4.3 Trial 2: Change in soil pH (ΔpH) over the trial period in response to nitrophosphate, MAP(33) and MAP(33)+ application

In Chapter 5 the increase in biomass (above and below ground) with increase in nitrophosphate and MAP(33) application is discussed in detail. However, because biomass increased with nitrophosphate and MAP(33) application (Chapter 5) the nutrient demand was higher, which drove the uptake of P and NH_4^+ leaving less $Ca(H_2PO_4)_2.H_2O$ to be hydrolyses to H_3PO_4 and NH_4^+ to be nitrified.

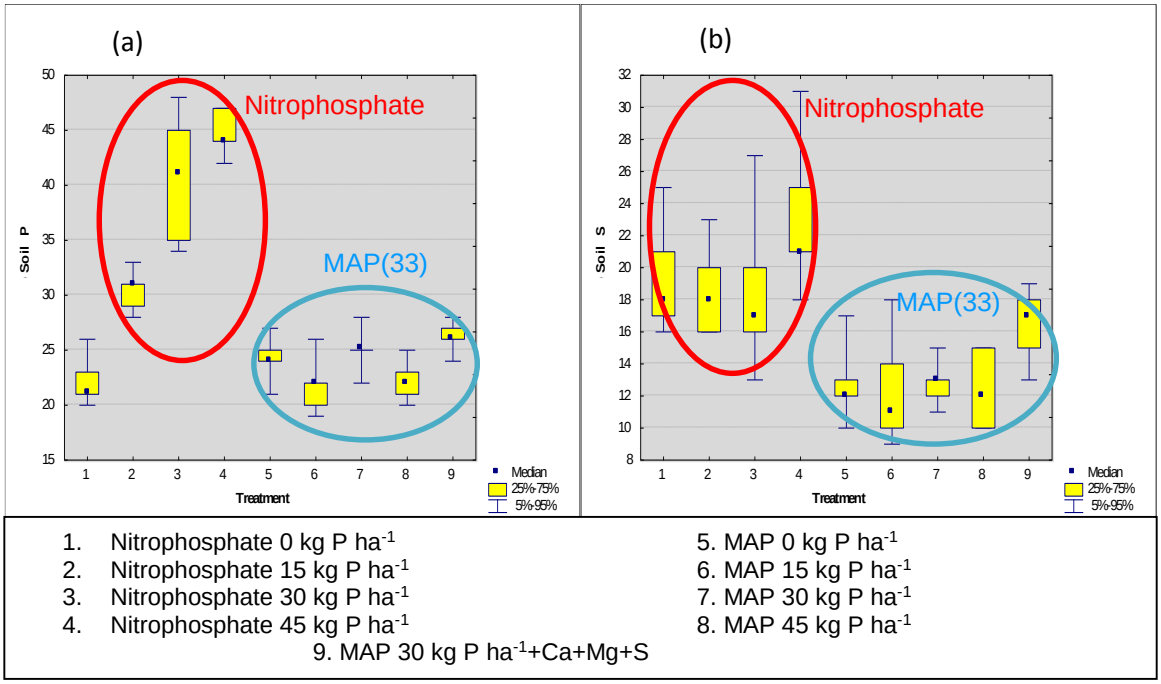
The different nitrophosphate and MAP(33) treatments had no significant effect ($P<0.05$) on the residual Ca in the soil (Table 4.2). However, it was found that the residual Mg concentration was only significantly higher ($P<0.05$) for the nitrophosphate treatment applied at 45 kg P ha^{-1} (Table 4.2). This was probably because more Mg (2.3 kg Mg ha^{-1}), associated with the nitrophosphate, was applied at the highest nitrophosphate application rate treatment. Potassium to be higher on the controls.

Table 4.2 Residual soil K, Ca and Mg concentration (mg kg^{-1}) determined after harvest for trial 2 (validation trial)

		Phosphorus applied (kg ha^{-1})			
	Fertiliser	0	15	30	45
K (mg kg^{-1})	Nitrophosphate	65^{bc}	62^{abc}	61^{abc}	59^{abc}
	MAP(33)	70^{c}	51^{ab}	58^{abc}	50^{a}
	MAP(33)+			50^{a}	
	P-value	0.13			
	LSD$_{(\alpha\text{-}0.05)}$	12			
Ca (mg kg^{-1})	Nitrophosphate	100^{a}	91^{a}	104^{ab}	122^{ab}
	MAP(33)	95^{a}	99^{ab}	112^{ab}	113^{ab}
	MAP(33)+			111^{ab}	
	P-value	0.18			
	LSD$_{(\alpha\text{-}0.05)}$	20			
Mg (mg kg^{-1})	Nitrophosphate	30^{ab}	28^{a}	31^{ab}	37^{c}
	MAP(33)	29^{ab}	32^{abc}	34^{abc}	34^{bc}
	MAP(33)+			33^{abc}	
	P-value	0.04			
	LSD$_{(\alpha\text{-}0.05)}$	4			

*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

In Figure 4.4, the results show that nitrophosphate and MAP(33) had an effect on soil Bray extractable P (Figure 4.4a) and $Ca(H_2PO_4)_2$ extractable S (Figure 4.4b) concentration when applied at different rates. Nitrophosphate treatments increased Bray extractable P and $Ca(H_2PO_4)_2$ extractable S more than with MAP(33) application. Application rate also significantly affected the Bray extractable P for the nitrophosphate treatments (Figure 4.4a). A linear increase in soil Bray extractable P with increase in nitrophosphate application was observed, while no significant differences in the Bray extractable P content was found for the different MAP(33) application rates (Figure 4.4a). Calcium phosphate ($Ca(H_2PO_4)_2$) extractable S was not influenced by the application rate for both nitrophosphate and MAP(33) treatments, but higher $Ca(H_2PO_4)_2$ extractable S was recorded for nitrophosphate applied at 45 kg P ha^{-1} (Figure 4.4b).



1. Nitrophosphate 0 kg P ha^{-1}
2. Nitrophosphate 15 kg P ha^{-1}
3. Nitrophosphate 30 kg P ha^{-1}
4. Nitrophosphate 45 kg P ha^{-1}
5. MAP 0 kg P ha^{-1}
6. MAP 15 kg P ha^{-1}
7. MAP 30 kg P ha^{-1}
8. MAP 45 kg P ha^{-1}
9. MAP 30 kg P ha^{-1}+Ca+Mg+S

*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 4.4 Bray extractable P (a) and $Ca(H_2PO_4)_2$ extractable S (b) after harvest for different nitrophosphate, MAP(33) and MAP(33)+ application rates

Higher Bray extractable P and $Ca(H_2PO_4)_2$ extractable S for the nitrophosphate treatments could be explained in terms of nutrient absorption by root growth. Nitrophosphate resulted in lower pH which impaired root growth (Mengel and Kirkby 2001) and hence less P and S was absorbed by the plants that resulted in an increase in the Bray extractable P and $Ca(H_2PO_4)_2$ extractable S from the soil. Nitrophosphate also contained 4.97% of S (Table 3.1) that was added during fertilisation. In contrast, MAP(33) treatments significantly increased root growth (Chapter 5), which explored greater soil volume and absorbed higher amounts of P and S that depleted soil reserves more. This is also confirmed with the nutrient uptake results (Chapter 6) where MAP(33) had higher P and S uptake and more biomass production than the nitrophosphate treatments.

4.3 Conclusions

Results from soil samples taken after harvest indicated a lower soil pH for the nitrophosphate treatments than the MAP(33) treatments. The prime cause for this lower soil pH for the nitrophosphate treatments is postulated to be the release of

phosphoric acid. Even though nitrophosphate treatments received less urea than MAP(33) it still resulted in more acidification. Nitrophosphate also had higher Bray extractable P and $Ca(H_2PO_4)_2$ extractable S than for the MAP(33) treatments. Soil Bray extractable P increased linearly with increase in nitrophosphate application rate with the highest Bray extractable P recorded for the 45 kg P ha^{-1} treatment. The residual Mg concentration was only significantly higher for the nitrophosphate treatment applied at 45 kg P ha^{-1}. However, nitrophosphate and MAP(33) had no significant effect on the K and Ca levels.

CHAPTER 5

CHANGE IN GROWTH AND CHLOROPHYLL CONTENT OF WHEAT TREATED WITH NITROPHOSPHATE AND MAP(33)

5.1 Introduction

Phosphorus plays an important role in plant metabolism, cellular energy transfer, respiration and photosynthesis (Ozanne 1980). For optimum crop yield, plants require adequate P from a very early growth stage and throughout their lifecycle (Grant et al. 2001). Limited P supply adversely impacts plant growth and yield and therefore dry matter production is a good indicator of plant growth and potential grain yield of a crop (Wu and Bao 2011). Part of the objectives of this study were to measure the increase in wheat biomass (root and above ground) with increase in nitrophosphate and MAP(33) application.

Chlorophyll is a green pigment that enables plants to absorb light energy. It is found imbedded in thylakoid membranes of the chloroplasts where they facilitate the synthesis of carbohydrate during photosynthesis hence chlorophyll content impacts on the dry matter accumulation in plants. When leaves have high chlorophyll content, plants intercept more light and produce carbohydrates. To confirm this, several studies have found significant correlations between chlorophyll readings and grain yield (Follett et al. 1992; Guler 2009).

Chlorophyll can be used as a good indicator for nutrient status in plants (Shaahan et al. 1999) and it is measured with a Minolta[1] chlorophyll meter (model SPAD 502). It is denoted in SPAD units, which stands for Soil Plant Analysis Development (Hoel and Solhaug 1998). Measurements are taken non-destructively on healthy fresh leaves as drying leaves destroy chlorophyll and carotenoids (Schertz 1919). Nitrogen (Guler 2009), Mg, Fe (Barton 1970), S, Ca, Mn and Zn (Mengel and Kirkby 1987) are known to be associated with chlorophyll synthesis and are highly correlated with the chlorophyll content (Shaahan et al. 1999; Guler 2009). Knowing relationships between chlorophyll measurements and leaf nutrient content can help farmers predict the nutrient status of the crop and make decisions on when to topdress. Blackmer and Schepers (1995) studied the ability of the chlorophyll meter to detect plant N

deficiencies in corn (*Zea mays* L.) and found that it can assist with the on farm decision making process on when to supply N fertiliser.

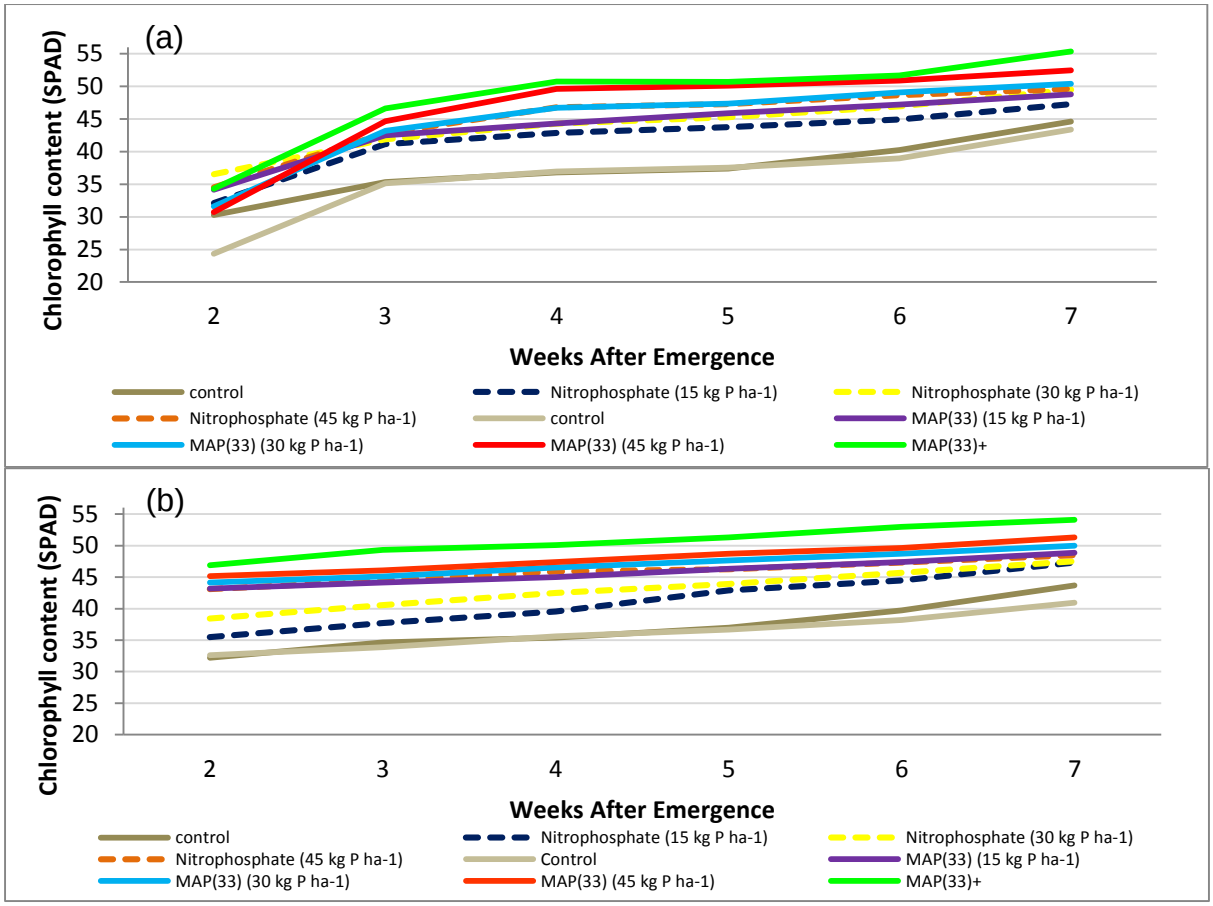
However, precautions must be taken when making decisions based on the chlorophyll content because SPAD readings are affected by irradiance. The lowest SPAD readings are measured at high irradiance and the highest readings at low irradiance (Hoel and Solhaug 1998). This was demonstrated by Hoel and Solhaug (1998) on winter wheat (*Triticum aestivum* L.) and *Oxalis acetosella* L plants. As such, time of the day and weather conditions influence SPAD readings.

In addition to chlorophyll measurements, fresh root volume, fresh and the dry mass of the roots and leaves were also measured to assess wheat growth. Plant roots have three main functions i.e. to anchor the plant, absorb water and nutrients. Root growth defines the extent to which a plant explores soil for water and mineral nutrients (Hsiao and Xu 2000) and therefore, its development give a good indication of the absorptive area and capacity of the root system to utilise soil nutrients (Van Tonder 2008). Furthermore, root growth impacts on the final yield of a crop. Leaves define the canopy size of a plant for capturing sunlight and carrying out photosynthesis to gain carbon and energy (Hsiao and Xu 2000) and are therefore a good indicator of plant growth and potential grain yield (Wu and Bao 2011).

5.2 Results and discussion

5.2.1 Chlorophyll content (SPAD)

The chlorophyll content was significantly ($P < 0.05$) affected by the interaction between the fertiliser type and application rate (Appendix). Nitrophosphate treatments are denoted with broken lines and MAP(33) treatments with solid lines. The control treatments are denoted with brown colours and these consistently resulted in significantly lower chlorophyll content than the fertilised treatments. In both trials, increasing the P applied generally increased the chlorophyll content of wheat for both fertilisers, but the increases were more pronounced for the MAP(33) treatments (Figure 5.1).



*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 5.1 Chlorophyll content of wheat fertilised with nitrophosphate and MAP(33) in trial 1 (a) and trial 2 (b)

Increasing P application proportionally increased the chlorophyll content of the leaves (Figure 5.1). This could be due to the fact that P is a source of energy in the form of ATP (FSSA 2007) and therefore, increasing P increases the energy in the plant, which is necessary for biochemical reactions. These results concur with those by Van Nieuwenhuyse and Jones (1996) who found a strong curvilinear relationship (R^2 = 0.67) between total P concentration and chlorophyll concentration. The effect of P on chlorophyll was also investigated by Schertz (1919) and revealed that the lack of P reduces the daily variation of the chlorophyll components and narrows the absorption bands. MAP(33) applied at 30 kg P ha^{-1} that included secondary nutrients (MAP(33)+) tended to have higher chlorophyll readings (3-7 WAE) in both trials (Figure 5.1). The controls produced the lowest chlorophyll content, probably because the plants had little or no access to nutrients (Chapter 6) that promote chlorophyll synthesis such as N and Mg.

Nitrophosphate and MAP(33) were then compared with each other, by using the chlorophyll data from different application rates that was pooled together to calculate the average for each fertiliser treatment. These results are presented in Table 5.1. Wheat fertilised with MAP(33) consistently had a significantly higher chlorophyll content than the wheat fertilised with nitrophosphate, except at week 2 and 3 after emergence for Trial 1 (Table 5.1). The chlorophyll content of the wheat fertilised with MAP(33) was up to 11% (equation 5.1) higher (2 WAE) than the nitrophosphate fertilised wheat (Table 5.1):

$$\text{Chlorophyll content increase} = \frac{SPAD_{MAP(33)} - SPAD_{nitrophosphate}}{SPAD_{nitrophosphate}} \times 100 \quad (\%) \qquad (5.1)$$

Table 5.1 Chlorophyll content of nitrophosphate and MAP(33) fertilised wheat

		Chlorophyll content (SPAD)					
	Fertiliser	2 WAE	3 WAE	4 WAE	5 WAE	6 WAE	7 WAE
Trial 1	Control	27.29^{a}	35.23^{a}	36.89^{a}	37.46^{a}	39.61^{a}	44.00^{a}
	Nitrophosphate	33.36^{c}	40.31^{b}	42.69^{b}	43.43^{b}	45.19^{b}	47.78^{b}
	MAP(33)	30.20^{b}	41.37^{b}	44.41^{c}	45.22^{c}	46.54^{c}	48.75^{c}
	P-value	0.00	NS	0.01	0.00	0.02	0.02
	$LSD_{(\alpha\text{-}0.05)}$	0.67	1.67	1.22	1.15	1.08	0.79
Trial 2	Control	32.42^{a}	34.28^{a}	35.52^{a}	36.83^{a}	38.95^{a}	42.31^{a}
	Nitrophosphate	37.31^{b}	39.35^{b}	40.83^{b}	42.52^{b}	44.30^{b}	46.75^{b}
	MAP(33)	41.27^{c}	42.31^{c}	43.64^{c}	44.83^{c}	45.98^{c}	47.77^{c}
	P-value	0.00	0.00	0.00	0.00	0.00	0.01
	$LSD_{(\alpha\text{-}0.05)}$	0.64	0.58	0.93	0.58	0.74	0.73

NS – non significant
*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

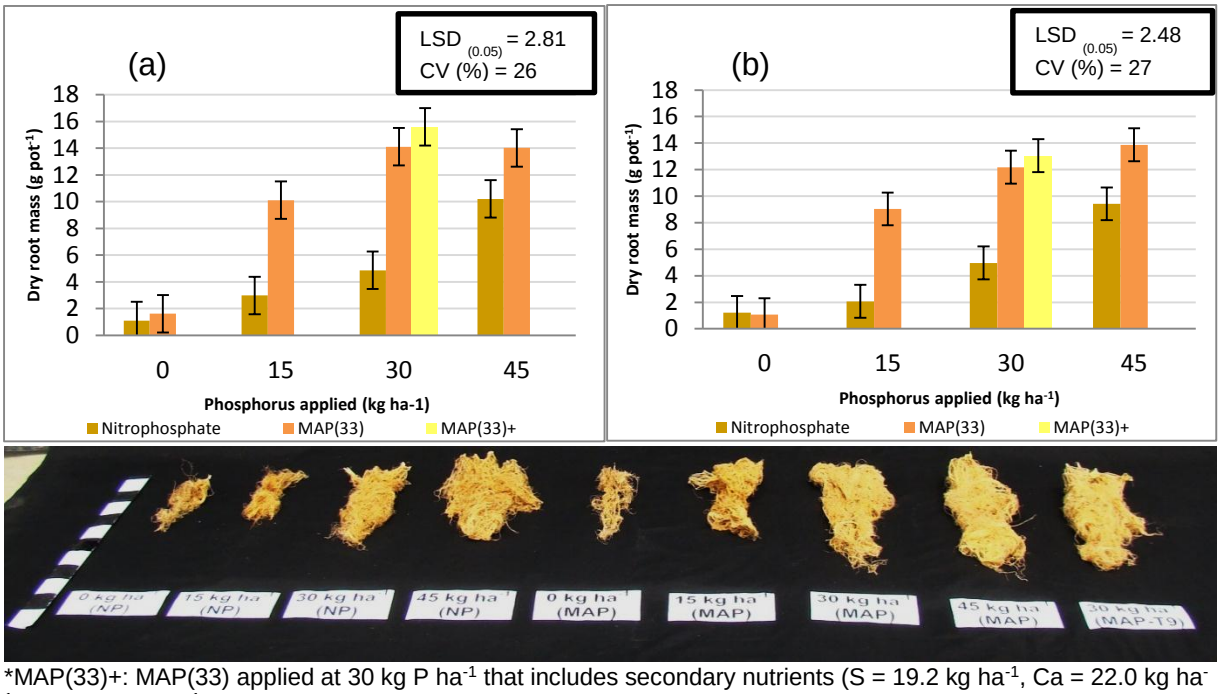
It was expected that the wheat fertilised with nitrophosphate will have a higher chlorophyll content than the MAP(33) fertilised wheat, because nitrophosphate contains additional Mg and S. However, these results suggest that the presence of the secondary nutrients (Mg and S) in nitrophosphate did not enhance chlorophyll production (Table 5.1), but the effect of nitrophosphate fertiliser on decreasing the soil pH (Chapter 4) that subsequently restricted the root growth might have affected nutrient uptake, including N, Mg and S (Chapter 6), that are critical for chlorophyll

synthesis. The decrease in soil pH effects therefore countermanded the benefits of secondary nutrients (Mg and S) (Barton 1970; Mengel and Kirkby 1987).

Higher chlorophyll accumulation by MAP(33) was due to the fact that plants developed a larger root system (Figure 5.2) that contributed to a higher absorption of nutrients, including N, Mg and S (Chapter 6), that are critical in chlorophyll synthesis (Barton 1970; Mengel and Kirkby 1987). Chlorophyll concentration also increases with fertiliser application rate due to an increase in N, P, Mg and S (Figure 5.1 a and b).

5.2.2 Root growth

The results of the dry root mass in response to nitrophosphate and MAP(33) applied at different application rates are presented in Figure 5.2.



*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 5.2 Dry root mass ($LSD_{(0.05)}$ presented as bars) of nitrophosphate and MAP(33) fertilised wheat in trial 1(a) and trial 2(b). Root photographs (c) taken after harvest

There was a significant interaction between fertiliser type and application rate (Appendix). For both fertiliser types root growth generally increased with application rate. The trend in both trials showed that MAP(33) treatments resulted in a higher root growth than nitrophosphate treatments (Figure 5.2). The controls had the lowest root

growth due to limited P supply. The increase in root growth with increase in P application is due to the fact that the higher P application increased the P availability for the plants (Mengel and Kirkby 2001). This was confirmed by results that showed an increase in P uptake with P application rate. These results are discussed in more detail in Chapter 6.

Another interesting result was that there were no statistical differences between MAP(33) applied at 30 kg P ha^{-1} and MAP(33) with secondary nutrients (MAP(33)+) applied at 30 kg P ha^{-1} (Figure 5.2). This suggests that the addition of secondary nutrients with MAP(33) did not affect root growth. A possible reason could be that S (19.2 kg ha^{-1}) and Mg (1.5 kg ha^{-1}) were added in small quantities to influence root growth or that S and Mg (Table 3.1) content of the soils are not limiting root growth. Soil S concentration of 13 mg kg^{-1} and Mg concentration of 28 mg kg^{-1} are generally regarded not very low for maize and wheat cultivation for example in New Zealand, ADAS in Britain soils are considered deficient in Mg when levels drop below 25 mg kg^{-1} available Mg (Craighead and Martin 2001). There were also no significant differences between MAP(33) applied at 30 and 45 kg P ha^{-1}. However, a significant difference was found between the 30 and 45 kg P ha^{-1} application rate treatment for nitrophosphate.

Nitrophosphate and MAP(33) treatments were then compared with each other by using the fresh root volume and dry root mass data from the different application rates. Trial 1 and 2 differed probably due to the increase in soil density during the filling of the pots (Chapter 3). The results of the individual measurements for the different application rates were pooled together to calculate the average for each fertiliser treatment and these results are presented in Figure 5.3. Fresh root volume was significantly ($P<0.05$) affected by fertiliser type. In Trial 1 the MAP(33) fertilised wheat resulted in 74% larger root volume (equation 5.2) when compared to the nitrophosphate fertilised wheat (Figure 5.3a), while in Trial 2 MAP(33) resulted in 21% larger root volume when compared with the nitrophosphate fertilised wheat (Figure 5.3b):

$$\text{Increase in fresh root mass} = \frac{\text{Fresh root mass}_{MAP(33)} - \text{Fresh root mass}_{nitrophosphate}}{\text{Fresh root mass}_{nitrophosphate}} (\%) \quad (5.2)$$

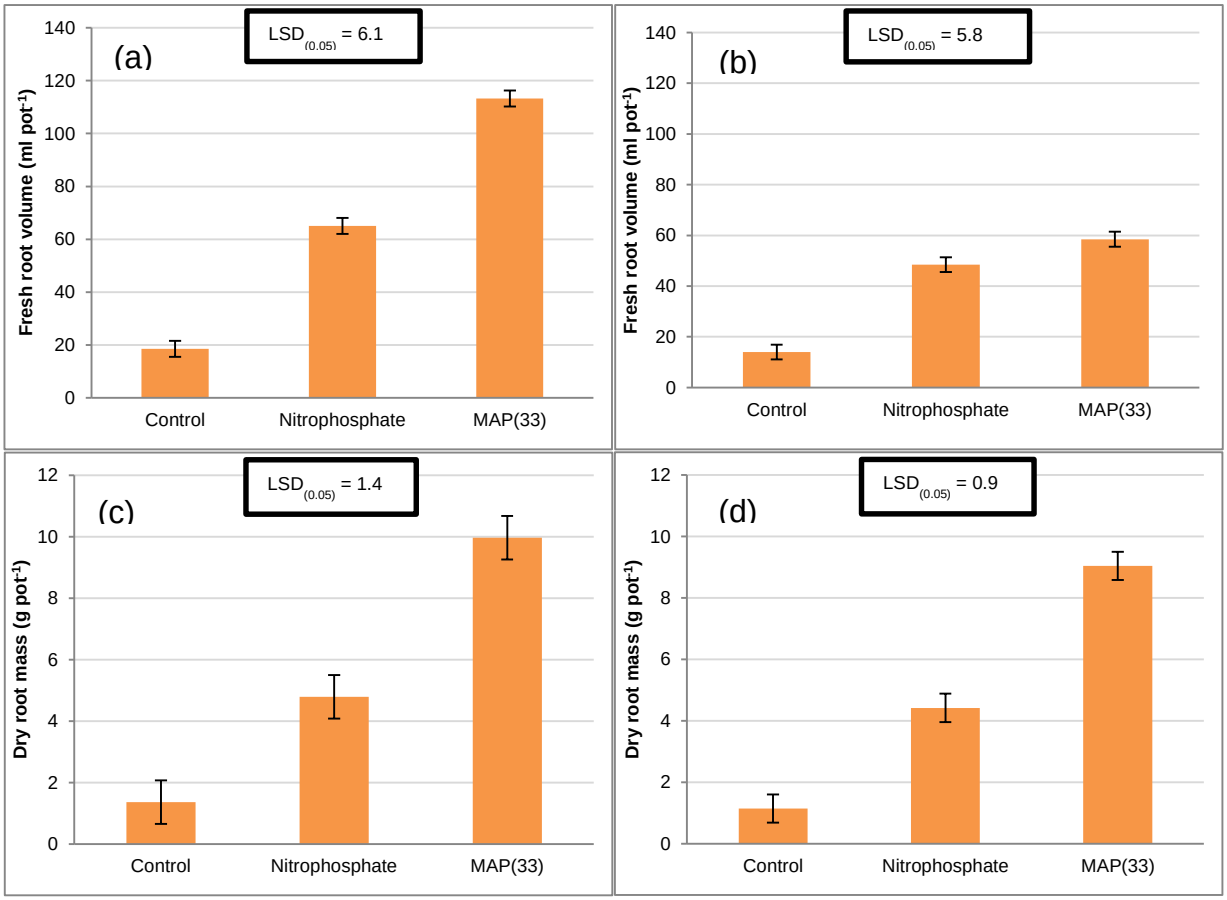


Figure 5.3 Fresh root volume ($LSD_{(0.05)}$ presented as bars) in trial 1(a) and trial 2(b) in response to nitrophosphate and MAP(33) application. Dry root mass in trial 1(c) and trial 2(d) of nitrophosphate and MAP(33) fertilised wheat

Dry root mass was also significantly ($P<0.05$) affected by fertiliser type (Figure 5.3). In Trial 1 (Figure 5.3c), MAP(33) fertilised wheat had 108% and in Trail 2 105% (Figure 5.3d) more roots (on mass basis) when compared to nitrophosphate fertilised wheat. The variability in the results for Trial 1 and 2 was higher for root volume than for root mass due to the fact that dry root mass is a more reliable and repeatable measurement than root volume (Burdette 1979).

In order to explain the difference in root growth between nitrophosphate and MAP(33) soil pH measurements were taken after harvest and the results are presented in Figure 5.4. The graph shows how the increase in soil pH enhances root growth (dry root mass) of potted wheat. A strong positive correlation was obtained between the pH and root mass ($R^2 = 0.81$). This correlation was strong despite the fact that it was calculated within a small pH range.

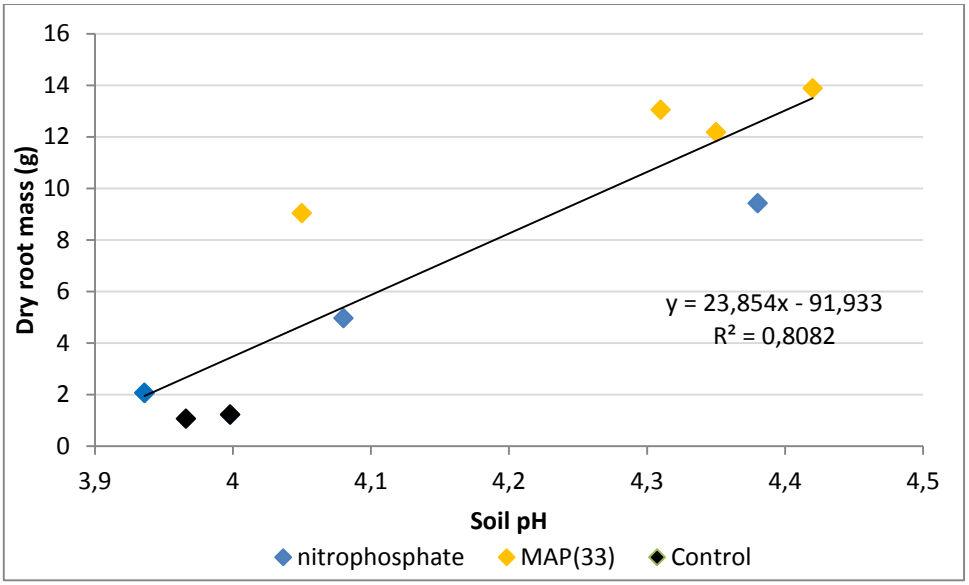
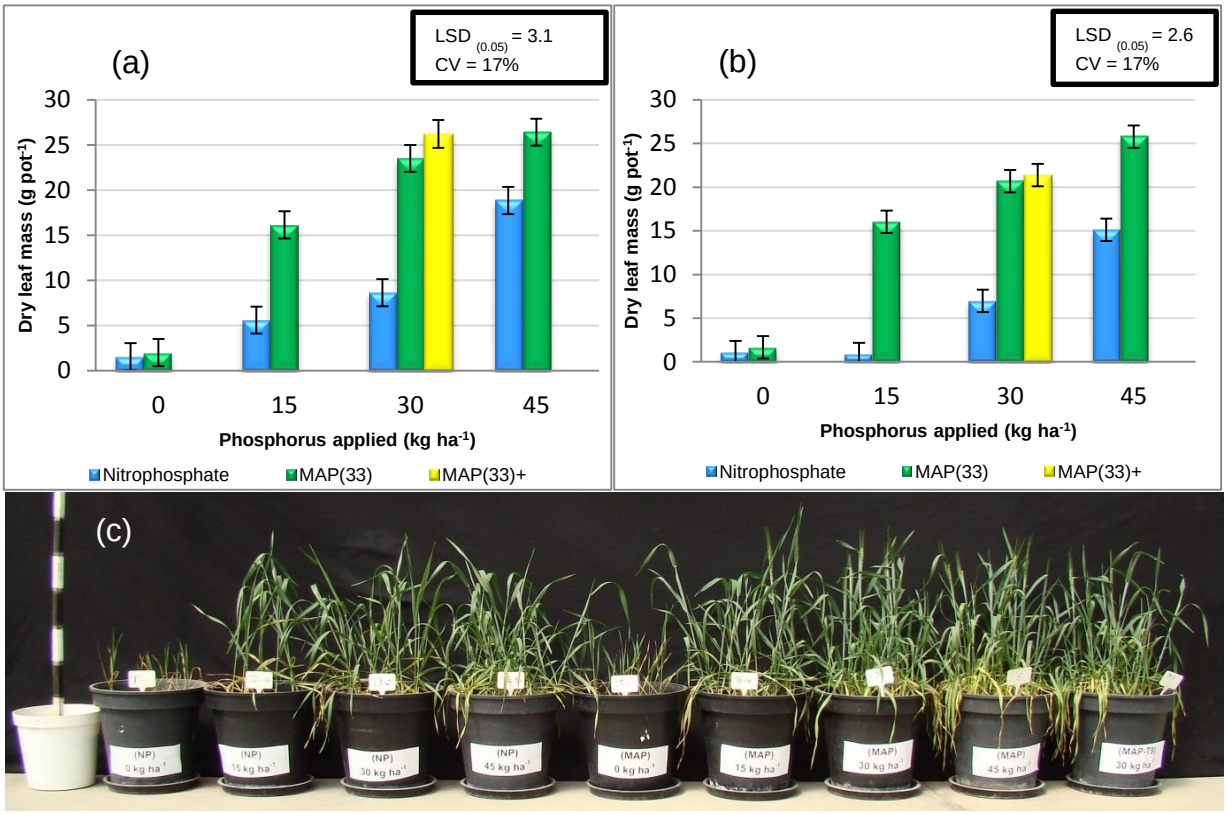


Figure 5.4 Relationship between soil pH and dry root mass

The pH of soil samples taken directly after harvest showed that MAP(33) fertilised soils had a higher soil pH and therefore root growth was not restricted while nitrophosphate predominantly produced a lower soil pH and dry root mass (Figure 5.4 and Chapter 4, Figure 4.2). Except for the 45 kg ha^{-1} nitrophosphate treatment that resulted in a higher soil pH, probably due to the release of OH^- and HCO_3^- (Chapter 4). However, the low dry root mass for the rest of the nitrophosphate treatments is explained by nitrophoshate that is being hydrolysed to form phosphoric acid that reduces soil pH and subsequently root growth. The reduction of root growth due to low soil pH obtained in this study concurs with the findings of Kerridge (1969) who found smaller root length at pH 4.0 than at pH 5.0. Limited wheat root growth as a result of soil acidity was also reported by Costa and Rosolem (2007). Howard and Adams (1965) saw a reduction in the growth rate of primary cotton roots at solution pH below 4.2 which is similar to the pH measured for the nitrophosphate treatment. Although Al and Fe in the soil were not measured, other studies have found that at lower pH the solubility of Al^{3+} and Fe^{3+} increases and result in higher concentrations that are toxic to plants (Haynes and Mokolobate 2001). Toxic levels (especially Al^{3+}) inhibit root growth through impedance of cell division and elongation (Wall et al. 2013). Restriction of root growth caused by Al^{3+} is also associated with the formation of callose where Al toxicity causes loss of the apical dominance making the growth of the main roots completely inhibited (Mengel and Kirkby 2001). When root growth is impaired, nutrient acquisition and water access are significantly reduced (Pinkerton and Simpson 1986) and this limit the plant growth rate.

5.2.3 Leaf growth

To determine the influence of fertiliser type (nitrophosphate and MAP(33)) applied at different rates on leaf growth, the dry leaf mass of the wheat was determined and the results are presented in Figure 5.5. A significant interaction was found between fertiliser type and application rate (Appendix).



*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 5.5 Dry wheat leaf mass ($LSD_{(0.05)}$ presented as bars) fertilised with nitrophosphate, MAP(33) and MAP(33) with secondary nutrients (MAP(33)+) at different applications rates in trial 1(a) and trial 2(b). Wheat plants (c) photographed at harvest

An increase in P application rate generally resulted in an increase in dry leaf mass for both nitrophosphate and MAP(33) treatments (Figure 5.5), with the maximum dry leaf mass recorded for the 45 kg P ha^{-1} applications and the lowest for the controls. This trend was similar in both trials. The overall trend indicated that leaf growth was more pronounced for the MAP(33) treatments and it consistently resulted in more leaf

growth than the nitrophosphate treatments (Figure 5.5). This was due to the increased root growth that resulted in higher nutrient and water uptake (Mengel and Kirkby 2001; Hsiao and Xu 2000). MAP(33) fertilised wheat (section 5.2.2) had more nutrient absorption than the nitrophosphate treatments. There were no statistical differences between MAP(33) applied at 30 kg P ha^{-1} and MAP(33) with secondary nutrients (MAP(33)+) applied at 30 kg P ha^{-1}.

Another important factor that needs to be considered is the solubility and availability of P in the different fertilisers. Analysis of the fertilisers showed that MAP(33) had 21% citric P compared to 8% for nitrophosphate (Chapter 3, Table 3.4). The citric acid test gives an indication of the solubility and plant availability of P (Krumm 1969). This suggests that under acidic conditions, which is typical of the soils used in this study, MAP(33) had more P available than nitrophosphate which could also have attributed to the higher leaf and root growth. Studies have shown that fertiliser solubility affects crop growth (Meelu et al. 1977; Hundal et al. 1979). Meelu et al. (1977) established that the response of wheat to P was directly related to the solubility of the fertiliser source when evaluating diammonium phosphate, urea ammonium phosphate, suphala (30% WSP), nitrophosphate (50% WAP) and SSP.

Nitrophosphate and MAP(33) were compared, to each other, by pooling the application rate data and the results are presented on Figure 5.6. Dry leaf mass was significantly ($P<0.05$) affected by fertiliser type. In Trial 1 (Figure 5.6a), MAP(33) fertilised wheat had a 96% (equation 5.3) and in Trial 2 (Figure 5.6b) a 167% increase in dry leaf mass when compared with the nitrophosphate treatment.

$$\text{Increase in dry leaf mass} = \frac{\text{Dry leaf mass}_{\text{MAP(33)}} - \text{Dry leaf mass}_{\text{nitrophosphate}}}{\text{Dry leaf mass}_{\text{nitrophosphate}}} \ (\%) \quad (5.3)$$

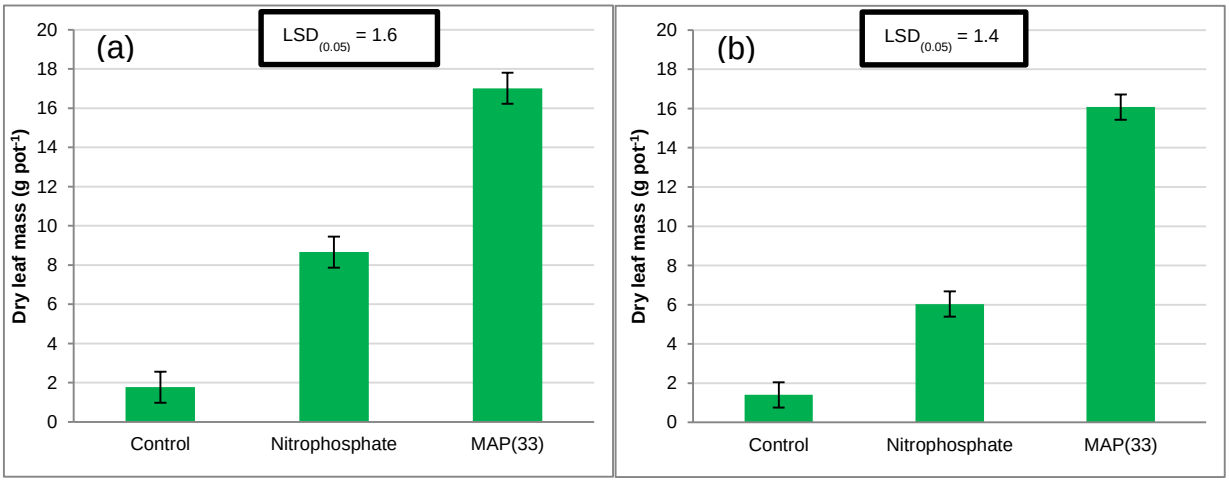


Figure 5.6 Dry leaf mass ($LSD_{(0.05)}$ presented as bars) of wheat as influenced by nitrophosphate and MAP(33) application in trial 1(a) and trial 2(b)

More leaf dry matter was produced in wheat fertilised with MAP(33) than with nitrophosphate (Figure 5.6), despite the fact that the later contains significant amounts of Ca (6%), Mg (0.4%) and S (5%). More root growth from the MAP(33) treatments allowed plants to take up more nutrients (Chapter 6), which facilitated leaf growth. Results from the previous section (section 5.2.2) showed that the root growth was less for the wheat fertilised with nitrophosphate than the wheat fertilised with MAP(33). Numerous studies have showed that there exist a direct link between root growth and nutrient uptake. Hsiao and Xu (2000) found that plants with more root growth explore greater soil volumes for water and nutrient uptake (Hsiao and Xu 2000). Greater root growth provides a larger absorptive area and the capacity to utilise soil nutrients (Van Tonder 2008). Good supply of nutrients and water supported the growth of the wheat canopy. On the other hand, nitrophosphate treatments resulted in less root growth with a subsequent reduction in nutrient uptake (Chapter 6) and hence leaf growth was reduced. Furthermore, higher chlorophyll content on the MAP(33) treatment (section 5.2.1) could have improved photosynthesis attributing to higher accumulation of dry matter.

5.3 Conclusion

Wheat plants fertilised with MAP(33) produced more biomass (root and leaf) than wheat plants fertilised with nitrophosphate. A major factor that contributed to reduced

biomass production on nitrophosphate was soil pH. Nitrophosphate treatments generally resulted in lower soil pH which restricted root growth and nutrient uptake. For MAP(33) treatments the soil pH increased, which resulted in more root growth enhancing nutrient and water absorption leading to improved wheat growth. Wheat fertilised with MAP(33) also had a higher leaf chlorophyll content than nitrophosphate fertilised wheat that resulted in more growth of the MAP(33) fertilised wheat.

CHAPTER 6

NUTRIENT UPTAKE WITH NITROPHOSPHATE AND MAP(33) APPLICATION IN WHEAT

6.1 Introduction

The optimum concentration of the appropriate nutrients is important to achieve high and quality yields in wheat. Seventeen essential nutrients are required to complete a plant's life cycle and include C, H, O, N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Ni, B, Mo and Cl (Mengel and Kirkby 2001; FSSA 2007; Pilon-Smiths et al. 2009). These are divided into macronutrients and micronutrients. Macronutrients (C, H, O, N, P, K, Ca, Mg and S) are required by plants in quantities greater than 1000 mg kg^{-1} dry weight of which the elements C, H and O represent 95% of the dry weight (Mengel and Kirkby 2001; Pilon-Smiths et al. 2009). Macronutrients form part of numerous plant components, including proteins, nucleic acids and chlorophyll, and are essential for processes such as energy transfer and the functioning of enzymes (FSSA 2007). The micronutrients (Fe, Mn, Zn, Cu, Ni, B, Mo and Cl), also referred to as trace elements, are required in minor quantities of less than 100 mg kg^{-1} dry weight (Mengel and Kirkby 2001; FSSA 2007; Pilon-Smiths et al. 2009) and play a critical role in enzyme systems (Mengel and Kirkby 2001; FSSA 2007) and oxidation-reduction reactions (FSSA 2007). There is another group of elements utilised by plants called beneficial nutrients and promote plant growth but they are not absolutely necessary for completion of the plant life cycle (Pilon-Smiths et al. 2009) and include cobalt (Co), selenium (Se), silicon (Si), sodium (Na), Al (Mengel and Kirkby 2001; Pilon-Smiths et al. 2009).

Plants take C and O up mostly from the atmosphere in the form of CO_2. It is speculated that plant roots can absorb C and O from the soil solution in the form of HCO_3, while H together with O is absorbed as water (Mengel and Kirkby 2001). The other macro- and micronutrients are absorbed mainly from the soil solution by roots of plants, although leaves can also absorb these nutrients in a lesser extent (Tucker 1999).

The fertilisers, nitrophosphate and MAP(33), used in this study contain both the essential nutrients, N, P while nitrophosphate also has Ca, Mg and S. In this chapter the objective of the study was to determine if nitrophosphate performed better than MAP(33) in improving nutrient uptake by wheat during vegetative growth. This

objective was met by analysing the nutrient concentration of the dry leaf matter after harvest.

The methodology for determining the optimum nutrient concentration for different plant growth stages dates back to the 1800s (Munson 1998). This procedure compares plant growth, relative growth or biomass production with the elemental concentrations of the entire plant or certain plant structures, such as leaves, stems, petioles, fruit or grain, sampled at different times or phenological stages (Kalra 1998). The concentration of the essential elements is expressed on a dry matter basis as either a percentage or grams per kilogram (g kg^{-1}) for the major elements, and for the micronutrients as parts per million (ppm) or milligrams per kilogram (mg kg^{-1}) (Kalra 1998).

The use of leaf analyses in wheat production is a standard practice to diagnose the nutrient status (deficient, optimum or toxic) of the crop (McCaulay 2011). Leaf analyses have been useful in relating elemental concentrations in the plant to growth response or yield and can also be used to determine soil nutrient availability and the percentage recovery of the applied nutrients in crop response experiments (Munson 1998). It can also identify and measure the potential toxic elements that may be found in plants, such as cadmium (Cd), mercury (Hg) and lead (Pb) (Risser and Baker 1990).

However, the limitation of plant analysis is that the measured concentration is affected by plant size and age due to the fact that nutrient concentration changes with the physiological growth stage of a plant (Bhaduri and Pal 2013). In older plants the dilution effect may be misleading where larger plants result in lower nutrient concentration while containing higher amounts of nutrients. To counter that, nutrient uptake (or nutrient content) is calculated by multiplying the specific nutrient concentration by dry leaf mass:

Specific nutrient uptake (mg) = Specific nutrient concentration (mg kg^{-1}) x dry leaf mass (kg) (6.1)

6.2 Results and discussion

6.2.1 Nitrogen

The N concentration of the dry leaf matter, for all treatments, ranged between 2.5-4.1% (Table 6.1), which fell in the sufficient range of 2.5-4.5% (Kalra 1998). However, N deficiency symptoms which were characterised by yellowing of lower leaves and stunted growth were observed for some of the control treatments in Trial 1 and 2 because these pots did not receive N fertilisation.

Table 6.1 Nitrogen concentration of leaf dry matter of potted wheat fertilised with nitrophosphate and MAP(33)

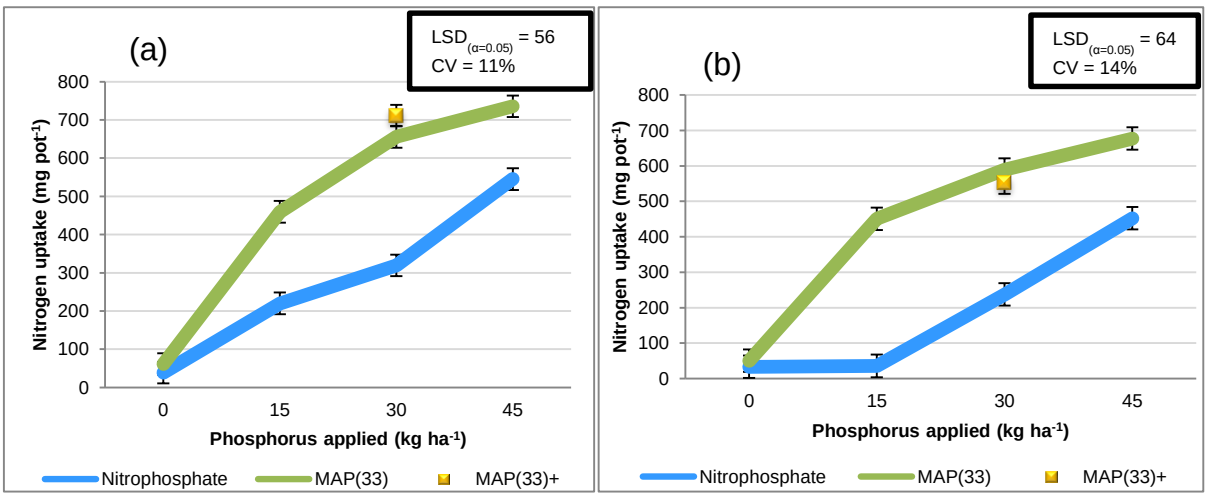
			Phosphorus applied (kg ha^{-1})			
	Fertiliser	Element	0	15	30	45
Trail 1	Nitrophosphate	N%	2.49^{a}	3.93^{d}	3.77^{d}	2.90^{bc}
	MAP(33)		3.06^{c}	2.90^{bc}	2.80^{bc}	2.82^{bc}
	MAP(33)+				2.71^{ab}	
Trial 2	Nitrophosphate	N%	2.95^{b}	4.12^{d}	3.48^{c}	3.02^{b}
	MAP(33)		3.01^{b}	2.81^{ab}	2.85^{ab}	2.63^{a}
	MAP(33)+				2.59^{a}	

Trial 1: $LSD_{(\alpha\text{-}0.05)}$ = 0.29; CV = 7%
Trial 2: $LSD_{(\alpha\text{-}0.05)}$ = 0.26; CV = 7%
*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

There were significant interaction effects between fertiliser type and application rate on N concentration. The N concentration of dry leaf matter decreased with increase in P application for MAP(33) treatments. However, the N concentration in the dry leaf tissue for the controls were lower because they did not receive any N fertiliser. In Trial 2, nitrophosphate applied at 15 kg P ha^{-1} resulted in the highest N concentration (4.1%) of the dried leaf matter and MAP(33) applied at 45 P ha^{-1} had the least (2.6%) (Table 6.1). This trend was similar in Trial 1. The decrease in dried leaf N with increasing P application could be explained in terms of the dilution effect where larger plants (Figure 5.5) result in lower nutrient concentration while containing higher nutrient amounts, this dilution effect was also observed by (Coetzee 2013). In Chapter 5 results for biomass are given and nitrophosphate applied at 15 kg P ha^{-1} had the lowest dry leaf mass yet the highest N concentration of the dried leaf matter and the reverse was true for MAP(33) applied at 45 kg P ha^{-1}, which confirms the dilution effect. There were no

statistical differences between MAP(33) applied at 30 kg P ha^{-1} and MAP(33) with secondary nutrients (MAP(33)+) applied at 30 kg P ha^{-1}. This was consistent with the same amount of biomass produced (Chapter 5) for MAP(33) applied at 30 kg P ha^{-1} and MAP(33)+ applied at 30 kg P ha^{-1}. However, nitrophosphate applied at 30 kg P ha^{-1} resulted in a significantly higher N concentration in the dried leaf matter than MAP(33)+ applied at the same rate (30 kg P ha^{-1}). These results are again consistent with the biomass produced, with less biomass produced for the wheat treated with the nitrophosphate (30 kg P ha^{-1}).

Nitrogen uptake results for the nitrophosphate and MAP(33) treatments are presented in Figure 6.1.



*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 6.1 Increase in nitrogen uptake ($LSD_{(0.05)}$ presented as bars) with nitrophosphate and MAP(33) application for trial 1(a) and trial 2(b)

For both trials (Figure 6.1a and b) N uptake was significantly higher for the MAP(33) treatments than the nitrophosphate treatments (Figure 6.1). However, the increase in N uptake for the MAP(33) treatments tended to asymptotically approach a maximum with P application, according to the law of yield return applied (Fox 1971), as indicated by the curvilinear plot between N uptake and MAP(33) applied. However, the N uptake for the nitrophosphate treatments appears to increase linearly over the range of nitrophosphate (0-45 kg P ha^{-1}) treatments, which signified that the demand for N by

the plants was still high and had not yet reached a maximum. This shows that the growth potential for plants treated with nitrophosphate had not been reached.

Both fertiliser treatments for both trials resulted in an increase in N uptake with an increase in P application. MAP(33) resulted in a higher N uptake for all application rates due to a larger root system (Chapter 5) that allowed for more N to be taken up. There were no statistical differences between MAP(33) applied at 30 kg P ha^{-1} and MAP(33) with secondary nutrients (MAP(33)+) applied at 30 kg P ha^{-1}.

6.2.2 Phosphorus

The P concentration of the dried leaf matter are presented in Table 6.2.

Table 6.2 Phosphorus concentration of leaf dry matter of potted wheat fertilised with nitrophosphate and MAP(33)

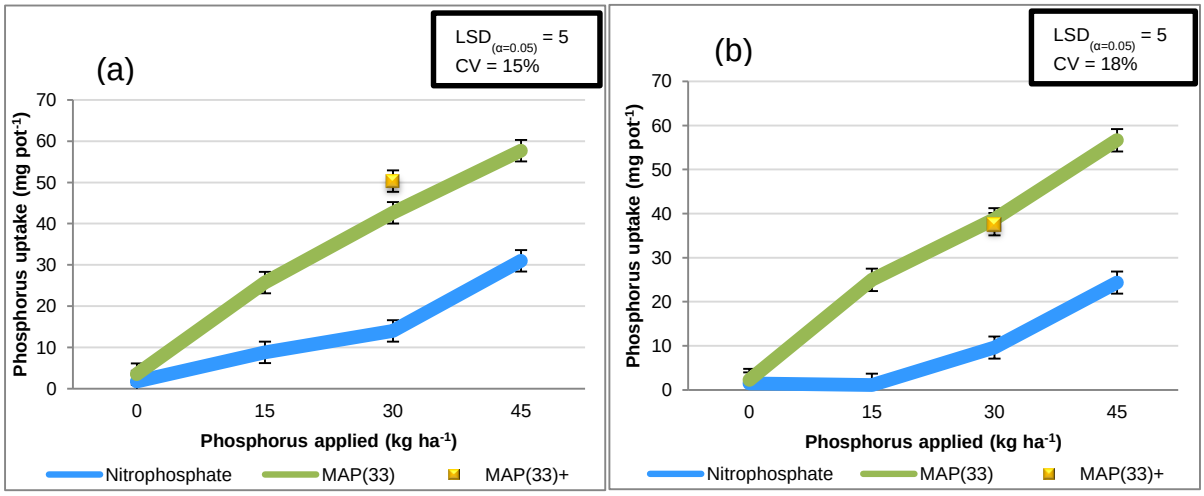
			Phosphorus applied (kg ha^{-1})			
	Fertiliser	Element	0	15	30	45
Trail 1	Nitrophosphate	P%	0.106^{a}	0.156^{b}	0.160^{bc}	0.164^{bc}
	MAP(33)		0.176^{bcd}	0.160^{bc}	0.182^{cd}	0.220^{e}
	MAP(33)+				0.192^{d}	
Trial 2	Nitrophosphate	P%	0.128^{a}	0.126^{a}	0.138^{a}	0.160^{b}
	MAP(33)		0.134^{a}	0.156^{b}	0.186^{c}	0.220^{d}
	MAP(33)+				0.176^{c}	

Trial 1: $LSD_{(\alpha\text{-}0.05)}$ = 0.025; CV = 11%
Trial 2: $LSD_{(\alpha\text{-}0.05)}$ = 0.016; CV = 8%
*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Phosphorus concentrations of the dry leaf matter ranged from 0.11 to 0.22% (Table 6.2) for all treatments. Concentrations below 0.15% are considered deficient and 0.20-0.75% sufficient according to Kalra (1998). In this study the control treatments and the treatments where nitrophosphate was applied at 15 kg P ha^{-1} and 30 kg P ha^{-1} (in Trial 2) had P concentrations below 0.15% that indicate a P deficiency (Table 6.2). For the control treatments P deficiency symptoms which were characterised by the reddish to purplish colouring of leaves and retarded growth were observed in both trials.

Type of fertiliser and application rate had an interaction effect on the P concentration of the dried leaf matter of wheat. Increasing the P application rate increased the P concentration in the dry leaf matter for both fertilisers in trials 1 and 2. MAP(33) applied at 45 kg P ha^{-1} resulted in the highest P concentration in the dry leaf matter (Table 6.2) where the P concentration of the dry leaf matter increased by 64 % (Trial 2) when compared to the control. This was due to the fact that large amounts of P were supplied to the plant coupled with an extensive root system (Chapter 5) that could absorb available P. There were no significant differences between MAP(33) applied at 30 kg P ha^{-1} and MAP(33) with secondary nutrients (MAP(33)+) applied at 30 kg P ha^{-1} which indicates the addition of secondary nutrients on the MAP(33)+ treatment did not enhance the P concentration of the dry leaf matter in this trial. However, these treatments had significantly higher P concentration in the dry leaf matter than the nitrophosphate treatment applied at 30 kg P ha^{-1} (Table 6.2).

Phosphorus uptake results are presented in Figure 6.2 and shows an increase in P uptake for nitrophosphate and MAP(33) at different application rates, with the maximum P uptake recorded for the MAP(33) applied at 45 kg P ha^{-1} (Figure 6.2).



*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 6.2 Increase in phosphorus uptake ($LSD_{(0.05)}$ presented as bars) with nitrophosphate and MAP(33) application for trial 1(a) and trial 2(b)

MAP(33) fertiliser treatments consistently resulted in higher P uptake than the nitrophosphate treatments for all application rates (Figure 6.2). This could be

explained in terms of the difference in root growth, P availability and biomass production between the two fertilisers. MAP(33) treatments resulted in more root growth than the potted wheat treated with nitrophosphate (Chapter 5). Therefore, the roots in the MAP(33) trial had a larger absorptive area and could also explore a larger soil volume (Hsiao and Xu 2000). In Chapter 4 results for the soil pH measured after harvest showed that the nitrophosphate treatments had a lower soil pH than MAP(33), hence there is a potential that the P was complexed by Al and Fe (Haynes and Mokolobate 2001; Mengel and Kirkby 2001) and because the P solubility of nitrophosphate is less than the P solubility of MAP(33) which makes P unavailable for uptake. Govil 1972; Meelu et al. 1977 and Hundal et al. 1979 showed that highly soluble fertilisers had higher P efficiency than the less soluble. More biomass was produced with MAP(33) than the nitrophosphate treatments and this increased the demand for P, because larger plants consume more nutrients.

Inconsistent results were found when MAP(33) applied at 30 kg P ha^{-1} was compared with MAP(33) with secondary nutrients (MAP(33)+) applied at 30 kg P ha^{-1}. In Trial 1, MAP(33)+ resulted in a significantly higher P uptake than the MAP(33) treatment. However, in Trial 2, there were no significant differences between MAP(33)+ and the MAP(33) treatments (Figure 6.2).

6.2.3 Potassium

The results of K concentration of the dry leaf matter are presented in Table 6.3. Potassium concentration between 1.5-5.5% in mature dried leaves are sufficient, with concentrations below 1.5% regarded as deficient and concentrations above 6% become excessive or toxic (Kalra 1998). In this study wheat plants had K concentrations between 2.1-3.5% which implies all plants had sufficient K.

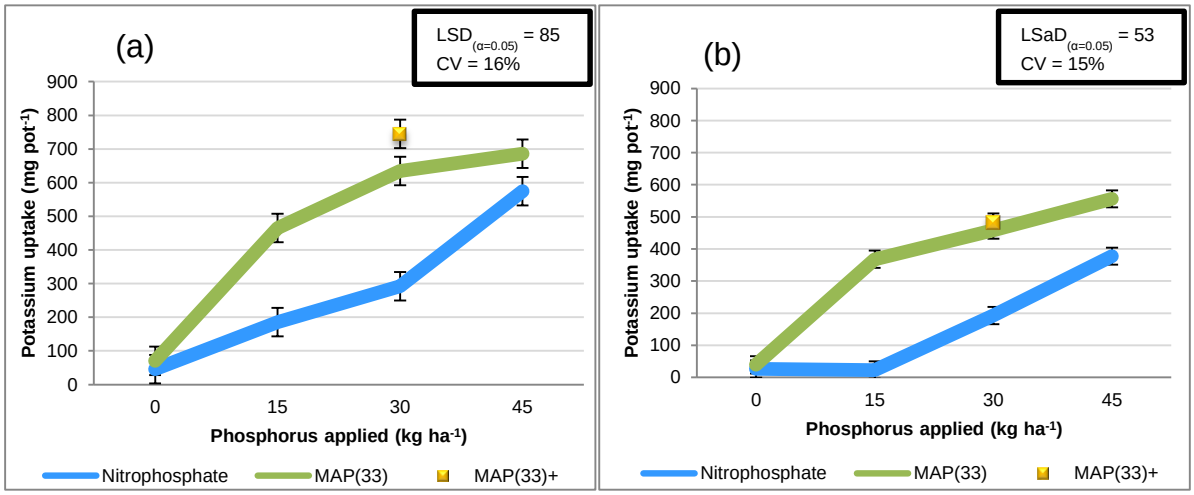
Table 6.3 Potassium concentration of leaf dry matter of potted wheat fertilised with nitrophosphate and MAP(33)

			Phosphorus applied (kg ha^{-1})			
	Fertiliser	Element	0	15	30	45
Trail 1	Nitrophosphate	K%	2.88^{bc}	3.30^{de}	3.36^{e}	3.05^{cd}
	MAP(33)		3.54^{e}	2.89^{c}	2.71^{ab}	2.61^{a}
	MAP(33)+				2.84^{abc}	
Trial 2	Nitrophosphate	K%	2.33^{ab}	2.50^{cd}	2.75^{e}	2.52^{d}
	MAP(33)		2.34^{bc}	2.30^{ab}	2.23^{a}	2.16^{a}
	MAP(33)+				2.27^{ab}	

Trial 1: $LSD_{(\alpha\text{-}0.05)}$ = 0.272; CV = 7%
Trial 2: $LSD_{(\alpha\text{-}0.05)}$ = 0.159; CV = 5%
*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

The interaction between fertiliser type and application rate significantly influenced the K concentration of the dry leaf matter. Nitrophosphate application rate increased the K concentration of the dried leaf matter (except at 45 kg P ha^{-1}) in both trials (Table 6.3), while the MAP(33) treatment generally decreased the K concentration of the dry leaf matter. The decrease in dried leaf K with the increase in MAP(33) application can be explained by a dilution effect, where larger plants (more biomass produced) result in lower nutrient concentration while containing higher nutrient amounts. Nitrophosphate treatments had higher K concentration than MAP(33) treatments. Increased K concentration of the dry leaf matter for the nitrophosphate was due to the smaller size (less biomass produced) of the wheat plants (Chapter 5). There were no statistical differences between MAP(33) applied at 30 kg P ha^{-1} and MAP(33) with secondary nutrients (MAP(33)+) applied at 30 kg P ha^{-1}.

Potassium uptake was calculated as the product of the K concentration of the dried leaf matter and the dry leaf mass. The results for the effect of nitrophosphate and MAP(33) on K uptake, compared at different application rates, are presented in Figure 6.3.



*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 6.3 Increase in potassium uptake ($LSD_{(0.05)}$ presented as bars) with nitrophosphate and MAP(33) application for trial 1(a) and trial 2(b)

Potassium uptake was significantly influenced by the interaction between fertiliser type and application rate. Increasing the application rate generally increased the K uptake for both fertilisers in both trials (Figure 6.3) with the maximum K uptake recorded for the MAP(33) applied at 45 kg P ha^{-1}. The MAP(33) treatments consistently caused more K to be absorbed than the nitrophosphate treatments for all application rates. This could be explained by the differences in soil pH between MAP(33) and nitrophosphate treatments (Chapter 4) that influenced root development. MAP(33) treatments resulted in higher K uptake, because it resulted in less acidic soils than nitrophosphate that resulted in more root growth. More biomass was also produced with the MAP(33) treatments than the nitrophosphate treatments (Chapter 5) that increased the K uptake due to the higher demand.

Inconsistent results were found between MAP(33) applied at 30 kg P ha^{-1} and MAP(33) with secondary nutrients (MAP(33)+) applied at the same rate (30 kg P ha^{-1}). In Trial 1, significantly more K was absorbed with the MAP(33)+ treatment than the MAP(33) treatment, while in Trial 2, there were no significant differences between the MAP(33)+ and MAP(33) treatments (Figure 6.3).

6.3.1 Calcium

Calcium concentration of the dried leaf matter results are presented in Table 6.4. In this study wheat plants had Ca concentrations of 0.3-0.5% (Table 6.4) and these levels

suggest that the plants in the trials were deficient in Ca despite the fact that nitrophosphate contains Ca. Mature dried leaf matter of plants containing 1-4% of Ca is regarded as sufficient, whereas concentrations below 0.5% are regarded as deficient and concentrations above 5% as toxic (Kalra 1998).

Table 6.4 Calcium concentration of leaf dry matter of potted wheat fertilised with nitrophosphate and MAP(33)

			Phosphorus applied (kg ha^{-1})			
	Fertiliser	Element	0	15	30	45
Trail 1	Nitrophosphate	Ca%	0.36^{a}	0.35^{a}	0.35^{a}	0.35^{a}
	MAP(33)		0.37^{a}	0.35^{a}	0.33^{a}	0.36^{a}
	MAP(33)+				0.36^{a}	
Trial 2	Nitrophosphate	Ca%	0.39^{b}	0.34^{ab}	0.34^{ab}	0.34^{ab}
	MAP(33)		0.49^{c}	0.34^{ab}	0.33^{a}	0.30^{a}
	MAP(33)+				0.30^{a}	

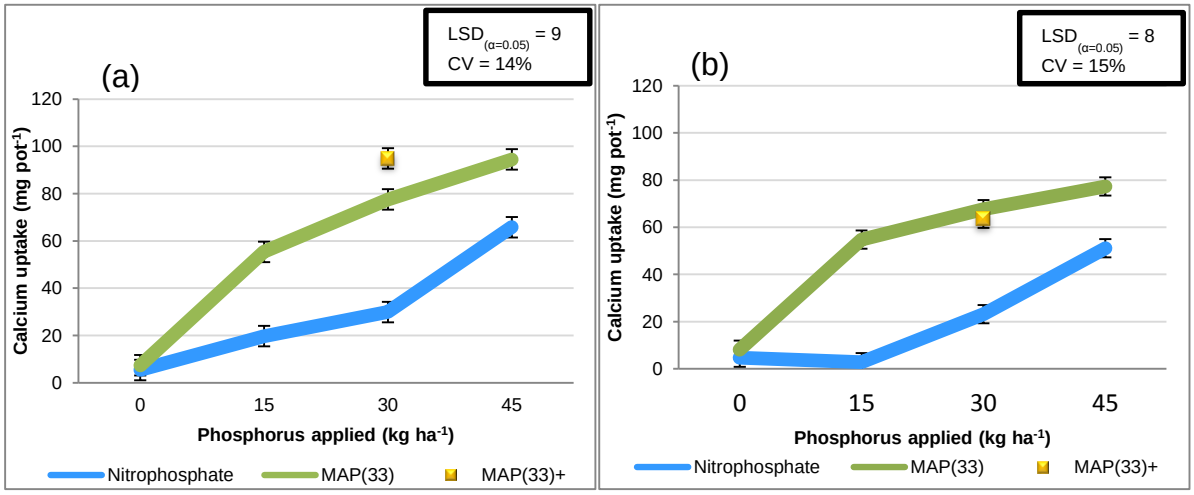
Trial 1: $LSD_{(\alpha\text{-}0.05)}$ = 0.047; CV = 10%
Trial 2: $LSD_{(\alpha\text{-}0.05)}$ = 0.061; CV = 13%
*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

In Trial 1, no significant differences were detected in the Ca concentration of the dried leaf matter for both fertiliser treatments (Table 6.4). While in Trial 2, the controls tended to have higher Ca concentrations than the fertilised wheat (Table 6.4). There were also no statistical differences between MAP(33) applied at 30 kg P ha^{-1} and MAP(33)+ applied at 30 kg P ha^{-1}. A possible reason could be that Ca (22 kg ha^{-1}) was added in small quantities to influence Ca concentration in the leaf tissue.

The additional amounts of Ca applied to the soil, with the nitrophosphate and MAP(33)+, treatments were relatively small. The optimum level of Ca required in the soil for grain crops ranges from 300-2000 mg kg^{-1} (FSSA 2007) and the Ca concentration of the soil used in this study was 79 mg kg^{-1} (Table 3.1). Therefore, a maximum of 33 kg Ca ha^{-1} was added when nitrophosphate was applied at 45 kg P ha^{-1}, which translates to 7.93 mg kg^{-1} of Ca that was supplemented to the soil. In the case of MAP(33)+ 22 kg Ca ha^{-1} was applied which translated to 5.29 mg kg^{-1}. Theoretically the Ca concentration of the soil would then increase to 87 mg kg^{-1} for nitrophosphate and 84 mg kg^{-1} for MAP(33)+, which was still too low for optimum

growth and hence plants did not respond to the Ca addition of the nitrophosphate and MAP(33)+.

Calcium uptake results are presented in Figure 6.4, which shows the influence of nitrophosphate and MAP(33) on Ca uptake compared at different application rates. Calcium uptake was significantly affected by the interaction between fertiliser type and application rate. Increasing the application rate of both fertilisers increased the Ca uptake in both trials (Figure 6.4). But the overall trend indicated that the initial Ca uptake was more pronounced for the MAP(33) treatments, that consistently resulted in a higher Ca uptake, than the nitrophosphate treatments (Figure 6.4).



*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 6.4 Increase in calcium uptake ($LSD_{(0.05)}$ presented as bars) with nitrophosphate and MAP(33) application for trial 1(a) and trial 2(b)

Higher Ca uptake for the MAP(33) treatment was due to the higher biomass production (Chapter 5) that increased the demand of nutrients. Inconsistent results were found between MAP(33) applied at 30 kg P ha^{-1} and MAP(33) with secondary nutrients (MAP(33)+) applied at 30 kg P ha^{-1}. In Trial 1, MAP(33)+ resulted in a significantly higher Ca uptake than the MAP(33), while in Trial 2, there were no significant differences between MAP(33)+ and MAP(33) (Figure 6.4).

6.3.2 Magnesium

The results of the effect of nitrophosphate and MAP(33) on the Mg concentration of the dried leaf matter compared at different application rates are presented in Table

6.5. The dry leaf matter of a mature plant, which is sufficient in Mg normally contains 0.25-1.00% Mg. If the dry leaf matter contains less than 0.20% it is regarded as deficient and Mg concentrations above 1.50% as toxic (Kalra 1998). In this study the Mg concentration of the dry leaf matter ranged from 0.15-0.45% (Table 6.5), which indicates that some treatments may have had Mg deficiency.

Table 6.5 Magnesium concentration of leaf dry matter of potted wheat fertilised with nitrophosphate and MAP(33)

			Phosphorus applied (kg ha^{-1})			
	Fertiliser	Element	0	15	30	45
Trail 1	Nitrophosphate	Mg%	0.20^{a}	0.19^{a}	0.19^{a}	0.19^{a}
	MAP(33)		0.39^{b}	0.37^{b}	0.40^{b}	0.45^{b}
	MAP(33)+				0.42^{b}	
Trial 2	Nitrophosphate	Mg%	0.20^{a}	0.18^{c}	0.16^{a}	0.15^{a}
	MAP(33)		0.22^{a}	0.15^{a}	0.17^{a}	0.16^{a}
	MAP(33)+				0.16^{a}	

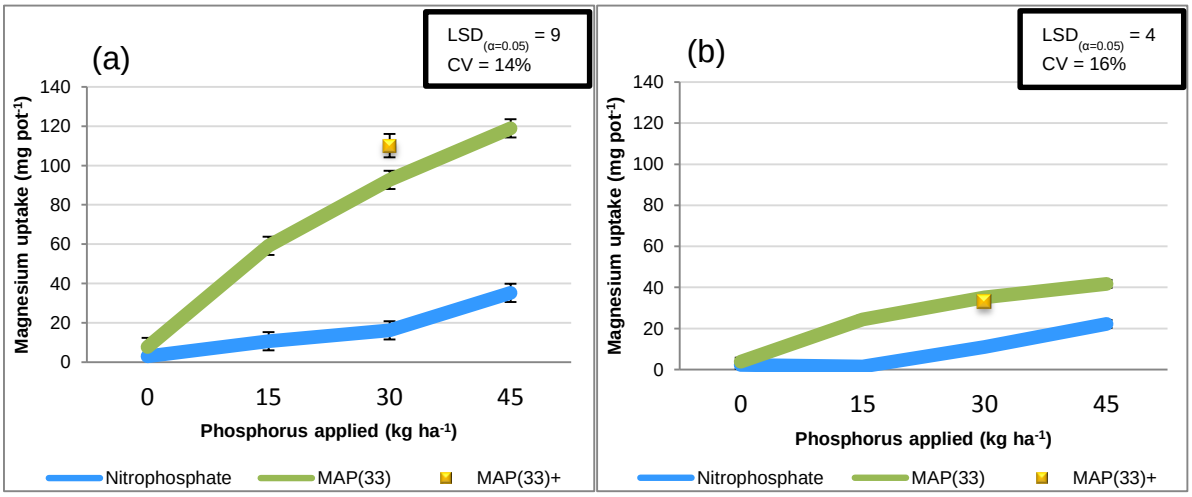
Trial 1: $LSD_{(\alpha\text{-}0.05)}$ = 0.049; CV = 12%
Trial 2: $LSD_{(\alpha\text{-}0.05)}$ = 0.017; CV = 8%
*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

There were no significant differences in the Mg concentration of the dry leaf matter with increase in the P applied. However, there were differences between nitrophosphate and MAP(33) treatments (Table 6.5), which is probably due to variation in the initial Mg content of the soil.

As with Ca, it was expected that the nitrophosphate and MAP(33)+ treatments will increase the Mg concentration of dried leaf matter more than with the MAP(33) treatments, due to the presence of Mg in the nitrophosphate fertiliser. However, the additional amount of Mg supplied to the soil with the nitrophosphate and MAP(33)+ application was small. The optimum concentration of Mg required in the soil for grain crops ranges from 80-300 mg kg^{-1} (FSSA 2007). However, the Mg concentration of the soil used in this study was 28 mg kg^{-1} (Table 3.1). Only 2.3 kg Mg ha^{-1} was added when nitrophosphate was applied at 45 kg P ha^{-1} and 1.5 kg Mg ha^{-1} when MAP(33)+ was applied. This translates to 0.55 mg kg^{-1} Mg for nitrophosphate and 0.36 mg kg^{-1} Mg for MAP(33)+ that was added to the soil. Theoretically the Mg concentration of the soil should increase to 28.55 mg kg^{-1} for nitrophosphate and 28.36 mg kg^{-1} for

MAP(33)+. This increase in the soil Mg concentrations was not enough for the significant uptake of Mg (Table 3.1).

Magnesium uptake results showing the influence of nitrophosphate and MAP(33) on Mg uptake applied at different rates are presented in Figure 6.5.



*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 6.5 Increase in magnesium uptake ($LSD_{(0.05)}$ presented as bars) with nitrophosphate and MAP(33) application for trial 1(a) and trial 2(b)

Magnesium uptake was significantly affected by the interaction between fertiliser type and application rate. Increasing the application rate generally increased Mg uptake for both fertilisers. Magnesium uptake increased with increase in P application rate for both fertilisers in both trials with the highest Mg uptake recorded for the MAP(33) applied at 45 kg P ha^{-1} (Figure 6.5). Bigger plants have higher demand for water and nutrients and this could have resulted in Mg uptake (Wilkinson et al. 1998).

The overall trend indicated that Mg uptake was more pronounced for the MAP(33) treatments and it consistently resulted in more Mg uptake than that for the nitrophosphate treatments (Figure 6.5). This was due to the fact that more biomass was produced with MAP(33) application than with the nitrophosphate application (Chapter 5) that resulted in an increase in the demand and absorption of Mg for the MAP(33) treatments.

Inconsistent results were found between MAP(33) applied at 30 kg P ha^{-1} and MAP(33)+ applied at 30 kg P ha^{-1}. In Trial 1, MAP(33)+ resulted in more Mg absorption than with the MAP(33) treatment. However, in Trial 2, there were no significant differences between MAP(33)+ and MAP(33) treatments (Figure 6.4).

6.3.3 Sulphur

The S concentration of the dry leaf matter for all treatments ranged between 0.26-0.43% (Table 6.6), which fall in the sufficient range of 0.25-1.00% (Kalra 1998). Sulphur concentration of the dry leaf matter was significantly affected by the interaction between fertiliser type and application rate. For MAP(33), the S concentration of the dry leaf matter generally decreased with an increase in rate for both trials (Table 6.6). For the nitrophosphate, no clear trends and inconsistent results were found. In Trial 1, the nitrophosphate applied at 15 and 30 kg P ha^{-1} treatments resulted in S concentration of the dry leaf matter significantly higher than the control and 45 kg P ha^{-1} treatments (Table 6.6) while no significant differences were found in Trial 2.

Table 6.6 Sulphur concentration of leaf dry matter of potted wheat fertilised with nitrophosphate and MAP(33)

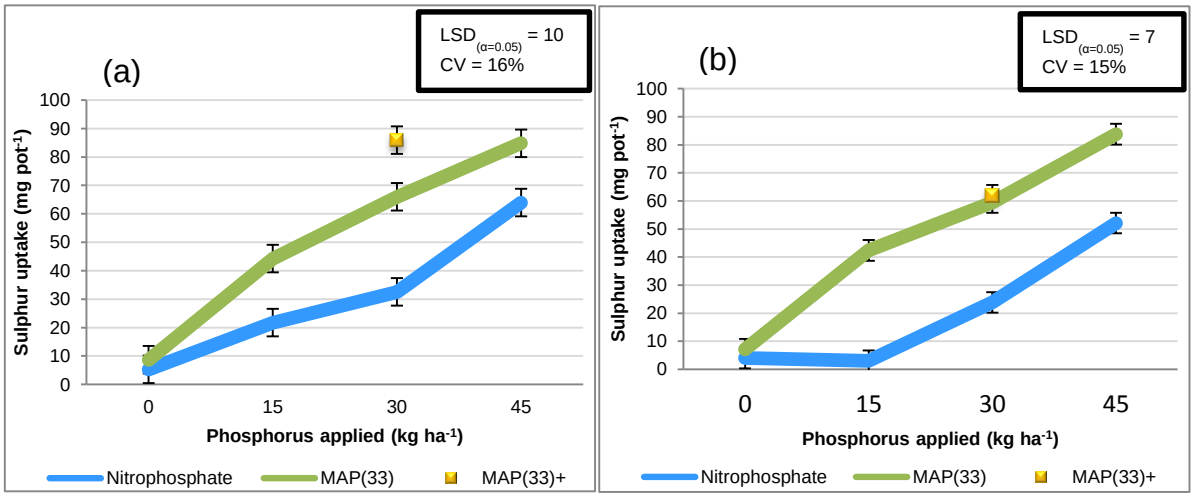
			Phosphorus applied (kg ha^{-1})			
	Fertiliser	Element	0	15	30	45
Trail 1	Nitrophosphate	S%	0.34^{b}	0.39^{c}	0.38^{c}	0.34^{b}
	MAP(33)		0.43^{d}	0.28^{a}	0.28^{a}	0.32^{b}
	MAP(33)+				0.33^{b}	
Trial 2	Nitrophosphate	S%	0.36^{c}	0.34^{c}	0.34^{c}	0.35^{c}
	MAP(33)		0.43^{d}	0.26^{a}	0.29^{ab}	0.33^{bc}
	MAP(33)+				0.29^{ab}	

Trial 1: $LSD_{(\alpha\text{-}0.05)}$ = 0.034; CV = 8%
Trial 2: $LSD_{(\alpha\text{-}0.05)}$ = 0.038; CV = 9%
*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

When comparing the two fertilisers, the dried leaf matter tend to have a higher S concentration for the nitrophosphate treatments than the MAP(33) treatments for both trials. This was probably due to the dilution effect, where more biomass is produced by the MAP(33) treated wheat plants (Chapter 5) that resulted in lower nutrient concentration, while containing higher nutrient amounts.

Inconsistent results were found between MAP(33) applied at 30 kg P ha^{-1} and MAP(33)+ applied at 30 kg P ha^{-1}. In Trial 1, the MAP(33)+ treatment resulted in significantly higher S concentration of the dry leaf matter than the MAP(33) applied at 30 kg P ha^{-1} treatment, while in Trial 2, there were no significant differences between the MAP(33)+ and MAP(33) treatments (Table 6.6).

Sulphur uptake was significantly affected by the interaction between fertiliser type and application rate (Appendix). Increasing the application rate of both fertilisers increased the S uptake in both trials. However, the increase was more pronounced for the MAP(33) treatments. This could be explained by the differences in the soil pH between the MAP(33) and nitrophosphate treatments (Chapter 4) that influenced root development. The MAP(33) treatments had higher soil pH while that of nitrophosphate was lower. This resulted in more root growth that could absorb more S. MAP(33) treatments also resulted in a higher biomass production than the nitrophosphate treatments (Chapter 5) that facilitated more S uptake due to the higher demand.



*MAP(33)+: MAP(33) applied at 30 kg P ha^{-1} that includes secondary nutrients (S = 19.2 kg ha^{-1}, Ca = 22.0 kg ha^{-1}, Mg = 1.5 kg ha^{-1})

Figure 6.6 Increase in sulphur uptake ($LSD_{(0.05)}$ presented as bars) with nitrophosphate and MAP(33) application for trial 1(a) and trial 2(b)

It is further postulated that the low soil pH caused by the nitrophosphate treatments (Chapter 4) impaired root growth (Mengel and Kirkby 2001) and hence had a detrimental effect on S uptake. This study concurs with the findings of Kerridge (1969) who found smaller root length at pH 4.0 than at pH 5.0. When root growth is impaired,

nutrient acquisition and water access are significantly reduced (Pinkerton and Simpson 1986) and this limit the plant growth rate.

In Trial 1, the MAP(33)+ treatment had a significantly higher S uptake than the MAP(33) treatment, while in Trial 2, there were no significant differences between the MAP(33)+ and MAP(33) treatments (Figure 6.6).

6.4 Micronutrients

6.4.1 Concentration

Wheat leaf dry matter Cu, Zn, Fe and B concentrations were generally lower for the nitrophosphate applied at 45 kg P ha^{-1}, while the highest concentrations were measured for the control treatments in both trials (Table 6.7 and Table 6.8).

Inconsistent results were found for the Mo concentration of the dried leaf matter for the nitrophosphate and MAP(33) treatment. In Trial 1, nitrophosphate treatment did not significantly affect the Mo concentration of the dried leaf matter, while in Trial 2, the control treatment had significantly higher Mo concentration in the dried leaf matter than the rest of the treatments. The higher micronutrient content in the control treatment can be explained by the dilution effect, where larger plants result in lower nutrient concentration while containing higher nutrient amounts. Wheat growth results showed that the control treatments produced less biomass than the 45 kg P ha^{-1} treatments that produced the highest biomass. Another factor that needs to be taken into account is the possibility that Cu, Zn, Fe and Mn cations can co-precipitate as phosphates metal complexes, rendering them less plant available. There was no significant differences between the Map(33)+ and MAP(33) treatments.

Table 6.7 Copper, manganese, zinc and iron concentrations of wheat leaf dry matter planted to a pot fertilised with nitrophosphate and MAP(33)

			Phosphorus applied (kg ha^{-1})					Phosphorus applied (kg ha^{-1})					Phosphorus applied (kg ha^{-1})					Phosphorus applied (kg ha^{-1})			
	Fertiliser	Element	0	15	30	45	Element	0	15	30	45	Element	0	15	30	45	Element	0	15	30	45
Trial 1	Nitrophosphate		5.8^{bc}	6.6^{cd}	6.6^{cd}	5.4^{ab}		249.4^{e}	194.8^{bc}	209.4^{cd}	212.6^{cd}		96.0^{c}	91.2^{c}	90.4^{c}	76.2^{b}		326.4^{d}	333.2^{d}	286.8^{cd}	253.4^{abc}
	MAP(33)	Cu (mg kg^{-1})	7.2^{d}	4.8^{a}	5.2^{ab}	5.6^{ab}	Mn (mg kg^{-1})	226.6^{de}	162.8^{a}	167.0^{a}	179.2^{ab}	Zn (mg kg^{-1})	116.8^{d}	65.8^{ab}	58.6^{a}	63.8^{ab}	Fe (mg kg^{-1})	339.6^{d}	210.2^{a}	221.4^{ab}	240.4^{abc}
	MAP(33)+				5.4^{ab}					177.2^{ab}					62.2^{a}					271.0^{bc}	
Trial 2	Nitrophosphate		6.4^{cd}	5.8^{bc}	5.6^{bc}	5.4^{b}		253.0^{d}	214.6^{c}	200.8^{c}	189.4^{bc}		101.8^{d}	89.6^{c}	79.2^{b}	67.6^{a}		351.2^{c}	287.2^{b}	288.4^{b}	257.8^{ab}
	MAP(33)	Cu (mg kg^{-1})	6.8^{d}	4.2^{a}	4.4^{a}	4.4^{a}	Mn (mg kg^{-1})	288.4^{e}	154.2^{a}	164.8^{ab}	155.4^{a}	Zn (mg kg^{-1})	117.2^{e}	67.4^{a}	64.4^{a}	64.0^{a}	Fe (mg kg^{-1})	426.2^{d}	249.8^{ab}	263.8^{ab}	278.2^{ab}
	MAP(33)+				4.2^{a}					166.2^{ab}					63.8^{a}					231.0^{a}	

Table 6.8 Boron and molybdenum concentrations of leaf dry matter of potted wheat fertilised with nitrophosphate and MAP(33)

			Phosphorus applied (kg ha^{-1})					Phosphorus applied (kg ha^{-1})			
	Fertiliser	Element	0	15	30	45	Element	0	15	30	45
Trial 1	Nitrophosphate		8.6^{b}	5.8^{a}	7.0^{ab}	5.6^{a}		3.41^{b}	3.72^{b}	2.98^{b}	3.62^{b}
	MAP(33)	B (mg kg^{-1})	8.4^{b}	5.4^{a}	5.4^{a}	6.2^{a}	Mo (mg kg^{-1})	3.69^{b}	3.28^{b}	2.85^{ab}	0.81^{a}
	MAP(33)+				6.4^{a}					3.63^{b}	
Trial 2	Nitrophosphate		10.2^{d}	7.8^{c}	7.8^{c}	7.4^{c}		2.89^{de}	2.42^{cd}	2.10^{bc}	2.22^{bc}
	MAP(33)	B (mg kg^{-1})	15.4^{d}	6.4^{bc}	4.4^{ab}	3.8^{a}	Mo (mg kg^{-1})	3.34^{e}	2.00^{bc}	1.19^{a}	1.71^{ab}
	MAP(33)+				4.0^{a}					1.27^{a}	

When comparing nitrophosphate and MAP(33) fertilisers, Mn and Zn concentrations of the dried leaf matter were significantly higher for the nitrophosphate than the MAP(33) treatment. This could also be explained in terms of the dilution effect where larger plants result in a lower nutrient concentration while containing higher nutrient amounts. In Chapter 5, the biomass production results showed that nitrophosphate produced significantly lower biomass (root and leaf) than the MAP(33) fertiliser.

6.4.2 Uptake

Micronutrient uptake was calculated as the product of micronutrient concentration in the dried leaves and dry leaf mass. In Figure 6.7 and Figure 6.8 results on the influence of nitrophosphate and MAP(33) on Cu, Mn, Zn, Fe, B and Mo uptake at different application rates are presented. Micronutrient uptake was significantly affected by the interaction between fertiliser type and application rate. Increasing the application rate generally increased Cu, Mn, Zn and Fe uptake for both fertilisers with the highest Cu, Mn, Zn and Fe uptakes recorded for the MAP(33) applied at 45 kg P ha^{-1} (Figure 6.7 and Figure 6.8). Boron (B) and Mo uptakes also increased with P application rate except for the MAP(33) applied at 45 kg P ha^{-1} (Figure 6.8).

The uptake of Cu, Mn, Zn and Fe were more pronounced with the MAP(33) treatments. It consistently resulted in higher Cu, Mn, Zn and Fe uptake than nitrophosphate for all application rates in both trials (Figure 6.7 and Figure 6.8). This was due to the increase in biomass production for the MAP(33) treatments (Chapter 5) that increased the demand for the nutrients more than the wheat plants treated with nitrophosphate. Increased uptake of micronutrients with the application rate could also be explained in terms of biomass because root and leaf growth increased when application rate was increased (Chapter 5).

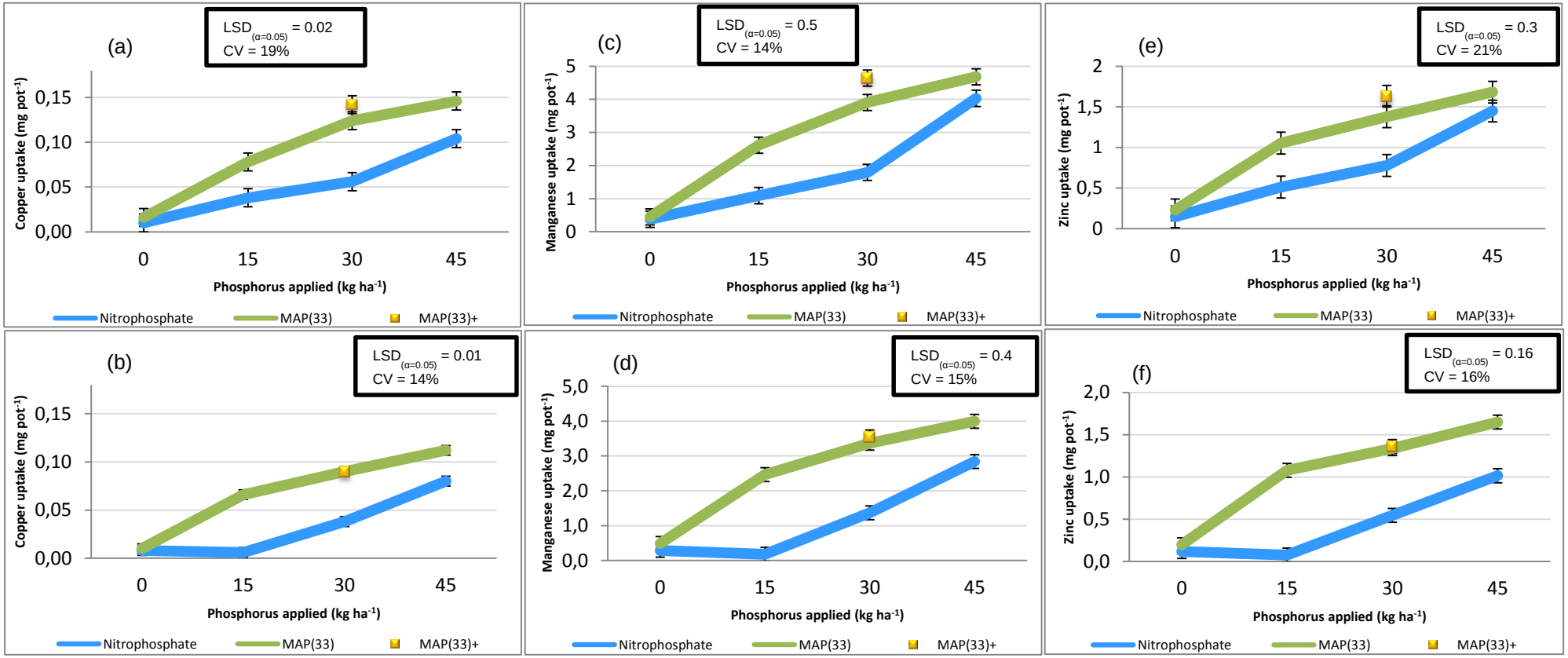


Figure 6.7 Increase in **c**opper (a and b), manganese (c and d) and zinc (e and f) uptake ($LSD_{(0.05)}$ presented as bars) of potted wheat fertilised with nitrophosphate and MAP(33)

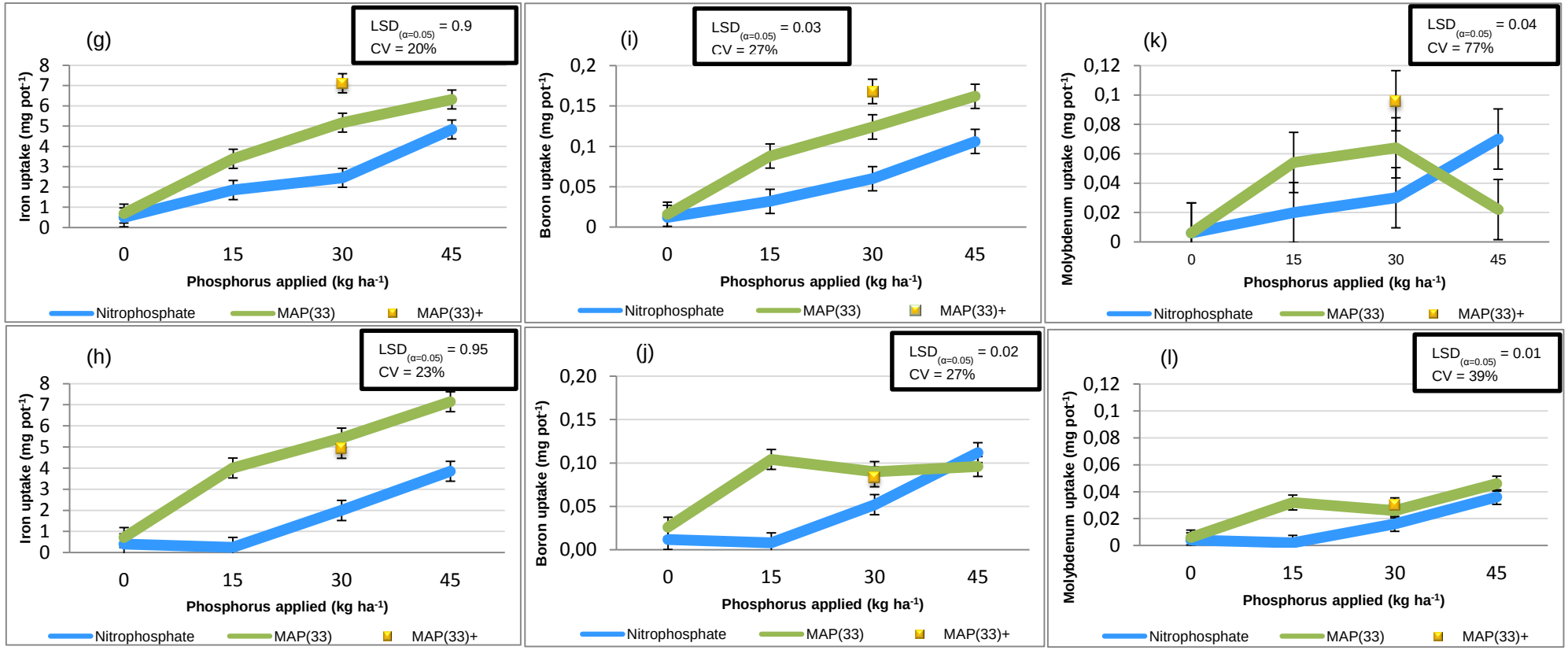


Figure 6.8 Increase in iron (g and h), boron (i and j) and molybdenum (k and l) uptake (LSD$_{(0.05)}$ presented as bars) of potted wheat fertilised with nitrophosphate and MAP(33)

Inconsistent results were found between MAP(33) applied at 30 kg P ha^{-1} and MAP(33)+ applied at 30 kg P ha^{-1}. In Trial 1 the MAP(33)+ treatment resulted in a significantly higher uptake of Cu, Mn, Zn, Fe, B and Mo than the MAP(33) treatment, while in Trial 2 there were no significant differences between MAP(33)+ and MAP(33) (Figure 6.7 and Figure 6.8). This was the same trend observed for the production of dry leaf mass (Figure 5.5) which confirms that micronutrient uptake was largely influenced by the biomass.

6.5 Conclusion

Wheat treated with MAP(33) resulted in a higher uptake of N, P, K, Ca, Mg, S, Cu, Mn, Zn and Fe than when nitrophosphate was applied. This was due to the differences in the soil pH between nitrophosphate and MAP(33) treatments that influenced root development. MAP(33) treatments increased the soils pH than higher than nitrophosphate, hence this resulted in more root growth and absorption of more nutrients. It is postulated that the nitrophosphate impaired root growth as a result of the low soil pH. MAP(33) produced more biomass than the nitrophosphate treatments which increased the nutrient uptake due to a higher demand. Inconsistent results were found between MAP(33) and MAP(33)+ applied at 30 kg P ha^{-1} for the macro- and micronutrient uptake.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Plant nutrition affects the productivity of wheat and to increase yields, the correct type of fertilisers and amount, taking into account the specific soil conditions, needs to be applied. However, in sandy soils it became a common practice to use large quantities of reduced nitrogen (N) such as urea, ammonia and ammonium, as pre-plant fertilisers for growing maize. This is increasingly causing wide spread problems, such as subsoil acidity in the areas of Free State, Mpumalanga and North West. Farmers blend MAP(33) and urea as pre-plant fertilisers for application in the top 20 cm of the soil (Bornman 2013). Nitrophosphate was therefore suggested as an alternative phosphate fertiliser to remedy the problems of low soil P, soil acidity and replace lost basic cations (Ca, Mg). The problem being that MAP(33), a source of P, which is pre-applied together with urea is believed to be resulting in soil acidification, accumulation of NH_4^+ and loss of cations. A study was therefore conducted with the aim to compare nitrophosphate with MAP(33) when used as pre-plant fertilisers in acidic sandy soils commonly found in commercial agriculture. A scenario was simulated where the acid forming fertilisers were applied to an already acid soil which received no liming to ameliorate the soil pH. To meet the objectives of the study a greenhouse experiment was conducted where the biomass, residual nutrient status and nutrient uptake of potted wheat plants fertilised with nitrophosphate and MAP(33) was compared.

Results from soil analyses sampled after harvest indicated that nitrophosphate and MAP(33) had a significant effect on the residual soil pH, P, S and Mg content of the soil. Soil treated with an MAP(33) and urea mix increased the soil pH while nitrophosphate resulted in lower soil pH. Lower soil pH could be explained by the hydrolysis of mono-calcium phosphate in nitrophosphate to dicalcium phosphate and phosphoric acid (Lindsay 1979).

$$Ca(H_2PO_4)_2.H_2O \rightleftharpoons CaHPO_4.H_2O + H_3PO_4 \qquad (7.1)$$

Phosphoric acid is released and has the potential to lower the soil pH. MAP(33) treatments and nitrophosphate applied at 45 kg P ha^{-1} increased the soil pH, due to

vigorous plant growth that produced higher biomass than the 15 and 30 kg P ha^{-1} nitrophopshate treatments. This caused a greater uptake of the reduced N, which is responsible for acidifying the soil when not utilised by plants (FSSA 2007).

Soil pH increased with MAP(33) application from the lowest (15 kg P ha^{-1}) to the highest (45 kg P ha^{-1}) application rate. This was despite the fact that MAP(33) is regarded as an acidifying fertiliser (FSSA 2007), because it contains NH_4^+ that decreases the soil pH during nitrification. However, nitrification often occurs when the NH_4^+ is not taken up by the plant (Bornman 2013). In this study wheat treated with MAP(33) absorbed most N (Chapter 6), possibly as NH_4^+. It is therefore postulated that the oxidation of NH_4^+ to nitric acid (HNO_3) was relatively low. More biomass was produced by wheat treated with MAP(33) than nitrophosphate that resulted in a higher absorption of nutrients including N. The gradual increase in soil pH for the MAP(33) treatments from the lowest to the highest application rate was proportional to the biomass produced when MAP(33) was applied at 15 to 45 kg P ha^{-1}, suggesting that more NH_4^+ was taken up as plant size increased. Furthermore, the pH increase for MAP(33) treatments and nitrophosphate applied at 45 kg P ha^{-1} treatment could be explained by the exudation of hydroxyl (OH^-) or hydrogen carbonate (HCO_3^-) ions from the roots when anions, such as HPO_4^- are taken up by the plants (Mengel and Kirkby 2001). These anions (OH^-, HCO_3^-) could raise the pH of the rhizosphere by up to 1 unit (Whitehead 2000; Rengel 2015).

In terms of P and S, nitrophosphate fertilised wheat resulted in higher soil residual P and S than MAP(33) fertilised wheat. Wheat fertilised with nitrophosphate had less developed roots due to the increase in soil acidity that caused less nutrient absorption, which could explain the large amount of P and S that remained in the soil not utilised. In contrast, wheat fertilised with MAP(33) had better root growth that explored a greater soil volume resulting in more P and S uptake leaving less in the soil.

Wheat growth responded significantly to the application of nitrophosphate and MAP(33). MAP(33) fertilised wheat consistently resulted in a higher chlorophyll content than nitrophosphate except at 2 and 3 weeks after emergence (WAE). It increased the chlorophyll content by up to 11% compared to nitrophosphate and this was due to the fact that wheat fertilised with MAP(33) developed a larger root system which absorbed higher amounts of nutrients including N, Mg and S which are critical for chlorophyll

synthesis. Nitrogen (Guler 2009), Mg (Barton 1970) and S together with Ca, Mn and Zn (Mengel and Kirkby 1987) are known to be associated with chlorophyll synthesis and are highly correlated with the chlorophyll content (Shaahan et al. 1999; Guler 2009). Increasing the P application rate also increased the chlorophyll content of wheat for both fertilisers, but the increment was more pronounced for the MAP(33) treatments. The increase in chlorophyll with MAP(33) treatment possibly increased photosynthesis that influenced biomass production. MAP(33) resulted in a higher chlorophyll content, because more P was taken up and the plants had less stress due to less damage to their root system.

MAP(33) fertilised wheat had 108% (first trial) and 105% (second trial) more root growth and, 96% (first trial) and 167% (second trial) more leaf growth compared to nitrophosphate fertilised wheat. It was also interesting to note that root growth was well correlated ($R^2 = 0.81$) with soil pH. The low soil pH caused by the phosphoric acid released, when nitrophosphate hydrolyses, resulted in impaired root growth and adversely affected nutrient uptake. Secondary nutrients in nitrophosphate therefore did not improve wheat growth because their effects were overridden by the lower soil pH. On the other hand MAP(33) fertilised wheat had more root growth, which explored greater soil volume and absorbed more nutrients and water. Furthermore the differences in the inherent and soluble P of the fertilisers probably influenced the results. Analysis of fertilisers (Table 3.2) showed that MAP(33) had higher citric soluble P (21%) than nitrophosphate (7.6%). Studies have shown that fertiliser solubility affects crop growth and work by Meelu et al. (1977) and Hundal et al. (1979) established that the response of wheat to P was directly related to the solubility of the fertiliser source when evaluating diammonium phosphate, urea ammonium phosphate, suphala (30% WSP), nitrophosphate (50% WAP) and SSP.

Nutrient uptake expressed as mg pot^{-1} was calculated as the product between the specific nutrient concentration in the dry leaf matter and the dry leaf mass. Nutrient uptake was significantly affected by the interaction between fertiliser and application rate. The highest nutrient uptake (S, N, P, K, Ca, Mg, Na, Cu, Mn, Zn and Fe), resulted from fertilising the wheat with 45 kg P ha^{-1} MAP(33). The uptake of the nutrients was largely influenced by biomass. Inconsistent results were obtained for B and Mo uptake

for both the first and second trial and therefore no concrete conclusions were drawn from these elements.

Nitrophosphate resulted in higher concentrations of S, N, K, Cu, Mn, Zn, Fe and Mo in the dry leaf matter than the MAP(33) treatment. This could be due to smaller plants, which has less biomass, have a higher nutrient concentration although the nutrient uptake was less than larger plants (dilution effect). However, P and Mg concentrations as well as nutrient uptake were significantly higher in MAP(33) fertilised wheat. It was deduced that MAP(33) increased the nutrient uptake as a result of greater root growth. A well-developed root system explores a greater soil volume for the absorption of water and mineral nutrients. Nitrophosphate did not take up more nutrients due to impaired root growth which was attributed to the low soil pH.

The results of this study suggests nitrophosphate is not the preferable fertiliser to substitute MAP(33) as a pre-plant fertiliser in soils with a low pH. The release and dissociation of phosphoric acid caused the pH of the soil to decrease and this overrides the benefits of NO_3^- and secondary nutrients contained in nitrophosphate. However, under less acidic soil conditions (pH 6.5) nitrophosphate has been found to be superior to MAP(33) (Mlalazi et al. 2013). Testing nitrophosphate and MAP(33) on Swiss chard in the near neutral soil, there was a significant difference ($p = 0.000292$) in dry mass yield for P source and nitrophosphate showed a mean relative yield of 199% over MAP(33) (Roberts et al. 2014). This implies more research should be done on different soils to safely conclude on which fertiliser is more effective. It is also recommended an incubation study to test the fertilisers in the soil without interference from growing plants. Use of a different N source other than urea to balance the N for treatments is recommended.

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APPENDIX

ANOVA table for soil analysis, growth, chlorophyll concentration and nutrient uptake of wheat fertilised with nitrophosphate and MAP(33).

Table 1 Comparison of Trial 1 versus Trial 2 based on the quality of wheat

		Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Fresh Root Volume (ml)	Trial 1 Mean	16.00	40.00	62.00	142.0	21.00	112.0	160.0	160.0	152.0
	Trial 2 Mean	14.00	22.00	70.00	88.00	14.00	68.00	60.00	92.00	112.50
	p-value	0.4714	0.0007	0.2623	0.0000	0.0528	0.0001	0.0000	0.0000	0.0007
Fresh Leaf Mass (g)	Trial 1 Mean	5.184	23.77	37.83	79.19	7.568	66.25	98.66	114.9	112.5
	Trial 2 Mean	6.378	16.00	42.61	74.32	5.340	73.13	94.02	119.2	99.40
	p-value	0.3080	0.0144	0.5500	0.4308	0.0022	0.3286	0.3953	0.6200	0.0178
Dry Root Mass (g)	Trial 1 Mean	1.096	2.978	4.870	10.21	1.624	10.11	14.11	14.02	15.61
	Trial 2 Mean	1.220	2.064	4.968	9.420	1.070	9.04	12.18	13.87	13.05
	p-value	0.5921	0.1409	0.9234	0.5718	0.0805	0.6151	0.1050	0.9297	0.2391
Dry Leaf Mass (g)	Trial 1 Mean	1.546	5.606	8.638	18.85	1.996	16.15	23.50	26.10	26.21
	Trial 2 Mean	1.128	0.890	6.990	15.11	1.680	16.02	20.69	25.76	21.39
	p-value	0.2363	0.0000	0.3394	0.0568	0.0237	0.9370	0.1400	0.7734	0.0008

Leaf Chlorophyll

Table 2 Comparison of Trial 1 versus Trial 2 based on the leaf chlorophyll of wheat

		Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
2 WAE	Trial 1 Mean	30.26	32.12	36.56	34.50	24.32	34.18	31.58	30.72	34.30
	Trial 2 Mean	32.22	35.48	38.42	43.10	32.62	43.16	44.14	45.14	46.90
	p-value	0.0809	0.0001	0.0076	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3 WAE	Trial 1 Mean	35.34	41.16	41.88	42.84	35.12	42.52	43.18	44.64	46.64
	Trial 2 Mean	34.66	37.74	40.58	44.42	33.90	44.14	45.10	46.08	49.34
	p-value	0.4368	0.0022	0.5988	0.1991	0.3758	0.0938	0.1035	0.0304	0.0419
4 WAE	Trial 1 Mean	36.82	42.86	44.30	46.78	36.96	44.30	46.74	49.62	50.76

	Trial 2 Mean	35.42	39.54	42.50	45.88	35.62	45.00	46.52	47.40	50.10
	p-value	0.1211	0.0584	0.3571	0.1959	0.1267	0.1966	0.7783	0.0489	0.5839
5 WAE	Trial 1 Mean	37.38	43.74	45.28	47.30	37.54	45.88	47.34	50.12	50.70
	Trial 2 Mean	37.00	42.90	43.92	46.24	36.66	46.32	47.62	48.72	51.30
	p-value	0.5077	0.2851	0.4315	0.1125	0.2456	0.6109	0.7317	0.1260	0.5905
6 WAE	Trial 1 Mean	40.26	44.92	46.92	48.66	38.96	47.22	49.08	50.88	51.66
	Trial 2 Mean	39.72	44.48	45.68	47.30	38.18	47.40	48.72	49.62	52.98
	p-value	0.6258	0.6815	0.3740	0.0383	0.3563	0.7704	0.7065	0.1095	0.2161
7 WAE	Trial 1 Mean	44.62	47.30	49.52	49.66	43.38	48.78	50.38	52.46	55.38
	Trial 2 Mean	43.66	47.32	47.52	48.50	40.96	48.86	49.96	51.28	54.10
	p-value	0.2610	0.9779	0.0326	0.0958	0.0291	0.8510	0.6411	0.1736	0.0865

Top Soil Analyses

Table 3 Comparison of soil analyses between Trial 1 (reference constant) and Trial 2 (mean)

		Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Bulk density	Trial 1	1469	1504	1446	1385	1437	1438	1435	1444	1462
	Trial 2 Mean	1393.6	1372	1420	1454.6	1361.4	1475.4	1462.6	1453.8	1456.6
	p-value	0.0045	0.0020	0.3948	0.0004	0.0171	0.0123	0.0480	0.2860	0.5542
pH	Trial 1	4.9	4.8	4.9	4.9	5.0	4.9	4.9	5.2	5.0
	Trial 2 Mean	4.0	3.94	4.08	4.49	3.97	4.54	4.61	4.55	4.85
	p-value	0.0000	0.0003	0.0002	0.0071	0.0000	0.0467	0.0606	0.0009	0.3994
Acid Saturation	Trial 1	0	0	0	0	0	0	0	0	0
	Trial 2 Mean	47.26	51.26	37.22	9.38	52.92	8.09	5.14	5.94	5.21
	p-value	0.0012	0.0039	0.0173	0.1015	0.0003	0.3739	0.2023	0.1878	0.3739
S	Trial 1	14	11	15	12	13	12	11	8	10
	Trial 2 Mean	19.4	18.6	18.6	23.2	12.8	12.4	12.8	12.4	16.4
	p-value	0.0296	0.0046	0.2048	0.0076	0.8712	0.8183	0.0533	0.0173	0.0040

P	Trial 1	17	24	28	39	22	17	28	23	28
	Trial 2 Mean	22.2	30.4	40.6	44.8	24.2	21.8	25.0	22.2	26.2
	p-value	0.0082	0.0018	0.0099	0.0039	0.0858	0.0161	0.0341	0.4050	0.0533
K	Trial 1	79	66	66	49	64	48	41	45	40
	Trial 2 Mean	65.2	61.6	61.0	59.8	70.2	62.2	59.8	53.6	73.6
	p-value	0.0065	0.2318	0.1256	0.0020	0.3916	0.0078	0.0006	0.1166	0.0200
Ca	Trial 1	148	97	103	119	90	91	98	115	109
	Trial 2 Mean	99.8	90.8	104.2	125.2	95.2	122.6	124.6	126.0	137.6
	p-value	0.0073	0.1769	0.6109	0.2730	0.5513	0.0070	0.0087	0.3352	0.0183
Mg	Trial 1	40	33	34	36	33	32	35	40	36
	Trial 2 Mean	29.8	28.4	31.4	37.2	28.6	37	36.8	36.6	40.4
	p-value	0.0087	0.0377	0.0329	0.4263	0.0129	0.0204	0.2859	0.1416	0.0829
Na	Trial 1	14	15	15	20	16	14	16	17	18
	Trial 2 Mean	17	20.2	19.8	20.8	20.6	20	19.2	18.2	24.8
	p-value	0.2587	0.0629	0.1469	0.7075	0.3056	0.0520	0.4351	0.51580	0.1951
Ca / Mg	Trial 1	2.26	1.79	1.85	2.02	1.66	1.73	1.71	1.75	1.85
	Trial 2 Mean	2.03	1.96	2.03	2.05	2.02	2.02	2.06	2.09	2.08
	p-value	0.0827	0.0027	0.0038	0.4326	0.0268	0.0017	0.0000	0.0090	0.0099
(Ca+Mg)/ K	Trial 1	5.29	4.48	4.70	7.10	4.40	5.84	7.41	7.84	8.21
	Trial 2 Mean	4.43	4.37	5.02	6.09	3.99	5.77	6.09	6.83	5.64
	p-value	0.0145	0.2108	0.1957	0.0042	0.0114	0.5759	0.0077	0.0399	0.0065
ECEC	Trial 1	1.33	0.99	1.03	1.10	0.95	0.90	0.95	1.09	1.02
	Trial 2 Mean	1.92	2.06	1.78	1.30	2.13	1.30	1.23	1.22	1.39
	p-value	0.0164	0.0087	0.0438	0.0250	0.0030	0.0150	0.0013	0.1287	0.0006

Nutrient Uptake

Table 4 Comparison of Trial 1 versus Trial 2 based on the nutrient uptake of wheat

		Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
S Uptake (mg/pot)	Trial 1 Mean	5.286	21.74	32.54	63.95	8.667	44.27	66.00	84.83	85.93
	Trial 2 Mean	4.024	3.004	23.83	52.12	7.123	42.38	59.47	83.79	62.05
	p-value	0.3446	0.0000	0.1596	0.0945	0.0897	0.6667	0.0836	0.8768	0.0002
N Uptake (mg/pot)	Trial 1 Mean	38.95	220.1	319.3	545.1	61.17	459.6	655.7	735.6	711.4
	Trial 2 Mean	33.38	35.93	237.6	452.3	50.61	450.7	589.2	677.1	553.1
	p-value	0.5763	0.0000	0.0903	0.0387	0.0803	0.7383	0.1745	0.2214	0.0000
P Uptake (mg/pot)	Trial 1 Mean	1.673	8.818	13.96	30.95	3.513	25.67	42.58	57.70	50.30
	Trial 2 Mean	1.441	1.136	9.62	24.36	2.251	24.97	38.74	56.63	37.58
	p-value	0.6156	0.0001	0.1386	0.0953	0.0168	0.7623	0.3454	0.7838	0.0006
K Uptake (mg/pot)	Trial 1 Mean	45.55	185.3	292.4	574.4	70.63	465.4	634.6	685.5	744.5
	Trial 2 Mean	26.33	23.01	192.8	377.6	39.26	367.9	458.4	556.0	483.7
	p-value	0.0799	0.0000	0.1067	0.0014	0.0002	0.0627	0.0003	0.0200	0.0000
Ca Uptake (mg/pot)	Trial 1 Mean	5.414	19.68	29.92	65.81	7.352	55.36	77.56	94.48	94.89
	Trial 2 Mean	4.678	2.810	23.21	51.17	8.137	54.73	67.72	77.32	63.58
	p-value	0.6243	0.0000	0.1818	0.0604	0.3084	0.8728	0.0893	0.0221	0.0000
Mg Uptake (mg/pot)	Trial 1 Mean	3.053	10.74	16.19	35.22	7.776	59.11	92.73	118.8	110.1
	Trial 2 Mean	2.268	1.474	11.03	22.24	3.726	24.38	35.08	41.74	33.4

	p-value	0.2819	0.0000	0.0493	0.0035	0.0010	0.0000	0.0000	0.0000	0.0000
Na Uptake (mg/pot)	Trial 1 Mean	10.45	31.48	46.87	69.33	11.66	56.95	83.93	120.2	121.8
	Trial 2 Mean	10.66	6.180	42.90	82.58	14.92	89.36	106.64	128.7	114.3
	p-value	0.9614	0.0007	0.5625	0.3018	0.0319	0.0133	0.0893	0.3546	0.5157
Cu Uptake (mg/pot)	Trial 1 Mean	0.009	0.037	0.056	0.102	0.014	0.076	0.121	0.147	0.141
	Trial 2 Mean	0.008	0.005	0.038	0.080	0.011	0.067	0.090	0.113	0.090
	p-value	0.6109	0.0000	0.0421	0.0555	0.0359	0.2482	0.0030	0.0197	0.0010
Mn Uptake (mg/pot)	Trial 1 Mean	0.376	1.089	1.793	4.026	0.451	2.614	3.907	4.678	4.641
	Trial 2 Mean	0.291	0.180	1.368	2.839	0.485	2.467	3.371	3.999	3.550
	p-value	0.3103	0.0000	0.1477	0.0125	0.3529	0.5072	0.0095	0.0371	0.0018
Zn Uptake (mg/pot)	Trial 1 Mean	0.147	0.512	0.778	1.449	0.233	1.055	1.378	1.680	1.630
	Trial 2 Mean	0.118	0.078	0.543	1.014	0.197	1.077	1.337	1.648	1.362
	p-value	0.3943	0.0000	0.0945	0.0382	0.1958	0.8180	0.7791	0.8101	0.0265
Fe Uptake (mg/pot)	Trial 1 Mean	0.495	1.847	2.452	4.835	0.681	3.388	5.174	6.309	7.113
	Trial 2 Mean	0.406	0.250	1.996	3.853	0.713	4.000	5.427	7.142	4.938
	p-value	0.4483	0.0000	0.3115	0.1621	0.7128	0.1296	0.4573	0.4074	0.0007
B Uptake (mg/pot)	Trial 1 Mean	0.013	0.032	0.060	0.104	0.017	0.088	0.124	0.163	0.168
	Trial 2 Mean	0.012	0.007	0.053	0.111	0.026	0.103	0.090	0.097	0.085
	p-value	0.8890	0.0001	0.5080	0.5649	0.0276	0.4619	0.0736	0.0029	0.0004
Mo Uptake	Trial 1 Mean	0.005	0.021	0.028	0.069	0.007	0.054	0.066	0.022	0.095

(mg/pot)	Trial 2 Mean	0.004	0.002	0.015	0.033	0.006	0.032	0.025	0.044	0.028
	p-value	0.2960	0.0003	0.2072	0.0043	0.3217	0.1201	0.0218	0.0272	0.0997

Leaf Analyses

Table 5 Comparison of Trial 1 versus Trial 2 based on the leaf analyses of wheat

		Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
S%	Trial 1 Mean	0.338	0.388	0.378	0.338	0.434	0.276	0.282	0.322	0.328
	Trial 2 Mean	0.356	0.338	0.344	0.346	0.426	0.264	0.290	0.326	0.290
	p-value	0.2693	0.0119	0.0241	0.4468	0.8480	0.4230	0.6152	0.7348	0.0058
N%	Trial 1 Mean	2.494	3.928	3.772	2.904	3.056	2.896	2.798	2.818	2.714
	Trial 2 Mean	2.946	4.118	3.480	3.022	3.010	2.814	2.848	2.626	2.594
	p-value	0.0369	0.0136	0.1786	0.3661	0.7964	0.6025	0.4730	0.1784	0.1249
P%	Trial 1 Mean	0.106	0.156	0.160	0.164	0.176	0.160	0.182	0.220	0.192
	Trial 2 Mean	0.128	0.126	0.138	0.160	0.134	0.156	0.186	0.220	0.176
	p-value	0.0590	0.0141	0.0004	0.6075	0.0555	0.5716	0.6454	1.0000	0.0922
K%	Trial 1 Mean	2.884	3.296	3.360	3.046	3.540	2.888	2.710	2.608	2.842
	Trial 2 Mean	2.328	2.504	2.748	2.518	2.338	2.296	2.230	2.160	2.266
	p-value	0.0093	0.0000	0.0003	0.0001	0.0001	0.0007	0.0014	0.0000	0.0010
Ca%	Trial 1 Mean	0.360	0.352	0.350	0.348	0.368	0.348	0.332	0.362	0.362
	Trial 2 Mean	0.394	0.344	0.340	0.340	0.488	0.342	0.328	0.300	0.298
	p-value	0.5052	0.7804	0.6666	0.6776	0.0263	0.7766	0.8281	0.0168	0.0025
Mg%	Trial 1 Mean	0.198	0.192	0.190	0.186	0.392	0.370	0.396	0.454	0.420
	Trial 2 Mean	0.196	0.176	0.162	0.148	0.222	0.152	0.170	0.162	0.156
	p-value	0.8465	0.1302	0.0252	0.0012	0.0026	0.0000	0.0000	0.0000	0.0000
Na%	Trial 1 Mean	0.724	0.568	0.562	0.362	0.584	0.356	0.358	0.466	0.464
	Trial 2 Mean	0.874	0.744	0.642	0.552	0.892	0.556	0.524	0.504	0.536
	p-value	0.5062	0.1747	0.3888	0.0045	0.0015	0.0062	0.0269	0.5288	0.1541
Cu	Trial 1 Mean	5.800	6.600	6.600	5.400	7.200	4.800	5.200	5.600	5.400
	Trial 2 Mean	6.400	5.800	5.600	5.400	6.800	4.200	4.400	4.400	4.200
	p-value	0.3706	0.0353	0.0203	1.0000	0.4714	0.1950	0.1114	0.0085	0.0278
Mn	Trial 1 Mean	249.4	194.8	209.4	212.6	226.6	162.8	167.0	179.2	177.2
	Trial 2 Mean	253.0	214.6	200.8	189.4	288.4	154.2	164.8	155.4	166.2
	p-value	0.8918	0.0585	0.4078	0.0601	0.0013	0.0563	0.8258	0.0360	0.2950

Zn	Trial 1 Mean	96.00	91.20	90.40	76.20	116.8	65.80	58.60	63.80	62.20
	Trial 2 Mean	101.8	89.60	79.20	67.60	117.2	67.40	64.40	64.00	63.80
	p-value	0.3715	0.7705	0.0088	0.1940	0.9695	0.7048	0.0718	0.9180	0.6711
Fe	Trial 1 Mean	326.4	333.2	286.8	253.4	339.6	210.2	221.4	240.4	271.0
	Trial 2 Mean	351.2	287.2	288.4	257.8	426.2	249.8	263.8	278.2	231.0
	p-value	0.3578	0.2271	0.9292	0.8625	0.0569	0.0099	0.0114	0.3088	0.0091
B	Trial 1 Mean	8.600	5.800	7.000	5.600	8.400	5.400	5.400	6.200	6.400
	Trial 2 Mean	10.200	7.800	7.800	7.400	15.40	6.400	4.400	3.800	4.000
	p-value	0.2792	0.0015	0.1411	0.0130	0.0058	0.3276	0.3016	0.0019	0.0039
Mo	Trial 1 Mean	3.408	3.716	2.980	3.622	3.686	3.284	2.846	0.812	3.630
	Trial 2 Mean	2.892	2.416	2.098	2.218	3.344	2.000	1.186	1.714	1.266
	p-value	0.4841	0.0677	0.2445	0.0062	0.6890	0.0775	0.0325	0.0161	0.1318

ANALYSES B: Comparing all Nine Treatments with each other

Yield & Quality

Table 6 Effect of treatments on the yield & quality of wheat

	Fresh Root Volume (ml)		Fresh Leaf Mass (g)		Dry Root Mass (g)		Dry Leaf Mass (g)	
	First Trial	Second Trial	First Trial	Second Trial	First Trial	Second Trial	First Trial	Second Trial
Descriptive Statistics								
Total n	45	45	45	45	45	45	45	45
Mean	96.111	59.111	60.646	58.939	8.2918	7.431	14.323	12.184
Std Dev	59.044	34.250	42.554	41.326	5.8048	5.255	9.876	9.417
CV%	61.43	57.94	70.17	70.12	70.01	70.72	68.95	77.29
Means								
Treatment 1	16.000 a	14.000 a	5.1840 a	6.3780 a	1.0960 a	1.220 a	1.546 a	1.1280 a
Treatment 2	40.000 b	22.000 a	23.766 b	15.996 b	2.9780 ab	2.064 a	5.606 b	0.8900 a
Treatment 3	62.000 c	70.000 b	37.826 c	42.614 c	4.8700 b	4.968 b	8.638 c	6.9900 b
Treatment 4	142.00 e	88.000 c	79.192 e	74.324 d	10.210 c	9.420 c	18.852 d	15.108 c
Treatment 5	21.000 a	14.000 a	7.5680 a	5.3400 a	1.6240 a	1.070 a	1.9960 a	1.6800 a
Treatment 6	112.00 d	68.000 b	66.246 d	73.130 d	10.110 c	9.036 c	16.148 d	16.018 c
Treatment 7	160.00 fg	60.000 b	98.664 f	94.022 e	14.112 d	12.178 d	23.498 e	20.692 d
Treatment 8	160.00 fg	92.000 cd	114.874 g	119.25 f	14.016 d	13.874 d	26.414 e	25.760 e
Treatment 9	152.00 ef	104.00 d	112.498 g	99.402 e	15.610 d	13.048 d	26.206 e	21.392 d
ANOVA Results – Treatment Effect								
p-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
$LSD_{(\alpha=0.05)}$	12.299	13.056	12.745	8.815	2.752	2.483	2.999	2.552
$LSD_{(\alpha=0.1)}$	10.239	10.869	10.610	7.338	2.291	2.067	2.497	2.125

Means within a column with the same letter(s) are not significantly different at the 95% probability level (Fisher's LSD test);

Leaf Chlorophyll

Table 7 Effect of treatments on the leaf chlorophyll of wheat

	Leaf Chlorophyll @ week 2		Leaf Chlorophyll @ week 3		Leaf Chlorophyll @ week 4	
	First Trial	Second Trial	First Trial	Second Trial	First Trial	Second Trial
Descriptive Statistics						
Total n	45	45	45	45	45	45
Mean	32.06	40.131	41.48	41.773	44.35	43.109
Std Dev	3.514	5.420	4.328	5.209	4.992	5.166
CV%	10.96	13.50	10.43	12.47	11.26	11.98
Means						
Treatment 1	30.260 b	32.220 a	35.340 a	34.660 a	36.820 a	35.420 a
Treatment 2	32.120 c	35.480 b	41.160 b	37.740 b	42.860 b	39.540 b
Treatment 3	36.560 e	38.420 c	41.880 bc	40.580 c	44.300 bc	42.500 c
Treatment 4	34.500 d	43.100 d	42.840 bc	44.420 d	46.780 d	45.880 de
Treatment 5	24.320 a	32.620 a	35.120 a	33.900 a	36.960 a	35.620 a
Treatment 6	34.180 d	43.160 d	42.520 bc	44.140 d	44.300 c	45.000 d
Treatment 7	31.580 bc	44.140 de	43.180 bc	45.100 de	46.740 d	46.520 de
Treatment 8	30.720 b	45.140 e	44.640 cd	46.080 e	49.620 e	47.400 e
Treatment 9	34.300 d	46.900 f	46.640 d	49.340 f	50.760 e	50.100 f
ANOVA Results – Treatment Effect						
p-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
$LSD_{(\alpha=0.05)}$	1.334	1.507	3.170	1.436	2.404	1.931
$LSD_{(\alpha=0.1)}$	1.110	1.254	2.639	1.195	2.001	1.608

Means within a column with the same letter(s) are not significantly different at the 95% probability level (Fisher's LSD test).

Table 8 Effect of treatments on the leaf chlorophyll of wheat (continue)

	Leaf Chlorophyll @ week 5		Leaf Chlorophyll @ week 6		Leaf Chlorophyll @ week 7	
	First Trial	Second Trial	First Trial	Second Trial	First Trial	Second Trial
Descriptive Statistics						
Total n	45	45	45	45	45	45
Mean	45.03	44.520	46.51	46.009	49.05	48.018
Std Dev	4.864	4.864	4.472	4.599	3.680	3.863
CV%	10.80	10.93	9.62	10.00	7.50	8.04
Means						
Treatment 1	37.380 a	37.000 a	40.260 a	39.720 b	44.620 a	43.660 b
Treatment 2	43.740 b	42.900 b	44.920 b	44.480 c	47.300 b	47.320 c
Treatment 3	45.280 bc	43.920 b	46.920 bc	45.680 c	49.520 cd	47.520 cd
Treatment 4	47.300 c	46.240 c	48.660 cd	47.300 d	49.660 cd	48.500 cde
Treatment 5	37.540 a	36.660 a	38.960 a	38.180 a	43.380 a	40.960 a
Treatment 6	45.880 bc	46.320 cd	47.220 cd	47.400 d	48.780 bc	48.860 de
Treatment 7	47.340 c	47.620 de	49.080 de	48.720 de	50.380 d	49.960 ef
Treatment 8	50.120 d	48.720 e	50.880 ef	49.620 e	52.460	51.280 f
Treatment 9	50.700 d	51.300 f	51.660 f	52.980 f	55.380	54.100 g
ANOVA Results – Treatment Effect						
p-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
$LSD_{(\alpha=0.05)}$	2.270	1.309	2.133	1.529	1.528	1.467
$LSD_{(\alpha=0.1)}$	1.889	1.090	1.776	1.273	1.272	1.221

Means within a column with the same letter(s) are not significantly different at the 95% probability level (Fisher's LSD test);

Top Soil

Table 9 Effect of treatments on the top soil

	ANOVA	Treatment Means

		p-value									
		Treatment Effect (df = 8)	1 (n=5)	2 (n=5)	3 (n=5)	4 (n=5)	5 (n=5)	6 n=5)	7 n=5)	8 (n=5)	9 (n=5)
Bulk Density	Second Trial	0.0149	1393.6 abc	1372.0 ab	1420.0 bcd	1458.7 d	1361.4 a	1479.0 cd	1465.5 cd	1450.5 cd	1461.0 bcd
pH	Second Trial	0.0000	3.998 a	3.936 a	4.080 ab	4.380 c	3.966 a	4.050 ab	4.350 c	4.420 c	4.310 bc
Acid Saturation	Second Trial	0.0098	47.256 b	51.262 b	37.224 ab	15.630 a	52.918 b	40.458 ab	12.847 a	14.850 a	26.074 ab
S	Second Trial	0.0238	19.400 bc	18.600 b	18.600 b	24.667 c	12.800 a	14.000 ab	12.500 ab	15.000 ab	18.000 abc
P	Second Trial	0.0000	22.200 a	30.40 b	40.60 c	44.33 c	24.20 a	20.00 a	26.50 ab	24.00 a	27.00 ab
K	Second Trial	0.1309	65.200 bc	61.600 abc	61.000 abc	58.667 abc	70.200 c	51.000 ab	58.000 abc	50.000 a	50.000 ab
Ca	Second Trial	0.1778	99.800 a	90.800 a	104.200 ab	121.667 b	95.200 a	99.000 ab	111.500 ab	113.000 ab	111.000 ab
Mg	Second Trial	0.0392	29.800 ab	28.400 a	31.400 ab	37.000 c	28.600 ab	32.000 abc	33.500 abc	34.000 bc	33.000 abc
Na	Second Trial	0.9281	17.000	20.200	19.800	21.667	20.600	26.000	17.000	20.500	22.000
Ca/Mg	Second Trial	0.9861	2.034	1.955	2.026	2.006	2.021	1.887	2.031	2.026	2.052
(Ca+Mg)/K	Second Trial	0.0000	4.426 a	4.366 a	5.019 b	6.078 cd	3.987 a	5.806 bcd	5.610 bc	6.664 d	6.455 cd
ECEC	Second Trial	0.0825	1.916 ab	2.060 b	1.776 ab	1.373 a	2.130 b	1.681 ab	1.214 a	1.246 a	1.419 ab
Exchangeable Acidity	Second Trial	0.0498	0.932 ab	1.128 bc	0.756 abc	0.217 a	1.150 bc	0.680 abc	0.160 ac	0.185 ac	0.370 abc

Means within a column with the **same letter(s)** or **no letters** at all are **not significantly** different at the 95% probability level (Fisher's LSD test).

Nutrient Uptake

Table 10 Effect of treatments on the nutrient uptake of wheat

		ANOVA p-value	Treatment Means								
		Treatment Effect (df = 8)	1 (n=5)	2 (n=5)	3 (n=5)	4 (n=5)	5 (n=5)	6 n=5)	7 n=5)	8 (n=5)	9 (n=5)
S Uptake (mg/pot)	First Trail	0.0000	5.286 a	21.74 B	32.54 c	63.95 e	8.667 a	44.27 d	66.00 e	84.83 f	85.93 f
	Second Trial	0.0000	4.024 a	3.004 A	23.826 b	52.117 d	7.123 a	42.380 c	59.473 de	83.788 f	62.045 e
N Uptake (mg/pot)	First Trail	0.0000	38.95 a	220.1 B	319.3 c	545.1 e	61.17 a	459.6 d	655.7 f	735.6 g	711.4 g
	Second Trial	0.0000	33.38 a	35.93 A	237.58 b	452.33 c	50.61 a	450.72 c	589.20 d	677.15 e	553.15 d
P Uptake (mg/pot)	First Trail	0.0000	1.673 a	8.818 B	13.96 c	30.95 e	3.513 a	25.67 d	42.58 f	57.70 h	50.30 g
	Second Trial	0.0000	1.441 a	1.136 A	9.616 b	24.356 c	2.251 a	24.974 c	38.738 d	56.634 e	37.584 d
K Uptake (mg/pot)	First Trail	0.0000	45.55 a	185.3 B	292.4 c	574.4 e	70.63 a	465.4 d	634.6 ef	685.5 fg	744.5 g
	Second Trial	0.0000	26.33 a	23.01 A	192.79 b	377.62 c	39.26 a	367.90 c	458.38 d	555.98 e	483.69 d
Ca Uptake (mg/pot)	First Trail	0.0000	5.41 a	19.68 B	29.92 c	65.81 e	7.35 a	55.36 d	77.56 f	94.48 g	94.89 g
	Second Trial	0.0000	4.68 a	2.81 A	23.21 b	51.17 c	8.14 a	54.73 c	67.72 d	77.32 e	63.58 d
	First Trail	0.0000	3.053 a	10.74 Ab	16.19 b	35.22 c	7.776 ab	59.11 d	92.73 e	118.81 f	110.08 f

Mg Uptake (mg/pot)	Second Trial	0.0000	2.268 a	1.474 A	11.029 b	22.240 c	3.726 a	24.378 c	35.078 d	41.742 e	33.360 d
Na Uptake (mg/pot)	First Trail	0.0000	10.45 a	31.49 B	46.87 bc	69.33 de	11.66 a	56.95 cd	83.93 e	120.16 f	121.82 f
	Second Trial	0.0000	10.66 a	6.18 A	42.90 b	82.58 b	14.92 a	89.36 bd	106.64 de	128.73 f	114.26 ef
Cu Uptake (mg/pot)	First Trail	0.0000	0.009 a	0.037 B	0.056 c	0.102 e	0.014 a	0.076 d	0.121 e	0.147 f	0.141 f
	Second Trial	0.0000	0.008 a	0.005 A	0.038 b	0.080 d	0.011 a	0.067 c	0.090 d	0.113 e	0.090 d
Mn Uptake (mg/pot)	First Trail	0.0000	0.376 a	1.089 B	1.793 c	4.026 e	0.451 a	2.614 d	3.907 e	4.678 f	4.641 f
	Second Trial	0.0000	0.291 a	0.180 A	1.368 b	2.839 c	0.485 a	2.467 c	3.371 d	3.999 e	3.550 d
Zn Uptake (mg/pot)	First Trail	0.0000	0.147 a	0.512 B	0.778 c	1.449 ef	0.233 a	1.055 d	1.378 e	1.680 f	1.630 ef
	Second Trial	0.0000	0.118 a	0.078 A	0.543 b	1.014 c	0.197 a	1.077 c	1.337 d	1.648 e	1.362 d
Fe Uptake (mg/pot)	First Trail	0.0000	0.495 a	1.847 B	2.452 b	4.835 d	0.681 a	3.388 c	5.174 d	6.309 e	7.113 e
	Second Trial	0.0000	0.406 a	0.250 A	1.996 b	3.853 c	0.713 a	4.000 cd	5.427 e	7.142 f	4.938 de
B Uptake (mg/pot)	First Trail	0.0000	0.013 a	0.032 A	0.060 b	0.104 cd	0.017 a	0.088 bc	0.124 d	0.163 e	0.168 e
	Second Trial	0.0000	0.012 a	0.007 A	0.053 b	0.111 d	0.026 a	0.103 cd	0.090 cd	0.097 cd	0.085 c
Mo Uptake (mg/pot)	First Trail	0.0004	0.005 a	0.021 Ab	0.028 abc	0.069 cd	0.007 a	0.054 bc	0.066 cd	0.022 ab	0.095 d
	Second Trial	0.0000	0.004 a	0.002 A	0.015 bc	0.033 d	0.006 ab	0.032 d	0.025 cd	0.044 e	0.028 d

Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).

Leaf Analyses

Table 11 Effect of treatments on the leaf analyses of wheat

		ANOVA p-value	Treatment Means								
		Treatment Effect (df = 8)	1 (n=5)	2 (n=5)	3 (n=5)	4 (n=5)	5 (n=5)	6 n=5)	7 n=5)	8 (n=5)	9 (n=5)
S%	First Trail	0.0000	0.338 B	0.388 c	0.378 c	0.338 b	0.434 d	0.276 a	0.282 a	0.322 b	0.328 b
	Second Trial	0.0000	0.356 C	0.338 c	0.344 c	0.346 c	0.426 d	0.264 a	0.290 ab	0.326 bc	0.290 ab
N%	First Trail	0.0000	2.494 A	3.928 d	3.772 d	2.904 bc	3.056 c	2.896 bc	2.798 bc	2.818 bc	2.714 ab
	Second Trial	0.0000	2.946 B	4.118 d	3.480 c	3.022 b	3.010 b	2.814 ab	2.848 ab	2.626 a	2.594 a
P%	First Trail	0.0000	0.106 A	0.156 b	0.160 bc	0.164 bc	0.176 bcd	0.160 bc	0.182 cd	0.220 e	0.192 d
	Second Trial	0.0000	0.128 A	0.126 a	0.138 a	0.160 b	0.134 a	0.156 b	0.186 c	0.220 d	0.176 c
K%	First Trail	0.0000	2.884 Bc	3.296 de	3.360 e	3.046 cd	3.540 e	2.888 c	2.710 ab	2.608 a	2.842 abc
	Second Trial	0.0000	2.328 Ab	2.504 cd	2.748 e	2.518 d	2.338 bc	2.296 ab	2.230 a	2.160 a	2.266 ab
Ca%	First Trail	0.9516	0.360	0.352	0.350	0.348	0.368	0.348	0.332	0.362	0.362
	Second Trial	0.0000	0.394 B	0.344 ab	0.340 ab	0.340 ab	0.488 c	0.342 ab	0.328 a	0.300 a	0.298 a
Mg%	First Trail	0.0000	0.198 A	0.192 a	0.190 a	0.186 a	0.392 bc	0.370 b	0.396 bc	0.454 d	0.420 cd

	Second Trial	0.0000	0.196 D	0.176 c	0.162 abc	0.148 a	0.222 e	0.152 a	0.170 bc	0.162 abc	0.156 ab
Na%	First Trail	0.0132	0.724 C	0.568 abc	0.562 abc	0.362 a	0.584 bc	0.356 a	0.358 a	0.466 ab	0.464 ab
	Second Trial	0.0001	0.874 C	0.744 bc	0.642 ab	0.552 a	0.892 c	0.556 a	0.524 a	0.504 a	0.536 a
Cu	First Trail	0.0001	5.800 Bc	6.600 cd	6.600 cd	5.400 ab	7.200 d	4.800 a	5.200 ab	5.600 ab	5.4000 ab
	Second Trial	0.0000	6.400 Cd	5.800 bc	5.600 bc	5.400 b	6.800 d	4.200 a	4.400 a	4.400 a	4.200 a
Mn	First Trail	0.0000	249.4 E	194.8 bc	209.4 cd	212.6 cd	226.6 de	162.8 a	167.0 a	179.2 ab	177.2 ab
	Second Trial	0.0000	253.0 D	214.6 c	200.8 c	189.4 bc	288.4 e	154.2 a	164.8 ab	155.4 a	166.2 ab
Zn	First Trail	0.0000	96.00 C	91.20 c	90.40 c	76.20 b	116.80 d	65.80 ab	58.60 a	63.80 ab	62.20 a
	Second Trial	0.0000	101.80 D	89.60 c	79.20 b	67.60 a	117.20 e	67.40 a	64.40 a	64.00 a	63.80 a
Fe	First Trail	0.0000	326.4 D	333.2 d	286.8 cd	253.4 abc	339.6 d	210.2 a	221.4 ab	240.4 abc	271.0 bc
	Second Trial	0.0000	351.2 C	287.2 b	288.4 b	257.8 ab	426.2 d	249.8 ab	263.8 ab	278.2 ab	231.0 a
B	First Trail	0.0013	8.60 B	5.80 a	7.00 ab	5.60 a	8.40 b	5.40 a	5.40 a	6.20 a	6.40 a
	Second Trial	0.0000	10.20 D	7.80 c	7.80 c	7.40 c	15.40 d	6.40 bc	4.40 ab	3.80 a	4.00 a
Mo	First Trail	0.1465	3.408 B	3.716 b	2.980 b	3.622 b	3.686 b	3.284 b	2.846 ab	0.812 a	3.630 b
	Second Trial	0.0000	2.892 De	2.416 cd	2.098 bc	2.218 bc	3.344 e	2.000 bc	1.186 a	1.714 ab	1.266 a

Means within a column with the **same letter(s)** or **no letters** are **not significantly** different at the 95% probability level (Fisher's LSD test).

ANALYSES C: Comparing nitrophosphate and MAP(33) fertilisers with P application rate of product.

Table 12 Effect of fertiliser and P application rate on the growth of wheat

	Fresh Root Volume (ml)		Fresh Leaf Mass (g)		Dry Root Mass (g)		Dry Leaf Mass (g)	
	First Trial	Second Trial	First Trial	Second Trial	First Trial	Second Trial	First Trial	Second Trial
Descriptive Statistics								
Total n	40	40	40	40	40	40	40	40
Mean	89.13	53.50	54.17	53.88	7.38	6.73	12.84	11.03
Std Dev	59.91	31.69	40.65	41.03	5.48	4.98	9.466	9.351
CV%	66.10	59.23	75.05	76.15	74.24	74.05	73.74	84.76
Means – Fertiliser								
P1: Nitrophosphate + Urea	65.000 a	48.50 a	36.49 a	34.83 a	4.789 a	4.42 a	8.661 a	6.029 a
P2: MAP + Urea	113.25 b	58.50 b	71.84 b	72.93 b	9.966 b	9.04 b	17.01 b	16.08 b
ANOVA Results – Fertiliser Effect								
p-value	0.0000	0.0014	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
$LSD_{(\alpha=0.05)}$	6.058	5.817	6.686	4.290	1.414	0.921	1.587	1.290
$LSD_{(\alpha=0.1)}$	5.037	4.838	5.560	3.567	1.175	0.766	1.320	1.073
Means – P Application Rate								
AR1: 0	18.50 a	14.00 a	6.376 a	5.859 a	1.360 a	1.145 a	1.771 a	1.404 a
AR2: 15	76.00 b	45.00 b	45.01 b	44.56 b	6.544 b	5.550 b	10.88 b	8.454 b
AR3: 30	111.0 c	65.00 c	68.25 c	68.32 c	9.491 c	8.573 c	16.07 c	13.84 c
AR4: 45	151.0 d	90.00 d	97.03 d	96.79 d	12.11 d	11.65 d	22.63 d	20.43 d
ANOVA Results – Application Rate Effect								
p-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
$LSD_{(\alpha=0.05)}$	8.567	8.227	9.455	6.067	1.999	1.303	2.245	1.824

$LSD_{(\alpha=0.1)}$	7.124	6.841	7.863	5.045	1.662	1.083	1.867	1.517
Means – Interaction between Fertiliser and P Application Rate								
T1: Nitrophosphate + Urea	16.00 a	14.00 a	5.184 a	6.378 a	1.096 a	1.220 a	1.546 a	1.128 a
T2: Nitrophosphate + Urea + 15 P rate	40.00 b	22.00 a	23.77 b	16.00 b	2.978 ab	2.064 a	5.606 b	0.890 a
T3: Nitrophosphate + Urea + 30 P rate	62.00 c	70.00 b	37.83 c	42.61 c	4.870 b	4.968 b	8.638 b	6.990 b
T4: Nitrophosphate + Urea + 45 P rate	142.0 e	88.00 c	79.19 d	74.32 d	10.21 c	9.420 c	18.85 c	15.11 c
T5: MAP + Urea	21.00 a	14.00 a	7.570 a	5.340 a	1.624 a	1.070 a	1.996 a	1.680 a
T6: MAP + Urea + 15 P rate	112.0 d	68.00 b	66.25 d	73.13 d	10.11 c	9.036 c	16.15 c	16.02 c
T7: MAP + Urea + 30 P rate	160.0 f	60.00 b	98.66 e	94.02 e	14.11 d	12.18 d	23.50 d	20.69 d
T8: MAP + Urea + 45 P rate	160.0 f	92.00 c	114.9 f	119.2 f	14.02 d	13.87 d	26.41 d	25.76 e
ANOVA Results – Interaction between Fertiliser and P Application Rate								
p-value	0.0000	0.0000	0.0000	0.0000	0.0006	0.0000	0.0000	0.0000
$LSD_{(\alpha=0.05)}$	12.115	11.635	13.372	8.580	2.827	1.842	3.175	2.579
$LSD_{(\alpha=0.1)}$	10.075	9.675	11.120	7.135	2.351	1.532	2.640	2.145

Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).

Leaf Chlorophyll

Table 13 Effect of Fertiliser and P application rate on the leaf chlorophyll of wheat

	Chlorophyll (SPAD) @ 2 WAE		Chlorophyll (SPAD) @ 3 WAE		Chlorophyll (SPAD) @ 4 WAE	
	First Trial	Second Trial	First Trial	Second Trial	First Trial	Second Trial
Descriptive Statistics						
Total n	40	40	40	40	40	40
Mean	31.78	39.29	40.84	40.83	43.55	42.24
Std Dev	3.620	5.105	4.140	4.676	4.677	4.763
CV%	11.39	13.00	10.14	11.45	10.74	11.28
Means – Product						
P1: Nitrophosphate + Urea	33.36 a	37.31 a	40.31 a	39.35 a	42.69 a	40.83 a
P2: MAP + Urea	30.20 b	41.27 b	41.37 a	42.31 b	44.41 b	43.64 b
ANOVA Results – Product Effect						
p-value	0.0000	0.0000	0.2042	0.0000	0.0072	0.0000
$LSD_{(\alpha=0.05)}$	0.671	0.640	1.666	0.581	1.217	0.933
$LSD_{(\alpha=0.1)}$	0.558	0.532	1.385	0.483	1.012	0.776
Means – P Application Rate						
AR1: 0	27.29 a	32.42 a	35.23 a	34.28 a	36.89 a	35.52 a
AR2: 15	33.15 bc	39.32 b	41.84 b	40.94 b	43.58 b	42.27 b
AR3: 30	34.07 c	41.28 c	42.53 b	42.84 c	45.52 c	44.51 c
AR4: 45	32.61 b	44.12 d	43.74 b	45.25 d	48.20 d	46.64 d
ANOVA Results – Application Rate Effect						
p-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
$LSD_{(\alpha=0.05)}$	0.949	0.905	2.356	0.822	1.721	1.319
$LSD_{(\alpha=0.1)}$	0.789	0.753	1.959	0.683	1.431	1.097
Means – Interaction between Fertiliser and Application Rate						
T1: Nitrophosphate + Urea	30.26 b	32.22 a	35.34 a	34.66 a	36.82 a	35.42 a
T2: Nitrophosphate + Urea + 15 P rate	32.12 c	35.48 b	41.16 b	37.74 b	42.86 b	39.54 b
T3: Nitrophosphate + Urea + 30 P rate	36.56 e	38.42 c	41.88 bc	40.58 c	44.30 b	42.50 c
T4: Nitrophosphate + Urea + 45 P rate	34.50 d	43.10 d	42.84 bc	44.42 d	46.78 c	45.88 de
T5: MAP + Urea	24.32 a	32.62 a	35.12 a	33.90 a	36.96 a	35.62 a
T6: MAP + Urea + 15 P rate	34.18 d	43.16 d	42.52 bc	44.14 d	44.30 b	45.00 d
T7: MAP + Urea + 30 P rate	31.58 bc	44.14 de	43.18 bc	45.10 de	46.74 c	46.52 de
T8: MAP + Urea + 45 P rate	30.72 b	45.14 e	44.64 c	46.08 e	49.62 d	47.40 e

ANOVA Results – Interaction between Fertiliser and Application Rate						
p-value	0.0000	0.0000	0.8317	0.0000	0.3989	0.0012
$LSD_{(\alpha=0.05)}$	1.342	1.280	3.331	1.162	2.434	1.865
$LSD_{(\alpha=0.1)}$	1.116	1.064	2.770	0.966	2.024	1.551

Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).

Table 14 Effect of Fertiliser and P application rate on the leaf chlorophyll of wheat (continue)

	Chlorophyll (SPAD) @ 5 WAE		Chlorophyll (SPAD) @ 6 WAE		Chlorophyll (SPAD) @ 7 WAE	
	First Trial	Second Trial	First Trial	Second Trial	First Trial	Second Trial
Descriptive Statistics						
Total n	40	40	40	40	40	40
Mean	44.32	43.67	45.86	45.14	48.26	47.26
Std Dev	4.665	4.446	4.299	4.076	3.083	3.37
CV%	10.53	10.18	9.37	9.03	6.37	7.13
Means – Product						
P1: Nitrophosphate + Urea	43.43 a	42.52 a	45.19 a	44.30 a	47.78 a	46.75 a
P2: MAP + Urea	45.22 b	44.83 b	46.54 b	45.98 b	48.75 b	47.77 b
ANOVA Results – Product Effect						
p-value	0.0032	0.0000	0.0161	0.0001	0.0177	0.0078
$LSD_{(\alpha=0.05)}$	1.147	0.580	1.078	0.736	0.794	0.729
$LSD_{(\alpha=0.1)}$	0.953	0.482	0.896	0.612	0.660	0.606
Means – P Application Rate						
AR1: 0	37.46 a	36.83 a	39.61 a	38.95 a	44.00 a	42.31 a
AR2: 15	44.81 b	44.61 b	46.07 b	45.94 b	48.04 b	48.09 b
AR3: 30	46.31 b	45.77 c	48.00 c	47.20 c	49.95 c	48.74 b
AR4: 45	48.71 c	47.48 d	49.77 d	48.46 d	51.06 c	49.89 c
ANOVA Results – Application Rate Effect						
p-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
$LSD_{(\alpha=0.05)}$	1.621	0.820	1.524	1.041	1.123	1.031
$LSD_{(\alpha=0.1)}$	1.348	0.682	1.268	0.865	0.934	0.857
Means – Interaction between Fertiliser and Application Rate						
T1: Nitrophosphate + Urea	37.38 a	37.00 a	40.26 a	39.72 b	44.62 a	43.66 b
T2: Nitrophosphate + Urea + 15 P rate	43.74 b	42.90 b	44.92 b	44.48 c	47.30 b	47.32 c
T3: Nitrophosphate + Urea + 30 P rate	45.28 bc	43.92 b	46.92 bc	45.68 c	49.52 cd	47.52 cd
T4: Nitrophosphate + Urea + 45 P rate	47.30 c	46.24 c	48.66 cd	47.30 d	49.66 cd	48.50 cd
T5: MAP + Urea	37.54 a	36.66 a	38.96 a	38.18 a	43.38 a	40.96 a
T6: MAP + Urea + 15 P rate	45.88 bc	46.32 c	47.22 cd	47.40 d	48.78 bc	48.86 de
T7: MAP + Urea + 30 P rate	47.34 c	47.62 d	49.08 de	48.72 de	50.38 d	49.96 ef
T8: MAP + Urea + 45 P rate	50.12 d	48.72 d	50.88 e	49.62 e	52.46 e	51.28 f
ANOVA Results – Interaction between Fertiliser and Application Rate						
p-value	0.3928	0.0001	0.0570	0.0002	0.0082	0.0000
$LSD_{(\alpha=0.05)}$	2.293	1.159	2.156	1.472	1.588	1.457
$LSD_{(\alpha=0.1)}$	1.907	0.964	1.793	1.224	1.320	1.212

Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).

Top Soil

Table 15 Effect of fertiliser and P application rate on the top soil of wheat

		p-value			LSD$_{(\alpha=0.05)}$ significant differences		
		Application rate (AR)	Fertiliser (F)	Interaction F x AR	Application rate (AR)	Fertiliser (F)	Interaction F x AR
Bulk Density	**Trial 2**	0.0085	0.1412	0.0648	AR1 < AR3, AR4 AR2 < AR4		T5 < T3, T4, T6, T7, T8 T2 < T4, T6, T7, T8 T1 < T4
pH	**Trial 2**	0.0000	0.0646	0.1163	AR4 > AR1, AR2, AR3 AR3 > AR1, AR2		T4, T7, T8 > T1, T2, T3, T5, T6
Acid Saturation	**Trial 2**	0.0015	0.2661	0.3035	AR1, AR2 > AR3, AR4		T4, T7, T8 < T1, T2, T5
S	**Trial 2**	0.3184	0.0010	0.8162	AR1 < AR4	F1 > F2	T4 > T2, T3, T5, T6, T7, T8 T5 < T1, T2, T3
P	**Trial 2**	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4	F1 > F2	T1 < T2, T3, T4 T2 < T3, T4 T2 > T5, T6, T8 T3, T4 > T5, T6, T7, T8
K	**Trial 2**	0.0312	0.2530	0.3500	AR1 > AR4		T5 > T6, T8 T1 > T8
Ca	**Trial 2**	0.0686	0.9292	0.7133	AR4 > AR1, AR2		T4 > T1, T2, T5
Mg	**Trial 2**	0.0137	0.7939	0.4035	AR4 > AR1, AR2		T4 > T1, T2, T3, T5 T2 < T8
Na	**Trial 2**	0.6492	0.6288	0.6715			
Ca/Mg	**Trial 2**	0.7159	0.8336	0.9798			
(Ca+Mg)/K	**Trial 2**	0.0000	0.0120	0.0147	AR4 > AR1, AR2, AR3 AR 3 > AR1, AR2		T1, T2 < T3, T4, T6, T7, T8 T3 < T4, T8 T5 < T3, T4, T6, T7, T8 T7 < T8
ECEC	**Trial 2**	0.0219	0.2599	0.3427	AR4 < AR1, AR2		T2, T5 > T4, T7, T8
Exchangeable Acidity	**Trial 2**	0.0131	0.2968	0.3517	AR4 < AR1, AR2		T4 > T1, T2, T5 T2, T5 > T7, T8

Codes for Fertiliser are F1 = Nitrophosphate + Urea and F2 = MAP + Urea

Codes for Application rate are AR1 = None, AR2 = 15, AR3 = 30 and AR4 = 45

Codes for the Interaction (F x AR) are T1 = Nitrophosphate + Urea, T2 = Nitrophosphate + Urea + 15 P, T3 = Nitrophosphate + Urea + 30 P, T4 = Nitrophosphate + Urea + 45 P, T5 = MAP + Urea, T6 = MAP + Urea + 15 P, T7 = MAP + Urea + 30 P and T8 = MAP + Urea + 45 P

Nutrient Uptake

Table 16 Effect of fertiliser and P application rate on the nutrient uptake of wheat

		p-value			$LSD_{(\alpha=0.05)}$ significant differences		Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).
		Application rate (AR)	**Fertiliser (F)**	**Interaction F x AR**	**Application rate (AR)**	**Fertiliser (F)**	**Interaction F x AR**
S Uptake (mg/pot)	**Trial 1**	0.0000	0.0000	0.0015	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2b, T3c, T4e, T5a, T6d, T7e, T8f
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2a, T3b, T4d, T5a, T6c, T7e, T8f
N Uptake (mg/pot)	**Trial 1**	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2b, T3c, T4e, T5a, T6d, T7f, T8g
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2a, T3b, T4c, T5a, T6c, T7d, T8e
P Uptake (mg/pot)	**Trial 1**	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2b, T3c, T4e, T5a, T6d, T7f, T8g
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2a, T3b, T4c, T5a, T6c, T7d, T8e

		p-value			$LSD_{(\alpha=0.05)}$ significant differences		Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).
		Application rate (AR)	**Fertiliser (F)**	**Interaction F x AR**	**Application rate (AR)**	**Fertiliser (F)**	**Interaction F x AR**
K Uptake (mg/pot)	**Trial 1**	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2b, T3c, T4e, T5a, T6d, T7ef, T8f
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2a, T3b, T4c, T5a, T6c, T7d, T8e
Ca Uptake (mg/pot)	**Trial 1**	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2b, T3c, T4e, T5a, T6d, T7f, T8g
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2a, T3b, T4c, T5a, T6c, T7d, T8e
Mg Uptake (mg/pot)	**Trial 1**	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2ab, T3b, T4c, T5ab, T6d, T7e, T8f
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2a, T3b, T4c, T5a, T6c, T7d, T8e
Na Uptake (mg/pot)	**Trial 1**	0.0000	0.0000	0.0009	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2b, T3bc, T4de, T5a, T6cd, T7e, T8f
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4	F1 < F2	T1a, T2a, T3b, T4c, T5a, T6cd, T7d, T8e

		p-value			$LSD_{(\alpha=0.05)}$ significant differences		Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).
		Application rate (AR)	**Fertiliser (F)**	**Interaction F x AR**	**Application rate (AR)**	**Fertiliser (F)**	**Interaction F x AR**
					AR3 < AR4		
Cu Uptake (mg/pot)	**Trial 1**	0.0000	0.0000	0.0004	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2b, T3c, T4e, T5a, T6d, T7f, T8g
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2a, T3b, T4c, T5a, T6c, T7d, T8e
Mn Uptake (mg/pot)	**Trial 1**	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2b, T3c, T4e, T5a, T6d, T7e, T8f
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2a, T3b, T4c, T5a, T6c, T7d, T8e

Table 17 Effect of Fertiliser and P application rate on the nutrient uptake of wheat (continue)

		p-value			$LSD_{(\alpha=0.05)}$ significant differences		Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).
		Application rate (AR)	**Fertiliser (F)**	**Interaction F x AR**	**Application rate (AR)**	**Fertiliser (F)**	**Interaction F x AR**
Zn Uptake (mg/pot)	**Trial 1**	0.0000	0.0000	0.0202	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2b, T3c, T4ef, T5a, T6d, T7e, T8f

		p-value			$LSD_{(\alpha=0.05)}$ significant differences		Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).
		Application rate (AR)	**Fertiliser (F)**	**Interaction F x AR**	**Application rate (AR)**	**Fertiliser (F)**	**Interaction F x AR**
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2a, T3b, T4c, T5a, T6c, T7d, T8e
Fe Uptake (mg/pot)	**Trial 1**	0.0000	0.0000	0.0027	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2b, T3b, T4d, T5a, T6c, T7d, T8e
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2a, T3b, T4c, T5a, T6c, T7d, T8e
B Uptake (mg/pot)	**Trial 1**	0.0000	0.0000	0.0081	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2a, T3b, T4cd, T5a, T6c, T7d, T8e
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR2 < AR3, AR4 AR3 < AR4	F1 < F2	T1a, T2a, T3b, T4c, T5a, T6c, T7c, T8c
Mo Uptake (mg/pot)	**Trial 1**	0.0001	0.2579	0.0000	AR1 < AR2, AR3, AR4		T1a, T2a, T3a, T4b, T5a, T6b, T7b, T8a
	Trial 2	0.0000	0.0000	0.0000	AR1 < AR2, AR3, AR4 AR4 > AR2, AR3	F1 < F2	T1a, T2a, T3b, T4d, T5a, T6cd, T7c, T8e

Codes for Fertiliser are F1 = Nitrophosphate + Urea and F2 = MAP + Urea

Codes for Application rate are AR1 = None, AR2 = 15, AR3 = 30 and AR4 = 45

Codes for the Interaction (F x AR) are T1 = Nitrophosphate + Urea, T2 = Nitrophosphate + Urea + 15 P, T3 = Nitrophosphate + Urea + 30 P, T4 = Nitrophosphate + Urea + 45 P, T5 = MAP + Urea, T6 = MAP + Urea + 15 P, T7 = MAP + Urea + 30 P and T8 = MAP + Urea + 45 P

Leaf Analyses

Table 18 Effect of aroduct and P application rate on the leaf analyses of wheat

		p-value			$LSD_{(\alpha=0.05)}$ significant differences		Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).
		Application rate (AR)	**Fertiliser (F)**	**Interaction F x AR**	**Application rate (AR)**	**Fertiliser (F)**	**Interaction F x AR**
S%	**Trial 1**	0.0002	0.0015	0.0000	AR1 > AR2, AR3, AR4	F1 > F2	T1b, T2c, T3c, T4b, T5d, T6a, T7a, T8b
	Trial 2	0.0000	0.0570	0.0001	AR1 > AR2, AR3, AR4 AR4 > AR2		T1c, T2c, T3c, T4c, T5d, T6a, T7ab, T8bc
N%	**Trial 1**	0.0000	0.0000	0.0000	AR1, AR4 < AR2, AR3	F1 > F2	T1a, T2c, T3c, T4b, T5b, T6b, T7ab, T8b
	Trial 2	0.0000	0.0000	0.0000	AR2 > AR1, AR3, AR4 AR3 > AR4	F1 > F2	T1b, T2d, T3c, T4b, T5b, T6ab, T7ab, T8a
P%	**Trial 1**	0.0000	0.0000	0.0011	AR1 < AR2, AR3, AR4 AR4 > AR2, AR3	F1 < F2	T1a, T2b, T3bc, T4bc, T5bc, T6bc, T7c, T8d
	Trial 2	0.0000	0.0000	0.0002	AR4 > AR1, AR2, AR3 AR3 > AR1, AR2	F1 < F2	T1a, T2a, T3a, T4b, T5a, T6b, T7c, T8d

Table 19 Effect of Product and P application rate on the leaf analyses of wheat (continue)

		p-value			$LSD_{(\alpha=0.05)}$ significant differences		Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).
		Application rate (AR)	**Fertiliser (F)**	**Interaction F x AR**	**Application rate (AR)**	**Fertiliser (F)**	**Interaction F x AR**
K%	**Trial 1**	0.0023	0.0032	0.0000	AR4 < AR1, AR2, AR3	F1 > F2	T1bc, T2de, T3e, T4cd, T5e, T6bc, T7ab, T8a
	Trial 2	0.0600	0.0000	0.0014	AR3 > AR1, AR4	F1 > F2	T1ab, T2bc, T3d, T4c, T5ab, T6a, T7a, T8a

		p-value			$LSD_{(\alpha=0.05)}$ significant differences		Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).
		Application rate (AR)	**Fertiliser (F)**	**Interaction F x AR**	**Application rate (AR)**	**Fertiliser (F)**	**Interaction F x AR**
Ca%	**Trial 1**	0.7094	1.0000	0.8598			T1a, T2a, T3a, T4a, T5a, T6a, T7a, T8a
	Trial 2	0.0000	0.5271	0.0274	AR1 > AR2, AR3, AR4		T1b, T2ab, T3ab, T4ab, T5c, T6ab, T7a, T8a
Mg%	**Trial 1**	0.1734	0.0000	0.0729	AR2 < AR4	F1 < F2	T1a, T2a, T3a, T4a, T5b, T6b, T7b, T8c
	Trial 2	0.0000	0.1554	0.0012	AR1 > AR2, AR3, AR4		T1c, T2b, T3ab, T4a, T5d, T6a, T7b, T8ab
Na%	**Trial 1**	0.0206	0.0486	0.1656	AR1 > AR2, AR3, AR4	F1 > F2	T1c, T2abc, T3abc, T4ab, T5bc, T6a, T7a, T8ab
	Trial 2	0.0001	0.0867	0.4651	AR1 > AR2, AR3, AR4		T1c, T2bc, T3ab, T4ab, T5c, T6ab, T7a, T8a
Cu	**Trial 1**	0.0208	0.0831	0.0000	AR1 > AR2, AR4		T1bc, T2cd, T3cd, T4ab, T5d, T6a, T7ab, T8ab
	Trial 2	0.0000	0.0003	0.0129	AR1 > AR2, AR3, AR4	F1 > F2	T1cd, T2bc, T3bc, T4b, T5d, T6a, T7a, T8a
Mn	**Trial 1**	0.0000	0.0000	0.7236	AR1 > AR2, AR3, AR4	F1 > F2	T1e, T2bc, T3cd, T4cd, T5de, T6a, T7a, T8ab
	Trial 2	0.0000	0.0013	0.0001	AR1 > R2, AR3, AR4	F1 > F2	T1d, T2c, T3c, T4bc, T5e, T6a, T7ab, T8a
Zn	**Trial 1**	0.0000	0.0005	0.0000	AR1 > AR2, AR3, AR4	F1 > F2	T1c, T2c, T3c, T4b, T5d, T6ab, T7a, T8ab
	Trial 2	0.0000	0.0096	0.0000	AR1 > AR2, AR3, AR4 AR2 > AR3, AR4	F1 > F2	T1d, T2c, T3b, T4a, T5e, T6a, T7a, T8a
Fe	**Trial 1**	0.0005	0.0022	0.0091	AR1 > AR2, AR3, AR4	F1 > F2	T1c, T2c, T3bc, T4ab, T5c, T6a, T7a, T8ab
	Trial 2	0.0000	0.5202	0.0171	AR1 > AR2, AR3, AR4		T1b, T2a, T3a, T4a, T5c, T6a, T7a, T8a
B	**Trial 1**	0.0001	0.3609	0.3596	AR1 > AR2, AR3, AR4		T1b, T2a, T3ab, T4a, T5b, T6a, T7a, T8a
	Trial 2	0.0000	0.1702	0.0000	AR1 > AR2, AR3, AR4		T1d, T2c, T3c, T4c, T5e, T6bc, T7ab, T8a

		p-value			$LSD_{(\alpha=0.05)}$ significant differences		Means within a column with the **same letter(s)** are **not significantly** different at the 95% probability level (Fisher's LSD test).
		Application rate (AR)	**Fertiliser (F)**	**Interaction F x AR**	**Application rate (AR)**	**Fertiliser (F)**	**Interaction F x AR**
Mo	**Trial 1**	0.0936	0.0669	0.0507	AR4 < AR2, AR3		T1b, T2b, T3b, T4b, T5b, T6b, T7, T8a
	Trial 2	0.0000	0.0426	0.0413	AR1 > AR2, AR3, AR4 AR2 > AR3	F1 > F2	T1de, T2cd, T3bc, T4bc, T5e, T6bc, T7a, T8ab

Codes for Fertiliser are F1 = Nitrophosphate + Urea and F2 = MAP + Urea

Codes for Application rate are AR1 = None, AR2 = 15, AR3 = 30 and AR4 = 45

Codes for the Interaction (F x AR) are T1 = Nitrophosphate + Urea, T2 = Nitrophosphate + Urea + 15 P, T3 = Nitrophosphate + Urea + 30 P, T4 = Nitrophosphate + Urea + 45 P, T5 = MAP + Urea, T6 = MAP + Urea + 15 P, T7 = MAP + Urea + 30 P and T8 = MAP + Urea + 45 P