IMRAN ASHRAF

Phosphate ageing in soil and bioavailability of aged phosphates

A thesis submitted for the requirement of the Doctoral Degree in Agriculture from the Faculty of Agricultural Sciences, Nutritional Sciences, and Environmental Management, Justus Liebig University Giessen

édition scientifique
VVB LAUFERSWEILER VERLAG

1. Auflage 2015

1st Edition 2015

Printed in Germany

édition scientifique
VVB LAUFERSWEILER VERLAG

STAUFENBERGRING 15, D-35396 GIESSEN
Tel: 0641-5599888 Fax: 0641-5599890
email: redaktion@doktorverlag.de

www.doktorverlag.de

Institute of Plant Nutrition
Justus Liebig University Giessen
Prof. Dr. Sven Schubert

Phosphate ageing in soil and bioavailability of aged phosphates

A thesis submitted for the requirement of the Doctoral Degree in Agriculture from the Faculty of Agricultural Sciences, Nutritional Sciences, and Environmental Management,
Justus Liebig University Giessen

Submitted by

Imran Ashraf

Faisalabad, Pakistan

Giessen 2015

Date of thesis disputation: 02-12-2015

Examination Commission

Chairperson:	Prof. Dr. Gertrud Morlock
Supervisor:	Prof. Dr. Sven Schubert
Co-supervisor:	Prof. Dr. Jan Siemens
Examiner:	Prof. Dr. Rod Snowdon
Examiner:	Prof. Dr. Sylvia Schnell

Dedication

This dissertation is dedicated to Tahira Parveen and Muhammad Ashraf, my mother and father, for their endless love, efforts, wishes, and support, Shabana Kouser and Taj Din, my aunt and uncle, for their moral support and encouragement, Nustrat Bibi, my aunt, for her unimaginable affection with wishes of her good health, Shamaila, my life partner, for her love, unshakeable trust and believe in me, and Aayan, my son, for bringing smiles and happiness to me with wishes of his bright future.

Contents

1 Introduction

1.1 Phosphorus

Phosphorus (P) is one of the essential elements for plants and animals (Ragothama, 1999). It is a plant macronutrient and the second most frequently limiting nutrient for plants after nitrogen (Schachtman *et al*., 1998). P deficiency in plants is a widespread problem, especially in highly weathered acid soils (Fageria and Baligar, 2001; Faye *et al*., 2006) and in calcareous soils (Marschner, 1995). In these soils, crop production relies highly on the application of phosphorus fertilizers (Cordell *et al*., 2009). P fertilizers are produced from rock phosphate; whose reserves are very limited (Vance *et al*., 2003; Konig *et al*., 2008; Cordell *et al*., 2009; Gilbert, 2009). P deficiency is one of the greatest limitations in modern agricultural production (Runge-Metzger, 1995; Lynch and Brown, 2008).

1.2 Phosphorus functions in plants

P is involved in very important processes in plants such as photosynthesis, respiration and energy transfer. It is a key component of DNA and RNA, where it is present as phosphate group, attached to the nitrogenous base and the sugar molecule. It is a key component of cell membranes in the form of phospholipids. It is part of the energy currencies of the cell such as ATP, ADP, and NADP(H) and other nucleotide triphosphates and diphosphates. The pyrophosphate bond in these nucleotide phosphates ensures the release of energy via hydrolysis as required (Theodorou and Plaxton, 1993; Mengel and Kirkby, 2001; Vance *et al*., 2003).

1.3 Phosphate dynamics in the soil-plant system

P is present as phosphate ion (PO_4^{2-}) in soils and plants. It takes part in chemical reactions in the form of phosphate ion. These phosphates are present as primary orthophosphate ($H_2PO_4^-$) and secondary orthophosphate (HPO_4^{2-}) in the soil solution, depending on the pH of the soil solution. These orthophosphates are in equilibrium conditions with each other. This equilibrium condition shifts more towards primary orthophosphates at low pH and towards secondary orthophosphate at high pH. Plants can only take up P from soil solution, where phosphate is present in very low concentrations in most of the soils (Bieleski, 1973; Hinsinger, 2001). This is due to strong retention of phosphate ions with soil particles. The total P may be high in most soils but unavailable due to strong retention of phosphate in acid

soils (via adsorption, occlusion, and precipitation) and calcareous soils (via precipitation). Most of the applied P becomes immobile after P fertilization (Holford, 1997).

There are four major pools of soil-P compounds which contribute to soil-solution P as shown in Figure 1. The first one is the adsorbed P pool. At low pH, phosphate is adsorbed at the surfaces of iron oxides (Fe oxides) and aluminum oxides (Al oxides) (see detail 1.4). This pool contributes to soil-solution P as the pH of soil increases. This process is called desorption. An increase in the concentrations of organic anions in the soil results in P desorption (Hinsinger, 2001; Qayyum *et al*., 2015). The second pool of the P compounds in the soil consists of occluded P (see detail 1.5). This P returns to the soil solution after reduction of oxide minerals. It is one the strongest-bound P forms in soils, thus its contribution to the soil-solution P is very small.

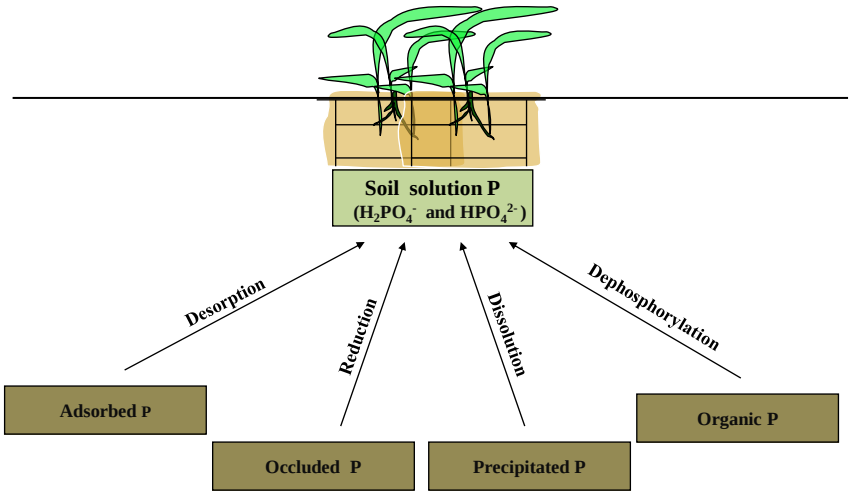

Figure 1: Various P fractions in the soil releasing orthophosphate ions into the soil solution (modified after Mengel and Kirkby, 2001)

The third pool is precipitated P. Phosphate ions are precipitated with iron (Fe^{2+} and Fe^{3+}) and aluminum ions (Al^{3+}) at low pH and with calcium ions (Ca^{2+}) at high pH present in the soil solution. These precipitates can be amorphous and crystalline. The P bound with crystalline forms is also very tightly fixed; hence, P release is very slow. The precipitated P is turned back into the soil solution by dissolution (Hossner *et al*., 1973; Mengel and Kirkby, 2001; Kochian *et al*., 2004; Vance *et al*., 2003; Sims and Pierzynski, 2005). The fourth soil-P pool

consists of organic P. This fraction comes from dead remains of plants and microbial masses. Most of the phosphates are in the form of inositol phosphates while other P compounds, present in phospholipids and nucleic acids, contribute very little due to their quick immobilization by soil microbes. This organic P is made available to plants by dephosphorylation carried out by microbes and plant roots by releasing phosphatases (Holford, 1997; Vance *et al*., 2003; Cordell *et al*., 2011; Gerke, 2015a). Organic P may constitute 20-80% of total soil P (Dalal, 1977; McLaughlin *et al*., 1990). This pool contributes significantly to the soil-solution P (Steffens *et al*., 2010).

Primary and secondary minerals of P are minute resources of P supply in soils. Such minerals include variscites, strengites, and apatites. In acid and calcareous soils, their contribution to the soil-solution P is very low (Lindsay *et al*., 1989; Dou *et al*., 2009). The other natural resource of P are the rock phosphates, which are mostly in the form of apatites. For the last six decades, these have been the main source of P fertilizers. After the green revolution, the intense P fertilization around the globe has resulted in depletion of the rock-phosphate reserves. It is expected that peak P-fertilizer production will occur within the next two decades (Cordell *et al*., 2009; Gilbert, 2009). So the prices of P fertilizers and ultimately the food prices are expected to be high. Under this scenario of limited P resources, efficient use of P becomes inevitable (Steen, 1998; Konig *et al*., 2008; Vance *et al*., 2003).

Phosphate is highly immobile in the soil solution. It is transported to the plants by diffusion. Mass flow contributes very little to P uptake (Bhat and Nye, 1974; Barber, 1995; Jungk and Claassen, 1997; Kirkby and Johnston 2008). P is mobile within plants in the form of orthophosphate ions. Under P-deficient conditions, P from lower leaves moves to upper leaves. The color of older leaves turns darkish green and the stem color may turn reddish. This is due to increased concentrations of anthocyanins under P deficiency (Bould *et al*., 1983; Bergmann, 1992).

1.4 Phosphate adsorption

Adsorption is a process in which ions from the soil solution are bound to the surface of soil particles i.e. soil minerals. Theses ions (solute particles) are attached with the solid surface at an interface between the liquid and the solid medium (Stumm, 1992). These ions are called adsorbates and the soil particles are called adsorbents. The attachment involves mainly covalent bonds, ligand exchange and ion exchange. Adsorption plays a vital role in retaining

these ions within the rhizosphere which prevents leaching into lower soil profiles. This happens to ions, which are very weakly adsorbed such as nitrate. On the other hand, strongly adsorbed ions, such as the phosphate, become unavailable to plants. The phosphate ion is adsorbed more strongly than other anions in the soil.

Soil minerals have reactive functional groups at their surfaces. These functional groups exhibit charge which may be permanent and variable (pH-dependent). These functional groups are called surface functional groups. These play a vital role in the adsorption (Sposito, 1989). The adsorption of ions also depends on the degree of crystallinity of the adsorbent mineral. The amorphous mineral surface adsorbs the adsorbate more strongly and in more quantity than a crystalline surface due to their higher number of reactive sites per unit area (Pagel and van Huay, 1976; Burnham and Lopez-Hernanads, 1982).

In acid soils (at low pH), phosphate is adsorbed at the surfaces of Fe oxides (Goethite, Ferrihydrite, Hematite, Akaganeite, Feroxyhyte), Al oxides (Gibbsite, Diaspore, Boehmite) and clay minerals (Taylor, 1987; Hsu, 1989; McKenzie, 1989; Schulze, 1989; Tejedor-Tejedor and Anderson, 1990; Bleam *et al*., 1991; Schwertmann and Cornell, 1991; Gerke and Hermann, 1992; Persson *et al*., 1996; Schulze *et al*., 1999; Arai and Sparks, 2001).

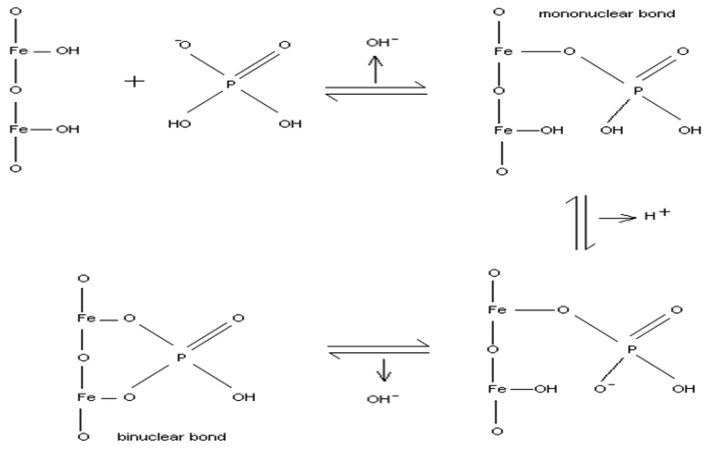

Figure 2: Phosphate adsorption at the surface of Fe oxides (modified after Parfitt, 1978)

The adsorption of the phosphate ions occurs by ligand exchange (Figure 2). The phosphate ion acts as a ligand. It is attached covalently to the Fe-oxide surface, which has net positive

charge due to removal of OH^-. This is called mononuclear adsorption. This mononuclear-bound phosphate is further attached to the Fe oxide surface at a different place due to removal of one more OH^-. This binuclear-bound phosphate is very strongly adsorbed and its availability to plants is small (Hingston *et al*., 1974; Parfitt and Smart, 1978; Barekzai and Mengel, 1985; Parfitt, 1978). The phosphate adsorption is pH-dependent as the removal of OH^- is involved. This adsorption is favored at low pH while desorption occurs as the pH increases (Haynes, 1984).

1.5 Phosphate occlusion

Phosphate adsorbed at the surfaces of Fe and Al oxides may further bind to amorphous hydrated Fe and Al oxides and amorphous aluminosilicates (Huang and Schnitzer, 1986; Lambers *et al*., 2006). This phosphate is called occluded phosphate. The phosphate ions are trapped within the matrix of amorphous oxide and amorphous aluminosilicates (Ottow *et al*., 1991). The occluded phosphate is very strongly fixed and is unavailable to plants (Walker and Syers, 1976; Wada, 1985). The principle of phosphate occlusion is shown in Figure 3. Phosphate is adsorbed at the surface of the Fe oxide and then further binds covalently to the amorphous hydrated Fe oxides, resulting in phosphate occlusion.

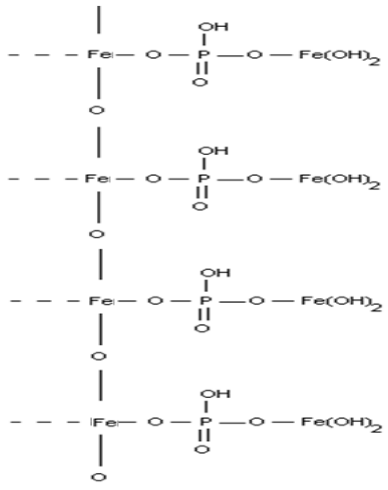

Figure 3: Principle of phosphate occlusion: Phosphate ions occluded by the Fe oxides (modified after Ottow *et al*., 1991)

1.6 Phosphate ageing

When acid soils are fertilized with P, most of the phosphate is adsorbed and then becomes occluded, termed phosphate ageing (Parfitt *et al*., 1975; Parfitt and Smart, 1978). It is the process in which phosphate is converted into occluded form with time, which appears mostly in acid soils.

1.7 Cluster roots and phosphorus bioavailability

Plant species show various adaptations to enhance P uptake from the soil under P starved conditions (Richardson *et al*., 2007). These adaptations may include alterations in root growth, increase in root hair density, topsoil foraging, formation of specialized roots, increase in release of various organic compounds via roots and formation of mycorrhizal associations (Fitter, 1985; Gerke, 1994; Keerthisinghe *et al*., 1998; Gerke *et al*., 2000; Lynch, 2005; Hill *et al*., 2006; White and Hammond, 2008; Fang *et al*., 2009; Brundrett, 2009; Jansa and Gryndler, 2010; Gerke, 2015b).

One of the forms of specialized roots is cluster roots. The cluster roots are lateral roots having bottle brush-like clusters of rootlets (Johnson *et al*., 1996; Watt and Evans, 1999; Lamont, 2003). Their role in utilizing soil P under P-deficient conditions has been well documented. These roots are typically found in soils with a low concentration of plant-available P, though some plant species can form them even under adequate plant-available soil P (Watt and Evans, 2003), though their inverse relationship persists (Shen *et al*., 2003). Low plant-internal P status triggers the formation of the cluster roots (Neumann and Martinoia, 2002). The strong interception of the cluster roots with soil helps in increased nutrient uptake (Gould, 1998).

Due to large mats of rootlets, the surface area of roots is substantially increased and these are in contact with a large volume of rhizosphere in a very small area. The quantity of plants exudates released per unit area by the cluster roots is much higher than by other root types. The cluster roots are present in many families of plants. White lupin (*Lupinus albus* L.) has been extensively used for the study of the cluster roots.

The cluster roots release root exudates such as carboxylates (mainly citrate, oxalate, oxaloacetate, malate, malonate, lactate and succinate), protons (H^+), phosphatases and phenolics (Neumann *et al*., 1999; Hinsinger, 2001; Roelofs *et al*., 2001; Yan *et al*., 2002; Zhu *et al*., 2005). Most of organic acids are present in dissociated forms within plants due to their

low dissociation constant as compared to neutral pH of the plant cells (Jones, 1998; Ryan *et al*., 2001). Therefore, they are released in the form of ions into the soil. Organic anions contribute to P mobilization by displacing the phosphate from adsorbing sites and chelating the metal ions which can adsorb P, and form soluble complexes with P (Gardner *et al*., 1983; Dinkelaker *et al*., 1989; Jones, 1998; Neumann and Römheld, 1999; Kirk, 1999; Hinsinger, 2001; Ryan *et al*., 2001; Shen *et al*., 2003; Wang *et al*., 2007; Wang *et al*., 2015).

Proton secretion decreases the soil pH and mobilizes Ca-bound P (Gardner *et al*., 1983; Dinkelaker *et al*., 1989; Kirk, 1999; Neumann and Römheld, 1999; Hinsinger *et al*., 2003; Shen *et al*., 2004; Tang *et al*., 2004). The secretion of phosphatases helps in the solubilization of organic P (Dinkelaker *et al*., 1997; Li *et al*., 1997; Gilbert *et al*., 1999; Neumann *et al*., 1999; Neumann *et al*., 2000; Richardson *et al*., 2000; George *et al*., 2004). Phenolics may mobilize occluded phosphate by reduction of the mineral oxides, and inhibit microbial growth (Lamont, 1972; Neumann *et al*., 2000; Weisskopf *et al*., 2006).

1.8 Objectives of the study

Occluded P is an important soil-P fraction particularly in highly weathered acid soils. It can contribute substantially to the soil-solution P under P-deficient conditions of acid soils by making it bioavailable. Plants with specialized roots such as the cluster roots may be able to mobilize and utilize this occluded phosphate by reducing the mineral oxides. The attention behind this study was to understand and investigate the dynamics of aged P in arable soils under controlled conditions with the following objectives:

1. To better understand the process of phosphate ageing.
2. To investigate the kinetics of phosphate ageing and phosphate adsorption in two different soils.
3. To investigate the relationship between applied and aged P in soil.
4. To investigate the bioavailability of aged P.

To achieve these objectives, it was hypothesized:

1. Phosphate-ageing increases with time.
2. Phosphate occluded by Fe oxides is plant-available.
3. Phosphate occluded by Al oxides is not plant-available.

2 Material and Methods

2.1 Phosphate ageing in a Luvisol topsoil and a Ferralsol

2.1.1 Soils

A Luvisol topsoil and a Ferralsol were selected to study the phosphate-ageing process. The chosen soils for the incubation experiment were collected from two sites in Hesse, Germany. Physicochemical characteristics of these soils are given in Table 1. Two other pH levels of these soils were adjusted according to a pre-experiment (see below).

2.1.2 Pre-experiment: pH buffer-curve experiment

The objective of this experiment was to find out how much acid or base had to be applied to adjust the pH of the soils. In the soil incubation experiment, each soil used had two pH levels i.e. 7.2 and 5.5. The pH of the Luvisol topsoil was 7.2; its pH was reduced to pH 5.5 by adding H^+ as HCl. The pH of the Ferralsol was 5.5; hence, its pH was increased to 7.2 by adding OH^- as NaOH.

Two hundred g of each soil (≤ 2 mm) were filled into small plastic pots. Various concentrations of H^+ (i.e. 0, 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20 mmol kg^{-1} soil) were applied to the Luvisol topsoil. Similarly, various concentrations of OH^- (i.e. 0, 1, 2, 3, 4, 5, 7, 10 mmol kg^{-1} soil) were applied to the Ferralsol. Each treatment had three replications. Soil moisture was maintained at 60% of maximum water-holding capacity. These pots were placed in a growth chamber at 25°C. After 1-week incubation, soil samples were dried at 40°C and were ground to measure pH.

Table 1: Physicochemical properties of the soils used in experiments.

Parameter	**[1]Ferralsol**	**[2]Luvisol topsoil**	**[3]Luvisol subsoil**
pH, 0.01 M $CaCl_2$	5.5	7.2	7.2
Total C, mg kg^{-1} soil	3300	17500	2800
Total N, mg kg^{-1} soil	200	1700	200
Total S, mg kg^{-1} soil	800	700	200
CAL P, mg kg^{-1} soil	2.32	39.53	5.94
[4]Fe oxide-adsorbed P, mg kg^{-1} soil	134.35	23.63	5.21
[4]Al oxide-adsorbed P, mg kg^{-1} soil	5.03	1.50	2.72
[4]Fe oxide-occluded P, mg kg^{-1} soil	5.76	1.52	0.94
[4]Al oxide-occluded P, mg kg^{-1} soil	1.17	not detectable	not detectable
CAL K, mg kg^{-1} soil	6.67	169.01	38.81
Exch. Mg, mg kg^{-1} soil	110.5	63.3	166.0
DTPA Cu, mg kg^{-1} soil	not detectable	1.58	0.60
DTPA Mn, mg kg^{-1} soil	11.10	20.20	11.03
DTPA Fe, mg kg^{-1} soil	12.20	57.60	34.65
Oxalate Fe, g kg^{-1} soil	2.20	1.90	1.42
Dithionite Fe, g kg^{-1} soil	16.50	5.70	5.92
Oxalate Al, g kg^{-1} soil	0.90	0.60	1.26
Dithionite Al, g kg^{-1} soil	1.70	0.50	1.27
CEC, cmol kg^{-1} soil	3.20	15.20	9.80
Sand, g kg^{-1} soil	304	88	479
Silt, g kg^{-1} soil	395	668	345
Clay, g kg^{-1} soil	301	245	176
Water-holding capacity, %	31.6	33.3	30.0
Horizon	(mixture of horizons)	(0 – 25 cm)	(80 – 120 cm)
Texture	Clay loam	Silt loam	Loam

[1]The Ferralsol is a mixture of various soil horizons. It was collected from Lich (Vogelsberg area) in central Hesse, Germany.

[2]The Luvisol topsoil has developed from loess and was collected from a farmer's field in Hünfeld, Hesse, Germany.

[3]The Luvisol subsoil was collected from Kleinlinden near Giessen, Hesse, Germany.

[4]P fractionation according to Chang and Jackson (1957).

2.1.3 Soil-incubation experiment

The Luvisol topsoil and the Ferralsol were incubated for 1 d, 3 months, and 6 months in a growth chamber at 25°C. There were two pH levels i.e. 7.2 and 5.5 of each soil and two P levels i.e. 0 and 100 mg kg^{-1} soil as KH_2PO_4.

Factors	**levels**
Soil	Luvisol topsoil, Ferralsol
P	0, 100 (mg kg^{-1} soil)
pH	7.2, 5.5
Incubation time	1d, 3 months, 6 months

Soils were incubated in plastic pots, each having 1 kg of soil. N and K were applied as plants were grown after the 6-months incubation. After completion of each incubation time, soil samples were dried at 40°C and then were ground for analyses. Soil parameters studied were pH, CAL P, and P fractions.

Table 2: Nutrients applied to soils at the start of soil incubation

Nutrient	**Amount (mg kg^{-1} soil)**	**Compound**
N	200	NH_4NO_3
K	250	KCl + KH_2PO_4
P (P+ treatments)	100	KH_2PO_4
Mg	50	$MgSO_4$

2.1.4 Plant growth experiment

Maize (*Zea mays* L. cv. Amadeo) and white lupin (*Lupinus albus* L. cv. Amiga) were grown in 6 months-incubated soils. Soil treatments were the same as in the incubation experiment i.e. two soil types, two pH levels and two P levels. Plants were grown in a growth chamber at 60% relative humidity and 16 h light time. The temperature at day time was 23°C and at night was 16°C. The light intensity was 700 $\mu E\ m^{-2}\ s^{-1}$. The lamps used for light were HQI-T 400 W/D q968 (made by OSRAM POWERSTAR, Germany). Plants were sown in pots. Each pot had 1 kg of soil with two plants. Micronutrients were also applied as given in Table 3. Water content was maintained at 60% of maximum water-holding capacity. After 6 weeks, plants were harvested. Soil (pH, CAL P) and plant parameters (fresh mass, dry mass, and P content) were determined.

Table 3: Nutrients applied to soils before plant sowing.

Nutrient	Amount (mg kg^{-1} soil)	Compound
Cu	5	$CuSO_4$
Mn	20	$MnSO_4$
Zn	10	$ZnSO_4$
B	1	H_3BO_3
Mo	0.2	$(NH_4)_6Mo_7O_{24}$

2.2 Phosphate kinetics in the Luvisol topsoil and the Ferralsol

2.2.1 Soil incubation I

The soils (the Luvisol topsoil and the Ferralsol) were incubated for 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h in pots at 25°C in the growth chamber. Each pot had 1 kg of soil. Potassium dihydrogen phosphate (KH_2PO_4) was used as P source at a rate of 100 mg P kg^{-1} soil. There were four replications. Water content was maintained at 60% of maximum water-holding capacity of soils. Incubation was done at 25°C in the growth chamber.

2.2.2 Soil incubation II

The soils (the Luvisol topsoil and the Ferralsol) were incubated for 24 h in pots. There were various P levels i.e. 0, 100, 150, 200, 250, 500 mg P kg^{-1} soil. Each pot had 1 kg of soil. Incubation was done at 25°C in the growth chamber. P was applied as potassium dihydrogen phosphate (KH_2PO_4). There were three replications. Water content was maintained at 60% of maximum water-holding capacity of soils.

2.2.3 Parameters studied

After completion of incubation, soil samples were dried at 40°C and then were ground for CAL-P analysis.

2.3 Phosphate ageing in a Luvisol subsoil amended with Fe oxide (Goethite) and Al oxide (Gibbsite)

2.3.1 Material

The Luvisol subsoil was investigated to study the effect of the Fe and Al oxide on the phosphate ageing. Physicochemical characteristics of this soil are given in Table 1.

2.3.2 Pre-experiment: pH buffer-curve experiment

In the soil-incubation experiment, there were three pH levels of the Luvisol subsoil. The pH of this soil was 7.2, while the other two pH levels (5.2 and 4.6) were adjusted. The objective of this pre-experiment was to find out how much acid had to be applied to adjust the pH values of the Luvisol subsoil. Two hundred g soil were filled into small plastic pots. Various concentrations of H^+ (i.e. 0, 1, 2, 3, 4, 5, 7, 10, 12, 14, 16, 18, 20 mmol kg^{-1} soil) were applied as HCl. Each treatment had three replications. Water content was maintained at 60% of maximum water-holding capacity. These pots were put into a controlled growth chamber at 25°C. After two-week incubation, soil samples were dried at 40°C and were ground to measure pH.

2.3.3 Synthesis of Goethite and Gibbsite

Goethite and Gibbsite minerals are oxides of Fe and Al, respectively. These minerals were prepared according to a method described by Schwertmann and Cornell (1991, Chap. 5, method 4). For Goethite, 100 mL of 1 M $Fe(NO_3)_3$ solution were filled into a 2 L polyethylene flask, then 180 mL of 5 M KOH solution were added under rapid stirring. Red brown ferrihydrate precipitated. Water was added to fill up to 2 L and the flask was kept at 70°C for 60 h. Then the suspension was washed with deionized water by centrifugation (3840 *g* for 5 min) and the mineral was dried at 40°C and was ground (≤ 1 mm). Gibbsite was prepared in the same way by using $Al(NO_3)_3$ solution.

2.3.4 Soil incubation

For this experiment, the Luvisol subsoil was incubated in plastic buckets. Each bucket had 3 kg of soil. There were three pH levels, i.e. 7.2, 5.2, 4.6 and two P levels i.e. with P (P+) and without P (P-). In P+ treatments, 200 mg P kg^{-1} soil were applied as KH_2PO_4. Goethite and

Gibbsite minerals were added as P adsorbents at the rate of 300 mmol Fe and Al kg^{-1} soil. There were four replications per treatment. The soils were incubated for 1 week, 3 months, and 6 months, respectively, at 25°C in a growth chamber. Soil water-content was maintained at 60% water-holding capacity throughout the incubation period.

Factors	**levels**
P	0, 200 (mg kg^{-1} soil)
pH	7.2, 5.2, 4.6
P adsorbents	Control, Fe oxide, Al oxide
Incubation time	1 week, 3 months, 6 months

2.3.5 Parameters studied

Soil parameters studied were pH, CAL P, oxalate-extractable Fe and Al, dithionite-extractable Fe and Al, and P fractions.

2.4 Bioavailability of Fe oxide and Al oxide-occluded phosphate

2.4.1 Synthesis of Fe oxide and Al oxide-occluded phosphate

Fe oxide and Al oxide-occluded phosphate were synthesized by mixing the P solution to freshly prepared Fe and Al oxides (see Chapter 2.3.3). For synthesis of Fe oxide-occluded phosphate, 100 mL of 1 M $Fe(NO_3)_3$ solution were filled into a 2 L polyethylene flask, then 180 mL of 5 M KOH solution were added under rapid stirring. Red brown ferrihydrate precipitated. Then 500 mL of 1 M KH_2PO_4 were added. Water was added to a volume of 2 L and the flask was kept at 70°C for 60 h. Then the suspension was washed with deionized water by centrifugation (3840 *g* for 5 min) and the mineral was dried at 40°C and ground. Dried mineral was washed sequentially with different extractants (see Chapter 2.5.1.5 and Table 5) to remove all P fractions except the occluded phosphate. Al oxide-occluded phosphate was synthesized in the same way using $Al(NO_3)_3$ solution.

2.4.2 Plant-growth experiment

In this experiment, maize (*Zea mays* L. cv. Amadeo) and white lupin (*Lupinus albus* L. cv. Amiga) were cultivated in the Luvisol subsoil in pots. Each pot had 1 kg of soil with one plant. Ten mg P kg^{-1} soil were applied as Al oxide-occluded P, Fe oxide-occluded P, and $Ca(H_2PO_4)_2$. Plants were cultivated under controlled climatic conditions with 16 h light time. Temperature at day was 25°C and at night was 18°C. The light intensity was 500 $\mu E\ m^{-2}\ s^{-1}$.

The lamps used for light were HQI-T 400 W/D q968 (made by OSRAM POWERSTAR, Germany). Macro and micronutrients were also applied (Table 4). Soil water-content was maintained at 60% of maximum water-holding capacity. There were four replications. Plants were harvested 35 d after sowing.

Table 4: Nutrients applied to soil before plant sowing.

Nutrient	Amount (mg kg^{-1} soil)	Compound
N	200	NH_4NO_3
K	250	KCl
Mg	50	$MgSO_4$
Cu	5	$CuSO_4$
Mn	20	$MnSO_4$
Zn	10	$ZnSO_4$
B	1	H_3BO_3
Mo	0.2	$(NH_4)_6Mo_7O_{24}$

2.4.3 Parameters studied

Soil parameters: occluded P

Plant parameters: fresh and dry mass, shoot and root P-content

2.5 Analyses

2.5.1 Soil analyses

2.5.1.1 pH

Ten grams finely ground soil (≤ 2 mm) were filled into a small glass tube and 25 mL 0.01 M $CaCl_2$ were added. The suspension was shaken with hand for 3-4 s and was kept with opened lid for 15 min. This process was repeated five times. The pH meter (CG 805) was calibrated with standard buffer solutions, having pH 7 and 4. The pH was recorded by immersing a pH electrode (glass electrode) into clear solution until pH meter showed constant value (Grewling and Peech, 1960).

2.5.1.2 Calcium-acetate-lactate-extractable P (CAL P)

Phosphate was extracted with a buffered solution (pH 4.1) of calcium lactate, calcium acetate and acetic acid. CAL P is regarded as plant-available soil P.

Five grams soil (≤ 2 mm) were filled into a plastic bottle and one spoon of coal was added. Then 100 mL CAL-extraction solution were added and the suspension was shaken for 2 h. After filtration, 20 mL filtrate were filled into a 25 mL flask. One milliliter conc. HNO_3 was added. Then, after mixing, 4 mL vanadate-molybdate reagent were added and P was determined with a spectrophotometer (Zeiss photometer) at 406 nm (Schüller, 1969).

2.5.1.3 Oxalate-extractable Fe and Al

Fe and Al oxides are present in soil as amorphous and crystalline forms. Amorphous Fe and Al are extracted with an oxalate solution. Two grams of soil were filled into a bottle and 100 mL oxalate solution (mixture of oxalic acid and ammonium oxalate) were added and the suspension was shaken in a dark room for 1 h. After filtration, Fe was determined using the atomic absorption spectrophotometery (AAS) at 248.3 nm and Al was determined at 309.3 nm (McKeague and Day, 1966). The atomic absorption spectrophotometer used was Spectra AA 220FS made by VARIAN.

2.5.1.4 Dithionite-extractable Fe and Al

Amorphous and crystalline (combined) Fe and Al were extracted with sodium dithionite. Two grams of soil were filled into a 100 mL centrifuge bottle and 40 mL of 0.3 M Na-citrate and 10 mL 1 M $NaHCO_3$ were added. This suspension was heated at 70-80°C in a water bath with rapid mixing. One gram solid sodium dithionite was added, followed by further heating for 5 min. After centrifugation (3840 *g* for 5 min) and filtration, the supernatant was used to determine Fe and Al. Fe was determined using the atomic absorption spectrophotometery (AAS) at 248.3 nm and Al was determined at 309.3 nm (McKeague and Day, 1966). The atomic absorption spectrophotometer used was Spectra AA 220FS made by VARIAN.

2.5.1.5 Fractionation of soil P

2.5.1.5.1 Extraction

Soil-P fractionation was carried out using an established sequential extraction method (Chang and Jackson, 1957). The extractants and the procedure are described as follows.

Table 5: Extractants used for the sequential extraction of various P fractions in soil.

P fraction	Extractant	Extraction time
Water-soluble P	1 M NH_4Cl	30 min
Al P (Al-bound P)	0.5 M NH_4F	1 h
Fe P (Fe-bound P)	0.1 M NaOH	17 h
Ca P (Ca-bound P)	0.5 M H_2SO_4	1h
Fe oxide-occluded P	0.3 M Na-citrate + 1 g Na-dithionite	30 min
Al oxide-occluded P	0.5 M NH_4F	1h

Water-soluble P: Finely ground (≤ 1 mm) 5 g soil were filled into a centrifuge flask and extracted with 50 mL of 1 M NH_4Cl for 30 min with constant shaking. After the centrifugation (3840 *g* for 5 min), the supernatant was saved to determine the water-soluble P while precipitate was further processed to extract Al-bound P (Al P) after washing twice with 25 mL of 10 M NaCl solution.

Al P: The soil sample after the extraction of the water-soluble P was washed twice with double-distilled water and was extracted with 50 mL of neutral 0.5 M NH_4F shaking for 1 h. After the centrifugation (3840 *g* for 5 min), the supernatant was saved to determine the Al P while the precipitate was further processed to extract Fe-bound P (Fe P) after washing twice with 25 mL of 10 M NaCl solution.

Fe P: The soil sample after the extraction of the Al P was washed twice with double-distilled water and was extracted with 50 mL of 0.1 M NaOH shaking for 17 h. After the centrifugation (3840 *g* for 5 min), the supernatant was saved to determine the Fe P while the precipitate was further processed to extract Fe oxide-occluded P after washing twice with 25 mL of 10 M NaCl solution.

Fe oxide-occluded P: The soil sample after the extraction of the Fe P was washed twice with double-distilled water and was extracted with 40 mL of 0.3 M sodium citrate and 1 g sodium

dithionite ($Na_2S_2O_4$). The suspension was heated in a water bath at 90°C for 15 min with constant shaking. After the centrifugation (3840 *g* for 5 min), the supernatant was saved to determine the Fe oxide-occluded P while the precipitate was further processed to extract Al oxide-occluded P after washing twice with 25 mL of 10 M NaCl solution.

Al oxide-occluded P: The soil sample after the extraction of the Fe oxide-occluded P was washed twice with double-distilled water and was extracted with 50 mL of neutral 0.5 M NH_4F by shaking for 1 h. After the centrifugation (3840 *g* for 5 min), the supernatant was saved to determine the Al oxide-occluded P (Chang and Jackson, 1957).

2.5.1.5.2 Determination of P

The P concentrations in the clear supernatants were determined using the blue molybdate method (Murphy and Riley, 1962).

2.5.2 Plant analyses

2.5.2.1 P content

At 105°C-dried plant sample of 0.5 g was ashed in a porcelain crucible at 520°C in a muffle furnace for one night. Then 2 mL double-distilled water and 5 mL of 5 M HNO_3 were added into the crucible and the solution was constantly heated and transferred over a white band filter into a 50 mL volumetric flask. The P concentration was measured using the yellow method with a spectrophotometer (Zeiss photometer) at 450 nm (Allen *et al*., 1974).

2.6 Statistics

Statistical package Sigma Plot 11 was used to check the significance of different treatments at 5% probability. Analysis of variance (ANOVA) and Fisher's LSD test were performed to compare the treatment means. The standard error of the mean (SE) and standard deviation (SD) were calculated with Microsoft Excel 2007.

3 Results

3.1 Phosphate ageing in the Luvisol topsoil and the Ferralsol

3.1.1 pH buffer-curves for the soils

The Luvisol topsoil and the Ferralsol were used in the soil incubation experiment. In this experiment, each of the soils had two pH levels i.e. 7.2 and 5.5. One pH level of each soil was adjusted. These adjusted pH levels were achieved by addition of H^+ and OH^- to the soils and amounts of these ions required for adjustment of pH 7.2 and 5.5 were found from the pH buffer-curves of these soils (Figure 4 and Figure 5).

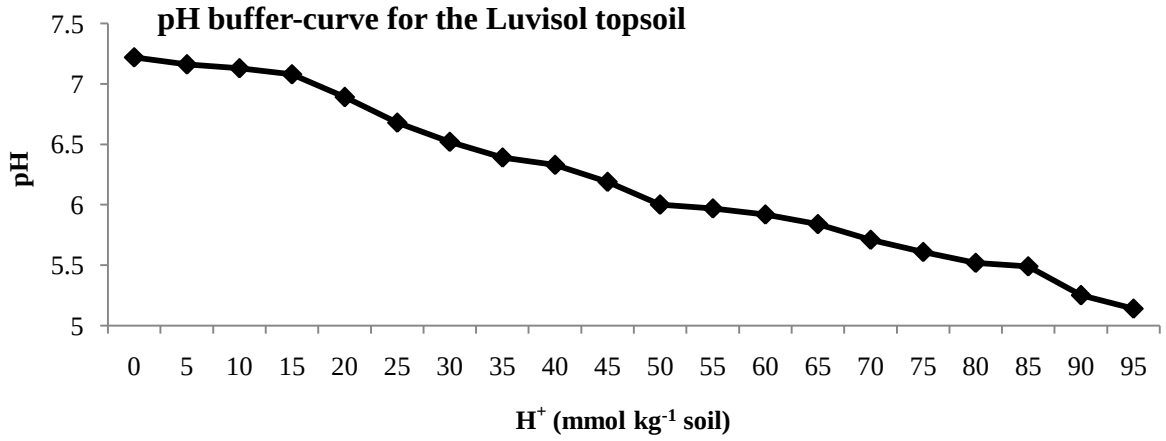

Figure 4: Effect of H^+ addition (applied as HCl) on pH of the Luvisol topsoil after 1 week-soil incubation. Values are the arithmetic means of two replicates.

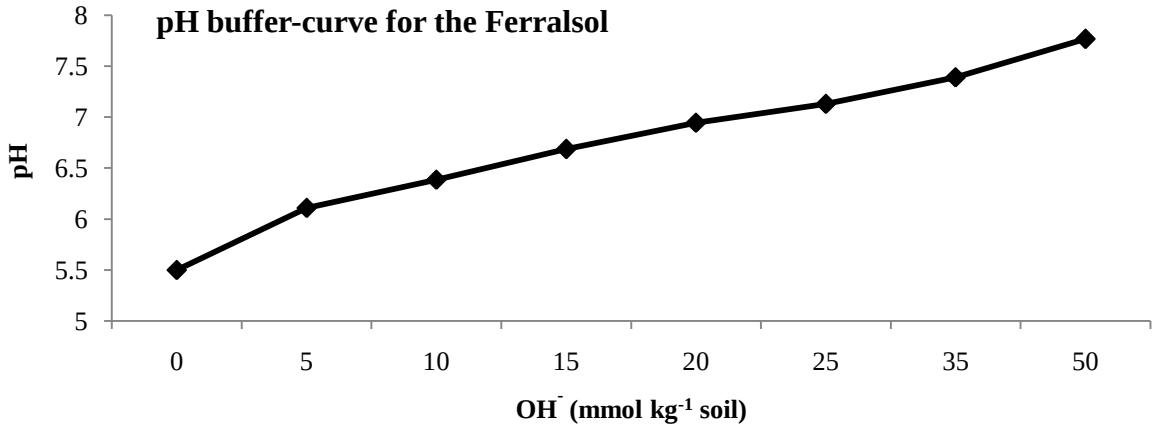

Figure 5: Effect of OH^- addition (applied as NaOH) on pH of the Ferralsol after 1 week-soil incubation. Values are the arithmetic means of two replicates.

Figure 4 shows the pH buffer curve for the Luvisol topsoil. Various amounts of H^+ were applied as HCl to decrease the pH. Figure 5 shows the pH buffer-curve for the Ferralsol. Various amounts of OH^- were applied as NaOH to increase the pH.

The Luviosl topsoil had pH 7.2; in the soil-incubation experiment, its other pH level (5.5) was adjusted by adding 93 mmol H^+ kg^{-1} soil in the form of HCl. The Ferralsol had pH 5.5; in the soil-incubation experiment, its other pH level of 7.2 was adjusted by the addition of 32 mmol OH^- kg^{-1} soil in the form of NaOH.

3.1.2 pH of soils after soil incubations

Figure 6 shows the pH values of the soils (the Luvisol topsoil and the Ferralsol) after 1 d, 3 months, and 6 months of soil incubation. In the Luvisol topsoil at pH 5.5 (adjusted pH), the pH was below the adjusted value after 1 d-soil incubation and there was a slight non-significant increase with time in P+ and P- treatments. At pH 7.2 (non-adjusted pH), there was a slight non-significant decrease in the pH in P- treatment after 6 months while vice versa at P+ treatment.

In the Ferralsol at pH 5.5 (non-adjusted pH), there was a slight increase in pH with time. At pH 7.2 (adjusted pH), the pH was decreased with time. However, these pH changes of soils with time were statistically non-significant.

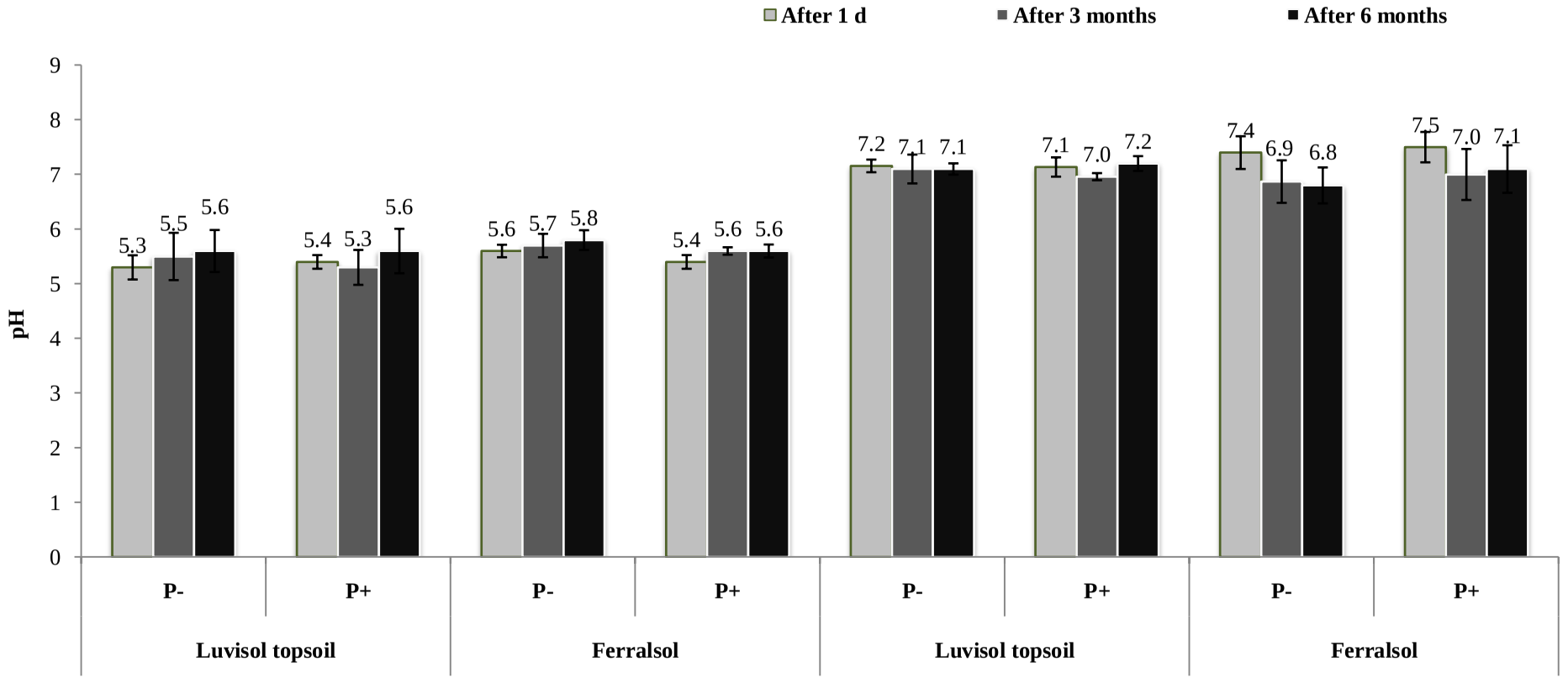

Figure 6: pH of the soils after 1 d, 3 months, and 6 months of incubation of the Luvisol topsoil and the Ferralsol in the treatments without P application (P-) and with 100 mg P kg^{-1} soil in form of KH_2PO_4 (P+). Values are the arithmetic means of three replicates ± SD.

3.1.3 Effect of P application, pH, incubation time, and soil type on the CAL-P concentrations in the soils

CAL P data after various incubation times (1 d, 3 months, and 6 months) are described in Figure 7. P fertilization had a significant effect on CAL-extractable P concentrations after 1 d, 3 months, and 6 months of soil incubation. The CAL-P concentrations were significantly higher in the P+ treatments (100 mg P kg^{-1} soil) than in the P- treatments (without P application) in both of the soils.

The effects of pH on the CAL-P concentrations were non-significant after 1 d of soil incubation (Figure 7A). After three months, pH had a significant effect on the CAL-P concentrations in the P- treatments. In the Luvisol topsoil, the CAL-P concentrations were significantly lower at pH 7.2 than at pH 5.5 in the P- treatments while in the Ferralsol the CAL-P concentrations were significantly higher at pH 7.2 than at pH 5.5 in the P- treatments. (Figure 7B). Similar results regarding the CAL-P concentrations were found after 6 months of soil incubation in the P- treatments in both of the soils. In the P+ treatment, the CAL-P concentration was significantly lower at pH 7.2 than at pH 5.5 in the Luvisol topsoil. The difference in the CAL-P concentration was non-significant in P+ treatments in the Ferralsol (Figure 7C).

The soils had a significant effect on the CAL-P concentrations after 1 d (Figure 7A). The CAL-P concentrations were significantly higher in the Luvisol topsoil than in the Ferralsol. These were very low in the Ferralsol. In this soil, most of the applied P (more than 90%) was not extractable with the CAL solution. Similar results regarding the CAL-P concentrations were found after 3 and 6 months of soil incubation (Figure 7: B and C).

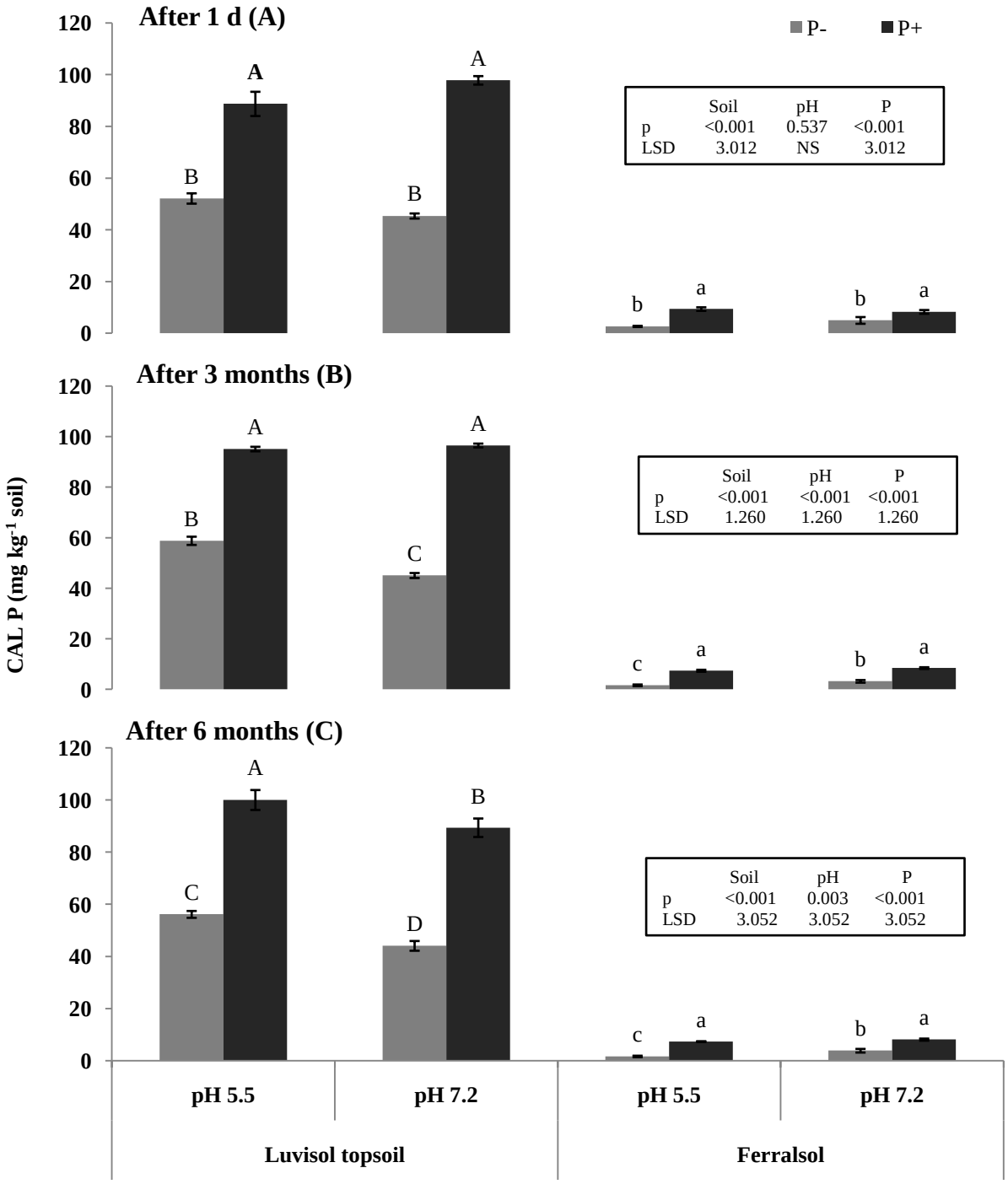

Figure 7: Effect of P application, pH, and soil type on the CAL-P concentrations in the Luvisol topsoil and the Ferralsol after 1 d (A), after 3 months (B), and after 6 months (C). Values are the arithmetic means of three replicates ± SE. Columns with different letters indicate significant difference at 5% level. In the legend, P- = 0 mg P kg^{-1} soil and P+ = 100 mg P kg^{-1} soil.

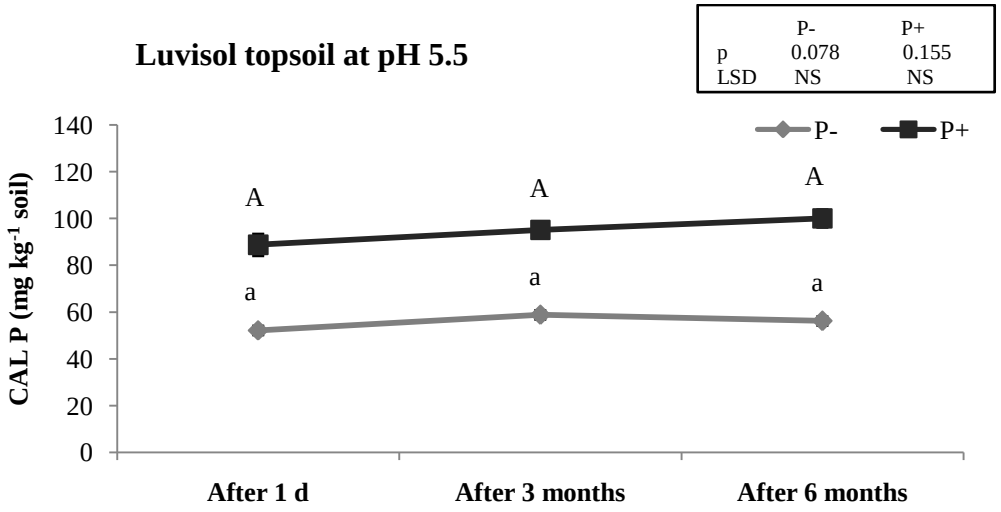

Figure 8: Effect of various incubation times on the CAL-P concentrations in the Luvisol topsoil at pH 5.5. Values are the arithmetic means of three replicates ± SE. Columns with different letters indicate significant difference at 5% level. In the legend, P- = 0 mg P kg^{-1} soil and P+ = 100 mg P kg^{-1} soil.

In the Luvisol topsoil at pH 5.5, there was no change in the CAL-P concentrations with time in the P+ treatment and a similar trend was observed in the P- treatment (Figure 8).

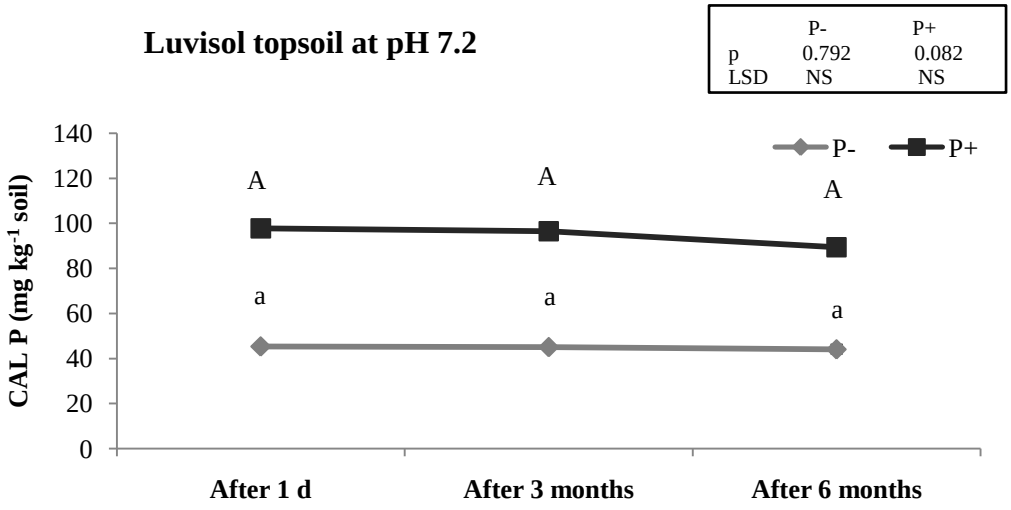

Figure 9: Effect of various incubation times on the CAL-P concentrations in the Luvisol topsoil at pH 7.2. Values are the arithmetic means of three replicates ± SE. Columns with different letters indicate significant difference at 5% level. In the legend, P- = 0 mg P kg^{-1} soil and P+ = 100 mg P kg^{-1} soil.

At pH 7.2 in the Luvisol topsoil, there was no significant change in the CAL-P concentrations with time in the P+ treatment while there was almost no change in the CAL-P concentrations in both P+ and P- treatments (Figure 9).

In the Ferralsol at pH 5.5 (Figure 10), the CAL-P concentration was significantly decreased after 3 months in the P+ treatment while in the last three months, the change was non-significant. Similar trend was observed in the P- treatment but the differences were non-significant.

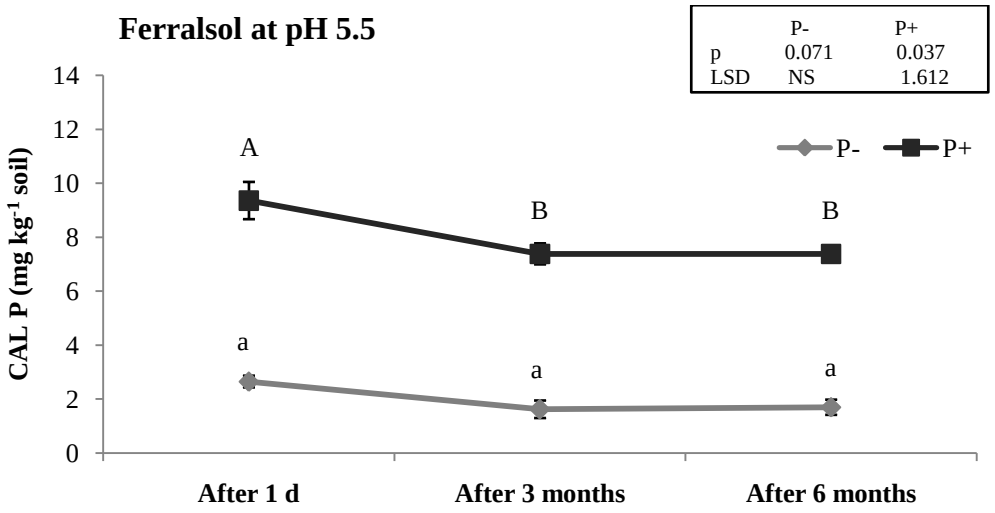

Figure 10: Effect of various incubation times on the CAL-P concentrations in the Ferralsol at pH 5.5. Values are the arithmetic means of three replicates ± SE. Columns with different letters indicate significant difference at 5% level. In the legend, P- = 0 mg P kg^{-1} soil and P+ = 100 mg P kg^{-1} soil.

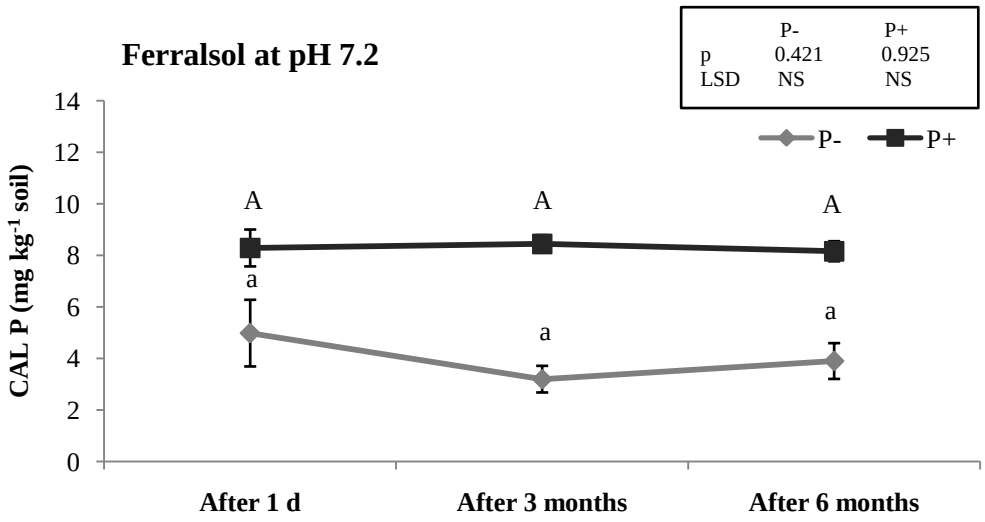

Figure 11: Effect of various incubation times on the CAL-P concentrations in the Ferralsol at pH 7.2. Values are the arithmetic means of three replicates ± SE. Columns with different letters indicate significant difference at 5% level. In the legend, P- = 0 mg P kg^{-1} soil and P+ = 100 mg P kg^{-1} soil.

At pH 7.2 (Figure 11), there was no significant change in the CAL-P concentration with time in both P treatments. In the P- treatments, there was a non-significant decrease in the CAL-P concentration after 3 months.

3.1.4 Effect of P, pH, and soil type on dry mass of maize and white lupin

Plant dry mass (shoot plus root) was significantly influenced by the soil type and P application. However, change in the pH did not affect the dry mass significantly except in the Luvisol topsoil, where maize had significantly higher dry mass when was grown at pH 7.2 than when was grown at pH 5.5 in the P+ treatments. Maize dry mass was significantly decreased in the Ferralsol as compared to the Luvisol topsoil. Similarly, the dry mass was decreased in the P- (without P application) as compared to P+ (100 mg P kg^{-1} soil).

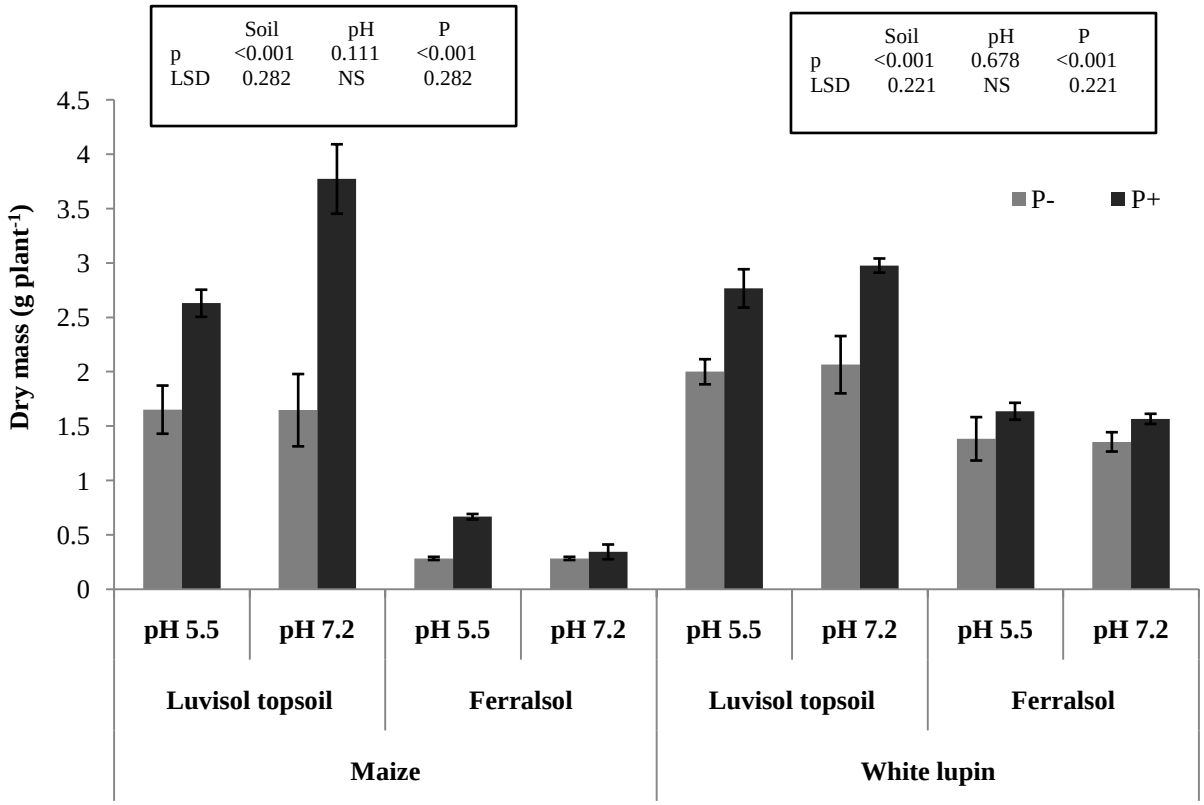

Figure 12: Effect of P application, pH, and soil type on the dry mass of maize and white lupin (shoot plus root). Values are the arithmetic means of three replicates ± SE. In the legend, P- = 0 mg P kg^{-1} soil and P+ = 100 mg P kg^{-1} soil.

The maximum effect of the P application was found in maize grown in the Luvisol topsoil at pH 7.2, while the minimum effect was found in the Ferralsol. Similar results were found in the case of white lupin. However, the differences between the soil types were small for white lupin relative to maize (Figure 12).

White lupin had significantly higher dry mass than maize when grown in the Ferralsol. In the Luvisol topsoil, the differences in the dry masses of maize and white lupin were statistically non-significant.

3.1.5 Effect of P, pH, and soil type on P content of maize and white lupin

There was a significant effect of the P application and the soil type on the P content (shoot plus root) of maize and white lupin in the Luvisol topsoil. The maximum effect on the P content was observed in maize.

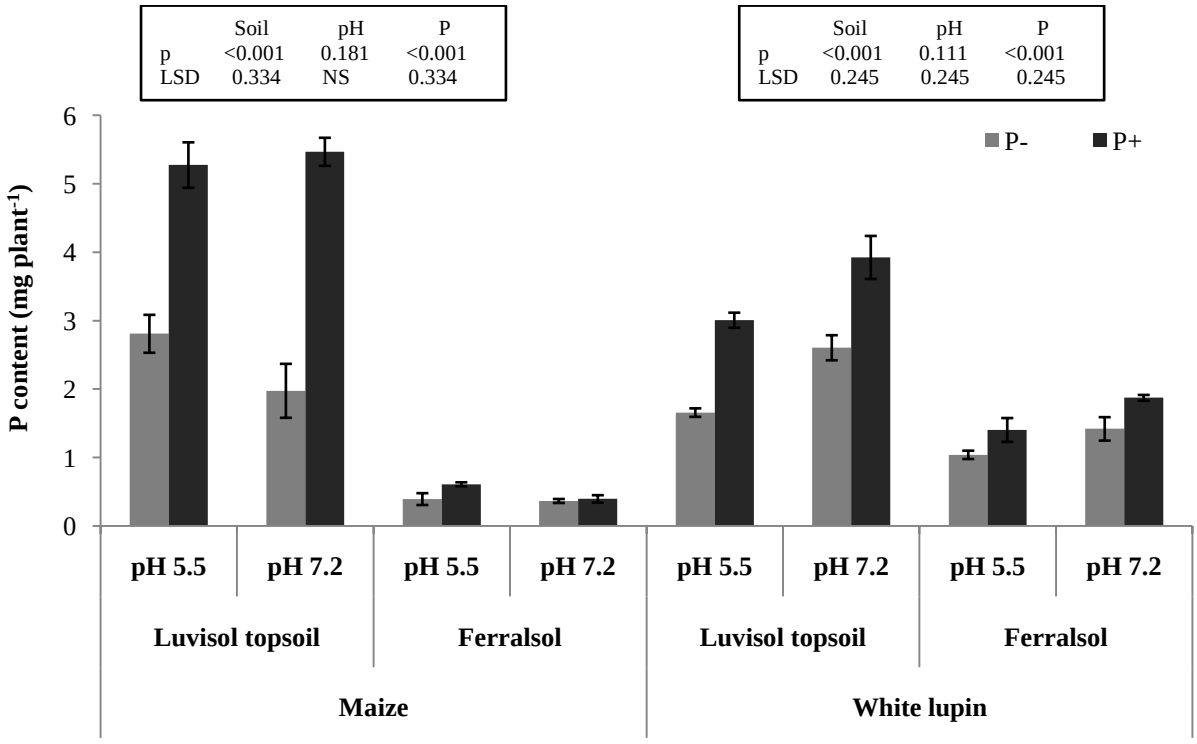

Figure 13: Effect of P application, pH, and soil type on the P content of maize and white lupin (shoot plus root). Values are the arithmetic means of three replicates ± SE. In the legend, P- = 0 mg P kg^{-1} soil and P+ = 100 mg P kg^{-1} soil.

In the Ferralsol, plants grown in the P+ treatments had higher P content than the plants grown in the P- treatments but these differences were statistically non-significant (Figure 13). A significant effect of pH on the P content was found when maize was grown in the Ferralsol (P+) where maize had a higher P content at pH 5.5 than at pH 7.2.

The pH had a significant effect on the P content of white lupin when it was grown in the Luvisol topsoil without P application (P-). Plants grown at pH 7.2 had a higher P content than those grown at pH 5.5. A similar trend was found in other treatments but these differences were statistically non-significant.

Plants grown in the Luvisol topsoil had a higher P content than those grown in the Ferralsol. The maximum contents were observed in the P+ treatments. Maize had a higher P content than white lupin when it was grown in the Luvisol topsoil with P application (P+). The maximum difference was observed at pH 5.5. In the P- treatments, the differences were non-significant. White lupin had a significantly higher P content than maize when grown in the Ferralsol at both pH and P levels.

3.1.6 Occluded-P concentrations in the soils before and after the cultivation of maize and white lupin

Maize and white lupin were cultivated in the 6 months-incubated soils. Occluded-P concentrations in soils before sowing and after harvest are shown in Figure 14. The changes in the occluded-P concentrations in the soil due to the plant cultivation were statistically non-significant in all treatments.

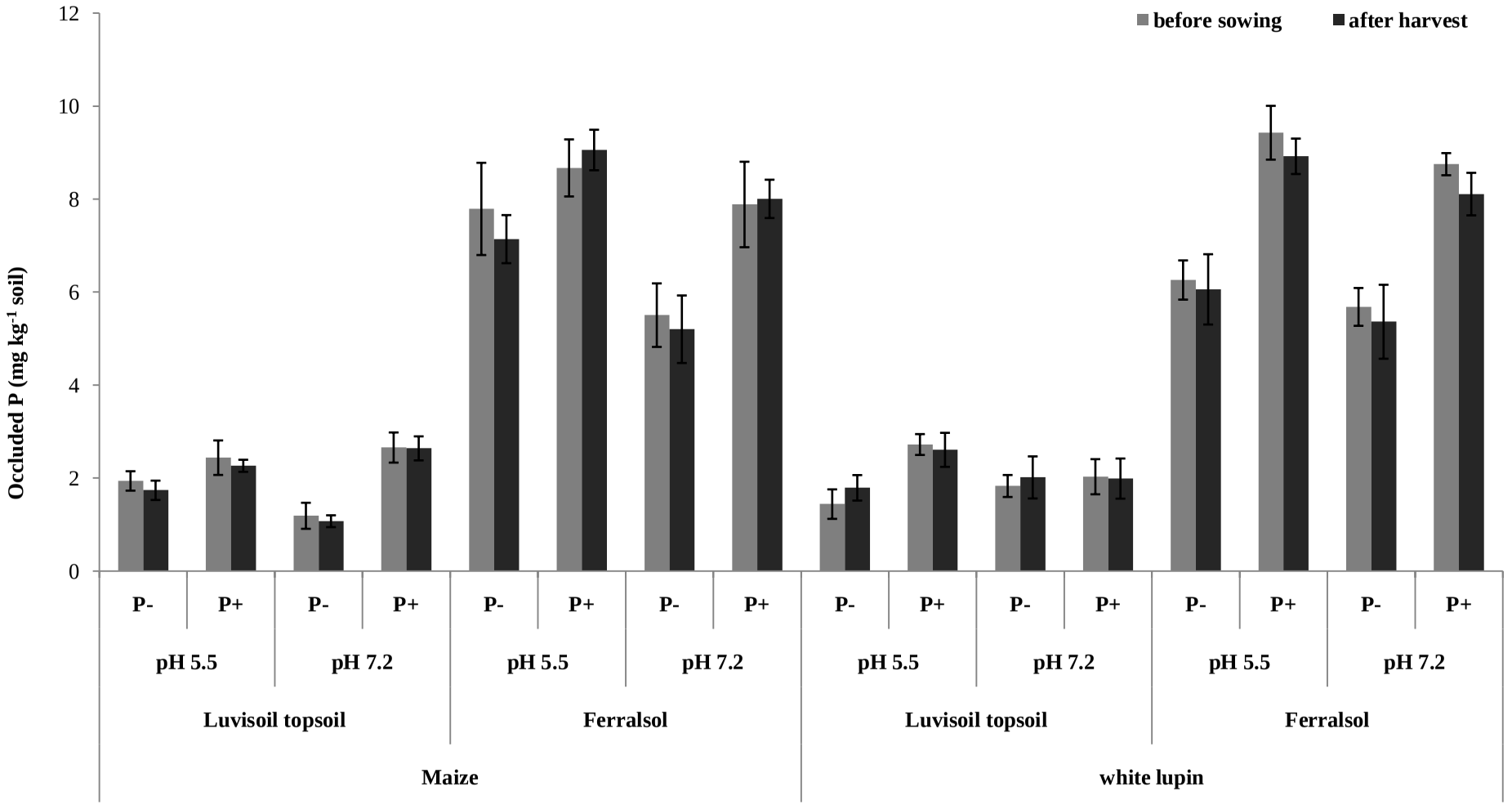

Figure 14: Occluded-P concentrations in soils before sowing and after harvest of maize and white lupin. Values are the arithmetic means of three replicates ± SE.

3.2 Phosphate kinetics in the Luvisol topsoil and the Ferralsol

3.2.1 Phosphate kinetics after various time intervals

Soils were incubated for various periods to study the phosphate kinetics in the Luvisol topsoil and the Ferralsol. Figure 15 shows the CAL-P concentrations in the soils after incubations with 100 mg P kg^{-1} soil (P+) and without P (P-) at various periods ranging from 0.5 h to 24 h. The Ferralsol had less CAL-P concentration than the Luvisol topsoil. The Figure 15 depicts that even after 0.5 h, most of the added P in the Ferralsol had become non-CAL-extractable and it decreased further after 1 h but then remained constant. In the P- treatment, changes in the CAL-P concentrations were non-significant. The Luvisol topsoil did not adsorb phosphate in the P+ treatment immediately. After 8 h of incubation, the CAL-P concentration decreased significantly. In the P- treatments, changes in the CAL-P concentrations with time were non-significant.

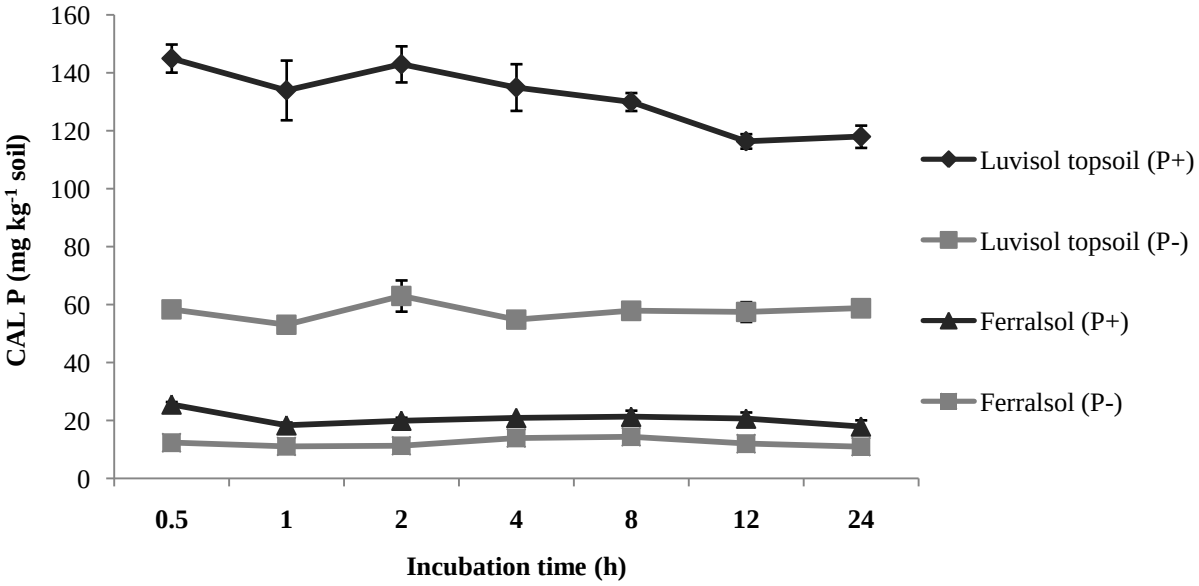

Figure 15: Effect of time on the CAL-P concentrations in the Luvisol topsoil and the Ferralsol. Values are the arithmetic means of four replicates ± SE. In the legend, P- = 0 mg P kg^{-1} soil and P+ = 100 mg P kg^{-1} soil.

3.2.2 Phosphate kinetics after various levels of P application

The Luvisol topsoil and the Ferralsol were incubated for 1 d with various levels of P application. Figure 16 shows the CAL-P concentrations in the soils when different levels of P were applied. There was a linear increase in the CAL-P concentration as the applied-P

concentration increased. Each P level had a significant effect on the CAL-P concentration except when 200 mg P kg^{-1} soil were applied in both soils.

Figure 17 presents net fixed P in the soils after 1 d when various P levels were applied. It shows that most of the P applied in the Ferrasol was aged, in contrast to the luvisol topsoil.

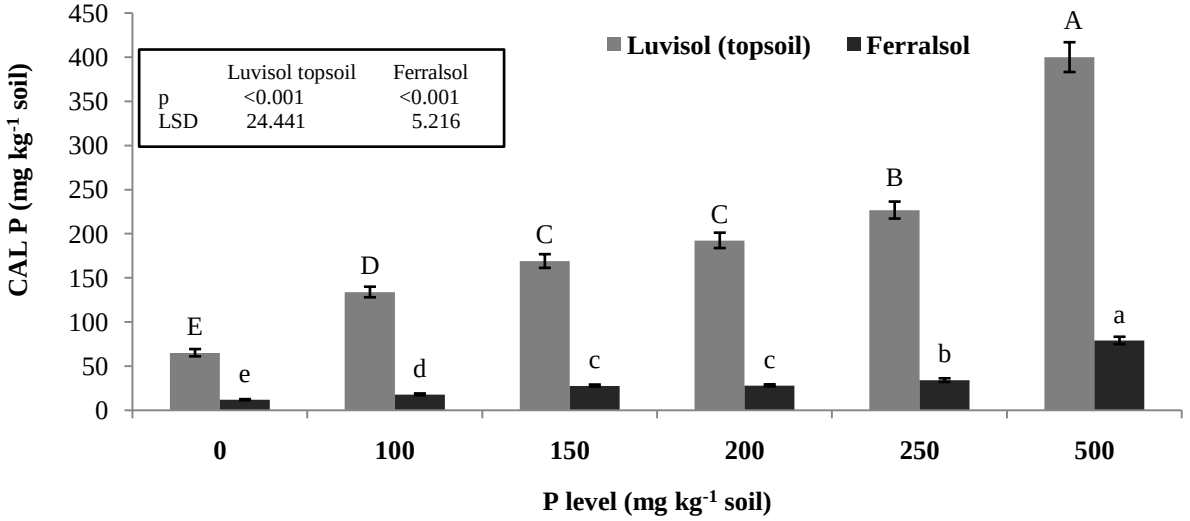

Figure 16: CAL-P concentration after 1-d incubation of the Luvisol topsoil and the Ferralsol at various levels of P application. Values are the arithmetic means of three replicates ± SE.

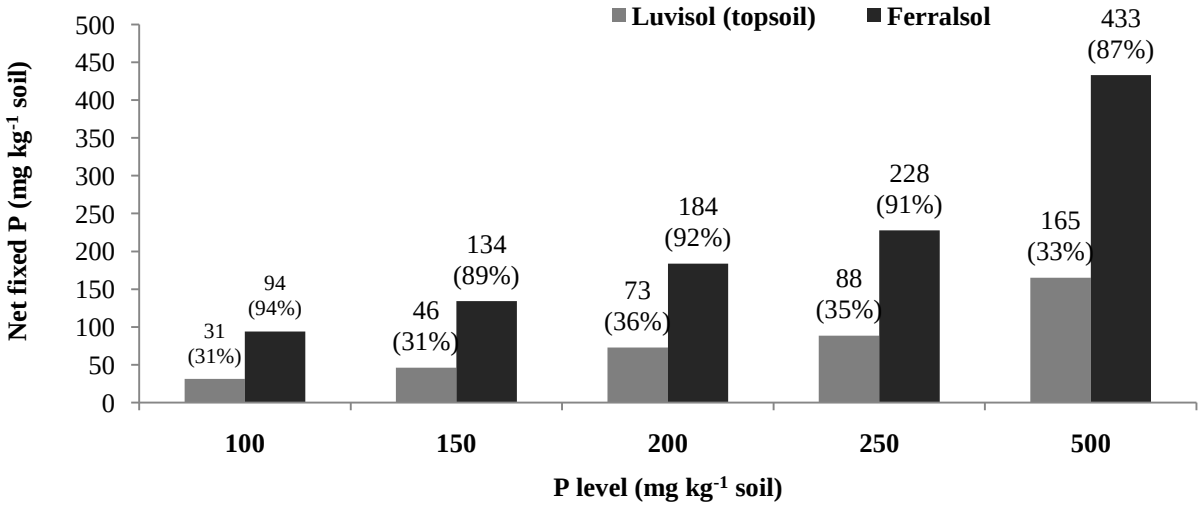

Figure 17: Net-fixed P after 1-d incubation of the Luvisol topsoil and the Ferralsol at various levels of P application. Values are the arithmetic means of three replicates. Net fixed P was calculated by subtracting CAL-P concentration of control treatment from the other treatment and was followed by subtraction from P applied.

3.3 Phosphate ageing in the Luvisol subsoil amended with Fe oxide (Goethite) and Al oxide (Gibbsite)

3.3.1 pH buffer-curve for the Luvisol subsoil

In the soil incubation experiment, the Luvisol subsoil had three pH levels i.e. 7.2, 5.2 and 4.6. The latter two pH levels were achieved by the addition of H^+ into the soil and amounts of these ions required for the adjustment of pH 5.2 and 4.6 were found from the pH buffer-curve of the soil (Figure 18). The Luviosl subsoil had pH 7.2; in the soil-incubation experiment, its other pH levels of 5.2 and 4.6 were adjusted by adding 135 and 170 mmol H^+ kg^{-1} soil in the form of HCl, respectively.

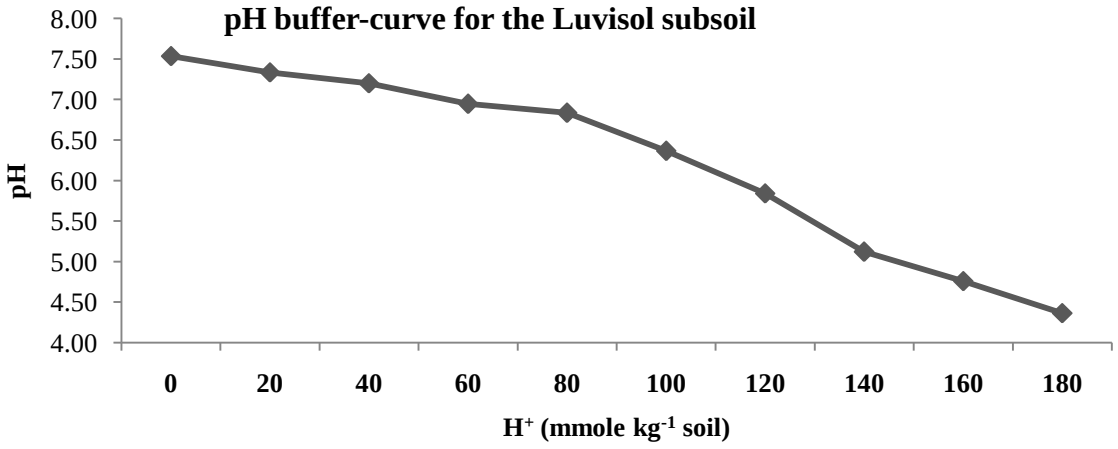

Figure 18: Effect of H^+ (applied as HCl) on the pH of the Luvisol subsoil after 1 week-soil incubation. Values are the arithmetic means of two replicates.

3.3.2 The X-ray diffraction analyses of Fe and Al oxides

Fe and Al oxides were used as P adsorbents to investigate the phosphate ageing in the incubation experiment of the Luvisol subsoil and for the synthesis of occluded phosphates. Phosphates occluded by Fe and Al oxides were used to investigate the bioavailability of occluded phosphates by maize and white lupin. The X-ray diffraction analyses of the Fe and Al oxides are shown in Figure 19 and Figure 20, respectively. Match, a computer software, was used to identify the minerals.

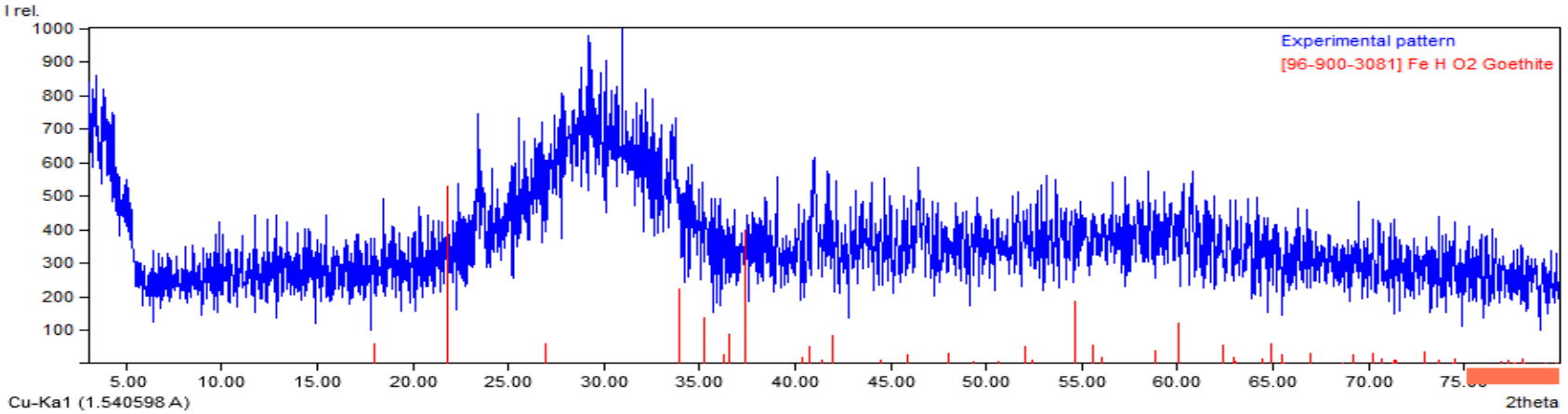

Figure 19: The X-ray diffraction analysis of Fe oxide

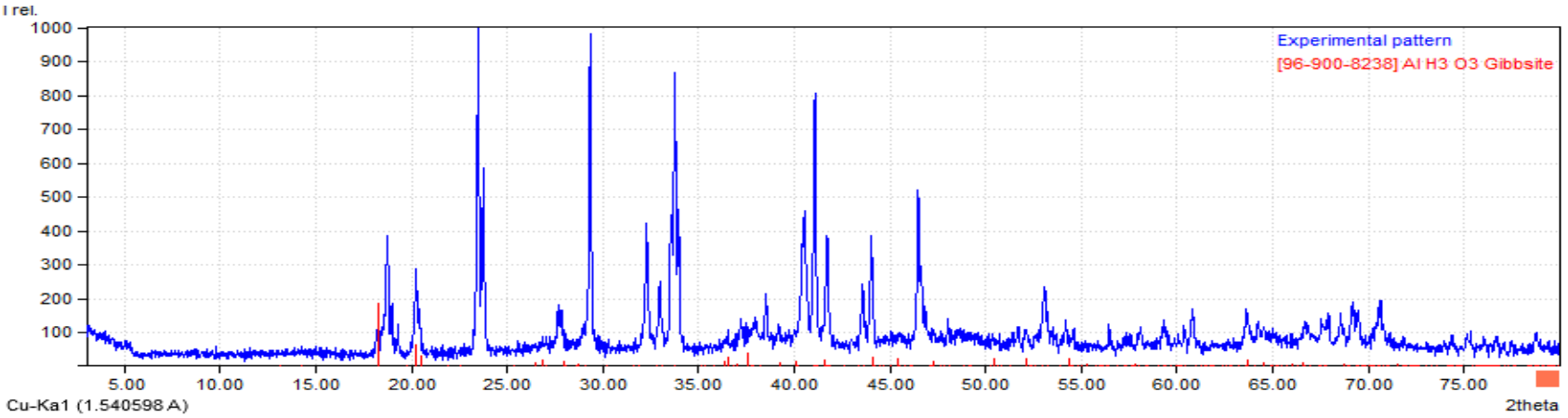

Figure 20: The X-ray diffraction analysis of Al oxide

3.3.3 Effect of P application, pH, phosphate adsorbent, and incubation time on the CAL-P concentrations in the Luvisol subsoil

After 1 week-soil incubation, the CAL-P concentrations were significantly affected by the P application, pH, and phosphate adsorbent. There were two P levels, three pH levels, and three P adsorbents as shown in Figure 21. The CAL-P concentrations were significantly higher in the P+ treatments than in the P- treatments. The CAL-P concentrations were significantly higher at pH 5.2 and pH 4.6 than at pH 7.2. The difference in the CAL-P concentrations between pH 5.2 and pH 4.6 was statistically non-significant. The CAL-P concentrations were significantly decreased when the Fe and Al oxide were applied. The maximum reduction in the CAL-P concentration was found in Al oxide-applied treatment, which was significantly different when the Fe oxide was applied. Similar results regarding the CAL-P concentration were found after 3 and 6 month-soil incubations.

Figure 21 shows the changes in the CAL-P concentration in the Luvisol subsoil with time when it was incubated for 1 week, 3 months, and 6 months. There was a significant effect of incubation time on the CAL-P concentrations in the soil when P was applied.

At pH 7.2 with P application (P+), there was a significant decrease in the CAL-P concentrations after 3 month-soil incubation when no phosphate adsorbent was applied. Similar results were observed when Al oxide was applied. There was a slight non-significant decrease in CAL-P concentrations when the Fe oxide was applied. A similar trend was found when the CAL-P concentrations after 1 week-soil incubation were compared with the CAL-P concentrations after 6 month-soil incubation. The CAL-P concentrations after 6 month-soil incubation were increased when these were compared with the CAL-P concentrations after 3 month-soil incubation. This increase was significant when no adsorbent was applied and when the Al oxide was applied. There was a slight non-significant increase in the CAL-P concentrations when the Fe oxide was applied. In the P- treatments, changes in the CAL-P concentrations with time were non-significant.

There was a significant decrease in the CAL-P concentrations after 3 month-soil incubation and 6 month-soil incubation when these were compared with the CAL-P concentrations after 1 week-soil incubation in the P+ treatments at pH 5.2. These were further decreased in the last 3 months but these differences were non-significant. In the P- treatments, changes in the

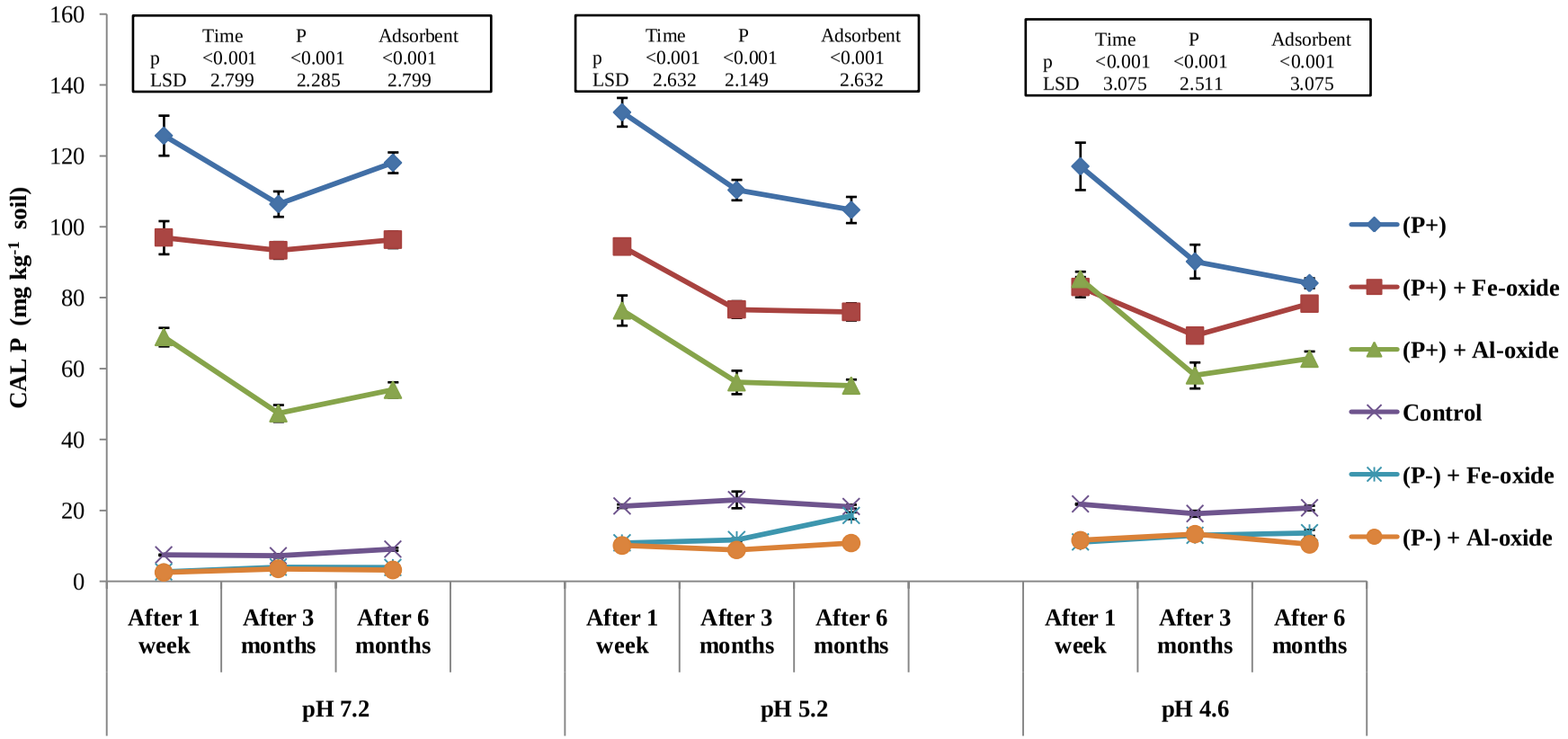

Figure 21: Effect of P application, pH, and phosphate adsorbents on the CAL-P concentrations in the Luvisol subsoil after various incubation times. Values are the arithmetic means of four replicates ± SE. In the legend, P- = 0 mg P kg^{-1} soil, P+ = 200 mg P kg^{-1} soil, Fe oxide = 300 mmol Fe kg^{-1} soil, and Al oxide = 300 mmol Al kg^{-1} soil.

CAL-P concentrations with time were non-significant except in the last 3 months when the Fe oxide was applied, where it was significantly increased.

At pH 4.6, the CAL-P concentrations were significantly decreased after 3 month-soil incubation. There was a slight non-significant increase in the CAL-P concentrations in the last 3 months when the Fe and Al oxide were applied while there was no significant decrease in the CAL-P concentration when phosphate adsorbent was not applied. In the P- treatments, the changes in the CAL-P concentrations with time were non-significant.

Most of the applied Fe and Al were present in the form of crystalline oxides after 6 months of soil incubation. Their concentrations were determined in the treatments where P was applied (Figure 22).

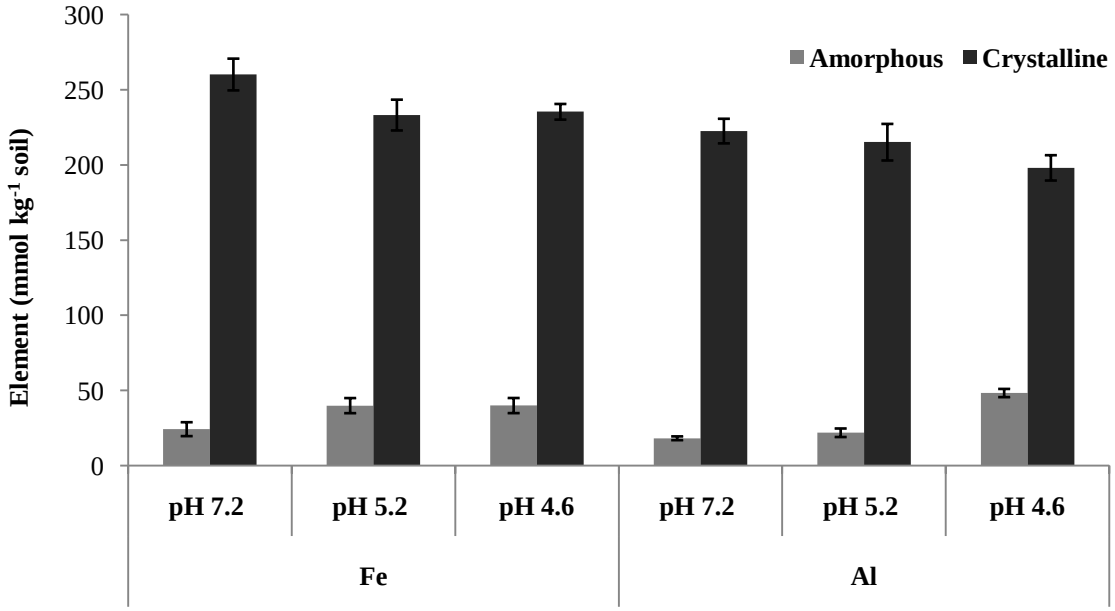

Figure 22: Concentrations of amorphous and crystalline Fe and Al in the 6 month-incubated Luvisol subsoil when 300 mmol Fe and Al kg^{-1} soil were applied as Fe and Al oxides respectively, and 200 mg P kg^{-1} soil were applied. Values are the arithmetic means of four replicates ± SD.

3.3.4 Effect of pH and P adsorbent on the occluded-P concentrations after 6 month-incubation of the Luvisol subsoil

Figure 23 shows the occluded-P concentrations affected by pH and phosphate adsorbents in the soil after 6 months of incubation. The occluded-P concentrations were significantly higher

where Al and Fe oxides were applied than where no adsorbent was applied (control) at all pH levels. There was a significant difference in the occluded-P concentrations between the Al and Fe oxides at pH 4.6, while the differences at pH 7.2 and 5.2 were non-significant. In the control treatment, the differences in the occluded-P concentrations were non-significant among all three pH levels. The differences in the occluded-P concentrations were significantly higher at pH 4.6 than at pH 7.2 where the Al and Fe oxides were applied. The differences in the occluded-P concentrations were non-significant between pH 7.2 and pH 5.2, and, between pH 5.2 and 4.6, where the Al and Fe oxides were applied.

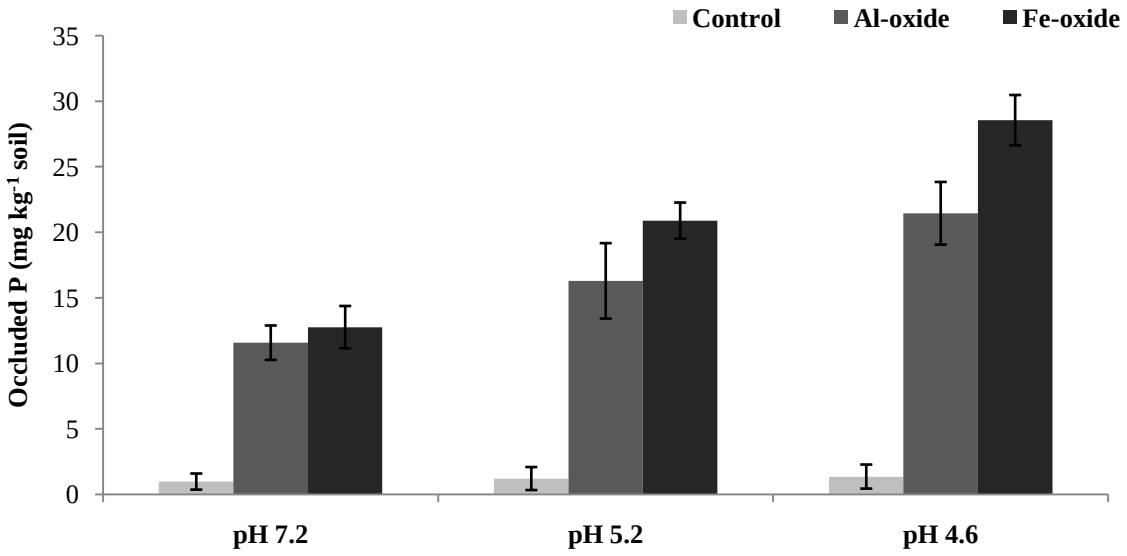

Figure 23: Occluded-P concentrations (Al oxide-occluded P + Fe oxide-occluded P) in the 6 month-incubated Luvisol subsoil when 200 mg P kg^{-1} soil was applied. Values are the arithmetic means of four replicates. The applied concentrations of Al and Fe oxides were 300 mmol kg^{-1} soil. Values are the arithmetic means of four replicates ± SD.

3.4 Bioavailability of Fe oxide and Al oxide-occluded phosphates

3.4.1 Effect of various P sources on dry mass of maize and white lupin

In the present study, 10 mg P kg^{-1} soil were applied as Al oxide-occluded P, Fe oxide-occluded P, and calcium dihydrogen phosphate. The Al oxide-occluded P and Fe oxide-occluded P were synthesized before the experiment.

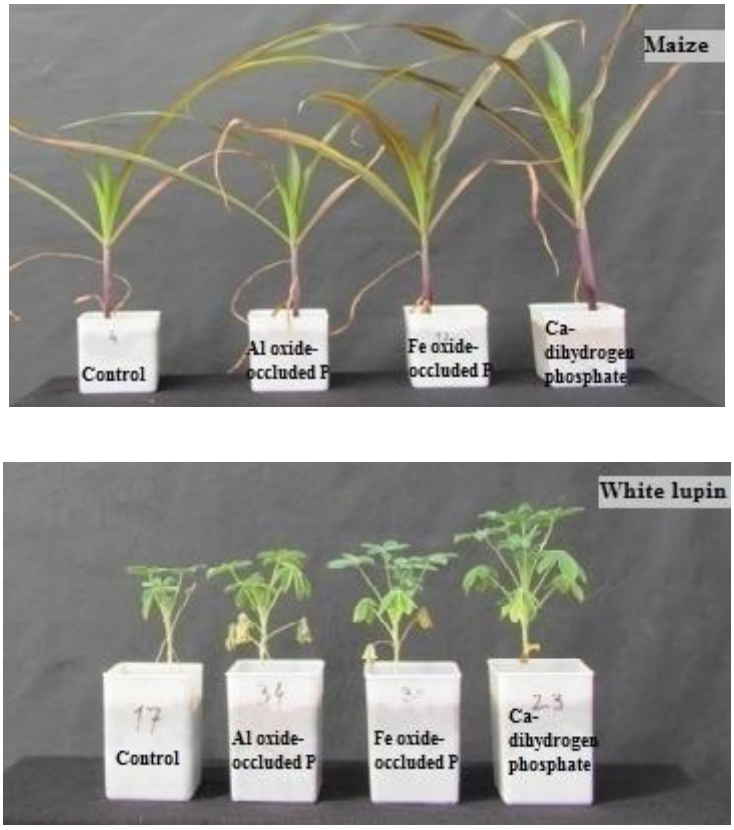

Figure 24: Maize and white lupin before harvest, cultivated for 35 d. 10 mg P kg^{-1} soil were applied as Al oxide-occluded P, Fe oxide-occluded P, and calcium dihydrogen phosphate.

The data for dry masses (shoot plus root) of maize and white lupin affected by various P sources are shown in Figure 25. Maize had the highest dry mass when P was applied as calcium dihydrogen phosphate, which was significantly different from Al oxide-occluded P, Fe oxide-occluded P, and control treatment. The differences among control, Al oxide-occluded P, and Fe oxide-occluded P were statistically non-significant.

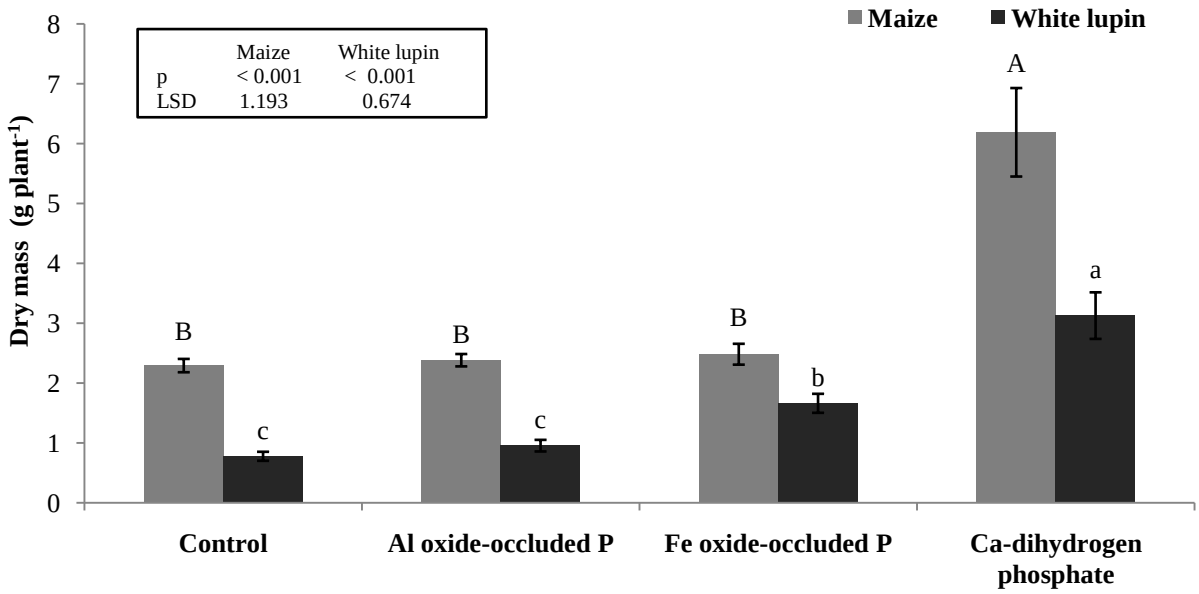

Figure 25: Effect of various P sources on the dry mass of maize and white lupin cultivated for 35 d (shoot plus root). 10 mg P kg^{-1} soil were applied as Al oxide-occluded P, Fe oxide-occluded P, and calcium dihydrogen phosphate. Values are the arithmetic means of four replicates ± SE. Columns with different letters indicate significant difference at 5% level.

White lupin also had the highest dry mass when P was applied as calcium dihydrogen phosphate, significantly different from Al oxide-occluded P, Fe oxide-occluded P, and control treatment. For white lupin, there was a significant increase in the dry mass when P was applied as Fe oxide-occluded P as compared to Al oxide-occluded P and control treatment. The difference between control and Al oxide-occluded P treatments was non-significant. Maize had significantly higher dry mass than white lupin in each treatment.

3.4.2 Effect of various P sources on P content of maize and white lupin

Maize had a significantly higher P content than white lupin. P contents of both maize and white lupin were significantly increased when P was applied as calcium dihydrogen phosphate. In maize, the differences in P content among control, Al oxide-occluded P, and Fe oxide-occluded P were non-significant (Figure 26).

White lupin had a significantly higher P content after application of Fe oxide-occluded P than in the treatments of control and Al oxide-occluded P. P contents of plants treated with the Al oxide-occluded P were statistically not different from the control treatment.

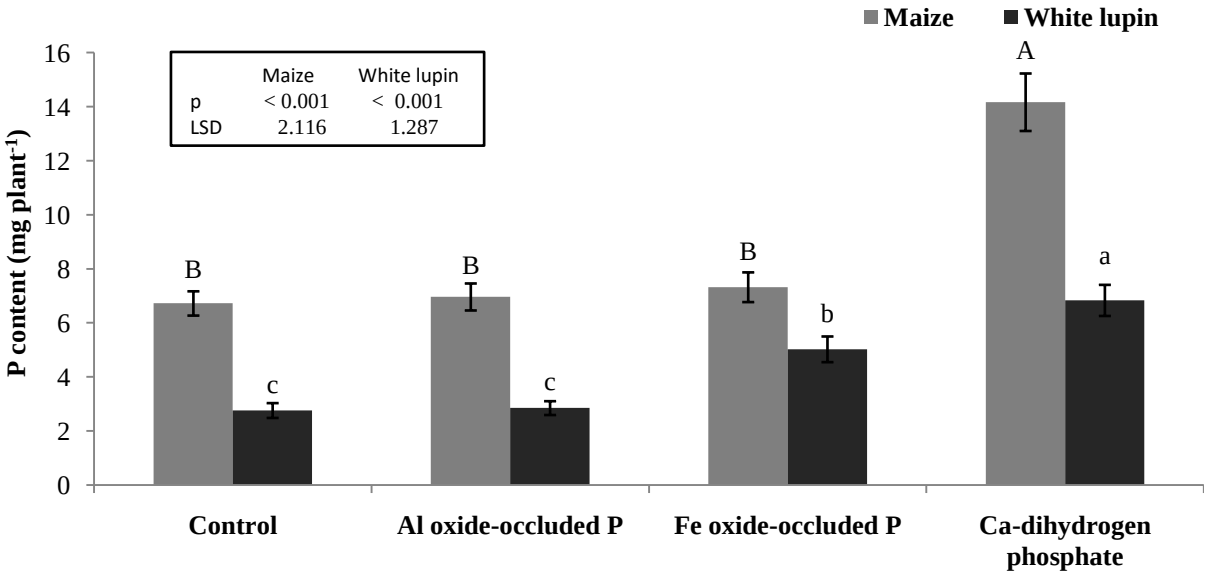

Figure 26: Effect of various P sources on the P content of maize and white lupin cultivated for 35 d (shoot plus root). 10 mg P kg^{-1} soil were applied as Al oxide-occluded P, Fe oxide-occluded P and calcium dihydrogen phosphate. Values are the arithmetic means of four replicates ± SE. Columns with different letters indicate significant difference at 5% level.

3.4.3 Changes in occluded-P concentrations in the soil after cultivation of maize and white lupin

Maize and white lupin were cultivated with the occluded P (10 mg P kg^{-1} soil), which was applied as the Al oxide-occluded P and Fe oxide-occluded P. There was a slight non-significant decrease in the occluded-P concentrations when maize was cultivated with the Al and Fe oxide-occluded P (Figure 27). The maximum reduction in the occluded-P concentration occurred for the Fe oxide-occluded P where 88% of the applied occluded-P was present in the soil after the plant harvest (Figure 28). In the Al oxide-occluded P treatment, 94% of the applied occluded-P was present after the harvest where maize was cultivated.

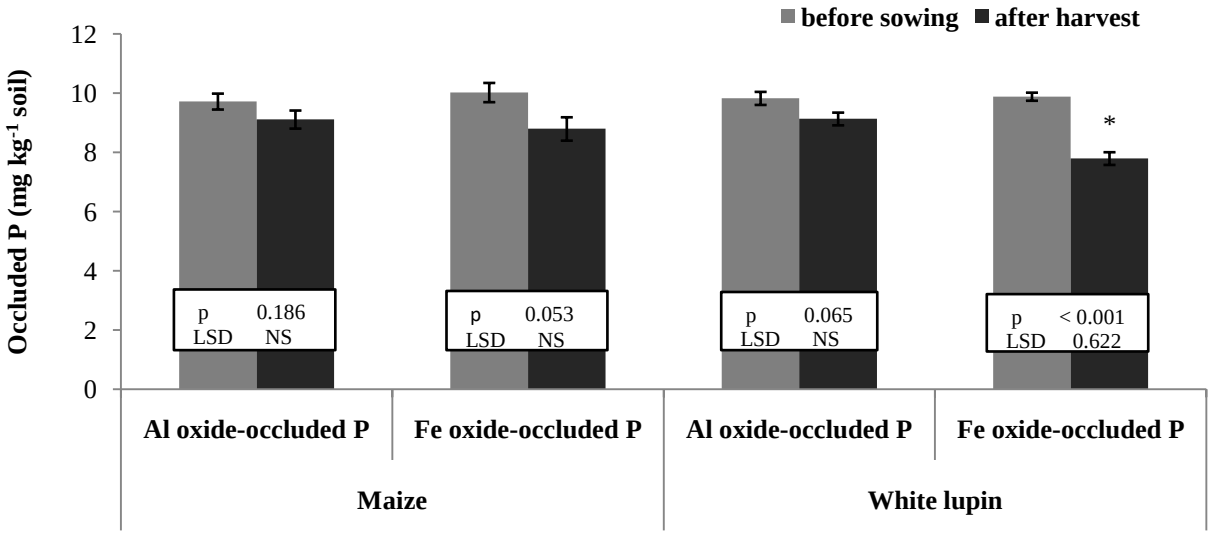

Figure 27: Occluded-P concentrations in the soil before sowing and after harvest of maize and white lupin. Values are the arithmetic means of four replicates ± SE. Columns with asterisk (*) indicate significant difference at 5% level.

Figure 28: Occluded P-concentrations after maize and white lupin cultivation. Values are the arithmetic means of four replicates. These were calculated by dividing occluded P after harvest with occluded P before sowing and multiplying by 100.

In the case of the white lupin cultivation, the Fe oxide-occluded P concentration in the soil was significantly decreased (Figure 27). After the harvest, 79% of the applied occluded-P was present in soil (Figure 28). There was a slight non-significant decrease in the Al oxide-occluded P concentration in the soil after the harvest (Figure 27).

4 Discussion

4.1 Phosphate ageing in soils

It was hypothesized that phosphate ageing in soils increases with time. The soils (the Luvisol topsoil, the Luvisol subsoil, and the Ferralsol) used for this study had different physicochemical characteristics (Table 1). The objective to use the soils having different physicochemical characteristics was to understand the effects of varying aspects of soil composition, which play a vital role in phosphate ageing (Sparks, 1995; Arai and Sparks, 2007), shown in Figure 2 and Figure 3. The Ferralsol, a clay loam and a highly weathered soil, had lower pH and had higher concentrations of Fe and Al oxides than the Luvisol topsoil (a silt loam soil) and the Luvisol subsoil (a loam soil) (Table 1). Further, the adjustment of various pH levels for each of the soils was to investigate the effect of H^+ and OH^- concentrations in the soil solution on the phosphate ageing because phosphate adsorption and occlusion processes are highly pH-dependent in soils (Sanchez and Uehara, 1980; Sparks, 1995). pH buffer-curves were established to assess the required H^+ and OH^- concentrations for adjustments of pH of the soils used in the investigation of phosphate ageing.

In the soil incubation experiments (Figure 7 and Figure 16), most of the applied P was aged in the Ferralsol (not extractable with the CAL method). The CAL method can extract the soil-solution phosphates, phosphates bound by Coulomb force, and adsorbed phosphates in the soil. This method cannot extract strongly held P, such as occluded and precipitated P. The CAL P is regarded as plant-available P. It is inversely related to aged-P concentrations in the soil. The higher aged-P concentrations in the Ferralol were due to the presence of higher concentrations Fe and Al oxides than in the other two soils i.e. the Luvisol topsoil and the Luvisol subsoil (Table 1). These oxides contribute to phosphate ageing due to the presence of net positive charge. The phosphate ageing is directly related to the concentrations of these metal oxides in the soil.

The P application not only increased the CAL-P concentrations in the soils but also the concentration of aged P. The strong correlation between applied P and aged P in the soils was due to high concentration of P present in the soil solution. As the soils were fertilized with P, the P concentration was increased in the soil solution, which led to an increase in aged-P concentration. Zhang *et al*. (2004) reported a substantial increase in plant-non-available P fractions after P fertilization in soils. In another study, Park *et al*. (2004) investigated the

impact of long-term compost and P fertilization on soil P status in a paddy cropping system. They found that the application of compost and P fertilizers resulted in an increase in the total and plant-available P in the soil. In the treatments where P was not applied (without P application) to the soils, the low concentrations of CAL P were due to the fact that the soils were deficient in P. In the Luvisol subsoil (Figure 21), the significant higher CAL-P concentrations found in the P+ treatments than in the P- treatments, were due to the fact that applied P (200 mg P kg^{-1} soil) was partly present in the soil solution which was extractable with CAL method. This was applicable to all incubation periods. The P- treatments were deficient in the CAL-P concentrations as no P was applied in these treatments.

Soil-phosphate adsorption and occlusion are pH-dependent processes. These are inversely related to soil pH value. The lower the soil pH, the higher are the phosphate adsorption and occlusion (phosphate ageing) and vice versa. Various investigations have shown this relationship (Hartikainen, 1981; Hartikainen & Yli-Halla, 1996). At low soil pH, surface functional groups of oxides present net positive charge and therefore, adsorb and ultimately occlude phosphate. In the present study, the effect of pH on the CAL-P concentrations in the soils was statistically non-significant (Figure 7 and Figure 21). These results are in contradiction to previous studies. This may be due to the fact that under natural soil environmental conditions, the low soil pH is related with high concentrations of the Fe and Al oxides. These oxides play a direct major role in phosphate ageing, not the high H^+ concentration in the soil. Further, basic cations such as Ca^{2+}, Mg^{2+}, K^+, and Na^+ are leached down to lower soil profiles in highly weathered soils, and as a result, soil pH decreases. In the present study, various soil-pH levels were adjusted by addition of H^+ and OH^- but the chemical composition of soil was not changed as it happens during weathering. The relative concentrations of H^+ and OH^- in the soil solution play a role in phosphate ageing mainly when the phosphate adsorbents are present in the soils. Low pH is the characteristic of acid soils, such as the Ferralsol, which is the result of leaching of basic cations due to high rainfall, presence of acidic parent material, and intensive cropping. The increase in the concentrations of CAL P in the Luvisol topsoil at pH 5.5 after 3 and 6 months of soil incubation might be due to addition of H^+ (used to adjust the pH) to the soil, which resulted in the release of some Ca-bound P. The significant decreases in the CAL-P concentrations in the Ferralsol were due to its low pH (Figure 7, Figure 15, and Figure 16). At low soil-pH, phosphate ageing is more than at high soil-pH. In the Luvisol subsoil (Figure 21), the increase in the CAL-P concentrations in the P- treatments at lower pH levels (pH 5.2 and 4.5) might also be due to

the release of some of Ca-bound P, as the H^+ were added to adjust these pH levels. After three and six months of soil incubations, the effect of pH was statistically non-significant. This was the due to the same reason that pH plays its role in phosphate ageing under the appropriate soil chemical composition.

The significant decrease in CAL-P concentrations after 3 and 6 months of soil incubations in the Ferralsol at pH 5.2 is suggested to be due to an increase in aged-P concentration with time where P was applied (Figure 10). In the last three months, the non-significant decrease in CAL-P concentration might be due to a decrease in the rate of phosphate ageing, which in turn, might be due to very low P concentration in the soil solution. In the P- treatments, the CAL-P concentrations remained unchanged due to low P concentrations in the soil solution as various soil-P fractions are in a continuous equilibrium conditions with the soil-solution P. At pH 7.2 (Figure 11), the non-significant trend in the CAL-P concentrations in 6 months was due to desorption of some of the adsorbed P as OH^- were used to adjust its pH while in the Luvisol topsoil (Figure 8), at pH 5.5, the H^+ addition may have resulted in the release of Ca-bound P. At pH 7.2 (Figure 9), a non-significant change in the trend of the CAL-P concentrations was expected because at high soil pH, adsorption plays very little role. From these results, it can be concluded that soil incubation for 6 months to investigate the phosphate ageing might not be long enough because the rate of phosphate ageing was found high when the soils were freshly fertilized with P. The total quantity of the aged P increased with time but rate of the phosphate ageing decreased with time. This phenomenon is well illustrated in Figure 15. The Ferralsol had aged most of the applied P even within 30 min after the start of the incubation. Figure 16 and Figure 17 show the similar results where more than 90% of the applied P was aged in the Ferralsol.

In the Luvisol subsoil, the CAL-P concentrations were decreased with time, which meant aged-P concentration was increased where P was applied (Figure 21). The CAL-P concentrations remained unchanged with time where P was not applied. This was due to the fact that the soil solution-P concentrations were very small and soils were deficient in P. There was not enough P in the soils to be aged. At pH 7.2, the trend in the CAL-P concentrations remained unchanged with time in the P+ treatments. This was because the phosphate ageing (adsorption and occlusion) proceeds well at lower pH, such as at pH of 5.2 and 4.6, than at pH of 7.2. At pH 5.2 and 4.6, aged-phosphate concentrations increased with time, with the maximum after 6 months, but the rate of the phosphate ageing was higher in the first 3 months than was in the last 3months. This also explains that the P adsorbents in the

soils can age phosphate very quickly provided the P is present in the soil solution. These results are consistent with the findings of Moharami and Jalali (2015). They found a decrease in the availability of P and an increase in the adsorbed P in soils when soils were incubated for 56 d. Similar results were observed by Kafkafi *et al*. (1967); Madrid and Posner (1979); Beek and van Riemsdijk (1982); Okajima *et al*. (1983); Willett *et al*. (1988); and Fuller *et al*. (1993).

The adsorbents (Fe oxide as Goethite and Al oxide as Gibbsite), used to investigate the phosphate aging in the Luvisol subsoil, were synthesized in the laboratory. The main objectives to use them were to have enough adsorbent concentration in the soils, and to keep the Fe and Al oxides in equal concentrations to compare them. In the soils, under natural conditions, these oxides are not present in equal amounts. This makes a comparative investigation of aged P by these oxides difficult. At low soil pH, these oxides exhibit net positive charge, as their values for the point of zero charge (PZC) are high (Parks, 1967; Stumm and Morgan, 1981). The concentration of positive charge increases with a decrease in soil pH. The significant effect of the adsorbent on the CAL-P concentration was due their ability to adsorb P (Figure 21). Both oxides significantly aged P when P was applied (300 mmol Fe and Al kg^{-1} soil) (Figure 21). These results are consistent with previous studies demonstrated by Hingston *et al*. (1967); Yao and Millero (1996); Shang *et al*. (1992); and Chitrakar *et al*. (2006).They found a high P adsorption with higher concentrations of phosphate adsorbents (metal oxides) in the soils. The Al oxide adsorbed more phosphate than the Fe oxide. This was due to more positive charge on the Al oxides than the Fe oxides. The Al oxides exhibit more net positive charge than the Fe oxides at a given pH, as PZC for the Al oxides is 8.2–9.1 and for the Fe oxides is 6.5–8 (Arai and Sparks, 2007).

Most of the oxides applied to the Luvisol subsoil were in crystalline form (Figure 22). The amorphous forms of these oxides exhibit more surface charge than crystalline forms per unit area. This was one of the reasons that the aged-P concentrations were found less than were expected. Surprisingly, occluded-P concentrations were higher in the Fe oxide treatment than that of the Al oxide treatment, though the difference was statistically non-significant (Figure 23). This was due to the higher concentration of amorphous Fe oxide in the soils than the amorphous Al oxides as shown in Figure 22. It can also be concluded that the Fe oxides play a vital role in the phosphate occlusion and, hence, ageing but it needs further confirmation.

The results and findings from the soil-incubation experiments show that the rate of phosphate ageing in the soils decreased with time, though the total aged-P concentration increased. The first hypothesis of this study, which states that phosphate ageing increases with time, is not supported by the findings. Thus, it is not accepted.

Phosphorus exists in various fractions in the soils and these fractions are determined by a specific fractionate method. The important soil P fractions are soil-solution P, Ca-bound P, adsorbed P, occluded P and organic P. There are various methods to determine the plant-available P (soil test P) such as the CAL method (Schüller, 1969), the Olsen method (Olsen *et al.*, 1954), the Bray and Kurtz method (Bray and Kurtz, 1945), and the Mehlich method (Mehlich, 1984). The plant-available P is comprised of soil-solution P, P held through Coulomb forces (weak forces) and to some extent adsorbed P. In the present study, the CAL method was used to determine plant-available P as it can be used for a wide range of soils and is the commonly used method in Europe. Similarly, there are various methods of P fractionation used for the extraction of other P fractions in soil. Among these methods, the Chang and Jackson method (Chang and Jackson, 1957), the Kurmies method (Kurmies, 1972), the Syers method (Syers *et al.*, 1972) and the Hedley method (Hedley *et al.*, 1982) are widely used methods.

In the present study, soil P fractionation was carried out using an established sequential extraction method described by Chang and Jackson (1957). One of the problems associated with other methods is that these do not distinguish between Fe oxide and Al oxide-occluded P, though give a total estimation of occluded-P concentration in the soil. One of the main objectives of this study was to investigate the bioavailability of occluded phosphates, so therefore, the Chang and Jackson method of P fractionation was used because it is the only available method, which can differentiate between both occluded-P fractions i.e. the Fe oxide-occluded P and the Al oxide-occluded P. As it is an old method, the various modifications suggested by various authors later were also considered (Fife, 1959; Williams *et al.*, 1967; Hartikainen, 1979 and Bowman *et al.*, 1989). The problems of re-adsorption and re-precipitation of P by various reagents such as from NaOH and NH_4F solutions (Williams *et al.*, 1971) were controlled by washing the soil samples with saturated NaCl as was proposed by Ruttenberg (1992) and Kuo (1996). According to Jiang and Gu (1989), the re-adsorption and re-precipitation problems can be minimized by washing the soil samples with ethyl alcohol and saturated NaCl. These problems occur mostly in the calcareous soils because of their high concentrations of Ca^{+2} and various Ca compounds. The soils used for the study

were not calcareous in nature, so therefore this problem was not prominent (Pansu *et al*., 2001). The Hedley and Kurmies methods of P fractionation are more relevant when the organic P needs to be fractionated and differentiation in the occluded-P fractions is not required. The Chang and Jackson method was used only to extract the various P fractions in the soil samples, while the P concentrations in the clear supernatants were determined using the blue molybdate method described by Murphy and Riley (1962), as this method is widely used.

4.2 Bioavailability of occluded phosphates

It was hypothesized that phosphate occluded by Fe oxides is plant-available and phosphate occluded by Al oxide is not plant-available. With respect to plant growth in the pot experiment (Figure 12, Figure 13), the higher dry masses and P contents of the plants (both maize and white lupin) grown in the P+ treatments (where P was applied) were due to higher CAL-P concentrations in the soils than in the P- treatments (where P was not applied). The CAL-P concentrations were very low in the P- treatments. The maximum increase in the dry mass of maize due to P application at pH 7.2 was because near-neutral pH is optimum for maize growth. Maize performs well when the soil pH lies in the range of 5.5–7.5, while optimum pH for white lupin is 4.0–6.0. The minimum effects of P application on the dry masses and P contents of plants were found when grown in the Ferralsol. It is suggested that this was due to the ageing of the applied P in the Feralsol and as a result, plant-available P in the soil solution was very low. The significant effect of the soil type on the dry masses and P contents of plants was due different physicochemical characteristics of the soils. The Ferralsol is a highly weathered soil as compared to the Luvisol topsoil. The different chemical composition of these soils had affected the plant growth significantly. As the pH of Luvisol topsoil under natural conditions is 7.2, plants grown on this soil normally have more dry matter because this pH lies within the optimum pH range for most of the crop plants. Many plants are sensitive to high concentrations of soluble Fe and Al (oxides) present in the soils, which are the characteristics of the Ferralsol. The non-significant effects of pH on the plant-dry masses and P contents may be because under natural soil environmental conditions, a change in soil pH alters the chemical composition of soil. The low pH coupled with higher concentration of the Fe and Al oxides affects the plant growth differently as compared to only low pH. The plants were grown in 6 month-incubated soils, which had all P fractions in the soils. This can explain the non-significant changes in the occluded-P concentrations in the

Luvisol topsoil and the Ferralsol (Figure 14). In the presence of high concentrations of soil-solution and adsorbed P, plants could not utilize the occluded P. As the plants were grown only for 6 weeks, P other than the occluded fraction was sufficient for the plant growth for this period. The bioavailability of occluded P could only be investigated when the soils had almost no or very little soil-solution and adsorbed P fractions.

Under natural soil environmental conditions, the occluded phosphates are formed in highly weathered acid soils. The main problem associated with investigating the dynamics and bioavailability of occluded phosphates in these soils is the presence of other phosphate fractions, which are relatively easily available to plants. The occluded-phosphate concentrations in the soils are relatively low. Thus, their bioavailability can only be investigated thoroughly when other plant-available phosphate fractions are not present in the soils. Further, the occluded phosphates exist as the Fe oxide-occluded phosphate and the Al oxide-occluded phosphate. The relative comparison between the bioavailability of both of these occluded phosphate fractions is possible when equal concentrations exist in soil, which are not equal under natural conditions. Therefore, both Fe oxide and Al oxide-occluded phosphates were synthesized in the laboratory (see 2.4.1) and were applied with 10 mg occluded P kg^{-1} soil. The soil used for investigating the bioavailability of the occluded phosphates was the Luvisol subsoil, a P deficient soil, thus, other P fractions were in very low concentrations.

According to Rengel and Marschner (2005), Wissuwa (2005), and Pearse *et al*. (2006), plant species show various adaptations to acquire P from the soil, thus make available different P fractions. It was found that white lupin was able to utilize the occluded phosphate from Fe oxide but not from Al oxide, while maize was unable to utilize both occluded phosphate forms when they were cultivated for 5 weeks (Figure 26 and Figure 27). This was due to the presence of cluster roots in white lupin. According to Shen *et al.* (2005), the formation of the cluster roots is regulated by P status in shoot rather than P concentrations in soil. The soil used in the present study was a P-deficient Luvisol subsoil and further, soil was not fertilized with P at any stage of plant growth. The plants experienced P deficiency from early stages of their growth (Figure 24) and thus, there were conducive conditions for an enhanced growth of the cluster roots. These cluster roots released various phenolics into the soil under P starved conditions, which helped white lupin to utilize the phosphate occluded by the Fe oxide. The phenolics are hydroxy derivatives of aromatic hydrocarbons. One of their unique properties is being strong reducing agents. They reduce the metal atoms. Main phenolics secreted by plant

roots are isoflavonoids, piscidic acid, and salicylic acid (Grayston *et al*., 1996). Under P-deficient condition, the secretion of phenolics into the soil is increased. Weisskopf *et al*. (2006) investigated the impact of phosphate supply and root type on the isoflavonoid exudation. They found that isoflavonoid exudation was enhanced in the cluster roots under P-deficient conditions. Neumann *et al*. (2000) also reported that the exudation by the cluster roots is high.

These phenolics are involved in the reduction of Fe^{3+} to Fe^{2+}, present in the form of amorphous Fe oxide, which made these oxide soluble and left adsorbed phosphates in the soil. The solubility of reduced Fe oxides is high. The adsorbed phosphates, after reduction of Fe^{3+}, were utilized by the release of organic anions from the cluster roots. As maize has no cluster roots and secretes root exudates (phenolics in particular) in lower quantity than white lupin does, it was unable to utilize the occluded phosphates when grown for 5 weeks. Thus, the phenolics-release is very important in the availability of the occluded phosphates because of their role in the reduction of Fe^{3+} present in the amorphous hydroxyl skin. The adsorbed phosphates are available to both, maize and white lupin, as organic anions play a role in their release from the metal oxides. Most of the crop plant species can utilize the adsorbed phosphates. Jones and Darrah (1994), and Fox (1995) reported that the organic acid release accelerates the desorption of the aged phosphates in forest soils. Ae *et al*. (1990) investigated the piscidic acid-release from pigeon pea roots and found that piscidic acid is a strong chelator of Fe, can mobilize sparingly soluble phosphates. Most of previous studies indicate the combined role of organic acids and phenolics in mobilizing sparingly soluble phosphates (Parfitt, 1979; Gerke, 1992) because phenolics can not only reduce the metal atom present in the mineral oxides, but can also act synonymous to organic anions as some phenolics exhibit negative charge (Haider and Martin, 1975). These compete with the phosphate ions for adsorbing sites and release the adsorbed phosphates.

The findings from the plant growth experiments support both of the other hypotheses of this study. Phosphate occluded by Al oxides is not plant-available, is accepted while phosphate occluded by Fe oxides is plant-available, is partly accepted.

4.3 Concluding remarks

Based on the observations and findings of the experiments, it can be concluded that ageing of phosphates in soil is dependent on the nature and physiochemical characteristics of soil type, time duration, soil pH, nature of phosphate adsorbents and their degree of crystallinity, and soil P status. The highly weathered soil, the Ferralsol, was able to age applied phosphates due to low pH and high concentrations of Fe and Al, while other soils relatively less weathered, such as the Luviol topsoil and the Luvisol subsoil, did not age phosphates until 6 months of incubation. The aged-phosphates concenrations increased with time but the rate of the phosphate ageing decreased due to the depletion of the soil-solution phosphates over time. Phosphate ageing was enhanced significantly by the addition of phosphate-adsorbing materials into the soils such as the Fe and Al oxides. These oxides first adsorbed the applied phosphates and then occluded them in the soil. Phosphate ageing was rapid in the soils when soils were fertilized with P.

White lupin and maize were grown for 5 weeks to investigate the bioavailability of aged phosphates. White lupin was able to utilize phosphate occluded by the Fe oxides due the presence of cluster roots. These cluster roots released phenolics, which reduced the coated hydroxyl skin of amorphous Fe oxides on the occluded phosphates. White lupin could not utilize the phosphate occluded by the Al oxides. Maize could not utilize either of the occluded forms due to the absence of the cluster roots.

5 Summary

Phosphorus (P) is one of the most limiting plant nutrients. This limitation of P is due to strong retention of phosphate ions with soil particles. Thus, most of the applied P in soils becomes unavailable for plants. In acid soils, phosphate is adsorbed at the surfaces of Fe and Al oxides and then becomes occluded with time, termed phosphate ageing. The aged phosphate is highly unavailable to plants due to its very strong fixation. It is known that plant species have developed various adaptations to enhance P uptake from the soil under P starved conditions. One of these adaptations is the formation of cluster roots. The plant species with the cluster roots such as white lupin, release various exudates into the soil which may mobilize various P fractions in the soil. The objectives of this study were to better understand the process of the phosphate ageing by investigating the kinetics and relationship of the aged and applied P in the soil, and to investigate the bioavailability of the aged P. Soil incubation and plant growth experiments were carried out to achieve these objectives.

In the first experiment, a Luvisol topsoil and a Ferralsol were incubated for 1 d, 3 months, and 6 months in a growth chamber at 25°C. There were two pH levels i.e. 7.2 and 5.5 of each soil and two P levels i.e. 0 (P-) and 100 (P+) mg P kg^{-1} soil. P fertilization had a significant effect on CAL-extractable-P (CAL-P) concentrations after 1 d, 3 months, and 6 months of soil incubation. The CAL-P concentrations were higher in the P+ treatments than in the P- treatments in both of the soils. The soils had a significant effect on the CAL-P concentrations. The CAL-P concentrations were higher in the Luvisol topsoil than in the Ferralsol. These were very low in the Ferralsol. In this soil, most of the applied P (more than 90%) was not extractable with the CAL method. Maize (*Zea mays* L. cv. Amadeo) and white lupin (*Lupinus albus* L. cv. Amiga) were grown in the 6 month-incubated soils. Plants grown in the Luvisol topsoil had a higher P content than those grown in the Ferralsol. The maximum P contents were in the P+ treatments. Maize had a higher P content than white lupin when it was grown in the Luvisol topsoil with P application (P+). White lupin had a higher P content than maize when grown in the Ferralsol.

In the second experiment, the soils (the Luvisol topsoil and the Ferralsol) were incubated for 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h in pots at 25°C in the growth chamber. P was applied at the rate 100 mg kg^{-1} soil. The CAL-P concentration data showed that most of the added P in the Ferralsol had become non-CAL-extractable after 1 h. The Luvisol topsoil did not adsorb

phosphate in the P+ treatment immediately. After 8 h of incubation, the CAL-P concentration decreased. In the second part of this experiment, these soils were incubated for 24 h in pots with various P levels i.e. 0, 100, 150, 200, 250, 500 mg P kg^{-1} soil. The CAL P and aged P data showed that most of the P applied in the Ferralsol was aged, in contrast to the Luvisol topsoil.

In the third experiment, a Luvisol subsoil was incubated in plastic buckets. Each bucket had 3 kg of soil. There were three pH levels, i.e. 7.2, 5.2, 4.6 and two P levels i.e. with P (P+) and without P (P-). In the P+ treatments, 200 mg P kg^{-1} soil were applied as KH_2PO_4. Goethite (Fe oxide) and Gibbsite (Al oxide) minerals were added as P adsorbents at the rate of 300 mmol Fe and Al kg^{-1} soil. The soils were incubated for 1 week, 3 months, and 6 months, respectively, at 25°C in a growth chamber. The results showed that the aged-P concentrations were affected by the P application, phosphate adsorbent and time. The aged-P concentrations increased after 3 months of incubation in the P+ treatments. The aged-P concentrations were increased where Al oxide was applied as P adsorbent.

In the fourth experiment, maize (*Zea mays* L. cv. Amadeo) and white lupin (*Lupinus albus* L. cv. Amiga) were cultivated in the Luvisol subsoil in pots. Each pot had 1 kg of soil with one plant. Ten mg P kg^{-1} soil were applied as Al oxide-occluded P, Fe oxide-occluded P, and $Ca(H_2PO_4)_2$. These occluded phosphates were synthesized before the start of the experiment. The data from the P contents in plants and the occluded-P concentrations in soil after the plant cultivation showed that white lupin mobilized the Fe oxide-occluded P but not the Al oxide-occluded P, while maize was unable to mobilize both occluded P forms.

Zusammenfassung

Phosphor (P) ist einer der meisten limitierenden Pflanzennährstoffe. Durch die starke Bindung von Phosphationen an Bodenpartikel ist ein Großteil des gedüngten Phosphors nicht pflanzenverfügbar. In sauren Böden wird Phosphat an den Oberflächen von Eisen- und Aluminiumoxiden adsorbiert und mit der Zeit okkludiert. Dieser Prozess wird Phosphatalterung genannt. Durch diese starke Bindung ist das gealterte Phosphat sehr wenig pflanzenverfügbar. Es ist bekannt, dass einige Pflanzenarten verschiedene Strategien entwickelt haben, um unter P-Mangelbedingungen weiterhin P aus dem Boden aufnehmen zu können. Eine Strategie ist die Bildung von Proteoidwurzeln. Pflanzenarten wie die Weißlupine geben verschiedene Exsudate aus diesen Proteoidwurzeln in den Boden ab. Diese Exsudate können P aus unterschiedlichen P-Fraktionen im Boden mobilisieren. Ziel dieser Studie war es, die Prozesse der Phosphatalterung aufzuklären. Hierzu wurden die Kinetik und die Beziehung zwischen gealtertem und gedüngtem P im Boden untersucht. Außerdem wurde mittels Bodeninkubations- und Gefäßversuchen mit Pflanzen die Bioverfügbarkeit von gealtertem P bestimmt.

Im ersten Experiment wurde der Oberboden eines Luvisols und eines Ferralsols bei 25°C für 1 Tag, 3 Monate und 6 Monate in einer Klimmakammer inkubiert. Beide Böden wurden auf zwei pH-Werte eingestellt (pH 7,2 und pH 5,5) und erhielten unterschiedliche P-Düngermengen 0 (P-) und 100 (P+) mg P kg^{-1} Boden. Die P-Düngung hatte einen signifikanten Einfluss auf die CAL-extrahierbaren-P-Konzentrationen nach 1 Tag, 3 Monaten und 6 Monaten Inkubationsdauer. Die CAL-P-Konzentrationen waren in beiden Böden höher in den P+-Varianten im Vergleich zu den P--Varianten. Des Weiteren waren die Konzentrationen im Luvisol deutlich höher als im Ferralsol. Im Ferralsol war ein Großteil des gedüngten Phosphats (mehr als 90%) nicht CAL-extrahierbar.

Nach der sechsmonatigen Inkubationsdauer wurden Mais (*Zea mays* L. cv. Aamdeo) und Weiße Lupine (*Lupinus albus* L. cv. Amiga) auf diesen Böden kultiviert. Sowohl der Mais als auch die Weiße Lupine zeigten höhere P-Gehalte nach der Kultivierung auf dem Luvisol als nach der Anzucht auf dem Ferralsol. In den P+-Varianten wurden bei beiden Pflanzenarten höhere P-Gehalte als in den P-Varianten ermittelt. Nach Anzucht der P+-Varianten auf dem Luvisol wies der Mais höhere P-Gehalte als die Weiße Lupine auf. Jedoch zeigte die Weiße Lupine im Vergleich zum Mais in der P+-Variante auf dem Ferralsol höhere P-Gehalte.

Im zweiten Experiment wurden der Luvisol und der Ferrasol für 0,5 h, 1 h, 2 h, 4 h, 8 h, 12 h und 24 h in Gefäßen bei 25°C in der Klimakammer inkubiert. 100 mg P kg^{-1} Boden wurden jeweils appliziert. Ein Großteil des applizierten Phosphors war im Ferralsol bereits nach 1 h nicht mehr CAL-extrahierbar. Der Oberboden des Luvisols hatte das applizierte P nicht sofort adsorbiert, da ein Abfall des CAL-extrahierbaren Phosphors erst nach 8 h Inkubationsdauer messbar war.

Im zweiten Teil des Experiments wurden die Böden für 24 h in Gefäßen mit unterschiedlichen P-Mengen inkubiert (0, 100, 150, 200, 250, 500 mg P kg^{-1}). Die Messergebnisse für das CAL-P und für die Fraktion des gealterten Phosphors zeigen, dass das meiste applizierte P im Ferralsol gealtert war, nicht aber im Luvisol.

Im dritten Experiment wurden 3 kg eines Luvisol-Unterbodens in Plastikgefäßen inkubiert. Im Boden wurden drei unterschiedliche pH-Werte eingestellt (pH 7,2, pH 5,2 und pH 4,6) und zwei P-Düngestufen, mit P (P+) und ohne P (P-). In der P+-Variante wurden 200 mg P kg^{-1} Boden als KH_2PO_4 appliziert. Jeweils 300 mmol Fe oder Al kg^{-1} Boden wurden dem Boden als Goethit (Eisenoxid) oder Gibbsit (Aluminiumoxid) als P-Adsorbenten untergemischt. Die Böden wurde nach je 1 Woche, 3 Monaten und 6 Monaten Inkubation bei 25°C in der Klimakammer beprobt. Die Ergebnisse zeigen, dass die Fraktion des gealterten Phosphats durch die P-Applikation, die verwendeten Oxide und die Inkubationsdauer beeinflusst wurde. Die Fraktion des gealterten Phosphat erhöhte sich nach 3 Monaten in der P+-Variante und in der Aluminiumoxid-Variante.

In einem vierten Experiment wurden Mais (*Zea mays* L. cv. Amadeo) und die Weiße Lupine (*Lupinus albus* L. cv. Amiga) in einem Luvisol Unterboden (je 1 kg Boden pro Pflanze) in Plastikgefäßen kultiviert. 10 mg P kg^{-1} Boden wurden dem Boden in unterschiedlichen Phosphatformen untergemischt: Aluminiumoxid-okkludiertes P, Eisenoxid-okkludiertes P und $Ca(H_2PO_4)_2$. Diese okkludierten Phosphate wurden für das Experiment synthetisch hergestellt. Die Pflanzen wurden nach 35-tägiger Anzucht geerntet. Der Mais konnte sich weder das okkludierte Phosphat des Aluminiumoxids noch das okkludierte Phosphat des Eisenoxids aneignen. Die P-Gehalte der Weißen Lupine und die P-Konzentrationen im Boden zeigten jedoch, dass die Weiße Lupine in der Lage war, das okkludierte P aus Eisenoxid, jedoch nicht aus Aluminiumoxid, zu mobilisieren.

References

Ae, N., Arihara, J., Okada, K., Yoshihara, T., and Johansen, C. 1990. Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science 248: 477–480.

Allen, S. E., Grimshaw, H., Parkinson, J., and Quarmby, C. 1974. Chemical Analysis of Ecological Materials. John Wiley and Sons, New York, USA.

Arai, Y., and Sparks, D. L. 2007. Phosphate reaction dynamics in soils and soil minerals: a multiscale approach. Advances in Agronomy 94: 135–179.

Arai, Y., and Sparks, D. L. 2001. ATR-FTIR spectroscopic investigation on phosphate adsorption mechanisms at the ferrihydrite-water interface. Journal of Colloid and Interface Science 241: 317–326.

Barber, S. A. 1995. Soil Nutrient bioavailability. A Mechanistic Approach. 2nd Ed. John Wiley, New York, USA.

Barekzai, A., and Mengel, K. 1985. Alterung von wasserlöslichem Düngerphosphat bei verschiedenen Bodentypen. Zeitschrift für Pflanzenernährung und Bodenkunde 148: 365–378.

Beek, J., and van Riemsdijk, W. H. 1982. Interactions of orthophosphate ion with soil. In: Bolt, G. H. (ed) Soil Chemistry, Physico-Chemical Models. Elsevier Science Publication, Co., Amsterdam, The Netherlands. 259–284.

Bergmann, W. 1992. Nutritional Disorders of Plants. Visual and Analytical Diagnosis. Gustav Fischer, Jena, Germany.

Bhat, K. K. S., and Nye, P. H. 1974. Diffusion of phosphate to plant roots in soil. Plant and Soil 41: 365–382.

Bieleski, R. L. 1973. Phosphate pools, phosphate transport, and phosphate availability. Annual Review of Plant Physiology 24: 225–252.

Bleam, W. F., Pfeffer, P. E., Goldberg, S., Taylor, R. W., and Dudley, R. 1991. A ^{31}P solid-state nuclear magnetic resonance study of phosphate adsorption at the boehmite/aqueous solution. Langmuir 7: 1702–1712.

Bould, C., Hewitt, E. J., and Needham, P. 1983. Diagnosis of Mineral Disorders in Plants. Volume 1: Principles. HMSO, London, UK.

Bowman, R. A. 1989. A sequential extraction procedure with concentrated sulfuric acid and dilute base for soil organic phosphorus. Soil Science Society of America Journal 53: 362–366.

Bray, R. H., and Kurtz, L. T. 1945. Determination of total, organic, and available forms of phosphorus is soils. Soil Science 59: 39–45.

Brundrett, M. C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil 320: 37–77.

Burnham, C. P., and Lopez-Hernandez, D. 1982. Phosphate retention in different soil taxonomic classes. Soil Science 134: 376–380.

Chang, S. C., and Jackson, M. L. 1957. Fractionation of soil phosphorus. Soil Science 84: 133–44.

Chitrakar, R., Tezuka, S., Sonoda, A., Sakane, K., Ooi, K., and Hirotsu, T. 2006. Phosphate adsorption on synthetic goethite and akaganeite. Journal of Colloid and Interface Science 298: 602–608.

Cordell, D., Rosemarin, A., Schröder, J. J., and Smit, A. L. 2011. Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. Chemosphere 84: 747–758.

Cordell, D., Drangert, J. O., and White, S. 2009. The story of phosphorus: global food security and food for thought. Global Environmental Change 19: 292–305.

Dalal, R. C. 1977. Soil organic phosphorus. In: Brady, N. C. (ed) Advances in Agronomy. Academic Press, New York, USA. 29: 83–117.

Dinkelaker, B., Hengeler, C., Neumann, G., Eltop, L., and Marschner, H. 1997. Root exudates and mobilization of nutrients. In: Rennenberg, H., Eschrich, W., Ziegler, H., (eds) Trees – contribution to modern tree physiology. Backhuys, Leiden, The Netherlands. 441– 452.

Dinkelaker, B., Römheld, V., and Marschner, H. 1989. Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). Plant, Cell and Environment 12: 285–292.

Dou, Z .X., Ramberg, C. F., Toth, J. D., Wang, Y., Sharpley, A. N., Boyd, S. E., Chen, C. R., Williams, D., and Xu, Z. H. 2009. Phosphorus speciation and sorption-desorption characteristics in heavily manured soils. Soil Science Society of America Journal 73: 93–101.

Fageria, N. K., and Baligar, V. C. 2001. Improving nutrient use efficiency of annual crops in Brazilian acid soils for sustainable crop production. Communications in Soil Science and Plant Analysis 32: 1303–1319.

Fang, Z., Shao, C., Meng, Y., Wua, P., and Chen, M. 2009. Phosphate signaling in Arabidopsis and Oryza sativa. Plant Science 176: 170–180.

Faye, I., Diouf, O., Guisse, A., Sene, M., and Diallo, N. 2006. Characterizing root responses to low phosphorus in pearl millet (*Pennisetum glaucum* L. R. Br.). Agronomy Journal 98: 1187–1194.

Fife, C. V. 1959. An evaluation of ammonium fluoride as a selective extractant for aluminium-bound soil phosphate: II. Preliminary studies on soils. Soil Science 87: 83–88.

Fitter, A. H. 1985. Functional significance of root morphology and root system architecture. In: Fitter, A. H., Atkinson, D., Read, D. J., Useher, M. B. (eds) Ecological Interactions in Soil-Plant, Microbes and Animals. Blackwell, London, UK. 87–106.

Fox, T. R. 1995. The influence of low molecular weight organic acids on properties and processes in forest soils. In: McFee, W. W., Kelly, J. M. (eds) Carbon forms and functions in forest soils. Soil Science Society of America, Madison, USA. 43–61.

Fuller, C. C., Davis, J. A., and Waychunas, G. A. 1993. Surface chemistry of ferrihydrite: Part2. Kinetics of arsenate adsorption and coprecipitation. Geochimica Cosmochimica Acta 57: 2271–2282.

Gardner, W. K., Barber, D. A., and Parberry, D. G. 1983. The acquisition of phosphorus by *Lupinus albus* L. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. Plant and Soil 70: 107–124.

George, T. S., Simpson, R. J., Hadobas, P. A., and Richardson, A. E. 2004. Expression of a fungal phytase gene in Nicotiana tobacum improves phosphorus nutrition in plants grown in amended soil. Plant Biotechnology Journal 3: 129–140.

Gerke, J. 2015a. Phytate (inositol hexakisphosphate) in soil and phosphate acquisition from inositol phosphates by higher plants. A review. Plants 4: 253–266.

Gerke, J. 2015b. The acquisition of phosphate by higher plants: Effect of carboxylate release by the roots. A critical review. Journal of Plant Nutrition and Soil Science 178: 351–364.

Gerke, J., Beißner, L., and Römer, W. 2000. The quantitative effect of chemical phosphate mobilization by carboxylate anions on P uptake by a single root. I. The basic concept and determination of soil parameters. Journal of Plant Nutrition and Soil Science 163: 207–212.

Gerke, J. 1994. Kinetics of soil phosphate desorption as affected by citric acid. Zeitschrift für Pflanzenernährung und Bodenkunde 157: 17–22.

Gerke, J. 1992. Phosphate, aluminium and iron in the soil solution of three different soils in relation to varying concentrations of citric acid. Zeitschrift für Pflanzenernährung und Bodenkunde 155: 339–343.

Gerke, J. and Hermann, R. 1992. Adsorption of orthophosphate to humic-Fe-complexes and to amorphous Fe-oxide. Zeitschrift für Pflanzenernährung und Bodenkunde 155: 233–236.

Gilbert, N., 2009. The disappearing nutrient. Nature 461: 716–718.

Gilbert, G. A., Knight, J. D., Vance, C. P., Allan, D. L. 1999. Acid phosphatase activity in phosphorus-deficient white lupin roots. Plant, Cell and Environment 22: 801–810.

Gould, S. F. 1998. Proteoid root mats bind surface materials in Hawkesbury Sandstone biomantles. Australian Journal of Soil Research 36: 1019–1031.

Grayston, S. J., Vaugham, D., and Jones, D. 1996. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5: 29–56.

Grewling, T., and Peech. 1960. Chemical soil tests. Cornell University Agricultural Experimental Station, USA. 960.

Haider, K., and Martin, J. P. 1975. Decomposition of specifically carbon-l4 labeled benzoic and cinnamic acid derivatives in soil. Soil Science Society of America Proceedings, Madison, USA. 39: 657–662.

Hartikainen, H. & Yli-Halla, M. 1996. Solubility of soil phosphorus as influenced by urea. Zeitschrift für Pflanzenernährung und Bodenkunde 159: 327–332.

Hartikainen, H. 1981. Effect of decreasing acidity on the extractability of inorganic soil phosphorus. Journal of the Scientific Agricultural Society of Finland 53: 16–26.

Hartikainen, H. 1979. Phosphorus and its reactions in the terrestrial soils and lake sediments. Journal of the Scientific Agricultural Society of Finland 51: 537–623.

Haynes, R. J. 1984. Lime and phosphate in the soil-plant system. Advances in Agronomy 37: 249–467.

Hedley, M. J., Stewart, J. W. B., and Chauhan, B. S. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Science Society of America Journal 46: 970–976.

Hill, J. O., Simpson, R. J., Moore, A. D., and Chapman, D. F. 2006. Morphology and response of roots of pasture species to phosphorus and nitrogen nutrition. Plant and Soil 286: 7–19.

Hingston, F. J., Posner, A. M., and Quirk, J. P. 1974. Anion adsorption by goethite and gibbsite. II. Desorption of anions from hydrous oxide. Journal of Soil Science 23: 16–26.

Hingston, F. J., Atkinson, R. J., Posner, A. M., and Quirk, J. P. 1967. Specific adsorption of anions. Nature 215: 1459–1461.

Hinsinger, P., Plassard, C., Tang, C. and Jaillard, B. 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant and Soil 248: 43–59.

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

Holford, I. C. R. 1997. Soil phosphorus: its measurement, and its uptake by plants. Australian Journal of Soil Research 35: 227–39.

Hossner, L. R., Freeouf, J. A., and Folsom, B. L. 1973. Solution phosphorus concentration and growth of rice (*Oryza Sativa* L.) in flooded soils. Soil Science Society of America Proceedings, Madison, USA. 37: 405–408.

Hsu, P. H. 1989. Aluminum oxides and oxyhydroxides. In: Dixon, J. B. and Weed, S. B. (eds) Minerals in Soil Environments. Book Ser. 1. Soil Science Society of America, Madison, USA. 331–378.

Huang, P. M., and Schnitzer, M. 1986. Interactions of soil mineral with natural organics and microbes. Special publication 17. Soil Science Society of America, Madison, USA. 159–221.

Jansa, J., and Gryndler, M. 2010. Biotic environment of the arbuscular mycorrhizal fungi in soil. In: Koltai, H., and Kapulnik, Y., (eds) Arbuscular Mycorrhizas: Physiology and Function, 2nd Ed. Springer, Heidelberg, Germany. 209–236.

Jiang, B., and Gu, Y. 1989. A suggested fractionation scheme of inorganic phosphorous in calcareous soils. Fertilizer Research 20: 159–165.

Johnson, J. F., Vance, C. P., and Allan, D. L. 1996. Phosphorus deficiency in *Lupinus albus* (altered lateral root development and enhanced expression of phosphoenol pyruvate carboxylase). Plant Physiology 112: 31–41.

Jones, D. L. 1998. Organic acids in the rhizosphere. A critical review. Plant and Soil 205: 25–44.

Jones, D. L., and Darrah, P. R. 1994. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant and Soil 166: 247–257.

Jungk, A., and Claassen, N., 1997. Ion diffusion in soil-root system. Advances in Agronomy 61: 53–110.

Kafkafi, U., Posner, A. M., and Quirk, J. P. 1967. Desorption of phosphate from kaolinite. Soil Science Society of America Proceedings, Madison, USA. 31: 348–353.

Keerthisinghe, G., Hocking, P. J., Ryan, P. R., and Delhaize, E. 1998. Effect of phosphorus supply on the formation and function of proteoid roots of white lupin (*Lupinus albus* L.). Plant, Cell and Environment 21: 467–478.

Kirk, G. J. D. 1999. A model of phosphate solubilization by organic anion excretion from plant roots. European Journal of Soil Science 50: 369–378.

Kirkby, E. A., and Johnston, A. E. 2008. Soil and fertilizer phosphorus in relation to crop nutrition. In: White, P. J. and Hammond, J. P., (eds) The Ecophysiology of Plant-phosphorus Interactions. Springer, Dordrecht, The Netherlands. 177–223.

Kochian, L. V., Hoekenga, O. A., and Pineros, M. A. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus deficiency. Annual Review of Plant Biology 55: 459–493.

Konig, N. B. J., Ittersum, M. K. V., Box, G. A., Boekel, M. A. J. S. V., Brandenburg, W. A., Broek, J. A. V. D. V., Goudriaan, J., Hofwegen, G. V., Jongeneel, R. A., Schiere, J. B., and Semies, M. 2008. Long-term global availability of food: continued abundance or new scarcity? NJAS-Wageningen Journal of Life Sciences 55: 229–292.

Kuo, S. 1996. Phosphorus. In: Sparks, D. L., Page, A. L., Helmke, P. A., Loeppert, R. H., Soltanpour, P. N., Tabatabai, M. A., Johnston, C. T., and Sumner, M. E. (eds)

Methods of Soil Analysis, Part 3, 3rd Ed. Soil Science Society of America, Madison, USA. 869–920.

Kurmies, B. 1972. Zur Fraktionierung der Bodenphosphate. Die Phosphorsäure 29: 118–151.

Lambers, H., Shane, M. W., and Cramer, M. D. 2006. Root structural and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. Annals of Botany 98: 693–713.

Lamont, B. 2003. Structure, ecology and physiology of root clusters: a review. Plant and Soil 248: 1–19.

Lamont, B. 1972. The effect of soil nutrients on the production of proteoid roots by Hakea species. Australian Journal of Botany 20: 27–40.

Li, M., Osaki, M., Rao, I. M., and Tadano, T. 1997. Secretion of phytase from the roots of several plant species under phosphorus-deficient conditions. Plant and Soil 195: 161–169.

Lindsay, W. L., Velk, P. L., and Chien, S. H. 1989. Phosphate minerals. In: Minerals in Soil Environments. Soil Science Society of America, Madison, USA. 1089–1130.

Lynch, J. P., and Brown, K. M. 2008. Root strategies for phosphorus acquisition. In: White, P. J., and Hammond, J. P. (eds) The Ecophysiology of Plant-phosphorus Interactions. Springer, Dordrecht, The Netherlands. 83–116.

Lynch, J. P. 2005. Root architecture and nutrient acquisition. In: Nutrient Acquisition by Plants: An Ecological Perspective. Springer, Berlin, Germany. 181: 147–184.

Madrid, L., and Posner, A. M. 1979. Desorption of phosphate from goethite. Journal of Soil Science 30: 697–707.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. Academic Press, London, UK. 454–461.

McKeague, J. A., and Day, J. H. 1966. Dithionite and Oxalate- extractable Fe and Al as aids in differentiating various classes of soils. Canadian Journal of Soil Science 46: 13–32.

McKenzie, R. M. 1989. Manganese oxides and hydroxides. In: Dixon, J. B., and Weed, S. B. (eds) Minerals in Soil Environments. Book Ser. 1. Soil Science Society of America, Madison, USA. 439–465.

McLaughlin, M. J., Baker, T. G., James, T. R., and Rundle, J. A. 1990. Distribution and the forms of phosphorus and aluminium in acidic topsoils under pastures in southeastern Australia. Australian Journal of Soil Research 28: 371–385.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2-extractant. Communications in Soil Science and Plant Analysis 15: 1409–1416.

Mengel, K., and Krikby, E. A. 2001. Principles of Plant Nutrition. Kluwer Academic Publishers, The Netherlands. 454–464.

Moharami, S., and Jalali, M. 2015. Effect of time on the sorption and distribution of phosphorus in treated soil with minerals and nano particles. Environmental Earth Sciences 73: 8599–8608.

Murphy, J., and Riley, J. P. 1962. A modified single solution method for determination of phosphates in natural waters. Analytica Chimica Acta 27: 31–36.

Neumann, G., and Martinoia, E. 2002. Cluster roots–an underground adaptation for survival in extreme environments. Trends in Plant Science 7: 162–167.

Neumann, G., Massonneau, A., Langlade, N., Dinkelaker, B., Hengeler, C., Römheld, V., and Martinoia, E. 2000. Physiological aspects of cluster root function and development in phosphorus-deficient white lupin (*Lupinus albus* L.). Annals of Botany 85: 909–919.

Neumann, G., and Römheld, V. 1999. Root excretion of carboxylic acids and protons in phosphorus-deficient plants. Plant and Soil 211: 121–130.

Neumann, G., Massonneau, A., Martinoia, E., and Römheld, V. 1999. Physiological adaptations to phosphorus deficiency during proteoid root development in white lupin. Planta 208: 373–382.

Okajima, H., Kubota, H., and Sakuma, T. 1983. Hysteresis in the phosphorus sorption and desorption processes of soils. Soil Science and Plant Nutrition 29: 271–283.

Olsen, S. R., Cole, C. V., Watanabe, F. S., and Dean, L. A. 1954. Estimation of Available Phosphorus in Soils by Extracting with Sodium Bicarbonate, USDA circ. 939; U.S. Government Printing Office, Washington D.C., USA.

Ottow, J. C. G., Prade, K., Bertenbreiter, W., and Jack, V. A. 1991. Strategies to alleviate iron toxicity of wetland rice on nutritionally poor and/or acid sulphate soils. In: De Turck, P. and Ponnamperuma, F. N. (eds) Rice Production on Acid Soils in the Tropics. Institute of Fundamental Studies, Kandy, Sri Lanka. 205–211.

Pagel, H., and van Huay, H. 1976. Important parameters of phosphate adsorption curves from tropical and subtropical soils and their changes resulting from P application. Archiv für Acker- und Pflanzenbau und Bodenkunde 20: 765–778.

Park, M., Singvilay, O., Shin, W., Kim, E., Chung, J., and Sa, T. 2004. Effects of long-term compost and fertilizer application on soil phosphorus status under paddy cropping system. Communications in Soil Science and Plant Analysis 35: 1635–1644.

Parks, G. A. 1967. Aqueous surface chemistry of oxides and complex oxide minerals-Isoelectric point and zero point of charge. In: Gould, R. F. (ed), Equilibrium Concepts in Natural Water Systems. American Chemical Society, Washington D.C., USA. 121–160.

Parfitt, R. L. 1979. The availability of P from phosphate-goethite bridging complexes. Description and uptake by ryegrass. Plant and Soil 53: 55–65.

Parfitt, R. L. 1978. Anion adsorption by soils and soil materials. Advances in Agronomy 30: 1–50.

Parfitt, R. L., and Smart, R. S. C. 1978. The mechanism of sulfate adsorption on iron oxides. Soil Science Society of America Journal 42: 48–50.

Parfitt, R. L., Atkinson, R. J., and Smart, R. S. C. 1975. The mechanism of phosphate fixation by iron oxides. Soil Science Society of America Proceedings, Madison, USA. 39: 837–841.

Pansu, M., Gautheyrou, J., and Loyer, J. Y. 2001. Soil analysis – sampling, instrumentation and quality control. Balkema Publishers, Lisse, France. 489.

Pearse, S. J., Veneklaas, E. J., Cawthray, G., Bolland, M. D. A., and Lambers H. 2006. Triticum aestivum shows a greater biomass response to a supply of aluminium phosphate than Lupinus albus, despite releasing fewer carboxylates into the rhizosphere. New Phytologist 169: 515–524.

Persson, P., Nilsson, N., and Sjoberg, S. 1996. Structure and bonding of orthophosphate ions at the iron oxide-aqueous interface. Journal of Colloid and Interface Science 177: 263–275.

Qayyum, M. F., Ashraf, I., Abid, M., Steffens, D. 2015. Effect of biochar, lime, and compost application on phosphorus adsorption in a Ferralsol. Journal of Plant Nutrition and Soil Science 178: 576–581.

Ragothama, K. G. 1999. Phosphate acquisition. Annual Review of Plant Physiology and Plant Molecular Biology 50: 665–693.

Rengel, Z., and Marschner, P. 2005. Nutrient availability and management in the rhizosphere: exploiting genotypic differences. New Phytologist 168: 305–312.

Richardson, A. E., George, T. S., Jakobsen, I., and Simpson, R. J. 2007. Plant utilization of inositol phosphates. In: Turner, B. L., Richardson, A. E., and Mullaney, E. J. (eds) Inositol phosphates: linking agriculture and the environment. CABI, Wallingford, UK. 242–260.

Richardson, A. E., Hadobas, P. A., and Hayes, J. E. 2000. Acid phosphomonoesterase and phytase activities of wheat (*Triticum aestivum* L.) roots and utilization of organic phosphorus substrates by seedlings grown in sterile culture. Plant, Cell and Environment 23: 397–405.

Roelofs, R. F. R., Rengel, Z., Cawthray, G. R., Dixon, K. W., and Lambers, H. 2001. Exudation of carboxylates in Australian Proteaceae: chemical composition. Plant, Cell and Environment 24: 891–903.

Runge-Metzger, A. 1995. Closing the cycle: obstacles to efficient P management for improved global security. In: Tiessen, H. (ed) Phosphorus in the global environment. Wiley, Chichester, UK. 27–42.

Ruttenberg, K. C. 1992. Development of a sequential extraction method for different forms of phosphorous in marine sediments. Limnology and Oceanography 37: 1460–1482.

Ryan, P. R., Delhaize, E., and Jones, D. L. 2001. Function and mechanism of organic anion exudation from plants. Annual Review of Plant Physiology and Plant Molecular Biology 52: 527–560.

Sanchez, P. A., and Uehara, G. 1980. Management consideration for acid soils with high phosphorus fixation capacity. In: Knasawneh, F. E., Sample, E. C., and Kamrath, E. J., (eds) The role of phosphorus in agriculture. American Society of Agronomy, Madison, USA. 471–514.

Schachtman, D. P., Reid R. J., and Ayling S. M. 1998. Phosphorus uptake by plants: from soil to cell. Plant Physiology 116: 447–53.

Schüller, H. 1969. Die CAL- Methode, eine neue Methode zur Bestimmung des pflanzenverfiigbaren Phosphates in Boden. Zeitschrift für Pflanzenernährung und Bodenkunde 123: 48–63.

Schulze, D. G., and Bertsch, P. M. 1999. Overview of synchrotron x-ray sources and synchrotron x-rays. In: Schulze, D. G., Stucki, J. W. and Bertsch, P. M. (eds) Synchrotron X-ray Methods in Clay Science. Clay Minerals Society, Boulder, USA. 1–18.

Schulze, G. 1989. An introduction to soil mineralogy. In: Dixon, J. B. and Weed, S. B. (eds) Minerals in Soil Environments. Soil Science Society of America, Madison, USA. 1–34.

Schwertmann, U., and Cornell, R. M. 1991. Iron Oxides in the Laboratory. Chap. 5.2.1. WILEY-VCH, Weinheim, Germany.

Sims, J. T., and Pierzynski, G. M. 2005. Chemistry of phosphorus in soil. In: Tabatabai, A. M., and Sparks, D. L. (eds) Chemical processes in soil. Book seri. 8. Soil Science Society of America, Madison, USA. 151–192.

Shang, C., Stewart, J. W. B., and Huang, P. M. 1992. pH effect on kinetics of adsorption of organic inorganic phosphates by short-range ordered aluminum and iron precipitates. Geoderma 53: 1–14.

Shen, J., Li, H., Neumann, G., and Zhang, F. 2005. Nutrient uptake, cluster root formation and exudation of protons and citrate in Lupinus albus as affected by localized supply of phosphorus in a split roots ystem. Plant Science 168: 837–845.

Shen, H., Yan, X. L., Cai, K. Z., and Matsumoto, H. 2004. Differential Al resistance and citrate secretion in the tap and basal roots of common bean seedlings. Plant Physiology 121: 595–603.

Shen, J., Rengel, Z., Tang, C., and Zhang, F. 2003 Role of phosphorus nutrition in development of cluster roots and release of carboxylates in soil-grown *Lupinus albus*. Plant and Soil 248: 199–206.

Sparks, D. L. 1995. Sorption phenomena on soils. In: Environmental Soil Chemistry. Academic Press, San Diego, USA. 99–139.

Sposito, G. 1989. The Chemistry of Soils. Oxford Univ. Press, New York, USA.

Steen, I. 1998. Phosphorus availability in the 21st century: management of a non-renewable resource. Phosphorus and Potassium 217: 25–31.

Steffens, D., Leppin, T., Luschin-Ebengreuth, N., Yang, Z. M., and Schubert, S. 2010. Organic soil phosphorus considerably contributes to plant nutrition but is neglected by routine soil testing methods. Journal of Plant Nutrition and Soil Science 173: 765–771.

Stumm, W. 1992. Chemistry of the Solid–Water Interface. Wiley, New York, USA.

Stumm, W., and Morgan, J. J. 1981. Aquatic Chemistry. John Wiley and Sons, New York, USA.

Syers, J. K., Smillie, G. W., and Williams, J. D. H. 1972. Calcium fluoride formation during extraction of calcareous soils with fluoride. Implications to inorganic P fractionation schemes. Soil Science Society of America Proceedings, Madison, USA. 36: 20–25.

Tang, C., Drevon, J. J., Jaillard, B., Souche, G., and Hinsinger, P. 2004. Proton release of two genotypes of bean (*Phaseolus vulgaris* L.) as affected by N nutrition and P deficiency. Plant and Soil 260: 59–68.

Taylor, R. M. 1987. Non-silicate oxides and hydroxides. In: Newman, A. C. D. (ed) The Chemistry of Clays and Clay Minerals. Longman Group Ltd., Harlow, Essex, UK. 129–201.

Tejedor-Tejedor, M. L., and Anderson, M. A. 1990. Protonation of phosphate on the surface of goethite as studied by CIR-FTIR and electrophoretic mobility. Langmuir 124: 79–110.

Theodorou, M. E., and Plaxton, W. C. 1993. Metabolic adaptations of plant respiration to nutritional phosphate deprivation. Plant Physiology 101: 339–344.

Vance, C. P., Uhde-Stone, C., and Allan D. L. 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a non-renewable resource. New Phytologist 157: 423–447.

Wada, K. 1985. The distinctive properties of andisols. Advances in Soil Science 2: 174–229.

Walker, T. W., and Syers, J. K. 1976. The fate of phosphorus during pedogenesis. Geoderma 5: 1–19.

Wang, Y., Chen, X., Whalen, J. K., Cao, Y., Quan Z., Lu, C., and Shi, Y. 2015. Kinetics of inorganic and organic phosphorus release influenced by low molecular weight organic acids in calcareous, neutral and acidic soils. Journal of Plant Nutrition and Soil Science 178: 555–566.

Wang, D., Marschner, P., Solaiman, Z., and Rengel, Z. 2007. Growth, P uptake and rhizosphere properties of intercropped wheat and chickpea in soil amended with iron phosphate or phytate. Soil Biology and Biochemistry 39: 249–256.

Watt, M., and Evans, J. R. 2003. Phosphorus acquisition from soil by white lupin (*Lupinus albus* L.) and soybean (*Glycine max* L.) species with contrasting root development. Plant and Soil 248: 271–283.

Watt, M., and Evans J. R. 1999. Proteoid roots: Physiology and development. Plant Physiology 121: 317–323.

Weisskopf, L., Abou-Mansour, E., Fromin, N., Tomasi, N., Santelia, D., and Edelkott, I. 2006. White lupin has developed a complex strategy to limit microbial degradation of the secreted citrate required for phosphate nutrition. Plant, Cell and Environment 29: 919–927.

White, P. J., and Hammond, J. P. 2008. Phosphorus nutrition of terrestrial plants. In: White, P. J., and Hammond, J. P. (eds) The Ecophysiology of Plant-Phosphorus Interactions. Springer, Dordrecht, The Netherlands. 51–81.

Willett, I. R., Chartres, C. J., and Nguyen, T. T. 1988. Migration of phosphate into aggregated particles of ferrihydrite. Journal of Soil Science 39: 275–282.

Williams, J. D. H., Syers, J. K., Harris, R. F., and Armstrong, D. E. 1971. Fractionation of inorganic phosphate in calcareous lake sediments. Soil Science Society of America Proceedings, Madison, USA. 35: 250–255.

Williams, J. D. H., Syers, J. K., and Walker, T. N. 1967. Fractionation of soil inorganic phosphate by a modification of Chang and Jackson's procedure. Soil Science Society of America Proceedings, Madison, USA. 31: 736.

Wissuwa, M. 2005. Combining a modeling with a genetic approach in establishing associations between genetic and physiological effects in relation to phosphorus uptake. Plant and Soil 269: 57–68.

Yan, F., Zhu, Y., Müller, C., Zörb, C., and Schubert, S. 2002. Adaptation of H^+-pumping and plasma membrane H^+ ATPase activity in proteoid roots of white lupin under phosphate deficiency. Plant Physiology 129: 50–63.

Yao, W., and Millero, F. J. 1996. Adsorption of phosphate on manganese dioxide in sea water. Environmental Science and Technology 30: 536–541.

Zhang, T. Q., MacKenzie, A. F., Liang, B. C., and Drury, C. F. 2004. Soil test phosphorus and phosphorus fractions with long-term phosphorus addition and depletion. Soil Science Society of America Journal 68: 519–528.

Zhu, Y., Yan, F., Zörb, C., and Schubert, S. 2005. A link between citrate and proton release by proteoid roots of white lupin (*Lupinus albus* L.) grown under phosphorus-deficient conditions? Plant and Cell Physiology 46: 892–901.

Declaration/ Erklärung

“I declare that the dissertation here submitted is entirely my own work, written without any illegitimate help by any third party and solely with materials as indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At all times during the investigations carried out by me and described in the dissertation, I have followed the principles of good scientific practice as defined in the “Statutes of the Justus Liebig University Giessen for the Safeguarding of Good Scientific Practice.”

“Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten. ”

Imran Ashraf

Giessen, Germany

Acknowledgments

I am highly thankful and deeply indebted to my supervisor Prof. Dr. Sven Schubert for providing me opportunity to work as a doctoral fellow in the Institute of Plant Nutrition, Justus Liebig University Giessen, Germany. I am highly grateful to him for his kind supervision, constructive criticism, valuable suggestions, necessary guidance, and sympathetic attitude. His style of mentorship has truly improved my abilities as an independent researcher. I am greatly thankful to Prof. Dr. Jan Siemens for being my co-supervisor and reviewing this manuscript.

My sincere gratitude goes to Prof. Dr. Diedrich Steffens for his useful inputs, timely guidance, valuable suggestions, sympathetic attitude, and critical comments. I am truly grateful and deeply indebted to him. I would like to pay my special thanks to Roland Pfanschilling and Corinna Alles for their help during analyses and experimentation, respectively. I am also thankful to Christina Plachta and Sigrid Beckermann for their kindness and moral support.

Heartiest thanks to Fraziska, Stefan, Johanna, Daniel, Phillip, Alexandra, Farooq, Abdul Wakeel, M Farooq, Faiq, Ahmed, Atiq, Nesar, Ammara, M Saqib, Daniela, Prof Kumar, Mr Wilming, Ms Rödiger, Ms Tripp, Ms Weimer, and Ms Langer for their friendliness and having memorable time in the institute.

Special thanks to Haider, Uzman, Imran, Shams, Rauf, Shahbaz, Wajid, Naqeeb, Shahid, Sahito, Sajid, Athar, Ijaz, Habib, Adnan, Aslam, Murtaza, and Farhan for their moral support, nice discussions, and having memorable time in Giessen.

I am highly thankful to my siblings and cousins especially Kamran, Adnan, Nafeesa, Sidra, Moon, Ghazala, Hina, Abu Bakar, Asma, Mashail, Zuha, Shahid, Khalil, Aamir, Faisal, Ali, and Ahmed for their moral support, encouragement, and well wishes during my study.

Finally yet importantly, I am highly obliged to the Higher Education Commission (HEC) of Pakistan and the German Academic Exchange Service (DAAD) for providing me financial support to carry out my doctoral study in Germany.

Imran Ashraf

15
P
Phosphorus
39.97

édition scientifique
VVB LAUFERSWEILER VERLAG

VVB LAUFERSWEILER VERLAG
STAUFENBERGRING 15
D-35396 GIESSEN

Tel: 0641-5599888 Fax: -5599890
redaktion@doktorverlag.de
www.doktorverlag.de

ISBN: 978-3-8359-6397-9