The solubility of native and applied zinc in the soil as affected by liming and type of inorganic phosphate fertiliser

By

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DECLARATION

I, Akanyang Larona Kabelo declare that the dissertation, which I hereby submit for the degree MSc. Soil Science at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE:……..

DATE:…….. ……

DEDICATION

This work is dedicated to my parents; Sekokiane Makgorotlhe and Seipone Modisaemang who supported and motivated me to continue with my studies before passing on in 2002. This work is also dedicated to my sister, Ketshephaone, my brother, Goitsemodimo, my nephew, Abang and my niece Masedi Kabelo with the deepest love and support they had for me throughout my studies.

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ABSTRACT

The prevalence of Zinc (Zn) deficiency in plants is a common global phenomenon. Soils with inherent low Zn contents are found, but it is often induced by high carbonate or organic matter contents in soils, high soil pH, or heavy phosphate fertilisation and often by a combination of the latter two. The effects of the latter are the most researched due to their negative effects on the mobility and plant-availability of Zn. The negative phosphate–Zn interactions are caused by several chemical factors in soils and physiological factors in plants. The objectives of this study were: a) to examine the impacts of different phosphate fertilizers sources and lime on the solubility of both native and applied zinc in soils based on different chemical fractions. b) To study the impact of these amendments on the diffusion of applied zinc from fertiliser bands.

The study was carried out in two highly weathered red apedal soils of the Hutton form, but different textural classes: sandy loam and clay. Three phosphate sources: Mono-ammonium phosphate (MAP), diammonium phosphate (DAP) and dicalciumphosphate (DCP) were co-applied individually with $ZnSO_4$ in simulated fertiliser bands to both soils in their unlimed and limed states. This resulted in eight fertiliser treatments combinations together with controls. A sequential extraction procedure was used to determine the amounts of Zn in different chemical pools.

The study showed that a large proportion of the native Zn was in acid extractable Zn fraction representing the Mn + Fe bound fractions while applied Zn was largely in NH_2OH fraction. Liming caused very big increases the zinc concentrations in the NH_2OH, $Mg(NO_3)_2$ and NH_2OH*HCl fractions in the extraction sequence in the applied Zn in both the clay and sandy loam soils. These represent the labile (soluble + adsorbed) zinc fraction and the zinc sorbed to sesquioxides. The co-application of both ammonium phosphates and lime in the fertilizer bands increased or decreased sum of fractions depending on the type of phosphate fertilisers. MAP extracted higher native Zn concentrations than DAP and DCP. The application of calcium phosphate (DCP) in the limed soils very strongly reduced both the Zn concentration in this fraction in the

band and movement of Zn from the band into the surrounding soil. This shows the very strong impact of the combination of liming/somewhat higher pH and co-application of a calcium phosphate on Zn in the soil.

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Chapter 1: General Introduction

The importance of zinc (Zn) as an essential nutrient was established as early as the 1930s (Welch, 1993; Nielsen, 2012). Since then, research aimed to understand its role as an essential plant nutrient and importance for human and animal health have continued. In both humans and animals Zn assists in the catalysis of at least 100 enzymes, promoting protein folding and managing gene expressions (Hortz and Brown, 2004; Hambidge and Krebs, 2007; Alloway, 2008b). In human health, zinc deficiency is experienced mostly in children, pregnant women and breastfeeding mothers manifested as weakened immune systems, poor brain development, stunted growth and respiratory problems in infants and toddlers (Gibson, 2012).

Zinc deficient soils, leading to Zn deficiency in humans is a worldwide concern (Ramzan et al., 2014). Zinc deficiency is reportedly the most common deficiency amongst the micronutrients. Zhao et al. (2016) highlighted that Zn deficiency is ranked fifth on the list of all factors causing human illness and death in developing countries. Zinc deficiency is especially experienced in developing countries in Asia and Africa where diet are cereal dominant (Cakmak, 2008). Around 400 million people in Sub-Saharan Africa, for example, are at risk of Zn deficiency, especially children under 5 years of age (Hortz and Brown, 2004; Gibson, 2006). In Southern Africa, Zn deficient soils are common (Van der Waals and Laker, 2008).

Around 1934, zinc sulphate ($ZnSO_4$) was used in the treatment of so-called “white bud” in maize (Nielsen, 2012). Since then Zn fertilisers have been made available in different compounds; namely (1) inorganic compounds, (2) synthetic chelates and (3) natural organic complexes. However, water-soluble $ZnSO_4$, is still the most popular and preferred zinc source. Less soluble compounds are also often used, for example, ZnO (Mortvedt and Gilkes, 1993).

Zn is only needed in small quantities and is commonly combined with phosphate fertilisers such as mono-ammonium phosphate (MAP), diammonium phosphate (DAP) and superphosphate (SSP) for easy application (Montalvo et al., 2016). Van Biljon et al. (2010) mentioned that this has also been the case in South Africa since the 1960s when serious Zn deficiencies were first identified in maize in the country. However, some researchers have argued that this is not ideal, since problems such as the separation of particles during manufacturing, handling and transportation have been encountered (Mortvedt, 1991). To overcome the segregation problem, an alternative approach involving co-granulation of phosphate and Zn fertilisers was introduced (Degryse et al., 2015). This, however, often lowers the effectiveness of Zn fertilisers due to the formation of less soluble/insoluble Zn phosphate compounds (Montalvo et al., 2016). Furthermore, certain soil properties aggravate this problem even further. Soil properties that affect Zn sorption and/or precipitation are pH, clay type and content, soil texture, soil organic matter content, calcium carbonate content, presence of sesquioxides and soluble phosphate level (Alloway, 2008a; Singh et al.,2008; Van Biljon et al., 2010). Alteration in these soil physiochemical properties influence the fate of both applied and native Zn (Kiekens, 1990; Shuman, 1991; Finzgar et al. 2007). It has been demonstrated that highly weathered soil with the presence of kaolinite and Fe and Al oxides retain Zn in unavailable forms (residual Zn / Fe and Al occluded Zn) (Saffari et al., 2009; Preetha and Stalin, 2014).

Concentrating phosphate fertiliser in a band can locally accentuate not only acidification but also alter soil conditions. During band placement of fertilisers, the surrounding soil goes through varying stages of acidification depending on the fertilisers used (Isensee and Walsh, 1971; 1972). These changes in soil condition are known to enhance Zn sorption (Degryse et al., 2009). The proposed mechanisms are as follows: increased acidity in the band results in an increase in positive charges on certain soil colloids, for example, iron oxides, which favours phosphate sorption. This, in turn, enhances the sorption of Zn (Strawn et al., 2015). Some phosphate fertilisers have greater soil acidification impacts than others (Manoharan, 1997).

The desorption of both native and applied Zn is also hampered by reactions in the fertiliser bands (Gworek and Mocek, 2003; He et al., 2013; Sharma and Kumar, 2016). Soil reactions triggered during band placement do not only affect the Zn transformation in the soil, but also regulate the diffusion of Zn from the point of application.

Although phosphate interactions with Zn have received renewed attention in recent years, there still remain outstanding questions regarding the mechanisms of Zn - phosphate retention in the soil. For instance, different effects of different phosphate compounds on Zn in soil has also not received much attention. Phosphate is commonly applied in the field as mono-ammonium phosphate (MAP), monocalcium phosphate, diammonium phosphate (DAP) and dicalcium phosphate (DCP). These compounds are chemically different and the fundamental question is if this has an impact on the solubility of native Zn and applied Zn due to their differences in pH which alters the micro-evironment surrounding a phosphorus fertiliser granule.

Aim

Based on the above-identified research gaps, the overall aim of this study is to examine the transformation of native Zn as well as applied Zn (as $ZnSO_4$) in two contrasting soils (in terms of chemistry and mineralogy) as affected by the type of applied inorganic phosphate fertiliser and liming.

Hypotheses

This study was conducted to test the following hypotheses:

1. The combined effects of liming (higher pH) and localised high levels of phosphate have a higher suppressive effects on zinc solubility than the effects of the above two factors independently.
2. The stable chemical zinc form (acid extractable fraction) controls the fate of Zn under concentrated P applications (band placement) than other Zn fractions.
3. The efficiency of DCP as a source of phosphorus lowers the mobility, and hence extractability, of Zn in limed conditions.

Objectives of this study were:

1. To determine the influence of treatment combinations (phosphate fertilisers type and liming) on the solubility of native Zn
2. To examine the influence of liming and types of inorganic phosphate fertiliser on the solubility of Zn applied as water-soluble $ZnSO_4$.
3. To assess diffusion of applied Zn in a simulated fertilisers band as affected by phosphate fertilisers in limed and unlimed soil.

Thesis outline

Chapter one covers the introduction that includes the problem statement and provides the objectives of the study. Chapter two is the literature review which describes outcomes of previous and recent similar studies. Chapter three covers the methodology, materials and description of soils and fertilisers used in this study to achieve the objectives. Chapter four provides information on certain characteristics of the soils used in the study. Chapter 5 deals with the effects of monoammonium phosphate (MAP), diammonium phosphate (DAP) and dicalcium phosphate (DCP) fertilisers on the solubility of native Zn, with or without applied lime. Chapter 6 deals with the effects MAP, DAP fertilisers on the solubility of applied Zn, with or without applied lime. Chapter 7 covers the mobility and diffusion of band applied Zn and enrichment of the surrounding soil with Zn. Chapter 8 summarises the study, concludes it and lays down recommendations.

Chapter 2: Literature review

2.1 Geographic distribution of Zn deficiency in Africa

Harsh climatic conditions, such as low rainfall, high temperatures and poor quality of soils are usually experienced in the sub-Sahara Africa (Van der Waals and Laker, 2008). Generally, nutrients deficiency is an indication of low soil fertility which leads to lower quality crop production. Various authors have indicated the presence of Zn deficiency in different regions of Africa. Some of these studies include from South Africa (Van der Waals and Laker, 2008), Zimbabwe (Tagwira et al., 1993a; 1993b), Morocco (Ryan et al., 1995), Nigeria (Agbenin, 1998; Egwu and Agbenin, 2013), Zambia (Chirwa and Yerokun, 2012), and Mozambique (Ricardo and Russell, 2006).

The studies from Zimbabwe indicated that most Zimbabwean soils inherently have low levels of Zn. From 120 soil profiles in 19 locations, 32% had less than 1 mg kg^{-1} of plant - available Zn. According to the authors, one of the main reasons for such low Zn content of the soils is attributed to the parent materials. Ryan et al. (1995) reported Zn deficiency of rain-fed maize planted in calcareous soils in the dry region of Morocco, was caused by high P fertilizer applications. Studies conducted by Ricardo and Russell (2006) on ten agro-ecological zones in the major crop producing areas of Mozambique, (dominated by Oxisols, Alfisols, and Ultisols with high clay content), reported low Zn content ranges of 0.27 – 4.15 mg kg^{-1}. Other studies by Chirwa and Yerokun (2012) in Zambia and Agbenin (1998) and Egmu and Agbenin (2013) in Nigerian savanna soils also reported Zn deficiency.

Studies conducted in South Africa by Herselman (2007) reported low EDTA extractable Zn concentrations (0.06 – 2.8 mg kg^{-1}) in Northern Cape, North West province, coastal areas of Western Cape and KwaZulu Natal as well as North-Eastern Free State. Similarly, a review by Van der Waals and Laker (2008) reported Zn deficiency throughout the maize belt of South Africa, which is dominated by sand. Generally liming and high phosphate application rates cause low Zn uptake by plants since they change the pH of the soil initiating some soil reactions which aggravate Zn unavailability (Laker, 1967).

2.2 Occurrence and abundance of natural/native Zinc in soils

2.2.1 Influence of geology and climate on the soil zinc content of soils

Different studies showed virtually the same estimates of average total Zn concentrations on the earth, namely 64 mg kg^{-1} (Storey 2007), 75 mg kg^{-1} (Reimann et al. 2014), 71 mg kg^{-1} (Chesworth,1991) and 70 mg kg^{-1} (Mitsios and Danalatos, 2006). Zinc also exists in various rocks and minerals at different concentrations, such as igneous (granite: 40 mg kg^{-1}, basalt: 100 mg kg^{-1}) and sedimentary rocks (limestone: 20 mg kg^{-1}, sandstone: 16mg kg^{-1}, shale: 95 mg kg^{-1}) (Storey, 2007). Most Zn enters soils through weathering processes of rocks (Mitsios and Danalatos, 2006) and volcanic activities (Orhue and Frank, 2011). Thus parent material significantly impacts on the distribution and concentrations of Zn and other trace elements (Alloway, 2008a). During weathering, the Zn is released to silicates, carbonates and sulphides to form Zn minerals such as sphalerite (ZnS), smithsonite ($ZnCO_3$), willemite ($ZnSiO_4$) and franklinite ($ZnFe_2O_4$) (Vodyanitskii, 2010). The major sources of natural Zn in the soil are sphalerite (ZnS) and wurtzite (Zn, Fe)S), but other sources, such as smithsonite ($ZnCO_3$) exist in the soil (Kiekens, 1990).

Zinc occurs in only one oxidation state namely Zn^{2+} compared to other micronutrients (Fe, Mn and Mo) that have 2 or more oxidation states (Essington, 2004; Storey, 2007). Therefore, due to this, Zn has simple solution chemistry and is usually bound with clays, hydrous oxides and organic matter (Whitehead, 2000). Some Zn deficiency zones are defined by certain climatic conditions, such as extreme temperature and rainfall, and the plants in that area. Studies showed that Zn deficiency occurs in arid and semi-arid climatic conditions (India), paddy soils with poor drainage (China), acidic sandy soil with good drainage (South-Eastern USA), tropics (Brazil, Chad and Philippines) and aeolian, calcareous, acidic, leached sands (Australia) (Welch, 1993). In South Africa the most severe Zn deficiencies occur in the sandy soils that dominate the western parts of the so-called “Maize quadrangle”, namely the North western Free State and Northwest province (Van der Waals and Laker, 2008).

Mostly Zn deficiency is experienced in alkaline, calcareous and limed soils, organic and peat soils, acidic leached sandy soils and paddy soils. The latter is found under rice cultivation, where Zn binds with sulphide and amorphous ferrous oxides to form sphalerite and wurtzite (Hafeez et al., 2013). Calcareous soils are usually in the arid and semi-arid areas. The high pH of these soils enhances the formation of Zn carbonate (Malakouti, 2007; Ryan et al., 1995).

2.2.1.1 Influence of weathering on Zn distribution between secondary minerals

Zn^{2+} forms bonds with clay minerals and secondary Fe and Mn oxides and oxyhydroxides (Kiekens, 1990). Weathering processes can be categorized into three stages: early, intermediate and late. In the early stage, the primary minerals, such as quartz, muscovite and albite, dominate (Essington, 2004). These minerals contain nutrients which are not available for plant uptake. In the intermediate stage, secondary minerals, transformed from primary minerals by carbonation, hydrolysis, hydration and redox reactions are comprised mainly by 2:1 clay minerals such as smectites. The nutrients adsorbed to these can then be taken up by plants. In the late stage, which involves formation of 1:1 clay minerals such as kaolinite, and oxides of Fe and Al, nutrients become unavailable due to soil conditions such as low pH and reduced environments (Huang et al., 2012). Highly weathered soils are generally highly leached, therefore they contain lower amounts of zinc. Furthermore, Zn also precipitates with Fe oxides and silicon to form sparingly soluble minerals (Brady and Weil, 2014).

2.2.2 Anthropogenic influences of Zn distribution in soil

Non-agricultural practices

Zinc is not only released to the environment through weathering processes. Several human-induced practices and activities contribute to and influence the distribution of Zn in the soil (Orhue and Frank, 2011). These practices either overload or deplete the Zn in the soil. Since these practices take place over years, they may influence geographic Zn distribution patterns. Mitsios and Danalatos (2006) mentioned human-

induced activities, such as Zn smelting processes and agricultural activities, which release more Zn to the atmosphere than natural processes.

Zinc has several uses in various industries. The most common one is galvanisation, whereby zinc is mixed with steel to avoid corrosion (Frassinetti et al., 2006). Others include its use in the manufacturing of paint, cosmetics and various alloys, as well as in the production of medicines (Prasad et al., 2005). Zn mining, smelting and processing add Zn emissions to the atmosphere through dust and smoke, together with the burning of coal and waste ignition (Vodyanitskii, 2010; Kabala and Singh, 2001). The same authors observed a high concentration of about 1390 mg kg^{-1} Zn in the soil around a Zn smelter in Canada. In addition, urban soils are subjected to Zn produced from the weathering of vehicle tyres (Reimann et al., 2014). Tapadar and Jha (2015) found that Zn was the most dominant metal in both disturbed dump soils from an open cast coal mine of Ledo Colliery in India and in undisturbed forest soils. The highest level recorded was 160 mg kg^{-1} in disturbed mine soils and around 70 mg kg^{-1} in the forest soils in India.

Agricultural practices

Agricultural management activities such as, application of fertilisers and sewage sludge, use of herbicides and pesticides and irrigation with sewage water can impact on the Zn content of soils. Pesticides and fertilisers containing Zn impurities may load excessive Zn in the soil. It has been observed that in some pesticides and fertilisers, Zn impurities can be as high as 25%, superphosphate being an example (Kiekens, 1990). In contrast, phosphate fertilisers have been proven to decrease the Zn solubility in soil (Reimann et al., 2014). Sewage sludge has been observed to increase Zn levels in the soil (Antoniadis et al., 2007; Khaled, 2004). It has been estimated that sewage sludge can contain average Zn contents of 1500 - 4100 mg kg^{-1} (Alloway, 2008c) or 72 -16,400 mg kg^{-1} (Stover et al., 1976) depending on the source of sludge (municipal or industrial) and treatments employed.

2.3 The fate of applied Zn to the soil

Due to varied conditions of the soil as influenced by different chemical and physical soil properties, Zn elements end up in either available or unavailable forms.

2.3.1 Sources of Zinc

To correct zinc deficiency in plants, organic and inorganic fertilisers are applied (Singh, 2005). Zinc fertilisers are used to increase the Zn concentrations in soil and hence enhance plant uptake and increased crop yields (Rafique et al., 2015). Moreover, there is increased use of Zn foliar application in Sub – Sahara Africa (Joy et al., 2015), particularly in fruit production. There are different Zn sources which can be used to correct inadequate levels of soil and plant zinc. They can be in (i) organic form: (animal manure, sewage sludge), (ii) inorganic (zinc sulphate ($ZnSO_4$), zinc oxide (ZnO), and zinc nitrate ($Zn(NO_3)_2$) and synthetic chelates (ZnEDTA, Zn- citrate, ZnHEDTA) forms (Shuman, 1998, Alloway, 2008a). Modaihsh (1990) found that Zn from ZnEDTA diffused readily in all soils, moving 20-25 mm from the layer where it was applied within three days. In contrast, the diffusion of Zn from $ZnSO_4$ was limited in all soils, being confined to within 5 mm from the layer where it was applied even after 13 days. A changed environment of altered soil conditions and less contact between the soil and $ZnSO_4$ enhances this problem. For instance, Zn fixation usually occurs in soils with high pH, but also in acid clay soils. Modaish (1990) found that diffusion of Zn was lowest in the soil with the highest clay content and CEC, despite having a relatively low pH.

Water solubility of Zn fertilisers is the key parameter in fertiliser effectiveness and efficiency (Amrani et al., 1999) and it varies depending on the source due to the variation on the proportion of H_2SO_4 being applied during manufacturing (Table 2.1) (Ahmad et al., 2012). Soluble fertilisers outperform the least soluble fertilisers (Westfall et al., 1999). However, apart from water solubility, the performance of Zn sources in releasing Zn depends on the pH and other soil conditions (Milani et al., 2015). Normally, Zn fertilisers which are 40 -50% water soluble are able to supply sufficient

Zn to plants (Slaton et al., 2005). Mortvedt (1991) also showed that the particle size of Zn fertiliser granules determines the efficiency of Zn sources.

Table 2.1: Some commonly used sources of Zn

Zinc Sources	**Zn content (%)**	**Solubility in H_2O**
Zinc sulphate monohydrate ($ZnSO_4.H_2O$)	36	~ 100% soluble
Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$)	22	~100% soluble
Zinc oxysulphate ($ZnSO_4$ ZnO)	20-50	Variable
Zinc oxide (ZnO)	50-80	Sparingly soluble
Zinc carbonate ($ZnCO_3$)	50 -56	Insoluble
Zinc chloride ($ZnCl_2$)	50	Soluble
Zn chelate (Na_2 Zn EDTA)	14	100% soluble
Zn chelate (Na Zn HEDTA)	6 -10	100% soluble
Sewage sludge	150 – 1200 mg/kg	

Source: (Alloway, 2008a)

When choosing which Zn sources to use, several factors have to be considered, including water solubility, the cost, Zn content and method of application, the latter being the latest discovered. Zinc sulphate is most commonly used due to its availability, high water solubility and being less expensive and easily applicable (Hafeez et al., 2013; Menon and Rahman, 1995; Shaver and Westfall, 2005). Zinc oxide is fairly widely used, but is less preferred than zinc sulphate due to its lower solubility in water. Some authors concluded that zinc oxide is effective in releasing Zn, since it dissolves slowly and hence maintains adequate plant-available concentrations of Zn over long periods of time (Mortvedt, 1991). One of the disadvantages of ZnO is that it is not effective in alkaline or limed soil (Milani et al., 2015).

Zinc sulphate ($ZnSO_4$) exists as monohydrate and heptahydrate which have 36% and 22% of Zn, respectively (Table 2.1) (Alloway, 2008a). Westfall et al. (1999) concluded that water solubility of Zn sources increases the Zn uptake by the plants after findings which showed that $ZnSO_4$ produced more dry matter of maize compared to other sources of Zn. Zinc oxide has a high Zn content (up to 80%) but has low solubility in water. Zinc oxide can be as effective as $ZnSO_4$ in acidic soil conditions (Mcbeath and McLaughlin, 2014). Breannan and Bolland (2006) found that in high pH soil, Zn sulphate is more soluble than zinc oxide while in low pH soil conditions they perform the same. To address the differences and challenges brought by these two Zn sources, zinc oxysulfate was developed. This is a combination of ZnO and $ZnSO_4$, created by partly acidifying ZnO by sulphuric acid. It contains an average Zn content of 52 % (Menon and Rahman, 1995).

Chelates are often preferred as sources of Zn over the normal fertilisers due to their high mobility and high release of Zn (Alvarez, 2007). EDTA and DTPA are known for creating stable complexes with Zn, thus maintaining it in plant–available form, especially in alkaline and calcareous soils (Almendros et al., 2015). Synthetic chelates are considered more efficient in alkaline soil conditions compared to $ZnSO_4$. (Zhao et al., 2016). Na_2Zn–EDTA is preferred, compared to CaZn-EDTA, due to lower competition of Na than Ca for exchange sites with Zn (Alloway, 2008b). According to international literature natural chelates can also be used to provide Zn, but they are less effective due to unstable complexes (Alloway, 2008a). Pot experiments by Barnard et al. (1990) at the University of Pretoria on sandy soil to which high lime levels were applied found that both $ZnSO_4$ and ZnEDTA applications failed to increase maize shoot growth and the Zn contents of the maize topgrowth, while Zn chelates produced using coal-derived humic acids and fulvic acids successfully increased both. In the case of Zn content the difference was very big.

2.3.2 Zinc application practices: broadcast versus band placement

Different zinc fertilisers can be applied as a side dressing (bands), blended together with or as coating on dry commonly used fertilisers (Shaver and Westfall, 2005). Application methods of Zn can decrease or increase Zn uptake by plants (Soper et al., 1989). Zinc applied to the soil as a granular fertiliser at the correct rate is by far the more economical way to ensure that the plant has adequate zinc through its lifecycle than foliar applications (Mengel and Kirkby, 2004). Cakmak et al. (1999) emphasized the role of application methods on the effective release of Zn to plants. Granular Zn fertilisers can be broadcasted or band placed (Mengel and Kirkby, 2004). In the broadcast placement method, the fertilisers are homogeneously mixed with the soil for even distribution, but due to contact with a large volume of soil, Zn fixation into unavailable forms is high such as occluded to oxides and silicates clays (Degryse et al., 2015).

Broadcasting Zn has also been found to result in greater adsorption of Zn due to increased contact with sorption exchange sites and poor plant root interception (Alloway, 2008c). Conversely, band placement reduces the adsorption of Zn due to less contact between the applied Zn and hence increases uptake of the element. Band placement requires placing fertilizers at a certain distance and depth close to the plant root. This was shown by McBeath and McLaughin (2014) in a study to determine the efficiency of zinc oxide as fertiliser. They observed that the band placed insoluble Zn oxide performed poorer as compared to broadcasted ZnO. Degryse et al. (2015) also found that the plant-availability of Zn from insoluble ZnO is better when it is broadcasted than when the band placed. On the other hand, Zhao et al. (2016) clearly observed that $ZnSO_4$ applied in a band is more extractable and more diffusive as compared to broadcast application.

Liming also influences the microenvironment around fertiliser granules due to its effect of increasing the soil pH. As pH increases, the ionic composition of the surrounding soil also changes (Isensee and Walsh, 1971, 1972). At higher pH, the Zn concentration in the soil solution decreases because Zn adsorption is increased (Rutkowska et al.,

2015). The presence of calcium carbonate also aggravates Zn adsorption as it binds to calcite surfaces (Dong and Wasylenki, 2016).

2.3.3 Soil properties influencing solubility of zinc in soil

The solubility of minerals in soil has been a central subject in soil fertility as it influences the efficiency of fertilisers as well as their management. Zinc solubility in soils has been given more attention with regards to contamination than in regard to Zn deficiency. Zinc solubility is mostly indicated by relating total concentrations of Zn in the soil to free Zn^{2+} activities (Catlett et al., 2002). There is a direct association between the Zn^{2+} activity and proton activity, thus as pH increases Zn solubility decreases (Singh et al., 2008).

a) Soil pH

It is well documented that pH plays a significant role in Zn solubility in the soil since it has an impact on the concentrations and activities of Zn^{2+} (Singh et al., 2008; Girija et al., 2013; Rutkowska et al., 2015). Zn availability is lowered by an increase in pH in that it enhances Zn retention (Moraghan and Mascagni, 1991; Martinez and Motto, 2000). High pH affects Zn availability in three ways; firstly, there is more specific Zn sorption because of an increase in negative charge. Secondly, there is an increase in dominance of hydrolysed Zn forms, namely $ZnOH^+$ at pH 7.7–9.0 (Harter, 1991) and $Zn(OH)_2$ in calcareous soil above pH 9.0 (Foth and Ellis, 1997). Thirdly, due to the formation of insoluble complexes with calcium carbonate and iron oxides (Alloway, 2008a; Van Biljon et al., 2010).

Farrah and Pickering, (1976) observed in their results when studying zinc sorption by minerals that at high pH the sorption of Zn^{2+} by clay also increases. They suggested three possible explanations for this pH effect, namely (i) less competition for active exchange sites from protons, and (ii) easy binding of Zn on exposed new sites due to the expansion of clay minerals as a result of OH^- ion adsorption, and (iii) more negative charges. The findings of Finzgar et al. (2007) showed that Zn sorption occurred at pH values of 5–6.5 while precipitation reactions took place at pH 6–7.

At low pH less than 7.7, Zn^{2+} is the abundant Zn species. Orhue and Frank, (2011) in their review, stated that in strongly acidic soil, Zn is less strongly bound when compared to weakly acidic soils. pH also has an effect on other soil properties such as an increased number of negative adsorption sites of soil colloids like clay minerals and organic matter, which are then available for easy Zn adsorption. This phenomenon in clays is due to the fact that as pH decreases, the functional groups (silanol and aluminol) protonates, leading to less retention of metals (Abollino et al., 2003).

b) Soil texture

The coarseness and the fineness of soil tend to regulate Zn availability in the soil. The particle size distribution of soil, i.e. the proportion between sand, clay and silt determines how vulnerable Zn is to leach and provides an estimation of Zn mobility (Rieuwerts et al., 1998). For instance, Zn deficiencies are critical in sandy soils with low organic matter levels due to inherent low Zn levels in such soils (Laker, 2005). The low affinity of sandy soils for Zn is caused by its properties such as the low amount of clay content, less Fe and Al oxides and lower organic matter (Zhang et al., 2006). Another characteristic of sandy soil is low water holding capacity which leads to a high rate of leaching, thus aggravating the problem of Zn deficiency (Alloway, 2008a). Laker (2005) also mentioned that sandy soils are much more prone to zinc deficiencies than medium –textured and clayey soils. Studies have shown that sandy soils low in organic matter content have lower bonding energies and adsorptive capacities (Behera et al., 2011). Clay soils, due to their high cation exchange capacity tend to retain more Zn than sandy soils, so an increase in clay content might increase available Zn (Hafeez et al., 2013).

c) *Organic matter*

One of the chemical functions of organic matter (OM) in the soils is to increase the cation exchange capacity of the soil (Brady and Weil, 2014). Organic matter contains functional groups which make good ligands for Zn and other metals (Ashworth and Alloway, 2004). Organic matter can have both negative and positive effects on Zn solubility through the formation of both soluble and insoluble organo- Zn complexes.

Organic matter has an impact on Zn solubility in four ways: i) Zn reacts with humified organic matter to form insoluble Zn - humic compounds, ii) Zinc reacts with organic acids such as amino and aliphatic acids, to form soluble compounds (Whitehead, 2000), thus enhancing Zn mobility and hence availability for plant uptake, iii) Ligands from root exudates may bound with Zn in the form of chelates. iv) Soil microbial activities during decomposition produce organic acids which cause dissolution of adsorbed Zn (Wei et al., 2006).

Wei et al. (2006) observed a positive relationship between organic matter and Zn availability, with an increase in organic matter leading to a high concentration of plant-available Zn after 18 years of cropping. Behera et al., (2011) also recorded a significant correlation between extractable Zn and organic matter content. This is in agreement with Sharma et al. (2014) and Fan et al. (2016) who concluded that due to organic acids produced during decomposition of organic matter, Zn retention tends to be less after application of OM. Shukla (1971) argued that OM has indirect effects on Zn availability since it changes the status of other soil parameters such as pH and Ca/Mg contents which might have an impact on the solubility of Zn. Alloway (2008b) highlighted that peat and muck soils may contain lower amounts of soluble Zn due to their low native Zn contents or formation of stable insoluble Zn-humic complexes.

d) Clay minerals

Clay minerals are the major adsorbents of Zn (Gworek and Mocek, 2003; Tlustos et al., 2005) through inner–sphere complexation (Rieuwerts et al., 1998). Under acidic conditions, the exposed hydroxyl groups are protonated, thus creating a positive charge in the soil colloids while in neutral or alkaline soil conditions, there will be deprotonation of surface hydroxyl groups. The affinity between these components and trace metals is influenced by the crystallinity of the minerals and differs among trace metals (Tiller et al., 1984). In ion exchange, Zn^{2+} has almost the same radius as Fe^{2+} and $Mg^{2+,}$ thus, it is easy for Zn^{2+} to also replace the Al^{3+} in the octahedral layer during isomorphous substitution (Mengel and Kirkby, 2004). Sipos et al., (2008) noted the part played by the ion exchange process during the binding of Zn by clay minerals. It

was found that 57% and 19% of total Zn immobilised by samples containing montmorillonite and vermiculite respectively were due to ion exchange. Smectites have been proven to have higher affinity for Zn as compared to kaolinite (Girija et al., 2013).

e) Presence of iron and aluminium oxides and hydroxides

Hydroxides of Fe, Mn and Al occur under oxidising conditions in the soil as coatings on clays (Orhue and Frank, 2011) or on particles (Stanton and Burger, 1967). Hydrous oxides have a significant impact on Zn retention as indicated by numerous studies, as they bind Zn on their surfaces (Stanton and Burger, 1967; Harter, 1991). Zinc can be strongly bound by Al, Fe and Mn oxides and hydroxides through any of inner sphere and outer sphere complexation, ion exchange, co-precipitation or crystal lattice absorption (Shuman, 1991; Sparks, 2003; Storey, 2007; Ryan et al.,2013) or by forming covalent or ionic bonds depending on the type of complexation directly or indirectly to specific sites. This increases Zn retention in acidic soil (Bolan et al, 2003; Essington, 2004; Behera et al. 2011). Oxides of Fe and Mn are more capable of adsorbing Zn than Al oxides (Basta and Gradwohl, 2000). The strength of Zn binding to oxides increases as oxide crystallinity increases. The higher crystallinity of the oxide minerals, the greater Zn affinity (Ryan et al., 2013). Stanton and Burger (1967) concluded that zinc adsorption increases as iron oxide crystallinity increases, more especially with multivalent negatively charged phosphate ions in the soil that acts as bridge between the iron oxides and zinc.

Different Fe oxides in the soil adsorb Zn differently, depending on the number of surface hydroxyl groups. For example, goethite with more of those groups than hematite adsorbs more Zn (Whitehead, 2000). Ryan et al. (2013) found that increased affinity of Fe oxides for Zn is greatly influenced by factors such as the state of crystallinity and surface area. Co-precipitation of zinc onto iron oxides is also a possible mechanism involved in Zn retention (Alloway, 2008a). Zinc deficiency is often found together with iron deficiency in high pH soils because they are rendered unavailable by similar mechanisms in such soils (Mousavi et al., 2012).

f) Presence of carbonates (CO_3)

Zn is bound to carbonates in a similar way to Fe and Mn oxides but co–precipitation usually is the major Zn retention mechanism in soils with free carbonates, particularly at high Zn content levels (polluted zones). This fixation usually occurs in alkaline soil due to the formation of minerals such as smithsonite ($ZnCO_3$) and hydrozincite ($Zn_5(OH)_6(CO_3)_2$), which control the solubility of Zn (Storey, 2007; Ryan et al.,2013). Many studies have proven that carbonates have a negative impact on the solubility of Zn by changing the pH or providing exchange sites on the surface (Kabata – Pendias, 2001). As the pH increases in calcareous soils, Zn reacts with OH^- and is adsorbed by $CaCO_3$ (Mortvedt et al., 1991, Martinez and Motto, 2000). In addition, Ca^{2+} competes with Zn^{2+} for exchange sites. Calcium carbonate surfaces are known to be good exchange sites for metal – surface processes (Zhang et al., 2006).

g) Presence of other micronutrients and macronutrients

Studies have shown that zinc interacts with micronutrients such as Cu, Pb, Fe and Ni in a positive or negative way (Menon and Graham, 1995; Rieuwerts et al., 1998). These elements influence the movement, distribution and availability of Zn in the soil and plants. Interactions such as Zn–Fe, Zn–Cu and Zn–Ni have negative impacts on Zn availability (Kabata – Pendias, 2001). Zinc and copper, due to their similar atomic radii, compete for the same exchange sites, thus the Cu ion strongly hinders Zn adsorption (Schulin et al. 2010). Literature has noted that Na^+ promotes more Zn adsorption as compared to K^+ (Girija et al. 2013). This effect of cations on Zn sorption depends on the pH of the soil.

Several authors observed antagonistic and synergistic behaviour between nitrogen and zinc (Shuman, 1998; Alloway, 2008a). Sajad et al., (2014) investigated the synergistic behaviour of this interaction on the quality of maize fodder by applying different rate of nitrogen fertilisers (NH_4) combined with 5kg and 10 kg Zn ha^{-1}. Their results showed that the crude protein yield increased with an increase in nitrogen and Zn application rate, indicating a positive synergism. Nitrogen fertilisers, more especially ammonium types, have a strong tendency of acidifying the soil. Therefore,

change of pH due to N- fertilisers can increase or decrease Zn mobility depending on the conditions of the soil, i.e. whether is alkaline or not (Zhao et al.,2016).

2.3.4 Mechanisms involved in Zn transformations in soils

Due to the heterogeneity of soils, there are different mechanisms involved in the retention of Zn in the soil as influenced by chemical, mineralogical and physical properties. Transformation of Zn is governed by equilibrium constants (Kiekens, 1990). These equilibrium shifts are controlled by mechanisms involved in Zn distribution which are: sorption (adsorption –desorption), precipitation, surface complexion and dissolution. However, there are still major controversies regarding which mechanisms are involved in the Zn retention due to phosphate (Agbenin, 1998). Furthermore, literature confirms that adsorption of Zn mainly occurs at soil pH 5 to 6.5. Above this soil pH, precipitation and surface complexation mechanisms seem to control Zn solubility (Rieuwerts et al., 1998). Elsokkary (1979) suggested that Zn which recede in the soil solution could go into two different mechanisms, namely either specific adsorption or precipitation. On the other hand, other authors suggested that another mechanisms such as surface complexion may play a part (Agbenin, 1998). The Zn retention mechanisms are as follows:

a) Desorption–sorption of Zn

Sorption processes take place at the solid/solution interface. The sorption mechanism is considered to be the most important solid-liquid phase process which has the biggest impact on soil fertility and fertiliser efficiency (Imitiaz et al., 2006). Sorption is the umbrella name for adsorption, desorption and absorption mechanisms which involve the movement, diffusion and removal of solutes from the liquid into the sorbent phase (Essington, 2004; Girija et al., 2013). Organic matter, clay minerals and Fe/Al/ Mn oxides are soil constituents that have a huge effect on the sorption of Zn. Previous studies demonstrated that in acidic soil, Zn adsorption is due to cation exchange while in high pH soils organic ligands play an important role (Kiekens, 1990). Girija et al. (2013) mentioned that zinc can be adsorbed specifically and non-specific. The latter, which is inner sphere complexation, involves sorption of Zn^{2+} and OH^- in the inner

structure of organic and inorganic soil colloids, especially oxides and hydroxides (Sparks, 2003). Specific Zn adsorption usually occurs in natural areas where Zn contents are low (Rieuwerts et al., 1998). Zn is the most preferred micro-element in adsorption by 2:1 clay minerals compared to other trace metals such as Pb, Cu and Cd. (Alloway, 1990.) Zn in inner sphere complexes is less readily available, compared to Zn in outer sphere complexes (Sparks, 2003).

b) Precipitation

Similar to adsorption/ desorption reactions, precipitation/dissolution reactions play a role in Zn retention on the soil (Pardo, 1999). Phosphates and carbonates can precipitate with Zn to form insoluble compounds (Rieuwerts et al., 1998). Zinc is involved in heterogeneous nucleation, i.e. surface induced precipitation. Zn can also precipitate on mineral surfaces (Schelegel and Manceau, 2006; Hettiarachchi et al., 2008). Research suggested that surface induced precipitation may be the main mechanism controlling zinc solubility, particularly in alkaline soils (Sadiq 1991; Basta and Tabatabai, 1992). In acidic soils, it may occur where there are high concentrations of Zn (Basta and Gradwohl, 2000). High concentrations of Zn lead to rapid sorption rates of Zn onto soil colloids depending on the number of sorption sites (Pèrez-Novo et al., 2011). The rate slowly decreases due to precipitation at the soil-solution interface (Sadiq, 1991). Likewise, Zn minerals such as franklinite ($ZnFe_2O_4$), willemite ($ZnSiO_4$) and hemimorphite ($Zn_4SiO_7\ (OH)_2$) contribute to Zn inefficiency (Manceau et al., 2000). Agbenin (1998) stated that when P is involved, no single mechanism can clearly explain P-induced Zn retention. Even though there is sufficient information on the solid minerals which control Zn solubility, the majority of the work was conducted in smelter–contaminated areas where there are high concentrations of zinc.

2.3.5 Zinc diffusion in soil

The two mechanisms responsible for the mobility of an ion are (i) mass flow and (ii) diffusion (Frassinetti et al., 2006; Hooda, 2010; Nazif et al., 2015). Diffusion is the movement of ions in static water in the soil along a concentration gradient, from high to lower concentration (Van der Watt & Van Rooyen, 1995), while mass flow is the

movement of ions along with moving water (Brady and Weil, 2014). Zinc is predominantly transported by diffusion (Moraghan and Mascagni, 1991).

Zinc is transported by diffusion to plant roots for plant uptake. Although effects of soil moisture content on the movement are less pronounced since the plant adapts, depending on the different moisture regimes, soil water content affects the rate of Zn diffusion (Moraghan and Mascagni, 1991). The chemical fractions in the soil reflect the Zn solubility, hence the Zn mobility. Aucamp (2000) found that the mobility of Zn increased “dramatically” at a soil pH (Water) of below 5.0, while for elements like Cu and Ni this happened only near a pH of 4.0. Modaish (1990) on the other hand highlighted that high soil pH and presence of $CaCO_3$ did not affect the diffusion of Zn when chelated with EDTA. However, high soil pH and presence of $CaCO_3$ both had strong negative effects on the diffusion of Zn from $ZnSO_4$. It is reasonable to expect a correlation between the chemical extractability of an element and its mobility in the soil. Understanding the transformation of Zn into different chemical pools can assist in providing an understanding of Zn diffusion rates in soils. Thus, the rate and degree to which applied Zn is removed from the pool with high solubility affects the diffusion of Zn in soils.

The mobility of Zn is usually expressed as the mobility index (MI) or mobility factor (MF). (Aucamp, 2000; Kabala and Singh, 2001; Gworeck and Mocek, 2003; Mao and Rao, 1997; Osakwe, 2010; Lei et al., 2009; Ngole, 2011). High MF values indicate that a particular ion has high mobility and availability (Aucamp, 2000; Mao and Rao, 1997; Osakwe, 2010; Lei et al., 2009; Ngole, 2011).The mobility factor/mobility index parameter is usually expressed as the proportion of non–specifically bound fractions to the sum of all fractions (Kabala and Singh, 2001). It is used to predict the mobility and availability of Zn in different chemical forms. It is calculated as Equation 2.2.

$$MI = \left(\frac{F1 + F2}{F1 + F2 + F3 + F4 + F5}\right) X\ 100 \qquad \text{Eq. 2.2}$$

(F1 + F2) is assumed to be the sum of weakly bound Zn chemical forms, such as water-soluble and exchangeable fractions while F3, F4 and F5 is assumed to be specifically bound Zn fractions. Some researchers include organic bound Zn in the weakly bound Zn chemical forms (Gworek and Mocek, 2003).

In pollution studies, extraction by 1M NH_4NO_3 is usually considered to be the most suitable extractant by means of which to extract the (water-soluble + adsorbed) fraction (Schloeman, 1994), but other weak extractants can also be used. The total concentration of an element is usually determined by the XRF technique, as was for example done in a master's study at the University of Pretoria by Aucamp (2000). This is usually considered to be the best indicator of total concentration. Where sequential extraction is done the sum of all fractions can alternatively be taken as the total concentration (Kabala and Singh, 2001; Osakwe and Okolie, 2015). Aucamp (2000) calculated the mobility factor as

$$MF = \left(\frac{Extractable\ concentration - as\ extracted\ with\ NH4NO3}{Total\ content\ determined\ by\ means\ of\ XRF}\right) * 100 \qquad \text{Eq. 2.3}$$

High MF values indicate that a particular ion has high mobility and availability (Mao and Rao, 1997; Osakwe, 2010; Lei et al., 2009)

Several researchers have used MF/MI to estimate the amount of the plant-available Zn (Gworek and Mocek, 2003; Kabala and Singh, 2001; Ngole, 2011; Faith et al., 2014). Ngole (2011) found that MF calculated with Equation 2.3 was more than 70% reliable in predicting Cu uptake by carrots, for example. Other researchers used it to determine potential off-site pollution hazards (e.g. Aucamp, 2000). Gworek and Mocek (2003) compared the two sequential extraction methods (McLaren and Crawford and Tessier et al) on which to base MF for different genetic soil horizons. It was found that the McLaren and Crawford (1973) method produced MF values ranging between 14% - 54%, while the Tessier et al. (1979) method had lower MF values ranging between 6% - 28%.

2.4 Zn-P interaction due to co-granulation of Zn fertilisers as blends with P fertilisers

Since Zn is needed in small quantities by plants, it is impractical to apply it alone to soil. In order to obtain easy and efficient application and distribution it is usually mixed with macronutrients such as nitrogen (N), potassium (K) and phosphorus (P) (Degryse et al., 2015), the latter being the most popular fertiliser combination. Zinc is usually mixed with the following phosphate sources; mono-ammonium phosphate (MAP), diammonium phosphate (DAP) or single superphosphate (SSP). Several studies have paid much attention to Zn-P due to their huge antagonistic effects on each other (Marschner, 1995; Fageria, 2001). The interaction is often called “P induced Zn deficiency” (Gianquinto et al., 2000) and is experienced in both plants, including top growth and roots (Thompson, 1996; Zhu et al. 2001; Fageria, 2001; Weldua et al., 2012; Drissi et al., 2015), and soils (Agbenin, 1998). Much emphasis is on Zn–P antagonism around the rhizosphere and in plants (Zhang et al., 2014). In plants, the interaction is usually characterised by Zn accumulation in the roots, dilution in above-ground plant tissue and P toxicity (Kabata – Pendias, 2001). The knowledge of this interaction in the soil is limited (Mousavi et al., 2012; Mousavi, 2011).

2.4.1 Impact of phosphorus fertilisers as blends with Zn sources on the Zn availability in the soil

Even though the P-Zn mixture application method is more useful and inexpensive than applying Zn alone, it can bring availability and solubility challenges to one or both elements in the soil. (Mortvedt, 1991). Application of phosphate fertilisers could change some of the soil properties such as surface charge, pH and available P, leading to direct interaction with Zn (Pardo, 1999; Yan et al., 2015). There is still contradictory information and uncertain issues surrounding the P–induced Zn retention mechanism. Some authors suggest that application of P fertilisers promote Zn redistribution in various chemical forms (Figure 2.1) (Mandal and Mandal, 1990). On the other hand, the formation of Zn-phosphate compounds in the soil contributes to Zn retention in the soil (Kassir et al., 2012). However, Agbenin (1998), Harter (1991)

and Barrow (1987) highlighted that at different soil pH levels, the presence of P in the soil tends to decrease, increase or has no effect on zinc availability.

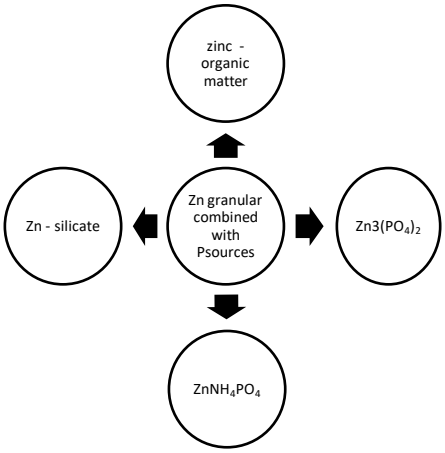

Figure 2.1: Possible soil reactions when Zn – P sources enter the soil.

Source: Mortvedt, 1991

The blending of Zn and P fertilisers bring disadvantages such as physical segregation during manufacturing, transportation and application (Milani et al., 2015). Therefore, co- granulation was introduced to address the problem. However, it brings about some changes in the chemical properties of both fertilisers. Usually, 1–5 % of Zn in the form of an inorganic Zn source is coated on granular P fertiliser, depending on the requirement for a particular environment (Lombi et al., 2004). MAP and DAP, due to their higher P concentrations and solubility have been more widely utilised than superphosphate lately (Alloway, 2008a). In most cases, P is band placed to reduce its high degree of fixation in the soil. Since Zn is often co-granulated with P, it becomes fixed too. For phosphorus, this method is very effective, but for Zn it may be a different situation. Ammonium phosphate fertilisers create an acidic environment around the fertiliser granule zone, particularly in the band placed method, due to acidification process during nitrification of the ammonium. This encourages an environment conducive to the occurrence of Zn deficiency due to changes in soil parameters (Degryse et al., 2009; Mousavi et al., 2012). MAP and DAP have different Zn contents, namely 10.3 ± 2.6 and 386 ± 17 mg kg $^{-1}$, respectively (Ahmad et al., 2012).

Research conducted by Degryse et al., (2015) clearly indicated that the pH of both soil and of zinc carrier plays a crucial role in the effectiveness of the zinc carrier and in Zn movement. Zn was applied as a $ZnSO_4$ solution at a content level of 0.35mg per petri dish. It was then coated on DAP and MAP fertiliser granules. Using the zinc visualisation method, the results showed that after 28 days of incubation in acid soils, $ZnSO_4$ and MAP + Zn have similar, relatively large diffusive circles (Figure 2.2). In the neutral soil $ZnSO_4$ applied alone gave a somewhat smaller circle than in the acid soil, while MAP + Zn gave a much smaller circle. In calcareous soil at high pH$ZnSO_4$ applied alone diffused, but less than in the neutral soil and much less than in the acid soil whereas, when combined with MAP fertiliser, there was no observable diffusion. In the case of DAP + Zn there was no observable diffusion in any of the soils.

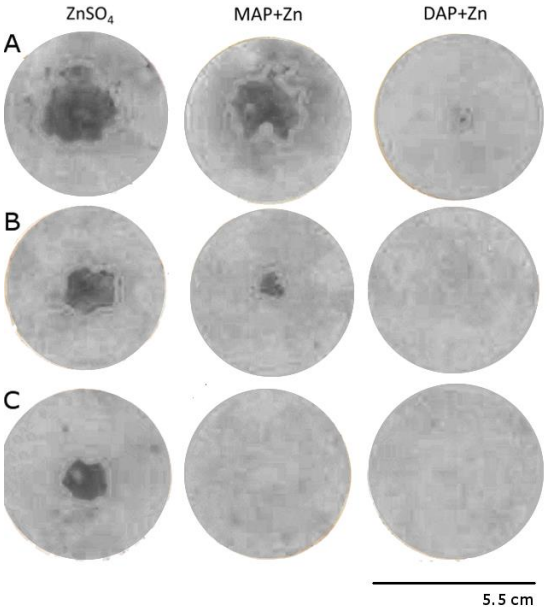

Figure 2.2: High Zn Zone (dark) around Zn fertilisers after 28 days with soil in (A) acidic, (B) near neutral and (C) calcareous soil for Zn applied as ZnSO4, Zn –coated MAP and Zn – coated DAP.

Source: Degryse et al. (2015.)

2.5 Speciation and fractionation of Zn in soil

Zinc and other heavy metals, such as copper and lead, occur in different chemical pools in the soil, depending on the chemical and physical properties of the soil. This

greatly influences their mobility as well as availability (Kabala and Singh, 2001). Zinc species in the soil control Zn distribution and its fate. Zinc solubility in the soil is attributed to different Zn transformed forms (Okoro et al., 2012). Most Zn in soil is in an insoluble state (Singh et al, 2013). Sequential extraction (SE) procedures provide good estimation of Zn contents in different chemical forms, and in addition, showing the impact of soil properties on the different Zn phases.

Soil management activities play a huge role in influencing the distribution of different Zn fractions. For instance, the addition of sludge may alter the available Zn and Zn complexed by organic matter. This was clearly confirmed by Regmi et al. (2010) whose results indicated significant increases in the available and organic matter bound Zn fractions in a biological farming system as compared to a conventional farming system.

2.5.1 Chemical forms of zinc and their distribution in the soil

Literature has shown that zinc may occur in different pools or complexes in the soil (Singh et al., 2013). The five most commonly distinguished phases include: (i) Zn present in the soil solution, (ii) zinc in exchangeable form (non –specifically adsorbed), (iii) zinc complexed by organic ligands, (iv) zinc occluded in Fe/Al/ Mn oxides and carbonate, (v) Zn in primary minerals and silicate clays (Viets, 1962; Shuman 1979; Nielsen et al., 1986; Kiekens, 1990; Garcia-Sanchez et al., 1999; Finzgar et al., 2007). Zinc fractionation has become a more useful tool for providing information on the mobility or solubility of Zn compared to the determination of total Zn concentrations only (Imitiaz et al., 2006). Several factors regulate the process of sequential extraction (SE), the method used for zinc fractionation such as the counter ions in the extractant solution, the buffer capacity of the reagent and extraction time.

a) Water soluble and exchangeable zinc

This fraction accounts for less than 10% (< 1mg kg^{-1} Zn) of the total Zn content in the soil (Shuman, 1991; Rauret, 1998). However, it is the most important fraction for plant growth as it provides available Zn in the soil solution (Ahmad et al., 2012). This fraction is helpful in soil tests and analyses as it provides the estimation of plant available Zn. The soluble and exchangeable fractions are the most useful to the plants because it

is where all soil chemical processes take place, particularly in the solution phase (Fathi et al., 2014). The water-soluble fraction is usually insignificant, particularly in the absence of evaporites (Filgueiras et al., 2002), with the exchangeable fraction making the biggest contribution to this fraction. The water-soluble + exchangeable fraction is the first to be extracted in the sequential extraction procedures. The exchangeable Zn fraction is weakly held by electrostatic forces thus it can easily be released by ion exchange process (Milivojevic et al., 2011). Chemical environment changes, such as pH, can easily shift the sorption processes of the exchanged Zn in the soil solution (Ahnstrom and Parker, 1999).

Soil moisture plays a vital role in the soil solution as it facilitates Zn movement (Shuman, 1991). Weak extractants such as neutral salts solutions ($CaCl_2$, MgCl, NH_4OAc, $Mg(NO_3)_2$, $NaNO_3$, $Ca(NO_3)_2$) are used to release the Zn which is in exchange sites and the soil solution (Narwal et al., 1999; Ahnstrom and Parker, 1999 Milivojevic et al., 2011). These salts can easily substitute weakly bound Zn. Nitrate salt solutions are commonly used and preferred because they facilitate the cation exchange process only without any other metal complexation involved (Filgueiras et al., 2002).

b) Organic matter and carbonate bound Zn

Organic matter and carbonates are the major Zn retainers after sesquioxides (Okoro et al., 2012). Zinc released in these forms is usually intermediate and can easily desorb to soil solution since it is weakly bound into these soil components. Their extraction usually comes second after the above fraction in the sequential extraction steps and in the importance of releasing available Zn. Zn– carbonate usually has an impact in calcareous soil (Elsokkary, 1979; Saffari et al., 2009). This carbonate fraction is highly dependent on pH and easily release or bind Zn when environment changes occur (Filgueiras et al., 2002). Zinc in the organic matter is either complexed or chelated. The common reagents used to extract Zn in this chemical pool are hydrogen peroxide and sodium acetate, but these reagents are not specific for carbonates dissolution only (Tessier et al., 1979).

c) Zn occluded in sesquioxides

Hydroxides and oxides of Fe, Al and Mn have negative effects on Zn availability, particularly in low pH soils since they are the main adsorbents of Zn in such soils (Sungur et al., 2015). Zinc can form both outer and inner sphere insoluble compounds with Fe-Mn oxides, depending on their physicochemical properties. Several mechanisms are involved in the retention of Zn by these sesquioxides, such as surface complex formation, ion exchange, sorption and penetration of lattices (Agbenin, 2003). The reducible fraction (Mn, Fe, Al -Zn) seems to be second strongest after the residual fraction to retain Zn in the soil. pH is the most important parameter affecting this fraction since at the low pH, there is an abundance of Fe/Al oxides which may be involved in inner sphere and outer sphere surface complex reactions with Zn, depending on the type of oxides (Scheinost et al., 2002). Hydroxylamine hydrochloric acid is the reagent mostly used for releasing Zn from these oxides (Zimmerman and Weindorf, 2010). However, it may be also release organic matter bound Zn (Ahnstrom and Parker, 1999). The acidic state of the extracting solution is most important to prevent the reagent to dissolve both organic matter bound and residual Zn. If the pH is below 1.5, it may also dissociate residual Zn (Filgueiras et al., 2002).

d) Zn in primary and secondary minerals

This constitutes the residual Zn fraction. Numerous studies indicated that this fraction contains most Zn, almost 80–90 % of the total Zn (Finzgar et al., 2007; Saffari et al. 2009). It dominants in polluted/contaminated, natural and agricultural soils (Regmi et al., 2010). The Zn here is in an inactive form and unavailable to plants (Preetha and Stalin, 2014). It is held on alumino-silicate minerals, resistant sulphides and stable organic matter (Milivojevic et al., 2011). The influence of parent material is exhibited in the residual fraction (Liang, 1991).

2.5.2 Determination of zinc in soil by means of single extractions

Single extraction is where one reagent is used to release Zn while sequential extraction is where several reagents are used in step-wise procedures (Kabala and

Singh, 2001). The zinc status of the soils can be determined by either indirect (chemical extractions) or direct (use of instrument techniques) methods (Sipos et al., 2008). Soil tests are usually used for the determination of available Zn while plant analysis gives Zn concentrations in the plant tissues (Prasad et al., 2005). Most laboratories use multi-elemental extractants to do Zn analysis in the soil. The difference in type and strength of these extractants is particular for each country due to environmental differences (Rauret et al., 1999). These extractants may be dilute acid (0.1-1 M HCl, 0.43–2 M HNO_3 or 0.0125 M H_2SO_4), weaker acids with chelating agents (EDTA, DTPA), dilute unbuffered salt solutions (KCl, $CaCl_2$ and $NaNO_3$) or buffered solutions (NH_4-acetate, buffered at pH 7 or 8) (Sims and Johnson, 1991; Rauret et al., 1999). These reagents can be used in combination or just single.

Extractants which are commonly used for single extractions include diethylenetriaminepentaaceticacid (DTPA), ethylenediaminetetraacetic acid (EDTA), Mehlich 1, Mehlich 3 and hydrochloric acid (Shuman, 1998). DTPA and EDTA are mostly used to determine plant available Zn in neutral and calcareous soils (Wang et al., 2005). DTPA is most widely used to determine the plant available Zn on surface soils (Chahal et al., 2005). Behera et al. (2011) found that DTPA extracted the lowest amount of extractable Zn (1% to 2.77%) while HCl, Mehlich 1 and Melich 3 managed to extract the highest amount Zn of 3.30 % - 6.29% from acidic soils in India. Relatively, high concentrations of extractable Zn are expected from HCl and Mehlich 3 extractants as they are able to do desorption of Zn from iron and manganese oxides, carbonates and to solubilise organic–Zn complexes forms.

A disadvantage of single extraction is that it focuses on the exchangeable and soluble Zn only while ignoring other Zn pools. Another concern highlighted by Wang et al., (2005) was that single extraction can extract adsorbed Zn, hence giving a false prediction of available Zn. For instance, 0.l M HCl has the ability to extract some of the residual and the carbonates occluded Zn (Zimmerman and Weindorf, 2010). To overcome the problem of differences brought about by the use of different extractants in determining zinc content, sequential extraction seems to be a more reliable and

better method as it focuses on the all on the phases of Zn (Ngole, 2007). It is, however, not practical for routine analysis of soils for fertiliser recommendations.

2.5.3 Fractionation of zinc by sequential extraction

2.5.3.1 Overview

Soil scientists use different approaches to increase understanding of the desorption process of metals, including Zn, from solid phases (Rauret et al., 1999). This is due to the fact that most studies have discovered that determination of total Zn content does not represent the available-Zn and its association with soil phases (Ngole, 2007; Prasad et al., 2005; Sungur et al., 2015). Total zinc content also does not provide sufficient information on the environmental behaviour and plant nutrition value of Zn (Alvarez and Gonzalez, 2006). Sequential extraction is the analytical technique used to quantify and identify different forms of an element associated with solid phases (Gworek and Mocek, 2003). The main purpose of fractionation by applying sequential extractions is to determine Zn distribution associated with different chemical forms (Kiekens 1990). Various soil chemical processes such as redox reactions, complexation and ion exchange are involved in Zn transformations, therefore sequential extraction uses these chemical reaction processes to distinguish various Zn chemical forms (Gworek and Mocek, 2003).

Sequential extraction has been used as a fundamental method to assess and quantify elements, particularly trace elements, in polluted soils, water, and sediments in the early 1980's (Rauret et al., 1999). In this technique, a series of chemical reagents at different strengths are used in sequence to extract a particular element associated with certain solid phases, starting with the weakest extractant to the strongest (Plekhanova and Bambusheva, 2010; Ramzan et al., 2014). Different extracting reagents target different Zn ligands in the soil. Two or more reagents can be used in an individual extractant to release Zn, depending on the concentration of the reagents, soil conditions and availability of the reagent (Iwegbue et al., 2007). Extraction methods behave differently in releasing Zn, depending on the properties of the dominant soils in an area, i.e. a method found to be suitable in a certain area, may not

perform well in other areas (Chahal et al., 2005). One of the advantages of SE is that it takes into account the Zn bound with different components which differ in affinity and binding abilities (Baranimotlagh and Gholami, 2013).

2.5.3.2 Sequential extraction (SE) techniques

Various SE procedures have been developed and accepted globally by different researchers (Zimmerman and Weindorf, 2010). The first SE techniques were initiated by McLaren and Crawford (1973) and Tessier (1979). Then many other researchers followed, including but not limited to, Tessier et al., (1979), Sposito et al., (1982) and Shuman (1985). Tessier (1979) modified the McLaren and Crawford (1973) copper extraction method to extract other trace metals apart from Cu. This method was widely used and recognised (Osakwe and Okolie, 2015). The Tessier procedure consists of five steps. These steps consist of the extractants that release trace metals from the exchangeable site, carbonate, Fe–Mn oxides, organic matter and primary and secondary minerals (Sepahvand and Forghani, 2012). Shuman (1985) modified the above procedures by firstly separating the Fe oxides into the amorphous and crystalline oxide fractions, secondly, separating Mn oxides from Fe oxides, thirdly, introducing sodium hypochlorite (NaOCl) to extract metals occluded into organic matter and lastly by introducing the extraction of trace metals from sands. Singh (1988) extended the Tessier procedure to 7 steps. Thus, the SE usually consists of the three to seven successive steps of fractionation (Table 2.2) (Tlustos et al., 2005).

Table 2.2: Some original and modified sequential extraction methods as proposed by the different researches.

Sequential extraction procedure	**Fractions**	**Reagents**
Tessier et al.,(1979)	Exchangeable	8ml of 1 *M* $MgCl_2$; pH 7.0 OR NaOAc; pH 8.2
	Bound to carbonates	8ml of 1 *M* NaOAc; pH 5.0
	Bound to Fe –Mn oxides	20ml of 0.3 *M* $Na_2S_2O_4$ + 1 *M* CH_3COONa +CH_3COOH
	Bound to organic matter	3ml of 0.02 *M* HNO_3 + H_2O_2 and 3.2 *M* CH_3COONH_4 in 20% HNO_3
	Residual	HCl + HNO_3 at ratio of 3:1 (HF – $HClO_4$ mixture)
Shuman (1985)	Exchangeable	40ml 1 *M* $Mg(NO_3)_2$; pH 7
	Organic matter	0.7 *M* NaOCl pH 8,5
	Manganese oxides	0.1 *M* NH_2OH-HCl pH 2
	Amorphous Fe oxides	0.2 *M* $(NH_4)_2C_2O_4.H_2O$ + 0.2 *M* $C_2H_2O_4$ pH 3
	Crystalline Fe oxides	Same reagents as amorphous Fe oxides + 0.1 *M* ascorbic acid
	Metals in sands	0.11 *M* $Na_4P_2O_7.10H_2O$
McLaren and Crawford procedure (Gworek and Mocek 2003)	Non –specifically bound (water + easily exchangeable)	0.05 *M* $CaCl_2$
	Specifically adsorbed and less exchangeable	25% CH_3COOH
	Specifically bound to organic matter	0.1 *M* $K_4P_2O_7$
	Occluded in oxides	1.0 *M* $C_2H_2O_4$ + 0.175 *M* $(NH_4)_2C_2O_4$
	Residual	30% HF and $HClO_4$
BCR procedure (Rauret et al., 1999)	Exchangeable, water and acid soluble	40 ml 0.11 *M* CH_3COOH
	Reducible	40 ml 0.5 *M* NH_2OH-HCl
	Oxidisable	10 ml 8.8 *M* H_2O_2 or 50ml
	Residual	HCl + HNO_3 digestion

The naming and the order of fractions are specific for each procedure. For instance, extraction Step II, for the McLaren and Crawford and Community Bureau of Reference (BCR) methods were specially included to extract (adsorbed + less exchangeable)

fraction in the former method and the reducible fraction in the latter method, respectively while for the Tessier procedure it was to extract the carbonate bound fraction (Table 2.2).

Variations in SE procedures (pH of the extractants, contact time and volume ratio between the soil and extractants and particle size and number and order of sequences) (Table 2.2) questioned their efficiency in releasing trace metals from specific fractions (Tlustos et al., 2005). Several researchers argue about the credibility of SE procedures to identify Zn fractions since they are still experiencing problems, such as over and underestimating of element concentrations in specific fractions due to dissolution and re-adsorption processes during extraction, changes in oxidation state and removal of unwanted elements (Scheinost et al., 2002). This lack of uniformity in extraction steps enhances difficulties in comparing outcomes of different SE methods or even within same SE procedure in different cases (Zimmerman and Weindorf, 2010). Comparing the McLaren and Crawford and Tessier et al. extraction methods, Gworek and Mocek (2003) found that the latter released more Zn from certain fractions than the former. Another problem associated with SE is the dissolution of non-targeted chemical pools. For instance, NaOAc reagents have the tendency to attack other fractions instead of the carbonate phase only (Filgueiras et al., 2002).

An attempt was made by Rauret et al. (1999) to standardise or harmonise sequential extractions procedures. They established a SE procedure called Community Bureau of Reference (BCR). This is a three-step extraction technique which focuses on i) water + exchangeable + acid soluble, ii) reducible fraction + Fe/ Mn oxides, iii) organic matter (Igwbue et al., 2007; Okoro et al., 2012). The BCR method tried to harmonise pH and chemical reagent strength, analysis of reagents and blank samples and changes in the extraction procedure (Rauret et al., 1999). However, this method still presents the same contradictions as the previous ones regarding the differences in data. For instance, using both the BCR and Tessier approaches in a study by He et al. (2013), found that 40.1% of Fe oxide occluded Zn was removed by the Tessier approach compared with only 19.4% by the BCR method, even though hydroxylamine

reagent was used in both techniques. The reason might have been the difference in reagent strength (0.04 *M* for the Tessier approach and 0.5 *M* for the BCR method).

Since there is no standard procedure for identification of Zn, or any trace metal fraction by SE (He et al., 2013), for effective sequential extraction, the strength of chemicals used is important. The extraction steps should begin with weak extractants, followed by stronger, ending with the most aggressive one (Fathi et al., 2014; Mahmoud Soltani et al., 2015). The appropriate sequence of reagents as indicated by Rauret et al. (1999) is as follows: Unbuffered salts – weak acids – reducing reagents – oxidising reagents – strong acids. The sequence order, as well as reagents, should be followed rigidly to avoid misinterpretation of Zn fractions (Kabala and Singh, 2001).

2.5.4 Zinc fractions in various soil types

The majority of research on Zn speciation using sequential methods has been carried out in polluted areas (Finzgar et al., 2007). The performance of these extractants was clear in the polluted soil (Sims and Johnson, 1991). Kabala and Singh (2001) fractionated Zn, Pb and Cu in a soil profile near a Cu smelter. They found that the residual Zn fractions were dominating in the sub-surface horizon (about 95% of the total Zn in sandy and 45% in silty soils). He et al. (2013) who followed the four sequential extraction techniques found similar results which showed that 40.3 -68.5% of the total Zn in a polluted soil was in residual form.

The same trend of the more stable fractions containing high Zn concentrations was also recorded in natural/uncontaminated soils by Chirwa and Yerokun (2012). Their study was conducted in selected agricultural Zambian soils which showed that >70% of Zn was found in the residual and oxide fractions. Similar results of high zinc content in residual fractions were observed in soils under both biological and conventional farming systems in Australia (Regmi et al., 2010). Likewise, $ZnSO_4$ applied to 55 soil samples collected from maize fields produced high Zn concentrations in the residual fraction, with mean values of 330.39 -352.86 mg kg^{-1} (Preetha and Stalin, 2014). In their work, several Zn rates (0, 1.25, 2.50, 5.00, 7.50, 10.00 kg Zn ha^{-1}) in the form of

zinc sulphate were applied on four groups of soils with different plant available Zn for 30 days.

The sequential method can be useful in determining Zn distribution in soils differing in pH levels. Fathi et al. (2014) studied Zn and Cu distribution in alkaline and acidic soils of Iran. Their study revealed the distribution of Zn in decreasing order as follows: residual > carbonate > crystalline Fe oxide > amorphous Fe oxide. Differences existed in the unstable Zn fractions (the exchangeable and manganese – occluded Zn), where Zn was undetected in alkaline (high pH calcareous soils) whereas in acidic soils these Zn fractions were having 1.2 – 2% and 1.2 – 1.85% of Zn respectively. This indicated the impact of carbonate on Zn adsorption. Other studies by Chowdhury et al. (1997) in selected New Zealand soils, mainly silt loam soils, reported similar trends, the residual fraction Zn fraction dominated the soil (40% of the total Zn).

Effects of time on the Zn distribution in submerged soils in cultivated rice fields were demonstrated by Mahmoud Soltani et al. (2015). Incubation time had a significant effect on the occurrence of Zn in different fractions. Zinc contents in the exchangeable, organic matter and crystalline fractions decreased in 60 days, but the Zn in the amorphous Fe oxide, manganese occluded and residual fraction increased. The sequence of dominance of the various Zn fraction changed with time also changed with time of incubation. After 30 days, the increasing Zn order was: exchangeable < crystalline sesquioxide < manganese oxide < organic matter –Zn < amorphous sesquioxide < residual. While after 60 days, the trend according to increasing order was: exchangeable < manganese oxide < crystalline sesquioxide < organic matter – Zn < amorphous sesquioxide < residual.

2.5 Research gaps and conclusion

The high incidence of zinc deficiency has become a global problem. Several soil properties and soil reactions aggravate Zn insufficiency in the soils. Zinc fertilisation has been used to overcome and raise Zn contents enough for plant uptake. However, the method of application and types of Zn fertilisers applied, seem to affect the solubility and availability of Zn. Phosphate fertilisation has an effect on the Zn contents

either native or applied. However, there is less knowledge of this impact on Zn in the soil, especially in areas of high localised Zn-P applications areas (bands), since most studies were conducted to find the effects of Zn–P interactions in soils and plants where broadcast applications were made.

For a better understanding of the fate of Zn in the soil, several techniques can be used for determination of Zn distribution. Both chemical analysis and imaging can provide a good estimation of Zn and its species in different chemicals forms. Single and sequential extractions are in use for determination of Zn in different chemical forms. Most studies emphasize the difficulty of the identification of Zn species and their distribution, hence development of many methods/ tests to overcome those challenges to trace its mobility (Barna et al., 2007). Due to the differences (soil mass, temperature, extracted volume), various sequential extraction techniques exit. Most researchers modified the original methods to cater to their environmental conditions and availability of chemical reagents since there is no standard protocol / procedure. These techniques are usually practised in polluted areas or areas with high Zn concentrations. Limited information is available on the chemical forms of zinc in natural and agricultural soils.

The most common challenge of these extractions is that they do not mimic the soil-plant root system, therefore, the chemical extractions are always indirect inferences of (bio) chemical reactions involving organisms. Thus, there is grinding and sieving of soils which lead to the destruction of soil structure, decrease in microbial activities and removal of debris and plant material. Another challenge is the loss of soil during extraction procedures, but they are still the most reliable methods to quantify Zn concentrations in different phases as influenced by different soil physicochemical properties (Tlustos et al., 2005). The current study will attempt to address some gaps by addressing the problems of inconsistency and variations of extraction methods.

Chapter 3: General Materials and Methods

3.1 Soils

The study was conducted on two soils with different textures, namely a sandy loam soil and a clay soil. Both were top soil samples collected from the Hutton form (Soil Classification Working Group, 1991), characterized by a uniformly red apedal subsoil. Both soils were strongly acidic. The soil selection was based on the findings of an unpublished honours study by the author, which indicated that a coarse-textured soil exhibit Zn deficiency more than a fine-textured soil. In addition, these soils represent contrasting properties known to influence Zn availability to plants and provided the acidity effect on Zn solubility (Alloway, 2008a). The sandy loam soil was collected from Bapsfontein, east of Johannesburg. The clay soil was collected from a long-term maize trial (control treatment) at the University of Pretoria's Hatfield Experimental farm which has received different combinations of NPK fertilisers at different rates since 1939 (Nel et al., 1996). The soil was air –dried and ground to pass through a 2 mm sieve.

3.2: Soil chemical and mineralogical characterization

The soils were characterized by means of normal routine soil analyses (Table 3.1). Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) using a Spectro Genesis was performed on the 1*M* NH_4OAc extracts (for Ca, Mg, K and Na), 0.02 *M* EDTA (Al, Fe, Mn, Zn and Cu), Bray-1 (P) and 1 *M* KCl.

Table 3.1: Methods used to determine physico- chemical properties of the experimental soils

Soil analyses	Methods / extractant	References
Soil texture analysis	Bouyoucos (hydrometer)	Gee and Bauder, 1986
pH(Water)	Water, 2.5:1 to- soil ratio	Coleman and Thomas, 1967
pH (KCl)	1 *M* KCl solution 2.5:1 to-soil ratio	FSSA,1974
Electrical Conductivity	2.5:1 water- to- soil ratio	Rhoades,1982
Soil organic matter	Dichromate oxidation (Walkley –Black method)	Nelson and Sommers, 1982
Exchangeable and soluble basic cations	1 *M* NH_4OAc extractable	Chapman, 1965
Micronutrients	0.02 *M* EDTA extractable	Lindsay and Norvell, 1978
Available P	P Bray -1	Bray and Kurtz, 1954
Exchangeable acidity	1 *M* KCl on volume basis	Thomas, 1982
Clay mineralogy	X-ray diffraction (XRD)	
Total elemental analysis	X-ray fluorescence (XRF)	

Determination of sesquioxides forms

The removal of free iron oxides was done before and after liming by the dithionite-citrate method whereby 0.5 g of soil samples were shaken with 25ml of sodium citrate solution and 0.4 g of sodium dithionite was added. The samples were shaken for 16 hours and then centrifuged and filtered. Acid ammonium-oxalate extractable Al, Fe and Mn were also determined (which gives the amorphous or “active” forms of Al, Fe and Mn). In the Acid ammonium oxalate method (in the dark), 0.250 g of soil was weighed and then 10ml of the acid ammonium oxalate solution was added to the weighed soil samples. The soil samples were shaken for 4 hours in the dark. The samples were also centrifuged and filtered. All extracts from the samples were analysed by ICP- OES. Calculation were done as specified in the methods to determime Fe, Al, Mn and Si concentrations (Courchesne and Turmel, 2007).

3.3 Cation Exchange Capacity (CEC) determination

The unbuffered $BaCl_2$ compulsive method (Gillman and Sumpter, 1986) was used since it is a direct and precise CEC method at field conditions. The following changes were made from the procedure of the above method: 1) concentrations of $BaCl_2.H_2O$ solutions were changed (2) combination of ammonium chloride and barium chloride, (3) using only one level of concentration for $Mg(NO_3)_2$ and finally using of ethanol to wash away the cations. The steps were also slightly modified (shaking time and number of decantation). The modified $BaCl_2$ compulsive method is described below:

3.3.1 Saturating the soil with barium chloride

Two grams of sieved soil was weighed and added into a 50 ml centrifuge tube. Then 10 ml of deionised water was added. The samples were shaken for 1 hour, centrifuged for 10mins at 3000rpm. The supernatant was decanted and discarded. Next was the addition of 20 ml ethanol, centrifuged for 10 min, and the supernatant was decanted and discarded. These steps were to remove soluble anions, especially sulfate, that can precipitate with Ba^{2+}. Addition of 0.2 *M* of combined $BaCl_2/NH_4Cl$ into the samples followed. The samples were shaken for 2 hours, centrifuged, the supernatant decanted and filtered into a vial (Step A). Then a second solution of barium chloride (0.05 *M* $BaCl_2$) was added to the soil, shaken for 5 minutes and then centrifuged and filtered into another vial (Step B). Another solution of barium chloride with a concentration of 0.002 *M* was added to the soil, shaken, centrifuged and filtered into another vial (Step C). The latter step was repeated twice. The EC and pH of the solution were then measured.

3.3.2 Removal of $BaCl_2$ by magnesium nitrate $(MgNO_3)_2$

The weight of the soil from the previous step was measured for entrained solution calculation. Then 20ml of 0.005 *M* $Mg(NO_3)_2$ was added, shaken for 1 hour and centrifuged for 10 minutes and then filtered into a vial (Step D). Filtration was done here to completely remove the suspended soil. This step was repeated twice for further washing out the Ba^{2+} ions from the soil.

All extracts from all the steps were analysed by ICP- OES.

3.3.3 Calculations

Only Ba^{2+} analyses from steps C and D were used to calculate CEC. The carry-over Ba^{2+} of the entrained solution was calculated from the extraction of the A and B vials. The concentration of Ba^{2+} was presented in cmol $_{(+)}$ kg^{-1}

3.4 Preparation of different soil pH levels (Preliminary trial)

Since the pH is a crucial factor in Zn solubility, the study was conducted in acidic and neutral soil conditions. Two pH levels of each soil were used: natural pH and pH (water) 6.0, attained by means of liming. The target was to lime soils to pH (water) 6.0 as indicated in the literature review as the optimal pH for plant growth (Martinez and Motto, 2000). South African studies have shown that zinc availability decreases dramatically when the pH (water) of sandy soils goes above 5.5 (Laker, 1967). Firstly, lime neutralization curves were established for each soil. This was necessary to derive the amounts of calcium carbonate required to bring each soil to the specified pH (water) of 6.0, which is equivalent to pH (KCl) 5.0. To create lime neutralization graphs, a preliminary trial was conducted to determine the amounts of lime needed to bring the pH of each for the soil to the target value.

The following procedure was followed: 10g of soil was weighed into 50 cm^3 centrifuge tubes. 0, 5, 10, 15, 20 or 25 ml of deionized water plus 25, 20, 15, 10, 5 or 0 ml of 0.02 M $Ca(OH)_2$ were added so as to always give a total of 25 ml liquid added to a soil sample (Table 3.2). The tubes were shaken for 30 minutes and left to settle for another half an hour. Then the pH was measured. After that, a graph of pH versus volume 0.02 M Ca $(OH)_2$ was constructed for each soil. The graphs provided the amount of 0.02 M Ca $(OH)_2$ required to bring each soil to the desired pH (i.e. 6.0 in water). From this, the amounts of $CaCO_3$ required to raise the pH of 5000 g of soil to 6.0 was found to be 2 g and 8 g for the sandy loam and clay soil respectively. Analytical grade $CaCO_3$ was used as a liming material. The limed soils were incubated to react so as to reach the targeted pH values. Samples were monitored each day after 3 days onwards,

measuring pH in order to assess if the desired pH was reached. If not, the soils were allowed to incubate for longer. It was found that both soils reached the targeted pH in 7- 8 days.

Table 3.2: Ratio of deionized water and 0.02 *M* Ca $(OH)_2$ in each centrifuge tube

Tube No	Deionized water (ml)	0.02 *M* $Ca(OH)_2$ (ml)
1	0	25
2	5	20
3	10	15
4	15	10
5	20	5
6	25	0

With the amount of limestone needed to reach the target pH known for each soil, as well as the reaction time to reach the target pH, two 500 g homogenized bulk samples per soil (one unlimed and one limed) were prepared. The bulk samples were stored in polythene buckets and used in the subsequent experiments.

3.5 P and Zn sources

There are several zinc and phosphorus sources commercially available for correcting P and Zn deficiencies. In this study, monoammonium phosphate (MAP), diammonium phosphate (DAP) and calcium hydrogen phosphate also known as dicalcium phosphate (DCP) were used as phosphate sources (Appendix E). Zinc sulphate ($ZnSO_4$) was used as a zinc source (Appendix F). All fertilisers used were analytical reagent (AR) grade. In this study, commercial fertilisers were not used to avoid additional acidity and impurities which might have an effect on the zinc – soil reactions.

3.6 Technique to simulate fertiliser band placement in the field

During commercial fertilisers' production processes, Zn is usually co–granulated to macronutrients due to its low requirement for plant growth. Zinc is blended with P fertilisers at rates of 0.5, 1.0, 1.5 or 5%, depending on regional soil conditions or agro-ecological zones. According to Mengel and Kirkby (2004), 20 to 80 kg P ha^{-1} is the most commonly recommended application rates, depending on the plant-available P level of the soil and the crop to be planted. In this study, an equivalent application rate

of 40 kg P ha^{-1} with 5% of Zn co-applied with the P fertiliser (Hettiarachchi et al., 2008) was used in a simulated band placement method. For simulating the band, 80 g of previously prepared unlimed and limed soil was weighed into Petri dishes of 9.0 cm diameter ensuring a flat surface to a depth of 1 cm. Depending on the soil texture and bulk density, 80 g was enough to fill the petri dish. A circular mass of soil was punched from the centre of each Petri dish (5 g) (Figure 3.1b). Zinc-enriched soil was mixed with the amount of P fertiliser that had to be applied and placed back in the centre to act as a band (explained in Section 3.7).

3.7: Establishment and homogeneity of Zn application levels

It was found that only 0.5 mg Zn per petri dish was needed to be band placed. The circular cavities made in the centre of the Petri dishes for the placement of the simulated fertiliser bands had diameter of were 2 cm and 1 cm deep. Approximately 5 g of soil was extracted from this area, depending on the bulk density of each soil. This amount was too small to be measured accurately with available scales. Therefore, the soil mass had to be increased for easy establishment of a specific Zn level.

In order to do this, larger bulk samples (500 g) were enriched with Zn first. The scale was enlarged to 500 g to bring the Zn needed to 45.29 mg (90.58 mg kg^{-1}) Zn for the sandy loam soil and 44.25 mg for the clayey soil (88.5 mg kg^{-1} Zn). This was still a small amount relative to 500g, and the risk existed that large variations might exist between replicates taken from the bulk samples. In order to ensure homogeneous mixing of Zn the following were done: 1) Reagent grade $ZnSO_4$ was crushed into a powder state to facilitate the mixing; 2) The soil (combined with Zn fertiliser in bulk samples) was thoroughly mixed in the containers (Figure 3.1a) by shaking it. These were dry mixtures, no water was added because this would have set the Zn reactions with soil in motion. To confirm homogenous distribution of zinc on the samples, 10g samples from the mixed soil were extracted with 30 ml of 0.02 *M* EDTA and Zn contents were determined by ICP-OES. If there was variability in the Zn content distribution, the samples were shaken again and measured until homogeneity was achieved. Data showed that all eight zinc bulk samples were well homogenised with

little variability (indicated by low CV's (Appendix A). In total, eight zinc bulk soil samples were created (four limed and unlimed bulk samples mixed with $ZnSO_4$) (Figure 3.1a).

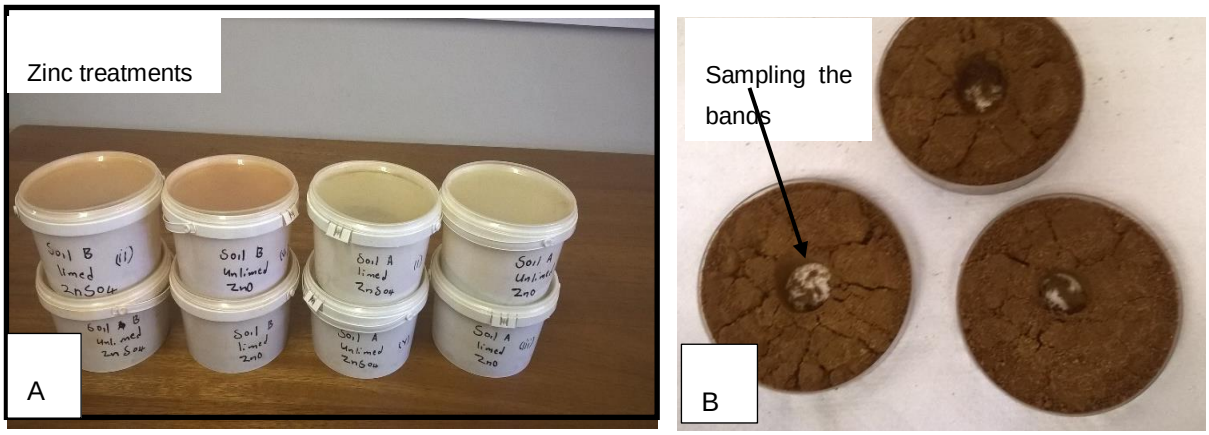

Figure 3.1: a) The 500g homogenized Zn enriched soil. b) The centre of the soils in a petri dish where the bands were established.

3.8 Incubation study

The experiment was set up in a manner similar to that described by Lombi et al. (2004). Before the placement of the fertilisers, the soil was wetted to 60 % of field capacity and kept at that water level throughout the experiment. The dishes were sealed with parafilm and incubated in a constant temperature room at 25°C overnight (Figure 3.2a). After that, the 5 g of soil was punched out as shown in Figure 31b. Then, 5 g of the Zn enriched soil was mixed with the equivalent of 9.8 mg P in the form of the above-mentioned P sources. Afterwards, the mixture of zinc-enriched soil and the various P sources were applied in the circular cavity in the soil in each petri. After treatment application, the petri dishes were closed, sealed again with parafilm and put in plastic zip-lock bags to prevent moisture loss (Figure 3.2b). They were then incubated in the constant temperature room (25°C) in a dark cupboard for a period of 60 days. After the incubation period, the samples were opened for sampling (Figure 3.1b). The moisture content was monitored and where there was need water was added.The experiments were arranged in a Completely Randomised Design with three replications.

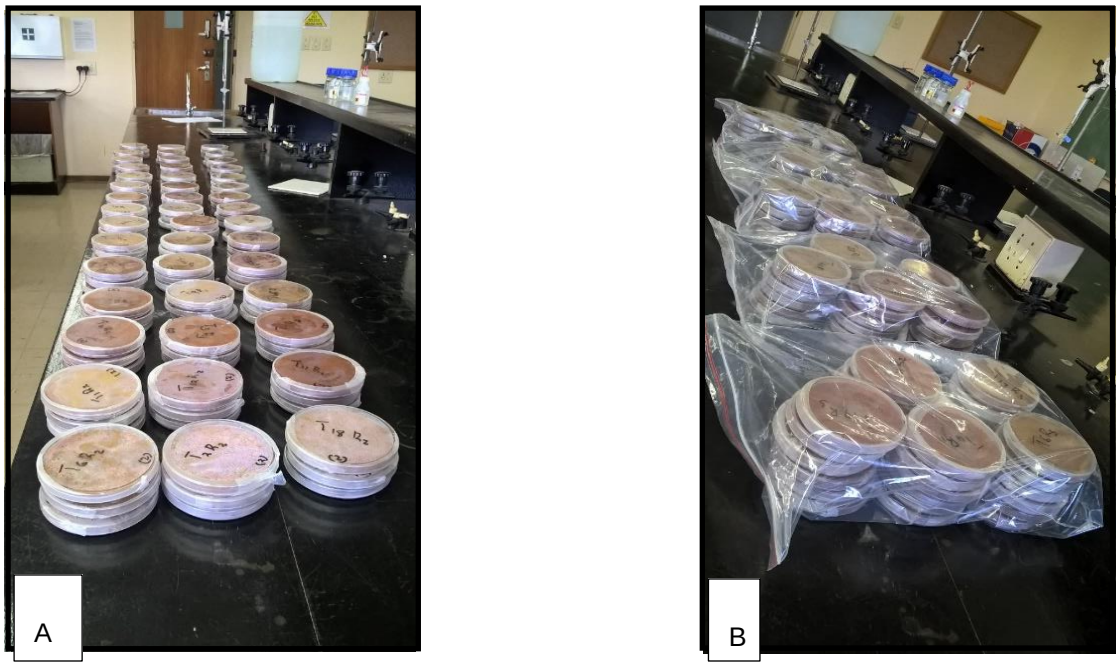

Figure 3.2: a) Prepared soil treatments closed by parafilm, b) Samples in Ziplock plastic bags to prevent further moisture loss.

3.9 Identification of minerals and elemental composition by X-Ray techniques

3.9.1 Clay preparation and separation for X-ray diffraction (XRD)

The XRD method was carried out on < 2 µm soil particles (clay fraction). To obtain < 2 µm soil particles from the soil, the samples had to be dispersed and the clay separated from the rest of the soil by the method which was modified in the Department of Plant and Soil Sciences at the University of Pretoria, where the study was conducted (Appendix B).

3.9.2 X-Ray Diffraction (XRD) analysis

The analyses were performed at the Department of Geology, University of Pretoria, on a Panalytical X'Pert PRO X-ray diffractometer in θ–θ configuration, equipped with a Fe filtered Co-Kα radiation (1.789Å) and with an X'Celerator detector and variable divergence- and fixed receiving slits. Samples were prepared according to the standardized Panalytical backloading system, which provides a nearly random distribution of the particles. The XRD analyses were done on the clay fraction only.

The data were collected in the range 5°≤2θ≤90° with a step size 0.008° 2θ and a 13-s scan step time. The phases were identified using X'Pert Highscore plus software. The diffractograms were matched with peak values of those in the mineral database for identification (Wiebke Grote, email communication).

3.9.3 X-Ray Fluorescence (XRF) analysis

The whole <2 mm soil samples were used for determination of elemental composition. The samples were pulverised in a tungsten–carbide mill. The samples were then desiccated in an oven at a temperature of 110°C and 1000°C for at least one hour. This was used to calculate the percentage loss on ignition. Determination of major elements was done on samples heated at 1000°C. 1 g of the heated samples was mixed with 6 g of lithium tetraborate ($Li_2B_4O_7$) and pelletized at 1000°C in a muffle furnace automated fluxer. The samples were then stored in a desiccator for analysis. For trace elements, polyvinyl alcohol (binder) was added to 20 g of samples dried at 110°C samples. A pressure of 20 ton cm^{-2} was used to pelletize the mixture for two minutes. The samples were then desiccated again at 110°C. XRF analysis for both major and minor elements was done using an ARL 9400XP + Wavelength dispersive XRF Spectrometer (Loubser and Verryn, 2008).

3.10 Statistical analysis

Each treatment was replicated three times. The data were subjected to analysis of variance (ANOVA). Multiple comparisons of least squares means were done using the LSD test (Duncan) to indicate significant differences. The level of significance was alpha < 0.05.

CHAPTER 4: Chemical and mineralogical characterization of experimental soils

4.1 Introduction

Soil properties play an important role in regulating zinc speciation and mobility (Rutkowska et al., 2015). These properties, such as CEC, pH, texture, mineralogy and organic matter content control soil solution chemical reactions such as sorption, precipitation and surface complexation (Catlett et al., 2002; Agbenin, 2003). Surface charges (permanent and variable charges), supplied by soil organic matter, sesquioxides and clay minerals provide sorption sites for Zn (McBride, 1991). Physical properties, such as soil texture also have a huge impact on the soil Zn solubility (Sharma et al., 2014; Wei et al., 2006).The aim of this chapter was to investigate the characteristics of the experimental soils and predict their influence in the zinc solubility and mobility.

4.2 Materials and Methods

Details of the procedures used for characterization of soil parameters are presented in Chapter 3, Sections 3.2 and 3.3.

4.3 Results and discussion

4.3.1 Soil texture

The study included coarse- and fine-textured soils (Figure 4.1). The results of the particle size analyses of the selected soils demonstrated that the soils fitted the intention to use soils with different textures.

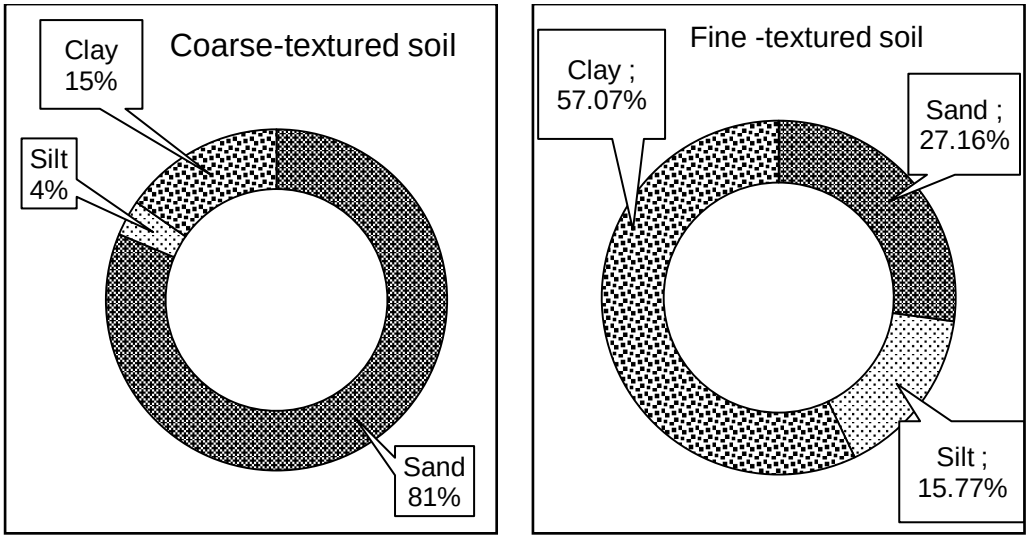

Figure 4.1: Particle size distribution of the experimental soils

The textural classes of the soils were sandy loam and clay soil respectively (Brady and Weil, 2014). The sandy loam soil has developed from quartzite rock, which mainly contains quartz. High quantities of silica (Si) content were, as it would be expected, observed in the sandy loam soil as reflected by the X-Ray Fluorescence results in Table 4.3.

4.3.2 Mineralogical analysis (XRD)

The XRD revealed that the clay fraction of these soils had five crystalline minerals, viz., quartz (SiO_2), goethite (FeOOH), hematite (Fe_2O_3), anatase (TiO_2), and the clay mineral kaolinite. This is not surprising since highly weathered soils have high sesquioxide contents (Antoniadis et al., 2018). Kaolinite was the most abundant clay mineral with the highest weight percentage (wt. %), ranging from 67.83 to 76.84% (Figure 4.2). The abundance of this mineral is also a reflection that these soils were at an advanced stage of weathering, more especially the clay fraction (Moraetis et al., 2016; Strawn et al., 2015). The dominance of kaolinite in the clay fraction is the reason for the low CEC of the clay soil, despite having a clay content of 55%. The clay fractions of both soils have a similar percentage of kaolinite (Figure 4.2). Another indication of the high degree of weathering of these soils is the presence of crystalline forms of Fe and Ti oxides. The clay fraction of the sandy loam mainly contained

goethite whereas the clay soil had both goethite and hematite. Hematite is responsible for the red colour of clay soils (Strawn et al., 2015). Goethite as the most stable and common Fe oxide in the soils was present in similar proportions (11.94, 9.64, 12.84 and 10.25 wt %) in both sandy loam and clay. Clay soil treated with magnesium chloride did not reveal the anatase mineral (Figure 4.3b). White (2006) stated that Ti oxides are resistant against weathering and hence their presence in these weathered soils is not surprising. The clay fractions of both soils also contained colloidal quartz, further underlining the highly weathered nature of the soils.

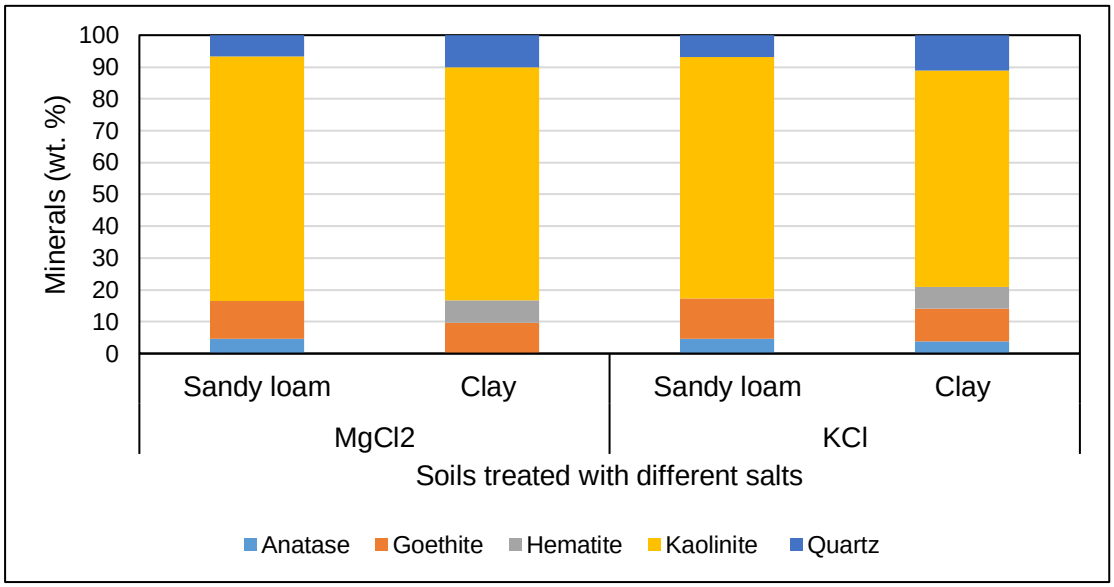

Figure 4.2: Semi-quantitative XRD analysis of clay fractions as determined by X-Ray diffraction (XRD). Soils were treated with two salt solutions $MgCl_2$ and KCl

The absence of smectite was indicated by no shifting of the peaks after treatment with ethylene glycol (Matini et al. 2011). The peak intensity did not fall upon K saturation at 25°C, which would have indicated the presence of vermiculite (Appendix C). The K-saturated clays collapsed with exposure to 550°C, reflecting the absence of pedogenic chlorite (Appendix C). In addition, the disappearance of peaks after heat treatment confirmed the presence of kaolinite (Matini et al. 2011). The peaks 1 ranging from 7.14 Å to 1.48 Å are attributed to kaolinite (Figure 4.3). X-ray diffractograms of both soils

revealed that the higher peak recorded was quartz, with the reflection of the intensity of 3.32 Å.

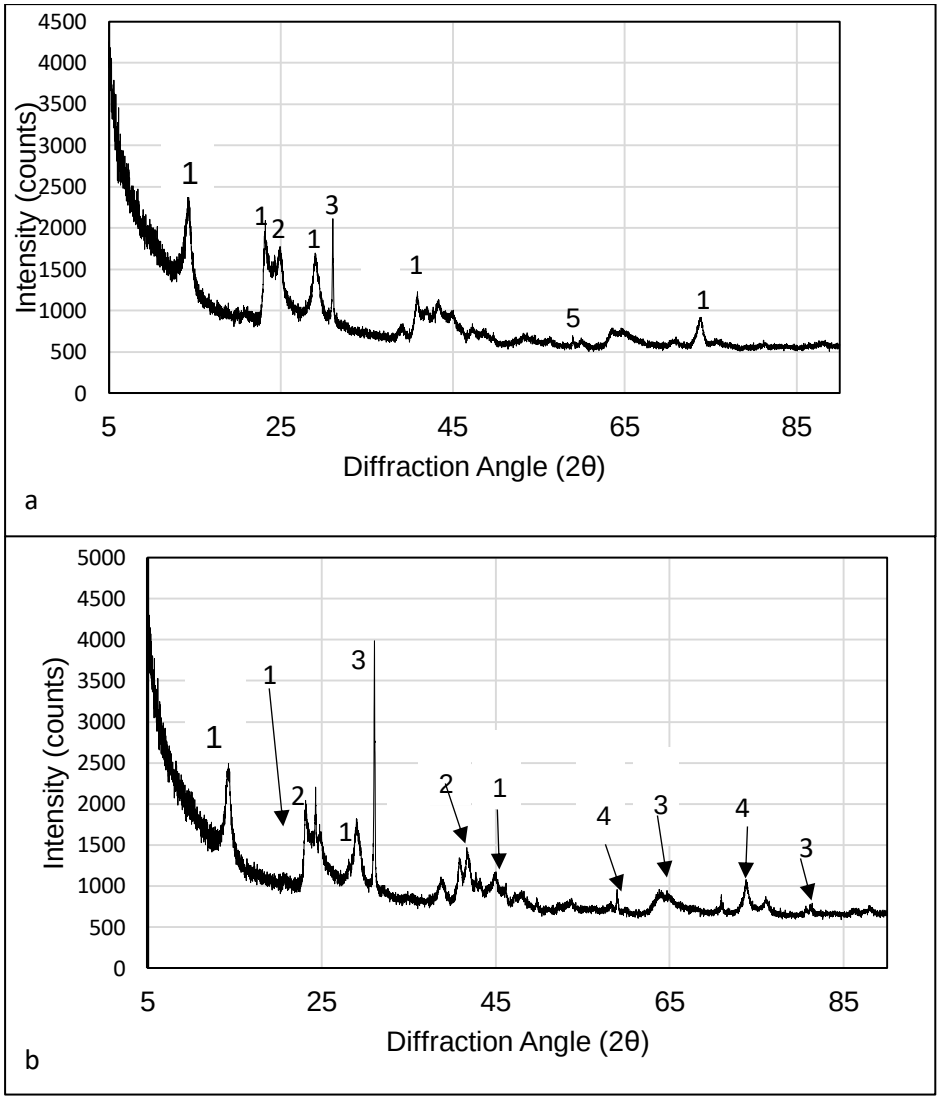

Figure 4.3: X-ray diffractograms of the clay fractions of (a) Sandy loam, (b) Clay; 1= kaolinite, 2= goethite, 3= quartz, 4= hematite and 5= anatase

4.3.3 Basic soil chemical analysis

The soils showed strongly acidic conditions with pH (KCl) of 4.0 and 3.5 for the sandy loam and clay soils respectively. The sandy loam soil had a pH (water) of 4.9 while clay soil had a pH (water) of 4.2 (Table 4.1).). It is general knowledge that normal soils almost all have a difference of approximately 1 pH unit between pH (KCl) and pH (water). Both soils developed under approximately 700 mm rainfall. High rainfall has

a tendency of leaching of basic cations over a long period of time. This might have attributed to the high acidity of these soils (Environomics, 2007; Rethman et al., 2007; Singh et al., 2017).

Regarding the limed soils, the near neutral pH (water) of 6.1 of these soils indicated that the application of lime on the bulk soil samples effectively neutralised the acidity during soil preparation. This also shows that the amount of lime added was sufficient to raise the pH to the target levels. The soils of Eastern South Africa are old and especially the sandstone derived soils are dystrophic (highly leached). Therefore, these soils, in general, contain very little soluble salts and have low base status (little exchangeable Ca, Mg, K and Na) (Table 4.1). The cation exchange capacity (CEC) of the sandy loam soil was 3.5 $cmol_c\ kg^{-1}$ which was lower than that of the clay soil (6.1 $cmol_c\ kg^{-1}$), as would expected. High soil acidity and the presence of kaolinite mineral (Figure 4.2) have contributed to the low CEC values of the soils (Singh et al., 2017). The CEC of soils was increased due to the addition of lime, 3.5 to 3.7 $cmol_c\ kg^{-1}$ in the sandy loam and 6.1 to 9.9 $cmol_c\ kg^{-1}$ in the clay soil. Low clay content of sandy loam soil contributed to the minimal increase on the retention of cations.

Table 4.1: Selected chemical properties of limed and un-limed study soils

	Sandy loam		**Clay**	
Chemical analysis	Un-limed	Limed	Un-limed	Limed
pH (KCl)	4.00	5.10	3.50	5.10
pH (H_2O)	4.90	6.10	4.20	6.10
EC (d S/m)	0.10	0.10	0.10	0.20
CEC ($cmol_c\ kg^{-1}$)	3.50	3.70	6.10	9.90
Ca (mg kg^{-1})	121	312	48.2	647
Mg (mg kg^{-1})	24.1	27.4	10.0	12.3
K (mg kg^{-1})	51.0	60.7	69.4	77.9
Na (mg /kg^{-1})	6.40	10.2	6.60	13.3
Organic matter content (%)	0.30	-	0.90	-

The base cations, viz. calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) were in low quantities as seen in Table 4.1. Since the soils have low pH, protons (H^+) and to some extent aluminium (Al^{3+}) and manganese (Mn^{2+}) replaced these cations. In contrast to unlimed soils, in limed soils, the base cation contents increased since H+ was substituted and lowered and Ca^{2+} and Mg^{2+} replaced the hydrogen ions on

the exchange sites (Behera et al., 2011; Singh et al., 2017). Both soils had low organic matter content, less than 1%.

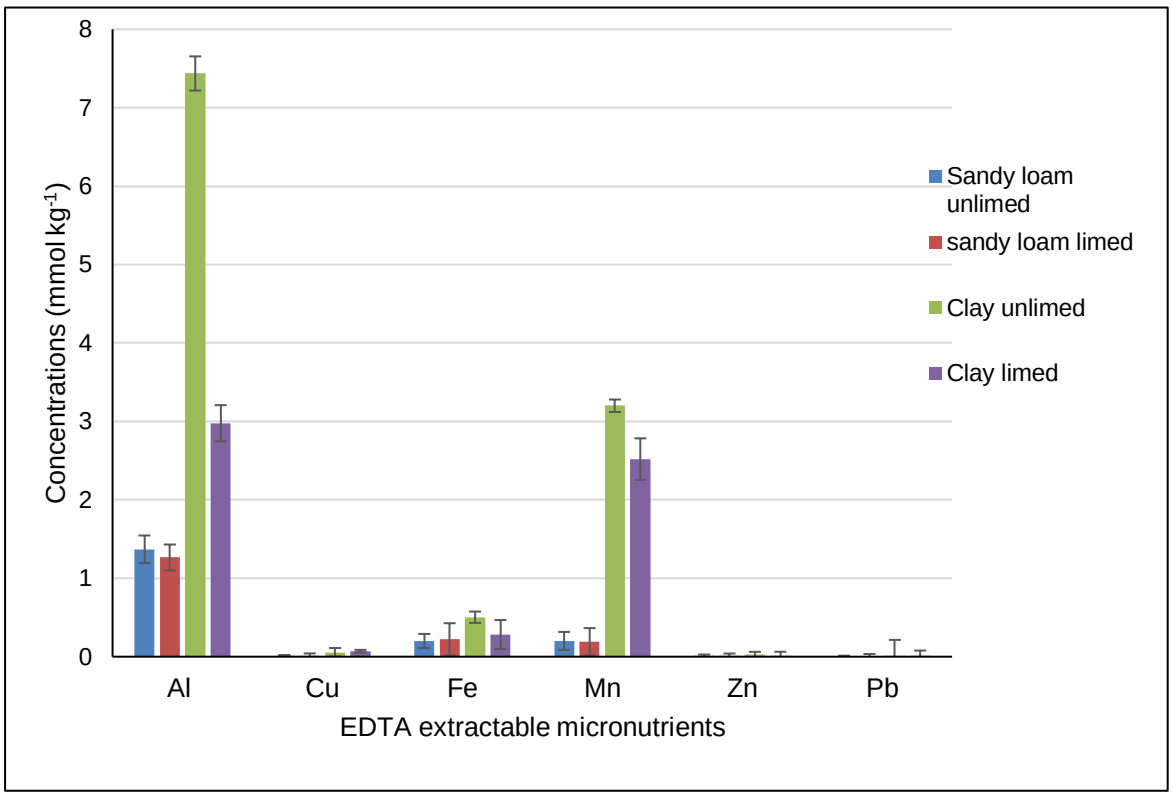

Figure 4.4: EDTA extractable Al, Cu, Fe, Mn, Zn and Pb which has an influence in Zn sorption on both limed and unlimed sandy loam and clay soils

Aluminium (Al) was the most abundant element extracted by EDTA in these soils, followed by manganese (Mn) and iron (Fe) (Figure 4.4). The clay soil in both limed and unlimed status contained higher concentrations of Al and Mn than the sandy loam. The lower extractable concentrations of Fe (0.20 – 0.50 mmol kg $^{-1}$) was not surprising since the soils have iron oxides which are stable in highly weathered soil. The sandy loam soil released lower amounts of the microelements compared to clay soil, regardless of the pH status. Copper (Cu), Zinc (Zn) and Lead (Pb) levels were very low in both soils at all pH status, some even below the detection level. Inherently, the parent material could have contained low concentrations of these elements.

4.3.4 Reductive analysis

Generally, these soils had relatively low concentrations of amorphous and non-crystalline sesquioxides (Table 4.2). The dithionite extractable sesquioxide (Al_d, Fe_d and Mn_d) values are higher than the oxalate extractable (Al_a, Fe_a and Mn_a) values in both soils. This was expected since the dithionite–citrate–bicarbonate (DCB) extracted sesquioxide, which constituted of "free" Fe and Al oxides are abundant in ageing soils (Antoniadis et al., 2018). On the other hand, ammonium oxalate released amorphous and poorly crystalline sesquioxides (McKeague and Day, 1966). The sandy loam soil did not release any form of Mn, while the clay soil had relatively high percentages of all forms of sesquioxides. Maniyunda et al., (2015) indicated that clay soils have a significant difference in the content, mobility and distribution of amorphous and non-crystalline Fe and Al forms. The high percentages of dithionite extractable Fe (at 6.3%) are worth noting because it explains the presence of iron minerals (hematite and goethite) in the study soils as identified by X-ray diffraction analysis, depicted by Figure 4.3. this shows a fair degree of ferralitisation. Good drainage and low organic matter content are two factors which promote the crystallisation of Fe oxides. Repeated oxidation and reduction and the presence of organic material favour amorphous fraction of sesquioxides.

Table 4.2: Distribution of different forms of Fe, Al and Mn in the soils, both limed and unlimed

Soil type	**Element content (%)**					
	Al_d	Fe_d	Mn_d	Al_a	Fe_a	Mn_a
Sandy Loam (Un-limed)	0.27	1.66	0.00	0.04	0.03	0.00
Sandy Loam (Limed)	0.25	1.50	0.00	0.04	0.04	0.00
Clay (Un-limed)	0.31	6.30	0.05	0.08	0.09	0.02
Clay (Limed)	0.29	6.39	0.05	0.08	0.11	0.02

d =dithionite –citrate method; a = acid ammonium oxalate method

4.3.5: Total elemental analysis (XRF)

Silicon (Si) was the dominant element in both soils with the proportions of 90.7 % and 75.8% in the sandy loam and clay soils respectively (Table 4.3). This was expected soil since the clay soil is derived from diabase rock which contains high percentages of iron-rich minerals like augite (little quartz), while the sandy loam soil is derived from quatrzite which contains a high level of quartz. The XRF analyses demonstrated that both soils consist of the high content of Si^{4+}, Al^{3+} and Fe^{2+} in their total elemental composition (Table 4.4).

Table 4.3: Elemental composition expressed as oxides (%)

Oxides compounds	Sandy Loam	Clay
Al_2O_3	4.09	9.47
Fe_2O_3	2.14	7.99
TiO_2	0.31	0.69
MnO	0.01	0.06
MgO	0.04	0.15
CaO	0.04	0.04
K_2O	0.21	0.52
P_2O_3	0.06	0.08

Table 4.4: Elements present in the study soils as determined by X-Ray fluorescence spectrometry (XRF) (mmol kg^{-1})

Elements	Sandy Loam	Clay
Si^{4+}	15094	12614
Al^{3+}	802	1857
Fe	268	1001
Mn	1.85	7.80
Mg	10.7	36.2
Ca	6.31	7.95
Cu	10.0	40.0
Pb	2.00	7.00

4.3.6 Zn and P analyses

It was ideal for this study to have soils with low Zn and P values so that the response of applied Zn and P and their effects could be studied better. The soils had total Zn contents of 21 and 33 mmol kg^{-1} for the sandy loam and clay soils respectively (Table 4.5). This showed that these soils inherently had low total Zn contents. The solubility

of Zn (as reflected by EDTA) was low, with the sandy loam soil having 0.44 mg kg^{-1} while the clay soil had 1.35 mg kg^{-1}. The critical soil levels for the occurrence of Zn deficiency is 0.6-2.0 mg kg^{-1} depending on the extraction method. Ruffo et al., (2016) stated that critical soil Zn levels varied between 0.8 – 1.17 mg kg^{-1} as extracted by EDTA. This implies that these soils had a very low level of available Zn. Liming of the soil did not have any effect on total Zn content, but it decreased the available Zn as expected. Limed soils decrease the solubility of Zn due to an increase of pH-dependent negative charges as well as precipitation. Another explanation could be the minerals residing in these soils become less soluble in neutral pH since most minerals have the least solubility at neutral pH. Therefore, there was precipitation as carbonates increase pH hence decrease solubility.

Table 4.5: Total and EDTA extractable- zinc and phosphate in studied soils

Analysis	**Sandy Loam**		**Clay**	
	Un-limed	Limed	Un-limed	Limed
Total Zn (mmol kg^{-1})	21.0	22.0	33.0	33.0
EDTA- Zn (mg kg^{-1})	0.44	0.30	1.35	0.65
Total P (mmol kg^{-1})	8.82	Bd	11.6	5.92
Bray-1 P (mmol kg^{-1})	0.67	0. 91	Bd	Bd

Bd = below detection limit

Phosphorus was low in concentrations in both soils. The unlimed sandy loam soil had a total phosphorus content of 8.82 mmol kg^{-1} while clay soil had 11.55 of mmol kg^{-1} (Table 4.5). Bray-1 extractable P was very low, with the sandy loam soil having less 1 mg kg^{-1} regardless of liming. The clay soil did not release any Bray -1 extractable P. In acid soil (< pH 4), Al and Fe oxides act as phosphate scavengers and thus react strongly with H_2PO_4 thus making it unavailable to plants. Bray-1 extractable P in the limed sandy loam soil increased slightly from 0.67 to 0.91 mg kg^{-1}. For the limed clay soil, no trend could be observed because the values were below the detection limit.

4.3.7 Exchangeable acidity, H^+ and Al^{3+}

The soils had titratable exchangeable acidity (Al^{3+} and H^+) of 0.75 $cmol_c\ kg^{-1}$ for the unlimed sandy loam soil and 4.07 $cmol_c\ kg^{-1}$ for the unlimed clay soil. The exchangeable acidity of the unlimed clay soil is composed of slightly higher hydrogen ions (2.25 $cmol_c\ kg^{-1}$) than aluminium (1.82 $cmol_c\ kg^{-1}$) (Table 4.6). A similar trend was observed with the unlimed sandy loam soil, with 0.44 $cmol_c\ kg^{-1}$ and 0.31 $cmol_c\ kg^{-1}$ for H^+ ions and exchangeable Al^{3+} respectively. There was a positive correlation between exchangeable acidity and exchangeable Al^{3+} and H^+ in both soils which means their increase cause acidity to increase. It was expected since exchangeable acidity is the sum of exchangeable protons and Al. Liming reduced all the acidity parameters very sharply, as would be expected.

Table 4.6: Components of acidity in the soils

Analysis	**Sandy Loam**		**Clay**	
	Un-limed	Limed	Un-limed	Limed
Titratable acidity ($cmol_c\ kg^{-1}$)	0.75	0.26	4.07	0.30
Exchangeable Al^{3+} ($cmol_c\ kg^{-1}$)	0.31	0.05	1.82	0.13
Exchangeable H^+ ($cmol_c\ kg^{-1}$)	0.44	0.21	2.25	0.17

4.4 Prediction of Zn's fate depending on the aforementioned soil properties

The soil properties discussed above play a significant role in the Zn occurrence in the soils. According to the mineralogical and chemical analysis, both soils are at advanced stage of weathering, as shown by (1) abundance of kaolinite clay mineral, (2) low organic matter and 3) presence of Fe and Ti oxides (Rieuwerts, 2007). At this stage, soil conditions tend to be unfavourable for the solubility of most elements, including Zn. Kaolinite is known to have lower affinity to Zn and possesses low CEC. High soil acidity (low pH) had a huge influence on the surface charge of these soils. The four occurring oxides in the study soils have a point of zero charge (PZC) at the following

pH; SiO_2 (pH 2), Fe_2O_3 (pH 8.5), FeOOH (pH 7.5 -9) and Al_2O_3 (pH 9.8) (Strawn et al., 2015). Since the pH of the soils is lower than the ZPC of the latter three minerals, it means they possess a positive charge whereas SiO_2 carries a negative charge (Yu, 1997). Kaolinite has a ZPC at pH 4.7 and since the pH values of the unlimed soils are below this ZPC. Kaolinite will in these soils have a positive charge. However, quartz has a low surface area and hence reactivity. Therefore, the chemical and mineralogical analyses indicate two soils with poor cation retention ability. With these chemical conditions, native Zn is likely to reside mostly in the residual fraction and in specifically adsorbed fractions (occluded in sesquioxides and on the edge of kaolinite clay). Casagrande et al., (2008) also found that of low-solubility Zn is in abundance in highly weathered soils. Furthermore, the low organic matter contents exhibited by these study soils aggravate Zn retention. In the limed soils, depending on the time of incubation and pH limed to, the soils should have a higher affinity for Zn. Research indicated that addition of $CaCO_3$ increased the Zn retention capacity on red earth soils (Zhang et al., 2014).

Due to its variable charge surface, especially the clay soil is expected to have a higher phosphate adsorption capacity. The adsorbed phosphate tends to bind Zn due to increased negative surface charge hence more exchange sites for Zn adsorption (Agbenin, 1998). Iron oxides have a high specific affinity for oxyanions such as phosphate. Added zinc is expected to be retained in the less exchangeable chemical fractions. Due to the pH levels of the unlimed soils, which is below 5, the Zn adsorption rate might be less compared to in limed soils.

Chapter 5: The Influence of phosphate source and liming on native Zn

5.1 Introduction

Sorption and desorption processes regulate the fate of both native and applied elements in soil environments (Agbenin, 2003; Agib and Jarkass, 2008). These reactions at the solid-water interface affect the solubility, bioavailability and transport mechanisms, such as diffusion of trace elements, including Zn (Ford and Sparks, 2000). As result of the factors mentioned, phosphate fertilisation, liming and soil management will not only impact the applied Zn, but potentially also impact native Zn in the soil. Zinc is known to bind at specific sites with sesquioxides (Catlett et al., 2002), aluminosilicates (Dahiya et al., 2005) and to form complexes with carbonates (Singh et al. 2008) and phosphates (Wang and Harell, 2005) depending on the soil properties such as pH, organic matter content, presence of sesquioxides and carbonates (Singh et al., 2008; Perez-Novo et al., 2011; Preetha and Stalin, 2014; Antoniadis et al., 2018). Phosphate induced Zn sorption is one of the Zn retention mechanisms which increase rapidly due to continuous application of phosphate fertilisers to improve crop yields. Furthermore, there is dilemma in the efficiency of different phosphate fertilisers providing the nutrient. Therefore, examine phosphate impact particularly co-applied with lime will provide understanding on the fate of native Zn. The aim of the study reported in this chapter therefore was to determine the influence of liming and of P, applied in the form of different P sources, on the native Zn fractions found in the two experimental soils

5.2 Materials and Methods

5. 2.1 Zn fractionation procedure

The Zn fractionation was performed on the incubated phosphate band described in Chapter 3, Section 3.6. In this chapter, no Zn was applied and the response of native Zn to the different phosphate fertilizers was followed with a sequential extraction after a fixed incubation time (60 days). The sequential extraction (SE) procedure in this

study was modified from the proposed methods by several authors as indicated in Table 5.1. Some modified processes performed include:

i. The use of dialysis membrane tubes to minimise loss of colloidal particles during the extraction steps.

ii. The use of one volume amount (50ml) for all extractants, with varied concentrations

iii. Adopting a uniform time of extractions for all extractions (24 hours).

iv. Acid extractable Zn fraction extraction is done by digestion the residue with 25 ml of aqua regia on a hot plate and topping up to 50 ml using distilled water before analysis.

v. Residual Zn is the defined as the difference between XRF and sum of fractions

Table 5.1: Summary of chemical extraction steps, associated fractions, applied reagents and recommending reference source as used in this study

Steps	Chemical pool	Associated fraction	Applied Reagents	References
1	$Mg(NO_3)_2$ extractable (**$Mg(NO_3)_2$**)	Soluble plus exchangeable	1 *M* $Mg(NO_3)_2$	Shuman, 1985; Preetha and Stalin, 2014
2	$NH_2OH.HCl$ extractable (**NH_2OH**)	Mn + Fe oxide bound (without HCl)	0.25 *M* NH_2OH	BCR method,(Rauret et al.,1999)
3	$NH_2OH*HCl$ extractable (**$NH_2OH*HCl$**)	Mn + Fe oxide bound (with HCl)	0.25 *M* $NH_2OH*HCl$, 0.25 M HCl, pH 2	Ross et al., 1985
4	NaOAc extractable (**NaOAc**)	Carbonate bound Zn	1 *M* NaOAc, pH 5	Tessier et al., 1979
5	Oxalate – extractable (**Oxalate**)	Amorphous sesquioxides occluded Zn	0.2 *M* $(NH_4)_2C_2O_4.H_2O$ + 0.2 *M* $C_2H_2O_4$ pH 3	Shuman 1985; Mckeague and Day, 1966, Chowdhury et al.,1997
6	Acid extractable Zn	Remaining acid soluble Zn (Aq-reg-Zn)	HNO_3 – HCl digestion	Tessier et al., 1979, BCR,1999;
7.	Residual Zn	Remaining Zn, not chemically extractable	Difference between Zn-XRF and sum of fractions	Logical addition made in this study

Parameters in bold are the abbreviations used in the discussion for the various Zn fractions throughout the chapter.

5.2.2 Sequential extraction process

The sample was subjected to the discussed sequential extraction (Figure 5.1). The Zn extraction was conducted on 5 g soil from each band. The choice of chemical extractants was based on their relative strengths in releasing Zn from insoluble compounds (Saffari et al., 2009). This has been discussed in Chapters 2 and 3.

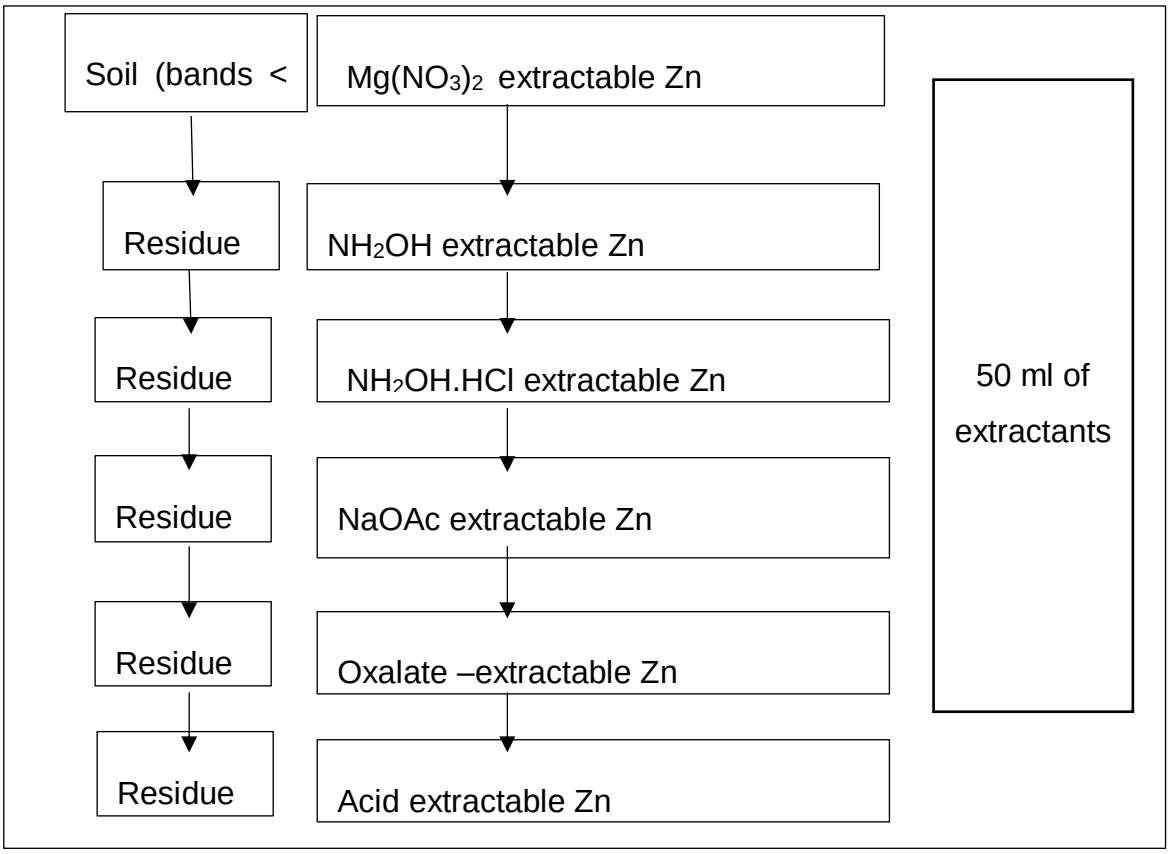

Figure 5.1: Flow diagram of sequential extraction steps

After 60 days of incubation, the bands were sampled from the incubated soil as illustrated in Figure 5.2. Moist soil samples of approximately 5 g were collected and placed in dialysis tubes (SnakeSkin® tubing with a capacity of 3.7 ml. cm^{-1}, pore size – 10 kDa, diameter – 25mm). The ends tied up by cable ties as depicted in Figure 5.3. The dry mass of samples was less than 5 g which was used in calculations. The dialysis tubes were immersed in the different extractants (50 ml) of the sequential extraction in the 100ml Schott bottles (Figure 5.4). The experiment was carried out in a controlled temperature room at 25°C. After 24 hours, extracted solutions were analysed for Zn. From the fourth step onwards, the solution was centrifuged and membrane filtered before ICP – AES analysis. The reason was that the tubes started to tear.

Figure 5.2: Petri dishes with fertiliser band removed for analyses.

Figure 5.3: The sampled band in dialysis tubes

The dialysis bags with soil were weighted prior to the start of extraction and subsequently weighted after each extraction to correct for the entrained solution and carry –over of Zn to the following extraction.

Figure 5.4: Dialysis membrane tubes- containing soil immersed in one of the extractants

5.3. Results and Discussions

5.3.1 The overall chemical extractability of native Zn

The sum of the sequential extraction was compared to Zn determined by X-Ray Fluorescence (Zn-XRF) as a means to understand the overall chemical extractability of native Zn (Figure 5.5). It is expected that Zn-XRF represents total Zn in the soil. The native Zn extracted from both soils by the sequential extraction procedure was very low relative to Zn-XRF (Figure 5.5). For the sandy loam soil, the Zn-XRF was 22 mg kg^{-1}, but the sum of Zn extracted by SE ranged from 2.0 mg kg^{-1} – 6.5 mg kg^{-1}, i.e. only 9.0 – 30% of total native Zn as determined by XRF. For the clay soil, the sum of the Zn extracted by SE being 7.0 mg kg^{-1} – 13.7 mg kg^{-1}, i.e. 21 -24% of the total Zn as determined by XRF since it had total Zn of 33 mg kg^{-1}. The SE procedure was more efficient in recovering Zn from the clay soil than from the sandy loam soil. The impact of added phosphates on native Zn was also noticeable. In the case of the loamy sand soil, the sum of fractions increased after phosphate application while in clay soil sum of fractions increased only after application of MAP, but not with application of DAP and DCP. Overall chemical extractability seemed to decrease in the order of MAP > DAP = DCP, for the sandy loam unlimed, clay limed and clay unlimed soils. For the

limed sandy loam soil, the overall chemical extractability was the DAP treatment was the lowest.

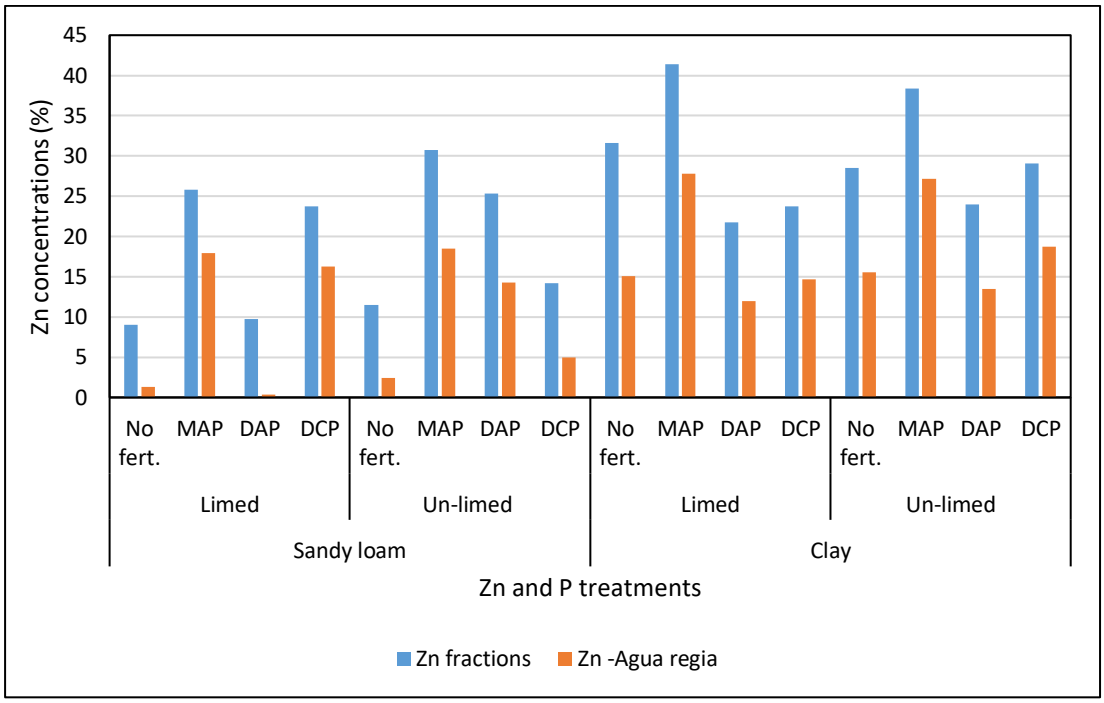

Figure 5.5: The sum of the various Zn fractions relative to Zn XRF, as well as a single aqua regia extractable Zn relative to Zn-XRF. The XRF levels for the sandy loam unlimed was 21 mg/kg and 22 for the lime sandy loam. The limed and unlimed clay soil had the same Zn XRF levels of 33 mg/kg.

A single once-off aqua regia digestion also mirrored the trend of the sum of fractions. For the sandy loam soil, less than 4.0 mg kg^{-1} of native Zn was recovered by the aqua –regia method while in the clay soil it ranged from 3.96 to 9.17 mg kg^{-1}. The possible explanation for this low extractability of native Zn is that it resided largely with silicon in these soils, and the aqua-regia method is not effective in releasing Zn occluded in the silicate clays. Some studies indicate that aqua regia extraction is insufficient in the recovery of Co, Cd, Cr, Ni and Ba since it does not destroy the silicate structure completely (Hseu, 2004; Gaudino et al., 2007). Hseu (2004) recommended the addition of hydrofluoric acid (HF) to enhance the destruction of silicate minerals as it binds with silicate to form SiF_4 leading to more complete digestion.

The second explanation could be that hopeite ($Zn_3(PO_4)$), franklinite ($ZnFe_2O_4$), willemite (Zn_2SiO_4) (Ford and Sparks, 2000) as possible Zn solid phases exert a control on Zn solubility. Based on the highly weathered study soils (Chapter 4), willemite and franklinite are two possible minerals which potentially can control Zn solubility. In theory, willemite is less soluble, between a pH of 3 to 6, than franklinite (Figure 5.6). Thus, the low recovery of native Zn might be because Zn resides in willemite. The solubility of franklinite is high in acidic conditions. Goethite and hematite keep Fe^{3+} activity lower than franklinite, therefore it is more likely that goethite and hematite will control Fe^{3+} activity (Lindsay, 1991). Sadiq (1991) also noted that hopeite is sparingly soluble in low pH soil conditions and could influence the fate of Zn with time.

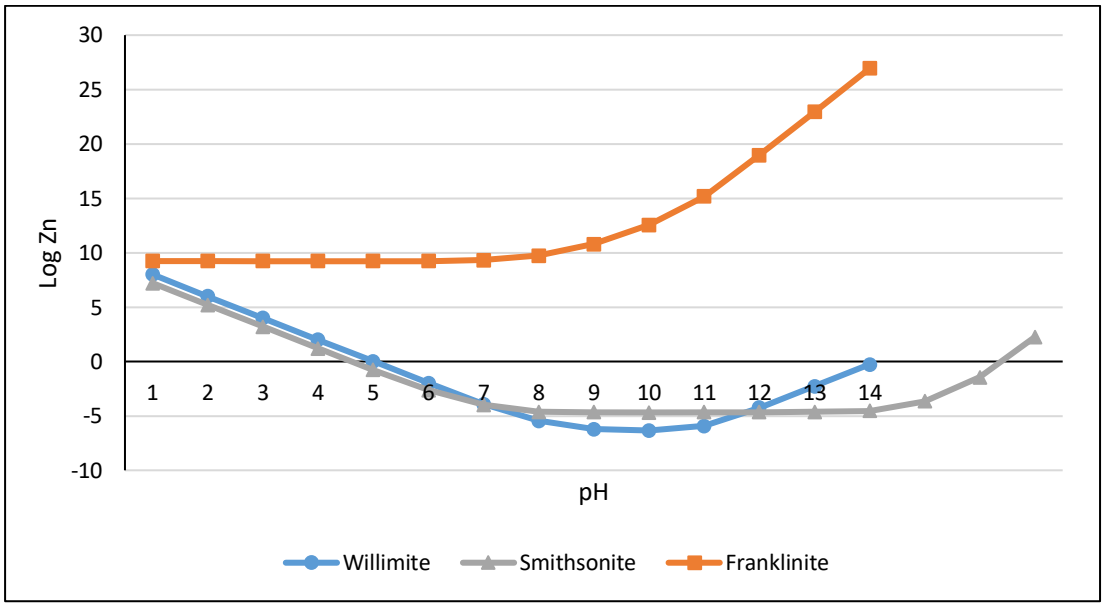

Figure 5.6: The solubilities of zinc minerals over a range of pH

5.3.2 Effect of phosphate fertilisers and liming on the chemical fractions of sandy loam soil

Zinc distribution between various chemical forms (mg kg^{-1}) is presented in Table 5.2 and. In spite of the overall low chemical extractability of native Zn, the influence of the ammonium phosphate and lime was evident. The highest mean concentrations were recorded for the NH_2OH*HCl (0.71 mg kg^{-1}), oxalate (0.66 mg kg^{-1}) and acid extractable - Zn fractions (2.04 mg kg^{-1}) (Table 5.2). Thus, most native Zn in this soil

is specifically bonded with sesquioxides and resides mostly in the silicate structure. The low values of the sum of fractions supported the low chemical extractability of native Zn in this soil.

Looking at phosphate amendments, MAP resulted in higher overall chemical extractability of Zn as reflected by the sum of fractions, with the limed and unlimed sandy loam soils having a total of 5.68 mg kg^{-1} and 6.46 mg kg^{-1} respectively (Table 5.2). This implies that MAP increased Zn solubility more than the other two sources of phosphate. That is, it seemed that the treatment of soil with MAP moved some of the Zn from fractions that are not chemically extractable to chemical extractable forms. However, MAP was not significantly different from the other two phosphate sources expect in the limed soil under acid extractable fraction which was statistically higher than other phosphate source (Table 5.2). Another observation was that in the case of the DAP treatment for the sandy loam soil, liming seemed to decrease the chemical extractability of Zn. The sum of the fractions decreased from 5.32 mg kg^{-1} to 2.15 mg kg^{-1} after liming. For DCP it was opposite. The opposite effect of DCP treatment might due to Ca which is constituent of this source.

Table 5.2: Native Zn concentration (mg kg^{-1}) in various chemical fractions as affected by the type of phosphate fertilizer and liming for the sandy loam soil

Soil treatments		$Mg(NO_3)_2$	NH_2OH	NH_2OH *HCl	NaOAc	Oxalate	Acid-extractable	Sum of fractions
Limed	No fert.	0.33^{c}	0.29^{f}	0.39^{a}	0.31^{cd}	0.38^{ab}	0.28^{a}	1.98
	MAP	0.09^{ab}	0.10^{bc}	1.02^{c}	0.03^{a}	0.50^{b}	3.94^{b}	5.68
	DAP	0.05^{a}	0.15^{cd}	0.96^{c}	0.50^{d}	0.41^{ab}	0.08^{a}	2.15
	DCP	0.14^{b}	0.13^{bc}	1.00^{c}	0.08^{ab}	0.29^{a}	3.58^{b}	5.22
Un-limed	No fert.	0.39^{c}	0.06^{ab}	0.42^{a}	0.15^{abc}	0.89^{c}	0.51^{a}	2.42
	MAP	0.40^{c}	0.20^{de}	0.89^{bc}	0.10^{ab}	0.98^{c}	3.89^{b}	6.46
	DAP	0.33^{c}	0.03^{a}	0.53^{ab}	0.48^{d}	0.95^{c}	3.00^{b}	5.32
	DCP	0.05^{a}	0.26^{ef}	0.45^{a}	0.27^{bc}	0.90^{c}	1.05^{a}	2.98
	Mean	**0.22**	**0.15**	**0.71**	**0.24**	**0.66**	**2.04**	**4.03**

No fert. = no fertilisers were added. Means with the same letter are not significantly different. The significance has been performed within each extractant, across all soil treatments. Values followed by the different letter within each treatment are significantly different from each other $\alpha<0.05$

Distribution of Zn chemical fractions varied according to the type of phosphate fertiliser and liming status. The highest percentage of Zn were recorded for the acid extractable fraction –Zn in MAP +lime, MAP + un-limed, DCP +limed and DAP + un-limed.The total proportions (%) of the Zn fractions in both limed and unlimed sandy loam are presented in Appendix H. Although, the sandy loam soil was expected to have low Zn concentration due to the low Zn affinity of sandy soils (Laker, 2005), the amount of clay was 15% and the fact that its clay fraction contains some goethite might contribute to the abundance of native Zn in the silicate clays. In addition, literature suggested that there is minimal isomorphous substitution involved with kaolinite due to its non-expansive property and well–arranged structure, it is possible that Zn may only be retained at the edges of the kaolinite (Patel et al., 2002). The other dominant chemical fractions were the NH_2OH and NH_2OH*HCl extractable fractions, which had the highest Zn concentrations in the unlimed soil where no phosphate was applied and in the limed soil when DAP was applied respectively.

The effect of liming on the distribution of chemical fractions of the sandy loam soil varied according to the specific treatments. Liming on its own had a negligible effect on the $Mg(NO_3)_2$ extractable fraction of native Zn compared with the control treatment where no fertiliser was added. However, where phosphate fertilisers were applied, there was a reduction in $Mg(NO_3)_2$ extractable Zn compared with the control treatments. MAP, DAP and DCP application decreased this most bioavailable Zn fraction. Agbenin (1998) also found that applied phosphate reduced the exchangeable Zn in sandy loam soils. In the case of MAP and DAP, a signature of the combined effect of liming and phosphate fertilizer was even a further decrease in the $Mg(NO_3)_2$ fractions and the oxalate fractions (Zn occluded in the Fe and Mn oxides and amorphous and non–crystalline sesquioxides).

The fraction that was increased the most by liming was the acid extractable fraction, which represents Zn of low solubility. The increase in the acid extractable fraction when the soils were limed suggests that the increase in alkalinity favoured the formation of the Zn solid phase that is resistant to chemical reduction (NH_2OH and oxalate extractable forms).

The NaOAc and NH_2OH extractable fractions were also decreased by lime combined with MAP and DCP. The phosphate fertilisers negatively affected these chemical pools further, except the NaOAc fraction in the DAP treatment. The DAP fertiliser behaved differently from the other two phosphate fertilisers in the sandy loam soil, as it was the only phosphate fertiliser which increased concentrations of NaOAc extractable Zn in both limed and unlimed conditions. This could be explained by the fact that DAP is known to raise the pH of the soils. This chemical pool contains Zn which can easily desorb into the soil solution and then can be held on the exchange sites. Furthermore, Zn solubility in weathered soils is controlled mainly by interacting with iron oxides and clay minerals rather than by carbonates (Egwu and Agbenin, 2013).

5.3.3 Effect of phosphate fertilisers and liming on the chemical fractions of clay soil

Native Zn concentrations in the clay soil were generally higher as compared to the sandy loam soil, as shown in Table 5.3 However, similar to sandy loam, the acid-extractable Zn fraction was dominant with the highest mean of 5.96 (Figure 5.3). This is not surprising since because of the higher surface area clay is known to increase the affinity of soil for Zn (Hafeez et al., 2013). The clay fraction of this soil also contained both hematite and goethite (Chapter 4, Figure 4.2). The appreciable iron enrichment was also evident in the XRF analyses (Table 4.3 and 4.4). Based on all, this enhanced the Zn retention was expected. The highest values were for the MAP treatment as it recorded 13.65 mg kg^{-1} for the limed and 12.67 mg kg^{-1} for the unlimed clay soil (Table 5.3). In addition, MAP showed significant difference from other phosphate amendments in the acid, $Mg(NO_3)_2$ and $NH_2OH*HCl$ and NH_2OH extractable fractions depending on the liming status. For instance, MAP treatment was significantly higher than both the DAP and DCP treatments in regard to the acid extractable fraction in both limed and unlimed conditions. DAP produced almost the same sum values in both lime status while DCP gave a high sum value in the unlimed clay soil (9.58 mg kg^{-1}) than in the limed clay soil (7.84 mg kg^{-1}), but in most cases there were no significant difference between P except in some unlimed treatments (Table 5.2).

Table 5.3: Native Zn concentration (mg kg^{-1}) in various chemical fractions as affected by type of phosphate fertilizer and liming for the clay soil

Soil treatments		$Mg(NO_3)_2$	NH_2OH	NH_2OH* HCl	NaOAc	Oxalate	Acid extractable	Sum of fractions
Limed	No fert.	0.25^{b}	0.58^{c}	1.58^{c}	1.30^{d}	1.76^{b}	4.96^{ab}	10.43
	MAP	0.09^{ab}	0.70^{c}	2.36^{d}	0.48^{ab}	0.85^{a}	9.17^{c}	13.65
	DAP	0.01^{a}	0.26^{b}	1.60^{c}	0.43^{a}	0.92^{a}	3.96^{a}	7.18
	DCP	0.10^{ab}	0.19^{ab}	1.37^{bc}	0.48^{ab}	0.86^{a}	4.84^{ab}	7.84
Un-limed	No fert.	0.89^{c}	0.08^{a}	0.96^{ab}	0.79^{c}	1.56^{b}	5.14^{ab}	9.42
	MAP	1.43^{d}	0.04^{a}	0.85^{a}	0.64^{abc}	0.76^{a}	8.95^{c}	12.67
	DAP	0.81^{c}	0.10^{ab}	0.85^{a}	0.69^{bc}	1.01^{a}	4.44^{ab}	7.90
	DCP	0.91^{c}	0.09^{ab}	0.82^{a}	0.71^{bc}	0.86^{a}	6.19^{b}	9.50
	Mean	**0.56**	**0.25**	**1.30**	**0.69**	**1.07**	**5.96**	

No fert. = no P fertilisers were added. Means in columns with the same letter are not significantly different. The test for significance has been performed within each extractant across all treatments. Values followed by the different letter within each treatment are significantly different from each other $\alpha<0.05$

The impacts of lime and phosphate amendments were overshadowed by the effect of clay content since the dominance of the acid extractable Zn fraction was distinguishable in each treatment. Due to high iron content, kaolinite act as surface for iron oxides to precipitate. The acid extractable fraction was dominant in all treatments regardless of the applied phosphate fertiliser and lime status. This fraction had Zn concentrations as high as 72%, 66% and 64% (of extractable Zn) for MAP+ unlimed, MAP + limed and DCP + unlimed respectively and as low as 48% for No fert +limed and 54% for No fert, unlimed and DAP+ limed. This is the fraction resistant to chemical reduction and means vast quantities of native Zn was possibly bound into clay minerals. Because of the abundance of kaolinite it is possibly the soil constituent where most of the native Zn resides (Finzgar et al., 2007).

Other fractions which followed the acid extractable fraction in amounts of zinc extracted were NH_2OH*HCl and oxalate extractable Zn. The clay soil had greater levels of dithionite extractable iron than sandy loam soil (Chapter 4, Table 4.2). It is

known that Zn associates strongly with iron oxides and it was, therefore, expected that the chemically reduceable fractions of the clay soil will be greater than in the sandy loam soil. High percentages of sesqui-oxides in this soil explain the dominance of the three Zn fractions that employ chemical reduction to extract Zn (Mandal and Mandal, 1990; Li and Shuman, 1996). The NH_2OH*HCl extractable Zn was increased by the application of lime. A similar observation was seen in the oxalate extractable fraction except for the DAP treatment. The phosphate amendment did reduced the oxalate extractable fraction for both limed and unlimed treatments. Similar findings were reported by Fathi et al. (2014) who fractionated native Zn from greenhouse soils which undergo continuous macronutrients fertilisation. However, there were no phosphate fertilisers applied.

In contrast, the NaOAc, NH_2OH and $Mg(NO_3)_2$ extractable fractions were the lowest extracted native Zn fractions in the clay soil. Since the soils were highly weathered, these fractions were least expected to have high native Zn concentrations. For instance, due to low CEC values (Chapter 4, Table 4.1), the $Mg(NO_3)_2$ extractability was low. Application of lime reduced the $Mg(NO_3)_2$ extractable Zn concentrations. This correlates well with studies which demonstrated that high pH decreased the available/ exchangeable Zn (Harter, 1991; Pardo and Guadalix, 1996; Singh et al., 2008). Similarly, application of phosphate fertilisers to the limed clay soils decreased the exchangeable Zn, i.e the ($Mg(NO_3)_2$ extractable Zn. This support the findings of other studies that showed that increasing pH had a negative effect on the exchangeability of Zn (Martinez and Motto, 2000; Girija et al., 2013). Another observation was that both phosphate fertilisers reduced the NaOAc extractable Zn in both lime and unlimed clay soil.

5.3.4 Comparison of the treatment effects on the Zn fractions in these soils

Similarities

The most pronounced similarity between the two soils was that the acid extractable Zn fraction contributed a large proportion of native Zn concentrations. Hence, it was dominating across almost all treatments in both lime status. This means that native Zn is partitioned with silicate clays in these soils or at least with soil minerals that are not

susceptible to chemical reduction. The dominance of this Zn occluded in the silicate clays (acid extractable –Zn) particularly in the clay soil reflected the crucial role played by the clay (kaolinite) present in the soil. Both soils had low percentages of NaOAc, NH_2OH and Mg $(NO_3)_2$ extractable Zn. Applied lime increased the Zn concentration of NH_2OH*HCl extractable Zn while decreased the Mg $(NO_3)_2$ extractable Zn. This was expected as high pH decreases Zn solubility. Tagwira et al. (1993b) and Mandal and Mandal (1990) also demonstrated that the application of lime together with phosphate aggravates the problem of Zn fixation. Another similarity was in regard to the impact of phosphate, which increased the concentrations of the acid extractable Zn fraction in unlimed soils. MAP recorded the higher value of the sum of fractions than the other two phosphate sources. The pH of this fertiliser could be attributed to this effect. These similarities of DAP and DCP in might be due to the fact that both the study soils are highly weathered in nature.

Differences

The differences exhibited were mainly with regard to the distribution of certain chemical Zn fractions as influenced by applied lime and phosphate fertilisers. In the clay soil, acid extractable Zn dominated all treatments, but in the sandy loam soil, the oxalate and NH_2OH*HCl extractable fractions dominated in the no fertiliser, unlimed and DAP treatments respectively. Another difference was observed for the NH_2OH and NH_2OH*HCl fractions, in the clay soil, lime application increased the Zn concentrations while in the sandy loam soil, the increase was slight for both fractions except in the DCP treatments where Zn concentrations in these fractions decreased. Regarding phosphate application in the limed clay soil, there was a slight increase while in unlimed clay soil, there was no effect at all in both fractions. The DAP treatment had a more pronounced effect on the NH_2OH*HCl extractable Zn fraction of the sandy loam soil than in the clay soil compared to the other phosphate fertiliser. This increase was only observed for the limed sandy loam and not for the unlimed.

5.4 Conclusion

Native Zn overall showed very low chemical extractability in both soils. The acid extractable fractions were never greater than 45% of the total Zn (XRF extractable

Zn). Most of the Zn extracted resided in the acid extractable fraction, especially in the clay soil. In the case of MAP and DCP, the Zn content of different fractions for the un-limed sandy loam was in the order acid extractable > oxalate > NH_2OH*HCl whereas in the limed sandy loam soil and clay soil the relative contents were acid extractable > NH_2OH*HCl > oxalate. Regarding the effect of phosphate, irrespective if the soil, when MAP applied together with lime, resulted in the highest acid extractable Zn content and also the highest sum of fractions.

CHAPTER 6: The Influence of phosphate source and liming on applied Zn

6.1 Introduction

Due to the low requirement of Zn by plants, Zn sources are usually co-granulated or co-applied with major nutrients, particularly phosphate (Degryse et al., 2015), to address the issue of uniformity in distribution and application. However, the efficiency of these Zn sources is generally compromised, resulting in decreased solubility of applied Zn (Sauerbeck and Helal, 1990; Gianquinto et al., 2000). This is because co–granulation and co-application alter the chemical environment around the fertiliser granules and trigger reactions which impact negatively on Zn solubility (Degryse et al., 2009). As outlined in chapter 2 and explained in the chapter 5 and other studies (Agbenin, 1998; Pardo, 1999; Selim, 2015), the application of phosphate fertilisers' shift Zn equilibria in the soil and hence affects the Zn sorption and release of it in the soil.

Studies have demonstrated that band placement of co-granulated Zn sources decreases the solubility of applied Zn due to the formation of sparingly soluble Zn phosphate minerals (Hettiarachchi et al., 2008). Furthermore, localised dissolution of clay minerals caused by the concentration of the fertiliser in the band also plays a role. Fate and efficiency of added Zn are becoming of increasing interest in order to obtain sustainable food supply, particularly in developing countries. This chapter focuses on the solubility of added Zn as $ZnSO_4$ when co-applied with different commonly used inorganic phosphate fertilisers in limed and unlimed soils.

6.2 Materials and methods

The same Zn fractionation procedure was used as detailed in Chapter 5, Sections 5.2.1 and 5.2.2. The same soils and fertilizers were used as in Chapter 5. Each soil was subjected to 8 treatment combinations. The factorial design for each soil was as follows: 2 liming levels (limed soil and unlimed soil) X 4 phosphate sources (including no phosphate) X 3 replicates. Chapter 3, Sections 3.5, 3.6, 3.7 and 3.8 provided the procedures for incubation study and application of Zn fertiliser.

6.3 Results and discussion

6.3.1 The Overall chemical extractability of applied Zn

Similar to native Zn, added Zn was expected to have low extractability from the experimental soils due to their highly weathered nature. More applied Zn was recovered in the limed treatments, particularly in the sandy loam soil with the highest for both soils being 56.7% from DAP + $ZnSO_4$ treatment (Figure 6.1). This appears to indicate that liming enhanced overall Zn extractability. However, in Chapter 7, it will be shown that this was actually due to the fact that liming inhibited movement of Zn out of the fertilizer bands and retained it in the bands. In contrast, unlimed treatments recorded very low Zn extractability from the bands as shown in Figure 6.1, thus, in the absence of lime, the formation of Zn solid phases that is not acid soluble was favoured. More applied Zn was extracted from the sandy loam soil than the clay soil. This was not surprising as clay soil is known to have high affinity and higher bonding energies to retain Zn (Behera et al., 2011).

Moreover, the clay soil contained a higher amount of crystalline forms of Fe, Al and Mn than the sandy loam soil (Table 4.2). This means, the clay soil had much more crystalline iron oxides since it had hematite and goethite (Figure 4.2). However, iron oxides crystals are of small size. Thus, there are more sorption sites for Zn in the clay soil. These could also be the reason why for the unlimed treatments, there was a large difference between the two soils. In the unlimed sandy loam soil, phosphate amendments increased the overall amount of Zn extracted due to higher retention of Zn in the band. In the clay soil, there was slight effect on the DCP treatment which gave 8.4 % of chemically extractable Zn.

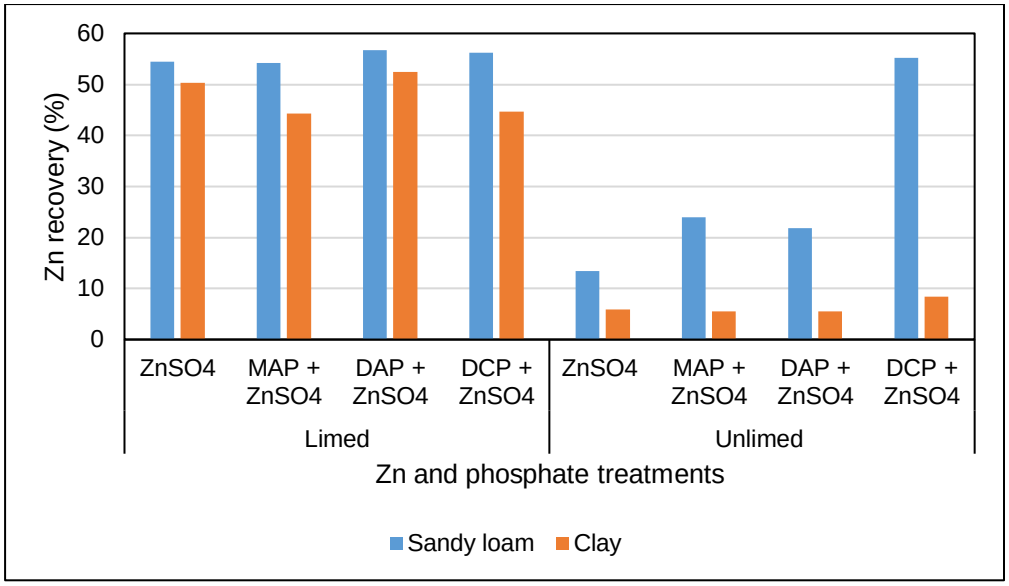

Figure 6.1: Applied Zn recovery as influenced by various treatment combinations

6.3.2. Treatment effects on the relative distribution of applied Zn between various chemical fractions in the fertiliser bands

Effect on the sandy loam

Comparison of the treatments where lime was only applied (no P fertilisers was applied) with where soils were unlimed, showed that the impact of the lime alone was a huge shift from Zn in the $Mg(NO_3)_2$ extractable fraction to the NH_2OH extractable fraction (Table 6.1). The total proportions (%) of the Zn fractions in both limed and unlimed sandy loam are presented in Appendix I. The combined percentages for the fractions are about the same but in $Mg(NO_3)_2$ extractable fraction, lime application decreased the Zn concentrations from 55% to 30% while in the NH_2OH extractable fraction, the Zn concentrations were increased from 20% to 30%. The enrichment of the NH_2OH fraction for the limed soils showed that some applied Zn transformed to solid phases that is not water soluble and exchangeable but only extractable with a stronger extractant exploiting chemical reduction to release it. The evidence point to sorbed Zn to iron and Mn oxides. This Zn fraction bound to Fe and Mn oxide (without acid) in limed soils was the most dominant fraction in both soils with r^2 of 0.99. This

means 99% of variability was due to hydroxylamine as an extractant. These reported results are in good agreement with other research that found that liming reduced the uptake of applied Zn drastically (Pardo and Guadalix, 1996; Finzgar et al., 2007). Limed treatments as expected produced higher values of sum of fraction than unlimed treatments looking at the sum of fractions (Table 6.1). This showed that the applied Zn did not transform into solid phases that is only acid soluble presumably a Zn -silicon solid phase or interaction.

Table 6.1: Applied Zn concentration (mg kg^{-1}) in various chemical fractions as affected by the type of phosphate fertilizer and liming for the sandy loam soil

Soil treatments		$Mg(NO_3)_2$	NH_2OH	NH_2OH* HCl	NaOAc	Oxalate	Acid extractable	Sum of fractions
Limed	No fert	19.33^{b}	28.64^{b}	8.53^{cd}	1.52_{f}	2.06^{d}	2.97^{bcd}	63.05
	MAP	18.47^{b}	30.90^{bc}	10.97^{de}	0.72^{d}	0.85^{ab}	1.92^{ab}	63.83
	DAP	19.89^{b}	24.62^{b}	14.81^{f}	1.06^{e}	0.74^{a}	4.33^{cd}	65.45
	DCP	12.22^{a}	36.19^{b}	12.19^{ef}	0.91^{e}	1.58^{c}	2.10^{ab}	65.19
Un-limed	No fert	9.53^{a}	3.22^{a}	2.04^{a}	0.40^{b}	0.97^{ab}	1.14^{ab}	17.3
	MAP	12.68^{a}	6.47^{a}	5.88^{bc}	0.53^{c}	0.75^{a}	2.74^{bc}	29.05
	DAP	9.16^{a}	7.34^{a}	4.44^{ab}	0.27^{a}	0.68^{a}	4.70^{d}	26.59
	DCP	21.66^{b}	29.41^{c}	10.90^{de}	0.50^{bc}	1.16^{b}	0.53^{a}	64.16
	R-squared	0.95	0.99	0.99	0.95	0.97	0.97	
	CV (%)	13.4	11.8	10.2	20.8	0.6	13.8	

No fert= no fertilisers were added. The values of R-squared and CV give the variation across treatment combination. Means with the same letter are not significantly different. The significance has been performed within each extraction, across all soil treatment. Values followed by the different letter within each treatment are significantly different from each other $P<0.05$

The effect of phosphate involves comparisons for the unlimed soil between the control, where no P fertiliser was applied, and where P fertilisers were applied (Table 6.1). All P sources brought about a reduction in the contribution of the NaOAc and oxalate extractable Zn fractions while for other remaining fractions, DCP increased the $Mg(NO_3)_2$ NH_2OH and NH_2OH*HCl extractable Zn fractions.

In the case of the calcium phosphate (DCP) there was a large increase for 26.19 mg kg^{-1} to the NH_2OH extractable fraction compared to the 3.25 and 4.12 mg kg^{-1} of DAP and DCP respectively (Table 6.1). Thus, these may reflect and impact by calcium. In the case of DAP there was also a shift towards the least soluble acid extractable fraction, while with DCP this fraction was completely eliminated. The effect of phosphates was reflected on the sum of fractions because the No fertilisers applied treatments have lower Zn concentrations compared to treatments where phosphate fertilisers were applied in unlimed soil conditions. Thus P application, in general, increased the binding of applied Zn into this relatively strongly bound fraction of Zn associated with sesquioxides. This conclusion is in agreement with Stanton and Burger (1967), Brady and Weil (2014) and Strawn et al. (2015). This negative effect of phosphate application on the amount of Zn in this fraction was in line with published results, such as those of Mandal and Mandal (1990), who found that applied phosphate fertiliser (KH_2PO_4) decreased the exchangeable and water-soluble Zn concentrations.

The impacts of lime or P applications alone, all lime + phosphate treatments brought shifts to the applied Zn fractions. This involves comparison of the control, where no lime or phosphates was applied, with treatments where both lime and P were applied. (Table 6.1). Sandy loam soil recorded higher values of chemically extractable Zn, DAP in limed treatment being the highest with 65.45 mg kg^{-1} (Table 6.1) while in clay soil, MAP in limed treatment gave the highest of 47.44 mg kg^{-1} as sum of fraction (Table 6.2). On the $Mg(NO_3)_2$ extractable Zn fraction, where MAP or DAP were applied in the presence of lime the Zn concentrations were about the same with Zn concentration of about 30%. Furthermore, there was no significant difference between the lime alone, MAP and DAP treatments (Table 6.1). In the case where the calcium phosphate (DCP) was applied there was reduction to the contribution of the $Mg(NO_3)_2$ extractable Zn fraction, only about 20% was extracted. Since it means that in this case the DCP and lime together supplied more calcium than in the other P treatments and where lime alone was applied, it may further support the indication of a strong calcium effect on the relative distribution of Zn between different fractions.

Again the reduction in the contribution of the $Mg(NO_3)_2$ fraction was accompanied by strong shifts towards the NH_2OH extractable fraction for all the lime + P combinations. NH_2OH extractable fraction also gave abundant Zn more than other fractions as influenced by both lime and phosphate amendments. There was increase in Zn concentration extracted NH_2OH from MAP and DCP fertilisers while DAP decreased the Zn concentration (Figure 6.1). However, they were not significantly different from each other. The NaOAc, oxalate and acid extractable Zn fractions gave low percentages of added Zn. These are chemical pools of Zn in which it is reportedly unavailable to plant uptake. Furthermore, applied Zn could not have been occluded in the silicates clays because it cannot move into the structure of an already formed clay mineral. Second, there is basically no smectites in the soils (Chapter 4) and isomorphic substitution of this kind is absent in kaolinite. Therefore, the only way that applied Zn could have ended up in this fraction was by the formation of extremely resistant and insoluble zinc minerals associated with iron oxide in the soil.

Effect on the clay soil

Application of lime brought major shift of the chemically extractable Zn in almost all fractions. The most contribution was from NH_2OH extractable Zn fraction where Zn concentration increased as well as in the NH_2OH*HCl. The dominance of Fe oxides bound Zn could possibly be related to the much higher sesquioxide content of the clay soil and the presence of hematite in this soil, indicating that some sesquioxides may possibly occlude Zn very strongly. On contrary, acid extractable Zn and $Mg(NO_3)_2$ extractable Zn fraction was decreased by addition of lime. However, the abundance of acid-extractable Zn in some of the unlimed clay treatments was surprising looking at the duration of the incubation period. This could be attributed to high Si^{4+} content of both soils as the anion has the ability to inhibit phosphate adsorption on surfaces as well as masking some clay minerals such as kaolinite hence minimising some properties responsible for Zn adsorption (Qian et al., 1996). Limed soil recorded higher applied Zn concentrations than unlimed soil as indicated by sum of fractions values (Table 6.2). This means application of lime in the clay soil discourage Zn solubility.

Table 6.2: Applied Zn concentration (mg kg^{-1}) in various chemical fractions as affected by the type of phosphate fertilizer and liming for the clay soil

Soil treatments		$Mg(NO_3)_2$	NH_2OH	NH_2OH* HCl	NaOAc	Oxalate	Acid extractable	Sum of fractions
Limed	No fert	14.33^{c}	30.70^{c}	16.07^{b}	1.54^{b}	2.60^{d}	7.15^{b}	41.69
	MAP	13.48^{c}	17.50^{b}	23.34^{c}	1.04^{ab}	2.25^{cd}	7.33^{b}	47.44
	DAP	12.96^{c}	31.87^{c}	20.43^{c}	1.11^{ab}	1.73^{bc}	6.87^{b}	43.10
	DCP	8.28^{b}	32.45^{c}	14.45^{b}	3.29^{c}	2.61^{d}	4.42^{a}	33.05
Un-limed	No fert	5.11^{ab}	0.91^{a}	1.92^{a}	0.96^{ab}	1.10^{a}	6.72^{b}	15.8
	MAP	5.36^{ab}	1.93^{a}	2.65^{a}	0.88^{ab}	1.34^{ab}	4.16^{a}	14.39
	DAP	7.01^{ab}	1.56^{a}	3.46^{a}	0.53^{a}	1.03^{a}	2.67^{a}	14.70
	DCP	4.78^{a}	1.36^{a}	2.10^{a}	0.79^{ab}	1.13^{a}	9.59^{c}	18.39
	R-squared	0.95	0.99	0.99	0.95	0.97	0.97	
	CV (%)	13.4	11.8	10.2	20.8	0.6	13.8	

No fert = no fertilisers were added. The values of R-squared and CV give the variation across treatment combination. Means with the same letter are not significantly different. The significance has been performed within each extraction, across all soil treatments. Values followed by the different letter within each treatment are significantly different from each other $\alpha<0.05$

Phosphate amendment alone, without lime did have minor shift on the chemically extractable fractions. The fractions which showed some shift were the acid extractable Zn and $Mg(NO_3)_2$ extractable Zn fractions. The acid extractable Zn was decreased by application of MAP and DAP while DCP increased it. For the $Mg(NO_3)_2$ extractable Zn fraction it was vice versa. Thus, MAP and DAP enhanced Zn solubility while DCP aggravate the solubility of Zn in the soil. DCP was significantly different from DAP and MAP in the above-mentioned fractions (Table 6.2). The reason for this could be that since DCP contains Ca^{2+} which, if released, can compete with Zn for the few negatively charged exchange sites. In addition, the pH of MAP and DAP might had a great impact.

The amendment of clay soil by lime and phosphate fertilizers has brought slight shift on the chemically extractable fractions. MAP combined with lime decreased the

NH_2OH extractable Zn significantly while increased the NH_2OH*HCl extractable Zn (Table 6.2). DAP and DCP insignificantly differed from the lime + ZnSO4 treatment which is control (Table 6.2). DCP extracted significantly low applied Zn concentrations in the acid extractable and $Mg(NO_3)_2$ extractable Zn fractions compared to the MAP and DAP treatments but in other fractions, it has produced significantly higher values than other phosphate treatments. NaOAc, oxalate and acid extractable fractions extracted very low applied Zn concentrations. Thus, the combined effect of lime and phosphate faciliated the breakdown of Zn ions from the exchangeable and occlusion sites to the soil solution. It reduced the affirnity of Zn to the carbonates, sesquioxides and silicate clays. In view of the substantial sesquioxide contents of the soils used in the study, it is probably not unexpected that such a large proportion of the applied Zn ended up in these fractions.

6.4: Conclusion

Regardless of soil type or phosphate treatment, liming the soil prior to the application of Zn and phosphate resulted in most of it been partitioned into non-exchangeable forms that require reductants to liberate. Limed soils produced higher Zn concentrations compared to their counterparts as shown by sum of fractions. Soil texture also had impact on the added Zn concentrations as Sandy loam soil extracted high applied Zn concentrations compared to clay soil. This can be attributed to the affinity to Zn. MAP, DAP and DCP brought shifts on the chemical Zn fractions depending on the soil type. In

CHAPTER 7: Zn diffusion from Zn –P bands in soils

7.1 Introduction

Zn diffusion in soils from both granular and fluid fertilisers has been investigated by several researchers (Hettiarachchi et al., 2008; Haslett et al., 2001). It has been established that, due to the high water solubility of $ZnSO_4$, Zn has a higher diffusion rate than from the less soluble ZnO, but much lower compared to ZnEDTA (Wessels, 2014). This suggests that the solubility of the Zn solid phase is a determining factor in the diffusion of Zn, also that complexation in solution plays an important role. However, soil properties will likely play the determining role when it comes to diffusion when Zn is applied, on its own, as a soluble source. Apart from the solubility of Zn compounds and soil properties, co-application of Zn and phosphorus will also affect diffusion (Agbenin, 1998). Degryse et al., (2015) assessed Zn diffusion where it was co-granulated with phosphate fertilisers. Their findings showed that $ZnSO_4$ applied alone diffused more than when applied with either mono-ammonium phosphate (MAP) or di-ammonium phosphate (DAP). $ZnSO_4$ co-granulated with DAP had the least /no diffusion at experimental pH values of 6.1 and 7.7. This is attributed to the less acidic environment that DAP create compared to MAP. In order to understand and predict the mobility and accumulation of metals in the soil, various relative indices have been developed such as mobility and/or enrichment factors.

This chapter has three objectives, namely

1. Investigating Zn diffusion from a simulated band as affected by type of phosphate fertiliser and liming using a Solubility Factor (SF) and Enrichment Factor (EF)

2. Establish if the chemical extractability of Zn (as reflected by SF) of the fertiliser band is indeed a predictor of Zn mobility by comparing it to a factor reflecting diffusion of Zn (EF).

7.2 Materials and methods

7.2.1 The Solubility Factor (SF) concept of Zn

This is an index where an extraction represents the chemical extractability of an element. The water-soluble plus the exchangeable fraction of an element is typically used as an indicator of the mobility of the element since extraction by means of a suitable weak salt solution is often taken as the mobile fraction (Kabala and Singh, 2001; Osakwe and Okolie, 2015). The SF used in the study is as follows:

$$SF = \left(\frac{MgNO3\ extractable\ Zn}{Sum\ of\ seguential\ extracted\ fractions}\right) * 100 \qquad \text{Eq. 7.1}$$

In this study, the exchangeable Zn fraction ($Mg(NO_3)_2$ extractable Zn), as the most mobile and a non- specifically bound fraction, and the sum of all the Zn extracted during sequential extraction were used to calculate solubility factor in fertiliser band. XRF determined Zn was not used, because those values differed by an order of magnitude from the sum of the sequentially extracted fractions, as explained in Chapter 5 and could thus not logically be used. Mobility factors were calculated only for inside the applied fertiliser bands and not for the rings around the bands. This was done because the objective was to find whether MF was a suitable parameter with which to predict how much applied Zn would move out of a fertiliser band and how far it would move from the band.

It has been suggested that higher SF values indicate that a particular element (i) is more bio-available (Ngole, 2011) and (ii) has greater mobility (Mao and Rao, 1997; Aucamp, 2000; Lei et al., 2009). However, the role of physico-chemical processes for example diffusability of ions in the soil cannot be directly assessed with chemical extractants. Indices based on chemical extractants are technically indirect inferences because only chemical solubility is assessed.

7.2.2 Enrichment Factor (EF) concept

In order to obtain a more direct measure of diffusion, the extent of Zn enrichment away from fertiliser band was determined. An EF shows the relative enrichment or depletion of an element or compound in soil compared to the background/control (Barbieri, 2016).

In this study, EF was calculated for samples collected from each fertilizer band:

$$EF = \frac{MgNO3\ Zn\ concentration\ for\ each\ ring - Background\ MgNO3\ Zn}{Background\ MgNO3\ Zn} \quad \text{Eq. 7.2}$$

7.2.3 Determination of Zn diffusion from simulated co-applied Zn-P fertiliser bands

Selection of samples

The samples used in this study were based on the experiment in Chapter. Due to large number of treatment combinations, it was not possible to determine Zn diffusion and enrichment for both fertiliser bands, inner ring and outer rings as shown in Figure 7.1. Therefore, only samples that had reasonable levels of Zn solubility i.e. Soil treatments which had $Mg(NO_3)_2$ extractable Zn > 5 mg kg^{-1} were selected. The solubility index of these treatments was also calculated (Appendix G).

7.2.4 Sampling strategy across the fertilizer band

Soil samples were collected in the fertilised band and in concentric circles (rings) around it (Figure 7.1). The rings were sampled using apparatus with various diameters (Appendix D). The sampled soils were weighed, air-dried and Zn was extracted with 1M $Mg(NO_3)_2$ solution. The extraction method is detailed in Chapter 5, Section 5.2.1.

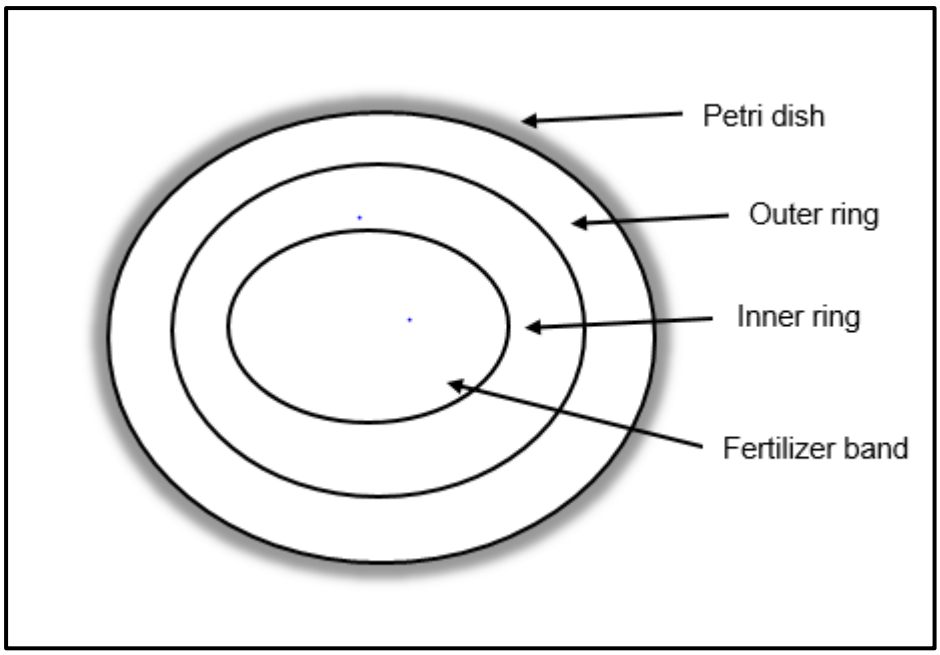

Figure 7.1: Diagram representing the sampling technique. The fertilizer band had a diameter of 1.75cm, The band + inner ring had a diameter of 2.8 cm and the band + inner ring +outer ring had a diameter of 5.0 cm

7.3 Results and Discussion

7.3.1 Effect of ammonium and calcium phosphate on Zn movement from fertiliser bands

Sandy loam soil

In general, only a small proportion of the zinc in the band diffused into the surrounding soil in the sandy loam soil (Figure 7.2). Very little Zn moved more than 1 cm from the point of application. The fertilizer band had substantially higher $Mg(NO_3)_2$ exchangeable Zn concentrations, followed by inner rings and outer rings respectively. For instance, $Mg(NO_3)_2$- Zn concentrations in the sandy loam were 21 mg kg^{-1}, and 5.6 mg kg^{-1} in the fertiliser band and inner ring respectively (Figure 7.2). The outer ring recorded a very low $Mg(NO_3)_2$ exchangeable Zn concentrations as low as 0.02 mg kg^{-1}.

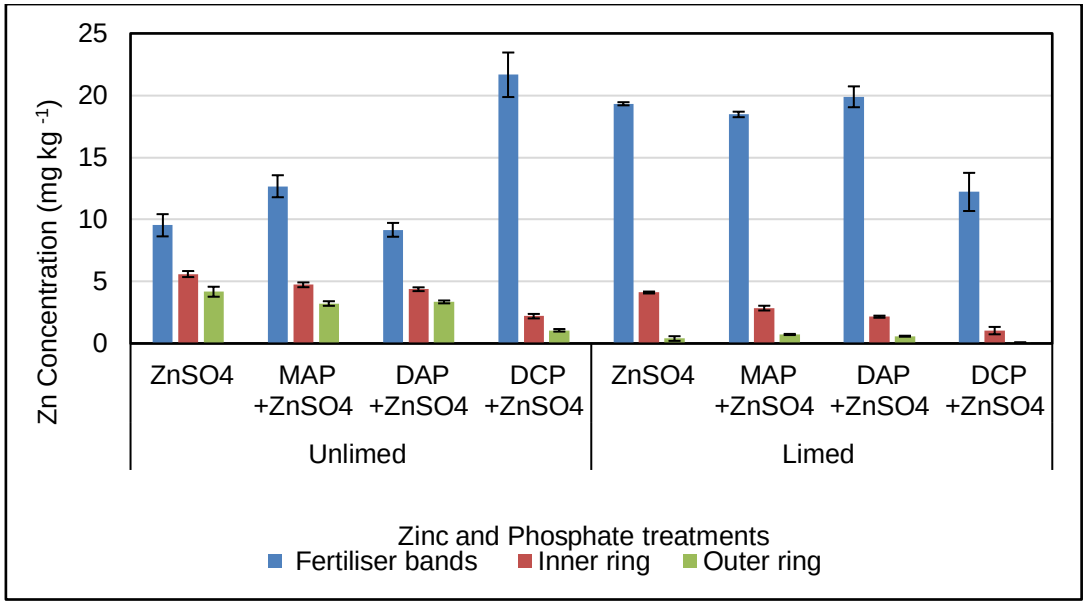

Figure 7.2: $Mg(NO_3)_2$ extractable Zn content (mg kg $^{-1}$) in the fertilizer band and subsequent soil rings for sandy loam soil. Errors bars represent the standard deviation

In all limed treatments, the Zn concentrations in inner and outer rings were lower than in unlimed treatments. In other words, much less applied zinc moved out of the fertilizer bands into the surrounding soil where lime was applied than where lime was not applied. This correspond with the finding that much higher total (sum) proportions (%) of applied Zn were recovered inside the fertilizer bands where lime was applied than where lime was not applied. Because the lime reduced the movement of Zn out of the bands, more Zn stayed inside the bands. Only DCP was an exception as it produced high amount of extractable Zn in unlimed soil. This could be explained by the pH of the fertilizer which neutralized the acidity of the soil to a pH desirable for Zn solubility.

Added phosphate reduced the Zn concentrations in both inner and outer rings more relative to those in the fertilizer bands, compared with where no P was applied in the fertilizer bands. This again shows that P application in the fertilizer bands reduced the movement of Zn out of the bands where it was applied. The Zn concentrations where P was applied in the inner rings in both limed and unlimed soils were in the order MAP>DAP>DCP. In other words, the degree of restriction of movement of Zn from the fertiliser bands by the different P fertilisers were in the order DCP>DAP>MAP. DCP dissolves slowly hence low change in the chemical composition of the soil around the

granules. Another possibility may be that Zn may have diffused to the inner and outer rings but was retained in other fractions.

Clay soil

A lower rate of Zn diffusion is expected for clay soils, with a large surface area that can attenuate Zn (Modaihsh, 1990; Hippler et al., 2015). The clay soil also showed the same trend as in the sandy loam soil with the $Mg(NO_3)_2$ exchangeable Zn content being higher in the fertiliser bands than in the inner and outer rings (Figure 7.3).

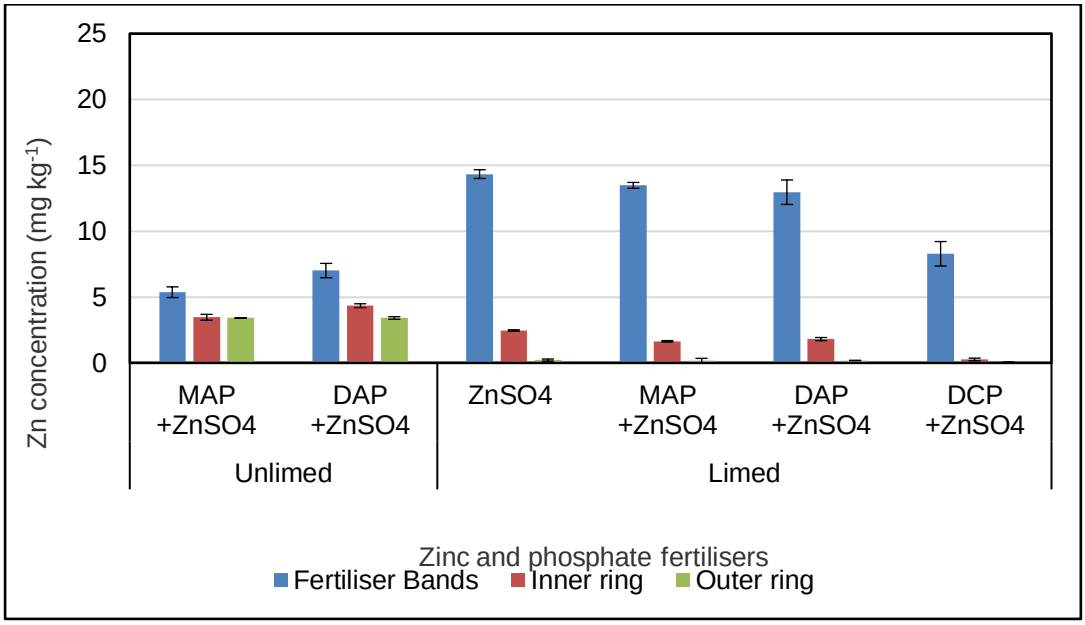

Figure 7.3: $Mg(NO_3)_2$ extractable Zn content (mg kg^{-1}) in the fertilizer band and subsequent soil rings for the clay soil.

The liming impact was noticeable as it reduced the Zn movement out of the fertiliser bands to the inner and outer rings than where no lime was applied. It again corresponded with the much higher Zn concentrations retained in the fertiliser bands where lime was applied than where it was not applied. In limed soils, calcium phosphate compounds are formed and these compounds are relatively water soluble. The Mg $(NO_3)_2$ extractable Zn levels in the band of the unlimed clay soil were much lower than in the sandy loam soil. For instance, MAP and DAP in unlimed clay had 5.4 and 7.0 mg kg^{-1} Zn respectively (Figure 7.2) while their counterparts in the limed clay had 13.5 mg kg^{-1} and 13.0 mg kg^{-1} (Figure 7.3). There was minimal Zn movement

beyond the inner rings into the outer rings in the limed clay soil, giving the $Mg(NO_3)_2$ extractable Zn values of barely above the detection limit.

7.3.2 Zn Enrichment Factors (EF) across the rings around the fertiliser band

Enrichment factors indicate the enrichment of a soil with a specific element relative to a background value for that element at the site where the study is conducted (Loska et al., 2005; Uduma and Awagu, 2013; Ngole, 2011). Thus EF values are relative values that depend on the background value for that element and not absolute values. A relatively high absolute enrichment can have a relatively low EF value if the background value for that element at that site is high. EF is usually used to study pollution with heavy metals (Loska et al., 2005; Uduma and Awagu, 2013; Ngole, 2011). Ngole (2011) tried to use EF to predict uptake of Cu by carrots, but its reliability as predictor was only about 10%, compared to over 70% for MF. In the present study, enrichment factors were used to determine the enrichment of Zn that occurred in the rings around the fertiliser bands, as a result of diffusion away from the fertilizer band, relative to the background Zn level in each of the two soils.

The inner rings of both unlimed soils showed Zn enrichment factors greater than 2, but lower than 4, where MAP and DAP were applied (Table 7.1). In the sandy loam soil the EF was higher than this (4.60) where no P was applied and much lower (1.20) where DCP was applied. In the unlimed clay soil no data were collected for where no P was applied and for where DCP was applied. In both limed soils EF was also in the range between 2 to 4 where no P was applied and in the clay soil also where MAP was applied. Where all three P sources were applied to the sandy loam soil and where DAP or DCP was applied to the clay soil the EF values were below 2. In both limed soils the EF values were extremely low where DCP was applied (<1). Thus the application of phosphate fertilisers decreased the EF values compared to where no P was applied. This could be expected in view of the impacts of P on Zn fractions in Chapter 6 and the MF values. Different phosphate fertilisers showed an interesting decreasing gradient in Zn enrichment following the order of No P>MAP>DAP>DCP in all cases.

The outer rings also showed EF values between 2 and 4 in the treatments for which data were collected in both unlimed soils, except in the DCP treatment in the sandy loam soil, which had an extremely low EF value (0.06) Both limed soils showed negative EF values for all treatments, including where no P was applied. It was found that liming had a strong negative effect on the concentration of $Mg(NO_3)_2$ extractable native Zn in both soils (Chapter 5). Thus in the limed soils the $Mg(NO_3)_2$ concentrations would have been lower than the background values in the controls where no lime was applied. For instance, in native Zn, addition of lime decreased the Zn concentrations extracted from fractions while in unlimed treatments, it was the opposite. Thus, it could be that liming resulted in the precipitation of exchangeable Al (Table 4.6) and oxalate extractable Fe (Table 4.2), hence possibly created more surfaces for sorption.

The EF values found in this study were lower compared to the EF values that are generally reported in literature, e.g. by Loska et al. (2005) and Uduma and Awagu (2013). However, those studies were conducted in polluted areas, such as zinc mine smelters where Zn concentrations were very high. I an agricultural soil amended with sewage sludge Ngole (2011) found EF values for Cu ranging between only 0.05 and 0.21. She quotes publications that rate EF values lower than 3 as “minor”.

Table 7.1 Calculated Enrichment factors of Zn for the various treatments

Soils	Treatments	Enrichment factor	
		Inner rings	Outer ring

Unlimed Sandy loam	$ZnSO_4$	4.60	3.17
	MAP +$ZnSO_4$	3.73	2.22
	DAP +$ZnSO_4$	3.37	2.35
	DCP +$ZnSO_4$	1.20	0.06
Limed sandy loam	$ZnSO_4$	3.11	-0.61
	MAP +$ZnSO_4$	1.85	-0.28
	DAP +$ZnSO_4$	1.15	-0.42
	DCP +$ZnSO_4$	0.04	-0.94
Unlimed Clay	MAP +$ZnSO_4$	2.46	2.41
	DAP +$ZnSO_4$	3.34	2.12
Limed clay	$ZnSO_4$	2.46	-0.90
	MAP +$ZnSO_4$	2.09	-0.52
	DAP +$ZnSO_4$	1.45	-0.81
	DCP +$ZnSO_4$	0.64	-1.00

As stated earlier,with solubility factor was not a direct measurement. The enrichment of soils outside the fertiliser band is directly the result of diffusion. MF was calculated from data from within the fertiliser band to indicate the potential movement of applied Zn from the band into the surrounding soil. As indicated earlier, it is derived from the relationship between the most mobile Zn fraction and total Zn in the band. EF outside the band is derived from the ratio between two determinations of the least tightly bound Zn in the soil, namely the actual Zn content and a background value. In order to establish whether MF was actually a reliable indicator/predictor of the mobility of Zn it was important to determine whether there was a strong positive relationship between MF in the bands and EF in the surrounding circles.

For both soils highly significant linear relationships between MF and EF were found for both the inner and outer circles. The coefficients of determination (r^2 values) ranged between 0.75 and 0.85 (Figures 7.4 and 7.5). It is important to note that these relationships were independent of lime and P treatments. This indicates that MF values for Zn in the bands were reliable predictors of Zn mobility and thus of Zn movement out of the rings. In essence, it means that chemical extractability is a predictor of Zn diffusion. However, it cannot 100% predict it, depending on the soil type, chemical extractability cannot predict Zn diffusion.

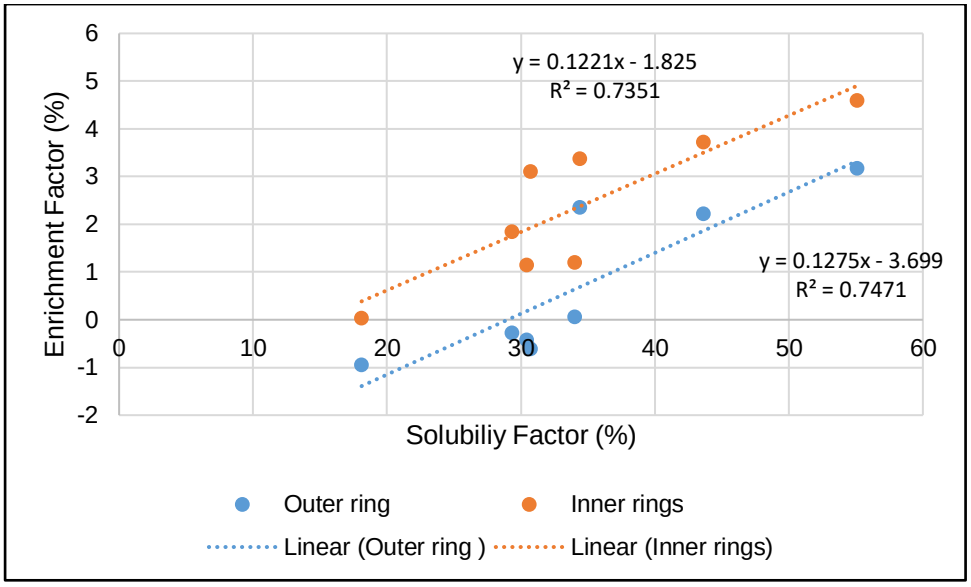

Figure 7.4: The relationship between SF and EF calculated for both inner and outer rings in sandy loam soil

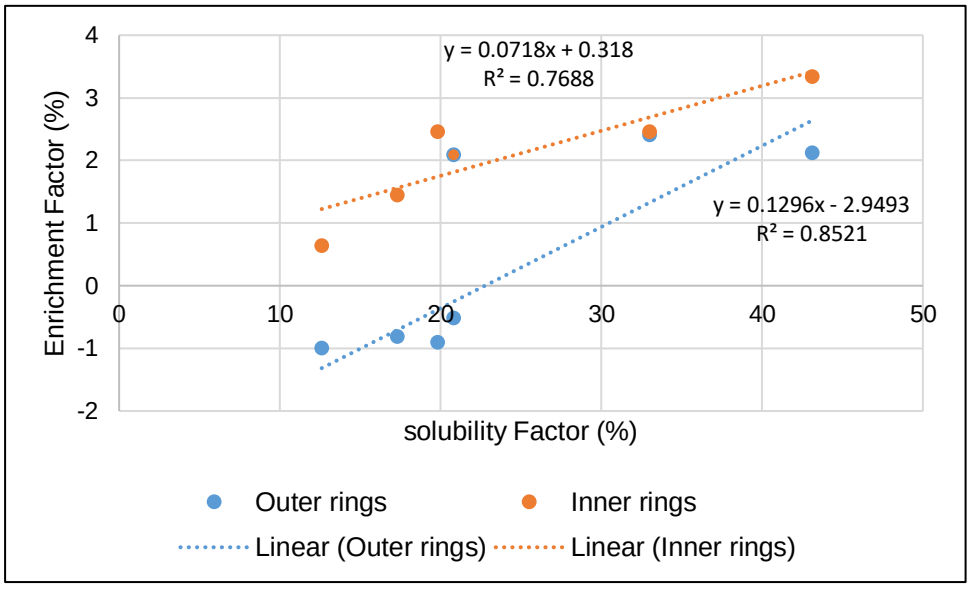

Figure 7.5: The relationship between SF and EF calculated for both inner and outer rings in clay soil

7.4 Conclusion

Chemical extractability of Zn can estimate the movement of Zn in the sandy loam and clay soils amended by phosphate fertiliser and liming. Applied phosphate fertilisers

decreased the movement of Zn away from the point of application, particularly in the outer rings. Similarly, liming decreased the diffusion of Zn to the outer rings as well. The SF and EF values were greater than 2, this means there is moderate Zn enrichment in the soils. Solubility factor can be a reliable indicator of potential of Zn diffusion in the soil.

CHAPTER 8: General discussion, Conclusions and Recommendations

8.1 General discussion

Decreasing the gap between total and plant-available zinc is the key issue to alleviate plant-available Zn deficiency in the soil. This could be done by understanding the chemical behaviour and mechanisms which determine Zn retention in the soil. The fate and solubility of the Zn in the soil are regulated by several chemical reactions, such as sorption mechanisms, precipitation/dissolution reactions and surface complexion. These reactions are greatly impacted by different chemical and mineralogical conditions in the soil. Phosphate modifies the surface characteristics of the soils, hence influencing the formation of Zn complexes which were more attracted to the surface (Strawn et al., 2015). Phosphate increases adsorption sites hence increases the exchange sites available to zinc. The aim of the study was to examine the diffusion of both native and applied zinc in two texturally contrasting soils with phosphate and lime amendments.

It was anticipated that there would be strong Zn retention in the soils, based on the characteristics of the soils included in the study (Chapter 4) and the effects of the amendments (lime and ammonium and calcium phosphate fertilisers). The soils were highly weathered, thus having relatively low CEC and organic matter content, the presence of kaolinite and crystalline Fe and Al oxides and low pH. Zinc behaved in contrasting manners, depending on whether it was native or applied. Native Zn had low chemical extractability compared to the applied Zn and occurred mainly in the acid extractable fraction, indicating that it was mainly occluded in the alumina-silicate clays. This supported one of the hypotheses of this study which stated that the stable chemical Zn controls the fate of Zn in the concentrated phosphate applications. However, this was not the case with applied Zn. Most of the applied Zn resided in the less tightly bound non–specifically sorbed fractions, particularly in the $NH_2OH.HCl$ extractable chemical pool. This is Zn adsorbed and complexed to amorphous iron and manganese oxides. The main reason for this is the dominant presence of kaolinite and

sesquioxides in the clay fractions of both soils. These have a high affinity for Zn (Behera et al. 2011).

A possible factor responsible for the above differences between native and applied Zn could be time. For the zinc to be occluded in alumina-silicate clays, it must be built into the structure of the clay minerals by means of isomorphous substitution, and that takes long enough time. The experimental incubation time of the applied Zn was not long to enable completion of that process. This implies that farmers can benefit well from applied Zn at least during the first two months after application.

Although, the inherent characteristics of the studied soils were conducive for Zn retention, an addition of lime and ammonium and calcium phosphate fertilisers' further decreased the amount of Zn in the loosely held water soluble and exchangeable forms extracted. These findings supported the first hypothesis which stated that the combined effects of liming (higher pH) and localised high levels of phosphate cause higher suppressive effects on zinc solubility than the effects of the above two factors independently. However, it depended whether it was applied or native Zn. Native zinc occurred in less soluble chemical pools after application of phosphate fertilisers, regardless of whether the soil was limed or not, especially in the clay soil. This means that the clay soil provided more charges to retain Zn than the sandy loam soil. Furthermore, the high sorption affinity of the experimental soils for P impacted on the solubility of native Zn. The PO^{-3}_4 species had a high affinity for sorption to the Fe and Al hydroxides on the soil surface, and being multivalent also had a high affinity for Zn, thus forming a chemical bridge between the soil surface and Zn (Stanton and Burger, 1967; Essington, 2004).

The ammonium phosphates (MAP and DAP) showed similar behaviour in controlling the solubility of both applied and native Zn. These two phosphates decreased the solubility of native Zn while enhancing the desorption of applied Zn from insoluble stable fractions. Ammonium (NH_4^+), one of their constituents is nitrified in the soil and during this process acid is produced. This clearly mitigates their effect on MF in the limed soils. In contrast, dicalcium phosphate (DCP) does not have this effect and this was seen clearly in its drastic negative effect on MF in the limed soils, giving the often

reported effect that liming and P application in combination produce very negative effects on plant-availability of Zn in soils.

Applied Zn was weakly adsorbed to soil surfaces hence the diffusion was affected. Diffusion and distribution of Zn from the point of application was restricted by the lime and phosphate amendments. Liming increased the $(MgNO_3)_2$ exchangeable Zn concentration in the fertiliser bands but decreased the on in the inner and outer rings. In the bands, the phosphate fertilisers have less contact with the soil hence less fixation. Thus the microenvironment of the surrounding soil is still conducive for Zn availability. This was the case with applied phosphate as it decreased the exchangeable Zn concentrations. The diffusion of Zn to the outer rings was very low particularly in the limed soils since it produced negative values. This means applied Zn did not diffuse to the outer rings. This results agreed well with Zn enrichment factor values which were reduced by the application of phosphate fertilisers and were higher in the unlimed soils than limed. The outer rings also produced low EF values compared to inner rings. Therefore, it can be deduced that there is a relationship between the Zn diffusion and Zn transformation i.e. the more Zn is transformed into sparingly soluble compounds the lower the diffusion rate. The mobility factor is a good indicator of the potential ability of Zn to move in soils and it correlated well with EF values regardless of the soil type.

8.2 Conclusions

Zinc solubility and diffusion in the studied soils were mainly affected by the lime and different inorganic phosphate fertilisers. Thus, more negative charges caused by the increased pH due to liming and PO_4^{-3} creating bridges for Zn exchange sites. Furthermore, the differences due to these amendments were also revealed between native and applied zinc. High insolubility of Zn was more in native Zn than applied Zn. It was predicted that there will be higher phosphate adsorption due to the characteristics exhibited by the soils, in turn, the adsorbed phosphate increase the sorption sites for Zn hence more Zn will be adsorbed. This was case in native Zn. Most applied Zn concentration resided on non –specifically chemical forms. Thus, it can be exchanged in the soil solution and be available for plant uptake.

To increase the mobility of applied Zn, farmers should avoid co-applying phosphate and zinc fertilisers or increasing soil pH to a level where it is inhibited. In the latter case, the effect can be mitigated somewhat by using ammonium phosphates instead of calcium phosphates. In these phosphates, MAP extracted more native Zn concentration compared to others. Thus, it increased native Zn solubility. It was seen that DCP aggravate the Zn solubility in the soil. It was demonstrated from the study that unlimed soils enhanced the diffusion of Zn to the outer rings. Application of phosphate fertilisers more especially MAP and DAP reduced the movement of Zn from the bands to the rings. This is was also shown by the low values of enrichment factor for each treatment. Knowledge of the sequential extraction was useful, as it helped in identifying Zn binding sites and evaluating its solubility and hence bioavailability. It can be concluded that stable chemical forms of Zn controls its solubility particularly the native Zn. For applied Zn, amending the soils by lime and inorganic fertilisers played a vital role in its solubility.

8.3 Recommendations

For this study, the following recommendations are made:

- Much longer extraction time should be used when using dialysis tubes for fractionation.
- Further research is needed into zinc mineralogy to establish whether there is the formation of hopetite and franklinite, minerals involved the Zn-P interactions.
- For a better understanding of soil chemistry around the bands where phosphate and zinc are involved, much more detailed research is needed whereby it is not limited to the agricultural concentration levels or limits of these two elements.
- This dissertation indicated that even though analytical methods are useful in predicting Zn availability, it is not enough to use them alone. Spectroscopic imaging techniques can be used to facilitate better understanding of the fate of Zn and other micronutrients in soil.

- More research is still needed on the effects of phosphate fertilisers on residual Zn since most researchers indicated that Zn can be released over several seasons.

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Appendix

Appendix A: Zinc content in the homogenised soil

	Zn mg /l	Zn mg/kg	Mean	STDev	CV%
Sandy (limed)	33.44	100.32	103.69	6.42	6.19
Sandy (limed)	33.22	99.66			
Sandy (limed)	37.03	111.09			
Clay (limed)	30.48	91.44	90.24	1.05	1.17
Clay (limed)	29.94	89.82			
Clay (limed)	29.82	89.46			
Clay (unlimed)	29.88	89.64	89.96	0.83	0.92
Clay (unlimed)	30.3	90.9			
Clay (unlimed)	29.78	89.34			
Sandy (unlimed)	30.31	90.93	91.76	1.52	1.65
Sandy (unlimed)	31.17	93.51			
Sandy (unlimed)	30.28	90.84			

Appendix B. Procedure for clay preparation and separation for XRD

Removal of organic matter

For medium –textured to clayey soil a mass of 100 g is needed for this procedure. If a soil sample has a low clay content, then the soil mass could be increased to 150 – 200 g. Thus for this study, 100 g was weighed for both soils. The weighed soil was transferred into a 250 ml Schott bottle. Then deionised water was added to make a saturated paste. Then, 5ml hydrogen peroxide (H_2O_2) was added to the bottle and placed in a preheated bath at 60°C. Soil frothed and as soon as frothing ceased, H_2O_2 has added again until there was no more froth generation.

Sample dispersion and flocculation

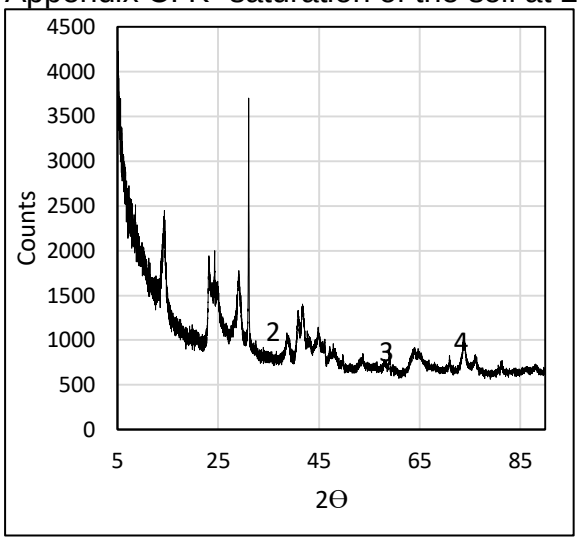
For dispersion, the solution was transferred to 250ml Schott bottle, 10 ml of Calgon plus 150 ml de-ionised water was added. The mixture was shaken for one hour by a mechanical shaker. Then it was dispersed by an electric mixer for five minutes. After that, the soil solution was washed into a 1 L sedimentation cylinder through 53 µm sieve. Deionised water was used to top up to the 1 L mark. Stokes 'law formula was used to calculate the standing time. Calculated time was 6 hours. Then clay suspension was transferred into a beaker for sample flocculation.

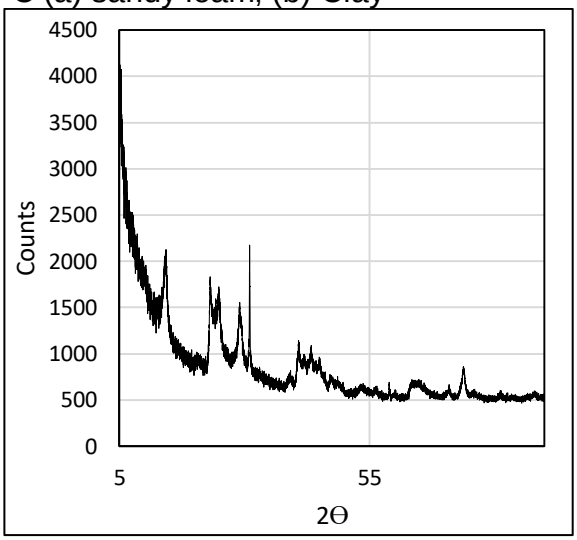
To flocculate the clay, 0.1 M of $MgCl_2$ and KCl solutions were added to the clay. After 60 minutes, the clear supernatant was removed by a syringe. Caution was practised not to lose soil. Then the clay solution was washed with 200 ml of deionised water to remove the salts. The mixture was left overnight to allow the clay to settle out and the supernatant was decanted and discarded. To further enhance removal of salts, centrifugation of the clay suspension was done and the supernatant was discarded. The process was repeated two times. Then the EC of the clay solution was measured. If it was < 50 mS m^{-1}, it was considered to have low enough salt content.

Salt removal by membrane dialysis

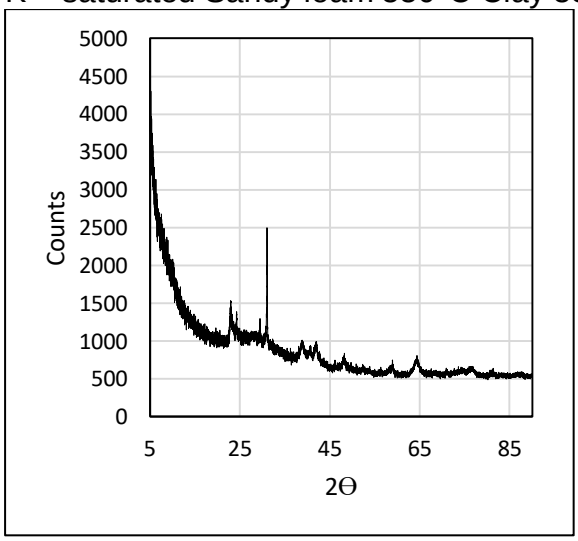
If the clay solution had EC >50 mS m^{-1}, a membrane dialysis method was applied to remove excess salts. The clay suspension was transferred into dialysis tubes. The tubes were then put into deionised water for two hours. After two hours, the water was changed. If the salt content was still high (above 50 mS m^{-1}), membrane dialysis was repeated until the low salt content was reached. The clay fractions that were treated as Mg saturation and K saturation as indicated above were air dried at 25°C for 7 days and heated to 550°C for 2 hours. Glycerol solvation was also carried out in the clay samples.

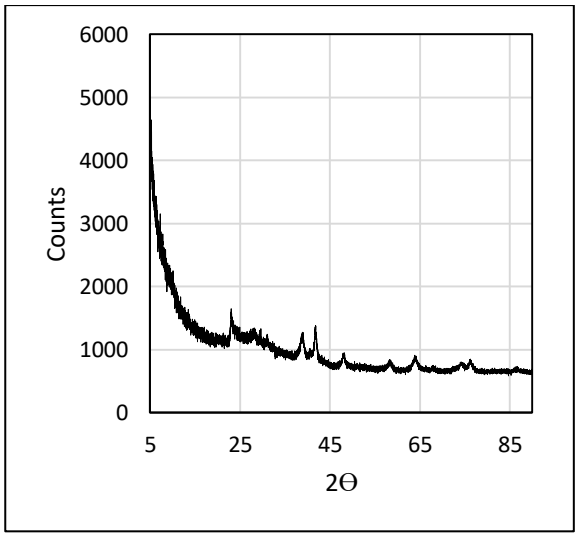
Appendix C: K- saturation of the soil at 25°C (a) sandy loam, (b) Clay



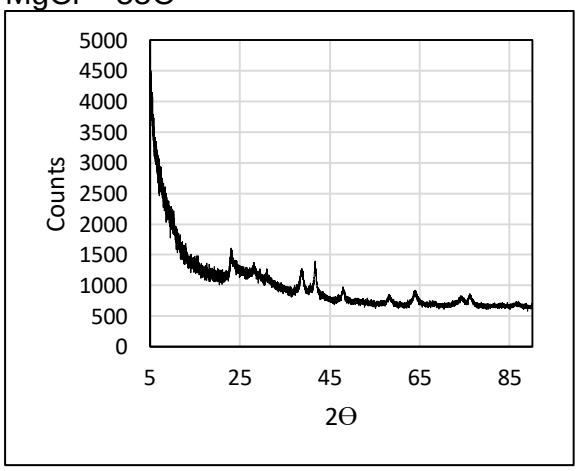


K – saturated Sandy loam 550°C Clay 550°C.





MgCl – 55C



Appendix D: Apparatus used for extracting rings in different sizes

Appendix E: Specifications of P analytical reagent grade sources which might have an effect on Zn (provided on the label)

	Mono-ammonium phosphate (MAP)	Diammonium phosphate (DAP)	Dicalcium phosphate (DCP)
Chemical name	Ammonium dihydrogen phosphate	diammonium hydrogen phosphate	Calcium hydrogen phosphate
Formula	$(NH_4)H_2PO_4$	$(NH_4)_2HPO_4$	$CaHPO_4$
Solubility			Insoluble in water, alcohol
Molecular wt	115.02	132.05	136.06
Insoluble matter	< 0.05%	0.05 %	
pH (5%; water, 25°C)	3.8 -4.4	7.6 – 8.2 (100g/l, H_2O,20°C)	
Assay	99%	> 99.0%	Min. 98 – 105%
Iron (Fe) (%)	0.001	0.001	Max 0.04%
Lead (Pb) (%)	< 0.0005	0.0005	Max 0.004%
Nitrate (NO3) (%)	< 0.001	0.001	
Magnesium (Mg) (%)	<0.0005	< 0.0005	
Potassium (K) (%)	< 0.005	< 0.001	

Appendix F: Composition and Certificate analysis of Zn analytical reagent grade

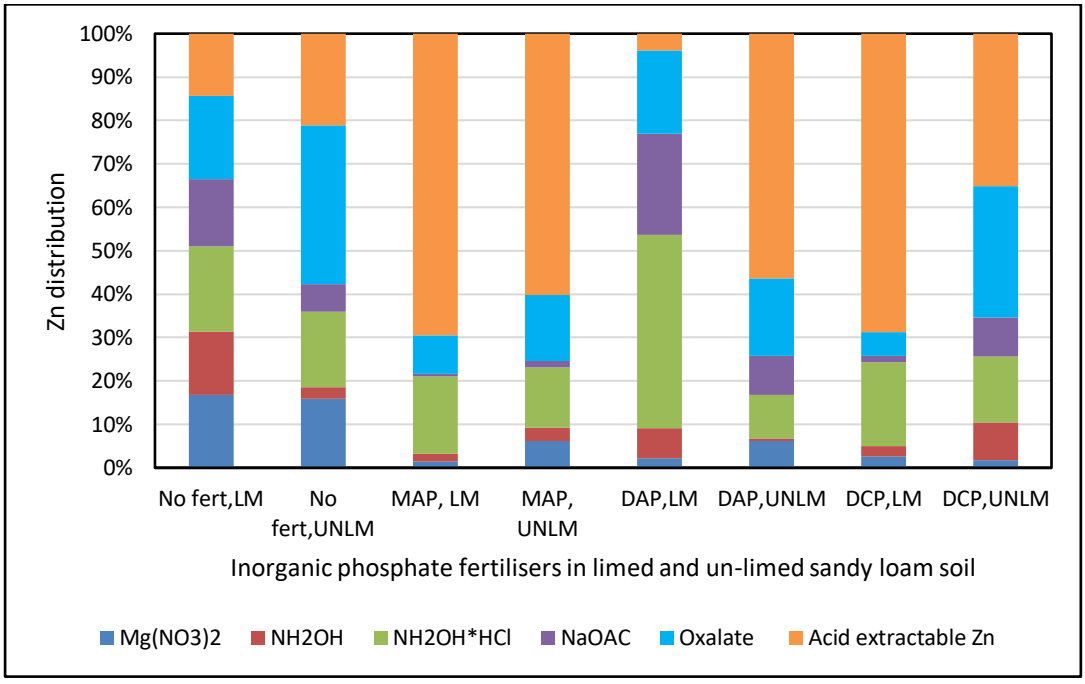
	Zinc sulphate
Chemical Formula	$ZnSO_4.7H_2O$
Molecular wt	287.54
Appearance	Whitish
Particle Size	Granular
Assay	99-104%
Arsenic (As)	0.00005%
Cadmium (Cd)	0.0001%
Chlorine	0.005%
Copper (Cu)	0.0005%
Iron (Fe)	0.001
Manganese (Mn)	0.002
Lead (Pb)	0.0007
Sodium (Na)	0.01
Sulphate (SO4)	0.01

Appendix G: solubility Index (SI) of Zn combined with phosphate in limed and unlimed clay and sandy loam soil

Soil treatment	Zinc sources	Phosphate sources	Clay SI (%)	Sandy loam SI (%)
Unlimed	$ZnSO_4$	No P added	4.7	10.2
		MAP	5.0	13.7
		DAP	6.8	9.6
		DCP	-	23.6
	ZnO	No P added	-	12.1
		MAP	5.1	7.4
		DAP	4.9	8.6
		DCP	4.6	19.4
Limed	$ZnSO_4$	No P added	15.6	21.1
		MAP	14.7	20.2
		DAP	14.1	21.7
		DCP	8.9	13.2
	ZnO	No P added	12.9	23.1
		MAP	12.5	20.3
		DAP	8.4	18.8
		DCP	8.8	9.4
Mean			**9.1**	**15.8**
Standard deviation			**4.1**	**5.8**

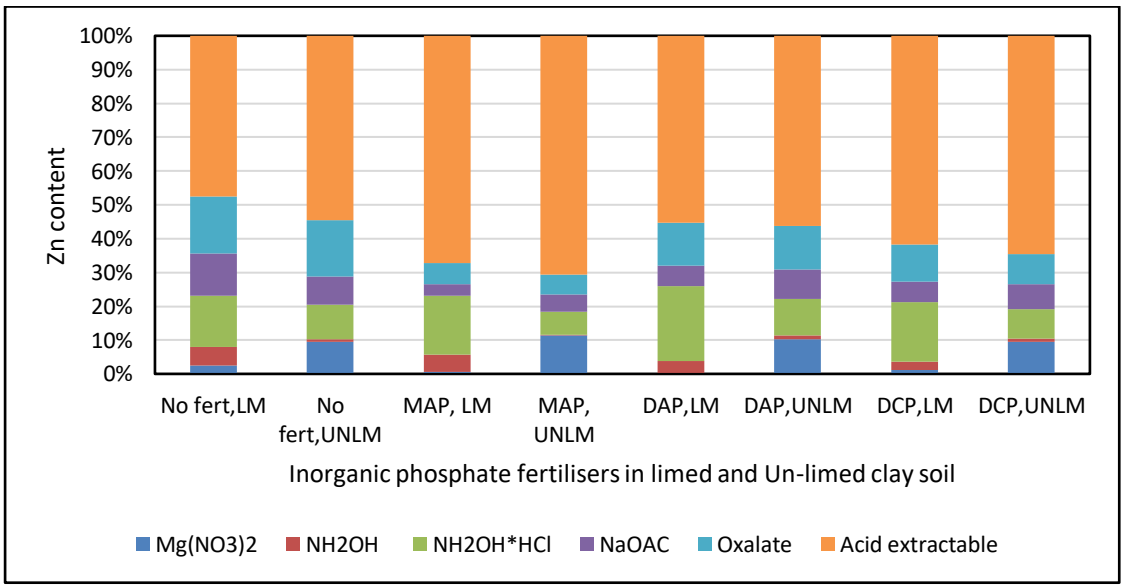
Appendix H: Relative distribution (%) of chemically extractable native Zn as affected by phosphate fertilisers in unlimed and limed soils

Sandy Loam



No fert: no fertiliser applied, UNLM: Un-limed, LM: limed

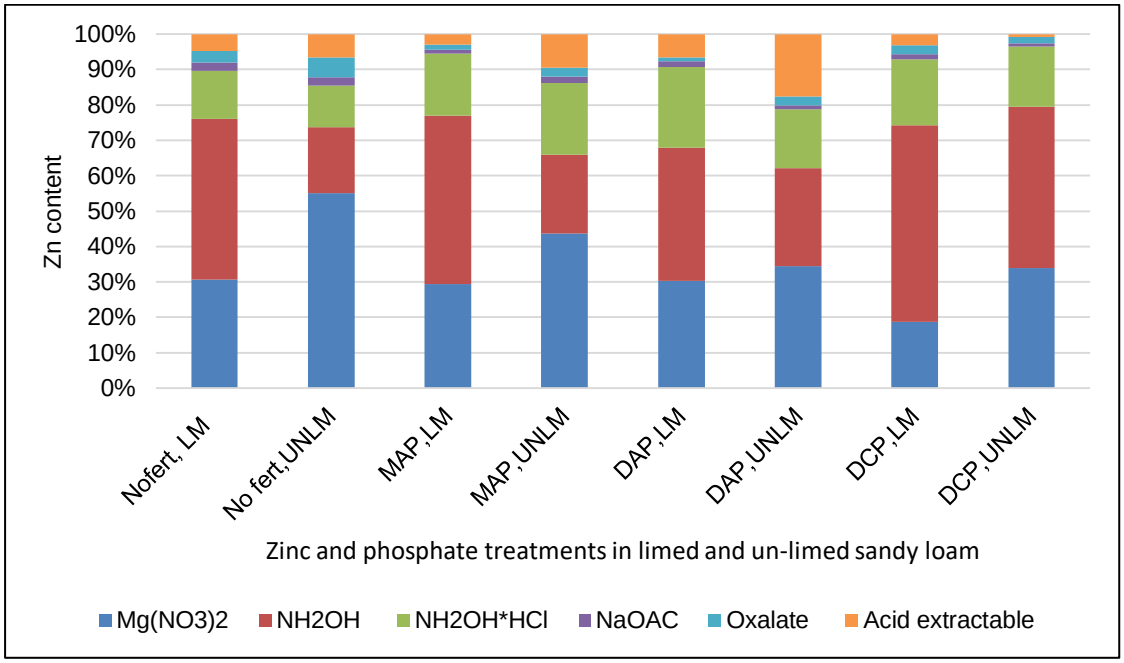
Clay soil



No fert: no fertiliser applied, UNLM: Un-limed, LM: limed

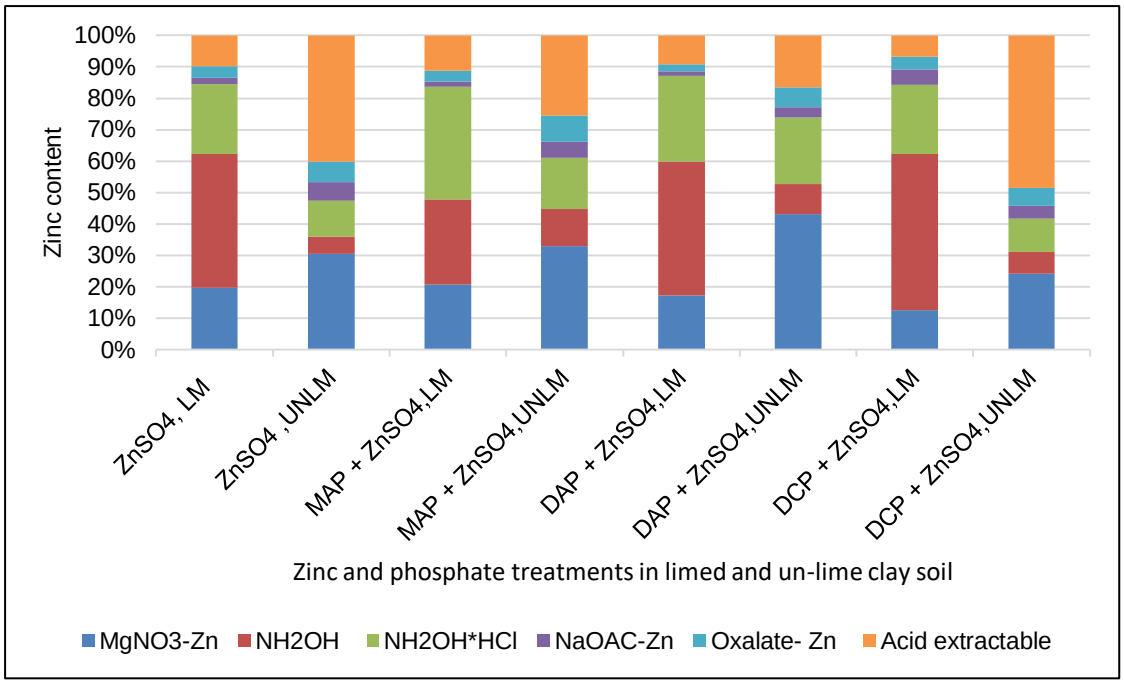
Appendix I: Relative distribution (%) of chemically extractable applied Zn as affected by phosphate fertilisers in unlimed and limed soils

Sandy loam



No fert: no fertiliser applied, UNLM: Un-limed, LM: limed

Clay soil



No fert: no fertiliser applied, UNLM: Un-limed, LM: limed