

RESPONSE OF A SANDY SOIL AND MAIZE
PLANTS TO ZINC FERTILIZERS

by

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TABLE OF CONTENTS

ABSTRACT	vi
UITTREKSEL	viii
DECLARATION	x
ACKNOWLEDGEMENTS	xi
CHAPTER 1 INTRODUCTION	1
1.1 Motivation	1
1.2 Aim	3
CHAPTER 2 LITERATURE REVIEW	4
2.1 Introduction	4
2.2 Forms and reactions of zinc in soil	4
2.3 Uptake, translocation and functions of zinc in plants	7
2.4 Zinc nutrition of crops, animals and humans	10
2.5 Zinc fertilizers and their properties	12
2.6 Evaluation of soils' zinc fertility status	14
2.6.1 Plant analysis	14
2.6.2 Soil analysis	15
2.7 Extraction and determination of plant available zinc	18
2.7.1 Extraction methods	18
2.7.1.1 Dilute hydrochloric acid method	18
2.7.1.2 Mehlich I method	19

2.7.1.3 DTPA-TEA method	20
2.7.1.4 EDTA method	20
2.7.1.5 Ambic II method	21
2.7.2 Determination methods	21
2.7.2.1 Atomic absorption spectroscopy	21
2.7.2.2 Inductively coupled plasma emission spectroscopy	22
2.8 Conclusion	23
 CHAPTER 3 MATERIALS AND METHODS	 24
3.1 Study soils and zinc sources	24
3.2 Incubation experiment	26
3.2.1 Experimental layout and treatments	26
3.2.2 Extraction reagents and procedures	28
3.2.2.1 Dilute hydrochloric acid method	28
3.2.2.2 Mehlich I method	28
3.2.2.3 DTPA-TEA method	28
3.2.2.4 NaEDTA method	29
3.2.2.5 Ambic II method	29
3.3 Glasshouse experiment	29
3.3.1 Experimental site	29
3.3.2 Experimental design and layout	30
3.3.3 Soil treatment and agronomic practices	31
3.3.3.1 Soil treatment	31
3.3.3.2 Pot preparation	33

3.3.3.3 Irrigation	33
3.3.3.4 Glasshouse management	33
3.3.4 Measurement and analysis	34
3.3.4.1 Aerial plant parameters	34
3.3.4.2 Subsoil plant parameters	34
3.3.4.3 Soil sample preparation and analysis	35
3.3.4.4 Plant sample preparation and analysis	35
3.3.5 Statistical analysis	36
 CHAPTER 4 EFFECT OF ZnSO₄, ZnO AND ZnEDTA APPLICATION ON PLANT AVAILABLE ZINC IN SANDY SOIL WHEN DETERMINED WITH VARIOUS EXTRACTANTS	 38
4.1 Introduction	38
4.2 Procedure	39
4.3 Results	39
4.3.1 Main effects	41
4.3.1.1 Zinc source	41
4.3.1.2 Application rate	41
4.3.1.3 Extraction method	42
4.3.2 Interactions	43
4.3.2.1 Zinc source and application rates	43
4.3.2.2 Extraction methods and zinc sources	44
4.3.2.3 Extraction methods and application rates	45
4.4 Discussion	47
4.5 Conclusion	50

CHAPTER 5 EFFECT OF ZnSO₄, ZnO AND ZnEDTA APPLICATION ON AERIAL- AND SUBSOIL PLANT PARAMETERS DURING THE EARLY GROWTH AND DEVELOPMENT OF MAIZE IN A SANDY SOIL	51
5.1 Introduction	51
5.2 Procedure	52
5.3 Results	52
5.3.1 Number of leaves	55
5.3.2 Stem thickness	58
5.3.3 Plant height	61
5.3.4 Leaf area	64
5.3.5 Dry mass	67
5.3.6 Root mass	70
5.3.7 Root length	73
5.4 Discussion	76
5.5 Conclusion	79
CHAPTER 6 EFFECT OF ZnSO₄, ZnO AND ZnEDTA APPLICATION ON THE ZINC CONTENT OF A SANDY SOIL AND MAIZE PLANTS	80
6.1 Introduction	80
6.2 Procedure	81
6.3 Results	81
6.3.1 Plant available zinc content of a sandy soil	84
6.3.1.1 Main effects	84
6.3.1.2 Interactions	84
6.3.2 Zinc concentration in the maize plants	90
6.3.2.1 Main effects	90

3.6.2.2 Interactions	90
6.3.3 Zinc uptake response by the maize plants	93
6.3.3.1 Main effects	93
6.3.3.2 Interactions	95
6.4 Discussion	97
6.4.1 Plant available zinc content of soil	98
6.4.2 Zinc concentration in and uptake by maize plants	99
6.5 Conclusion	103
CHAPTER 7 SUMMARY, SYNTHESIS AND RECOMMENDATIONS	104
REFERENCES	109

ABSTRACT

Maize in Southern Africa is the most important crop for animal and human nutrition. Soil fertility, its management and understanding have an unmistakable role to play in modern agriculture. Maize is prone to zinc deficiency and is known to decrease yield as well as lowering nutritional value. Zinc is reported to be one of the most important micronutrients for the growth and development of maize.

An incubation and glasshouse experiment was conducted to evaluate the response of plant available zinc in sandy soil when fertilized with ZnSO_4 , ZnO and ZnEDTA at different rates. For this purpose a range of extractants were used: HCl , Mehlich I, DTPA, EDTA and Ambic II. In the incubation experiment, two almost similar sandy soils differing only in acidity were treated with the three zinc fertilizers to increase the zinc content with 0 mg kg^{-1} , 1 mg kg^{-1} , 2 mg kg^{-1} , 3 mg kg^{-1} and 4 mg kg^{-1} . Each treatment was repeated five times. Fertilizers were applied as a solution, and after application soil went through three wetting and drying cycles before plant available zinc was determined in them.

In the mentioned glasshouse experiment maize was planted in 40.5 L pots using a complete randomized block design. The same zinc fertilizers were used as for the incubation experiment but application rates differed. One of the soils used for the incubation experiment was selected and treated to increase its zinc content with 0 mg kg^{-1} , 0.5 mg kg^{-1} , 1 mg kg^{-1} , 2 mg kg^{-1} and 4 mg kg^{-1} . Phosphorus and nitrogen were added to the soil at a constant rate. Fertilizers were dissolved in water and applied as a solution on soil before thoroughly mixed. Maize were planted 50 mm deep and soil was maintained at drained upper limit during the growing period. During the five week growing period stem thickness, plant height and number of leaves were measured weekly while leaf area, root length, root mass and plant available zinc were measured at the end of the growing period. The experiment was repeated at two planting dates. After the growing period soil was sampled for zinc and phosphorus analysis.

Concerning zinc source used, ZnSO_4 was superior followed by ZnEDTA and ZnO in most of the measured plant parameters as well as plant available zinc content. Plant available zinc content at the end of the incubation experiment differed between the two soils. Extraction methods used to determine plant available zinc content led to different values. For both soils used in the incubation experiment Ambic II, DTPA and EDTA tend to extract more zinc than HCl and Mehlich I.

Zinc fertilizers and application rates had a significant effect on plant parameters in the glasshouse experiment. The two plantings differed from each other. The effect of ZnO and

ZnEDTA on aerial and subsoil growth parameters was not consistent throughout the glasshouse experiment. Most of the plant parameters showed an impaired development at increasing application rates. This phenomenon however did not occur in the plant available zinc content at the end of the growing period. Extraction method used to determine plant available zinc content at the end of the glasshouse experiment differed. However, the order differs from the results obtained in the incubation experiment. For both experiments the Ambic II and EDTA methods tend to extract the highest amount of zinc from the soil. Zinc source and application rate had a significant effect on both the concentration and uptake of zinc in/by maize. Again ZnSO_4 was superior in increasing uptake and concentration of zinc by/in maize, with ZnO and ZnEDTA being inconsistent.

Considering the reasons for this study it is clear that ZnSO_4 was superior over ZnO and ZnEDTA. This could be attributed that with ZnEDTA and ZnO there were no compensation for the S in ZnSO_4 . Furthermore the ZnEDTA used was synthetically prepared and may be less effective than natural products. Zinc fertilizer and application rate also proved to have an effect on plant available zinc content and maize growth response.

Keywords: zinc source, extraction method, plant parameters, uptake, concentration

UITTREKSEL

Mielies is die belangrikste gewas in Suidelike Afrika vir die voeding van mens en dier. Grondvrugbaarheid, die bestuur en begrip daarvan het daarom 'n onmisbare rol in moderne landbou. Mielies is sensitief vir sinktekorte en word gekenmerk deur verlaagde opbrengs sowel as laer voedingswaarde van grane. Daar word berig dat sink een van die belangrikste mikro-elemente vir die groei en ontwikkeling van mielies is.

'n Inkubasie- en glashuiseksperiment is uitgevoer om die reaksie van plantbeskikbare sink in sandgrond wanneer bemes word met ZnSO_4 , ZnO en ZnEDTA teen verskillende peile te ondersoek. Vir die doel is 'n reeks ekstraheermiddels gebruik: HCl , Mehlich I, DTPA, EDTA en Ambic II. In die inkubasie-eksperiment is twee soortgelyke sandgronde, wat slegs verskil in suurheid, behandel met die drie sinkbemestingstowwe om die sinkinhoud van die grond te verhoog met 0 mg kg^{-1} , 1 mg kg^{-1} , 2 mg kg^{-1} , 3 mg kg^{-1} en 4 mg kg^{-1} . Elke behandeling is vyf keer herhaal. Bemestingstowwe is toegedien as 'n oplossing en na toediening het die grond deur drie benatting- en drogingsiklusse gegaan voor plantbeskikbare sink daarin bepaal is.

In die bogenoemde glashuiseksperiment is mielies geplant in 40.5 L potte deur 'n volledige ewekansige blok ontwerp te gebruik. Dieselfde sinkbemestingstowwe as vir die inkubasie-eksperiment is gebruik maar toedieningspeile het wel verskil. Slegs een van die gronde in die inkubasie-eksperiment is vir die glashuiseksperiment gebruik en dié se sinkinhoud is met 0 mg kg^{-1} , 0.5 mg kg^{-1} , 1 mg kg^{-1} , 2 mg kg^{-1} en 4 mg kg^{-1} verhoog. Die grond is ook met fosfor en stikstof teen 'n konstante peil bemes. Alle kunsmis is opgelos in water voordat dit aan die grond toegedien is, daarna is grond deeglik gemeng. Mieliesaad is in die middel van die pot 50 mm diep geplant. Gedurende die eksperiment is grond nat gehou teen die boonste grens van plantbeskikbare water. Stamdikte, planthoogte en aantal blare is op 'n weeklikse basis gemeet gedurende die vyf week groeiperiode terwyl blaaroppervlakte, wortellengte, wortelmassa en plantbeskikbare sink aan die einde van die groeiperiode bepaal is. Na die groeiperiode is grondmonsters gebruik vir fosfaat en sink ontledings.

Vir die meeste plantparameters het ZnSO_4 beter gedoen, gevolg deur ZnEDTA en ZnO . Plantbeskikbare sink aan die einde van die inkubasie-eksperiment het verskil tussen die twee gronde. Ekstraksiemetodes wat gebruik is vir die bepaling van plantbeskikbare sink het tot verskillende waardes gelei. Vir beide die gronde in die inkubasie-eksperiment het Ambic II, DTPA en EDTA meer plantbeskikbare sink ge-ekstraheer as HCl en Mehlich I.

Sinkbemestingstowwe en toedieningspeile het 'n betekenisvolle effek op plantparameters in die glashuiseksperiment gehad. Die twee plantdatums het ook betekenisvol van mekaar

verskil. Die effek van ZnO en ZnEDTA op bo- en ondergrondse groeiparameters was nie konstant gedurende die glashuisekperiment nie. Meeste van die plantparameters het swakker ontwikkeling getoon met 'n toename in toedienningspeile. Hierdie verskynsel het egter nie voorgekom in die plantbeskikbare sink aan die einde van die groeiperiode nie. Daar was wel betekenisvolle verskille tussen ekstraksiemetodes wat gebruik is vir die bepaling van plantbeskikbare sink aan die einde van die glashuisekperiment. Die volgorde het egter verskil van dié in die inkubasie-eksperiment. Vir beide die eksperimente het dié Ambic II en EDTA metodes groter hoeveelhede sink ge-ekstraheer vanuit die grond. Sinkbron en toedienningspeil het 'n betekenisvolle effek op beide die konsentrasie en opname van sink in/deur mielies getoon. Weereens was ZnSO₄ beter betreffende die opname en konsentrasie van sink in/deur mielies, met ZnO en ZnEDTA wat nie 'n konstante reaksie getoon het nie.

Na aanleiding van die redes vir hierdie studie is dit duidelik dat ZnSO₄ beter was as ZnO en ZnEDTA. Dit sou toegeskryf kan word dat met ZnEDTA en ZnO daar geen kompensasie was vir die S in ZnSO₄. Verder is sintetiese bereide ZnEDTA gebruik wat minder effektief mag wees as natuurlike produkte. Sinkbemestingstof en toedienningspeil het wel 'n effek op plantbeskikbare sink en mielie-ontwikkeling gehad.

Sleutelwoorde: sinkbron, ekstraksiemethode, groeiparameters, opname, konsentrasie

DECLARATION

I declare that this dissertation, hereby submitted for the Magister Scientiae Agriculturae degree at the University of the Free State, is my own independent work and has not previously been submitted to any other University. I furthermore cede copyright of this dissertation in favour of the University of the Free State.

Cornelis Frederick Wessels

Date

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CHAPTER 1

INTRODUCTION

1.1 Motivation

Maize is the largest produced field crop and the most important source of carbohydrates in the southern African region. South Africa is the main maize producer in Africa with most production localized in the Free State, followed by Mpumalanga, North West and KwaZulu-Natal provinces (Abstract of agriculture statistics, 2014). Annually South Africa produces approximately 10 to 12 million tons of maize on approximately 2.5 million hectares of land. Almost half of the production consists of white maize, for human food consumption (BFAP, 2011).

The average maize yield in South Africa has increased 3.5 times in the past 50 years, from an average of less than 1 t ha⁻¹ to 3.5 t ha⁻¹, with a yield average of 4.2 t ha⁻¹ over the last 5 years. This increase is due to the development of better fertilizer practices as well as improvements in soil tillage practices, plant breeding, weed management and pesticides. Farmers in South Africa are well informed about the use of macro-nutrients like nitrogen, phosphorus and potassium, but the importance of micronutrients goes sorely unnoticed (MIG, 2011).

As mentioned, about half of the produced maize in South Africa is being used for human food consumption. From this perspective the quality of maize grain is very important. The South African Government Gazette in 2003 stipulated that maize should contain an average of at least 18.55 mg Zn kg⁻¹ un-sieved maize meal. However, the South African Grain Laboratory reported in a survey by the Maize Trust that the average zinc levels in South African maize were only 12.35 and 13.47 mg Zn kg⁻¹ for 2003/2004 and 2004/2005 seasons, respectively (Van Biljon *et al.*, 2010).

Good quality maize grain depends on proper plant nutrition, hence good soil fertility. In South Africa the need to research soil fertility has been pointed out by Barnard & Du Preez in 2004. Soil fertility can be managed by appropriate fertilization. Essential plant nutrients comprise of macronutrients (N, P, K, Ca, Mg and S) and micronutrients (Fe, Mn, Zn, Cu, Ni, B, Mo and Cl) (FSSA, 2007). In most circumstances the macronutrients are well managed, but this is not necessarily the case with micronutrients.

Maize has a high demand for zinc relative to other crops. In many countries it is the crop most likely to show zinc deficiency symptoms (Camberato & Maloney, 2012). Around the

world, soils most prone to show zinc deficiency are sandy soils and calcium-enriched soils (Mousavi *et al.*, 2013). Sandy soils contribute largely to maize production in South Africa which aggravates the zinc deficiency problem.

It is not surprising that several reports indicated low levels of zinc in maize grain which could be detrimental to humans and animals. In South Africa there are some notable examples of health disorders caused by micronutrients (Laker, 1979). In many soils throughout the country there have been found to be a low level of micronutrients (Herselman & Steyn, 2001).

These low levels might be an indication of insufficient plant available zinc in South African soil despite fertilization. A possible explanation therefore may be that inappropriate fertilizer sources are used or improved cultivars are not able to use soil and/or fertilizer zinc efficiently. The low levels of zinc in maize grain are alarming as it influences plant, animal and human health.

Zinc has a wide range of functions in plants and is required for protein synthesis, gene regulation, structure and integrity of bio-membranes, the protection of cells from oxidative damage, as well as many other roles (Bell & Dell, 2008). During zinc deficiency, protein synthesis is lowered due to low levels of RNA, because zinc plays an essential role in RNA polymerase. The role of zinc fingers in DNA transcription and gene regulation also shows the importance of zinc in protein synthesis. Because of the importance of zinc in protein synthesis, it results in high zinc requirements in meristematic tissues of plants (Bell & Dell, 2008).

Zinc deficiency reduces photosynthesis, but the exact cause is unknown. In plants such as maize, the zinc dependent enzyme carbonic anhydrase is required for photosynthesis to provide HCO_3^- as a substrate for phosphoenolpyruvate carboxylase. Zinc is important for plants in their structure and function of bio-membranes. This micronutrient also plays an important role in the generation and detoxification of reactive oxygen species (Cakmak, 2000). Some of the symptoms of zinc deficiency in plants are caused by the oxidative degradation of the growth hormone, auxin (Bell & Dell, 2008).

Many of the functions of zinc in plants also apply to animals and humans. In both, zinc is important for zinc-metallo enzymes and also for zinc fingers in DNA. Thus, zinc is essential for DNA and protein synthesis, cell division and growth. Zinc also has a neurological function and is required for male and female reproduction (Bell & Dell, 2008). Immune functions are also zinc regulated and deficiencies impair resistance to infection (Walker & Black, 2004). According to Ho (2004), zinc may also be important in host defence against cancer.

The South African fertilizer industry incorporates zinc in fertilizer mixtures, especially for maize production. For many years these mixtures were homogeneous products, implying that each granule has the same zinc content. In later years, however, the fertilizer industry changed to physical mixtures containing granules of a zinc source. The latter mixtures may result in uneven and/or insufficient application of zinc. Furthermore, the kind of zinc source incorporated in the fertilizer mixtures used either earlier or nowadays is usually unknown.

With a decrease in organic matter in soil over time, as well as the introduction of higher yielding cultivars, it became necessary to increase research on micronutrients. The supply of micronutrients from the soil decreased, while the demand from the crop increased. It is important to do calibration studies over time to determine threshold values for different micronutrients in the soil and in the plant itself (Van Biljon, 2009).

Against this background, an investigation on zinc fertilizer sources and their efficiency to stimulate growth of the maize plant in sandy soil is justified.

1.2 Aim

The major aim with this research was to study the response of a sandy soil and maize plants to zinc fertilizer sources. Specific objectives were to establish:

1. Using an incubation study, how the application of zinc sulphate (ZnSO_4), zinc oxide (ZnO) and zinc EDTA at different rates affected the plant available zinc in a sandy soil when determined with a range of extractants like diluted hydrochloric acid, DTPA, Mehlich I, Ambic II and disodium EDTA.
2. In a glasshouse study, how the application of zinc sulphate, zinc oxide and zinc chelate at different rates affected the maize growth in a sandy soil during the five week period after planting (including germination and emergence).
3. By using the glasshouse data, whether the maize plant growth is related or not related to plant available zinc in the soil, as determined by the same range of extractants selected for the incubation study.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The major aim with this research was to study the response of a sandy soil and maize plants to zinc fertilizer sources. This literature review therefore focuses on aspects involving the forms and reactions of zinc in soils as well as the uptake, translocation and functions of zinc in plants. Then zinc nutrition of crops, animals and humans are addressed concisely. Attention is also given to zinc fertilizers and their properties before the evaluation of soils' zinc fertility status is discussed. Then the focus shifts to the extraction and determination of plant available zinc.

2.2 Forms and reactions of zinc in soils

The zinc content in the lithosphere average about 80 mg kg⁻¹. Igneous rock contains 70 mg Zn kg⁻¹, while shale contains 95 mg Zn kg⁻¹, limestone 20 mg Zn kg⁻¹ and sandstone 16 mg Zn kg⁻¹. The parent material from which a soil originates will have therefore a direct influence on its zinc concentration. Thus the total zinc concentration of soil varies between 10 and 300 mg kg⁻¹ with an average of about 50 mg kg⁻¹ (Havlin *et al.*, 1999).

The total zinc content of soil exists of five different forms. These forms influence the availability of zinc for plants. Zinc can occur as free and complex ions in soil solutions, as non-specific and specific adsorbed cations, as ions occlude mainly in soil carbonates and hydrous oxides, in biological residue and living organisms and lastly in the lattice structure of primary and secondary minerals (McLaren & Crawford, 1973). Distribution of zinc in these forms varies widely among soils as a result of differences in their parent material and hence mineralogy and organic matter content (Lyengar *et al.*, 1981). Non-specific adsorption of zinc in soil forms ionic bonds. These non-specific adsorbed cations usually are referred to as exchangeable cations (Sposito, 1981). Specific adsorption on soil takes place when zinc reacts with electron donors from bonds with relatively high covalency. Zinc in soil can be adsorbed specific to organic matter, phyllosilicates and hydrous oxides of aluminium, iron and manganese (Udo *et al.*, 1970).

The distribution of zinc between the different forms mentioned above is governed by the equilibrium constants of the corresponding reactions in which zinc is involved. These reactions which comprise inter alia of precipitation, dissolution, mineralization, immobilization, adsorption and desorption determine ultimately the concentration of zinc in the soil solution. Zinc in the soil solution plays a vital role in supplying plant requirements.

Most of the plants' zinc requirements are supplied by root absorption from the soil solution (Kieken, 1995).

The pools and relating reactions that determine the availability of zinc in soil for plant uptake are illustrated in Figure 2.1. Zinc in the soil solution is controlled by the solution pH and the zinc adsorbed on clay and organic surfaces in soil. When primary and secondary minerals dissolve it provides zinc to the soil solution, which is then adsorbed onto the CEC and/or incorporated into the microbial biomass and complexed by organic compounds in the soil solution. Some of the zinc in the soil solution can be adsorbed by soil but can again become plant available through desorption. Decaying plant and animal residues can also contribute to soil organic matter which can increase zinc in solution through mineralization. Zinc is taken up from the soil solution by plant roots (Havlin *et al.*, 1999).

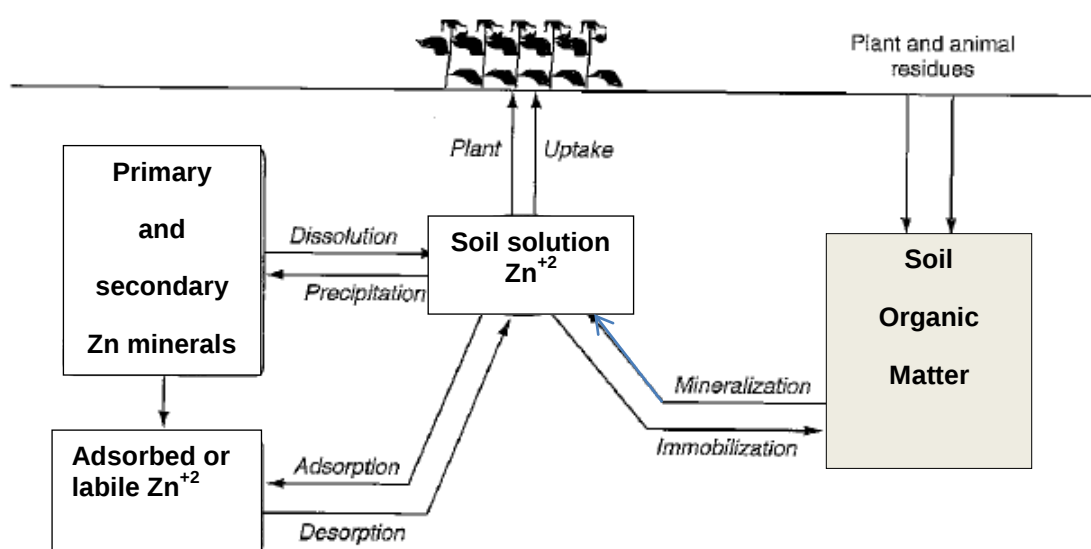


Figure 2.1 Diagrammatic representation of zinc cycling in soils (Havlin *et al.*, 1999).

Usually, the zinc concentration in the soil solution is very low and ranges between 2 and 70 $\mu\text{g kg}^{-1}$ (Havlin *et al.*, 1999). About 50% of the zinc in solution is complexed by organic material. Above pH 7.7, ZnOH^+ becomes the most abundant in the soil solution. Diffusion is the dominant mechanism for transporting zinc as Zn^{2+} to plant roots. The diffusion of chelated Zn^{2+} can be significantly greater than that of unchelated Zn^{2+} (Havlin *et al.*, 1999).

The solubility of zinc is highly pH dependent and decreases 100 - fold for each unit increase in pH. The relationship can be demonstrated by the following equation:



In the range of pH 5 to 7, a thirty-fold reduction of zinc concentration has been observed. Most zinc deficiencies related to pH occurs in neutral and calcareous soils. At high pH, Zn^{2+} precipitates as insoluble amorphous soil Zn, ZnFe_2O_4 and/or ZnSiO_4 , which reduces Zn^{2+} in soils. Liming of acidic soils low in zinc will reduce the uptake of Zn^{2+} , this is related to the change in soil pH and its effect on Zn^{2+} solubility. Increasing pH increases the adsorption of Zn^{2+} by clay minerals, Al/Fe oxides, organic matter and CaCO_3 (Havlin *et al.*, 1999).

The mechanism of Zn^{2+} adsorption to oxide surfaces is likely to take place in soil. Such adsorption is considered an extension of the oxide surface resulting in retention of Zn^{2+} . Adsorption of Zn^{2+} can also occur where Zn^{2+} is less firmly held and can be replaced by other cations. The CEC of montmorillonite, illite and kaolinite is directly related to the adsorption of Zn^{2+} by clay minerals. Zinc is strongly adsorbed by magnesite and lesser to dolomite and calcite. It appears that zinc is adsorbed into the crystal surface at sites in the lattice normally occupied by Mg atoms in magnesite and dolomite. The strong adsorption of zinc by carbonates is partly responsible for the reduced plant availability of zinc in calcareous soils (Havlin *et al.*, 1999).

Stable complexes are formed in soil between Zn^{2+} and organic matter components. The humic and fulvic acid fraction are prominent in zinc adsorption. Reaction with organic matter can be divided into three classes:

1. Immobilization of high molecular weight organic substances
2. Solubilization and mobilization by short-chain organic acids and bases
3. Complexation by initially organic substances that then form insoluble salts

Depending on the characteristics and amount of organic matter involved, the reaction of organic matter and Zn^{2+} can be expected to vary. If reaction 1 and/or 3 is dominant the availability of zinc will be reduced. Conversely the formation of soluble chelated zinc compounds will enhance zinc availability by keeping Zn^{2+} in solution (Havlin *et al.*, 1999).

The amount of dissolved zinc per unit volume soil plus the amount of surface-bound zinc per unit volume of soil that is in rapid equilibrium with the dissolved zinc is referred to as the labile zinc (Corey, 1990). Labile zinc consist mainly of free and complexed zinc in soil solution, which provide the intensity of the soil to supply zinc to plants, and the non-specific

absorbed zinc, which provide the capacity of soil to replenish this micronutrient into the soil solution (Reed & Martens, 1996).

Zinc that exists in forms that are tightly bonded to the soil surface which is in contact with the soil solution is in a non-labile form and is not available for plant uptake. Portions of the labile forms revert to non-labile forms with time and portions of the non-labile forms revert to the labile forms upon weathering. The reversion of zinc from the non-labile to the labile form is very slow and rarely would supply in crop needs during a growing season (Reed & Martens, 1996).

The labile and non-labile forms of zinc amounts to the total zinc in soil. To determine total zinc in soil, it requires either complete destruction of the inorganic and organic soil fractions or partial destruction for the zinc extraction process (Jackson, 1958). In this literature study we focus on plant available zinc of which the concentration is lower than total zinc in soils.

2.3 Uptake, translocation and functions of zinc in plants

The uptake of zinc by plant root occurs primarily through the absorption of Zn^{2+} from the soil solution (Alloway, 2004). There is considerable disagreement in the literature as to whether Zn uptake is active or passive (Mengel & Kirkby, 1987). Kochian (1993) proposed that the transport of zinc during uptake takes place across the plasma membrane towards a large negative electrical potential so that the process is thermodynamically passive. In grasses the non-protein amino acid called phytosiderophores form a complex with zinc and transport it to the outer face of the root cell plasma membrane. During zinc deficiency these phytosiderophores are released from the roots as a result (Kochian, 1993). However, uptake of zinc is inhibit by other metal cations, including Cu^{2+} , Fe^{2+} and Mn^{2+} possibly because of competition for the same carrier site in the casparian bands or plasmalemma. The antagonistic effect is especially prevalent with Cu^{2+} and Fe^{2+} (Havlin *et al.*, 1999). Zinc uptake is also reduced by low temperature and metabolic inhibitors (Bowen, 1969).

The form in which zinc is translocated from the roots to the upper part of the plant is not known (Mengel & Kirkby, 1987). Tiffen (1967) reported that zinc is slightly cathodic in tomato exudates and concluded that it is not translocated as citrate, as zinc citrate complexes are anodic. The translocation of zinc in plants is not great (Mengel & Kirkby, 1987) and when the zinc supply is high, zinc accumulates in root tissue. In older leaves zinc becomes very immobile (Rinnie & Langston, 1960). The rate of transport to younger tissues is particularly inhibited in zinc deficient plants (Loneragan, 1975).

Very little is known about the mechanism(s) by which zinc is translocated from the vegetative parts of plants to their seed during the reproductive phase (Martens & Westermann, 1991). However, either soil or foliar applications of zinc increased zinc levels in grain of maize and wheat (Yilmaz *et al.*, 1997; Cakmak, 2008; Wang *et al.*, 2012; Velu *et al.*, 2014). This aspect will be dealt with later on in more detail.

The micronutrient zinc has an important function in the enzyme systems of plants. Zinc as Zn^{2+} resembles Mn^{2+} and Mg^{2+} in some enzyme systems, in that it brings the binding and conformation between enzyme and substrate (Mengel & Kirkby, 1987). Until recently the only authenticated enzyme specifically activated by Zn^{2+} was carbonic anhydrase. This enzyme promotes hydrolysis and hydration reactions involving carbonyl groups (Sandmann & Goger, 1983).

Other important enzymes containing zinc include alcohol dehydrogenase, superoxide dismutase and RNA polymerase (Vallee & Wacker, 1970). Zinc is closely involved in the nitrogen metabolism of plants. During zinc deficiency in plants, protein synthesis and protein levels are drastically reduced and amino acids and amides are accumulated (Mengel & Kirkby, 1987).

Plants suffering from zinc deficiency often show chlorosis in the interveinal areas of the leaf. These areas are pale green, yellow or white. In maize, chlorotic bands form on either side of the midrib of the leaf (Mengel and Kirkby, 1987). Zinc deficiency in plants is closely related to the inhibition of RNA synthesis. This prevents the normal development of chloroplast grana and vacuoles are developed in them (Thomson & Weier, 1962). Zinc deficiency is also characterized by short internodes and chlorotic areas in older leaves (Mengel and Kirkby, 1987). Consequently crop yields are drastically reduced.

Many researchers reported that one of the most common causes of zinc deficiency in crops are high levels of soil phosphate (Alloway, 2004). Due to the growth enhancement from high phosphorus levels the plant uptake of zinc decreases sharply. However, extractable zinc in soil is either not at all or slightly decreased by a high phosphorus supply (Marschner, 1993). Phosphorus also has an inhibiting effect on the absorption of zinc by roots and the translocation of zinc from roots to shoots (Alloway, 2004). The reduced uptake of zinc by plant roots can be explained by four possible mechanisms: (i) infection of roots with vesicular arbuscular mycorrhizae is suppressed by a high concentration of phosphorus; (ii) cations added with phosphate salts may inhibit zinc absorption from the soluble fraction; (iii) hydrogen ions generated by phosphate salts may also inhibit zinc absorption; and (iv) phosphorus enhances the adsorption of zinc onto soil constituents (Alloway, 2004).

The reduced translocation of zinc by phosphorus in plants can be explained by several possible mechanisms which include: (i) the inhibition of translocation of zinc from roots to shoots; (ii) reduction in the amount of soluble zinc; (iii) binding of zinc by phosphorus-containing phytate; and (iv) leakage of phosphorus from membranes (Loneragan & Webb, 1993).

It is often suggested therefore that phosphate affects the physiological availability of zinc in plant tissue (Mengel & Kirkby, 1987). Nitrogen affects the zinc status of crops by promoting plant growth and by changing the pH of the root environment (Alloway, 2004). In most soils, nitrogen is the most limiting factor influencing plant growth. Crops often respond to zinc and nitrogen application together but not zinc alone. Different nitrogen fertilizers differ also in their influence on soil pH and therefore influence zinc availability (Alloway, 2004).

Several macronutrients such as calcium, magnesium, potassium and sodium are known to inhibit zinc absorption in solution culture experiments but in soil these nutrients main effect seems to be through their influence on soil pH (Alloway, 2004). With low levels of calcium it was found that potassium and magnesium inhibit the absorption of zinc but the effect disappeared with the increase of the calcium concentration (Alloway, 2004).

The main interaction between zinc and other micronutrients is those with copper, iron, manganese and boron (Alloway, 2004). An interaction between zinc and copper occur through the competitive inhibition of absorption. This is due to the sharing of the same site for root absorption. Copper also affects the redistribution of zinc in plants (Alloway, 2004). Under conditions of iron deficiency, increased zinc uptake by plants and hence the zinc concentration in shoots can be considerably increased. Zinc deficiency also shows an increasing iron concentration in the shoots of plants (Alloway, 2004). It has been found that high levels of manganese in combination with high iron may inhibit the absorption of zinc by rice in flooded soils and enhance zinc deficiency. Due to the impaired membrane function in the roots of zinc deficient plants, these plants absorb high concentrations of boron (Alloway, 2004).

The occurrence of zinc deficiency symptoms may also relate to climate conditions. In areas with cool and wet spring seasons zinc deficiency may occur (Lucas & Knezek 1972). This can be explained to some extent on the restricted root development in cool soils and the inhibition of microbiological activity that directly influence the release of zinc from organic material.

Zinc toxicity is rarely encountered in practice. The main problems occur near deposits of zinc ore, ore mines and lead or zinc smelting plants, where the stack gasses contain considerable amounts of zinc (Bergmann, 1992). Rainwater collected and stored in galvanized roofs and gutters can also raise the zinc content of the soil if used for watering. Heavy applications of slurry from piggeries, repeated application of sewage sludge and composted domestic wastes to improve soil structure can also raise the zinc levels in the soil over years. Abnormally high zinc levels have also been reported in soils near roads. Soils with high levels of zinc can be cured by raising the pH. Liming is therefore the most economical approach to avoid toxicity in plants.

In soil, zinc levels above 10 mg kg^{-1} extracted by the DTPA method is considered potentially harmful in acid soil. Total zinc concentration in soil usually falls in the range of 10 - 300 mg kg^{-1} , with concentrations above 150 mg kg^{-1} regarded as high and this may cause reduced plant growth (Landon, 1991). Levels of 150 to 200 mg kg^{-1} in dry matter of plant tissue are considered as toxic as stated by Sauerbeck (1982).

Takkar and Mann (1977) found that maize show zinc toxicity at a 60 day growing stage with a zinc level exceeding 81 mg kg^{-1} dry weight. According to Bergmann (1992), maize yield losses remain insignificant until the zinc level rises to 550 mg kg^{-1} dry weight. Maize can tolerate zinc levels of 238 mg kg^{-1} dry weight without loss of yield.

Zinc toxicity results in a reduction in root growth and leaf expansion which is followed by chlorosis (Mengel & Kirkby, 1987).

2.4 Zinc nutrition of crops, animals and humans

One of the most common micronutrient deficiencies is that of zinc and therefore zinc is becoming an increasingly significant factor in crop production (Mengel & Kirkby, 1987). The susceptibility of a crop to zinc deficiency varies from crop to crop and also between cultivars. Sensitive crops include maize, hops, flax and beans, while crops that are moderately sensitive are potatoes, tomatoes and lucerne. Crops that are insensitive include oats, barley, wheat and rye (Viets *et al.*, 1954).

In most soils the total zinc content of the soil exceeds the requirement of crops, but availability is the important limiting factor (Mengel & Kirkby, 1987). The mobility of the zinc in relation to its availability is also an important factor influencing the uptake of this micronutrient from soil. Research done by Elangwhary and coworkers in 1970 reported that 95% of zinc moves by diffusion. Therefore a diffusion gradient occurs at root depletion

zones, similar to phosphate (Barber et al., 1963). Factors in soil such as compaction therefore reduce the availability of zinc (Mengel & Kirkby, 1987).

The availability of zinc for crop nutrition can be affected by many factors as described earlier. However, due to the availability of new high yield cultivars the problem of zinc deficiencies in especially sensitive crops like maize may further be aggravated. Improved maize cultivars progressively deplete the available soil zinc pools. This depletion of available zinc pools by large off take in agriculture produce may occur to a greater extent in soil with adverse chemical properties like high levels of CaCO_3 and low levels of organic matter and soil water. In such soils, especially when sandy in texture the supply of zinc to roots would be lower than the roots capacity to take up zinc (Cakmak, 2008).

The use of zinc fertilizers to increase the zinc contents in plants with the aim to increase zinc in grain is of great importance. A proper fertilizer strategy could be a rapid solution to the problem and can be considered an important complementary approach to the on-going breeding programs (Cakmak, 2008). Research done on the increasing of zinc in grain is very rare, although a large number of studies were done on the role of soil and foliar applied zinc fertilizers in the correction of zinc deficiency and increasing plant growth and yield (Martens & Westermann, 1991).

Research done on wheat with zinc fertilizers showed an improvement not only in productivity, but also grain zinc concentration (Yilmaz et al., 1997). The most effective method to increase the zinc concentration in grain was with soil and foliar applications. Zinc fertilizers increased the zinc concentration in grain 3.5-fold in comparison to where no zinc was applied. Research done on maize also shows zinc in grain increase between 4% and 16% with soil applied zinc (Wang et al., 2012). Again soil and foliar zinc applications promote zinc accumulation much more than soil applied alone. Knowledge of the different forms of zinc fertilizer and timing of foliar application is crucial for enhancing grain zinc (Velu et al., 2014). Compared to the other forms of zinc fertilizer, the application of zinc sulphate was the most effective way to increase grain zinc (Velu et al., 2014).

Increasing seed concentration of zinc by soil and/or foliar application of zinc also promotes several agronomic benefits for crop production. By applying zinc fertilizer to plants in potentially zinc deficient soils, uptake and accumulation of phosphorus can be reduced, which forms phytate in grain. This effect of zinc fertilization may result in better bio-availability of zinc in the human digestive system (Cakmak, 2008).

As mentioned before zinc in animals is critical for zinc-metallo enzymes and also for zinc fingers in DNA. Important processes which involve zinc include; protein synthesis, cell division, reproduction, immune functions and growth (Ho, 2004; Walker & Black, 2004; Bell & Dell, 2008).

The total amount of zinc in grain is not always a good indicator of its bioavailability in the human digestive system. Besides high levels of zinc in grain, the bioavailability of zinc is an important nutritional aspect. One of the factors decreasing the zinc bioavailability is phytate (Egli *et al.*, 2004).

Many data in literature refers to the uptake and removal of zinc by crops in the trend of 60 to 300 g Zn ha⁻¹. These values differ between crops and the cultivation practices followed (Alloway, 2004). For example, research done with maize in Queensland, Australia showed zinc removal by grain amounts 150 g ha⁻¹ under irrigation compared to 70 g ha⁻¹ under dryland (Department of Primary Industries & Fisheries, 2007). A maize crop yielding 9.5 t ha⁻¹ of grain, grown in North America, could be expected to remove 380 g Zn ha⁻¹ in the grain and stover (IFA, 1992).

Optimum zinc levels can be maintained in soil with fertilization when the amount of zinc removed by a crop is known. Soil application of zinc is typically in the range of 4.5 - 34 kg Zn ha⁻¹. Higher applications than the typical range are often used for crops which are particularly sensitive to zinc deficiency, such as maize. (Martens & Westermann, 1991). Zinc containing fertilizers can be broadcast or band placed. Band application of zinc is more effective for maize and therefore reduces the amount of fertilizer required. For example, a zinc containing fertilizer band placed at 0.34 - 1.34 kg Zn ha⁻¹ gave a grain yield equal to when the same fertilizer was broadcasted at 26.9 kg Zn ha⁻¹ (Martens *et al.*, 1973).

A foliar application can be used on maize but often requires several applications. In most cases this is only used in emergencies to prevent major yield losses and zinc can be applied at a rate of 11 kg Zn ha⁻¹ (International Lead Zinc Research Organisation, 1975).

2.5 Zinc fertilizers and their properties

When a soil is fertilized with zinc the purpose is to increase the water soluble zinc fraction in the soil. This will improve the amount of zinc available to plants. However, there are numerous zinc fertilizers on the market and in many cases they are accompanied by unsubstantiated claims as to the level of zinc available for plant uptake (Gangloff *et al.*, 2000). Recent studies report that highly water soluble zinc fertilizers are the most effective to correct zinc deficiencies in soils and hence crops (Amrani *et al.*, 1999). Thus it is widely

accepted that a zinc fertilizer of which 40 - 50% of the total zinc is water soluble will meet the zinc requirements of crops. There is a high correlation between the water solubility of a zinc fertilizer, zinc uptake and plant growth (Amrani *et al.*, 1999). Zinc concentrations in plant tissue have also been reported to decrease as the water solubility of zinc fertilizer declines (Slaton *et al.*, 2005). For example zinc oxide dissolves poorly in water (Figure 2.2).



Figure 2.2 A picture showing the low solubility of ZnO in water.

Zinc deficiency can severely impair crop growth and decrease yield, but it can also be easily and economically corrected by applying zinc fertilizers (Westfall & Gilkes, 1999). The most important factor is which fertilizer source will result in the best increase of plant available zinc in soil to increase yield.

Five groups of different zinc sources are commercially used as fertilizers today. These vary in their zinc content, price and effectiveness in crops (Alloway, 2004).

1. Inorganic sources include zinc oxide, zinc carbonate, zinc sulphate, zinc nitrate and zinc chloride. Of these products zinc sulphate is most commonly used as fertilizer around the world and is available in crystalline and granular form. The granular form has a lower solubility and is not as effective immediately after application (Mortvedt & Gilkes, 1993).
2. Synthetic chelates are generally formed by combining a chelate, such as EDTA with metal ions. The stability of the bonding between the chelate and the metal determines the availability of the metal to the plants. ZnEDTA is the most widely used chelated source of zinc. Other chelates, for example zinc citrate, are also used. It is less expensive than ZnEDTA but it is also less stable. Chelates such as ZnEDTA are regarded as being the most effective sources of plant micronutrients. It is considered that ZnEDTA is 2 to 5 times more effective than zinc sulphate. Chelates can be applied to the soil or used as a foliar spray (Mortvedt & Gilkes, 1993).

3. Natural organic complexes are usually manufactured by reacting zinc salts with organic byproducts from paper pulp manufactures. Organic compounds include lignosulphonates, phenols and polyflavoniods (Mortvedt & Gilkes, 1993).
4. Zinc can also be fertilized as ammoniated zinc sulphate. This is an inorganic complex that is a source of nitrogen, zinc and sulphur. It is often combined with ammonium polyphosphate as starter fertilizers (Mortvedt & Gilkes, 1993).
5. Organic sources comprise inter alia of animal wastes contain small quantities of plant available zinc. The concentration of zinc in these sources range from 0.01 to 0.05 %. With large manure applications or repeated applications sufficient amounts of zinc can be provided. Municipal waste varies greatly in zinc concentration depending on the source, with an average of 0.05% (Havlin *et al.*, 1999).

The high water solubility of zinc sulphate ($\text{ZnSO}_4 \cdot 2\text{H}_2\text{O}$), has proven to be a reliable and popular source of zinc fertilizer. However as mentioned above there are many other forms of zinc fertilizer commercially available (Shaver *et al.*, 2007). Many fertilizer mixtures on the market in South Africa contain sufficient amounts of zinc, but it is unknown whether the zinc in these mixtures is available for plant uptake. This is because the form in which zinc does occur in these mixtures is not known. Zinc fertilizers that are mainly used in South Africa's agricultural sector are zinc sulphate, zinc oxide and zinc EDTA.

2.6 Evaluation of soils' zinc fertility status

Several techniques are commonly employed to assess the zinc status of soils: (i) nutrient deficiency symptoms of plants; (ii) tissue analysis from plants growing in soil; (iii) biological test where plants' growth is used as a measure of soil fertility; and (iv) soil analysis (Havlin *et al.*, 1999). However, soil and plant analyses are due to their quantitative nature the techniques favoured by scientists, advisors and farmers to ensure optimum productivity of cropping systems. In this section plant analysis of zinc will be addressed concisely and soil analysis of zinc in much more detail.

2.6.1 Plant analysis

At the beginning of the 1800's plant analysis was used by the French botanist Th. De Saussure to determine the nutrient requirements of plants. Repeated attempts have been made since then to use plant analysis for the determination of the nutrient status with the

ultimate aim to estimate the amounts of fertilizers needed for optimum growth (Bergmann, 1992).

However, the use of only plant analysis is not an alternative to soil testing, but is a necessary supplementary or complementary method to soil testing. With the use of plant analysis, the effects of zinc nutrition can be established on the concentration of zinc and other nutrients in plants. This information leads to a better understanding of the uptake of zinc from the soil by the plant and hence the translocation in the plant. Therefore it is critical in modern agricultural that plant analysis and soil testing go hand in hand to ensure better understanding about crop nutrition and soil fertility.

The knowledge of the nutrient levels in the soil is less important than knowing the quantities of nutrients actually assimilated by the plants, and their concentration in the actively growing tissue are of decisive importance for growth and development of plants (Bergmann, 1992).

2.6.2 Soil analysis

All soils contain measurable concentrations of micronutrients, but these concentrations may vary widely and many factors influence these levels. The availability of micronutrients such as zinc for uptake by plants or movement in soil depends on a range of soil properties (Alloway, 2004).

The concentration of total zinc in soil, according to Alloway (2004) shows an average of 55 mg kg⁻¹. However, Kiekens (1995) reported a typical range of total zinc in soil of 10 - 300 mg kg⁻¹ with a mean of 50 mg kg⁻¹. According to researchers all over the world there is a clear trend of lower zinc concentrations in sandy soils and higher zinc concentrations in soils with higher clay content (Alloway, 2004). Sandy soils therefore are more prone for zinc deficiency under crop production.

In studies many chemical extraction procedures have been proposed to estimate the plant availability of zinc in soil. After all the research there is still no agreement as to which extractant most accurately estimates the labile, or the bioavailable zinc (Leleyter *et al.*, 2012). It is therefore very important to understand the principles on which the different extraction methods are based to successfully determine the plant available zinc in soils.

At first single element tests were developed and used to estimate the plant availability of zinc in soil. An example is the 0.1 M HCl extractant test for zinc. Later, a test for the simultaneous extraction of micronutrients was initiated with the development of the DTPA-TEA (diethylenetriaminepentaacetic acid-triethanolamine) extractant to estimate the plant availability of Cu, Fe, Mn and Zn in soil (Lindsay & Norvell, 1978).

Today scientists use soil tests that are developed for simultaneous extraction of micronutrients and macronutrients in a single method. Examples are the Mehlich III and DTPA-AB (diethylenetriaminepentaacetic acid- NH_4HCO_3) procedures (Mehlich, 1984). The use of simultaneous extraction for the determination of micronutrients and macronutrients is desirable for rapid conveyance of soil test data to crop producers at a reasonable cost. Extractants used currently for zinc analysis are chelating agents, inorganic acids and a combination of chelating agent, acid and salts. Amounts of extractable zinc solubilized from the soil by these extractants depend on the concentration of extraction solution ratio, extraction time, extraction temperature, type of vessel and shaker, and shaker speed (Sorensen *et al.*, 1971).

An ideal soil test procedure should be rapid, reproducible and correlate reliably with responses in plant yield, plant zinc concentration or zinc uptake. The extractant should be selected to solubilize amounts of zinc that are proportional to the amount that will be absorbed by plants during a single growing season, and also should be effective over a wide range of soil types (Reed & Martens, 1996). It is also important that the amount of zinc that is extracted by the chemical reagent can be checked against critical values. These values are derived from the responses of a specified crop to zinc in field experiments on relevant soil types. Field calibration is necessary to determine critical levels of extractable zinc that separate soils into deficient and sufficient categories (Reed & Martens, 1996).

Depending on the method of action, reactants fall into different categories. Those which employ salts as CaCl_2 or $\text{Ca}(\text{NO}_3)_2$ in order to leach cations adsorbed onto solid materials, due to the negative charge on soil particles. Secondly, techniques using an acid solution in order to simulate the effect of an acid input are used because low pH favours the dissociation of the existing complexes. The third category uses complexing or reducing agents such as EDTA (Alvaraz *et al.*, 2006).

It is noteworthy that most of the extraction methods proposed in literature employ acid solutions or chelating agents. Neutral salt solutions extract little or no zinc from the soil, and correlate poorly with crop response (Stanton, 1964).

Different extraction conditions lead to a variety of different amounts of zinc solubilized by a specific soil test. Calibration of a specific soil test with crop response is therefore very important. Calibration data for a soil test apply solely to soil test values obtained with the extraction conditions used during the calibration (Reed & Martens, 1996).

Above mentioned aspects are illustrated in Table 2.1 where lower critical zinc concentrations for different scenarios are given. Based on these values soils can be divided in those that have sufficient and insufficient levels of zinc for crop production. It is noteworthy that most of the values are either 2 mg Zn kg⁻¹ or lower. Soils with a zinc concentration lower than 2 mg kg⁻¹ can therefore be regarded as soil on which selected crops may experience zinc deficiencies.

Table 2.1 Lower critical zinc concentrations in soil as established with various extractants for different crops (Alloway, 2004)

Soil extractant	Lower critical concentration (mg Zn kg ⁻¹) and crop
DTPA	0.1 - 1.0 (All crops)
Mehlich I	1.1 (Average all crops)
Mehlich I	0.5 - 3.0 (Rice)
0.05 M HCl	1.0 (Rice)
0.1 M HCl	1.0 - 5.0 (All crops)
0.1 M HCl	1 - 7.5 (Several crops)
0.1 M HCl	2.0 (Rice)
DTPA-AB	0.9 (Sensitive crops)
DTPA (0.005 M, pH 7.5)	0.13 (Subterranean clover - sandy soil)
DTPA	0.55 (Subterranean clover - clay soil)
DTPA	0.48 (Chickpea)
DTPA	0.60 (Maize)
DTPA	0.65 (Pearl millet)
DTPA	0.65 (Wheat, rice)
DTPA	0.76 - 1.24 (Rice)
DTPA	0.5 (Rice)
EDTA	1.5 (Rice)

Soil analysis can be carried out at any time and has therefore an advantage over plant analysis. Soils differ widely, and it is thus necessary to take an adequate number of subsamples over the area being investigated. Soil samples must be placed in a clean plastic bag to prevent contamination. In all stages of soil analysis, it is essential that any contamination of the soil samples or solutions with zinc is avoided. A small amount of contamination can be enough to give an extractable zinc value that is sufficient for plant needs, when in fact the soil is deficient in the micronutrient (Alloway, 2004).

Firstly, it is important that zinc contamination during the extraction of zinc from soil samples should be avoided to ensure accurate extractable zinc data. Materials that may cause zinc contamination include galvanized containers, cast iron mortars, rubber stoppers, brass screens and other metal utensils should be avoided during preparation of soil samples for extractable zinc analyses (Eik & Gelderman, 1988). Extraction solutions should be prepared in acid-washed glassware or plastic containers to prevent zinc contamination. Reagents and water used during extraction may be a source of zinc contamination and therefore highly pure deionized water should be used to prevent contamination. Leggett and Argyle (1983) reported that drying temperature affects the amounts of zinc extracted from soils. Hammes and Berger (1960) explained that zinc is released during drying due to reduction of manganese in hydrous oxides and to alteration of functional groups in organic matter. Grinding of the soil sample leads to a smaller particle size, increases the surface area and therefore increases amounts of extractable zinc (Severson *et al.*, 1979). Variations in drying and grinding conditions lead to large differences in the amount of zinc solubilized by a specific soil test. Calibration data for a soil test is therefore very important.

2.7 Extraction and determination of plant available zinc

The most commonly extraction methods employed for the determination of plant available zinc in soil will be addressed firstly in some detail. Then the determination of zinc in the extraction solutions will be discussed concisely.

2.7.1 Extraction methods

2.7.1.1 Dilute hydrochloric acid method

The dilute HCl method was developed by Nelson *et al.* in 1959. Hydrochloric acid with a concentration of 0.1 M is used in this extraction. This method has been used for much longer than other tests for determining the plant available zinc in soil. Use of the 0.1 M HCl method to determine the need for zinc fertilization of maize on slightly acid, sandy textured Alabama soils was first reported by Wear and Sommer (1948). In the north central region of the USA

this extraction method is still widely used to evaluate the plant available zinc status of neutral and acid soils (Whitney, 1988). Because the 0.1 M HCl dissolves CaCO_3 that coincide with release of occluded zinc in calcareous soil provides this method not a satisfactory estimate of plant available zinc in these soils. This is because the extracted zinc is usually not fully available for plant uptake (Trierweiler & Lindsay, 1970). Also CaCO_3 in soil samples lead to the neutralization of the 0.1 M HCl extractant solution (Nelson *et al.*, 1959). Since this method was first used it has undergone a variety of modifications. Changes in soil mass-to-extraction solution ratio, shaking vessel time, and the type of shaking have been made to improve the extraction (Wear & Sommer, 1948). Research has shown that, for this extraction method, a 0.1 M HCl extractable zinc level of less than 2.0 mg kg^{-1} indicates that zinc application is needed for optimum production of maize and sorghum. Cox and Wear (1977) also stated the importance of the shaking time, when the solution was shaken for 15 rather than 30 minutes the critical level for the soil test for maize production was only $0.5 \text{ mg Zn kg}^{-1}$.

2.7.1.2 Mehlich I method

Nelson *et al.* (1959) initially developed this method to estimate levels of plant available phosphorus in soils. However, by 1974 this extractant was used in many soil testing laboratories to measure Ca, Mg and K (Sabbe & Breland, 1974). The Mehlich I method was first calibrated by Cox in 1968 to determine the level of plant available micronutrients in soil. Research done by Wear and Evans in 1968 led to the use of the Mehlich I method as an extractant for the determination of plant available zinc (Reed & Martens, 1996). Very important is that Wear and Evans (1968) showed that Mehlich I extractable zinc correlated more closely with zinc uptake by maize plants than did either 0.1 M HCl or 0.05 M EDTA extractable zinc. Perkins (1970) also reported good correlation between zinc in leaf blades of maize plants and Mehlich I extractable zinc.

This procedure has been referred to as the double acid, dilute double acid and dilute HCl- H_2SO_4 method since the extraction solution consists of 0.05 M HCl and 0.125 M H_2SO_4 . By shaking the soil with an acid solution such as the Mehlich I extractant, structural zinc which is not in contact with the soil solution zinc may be extracted (Martens & Lindsay, 1990). The extraction of non-labile zinc can be decreased by using a short extraction period of 5 minutes (Cox, 1968).

Perkins (1970) reported that Mehlich I extractable zinc in soil range from negligible to 7.6 mg kg^{-1} . Research done in south-eastern USA with the Mehlich I method showed a critical zinc

level $0.8 \text{ mg Zn kg}^{-1}$ for maize production. This value separate soil with a CEC of less than $7.5 \text{ cmol}_c \text{ kg}^{-1}$ into zinc-sufficient and zinc-deficient categories (Cox & Wear, 1977).

2.7.1.3 DTPA-TEA method

The DTPA-TEA (Diethylenetriaminepentaacetic acid-triethanolamine) method was developed by Lindsay and Norvell (1978) to identify near-neutral and calcareous soils with insufficient levels of the plant available micronutrients copper, iron, manganese and zinc. Previously the method was referred to as the DTPA method. Today it is called the DTPA-TEA method to prevent confusion with the DTPA-AB method, which was developed by Soltanpour and Schwab in 1977. DTPA is the chelating agent used in this method and was selected because it has the most favourable combination of stability constants for simultaneous complexation of Cu, Fe, Mn and Zn (Lindsay & Norvell, 1978).

Since zinc deficiencies are common on calcareous soils, the extractant was designed to avoid excessive dissolution of CaCO_3 with the release of occluded zinc. This precaution is very important because the occluded zinc in CaCO_3 is normally not available for absorption by plant roots. Excessive dissolution of CaCO_3 is prevented by inclusion of Ca^{2+} as CaCl_2 in the extraction solution and by buffering the solution at pH 7.3 with TEA $[(\text{HOCH}_2\text{CH}_2)_3\text{N}]$.

Extractable zinc, using the DTPA-TEA method, was measured in 77 agricultural surface soils in Colorado and ranged from 0.17 to 11.5 mg kg^{-1} (Lindsay & Norvell, 1978). A level of less than $0.8 \text{ mg Zn kg}^{-1}$ in near-neutral and calcareous soils indicated inadequate zinc for maize production. The DTPA-TEA method could also be used to evaluate the plant available zinc status of acidic soils when soil pH along with the level of DTPA-TEA extractable zinc is considered (Lindsay & Norvell, 1978).

2.7.1.4 EDTA method

The EDTA (Ethylenediaminetetraacetic acid) extraction method is one of the most widely used because of its high extraction capacity (Sahuquillo *et al.*, 2003). EDTA is assumed to extract metals on exchange sites of both inorganic and organic complexes. The leaching of EDTA seems less questioned than HCl leaching. Most scientists use an EDTA concentration of 0.05 mol L^{-1} , even concentrations of 0.02 mol L^{-1} have been reported (Gismera *et al.*, 2004). This reactant for extraction can be used for the determination of Zn, Mn, Cu and Co.

Sequential extraction procedures give information about the mineralogy and also enables the differentiation of mobile and residual fractions, with the advantage of characterizing the different labile fractions (Leleyter & Baraud, 2006). In sequential extraction methods

generally three extractants are used. The earlier ones are the least aggressive and more specific, subsequent extractants are progressively more destructive. These sequential extraction procedures are a useful tool for solid speciation of particle elements, to study the origin, fate, biological and physicochemical availability and transport of absorbed elements (Leleyter *et al.*, 2012). Different compounds for EDTA can be used, however di-ammonium EDTA is generally used.

2.7.1.5 Ambic II method

This method was developed by Van der Merwe *et al.* (1984) when they modified the ISFEI extraction method of Hunter (1974). Prior to this, Farina (1981) reported on the simplicity and effectiveness of the Hunter system as well as assessing the possibility of adopting and implementing the system in South African laboratories. Although the Ambic II method is widely used in South African laboratories today, literature on the subject is lacking, especially for the determination of zinc in soil.

Initially, the method was developed to determine phosphorus in a wide range of South African soils. However, the extractant was found to be suitable for the determination of K, Ca, Mg, Cu, Zn, Fe and Mn (Van der Merwe *et al.*, 1984). The main difference between the Ambic I and Ambic II methods, is that extraction in the case of Ambic II is: (i) based on a volume of soil; (ii) no acid is used to clarify coloured solutions; (iii) di-sodium EDTA is used instead of di-ammonium EDTA; and (iv) the sample is stirred instead of using reciprocal shaking.

2.7.2 Determination methods

2.7.2.1 Atomic absorption spectroscopy

Once zinc is in solution the concentration thereof can be determined with absorption spectroscopy. This method uses the absorption of light to measure the concentration of gas-phase atoms. Samples are usually liquids therefore the analyte atoms or ions must be vaporized in a flame or graphite furnace. When ultraviolet or visible light is shone on the atoms, it absorbs light and makes transitions to higher electronic energy levels. From the amount of absorption the analyte concentration can be determined. Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentrations (Brian, 2000).

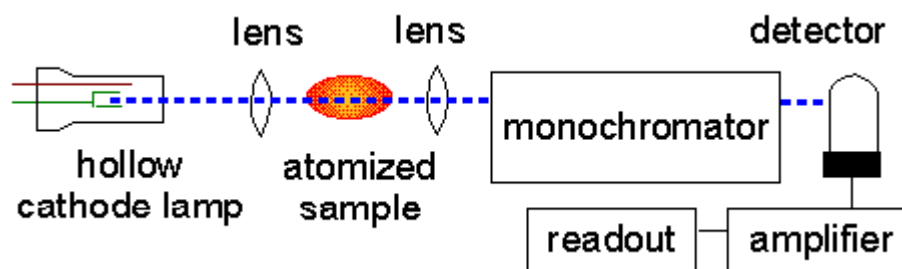


Figure 2.3 Schematic illustration of an atomic absorption spectroscopy setup (Brian, 2000).

The basic setup of an atomic absorption spectroscopy and the important components are shown in Figure 2.3. First light must be shone by the hollow cathode lamp through the atomized sample. The monochromator then transmits a mechanically selectable narrow band of wavelengths of light. The detector then recovers information of interest contained in a modulated wave, next the amplifier increases the power of the signal to create a readout.

For the light source a hollow-cathode lamp of the relevant element is used. Lasers are also used in research instruments. Analyte atoms must be in the gas phase and therefore ions or atoms must undergo desolvation and vaporization in a high-temperature source like a flame or graphite furnace. The flame atomic absorption meter is widely used but can only analyze solutions. The graphite furnace atomic absorption meter can measure solutions, slurries or solid samples (Brian, 2000).

2.7.2.2 Inductively coupled plasma emission spectroscopy

Inductively coupled plasma atomic spectroscopy (ICP-AES), also referred to as inductively coupled plasma optical emission spectrometry (ICP-OES), is an analytical technique used for the detection of trace metals and some non-metals in solution.

Samples are nebulized into plasma where the temperature is sufficiently high to break chemical bonds, liberate elements present and transform them into a gaseous atomic state. A number of atoms pass into the excited state and emit radiation. Radiation and the frequency of the element are characterized and when known they are used for identification purposes. The intensity of the radiation is proportional to the concentration of that element within the solution and can be used for quantitative purposes (Stafansson *et al.*, 2007).

2.8 Conclusion

The importance of zinc for crops, animals and humans nutrition has been stated by many researchers in the past. Zinc has a very important role to play in South African agriculture, especially for maize production on sandy soils. This is because maize is very sensitive for zinc deficiency and low plant available zinc levels are also characteristic of sandy soils. Zinc fertilizers therefore are essential in supplying zinc to maize plants. A good knowledge about the cycling of zinc in the soil-plant system is therefore critical. When focusing on soil and its interaction with zinc, it shows that soil and its characteristics influence plant available zinc content greatly. Therefore, it is important to use relevant methods of analysing zinc in the system. Soil and plant analysis have an unmistakeable role to play in measuring zinc. As stated above it is clear that there is a difference between extraction methods and its ability to determine plant available zinc in soil.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study soils and zinc sources

A survey was conducted on uncultivated land near Wesselsbron in the Free State Province for the selection of suitable soil for this study. The major criteria were that the soil must be from a physical and chemical condition representative of the sandy ones used for maize production but its level of plant available zinc should be low.

A representative soil sample from each of five locations were collected for analysis. This was done by sampling 10 randomly selected spots with an Edelman auger at a depth of 0 - 300 mm from a 100 m² area. The 10 subsamples of a locality were thoroughly mixed before a composite sample was taken, air-dried, sieved and analysed.

The five composite samples were analysed at the laboratory of the ARC-Small Grain Institute near Bethlehem. Standard methods (The Non-affiliated Soil Analysis Working Committee, 1991) were used for the determination of particle size distribution (Hydrometer), organic C (Walkley-Black), pH (1:2.5 KCl suspension), extractable P (Bray 1), exchangeable Ca, Mg, K and Na, as well as CEC (1 mol dm⁻³ NH₄OAc at pH 7), extractable Zn (0.1 mol dm⁻³ HCl) and exchangeable acidity (1 mol dm⁻³ KCl).

Analysis showed that two of the five soils met the criteria and were therefore selected. The two soils are sandy with relatively low levels of plant available zinc (Table 3.1). These soils mainly differ with respect to soil acidity as indicated by pH 4.5 for soil A vs 4.8 for soil B and exchangeable acidity 0.3 cmol_ckg⁻¹ for soil A vs 0 cmol_ckg⁻¹ for soil B.

Table 3.1 Some physical and chemical properties of the two soils selected for the study

Soil	A	B
Particle size: (%)		
Sand	95	95
Silt	4	3
Clay	1	2
Organic C (%)	0.1	0.1
pH (KCl)	4.5	4.8
Extractable P (mg kg^{-1})	9.3	9.1
Extractable cations: (mg kg^{-1})		
Ca	165	213
Mg	54	53
K	121	127
Na	0.6	0.4
CEC ($\text{cmol}_c \text{ kg}^{-1}$)	1.9	1.8
Extractable Zn (mg kg^{-1})	0.8	0.2
Exchangeable acidity ($\text{cmol}_c \text{ kg}^{-1}$)	0.3	0

The zinc sources selected for this study were zinc sulphate (Table 3.2), zinc oxide (Table 3.3) and ethylenediaminetetraacetic acid zinc-disodium complex (Table 3.4), hereafter refer to as ZnSO_4 , ZnO and ZnEDTA respectively. These three sources are the most widely used in agriculture to increase plant available zinc levels in soil (Section 2.5). Based on their molecular mass, the concentration of zinc in ZnSO_4 , ZnO and ZnEDTA are 22.7%, 80.3% and 14.0%, respectively.

Table 3.2 Certificate of analysis of zinc sulphate (ZnSO_4)

Formula: $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ **Mol. Wt.:** 287.54

Assay	99.5%
Chloride (Cl)	0.0004%
Substances not precipitated by $(\text{NH}_4)_2\text{S}$	0.2%
Lead (Pb)	0.001%
Iron (Fe)	0.0005%
Manganese (Mn)	0.0003%
Ammonium (NH_4)	0.001%
Arsenic (As)	0.0002%

Table 3.3 Certificate of analysis of zinc oxide (ZnO)**Formula:** ZnO**Mol. Wt.:** 81.38

Assay	99.5%
Chloride	0.002%
Sulphate (SO ₄)	0.01%
Iron (Fe)	0.0005%
Lead (Pb)	0.01%
Oxidisable matter (O)	0.0025%
Arsenic (As)	0.0004%
Loss on ignition	1.0%

Table 3.4 Certificate of analysis of ethylenediaminetetraacetic acid zinc-disodium complex (ZnEDTA)**Fomula:** C₁₀H₁₂N₂Na₂O₈Zn**Mol Wt.:** 399.59

Chemical name	Ethylenediaminetetraacetic acid zinc-disodium complex
Appearance	White powder
Chelated zinc	14.00%
pH value	6.0 ± 0.7
Dissolubility	About 1000 g L ⁻¹ (20 °C)
Bulk density	625 Kg m ⁻³

3.2 Incubation experiment

3.2.1 Experimental layout and treatments

A representative and sufficient amount of soil A and of soil B was collected, air-dried and sieved through a 5 mm sieve to remove all unwanted plant material. An electronic scale was then used to weigh 400 g samples of the two different soils into 500 ml plastic containers. For each soil 75 containers were filled (Figure 3.1a). The soil within the plastic containers were treated with ZnSO₄, ZnO and ZnEDTA to increase their zinc content with 0 mg kg⁻¹, 1 mg kg⁻¹, 2 mg kg⁻¹, 3 mg kg⁻¹ and 4 mg kg⁻¹ (hereafter refer to application rate 1 to 5), respectively. This implies five replications per application rate (AR) or treatment for each soil.

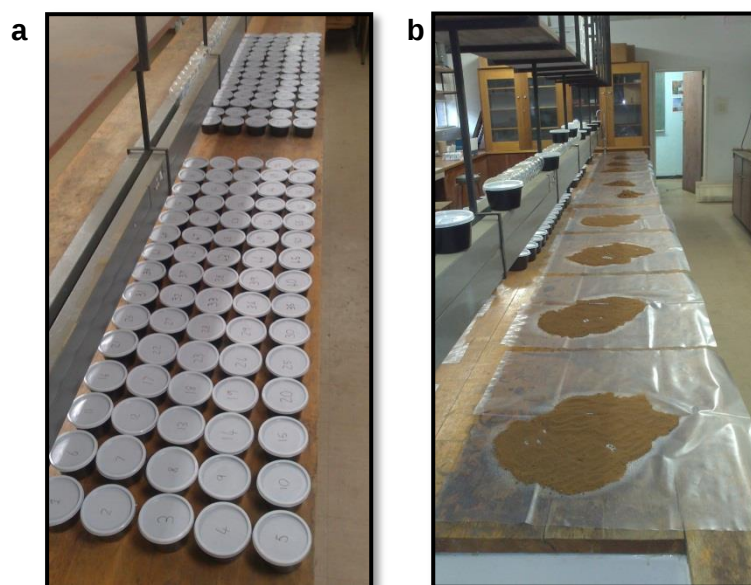


Figure 3.1 Plastic containers filled with 400 g soil (a) and plastic sheets with soil used to facilitate the application of the zinc solutions and mixing of soil (b).

Sufficient volumes of ZnSO_4 , ZnO and ZnEDTA solutions containing appropriate amounts of zinc for the different treatments were prepared. The soil within a plastic container was poured out on an uncontaminated plastic sheet (Figure 3.1b). Then 60 ml of the relevant solution was sprayed on the soil using a sprayer connected to a burette to ensure precision application. After application the soil was mixed properly and then returned to the plastic containers. Containers were closed to keep the soil at 80% of field capacity. After two weeks the containers were opened for the soil therein to be dried at room temperature. After drying, the soil was again thrown out on uncontaminated plastic sheets and sprayed with distilled water to 80% of field capacity. Thereafter the soil was mixed again and returned to their original container. The soil went through three wetting and drying cycles, including the zinc application.

Following the wetting and drying cycles, the soil was air-dried, sieved through a 2 mm sieve and kept in the corresponding container. The soil within each of the 150 containers were then analysed for plant available zinc with different extraction methods as described in the next section.

3.2.2 Extraction reagents and procedures

Only a concise description of each method's extraction reagents and procedures are given. The principles of these methods were addressed earlier in Section 2.7. More detailed discussion of these methods can be found also in The Non-affiliated Soil Analysis Working Committee (1991) and Reed and Martens, (1996).

3.2.2.1 Dilute hydrochloric acid method

For 1 L of 0.1 M HCl extraction solution, 8.33 cm³ of concentrated HCl (12 M) was added to 800 cm³ of deionized water, diluted to 1 L with deionized water, and mixed.

Next, 5 g of the sieved soil was placed in a 50 cm³ Erlenmeyer flask, 20 cm³ of the HCl extraction solution was added and the mixture was then shaken on a reciprocal shaker at 180 cycles per minute. After shaking for 30 minutes the suspension was filtered through a Whatman no. 42 filter paper. The amount of extractable zinc in the filtrate was then determined by atomic absorption spectrometry.

3.2.2.2 Mehlich I method

The Mehlich I extraction solution consists of 0.05 M HCl and 0.0125 M H₂SO₄. For 1 L of Mehlich I extraction solution, 4.17 cm³ of concentrated HCl (12 M) and 0.70 cm³ of concentrated H₂SO₄ (17.8 M) were added to 800 cm³ deionized water and diluted to 1 L.

Five gram of the sieved soil was then placed in an Erlenmeyer flask, 20 cm³ of the Mehlich I extraction solution was added. The mixture was shaken for 5 minutes on a reciprocal shaker at 180 cycles per minute with a stroke length of 4.5 cm. After shaking, the suspension was filtered through a Whatman no. 42 filter paper. The amount of extractable zinc in the filtrate was then determined by atomic absorption spectrometry.

3.2.2.3 DTPA-TEA method

The DTPA-TEA extraction solution consists of 0.005 M DTPA, 0.01 M CaCl₂, and 0.1 M TEA adjusted to a pH of 7.3 with HCl. For 10 L of this solution, 149.2 g of the reagent grade TEA, 19.67 g of DTPA and 14.7 g of CaCl₂•H₂O were dissolved in 200 cm³ deionized water. Sufficient time was left for DTPA to dissolve before diluted to 9 L with deionized water. The pH of the solution was then adjusted to 7.3 with 1.0 M HCl while stirring before diluted to 10 L with deionized water.

Ten gram of the sieved soil was weighed into a 125 cm³ conical flask, 20 cm³ of the DTPA-TEA extraction solution was added. The flask was then covered with stretchable Parafilm before the mixture was shaken on a horizontal shaker with a stroke length of 8 cm at a speed of 120 cycles per minute for 2 hours. After two hours of shaking, the suspensions were filtered through a Whatman no. 42 filter paper. The amount of extractable zinc in the filtrate was then determined by atomic absorption spectrometry.

3.2.2.4 NaEDTA method

To prepare the 0.02 mol dm³ NaEDTA extraction solution, 7.5 g of NaEDTA was dissolved in 1 L of deionized water. The solution was kept at a temperature of 20°C. Five gram of soil was weighed and placed in an extraction bottle. Then 15 cm³ of the extraction solution was added where after the bottle was sealed and shaken horizontally for 60 minutes at 180 oscillations per minute in a reciprocating shaker. After shaking, samples were centrifuged for 50 minutes at 2 000 rpm. The suspension was then filtrated through Whatman no 40 paper into suitable containers. The amount of extractable zinc in the filtrate was then determined by atomic absorption spectrometry.

3.2.2.5 Ambic II method

For the preparation of the Ambic II solution 197.6 g ammonium bicarbonate, 37.2 g disodium EDTA and 3.7 g of ammonium fluoride were dissolved in 5 L deionised water. Then 100 cm³ of Superfloc was added. Superfloc was prepared by slowly adding 5 g of Superfloc N-127 to 1 L lukewarm deionized water while stirring at 400 rpm. Solution was mixed well and made up to 10 L. After allowing it to stand overnight the pH was adjusted to 8.0 with concentrated ammonia solution.

For the procedure 2.5 cm³ of sieved soil was scooped into a sample cup adding 25 cm³ of the extracting solution. The sample was stirred for 10 minutes at 400 rpm before filtered through a Whatman no 1 filter paper. The amount of extractable zinc in the filtrate was then determined by atomic absorption spectrometry.

3.3 Glasshouse experiment

3.3.1 Experimental site

The study was conducted in a glasshouse situated at the University of the Free State, Bloemfontein, South Africa.

3.3.2 Experimental design and layout

Two pot experiments were conducted in the 2013/2014 growing season. Maize was planted 4 January 2014 for the first planting and on 22 February 2014 for the second planting. For both plantings a two factor, randomized block design was used. Zinc fertilizer and application rate were the two factors selected for this experiment (Figure 3.2).

Block 1

S1	O3	E4	S2	O2	E2	E5	S5	O5	O1	E3	S4	S3	O4	E1
----	----	----	----	----	----	----	----	----	----	----	----	----	----	----

Block 2

O4	E1	S3	S1	O3	E4	S2	O2	E2	E5	S5	O5	O1	E3	S4
----	----	----	----	----	----	----	----	----	----	----	----	----	----	----

Block 3

E3	S4	O1	O4	E1	S3	S1	O3	E4	S2	O2	E2	E5	S5	O5
----	----	----	----	----	----	----	----	----	----	----	----	----	----	----

Block 4

S5	O5	E5	E3	S4	O1	O4	E1	S3	S1	O3	E4	S2	O2	E2
----	----	----	----	----	----	----	----	----	----	----	----	----	----	----

Block 5

O2	E2	S2	S5	O5	E5	E3	S4	O1	O4	E1	S3	S1	O3	E4
----	----	----	----	----	----	----	----	----	----	----	----	----	----	----

Figure 3.2 Experimental layout consisting of a two factor randomized block design. The two factors were the three zinc sources ZnSO_4 (S), ZnO (O) and ZnEDTA (E) combined with five application rates to increase the zinc level in the soil with 0 mg kg^{-1} (1), 0.5 mg kg^{-1} (2), 1 mg kg^{-1} (3), 2 mg kg^{-1} (4) and 4 mg kg^{-1} (5).

3.3.3 Soil treatment and agronomic practices

3.3.3.1 Soil treatment

The glasshouse study was conducted only with soil B (Table 3.1). A sufficient amount of this soil was collected, air-dried and sieved through a 2 mm sieve to remove all unwanted plant material. For every zinc treatment 800 kg of air-dried soil was weighed and spread on a clean concrete floor (Figure 3.3). Then the appropriate amount of zinc fertilizer source (ZnSO_4 , ZnO or ZnEDTA) together with NH_4NO_3 as nitrogen source and H_3PO_4 as phosphorus source were dissolved in 20 L of water and sprayed evenly over the soil.

The intention with these treatment applications (Table 3.5) were to increase the zinc content of the soil with 0 mg kg^{-1} , 0.5 mg kg^{-1} , 1 mg kg^{-1} , 2 mg kg^{-1} and 4 mg kg^{-1} (hereafter refer to application rates 1 to 5), respectively and that all of the zinc treated soils have levels of 20 mg kg^{-1} N and 25 mg kg^{-1} P. These application rates differed somewhat from those used in the incubation experiment (See section 3.2.1).



Figure 3.3 Soil spread out for treatment with soluble fertilizer sources.

After spraying, the soil was turned over several times to ensure proper mixing between dissolved chemicals and soil particles. The treated soils were then air-dried and stored in plastic bags for pot preparation.

Table 3.5 Application rates for zinc, nitrogen and phosphorus fertilizer sources per 800 kg of soil

Soil treatment & zinc fertilizer	Mass of Zinc fertilizer (g)	Mass of nitrogen fertilizer (g)	Volume of phosphorus fertilizer (cm ³)
ZnSO ₄ AR 1	0	50	30
ZnSO ₄ AR 2	1.7591	50	30
ZnSO ₄ AR 3	3.5180	50	30
ZnSO ₄ AR 4	7.0361	50	30
ZnSO ₄ AR 5	14.0721	50	30
ZnO AR 1	0	50	30
ZnO AR 2	0.4979	50	30
ZnO AR 3	0.9958	50	30
ZnO AR 4	1.9915	50	30
ZnO AR 5	3.9831	50	30
ZnEDTA AR 1	0	50	30
ZnEDTA AR 2	2.8571	50	30
ZnEDTA AR 3	5.7143	50	30
ZnEDTA AR 4	11.4286	50	30
ZnEDTA AR 5	22.8571	50	30

3.3.3.2 Pot preparation

A total of 75 polyethylene pots were used for each planting. The pot's dimensions were 0.34 x 0.34 m (length x width) with a height of 0.35 m and a total volume of 40.5 L.

Each polyethylene pot was filled with its corresponding treated soil to 80 mm below the brim of the pot, which was the planting depth. All the pots were wetted with distilled water to ensure the soil is at drained upper limit (θ_{DUL}). After wetting, six maize seeds (cultivar DKC 78-45 BRGEN) were planted in a row, in the middle of each pot. Another 50 mm layer of soil was then added on top of the seeds as to allow for a 30 mm clearing from the top of the pot's brim. This 30 mm clearing was sufficient for water application. After emergence the seedlings were thinned to three plants per pot.

3.3.3.3 Irrigation

During maize growth the soil moisture was maintained at θ_{DUL} for the specific soil. Drained upper limit was determined by weighing five pots with the required amount of dry sieved soil before wetting it to saturation. After saturation the pots were left to drain freely for 48 hours. The pots were then again weighed to obtain the average θ_{DUL} of the soil.

With the use of an electronic scale planted pots were weighed throughout the experiment to determine the amount of water required for sufficient irrigation. Distilled water was used for irrigation to avoid any nutrient application through municipal water.

3.3.3.4 Glasshouse management

During the five weeks of the experiment, the glasshouse was programmed to ensure optimum growth conditions for maize. Temperatures were maintained at 20°C during the night and at 32°C during the day. Disease and insect outbreak was visually monitored on a regular basis during the five week vegetative growth period.

3.3.4 Measurement and analysis

3.3.4.1 Aerial plant parameters

- Stem thickness:* Each plant's stem thickness was measured, in millimetre (mm), on a weekly basis 10 mm above the soil surface. Stem thickness was measured perpendicular with the main vein of the outer leaf sheath.
- Plant height:* Measured from the soil surface to the highest point of the maize plant, in its natural position (Figure 3.4a). Plant height was measured once a week in metre (m).
- Number of leaves:* The number of fully developed leaves was counted on a weekly basis. Only leaves with a fully devolved ligule or leaf collar were considered as fully developed.
- Leaf area:* After the five week growth period, leaves were cut from the stem behind the collar of each plant. With the use of the LICOR 3000 leaf area meter the leaf area of all the photosynthetic active leaves was determined. Leaf area was measured in square centimetre (cm²).
- Dry mass:* Following determination of leaf area the whole plant was cut in pieces (+/- 25mm) and placed in a paper bag and dried in an oven at 50 °C for 1 week. After drying, each plant was individually weighed to obtain its dry mass in grams (g).

3.3.4.2 Subsoil plant parameters

- Root length:* After harvesting the aerial plant material, pots were washed with water to recycle all the plant roots in each pot for analysis (Figure 3.4b). A modified infrared root line intersection counter was used to determine the length of the roots for each pot in metre (m).
- Root mass:* All of the roots from each pot were placed in paper bags and dried in an oven at 50°C for one week. After drying, the roots were weighed with an electronic scale to determine the mass in gram.

3.3.4.3 Soil sample preparation and analysis

After soil sampling, all the samples were air dried, ground and sieved through a 2 mm stainless steel sieve to ensure homogeneous samples.

Pre-plant samples: Three samples were randomly taken per treatment to determine the average zinc status of the treated soil, before planting. These samples were collected for zinc and phosphorus analysis.

Post-harvest samples: Four evenly spaced samples were taken over the full depth of the pot, at termination of the experiment (Figure 3.4c). Samples for each pot were combined and thoroughly mixed to prepare only one representative sample. These samples were also used for zinc and phosphorus analysis.

Extraction and determination methods employed for zinc and phosphorus analysis in pre-plant and post-harvest soil samples.

3.3.4.4 Plant sample preparation and analysis

After plant samples were oven-dried, they were finely milled mechanically for preparation of chemical analysis.

Nitrogen in the aerial plant parts was determined by combustion of the milled samples in a Leco TruSpec® CN analyser according to instructions of the manufacturer. For the determination of P, K, Ca, Mg, Cu, Fe, Mn, Zn, B and Na the milled plant samples were incinerated at 500°C for at least 3 hours, allowed to cool before wetted with concentrated nitric acid and then incinerated for another hour. After cooling, 10 ml of a 1:2 distilled water to nitric acid solution was added to the silica crucibles, heated on a sand bath and when warm enough washed over into a 100 ml volumetric flask with distilled water. The P in this solution was determined colorimetrically while the other nutrients were determined with atomic absorption spectrometry, as prescribed by manufacturers (Palic *et al.*, 2000).

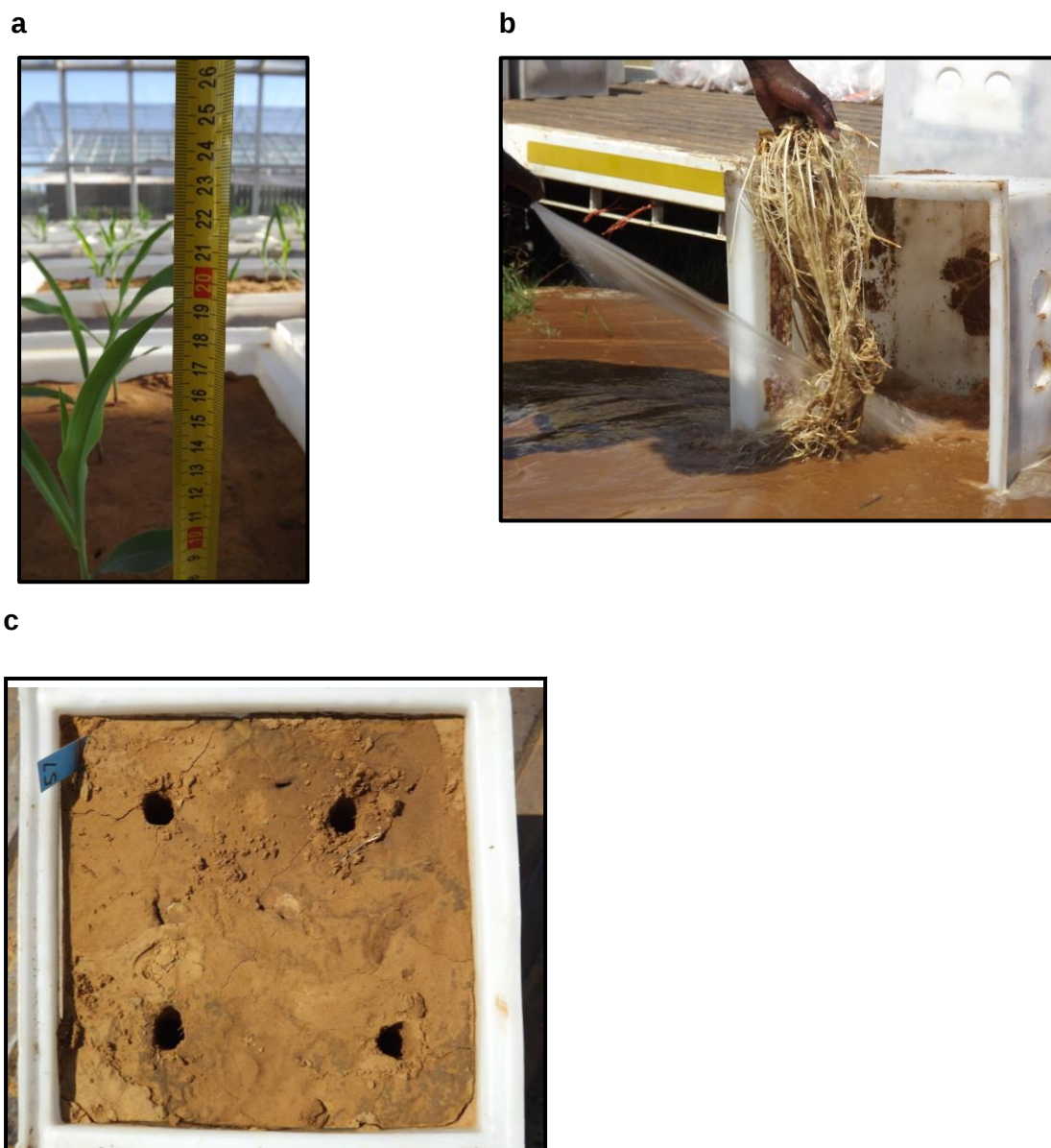


Figure 3.4: Plant height measured to the highest point of the plant (a), roots carefully washed from the soil with water (b) and evenly spaced soil samples taken from each pot with an augur after the five week growth period.

3.3.5 Statistical analysis

Incubation experiment

Data were subjected to statistical analysis using the statistical software GenStat (Payne *et al.*, 2012). A three-factor completely random design (CRD) with a factorial combination was employed for analysis of variance (ANOVA). Assumptions for ANOVA were satisfied and the least significant difference (LSD) was calculated at $P \leq 0.05$. This allowed for treatment means to be compared using the Tukey's multiple comparison test.

Glasshouse experiment

A two-factor randomized block design (RBD) with a factorial combination was employed for analysis of variance (ANOVA). Assumptions for ANOVA were satisfied and the least significant difference (LSD) was calculated at $P \leq 0.05$. This allowed for treatment means to be compared using the Tukey's multiple comparison test.

CHAPTER 4

EFFECT OF ZnSO₄, ZnO AND ZnEDTA APPLICATION ON PLANT AVAILABLE ZINC IN SANDY SOIL WHEN DETERMINED WITH VARIOUS EXTRACTANTS

4.1 Introduction

The concentration of zinc in the soil solution plays a vital role in supplying sufficient zinc to plants. Reactions that influence the zinc concentration in the solution and hence its availability for plant uptake are precipitation, dissolution, mineralization, immobilization, adsorption and desorption (Kiekens, 1995).

Zinc fertilizers are applied to soil to increase the zinc soluble fraction in the soil to increase the amount of zinc available to plants. The effectiveness of a zinc compound as fertilizer depends on its solubility, bioavailability and its effect of distribution in the soil profile (McBeath & McLaughlin, 2014). There are however numerous zinc fertilizers on the market and in many cases they are accompanied by unsubstantiated claims as to the level of availability for plant uptake (Gangloff *et al.*, 2000). Zinc fertilizers that are mainly used in South Africa's agricultural sector are zinc sulphate, zinc oxide and zinc EDTA.

The availability of micronutrients such as zinc for uptake by plants or movement in soil depends on a range of soil properties (Alloway, 2004). According to researchers all over the world there is a clear trend of low zinc concentrations in sandy soils compared to that of clayey soils (Alloway, 2004). Sandy soils are therefore more prone for zinc deficiency under crop production.

Different extraction conditions lead to a variety of different amounts of zinc solubilized by a specific soil test. Calibration of a specific soil test is therefore very important. Calibration data for a soil test applies solely to soil test values obtained with the extraction conditions used during the calibration (Reed & Martens, 1996). A positive correlation between the nutrient concentration determined by the method and the nutrient quantity taken up by plants is fundamental when choosing a suitable extractant (Lopes & Abreu, 2000).

Depending on the method of action, reactants fall into different categories. Firstly, those which employ salts such as CaCl₂ or Ca(NO₃)₂ in order to leach cations adsorbed onto solid materials, due to the negative charge on soil particles. Secondly, techniques using an acid solution in order to simulate the effect of an acid input are used because low pH favours the dissociation of the existing complexes. The third category uses complexing or reducing agents such as EDTA (Alvarez *et al.*, 2006).

At first, single element availability tests were developed and used to estimate the availability of zinc in the soil. An example is the 0.1 M HCl extractant test for zinc. Later, simultaneous extraction of microelements was initiated with the development of the DTPA-TEA (diethylenetriaminepentaacetic acid-triethanolamine) extractant to estimate the availability of Cu, Fe, Mn and Zn (Lindsay & Norvell, 1978).

Today, some scientists use soil tests that are developed for simultaneous extraction of micronutrients and macronutrients in a single method. Examples are the Mehlich III and DTPA-AB (diethylenetriaminepentaacetic acid- NH_4HCO_3) procedures (Mehlich, 1984). The use of simultaneous extraction for the determination of micronutrients and macronutrients is desirable for rapid conveyance of soil test data to the crop producers at a reasonable cost.

In this study the focus will be on five of the most well-known extraction methods used in South Africa, to determine plant available zinc in soil. They are the HCl, Mehlich I, DTPA-TEA, NaEDTA and the Ambic II extraction methods.

4.2 Procedure

Two very similar sandy soils, soil A and soil B (See section 3.1 for soil properties) were used in an incubation study. Both soils were treated with ZnSO_4 , ZnO or ZnEDTA to increase their zinc content with 0 mg kg^{-1} , 1 mg kg^{-1} , 2 mg kg^{-1} , 3 mg kg^{-1} and 4 mg kg^{-1} , referred to as application rate (AR) 1 to 5. Zinc was applied to the soil as a solution using a sprayer. Each treatment was mixed properly after application. Soil went through three wetting and drying cycles before the determination of plant available zinc. For the determination of zinc the following extraction methods were used: HCl, Mehlich I, DTPA –TEA, NaEDTA and Ambic II. For details on materials and methods refer to Chapter 3, Section 3.1 and 3.2.

4.3 Results

At the termination of the incubation study soil A and soil B differed significantly ($p < 0.01$) in plant available zinc content. Soil A had an average zinc content of 3.19 mg kg^{-1} soil which was significantly higher than that of soil B with an average zinc content of 2.50 mg kg^{-1} soil ($\text{LSD} = 0.11$). In this section the results of the two soils will therefore be presented separately because of this significant difference in zinc content between them.

Analysis of variance on the zinc contents of soil A (Table 4.1) showed significant differences ($p < 0.01$) for all main effects, viz. extraction method, zinc source and application rate. All the interactions between the main effects also showed significant differences ($p < 0.01$). Similar outcomes were obtained by analysis of variance on the zinc content of soil B (Table 4.1). For

both soils, treatment replications did not differ significantly with p values of 0.77 for soil A and 0.16 for soil B.

The focus of this section will therefore be on the interactions of the main effects concerning each soil's zinc content. However, firstly the influence of the main effects on the zinc content of both soils will be addressed concisely.

Table 4.1 Summary of analysis of variance for Soil A and Soil B

Soil A

Source ¹	d.f.	s.s	m.s.	v.r.	F pr.
EXM	4	26.15794	6.53948	114.12	<.0001
ZNS	2	137.73802	68.86901	1222.87	<.0001
AR	4	322.57655	80.64414	1431.96	<.0001
EXM*ZNS	8	10.57372	1.32171	23.47	<.0001
EXM*AR	16	5.13094	0.32068	5.69	<.0001
ZNS*AR	8	107.91538	13.48942	239.53	<.0001
EXM*ZNS*AR	32	10.23392	0.31981	5.68	<.0001
Residual	300	16.89520	0.05632		
Total	374	6.37.22166			

Soil B

Source ¹	d.f. (m.v.)	s.s	m.s.	v.r.	F pr.
EXM	4	28.38365	7.09591	115.72	<.0001
ZNS	2	111.85849	55.92925	1227.38	<.0001
AR	4	349.30693	2.66337	58.45	<.0001
EXM*ZNS	8	21.30693	2.66337	58.45	<.0001
EXM*AR	16	20.12884	1.25805	27.61	<.0001
ZNS*AR	8	52.13763	14.39264	315.85	<.0001
EXM*ZNS*AR	32	52.31763	1.63493	35.88	<.0001
Residual	299(1)	13.62488	0.04557		
Total	373(1)	711.72581			

¹EXM = Extraction method, ZNS = Zinc source and AR = Application rate

4.3.1 Main effects

4.3.1.1 Zinc source

The effect of zinc source on the average zinc content of Soil A and Soil B is illustrated in Figure 4.1.

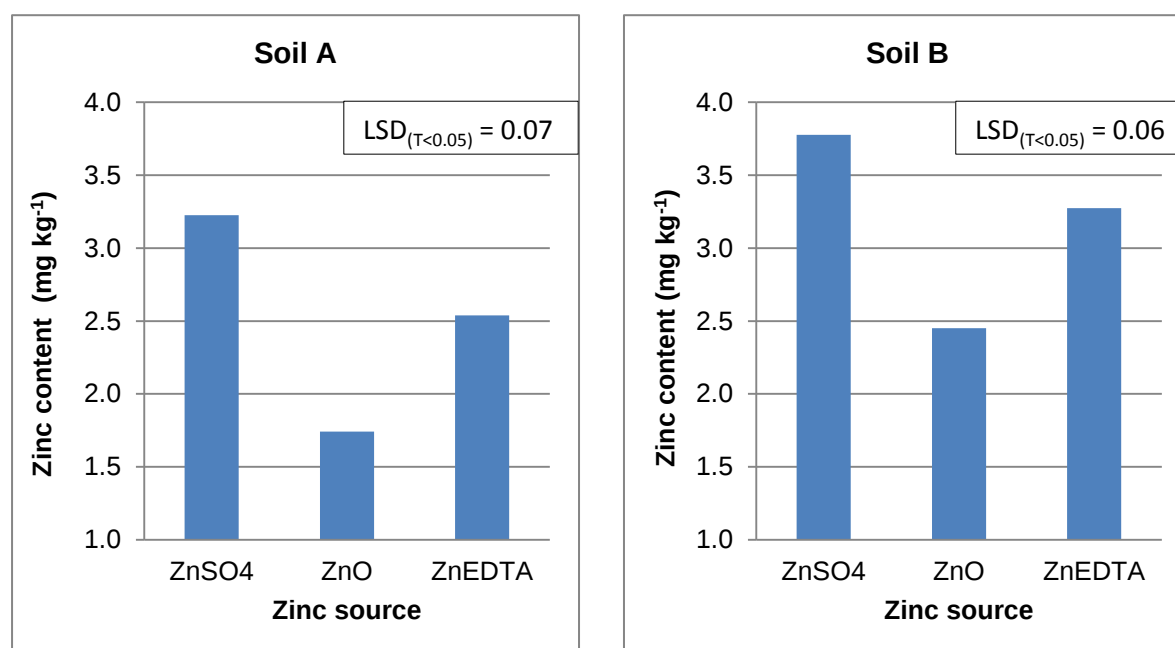


Figure 4.1 Average zinc content of soil A and soil B as affected by zinc source.

When soil A was treated with ZnSO₄, it increased the average zinc content to 3.22 mg kg⁻¹, compared to ZnO which increased the zinc content to 1.74 mg kg⁻¹, and ZnEDTA to 2.54 mg kg⁻¹. Thus ZnSO₄ was 1.85 and 1.27 times more effective to increase zinc than ZnO and ZnEDTA, respectively.

A similar pattern in zinc content was found for soil B. In this soil ZnSO₄ was however only 1.54 and 1.13 times more effective to increase zinc in soil than ZnO and ZnEDTA, respectively.

4.3.1.2 Application rate

The effect of application rate on the average zinc content of soil A and soil B is displayed in Figure 4.2.

An increase in application rate showed a sharp increase in the zinc content of soil A. The highest zinc content was found at AR 5 which was 3.03 times higher than the zinc content at AR 1 where no fertilizer was added. From a fertilizer perspective the amount of zinc fertilizer

added did not meet the desired zinc level in soil. For example soil not fertilized had a zinc content of 1.26 mg kg^{-1} , while soil fertilized to increase the zinc content with 4 mg kg^{-1} had a zinc content of only 3.83 mg kg^{-1} . If all the applied zinc remained plant available the zinc content of the soil should be 5.26 mg kg^{-1} . The effectiveness of the zinc fertilizers over the application rates ranged between 55% and 65%.

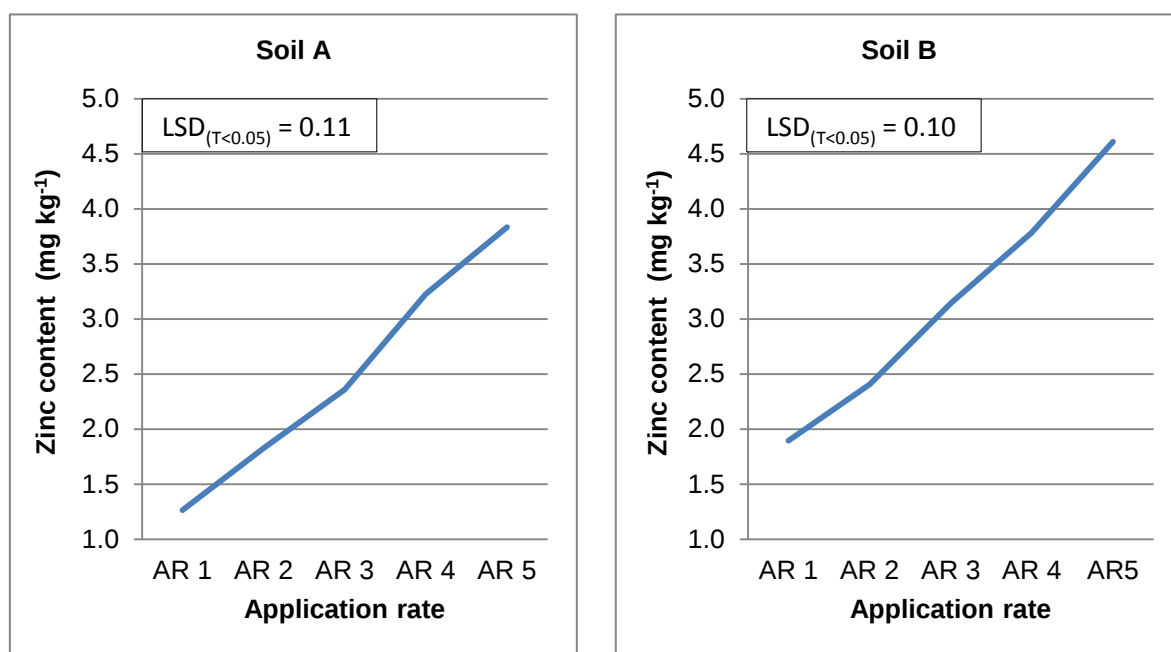


Figure 4.2 Average zinc content of soil A and soil B as affected by application rate.

Soil B, similar to soil A showed a sharp increase in zinc content with an increase in applied zinc. However, the range of effectiveness of the zinc fertilizers over the application rates was somewhat wider, 51% to 68%.

4.3.1.3 Extraction method

The average amount of zinc extracted from soil A and soil B with different extraction methods is depicted in Figure 4.3.

The extractable zinc determined in soil A with the Ambic II method was significantly greater than that determined with the other four methods. However, the zinc extracted by the EDTA and DTPA methods did not differ significantly from one another. The Ambic II method extracted 37% more zinc than the Mehlich I method, which gave the lowest value. In soil B the EDTA method extracted a significantly greater amount of zinc than the other four methods which was not the case in soil A. However, the Mehlich I method gave the lowest zinc value, like in soil A.

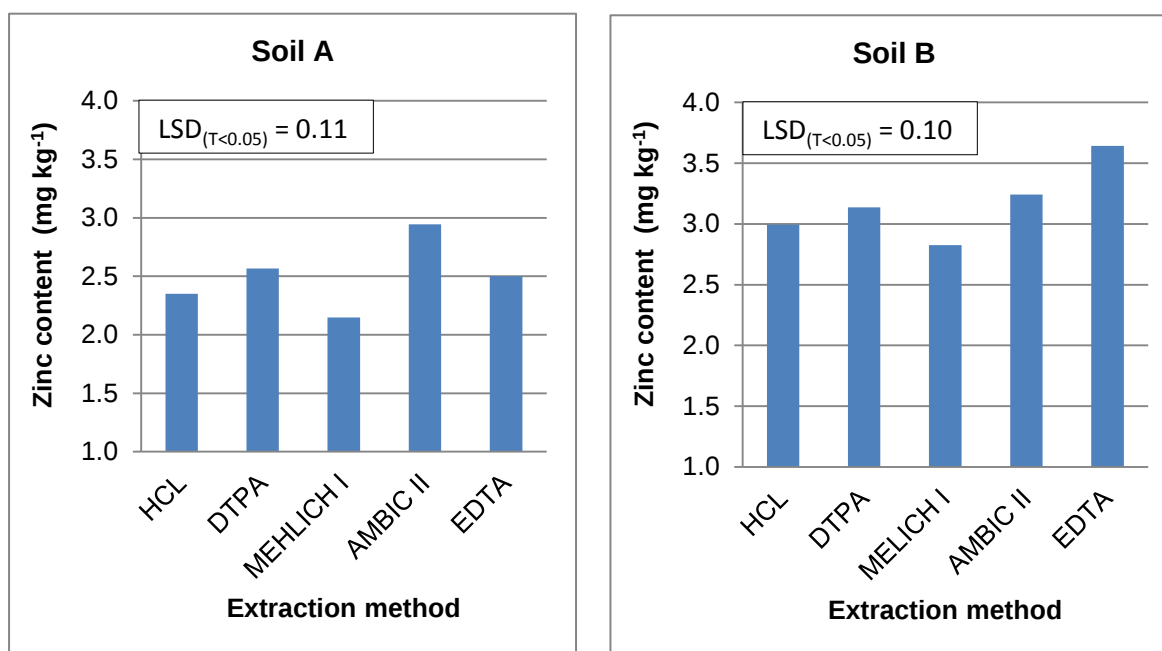


Figure 4.3 Average zinc content of soil A and soil B as determined with different extraction methods.

4.3.2 Interactions

4.3.2.1 Zinc sources and application rates

The interaction effects of zinc sources and application rates on the average zinc contents of soil A and soil B are presented in Figure 4.4.

The zinc content of soil A increased almost linear with increasing rates of ZnSO_4 and ZnEDTA application. This was not the case with ZnO because the zinc content of AR 4 and AR 5 were similar. Based on equivalent rates of zinc application ZnO was the least effective and ZnSO_4 the most effective to increase this soil's zinc content. For example at AR 3 ZnO , ZnEDTA and ZnSO_4 resulted in zinc content of 1.82, 2.48 and 2.78 mg kg^{-1} , respectively.

A similar pattern evolved for the zinc content of soil B due to the interaction effects of zinc sources and application rates. For this soil at AR 3 resulted ZnO , ZnEDTA and ZnSO_4 in zinc content of 2.46, 3.30 and 3.66 mg kg^{-1} , respectively.

These zinc contents are higher than those recorded in soil A, despite that soil B had a lower zinc content in its control treatment than soil A.

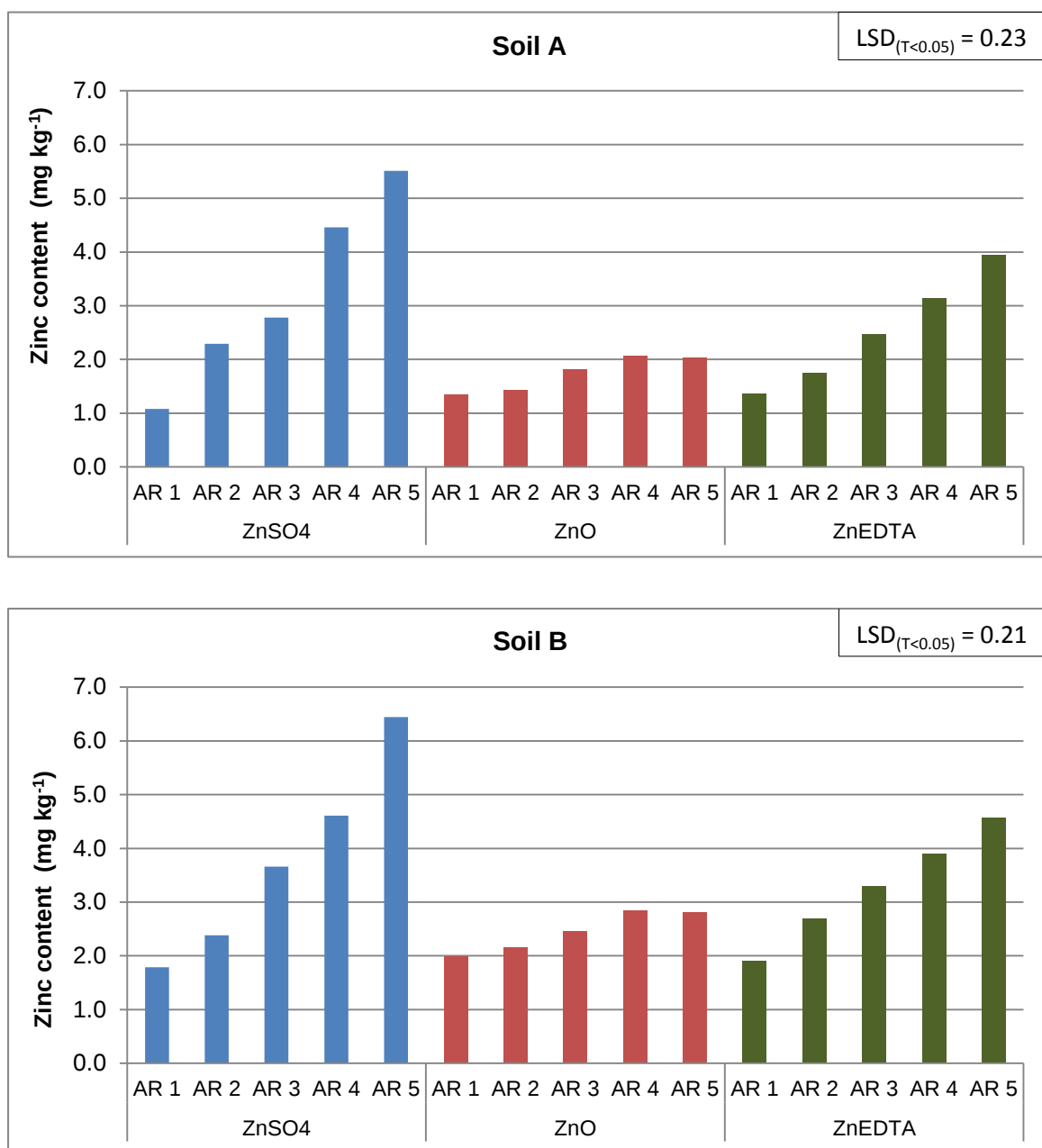


Figure 4.4 Interaction effect of zinc source and application rate on average zinc content of soil A and soil B.

4.3.2.2 Extraction methods and zinc sources

The interaction effects of extraction methods and zinc sources on the average zinc contents of soil A and soil B are shown in Figure 4.5.

The ZnSO₄-treated soil A, when analysed with the Ambic II method, resulted in a significantly higher zinc content than all other interactions between extraction method and

zinc source. For each of the extraction methods, soil treated with ZnSO_4 gave the highest zinc content, followed by ZnEDTA- and ZnO-treated soils. The zinc contents of soil treated with ZnSO_4 and ZnEDTA did not differ significantly from one another if the DTPA method was employed for extraction. For all the other extraction methods there were no significant differences between the zinc content of soil fertilized with the three zinc sources.

All soil treated with either ZnSO_4 or ZnO, and extracted with the HCl, DTPA and EDTA methods did not differ significantly in zinc content. Likewise, the zinc contents of Zn-EDTA soils when extracted by the DTPA and HCl methods did not differ significantly.

In soil B, regardless of extraction method, mirrored the pattern of its zinc content that coincide with zinc source like those in soil A. However soil treated with ZnSO_4 , and extracted with the EDTA method rather than the Ambic II method, resulted in significantly higher zinc content than all the other interactions between extraction method and zinc source. The zinc content of soil treated with ZnSO_4 , ZnO and ZnEDTA, did not differ significantly from one another when the Ambic II extraction method was employed. For all the other extraction methods there were significant differences between the soil fertilized with the different zinc sources.

All soils treated with ZnO and extracted with the HCl, DTPA, Mehlich I and EDTA methods did not differ significantly in zinc content. Likewise, the zinc content of ZnSO_4 -treated soil when extracted with the HCl, DTPA, Mehlich I and Ambic II methods, did not differ significantly. The zinc content of soil treated with ZnEDTA and extracted with the HCl and Mehlich I methods did not differ significantly. Also, soil treated with ZnEDTA and extracted with the DTPA and Ambic II methods did not differ significantly in zinc content.

4.3.2.3 Extraction methods and application rates

The interaction effects of extraction methods and application rates on the average zinc contents of soil A and soil B are depicted in Figure 4.6.

The zinc content of soil A regardless of the extraction method used increased almost linear when the application of zinc increased from AR 1 to AR 5. However, comparing equivalent application rates, then slight differences between the extraction methods are observed concerning their ability to extract zinc. It seems that the Mehlich I method was the least effective, followed by the HCl, EDTA, DTPA and Ambic II methods.

For soil B a very similar pattern as for soil A evolved, namely an almost linear increase in zinc content with higher rates of zinc application irrespectively of the extraction method employed. The exemption was with the Ambic II method where a very large amount of zinc

was extracted at AR 5 for unknown reason(s). If the latter is ignored then it appears when comparing equivalent application rates, that the EDTA method was the most effective, followed by the DTPA, HCl and either Mehlich I or Ambic II methods.

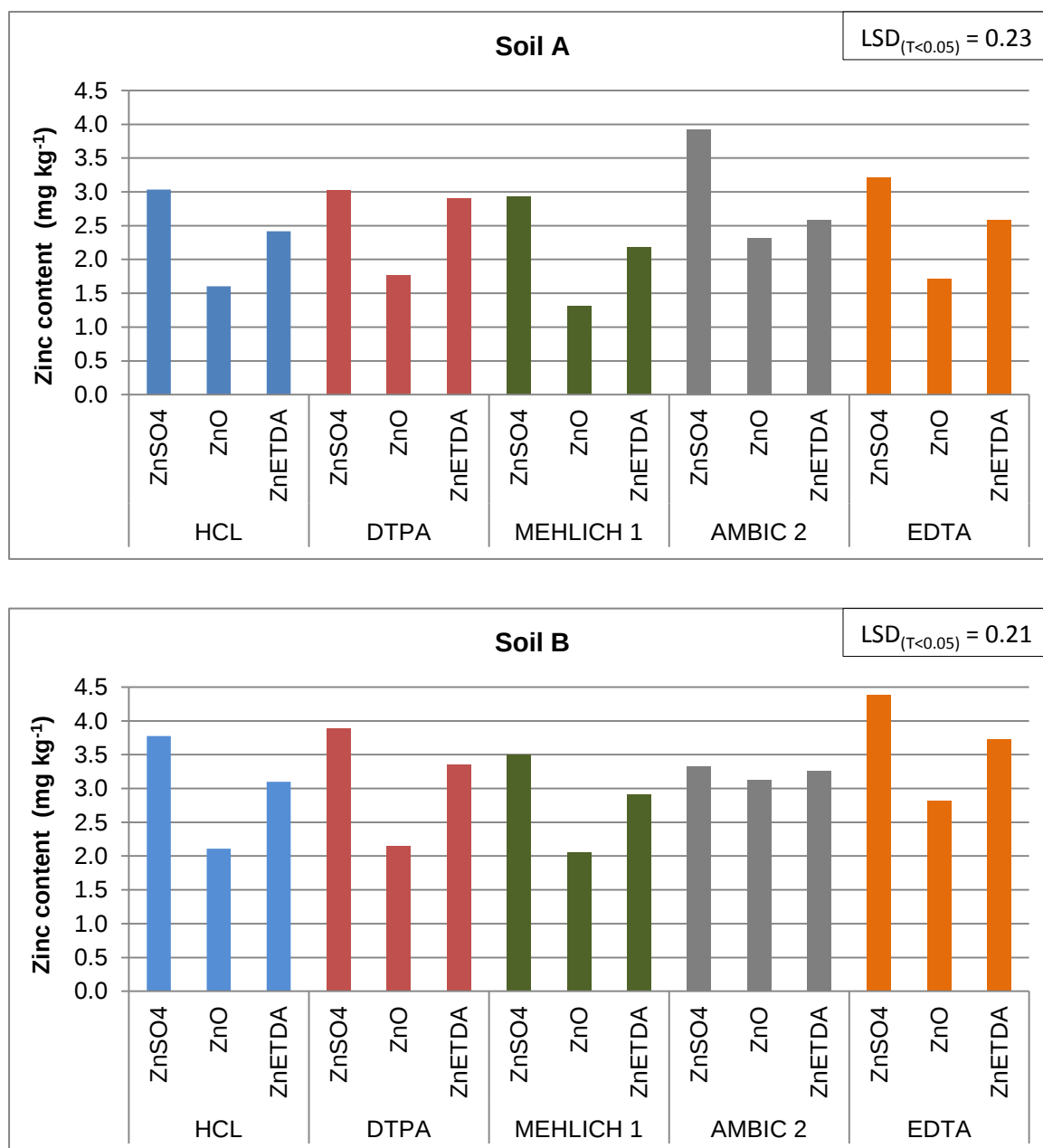


Figure 4.5 Interaction effect of extraction method and zinc source on average zinc content of soil A and soil B.

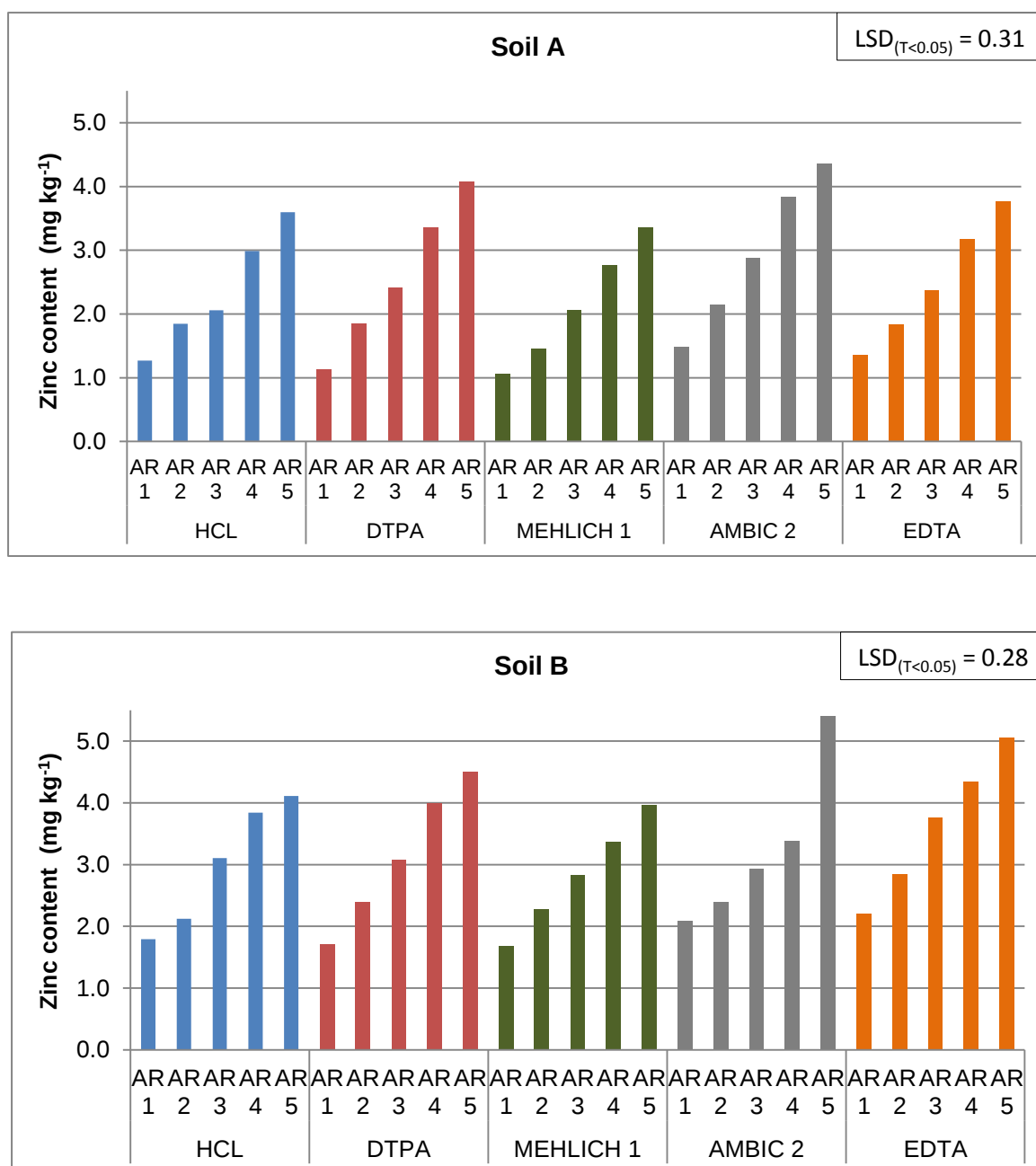


Figure 4.6 Interaction effect of extraction method and application rate on average zinc content of soil A and Soil B.

4.4 Discussion

Chelating agents are used to supply plants with nutrients such as Fe and to a lesser extent Zn and Mn (Wallace, 1963). It is considered that chelating agents such as ZnEDTA is more stable in maintaining the nutrients in a more available form for plants (Cheng *et al.*, 1972). After the application of ZnEDTA in/on soil it is suggested that the majority of the ZnEDTA complex remain in solution for a few weeks and that a certain proportion of the Zn in the complex is not replaced if the pH of the soil is near neutrality (Norvell & Lindsay, 1969).

Chelated fertilizers are regarded as the most effective sources of micronutrients for plants. It is considered that ZnEDTA is 2 to 5 times more effective than zinc sulphate (Mortvedt & Gilkes, 1993). However, results from this study (Figure 4.1) show clearly that ZnEDTA increased the zinc content of both soils, but to a lower level than ZnSO₄. This outcome was not influenced by neither the rate at which the zinc was applied (Figure 4.4) nor the method used to extract the zinc from soil (Figure 4.5). Studies showed that zinc fertilizers with high water solubility are the most effective to correct zinc deficiency in soil and hence crops (Amrani *et al.*, 1999).

Results from a study done recently on the efficacy of zinc oxides as fertilizers by McBeath and McLaughlin (2014) showed that ZnO compared to ZnSO₄ fertilizers had very low water solubility and slow dissolution rate, because of a higher dissolution pH. During the application of the zinc solutions in this study, it was clearly observed that ZnO had low water solubility. The statement above of McBeath and McLaughlin (2014) therefore supported the results shown in Figure 4.1, 4.4 and 4.5.

More soluble sources of zinc tend to be more readily available for uptake by plants due to the fact that zinc is a diffusion-limited nutrient. Soluble zinc sources like ZnSO₄ tend to be more plant available because they allow increased movement of fertilizer away from the application point (Amrani *et al.*, 1999). The low solubility of ZnO depends on the physical and chemical nature of the product, which is influenced by the manufacturing process (McBeath & McLaughlin, 2014). The composition of the zinc source may also have an influence on the soil reaction. Zinc oxide tends to have a large degree of heterogeneity in particle morphology in comparison to ZnSO₄ which has a very consistent composition (McBeath & McLaughlin, 2014).

A rise in plant available zinc levels is expected when soil is fertilized, with higher zinc rates as in this study (Figure 4.2). For both soils the increase in zinc content that coincide with higher application rates was not affected by either the zinc source (Figure 4.4) or the extraction method (Figure 4.5). No relevant literature was found on the effect of zinc application rates on zinc content in soil. However, a sharp increase in phosphorus levels was noticed with the increase in phosphorus fertilization rate (Westermann, 1977). Moura *et al.* (2013) reported a linearly increase in boron content in fertilized soil with dosages of 0 to 6 kg ha⁻¹.

For the development of soil tests to determine plant available zinc in soil, the extractant used should be selected to solubilize the amount of zinc proportional to the amount of nutrient adsorbed by plants during a single growing season. These tests must also be effective over

a wide range of soil types (Reed & Martens, 1996). Different extraction conditions lead to a variety of different amounts of zinc solubilized by a specific soil test (Reed & Martens, 1996).

Each extraction method correlates the best to zinc's plant availability under specific soil conditions. The HCl method is being used to evaluate the zinc status in neutral and acid soils (Whitney, 1988). Soil tests like the EDTA, Ambic II and Mehlich I methods can be used for zinc extraction on all soils except those in which free lime occurs (Wear & Evans, 1968). The DTPA method correlates best with plant available zinc in neutral to calcareous soils (Lindsay & Norvell, 1978). Both the soils used in this study have a pH below 5, and therefore should the DTPA method not be the ideal extraction method for the determination of zinc.

Through calibrations of soil tests, critical values have been established which divide soil between a sufficient and insufficient zinc level for a specific crop. For each specific extraction method and crop these critical values differ (Alloway, 2004). For example, the critical value for rice using the DTPA extraction method is 0.76 - 1.24 mg Zn kg⁻¹ while the critical value for the Mehlich I method is 0.5 - 3.0 mg Zn kg⁻¹ and 1.5 mg Zn kg⁻¹ for EDTA (Alloway, 2004). Although the amount of zinc extracted by the different extraction methods differ, it is also important to keep in mind the critical value of each method for comparison between the methods.

Research done by Khatri-Chhetri and Schulte (1984) to evaluate five extraction methods to predict the uptake of zinc by maize reported a higher extractable zinc content using the DTPA method, followed by the EDTA and HCl method. The DTPA method was considered the most suitable method, because of its potential for using the same extractant in the determination of Fe, Cu and Mn. It was found that the HCl and Mehlich I extractants were less efficient in estimating plant available zinc, and the extractant most indicative of plant available zinc was DTPA (Sarto Mansano *et al.*, 2011). In a sandy soil, significant correlations were found between Mehlich I and DTPA extractants (Ortiz *et al.*, 2007). However, Muraoka *et al.* (1983) observed superiority of EDTA and DTPA over HCl extractants.

However, in this study it was found that with soil A the Ambic II method was superior in the amount of zinc extracted, followed by the EDTA, DTPA, HCl and Mehlich I methods (Figure 4.3). This sequence changed somewhat for soil B since the most zinc was extracted with the EDTA method, followed by the Ambic II, DTPA, HCl and Mehlich I method. Neither the zinc source nor the application rate (Figure 4.6) changed this trend. Unfortunately, the zinc values of the two soils could not be related to plant uptake since the incubation study was done in a laboratory.

4.5 Conclusion

All treatment factors, namely soil, zinc source, application rate and extraction method affected the concentration of zinc in soil. The average zinc content of soil A was significantly higher than that of soil B. The most efficient zinc source to increase zinc content was ZnSO_4 , followed by ZnEDTA and ZnO. As expected with increased zinc source dosages, an increase in soil zinc content was found. Significant differences were observed between the extraction methods HCl, Mehlich I, DTPA, EDTA and Ambic II. For soil A the Ambic II method and for soil B the EDTA method were the superior ones concerning the amount of zinc extracted, followed by the DTPA, HCl and Mehlich I methods irrespectively of the soil.

CHAPTER 5

EFFECT OF ZnSO₄, ZnO AND ZnEDTA APPLICATION ON AERIAL- AND SUBSOIL PLANT PARAMETERS DURING THE EARLY GROWTH AND DEVELOPMENT OF MAIZE IN A SANDY SOIL

5.1 Introduction

One of the most common micronutrient deficiencies is that of zinc and therefore zinc is becoming an increasingly significant factor in crop production (Mengel & Kirkby, 1987). The susceptibility of a crop to zinc deficiency varies in different crops and also between cultivars. Sensitive crops include maize, hops, flax and beans, while crops that are moderately sensitive are potatoes, tomatoes and lucerne. Crops that are insensitive include oats, barley, wheat and rye (Viets *et al.*, 1954).

Maize is the largest produced field crop and the most important source of carbohydrates in the southern African region. Currently South Africa is the main maize producer in Africa (BFAP, 2011). Approximately half of the maize produced in South Africa is used for human food consumption. From this perspective the quality of maize grain is extremely important. Good quality maize grain depends on proper plant nutrition, hence good soil fertility.

Around the world, soils most prone to show zinc deficiency are sandy soils and calcium - enriched soils (Mousavi *et al.*, 2013). Sandy soils contribute largely to maize production in South Africa which aggravates the zinc deficiency problem.

The purpose of zinc fertilization is to increase the water soluble zinc fraction in soil. This will improve the amount of zinc available for uptake by plants. However, there are numerous zinc fertilizers on the market and in many cases they are accompanied by unsubstantiated claims as to the level of zinc availability for plant uptake (Gangloff *et al.*, 2000). Zinc fertilizers that are mainly used in South Africa's agricultural sector are zinc sulphate, zinc oxide and zinc EDTA.

The focus with this study will be to establish how the application of zinc sulphate, zinc oxide and zinc EDTA, at different rates, affected maize growth in a sandy soil during the five week period after planting. Various aerial (number of leaves, stem thickness, plant height, leaf area and dry mass) and subsoil (root mass and root length) plant parameters will be used as indicators of maize growth.

5.2 Procedure

Two pot experiments were conducted in the 2013/2014 growing season. Maize was planted in a glasshouse at the University of the Free State using a two factor, randomized block design. Only soil B was used for this experiment; see Table 3.1 for soil properties. This soil was air-dried and sieved before the application of fertilizers. The relevant zinc fertilizer (ZnSO_4 , ZnO and ZnEDTA) together with H_3PO_4 and NH_4NO_3 as phosphorus and nitrogen sources were dissolved in 20 L of water and sprayed over the soil. Soil was treated to increase the zinc content with 0 mg kg^{-1} , 0.5 mg kg^{-1} , 1 mg kg^{-1} , 2 mg kg^{-1} and 4 mg kg^{-1} (AR 1 - AR 5), and $20 \text{ mg kg}^{-1} \text{ N}$ and $25 \text{ mg kg}^{-1} \text{ P}$ per treatment.

During the vegetative growth period, the maize was measured weekly for number of leaves, stem thickness and plant height. Readings started one week after emergence. At termination of the experiment leaf area, dry mass, root mass and root length was measured using the relevant plant material.

For the full discussion on materials and methods see Section 3.3.

5.3 Results

The two plantings differed significantly ($p < 0.001$) concerning the parameters measured as indicators of maize growth. For this chapter the data for the two plantings will therefore be presented separately, referred to as first planting (planted at 4 January 2014) and second planting (planted at 22 February 2014).

A summary of analyses of variance, showing the effects of zinc source and application rate on growth parameters measured weekly during the first and second plantings, is presented in Table 5.1. A summary of analyses of variance, showing the effects of zinc source and application rate on growth parameters measured at termination of the first and second planting, is presented in Table 5.2.

With inspection of the statistical data in Table 5.1 it was clear that all the main effects, as well as interactions differed significantly over time. For the discussion of the number of leaves, stem thickness and plant height the focus will therefore be on the main effects and interactions, but not on interactions influenced by the time factor. As expected repeated measurement (number of leaves, stem thickness and plant height) increase over time. These plant parameters also showed no downturn at any stage of early growth and development. Thus the discussion of the results will focus on the average plant parameters for repeated measurements over the five week growth period.

Table 5.1 Summary of analyses of variance showing the effects of zinc source and application rate on growth parameters measured weekly during the first and second plantings of maize

Source ¹	Number of leaves	Stem thickness	Plant height
First Planting			
ZNS	ns	*	*
AR	ns	*	*
ZNS×AR	ns	ns	ns
W	*	*	*
W × ZNS	ns	*	ns
W × AR	ns	ns	ns
W × ZNS × AR	ns	ns	ns
Second Planting			
ZNS	*	*	*
AR	*	*	*
ZNS×AR	*	*	*
W	*	*	*
W × ZNS	*	*	*
W × AR	ns	ns	*
W × ZNS × AR	*	ns	ns

¹ZNS = Zinc source, AR = Application rate and W = Weeks after planting

*= significant ($P \leq 0.05$), ns = not significant.

Table 5.2 Summary of analyses of variance showing the effects of zinc source and application rate on plant parameters at the termination of the first and second plantings of maize

Source ¹	Leaf area	Dry mass	Root mass	Root length
	First planting			
ZNS	*	*	ns	ns
AR	*	*	*	ns
ZNS×AR	ns	ns	*	ns
	Second planting			
ZNS	*	*	ns	*
AR	*	*	ns	ns
ZNS×AR	*	*	ns	ns

¹ZNS = Zinc source, AR = Application rate and W = Weeks after planting

*= significant ($P \leq 0.05$), ns = not significant.

5.3.1 Number of leaves

Main effects

The main effects of zinc source, application rate and weeks after planting on the number of leaves for both plantings are displayed in Figure 5.1. In the first planting zinc source had no significant influence on the number of leaves. Significant differences in the number of leaves were observed however in the second planting. Soil fertilized with ZnSO₄ resulted in a higher number of leaves than soil fertilized with ZnO and ZnEDTA. Although there were no significant differences recorded in the first planting, results for the first planting show a similar trend as for the second planting.

During the first planting application rate had no significant effect on the number of leaves. This was not the case for the second planting where the number of leaves was significantly higher at AR 5 than at all other application rates. Noteworthy for both plantings is that the lowest number of fully developed leaves was counted at AR 4 which was lower than at all the other application rates.

For both plantings there was a clear increase in number of leaves over time. This increase in number of leaves differed significantly between weeks after plantings.

Interactions

Figure 5.2 illustrates the interaction effects of zinc source and application rate on number of leaves for both plantings. In the first planting the number of leaves was not affected by this interaction which was not the case for the second planting. For the latter planting soil fertilized with ZnO at AR 4 had a significant lower number of leaves than all the other treatments combinations.

The number of leaves was higher in the first planting than the second planting when comparing similar treatment combinations. Despite these differences between the first and second planting it is clear that for both plantings there was an increase in the number of leaves from AR 1 to AR 2, regardless of the zinc source applied.

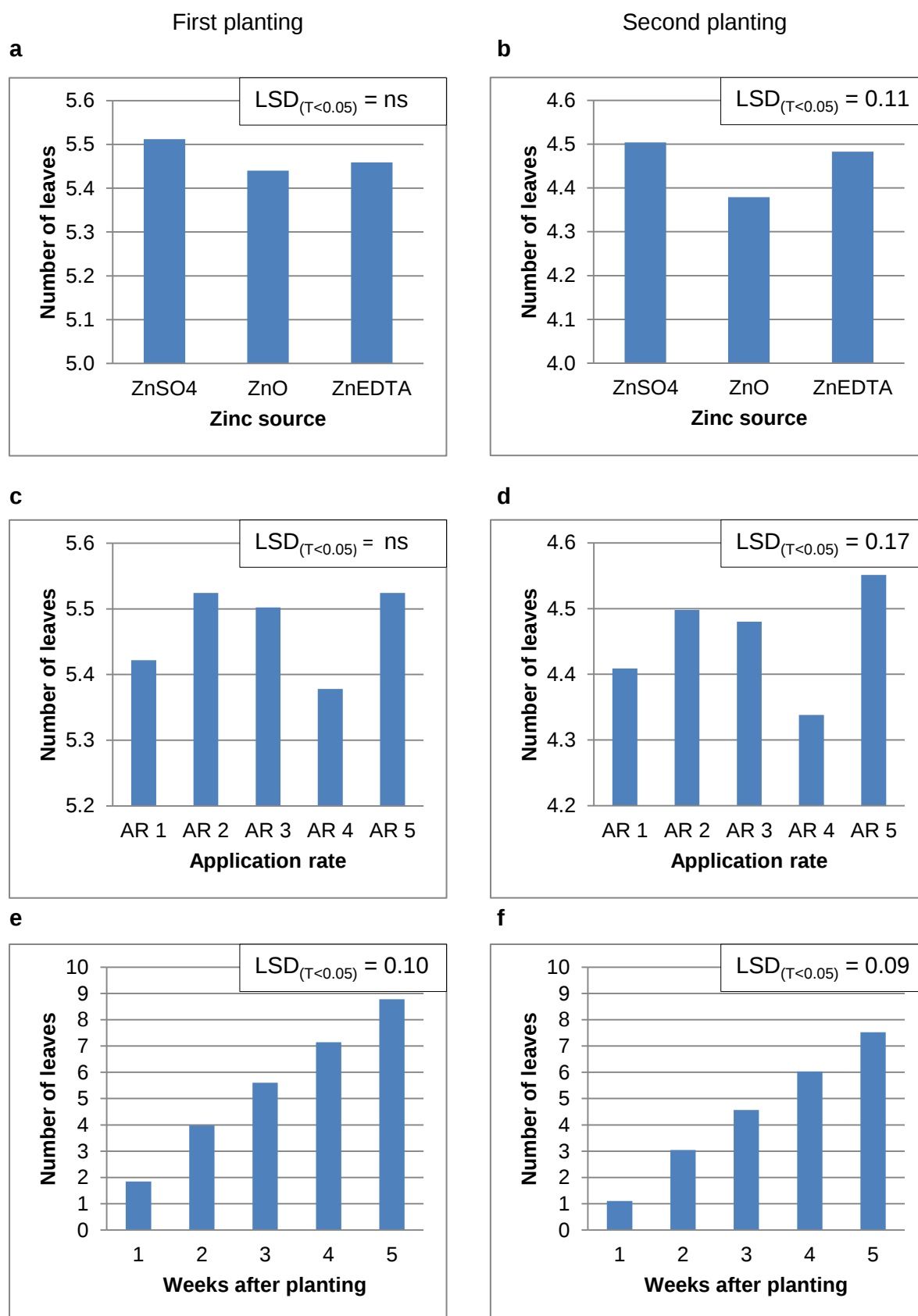
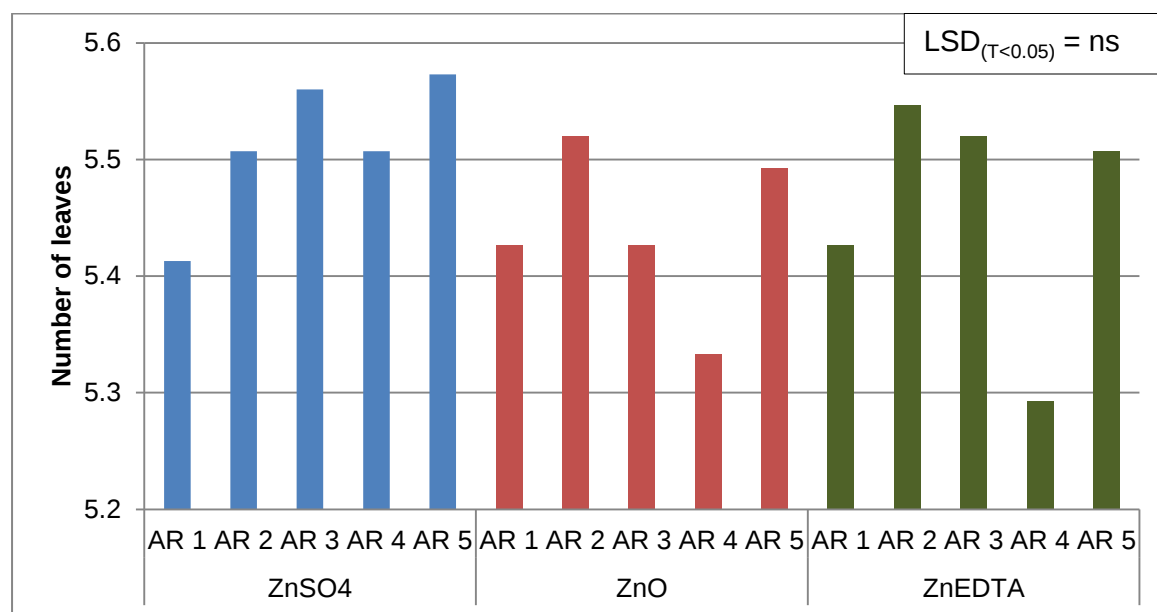


Figure 5.1 Main effects of zinc source (a and b), application rate (c and d) and weeks after planting (e and f) on the number on leaves for both plantings.

a



b

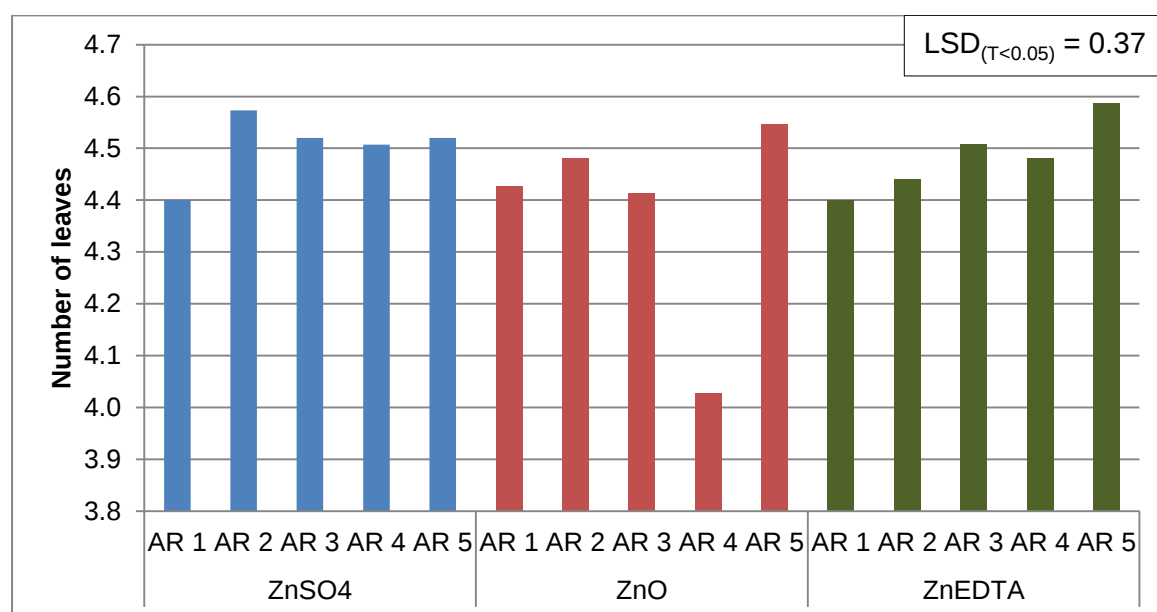


Figure 5.2 Interaction effects of zinc source and application rate on the number of leaves for the first (a) and second (b) planting.

5.3.2 Stem thickness

Main effects

The main effects of zinc source, application rate and weeks after planting on stem thickness are presented in Figure 5.3. Zinc source showed significant effects on stem thickness for both plantings. Soil treated with ZnSO_4 gave the largest stem thickness when comparing it to soil treated with the other two zinc sources. The maize grown in soil treated with ZnSO_4 had a stem thickness of 13.32 mm and 10.87 mm for the first and second plantings, respectively. This is for the first planting 0.58 mm and 0.71 mm for the second planting thicker than the stems from either ZnO- or ZnEDTA-treated soils.

The effect of application rate on stem thickness shows with analysis of variance significant differences for the first ($p = 0.010$) and second ($p = 0.017$) planting. However, when using the Tukey - Kramer LSD comparison test, for comparison between treatments, there was no significant difference between application rates. For the second planting AR 5 resulted in the largest stem thickness. For both plantings at AR 4 a clear drop in stem thickness was measured when comparing these values to those of the other application rates. A comparison of the two plantings showed the same trend of an increase in stem thickness from AR 1 to AR 2.

For both plantings the stem thickness increased with time.

Interactions

The interaction effects of zinc source and application rate on stem thickness for both plantings are shown in Figure 5.4. Stem thickness in the first planting was larger in comparison with that in the second planting. Soil treated with ZnO at AR 4 had the thinnest stems in the second planting. However, both the plantings show a similar trend in stem thickness with regard to zinc source and application rate interaction effects.

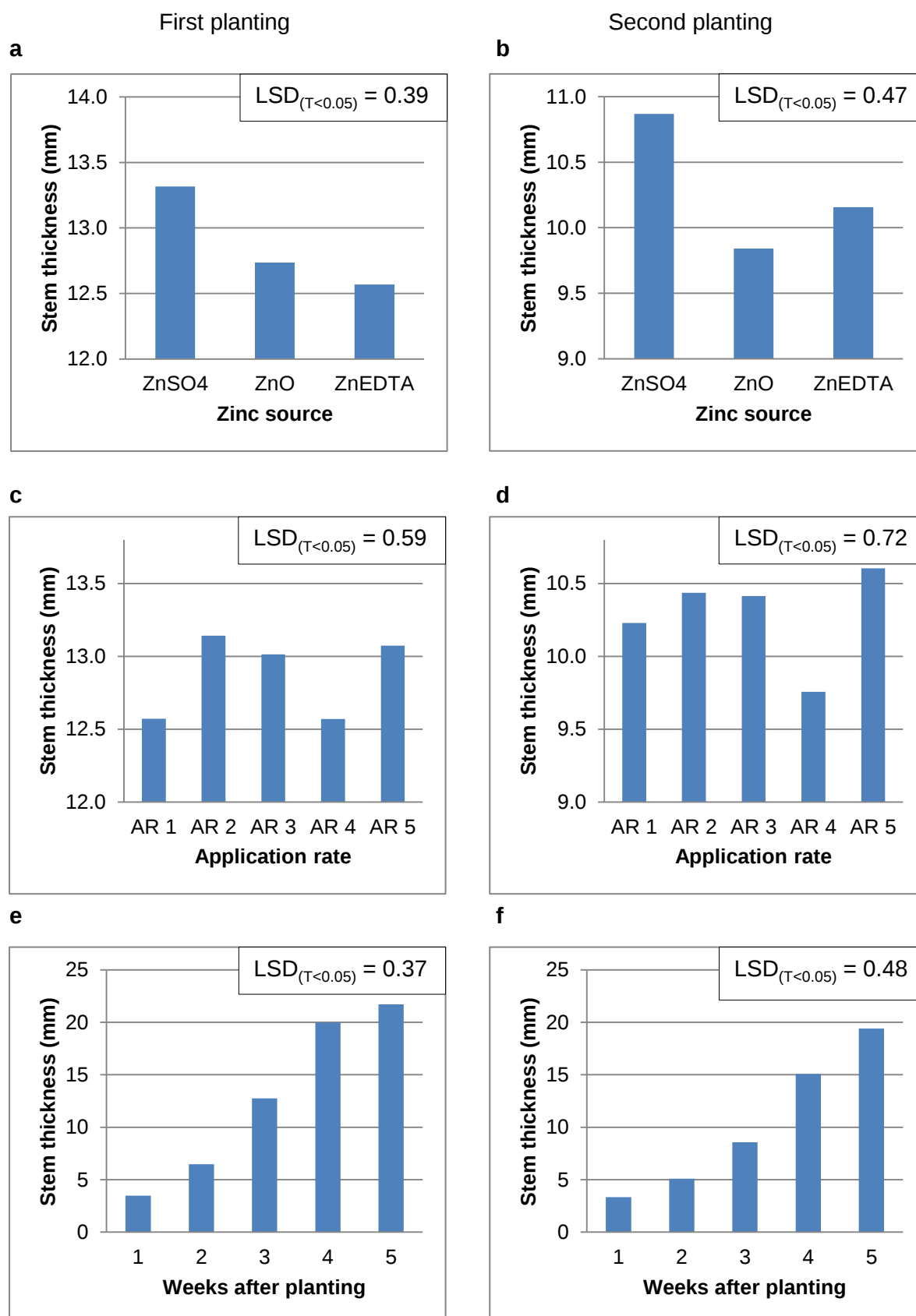
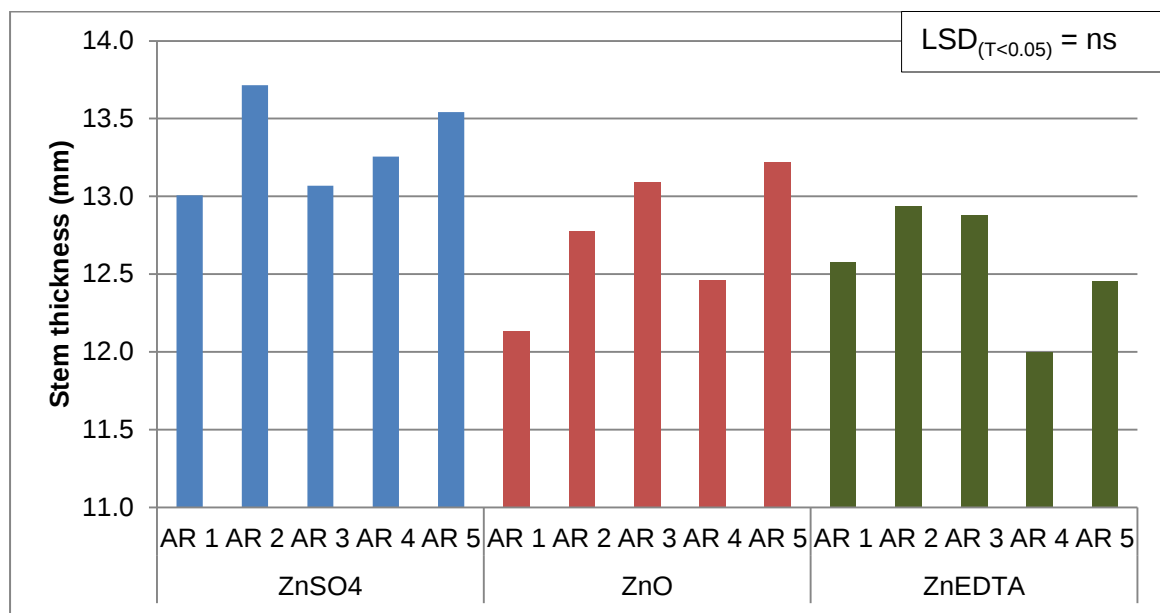


Figure 5.3 Main effects of zinc source (a and b), application rate (c and d) and weeks after planting (e and f) on stem thickness for both plantings.

a



b

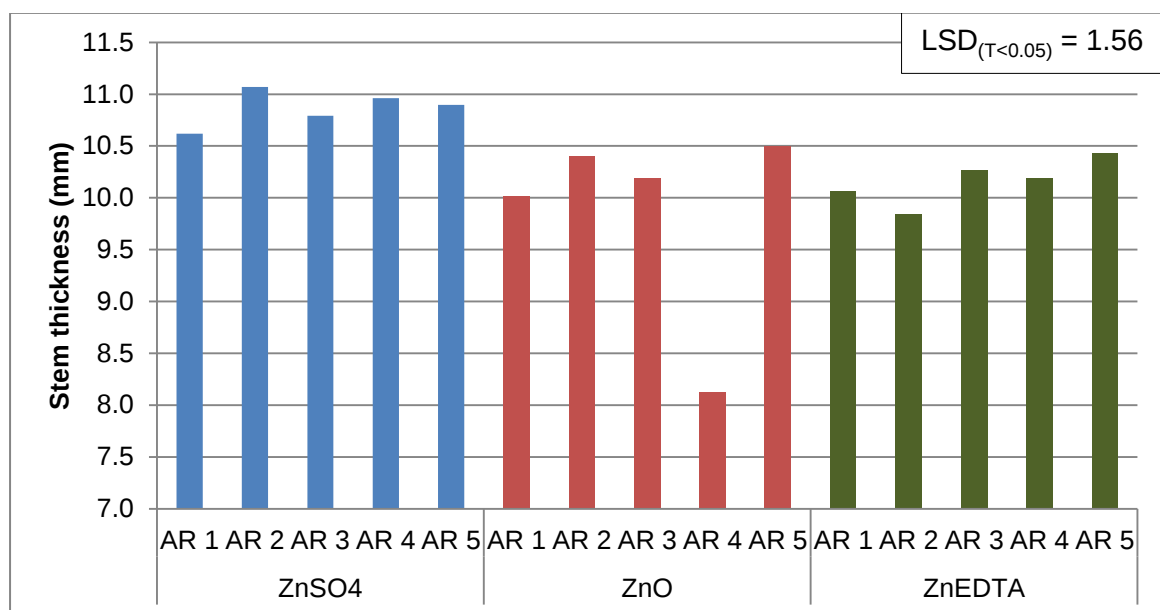


Figure 5.4 Interaction effects of zinc source and application rate on stem thickness for the first (a) and second (b) planting.

5.3.3 Plant height

Main effects

The main effects of zinc source, application rate and weeks after planting on plant height are illustrated in Figure 5.5. Plant height was affected significantly in the first ($p = 0.028$) and second ($p < 0.001$) planting by zinc source. The effect of ZnSO_4 on plant height was greater than that of ZnO and ZnEDTA for both the plantings.

The application rate influenced plant height significantly in the first ($p = 0.006$) and second ($p < 0.001$) plantings. For both plantings the tallest plants were measured at AR 5, followed by those of AR 2. Maize planted in soil fertilized at AR 5 were 0.0365 m and 0.0382 m higher than maize planted in soil fertilized at AR 1 for the first and second plantings, respectively. Although there was a difference between the two plantings, both plantings showed a similar trend in plant height with application rate.

For both plantings there were significant differences in plant height between weeks after planting. There was a clear increase in plant height over time for the first and second plantings.

Interactions

The interaction effects of zinc source and application rate on plant height for both plantings are demonstrated in Figure 5.6. This interaction had no significant effect on plant height in the first planting ($p = 0.997$). However, the plants of soil treated with ZnO at AR 4 were shorter than the plants of all other treatments in the second planting. Both plantings show a similar trend in plant height with regard to the zinc source and application rate interaction.

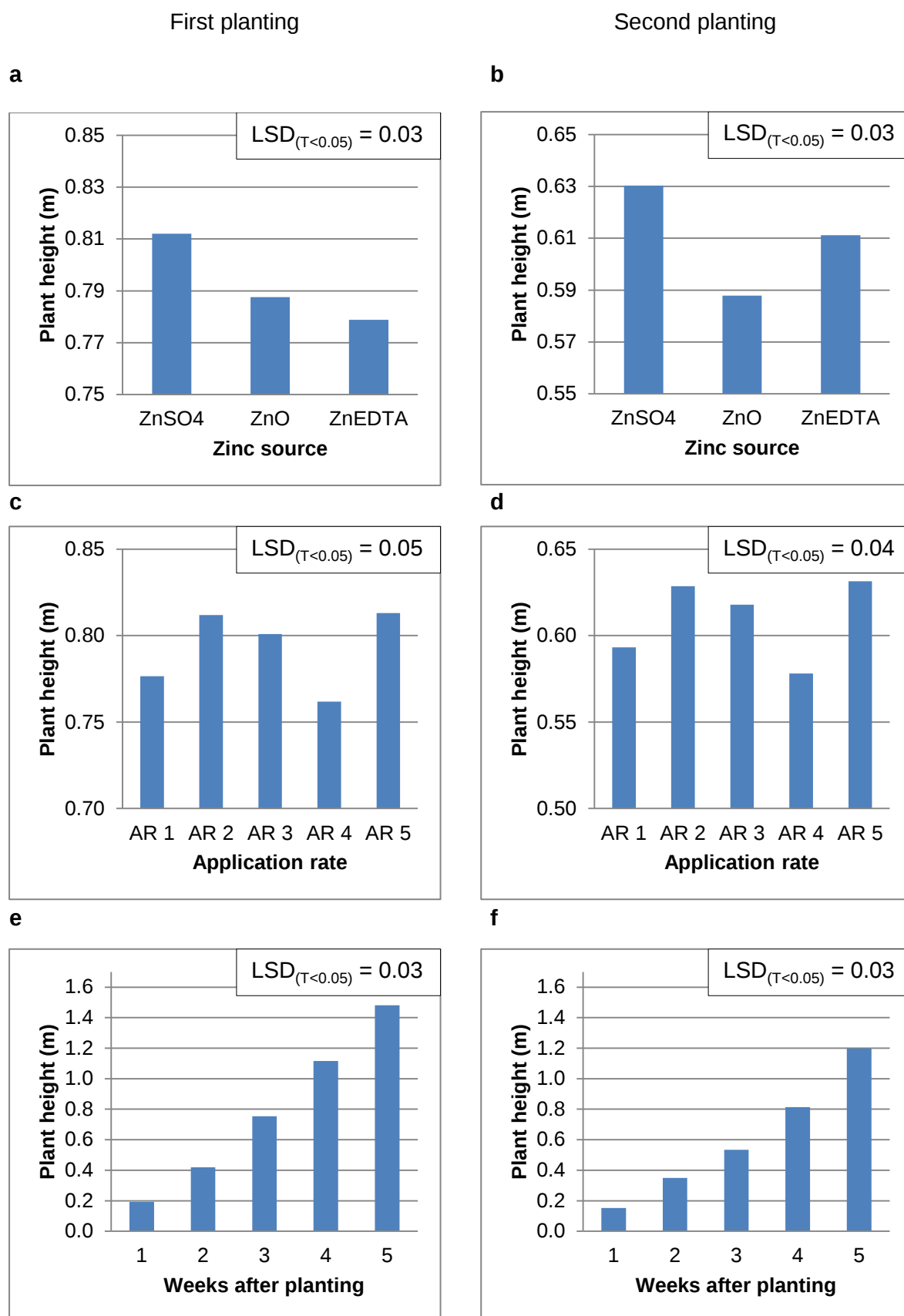
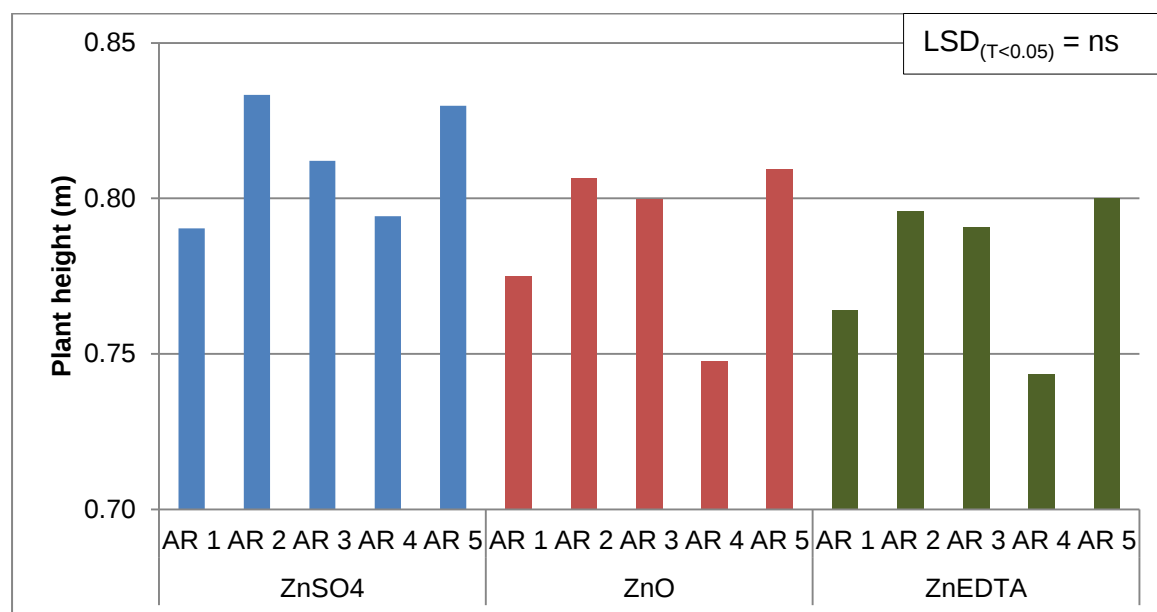


Figure 5.5 Main effects of zinc source (a and b), application rate (c and d) and weeks after planting (e and f) on plant height for both plantings.

a



b

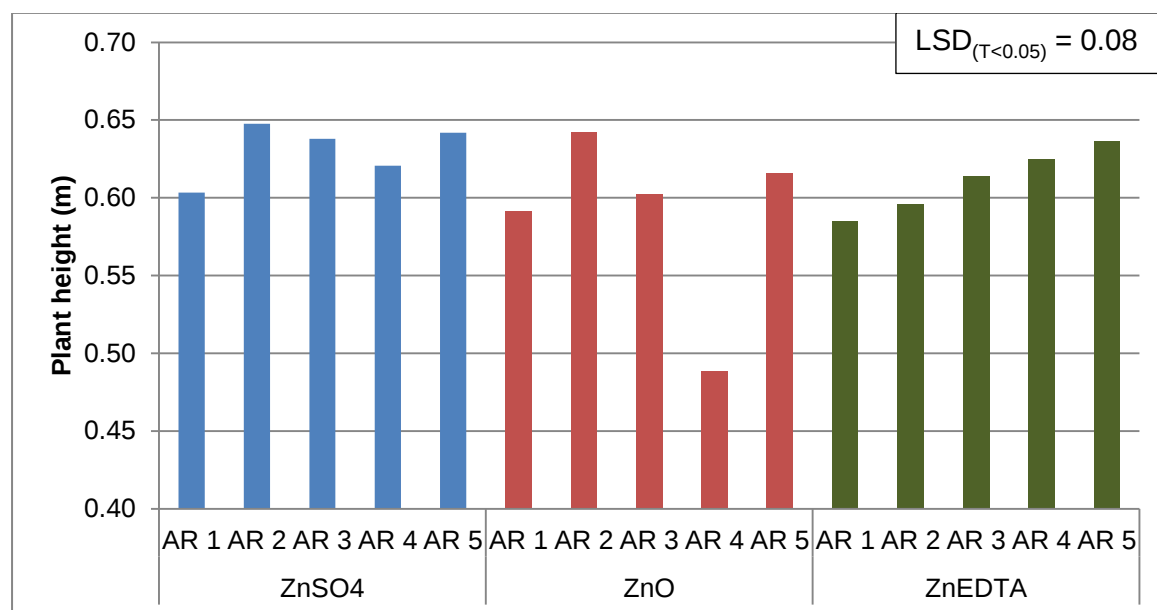


Figure 5.6 Interaction effects of zinc source and application rate on plant height for the first (a) and second (b) planting.

5.3.4 Leaf area

Main effects

The main effects of zinc source and application rate on leaf area for both plantings are shown in Figure 5.7. Significant differences in leaf area were measured due to the zinc sources used ($p < 0.001$). Soil treated with ZnSO_4 resulted in the largest leaf area for both plantings. The leaf area of plants from the ZnSO_4 -treated soil was in the first planting 9.5% and in the second planting 15.1% larger than the leaf area of plants from either the ZnO- or ZnEDTA-treated soils.

Application rate influenced leaf area significantly in the first ($p < 0.001$) and second ($p < 0.008$) plantings. For both plantings the leaf area at AR 1 and AR 4 was lower than at the other application rates. When comparing the two plantings a similar trend in leaf area is observed with respect to application rates. Although the trend is similar, the first planting produced a leaf area of about 1000 cm^2 larger than the second planting.

Interactions

The interaction of zinc source and application rate did not influence leaf area significantly in the first planting ($p = 0.887$) but in the second planting ($p < 0.001$) it did. Soil treated with ZnO at AR 4 resulted in the smallest leaf area of all the treatment combinations (Figure 5.8). Regardless of the zinc source, similar trends were observed, namely an increase in leaf area from AR 1 to AR 3, followed by a clear drop in leaf area at AR 4 where after the leaf area increase again at AR 5.

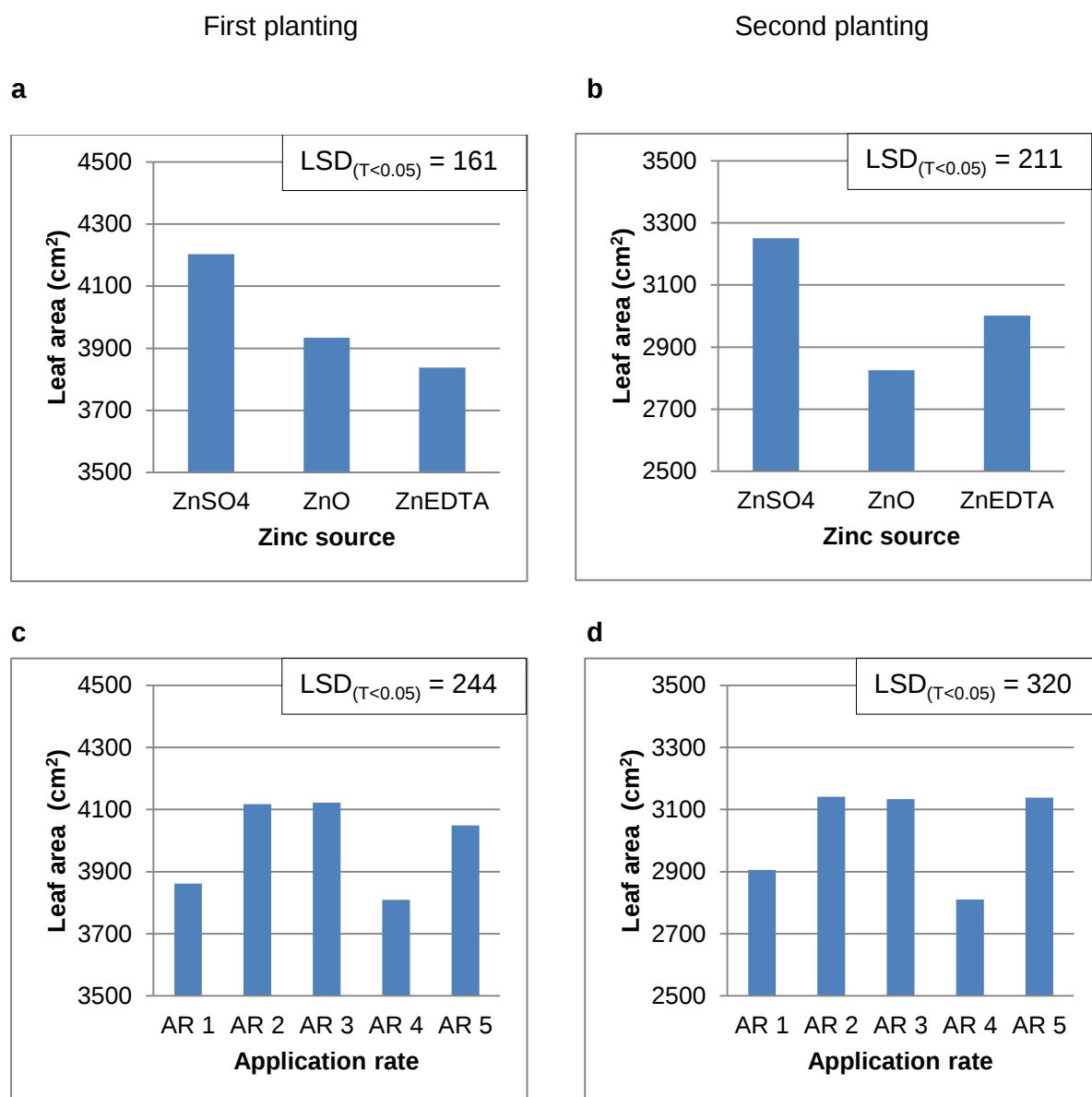
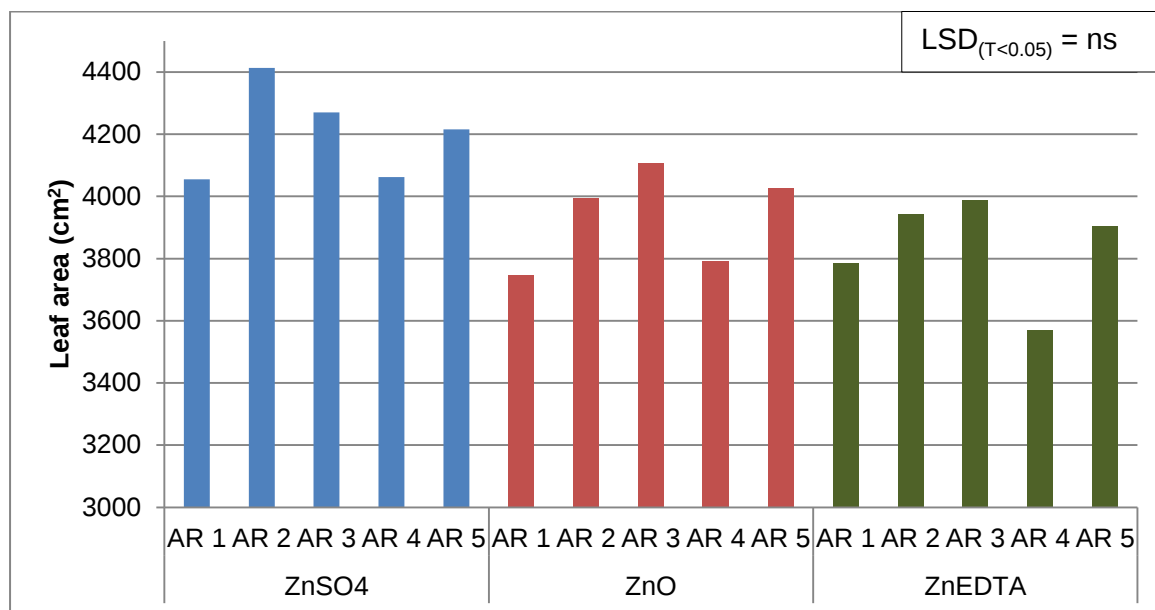


Figure 5.7 Main effects of zinc source (a and b) and application rate (c and d) on leaf area for both plantings.

a



b

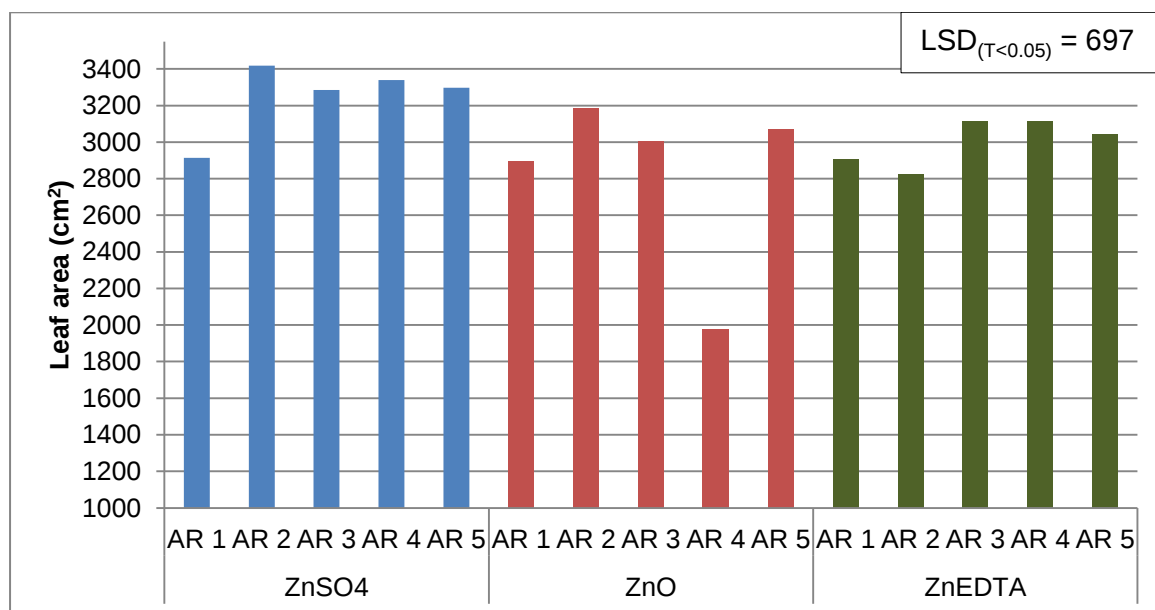


Figure 5.8 Interaction effects of zinc source and application rate on leaf area for the first (a) and second (b) planting.

5.3.5 Dry mass

Main effects

The effects of zinc source and application rate on dry mass are depicted in Figure 5.9. Zinc source affected dry mass significantly for both plantings ($p < 0.001$). A higher dry mass resulted from ZnSO_4 -treated soil than from either the ZnO - and ZnEDTA -treated soils. This difference in dry mass was about 15.7% for the first planting and 23.1% for the second planting,

The application rate influenced dry mass significantly in the first ($p = 0.020$) and second ($p = 0.011$) plantings. However, when comparing treatment means, using Tukey-Kramer LSD comparison test, there was no significant differences in dry mass between application rates for the first planting. The dry mass at AR 1 and AR 4 was clearly lower than at the other application rates. Although there is a significant difference between the plantings, both the plantings show a similar trend in dry mass regarding application rate.

Interactions

Analyses of variance calculated for the first ($p = 0.541$) and second ($p < 0.001$) plantings showed that dry mass was significantly influenced by the interaction of zinc source and application rate. Soil treated with ZnO at AR 4 resulted in the lowest dry mass of the treatment combinations (Figure 5.10). This applies for both plantings which show similar trends. For most of the zinc sources there was an increase in dry mass from AR 1 to AR 3, followed by a clear drop in dry mass at AR 4 where after the dry mass increased at AR 5.

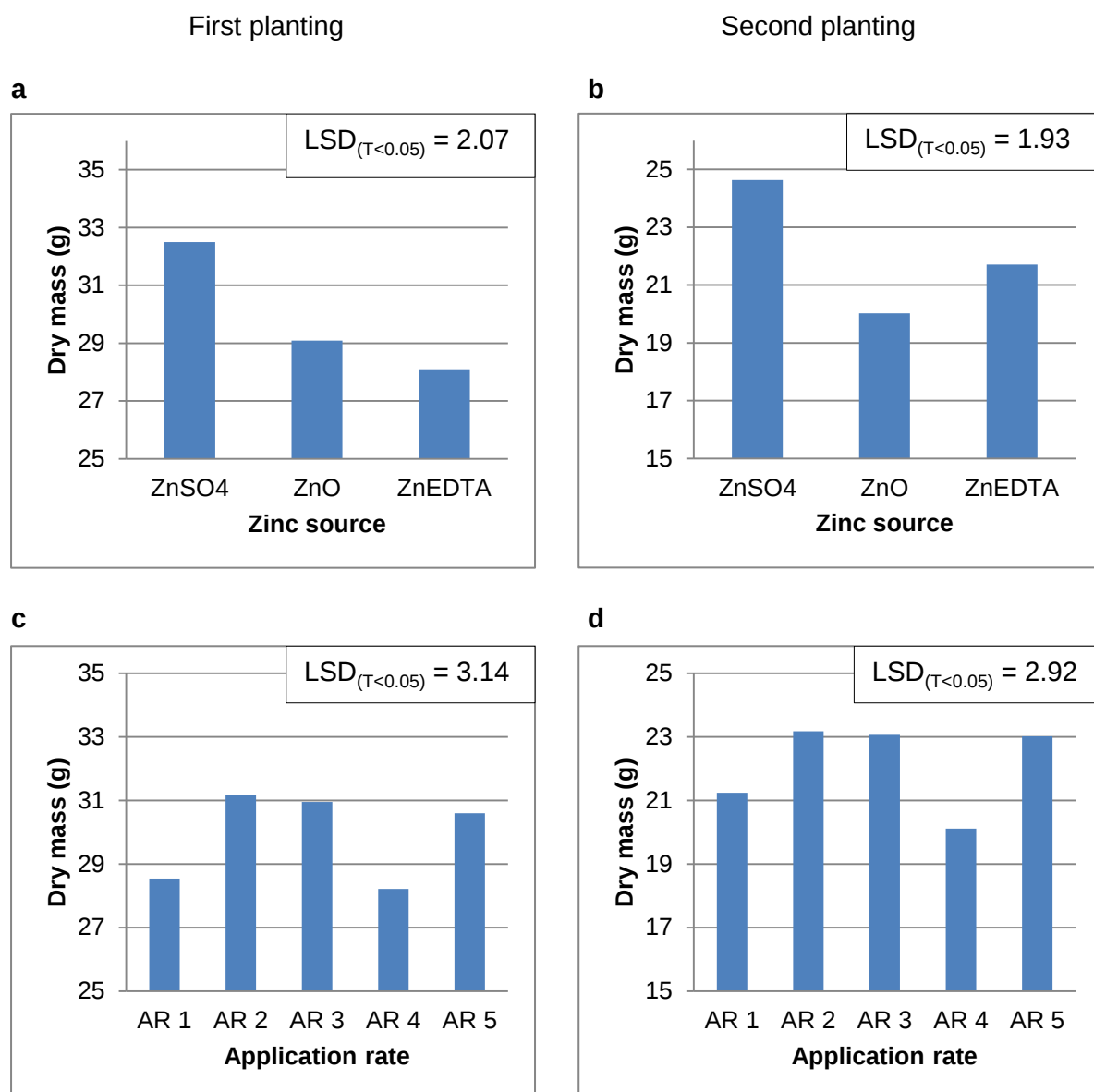
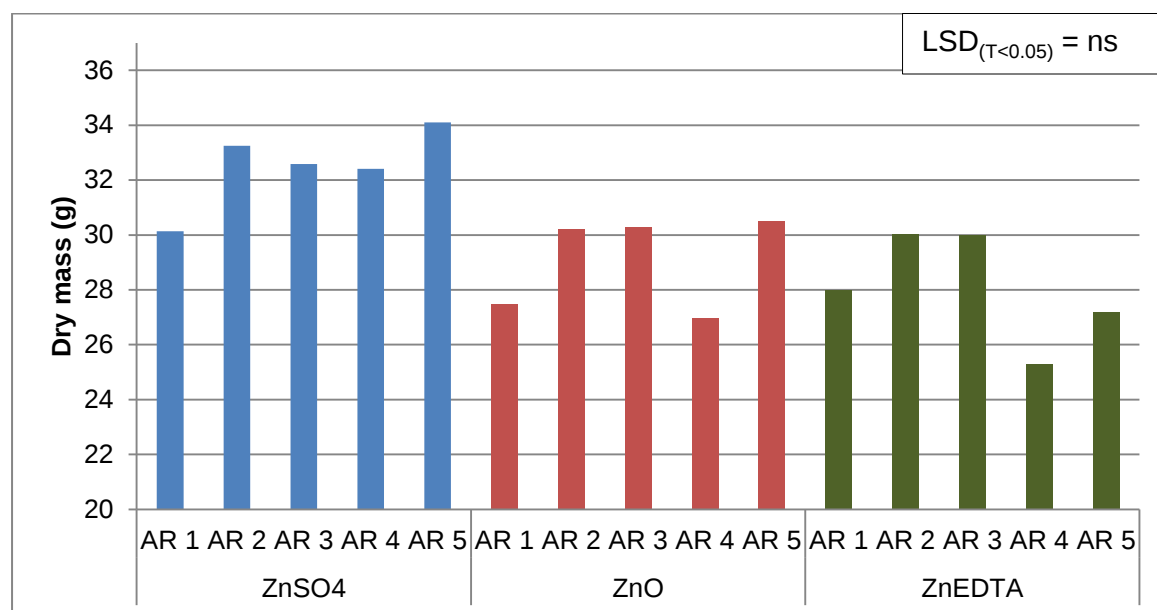


Figure 5.9 Main effects of zinc source (a and b) and application rate (c and d) and on dry mass for both plantings.

a



b

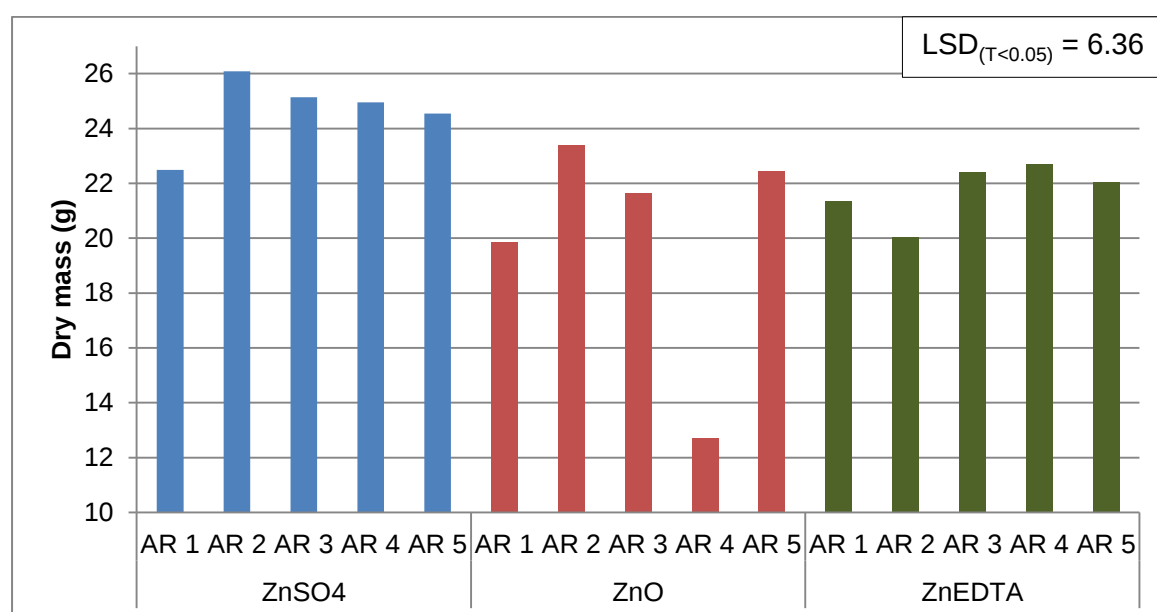


Figure 5.10 Interaction effects of zinc source and application rate on dry mass for the first (a) and second (b) planting.

5.3.6 Root mass

Main effects

The effects of zinc source and application rate on root mass are shown in Figure 5.11. Zinc source had no significant influence on root mass in the first ($p = 0.331$) and second ($p = 0.064$) plantings.

However, application rate influenced root mass significantly for the first planting ($p = 0.044$) which was not the case for the second planting ($p = 0.415$). The largest root mass for the first planting was measured at AR 5, 2.57 g higher than at AR 1. Both plantings showed a similar trend in root mass as result of the application rates employed. The root mass for both plantings was similar.

Interactions

The interaction of zinc source and application rate showed that root mass was significantly affected for the first planting ($p = 0.003$), but not for the second planting ($p = 0.678$). Soil treated with ZnO at AR 1 and AR 4 resulted in the lowest root mass for the first planting, with AR 4 the lowest for both plantings (Figure 5.12). However, soil treated with ZnO at AR 5 and ZnSO₄ at AR 5 resulted in the highest root mass for the first planting and second planting, respectively. Both plantings showed similar trends regarding root mass. For most of the zinc sources there was an increase in root mass from AR 1 to AR 3, followed by a clear drop in root mass at AR 4 where after root mass increased at AR 5.

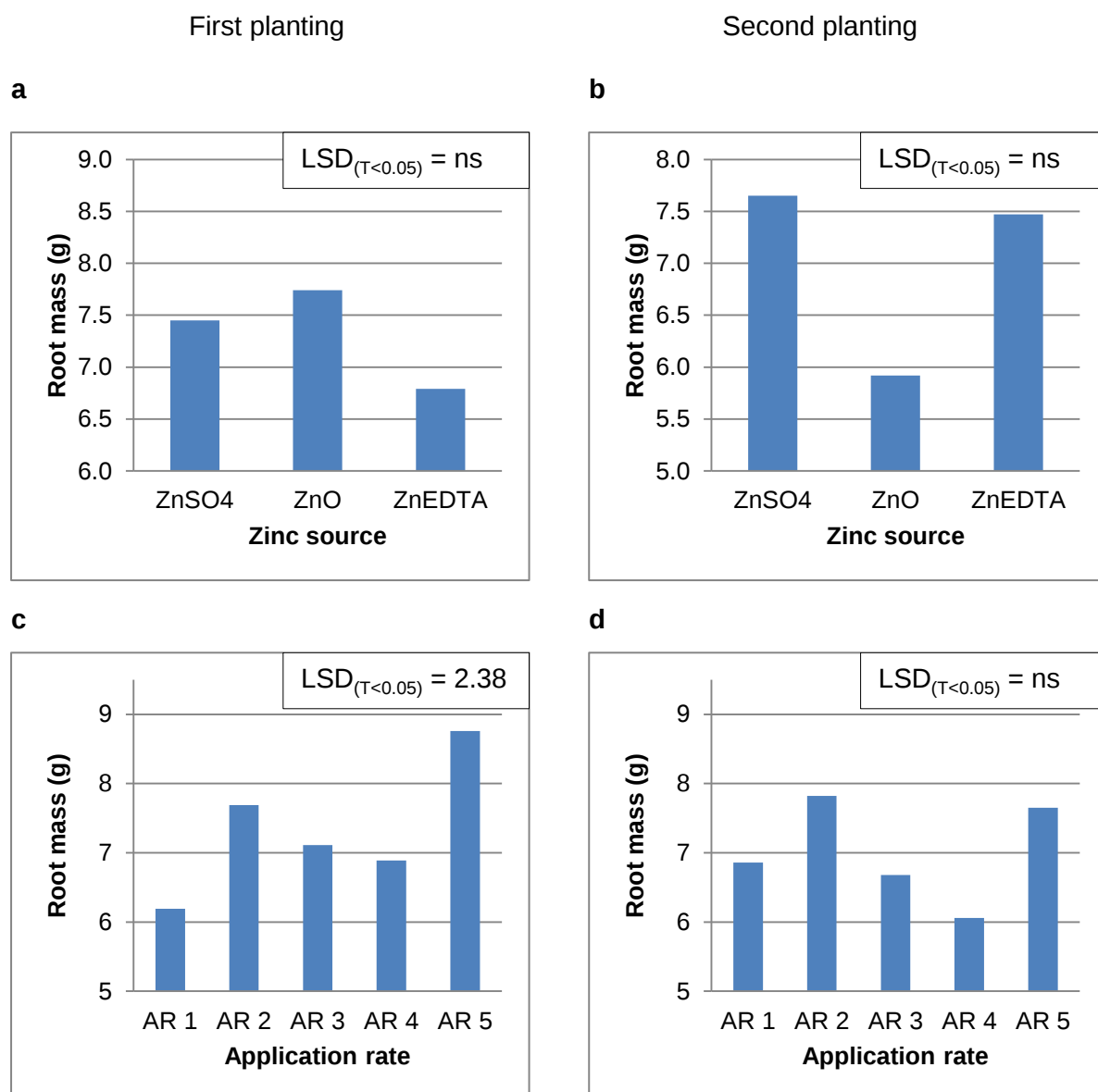
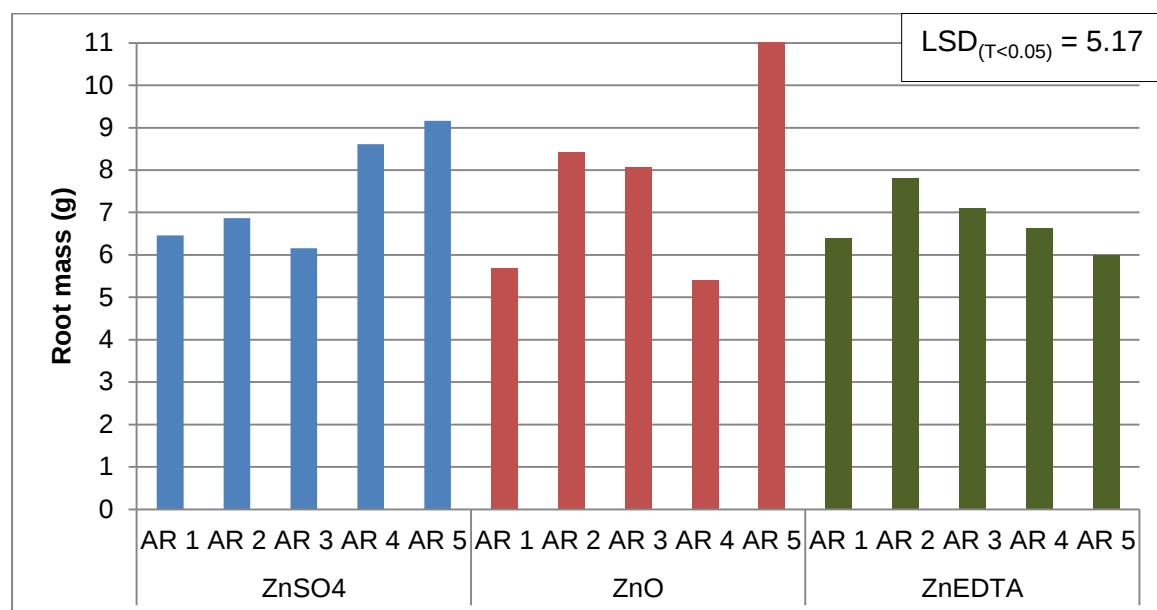


Figure 5.11 Main effects of zinc source (a and b) and application rate (c and d) on root mass for both plantings.

a



b

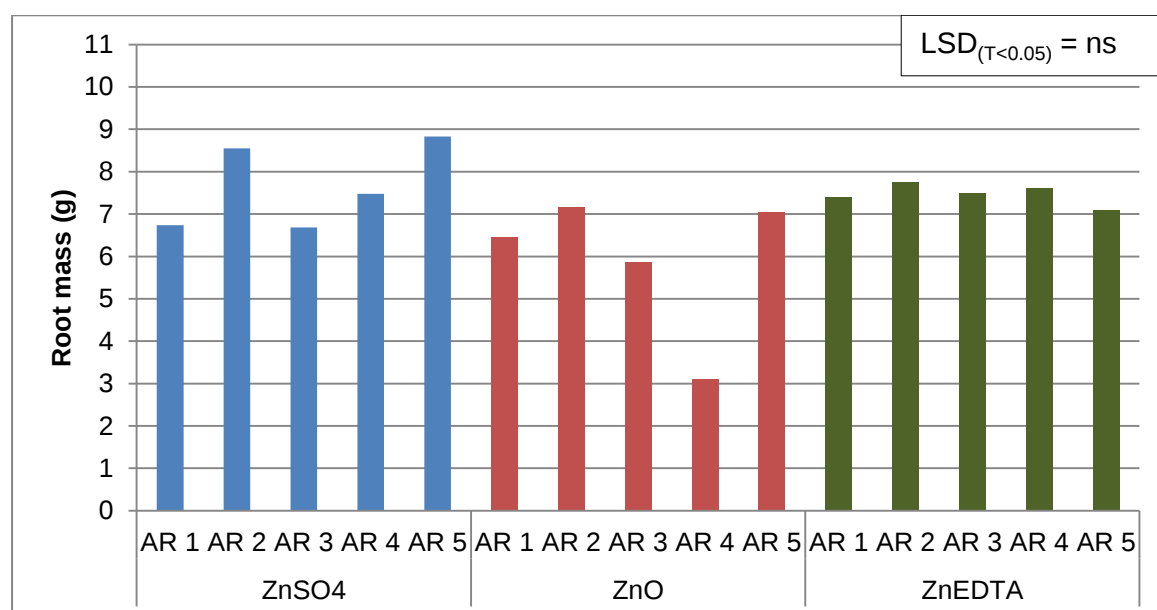


Figure 5.12 Interaction effects of zinc source and application rate on root mass for the first (a) and second (b) planting.

5.3.7 Root length

Main effects

The effects of zinc source and application rate on root length are displayed in Figure 5.13. Root length was significantly affected by zinc source for the first planting ($p = 0.018$) but not for the second planting ($p = 0.712$). Soil treated with ZnSO_4 resulted in the largest root length, followed by soil treated with ZnEDTA and then soil treated with ZnO . Although there are significant differences in root length between the two plantings, both plantings showed similar trends with regard to the zinc source used.

Application rate had no significant effect on root length for the first ($p = 0.769$) and second ($p = 0.069$) plantings. Although there are significant differences in root length between the two plantings, both plantings show similar trends in root length with regard to application rate. For both plantings there was a clear increase in root length from AR 1 to AR 2, followed by a clear drop at AR 3 where after root length increase at AR 5.

Interactions

The interaction effects of zinc source and application rate on root length for both plantings are illustrated in Figure 5.14. Analysis of variance calculated for this interaction showed no significant effects for the first ($p = 0.323$) and second ($p = 0.822$) plantings with respect to root length.

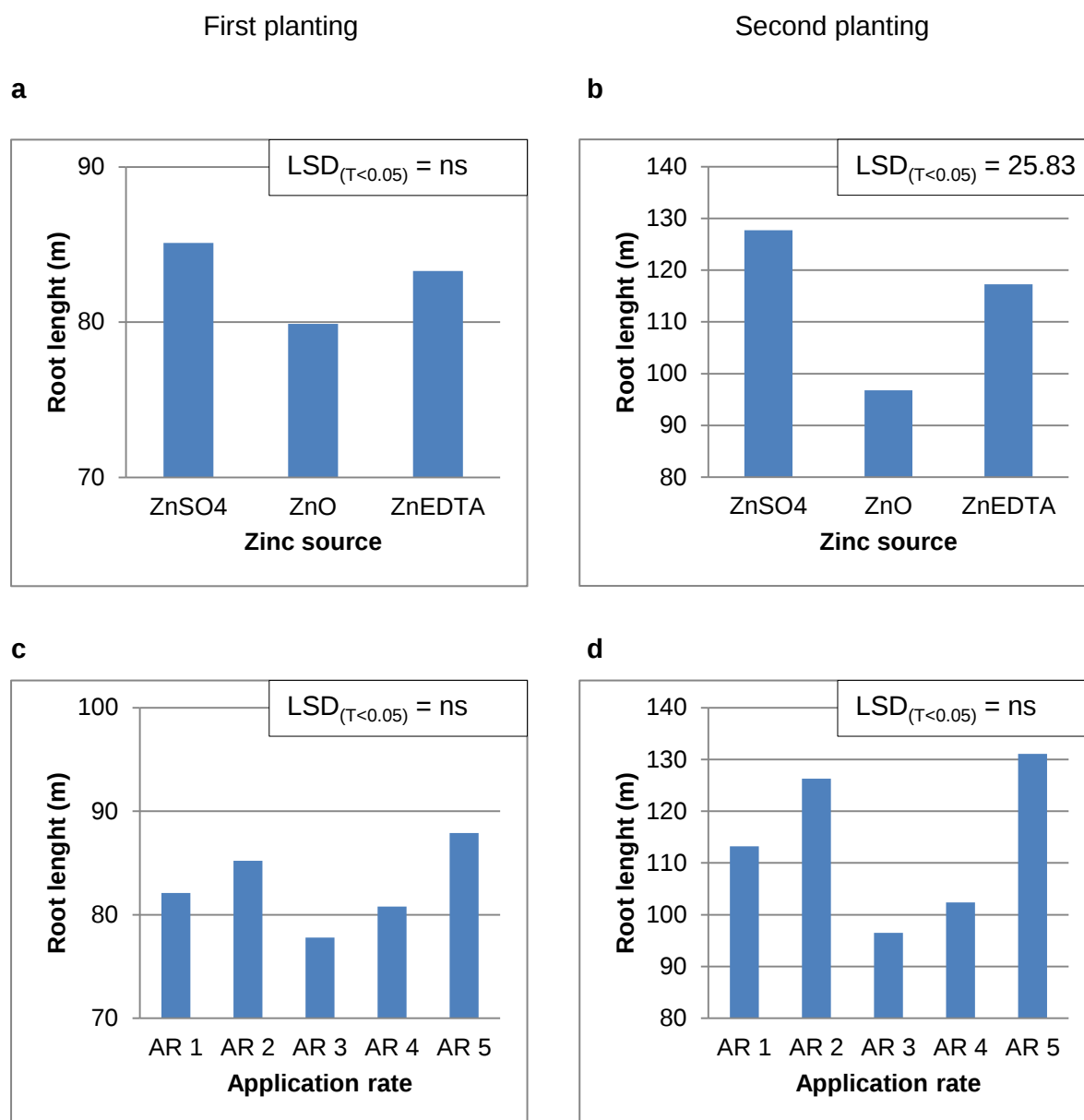
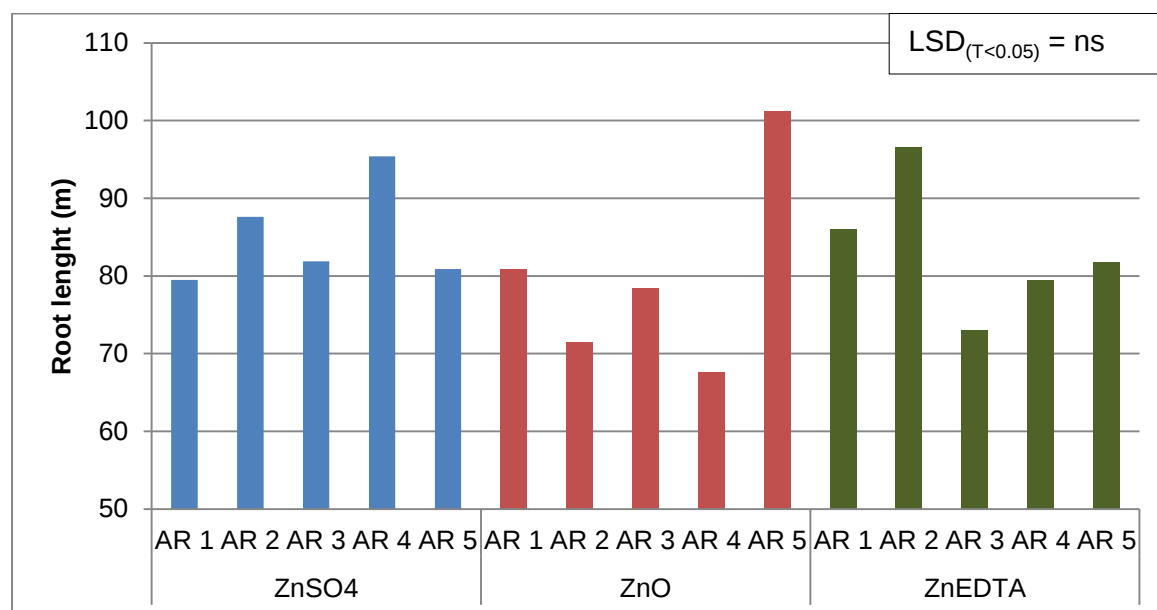


Figure 5.13 Main effects of zinc source (a and b) and application rate (c and d) on root length for both plantings.

a



b

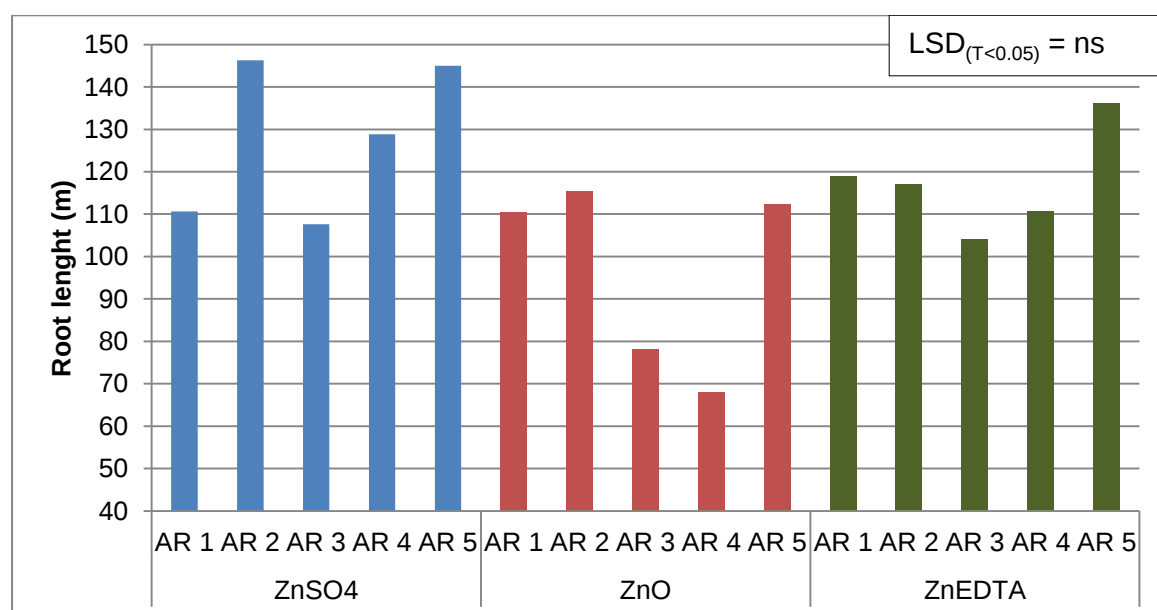


Figure 5.14 Interaction effects of zinc source and application rate on root length for the first (a) and second (b) planting.

5.4 Discussion

Research done on zinc fertilizers by Maftoun and Karimian (1989) reported a significant increase in plant height and dry matter as a result of the zinc source that was applied. This statement was confirmed by work done by Hosseini and co-workers (2007). Maftoun and Karimian (1989) also observed that plants supplied with zinc produced higher stems and leaf dry weights than those grown without zinc. The stimulation of stem and leaf growth exhibited by zinc fertilization is similar to previous reports by other researchers (Prasad & Sinha, 1981; Singh *et al.*, 1983). Maftoun and Karimian (1989) found an increase of 36% and 40% in stem and leaf dry weights, respectively when Zn levels were increased from 0 mg per pot to 20 mg per pot. Wang *et al.* (2009) reported that under well-watered conditions, the application of zinc increased dry mass of shoots and total plant weight by 78% and 52%, respectively. Research focused on the foliar application of zinc as oxysulfate showed that the grain yield of maize increased more than 2 t ha⁻¹ when fertilizer is applied at a rate of 1 kg ha⁻¹ (Potarzycki & Grzebisz, 2009).

Zinc deficiency symptoms such as stunted plants are often observed in maize plants grown in the field (Liu *et al.*, 1993). This can be a possible explanation of the smaller maize plants at AR1, since the soil in this study had a low zinc level. It is well documented by plant physiologists that zinc exerts a great influence on basic plant life processes such as nitrogen metabolism, more specific the uptake of nitrogen which influences protein quality. Photosynthesis together with chlorophyll synthesis and carbon anhydrase activity is also influenced by insufficient zinc (Alloway, 2004; Cakmak, 2008). All these processes are known to influence parameters like plant height, stem thickness and number of leaves.

Research completed on zinc fertilizer placement for maize found a positive response of root growth with the application of zinc (Zhang *et al.*, 2013). Haines (2002) also stated that little is known about the response of maize root plasticity to localized zinc enrichment in soil. Zhang *et al.* (2013) concluded that a mismatch between maize root distribution and zinc availability in soil may constrain the uptake of zinc by maize roots if there was no response to zinc enrichment. Research done on wheat showed that this crop's root growth significantly increased with zinc application (Holloway *et al.*, 2010). Results reported by Zhang *et al.* (2013) indicated a significant difference in root dry mass, root length and root surface area between the control and zinc application treatments.

When comparing zinc sources, Moraghan (1996) found that uptake of zinc by navy beans from ZnSO₄, Zn lignosulfonate and ZnEDTA was about the same when these fertilizers were mixed with the soil, but ZnEDTA was superior when band placed. Lahav and Hochberg

(1975) reported that the zinc of ZnEDTA was not fixed and remains mobile in soil. Several other workers reported that zinc chelation increase the transport and movement of zinc to plant roots (Elgawhary *et al.*, 1970). Maftoun and Karimian (1989) concluded that ZnEDTA was a more efficient source of zinc fertilizer for maize in arid regions with calcareous soils than ZnSO₄. The better response of maize to ZnEDTA might be attributed to less fixation and hence greater transport of zinc to plant roots. At equal rates of zinc application, chelates such as ZnEDTA have been shown that their zinc is more plant available than the zinc in inorganic sources like ZnSO₄ and ZnO (Wallace & Romney, 1970; Boawn 1973). This statement was not supported by the results of this study. Since ZnSO₄ was superior to ZnEDTA.

Goos and co-workers (2000) compared the availability of three zinc sources to maize under greenhouse conditions. They reported that the controls showed typical zinc deficiency symptoms and stunted growth. However, the growth response of maize to zinc depended greatly on how the zinc sources were applied. When added sources were mixed with the bulk of the soil, there was a good response to all of them, with even the lowest rate being adequate for near-maximum dry matter production. For most parameters measured in this study it was clear that there was a near-maximum response to zinc fertilizer released at low application rates.

Results published by Goos *et al.* (2000) showed that the effect of ZnEDTA on dry matter production of maize was superior to that of ZnSO₄ for the first planting but not for the second planting. It is noteworthy that the results of this study showed an increase in dry mass production when the application of ZnEDTA was raised to AR 3, followed by a decrease to AR 4 before increasing again to AR 5. This trend was observed for most of the parameters measured in this study. Maftoun & Karimian (1989) also found that stem and leaf dry weights were somewhat suppressed with higher ZnEDTA and ZnSO₄ application rates. This could possibly be due to induced P, Mn, Fe or Cu deficiencies (Maftoun & Karimian, 1989). However, application rates employed by Maftoun & Karimian (1989) were significantly higher than the application rates used in this study.

The effectiveness of various zinc sources for plant growth has been reviewed by several researchers (Wallace & Romney, 1970; Giordano & Mortvedt, 1972; Lindsay, 1972; Murphy & Walch, 1972). They reported that under glasshouse conditions, chelated forms of zinc were usually more effective than inorganic forms of zinc. Brown and Krantz (1966) noted that maize responded equally well to ZnSO₄ and ZnEDTA when broadcasted. However, when band placed, ZnEDTA was more effective than ZnSO₄.

Diddowson and Watts (1977) concluded that when ZnSO_4 and ZnEDTA are applied to calcareous soil the dry matter production of maize was similar with the two sources. However, Shukla and Morris (1967) reported that ZnSO_4 and ZnO were equally effective or superior to chelated zinc in increasing the zinc concentration and uptake of maize in a glasshouse study. Also in field studies it was shown that inorganic zinc sources increase yield of maize (Schnappinger *et al.*, 1969).

Research done by Westfall and Gilkes (1999) reported that the water solubility of fertilizers appears to be the key to zinc availability for crops on soils low or deficient in zinc. Zinc oxide has the lowest water solubility of three sources used in this study. Results show clearly that plant reaction was lowest on soil fertilized with ZnO , followed by soil fertilized with ZnEDTA and the ZnSO_4 . However, depending on soil type (i.e. soil with a pH below 5.5) or other considerations, water insoluble fertilizers such as ZnO can be equally effective in improving plant growth when soil is zinc deficient (Alloway, 2004).

No good reason(s) could be found in literature for the poor plant reactions of maize at AR 4. However, it can be speculated that the addition of zinc to soil at higher rates could lead to an increasing availability of zinc to the maize plants. When the addition of zinc exceeded a critical rate the effects of adsorption were superior which could lead to a decrease in plant reaction. Adsorption plays a very important role in the cycling of zinc in the soil. Several mechanisms control the concentration of zinc in the soil solution, and hence the amount of zinc which is immediately available to plants roots, and also amounts of zinc in labile forms which can be desorbed and become available to plants (Alloway, 2004). The mechanisms involved in the adsorption of zinc ions on solid surfaces include: cation exchange, specific adsorption, binding to organic matter, chemisorption and precipitation (Alloway, 2004).

5.5 Conclusion

Zinc source and application rate had a significant effect on both the aerial and subsoil growth parameters of maize that were measured during both the plantings. For most parameters ZnSO_4 was superior, followed by ZnEDTA and ZnO . Thus the choice of zinc source proved to be of great importance for the enhancement of the vegetative growth and development of maize in the particular sandy soil. Regardless of the zinc source used, the reaction of the maize plants as indicated by most of the measured aerial and subsoil growth parameters improved from an equivalent application rate of 0 mg Zn kg^{-1} to 1 mg Zn kg^{-1} . However, for unknown reason(s) a further increase of zinc application to 2 mg kg^{-1} inhibited plant reaction which was not the case with a 4 mg kg^{-1} zinc application. This phenomenon warrants further investigation. The reaction of the maize plants to the different treatment combinations was better for the first planting than for the second planting, probably on account of shorter daylight hours for the second planting than the first planting.

CHAPTER 6

EFFECT OF ZnSO_4 , ZnO AND ZnEDTA APPLICATION ON THE ZINC CONTENT OF A SANDY SOIL AND MAIZE PLANTS

6.1 Introduction

Application of zinc sources at different rates has usually an influence on the plant available zinc content of soil as well as the concentration and uptake of zinc by maize. Quantification of such response is therefore essential in the evaluation of the efficiency of zinc fertilizers.

To determine plant available zinc in soil, a wide range of extractants that differ in chemical composition are used. Researchers who developed these extractants anticipated that it would lead to better estimation of plant available zinc in soil. After many studies there is still no agreement as to which extractant most accurately estimates the labile, or the bioavailability, of zinc (Leleyter *et al.*, 2012).

Although soil analysis can be used to evaluate the response of soil to zinc fertilizer, analysis of the maize plant will help explain this influence better. The knowledge of nutrients actually assimilated by the plants, and their concentration in the actively growing tissue are of decisive importance for growth and development of plants (Bergmann, 1992). The uptake of zinc takes place primarily through the absorption of Zn^{2+} from the soil solution by the roots (Alloway, 2004). There is considerable disagreement in the literature as to whether Zn uptake is active or passive (Mengel & Kirkby, 1987).

The form in which zinc is translocated from the roots to the upper part of the plant is not known (Mengel & Kirkby, 1987). Tiffen (1967) reported that zinc is slightly cathodic in tomato exudates and concluded that it is not translocated as citrate, as zinc citrate complexes are anodic. The translocation of zinc in plants is not good (Mengel & Kirkby, 1987). When zinc supply is high, zinc accumulates in root tissues. Especially in older leaves zinc becomes very immobile (Rinnie & Langston, 1960). The rate of transport to younger tissues is particularly inhibited in zinc deficient plants (Lonergan, 1975).

A nutrient interaction occurs when the supply of one nutrient affects the absorption and/or utilization of another nutrient (Morris *et al.*, 2007). Therefore, nutrient interactions may occur either in the soil, at the root surface or within the plant and can accordingly be categorized into two major categories. The first category describes interactions that occur as a result of the chemical bond that develops between ions (Fageria, 2001). The second category describes the interaction between those ions with similar chemical properties. These ions will

consequently compete for the site of adsorption, absorption, transport and function on plant root surfaces and/or within plant tissue (Robson & Pitman, 1983; Marschner, 1995; Fageria, 2001). Nutrient interactions may be positive (synergistic), negative (antagonistic) or may even have no effect on each other at all (Fageria, 2001).

In this study the focus will therefore be to establish how the application of zinc sulphate, zinc oxide and zinc EDTA at different rates affected the plant available zinc content in sandy soil and also the zinc concentration and uptake in/by maize plants after a five week growing period.

6.2 Procedure

Two pot experiments were conducted in the 2013/2014 growing season. Maize was planted in a glasshouse at the University of the Free State using a two factor, randomized block design. Only soil B was used for this experiment; see Table 3.1 for soil properties. This soil was air-dried and sieved before the application of fertilizers. The relevant zinc fertilizer (ZnSO_4 , ZnO and ZnEDTA) together with H_3PO_4 and NH_4NO_3 as phosphorus and nitrogen sources were dissolved in 20 L of water and sprayed over sufficient soil for a treatment. Soil was treated to increase the zinc content with 0 mg kg^{-1} , 0.5 mg kg^{-1} , 1 mg kg^{-1} , 2 mg kg^{-1} and 4 mg kg^{-1} (AR 1 - AR 5), and 20 mg kg^{-1} N and 25 mg kg^{-1} P per treatment.

At termination of the experiment four evenly spaced soil samples were taken over the full depth of the pot. Samples were thoroughly mixed, air-dried and sieved for the preparation of analysis. These samples were used for zinc and phosphorus (Ambic II) analysis. Soil samples were analysed for plant available zinc using: HCl, Mehlich I, DTPA-TEA, NaEDTA and Ambic II extraction methods. Every plant sample consisted of all three maize plants per pot. After plant samples were oven-dried, they were finely milled mechanically for the preparation of chemical analysis. The milled samples were used for the determination of N, P, K, Ca, Mg, Cu, Fe, Mn, Zn and B.

For the full discussion on materials and methods see Section 3.3.

6.3 Results

At the termination of the glasshouse study both plantings showed similar results in measured soil zinc content ($p = 0.669$). However results will be presented separately for the first and second planting, hence the discussions in previous chapters. For both plantings application rate accounts for the most variation in zinc content, followed by extraction method and zinc source.

Analysis of variance on the zinc content of the first planting (Table 6.1) showed significant differences ($p < 0.01$) for all main effects, viz. extraction method, zinc source and application rate. Interaction between zinc source and application rate also showed significant differences ($p < 0.01$). Similar outcomes were obtained by analysis of variance on the zinc content of the second planting, with the exception to the significant difference found between the interaction of extraction method and application rate (Table 6.1).

Soil samples were also used for phosphorus analysis. Analysis of variance showed no significant difference in phosphorus content between samples ($p > 0.05$). Therefore, the effect of zinc source and application rate on this nutrient will not be discussed.

Concerning plant analysis, data showed significant differences between the first- and second plantings. Results will therefore be presented separately for the first and second plantings. Analysis of variance on the zinc concentration in plant material of the first planting (Table 6.2) showed significant differences ($p < 0.01$) for all main effects, viz. zinc source and application rate. Interaction between zinc source and application rate showed no significant differences ($p = 0.09$). Analysis of variance of the second planting showed similar results, however zinc source showed no significant differences on zinc concentration in plant material for the second planting (Table 6.2). Analysis of variance on zinc uptake by maize plants of the first and second planting (Table 6.2) showed significant differences ($p < 0.01$) for all main effects as well as the interaction between zinc source and application rate for both plantings.

The focus of this section will therefore be on the main effects, as well as interactions, concerning each planting's soil plant available zinc content as well as the zinc concentration and uptake in/by maize plants. Firstly, the influence of the main effects and interactions on the plant available zinc content in soil of both plantings will be addressed, followed by the influence of the main effects and interactions on the zinc concentration in maize plants and the uptake of zinc by maize plants.

Table 6.1 Summary of analyses of variance showing the effects of extraction method, zinc source and application rate on zinc content in soil at the end of the five week growing period for the first and second plantings of maize

Source ¹	First Planting	Second planting
EXM	*	*
ZNS	*	*
AR	*	*
ZNS*AR	*	*
EXM*ZNS	ns	ns
EXM*AR	ns	*
EXM*ZNS*AR	ns	ns

¹EXM = Extraction method, ZNS = Zinc source and AR = Application rate

*= significant ($P \leq 0.05$), ns = not significant.

Table 6.2 Summary of analyses of variance showing the effects of zinc source and application rate on zinc concentration and zinc uptake at the end of the five week growing period for the first and second plantings of maize

Source ¹	Zinc concentration	Zinc uptake per pot
First planting		
ZNS	*	*
AR	*	*
ZNS × AR	ns	*
Second planting		
ZNS	ns	*
AR	*	*
ZNS × AR	ns	*

¹ZNS = Zinc source and AR = Application rate

*= significant ($P \leq 0.05$), ns = not significant.

6.3.1 Plant available zinc content of a sandy soil

6.3.1.1 Main effects

The main effects of zinc source, application rate and extraction method on the plant available zinc content of the sandy soil for both plantings are displayed in Figure 6.1.

The average zinc content of the soil at the termination of the experiment for both plantings was similar. For both plantings soil fertilized with ZnSO_4 was superior in zinc content, followed by ZnEDTA and ZnO . Despite these differences between the zinc sources, the plant available zinc content resulted with either ZnSO_4 or ZnO were similar for both plantings, but this was not the case with ZnEDTA .

The effect of application rate on zinc content was the same for the first and second plantings. Both showed an increase in zinc content from AR 1 to AR 5. Note the sharp increase in gradient due to that the application rate that was doubled every time.

The zinc extraction methods led to different zinc contents, however, both plantings showed similar trends. Soil extracted by the HCl extraction method resulted in the highest zinc content followed by extraction methods: Ambic II, EDTA, Mehlich I and DTPA for both the plantings. It is noteworthy that differences between the highest and lowest values is approximately $1.31 - 1.49 \text{ mg kg}^{-1}$ which is large considering the critical ranges for this micronutrient.

6.3.1.2 Interactions

Zinc source and application rate

The interaction effects of zinc source and application rate on the plant available zinc content of the sandy soil for the first and second plantings are presented in Figure 6.2.

Both the first and second plantings showed similar results. For ZnSO_4 and ZnEDTA there was a clear increase in zinc content from AR 1 to AR 5. However, soil fertilized with ZnO at AR 3 showed a drop in zinc content for both plantings. Of the three zinc sources, ZnSO_4 was superior in increasing the zinc content, followed by ZnEDTA and ZnO for both the plantings. For both plantings soil fertilized at AR 1 to AR 2 with all zinc sources showed a slight increase in zinc content, this was not the case with the increase in zinc content in soil fertilized at AR 4 to AR 5.

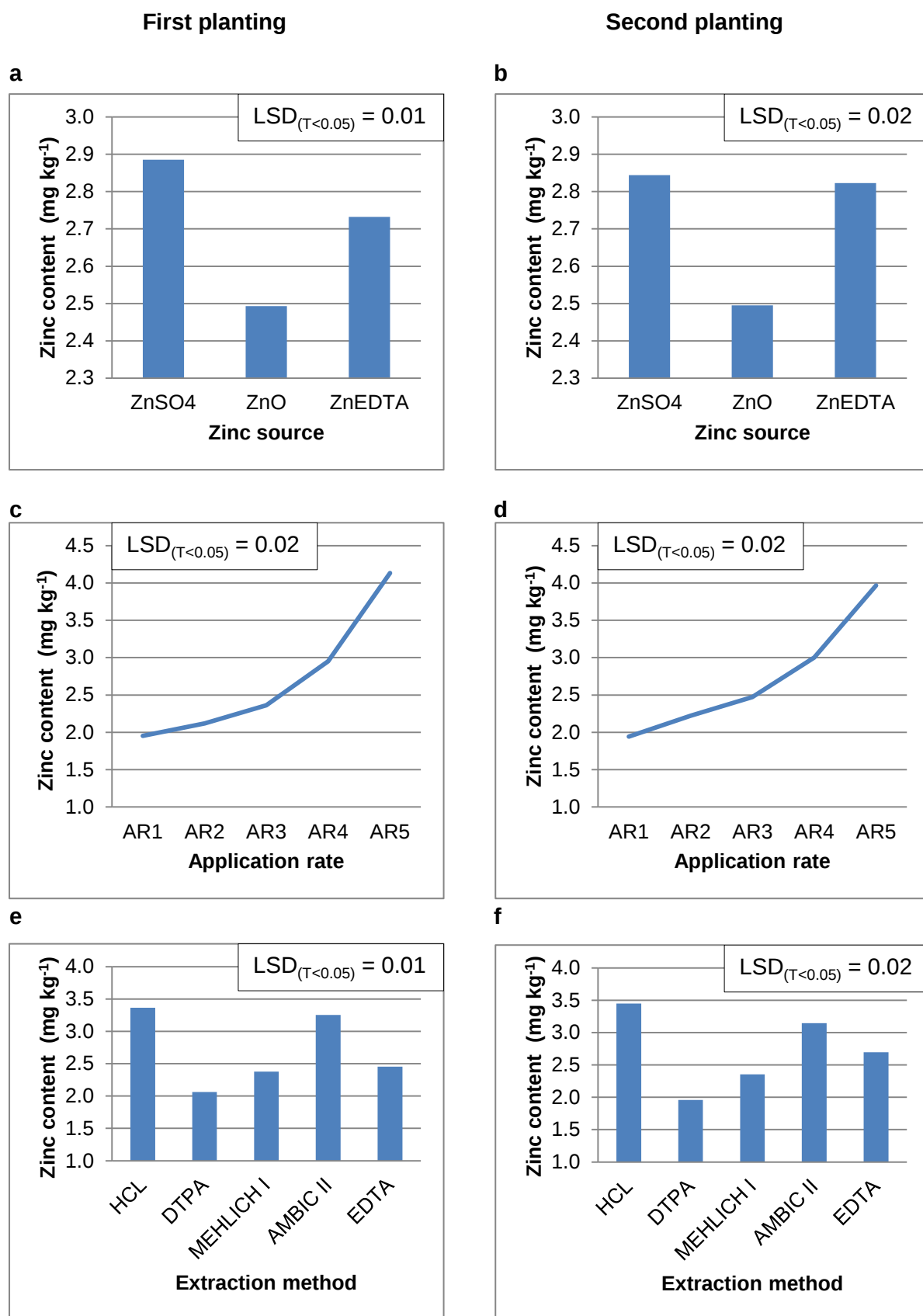
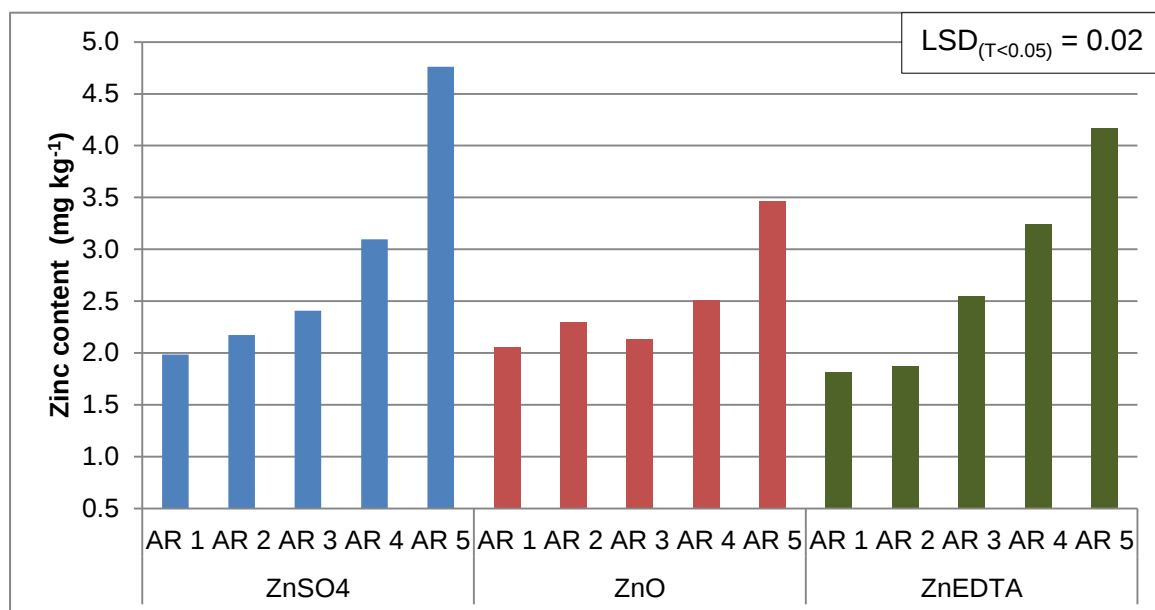


Figure 6.1 Main effects of zinc source (a and b), application rate (c and d) and extraction method (e and f) on the average plant available zinc content of the sandy soil.

a



b

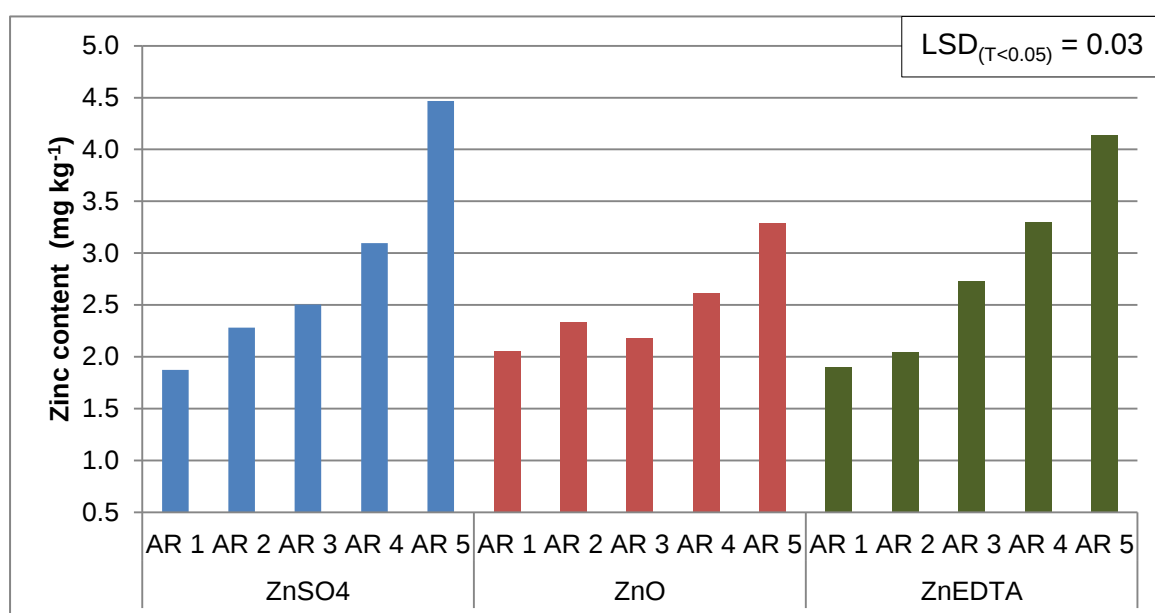


Figure 6.2 Interaction effects of zinc source and application rate on the average plant available zinc content of the sandy soil for the first (a) and second (b) planting.

Extraction method and zinc source

The interaction effects of extraction method and zinc source on the plant available zinc content of the sandy soil for the first- and second planting are presented in Figure 6.3.

The ZnSO₄-treated soil for the first planting analysed with the HCl method resulted in the highest zinc content. This zinc content was higher than all the other interactions between extraction method and zinc source. For each of the extraction methods, soil treated with ZnSO₄ gave the highest zinc content, followed by the ZnEDTA- and ZnO-treated soils. Although there are differences between soil treated with different zinc sources and the extraction method used to determine plant available zinc, analysis of variance showed no significant differences ($p = 0.72$) between interactions for the first planting.

The effect of extraction method and zinc source for the second planting showed similar results when comparing to the first planting. However, soil treated with ZnEDTA analysed with the HCl and Mehlich I methods resulted in the highest zinc content for the specific extraction method. Although there were differences between soil treated with different zinc sources and extraction method used to determine plant available zinc, analysis of variance showed no significant differences ($p = 0.31$) between interactions for the second planting also.

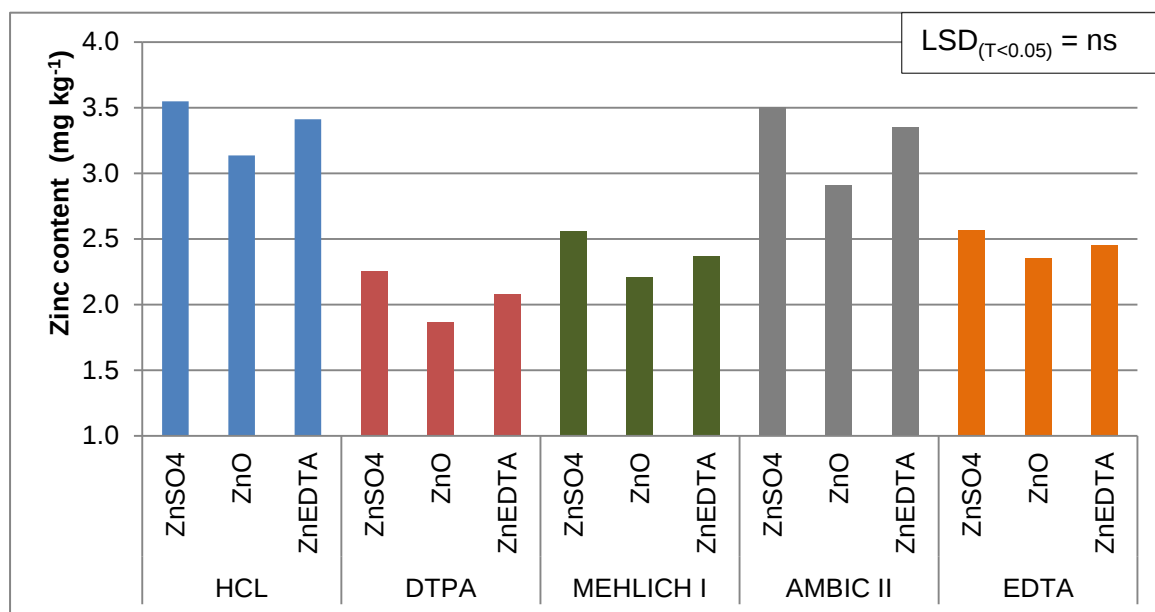
Extraction method and application rate

The interaction effects of extraction method and application rate on the plant available zinc content of the sandy soil for the first- and second planting are presented in Figure 6.4.

The zinc content of the first planting, regardless of the extraction method used, increased almost linear when the application of zinc increased from AR 1 to AR 5. The exception was when soil treated at AR 1 was analysed with the EDTA method. However, when comparing equivalent application rates, slight differences between the extraction methods are observed concerning their ability to extract zinc. It seems that the DTPA method was the least effective, followed by the EDTA, Mehlich I, Ambic II and HCl methods.

For the second planting, a similar pattern as for the first planting evolved. It also shows a similar increase in zinc content for all extraction methods from AR 1 to AR 5. There is also a clear difference between the extraction methods' ability to extract zinc.

a



b

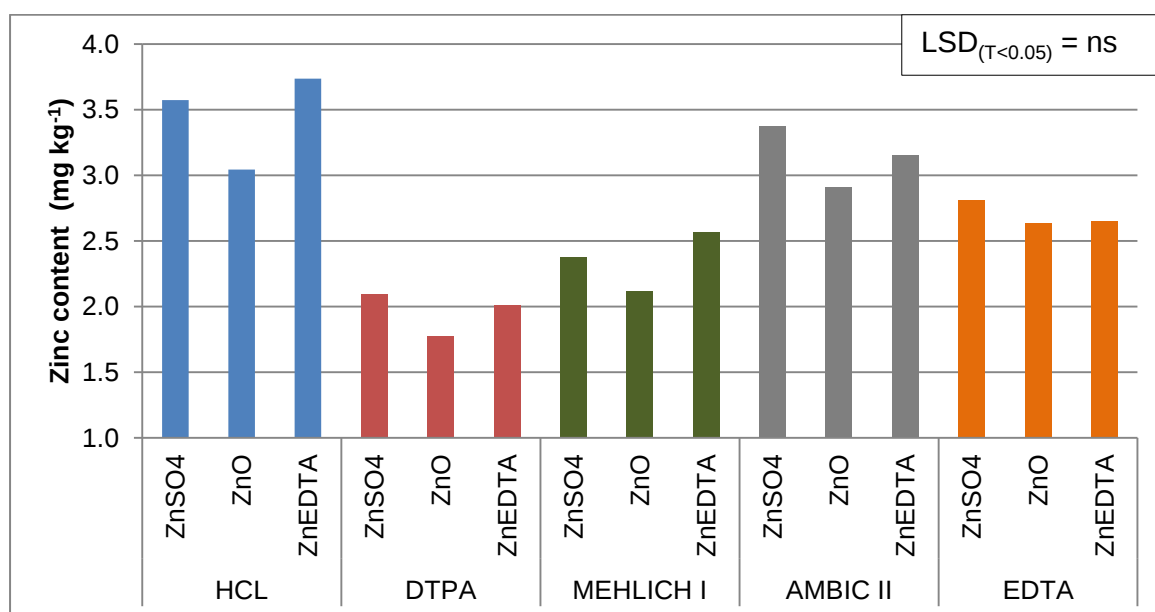
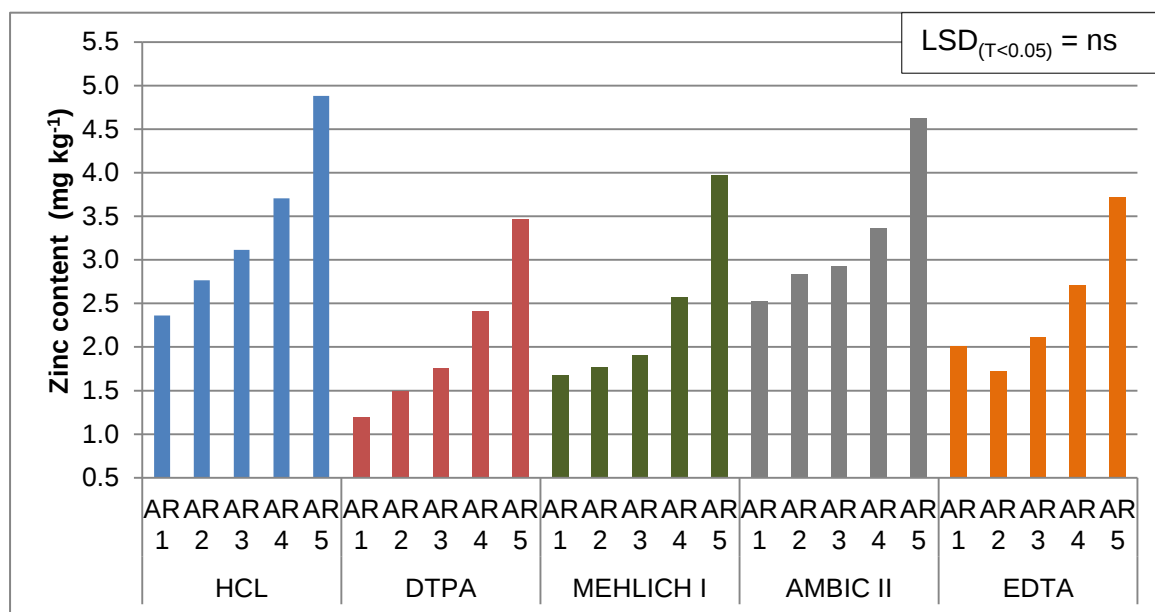


Figure 6.3 Interaction effects of extraction method and zinc source on the average plant available zinc content of the sandy soil for the first (a) and second (b) planting.

a



b

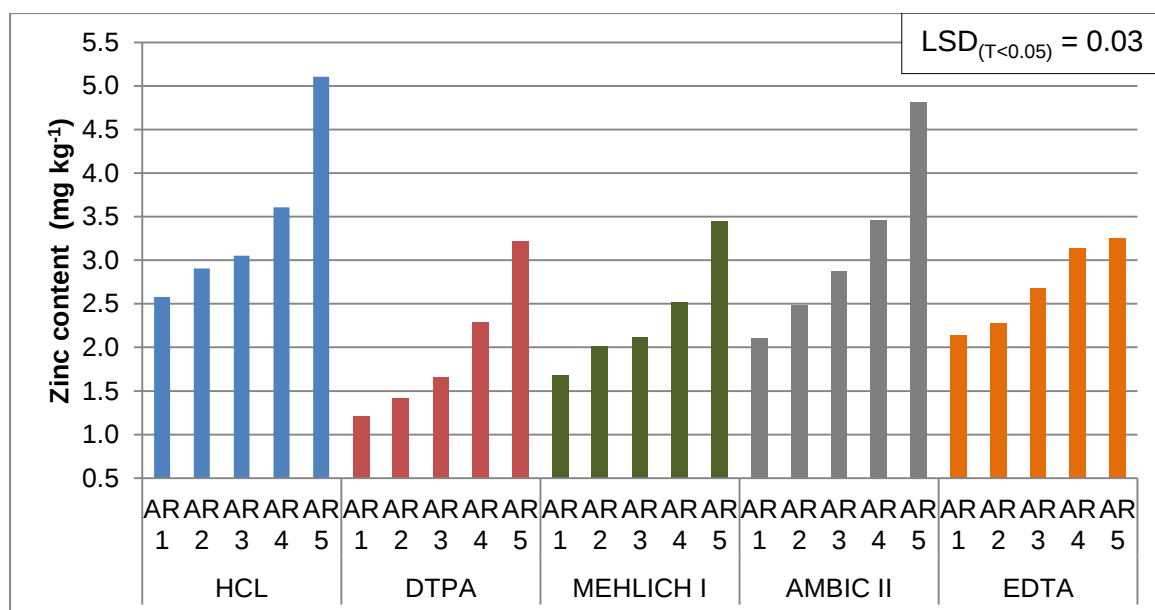


Figure 6.4 Interaction effects of extraction method and application rate on the average plant available zinc content of the sandy soil for the first (a) and second (b) planting.

6.3.2 Zinc concentration in the maize plants

6.3.2.1 Main effects

The main effects of zinc source and application rate on the zinc concentration in maize plants for both plantings are displayed in Figure 6.5.

The effect of zinc source on the zinc concentration in maize plants showed significant differences for the first planting ($p < 0.01$), but not for the second planting ($p = 0.223$). For the first planting zinc concentration increased in the maize plants of the ZnSO_4 treated soil to 71.1 mg kg^{-1} . This is 9.1 mg kg^{-1} higher than in the maize plants from soil fertilized with ZnEDTA. Soil treated with ZnO increased the maize plants' zinc concentration to 58.2 mg kg^{-1} . For the second planting the trend differed from the first planting. Soil treated with ZnSO_4 gave the highest zinc concentration in maize plants followed by soil treated with ZnO and ZnEDTA. Variation in zinc content amounts to 12 mg kg^{-1} for the first planting and 8.4 mg kg^{-1} for the second planting.

The effect of application rate on the zinc concentration in the maize plants showed significant differences for both plantings ($p < 0.01$). For the first planting a clear increase in concentration was found from AR 1 to AR 5. The gradient of the increase is sharper from AR 3 to AR 5. Maize plants grown in soil fertilized at AR 5 resulted in the highest zinc concentration, 63.49% higher than that of maize plants grown in soil where no zinc was added to the soil, viz. AR 1. The response of the maize plants for the second planting differed from the first planting. Initially there was an increase in the maize plants' zinc concentration from AR 1 to AR 2, followed by a drop to AR 3. From AR 3 the zinc concentration again increased to the peak at AR 5. Both the minimum and maximum value for zinc concentration in the second planting is lower than in the first planting. Despite the drop in zinc concentration at AR 3 and AR 4 maize plants grown in AR 1 soil gave the lowest zinc concentration and AR 5 soil the highest, a difference of 67.08%.

6.3.2.2 Interaction

The interaction effects of zinc source and application rate on the zinc concentration in maize plants for both plantings are displayed in Figure 6.6.

The effect of zinc source and application rate showed no significant differences in the maize plants' zinc concentrations for the first ($p = 0.09$) and second ($p = 0.11$) plantings. However, maize plants grown in soil fertilized with ZnSO_4 at AR 5 gave the highest zinc concentration for the first planting. The zinc concentration of maize plants grown in soil fertilized with ZnSO_4 increased from AR 1 to AR 5.

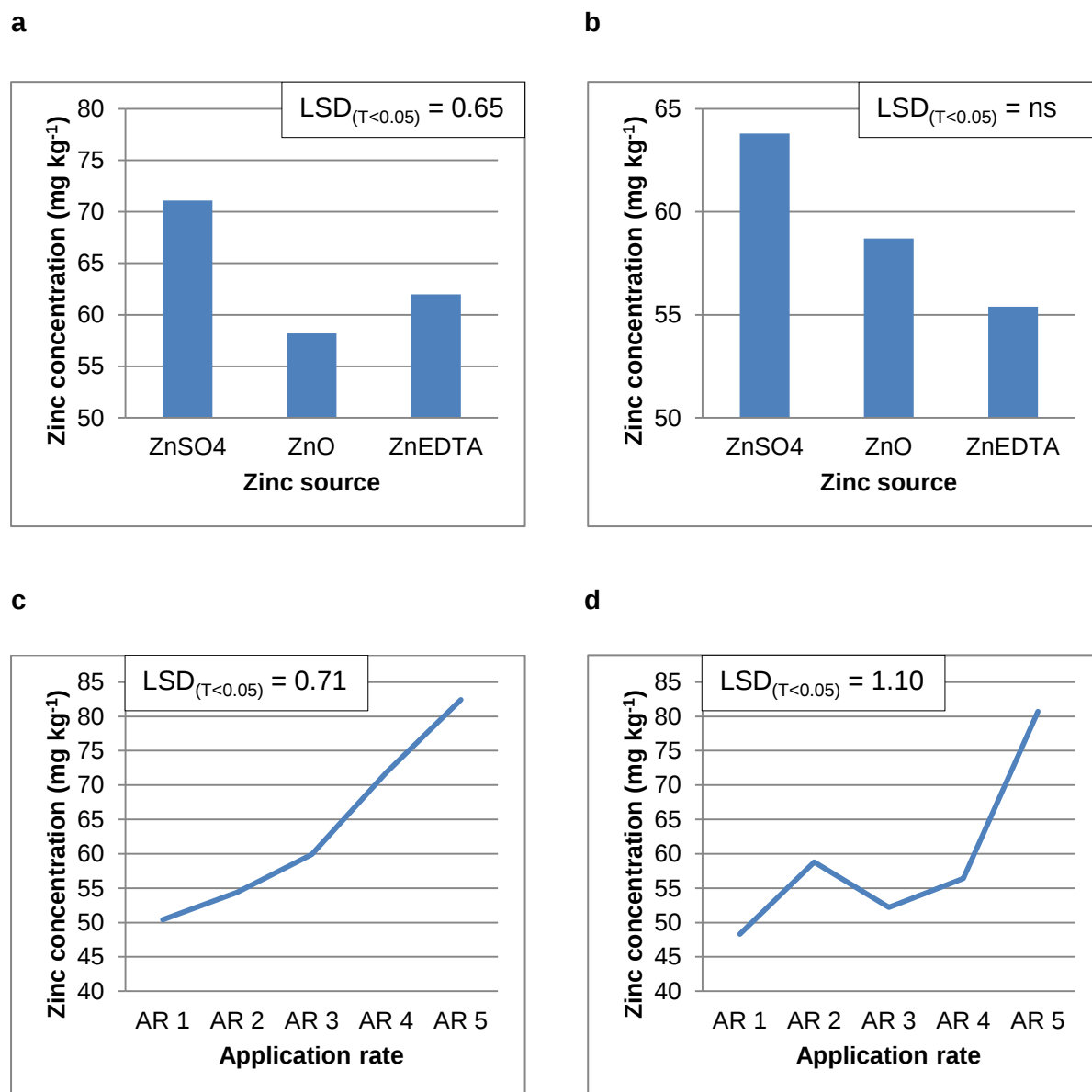
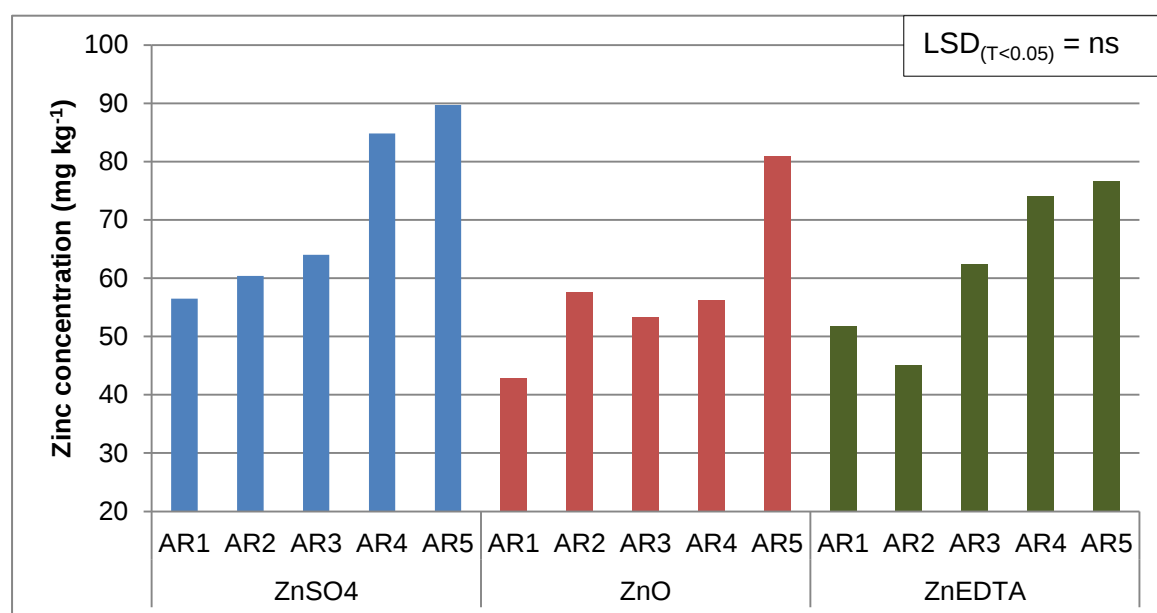


Figure 6.5 Main effects of zinc source (a and b) and application rate (c and d) on the average zinc concentration in maize plants for both plantings.

a



b

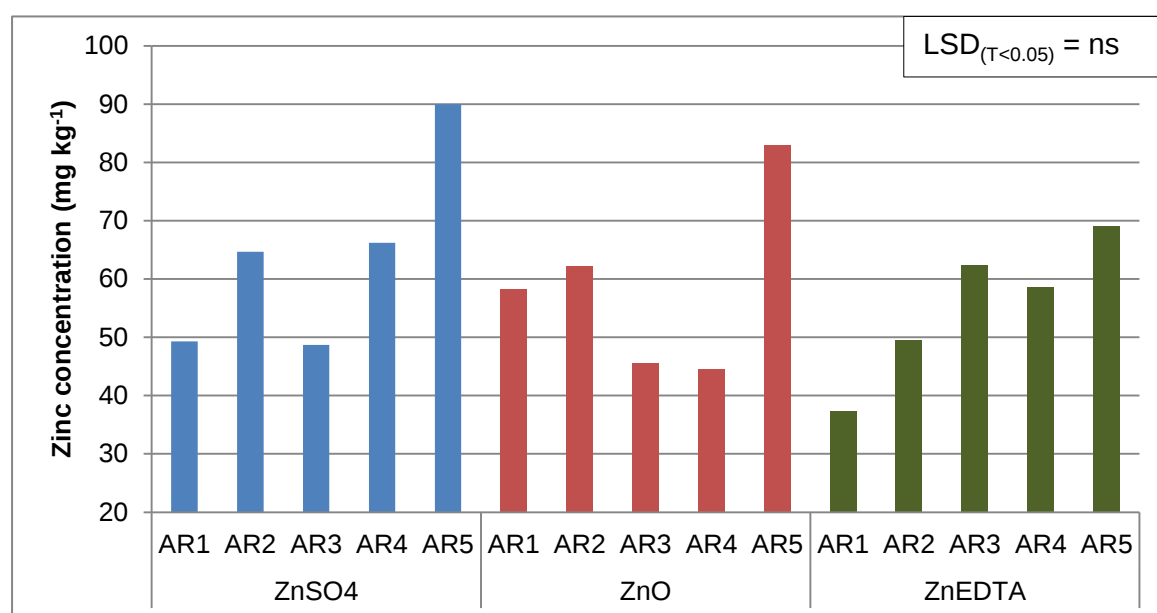


Figure 6.6 Interaction effects of zinc source and application rate on the average zinc concentration in the maize plants for the first (a) and second (b) planting.

For soil treated with ZnO a drop in zinc concentration of maize plants was found at AR 3 and AR 4. Maize plants from soil fertilized with ZnEDTA at AR 2 also showed a clear drop in zinc concentration. Despite the drop, the highest zinc concentrations in the maize plants realized when the sandy soil was fertilized with the three zinc sources at AR 5. Results differed for the second planting. Maize plants from soil treated with ZnSO₄ showed an initial increase in zinc concentration at low application rates, as in the first planting. However, maize plants from soil fertilized with ZnSO₄ at AR 3 showed a drop in zinc concentration. As in the first planting soil treated with ZnO initially increased the zinc concentration of maize plants, but at AR 3 their zinc concentration dropped. In contrast to the first planting the zinc concentration at AR 4 further dropped before increasing at AR 5. The effect of ZnEDTA and application rate on the zinc concentration of maize plants in the second planting differed from the first planting. Maize plants' zinc concentration increased from AR 1 to AR 3, followed by a drop at AR 4 and then increased again to AR 5

6.3.3 Zinc uptake response by the maize plants

6.3.3.1 Main effects

The main effects of zinc source and application rate on the zinc uptake by maize plants for both plantings are displayed in Figure 6.7.

The effect of zinc source on the zinc uptake by maize plants showed significant differences for the first planting ($p < 0.01$) and second planting ($p = 0.004$). For the first planting zinc uptake by maize plants grown in soil treated with ZnSO₄ was the highest, followed by those grown on soil treated with ZnEDTA and ZnO. Maize plants grown on soil treated with ZnSO₄ realized a zinc uptake of 4.73 mg per pot for the second planting, lower than the zinc uptake of 6.67 mg per pot for the first planting. However, zinc uptake by maize plants grown on soil treated with ZnO and ZnEDTA was similar for both plantings. Although not significantly, maize plants grown on soil treated with ZnEDTA had a slightly higher zinc uptake comparing to maize plants grown on soil treated with ZnO for the first planting and vice versa for the second planting. The zinc uptake by maize plants ranged with 1.58 mg per pot for the first planting, and with 1.17 mg per pot for the second planting.

The effect of application rate on the zinc uptake by maize plants showed significant differences for both plantings ($p < 0.01$). For the first planting zinc uptake by maize plants increased from AR 1 to AR 5. However, zinc uptake by maize plants for the second planting show a different trend. Initially zinc uptake increased from AR 1 to AR 2, followed by a drop in zinc uptake at AR 3 to AR 4 and then an increase to AR 5 again. For both plantings the highest and lowest uptake of zinc was found at AR 5 and AR 1 treated soil, respectively.

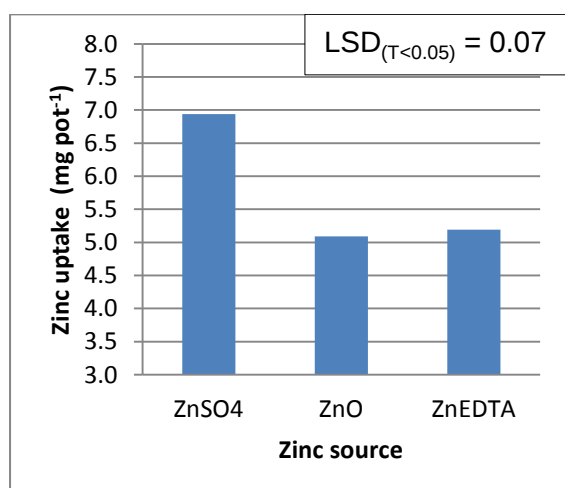
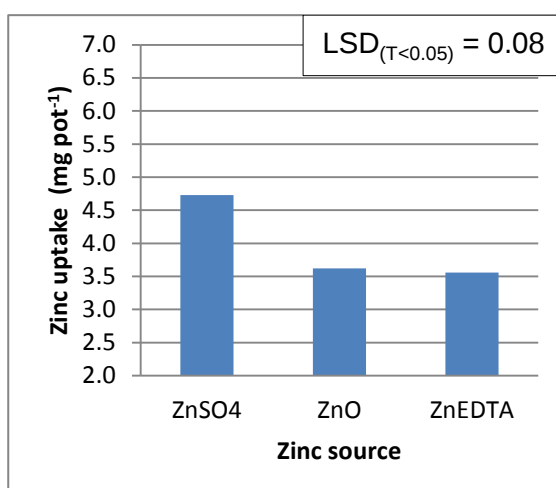
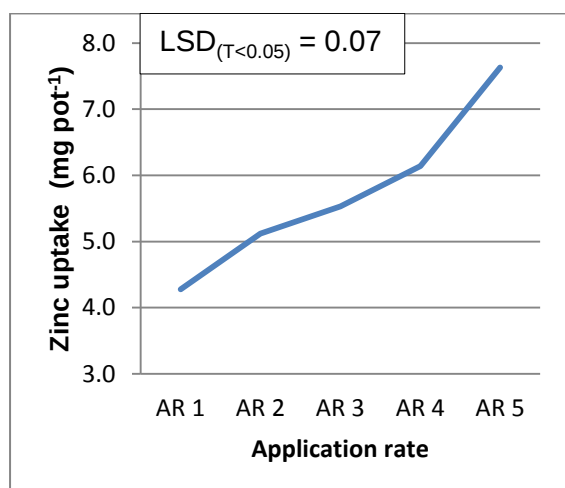
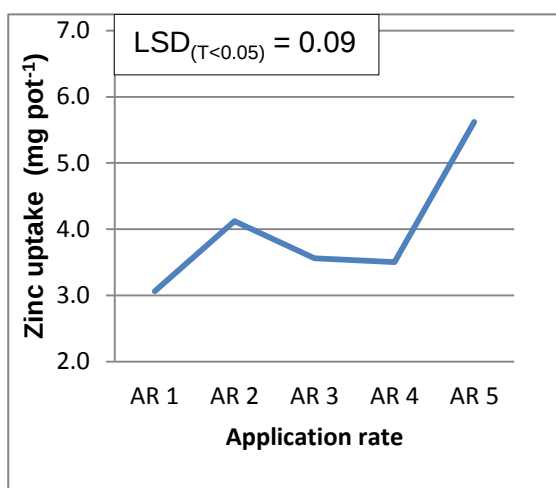
a**b****c****d**

Figure 6.7 Main effects of zinc source (a and b) and application rate (c and d) on the average zinc uptake by maize plants for both plantings.

6.3.3.2 Interaction

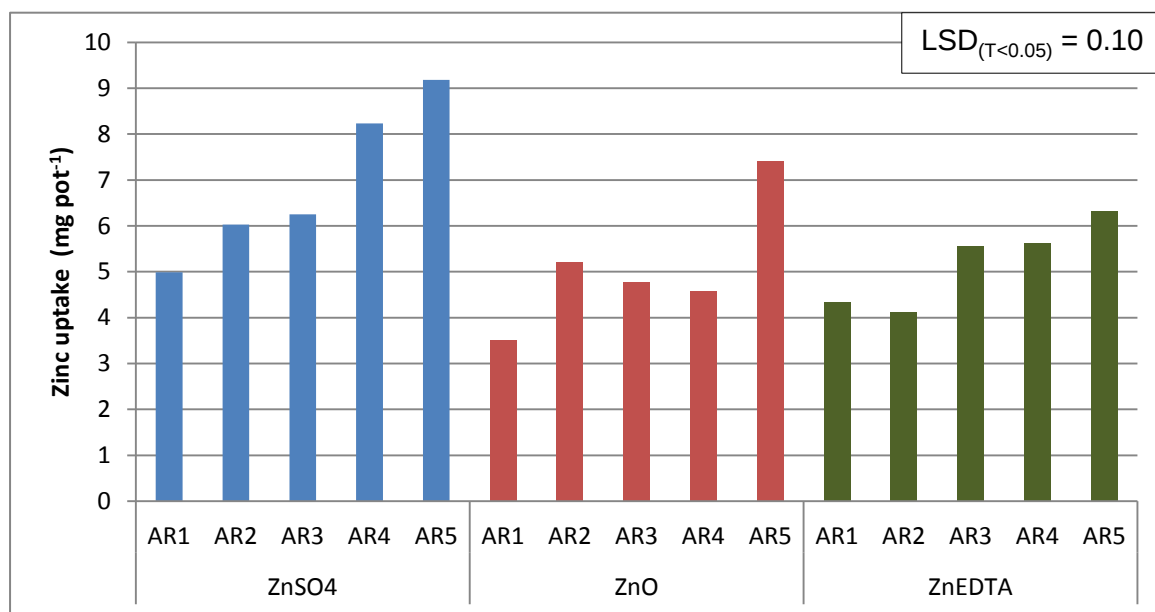
The interaction effects of zinc source and application rate on the zinc uptake by the maize plants for both plantings are displayed in Figure 6.8.

The effect of zinc source and application rate on the zinc uptake by maize plants showed significant differences for the first ($p < 0.01$) and second ($p = 0.01$) planting. For the first planting maize plants grown on soil treated with ZnSO_4 showed the highest zinc uptake per application rate when comparing to the other two zinc sources. The zinc uptake by maize plants grown on ZnSO_4 treated soil increased from AR 1 to AR 5 which was not the case for ZnO and ZnEDTA . Zinc uptake by maize plants grown on soil treated with ZnO increased initially, then dropped at AR 3 to AR 4 and again increased at AR 5. The effect of ZnEDTA on zinc uptake by maize plants showed a slight drop from AR 1 to AR 2 before uptake increased from AR 3 to AR 5.

The uptake of zinc by maize plants for the second planting differed significantly from the first planting. For the second planting zinc uptake from the ZnSO_4 and ZnEDTA treated soils increased from AR 1 to AR 5 with AR 2 and AR 4 the exceptions for ZnSO_4 and ZnEDTA , respectively. The zinc uptake by maize plants grown on soil treated with ZnO showed exactly the same trend as for the first planting.

It is clear that zinc uptake by maize plants was lower in the second planting than in the first planting. Concerning this zinc uptake in both plantings soil treated with ZnSO_4 was superior to soil treated with either ZnO or ZnEDTA . The zinc uptake by maize plants from the ZnO and ZnEDTA soils was almost the same.

a



b

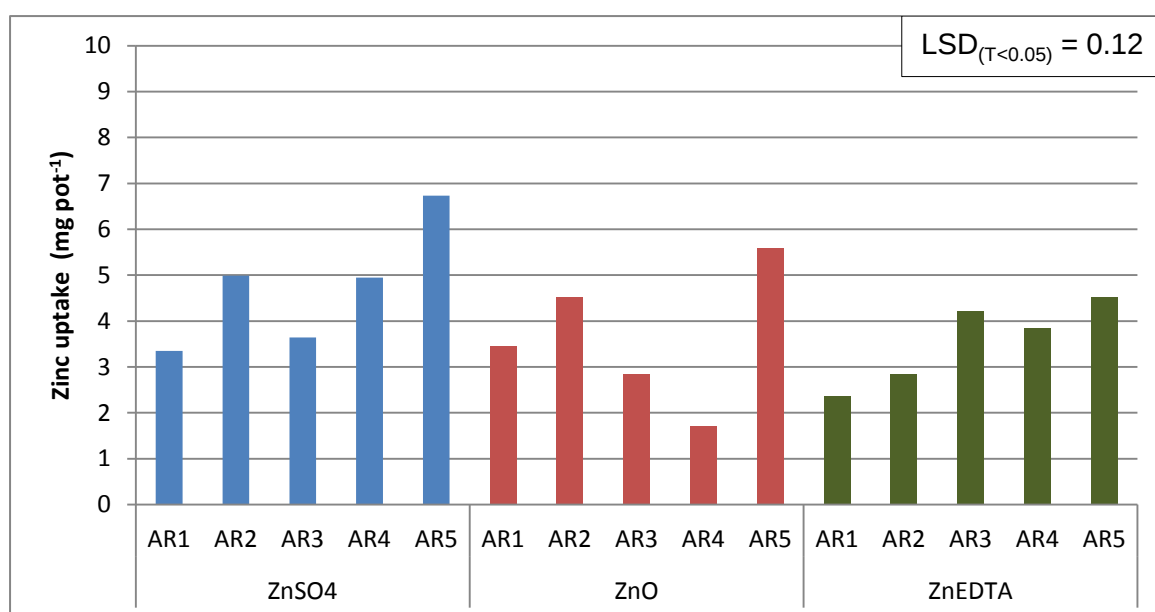


Figure 6.8 Interaction effects of zinc source and application rate on the average zinc uptake by maize plants for the first (a) and second (b) planting.

6.4 Discussion

The concentration and uptake of certain macro- and micronutrients is affected by either a synergistic (positive) or an antagonistic (negative) response (Sumner & Farina, 1986; FSSA, 2007) to the treatment factors, viz. zinc source and application rate according to Mulder's chart (Figure 6.9).

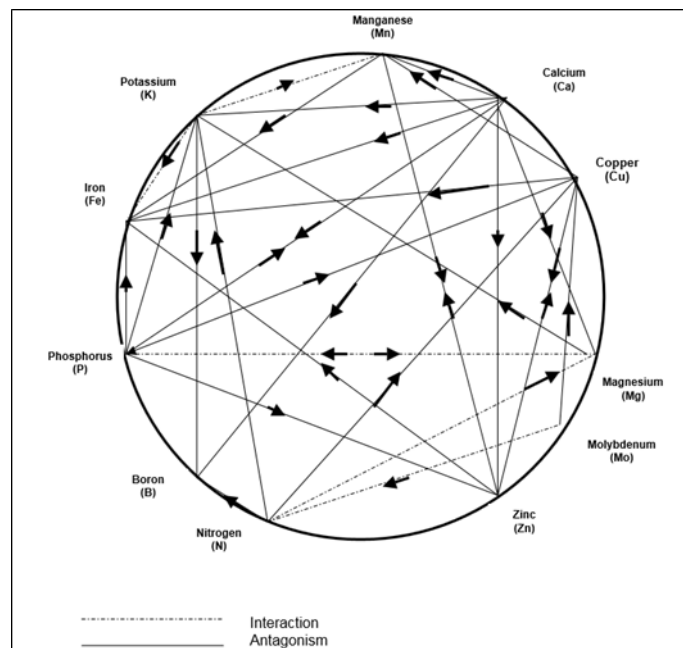


Figure 6.9 Mulder's chart showing the interaction and antagonistic effects between plant nutrients (FSSA, 2007).

The negative effect of high phosphate levels on zinc uptake is one of the most common causes of zinc deficiency in crops encountered around the world. Although this interaction with phosphate has been recognized for many years, the actual mechanisms responsible are still not completely understood. Several macronutrients such as calcium and magnesium are known to inhibit the absorption of zinc by plant roots, the main effect seem to be through their influence on soil pH. Interaction between zinc and copper occur due to sharing the same site for root absorption. Copper can also affect the redistribution of zinc within the plants. Zinc deficiency increases iron concentration in the shoots of maize plants due to the mechanisms involving acidification of the rhizosphere and release of reductants and phytosiderophores. High manganese in combination with high iron may inhibit the absorption of zinc also (Alloway, 2004)

6.4.1 Plant available zinc content of soil

Chelated fertilizers are regarded as the most effective sources of micronutrients for plants. It is considered that ZnEDTA is stable in maintaining the nutrients in a more available form for plants (Cheng *et al.*, 1972). However, results from this study (Figure 6.1) clearly show that ZnEDTA increased the zinc content of the sandy soil used in this study, but to a lower level than ZnSO₄. This outcome was not influenced by neither the rate at which the zinc source was applied (Figure 6.2) nor the method used to extract the plant available zinc from soil (Figure 6.3). Studies have shown that zinc fertilizers with high water solubility are the most effective to correct zinc deficiency in soil and hence crops (Amrani *et al.*, 1999).

More soluble sources of zinc tend to be more readily available for uptake by plants due to the fact that zinc is a diffusion-limited nutrient. Soluble zinc sources like ZnSO₄ tend to be more plant available because they allow increased movement of fertilizer away from the application point (Amrani *et al.*, 1999). This may be a reason why ZnSO₄ was superior in increasing zinc content over ZnO with low water solubility (McBeath & McLaughlin, 2014).

As expected, for both plantings higher zinc content was found at high application rates. It could be supported by research done by Zhang *et al.* (2013) who reported a linear increase in zinc content in fertilized soil.

As established by many researchers in the past, extraction method used to extract zinc from soil differs in the amount of zinc solubilized (Reed & Martens, 1996; Alloway, 2004). To choose the correct extraction method it is important to consider the specific soil properties and crop (Wear & Evans, 1968; Lindsay & Norvell, 1978; Whitney, 1988). Therefore critical values for soil tests differ and the importance of calibration of soil tests are unmistakable (Alloway, 2004).

Research done to evaluate zinc extraction methods predicting the uptake of by maize found higher extractable zinc content using the DTPA method, followed by the EDTA and HCl methods. The DTPA method was considered to be the most suitable method, because of its potential for using the same extractant in the determination of Fe, Cu and Mn (Khatri-Chhetri & Schulte, 1984). Sarto *et al.* (2011) stated that the HCl and Mehlich I extractants were less efficient in estimating plant available zinc. He also reported that the most indicative extractant was DTPA.

However, in this study it was found that for both plantings the HCl method was superior in the amount of zinc extracted, followed by the Ambic II, EDTA, Mehlich I, and DTPA methods (Figure 6.1). Although extractable zinc differed between extraction methods used, with calibration most of the extraction methods could be suitable to estimate soil zinc status.

6.4.2 Zinc concentration in and uptake by maize plants

Soil application of zinc fertilizer significantly affected nutrient concentration in maize plants (Hosseini *et al.*, 2007). Maftoun and Karimian (1989) reported that ZnEDTA was generally more effective than ZnSO₄ in increasing zinc concentration and zinc uptake by stems and leaves of maize. However, the relative effectiveness of various zinc sources for plant growth has been reviewed by several workers (Giordano & Mortvedt, 1972; Lindsay, 1972; Murphy & Walch, 1972). These reviews have reported that under glasshouse conditions, chelated forms of zinc were found to be more effective than inorganic forms of zinc (Wallace & Romney, 1970). The higher zinc concentration due to ZnEDTA application may be attributed to higher mobility and less fixation of zinc from ZnEDTA than from ZnSO₄ (Kumar & Singh, 1979). Lahav and Hochberg (1975) found that ZnEDTA was not fixed in soil and was quite mobile. Some researchers (Elangwhary *et al.*, 1970; Prasad *et al.*, 1976) have also found that zinc chelation increased the transport and movement of zinc to plant roots. However, Shukla and Morris (1967) reported that ZnSO₄ and ZnO were equally efficient or superior to chelated zinc in increasing the zinc concentration and uptake of maize in a glasshouse study, which correspond with parts of this study.

Maftoun and Karimian (1989) found that the uptake of zinc generally increases with increasing zinc rates and is mainly due to an increase in either dry matter yield or zinc concentration in various plant parts. Thus, maize plants with a high zinc uptake capacity do not necessarily have a high zinc concentration in their leaf or shoot tissue (Graham & Welch, 1996).

Important ratios between nutrients such as phosphorus/zinc could be used as an index of the zinc status of the plants as mentioned by Maftoun and Karimian (1989). Maftoun and Karimian (1989) found a decrease in the ratio of phosphorus/zinc with the application of zinc to soils. ZnEDTA is proven to be more effective in decreasing the phosphorus/zinc ratio.

Zinc is taken up by plant roots primarily as Zn²⁺ from the soil solution. Alloway (2004) stated that zinc uptake is mediated by a protein with a strong affinity for zinc. It is proposed that zinc transport occurs across the plasma membrane towards a large negative electrical potential so that the process is thermodynamically passive (Kochian, 1993). In maize Kochian (1993) found that non-protein amino acids called 'phytosiderophores' form a complex with zinc and transport it to the outer face of the root-cell plasma membrane. These phytosiderophores are released from the roots as a result of zinc deficiency. This complex is then transported to the cell via a transport protein (Alloway, 2004).

Furthermore, zinc uptake mainly takes place through direct root contact and is metabolically controlled. Extensive interactions take place between the uptake of zinc and other micronutrients (Alloway, 2004). These interactions were mentioned earlier.

In the plant, zinc is transported as either Zn^{2+} or bound to organic acids. This micronutrient is stored in the root tissues of plants and translocated to the shoot when needed. Zinc can also be partially translocated from old leaves to developing organs (Alloway, 2004).

For the interpretation of plant analysis results are based on the scientific principle that healthy plants contain predictable concentrations of essential elements. Plant analysis has evolved through years of research and experience to become an integral part of modern crop management (Campbell & Plank, 2000). Many researchers have offered schematics showing the relationship between maximum yield and concentration of essential elements (Ulrich & Hills, 1967; Brown, 1970; Dow & Roberts, 1982). Campbell and Plank (2000) added interpretation ranges to these relationships (Figure 6.10).

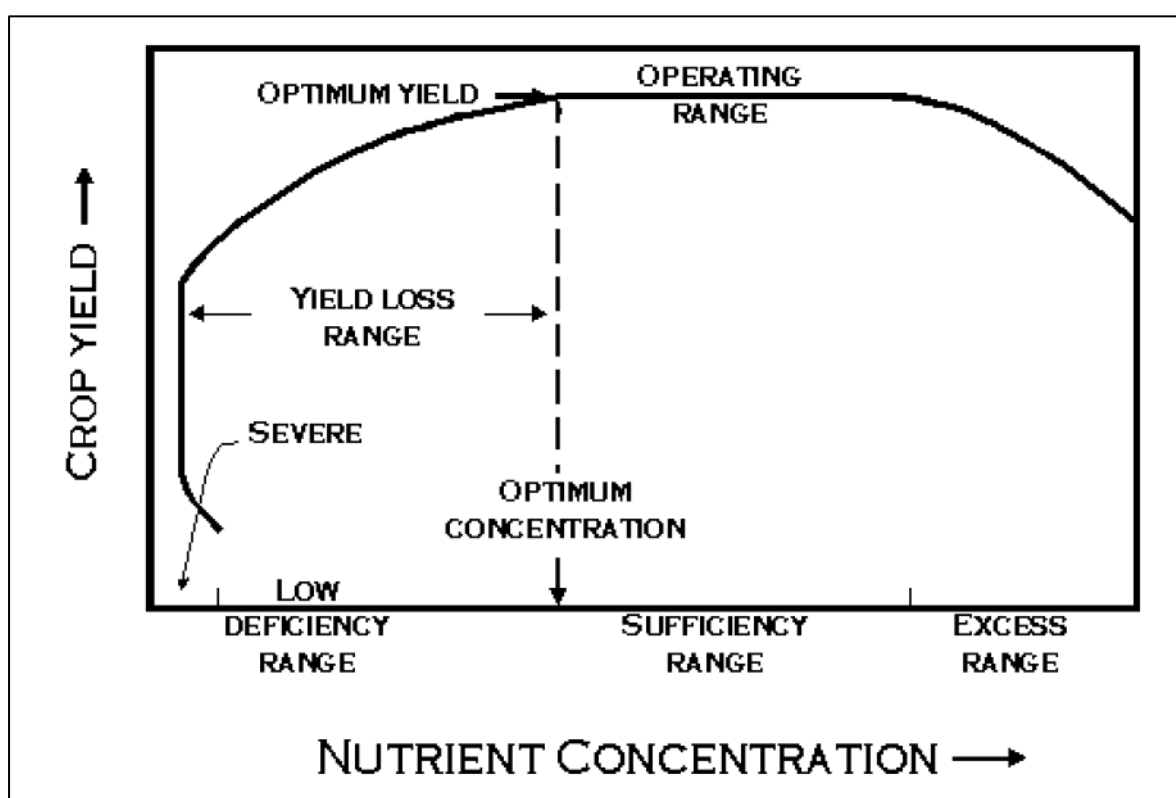


Figure 6.10 Schematic presentation of crop yield and nutrient concentration in plants (Campbell & Plank, 2000).

Thresholds (Table 6.3) will be used as reference to compare the concentration of nutrients in this study to standards delineated by Campbell and Plank (2000).

Sufficient zinc concentration in maize plants range between 20 and 70 mg kg⁻¹ (Campbell & Plank, 2000). Analysis of plant material obtained from the experiment for both the plantings ranged between 48.30 and 90.00 mg kg⁻¹ which shows that maize in this study did not show any form of zinc deficiency. Zinc concentrations in maize plants for both plantings showed similar results.

Nitrogen concentration in maize for the first planting seems to be lower than the second planting, with low values showing slight deficiency. Phosphorus analysis of plant material showed similar results for both plantings. Although maize plants showed no visible deficiency symptoms during the growing period for both plantings it is clear that plant analysis show values lower than 0.25% and can therefore be described as deficient. Potassium analysis of plant material show values higher than the sufficient range (2 - 3%). Analysis for the second planting is higher than for the first planting. Although the value can be characterized as toxic, plants showed no signs of toxicity. Calcium and magnesium concentrations in plant material were sufficient for both plantings. Values for the second planting seems to be slightly lower than for the first planting.

Concerning the plant analysis for micronutrients, both iron and manganese concentrations in plant material were sufficient for both plantings. Values for the first and second plantings were similar. Copper concentration seems to be deficient according to the critical content of lower than 5 mg kg⁻¹ (Campbell & Plank, 2000) for both plantings. However no deficiency symptoms were visible during the trial.

Table 6.3 Sufficiency reference ranges of nutrients for maize during early growth and development until before tasseling (Campbell & Plank, 2000), as well as measured nutrient concentration ranges for main treatments

Nutrient		Sufficiency reference ranges				Experiment ranges ¹					
		Deficient	Low	Sufficient	Toxic	First planting			Second planting		
						ZNS	AR	ZNS * AR	ZnS	AR	ZNS * AR
N	Percent (%)	< 3	-	3 – 4	> 4	2.83 – 3.07	2.74 – 3.19	2.56 – 3.28	3.46 – 3.57	3.30 – 3.66	3.23 – 3.80
P		< 0.25	0.25 – 0.3	0.3 – 0.5	> 0.5	0.20 – 0.21	0.19 – 0.20	0.19 – 0.21	0.21 – 0.23	0.21 – 0.23	0.18 – 0.24
K		< 2	-	2 – 3	> 3	4.20 – 4.33	3.93 – 4.51	3.84 – 4.67	6.21 – 6.32	6.02 – 6.42	5.65 – 6.55
Ca		< 0.4	-	0.25 – 0.8	> 0.8	0.33 – 0.34	0.32 – 0.35	0.27 – 0.36	0.29 – 0.31	0.27 – 0.34	0.25 – 0.36
Mg		< 0.25	-	0.15 – 0.6	> 0.6	0.31 – 0.34	0.29 – 0.36	0.27 – 0.40	0.25 – 0.26	0.25 – 0.27	0.24 – 0.28
Fe	(mg kg ⁻¹)	< 15	16 – 30	30 – 250	> 250	99.50 – 101.30	98.70 – 105.50	94.20 – 107.90	88.30 – 90.70	88.60 – 89.80	80.80 – 103.80
Mn		< 15	16 – 20	20 – 150	> 150	45.28 – 63.68	49.43 – 53.97	42.60 – 70.70	49.30 – 58.50	48.10 – 57.30	43.1 – 63.50
Zn		< 15	16 – 20	20 – 70	> 70	58.20 – 71.10	50.04 – 82.40	42.90 – 89.70	55.40 – 63.80	48.30 – 80.70	37.40 – 90.00
Cu		< 5	-	5 – 25	> 25	4.16 – 4.40	3.90 – 4.70	3.30 – 4.90	2.96 – 3.34	2.83 – 3.47	1.40 – 4.00

¹ZNS = Zinc source and AR = Application rate

6.5 Conclusion

Treatment factors namely zinc source, application rate and extraction method affected the plant available content of zinc in the sandy soil. However, no significant difference was found between the zinc content of both plantings. The most efficient zinc source to increase the sandy soil's zinc content was ZnSO_4 followed by ZnEDTA and ZnO . As expected, with increased zinc source dosage, an increase in soil zinc content was found. Significant differences were observed between the extraction methods HCl, Mehlich I, DTPA, EDTA and Ambic II. For both plantings, the HCl method was superior concerning the amount of zinc extracted, followed by the Ambic II, EDTA, Mehlich I and DTPA methods.

Zinc source and application rate had a significant effect on both the concentration and uptake of zinc in/by maize plants. For both plantings the effect of ZnSO_4 on increasing the zinc concentration in maize was superior to either ZnEDTA or ZnO . However, application of ZnEDTA resulted in slightly higher zinc concentration in the maize plants than application of ZnO for the first planting and vice versa for the second planting. The effect of application rate on zinc concentration in the maize plants differed between the two plantings. For the first planting zinc concentration increased with an increase in zinc application. However, for the second planting zinc concentration increased from AR 1 to AR 2 followed by a drop in concentration at AR 3 and AR 4. In both plantings the highest and lowest zinc concentrations were found at AR 5 and AR 1, respectively. The effect of zinc source and application rate on zinc uptake by maize showed similar results as the effect on zinc concentration. However, the effect of ZnEDTA and ZnO on zinc uptake by maize for both the plantings showed almost similar values. Zinc uptake is directly affected by dry mass. Therefore it is important to keep in mind that maize plants with a high zinc uptake do not necessarily have a high zinc concentration. This is known as the dilution effect.

CHAPTER 7

SUMMARY, SYNTHESIS AND RECOMMENDATIONS

Maize, as a source of carbohydrate, is the most important crop in southern Africa. Therefore the quality of maize is very important for animal and human nutrition. Good quality maize depends on proper plant nutrition, hence good soil fertility. Thus, understanding the management of soil fertility has an unmistakeable role to play in modern agriculture.

Zinc deficiency is known to decrease yield and produce maize with a lower nutritional value. A deficiency of zinc is often observed when maize is grown on sandy soils. This is because maize has a high demand for zinc when compared to other crops.

A study was therefore conducted to evaluate in an incubation and glasshouse experiment the response of plant available zinc in sandy soil when ZnSO_4 , ZnO and ZnEDTA are applied at different rates, using a range of extractants. In the mentioned glasshouse experiment maize's quantitative growth, nutrient concentration and nutrient uptake were also measured during the early growth of the crop.

For the incubation experiment two similar, low zinc content, sandy soils (soil A and soil B, Table 3.2) were used. The two soils mainly differed in pH (4.5 for soil A vs 4.8 for soil B) and acid saturation ($0.3 \text{ cmol}_c\text{kg}^{-1}$ for soil A vs $0.0 \text{ cmol}_c\text{kg}^{-1}$ for soil B). Both soils were treated with ZnSO_4 , ZnO or ZnEDTA to increase their zinc content with 0 mg kg^{-1} , 1 mg kg^{-1} , 2 mg kg^{-1} , 3 mg kg^{-1} and 4 mg kg^{-1} (AR 1 - AR 5). Each treatment was repeated five times. Zinc was applied to the soil as a solution using a sprayer. After application of fertilizers each treatment was properly mixed. Soil went through three wetting and drying cycles before plant available zinc content was determined using the extractants: HCl, Mehlich I, DTPA-TEA, NaEDTA and Ambic II. Main effects for the experiment were zinc source, application rate and extraction method.

For the glasshouse experiment 40.5 L pots were filled with a sandy soil (Soil B, Table 3.2). A randomized complete block design was used for the experiment. Main factors included zinc source (ZnSO_4 , ZnO and ZnEDTA), and application rate (0 mg kg^{-1} , 0.5 mg kg^{-1} , 1 mg kg^{-1} , 2 mg kg^{-1} and 4 mg kg^{-1}) referred to as AR 1 - AR 5. Each treatment was repeated five times. For the application of fertilizers the appropriate amount of fertilizer source together with NH_4NO_3 as nitrogen source and H_3PO_4 as phosphorus source, was dissolved in 20 L of water and sprayed evenly over the soil. After pots were filled, each pot with the relevant

treated soil, maize seeds were planted. Maize seeds were planted in a row, in the middle of each pot 50 mm deep. After planting, the soil was watered and maintained at drained upper limit for the duration of the experiment. Plants were allowed to grow for five weeks. Some plant parameters (stem thickness, plant height and number of leaves) were measured on a weekly basis while other plant parameters (leaf area, dry mass, root length and root mass) were measured at the end of the five week vegetative growing period. At termination of the experiment the soil's plant available zinc was also determined. The experiment was repeated at two planting dates.

From the results of the incubation experiment it's clear that all treatment factors namely soil, zinc source, application rate and extraction method affected the measured concentration of zinc in soil. After using a wide range of extractants (HCl, Mehlich I, DTPA-TEA, NaEDTA and Ambic II) for determination of plant available zinc content for both soils, the average zinc content of soil A was significantly higher than that of soil B. For both soils ZnSO_4 was the most efficient zinc source to increase plant available zinc content followed by ZnEDTA and ZnO. Water solubility of fertilizer is an important factor influencing its availability. Low water soluble zinc fertilizers such as ZnO tend to be less plant available. This statement is supported by this study, which clearly indicated a lower soil response to ZnO than ZnSO_4 .

The effect of increasing application rate on the plant available zinc content showed an almost linear increase in zinc content. Significant differences were observed between extraction methods used to determine plant available zinc content. The average plant available zinc content of soil A extracted by the Ambic II method was superior over the other extraction methods, followed by DTPA, EDTA, HCl and Mehlich I methods. Soil B extracted by the EDTA method was superior in plant available zinc content followed by the Ambic II, DTPA, HCl and Mehlich I methods. For both soils it's clear that using the Mehlich I method gave the lowest plant available zinc content followed by the HCl method.

Different extraction methods tend to extract different amounts of zinc. However, each extraction method is developed to determine plant available zinc under specific conditions and for a specific crop. In South Africa the HCl method is widely used to determine zinc content in agricultural soil while the DTPA method is one of the most used methods over the world. The DTPA method is popular for its ability to determine more than one element. Studies also indicated that the DTPA extraction method is suitable for the determination of zinc availability to maize in a wide range of soils.

Zinc source and application rate had a significant effect on both the aerial- and subsoil growth parameters of maize in the glasshouse experiment. Zinc plays a critical role in plants as proved by many researchers. This nutrient has an important function in the enzyme

systems of plants. Zinc is also very important in the nitrogen metabolism, chlorophyll synthesis and carbon anhydrase activity. All these processes are known to influence parameters like plant height, stem thickness and number of leaves.

For all plant parameters (number of leaves, stem thickness, plant height, leaf area, dry mass, root mass and root length) ZnSO_4 was superior, followed by ZnEDTA and ZnO . However, the effect of ZnO and ZnEDTA on aerial and subsoil growth parameters was not consistent between plantings. For most of the parameters in the first planting, the effect of ZnO was superior to ZnEDTA and vice versa for the second planting. The effect of ZnSO_4 being superior in increasing plant available zinc in the soil during the incubation experiment, supports the better plant reaction in the glasshouse experiment. Measured aerial and subsoil growth parameters increased from an equivalent application rate of 0 mg Zn kg^{-1} to $0.5 \text{ mg Zn kg}^{-1}$. However, for most of the parameters measured, further increase in application rate inhibited plant growth followed by an increase again. The reaction of maize plants to the different treatment combinations was better for the first planting, probably on account of shorter daylight hours for the second planting.

Plant available zinc content at termination of the glasshouse experiment was affected by all treatment factors including: zinc source, application rate and extraction method. No significant difference was found between the first and second planting. The most efficient zinc source to increase the zinc content was ZnSO_4 followed by ZnEDTA and ZnO . As expected an increase in zinc application rate led to an increase in zinc content. However, this effect diminished the possible explanation for inhibition in plant growth due to lower plant available zinc content at AR 4. As in the incubation experiment, the extraction method used led to a difference in zinc content. For both plantings the HCl method was superior followed by the Ambic II, EDTA, Mehlich I and DTPA methods. This order differs from the results obtained in the incubation experiment. The HCl extraction method was not superior in its ability to extract zinc when used in the incubation experiment. However, Ambic II and EDTA which follow HCl in their ability to extract zinc were superior in the incubation experiment. Therefore in both experiments the Ambic II and EDTA methods extracted high amounts of zinc. Results for the incubation experiment also showed a higher extractable zinc using the DTPA method than the Mehlich I method, which was not the case with the glasshouse experiment, although both methods extracted lower amounts of zinc than the Ambic II and EDTA methods for both experiments.

Zinc source and application rate had a significant effect on both the concentration and uptake of zinc in/by maize. Zinc is taken up by plant roots primarily as Zn^{2+} from the soil solution. Zinc is then translocated to the upper parts of the plant. The specific form in which

zinc is translocated is not known. It is known that translocation of zinc is not good in the plant. When zinc supply is high, zinc accumulates in root tissue. Zinc sulphate was superior in increasing the zinc concentration in maize as well as the uptake for both the plantings. This was also the case for most of the aerial plant parameters measured during the growing period as mentioned. Soil fertilized with ZnEDTA was superior in increasing maize's zinc concentration compared to soil treated with ZnO for the first planting. For the second planting, ZnO was superior over ZnEDTA. Zinc concentration in maize increased with the increase in application rate of the first planting. However, for the second planting maize's zinc concentration increased initially with the higher application of zinc, followed by a drop at AR 3. Zinc concentration in maize again increased when grown on soil treated at AR 3 to AR 5. This drop in zinc concentration correlates with the drop which the measured aerial plant parameters showed at AR 4 during the growing period.

The effect of zinc source and application rate on the zinc uptake of maize from soil showed similar results as the effect on zinc concentration in the crop. Soil treated with ZnSO_4 was superior in the amount of zinc absorbed from the soil. The effect of ZnEDTA and ZnO on zinc uptake for both plantings showed similar results. Zinc uptake is directly affected by dry mass. Therefore it is critical to take note that plants with a high zinc uptake do not necessarily have a high zinc concentration. The availability of zinc in plants is influenced by interactions with other nutrients. It is assumed that this complicated interaction may affect the concentration and uptake of zinc by maize in this study. Zinc concentration in maize at the termination of the glasshouse experiment ranged between the sufficient ranges for plant analysis. Zinc fertilizer increase zinc concentration in maize, which therefore could increase the nutritional value of the crop.

Although soil used for this study was low in zinc content, maize showed no sign of deficiency symptoms during the growing period. This could be explained by the fact that zinc application at the lowest rate was nearly enough to lift the zinc content of the soil above the critical level for maize. Maize during the early growth could also have the ability to extract sufficient amounts of zinc from soil at low zinc levels.

Considering the reasons for this study it is clear that ZnSO_4 was superior over ZnO and ZnEDTA. However, researches claim better soil and plant response to chelated fertilizers such as ZnEDTA. Although the majority of studies indicated that chelated fertilizers are superior to inorganic fertilizers, some studies have shown similar results to this study, which indicates that an inorganic fertilizer such as ZnSO_4 was equally effective or superior in increasing maize response.

This phenomenon could be attributed to the fact that with ZnEDTA and ZnO there were no compensation for the S which ZnSO₄ contained. Furthermore, the ZnEDTA used in this study was prepared synthetically and may therefore be less effective than natural chelated Zn products.

Zinc as micronutrient has a critical role to play in agriculture worldwide. Understanding the effect of zinc fertilizers on soil and maize is therefore very important. It can be concluded that ZnSO₄ was more effective in increasing the plant available zinc content of sandy soil and hence the early growth of maize in this study. Extraction methods differed in their ability to extract plant available zinc from soil. This is an important aspect to consider when using soil analysis to evaluate availability of zinc in soil for crop production.

It is recommended that:

- Further research should be done to establish correlation between different zinc extraction methods on a variety of soil types.
- Critical values of a representative range of zinc extraction methods should be established for maize production specific to local conditions.
- Effect of zinc application on the zinc concentration of maize grain warrants a thorough study locally.
- Field trials with maize must be performed to determine how zinc sources compare when environmental conditions are not controlled.
- Further investigation should be done to verify the impaired development of maize plant parameters at increasing application rates.

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