ZINC FERTILIZATION OF LENTIL IN SASKATCHEWAN TO INCREASE YIELD AND GRAIN ZINC CONTENT

A Thesis Submitted to the College of Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

in the Department of Soil Science

University of Saskatchewan

Saskatoon

By

Sarah Anderson

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ABSTRACT

Zinc (Zn) fertilization is considered an important agronomic strategy for global food security. Lentil production in Saskatchewan not only provides significant economic benefit for growers, but is marketed in several countries where human Zn deficiencies are common. The impact of Zn fertilization on lentil yield and Zn concentration deserves attention. Field experiments were conducted in 2013 to determine if Zn fertilization of lentil could increase yield, grain Zn concentration and its bioavailability for humans in three popular lentil cultivars: CDC Maxim (red), CDC Imvincible (small green) and CDC Impower (large green). The effects of three rates (0, 2.5 and 5 kg Zn ha^{-1}) of soil applied $ZnSO_4$ were examined at a site in the Brown soil zone identified as Zn deficient and a site in the Dark Brown soil zone that was identified as sufficient in soil Zn. In 2014, hard red spring wheat was seeded to assess the residual effects on a rotational crop. A companion pot study was conducted in a polyhouse that compared single rates of soil and foliar applied forms of Zn fertilizer: soil applied $ZnSO_4$, foliar applied Zn lignosulphonate, soil and foliar applied Zn chelated with EDTA. At the two field sites, soil applied $ZnSO_4$ fertilizer had no significant effect on lentil yield, grain Zn concentration, and predicted bioavailability of Zn for humans. Significant differences in residual DTPA-extractable Zn were generally not found among rates of applied $ZnSO_4$ fertilizer, and soil applied $ZnSO_4$ did not have residual benefits for spring wheat grown at either location in 2014. Migration of Zn into less labile soil fractions was identified as a factor contributing to this general lack of response to soil applied $ZnSO_4$ fertilizer. Based on results from the polyhouse study, chelated forms of Zn may be more effective than inorganic or organic-complexed forms of Zn in supplying Zn and improving predicted dietary bioavailability of lentils for humans. Phytate:Zn molar ratios were significantly decreased in all lentil cultivars fertilized with soil applied Zn that was chelated with EDTA (17.1) compared to when fertilized with soil applied $ZnSO_4$ (24.7). Overall, the responses of lentil to Zn fertilization were small and variable, such that significant economic benefits were not observed.

ACKNOWLEDGEMENTS

It is with great appreciation that I acknowledge my supervisor, Dr. Jeff Schoenau. This project benefitted greatly from his consistent leadership. Jeff's innate teaching abilities and enthusiasm for agriculture contributed greatly to a very fulfilling research experience. I would also like to extend my gratitude to my Advisory Committee: Drs. Albert Vandenberg, Derek Peak, and Fran Walley. Their attention to detail was second-to-none and their collective insights were incredibly helpful. Special thanks to my external examiner, Dr. Tom Warkentin, for his feedback and time dedicated to improving the final version of this thesis.

I had the privilege of working with wonderful research personnel from departments of both Soil and Plant Science. Very special acknowledgement is extended to the members of "Team Schoenau": Cory Fatteicher, Ryan Hangs, Tom King, Muhammad Maqsood, Noabur Rahman, Elliott Hildebrand, Blake Weiseth, Hasan Ahmed, Mikrigul Rehmut, Ron Urton, Jing Xie, Nancy Howse and Gourango Kar. Simply stated, this was a great group of individuals to work alongside. They all taught me a lot about being a grad student and I am incredibly grateful for their abundance of field and lab assistance. I would also like to thank several members of the Plant Science Field Lab for taking me under their wings. I am particularly grateful to Brent Barlow, Scott Ife, Devini DeSilva and Barry Goetz for their advice and resource support. I also would like to acknowledge the efforts of Jaret Horner, Thiago Prado, Stacey Wagenhoffer, and Mark Thompson. Very special thanks to Arun Shunmugam and Gene Arganosa for their time spent teaching me procedures of phytate analysis. Anoja Weerasinghe is also gratefully acknowledged for her contributions to lentil processing and fractionation procedures.

This research was made possible through financial contributions from the Saskatchewan Pulse Growers, Don Jacques Memorial Fellowship, Saskatchewan Ministry of Agriculture, and NSERC. Their investments in agricultural research are greatly appreciated. I would also like to recognize Joe Tindall with NexusAg Business Inc. for his contributing fertilizer resources and supplying product knowledge.

Finally, I would like to thank my family and friends for their continued support of my academic career. Thank you for all of your words and actions of encouragement!

DEDICATION

This thesis is dedicated to my family who cultivated my love of agriculture. It is also written in appreciation for all those who dedicate their livelihoods to putting food on the plates of people around the world.

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LIST OF ABBREVIATIONS

B	boron
BCR	Communities Bureau of Reference
CDC	Crop Development Centre
CRD	complete randomized design
cv	cultivar
DAS	days after seeding
DDFM	denominator degrees of freedom measured
DTPA	diethylene-triamine-penta acetic acid
EDTA	ethylene-diamine-tetra acetic acid
Fe	iron
Mn	manganese
N	nitrogen
NIST	National Institute of Standards and Technology
OC	organic carbon
OM	organic matter
P	phosphorus
RCBD	randomized complete block design
S	sulphur
SAS	Statistical Analysis Software
SEM	standard error of mean
SPG	Saskatchewan Pulse Growers
TAZ	total absorbed zinc
Zn	zinc
ZnO	zinc oxide
$ZnSO_4$	zinc sulphate

1. GENERAL INTRODUCTION

1.1 Micronutrient Malnutrition and Global Food Insecurity

With nine billion people anticipated to be competing for the planet's resources by the year 2050 (FAO, 2011), widespread and chronic hunger is a very real concern. The food security challenges associated with a rapidly expanding population are not limited to quantity of food resources, but also include the nutritional quality of food that can be produced. Advancements in agricultural technology and management during the period of the "Green Revolution", commencing during the 1960s, successfully prevented widespread starvation in many developing regions of the world (Bouis and Welch, 2010). Consequently, implementing agricultural practices that greatly increased crop yields in these countries also resulted in a surge of micronutrient malnutrition within the same local populations (Bouis and Welch, 2010).

Over one-third of the current world population is estimated as being at risk for deficiencies of one or more micronutrients (Bouis et al., 2012), with deficiencies of zinc (Zn) being among the most prevalent (Alloway, 2008). Human deficiencies of essential micronutrients, such as Zn, are often the result of insufficient nutrient concentration of staple cereal and legume grains in their diets. Food security can only exist when all members of a population have continuous physical and affordable access to enough safe food to support their daily caloric and nutritional requirements within their own dietary preferences (Roberts and Tasistro, 2012). Although agricultural systems have historically not prioritized human nutrition ahead of yield improvements and producer profit, there is a great opportunity for the agriculture industry to grow food that will source enough nutrition to help promote a healthy world population (Bouis and Welch, 2010). Fertility management will be a key agronomic strategy in achieving this goal. Zinc fertilization of crops is considered one cost-effective approach to help combat human Zn deficiency (Roberts and Tasistro, 2012).

The most substantial gains in production have historically taken place in cereal yields. Production of pulses—dried, edible seeds of leguminous crops—also significantly increased from 1965-1999, but it was not to the extent of cereals and was unable to keep up with the population growth in much of the developing world (Bouis et al., 2012). As such, an opportunity currently exists to further improve pulse production. Pulses, including lentils (*Lens culinaris* L.), are important staple foods and are incorporated into the diets of the populations of more than 100 countries (Thavarajah et al., 2011). Lentils are considered a relatively affordable, high protein whole-food source that is comprised of high levels of nutrients compared to other staple foods (AAFC, 2010; Thavarajah et al., 2011). Resulting from its current dietary value and opportunity for increased production, lentil has the potential to deliver adequate nutrition to developing countries and help alleviate human Zn deficiency.

1.2 Justification of Research

Inadequate Zn intake is a health concern for the populations of many of the countries importing Canadian lentils (Alloway, 2009; Schulin et al., 2009; White and Broadley, 2009). Driven by Saskatchewan producers, Canada is a world leader in lentil production and exports, with approximately 95% of Canada's lentil crop being grown in Saskatchewan in the two most recent cropping seasons (Statistics Canada, 2014). Saskatchewan lentil production carries economic importance and knowledge of lentil agronomic practices that will potentially increase yield and marketability will be beneficial for Saskatchewan lentil producers. The study described in this thesis explores the potential benefits of Zn fertilization of lentil grown in Saskatchewan soils. The specific goal is assessing yield and concentration of Zn in the harvested lentil grain in response to Zn fertilization. Although the pulse crop industry is beginning to recognize that there may be potential gains from considering Zn in the lentil fertility package, there has not been enough research conducted to clearly identify what rates, lentil genotypes, and soil types are most likely to produce a yield or quality response to Zn fertilization.

In addition to ascertaining yield benefits for Saskatchewan growers, the project has international market implications. This study specifically examines ways to promote a greater accumulation of Zn in exported lentil grain and could eventually help the industry build a case for marketing a premium and nutrient-rich lentil product to countries requiring more essential micronutrients in their diets. There is already some evidence to support that Zn fertilization can increase the concentration of Zn in lentil grain (Gulser et al., 2004; Zeidan et al., 2006). However, by further investigating its relationship to lentil genetics, soil type, and fertilization practices, the pulse crop industry may be able to more consistently guarantee minimum nutrient levels in exported lentils. In doing so, Saskatchewan lentil production has the potential to gain a significant advantage in the global pulse market.

The objectives of this research were to determine what rates of soil applied Zn fertilizer, lentil genotypes, soil properties and forms of amended Zn will initiate the greatest yield and grain nutrient responses in lentil. Secondly, to determine predicted bioavailability of Zn in lentil grain grown under various Zn fertilization regimes under both field and polyhouse conditions Thirdly, to determine the residual effects of Zn fertilization in a subsequent rotational wheat crop. The body of research contained within this thesis tested the following hypotheses:

(i) Differences in response to Zn fertilization will exist among lentil cultivars;
(ii) Increasing rates of soil applied $ZnSO_4$ will improve yield and grain Zn concentration of lentil;
(iii) Yield and grain Zn concentration will be influenced by the form of Zn fertilizer applied;
(iv) Zinc fertilization will improve the bioavailability of Zn in lentil grain;
(v) Application of Zn fertilizer will have residual benefits for spring wheat yield grown the subsequent year.

1.3 Organization of Thesis

The research contained in this thesis is organized in manuscript format. In addition to this general introduction and the literature review, presented in Chapter 2, three experiments are reported in Chapters 3-5. The first research chapter (Chapter 3) presents research with Zn fertilization conducted under field conditions. The objective of this study was to investigate the effects of different rates of soil applied zinc sulphate ($ZnSO_4$) on lentil yield and grain Zn concentration at two field locations within the lentil-growing region of Saskatchewan. Chapter 4 describes a polyhouse experiment that examined the effects of various forms of Zn fertilizer (sulfate salt, natural organic complex, and chelated forms), applied at a single rate, on lentil yield and nutrient concentration of three popular lentil cultivars. Using lentil grain samples from the field and polyhouse studies, Chapter 5 covers the results of measurements of extractable grain phytate concentration to assess the potential human bioavailability of Zn in harvested lentil grain. A synthesis of the research, general conclusions and recommendations for future work are presented in Chapter 6.

This thesis also includes several appendices that contain supplemental data. Appendix A covers a follow-up field study on the residual effects of Zn fertilizer on the yield of a spring wheat crop grown on the Zn-fertilized lentil stubble at the two field sites; appendices B present additional data that were collected corresponding to studies in each of the research chapters (Chapters 3-5). A simple economic analysis of the soil application of $ZnSO_4$ fertilizer is presented in Appendix E. Appendix F contains data of the nutrient concentrations of various lentil seed fractions typical of standard industry processing prior to human consumption.

2. LITERATURE REVIEW

2.1 Zinc in Soil and Plant Nutrition

2.1.1 Zinc content and distribution in soil

Whether it be via primary uptake or secondary consumption, all living organisms obtain zinc (Zn) from the soil. Consequently, an understanding of the distribution and behaviour of various Zn forms existing in different soil environments is crucial for the effective management of this important trace element. Global averages of total Zn content reported for non-contaminated soils range between 50 and 70 mg Zn kg^{-1} (Brennan, 2005; Alloway, 2009, 2008; Kabata-Pendias, 2011; Sharma et al., 2013). Chernozem soils were described by Kabata-Pendias (2011) as having a global mean total Zn content of 65 mg Zn kg^{-1}. When data from global soil surveys were synthesized and categorized based on soil texture, there was a consistent trend of total Zn content increasing with clay content (Alloway, 2008), indicating that coarse-textured (sand) soils are inherently low in total Zn compared to fine-textured (clay) soils. Differences in Zn content related to soil texture can be traced back to the soil's original parent material. Soils formed on shale or clay sediments are typically much higher in total Zn compared to soils that have developed on limestone, sandstone or dolomite (Alloway, 2008). Kabata-Pendias (2011) estimates that the average amount of Zn contained in the Earth's crust is the same as the mean total Zn content of global soils which, therefore, reinforces that the composition of parent material, and its associated weathering processes, strongly dictates the inherent Zn status of a soil. Due to the lack of availability to plants of some Zn fractions, total soil Zn content is not a direct indicator of potential plant response to Zn fertility. However, it is a useful measurement because the total Zn content can help to gauge the overall Zn status of a soil, including plant-available and unavailable forms.

Over fifty years ago, based on solubility, exchange reactions and chemical forms, Viets (1962) postulated five distinct soil fractions in which total soil Zn is distributed, including the following: (i) water-soluble, (ii) exchangeable, (iii) adsorbed, chelated or complexed, (iv) associated with secondary minerals or bound to insoluble metal oxides, and (v) structurally bound in primary minerals. Subsequent literature generally supports this classification, however, the exact categorization of these chemical pools, and where the boundaries between them occur, are still not consistent. For instance, more recent literature distinguishes Zn associated with soil organic matter as a distinct pool (Alloway, 2008) and either combines adsorbed and exchangeable Zn together as a single pool (Shuman, 1991), or shifts easily exchangeable forms of Zn into the soil solution pool and segregates specifically adsorbed Zn as its own fraction (Kabata-Pendias, 2011). Zinc bound in either primary or secondary minerals is also sometimes reported as a single pool (Shuman, 1991; Kabata-Pendias, 2011). The difficulty in developing a standard classification of soil Zn fractions may stem from the complexity of the soil system and an inability to capture the status of Zn pools through chemical measurements. The transformations between Zn forms are highly dynamic processes that are interrupted or initiated by events such as additions of nutrients, changes in soil moisture and pH, or the mineralization of organic matter (Brennan, 2005). As a result, the size and solubility of the pools differ among the individual fractions, vary among soil types, and are subject to constant fluctuations over time. Inconsistent descriptions of soil Zn fractions may also be attributed to a lack of universal soil extraction methodologies. Numerous sequential extraction procedures have been employed (Shuman, 1991) in an attempt to measure the size and plant availability of individual Zn fractions within particular soils. Results sometimes have limited comparability because the quantity and extractability of particular forms of soil Zn will vary with procedural factors such as duration of extraction and the concentration of extracting reagents. Despite variable sequential extraction procedures, most results support a general trend of decreasing fraction size but increasing Zn activity and plant-availability (Jones and Jacobsen, 2009). Spectroscopic methods of Zn speciation, such as use of synchrotron light, have mainly been applied to soils contaminated with zinc (Hamilton, 2014) but offer promise for assessment of zinc forms in non-contaminated agricultural soils.

The residual Zn fraction is typically the largest pool in unweathered soils (Shuman, 1991; Kabata-Pendias, 2011) and is mainly comprised of Zn tightly integrated within the crystal lattice structures of primary and secondary silicate minerals (Shuman, 1991). The minerals within the residual pool are highly resistant to weathering and, therefore, the Zn in this fraction is very stable and protected from direct plant uptake. Zinc bound to the primary minerals in soil parent material is not considered in reversible equilibrium with other pools (Viets, 1962). Instead, Zn ions are released from weathering parent material minerals directly into the soil solution fraction before either being taken up by plants or migrating into other soil fractions (Viets, 1962). In sequential extractions, the residual fraction is determined in the last extraction step once all other pools have been removed from the soil. Although hydrofluoric acid is the only extracting reagent strong enough to dissolve the most resistant primary and secondary silicate structures, its dangerous nature restricts its use in most laboratories (Hendershot et al., 2006). Consequently, total Zn content or size of the residual pool is typically determined by other pseudo-total digestion techniques using aqua regia (Žemberyová et al., 2006) or nitric or sulphuric acid followed by hydrogen peroxide with the understanding that, due to incomplete silicate break down, some Zn may remain unaccounted for (Hendershot et al., 2006).

In addition to being bound to secondary silicate minerals, considerable amounts of Zn can also be associated with other secondary minerals such as hydrous metal oxides and carbonates (Shuman, 1991). Many researchers (Shuman, 1991; Harter, 1991; Uygur and Rimmer, 2000; Kabala and Singh, 2001; Kabata-Pendias, 2004) recognize the critical role that this group of secondary minerals has on micronutrient reactions and retention within the soil. Iron (Fe) and manganese (Mn) oxides tend to be well distributed in most soils. Carbonate minerals only play a role in calcareous soils (Shuman, 1991; Uygur and Rimmer, 2000), but are important in prairie soils as are in Saskatchewan. Uygur and Rimmer (2000) revealed that the interactions between metal oxides and carbonates further influences the adsorption of Zn. The adsorption capacity of a soil for Zn is increased by Fe and Mn oxides because they have a large surface area, high affinity for metal ions such as Zn, and can also occur as coatings on soil particles (Shuman, 1991; Uygur and Rimmer, 2000; Kabata-Pendias, 2004) Although not readily plant available, the Zn in this fraction does react with other soil Zn pools (Viets, 1962) and may be more or less mobile depending on soil physicochemical factors (Kabala and Singh, 2001). Experimentation with a variety of reagents to extract Zn bound to Fe and Mn oxides has had varying degrees of success (Shuman, 1991). Hydroxylamine is a popular extracting reagent for the metal oxide pool but, because Mn oxides tend to dissolve easier than Fe oxides, hydroxylamine extractions significantly under-estimate the Zn in the Fe oxide fraction (Shuman, 1991). However, hydroxylamine in combination with acetic acid (Shuman, 1991) or hydroxylamine hydrochloride (Žemberyová et al., 2006) is reported to satisfactorily dissolve both Fe and Mn oxides.

Due to its combination of size and potential plant availability, the organic matter (OM) associated soil Zn pool is often considered the most important labile pool of Zn (Brennan, 2005).

Unfortunately, it is one of the more poorly understood soil pools in relation to micronutrient fertility due to its complex and dynamic nature. In Viets' (1962) original classification of soil Zn pools, the OM pool would fall into the fraction described as adsorbed, chelated, or complexed. This pool is in reversible, and sometimes rapid, equilibrium with the soil solution and exchangeable Zn fractions (Viets, 1962; Shuman, 1991). The extent of the Zn availability within the OM pool is contested within the literature. Because of its soil colloid properties, including a large surface area and net negative charge, soil OM can boost the reactivity and cation exchange capacity of a soil which increases the retention of Zn in the OM soil fraction. This adsorbed Zn may or may not become available for plant uptake. Zinc associated with some OM fractions is considered readily available as it has increased mobility through the soil due to chelation which protects Zn cations from interacting with other components of the soil matrix (Brady and Weil, 2008; McCauley et al., 2009). Plant available Zn has been positively correlated to the soil OM fraction (Zeng et al., 2011; Kumar and Qureshi, 2012). In contrast, Alloway (2008) reported that soils with very high levels of OM, such as peat soils, are commonly associated with Zn deficiency, due to a low content of Zn-bearing minerals. The formation of insoluble organic complexes is another potential fate of Zn cations in this fraction (Brady and Weil, 2008) and may explain contrasting perspectives on the availability of OM-bound Zn. The close interactions of the OM fraction with other soil pools (Kumar and Qureshi, 2012), in combination with the several Zn forms that exist within the OM pool, contribute to difficulty in chemical extraction of Zn from this pool (Shuman, 1991). A popular approach is to oxidize the OM via digestion with concentrated hydrogen peroxide (Shuman, 1991; Žemberyová et al., 2006) prior to extraction with another reagent such as ammonium acetate (Žemberyová et al., 2006). Shuman (1991) cautions that in attempting to oxidize OM, sulphides are also oxidized and, therefore, must be included within the extracted fraction. Zinc sulphides may be a significant fraction in poorly aerated soils.

Although the previously described soil Zn fractions play indirect roles in plant nutrition, it is only from the soil solution that plants are able to directly absorb Zn. Zinc within the soil solution can exist in free (Zn^{2+}) or hydrated ($ZnOH^{+}$) ionic forms and as soluble organic ligand complexes (Viets, 1962; Shuman, 1991; Alloway, 2009). Cationic Zn forms in soil solution are susceptible to being held onto negatively charged colloids by electrostatic forces (Shuman, 1991). The Zn ions that are easily desorbed are in continuous exchange with the soil solution and are considered to be labile forms (Viets, 1962; Alloway, 2009). Because the boundary between soil solution and easily exchangeable Zn is constantly shifting, the water soluble soil solution and exchangeable fractions are typically extracted together as a combined pool (Shuman, 1991). Furthermore, the soil solution fraction is often below analytical detection limits when extracted with water alone (Shuman, 1991). Weak acids or dilute salt solutions are often used in extraction procedures of this combined pool (Shuman, 1991). The soil solution and easily exchangeable fractions are crucial to the understanding of soil-derived plant nutrition because they account for the most readily plant-available forms of Zn. However, these fractions typically account for a very small proportion of a soil's total Zn content—even in soils with a very large amount of total Zn (Alloway, 2008). The fraction of plant-available Zn is highly regulated by a number of soil and environmental characteristics.

2.1.2 Factors controlling plant-availability of zinc

Soil properties such as high pH, elevated calcium carbonate content, low cation exchange capacity, low organic matter content, limited or excess water, and cool temperatures are consistently reported to be associated with crop Zn deficiency (Table 2.1, Gulser et al., 2004; Alloway, 2008, 2009; Shulin et al., 2009; White and Broadley, 2009).

Table 2.1 Summary of common factors contributing to Zn deficiency in plants (adapted from Gulser et al., 2004; Alloway, 2008, 2009; Shulin et al., 2009; White and Broadley, 2009).

Factor	Description
Total soil Zn	Referred to as "primary" Zn deficiencies and are most common in soils with a total Zn content <30 mg Zn kg^{-1} such as very sandy or peat soils
Soil pH	Solubility of Zn reversely proportional to soil pH. Deficiencies common in calcareous soils with pH >7
Soil OM	Very low OM soils (i.e. sandy soils) and very high OM soils (i.e. peat soils) are prone to Zn deficiency
Root Development	Compacted soils and cool temperatures in early spring have been associated with Zn deficiency
Nutrient interactions	An antagonistic micronutrient interaction is known to occur between Zn and Cu and "P-induced Zn-deficiency" is often observed with high phosphate application
Soil moisture	Flooded soils, such as rice paddies, are prone to Zn deficiency

Even when soil Zn status is considered sufficient through measurement of an extractable concentration, these soil factors can work to antagonize uptake of Zn by plants. Particularly in high pH, calcareous soils, plant-available Zn is commonly determined through extraction with diethylene-triamine-penta acetic acid (DTPA). The lower critical concentration of DTPA-extractable soil Zn that would indicate a crop would be responsive to additions of Zn is generally accepted to be approximately 0.50 mg Zn kg^{-1} soil (equivalent to 1.0 kg Zn ha^{-1} in top 0-15 cm of soil profile). Lower critical thresholds of DTPA-extractable soil Zn differ between soil testing laboratories and within the literature due to differences in crop and soil type, but tend to align closely to 0.5 mg Zn kg^{-1} (Table 2.2).

Table 2.2 Critical concentration values of DTPA-extractable soil Zn (mg Zn kg^{-1}) reported in literature and North American soil testing laboratories.

Critical Concentration of DTPA-Extractable Soil Zn (mg Zn kg^{-1})	Crop	Reference
0.1-1.0	Range for all crops	Alloway, 2008
0.48	Chickpea	
0.50	Rice Wheat	
0.65	Rice	
0.12-0.60	Clover	Brennan, 2005
0.24	Spring Wheat	
0.50	All crops	Cakmak, 1999
0.50	All crops	Singh et al., 1987
0.1-1.0	All crops	A&L Canada Laboratories Inc., 2011
0.1-0.6	All crops	Agvise Laboratories, 2012

Zinc is critical in plants for growth, improved stress tolerance, and chlorophyll production (Sharma et al., 2013). However, the nature of Zn and its relationship with the soil environment often presents challenges for uptake into plants. Gulser et al. (2004) explain that the relationship between Zn soil levels and plant uptake is complex and mitigated by interacting factors of plant genotype, soil properties and environmental conditions. Arid and semi-arid environments dominated by calcareous soils are particularly prone to Zn deficiencies (White and Broadley, 2009).

Uptake of Zn by plants is also restricted by its limited soil mobility and generally relies on root interception and micro-diffusion (White and Broadley, 2009). The relationship between the roots' ability to scavenge Zn from the soil and Zn concentration in the plant may give insight into a contributing factor of genotypic difference in Zn efficiency as a result of cultivar root system differences. Furthermore, mobility of Zn through the plant phloem is also restricted and, therefore, the edible portions of the plant that are nourished by the phloem are often low in Zn content (White and Broadley, 2009).

2.1.3 Interactions with other nutrients

Phytoavailability of Zn is also influenced by the soil status of other nutrients. Interactions between nutrients are often complex and can vary with crop species and cultivar and in response to environmental conditions (Welch and Graham, 2012). A nutrient interaction occurs when the supply of one nutrient suppresses (antagonistic) or enhances (synergistic) the uptake or utilization of another and can occur within the soil, particularly near the root surface, or within the plant (Fageria, 2001).

Phosphorus (P)-induced Zn deficiency is one of the most recognized antagonistic nutrient interactions with Zn. However, the underlying mechanisms of this interaction are not completely understood. It has been speculated that high levels of P in the soil form precipitates with Zn^{2+} ions but Lindsay (1972) indicated that even when P-Zn soil precipitates do form, they are as soluble as many commercial fertilizers that have been shown to have good availability. When P is available in excess, luxury uptake by the plant can also occur and this may result in an increase of dry matter production and a subsequent dilution effect caused by amplified P: Zn ratios within the plant (Singh et al., 1988; Fageria, 2001). High levels of P have also been found to inhibit the translocation of Zn from plant roots to shoot and grain portions (Dwivedi et al., 1975; Singh et al., 1988). Alloway (2008) suggests that the antagonistic impact that high amounts of soil P has on Zn uptake could be attributed to a reduction of abuscular mycorrhizae fungi infection of the roots. Abuscular mycorrhizae fungi play an important role in plant nutrition by increasing the surface area of roots to improve the uptake of many soil nutrients, including Zn.

From a yield perspective, nitrogen (N) fertilization has been found to have a synergistic effect with applications of Zn (Alloway, 2008). Because N is generally the strongest contributing nutrient to yield, it is reasonable that N and Zn fertilization together would boost yield more than

Zn fertilization alone. In very calcareous soils, N fertilization can have an acidifying effect which lowers the pH near the root zone and increases the availability of soil Zn (Alloway, 2008). Applications of S fertilizer are also reported to have the same soil acidifying effect and positive impact on Zn mobilization (Fageria, 2001). Gao and Grant (2011), however, found that grain Zn content of durum wheat was decreased in the majority of site years in response to N fertilization compared to the control treatment with no N fertilizer.

Zinc interactions with other micronutrients are sometimes more difficult to interpret. Iron and Zn interactions are of particular interest because Fe biofortification is another area of focus in the effort to diminish human micronutrient malnutrition. Gulser et al. (2004) found that Zn concentration in lentil grain was negatively correlated to the grain concentrations of Fe. Other studies have reinforced this negative interaction, indicated a synergistic effect between the two nutrients, or showed no interaction at all (Alloway, 2008). Interactions between Zn and copper (Cu) are generally considered to be negative (Welsh and Graham, 2012). Fageria (2001) reports that high levels of Cu tend to disrupt the absorption or translocation of other nutrients in plants. This is particularly true when Cu is in excess of Zn because the two micronutrients have similar chemical properties and compete for sites of absorption on the plant root (Alloway, 2008). High levels of boron (B) that can have toxic effects on plants can be partially controlled through Zn fertilization (Fageria, 2001). In a study in corn, the interaction between B and Zn was found to be negative in regards to tissue nutrient concentration but positive in terms of plant growth and dry matter production (Hosseini et al., 2007). As a result of their findings, Hosseini et al. (2007) recommended that corn, especially when grown on Zn deficient soils, be fertilized with Zn when soil levels of B are high.

2.1.4 Function of zinc in plant nutrition

Relative to macronutrients, Zn is only required by plants in small quantities, but it is a critical micronutrient for healthy plant function during essentially all stages of growth and development. Alloway (2008) reported that seeds with a high Zn concentration have improved vigour compared to those with a low levels of grain Zn. Severely Zn deficient plants are most classically characterized by “little leaf” syndrome which includes marked reductions in leaf size and abnormally shaped leaves (Marschner, 1986; Alloway, 2008). Due to shortened internodes, referred to as rosetting in dicotyledons, Zn deficient plants may appear stunted and also exhibit visible symptoms such as interveinal chlorosis, bronzing, upward leaf cupping (Marschner, 1986; Alloway, 2008; Hussain, 2012) and suppression of branching (Pandey et al., 2006). All of these symptoms associated with Zn deficiency (Fig. 2.1) result in a loss of net photosynthesis that could be as great as 50-70% (Alloway, 2008) which leads to subsequent reductions in yield. Alloway (2008) also explains that yield may be reduced by as much as 20% in marginally Zn deficient plants showing no visible symptoms.

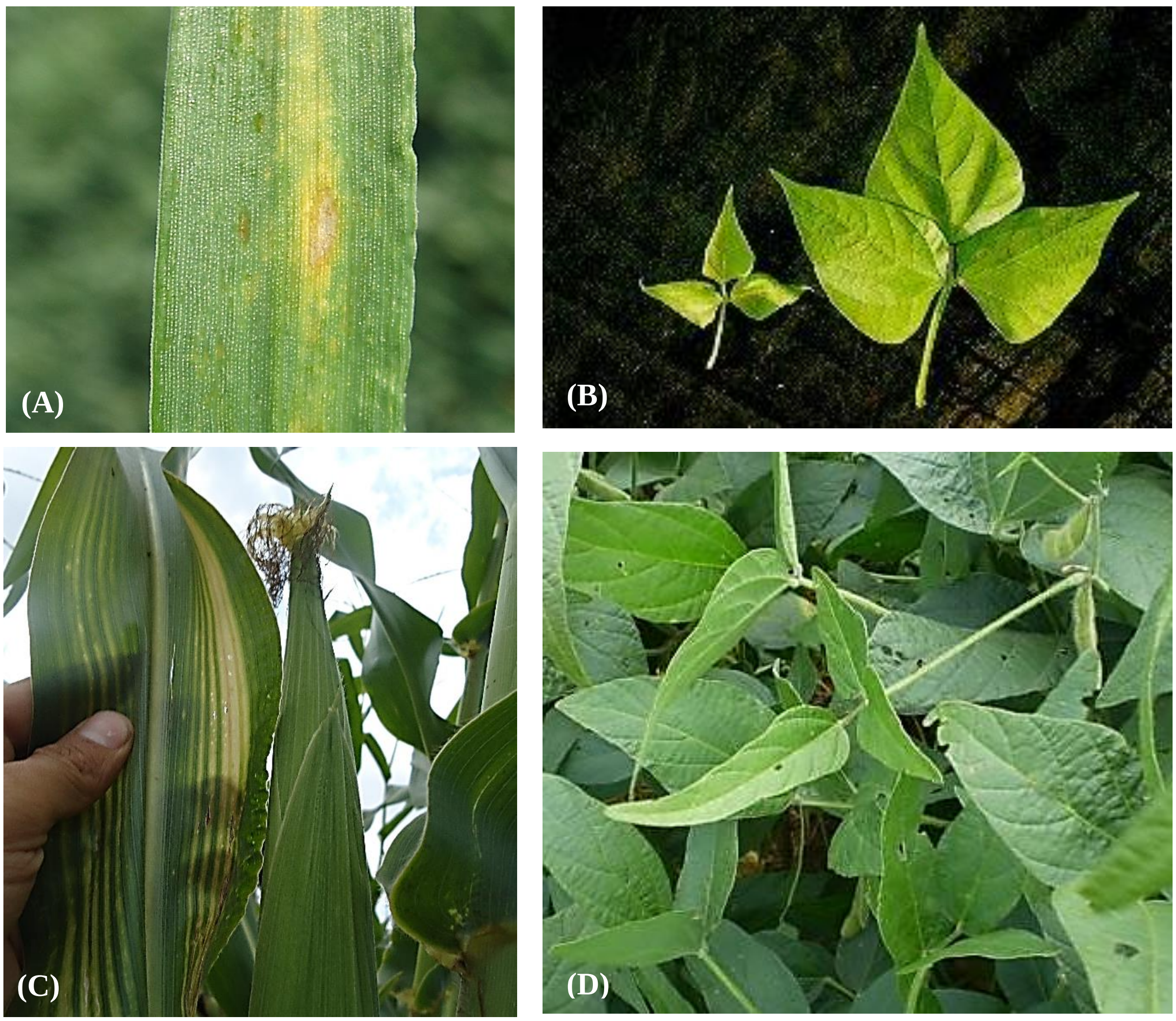

Fig. 2.1. Visual symptoms of zinc deficiency in various crops. (A) Necrosis and interveinal chlorosis in wheat (Sharma and Kumar/IPNI); (B) "little leaf" syndrome and upward cupping of leaves in Zn deficient common bean (left leaf) (Lavorenti/IPNI); (C) severe interveinal chlorosis in corn (Drissi/IPNI); (D) abnormally shaped leaves in soybean (Casarin/IPNI). Images reproduced with permission from IPNI, 2014.

Not only does Zn status play a role in plant photosynthesis from a leaf area perspective, it also has a critical function in carbohydrate metabolism of plants and their conversion of sugars to starch (Marschner, 1986; Alloway, 2008). Zinc is also a constituent of several plant enzymes which contributes to its role in metabolism as well as protein synthesis (Marschner, 1986). As evidenced by the visible symptoms of stunted growth and abnormally small leaves associated with Zn deficient plants, Zn has a function in auxin metabolism and growth regulation (Marschner, 1986). Sufficient levels of Zn may also help plants resist certain pathogenic infections and maintain the integrity of cells (Alloway, 2008). Zinc is required for pollen function and fertilization (Marschner, 1986; Pandey et al., 2006). Onset of flowering in lentil was delayed by 5-6 days when Zn was limiting (Pandey et al., 2006). Furthermore, Zn deficient plants produced lower seed yields and reduced the viability of the seeds by approximately 50% compared to plants with sufficient levels of Zn (Pandey et al., 2006).

2.3 Importance of Zinc in Human Health

2.3.1 Incidence of human zinc deficiencies

Human micronutrient deficiencies have historically been overshadowed by concerns of chronic hunger and malnutrition. Human malnutrition traditionally describes a condition where caloric intake is unsustainably low (Stein, 2010). Micronutrient malnutrition—sometimes referred to as a hidden hunger—can still exist in human diets with sufficient caloric intake but that are lacking adequate levels of one or more essential micronutrients (White and Broadley, 2009).

Zinc deficiency is one of the most widespread nutrient deficiencies in the world and affects over 30% of the world population (Alloway, 2009; Schulin et al., 2009; White and Broadley, 2009). Children are particularly sensitive to Zn deficiency and, according to the results of a meta-analysis of randomized controlled nutrition trials, approximately 4.4% of child deaths in Latin America, Africa, and Asia (between six months and five years of age) could be attributed to Zn deficiency (Fischer Walker et al., 2009). Fischer Walker et al. (2009) also correlated Zn deficiency to high percentages of child deaths resulting from diarrhea, malaria, and pneumonia. It has been suggested that the benefits of supplementing Zn to reduce the incidence of diarrhea are comparable to the impact of implementing water quality and waste disposal programs (Hotz and Brown, 2004).

2.3.2 Human zinc nutrition and function

Just as Zn is an essential element required by plants, it also plays vital roles in several aspects of human health and nutrition. A diverse range of catalytic reactions, structural functions, and cellular regulatory processes are all, in part, facilitated by Zn (Hotz and Brown, 2004; Stein, 2010). Required by more than 300 individual enzymes (Prasad, 2012), Zn is especially crucial for catalytic activities and is the only metal utilized by all six enzyme classes (Sharma et al., 2013). Involvement to some capacity in every major biochemical and metabolic pathway within the human body, cellular division, synaptic signaling, and DNA transcription are only a few examples of important cellular processes that require Zn participation (Hotz and Brown, 2004). Zinc is pervasive throughout the body and can be found within every constituent of human anatomy (Hotz and Brown, 2004).

From a broader human health perspective, the role that Zn plays in immune system function, growth and development, reproduction, and neurological development (Sharma et al., 2013) tends to garner the most attention in current research initiatives. Prasad (2012) recalled that patients, whom were later diagnosed as being Zn deficient, being observed and treated in medical clinics in Shiraz, Iran rarely lived past the age of 25 due to premature death caused by infections. The correlation between Zn deficiency and disrupted T-helper cell function was initially found in mice (Fraker et al., 1977). Since that discovery, researchers have continued to uncover linkages between human Zn status and immune system function. Zinc supplementation has led to significant reductions in the incidence of diarrhea and other infections (Fischer Walker et al., 2009) and some improvements in the growth of children (Bhandari et al., 2001). Studies of immune function response to Zn have frequently focused on child subjects; however, Prasad (2012) also reported a significant 66% decline in infections when elderly patients were supplemented with Zn. Although Zn has important health benefits at any stage of human life, its essentiality for healthy growth and development has led to childhood Zn nutrition studies being prioritized. Physical growth and sexual maturation responses to additions of Zn via diet or supplementation have been frequently documented (Halsted et al., 1972; Prasad, 1991 and 2012; Bhandari et al., 2001). In fact, the clear relationship between Zn and healthy physical and immunological development has led to the routine Zn fortification of commercial infant formulas (Gordon et al., 1981). In contrast, the relationship between Zn and improved neurological function in humans is less clear. Neurological symptoms such as mental lethargy, poor memory retention, and restricted attention and learning are commonly associated with Zn deficiency (Ashworth et al., 1998). Case studies briefly summarized by Gordon et al. (1981) and a nutrition trial by Ashworth et al. (1998) generally support the positive neurobehavioral role Zn plays in humans. However, Zn therapy has failed to improve childhood mental development index scores (Hamadani et al., 2001) or motor and language milestone scores (Surkan et al., 2013).

2.4 Bioavailability of Zinc in Human Diets

Bioavailability is a measure of the amount of the consumed nutrient that is intestinally absorbed (Sharma et al., 2013). Human Zn deficiency is most prevalent in regions where diets are dominated by staple cereal and legume grains low in bioavailable Zn (Schulin et al., 2009). Fish and meat products, which are rich in available Zn, tend to be limited in such diets (Schulin et al., 2009; White and Broadley, 2009). Cereal and legume grains are poor sources, relative to meat products, of bioavailable Zn not only because the concentration of Zn in the edible grain tends to be low, but also because they are high in myo-inositolhexaphosphate (phytate) which is a significant inhibitor of the absorption of Zn ions within the human intestine (Welch and Graham, 2012). Although there are several other anti-nutrients, such as fibers, lectins, and other heavy metals, that diminish the bioavailability of digested Zn (Welch and Graham, 2012), phytate concentration provides the most reliable indicator of dietary bioavailable Zn. The molar ratio of phytate:Zn is commonly used to evaluate the bioavailability of Zn in food items (Hotz and Brown, 2004). The relationships between phytate:Zn molar ratio, category of bioavailability, and estimation of the percent of bioavailable Zn is summarized in Table 2.1.

When diets are low in bioavailable Zn, humans are required to intake almost three times the amount of Zn to meet normative physiological requirements compared to diets with a high level of bioavailable Zn (Brown et al., 2001). If the issue of bioavailability cannot be overcome in these diets, they would need to consume greater quantities of staple food items to prevent micronutrient malnutrition. In regions where sufficient caloric intake is already an issue, diets with poor levels of bioavailable Zn place additional pressure on food security.

Table 2.3. Categorization of phytate:Zn molar ratios and its effect on the bioavailability of Zn (%) (adapted from Brown et al., 2001).

Phytate:Zinc Molar Ratio	Relative Bioavailability	Bioavailable Zinc (%)
<5	High	45-55
5-15	Moderate	30-35
>15	Poor	10-15

Miller et al. (2007) developed a more comprehensive mathematical modelling approach to quantitatively estimating the bioavailability of Zn in human diets. Unlike the molar phytate: zinc ratio, which calculates an estimate of bioavailability based on phytate and zinc concentrations in specific food items, the trivariate model predicts the total absorbed Zn (TAZ) over the course of an entire day (Miller et al., 2007). The reliability of this model in predicting absorptive Zn values was verified in human isotope tracing studies (Rosado et al., 2009). Single meal measurements give only limited insight into the dietary patterns of a population compared assessment an entire day of meals. Furthermore, this model can be used as a tool to evaluate the suitability of staple grains to be used in biofortification programs because it assumes the daily absorption of Zn at an intake level of 300 grams per day.

2.5 Strategies for Increasing Dietary Micronutrient Intake

Traditional strategies used to combat micronutrient malnutrition in vulnerable populations include diet diversification, supplementation and food fortification programs. The latter options have proven relatively successful, but are sometimes restricted in their administration, especially to rural populations in developing countries, due to cost, lack of infrastructure to facilitate safe and long term delivery, political instability, and inconsistent investment into supplementation and food fortification programs (Hussain, 2012). Diet diversification is advantageous in that it uses food as the delivery system which helps to streamline the process. However, incorporating a wider range of foods with higher amounts of bioavailable minerals, such as meat and fresh fruits and vegetables, may not be a realistic option for all populations due to limitations of personal economics, ingredient accessibility, and dietary preferences.

Perhaps a more sustainable approach would be to utilize a food delivery system that enhances the micronutrient concentration in existing staple grains and food items of a given population. Biofortification is a strategy that targets increased accumulation and availability of micronutrients in staple grains through crop genetic or agronomic approaches (Schulin et al., 2009; White and Broadley, 2009). Bouis and Welch (2010) demonstrated great potential for biofortification through conventional crop breeding by identifying several staple foods whose best source of genetic material had micronutrient contents much higher than even the nutritional targets for those nutrients. Genetic biofortification, through conventional breeding or transgenic approaches, has also been supported as a viable means of increasing the micronutrient content in food by several others (Sharma et al., 2013; Impa and Johnson-Beebout, 2012; Bouis et al., 2010; Cakmak et al., 1999). Cakmak (2008), however, cautions that breeding for Zn-efficient traits in crops can be hampered by soil factors and a lack of available Zn. Furthermore, widespread adoption of Zn-efficient cultivars without Zn fertilization may not be sustainable in the long-term due to rapid depletion of the already limited soil Zn in regions growing Zn-efficient crops (Alloway, 2008; Cakmak, 2008).

Zinc fertilization, a method of agronomic biofortification, is one of the most straightforward means of enhancing Zn concentration in the grain (Schulin et al., 2009). When Zn was applied at higher rates than required for targeted yields, increased bioavailability of Zn was measured in the grain of wheat (Cakmak, 2008), flax (Moraghan, 1980) and pea (Peck et al., 1980). Zeidan et al. (2006) found that grain Zn of lentil grown in Egypt significantly improved as a result of foliar Zn fertilization. Yields of crops grown on Zn deficient soils may also benefit from Zn fertilization. Lentil crops grown on Zn deficient soils were almost 33% lower yielding compared to lentils grown with Zn fertilizer (Zeidan et al., 2006). Increased yields and Zn efficiency were observed by Gulser et al. (2004) when lentil crops grown in Turkey were fertilized with Zn compared to treatments not amended with Zn.

Although both strategies have merit, a complementary system that integrates genetics and agronomics may be the most effective. As White and Broadley (2009) point out, combining genetic biofortification efforts with fertilization has the added potential advantage of improving yield on relatively infertile soils in addition to increasing the accumulation of micronutrients in edible plant parts. Crop genotypes also differ in their ability to perform better under Zn deficient conditions relative to other cultivars (Zn efficiency) (Gulser et al., 2004). Several studies have reported cultivar differences in grain Zn concentration even when lentils have been grown under the same environmental conditions and in the same soil type (Thavarajah et al., 2009; White and Broadley, 2009; Gulser et al., 2004). A greater understanding of how to best stack cultivar selection with Zn fertilization may prove to generate the greatest benefits of increasing Zn uptake into edible grains and its human bioavailability while improving yields.

2.6 Forms and Methods of Application of Zinc Fertilizers

Application of fertilizer is generally the most commonly used agronomic practice used to manage and correct crop nutritional deficiencies. Reviews of scientific literature advocate soil applied Zn fertilizer as the most effective and frequently used fertilization method for correcting Zn deficiencies (Martens and Westermann, 1991). However, Zn amendment options are numerous and vary considerably in cost, form, nutrient content, potential plant availability, and, ultimately, effectiveness (Alloway, 2008; Morvedt, 1991). Zinc fertilizer sources can be categorized into the following three groups: (i) inorganic, (ii) synthetic chelates, and (iii) natural organic complexes (Mortvedt, 1991).

Zinc sulphate ($ZnSO_4$), an inorganic source of Zn, is the most widely used Zn fertilizer in the world (Alloway, 2008). The extensive use of $ZnSO_4$ is attributed to its widespread accessibility in fertilizer markets around the world and its low cost—relative to other forms of Zn (Martens and Westermann, 1991). Zinc sulphate is also highly water soluble which allows for its rapid dissolution into the soil solution and greater potential for immediate uptake within the year of application. Although very uncommon as an agronomic practice in North American, the high water solubility of $ZnSO_4$ also allows for the option of being dissolved in water and applied to the crop as a foliar spray. Degree of water solubility is an important factor when selecting granular and powder sources of Zn because it is related to crop response (Gangloff et al., 2002). Mortvedt (1992) found that solid Zn fertilizers were required to contain a minimum of 40% water soluble Zn in order to be effective. Zinc oxide (ZnO) products are another popular inorganic source of Zn. Due to its higher Zn concentration, ZnO can be applied at lower rates compared to $ZnSO_4$, but it is generally less effective in correcting Zn deficiencies during the season of application because it has lower water solubility (Mortvedt, 1991).

Although chelated forms of Zn are typically much more expensive than $ZnSO_4$ (Alloway, 2008), they often have higher agronomic and recovery efficiencies compared to inorganic forms of Zn because they can be effectively applied at much lower rates (Impa and Johnson-Beebout, 2012). Derived from the Greek word "chela" meaning claw, chelates are formed when chelating agents attach to metal cations, such as Zn^{2+}, using coordinate bonds (Morvedt, 1991). Once the Zn cation is integrated into the chelation ring, it is better protected from reacting with the soil and is less prone to becoming unavailable for plant uptake as a result of fixation or precipitation processes (Gangloff et al., 2002; Impa and Johnson-Beebout, 2012). Zinc chelated with ethylene-diamine-tetra acidic acid (EDTA), the most commonly used synthetically chelated micronutrient, forms a very stable chelate that is resistant to decomposition (Obrador et al., 2003; Alloway, 2008). Several researchers (Martens and Westermann, 1991; Gangloff et al., 2002; Obrador et al., 2003; Impa and Johnson-Beebout, 2012) have found that ZnEDTA has greater mobility in the soil and is more accessible for plant uptake which results in higher concentrations of plant tissue and grain Zn compared to $ZnSO_4$, despite being applied at a lower rate.

Natural organic complexes of Zn are formed when organic by-products, typically from wood and pulp industries, or citrates are reacted with Zn salts (Morvedt, 1991). For example, Zn lignosulphonate, a popular organically complexed source of Zn, is produced when $ZnSO_4$ is reacted with lignin wastes from paper manufacturing (Gangloff et al., 2002). Although these reactions may produce similar bonds to that of chelates, the properties and agronomic effectiveness of natural organic complexes are much more variable (Morvedt, 1991). Because this form of fertilizer is derived from a variety of by-product sources, the structure and stability of the resulting complexes is difficult to predict; however, it is generally accepted that the Zn complexes are much less stable compared to chelated Zn (Morvedt, 1991; Alloway, 2009).

The application and placement methods of the various Zn fertilizer sources are also important to consider when identifying fertilization strategies that will improve Zn uptake and agronomic efficiency. Zinc fertilizers can often be applied as either soil or foliar products. Foliar Zn application can be advantageous compared to soil application because it eliminates the risk of Zn interacting with the soil and becoming immobilized (Impa and Johnson-Beebout, 2012). However, its effectiveness relies on sufficient leaf absorption which can be hindered by the leaf cuticle layer restricting nutrient penetration (Shulin et al., 2009) or by inadequate leaf interception in the case of small leaf area (Martens and Westermann, 1991). Martens and Westermann (1991) also explain that repeat applications of foliar Zn are often required to correct moderate and severe Zn deficiencies due to their lower application rates compared to soil applied

Zn. Soil applied Zn fertilizers are typically surface broadcasted or broadcast and incorporated. Zhang et al. (2013) found that granular Zn fertilizer needs to be thoroughly incorporated into the soil profile, to depths of 15-30 cm, to maximize root interception and Zn uptake in corn. Other sources claim that banded Zn fertilizer is a more efficient placement method compared to broadcast options because adsorption of Zn onto soil surfaces is lower due to less soil-to-Zn contact (Alloway, 2009).

3. EFFECTS OF SOIL APPLIED ZINC SULPHATE ON LENTIL YIELD AND GRAIN ZINC CONCENTRATION UNDER FIELD CONDITIONS

3.1 Preface

Soil application of zinc sulphate ($ZnSO_4$) fertilizer is the most common zinc (Zn) fertilization practice around the world. Increased yield and nutrient concentration of staple grains has been reported in response to broadcast and incorporated $ZnSO_4$ fertilizer application. Many of the crop responses to $ZnSO_4$ reported in the literature have been observed with application rates much higher than would typically be recommended or considered economical for Saskatchewan producers. Field investigations of lentil response to Zn fertilization in Saskatchewan are limited. Therefore, the research described in this thesis chapter was conducted to evaluate the response of three popular lentil cultivars to three rates of soil applied $ZnSO_4$ fertilizer at two Saskatchewan field locations within the lentil growing region, and with contrasting soil properties and soil available Zn status.

3.2 Abstract

Saskatchewan lentil production provides a major portion of the world's growing demand for legume grains. Much of this demand originates in regions where human zinc (Zn) deficiencies arise as the result of insufficient Zn content of staple grains in their diets. A field experiment was conducted in 2013 to determine if Zn fertilization could increase yield and grain Zn concentration in three popular lentil cultivars—CDC Impower (large green), CDC Imvincible (small green) and CDC Maxim (red). The effects of three rates (0, 2.5, and 5 kg Zn ha^{-1}) of soil applied $ZnSO_4$ were examined at a site in the Brown Soil Zone identified as Zn deficient according to soil analysis, and one in the Dark Brown Soil Zone identified as being sufficient in levels of Zn. Rates of applied fertilizer $ZnSO_4$ used in this experiment were not found to significantly increase yield or grain Zn content in any of the three lentil cultivars tested. Residual levels of plant available Zn in soil did not significantly differ between rates of soil applied $ZnSO_4$ at either field location. Rapid fixation of applied Zn into soil fractions unavailable for plant uptake is suggested as a primary explanation for the lack of detected significant differences in lentil yield and grain Zn concentration, and residual amounts of soil Zn in response to increasing Zn fertilization rates.

3.3 Introduction

Soil applied Zn fertilizer has been reported as one of the most effective methods of increasing the concentration of Zn in the grains of cereals and lentil (Gulser et al., 2004). Zinc sulphate ($ZnSO_4$) is cited as one of the most commonly applied Zn fertilizer amendments (Alloway, 2009). Soil applications of Zn fertilizer help to provide nutrient supply when the risk of crop Zn deficiency is the greatest for a given soil—in cool, wet soil conditions, particularly in the case of early spring seeding, when root development is limited. Furthermore, early access to Zn can be advantageous to young seedlings because Zn triggers enzyme activations that are linked to improved stress tolerance and vigour (Sharma et al., 2013). Application of forms of Zn that are absorbed through the leaf is often delayed until the crop growth stage is more advanced and leaf area is larger as a way to increase interception of the foliar fertilizer. However, in some instances, early season Zn deficiencies have already limited crop growth and development to such an extent that they may not be able to be corrected by later season additions of Zn.

Although a supply of Zn can be beneficial to plants, it can be harmful when supply is too great (Sharma et al., 2013) and, therefore, understanding what rates of soil applied Zn fertilizer result in the greatest plant benefit is important agronomic information. Gulser et al. (2004) generally found improved yield and lentil grain Zn concentration with increasing rates of Zn fertilizer; however, at high rates there was such an increase in lentil biomass that the concentration of Zn in the grain was diluted. The objective of this field study is to examine the response of lentil yield and grain Zn content to three different rates of soil applied $ZnSO_4$. Measurements of the variability in response of three different lentil cultivars will also be investigated.

3.4 Materials and Methods

3.4.1 Site description and environmental conditions

Field trials at two separate site locations were established in the spring of 2013. The sites were selected to represent the range of Saskatchewan's lentil growing region. The two sites were a farm field in the Brown Soil Zone near Central Butte, SK and the Saskatchewan Pulse Grower (SPG) research land near Saskatoon, SK in the Dark Brown Soil Zone. Soil properties and baseline nutrient levels are summarized in Table 3.1.

Table 3.1. Summary of spring 2013 baseline soil properties from Central Butte and Saskatoon field site locations. Values are means of soil cores collected from the marked out control plots before any treatments or field operations were conducted**.**

Depth	Soil Property							
	pH	EC	OC	N†	P	K	S‡	Zn
(cm)		(dS m^{-1})	(%)	(kg ha^{-1})				
	----Central Butte----							
0-15	8.0	0.23	1.4	8.4	17.7	535.0	14.8	0.93
15-30	8.1	0.26	-	8.5	-	-	16.9	0.54
30-60	-	-	-	10.4	-	-	645.1	-
	----Saskatoon----							
0-15	7.1	0.26	2.6	11.0	38.4	504.2	13.1	3.7
15-30	7.2	0.13	-	10.0	-	-	9.7	2.2
30-60	-	-	-	17.9	-	-	29.8	-

† Nitrate, NO_3- N

‡ Sulphate, SO_4-S

The soil at the Central Butte site is a loam classified as an Orthic Brown Chernozem of the Ardill association. The Saskatoon site represents a clay loam soil of the Bradwell association and is classified as an Orthic Dark Brown Chernozem. According to ALS Ltd Saskatoon laboratory soil analysis and interpretation, the site at Central Butte is considered deficient in Zn for lentil and the application of Zn fertilizer was recommended; the site at Saskatoon had sufficient levels of Zn according to soil test results and no Zn fertilizer recommendation was made. The two sites were considered to have sufficient N for lentil production assuming inoculation and N fixation onset, marginal to sufficient P, sufficient S and levels of other micronutrients. The differences in Zn status, soil organic matter, pH, and environmental conditions (Table 3.2) between the two sites provides a good contrast of soil properties and environmental factors to address crop response to fertilization. Precipitation and temperature data for Central Butte and Saskatoon field sites was collected from Environment Canada weather stations situated near Elbow, Saskatchewan and Saskatoon, Saskatchewan, respectively. Environmental conditions at Saskatoon are wetter and slightly warmer compared to Central Butte, both historically and during the 2013 growing season. Both locations are representative of relatively cool spring temperatures that are typical of Saskatchewan.

Table 3.2. Comparison of mean monthly precipitation (mm) and temperature (ºC) during 2013 growing season to thirty year (1982-2012) means at Central Butte and Saskatoon field site locations

Month	Central Butte				Saskatoon			
	Mean Monthly Precipitation		Mean Monthly Temperature		Mean Monthly Precipitation		Mean Monthly Temperature	
	2013	HM†	2013	HM	2013	HM	2013	HM
	(mm)		(ºC)		(mm)		(ºC)	
May	28.7	53.1	12.0	10.7	15.2	44.6	13.0	11.9
June	82.0	68.5	14.1	15.7	115.9	72.7	15.5	16.8
July	38.1	57.2	17.1	18.5	35.2	66.8	17.4	19.3
Mean	49.6	59.6	14.4	14.9	55.4	61.4	15.3	15.9

† HM= Historical mean (1982-2012)

Variations between 2013 and thirty-year average temperatures were small. However, precipitation in 2013 at both sites was slightly lower than the historical mean in all months except June.

3.4.2 Baseline soil characteristics

Baseline soil samples were taken at each site prior to field operations and seeding at depths of 0-15 cm, 15-30 cm and 30-60 cm from control plots. Soil samples collected were air-dried and ground with a wooden rolling pin. The homogenized soil samples were sieved and the <2 mm fraction was retained and analyzed for various extractable nutrient levels and chemical properties (Table 3.1). Soil pH and electrical conductivity (EC) were measured in a 1:2 soil:water suspension (Nelson and Sommers, 1982) with a Calomel glass electrode assembly on a Beckman 50 pH meter (Beckman Coulter, Fullerton, CA, USA) and an Accumet AP85 pH/EC meter (Accumet, Hudson, MA, USA) respectively. Soil organic carbon (OC) was determined following the methodology of Wang and Anderson (1998) using a LECO C632 carbon combustion analyzer (LECO corporation, St. Josesph, MI, USA). Soil nitrate (NO_3^-) and sulphate (SO_4^{2-}) were extracted from samples using a 0.01M $CaCl_2$ extraction methodology described by Houba et al. (2000). Automated colorimetry was used to analyze the extracts for levels of NO_3-N and SO_4-S. Available phosphorus (P) and potassium (K) were measured on the soil depth sample of 0-15 cm using a modified Kelowna extraction procedure (Qian et al., 1994). Extracts were colorimetrically analyzed for P using a Technicon Autoanalyzer II segmented flow automated system (Technicon Industrial Systems, Tarrytown, NY, USA). Concentrations of K in extracts were analyzed using flame atomic absorption (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA). Plant available Zn was extracted from samples taken from the 0-15 cm and 15-30 cm depths of the soil profile using a 0.005M diethylene-triamine-penta acetic acid (DTPA) solution (Lindsay and Norvell, 1978).

In addition to plant-available Zn determined by DTPA extraction, a detailed characterization of soil Zn was conducted by sequentially fractionating soil Zn into various soil-Zn pools (Fig 3.1) following the modified BCR three-step sequential extraction procedure (Zemberyov et al., 2006). Acetic acid (0.11M) was used to extract Zn from the soil solution-carbonate-exchangeable fraction. The iron/manganese oxyhydroxide fraction was extracted next with freshly prepared 0.5M hydroxylamine hydrochloride. The organic-bound fraction was the last to be chemically extracted using concentrated hydrogen peroxide (8.8M) followed by 1.0M ammonium acetate adjusted to pH 2. Residual Zn included Zn not extracted in previous steps and levels were determined through a hydrogen peroxide-sulphuric acid digestion (Thomas et al., 1967). Flame atomic absorption was used to analyze Zn concentration in the extracts of all Zn fractions, including DTPA-extractable and residual Zn.

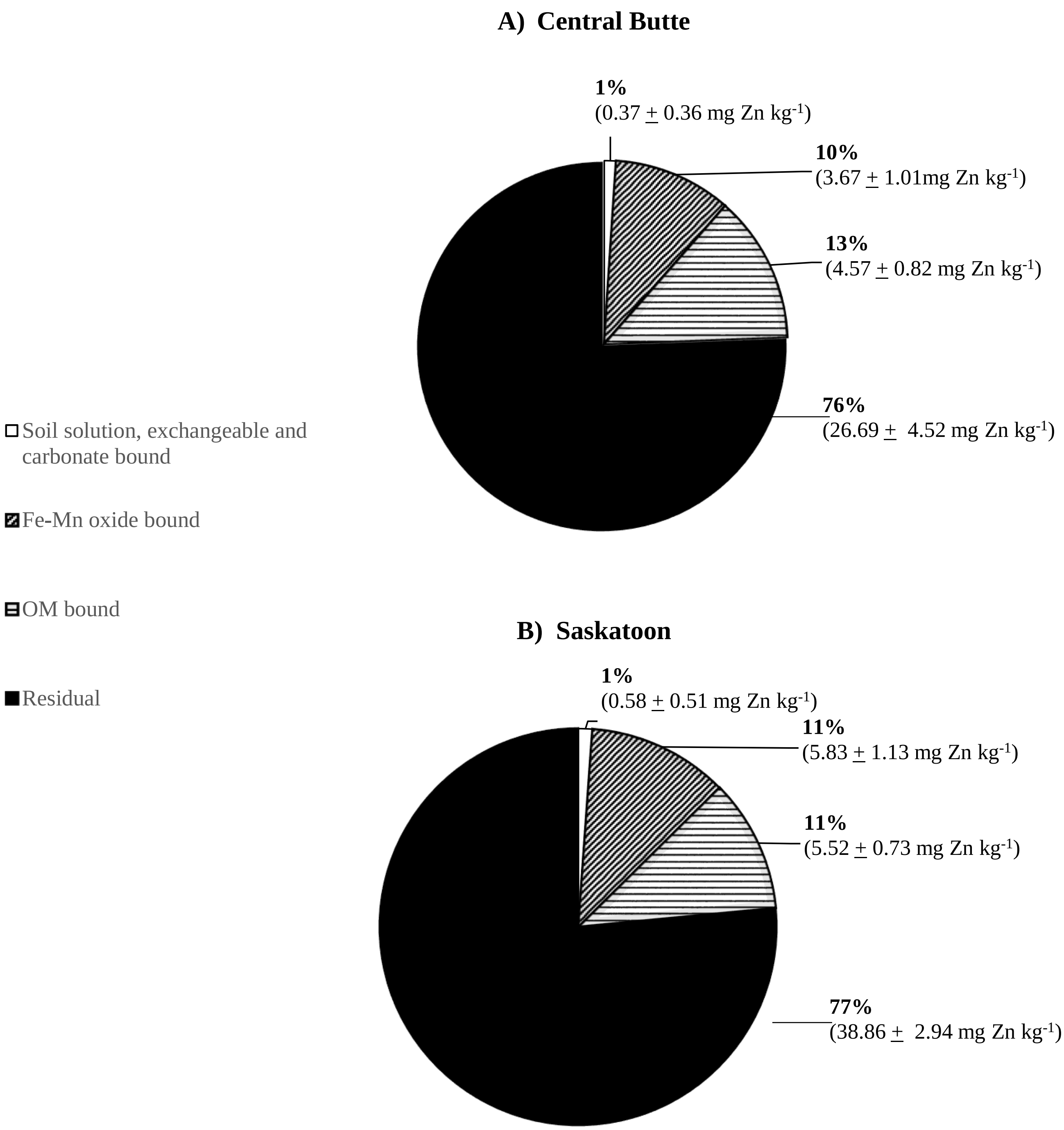

Fig 3.1. Baseline distribution of Zn (percentage of total and mg Zn kg^{-1} in the fraction), +/- standard deviation, in various soil fractions at (A) Central Butte and (B) Saskatoon sites as determined by BCR sequential extraction.

3.4.3 Experimental design and set-up

In order to assess the interaction between the effects of fertilizer and lentil cultivar, field trials were set up as a split-plot design with Zn fertilization rate as the main plot factor and lentil cultivar as the subplot variable. Subplots consisted of three seeded rows with cultivars being randomized throughout and replicated three times within each main plot. Main plots, consisting of fertilizer treatments, were randomized and replicated twice within the site landscape to achieve six treatment replications and a total of 54 subplots per site (Figure 3.2). Main plots were separated with a border crop of lentil, which was not fertilized or measured, with the purpose of buffering any fertilizer interactions between main plot treatments.

All seed used in the field trial was sourced from the Kernen Crop Research Farm near Saskatoon, SK and included popular imidazolinone-tolerant cultivars from the large green (cv. CDC Impower), small green (cv. CDC Imvincible) and small red (cv. CDC Maxim) lentil market classes. The appropriate quantity of seeds of each cultivar to target a seeding rate of 130 plants m^{-2}, once accounting for percent germination, was allocated to individual envelopes prior to field seeding. Granular inoculant, containing *Rhizobium leguminosarium* biovar *viceae,* was placed in the seed furrow at the time of seeding using Nodulator® XL inoculant (BASF Canada, Inc., 2013). Granular fertilizer treatments were weighed and blended into vials prior to being applied to the field. All plots received a base macronutrient blend of 28-26-0-0, sourced from urea and monoammonium phosphate, broadcast and incorporated at a rate of 100 kg product ha^{-1}. The addition of these nutrients ensured that differences between treatments were attributed to Zn rate rather than macronutrient deficiencies. The Zn fertilizer treatments included applications of 35.5% Zn content granular $ZnSO_4$ at rates of 0, 2.5, or 5 kg Zn ha^{-1}. Fertilizer applications were made one week prior to seeding by broadcasting onto the appropriate subplots followed by incorporation to a depth of approximately 5 cm, using a rototiller. Sites were seeded using a plot drill equipped with disc openers on 30 cm spacing. The Central Butte site was seeded on May 22, 2013 and the Saskatoon site was seeded on May 28, 2013. Pre-plant weed control at both sites was accomplished by an application of glyphosate at 0.75 litre active ingredient ha^{-1} and broadcast application of 5% ethafluralin (Edge™ Granular) herbicide in early May. In crop herbicide applications were made in accordance with product label guidelines and included Solo® (imazamox) at Central Butte and Odyssey® (imazamox and imazethapyr) at Saskatoon. A tank-mix of clethodim and pinoxaden for grassy weed control was made as a separate application. Disease control measures at Central Butte included the application of pyraclostrobin (Headline®) fungicide at 400 mL ha^{-1} at the beginning of lentil flowering. A late-season insecticide application of 82 mL ha^{-1} of lambda-cyhalothrin was made at the Saskatoon site in response to aphid pressure.

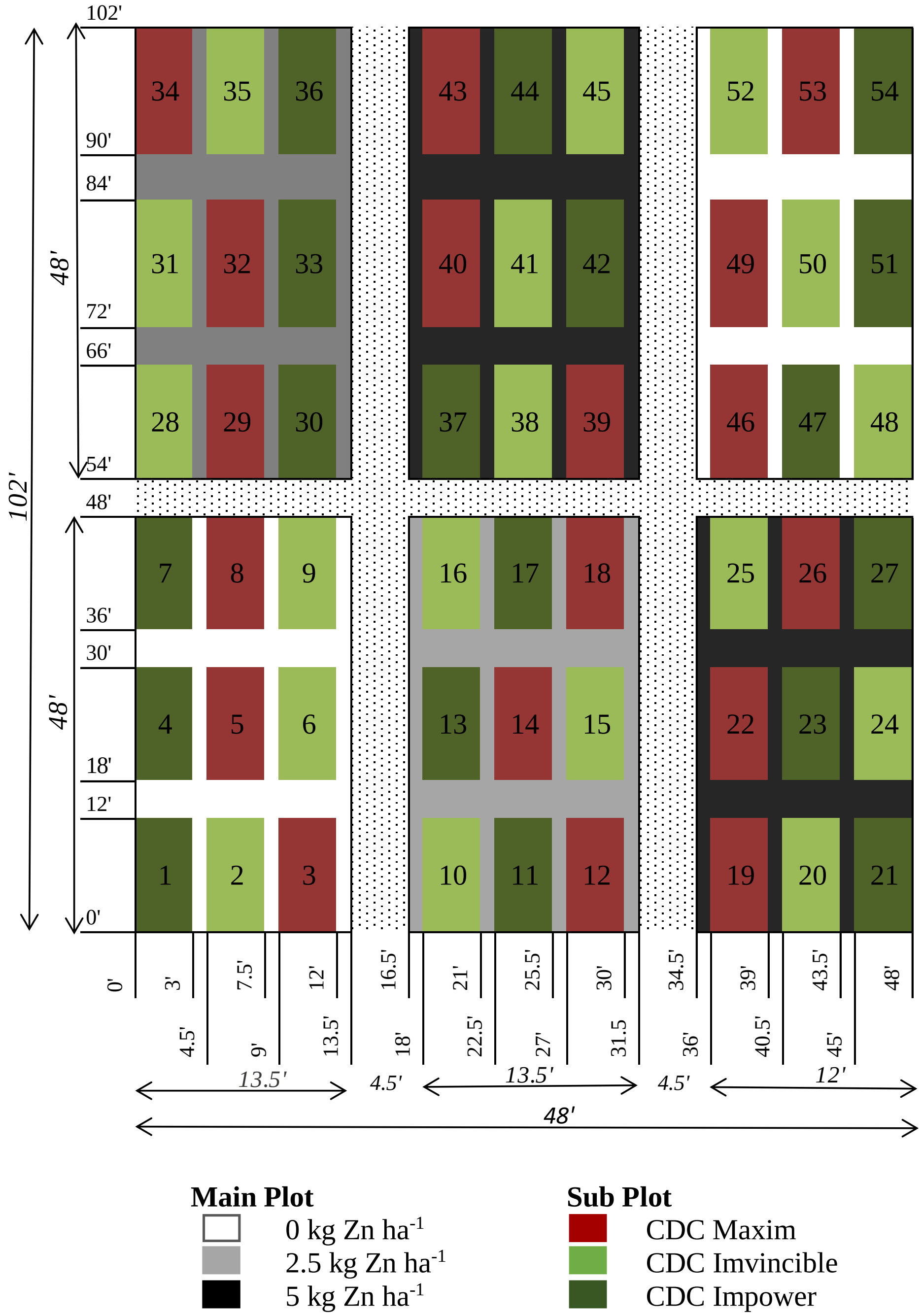

Fig 3.2. Diagram of split-plot design field layout specific to Central Butte field site; Saskatoon field site follows the same general layout with main plots positioned as appears in diagram but different in sub-plot randomization.

3.4.4 Harvest and analysis

Plot harvest was completed at maturity and took place on August 19, 2013 at the Central Butte site and on September 3, 2013 at the Saskatoon location. At harvest, one-meter row length samples, uncontaminated with soil, were removed from each sub-plot by hand. Samples were consistently removed from the center row of the sub-plot to avoid any cross plot effects. After samples were removed by hand, plots were dessicated with Reglone® dessicant (240 g L^{-1} diquat) and remaining lentils were mechanically harvested using a plot combine once all plots were sufficiently dry.

Yield analysis was conducted, and nutrient concentration of straw and grain was measured for the samples removed by hand. Once hand-harvested samples were dried at 35 °C and weighed, a random sub-sample was removed and threshed using a de-awning machine. The de-awning implement operates using rubber belting and, therefore, eliminates abrasive contact with metal parts which could pose a risk of Zn contamination. Sampled straw and threshed grain were then hand ground and a 0.25 g sub-sample was digested using the sulphuric acid-peroxide wet ashing technique described by Thomas et al. (1967). Nitrogen and P concentration in the digests was measured colorimetrically (Technicon AutoAnalyzer; Technicon Industrial Systems, Tarrytown, NY, USA). Zinc and Fe concentrations were also determined by analyzing the digests using atomic absorption (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alta, CA, USA). Quality assurance was completed by the inclusion of a National Institute of Standards and Technology (NIST) certified plant material standard of known Zn concentration every 40 samples, and ensuring that the sample value for Zn concentration obtained was within 5% of the NIST certified value. The threshed grain from the sub-samples was also weighed and added to the grain weight from the mechanically threshed bulk samples to determine total yield per treatment.

After plots were harvested, soil samples (0-15 cm) were removed from each sub-plot in the second week of September 2013 and again in the first week of May 2014. Soil cores were removed from two locations near the middle row of crop residue and bulked together. All samples were air-dried prior to being homogenized and passed through a 2 mm stainless steel sieve. Residual levels of plant-available Zn in the soil samples were determined by extraction with 0.005M DTPA following the protocol of Lindsay and Norvell (1978) and concentrations of Zn in the extracts were determined using the flame atomic absorption spectrometer.

Distribution of soil Zn fractions at the Central Butte site, within the 0-15 cm depth samples collected from cv. CDC Maxim sub-plots fertilized with 0 and 5 kg Zn ha^{-1}, was assessed using a modified version of the BCR three-step sequential extraction procedure (Žemberyová et al., 2006). Soil weights, extracting reagents, and shaking times followed the same protocol as Žemberyová et al. (2006); however, specific fractions were not extracted sequentially. In addition to the specific extraction protocols described by Žemberyová et al. (2006), an extraction with 0.5M HCl was included in an attempt to extract soil Zn bound to the carbonate fraction. Instead of separating the supernatant from the centrifuged soil, extract was filtered through a VWR® # 454 filter paper into 8 Dram vials and stored at 4 ºC before being analyzed using atomic absorption.

3.4.5 Statistical analysis

Statistical analyses were performed using the PROC MIXED procedure of SAS 9.4 for a split-plot design with experimental units arranged as a randomized complete block design (RCBD). The model used for analysis was $Y= \mu + block + A + e_1 + B + A*B + e_2$ where Y is the dependent variable which included either straw or grain yield (kg ha^{-1}) or Zn concentration in the straw, grain, or soil (mg Zn kg^{-1}); μ is the population mean for the dependent variable and e denotes a measure of error; block is considered a random effect; A denotes the main plot fixed effect of the fertilizer rate (kg ha^{-1}); B is the sub-plot fixed effect of the lentil cultivar and A*B is the effect of the interaction between the main and sub-plot factors as a fixed effect. The denominator degrees of freedom (DDFM) used the Satterthwaite method. Multi-treatment comparisons were made using the Tukey-Kramer method where significance was declared at $P<0.05$.

3.5 Results

3.5.1 Effect of zinc fertilization on lentil yield

Yield responses of lentil to soil applied $ZnSO_4$ fertilizer are reported as the mean values of six treatment replicates and are summarized in Table 3.3. In general, lentil yield was unresponsive to Zn fertilization. At the Central Butte location, the effects of cultivar (P=0.198) and Zn rate (P=0.929) on lentil biomass production were not significant. Significant differences among grain yields of any lentil cultivar, fertilized at any rate of Zn, were not detected.

Similarly, Zn fertilization at Saskatoon site had no significant effect on lentil grain or straw yield compared to the control treatment where no Zn fertilizer was applied. However, there was a significant cultivar effect on grain yield (P=0.004) and biomass production (P=0.001) at the Saskatoon site. CDC Impower, the large green cultivar, produced significantly higher amounts of straw and lower grain yields relative to the other two lentil cultivars. Grain and straw yields were not statistically different between the small red and small green cultivars.

Table 3.3. Effect of three rates of soil applied $ZnSO_4$ on grain and straw yield (kg ha^{-1}) of three lentil cultivars at two sites in 2013.

Site	Yield[†]	Zn Rate			SEM[‡]	P values		
		0	2.5	5		Rate (R)	Cultivar (C)	R*C Interaction
		(kg Zn ha^{-1})						
	--Grain--							
Central Butte	CDC Maxim	2992 aA	2953 aA	3116 aA	439.2	0.994	0.554	0.925
	CDC Imvincible	3131 aA	2909 aA	2826 aA				
	CDC Impower	2634 aA	2778 aA	2853 aA				
Saskatoon	CDC Maxim	4304 aA	4808 aA	4341 aA	281.7	0.536	0.004	0.667
	CDC Imvincible	4193 aA	4400 aA	4603 aA				
	CDC Impower	3817 aB	3855 aB	3571 aB				
	--Straw--							
Central Butte	CDC Maxim	2439 aA	2494 aA	2231 aA	275.5	0.929	0.198	0.588
	CDC Imvincible	2702 aA	2406 aA	2344 aA				
	CDC Impower	2652 aA	2606 aA	2950 aA				
Saskatoon	CDC Maxim	3300 bB	3705 abB	3370 abB	255.7	0.683	0.001	0.656
	CDC Imvincible	3743 abB	3640 abB	3997 abB				
	CDC Impower	4186 abA	4494 aA	4250 abA				

[†] Means with the same lower-case letter in the same row (within a cultivar) and with the same upper-case letter in the same column (within Zn rate), at a given site, are not significantly different (P>0.05) as determined by multi-treatment comparisons using the Tukey-Kramer method.

[‡] SEM= standard error of mean (rate x cultivar) with N=9 and R=6

3.5.2 Accumulation of zinc in lentil

Similar to yield, the concentration of Zn in lentil grain and straw (Fig 3.3) was generally unaffected by soil applied $ZnSO_4$ fertilizer. Although site data were analyzed independently of each other, there were some common patterns that appeared at both locations. Concentration of Zn in the grain consistently surpassed that which was measured in the lentil straw of any cultivar fertilized with any rate of Zn. Increasing rates of Zn fertilizer did not, however, result in significant differences among corresponding straw Zn concentration values at either site. The effect of Zn rate on grain Zn concentration at Central Butte (P= 0.595) and Saskatoon (P= 0.176) was non-significant. There were also no significant interactive effects between fertilization rate and lentil cultivar on Zn concentrations of lentil straw or grain in any of the cultivars at either Central Butte or Saskatoon locations.

At Central Butte, lentil cultivar had no significant effect on the accumulation of Zn in lentil grain (P= 0.186) or straw (P=0.471). Similarly, the effect of cultivar on the straw Zn concentration of lentil grown at Saskatoon was also non-significant (P= 0.540). However, the effect of cultivar on the concentration of Zn measured in the grain of lentil grown at Saskatoon was significant (P= <.0001). CDC Impower accumulated significantly higher concentrations of Zn in the grain compared to CDC Imvincible when no Zn fertilizer was applied. Concentrations of Zn in the grain at the Saskatoon site were higher than at the Central Butte site, similar to extractable available Zn in the baseline soil samples.

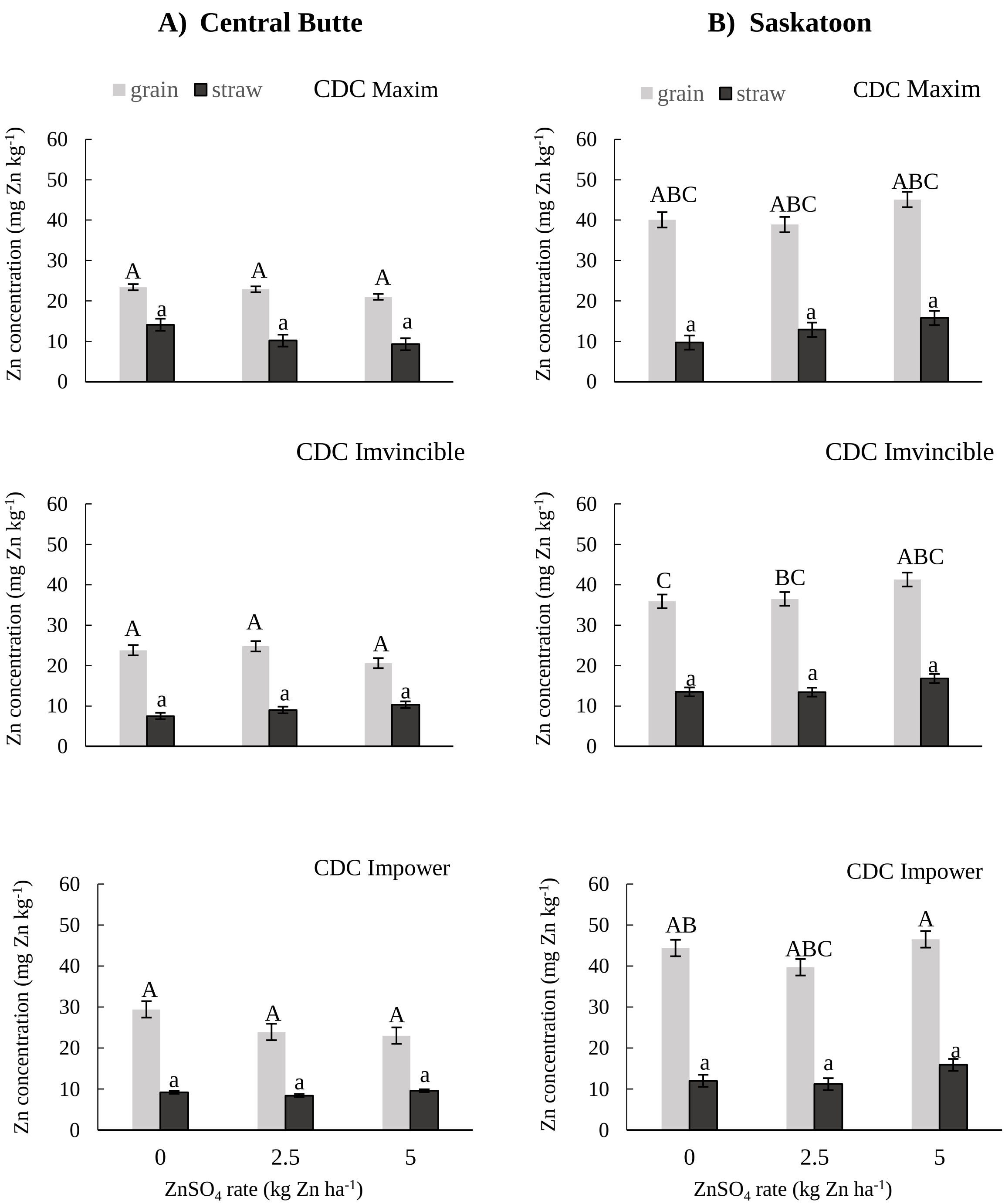

Fig. 3.3. Mean zinc concentration (mg Zn kg^{-1}) in lentil grain (grey coloured bars) and straw (black coloured bars) of three lentil cultivars at two field locations (Central Butte and Saskatoon SK) as affected by Zn fertilization with soil applied $ZnSO_4$. Error bars are standard errors of mean (rate x cultivar) with N=18 and R=6. Within a site location and for either grain (upper-case letters) or straw (lower-case letters), means with the same letters within a variety or fertilizer rate are not significantly different as determined by multi-treatment comparison using the Tukey-Kramer method (P>0.05).

3.5.3 Effect of zinc fertilization on soil zinc status

Rate of soil applied $ZnSO_4$ had no significant effect (P>0.05) on residual levels of plant available Zn in the soil at both site locations during either season of measurement: fall 2013 or spring 2014 (Fig. 3.4). There was a significant effect of cultivar (P=0.04) on residual soil Zn measured in the fall of 2013 at Central Butte where CDC Maxim subplots had significantly greater amounts of residual plant-available soil Zn compared to CDC Imvincible. However, this significant effect of cultivar was not detected in the spring of 2014 (P=0.34) and did not exist during either season at Saskatoon.

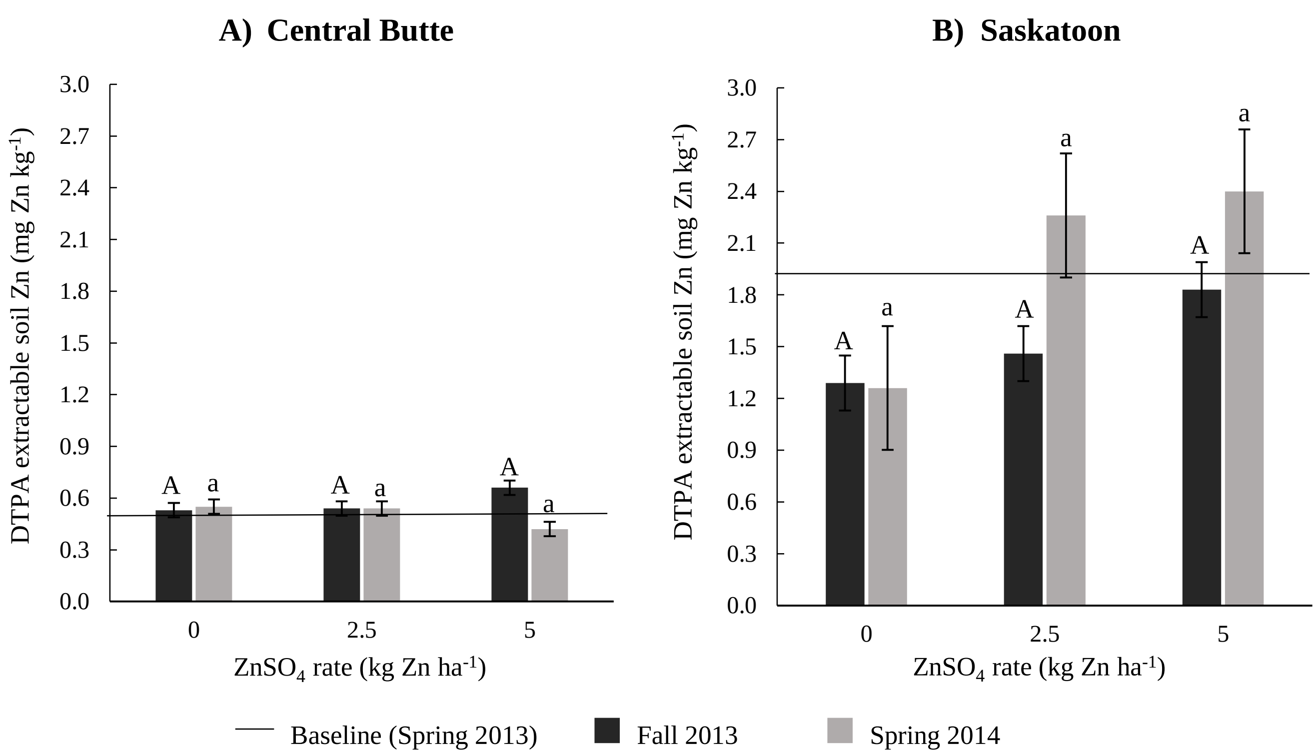

Fig. 3.4. Effect of Zn fertilization rate (kg Zn ha^{-1}) on residual levels of DTPA-extractable soil Zn (mg Zn kg^{-1}), reported as mean values of three lentil cultivars, measured post-harvest in the fall of 2013 (black coloured bars) and pre-seeding in the spring of 2014 (grey coloured bars) at (A) Central Butte, SK and (B) Saskatoon, SK. Error bars are standard errors of mean and the horizontal line represents baseline levels of DPTA-extractable Zn measured prior to seeding during the spring of 2013. Within a given site location, means with the same upper-case letter (fall 2013) and lower-case (spring 2014) are not significantly different (P>0.05) as determined by multi-treatment comparisons using the Tukey-Kramer method.

Reflective of native levels of soil Zn, soil at the Saskatoon location had greater amounts of DTPA extractable Zn compared to the Central Butte site when measured in either the fall of 2013 or the spring of 2014. With the exception of spring 2014 levels at Central Butte, there was a subtle, although non-significant, trend of higher levels of plant available soil Zn with increasing rates of soil-applied $ZnSO_4$ fertilizer. During the season of application (2013), residual levels of DTPA-extractable Zn, which were measured post-harvest, fell short of baseline amounts of soil Zn determined prior to seeding at Saskatoon. In the spring of 2014, additions of Zn fertilizer made in the spring of 2013 produced higher mean concentrations of plant available soil Zn. However, these differences were not significant compared to the control treatments where no Zn fertilizer was applied. At Central Butte, there was generally little deviation of soil Zn compared to baseline levels in either season of measurement, regardless of the application rate. Zinc sulphate applied at the 5 kg Zn ha^{-1} rate boosted the DTPA extractable level of soil Zn during the year of application in Central Butte, but when measurements of soil Zn were taken in the spring of 2014 they had dropped below baseline levels.

Regardless of fertilization rate or site location, much of the Zn in the soil existed in forms that are considered to be plant unavailable (non-labile), at least in the short-term. Differences in percent of unavailable soil Zn, determined by subtracting DTPA extractable Zn from the total digested Zn, were not significant ($P>0.05$) at either location. Analysis of raw data indicates that the highest percentages (proportions) of unavailable forms of Zn at Central Butte were measured within treatments that were fertilized with 5 kg Zn ha^{-1} (98.9%) whereas at Saskatoon, control treatments of 0 kg Zn ha^{-1} had the highest percentages of unavailable Zn (98.0%). When Zn was extracted from soils with reagents that did not include DTPA, a similar pattern existed where the large majority of Zn was distributed in soil fractions and forms that were unavailable for plant uptake. The distribution of Zn in Central Butte soil fractions without Zn fertilization and when fertilized with the highest rates of soil applied $ZnSO_4$ (5 kg Zn ha^{-1}) are illustrated in Figure 3.4.

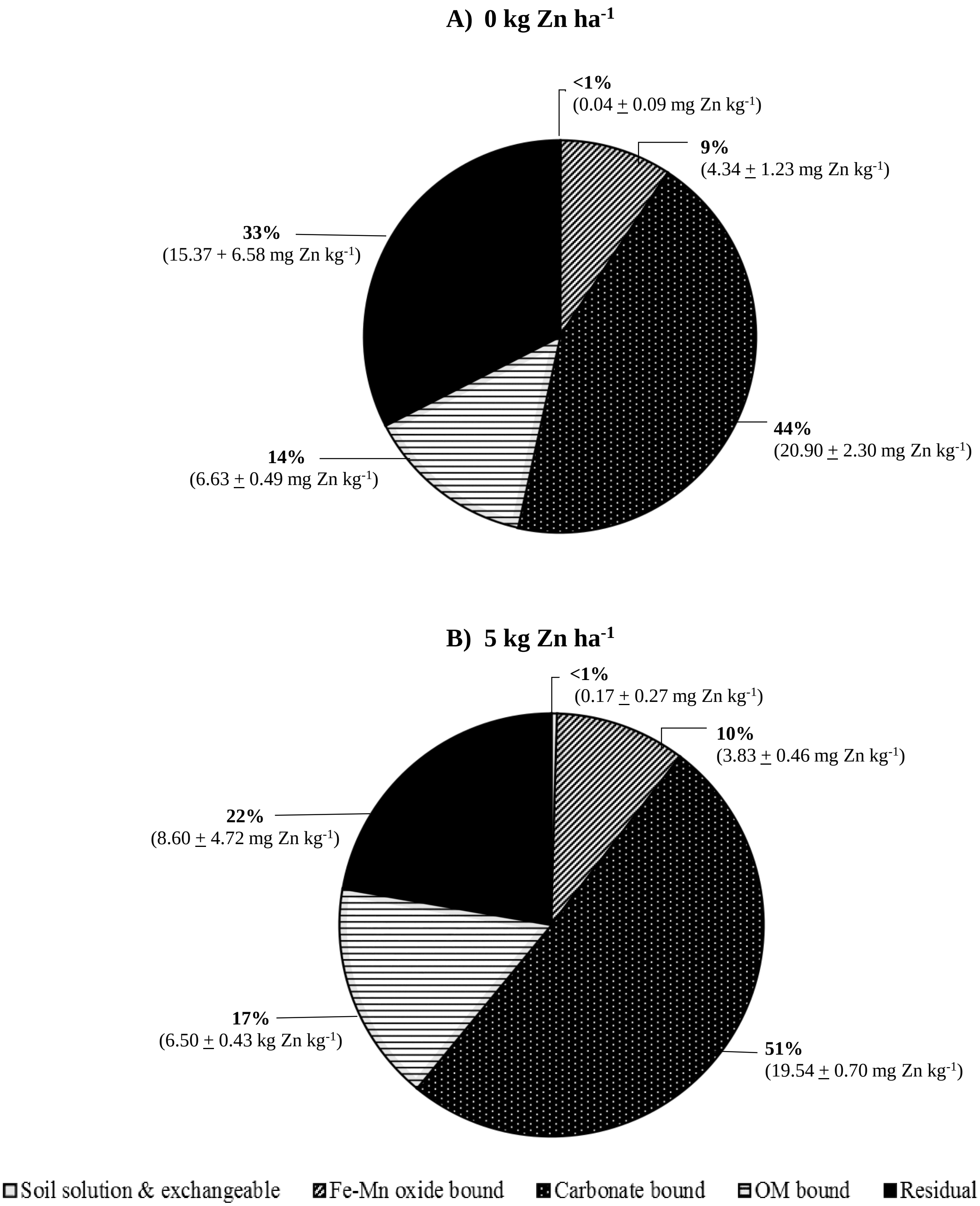
A) 0 kg Zn ha-1
<1%
(0.04 + 0.09 mg Zn kg-1)
9%
(4.34 + 1.23 mg Zn kg-1)
33%
(15.37 + 6.58 mg Zn kg-1)
44%
(20.90 + 2.30 mg Zn kg-1)
14%
(6.63 + 0.49 mg Zn kg-1)
B) 5 kg Zn ha-1
<1%
(0.17 + 0.27 mg Zn kg-1)
10%
(3.83 + 0.46 mg Zn kg-1)
22%
(8.60 + 4.72 mg Zn kg-1)
17%
(6.50 + 0.43 kg Zn kg-1)
51%
(19.54 + 0.70 mg Zn kg-1)
Soil solution & exchangeable
Fe-Mn oxide bound
Carbonate bound
OM bound
Residual

Fig 3.5. Distribution of Zn (percentage of total and mg Zn kg^{-1} in the fraction), +/- standard deviation, in various soil fractions at Central Butte, SK in CDC Maxim subplots fertilized with (A) 0 kg Zn ha^{-1} and (B) 5 kg Zn ha^{-1} as determined by non-sequential extraction using a modified BCR method.

3.6 Discussion

3.6.1 Lentil yield response to zinc fertilization rate

Soil-applied $ZnSO_4$ fertilizer, that was broadcast and incorporated prior to seeding, had no significant effect on lentil grain or straw yields at either Central Butte or Saskatoon. Concentrations of DTPA-extractable Zn in the soil of 0.50 mg Zn kg^{-1} is generally considered the critical soil Zn level required to maximize crop yield (Cakmak et al., 1999) and the level at or above that a response to added Zn fertilizer would not be anticipated. However, responses in crop yields have been detected as a result of Zn fertilizer applications when plant-available Zn has been measured as high as 1.0 mg kg^{-1} soil (Alloway, 2008). Therefore, there is some debate about critical extractable Zn levels in soil used to predict fertilizer Zn responses. Brennan (2005) observed that the critical yield response level of DTPA-extractable Zn for spring wheat, grown on calcareous soils (pH $\geq$7) of southwestern Australia, was 0.24 mg Zn kg^{-1} and determined lentil had a higher physiological Zn requirement compared to spring wheat. Although critical thresholds for soil Zn vary with soil properties and crop species, a yield response to Zn would not be expected at Saskatoon because the baseline level of DTPA-extractable Zn at this site was 1.85 mg kg^{-1} soil. However, the baseline level of DTPA-extractable Zn measured at Central Butte (0.47 mg kg^{-1} soil) fell below the critical threshold of soil Zn concentration reported in the literature and still failed to prompt an improvement in lentil yield. These results suggest that Zn fertilizer recommendations should not be exclusively based on soil-test values of plant available Zn as the current range of lower critical concentrations of soil Zn may be too general to accurately predict the yield response specific to a variety of combinations of crop, environmental, and management situations.

The crop response to Zn is certainly dependent on crop type. Increased yields have been observed in rice (Naik and Das, 2008; Shivay et al., 2008), corn (Singh et al., 1979), and wheat (Cakmak et al., 1999) grown on soils ranging in pH 7.2-8.8 and initial DTPA-extractable soil levels of 0.01-0.78 mg Zn kg^{-1} when soil applied $ZnSO_4$ had been broadcast and incorporated at rates ranging from 5-23 kg Zn ha^{-1}. Gulser et al. (2004), in field trials located in southeastern Turkey, reported increases in lentil yield in response to soil applications of $ZnSO_4$ fertilizer that were consistent in two cropping seasons across multiple lentil cultivars. Although the soil properties of these cited studies were aligned with the low OM, high pH, low extractable Zn nature of the soil at Central Butte, the rates of applied Zn were typically 2-4 times higher than those applied in the present study. The lack of yield response agreement between the present and previously reported studies could indicate that application rates of $ZnSO_4$ greater than 5 kg Zn ha^{-1} may be required to initiate a yield response in crops grown on calcareous soils with high fixation capacity and with limited available Zn. However, the yield results of this study are in agreement with findings of Singh et al. (1987) who reported no significant yield responses in several dryland annual crops, including lentil, to $ZnSO_4$ applied at 10 kg Zn ha^{-1} in 23 field trials across Saskatchewan, even when DTPA-extractable Zn levels were lower than 0.5 mg kg^{-1} soil. These combined findings support the assertion that Saskatchewan producers are unlikely to economically benefit from fertilizing lentil crops with soil applied $ZnSO_4$ (Appendix E).

3.6.2 Effect of zinc fertilization rate on zinc accumulation in lentil

Zinc concentration of lentil grain and straw measured at Central Butte and Saskatoon were generally within a typical range of concentration values (~20 to 40 mg Zn kg^{-1} grain; ~10 mg Zn kg^{-1} straw) reported in literature derived from lentil-based research on Saskatchewan soils (Thavarajah et al., 2009; Maqsood et al., 2013). Relative to Zn concentration in straw, lentil grain consistently accumulated higher amounts of Zn at both locations. This distribution of Zn in the plant could be partially explained by upward translocation of soil-derived Zn from the roots to the grain. However, because mobility of Zn through the phloem is generally considered to be limited (Havlin et al., 2014), the differences between lentil grain and straw Zn concentration may be more accurately explained through a dilution effect as a high level of straw production occurred at each location. Straw yield of CDC Impower was significantly higher than the straw production of the other two lentil cultivars grown at Saskatoon. The difference between grain and straw Zn concentrations is largest in CDC Impower with a greater proportion of total plant Zn being allocated to the lentil grain in the cultivar where lentil yield is the lowest which further supports the occurrence of a dilution effect in lentil straw. Greater lentil grain Zn concentration, across all cultivars and Zn fertilizer treatments, at Saskatoon compared to Central Butte is reflective of the native levels of plant available Zn within each soil, with Saskatoon site having DTPA extractable (labile) Zn that was 3-4 times higher than the Central Butte site. Furthermore, the higher levels of soil OM at the Saskatoon site may increase the mobility of Zn through the soil as Zn ions are able to form soluble complexes with OM (Kabata-Pendias, 2011).

3.6.3 Fate of zinc fertilizer in the soils

Soil applications of $ZnSO_4$ fertilizer did not significantly ($P>0.05$) improve the plant-available Zn status of soils at either Central Butte or Saskatoon (Fig. 3.3). This is in agreement with the overall lack of effect of Zn fertilization on yield or plant Zn concentrations. These results are contradicted by the findings of Kumar and Qureshi (2012) who, in a pot study conducted in India, demonstrated that DTPA-available Zn in soil was significantly enhanced with increasing rates of Zn fertilizer. However, the lowest Zn fertilization rates of the Kumar and Qureshi (2012) study were double the highest rates of Zn applied in the present study. Singh et al. (1987) also reported increased levels of DTPA-extractable soil Zn when Saskatchewan soils were fertilized with $ZnSO_4$ at 10 kg Zn ha^{-1}. This residual effect was reported in the third field season and therefore, does agree with the increased trend of DTPA-extractable Zn above baseline levels at Saskatoon that was delayed until the season following application. Although $ZnSO_4$ is a very soluble form of Zn that is considered highly available (Mortvedt, 1992), these results suggest that the effects of its application on plant-available Zn in these soils are minimal during the first year of application.

Future investigation of the effects of soil processes, including microbial activity and water dynamics, affecting plant-available Zn in soil are needed to explain the temporal effects of Zn fertilizer application. The results also suggest a spatial effect of Zn fertilizer placement. An extensive survey of soil samples collected along transects indicated that spatial distribution of Zn in Saskatchewan soils is highly variable even across very short distances (Singh, 1986). Although granular $ZnSO_4$ was bulk blended with larger quantities of granular macronutrients (urea and monoammonium phosphate) prior to being broadcast to aid in uniformity of application across the plot area, the low application rates of Zn inherently result in widely spaced placement of $ZnSO_4$ fertilizer granules. An application of liquid Zn fertilizer sprayed across the plot area would improve uniformity but is not a common practice used by producers. Unfortunately, highly variable DTPA-extractable soil Zn levels could be obtained, depending on whether or not a soil sample was removed from close proximity to a $ZnSO_4$ fertilizer granule. This could help account for the relatively high SEM values for measurements of residual levels of DTPA-extractable Zn in the Saskatoon soil. Furthermore, plants obtain Zn primarily through diffusion and root interception (White and Broadley, 2009), which indicates that better plant uptake and utilization of Zn will be achieved when Zn is placed very close to the seed due to poor mobility through the soil. Limited crop uptake of Zn during the first season of application has been reported when low rates of Zn were incorporated into the soil as intact $ZnSO_4$ fertilizer granules (Goos et al., 2000).

Compared to Saskatoon, DTPA-available Zn in the Central Butte soil deviated less from the baseline level. Perhaps this is indicative of an immediate and extensive fixation of the applied $ZnSO_4$ into insoluble forms in this soil. The results of a specific extraction procedure for soil Zn (Fig. 3.5) demonstrated that almost none of the total soil Zn is distributed within the soil solution (<1%). Relative to the control treatment, a higher amount of soil solution Zn was measured in subplots fertilized with 5 kg Zn ha^{-1}. However, this increase was negligible, and the largest changes in size of the soil Zn pools was measured in the residual fraction. Zinc held within the residual fraction decreased by 11% when 5 kg Zn ha^{-1} was applied. The majority of this Zn was reallocated to the carbonate-bound fraction which is also considered unavailable for plant uptake. Calcareous soils, such as that of Central Butte, have been noted for their large adsorption capacity of Zn on iron-coated carbonates (Uygur and Rimmer, 2000). The significant cultivar effect on residual levels of DTPA-extractable Zn in Central Butte soil measured during the fall of 2013 could be an anomaly, but might also suggest genetic differences amongst the different lentil cultivars to access soil Zn or promote its mobilization. Previous research has shown that the rhizosphere of wheat is able to secrete organic acids (Maqsood et al., 2011) that may aid in the mobility of Zn through the soil through the formation of soluble complexes. Although these differences are demonstrated at the species level, they could also exist at the cultivar level. Future research with a focus on differences in root systems between lentil cultivars and the effects of rooting characteristics on nutrient uptake could improve current understanding of micronutrient cycling.

3.7 Conclusion

Application of $ZnSO_4$ fertilizer through broadcast and incorporation of intact granules was not effective for increasing yield or grain Zn concentration in lentil. Three popular lentil cultivars grown at two Saskatchewan field sites did not respond to additions of Zn fertilizer at rates as high as 5 kg Zn ha^{-1}. Furthermore, varying rates of $ZnSO_4$ did not result in significant differences amongst residual levels of DTPA-extractable Zn. Migration of applied Zn into non-labile forms is considered a contributing factor and fertilizer placement strategies may also play a role in the results of this study. Despite the above-average growing conditions at both field sites and a soil at the Central Butte location that, based on DTPA extractable Zn less than 0.5 mg Zn kg^{-1}, was anticipated to be responsive to additions of Zn, Saskatchewan lentil growers at either of these locations would not have benefitted from Zn fertilization in the 2013 crop season. The results of this experiment reinforce field trials with Zn fertilization conducted in the 1980s in Saskatchewan, indicating limited crop response to applied Zn fertilizer.

4. EFFECTS OF ZINC FERTILIZER AMENDMENTS ON YIELD AND GRAIN ZINC CONCENTRATION UNDER CONTROLLED ENVIRONMENT CONDITIONS

4.1 Preface

The previous chapter demonstrated that varying rates of soil applied $ZnSO_4$ did not result in lentil yield or grain Zn concentration increases. However, the effects of other forms and application methods of Zn fertilization on yield and nutrient concentration of lentil remain uncertain. Therefore, the three lentil cultivars, described in Chapter 3, were amended with single rates of Zn fertilizer in a pot study. These amendments were derived from different Zn fertilizer sources and were applied as either soil or foliar treatments. Unlike the previously described field study which examined the effects of rate of applied Zn, this pot experiment was intended to explore potential differences amongst Zn fertilizer forms.

4.2 Abstract

The application of Zn fertilizer to lentil is an agronomic strategy that has the potential to improve yield and enhance grain Zn concentration. As the demand for nutrient-dense food increases, an understanding of the effectiveness of various Zn fertilizer sources and application methods becomes increasingly important. A pot study was conducted in a polyhouse at the University of Saskatchewan in 2013 to determine if Zn fertilizer applied to three popular Saskatchewan lentil cultivars could increase yield and concentration of Zn in the grain. The effects of soil and foliar applied Zn forms, including $ZnSO_4$, Zn chelated with EDTA, Zn Lignosulphonate, and a control with no addition of Zn, were evaluated. Forms of Zn were not found to significantly increase yield (P=0.828) or grain Zn concentration (P=0.708) in any of the lentil cultivars tested. Fertilization with soil applied $ZnSO_4$ resulted in significantly (P<.0001) higher amounts of residual available Zn in the soil compared to soil or foliar applied chelated Zn form. Soil fertilized with $ZnSO_4$ had 1.13 mg kg^{-1} DTPA-extractable Zn compared to 0.84 mg Zn kg^{-1} and 0.77 mg Zn kg^{-1} in the soil and foliar applied chelated Zn, respectively. This effect is attributed to the higher recommended application rate made for soil applied $ZnSO_4$.

4.3 Introduction

Producers are currently being faced with an ever-expanding micronutrient fertilizer product market. Micronutrients, including Zn, can be supplied in a variety of forms and may be either soil or foliar applied. Although product choice allows producers flexibility in their fertility management, all forms of Zn may not be equally beneficial in enhancing yield or increasing Zn concentration in the grain. Solubility, mobility and molecular size of the product are factors that strongly influence the bioavailability of Zn to plants. Generally, increased solubility of a granular Zn fertilizer results in improved crop response and Zn uptake (Mortvedt, 1992). Mortvedt (1992) also suggests that Zn uptake and crop production are significantly reduced when Zn fertilizer is less than 40% water-soluble. Method of application—either soil or foliar—also presents various challenges for Zn uptake. Effects of soil applied Zn fertilizers can be variable due to inherent soil variability. Availability of soil applied Zn to plants may be dependent on soil temperature, pH, moisture, and nutrient status (Gulser et al., 2004; Schulin et al., 2009; White and Broadley, 2009). Similarly, the efficacy of foliar applied Zn may be limited by the molecular size of a particular Zn form, which impacts the entry of the nutrient into the plant via the leaf (Schulin et al., 2009). Larger Zn molecules may be unable to efficiently penetrate the leaf barrier and gain access into the plant. Method of Zn application is not determined by its form, as Zn fertilizers of a given form can often be applied as either a soil or foliar amendment; however, some forms are better suited for uptake through the root system compared to absorption through the leaf and vice versa.

The objective of this study was to test the hypothesis that yield and grain Zn concentration will be influenced by the form of Zn fertilizer applied in a controlled environment experiment. The intent of this investigation was to examine and compare the response of three lentil cultivars to major formulations of Zn available to producers: salt, chelate and organic complex, not to compare the efficacy of different commercially available Zn fertilizer products.

4.4 Materials and Methods

4.4.1 Soil preparation and planting

Surface soil, to a depth of 15 cm, was collected from a farm field (SE35-20-4-W3) near Central Butte, SK in the fall of 2012 and thoroughly combined with equivalent surface soil collected in the spring of 2013 from the same location. The soil was collected from a field that was located about 3 km from the Central Butte field research trial described in Chapter 3. The soil texture was loam, classified as an Orthic Brown Chernozem of the Ardill association. The soil was non-saline and had a pH of 7.7 with a DTPA extractable Zn nutrient status of 0.73 mg Zn kg^{-1}. The site was managed as a chemfallow-canola-wheat rotation and at the time of soil collection, the field was wheat stubble.

Bulked soil was thoroughly air-dried and homogenized, avoiding any contact with metal tools or storage equipment to prevent potential Zn contamination from non-treatment sources. Large soil aggregates were pulverized with a wooden roller. Stones and other large debris were removed to establish a suitable seedbed and to ensure that soil primarily contributed to the weight of the pots. Otherwise, limited soil preparation occurred to ensure that the pot soil structural conditions mimicked those of the field as closely as possible. One-litre pots were lined with a cellulose coffee filter to help contain all the soil and applied nutrients within the pot. One kg of soil was then weighed into pot.

All soil received a base application of N, P, K, and S based on recommendations derived from soil test levels to ensure that Zn was the limiting nutrient factor and all other nutrient variables were held constant. According to soil test, micronutrients other than Zn were not identified as a limitation. Basal macronutrients were soil-applied as solutions to avoid any application inefficiencies that might be caused by adding small amounts of granular product to each pot. The source of each nutrient and its application rate is provided in Table 4.1.

Table 4.1. Summary of basal soil applied macronutrients in pot study.

Nutrient	Nutrient Source	Chemical Formula	Application Rate (nutrient)
N	Ammonium nitrate	NH_4NO_3	50 µg N g^{-1}
P	Monocalcium dihydrogen phosphate	$Ca(H_2PO4)_2$ H_2O	25 µg P g^{-1}
K	Potassium sulphate	K_2SO_4	61 µg K g^{-1}
S	Potassium sulphate	K_2SO_4	25 µg S g^{-1}

The basal macronutrients were soil applied to all pots and soil applied Zn treatments (Table 4.2) were incorporated into the soil by removing a small scoop of approximately 200 g of soil from the uppermost soil portion and then making the appropriate fertilizer applications. All nutrients were applied as solution opposed to intact granules. The removed soil was returned to the pot by placing it directly on top of the fertilizer to mimic a fertilizer band, approximately 5 cm deep, in the field. After all the pots received their soil applied basal macronutrient fertilizer amendments, 150 ml of deionized water was added to each pot to bring the soil moisture up to field capacity. Watered pots were left to stand overnight prior to seeding.

Table 4.2. Summary of Zn fertilizer treatments in pot experiment.

Treatment	Zn Application Method	Zn Application Rate (kg Zn ha^{-1})
Control	N/A†	0.000
$ZnSO_4$	Soil	2.500
7% Zn lignosulphonate	Foliar	0.246
9% Zn chelated with EDTA	Foliar	0.246
9% Zn chelated with EDTA	Soil	0.246

†N/A denotes not applicable

One of three lentil cultivars was seeded into each of the pre-fertilized pots—small green (cv. CDC Imvincible) and small red (cv. CDC Maxim) cultivars were seeded at the rate of eight seeds per pot and the large green cultivar (cv. CDC Impower) was seeded at a rate of 10 seeds per pot. A small increase in seeding rate for CDC Impower was used to compensate for a slightly lower percent germination relative to other cultivars. Once germination was evident, pots were moved on May 21, 2013 to a University of Saskatchewan polyhouse where they were grown under natural light and temperature conditions. Seedlings were thinned to three healthy seedlings per pot as soon as establishment was sufficient. Pots were repositioned randomly on a weekly basis.

4.4.2 Treatments

Treatment combinations of three lentil cultivars and five Zn fertilizer amendments were replicated six times for a total of 90 pots in the study. The selected cultivars—CDC Impower, CDC Imvincible, and CDC Maxim—currently represent the majority of the lentil production in Saskatchewan for imidazolinone-tolerant lentils in their respective market classes.

Although a wide variety of Zn-based micronutrient fertilizer products are currently commercially available, the scope of this experiment was limited to these five treatments in an attempt to cover the influence that different general forms of Zn fertilizer (salt, chelate, organic complex) have when applied either as a soil or foliar amendment. The commercial availability of $ZnSO_4$ as a foliar product is limited so a foliar application treatment of $ZnSO_4$ was not included in the study. A commonly available, 7% Zn lignosulphonate foliar product was used instead because it is formulated as organic ligands complexed with $ZnSO_4$. It represents a form of Zn that is available in Saskatchewan and that grain producers would normally foliar apply in their own field operations. The application rates for the 7% Zn lignosulphonate product and the 9% Zn chelated with EDTA treatments were determined on the basis of manufacturer recommendations. The regular recommended application rate for 7% Zn lignosulphonate, however, was adjusted to 0.246 kg Zn ha^{-1} from 0.213 kg Zn ha^{-1} to provide the same application rate of actual Zn among chelated products. This application rate did not exceed the highest rate recommended by the manufacturer of 0.425 kg Zn ha^{-1} for the 7% Zn lignosulphonate foliar product. The median field application rate for $ZnSO_4$ of 2.5 kg Zn ha^{-1} was selected as a recommended application rate for a soil applied Zn fertilizer salt, and also to avoid any potential for Zn toxicity that has been reported in some incidences for the 5.0 kg Zn ha^{-1} application rate in a previous study by Maqsood et al. (2013). Note that the application rates of Zn for the lignosulfonate and chelate products are about 10 times less than for the soil applied $ZnSO_4$. Both soil applied and foliar treatments were prepared and applied as solutions. For the soil applications of the Zn fertilizer as sulfate salts and synthetic chelates, the placement in a band was selected in an attempt to reduce the degree of possible fixation by soil constituents that was believed to be a factor resulting in reduced efficacy of the broadcasted $ZnSO_4$ in the field experiment described in Chapter 3.

4.4.3 Foliar fertilization

Foliar treatments were applied when the majority of lentil plants reached the 8th node stage and had not surpassed the 9th node stage. This stage corresponds to the appropriate herbicide timing as observed on the labels of products registered for use on imidazoline-tolerant lentil cultivars. Foliar applications at the later end of the stage range for herbicide application maximizes the surface area of aboveground biomass for foliar fertilizer interception. Furthermore, this timing represents the most typical application timing that producers would implement in the field as it allows for the Zn amendment to be tank-mixed with the herbicide and applied in a single pass.

Foliar fertilizer products were each separately dissolved into 1 L of deionized water—483 mg of 9% Zn chelated with EDTA and 621 mg of 7% Zn lignosulphonate. Assigned pots received 1 ml of the appropriate foliar treatment using a small plastic spray bottle (Figure 4.1), which equates to an applied water volume of 500 L ha^{-1}. It was determined that six complete pumps of the spray trigger was an accurate and repeatable application of 1 ml of both foliar solutions.

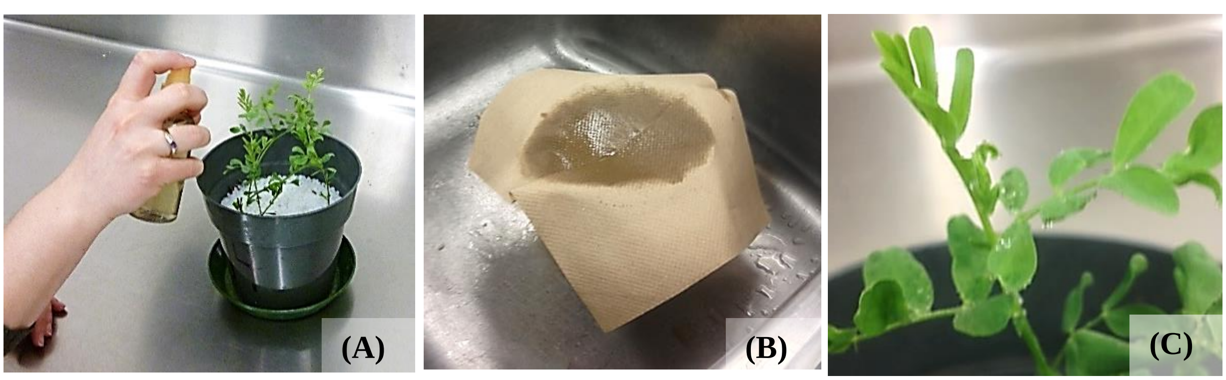

Fig. 4.1. Photos illustrating foliar application of Zn fertilizers: (a) dispensing foliar product with plastic spray bottle, (b) depiction of spray pattern, and (c) droplets of solution adhering to leaf surface.

4.4.4 Plant and soil analysis

Lentil plants were harvested in August upon reaching physiological maturity, 75 days after seeding (DAS). Stems were clipped at the soil interface and entire biomass per pot was placed into individual paper bags and left to air dry at 30 ^{o}C for several days. For each pot, lentil seeds were hand threshed and separated from the remaining biomass. Straw and seed yield were recorded prior to grinding individual samples using a hand-held plant grinder with stainless steel blades. Nutrient concentration of harvested lentil straw and grain was determined using a sulphuric acid-peroxide digest method (Thomas et al., 1967) as described in Chapter 3 and analyzed colorimetrically (Technicon AutoAnalyzer; Technicon Industrial Systems, Tarrytown, NY, USA) for N and P in grain and P only in straw. Digests were also analyzed for Fe and Zn using atomic absorption (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA). Soil was removed from each pot, air-dried and homogenized. Homogenized subsamples were obtained by using a wooden rolling pin to break up larger soil aggregates and passing the air-dried soil through a <2 mm stainless steel sieve. Residual levels of plant-available Zn were determined through diethylenetriaminepentaacetic acid (DTPA) extraction (Lindsay and Norvell, 1978) and analysis using atomic absorption spectroscopy as described in Chapter 3.

4.4.5 Statistical design and analysis

Statistical analyses were performed using the PROC MIXED procedure of SAS 9.4 for complete randomized design (CRD) using a two-factorial treatment arrangement. The model used for the analysis was: $Y = \mu + f_1 + f_2 + f_1 * f_2 + e$, where Y was an observation of the dependent variable which included either biomass (g), Zn concentration in the plant (mg Zn kg^{-1}) or DTPA-extractable Zn (mg Zn kg^{-1}) in soil; μ was the population mean for the variable; f_1 was the effect of the Zn fertilizer treatment as a fixed effect; f_2 was the effect of lentil cultivar as a fixed effect; $f_1 * f_2$ was the effect of the interaction between fertilizer and variety as a fixed effect; and e was the random error associated with the observation of the dependent variable. The denominator degrees of freedom (DDFM) was calculated using the Satterthwaite method. For all statistical analyses, significance was declared at $P<0.05$. Differences among the treatments were evaluated using a multiple comparison test following the Tukey-Kramer method.

4.5 Results

4.5.1 Yield

Zinc fertilization, either soil or foliar applied forms, did not contribute to increases in lentil grain or straw yield (Table 4.3). Grain yield differences between cultivars were also non-significant ($P>0.05$) and no response was observed to the various forms of applied Zn. Although Zn fertilization did not have a significant effect on straw yield ($P=0.579$), the effect of cultivar on straw yield was highly significant ($P=<.0001$). With the exception of CDC Maxim fertilized with foliar lignosulfonate, cultivars from the small green and red lentil market classes produced straw yields that were statistically the same. Regardless of Zn treatment, the straw production of CDC Impower was consistently significantly greater compared to the other lentil cultivars.

Table 4.3. Effects of various forms of Zn fertilizer on grain and straw yield (g pot^{-1}) of three lentil cultivars

Fertilizer	Cultivar	Yield (g pot^{-1}) †	
		Grain	Straw
Control	CDC Maxim	1.47 a	1.97 c
	CDC Imvincible	1.43 a	1.92 c
	CDC Impower	1.29 a	3.00 a
Soil $ZnSO_4$	CDC Maxim	1.45 a	1.92 c
	CDC Imvincible	1.38 a	1.79 c
	CDC Impower	1.37 a	2.93 a
7% Zn Foliar Lignosulphonate	CDC Maxim	1.32 a	2.19 bc
	CDC Imvincible	1.35 a	1.91 c
	CDC Impower	1.43 a	2.71 ab
9% Zn Foliar EDTA chelated	CDC Maxim	1.36 a	1.84 c
	CDC Imvincible	1.31 a	1.86 c
	CDC Impower	1.35 a	2.78 a
9% Zn Soil EDTA chelated	CDC Maxim	1.52 a	1.85 c
	CDC Imvincible	1.35 a	1.98 c
	CDC Impower	1.33 a	2.72 ab
SEM‡		0.08	0.12
Statistical Analysis		P values	
Fertilizer effect		0.828	0.579
Cultivar effect		0.309	<.0001
Fertilizer*Cultivar interaction effect		0.662	0.334

† Means with the same letter in the same column are not significantly different (P>0.05) as determined by multi-treatment comparisons using the Tukey-Kramer method.
‡ SEM= standard error of mean.

4.5.2 Effect of fertilizer on grain and biomass zinc concentration

Form of applied Zn had no significant effect on Zn concentration (mg Zn kg^{-1}) in grain (P=0.708) or straw (P=0.353) of the three lentil cultivars (Table 4.4), and overall, concentrations were similar among treatments. On average, CDC Maxim accumulated the most Zn in the grain; however, differences between cultivars were not significant (P>0.05).

Table 4.4. Effects of various forms of Zn fertilizer on grain and straw Zn concentration (mg Zn kg^{-1}) of three lentil cultivars

Fertilizer	Cultivar	Zn concentration (mg Zn kg^{-1}) †	
		Grain	Straw
Control	CDC Maxim	36.7 a	29.5 a
	CDC Imvincible	38.2 a	31.4 a
	CDC Impower	33.3 a	31.5 a
Soil $ZnSO_4$	CDC Maxim	36.2 a	24.4 a
	CDC Imvincible	35.3 a	29.1 a
	CDC Impower	33.7 a	32.2 a
7% Zn Foliar Lignosulphonate	CDC Maxim	41.0 a	30.1 a
	CDC Imvincible	38.4 a	30.3 a
	CDC Impower	34.9 a	31.5 a
9% Zn Foliar EDTA chelated	CDC Maxim	41.6 a	33.2 a
	CDC Imvincible	32.8 a	31.9 a
	CDC Impower	36.9 a	31.6 a
9% Zn Soil EDTA chelated	CDC Maxim	37.3 a	32.8 a
	CDC Imvincible	39.1 a	30.6 a
	CDC Impower	43.5 a	30.6 a
SEM‡		4.53	2.21
Statistical Analysis		P values	
Fertilizer effect		0.708	0.353
Cultivar effect		0.719	0.569
Fertilizer*Cultivar interaction effect		0.859	0.536

† Means with the same letter in the same column are not significantly different (P>0.05) as determined by multi-treatment comparisons using the Tukey-Kramer method.
‡ SEM= standard error of mean.

4.5.3 Effect of zinc fertilization on soil zinc removal

Total above-ground plant Zn uptake and removal (µg Zn pot^{-1}), a product of crop yield and Zn accumulation (concentration), by lentil cultivars was not significantly different when fertilized with various forms of Zn (P>0.05). Figure 4.2 illustrates that, in general, slightly greater amounts of Zn are removed by lentil straw than the grain, but differences in either plant component were not significant when comparisons between Zn treatments are made.

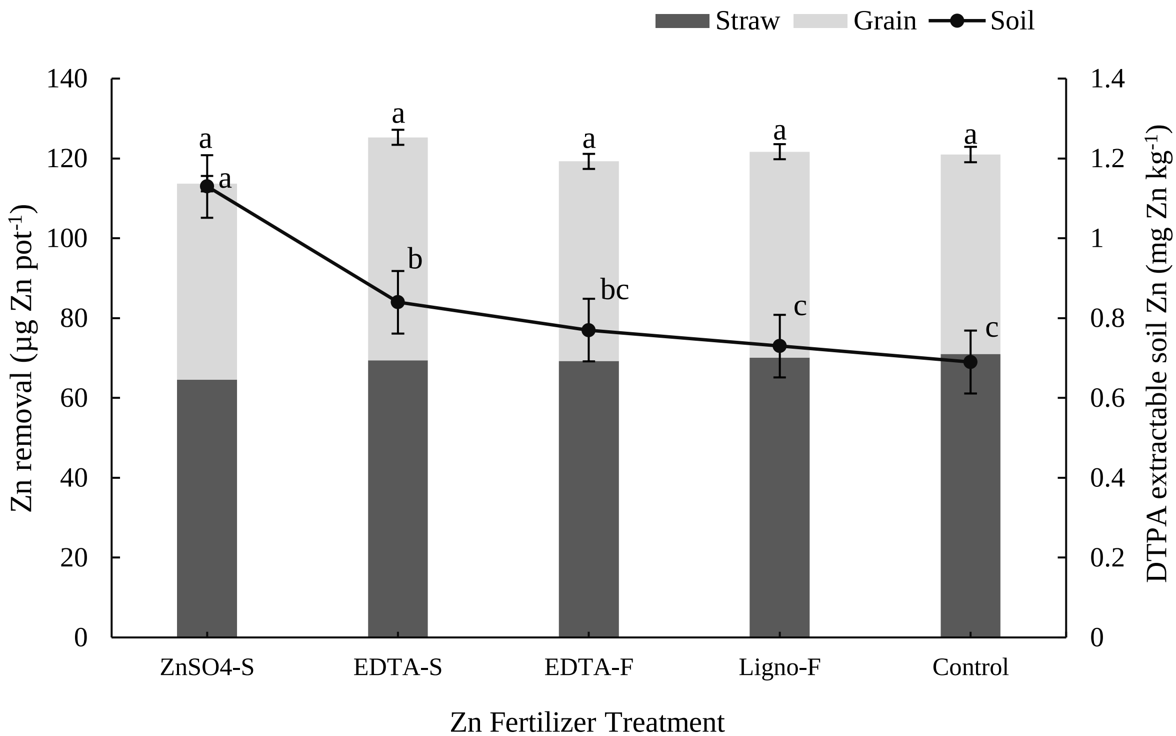

Fig 4.2. Comparison of Zn fertilizer treatment effects (mean of three cultivars) on residual DTPA extractable soil Zn (**µ**g Zn g^{-1}) levels and the total plant Zn uptake and removal (**µ**g Zn pot^{-1}) partitioned into Zn removed (**µ**g Zn pot^{-1}) in straw (dark grey bars) and grain (light grey bars). Zinc fertilizer treatments include ZnSO4-S= soil applied $ZnSO_4$; EDTA-S= soil applied 9% EDTA-chelated Zn; EDTA-F= foliar applied 9% EDTA-chelated Zn; Ligno-F= foliar applied lignosulphonate Zn; Control= no Zn fertilizer. Error bars are standard error of mean (SEM) of fertilizer treatment x total Zn removal with N=15 and R= 6. Means with different letters are significantly different (Tukey's HSD, P<0.05). Residual soil Zn levels are located on the secondary axis on the right-hand side of the figure and depicted by the line (SEM= 0.03)**.**

The effect of lentil cultivar on total Zn removal per pot was highly significant (P<.0001, Table 4.5). Significantly higher amounts of Zn were removed by CDC Impower compared to either CDC Maxim or CDC Imvincible. Although there were no significant differences in Zn removal via grain between lentil cultivars, significantly higher amounts of total removed Zn were driven by significantly higher Zn removal in the straw of CDC Impower (P<.0001). The effect of cultivar, however, had no significant (P=0.143) impact on levels of DTPA extractable soil Zn measured post-harvest (mg Zn kg^{-1}) (Table 4.4). In contrast, the effect of fertilizer form on the amount of residual soil Zn that was considered plant available was highly significant (P<.0001) (Fig. 4.2). Pots amended with soil applied forms of Zn, particularly $ZnSO_4$, had higher amounts of DTPA extractable Zn, followed by foliar forms of Zn and then the control treatment. Soil applied $ZnSO_4$ treatment resulted in the highest residual available soil Zn content, and significantly higher amounts of residual plant available soil Zn compared to all other Zn treatments. When Zn chelated with EDTA was soil applied, the DTPA extractable soil Zn was significantly greater than when foliar Zn lignosulphonate or no Zn fertilizer was applied.

Table 4.5. Zinc removal (μg Zn pot^{-1}) in lentil cultivars amended with different forms of Zn fertilizer

Cultivar	Zn Uptake and Removal (μg Zn pot^{-1}) †		
	Straw	Grain	Total
CDC Maxim	58.7 b	54.2 a	112.9 b
CDC Imvincible	58.1 b	50.1 a	108.2 b
CDC Impower	89.9 a	49.6 a	139.4 a
SEM‡	2.92	3.00	4.54
P-value	<.0001	0.49	<.0001

† Means with the same letter in the same column are not significantly different (P>0.05) as determined by multi-treatment comparisons using the Tukey-Kramer method.
‡ SEM= standard error of mean

4.6 Discussion

4.6.1 Comparisons of zinc form and application method on lentil response

Mean values of grain and straw Zn concentration, across all cultivars and Zn fertilizer treatments, were approximately 30 to 40 mg Zn kg^{-1}. These valus were within the range of lentil Zn concentration values reported in a pot study with Saskatchewan soils (Maqsood et al., 2013) and the grain Zn concentration measurements were also in close agreement with the values for lentil grown at Central Butte in the previously described field study (see Chapter 3). However, the concentrations of Zn in the straw from the pot study are more similar to the grain Zn values and are two to three times higher than those reported in the field experiment at Central Butte. This suggests that the dilution effect discussed in Chapter 3 (3.6.2) did not occur in the pot study and can be attributed to overall limitations on lentil growth in pots due to restricted volume for root exploration and nutrient competition as a result of soil volume constraints within the pot. With the exception of straw yield, the effect of cultivar on lentil yield or Zn accumulation in grain and straw was not significant. Regardless of Zn fertilizer treatment, significantly higher amounts of dry matter production were measured in the lentil cultivar CDC Impower. These results can be explained by genetic differences and inherent growth characteristics of the cultivars. CDC Impower is a later flowering, longer maturing and taller growing variety than CDC Imvincible and CDC Maxim, with average height being reported as 41, 33, and 34 cm, respectively (Government of Saskatchewan, 2014).

The form of Zn fertilizer did not have a significant effect on yield or Zn concentration in either grain or straw of any of the three lentil cultivars. These results are generally in disagreement with findings reported in literature for regions such as the Middle East and Asia, where Zn deficiencies are more common. A yield increase in lentil was reported in response to foliar applications of Zn chelated with EDTA made in a field study in Egypt (Zeidan et al., 2006). Compared to the present study, lentil was fertilized with higher rates of Zn during separate applications and at later stages of crop development in the experiment by Zeidan et al. (2006). This might suggest that application rate and timing of Zn fertilizer are more important factors affecting lentil yield response than form of Zn fertilizer. In agreement with the results of the present study, Zeidan et al. (2006) did not find a significant increase in lentil grain Zn concentration in response to foliar Zn application. Lentil responses to soil applied $ZnSO_4$ (5.7 kg Zn ha^{-1}) in field studies in Nepal were mixed as no yield response was reported but significant, albeit small, improvements in lentil grain Zn concentration were measured (Johnson et al., 2005).

Significant differences in yield and plant Zn concentration for other crops have been found in pot studies evaluating the effects of fertilization with various Zn forms. Corn grown in a pot experiment, with a soil of pH 8.3, and fertilized with chelated forms of Zn produced significantly more dry matter with higher tissue Zn concentration values compared to plants fertilized with Zn- amino acid sources (Obrador et al., 2003). The significant response differences between these two sources of Zn is attributed to the metal chelate resulting in higher amounts of Zn in labile forms in the water soluble and exchangeable soil Zn pools (Obrador et al., 2003). Results of the corn study are difficult to directly compare to the findings of the present study because the Zn chelate fertilizer source was created through mixing a combination of chelating agents (DTPA, EDTA and HEDTA, N-2-hydroxyethyl-ethylenedinitroltriacetate) and applied at much higher rates (20-40 kg Zn ha^{-1}). However, using rates of Zn more comparable to the present study, Goos et al (2000) found that fertilizing corn, grown in a greenhouse on low Zn (0.3 mg Zn kg^{-1}) and high pH (8.1) soils, with an EDTA-chelated form of Zn resulted in significantly higher dry matter yields and Zn uptake compared to other forms of Zn. The study also found similar yield and Zn accumulation responses between soil-applied $ZnSO_4$ and Zn lignosulphonate and suggested inorganic and organically-complexed forms of Zn were equal in their chemical availability when soil applied during the first year of application. This is further reinforced by Gangloff et al. (2002) who found no significant differences in efficiency between inorganic ($ZnSO_4$) and organically-complexed (Zn lignosulphonate) sources of Zn when soil applied but did find that chelated Zn (EDTA) was two to five times more effective in improving Zn concentration in corn tissue and dry matter yield. Direct comparison of $ZnSO_4$ and Zn lignosulfonate is not possible in the current study, as the $ZnSO_4$ was soil applied while the Zn lignosulfonate was foliar applied.

4.6.2 Zinc nutrient cycling

Despite different methods and rates of application of Zn fertilizer, the removal of Zn from soil did not significantly differ among Zn fertilizer treatments. As a product of lentil yield and Zn accumulation, significant differences in Zn uptake between forms was not an expected result based on lack of yield and Zn concentration differences. Zinc uptake in corn was highest when fertilized with Zn chelated with EDTA but total Zn removal did not differ between $ZnSO_4$ and Zn lignosulphonate forms (Gangloff et al, 2002). However, unlike the present study, the plants in the Gangloff et al. (2002) experiment were harvested prior to physiological maturity which could account for early season differences in Zn removal but not guarantee that differences among Zn fertilizer forms would exist if measured through the entire crop cycle once Zn accumulation was measured in grain yield.

Although Zn uptake did not vary among Zn fertilizer treatments, differences in residual DTPA-exactable soil Zn were observed (Fig. 4.2). Soil applied forms of Zn fertilizer retained the highest levels of DTPA-available soil Zn while foliar applied Zn forms did not differ from the control treatment. Pots fertilized with soil applied $ZnSO_4$ had the highest content of residual soil Zn. This result agrees with findings from Goos et al. (2000) who reported higher levels of residual DTPA-extractable Zn in soil fertilized with $ZnSO_4$ compared to Zn chelated with EDTA after the first season of application. Those results were attributed to significantly lower Zn uptake in corn fertilized with $ZnSO_4$ compared to Zn-EDTA. However, in the present study no such uptake differences existed and, therefore, it is more reasonable that residual levels of soil

Zn may be a factor of application rates of $ZnSO_4$, which were almost ten times higher than other Zn sources.

Results of this experiment suggest advantages in matching certain lentil cultivars with specific forms of Zn fertilizers. The effect of cultivar on Zn uptake was significant. Relative to other cultivars in the study, CDC Impower removed the highest amounts of Zn from the soil as a result of significantly higher straw yields (Table 4.5). Choosing fertilizer sources that leave higher amounts of DTPA-extractable Zn in the soil may be more sustainable for producers growing lentil with high rates of Zn removal. Zinc that is removed by lentil straw only has an opportunity to return to the soil pool if crop residues remain on the field and undergo microbial decomposition. However, particularly in many developing countries where it is common practice to harvest all plant components, it is much more likely that the Zn uptake in the straw is permanently removed from the field which may further exacerbate localized soil Zn deficiency.

4.7 Conclusion

Three cultivars of lentil grown on a calcareous soil with pH 7.7 and low extractable Zn levels (0.7 mg Zn kg^{-1}) were unresponsive to fertilizer applications using various Zn sources and application method. There were no significant differences in yield or Zn accumulation in either lentil grain or straw among Zn fertilizer treatments and differences in Zn uptake were a factor of a significant cultivar effect as opposed to a fertilizer response. Inorganic ($ZnSO_4$), organic-complexed (Zn lignosulphonate), and synthetic chelate (Zn-EDTA) forms of Zn were equivalent to unfertilized control treatments when applied at rates recommended by product manufacturers. Despite avoiding soil component interactions and fixation into unavailable forms, Zn applied as a foliar did not improve lentil yield or Zn concentration. Based on these findings, it is concluded that the described soil had a supply of Zn sufficient for lentil production and the lentil was able to obtain sufficient Zn to meet its physiological requirements. Given the similarity of the soil used in this study to the field study soil at Central Butte, where no responses were found, it also indicates potential limitations in the ability of the soil test DTPA extraction to identify critical levels of soil Zn for lentil response. In situations where Zn is required to remedy soil deficiencies, fertilizer applications of $ZnSO_4$ would have the greatest long-term impact because higher levels of residual DTPA-extractable Zn were observed that would potentially remain in the soil in available form for future crop use compared to other Zn fertilizer sources used in this study.

5. EFFECTS OF ZINC FERTILIZATION ON PREDICTED BIOAVAILABILITY OF ZINC IN LENTIL GRAIN

5.1 Preface

The previous chapters in this thesis have examined the effects of application rate and form of zinc (Zn) fertilizer applied on yield and the concentration of Zn in lentil grain and straw. Although various rates and forms of Zn did not significantly increase the total Zn content in the lentil grain, the effects of Zn fertilization on the bioavailability of Zn in the lentil grain still remains uncertain. This chapter addresses this issue by assessing the phytate content in the lentil grain and examining phytate zinc relationships. Phytate content in the lentil grain was used as a primary parameter in calculations that estimated Zn bioavailabilty. Using grain samples from the studies described in the previous two chapters, this chapter examines the potential for Zn fertilization to combat human Zn deficiency by enhancing the amount of intestinal-absorbable Zn in a staple legume grain.

5.2 Abstract

Human Zn deficiencies due to insufficient concentrations of Zn in staple legume and cereal grains may be compounded by anti-nutrients, such as phytate, binding with grain Zn and impeding its intestinal absorption. As such, phytate concentration and molar phytate:Zn ratios in staple grains can be useful predictors of the amount of Zn in a grain that is bioavailable for humans. Lentil grain, representing cultivars from three market classes (small red, cv. CDC Maxim; small green, cv. CDC Imvincible; large green, cv. CDC Impower), was harvested in 2013 from a polyhouse experiment that was established at the University of Saskatchewan (Saskatoon, SK) and field experiments conducted at Saskatoon and Central Butte, SK. Lentils grown in the polyhouse study were fertilized at a single rate using different Zn fertilizer sources and methods of application while in the field studies, different rates of soil-applied $ZnSO_4$ were used. The form of Zn fertilizer applied had a significant effect on Zn bioavailability in lentils. The Zn chelated with EDTA decreased grain phytate concentrations and improved predicted Zn bioavailability of lentil grain across all cultivars. Chelated Zn resulted in 6.2 mg g^{-1} of grain phytate when foliar applied and 6.5 mg phyate g^{-1} when soil applied compared to 8.9, 8.7, and 7.1 mg g^{-1} in lignosulphonate, $ZnSO_4$ and control treatments, respectively. Rate of $ZnSO_4$ applied in the field did not have a significant effect on bioavailability of Zn. Significant differences in phytate concentration and molar phytate:Zn ratios were observed between cultivars grown at Saskatoon and Central Butte field locations. The highest grain phytate concentrations (6.1 and 7.9 mg g^{-1}) and phyate:Zn molar ratios (27.5 and 20.9) were measured in CDC Imvincible at Centratl Bute and Saskatoon, respectively.

5.3 Introduction

Human micronutrient malnutrition, particularly as a result of Zn deficiency, is a major issue in human health and food security in some regions of the world. Low Zn concentration and poor bioavailability of the Zn in staple foods is largely responsible for compromised levels of absorbable Zn. Populations with diets consisting of primarily of cereal and legume grains are more susceptible to becoming Zn deficient compared to populations that consume a more varied diet that includes more meat, fruit and vegetables. Cereal and legume grains are poor sources of bioavailable Zn due to their high levels of anti-nutrients: myo-inositolhexaphosphate (phytate) in particular. Phytate is important in crop nutrition because it is the major storage form of phosphorus in the seed and typically represents 70-80% of total seed phosphorus (Chitra et al., 1995; Erdal et al., 2002). Phytate has also been shown to have anti-carcinogenic and positive cardiovascular effects when consumed by humans (Welch and Graham, 2012). However, it also irreversibly binds with Zn and carries the Zn ions through the gastrointestinal system without being absorbed (Welch and Graham, 2012). The concentration of phytate is, therefore, a valuable measurement in assessing Zn bioavailability. Molar ratios of phyate: Zn are often considered the best predictor of bioavailable Zn in food (Hotz and Brown, 2004), but a more recently developed trivariate mathematical model of the total daily absorbed Zn has also proven to be a useful tool in determining Zn bioavailability in staple foods (Miller et al., 2007; Hambidge, 2010).

Zinc fertilization has been shown to be effective in reducing grain phytate concentration and phytate: Zn molar ratios in a range of wheat cultivars (Erdal et al., 2002). The effects of Zn fertilization on phytate and Zn availability of other staple grains has not been thoroughly investigated. Using harvested lentil grain from experiments described in previous chapters, it is the intent of this study to examine what the impacts of Zn fertilization are on Zn bioavailability in lentil. The general research hypothesis is that lentil fertilized with Zn will have significantly reduced grain phytate and molar phytate:Zn ratios compared to lentil which has not been fertilized with Zn. An additional research objective includes gaining a better understanding of the genotype response of lentil grown in varying soil and environmental conditions to various forms and rates of Zn fertilizer.

5.4 Materials and Methods

5.4.1 Sample preparation

Lentil grain samples that were harvested from a field and a polyhouse experiment in 2013, outlined in Chapters 3 and 4, were prepared for analysis. Lentils grown in the polyhouse study were grown in pots filled with Ardill association soil fertilized with single rates of various Zn fertilizer forms including soil applied $ZnSO_4$, soil and foliar applied Zn chelated with EDTA, and foliar applied Zn lignosulphonate. Lentil grain samples from two field sites near Saskatoon and Central Butte, SK fertilized with three rates (0, 2.5 and 5 kg Zn ha^{-1}) of $ZnSO_4$ that were broadcasted and soil-incorporated prior to seeding, were cleaned and prepared for analysis. Details on this experiment are found in Chapter 3. In the polyhouse and field experiments, the same three lentil cultivars were grown and included cvs. CDC Impower, CDC Imvincible, and CDC Maxim, representing three of the major lentil market classes (large green, small green, and small red, respectively). Using a hand-held plant grinder with stainless steel blades, lentil grain samples were ground to a diameter less than 2 mm and stored in polyethylene vials prior to analyses.

5.4.2 Analysis of lentil grain zinc and phytate concentration

Zinc concentration was determined through a wet ashing technique (Thomas et al., 1967) where a 0.25 g subsample of ground lentil grain was digested using sulphuric acid and peroxide. The digested material was analyzed for Zn using atomic absorption spectroscopy (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA). Plant Zn concentration assessment quality control was ensured through inclusion of internal standards of known Zn concentration in every batch of samples analyzed.

Following a modified method of Gao et al. (2007), phytate was extracted from 50 mg of finely ground lentil grain using 1 ml of 0.8 M HCl. Weighed lentil grain fines and HCl extracting solution were placed in a 2 ml microfuge tube and thoroughly combined using a vortex before tubes were placed on shaker (Thermo Scientific™ Labquake™ Tube Shaker/Rotator, Thermo Fisher Scientific Inc.). Samples were allowed to continuously shake at room temperature for 16 h. The microtubes were removed from the shaker and placed in a centrifuge (20 min, 8,000 rpm; Thermo Scientifict™ Pico™ Microcentrifuge, Thermo Fisher Scientific Inc.) before 10 μl of the supernatant was transferred into a fresh microtube and combined with 740 μl of doubly distilled water and 250 μl of modified Wade's reagent (0.03% $FeCl_3 \cdot 6H_2O$ + 0.3% sulfosalicylic acid; Gao et al., 2007). Each microtube was placed on a vortex to ensure thorough mixing of the sample before a 200 μl aliquot was pipetted onto a microliter plate. Phytate was determined colorimetrically using a microplate spectrometer (Bio-Rad xMark™ Microplate Absorbance Spectrometer, Bio-Rad Laboratories Inc.) with absorbance measured at 490 nm. A standard calibration curve was generated from the readings of standard sodium phytate solutions (Sigma-Aldrich P-8810; St. Louis, MO, USA) and used to calculate the specific concentration of phytate within the extract. All samples were prepared and analyzed in duplicate with means reported and used in calculations.

5.4.3 Statistical analysis and calculations

The PROC MIXED procedure in SAS (version 9.4; SAS Institute, Cary, NC, USA) was used for all statistical analysis. Specific statistical procedures corresponded to the experimental designs and treatment arrangements for the experiments from which the lentil samples were obtained. Analysis of the lentil grain samples harvested from the field experiment (Chapter 3) were performed for a split-plot design with a treatment arrangement of randomized complete block design (RCBD). Lentil grain harvested from the pot study (Chapter 4) was analyzed according to the procedure for a complete randomized design (CRD) with a two-factorial treatment arrangement. The Tukey-Kramer method was used to make multi-treatment comparisons and differences between treatments were considered significant at $P<0.05$. Significantly different groupings were assigned using the pdmix800 SAS macro (Saxton, 1998).

Phytate: zinc molar ratios were calculated to qualitatively evaluate the Zn bioavailability of lentil grain grown under various Zn fertilizer regimes. The phyate: zinc molar ratios were calculated according to Hotz and Brown (2004) using the following equation:

$$(mg\ phytate/660)/(mg\ zinc\ /65.4) \qquad \text{(Eq. 5.1)}$$

Where 660 and 65.4 are the molecular weights of phytate and zinc, respectively.
Using the trivariate model of Zn absorption (Miller et al., 2007), lentil grain was evaluated for its potential fit in Zn biofortification programs and levels of bioavailable Zn were approximately quantified with the following equation:

$$TAZ = 0.5\left(A_{MAX} + TDZ + K_R\left(1+\frac{TDP}{K_p}\right) - \sqrt{\left(A_{MAX} + TDZ + K_R\left(1+\frac{TDP}{K_p}\right)\right)^2 - 4 * A_{MAX} + TDZ}\right) \qquad \text{(Eq. 5.2)}$$

Where TAZ (total daily absorbed Zn) is the dependent variable, TDZ (total dietary Zn) and TDP (total dietary phytate) are independent variables, and the three constant parameters include A_{MAX} (maximum absorption of Zn, 0.091), K_R (Zn-transport receptor binding equilibrium dissociation constant, 0.033), and K_P (Zn-phytate binding reaction equilibrium dissociation constant, 0.68) (Hambidge et al., 2010; Miller et al., 2007).

5.5 Results

5.5.1 Polyhouse study

Zinc fertilization had a significant effect (P=0.01) on phytate concentration (Fig. 5.1) and molar phytate: zinc ratios (Table 5.1) in lentil grain of all three cultivars. Zinc chelated with EDTA, either foliar or soil applied, resulted in the lowest grain phytate concentations (Fig. 5.1).

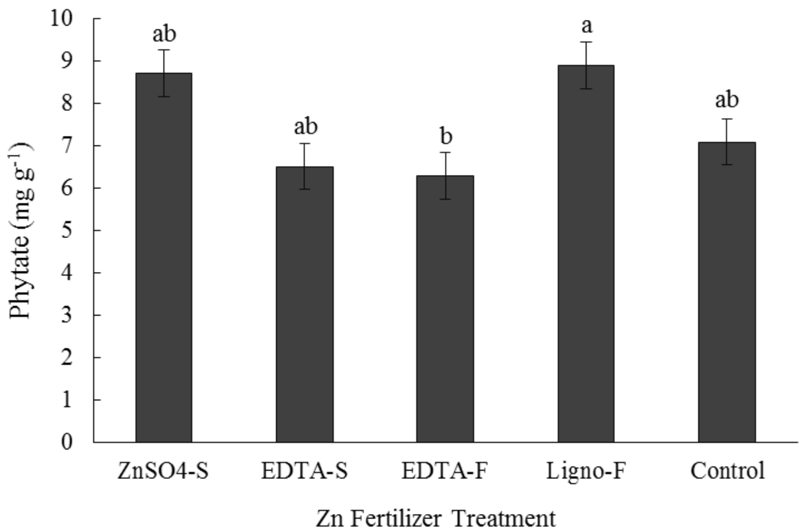

Fig 5.1. Effect of five Zn fertilizer treatments on phytate concentration (mg g^{-1}) in harvested lentil grain (mean of the three cultivars) grown under polyhouse conditions. S denotes soil applied while F denotes foliar applied. $ZnSO_4$ was applied at 2.5 kg Zn ha^{-1} while the other fertilizers were applied at 0.246 kg Zn ha^{-1}

Table 5.1. Effect of various forms of Zn fertilizer on Zn bioavailability in lentil grain (mean of three cultivars) as evaluated by molar phytate: Zn ratios and estimations of bioavailable Zn (mg Zn 300 g^{-1}) quantified using the trivariate model approach developed by Miller et al. (2007).

Fertilizer	Bioavailability Measurement †	
	Phytate: Zn Molar Ratio	Estimated Bioavailable Zn (mg Zn 300 g^{-1})‡
$ZnSO_4$	24.7 a	2.60 b
9% Soil EDTA chelated	17.1 b	3.08 a
9% Foliar EDTA chelated	18.0 ab	3.02 ab
7% Foliar Lignosulphonate	24.4 ab	2.69 ab
Control	20.2 ab	2.88 ab
SEM§	1.9	0.13
Statistical Analysis	P values	
Fertilizer effect	0.01	0.05
Cultivar effect	0.51	0.48
Fertilizer*Cultivar interaction effect	0.52	0.68

† Means with the same letter in the same column are not significantly different (P>0.05) as determined by multi-treatment comparisons using the Tukey-Kramer method.
‡ Means with the same letter in the same column are not significantly different (P>0.10) as determined by multi-treatment comparisons using the Tukey-Kramer method.
§SEM= standard error of mean

The rankings of molar phytate:Zn ratios (Table 5.1) amongst Zn fertilizer treatments follow a trend similar to that of concentrations of phytate in lentil grain. EDTA chelated forms of Zn resulted in the lowest mean phytate:Zn molar ratios in the lentil grain. Across all cultivars, lentil fertilized with soil applied Zn-EDTA had significantly lower phytate:Zn molar ratios compared to lentil fertilized with soil applied $ZnSO_4$. While not significant at P =0.05, the Zn fertilizer treatments did have a significant effect at P<0.10 on estimated bioavailable Zn provided by 300 g of lentil with groupings that matched those of phytate:Zn molar ratios. Significant correlations (P<0.10), of varying strength, existed between concentrations of Zn, phytate, and total phosphorus (P) in the lentil grain (Appendix D).

5.5.2 Field study

Application of $ZnSO_4$ had no significant effect on any of the lentil grain Zn bioavailability assessments in the field trials at the two sites. However, with the exception of phytate concentration in the lentil grain from the Saskatoon site, the effect of cultivar was significant ($P \leq 0.05$) for all indicators of Zn bioavailability in lentil seeds grown under field conditions at Central Butte and Saskatoon, SK sites (Table 5.2). CDC Imvincible had the highest concentrations of phytate in the grain at either site which resulted in higher phytate:Zn molar ratios and lower levels of estimated bioavailable Zn compared to other lentil cultivars. The differences between CDC Impower and CDC Maxim were not significant, but CDC Impower had generally improved predicated bioavailability relative to CDC Maxim.

Table 5.2. Phytate content (mg g^{-1}), phytate: Zn molar ratios and estimated bioavailable Zn (mg Zn 300 g^{-1}) in grain of three lentil cultivars harvested from field locations at Central Butte and Saskatoon, SK.

Location	Cultivar	Phytate Concentration (mg g^{-1})	Bioavailability Measurement[†] Phytate: Zn molar ratio	Bioavailability Measurement[†] Estimated Bioavailable Zn (mg Zn 300 g^{-1})
Central Butte	CDC Maxim	5.49 b	24.3 ab	2.50 ab
	CDC Imvincible	6.13 a	27.5 a	2.36 b
	CDC Impower	5.66 ab	22.9 b	2.60 a
	SEM[‡]	0.41	3.1	0.17
	Statistical Analysis		P values	
	Fertilizer effect	0.46	0.96	0.99
	Cultivar effect	0.05	0.03	0.03
	Fertilizer*Cultivar effect	0.61	0.51	0.42
Saskatoon	CDC Maxim	7.66 a	18.7 ab	2.96 ab
	CDC Imvincible	7.87 a	20.9 a	2.78 b
	CDC Impower	7.78 a	17.9 b	3.01 a
	SEM	0.27	1.0	0.08
	Statistical Analysis		P values	
	Fertilizer effect	0.36	0.36	0.30
	Cultivar effect	0.83	0.02	0.02
	Fertilizer*Cultivar effect	0.72	0.81	0.73

[†] Means with the same letter in the same column are not significantly different (P>0.05) as determined by multi-treatment comparisons using the Tukey-Kramer method.
[‡] SEM= standard error of mean

The phytate:Zn molar ratios across both locations fell within the range measured in the polyhouse study. In general, lentil grain with lower molar ratios of phytate:Zn and higher estimates of bioavailable Zn was produced at the Saskatoon site relative to grain grown at the Central Butte site. Correlations between Zn, phytate, and P concentrations in the lentil grain in the field study were low and not significant (P>0.05, Appendix D).

5.6 Discussion

5.6.1 Predicted zinc bioavailability of lentil grown in Saskatchewan

The results of this study demonstrate that differences in cultivar and source of Zn fertilizer influence phytate concentration and, therefore,predicted bioavailability of Zn in lentil grain. When ratios of molar phytate:Zn are used as a primary indicator of Zn bioavailability to humans, even the lowest mean ratio (17.1, mean of all lentil cultivars fertilized with soil applied Zn-EDTA) is >15 which would categorize the Zn bioavailability of lentil grain in this study as poor. These findings are in disagreement with the nutritional analysis of grain from 19 lentil genotypes grown in Saskatchewan in 2005 and 2006 (Thavarajah et al., 2009a). The mean grain phytate concentration of lentil grown in polyhouse and field conditions in the present study ranged from 5.1 to 8.9 mg g^{-1}. At the Saskatoon field site, the average phytate concentration in the grain of all lentil cultivars ranged from 7.5 to 8.2 mg g^{-1}, and was approximately double the concentration of phytate that was measured in the grain of lentil grown at a Saskatoon field site in an alternate study (Thavarajah et al., 2009a). When molar phytate:Zn ratios are calculated using mean grain Zn and phytate concentration values from the respective corresponding data set of Thavarajah et al. (2009, 2009a), the average bioavailability of Zn in the lentil grain would be categorized as moderate, as molar phyate:Zn ratios fell between 5 and 10 in both 2005 and 2006 at Saskatoon. Although there are similarities between these two studies in regards to crop type and site location, differences in lentil genotypes analyzed, environmental conditions in the growing seasons, and soil fertility make direct comparison difficult. More field trials may be required to better understand relationships between management practices, environmental conditions, and lentil genotypes and the impact they have on the nutritional quality of Saskatchewan lentils. Grain phytate concentration in the present study was determined colormetrically but in the research by Thavarajah et al. (2009a) it was determined using alternate methods. Analytical methods will need to be standardized in the future if Saskatchewan-grown lentils can be consistently guaranteed as a high source of bioavailable Zn to human populations facing widespread micronutrient deficiency.

5.6.2 Effect of zinc fertilization on predicted zinc bioavailability in lentil grain

Zinc fertilization with EDTA-chelated form reduced the grain phytate with apparent improvement of overall predicted bioavailability, as determined by decreased phytate:Zn molar ratios and increased estimates of bioavailable Zn per 300 g of lentils across all cultivars. Improved nutrient bioavailability in response to Zn fertilizer application has been found in other staple grains (Erdal et al., 2002; Wei et al., 2012; Lu et al., 2011). Accumulation of phytate in the grain is reportedly reduced under conditions of adequate Zn fertility because Zn diminishes the uptake of P by the roots (Cakmak, 2008). A reduction in P uptake by the plant results in less P being deposited and stored as phytate in the seed at the time of grain filling. Under Zn deficient conditions, increased P accumulation in plants has been attributed to a greater expression of P-transporter genes in the roots of barley plants (Huang et al., 2000).

Although a relationship between Zn fertility and reduced phytate accumulation in the grain has been recognized in the literature, Zn bioavailability differences in response to various Zn fertilizer sources is not well understood. In the present study, soil applied $ZnSO_4$ and foliar applied Zn lignosulphonate had the highest phytate:Zn molar ratios. Decreases in phytate accumulation in grain have been found in wheat fertilized with soil applications of $ZnSO_4$ (Erdal et al., 2002), but the applications rates were much higher (23 kg Zn ha^{-1}) than in the present study. Therefore, the suggestion made in previous chapters that soil applied $ZnSO_4$, at the rates used in this study, does not contribute greatly to Zn nutrition of lentil due to rapid soil fixation into unavailable forms, is supported by the results of the current experiment. The largest improvements to predicted Zn bioavailability were a result of Zn-EDTA fertilizer treatments. Foliar fertilization with Zn-EDTA also reduced phytate contencentration in polished rice, but to a lesser extent than foliar fertilization with $ZnSO_4$ (Wei et al., 2006). The ability of EDTA to more readily enter the plant compared to lignosulphonate is because EDTA is a very stable chelate in the soil. It also is a smaller molecule than lignosulphonate, and therefore better able to penetrate the leaf surface. This could be related to processes affecting phytate formation in the plant.

5.6.3 Effect of genotype and environment on predicted zinc bioavailability in lentil grain

The significant differences in Zn bioavailability observed between different lentil cultivars are consistent with genotype differences found in soybean (Raboy and Dickinson, 1984), wheat (Erdal et al., 2002; Hussain, 2012) and rice (Ning et al., 2009). The significant cultivar effect demonstrated in the present study is also in agreement with significant differences in lentil grain phytate concentration reported in other Saskatchewan field and growth chamber studies (Thavarajah et al., 2009a and 2010). Although correlations between grain phytate and Zn concentration were weak and non-significant at the two field locations, there is a consistent pattern at both sites where the cultivars with high phytate concentration also had lower Zn concentration in the grain. It is unclear if this is a consequence of the lentil cultivar having an enhanced ability to store phytate or a reduced ability to transport Zn into its seed.

The results of this study also indicate that soil and environmental factors play a role in grain phytate concentrations and the Zn bioavailability of lentils for humans. Mean phytate concentration was higher in lentil from the Saskatoon field site compared to Central Butte site, and also compared to those grown in the polyhouse using soil collected close to the Central Butte field site. The higher phytate concentration in lentil grain from Saskatoon site could be reflective of higher available phosphorus levels in the soil at the Saskatoon location. They were two times higher compared to those at Central Butte (Table 3.1). Increasing P nutrition in the soil has been found to increase phytate concentration in grain of wheat (Lu et al., 2011) and soybean (Raboy and Dickinson, 1984). Higher temperatures during the grain-filling period in lentil have also been found to increase phytate concentration in lentil seeds (Thavarajah et al., 2010). The mean growing-season temperature in 2013 was higher in Saskatoon than Central Butte (Table 3.2). The average phytate concentration was slightly lower in the polyhouse study compared to the Saskatoon field site. Despite lower soil phosphorus levels, the relatively warmer temperatures inside the polyhouse may have resulted in increased concentrations of grain phytate. Despite increased phytate concentrations, Saskatoon lentils had greater Zn bioavailability, as determined by lower molar phytate: Zn ratios and higher estimates of bioavailable Zn per 300 g of lentils, compared to lentils grown on the Central Butte soil in field or polyhouse. This is a consequence of the higher mean concentration of Zn in lentil grain grown at Saskatoon site, attributable to substantially higher inherent soil available Zn status of the Saskatoon site.

5.7 Conclusion

Results from this study indicate that phytate accumulation in lentil grain is influenced by Zn fertility, lentil genotype differences, and environmental conditions during the growing season. Zinc reduces the P uptake by lentil and decreases the accumulation of grain phytate. The nutritional analysis of lentil reinforces that low rates of soil applied $ZnSO_4$ fertilizer do not improve the supply of Zn to lentil beyond the soil's inherent ability to supply it because Zn fertilizer treatments of $ZnSO_4$ did not reduce the phytate concentration of lentils or improve the bioavailability of Zn for humans. Observed differences in Zn bioavailability and phytate accumulation of lentil grain amongst Zn fertilizer sources could reflect variations in molecular size of each product and the plant's ability to use the applied Zn and/or formation of phytate. Compared to other lentil cultivars, based on its lower apparent predicted bioavailable Zn concentration, CDC Imvincible would not be best suited for biofortification programs aimed to relieve widespread human Zn deficiency.

6. SYNTHESIS AND CONCLUSIONS

Zinc (Zn) is an essential micronutrient for the growth and development of all living organisms due to its primary role in enzyme function. The application of Zn fertilizer to crops to increase yield and also improve the nutritional quality of edible grains for human consumption is an important consideration in food security. However, much of the agricultural Zn research has occurred in regions outside of Canada, using fertilizer application rates of Zn that are often two to three times greater than typically recommended by micronutrient product manufacturers for producers on the Canadian prairies. Furthermore, the majority of Zn fertilizer response experiments have focused on staple cereal grains such as rice, wheat, or corn and have neglected lentil despite the rising demand for its consumption on a global level. Because Saskatchewan is a world leader in lentil production and exports most of its lentils to regions of the world with high incidence of human Zn deficiency, Saskatchewan-specific information on the yield and nutrient concentration effects of Zn fertilization of lentil is important. The research presented in this thesis addressed this gap, with the general goal of assessing the yield and nutritional quality responses of popular lentil cultivars (cv. CDC Maxim, CDC Imvincible, and CDC Impower) grown at two field locations within Saskatchewan, in response to rate of pre-plant broadcast zinc sulphate fertilizer. A polyhouse experiment was also conducted to examine the response of lentil to different forms of Zn fertilizers (sulphate salt, sulphonate complex, EDTA chelate) that were soil and foliar applied.

6.1 Summary of Findings

A primary objective of this research was to evaluate the response of lentil to different rates of soil-applied zinc sulphate ($ZnSO_4$, Chapter 3). The rates used in this study (0, 2.5 and 5 kg Zn ha^{-1}) reflect granular Zn fertilizer rates typically recommended for Saskatchewan crop producers. No significant differences in yield or Zn concentration of grain or straw were detected among treatments for any of the lentil cultivars ($P>0.05$) at two field sites that were selected to represent contrasting soil properties in the Dark Brown (Saskatoon) and Brown (Central Butte) soil zones. A crop response to addition of Zn was not demonstrated at the Central Butte site despite soil properties that would be indicative of Zn deficiency such as such as high pH, relatively low soil organic matter (OM) content, and levels of plant-available (DTPA-extractable) Zn that fell below the commonly accepted critical limit of 0.5 mg Zn kg^{-1} in soil. A possible explanation for the lack of response put forward, particularly at Central Butte, is the rapid fixation of the broadcast and incorporated $ZnSO_4$ onto soil particles and the transformation of labile Zn into non-labile forms. A lack of significant differences in the residual amount of DTPA-extractable Zn among Zn application rates further supports the suggestion that Zn fertilizer, soil-applied as $ZnSO_4$, quickly became unavailable for plant uptake. Furthermore, the results of a modified BCR extraction procedure demonstrated that the majority of native soil Zn was distributed in fractions unavailable for plant uptake. When $ZnSO_4$ fertilizer was applied, much of this Zn migrated to the carbonate-bound soil fraction within the first season of application.

The impacts of soil Zn fixation were confirmed in a subsequent field experiment that examined the residual value of $ZnSO_4$ fertilizer applied to lentil in 2013 on the yield and nutrient concentration of a rotational crop of hard red spring wheat grown in 2014. No significant differences in grain or straw yield among any rates of the previously applied fertilizer Zn were detected at either Saskatoon or Central Butte site locations. Soil applications of $ZnSO_4$ fertilizer in the 2013 crop season generally did not increase the size of the DTPA-extractable soil Zn pool for any rate of applied Zn in 2014.

Forms of Zn that can be foliar-applied do not have the opportunity to interact with the soil components that fix Zn into unavailable forms and, therefore, may be a superior approach to Zn fertilization. In a polyhouse experiment, single rates of an inorganic salt ($ZnSO_4$), an organic complex (Zn-lignosulphonate) and a synthetic chelate (Zn-EDTA) Zn fertilizer were either soil or foliar applied to lentil cultivars and compared to an unfertilized control. There were no significant differences ($P>0.05$) in yield or Zn concentration of lentil grain or straw in response to any of the Zn fertilizer treatments. There was, however, a significant effect ($P=<.0001$) of cultivar on straw yield, with higher production of straw by the lentil cultivar CDC Impower and, consequently, a significantly greater total Zn uptake ($P=<.0001$). The soil application of $ZnSO_4$ fertilizer resulted in significantly higher amounts of DTPA-extractable Zn in the soil compared to other Zn fertilizer treatments. Because the differences in total Zn removal between forms was not significant, higher levels of residual

Zn in the soil applied $ZnSO_4$ treatment were attributed to an application rate that was 10 times higher than the synthetic chelate or organic-complexed Zn treatments.

Although Zn fertilization did not significantly increase the concentration of Zn in the grain of lentil grown under field or polyhouse conditions, an additional objective of this research was to evaluate the effect on Zn fertilization on human bioavailability of Zn in lentils. Concentration of grain phytate, a major storage form of phosphorus in the legume and cereal seeds that irreversibly binds to Zn cations and hinders them unavailable for intestinal absorption, was measured in lentils grown in the field and polyhouse studies. Grain phytate concentration values were assessed individually and through their contribution to other measures of human Zn bioavailability including the phytate:Zn molar ratio and estimated bioavailable Zn per 300 g of lentils. Collectively, the results of this study indicated that Zn fertilizer amendments, environmental conditions, and cultivar differences influenced the accumulation of lentil grain phytate and, thus, its predicted level of Zn bioavailability for humans. Lentil cultivar had a significant effect ($P<0.05$) on human bioavailability of Zn for lentils grown in Central Butte and Saskatoon field locations. Form of fertilizer also significantly ($P<0.05$) influenced Zn bioavailability in lentils grown in the polyhouse. Chelated forms of Zn fertilizer, either soil or foliar applied, resulted the greatest improvements in dietary bioavailability of Zn in lentils. However, the reason for this is not known.

6.2 Research Implications and Recommendations

The results of this research demonstrate that a critical limit of <0.5 mg Zn kg^{-1} extractable by DTPA from soil, generally accepted for most crops as the threshold below which a significant yield response of lentil is anticipated, may not be accurate for all crops grown in all soil conditions. Based on the current findings, lentil appears to be a good scavenger of soil Zn even in soils with characteristics limiting the availability of Zn. Therefore, applications of Zn fertilizer based on soil test recommendations alone should be exercised with caution. Until a critical limit for soil Zn is clearly established for lentil grown under a variety of soil conditions reflective of those within Saskatchewan's lentil growing region, producers who suspect Zn deficiency at a particular field location should also use field history information, tissue testing, and comprehensive field scouting for visual deficiency symptoms to supplement soil test data. Due to the inherent spatial variability of plant-available Zn in soil, a blanket application of soil applied Zn fertilizer will be unlikely to result in a consistent response across an entire field area. Directed sampling of different field areas according to topography, texture, pH, organic matter content could be useful to identify regions of the field where zinc deficiency and fertilization response would most likely be encountered. Increasing the number of collected soil cores could also help reduce the chance of a soil test result being unrepresentatively skewed toward a low measured level of DTPA-extractable Zn.

Zinc sulphate that was soil applied as broadcast and incorporated intact granules in the field or as banded solution in the pot study, did not significantly improve the yield, Zn concentration, or predicted Zn dietary bioavailability of lentil. Soil applied $ZnSO_4$ would not be recommended as the primary choice among Zn fertilizer sources. Applications of foliar Zn in response to an identified crop deficiency would help buffer the risk of unnecessary "preventative" Zn fertilizer applications. Although $ZnSO_4$ left significantly higher amounts of residual plant-available Zn in the soil compared to other forms of Zn fertilizer, this would not warrant a preventative application of $ZnSO_4$ with the justification that additional Zn will be available for next year's crop. The residual value of soil applied $ZnSO_4$ on a rotational spring wheat crop was minimal and resulted in no significant yield increases.

Economic analysis of the field data for Zn response showed that Zn fertilization did not demonstrably increase economic return, due to the lack of yield responses to the Zn fertilizer applied. Yield response of lentil to Zn fertilization is too variable to allow much confidence in a consistent positive economic return on fertilizer investment. Unless a crop deficiency is clearly established, producers should not apply Zn fertilizer at the expense of other field operations that have more reliable agronomic efficacy. Producers need to allocate their crop expenses towards inputs and management practices best suited for their own regional and seasonal circumstances including drought or extreme moisture, disease and insect pressure, or difficult-to-control weeds. In the context of the 2013 crop season, lentil producers might have seen higher returns, due to improved lentil yield or quality, from investments in fungicide application, seed treatment, or new seed stock, relative to the application of Zn fertilizer.

6.3 Future Research Considerations

This thesis reinforced the complexity involved in making sound fertility decisions—particularly pertaining to those involving micronutrients such as Zn where a response is inherently variable. However, it would be premature to eliminate Zn fertilization as an effective yield or nutrient enhancing strategy for lentil production as there are still many Zn nutrient concepts that require further study. Future research that investigates various combinations of Zn form, application rate, product placement, and application timing on different soils in the field will help the industry better understand the most effective strategies for Zn fertilization. An emphasis on future research that examines critical limits of Zn in soil specific to legume crops grown under different soil and environmental conditions will also be valuable in reducing unnecessary Zn fertilizer applications. The crop year during which this research was conducted was one of the best on record for Saskatchewan agriculture, and therefore, did not provide the opportunity assess the response of lentil to Zn under conditions of stress. Because Zn plays a crucial role in enzyme function and stress tolerance, it would be valuable to determine if Zn fertilization could assist crops in recovering from in-season stress such as hail, flooding, drought or disease.

Although this research provided insight into the effects of Zn fertilization on the dietary bioavailability of Zn in lentils for humans, there is still much to discover about enhancing Zn and its nutritional value in lentils. Thavarajah et al. (2009a) suggested that determination of grain phytate concentration through colorimetric methods may overestimate the actual presence of grain phytate. A universally-accepted method of phytate determination will be essential to compare results between researchers and accurately track the progress being made towards improving the bioavailability of Zn to humans in staple foods. Some mechanisms require further elucidation. For example, the EDTA chelated form of Zn fertilizer appeared to be effective in improving predicted human bioavailable Zn in lentil in this study, but the mechanism is not clear. Bioavailable Zn was determined through the analysis of raw lentils but, in reality, humans consume cooked lentils that have undergone various processing. Further research into the post-harvest processing effects will help to better assess the bioavailable Zn nutritional value for humans.

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APPENDIX A: EFFECTS OF RESIDUAL ZINC FERTILIZER ON HARD RED SPRING WHEAT YIELD AND ZINC CONCENTRATION

A.1 Preface

The in-season yield, crop nutrient concentration, and soil effects of fertilizing lentil with Zn were examined in previous chapters of this thesis. A lack of response of lentil to Zn fertilization was generally attributed to the rapid transformation of soil-applied Zn sulfate fertilizer into unavailable forms of Zn in the soil. It is not clear if the non-labile forms of Zn will become increasingly plant available with time. Furthermore, the impacts of a fertility decision often outlast a single season and, therefore, the residual value of soil- applied Zn fertilizer application on subsequent crops warranted investigation. This chapter specifically examines the effects of varying application rates of soil applied $ZnSO_4$ to lentil cultivars grown in the 2013 crop season, on the yield and Zn concentration of hard red spring wheat grown in 2014.

A.2 Abstract

Wheat is an important staple grain for human consumption around the world and accounts for a large proportion of Saskatchewan's crop production. Therefore, understanding response of wheat to additions of micronutrient fertilizer made the year before to a lentil crop is of interest in the potential to improve yield and quality of spring wheat grown in Saskatchewan. Three popular lentil cultivars (cvs. CDC Maxim, CDC Imvincible, and CDC Impower) were grown at field sites near Central Butte and Saskatoon, SK in 2013 and fertilized with soil applied $ZnSO_4$ at rates of 0, 2.5, and 5 kg Zn ha^{-1}. The residual effects of the initial Zn fertilizer application made in 2013 were determined the following crop season through examination of the impacts on grain and straw yield and Zn concentration in the 2014 red hard spring wheat (cv. CDC Utmost) crop. Residual levels of DTPA-extractable Zn in soil were measured prior to seeding and post-harvest of spring wheat in the spring and fall of 2014, respectively. Spring wheat grain and straw yields among the previous season's fertilizer and lentil cultivar treatments were not significantly different at either field location. Rate of Zn fertilizer applied in 2013 also did not significantly influence residual DTPA-extractable Zn in the soil at the Central Butte site, where soil pH and carbonate content is higher. However, there was a significant effect of 2013 Zn fertilizer rate and lentil cultivar on DTPA-extractable Zn measured in the soil at Saskatoon post-harvest of spring wheat during the fall of 2014.

A.3 Introduction

In a traditional Saskatchewan cropping rotation, cereals such as spring wheat are generally seeded in the subsequent growing season following a pulse crop. Similar to lentil, wheat is also an important economic crop in this province as there is great export demand for high quality and nutritious wheat. A large portion of the world population relies on wheat as a staple grain in their diets and, therefore, ensuring adequate levels of Zn in wheat grain is potentially an important strategy to overcoming global human Zn deficiencies. Compared to lentil, wheat is reported to have lower Zn requirements (Gulser et al., 2004; Brennan, 2005). Research supports Zn fertilization as an agronomic method to increase the concentration of Zn in the edible grain in cereals (Cakmak, 2008; White and Broadley, 2009). White and Broadley (2009) suggested that a single application of soil applied Zn fertilizer may have persistent residual effects for several growing seasons. However, there has not been much research in this area to quantify this claim.

Information that is important for Saskatchewan producers making micronutrient fertility decisions may be obtained by assessing the residual value of a single application of soil applied $ZnSO_4$ at rates of 0, 2.5 and 5 kg Zn ha^{-1}. This investigation will evaluate the fate of Zn fertilizer across two crop seasons with the intent to better understand how an initial decision to make a soil application of Zn fertilizer in one cropping season to a pulse crop, such as lentil, can impact the following rotational spring wheat crop. It is the objective of this study to examine the residual effects of $ZnSO_4$ fertilizer that was soil-applied for various lentil cultivars in 2013, on the agronomic performance of hard red spring wheat that was grown in 2014.

A.4 Materials and Methods

A.4.1 Experimental set-up

In the spring of 2014, hard red spring wheat (*Triticum aestivum* L, cv. CDC Utmost) was direct seeded across the lentil plots utilized in the 2013 field experiment described in detail in Chapter 3. As previously described, sites were located in the Brown Soil Zone near Central Butte, SK and near Saskatoon within the Dark Brown Soil Zone. Site data was analyzed independently of one another and, therefore, specific site management practices differed between the two locations. Field management practices reflect the appropriate agronomic decisions and preferences for crop establishment and pest control within each region. Early season weed control was accomplished at both sites by a pre-plant application of glyphosate, made at least two days prior to seeding, at 0.75 litre active ingredient per ha. The Central Butte site was seeded on May 22, 2014 and the Saskatoon site was seeded on June 2, 2014. Both sites were seeded using field scale equipment with paired-row openers spaced 25 and 30 cm apart at Central Butte and Saskatoon, respectively. In order to study the residual effects of Zn fertilizer that was soil-applied in the previous growing season, no micronutrient fertilizer was applied at the time of seeding. However nitrogen and phosphorus, applied as granular urea (50 kg N ha^{-1}) and monoammonium phosphate (20 kg P_2O_5 ha^{-1}) respectively, was side-banded at the time of seeding to ensure that limitations in macronutrient fertility were not limiting the response of spring wheat to residual Zn fertilizer. Hard red spring wheat was seeded at a rate of 80 kg ha^{-1} at Central Butte and 100 kg ha^{-1} at Saskatoon. In-season control of weeds was accomplished through the application of herbicides at recommended rates in accordance with label directions. Bromoxynil/MCPA-ester tank-mixed with clodinifop was applied at the Central Butte field location and a herbicide application of thiencarbazone-methyl/pyrasulfotole/bromoxynil (Velocity m3®) was made at the Saskatoon site. A fungicide application, coinciding with herbicide timing, of propiconazole was made at Central Butte but not Saskatoon.

A.4.2 Baseline soil properties

Soil cores were removed, prior to seeding, from each of the six main plots at both site locations at depth increments of 0-15 cm, 15-30 cm and 30-60 cm. Three cores were taken from each plot. Once air-dried, thoroughly mixed soil samples were ground using a wooden rolling pin and passed through a 2 mm stainless steel sieve. These homogenized soil fractions from each soil core were analyzed for various chemical properties and extractable nutrients that are described in Table A.1.

Table A.1. Soil properties from Central Butte and Saskatoon locations. Values are means from soil cores collected from a middle subplot (cv. CDC Maxim) within each main plot in the spring of 2014 prior to seeding spring wheat.

Depth	Soil Property							
	pH	EC	OC	N†	P	K	S‡	Zn
(cm)		(dS m^{-1})	(%)			(kg ha^{-1})		
	---Central Butte---							
0-15	7.8	0.26	1.6	7.2	32.1	632.6	56.6	0.99
15-30	8.0	0.29	1.2	10.6	9.1	220.0	73.9	-
30-60	-	-	-	26.1	-	-	4782.8	-
	---Saskatoon---							
0-15	6.1	0.10	2.8	14.1	40.2	627.4	49.7	3.3
15-30	6.6	0.11	1.6	14.5	14.2	317.3	44.8	-
30-60	-	-	-	29.8	-	-	163.2	-

† Nitrate, NO_3- N
‡ Sulphate, SO_4-S

Utilizing a 1:2 soil: water suspension (Nelson and Sommers, 1982), soil pH was determined using a Beckman 50 pH meter (Beckman Coulter, Fullerton, CA, USA) with a Calomel glass electrode assembly. Electrical conductivity (EC) was determined on the same suspension using an AP85 pH/EC meter (Accumet, Hudson, MA, USA). Soil organic carbon

(OC) was determined using a LECO C632 combustion analyzer (LECO Corporation, St. Joseph, MI, USA) following the protocols outlined by Wang and Anderson (1998). Soil nitrate (NO_3^-) and sulphate (SO_4^{2-}) were extracted from soil sampled to a depth of 60 cm using a 0.01 *M* $CaCl_2$ extraction (Houba et al., 2000) and analyzed using automated colorimetry. A modified Kelowna extraction procedure (Qian et al., 1994) was used to measure available phosphorus (P) and potassium (K) in the 0-15 cm depth increment. Extracts were colorimetrically analyzed for P using a Technicon Autoanalyzer II segmented flow automated system (Technicon Industrial Systems, Tarrytown, NY, USA). Plant available Zn was extracted using a 0.005*M* diethylene-triamine-pentacetic acid (DTPA) solution (Lindsay and Norvell, 1978) from samples taken from the 0-15 cm and 15-30 cm depths of the soil profile. Concentrations of K and Zn in extracts were analyzed using flame atomic absorption (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA).

A.4.3 Harvest and analysis

Red hard spring wheat was harvested at both sites upon reaching physiological maturity: August 27, 2014 at Central Butte and September 9, 2014 at Saskatoon. Crop samples were removed from within each of the lentil cultivar sub-plots, across all six main plots established in the 2013 field studies. These samples were quadrats of one m^2, and were harvested by hand using a sickle and cutting to a stubble height of approximately 5 cm. The center of the sub-plot experimental units were targeted for harvest to ensure samples were removed from locations of broadcasted and incorporated pre-seed fertilizer applications made in the spring of 2013. Once the experiment was terminated in the fall of 2014, soil cores were collected from each sub-plot to a depth of 15 cm to determine residual levels of plant-available Zn determined through DTPA extraction (Lindsay and Norvell, 1978). Concentrations of Zn in the extracts were determined using flame atomic absorption (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA). Hand-harvested spring wheat samples were dried at 30 °C and weighed prior to being threshed using a stationary mechanical thresher. Harvested grain was cleaned and weighed to determine grain and straw yields (kg ha^{-1}). Ground sub-samples of wheat grain and straw were retained for future digestion and determination of nutrient concentration.

A.4.4 Statistical analysis

Statistical analyses of yield and Zn concentration in the straw and grain of spring wheat crops was conducted using SAS 9.4. The PROC MIXED procedure for a split-plot design with treatments arranged as a randomized complete block design (RCBD) was utilized with the following model used for analysis: Y= μ + block + A + e_1 + B+ A*B + e_2 where Y is the dependent variable which included either straw or grain yield (kg ha^{-1}), Zn concentration in the straw or grain (mg Zn kg^{-1}), or DTPA-extractable Zn in the soil (mg Zn kg^{-1}); μ is the population mean for the dependent variable and e denotes a measure of error; block is considered a random effect; A denotes the main plot fixed effect of the residual fertilizer rate (kg ha^{-1}); B is the sub-plot fixed effect of the previously cropped lentil cultivar and A*B is the effect of the interaction between the main and sub-plot factors as a fixed effect. The DDFM used the Satterthwaite method. Multi-treatment comparisons were made using the Tukey-Kramer method where significance was declared at $P<0.05$.

A.5 Results and Discussion

A.5.1 Residual effects of zinc fertilization on yield

Mean yields at both site locations ranged between 3430 and 3953 kg ha^{-1} for grain and 4444 and 6960 kg ha^{-1} for straw (Table A.2). Grain yields from Central Butte and Saskatoon were similar to each other and were both higher than grain yield averages reported for spring wheat grown within the same rural municipalities (Government of Saskatchewan, 2013). Rate of Zn fertilizer applied during the previous (2013) crop season had no significant effect on spring wheat yield of grain at Central Butte (P=0.111) or Saskatoon (P=0.990). Effects of $ZnSO_4$ fertilizer application rate on straw, or previously grown lentil cultivar on grain or straw yield of spring wheat, were also not significant at either site ($P>0.05$).

Spring wheat is generally considered less sensitive to soil Zn deficiencies and requires smaller quantities of Zn compared to lentil (Brennan et al., 2001). Based on the lack of yield and response to Zn fertilizer applied to lentil previously reported in Chapter 3 of this thesis, a significant yield response to residual Zn fertilizer was not expected in spring wheat. Spring wheat was also found to use residual Zn less effectively than Zn applied at the time of seeding relative to other legume and oilseed crop species grown in a growth chamber experiment (Brennan and Bolland, 2002). However, previous research has demonstrated a lasting yield response of spring wheat to a single application of Zn fertilizer in calcareous, Zn limited soils in South Australia and Turkey (Brennan, 2001; Alloway, 2008). Residual Zn has effectively improved wheat yields from three to seven years after an initial single soil broadcast and incorporated application of $ZnSO_4$ fertilizer in Turkey (Cakmak et al., reported in Alloway, 2008). The primary difference between these experiments and the present field study is a much higher rate of initial $ZnSO_4$ fertilizer application: 23-28 kg Zn ha^{-1} were applied to field sites in Turkey, which is approximately 5 times greater than the rates of Zn applied in the present study. Although Brennan (2001) found a decrease in the residual effectiveness of Zn fertilizer with time, a positive yield response in wheat was still reported approximately 10 years after the first application. Direct comparisons of these results with those of the present study cannot be made, however, because fertilizer application rates ranged from 9-22 kg Zn ha^{-1} applied as zinc oxide.

Table A.2. Residual effect of $ZnSO_4$ fertilizer rate (0, 2.5, and 5 kg Zn ha^{-1}) applied to three different lentil cultivars in May, 2013 on the grain and straw yield (kg ha^{-1}) of spring wheat (cv. CDC Utmost) grown in 2014 at Central Butte and Saskatoon locations.

Site	Lentil Cultivar Stubble	Zn Rate (kg Zn ha^{-1})			SEM‡	P values		
		0	2.5	5		Rate (R)	Cultivar (C)	R*C Interaction
				Grain Yield† (kg ha^{-1})				
Central Butte	CDC Maxim	3805 aA	3470 aA	3953 aA	190.1	0.111	0.698	0.623
	CDC Imvincible	3893 aA	3430 aA	3606 aA				
	CDC Impower	3719 aA	3677 aA	3908 aA				
Saskatoon	CDC Maxim	3797 aA	3441 aA	3713 aA	199.8	0.990	0.530	0.555
	CDC Imvincible	3654 aA	3941 aA	3780 aA				
	CDC Impower	3778 aA	3915 aA	3781 aA				
				Straw Yield (kg ha^{-1})				
Central Butte	CDC Maxim	4658 aA	4445 aA	4674 aA	346.2	0.714	0.967	0.972
	CDC Imvincible	4773 aA	4444 aA	4725 aA				
	CDC Impower	4533 aA	4553 aA	4874 aA				
Saskatoon	CDC Maxim	6516 aA	5769 aA	6358 aA	361.3	0.985	0.384	0.396
	CDC Imvincible	6216 aA	6583 aA	6353 aA				
	CDC Impower	6436 aA	6960 aA	6479 aA				

† Means with the same lower-case letter in the same row (within a cultivar) and with the same upper-case letter in the same column (within Zn rate), at a given site, are not significantly different ($P>0.05$) as determined by multi-treatment comparisons using the Tukey-Kramer method.

‡ SEM= standard error of mean (rate x cultivar) with N=9 and R=6

A.5.2 Residual effects of zinc fertilization on zinc concentration in spring wheat grain and straw

Table A.3. Residual effect of $ZnSO_4$ fertilizer rate (0, 2.5, and 5 kg Zn ha^{-1}) applied to three different lentil cultivars in May, 2013 on the grain and straw Zn concentration (mg kg^{-1}) of spring wheat (cv. CDC Utmost) grown in 2014 at Central Butte and Saskatoon locations.

Site	Lentil Cultivar Stubble	Zn Rate (kg Zn ha^{-1})			SEM‡	P values		
		0	2.5	5		Rate (R)	Cultivar (C)	R*C Interaction
		---Grain Zn Concentration†--- (mg Zn kg^{-1})						
Central Butte	CDC Maxim	22.2 aA	22.6 aA	15.8 aA	4.2	0.642	0.387	0.354
	CDC Imvincible	24.0 aA	24.2 aA	26.5 aA				
	CDC Impower	15.4 aA	24.2 aA	25.7 aA				
Saskatoon	CDC Maxim	34.3 abAB	35.6 abAB	33.1 abAB	5.0	0.673	0.312	0.001
	CDC Imvincible	35.6 abAB	32.2 abAB	23.9 bB				
	CDC Impower	21.0 bB	21.0 bB	45.1 aA				
		---Straw Zn Concentration--- (mg Zn kg^{-1})						
Central Butte	CDC Maxim	11.4 aA	12.4 aA	12.2 aA	2.0	0.685	0.398	0.562
	CDC Imvincible	14.2 aA	12.5 aA	14.9 aA				
	CDC Impower	14.6 aA	11.0 aA	10.0 aA				
Saskatoon	CDC Maxim	21.1 aA	14.2 aA	13.3 aA	3.7	0.236	0.625	0.05
	CDC Imvincible	24.1 aA	11.4 aA	15.5 aA				
	CDC Impower	10.4 aA	20.8 aA	11.1 aA				

† Means with the same lower-case letter in the same row (within a cultivar) and with the same upper-case letter in the same column (within Zn rate), at a given site, are not significantly different (P>0.05) as determined by multi-treatment comparisons using the Tukey-Kramer method.

‡ SEM= standard error of mean (rate x cultivar) with N=9 and R=6.

A.5.3 Residual effects of zinc fertilization soil Zn status

No significant differences (P>0.05) in DTPA-extractable Zn among rates of applied $ZnSO_4$ fertilizer were detected in soils at either Central Butte or Saskatoon during any sampling interval (Fig. A.1) When DTPA-extractable Zn was measured in soil post-harvest of spring wheat during the fall of the 2014, the mean values for Zn fertilizer rate did not deviate much from the baseline (spring 2013) level of DTPA-extractable Zn at Central Butte. This pattern was consistent with measurements taken during previous sampling seasons (fall 2013 and spring 2014). It could suggest that relatively small additions of Zn fertilizer are quickly transformed into forms of Zn not available to plants and therefore do not contribute greatly to soil solution and easily exchangeable pools of Zn that can be accessed by plants. Non-significant trends of DTPA-extractable soil Zn increasing with application rate of Zn fertilizer have been observed at the Saskatoon site during all seasons of measurement. At Saskatoon, under native Zn soil conditions, the pool of DTPA-available Zn generally decreased with time. However, when Zn fertilizer is applied at the 2.5 or 5 kg Zn ha^{-1} rates, the largest increase in plant-available Zn was measured the year following initial application (spring 2014) followed by a decrease when measured following the subsequent crop season (fall 2014). These results agree with the findings of Ma and Uren (2006) who found that the relative amounts of Zn unavailable for plant uptake and the rate at which water-soluble Zn transformed into more stable, less-reactive forms were higher during the first year of Zn fertilizer application and also increased with increasing rates of added Zn. Ma and Uren (2006) suggested that Zn fertilizer is more efficiently utilized for crop production when applied more frequently at lower rates compared to infrequent Zn applications at higher rates

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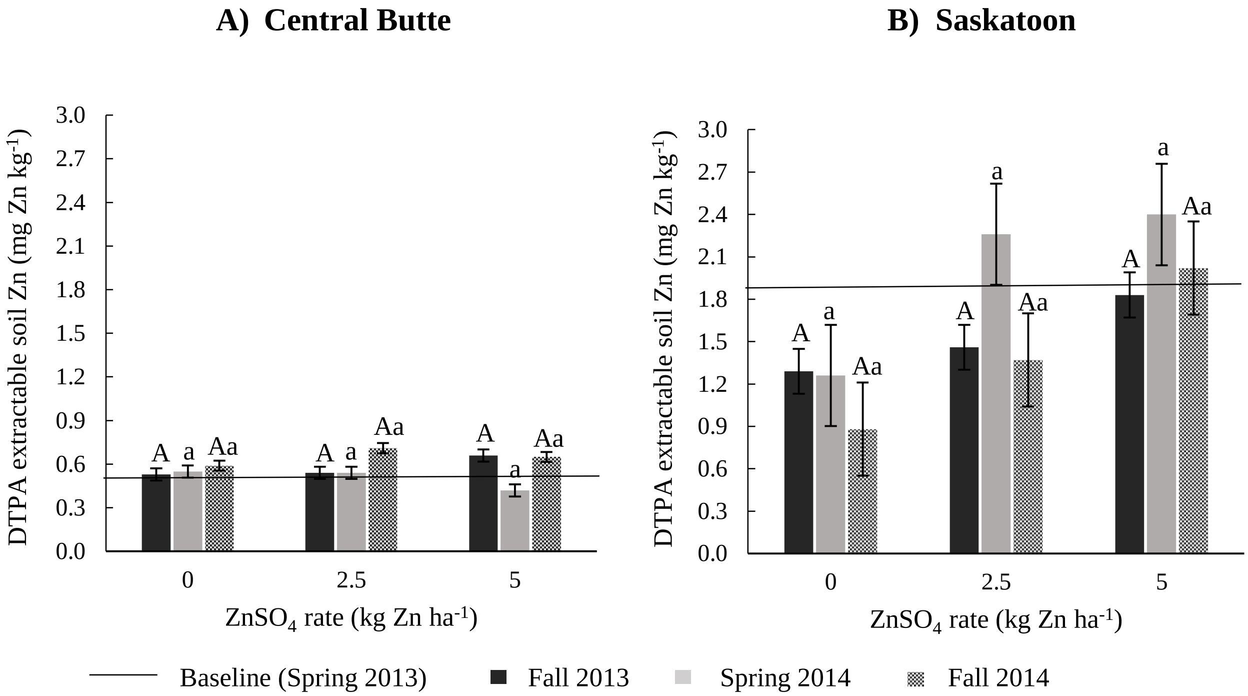

Fig.A.1. Effect of Zn fertilization rate (kg Zn ha^{-1}) on residual levels of DTPA-extractable soil Zn (mg Zn kg^{-1}) measured post-harvest in the fall of 2013 (black coloured bars), pre-seeding in the spring of 2014 (grey coloured bars), and post-harvest in the fall of 2014 (hatched bars) at (A) Central Butte, SK and (B) Saskatoon, SK. Error bars are standard errors of mean and the horizontal line represents baseline levels of DPTA-extractable Zn measured prior to seeding during the spring of 2013. Within a given site location, means with the same upper-case letter (fall 2013), lower-case (spring 2014) or combination of upper and lowercase (fall 2014) are not significantly different (P>0.05) as determined by multi-treatment comparisons using the Tukey-Kramer method.

Despite no significant differences in fall 2014 DTPA-extractable soil Zn among residual rates of applied $ZnSO_4$ fertilizer at Central Butte (P=0.882) and Saskatoon (P=0.120) locations, there was a significant interaction effect (rate x lentil cultivar) on DTPA-extractable Zn measured in soil during fall 2014 at Saskatoon (P=0.02, Fig. A.2).

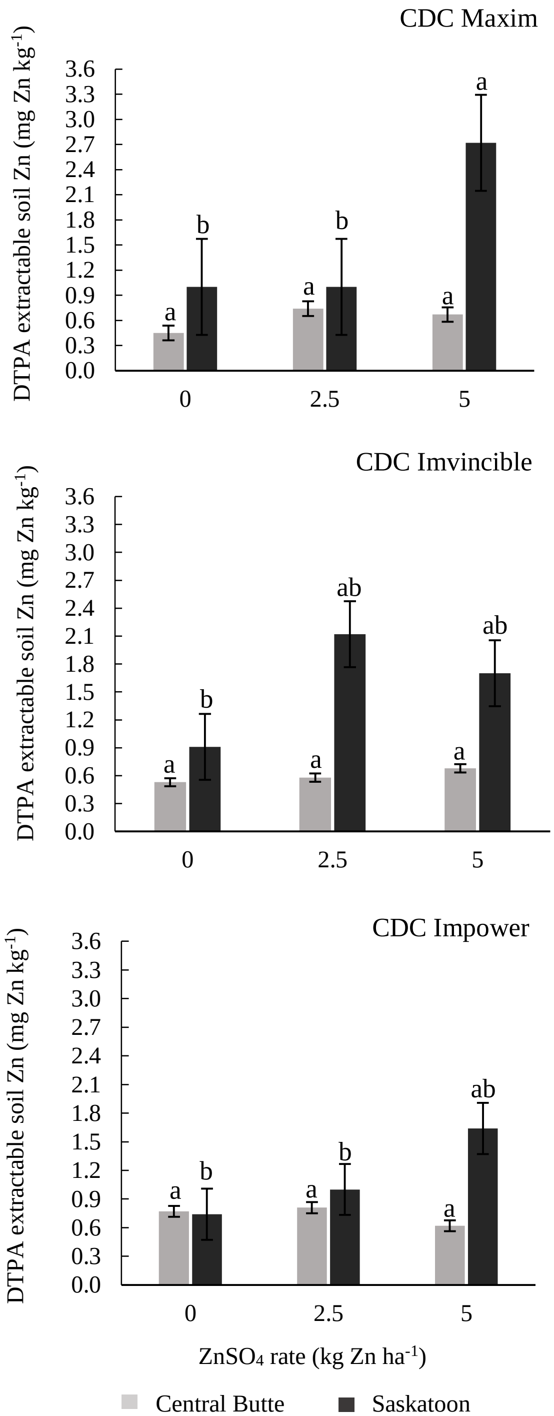

Fig. A.2. DTPA-extractable soil Zn (mg Zn kg^{-1}) measured post-harvest of spring wheat (cv. CDC Utmost) during fall 2014 at Central Butte (grey bars) and Saskatoon (black bars) field locations. Lentil cultivars (CDC Maxim, CDC Imvincible, and CDC Impower) and $ZnSO_4$ fertilization rates (0, 2.5 and 5 kg Zn ha^{-1}) describe treatments applied during the previous growing season (spring 2013). Error bars are standard error of the mean. Within a site location, means are compared between cultivars within a specific Zn rate or across Zn fertilizer rates within a specific cultivar; means with the same letters are not significantly different (P>0.05).

The pattern of DTPA-available Zn in the soil observed in fall 2014 could be reflective of respective differences in the amount of Zn released into soil by lentil residue produced in the previous growing season. Although there were no significant differences in straw Zn concentration among lentil cultivars grown in 2013 and fertilized with different rates of Zn, lentil fertilized with the highest rate of $ZnSO_4$ (5 kg Zn ha^{-1}) consistently accumulated the highest concentration of straw Zn. Furthermore, CDC Imvincible also had higher straw Zn content relative to other cultivars.

A.5.4 Additional statistical information

Table A.4. Standard deviation for grain and straw yield (kg ha^{-1}) of spring wheat (cv. CDC Utmost) grown in 2014 at Central Butte and Saskatoon locations that were previously seeded to three lentil cultivars fertilized with three rates (0, 2.5 and 5 kg Zn ha^{-1}) of soil applied $ZnSO_4$ at two sites in 2013 (corresponds to Table A.2).

Zn Rate (kg Zn ha^{-1})	Lentil Cultivar Stubble	Central Butte		Saskatoon	
		Grain	Straw	Grain	Straw
		(kg ha^{-1})			
0	CDC Maxim	308.1	504.1	730.0	901.1
	CDC Imvincible	363.0	453.3	475.0	978.6
	CDC Impower	598.1	702.6	474.7	747.2
2.5	CDC Maxim	372.6	619.8	277.6	513.9
	CDC Imvincible	351.6	450.0	421.7	510.9
	CDC Impower	655.4	1274.3	390.3	860.1
5	CDC Maxim	610.7	795.5	302.0	604.0
	CDC Imvincible	488.3	1325.2	505.9	1092.5
	CDC Impower	252.1	373.2	646.3	1377.7

Table A.5. Standard deviation for Zn concentration in grain and straw (mg Zn kg^{-1}) of spring wheat (cv. CDC Utmost) grown in 2014 at Central Butte and Saskatoon locations that were previously seeded to three lentil cultivars fertilized with three rates (0, 2.5 and 5 kg Zn ha^{-1}) of soil applied $ZnSO_4$ in 2013 (corresponds to Table A.3).

Zn Rate (kg Zn ha^{-1})	Lentil Cultivar Stubble	Central Butte		Saskatoon	
		Grain	Straw	Grain	Straw
		(mg Zn kg^{-1})			
0	CDC Maxim	8.0	6.1	10.5	16.9
	CDC Imvincible	11.9	2.8	14.0	3.9
	CDC Impower	5.4	6.8	12.9	6.2
2.5	CDC Maxim	9.6	3.2	9.3	8.8
	CDC Imvincible	5.5	3.5	12.3	4.9
	CDC Impower	13.2	4.2	6.0	12.5
5	CDC Maxim	8.4	6.8	6.2	6.9
	CDC Imvincible	14.6	4.7	10.8	7.7
	CDC Impower	11.9	3.7	13.3	6.9

Table A.6. Analysis of variance examining the relationship between fixed effects of residual fertilizer rate (0, 2.5 and 5 kg Zn ha^{-1}, broadcast and incorporate as $ZnSO_4$ in the spring of 2013) and lentil cultivar grown in 2013 and yield (kg ha^{-1}) and Zn concentration (mg Zn kg^{-1}) in grain and straw of spring wheat (cv. CDC Utmost) grown in 2014 at Central Butte and Saskatoon (corresponds to Tables A.2 and A.3).

Site	Dependent Variable		Fixed Effect	Num df†	F-value	P-value	SEM‡
Central Butte	Yield						
		Grain	Zn Fertilizer rate	2	2.31	0.111	109.8
			Cultivar	2	0.36	0.698	109.8
			Rate x Cultivar	4	0.66	0.623	190.9
		Straw	Zn Fertilizer rate	2	0.38	0.714	228.1
			Cultivar	2	0.03	0.967	199.9
			Rate x Cultivar	4	0.13	0.972	346.2
	Zn concentration						
		Grain	Zn Fertilizer rate	2	0.45	0.642	2.4
			Cultivar	2	0.97	0.387	2.4
			Rate x Cultivar	4	1.13	0.354	4.2
		Straw	Zn Fertilizer rate	2	0.43	0.685	1.2
			Cultivar	2	0.94	0.398	1.1
			Rate x Cultivar	4	0.75	0.562	2.0
Saskatoon	Yield						
		Grain	Zn Fertilizer rate	2	0.01	0.990	115.4
			Cultivar	2	0.64	0.530	115.4
			Rate x Cultivar	4	0.76	0.555	199.8
		Straw	Zn Fertilizer rate	2	0.02	0.985	208.6
			Cultivar	2	0.98	0.384	208.6
			Rate x Cultivar	4	1.04	0.396	361.3
	Zn concentration						
		Grain	Zn Fertilizer rate	2	0.45	0.673	3.6
			Cultivar	2	1.20	0.312	2.9
			Rate x Cultivar	4	5.76	0.001	5.0
		Straw	Zn Fertilizer rate	2	1.49	0.236	2.2
			Cultivar	2	0.47	0.625	2.2
			Rate x Cultivar	4	2.61	0.048	3.7

†Num df= numerator degrees of freedom

‡SEM= standard error of mean

Table A.7. Standard deviation for residual levels of DTPA extractable soil Zn (mg Zn kg^{-1}) at Central Butte and Saskatoon field locations. Zinc fertilization treatments (soil applied $ZnSO_4$ at rates of 0, 2.5 and 5 kg Zn ha^{-1}) were applied to three popular lentil cultivars at each site in the spring of 2013. Soil cores were removed and analyzed for DTPA extractable Zn post-harvest of the lentil crop in the fall of 2013, prior to seeding spring wheat in the spring of 2014 and post-harvest of the spring wheat crop in the fall of 2014 (corresponds to Figures 3.3. and D.1).

Zn Rate (kg Zn ha^{-1})	Soil Sampling Period	DTPA Extractable Soil Zn (mg Zn kg^{-1}) Central Butte	Saskatoon
0	Fall 2013	0.19	0.19
	Spring 2014	0.31	0.27
	Spring 2014	0.37	0.30
2.5	Fall 2013	0.22	0.49
	Spring 2014	0.09	2.29
	Spring 2014	1.95	0.96
5	Fall 2013	0.67	0.74
	Spring 2014	0.13	2.15
	Spring 2014	1.22	1.09

Table A.8. Standard deviation for residual levels of DTPA extractable soil Zn (mg Zn kg^{-1}) at Central Butte and Saskatoon field locations measured post-harvest of spring wheat crop in the fall of 2014. Zinc fertilization treatments (soil applied $ZnSO_4$ at rates of 0, 2.5 and 5 kg Zn ha^{-1}) were applied to three popular lentil cultivars (cvs. CDC Maxim, CDC Imvincible, and CDC Impower) at each site in the spring of 2013 (corresponds to Figure D.2).

Zn Rate (kg Zn ha^{-1})	Lentil Cultivar	DTPA Extractable Soil Zn (mg Zn kg^{-1}) Central Butte	Saskatoon
0	CDC Maxim	0.22	0.41
	CDC Imvincible	0.21	0.19
	CDC Impower	0.54	0.24
2.5	CDC Maxim	0.65	0.42
	CDC Imvincible	3.29	1.31
	CDC Impower	0.82	0.31
5	CDC Maxim	0.49	1.39
	CDC Imvincible	2.01	0.84
	CDC Impower	0.47	0.74

A.7 Conclusion

Application of soil applied $ZnSO_4$ fertilizer at rates of 0, 2.5 and 5 kg Zn ha^{-1} to a lentil crop grown in 2013 had no residual effect on increasing yield and Zn concentration of hard red spring wheat grown in the following 2014 crop season. Zinc released in crop residues may contribute more to the plant-available pool of Zn in the soil compared to Zn fertilization alone.

Grain yield and plant (DTPA)-available Zn in the soil were not significantly improved by residual $ZnSO_4$ fertilizer at Central Butte or Saskatoon. Only for one lentil cultivar (CDC Imvincible) and one site (Saskatoon), did Zn application in the spring of 2013 result in a higher available Zn content in the surface soil at the end of the study in the fall of 2014. These combined findings suggest that Zn fertilizer application decisions cannot be justified with the expectation of greatly enhanced soil Zn availability for utilization by crops grown following the year of application. Based on this study of the residual value of soil applied Zn fertilizer, producers in the Brown and Dark Brown soil zone should consider a direct application of Zn during the growing season if crop Zn deficiency is suspected and not rely on residual Zn fertilizer in the soil as a strategy to prevent the onset of Zn deficiency in hard red spring wheat.

APPENDIX B: EFFECTS OF SOIL APPLIED ZINC SULPHATE ON LENTIL YIELD AND GRAIN ZINC CONTENT UNDER FIELD CONDITIONS

B.1 Additional Statistical Information

Table B.1. Standard deviation for grain and straw yield (kg ha^{-1}) of three lentil cultivars fertilized with three rates (0, 2.5 and 5 kg Zn ha^{-1}) of soil applied $ZnSO_4$ at two sites in 2013 (corresponds with Table 3.3).

Zn Rate (kg Zn ha^{-1})	Lentil Cultivar	Central Butte Grain	Central Butte Straw	Saskatoon Grain	Saskatoon Straw
		(kg ha^{-1})			
0	CDC Maxim	640.3	409.2	1114.8	810.2
	CDC Imvincible	707.6	509.1	513.3	492.9
	CDC Impower	462.4	515.2	566.4	519.6
2.5	CDC Maxim	619.8	375.2	400.9	283.5
	CDC Imvincible	1051.2	741.2	665.8	721.6
	CDC Impower	1342.7	991.0	975.4	802.7
5	CDC Maxim	1066.7	785.7	573.5	462.6
	CDC Imvincible	421.5	403.6	645.7	457.6
	CDC Impower	696.7	405.8	394.2	723.3

Table B.2. Standard deviation for Zn concentration (mg Zn kg^{-1}) in grain and straw yield of three lentil cultivars fertilized with three rates (0, 2.5 and 5 kg Zn ha^{-1}) of soil applied $ZnSO_4$ at two sites in 2013 (corresponds with Figure 3.2).

Zn Rate (kg Zn ha^{-1})	Lentil Cultivar	Central Butte Grain	Central Butte Straw	Saskatoon Grain	Saskatoon Straw
		(mg Zn kg^{-1})			
0	CDC Maxim	7.3	17.3	3.9	2.6
	CDC Imvincible	6.9	2.2	3.8	5.1
	CDC Impower	8.0	5.1	4.4	4.9
2.5	CDC Maxim	3.8	5.3	3.6	3.0
	CDC Imvincible	4.5	3.1	3.7	5.9
	CDC Impower	2.6	1.2	4.7	3.6
5	CDC Maxim	4.8	1.6	4.2	5.7
	CDC Imvincible	2.3	5.7	2.9	5.9
	CDC Impower	3.4	3.8	3.5	9.2
Statistical Analaysis		P values			
	Rate	0.595	0.967	0.176	0.477
	Cultivar	0.186	0.471	<.0001	0.540
	Rate* Cultivar Interaction	0.423	0.660	0.458	0.882

Table B.3 Analysis of variance examining the relationship between fixed effects of fertilizer rate (0, 2.5 and 5 kg Zn ha^{-1}, pre-plant broadcast and incorporated as granular $ZnSO_4$) and lentil cultivar (cvs. CDC Maxim, CDC Imvincible, and CDC Impower), yield (kg ha^{-1}) and Zn concentration (mg Zn kg^{-1}) in grain and straw of lentil grown at Central Butte and Saskatoon field locations in 2013 (corresponds to Table 3.3 and Figure 3.3).

Site	Dependent Variable		Fixed Effect	Num df†	F-value	P-value	SEM‡
Central Butte	Yield						
		Grain	Fertilizer rate	2	0.01	0.994	359.3
			Cultivar	2	0.60	0.554	253.6
			Rate x Cultivar	4	0.22	0.925	439.2
		Straw	Fertilizer rate	2	0.08	0.929	193.6
			Cultivar	2	1.68	0.198	159.1
			Rate x Cultivar	4	0.71	0.588	275.5
	Zn concentration						
		Grain	Fertilizer rate	2	1.81	0.175	6.7
			Cultivar	2	0.98	0.382	6.7
			Rate x Cultivar	4	0.79	0.537	11.6
		Straw	Fertilizer rate	2	0.66	0.579	22.4
			Cultivar	2	4.51	0.017	13.8
			Rate x Cultivar	4	0.37	0.827	23.9
Saskatoon	Yield						
		Grain	Fertilizer rate	2	0.63	0.536	162.6
			Cultivar	2	6.14	0.004	162.6
			Rate x Cultivar	4	0.60	0.667	281.7
		Straw	Fertilizer rate	2	0.43	0.683	156.2
			Cultivar	2	8.98	0.001	147.6
			Rate x Cultivar	4	0.61	0.656	255.7
	Zn concentration						
		Grain	Fertilizer rate	2	3.27	0.176	1.7
			Cultivar	2	11.61	<.0001	1.2
			Rate x Cultivar	4	0.93	0.458	2.1
		Straw	Fertilizer rate	2	0.95	0.479	2.4
			Cultivar	2	0.70	0.505	1.7
			Rate x Cultivar	4	0.32	0.866	2.9

†Num df= numerator degrees of freedom

‡SEM= standard error of mean

APPENDIX C: EFFECTS OF ZINC FERTILIZER AMENDMENTS ON YIELD AND GRAIN ZINC CONTENT UNDER CONTROLLED CONDITIONS

C.1 Nutrient Composition of Lentil

Table C.1. Effects of various forms of Zn fertilizer on selected nutrient concentrations (mg kg^{-1}) within grain and straw lentil

Fertilizer	Cultivar	Grain				Straw		
		Zn	Fe	P	N	Zn	Fe	P
		(mg kg^{-1})						
Control	CDC Maxim	36.7 a	120.5 a	3637.4 a	18008.0 a	29.5 a	211.1 a	1110.8 abcde
	CDC Imvincible	38.2 a	110.6 a	3534.6 a	18221.0 a	31.4 a	183.6 a	922.6 bcde
	CDC Impower	33.3 a	94.0 a	2581.6 a	16831.0 a	31.5 a	248.8 a	1588.6 a
$ZnSO_4$	CDC Maxim	36.2 a	109.7 a	3148.7 a	15076.0 a	24.4 a	153.2 a	861.5 cde
	CDC Imvincible	35.3 a	115.4 a	3217.3 a	17023.0 a	29.1 a	266.3 a	884.7 cde
	CDC Impower	33.7 a	107.0 a	2551.9 a	16229.0 a	32.2 a	164.1 a	1373.7 abc
7% Foliar Lignosulphonate	CDC Maxim	41.0 a	95.5 a	3827.6 a	17011.0 a	30.1 a	219.4 a	1068.3 bcde
	CDC Imvincible	38.4 a	112.5 a	3751.6 a	19511.0 a	30.3 a	201.5 a	833.7 de
	CDC Impower	34.9 a	119.5 a	3251.7 a	20436.0 a	31.5 a	210.4 a	1370.3 abc
9% Foliar EDTA chelated	CDC Maxim	41.6 a	120.1 a	3006.4 a	14107.0 a	33.2 a	203.1 a	930.8 bcde
	CDC Imvincible	32.8 a	86.5 a	1777.2 a	9563.5 a	31.9 a	171.0 a	913.0 bcde
	CDC Impower	36.9 a	101.2 a	2431.8 a	15516.0 a	31.6 a	227.7 a	1430.1 ab
9% Soil EDTA chelated	CDC Maxim	37.3 a	98.1 a	2089.3 a	10347.0 a	32.8 a	176.3 a	784.6 e
	CDC Imvincible	39.1 a	97.8 a	2307.0 a	11527.0 a	30.6 a	183.6 a	724.8 e
	CDC Impower	43.5 a	119.7 a	3231.2 a	21295.0 a	30.6 a	225.2 a	1348.2 abcd
SEM‡		4.53	12.8	666.8	3495.7	2.21	37.3	104.4
Statistical Analysis		P values						
Fertilizer effect		0.708	0.939	0.166	0.239	0.353	0.949	0.061
Cultivar effect		0.719	0.848	0.725	0.292	0.569	0.626	<.0001
Fertilizer*Cultivar interaction effect		0.859	0.333	0.725	0.674	0.536	0.398	0.902

† Means with the same letter in the same column are not significantly different (P>0.05) as determined by multi-treatment comparisons (Tukey-Kramer)

‡ SEM= standard error of mean

C.2 Additional Statistical Information

Table C.2. Standard deviation for grain and straw yield (g pot^{-1}) of three lentil cultivars fertilized with various forms of Zn fertilizer (corresponds to Table 4.3).

Fertilizer	Cultivar	Yield (g pot^{-1})	
		Grain	Straw
Control	CDC Maxim	0.16	0.12
	CDC Imvincible	0.18	0.15
	CDC Impower	0.18	0.32
Soil $ZnSO_4$	CDC Maxim	0.10	0.14
	CDC Imvincible	0.10	0.14
	CDC Impower	0.29	0.47
7% Zn Foliar Lignosulphonate	CDC Maxim	0.14	0.58
	CDC Imvincible	0.22	0.19
	CDC Impower	0.32	0.19
9% Zn Foliar EDTA chelated	CDC Maxim	0.11	0.13
	CDC Imvincible	0.13	0.08
	CDC Impower	0.25	0.22
9% Zn Soil EDTA chelated	CDC Maxim	0.07	0.17
	CDC Imvincible	0.07	0.20
	CDC Impower	0.21	0.33

Table C.3. Standard deviation for Zn concentration (mg Zn kg^{-1}) grain and straw yield of three lentil cultivars fertilized with various forms of Zn fertilizer (corresponds to Table 4.4).

Fertilizer	Cultivar	Zn concentration (mg Zn kg^{-1})	
		Grain	Straw
Control	CDC Maxim	5.0	2.1
	CDC Imvincible	9.9	1.6
	CDC Impower	10.7	3.6
Soil $ZnSO_4$	CDC Maxim	9.8	7.0
	CDC Imvincible	6.3	2.1
	CDC Impower	5.3	5.5
7% Zn Foliar Lignosulphonate	CDC Maxim	19.1	11.1
	CDC Imvincible	10.5	3.1
	CDC Impower	5.5	2.9
9% Zn Foliar EDTA chelated	CDC Maxim	10.8	2.9
	CDC Imvincible	11.1	6.0
	CDC Impower	12.5	11.1
9% Zn Soil EDTA chelated	CDC Maxim	14.6	3.6
	CDC Imvincible	15.0	3.1
	CDC Impower	11.4	1.8

Table C.4 Analysis of variance examining the relationship between fixed effects of Zn fertilizer form (soil applied $ZnSO_4$, soil and foliar applied Zn chelated with EDTA, foliar applied Zn lignosulphonate and control treatment) and lentil cultivar (cvs. CDC Maxim, CDC Imvincible, and CDC Impower), yield (g pot^{-1}) and Zn concentration (mg Zn kg^{-1}) in grain and straw of lentil grown under controlled environment conditions (corresponds to Tables 4.3 and 4.4).

Dependent Variable		Fixed Effect	Num df†	F-value	P-value	SEM‡
Yield						
	Grain	Zn fertilizer form	4	0.37	0.829	0.04
		Cultivar	2	1.21	0.304	0.03
		Zn form x Cultivar	8	0.74	0.656	0.08
	Straw	Zn fertilizer form	4	0.69	0.603	0.06
		Cultivar	2	123.6	<.0001	0.05
		Zn form x Cultivar	8	1.24	0.289	0.11
Zn concentration						
	Grain	Zn fertilizer form	4	0.54	0.708	2.6
		Cultivar	2	0.33	0.719	2.0
		Zn form x Cultivar	8	0.49	0.859	4.5
	Straw	Zn fertilizer form	4	1.12	0.353	1.3
		Cultivar	2	0.57	0.569	0.99
		Zn form x Cultivar	8	0.88	0.536	2.2

†Num df= numerator degrees of freedom
‡SEM= standard error of mean

APPENDIX D: EFFECTS OF ZINC FERTILIZATION ON PREDICTED BIOAVAILABILITY OF ZINC IN LENTIL GRAIN

D.1 Additional Statistical Information

D.1.1 Polyhouse study

Table D.1. Standard deviation of phytate content (mg g^{-1}), phytate: Zn molar ratios and estimated bioavailable Zn (mg Zn 300 g^{-1}) in grain of three lentil cultivars grown in a polyhouse and fertilized with various forms of Zn (corresponds to Figure 5.1 and Table 5.1).

Zn Fertilizer	Phytate Content (mg g^{-1})	Bioavailability Measurement: Phytate: Zn molar ratio	Bioavailability Measurement: Estimated Bioavailable Zn (mg Zn $300g^{-1}$)
$ZnSO_4$	3.30	8.47	0.47
9% Soil EDTA chelated	2.20	5.26	0.50
9% Foliar EDTA chelated	1.85	6.35	0.53
7% Foliar Lignosulphonate	3.70	10.43	0.68
Control	2.64	7.55	0.56
Statistical Analaysis		**P values**	
Fertilizer	0.010	0.010	0.050
Cultivar	0.678	0.510	0.478
Fertilizer* Cultivar Interaction	0.147	0.502	0.680

Table D.2. Analysis of variance examining the relationship between fixed effects of Zn fertilizer form (soil applied $ZnSO_4$, soil and foliar applied Zn chelated with EDTA, foliar applied Zn lignosulphonate and control treatment) and lentil cultivar (cvs. CDC Maxim, CDC Imvincible, and CDC Impower) and grain phytate concentration (mg g^{-1}), phyate:Zn molar ratio, and estimated bioavailable Zn (mg 300 g^{-1}) of lentil grown under controlled environment conditons (corresponds to Figure 5.1 and Table 5.1).

Dependent Variable	Fixed Effect	Num df†	F-value	P-value	SEM‡
Grain Phytate Concentration	Zn fertilizer form	4	3.57	0.010	0.65
	Cultivar	2	0.39	0.678	0.51
	Zn form x Cultivar	8	1.57	0.147	1.1
Phytate:Zn molar ratio	Zn fertilizer form	4	3.57	0.010	1.9
	Cultivar	2	0.68	0.510	1.4
	Zn form x Cultivar	8	0.92	0.502	3.2
Estimated Bioavailable Zn	Zn fertilizer form	4	2.50	0.049	0.13
	Cultivar	2	0.74	0.478	0.10
	Zn form x Cultivar	8	0.71	0.680	0.23

†Num df= numerator degrees of freedom
‡SEM= standard error of mean

Table D.3. Relationships (Pearson correlation coefficients, r) among nutritional quality parameters of lentil grain grown under polyhouse conditions in 2013 and fertilized with different forms of Zn.

		Grain Zn	Grain P	Grain Phytate	Phytate:Zn molar ratio	Estimated Bioavailable Zn
		(mg Zn kg^{-1})	(mg P kg^{-1})	(mg g^{-1})		(mg Zn 300g^{-1})
Grain Zn	r	1.000	0.842	0.176	-0.399	0.552
(mg Zn kg^{-1})	p-value		<.0001	0.096	<.0001	<.0001
Grain P	r	0.842	1.000	0.459	0.804	0.223
(mg P kg^{-1})	p-value	<.0001		<.0001	<.0001	0.035
Grain Phytate	r	0.176	0.459	1.000	0.804	-0.699
(mg g^{-1})	p-value	0.096	<.0001		<.0001	<.0001
Phytate:Zn	r	-0.399	-0.030	0.804	1.000	-0.962
molar ratio	p-value	<.0001	0.781	<.0001		<.0001
Estimated Bioavailable Zn	r	0.552	0.223	-0.699	-0.962	1.000
(mg Zn 300g^{-1})	p-value	<.0001	0.035	<.0001	<.0001	

D.1.2 Field study

Table D.4. Standard deviation of phytate content (mg g^{-1}), phytate: Zn molar ratios and estimated bioavailable Zn (mg Zn 300g^{-1}) in grain of three lentil cultivars harvested from field locations at Central Butte and Saskatoon, SK (corresponds to Table 5.2).

Location	Cultivar	Phytate Content	Bioavailability Measurement	
			Phytate: Zn molar ratio	Estimated Bioavailable Zn
		(mg g^{-1})		(mg Zn 300g^{-1})
Central Butte	CDC Maxim	0.97	6.16	0.40
	CDC Imvincible	1.22	8.37	0.39
	CDC Impower	1.24	6.96	0.36
Saskatoon	CDC Maxim	1.07	3.89	0.31
	CDC Imvincible	0.98	3.67	0.26
	CDC Impower	1.13	3.16	0.25

Table D.5. Analysis of variance examining the relationship between fixed effects of Zn fertilizer rate (0, 2.5 and5 kg Zn ha^{-1}, pre-plant soil broadcast and incorporated as granular $ZnSO_4$) and lentil cultivar (cvs. CDC Maxim, CDC Imvincible, and CDC Impower) and grain phytate concentration (mg g^{-1}), phyate:Zn molar ratio, and estimated bioavailable Zn (mg Zn 300 g^{-1}) of lentil grown at field locations in Central Butte and Sasktoon in 2013 (corresponds to and Table 5.2).

Site	Dependent Variable	Fixed Effect	Num df†	F-value	P-value	SEM‡
Central Butte	Grain Phytate Concentration	Fertilizer rate	2	1.02	0.458	0.66
		Cultivar	2	3.24	0.049	0.41
		Rate x Cultivar	4	0.68	0.607	0.71
	Phytate:Zn molar ratio	Fertilizer rate	2	0.04	0.958	5.2
		Cultivar	2	3.87	0.029	3.1
		Rate x Cultivar	4	0.83	0.511	5.4
	Estimated Bioavailable Zn	Fertilizer rate	2	0.01	0.988	0.28
		Cultivar	2	3.94	0.027	0.17
		Rate x Cultivar	4	0.99	0.422	0.30
Saskatoon	Grain Phytate Concentration	Fertilizer rate	2	1.44	0.364	0.33
		Cultivar	2	0.19	0.826	0.27
		Rate x Cultivar	4	0.52	0.724	0.47
	Phytate:Zn molar ratio	Fertilizer rate	2	1.43	0.366	1.4
		Cultivar	2	4.22	0.021	1.0
		Rate x Cultivar	4	0.39	0.815	1.8
	Estimated Bioavailable Zn	Fertilizer rate	2	1.81	0.305	0.10
		Cultivar	2	4.50	0.017	0.08
		Rate x Cultivar	4	0.51	0.732	0.13

†Num df= numerator degrees of freedom
‡SEM= standard error of mean

Table D.6. Relationships (Pearson correlation coefficients, r) among nutritional quality parameters of lentil grain grown at field sites in Central Butte and Saskatoon, SK in 2013 and fertilized with three rates of soil applied $ZnSO_4$ (0, 2.5, and 5 kg Zn ha^{-1}).

		Zn Rate	Grain Zn	Grain P	Grain Phytate	Phytate:Zn molar ratio	Estimated Bioavailable Zn
		(kg ha^{-1})	(mg kg^{-1})		(mg g^{-1})		(mg Zn 300g^{-1})
---Central Butte---							
Zn Rate (kg Zn ha^{-1})	r	1.000	-0.375	-0.077	-0.476	-0.115	0.002
	p-value		0.005	0.580	0.0003	0.408	0.990
Grain Zn (mg Zn kg^{-1})	r	-0.375	1.000	0.316	0.128	-0.671	0.764
	p-value	0.005		0.020	0.357	<.0001	<.0001
Grain P (mg P kg^{-1})	r	-0.077	0.316	1.000	0.219	-0.096	0.147
	p-value	0.580	0.020		0.111	0.489	0.288
Grain Phytate (mg g^{-1})	r	-0.476	0.128	0.219	1.000	0.616	-0.528
	p-value	0.0003	0.357	0.111		<.0001	<.0001
Phytate:Zn molar ratio	r	-0.115	-0.671	-0.096	0.616	1.000	-0.971
	p-value	0.408	<.0001	0.489	<.0001		<.0001
Estimated Bioavailable Zn (mg Zn 300g^{-1})	r	0.002	0.764	0.147	-0.528	-0.971	1.000
	p-value	0.990	<.0001	0.288	<.0001	<.0001	
---Saskatoon---							
Zn Rate (kg Zn ha^{-1})	r	1.000	0.341	-0.326	-0.239	-0.371	0.386
	p-value		0.117	0.016	0.082	0.006	0.004
Grain Zn (mg Zn kg^{-1})	r	0.341	1.000	0.412	-0.139	-0.736	0.761
	p-value	0.117		0.002	0.318	<.0001	<.0001
Grain P (mg P kg^{-1})	r	-0.326	0.412	1.000	0.009	-0.287	0.258
	p-value	0.016	0.002		0.946	0.036	0.060
Grain Phytate (mg g^{-1})	r	-0.239	-0.139	0.009	1.000	0.756	-0.744
	p-value	0.082	0.318	0.946		<.0001	<.0001
Phytate:Zn molar ratio	r	-0.371	-0.736	-0.287	0.756	1.000	-0.991
	p-value	0.006	<.0001	0.036	<.0001		<.0001
Estimated Bioavailable Zn (mg Zn 300g^{-1})	r	0.386	0.761	0.258	-0.744	-0.991	1.000
	p-value	0.004	<.0001	0.060	<.0001	<.0001	

APPENDIX E: ECONOMIC ANALYSIS OF FERTILIZING LENTIL WITH SOIL APPLIED ZINC SULPHATE AND ITS RESIDUAL VALUE FOR SPRING WHEAT

Table E.1. Economic analysis comparing profitability of fertilizing different classes of lentil, grown at Central Butte, SK in 2013, with varying rates (0, 2.5 and 5 kg Zn ha^{-1}) of soil applied $ZnSO_4$. An explanation of calculation assumptions is provided in Table E.3.

Lentil Market Class†	**Red**			**Large Green**		
Zn Fertilizer Rate (kg Zn ha^{-1})	0	2.5	5	0	2.5	5
Region	Brown Soil Zone					
REVENUE PER HECTARE						
Average Yield (kg ha^{-1})	2992	2953	3116	2634	2778	2853
Average Market Price ($ kg^{-1})	0.46	0.46	0.46	0.48	0.48	0.48
Gross Revenue ($ ha^{-1})	1,376.32	1,358.38	1,433.36	1,264.32	1,333.44	1,369.44
EXPENSES PER HECTARE						
Variable Input Expenses						
Seed	23.00	23.00	23.00	44.00	44.00	44.00
Fertilizer						
- MAP (11-52-0-0)	34.44	34.44	34.44	34.44	34.44	34.44
- $ZnSO_4$	0.00	22.50	45.00	0.00	22.50	45.00
Chemical						
- Seed Treatment/Inoculant	6.67	6.67	6.67	6.67	6.67	6.67
- Herbicide/Fungicide	98.45	98.45	98.45	98.45	98.45	98.45
- Insecticide	6.08	6.08	6.08	6.08	6.08	6.08
- Dessicant	40.76	40.76	40.76	40.76	40.76	40.76
Machinery Operating Costs						
- Fuel	47.28	47.28	47.28	47.28	47.28	47.28
- Repair	23.02	23.02	23.02	23.02	23.02	23.02
Custom Work/Hired Labour	33.96	33.96	33.96	33.96	33.96	33.96
Crop Insurance Premium	51.32	51.32	51.32	51.32	51.32	51.32
Utilities and Miscellaneous	14.75	14.75	14.75	14.75	14.75	14.75
Interest on Variable Input Expenses	9.81	9.81	9.81	9.81	9.81	9.81
Total Variable Input Expenses	389.54	412.04	434.54	410.54	433.04	455.54
NET RETURNS PER HECTARE‡						
Gross Revenue- Expenses ($ ha^{-1})	**986.78**	**946.34**	**998.82**	**853.78**	**900.40**	**913.90**

†Average yield estimates for each lentil market class have been obtained from 2013 field experiment at Central Butte location; yield data for the red and large green market classes are represented by lentil cultivars CDC Maxim and CDC Impower, respectively (see Table 3.2).

‡Values are calculated based on mean yield estimates that are not significantly different (Table 3.2, P=0.925) and represent six treatment replicates with large standard deviation (Table B.3). Therefore, economic responses of lentil to Zn fertilization are highly variable and the reported net returns should be interpreted with caution.

Table E.2. Economic analysis comparing profitability of fertilizing different classes of lentil, grown at Saskatoon, SK in 2013, with varying rates (0, 2.5 and 5 kg Zn ha^{-1}) of soil applied $ZnSO_4$. An explanation of calculation assumptions is provided in Table E.3.

Lentil Market Class†	**Red**			**Large Green**		
Zn Fertilizer Rate (kg Zn ha^{-1})	0	2.5	5	0	2.5	5
Region	Dark Brown Soil Zone					
REVENUE PER HECTARE						
Average Yield (kg ha^{-1})	4304	4808	4341	3817	3855	3571
Average Market Price ($ kg^{-1})	0.46	0.46	0.46	0.48	0.48	0.48
Gross Revenue ($ ha^{-1})	1,979.84	2,211.68	1,996.68	1,832.16	1,850.40	1,714.08
EXPENSES PER HECTARE						
Variable Input Expenses						
Seed	23.00	23.00	23.00	44.00	44.00	44.00
Fertilizer						
- MAP (11-52-0-0)	34.44	34.44	34.44	34.44	34.44	34.44
- $ZnSO_4$	0.00	22.50	45.00	0.00	22.50	45.00
Chemical						
- Seed Treatment/Inoculant	6.67	6.67	6.67	6.67	6.67	6.67
- Herbicide/Fungicide	98.45	98.45	98.45	98.45	98.45	98.45
- Insecticide	6.08	6.08	6.08	6.08	6.08	6.08
- Dessicant	40.76	40.76	40.76	40.76	40.76	40.76
Machinery Operating Costs						
- Fuel	47.28	47.28	47.28	47.28	47.28	47.28
- Repair	23.02	23.02	23.02	23.02	23.02	23.02
Custom Work/Hired Labour	33.96	33.96	33.96	33.96	33.96	33.96
Crop Insurance Premium	51.32	51.32	51.32	51.32	51.32	51.32
Utilities and Miscellaneous	14.75	14.75	14.75	14.75	14.75	14.75
Interest on Variable Input Expenses	9.81	9.81	9.81	9.81	9.81	9.81
Total Variable Input Expenses	386.46	408.96	431.46	407.46	429.96	452.46
NET RETURNS PER HECTARE‡						
Gross Revenue- Expenses ($ ha^{-1})	**1,593.38**	**1,802.72**	**1,565.40**	**1,424.70**	**1,420.44**	**1,261.62**

†Average yield estimates for each lentil market class have been obtained from 2013 field experiment at Saskatoon location; yield data for the red and large green market classes are represented by lentil cultivars CDC Maxim and CDC Impower, respectively (see Table 3.2).

‡Values are calculated based on mean yield estimates that are not significantly different (Table 3.2, P=0.536) and represent six treatment replicates with large standard deviation (Table B.3). Therefore, economic responses of lentil to Zn fertilization are highly variable and the reported net returns should be interpreted with caution.

Table E.3. Description of cost assumptions incorporated into lentil production budget (Table E.1).

Item†	Description of Assumptions
Market Price	Average lentil price, within each market class, from crop year spanning August 2013-July 2014 (Saskatchewan Ministry of Agriculture, 2013)
Seed	Cleaned seed price was assumed as 50% higher than the average market value as calculated from the 2012 off-combine price up to and including price in early May, 2013; red = $0.46 kg^{-1}, large green=$0.48 kg^{-1} (Saskatchewan Ministry of Agriculture, 2012). Average seeding rates were based on estimates of thousand kernel weight and a targeted plant stand of 130 plants m^{-2}; red= 50 kg ha^{-1}, large green = 100 kg ha^{-1} (Saskatchewan Ministry of Agriculture, 2011)
Fertilizer	Monoammonium phosphate (MAP, 11-52-0-0) was applied at 42 kg ha^{-1} to supply 22 kg P ha^{-1} of maximum safe rate of seed placed phosphorus. Fertilizer was assumed to be purchased in the spring of 2013, when market value was $825 tonne^{-1}. Average price of $ZnSO_4$ per actual kg of Zn was estimated at $9.00 kg^{-1}. Fertilizer prices were sourced through a local crop input retail (G-Mac's AgTeam Inc.).
Chemical	Chemical values where derived from the Saskatchewan Ministry of Agriculture (2011). Although specific products are not described, only registered products and rates were used for calculating average costs. Application costs for Reglone® Dessicant (240 g L^{-1} *diquat*) were calculated based on an application rate of 1.48 L ha^{-1} priced at $24.50 L^{-1} and a surfactant cost of $4.50 ha^{-1} (G-Mac's AgTeam Inc.).
Machinery Operating Costs	Estimated fuel consumption for various field operations with diesel fuel price assumed at $0.87 L^{-1}. Annual repair costs have been estimated at 4% of average machinery investment (Saskatchewan Ministry of Agriculture, 2011)
Custom Work/Hired Labour	Estimates for Table E.1 derived from planning guide for crops grown in the Brown Soil Zone (Saskatchewan Ministry of Agriculture, 2011a); Table E.2 assume estimates made by Saskatchewan Ministry of Agriculture (2011)
Crop Insurance Premium	Average of Saskatchewan Crop Insurance Corporation (SCIC) premiums calculated as 70% of risk area coverage (Saskatchewan Ministry of Agriculture, 2011).
Interest on Variable Rate Expenses	Assume an interest rate of 4.75% on all cash operating costs within a six month period (Saskatchewan Ministry of Agriculture, 2011).

†Expenses are estimated values only and do not represent the production costs of every possible management scenario. Items such as, but not limited to, equipment value and depreciation, grain transportation to market, storage, management cost, and land value have not been included in the present economic analysis as they are highly variable amongst individual producers. A guideline for some omitted expenses can be found in Saskatchewan Ministry of Agriculture (2011, 2011a) planning guides for specialty crops and crops grown within the Brown Soil Zone.

APPENDIX F: EFFECT OF ZINC FERTILIZATION ON ZINC AND FE CONCENTRATION IN PROCESSED LENTIL GRAIN FRACTIONS

F.1 Zinc Concentration in Processed Lentil Grain Fractions

Table F.1 Mean Zn concentration (mg Zn kg^{-1}) in processed lentil grain fractions (whole seed, football, splits, and seed coat) of small red lentil (cv. CDC Maxim) grown at Central Butte and Saskatoon field locations in 2013 and fertilized with three rates (0, 2.5, and 5 kg Zn ha^{-1}) of soil applied $ZnSO_4$ fertilizer.

Site	Lentil Grain Fraction	Zn Fertilizer Rate (kg Zn ha^{-1})		
		0	2.5	5
		Zn concentration† (mg Zn kg^{-1})		
Central Butte	Whole seed	27.5 aA	24.1 aAB	20.5 aAB
	Football	24.4 aA	22.2 aAB	20.1 aAB
	Splits	26.0 aA	21.9 aAB	20.6 aAB
	Seed coat	14.7 bB	17.3 bAB	15.8 bAB
Saskatoon	Whole seed	40.6 aA	39.9 aA	43.5 aA
	Football	40.6 aA	39.2 aA	43.6 aA
	Splits	38.0 aA	39.1 aA	43.1 aA
	Seed coat	18.9 bB	15.5 bB	20.6 bB

†Means with the same lower case letter in the same row (within a lentil fraction) and the same upper case letter in the same column (within a Zn fertilizer rate) are not significantly different (P>0.05) as discussed by multi-treatment comparisons using the Tukey-Kramar method.

Table F.2 Standard deviation of Zn concentration (mg Zn kg^{-1}) in processed lentil grain fractions (whole seed, football, splits, and seed coat) of small red lentil (cv. CDC Maxim) grown at Central Butte and Saskatoon field locations in 2013 and fertilized with three rates (0, 2.5, and 5 kg Zn ha^{-1}) of soil applied $ZnSO_4$ fertilizer (corresponds to Table F.1).

Zn Rate	Central Butte				Saskatoon			
	Lentil Grain Fraction							
(kg Zn ha^{-1})	Whole seed	Football	Splits	Seed coat	Whole seed	Football	Splits	Seed coat
0	6.57	3.81	5.22	6.78	3.99	1.88	1.31	1.35
2.5	4.08	3.39	2.73	1.16	2.34	1.00	1.15	1.32
5	1.96	2.00	2.15	1.97	3.27	2.02	2.32	4.18

F.2 Iron Concentration in Processed Lentil Grain Fractions

Table F.3 Mean Fe concentration (mg Fe kg^{-1}) in processed lentil grain fractions (whole seed, football, splits, and seed coat) of small red lentil (cv. CDC Maxim) grown at Central Butte and Saskatoon field locations in 2013 and fertilized with three rates (0, 2.5, and 5 kg Zn ha^{-1}) of soil applied $ZnSO_4$ fertilizer.

Site	Lentil Grain Fraction	Zn Fertilizer Rate (kg Zn ha^{-1})		
		0	2.5	5
		Fe concentration† (mg Fe kg^{-1})		
Central Butte	Whole seed	72.2 aA	65.0 aA	61.7 aA
	Football	66.3 aA	60.1 aA	66.3 aA
	Splits	65.2 aA	58.0 abA	59.1 abA
	Seed coat	56.4 bA	55.7 bA	48.8 bA
Saskatoon	Whole seed	73.7 aAB	78.2 aA	73.7 aAB
	Football	74.9 abAB	76.8 abAB	78.4 abA
	Splits	71.5 bB	73.0 bAB	76.7 bAB
	Seed coat	41.4 cC	43.0 cC	43.6 cC

†Means with the same lower case letter in the same row (within a lentil fraction) and the same upper case letter in the same column (within a Zn fertilizer rate) are not significantly different (P>0.05) as discussed by multi-treatment comparisons using the Tukey-Kramar method.

Table F.4 Standard deviation of Fe concentration (mg Fe kg^{-1}) in processed lentil grain fractions (whole seed, football, splits, and seed coat) of small red lentil (cv. CDC Maxim) grown at Central Butte and Saskatoon field locations in 2013 and fertilized with three rates (0, 2.5, and 5 kg Zn ha^{-1}) of soil applied $ZnSO_4$ fertilizer (corresponds to Table F.3).

Zn Rate	Central Butte				Saskatoon			
	Lentil Grain Fraction							
(kg Zn ha^{-1})	Whole seed	Football	Splits	Seed coat	Whole seed	Football	Splits	Seed coat
0	6.72	1.79	1.57	19.9	2.26	1.92	1.89	2.36
2.5	3.46	2.64	2.69	3.42	1.43	1.35	3.27	4.87
5	3.76	3.19	4.16	12.5	3.43	3.55	2.44	1.77

F.3 Additional Statistical Information

Table F.5. Analysis of variance examining the relationship between fixed effects of fertilizer rate (0, 2.5 and 5 kg Zn ha^{-1}, pre-plant broadcast and incorporated as granular $ZnSO_4$) and processed lentil grain fraction of small red lentil (cv. CDC Maxim, grown at Central Bute and Saskatoon field locations in 2013), and Zn and Fe concentration (mg Zn kg^{-1}).

Site	Dependent Variable	Fixed Effect	Num df†	F-value	P-value	SEM‡
Central Butte	Zn Concentration	Zn Fertilizer Rate	2	0.70	0.563	2.3
		Lentil Fraction	3	17.79	<.0001	1.5
		Rate x Fraction	6	1.56	0.195	2.6
	Fe Concentration	Zn Fertilizer Rate	2	1.04	0.459	3.7
		Lentil Fraction	3	7.98	0.001	2.7
		Rate x Fraction	6	0.46	0.830	4.7
Saskatoon	Zn Concentration	Zn Fertilizer Rate	2	5.83	0.093	0.9
		Lentil Fraction	3	295.1	<.0001	0.8
		Rate x Fraction	6	0.76	0.605	1.3
	Fe Concentration	Zn Fertilizer Rate	2	9.06	0.001	0.7
		Lentil Fraction	3	444.7	<.0001	0.8
		Rate x Fraction	6	0.59	0.735	1.4

†Num df= numerator degrees of freedom

‡SEM= standard error of mean; reported as mean SEM values for Zn rate and rate x fraction effects in Central Butte as a result of one missing 0 kg Zn ha-1 treatment replicate (mean of three replicates versus four replicates in other Zn fertilizer treatments)