Cape Peninsula University of Technology

THE EFFECT OF ZINC AND SOIL PH ON GRAIN YIELD AND NUTRIENT CONCENTRATIONS IN SPRING WHEAT CULTIVATED ON POTTED SOIL

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DECLARATION

I, **Singbo Arnaud**, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed ______________________ Date: April 2018

DEDICATION

I dedicate this work to God Almighty the source of my strength.

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ABSTRACT

Zinc deficiency on various soil types have been reported in arable soils of sub Saharan Africa (SSA) including South Africa. A pot trial was conducted at the Cape Peninsula University of Technology, Wellington campus to investigate the interaction of different application rates of Zn at various soil pH on the grain yield and quality of spring wheat in a completely randomized factorial design replicated three times. The four soil pH tested were: pH_A: 5.1, pH_B: 5.6, pH_C: 6.1, pH_D: 6.6 which correspond to lime application at 0, 0.5, 1 and 1.5 t/ha. Five Zn rates (Zn1: 3.5; Zn2: 4.5; Zn3: 5.5 Zn4: 6.5, and Zn5: 7.5 mg /kg soil which correspond to Zn1: 7; Zn2: 9; Zn3: 11; Zn4: 13 and Zn5: 15 kg /ha) were applied at two (planting and flowering) growth stages. Yield and yield component data collected were analyzed using SAS version 9.2 and means were separated by Duncun's Multiple Range Test (DMRT). The results showed that grain yield and yield components were significantly affected by lime application pH_C (6.1): 1t/ha at planting. Zn application at planting had no significant effect on the grain yield and yield components. However, at flowering, the simultaneous increase of Zn along with increase in lime positively affected grain yield and yield components. Plant analysis showed that at both stages (planting and flowering), Zn application, especially at pH 6.6, significantly increased P, K, Ca, Na, Mg Fe, Cu and B concentrations in wheat grain, but the concentrations of N, Mn, Zn and protein remained unaffected. Zn application had no effect on most nutrients due to the presence of lime. While the absence of lime, Zn4: 6.5mg/kg (corresponding to 13kg/ha) significantly increased the nutrients. In addition, Zn3: 5.5mg/kg (corresponding to 11kg/ha) promoted Zn absorption by grain in all treatments.

***Key words*:** pH levels, stage, wheat, zinc levels.

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ABBREVIATIONS AND GLOSSARY

AM	Arbuscular mycorrhizae
ARC	Agricultural Research Council
B	Boron
Ca	Calcium
CA	Carbonic Anhydrase
Cu	Copper
DAP	Days After Planting
FAO	Food and Agriculture Organization of the United Nations
Fe	Iron
Ha	Hectare (unit of land area)
IAA	Indole Acetic Acid
K	Potassium
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
P	Phosphorus
PCA	Principal Component Analysis
pH	Measure of acidity or alkalinity
RNA	Ribonucleic acid
Zn	Zinc
ZnO	Zinc oxide

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CHAPTER ONE: INTRODUCTION

1.1 Background

Zinc (Zn) deficiency is one of the most common mineral deficiencies, touching up to a third of the world's population (Welch & Graham, 2004; Broadley *et al.,* 2007). Deficiency in Zn as well as other micro-nutrients is of increasing concern in both developed and developing countries. This is because it is associated with reduced work productivity and a decrease in gross national product (Bouis, 2003). A recent study conducted by Motadi *et al.,* (2015) in the Limpopo province of South Africa revealed that 43 % of preschool children aged 3 to 5 years show signs of zinc deficiency.

Zinc is needed in the activity of about 300 enzymes found in all major enzyme classes (Vallee & Falchuk, 1993; Broadley *et al.,* 2007). Due to the various roles played by this mineral in human physiology, its shortage affects a large amount of biochemical and physiological functions. Zn is involved in nearly all biological processes including growth, reproduction, immune responses, and neurobehavioral development (Heid, 2017). Therefore, its absence may cause a severe loss of weight, hindered skeletal development and sexual maturity, mental handicaps, dermatitis, recurrent infections, persistent diarrhea and chronic ulcers (Gibson, 2006). The most noticeable sign of zinc shortage is overall stunted growth especially in infants (Black, 1998; Heid, 2017).

Zinc deficiency in humans results from diets low in bio-available Zn. Cereals and leguminous plants are known to contain low Zn due to the fact that they are high in phytic acid, which then renders zinc inaccessible through the formation of poorly soluble Zn–phytate complexes (House, 1999; Frossard et al., 2000). Whereas fish, meat, vegetables and fruits contain appreciable concentrations of Zn due to their low phytate among other factors. Subsequently, Zn deficiency is more prominent in less privileged areas of developing nations, where people depend primarily on cereal and leguminous staple foods to survive and where the intake of animal and fish products is often limited due to marginal incomes or other reasons (Schulin *et al.,* 2009). Zinc deficiency is particularly worse in areas where this mineral's availability in the soil is low (Noulas *et al.,* 2018).

On a global scale, maize and wheat are the top two most important grain crops and South Africa is not an exception. Most of the wheat cultivars serve as a source of flour for making bread. The ever increasing world population necessitates a matching increase in the rate of food production to meet the food demand. Therefore, the productivity of crops needs to be enhanced since there is a scarcity of arable soils. Another cause of low crop production is the fact that the removal of nutrients from the soil is greater than their addition. Thus, many soils and crops suffer shortages in essential plant nutrients for production and productivity (Kabata-Pendias & Pendias, 2001).

The availability of most plant nutrients is greatly affected by soil pH (Jensen, 2010). In addition, soil pH affects the physical, chemical and biological properties and processes of the soil, and ultimately the growth and development of the plant. The nutrition, growth and yields of most crops augment as pH rises to an optimum level and diminish when soil pH is low. In fact, most plant nutrients are optimally available to plants within this 6.5 to 7.5 pH range which range is generally is compatible to plant root growth (Jensen, 2010).

The Food and Agriculture Organization of the United Nations (FAO, 2005) reveals that South Africa's cultivated soils show a severe organic matter deficit, are susceptible to wind erosion as well as acidification through cultivation and nitrogen fertilization.

Plant growth and reproduction are not complete without Zn due to the role of regulation it plays in a broad range of enzymes and important biochemical pathways (Kabata-Pendias & Pendias, 2001). To contribute to several interventions for improving hidden hunger, it is essential to consider the interaction between soil pH and Zn and its effect on wheat cultivation for high yield and better grain quality towards sustainable food and nutritional security.

1.2 Problem statement

South African soils are generally low in bio-available zinc (< 3 mg/kg) (ARC-ISCW, 2004). Even though the lowest Zn values are found in sand soil, the content ranges from 10-300 mg/kg with a mean of 64 mg/kg (Noulas *et al,* 2018). For the current study, a sandy soil was used because it is a challenging soil in the area which also accounts for 33 % of the total area under wheat production in South Africa (FAO, 2005). These soils have poor water holding capacity and nutrients; are infertile and typically leached. Moreover, high and low pH, high and low organic matter, calcareous, sodic, sandy, wetland or ill-drained and limed acid soils are reportedly Zn deficient (Takkar & Walker, 1993). Therefore, a deeper understanding and knowledge is needed on how other soil characteristics like soil pH can affect Zn fertilization for higher grain yield and better grain quality in the cropping system.

Mineral fertilizers are considered a good source of Zn, but it gets fixed quickly in the soil matrix, resulting in poor availability to plants (Zia *et al.,* 1999). A previous report says that nearly 90 % of the total Zn in the soil exists in residual fraction, with no importance to the ready-to-use fraction (Mandal *et al.,* 1988). Thus, it is important to increase the bio-availability of Zn to plants. This can be done by solubilizing fixed Zn, by reducing fixation of the applied Zn fertilizers, and/or by supplying Zn fertilizer at the precise moment its application would be effective to the plant.

Although, significant knowledge exists about the effects of Zn fertilization on wheat under climatic conditions of the Western Cape, there is limited information on the effect of soil pH on wheat response to Zn fertilization. The aim of the current study is therefore to investigate the extent to which soil pH combined with Zn fertilization influence grain yield and nutrient concentration in wheat.

1.3 Research question and hypotheses

This study aims to address the key question: "What is the influence of zinc on mineral contents of wheat, with emphasis on the improvement of zinc levels?" This major question is examined by exploring the following hypotheses:

1. High soil pH and close to neutral is favorable to high nutrient concentrations in wheat grain
2. Zinc application at flowering increases the grain yield and nutrient concentration in the grain of spring wheat
3. Zinc application at a higher rate than the recommended rate increases the grain yield and the nutrient concentration in spring wheat grain.
4. The higher the zinc rate application rate, the higher the zinc concentration in the grain at different soil pH levels.

1.4 Aim and objectives of the study

The broad aim of the current study is to examine the effects of soil pH on spring wheat in response to zinc fertilizer application. The specific objectives are:

- To evaluate the influence of soil pH on grain yield and on nutrient concentration in spring wheat grain

- To determine the optimum rate and time of zinc application in cultivation of spring wheat
- To determine the influence of soil pH and zinc application on soil chemical composition after harvesting the wheat crop.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

The application of N, P and K fertilizers, has been proven to lead to the increase in crop yield while very little consideration has been given to micro-nutrient fertilization (Brady & Weil*,* 2002). As a result, micro-nutrient insufficiency in crop plants have been a rising issue in different countries, including developed countries (Welch *et al*., 1991).

Among micro-nutrient deficiencies, Zn is the most common and regular in crops across the world, resulting in extreme reduction in yield and nutritional quality (Alloway, 2008). In addition, Zn deficiency in humans is a direct consequence of Zn deficiency in crops. According to the World Health Organization (2002) Zn deficiencies rank fifth in the leading causes of disease in developing countries (Alloway, 2008; Gunes *et al*., 2007). According to Cakmak (2002), adjustment with Zn fertilizer of a zinc-deficient soil increases Zn concentration in both the soil and wheat grain. Zhao *et al*., (2011) reported an increase in grain Zn concentration in response to the addition of Zn to the roots.

In the normal and vigorous growth and reproduction process of crops, Zn is one of the eight trace crucial elements. The remaining others are chlorine, manganese, molybdenum, iron, copper, boron, and nickel. They are referred to as 'essential trace elements' or micronutrients, because they are very often needed in minor concentrations (about 5-100 mg/kg) in the plant tissues. Many factors affect the total Zn content of soils, including composition of the soil parent material, inputs from atmospheric deposition, and input due to fertilization during agricultural production practices (Alloway, 2008).

2.2 Wheat

Wheat (*Triticum.aestivum*) is a cereal or grain crop belonging to the Poaceae family. It is an annual crop that reproduces by seed. The exact origins of the wheat plant are yet to be known. However, it is believed to have evolved from wild grasses, probably somewhere in the near East (DAFF, 2009). Presently, wheat is grown on more land area than any other commercial crop globally. It is one of the top five grain commodities in the world, ranking third after maize (corn) and rice respectively in production tonnage (Bareja, 2015). According to DAFF (2010), the average annual production of wheat in South Africa ranges from 1.5 to 3 million tons. Wheat contains minerals, vitamins and fats (lipids), and is very nutritious when combined with some animal protein. A wheat-based diet is higher in fiber than a meat-based diet (Johnson *et al*., 1978).

There are diverse botanical classification systems used for distinguishing varieties of wheat. Botanically, this means that the nomenclature or taxonomy of varieties of wheat differ depending on the information source used. However, the most cultivated varieties of wheat can be grouped into three broad species, namely diploid, tetraploid and hexaploid. The hexaploid species consist of the common wheat or bread wheat (*Triticum aestivum*) and spelt (*Triticum spelta*). Bread wheat is the most widely cultivated variety in the world. Although spelt is sometimes considered a subspecies of the closely-related common wheat, it is cultivated in limited quantities. In the tetraploid species are durum (*Triticum durum*), emmer (*Triticum dicoccon*) and khorasan (*Triticum turgidum sspturanicum*). Durum is the second most widely cultivated and used type of wheat. Both emmer and khorasan are ancient grain types that are rarely cultivated today. Lastly, the diploid species consist of Einkorn (*Triticum monococcum*), with wild and cultivated variants. Einkorn was domesticated at the same time as emmer wheat, but never reached the same importance (Cooper, 2015).

Moreover, within species, wheat can be further classified according to several criteria. In some instances, wheat is classified in terms of the season of growth. For example, in South Africa there is winter wheat and spring wheat. In other instances, wheat is classified based on the protein content. Typically, the content of bread wheat protein goes from 10 % in some soft wheat with high starch contents to 15 % in hard wheat. For this reason, the quality of the wheat's protein gluten is an essential factor. The gluten protein can determine the suitability of wheat for a particular meal. For example, the strong and elastic nature of the gluten in bread wheat allows dough to trap carbon dioxide during leavening, but elastic gluten interferes with the rolling of pasta into thin sheets.

Finally, wheat can also be classified based on the color of the grain, i.e. red, yellow or white. The phenolic compounds existing in the bran layer confer to many wheat varieties reddish-brown colours which are also transformed to pigments by browning enzymes. In addition to having a lesser content of phenolics and browning enzymes, white wheat is generally less mordant in taste than red one. The yellowish colour of durum wheat (source of semolina flour) is due to lutein (a carotenoid pigment), which can be corroded to a colorless form by enzymes present in the grain (Cooper, 2015).

Wheat, as previously indicated, is an important food source for all nations across all continents of the world. In South Africa, like other places, wheat is mainly used for human consumption and the remainder is used as animal feed (Makgoba, 2013). As a nutritional source for humans, the grain can be eaten either whole or in processed form. For animals, the bran from flour milling is an important livestock provender, whilst the germ is a valuable addition to provender concentrate. feed on the grain either coarsely or wholly ground. Some wheat is cut for hay. Before the stems elongate, wheat can be turned into pasture (DAFF, 2009)

Aside from its consumption uses, wheat also serves some commercial functions, particularly for the bread-making industry and breweries. Finely ground wheat grain is used as flour, and subsequently forms the base ingredient in most breads, pastries and pastas. Some wheat grain is also used in the making of alcoholic beverages such as beer. The alcohol from the industry can be made into explosives and synthetic rubber (DAFF, 2009). Finally, the straw from wheat post-harvest can be made into mats, carpets, baskets, packing material, and cattle bedding (DAFF, 2009).

2.2.1 Agronomic considerations

In grain crop production, it is important to understand key contributing factors affecting crop yield or growth potential. In wheat production, considerations must be given to criteria such as cultivars, soil, climatic and environmental conditions and nutrient requirements in order to ensure maximum grain yield. Firstly, selecting the right cultivated variety or cultivar to plant is one of the most important production decisions in wheat production (DAFF, 2010) When choosing the correct wheat cultivar to produce, the specific production area (i.e. the region or sub-region), the yield potential of the seed, the adaptability and disease profile of the seed, as well as other agronomic characteristics are all important considerations, as they can influence the production risk management and grain yield. In South Africa, there are three categories of commercial cultivars available, depending on the production area to be used. There are cultivars for dryland production for both northern and southern production areas, as well as irrigation cultivars (Makgoba, 2013). For example, in this experiment, Tankwa cultivar was used as it is one of the preferred bread wheat for the southern production areas on the Miller's preference list (DAFF, 2010).

The growing period of cultivars is also a key determinant of land suitability. The development of the wheat plant is a complex process as many of the life cycle stages overlap. Thus, it is possible to find one part of the plant developing, while another part may be dying. According to the development stage schemes developed by Feekes (Large, 1954) and Zadoks *et al*. (1974) (Table 2.1), the growth cycle entails the general developmental stages of the plant from germination to maturity, through emergence, the production of leaves, tillering, shoot elongation, flowering and the stages of grain ripening (Zadoks *et al*., 1974). However, different cultivars and shoots vary in the timing and duration of these developmental stages due to genotypic differences and differing responses to environmental conditions (McMaster, 2009; Simmons *et al*., 2017).

Table 2.1: Wheat plant growth description of the development stages

Stage of Development	Feekes	Zadoks	Description
Germination	No stage	01-07	
Emergence	No stage	09	First true leaf emerges through the coleoptile and tip is visible above the soil surface
Tillering	01-02	20-29	First tiller is visible
Internode elongation	06-07	31-36	First node is visible
Flag leaf or booting	08-10	39-49	Flag leaf growth is considered complete when the ligule is visible and new leaf comes out
Heading	10.1-10.5	50-58	First spikelet is visible
Anthesis	10.5.1-10.5.4	61-69	First anther (yellow) is visible on inflorescence
Physiological maturity	11.1-11.4	77-99	Once all components of the spike, internode tissue, and leaves have lost green color.

Adapted from Zadocks *et al*. (1974)

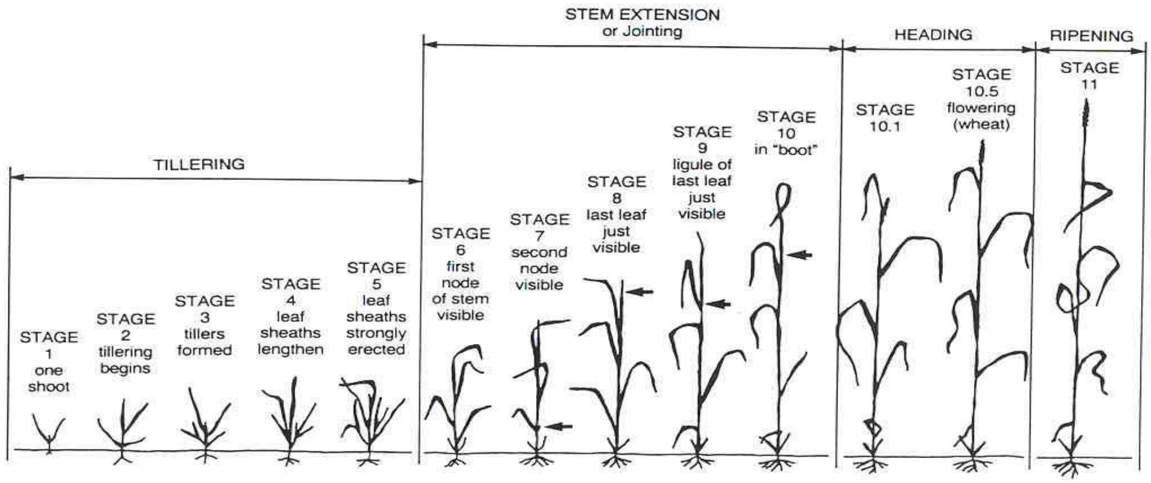


Figure 2.1: Life cycle of the wheat plant (Adapted from Stapper, 2007)

Secondly, the growth and development of wheat cultivars are also determined by soil, water supply, temperature and other environmental factors. The land characteristic or quality is a critical requirement for wheat growth. Generally, wheat fairs best in a well-drained fertile loamy to sandy loam soil with pH levels of 6 to 7.5. Acidic soils — which are associated with high Al^{3+} content — are harmful to wheat growth, especially during the early development stages, because they deplete other soil nutrients (DAFF, 2009). Moreover, highly saline soils, except for a few cases, have unfavorable effects on wheat growth and development.

Similarly, rainfall is an important climatic factor for wheat growth and development. The availability, or scarcity, of water supply can determine the difference between a high or low crop yield. According to the Department of Agriculture Forestry and Fishery (2009), on average, 600mm of water is required for wheat production annually in South Africa. However, the water requirements of wheat differ depending on other climatic conditions such as temperature, wind and humidity. Therefore, wheat grown in dry areas needs more water than that grown in humid/moist or cooler climates. Thus, in dry areas, moisture conservation techniques such as stubble mulching become necessary. Essentially, it is important to control the soil moisture levels in wheat fields. This is particularly important in South Africa during winter wheat production as most of the country gets summer rainfall. That is, lowering moisture application under irrigation during flowering, increasing during pod filling and stopping completely during ripening (DAFF, 2009). Another important climatic factor influencing the development of wheat is temperature. Generally, relatively warmer temperatures (between 22° and 34°C) are optimal for spring/ summer wheat growth and development, whilst winter wheat prefers cooler temperatures (between 5° and 25°C). Soil temperatures lower than 5°C are unfavorable for wheat production, particularly during seed germination.

Thirdly, and the last of the principal agronomic considerations for wheat growth relates to crop nutrition. Wheat, like other crops, needs a combination of essential macro and micro nutrients for development and growth. In terms of the macronutrients required by the plant, attention is limited to N, P and K; the three major nutrients or essential elements without which the crop cannot finish the cycle of its life. In instances where these macronutrients are deficient or unavailable in the soil naturally, they can be supplied to the plant in the form of fertilizers or manures supplied to maintain the fertility of the soil and to improve crop yield. Nitrogen fertilizers are typically applied through gently broadcasting directly on to the soil before or preferably during planting and lightly to avoid any direct contact with seed. Phosphorus deficiency is most often observed on acid soils, calcareous soils, and peat and muck soils. However, wheat and other cereal crops generally require less phosphorus as compared to other crop. Typically, potassium deficiency mostly occurs on acid sandy soils and on soils that have been heavily cropped. South African soils moderately contain potassium, which makes its deficiency rare in wheat production areas.

Aside from the NPK, there are important trace elements or micronutrients (zinc, manganese, iron, boron, chlorine, copper, molybdenum, and nickel) needed for the optimum growth and development of plants, including wheat. When one misses, symptoms are shown on the leaves. Early correction of deficiencies is required during plant growth to avoid possible yield losses. Under conditions where yield is limited by micronutrients, the numbers in table 2.2 will help to detect nutrient deficiency syndrome. Thus, availability of various macro and micronutrients in the soil makes crops grow and support the completion of their life cycle.

Table 2.2: Values of plant analysis of wheat at flag-leaf stage

Elements	Low(deficient)	Marginal	High(sufficient)
Nitrogen (%)	< 3,4	3,7–4,2	> 4,2
Phosphorus (%)	< 0,2	0,2–0,5	> 0,5
Potassium (%)	< 1,3	1,5	> 1,6
Sulfur (%)	< 0,15	0,15	> 0,4
Calcium (%)	< 0,1	0,2	> 0,2
Magnesium (%)	< 5,0	0,15	0,15–0,3
Copper (mg/kg)	< 20,0	5–10	10,0
Zinc (mg/kg)	< 30,0	20–70	> 70,0
Iron (mg/kg)	< 25,0	35–100	> 100,0
Molybdenum (mg/kg)	< 0,05	50–180	> 180,0
Boron (mg/kg)	< 6,0	0,05–0,1	> 0,1
		6–10	10,0

(Adapted from DAFF, 2010)

2.3 The role of zinc in plant nutrition

Among the micronutrients, Zn is one of the most vital in crop production and whose deficiencies have appeared the most common of all (Brown et al., 1993). Graham *et al*., (1992) suggested that Zn is needed in a certain critical amount for the comfort and growth of roots in the soil. According to Marschner (1995), its affinity with nitrogen, oxygen and especially sulfur (N-, O- and S-) helps to create complexes and ligands which are what its metabolic functions are based on. Zinc in this regard assumes both functional and structural roles in enzyme reactions. Even though several metallo-enzymes (beyond 70) contain Zn in a plant, it represents just a slight fraction of the total amount of Zn (Brown *et al*., 1993).

Zinc does not experience valency changes in plants. It's usually under insoluble forms associated with the cell walls, low molecular weight complexes, free ions and storage metallo-proteins. The presence of organic ligands or complexes with phosphorus can render Zn inoperative within cells. The water soluble proportion (i.e. free ions and low molecular weight complexes) under which Zn finds itself is majorly species-dependent and it goes from 58 to 91%. Under that form, it is highly active physiologically and serves as a better indicator of plant Zn status rather than total Zn content. The lower the Zn molecular weight complexes, the more soluble they are (Brown *et al*., 1993).

Moreover, the enzymes whose activities are zinc-based have Zn mostly bound through imidazole and cysteine. Srivastava and Gupta (1996) revealed the significance of Zn in many important enzyme systems, including but not limited to the transport of carbon dioxide in photosynthesis (carbonic anhydrase), protein synthesis (RNA polymerase), and starch formation (ribulose bi-phosphate carboxylase).

2.3.1 Physiological functions of zinc

Zinc, like other mineral micronutrients performs vital metabolic and cellular functions in plants. It has been found to play a role in plant metabolism by influencing the activities of plant enzymes involved in carbohydrate metabolism, protein synthesis, maintenance of cellular membranes, and the regulation of auxin (Brown *et al*.,1993).

Firstly, Zn plays a role in carbohydrate metabolism through its influence on photosynthesis and sugar transformations. It does so by activating and influencing the activities of enzymes like carbonic anhydrase (Hafeez *et al*., 2013). Based on the level of deficiency and the plant species, Zn shortage is able to drop photosynthesis of plant by 50 to 70%. The drop can be partly attributed to a reduction in carbonic anhydrase (CA) enzyme activity as well as a serious reduction in the abnormal structure of chloroplasts and the chlorophyll content. In dicotyledonous plants, the CA represents a bigger particle and holds more Zn as compared to monocotyledons such as cereal crops. Zinc is a component of CA. Thus, Zn stress in plants can cause a sharp decline in CA activity, which subsequently affects the carbon dioxide assimilation pathway. It is generally unclear whether CA is involved in photosynthesis in plants with C_3 metabolism such as wheat (C_3 plants) since CA activity has no direct relationship with photosynthetic absorption of CO_2 (Graham *et al*., 1992). Therefore, Zn content and CA activity are narrowly associated, even though it merely affects dry matter production and photosynthesis for minus activity. There is no CA of anhydrase when Zn shortage is severe. In contrast, CA may play a more key role in plants with C_4 metabolism like maize and sorghum. For this reason, Zn deficiency may have a more dramatic effect on the rate of photosynthesis in C_4 plants compared to C_3 plants, making C_4 plants highly sensitive to Zn deficiency (Marschner, 1995).

Moreover, Zn is important in the metabolism of starch since it influences the enzymes involved in the formation of sucrose (e.g. aldolase) and the activity of the enzyme starch synthetase. Thus, Zn deficiency adversely affects the starch content and the number of starch grains in these crops. Brown *et al*., (1993) suggests that Zn shortage harms the leaf to root transfer of sucrose. In the same way, some studies have shown the phloem loading of sucrose being restored through the correction of Zn deficiency. This can be attributed to the action of Zn in maintaining the integrity of bio-membranes (Brown et al., 1993).

Secondly, Zn influences protein synthesis by activating plant enzymes involved in this process (Hafeez *et al*., 2013). In Zn deficient plants, protein synthesis is affected through a decrease and the deformation of ribosomes and a decrease in RNA. For example, the quantity of free ribosomes and RNA has been shown to drastically drop in the meristem of rice seedlings due to Zn shortage (Brown *et al*.,1993). In addition to its necessity in the activity of the enzyme RNA polymerase, Zn also fights against the enzyme ribonuclease to preserve the ribosomal RNA. A typical indicator of Zn deficiency in higher plants is high levels of ribonuclease activity leading to a drastic reduction in RNA as the first consequence of Zn shortage. However, high Zn concentrations are necessary by meristematic tissue where cell division as well as synthesis of nucleic acid and protein is actively taking place due to the importance of Zn in protein synthesis (Brown *et al*.,1993). Therefore, a drop in RNA can also take place before the increase in ribonuclease activity. The primary influence of Zn on protein breakdown is found in its participation in functions of genetic material and stability.

Thirdly, Zn ensures the maintenance of the structure and function of bio-membranes in plants, albeit indirectly (Welch *et al*., 1982). Another way Zn sustains the firmness of cellular membranes is through the maintenance of ion transport systems' structural and the orientation of macromolecules. This same role is sometimes assumed through the interaction with phospholipids and sulphydryl groups of membrane proteins. Many interpret the loss of membrane integrity to be the earliest biochemical change as a result of the Zn deficit. Zinc also regulates the detoxification and regeneration of free oxygen radicals (O^2) that are likely to impair sulphydryl groups and membrane lipids. It does that by exercising an inhibitory action on membrane damage catalyzed by free oxygen radicals. Together with calcium, phosphorus, boron, and manganese, the major role of Zn in membranes is to protect membrane lipids and proteins from peroxidation caused by the free oxygen radicals (Graham *et al*.,1992).

Lastly, Zn is involved in the regulation of auxin synthesis and pollen formation. The most common, distinct and visible symptoms of Zn deficiency are stunted growth and ‘little leaf’ as results of disturbances in the metabolism of auxins, which are growth regulating compounds (Brown *et al*.,1993).

Moreover, flowering and seed production are terribly affected by Zn shortage in plants.
The increased formation of abscisic acid is probably responsible for the decrease in seed production in zinc-deficient plants by causing disruption of the development and physiology of pollen grains and anthers, irreparable damages of leaves and flower buds. It has been found that deficiency of Zn in the wheat crop is the cause of abnormal pollen grain small anthers (Alloway, 2008). Sharma *et al*., (1990) in study on maize, discovered the delay in the growth of pollen grains and tassels, anthers.

2.3.2 Zinc deficiency and its correction in wheat

Zinc deficiency, or the lack of plant available Zn poses great challenges in world food production. Research has linked Zn deficiency, among other things, to the type of cultivable soils. A wide range of different types of soils is recognized to affect and render crops zinc-deficients; zinc deficiency is generally recorded in calcareous, sandy, peat and muck soils; strongly weathered deep tropical soils; salt-affected soils; and gleysols (Hafeez *et al*., 2013). Plants respond greatly differently in their sensitivity/tolerance to Zn deficiency. Even on the same soil, some crops may suffer from Zn deficiency, while others are not affected. For instance, research has revealed a wide range of efficiency of Zn utilization displayed by wheat varieties.

Nonetheless, Zn deficiency in plants generally causes stunted growth and reduces grain yield and nutritional quality (Hafeez *et al*., 2013). In wheat, the visual symptoms of this deficiency are easily observable. At the early stage, the symptoms are shown on new leaves by reason of Zn being motionless. At mild deficiency in wheat, light green to white chlorotic and necrotic strips appear on either side of the leaf. When the shortage is advanced, the lower leaves go completely chlorotic and short, but of normal width (Hafeez *et al*., 2013). Sometimes, the leaves look oil-soaked, and the leaves often collapse in the middle as the necrosis continues. The mid-ribs and margins of the leaves remain green, although the edges may turn to red or brown in some cases. In a small shape, the leaves curve towards the top and generate interveinal chlorosis. Necrotic spots appear which later join each other to form brown necrotic and brittle patches on the upper leaf surface (Hafeez *et al*., 2013). The necrosis eventually wilts, bend and collapse in fairly old leaves on which it is more perceptible.

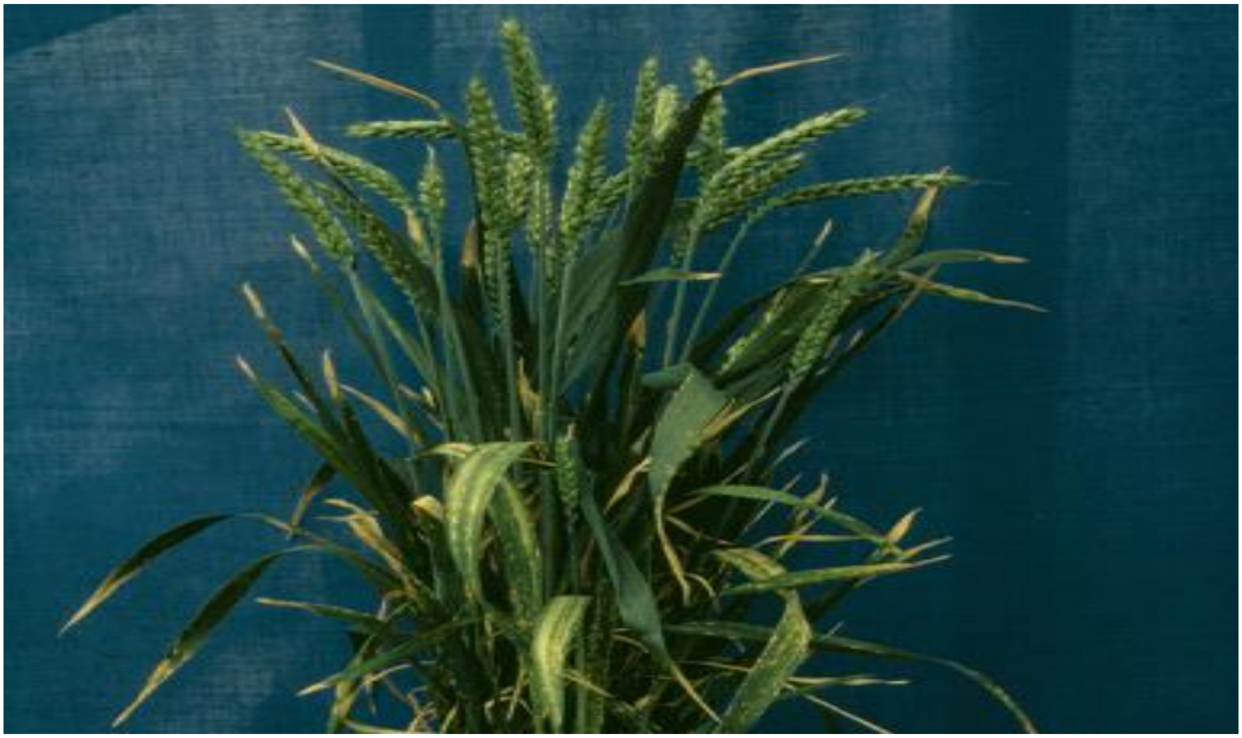


Figure 2.2: Chlorosis and necrotic spots on the leaves of zinc deficient wheat (Adapted Alloway, 2008)

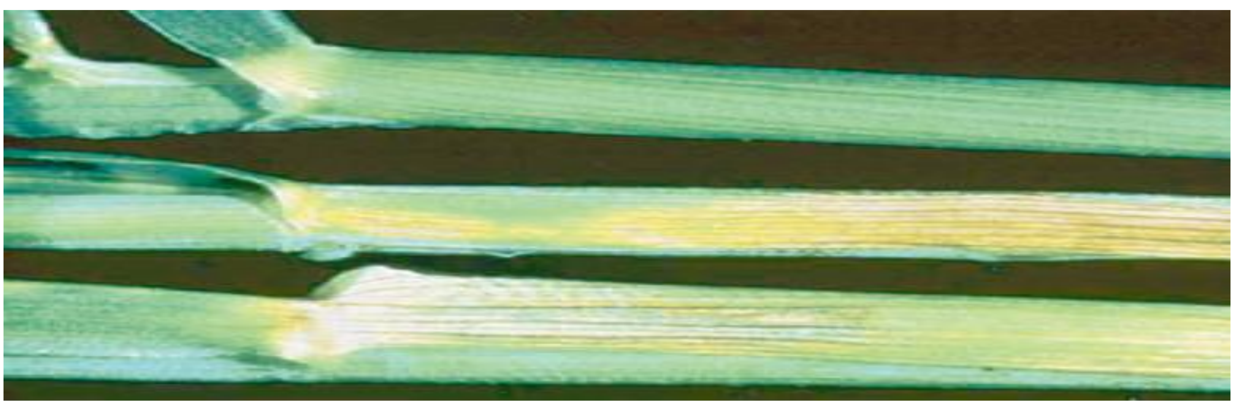


Figure 2.3: Interveinal chlorosis and necrotic patches on leaves of wheat (Adapted from Alloway, 2008**)**

Zinc deficiency can easily be corrected through the application of Zn fertilizers to the soil. A Zn compound can be broadcast and sprayed on the seedbed and/or incorporated into the topsoil. Zinc sulphate is the most commonly used fertilizer compound worldwide and is available in both crystalline monohydrate and heptahydrate forms. Other Zn compounds include zinc oxide (ZnO), zinc carbonate ($ZnCO_3$), zinc nitrate (Zn $(NO_3)_2$) and zinc chloride ($ZnCl_2$). The more soluble the source of Zn, such as zinc sulphate, the more rapidly it becomes plant-available after mixing into the soil.

Due to its significant residual effect, soil applications of Zn fertilizers can last as long as five to ten or more years. The efficacy of Zn fertilizers through soil application, tends to improve in the years following application when the Zn has been more thoroughly mixed into the topsoil through cultivation (Alloway, 2008).

2.3.3 Factors affecting zinc availability

Zinc is absorbed by plant roots primarily as Zn^{2+} from the soil solution or bound to organic acids with a strong affinity for Zn. It accumulates in root tissues and is translocated to the shoots when needed. There are various factors that affect Zn availability in soils, and subsequently are major causes of Zn deficiency in plants. These include soil pH, soils low in organic matter and cool soil temperatures among others (Alloway, 2008).

2.3.3.1 Soil pH

The soil environment, as previously indicated, affects wheat growth and development. However, the physical properties of soil alone are not necessarily vital for wheat growth. Rather, it is the soil’s capacity to supply nutrients (chemical properties) that has the biggest impact on wheat performance. Therefore, an important environmental condition and chemical characteristic of the soil which determines soil nutrient availability and subsequently affects the quality of plant growth is pH (Kluepfel *et al*., 2012). Soil pH is a measure of the relative acidity or alkalinity of the soil. The pH levels range from 0 to 14 with 7 as the neutral point. Thus, pH levels less than 7 indicate acidity, whereas pH levels greater than 7 indicate alkalinity. With regards to soil classifications, generally, soils are considered acidic below a pH of 5, and very acidic below 4. Conversely, soils are alkaline above a pH of 7.5 and very alkaline above 8 (Jensen, 2010).

The optimum soil pH for plant growth varies for crops. However, research has determined that the “ideal” soil pH level for most crops is close to neutral, within the range of 6.5 and 7.5. This range is considered desirable and very compatible to plant growth because it is within this range that most plant nutrients are optimally available (Jensen, 2010). Nonetheless, some crops have been found to grow well outside this optimum range; thus, in slightly acidic or alkaline soils. In general, however, the nutrition, growth and yields of most crops decrease where pH is too low and increase as pH rises to an optimum level (Table 1) (Smith & Doran, 1996).

Considering that soil pH directly affects plant nutrient availability (Kluepfel *et al*., 2012 and Hafeez et al., 2013), it can be problematic if pH levels are not carefully controlled. Nutrient deficiencies, low microbial activity and crop yield, and deterioration of environmental conditions have all been associated with poor soil pH management (Smith & Doran, 1996). Although the availability of various macro and micronutrients varies with the pH levels and from crop to crop, evidence suggests that pH has an influence on availability of plant nutrients.

For instance, soil pH may modify the uptake of Zn by influencing the activities of soil micro-organisms and changing the ability of the plant to absorb or transport to the tops, the stability of soluble and insoluble organic Zn complexes, the solubility of antagonistic ions, any rhizosphere effects among others. Thus, the solubility of Zn in soils is highly pH dependent, with solubility decreasing with increasing pH. As a result, Zn deficiencies are very common in calcareous soils, i.e. very alkaline (pH greater than 7.4) soils (Smith & Doran, 1996).

However, most nutrient deficiencies are easily prevented or corrected by keeping the soil at the optimum pH level, i.e. controlling the soil pH (Kluepfel *et al*., 2012). Thus, the right pH level not only affects the soil's physical, chemical and biological properties and processes, but the plant growth and yield potential.

2.3.3.2 Soil organic matter

The mobility and solubility of Zn are enhanced due to the organic matter in the soil, which sponsors the readiness of Zn by complexing the substances that fix zinc. Therefore, soils low in organic matter are unable to retain zinc and as a result tend to be more prone to deficiencies (Hafeez *et al*., 2013). The contribution of organic matter to micronutrients, particularly zinc, binding is highest when the predominant clay mineral is kaolinite and lowest when it is montmorillonite.

2.3.3.3 Soil temperatures

At the early growing season, zinc shortage is more pronounced due to low temperatures. High temperatures promote the proliferation of roots and the availability of zinc by enhancing organic matter's decomposition by the microbial activity (DAFF, 2010).

2.4 Zinc interaction with other plant nutrients

In the soil, and within the plant, micronutrients can interact with one another and some macronutrients. They may combine to cause an added effect in relation to the plant. These interactions may enhance or reduce plant growth. There have been many studies detailing how the interactions of zinc with other nutrients affect its availability from soils and plants' responses regarding zinc absorption, distribution or utilization. The subsequent discussion outlines the interactions of zinc with other important nutrients.

2.4.1 Zinc-Phosphorus interactions

The interaction between zinc and phosphorus, or zinc-phosphorus interactions, is an ongoing study as 'phosphorus-induced-zinc deficiency' increasingly becomes a major plant growth disorder. High soil phosphate levels are one of the most common causes of zinc deficiency in crops, although the actual mechanisms responsible for this interaction are still not completely understood (Hafeez *et al*., 2013). Some researchers attribute the cause of phosphorus-induced-zinc deficiency to phosphorus toxicity. In this respect, Marschner (1993) found that plant uptake of zinc generally decreased sharply with increased phosphorus supply (for example fertilizer phosphorus) in the soil content.

Huang et al. (2000) supported Marschner's (1993) findings but suggested enlightenment for the apparent relationship that exists between high concentrations of phosphorus and zinc deficiency in plants. Huang et al. (2000) and Marschner's (1993) found that the manifestation of the genes that encode the proteins that transport phosphorus is firmly controlled but dependent upon the phosphorus and zinc status of the plant. When zinc is lacking, it causes the cessation of phosphorus transporter proteins and engenders the buildup of very high concentrations of phosphorus in the plant.

Conversely, Loneragan and Webb (1993) posit two different theories of zinc-phosphorus interactions possibly responsible for phosphorus-induced-zinc deficiency, both of which focus on the dilution impact on zinc concentration in plant tops owing to growth responses to phosphorus. In the first instance, an increase in phosphorus applications (phosphate salts) decreases the zinc concentrations in plant shoots. This relationship commonly occurs when the amounts of both zinc and phosphorus in the soil are small, but the addition of phosphatic fertilizer sponsors plant growth to the point of causing the dilution of zinc concentrations in plant tissues and later escalates to zinc deficiency. The imbalanced concentration levels of phosphorus (high) and zinc (low) is the cause of "phosphorus-enhanced zinc requirement syndrome". In wheat for example, the major problem is when phosphorus is prevented from circulating because of its accumulation in old leaves (Loneragan & Webb, 1993).

In the second instance, zinc deficiency is induced by phosphorus (for example an increase in phosphorus application or phosphate salts) without diluting zinc concentrations in plant shoots. In such a case, zinc deficiency symptoms appear without Zn dropping. The increasing phosphorus concentrations has probably risen the internal Zn requirement of the plant (a "phosphorus-enhanced zinc requirement") (Loneragan & Webb, 1993). There are two likely explanations for this; either the phosphorus depresses zinc absorption (uptake) by roots or interferes with (slows down) the translocation of zinc from the roots to the shoots.

Still, other ways through which phosphorus can affect zinc absorption may include (i) arbuscular mycorrhizae; (ii) cations added with phosphate salts; (iii) H^+ ions generated by phosphate salts. The role of arbuscular mycorrhizae (AM) in the uptake of phosphorus by plants is well known. AM effectively increases the area of the root absorbing surface in the soil and this affects the absorption of all elements, not just phosphorus. Thus, relatively high concentrations of phosphorus suppress the development of mycorrhizae and subsequently reduce the uptake of other ions such as Zn^{2+}. Cations such as Ca, Mg and K, as well as H^+ ions generated by phosphate salts can also inhibit zinc absorption from solution (Alloway, 2008). Chaudhry and Loneragan (1972) reported that alkaline earth cations inhibited Zn^{2+} absorption by plants noncompetitively.

2.4.2 Zinc-Nitrogen interactions

Nitrogen appears to affect the zinc status of crops by both promoting plant growth and by changing the pH of the root environment. Nitrogen is known to be the principal factor limiting growth and yield. It is therefore expected and reported that the interactions between nitrogen and zinc fertilizers have enhanced yield. For example, crops often respond to zinc and nitrogen together but not to zinc alone. The application of nitrogen fertilizers in the absence of, or in soils low in zinc can lead to zinc deficiency by affecting zinc absorption through changing the soil pH. Nitrogen fertilizers such as ammonium sulphate ($(NH4)_2SO_4$) have an acidifying effect on soils and so lead to an increase in the availability of zinc to crops in soils of relatively high pH status. Conversely, nitro-chalk (Ca $(NO_3)_2$) can increase the soil pH and reduce zinc availability (Hafeez *et al*., 2013).

2.4.3 Interactions of zinc with other macronutrients

Studies have found the absorption of zinc by the roots to be hindered by macronutrients such as calcium, magnesium, potassium and sodium in solution culture experiments (Hafeez et al., 2013). In soils, however, they most likely affect zinc through soil pH. For example, applications of gypsum ($CaSO_4$), which decrease the soil pH, increases the zinc content of plants. Yet, the equivalent amount of calcium applied as calcium carbonate ($CaCO_3$), increase the pH and decrease the zinc content of plants (Hafeez *et al*., 2013).

Potassium and magnesium have been shown to inhibit zinc absorption in solutions with low levels of calcium; although once the calcium concentration increases, the effects disappear. Ramon and Villemin (1989) reported that maize responded to zinc and potassium applications, with a significant response to zinc at all levels of potassium.

2.4.4 Interactions of zinc with other micronutrients

Zinc is known to interact with copper, iron, manganese and boron. Zinc interacts with copper in several ways, but it is often competitively antagonistic (Hafeez *et al*., 2013). They mutually inhibit the absorption of the other, indicating that both are absorbed through the same mechanism or carrier sites. Copper nutrition can also affect the redistribution of zinc within plants. When either element is less present in the soil, application of the other will exacerbate deficiency in the plant. In copper deficient plants, the senescence of the oldest leaves and the export from them of nitrogen, copper and zinc was delayed compared with plants with adequate copper.

Iron-zinc interactions are equally as complex as those between zinc and phosphorus. Increasing zinc supplies to plants has often produced conflicting observations. In some cases, higher zinc concentration increases iron status; while in others, it decreases iron and/or have no effect on it (Loneragan & Webb, 1993). Zinc concentrations and absorption in shoots greatly increases in situations where iron is deficient (Hafeez *et al*., 2013). In dicotyledons, the acidification of the rhizosphere resulting from iron deficiency is probably responsible for higher zinc absorption.

2.5 Conclusion

Overall, zinc is as important to humans as it is to plants. Because of its great contribution and participation in plant growth and reproduction, zinc deficiency can directly affect human health and development. Available literature has shown that zinc is an essential plant nutrient for all types of crops, including wheat. When it is deficient in plants, an application of zinc fertilizer is necessary for healthy crop growth and higher yields (Rengel et al., 1999; Frossard *et al*., 2000; Graham *et al*., 2001; Welch & Graham, 2004).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Research location

The present study was conducted at the Wellington campus of the Cape Peninsula University of Technology (CPUT) located at 33°37'53.39" S, 19°00'35.56" E. The average midday temperatures for Wellington range from 16.5°C in July to 28.8°C in February. The region is coldest during July when the mercury drops to 5.7°C on average during the night. (SA Explorer, 2017).

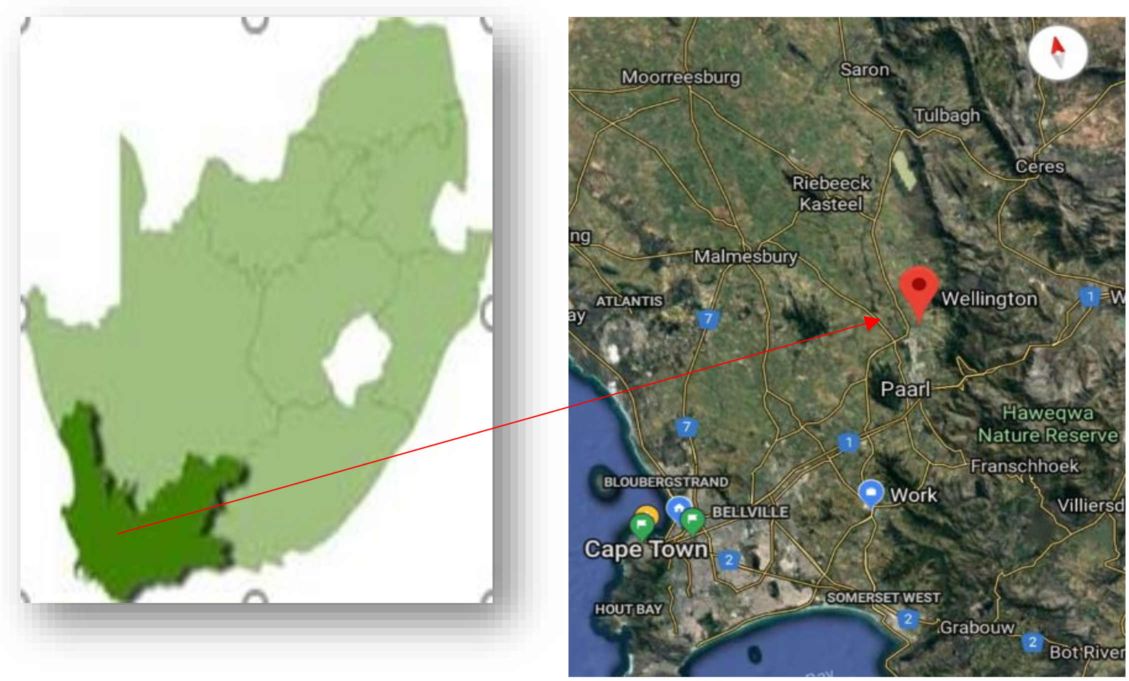


Figure 3.1: South Africa's map showing Wellington in Western Cape province

3.2 Materials

3.2.1 Wheat cultivar

TANKWA, a spring wheat variety that is popular among the Western Cape farmers was used for this trial. This wheat has been listed on the Miller's preference list of preferred bread wheat in the southern production areas since 2009/2010 (DAFF, 2009). The seeds were obtained from the Agricultural Research Council (ARC), Small Grain Institute in Stellenbosch. Before planting, seed viability was tested to ensure that all seeds have a high germination rate.

3.2.2 Chemical fertilizer

The following fertilizers were used: Urea at 46 % as source of nitrogen; Murate of potash containing 203g and phosphorus in 1000g; Potassium oxide at 50 % of potassium; Zinc Sulfate ($ZnSO_4$) at 33 % of zinc; and Lime.

3.3 Methods

3.3.1 Preparation of soil and lime material

The study was a pot experiment, conducted in pots of 10kg capacity each. The soil was collected on the 16th February 2016 to a depth of 15 cm from an arable field, at CPUT Wellington campus and that had been under fallow land for nearly 5 years. Due to the considerable clay content in the soil, a mixture of sand and soil was used in a ratio of 1:1. In addition, the mixture of sand and soil was filled in pots with four drainage holes at the bottom to ensure drainage to prevent accumulation of salts in the growing medium as well as water stress. The sand-soil-lime mixture was passed through a 2mm sieve. Calcitic lime was mixed and thoroughly combined with the sand-soil mixture to make up four application levels of 0, 0.5, 1, and 1.5 ton of lime /ha. The calculation of the quantity of lime or any fertilizer applied to a pot was worked out following the method described by Khan (2013). We first determined the mass of soil collected from an area of one hectare and at a depth of 15cm, by the formula: [mass = Volume (Area X depth) X bulk density].

[mass = 10,000m^2 X 0.15m X 1300 kg/m^3] = 1,950,000 kg of soil.

Consequently, 1kg of a certain fertilizer per hectare corresponds to 1kg of the fertilizer per 1,950,000 kg of soil, which also corresponds to 5.13mg of the fertilizer per 10kg of soil per pot; subsequently, 5.13 was multiplied by the required amount of each fertilizer and applied to each pot. The sand-soil-lime mixture was watered twice a week for one month to allow decomposition. Samples of the soil mixture were then sent to Bemlab (Gant's Sentrum, 16 Van Der Berg Cres, Strand, Cape Town, 7140, South Africa) for physicochemical analysis.

Table3.1: Application levels of lime used in the experiment and the consequent average soil pH recorded

Lime application rate (ton/ha)	0	0.5	1	1.5
Soil pH achieved	5.1	5.6	6.1	6.6

3.3.2 Characterization of soil-sand-lime mixtures

The soil was air dried, passed through a 2 mm sieve for determination of stone fraction (weight/weight basis) and analysed for pH (1.0 M KCl), P (Bray II), total extractable cations namely K, Ca, Mg and Na (extracted at pH = 7 with 0.2 M ammonium acetate), organic matter by means of the Walkley-Black method (The Non-affiliated Soil Analyses Work Committee, 1990) and trace elements namely Cu, Mn, Fe and Zn were extracted using 0.02M Disodium EDTA. Phosphorus (Olsen) was extracted using 0.5M Sodium bicarbonate solution using the method described by The Non-affiliated Soil Analyses Work Committee (1990). The extracted solutions were analysed with a Varian ICP-OES. Salinity was determined by measuring the resistance of saturated paste in an electrode cup according to the method described by The Non-affiliated Soil Analyses Work Committee (1990). Extractable acidity was extracted with 1M KCl and determined through titration with 0.05 M NaOH (Non-affiliated Soil Analyses Work Committee, 1990).

3.3.3 Experimental design and layout.

The wheat plants were grown at four different soil pH levels by adding lime at four different rates (pH_A: No lime, pH_B: 0.5t/ha, pH_C: 1t/ha and pH_D: 1.5t/ha). The plants were then subjected to five different levels of zinc (rate of application: Z_1: 3.5, Z_2: 4.5, Z_3: 5.5, Z_4: 6.5, Z_5: 7.5 mg Zn/kg soil), applied at 2 different periods (time of application: at planting (P); at flowering (F)).

The experimental design was a 3-factor factorial of soil pH, zinc levels and planting stage, in a completely randomized design with three replicates. However, an experimental unit was made up of one pot and for this reason the whole experiment was made up of 120 experimental units/pots (5 Zn levels x 2 times of application x 3 replicates x 4 pH levels).

Lime Application or pH levels in two wheat growth stages

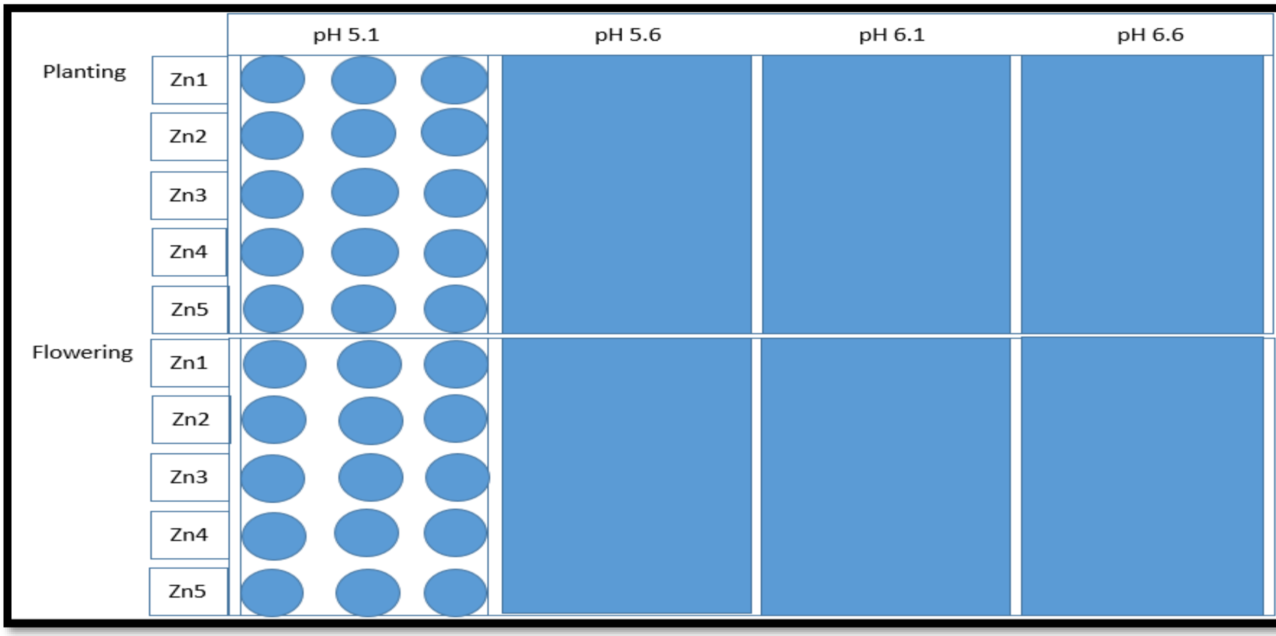


Figure 3.2: Experimental layout

3.3.4 Trial management

Wheat seedlings were raised in the nursery and strong and healthy seedlings were transplanted into potted soils treated with different concentration of lime on the 12th of May 2016. After transplanting, basal fertilizers were applied to every pot at the rate of 160 kg N ha^{-1} as urea, 30 kg P ha^{1} as super phosphate and 80 kg K ha^{-1} as a muriate of potash. Later, top dressing of 60 kg N ha^{-1} was supplied in equal two split doses during crop growth at 20 days after transplant and at the early flowering stage.

120 pots were divided into two 60 pots each with first Zn application at transplanting followed by second application at flowering stage. The application rate of fertilizer is according to the guidelines of the test crop per hectare as outlined by the National Department of Agriculture and Forestry and Fishery (DAFF, 2009). Adequate supply of moisture is required during the growing phase to ensure even growth and proper development. Pots were irrigated twice a week during the vegetative stage and three times per week during the reproductive stage to prevent water stress. Weeds were removed manually once every two weeks; where weed growth was severe, it was removal was immediate.

3.3.5 Data collection and grain analyses

The plants were harvested 163 weeks after planting. In the course of the experiment, two types of data related to yield components and grain nutritional quality were recorded. Regarding the yield components, the tillers per plant were counted on the 52^{th} day after planting (tillering). At harvest (maturity stage), plant height was measured from the soil surface up to the highest point of the longest leaf. The whole plant (heads, shoot and root) was collected, washed and dried in an oven at 80°C for 48 hours in paper bags to determine dry matter (g/plant). The grains were removed from the heads or spikes by hand to determine the grain yield per plant (g) and the harvest index was determined (dry biomass grains [g]/dry biomass whole plant [g] * 100). The grain nutritional quality, nutrients concentration, the protein contents and Fe/Zn ratio were also determined.

After sampling, the grains were dried over night at 70ºC in an oven. The dried leaves were then milled and ashed at 480ºC, shaken up in a 50:50 HCl (32%) solution for extraction through filter paper (Campbell & Plank, 1998; Miller, 1998). The cation (K, Ca, Mg and Na) and micro nutrient (B, Fe, Zn, Cu, Mn) content of the extract was measured with a Varian ICP-OES. Total N content of the ground grains was determined through total combustion in a Leco N-analyser. The protein contents were calculated by multiplying grain N concentration by the factor 5.7 (Zörb *et al*., 2010).

3.3.6 Statistical procedure

The experimental design was a completely randomized design with three replications. The treatment design was a factorial arrangement with three factors, pH with 4 Levels, zinc with 5 levels and two physiological stages (planting and flowering). An analysis of variance (ANOVA) was performed on the data using General Linear Models Procedure (PROC GLM) of SAS software (Version 9.2; SAS Institute Inc, Cary, USA). Shapiro-Wilk test was performed on the standardized residuals from the model to verify normality (Shapiro & Wilk, 1965). Fisher’s least significant difference was calculated at 5% level to compare treatment means (Ott & Longnecker, 2001). A probability level of 5% was considered significant for all significant tests. Pearson product moment correlations were performed using Correlation Procedure (PROC CORR) of SAS software (Version 9.2; SAS Institute Inc, Cary, USA). Principal component analysis was conducted for both data sets combined to investigate the relationship between the factors (pH and Zn) and variables, using XLSTAT (Version 2015.1.03.15485, Addinsoft, Paris).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Soil

4.1.1 Physio-chemical properties and nutrient status of initial and post-harvest soil

The results of the soil analysis of the initial soil revealed that it was a sandy soil and acidic (pH 5.1). The soil tests also revealed a Zn content of 2.2 mg/kg (Table 4.1). According to Alloway (2008), sandy soils with a low Zn content of 10 – 30 mg/kg are likely to cause Zn deficiency in crops. In addition, the Zn content of this soil is a little above the lowest value of the range of critical values of Zn for upland crops (which is 2 to 5mg/kg) reported by Fageria (2009).

Table 4.1: Chemical analysis of the soil (control) before planting

	(mg/kg)		(cmol/kg)			(mg/kg)					
pH(KCl)	P	K	Na	Ca	Mg	Cu	Zn	Mn	B	Fe	S
5.1	26	20	0.07	1.03	0.37	0.9	2.2	18.3	0.17	204	15.26

However, the result of the analysis of the post-harvest soil, showed the performance of the physio-chemical properties and nutrients present in soil as affected by different levels of lime and Zn application as well as stages of Zn application (Appendix 1). There were discrepancies in the soil pH and some nutrients for the different treatments. The majority of the nutrients increased in the post-harvest soil in comparison to the initial soil. The results of the soil analysis showed that lime affected more the availability of the nutrients in the post-harvest soil. The nutrients that experienced major variations are discussed below.

Table 4.2 shows the amount of Ca available in the post-harvest soil as affected by lime and Zn application. Zn application had no influence on the soil Ca after harvest in both times of Zn application, while the lime significantly increased Ca concentration in the post-harvest soil for both times of application of the mineral ($p < 0.05$). However, the amount of Ca present in the soil when Zn was applied at planting was 200 to 300mg/kg greater than when Zn was applied at flowering.

Table 4.2 Ca concentration in the post-harvest soil for both application times (planting and flowering)

Stage	Zn levels (mg/kg)	pH levels 5.1	5.6	6.1	6.6	Average
Planting	Zn1	0.82	0.76	1.06	1.38	1.005
	Zn2	0.95	0.75	1.16	1.04	0.975
	Zn3	0.99	0.88	1.05	1.08	1
	Zn4	0.77	0.94	1.08	1.1	0.9725
	Zn5	0.9	0.86	0.92	1.16	0.96
	Average	0.886	0.838	1.054	1.152	
Flowering	Zn1	0.64	0.67	1.1	0.63	0.95
	Zn2	0.61	0.65	0.79	1.7	0.975
	Zn3	0.64	0.66	0.71	0.72	1.025
	Zn4	0.5	0.56	0.94	0.79	1.775
	Zn5	0.59	0.55	0.77	0.85	1.45
	Average	0.596	0.618	0.862	0.938	

Significance between means was tested at $p < 0.05$

Table 4.3 shows the amount of K available in the post-harvest soil as affected by lime and Zn application. Zn application had no influence on the soil K after harvest at both stages of Zn time of application. Increasing lime significantly increased K concentration in the post-harvest soil when Zn was applied at planting and not when Zn was applied at flowering. The amount of K present in the soil when Zn was applied at planting was also greater than when Zn was applied at flowering.

Table 4.3: K concentration in the post-harvest soil for both application times (planting and flowering)

Stage	Zn levels (mg/kg)	pH levels 5.1	5.6	6.1	6.6	Average
Planting	Zn1	10	9	10	13	10.5
	Zn2	9	9	13	12	10.75
	Zn3	11	10	13	10	11
	Zn4	10	9	11	10	10
	Zn5	12	11	10	15	12
	Average	10.4	9.6	11.4	12	
Flowering	Zn1	11	11	12	10	11
	Zn2	10	12	9	9	10
	Zn3	8	11	13	12	11
	Zn4	8	9	11	10	9.5
	Zn5	8	9	10	9	9
	Average	9	10.4	11	10	

Significance between means was tested at $p < 0.05$

Table 4.4 shows the amount of Mg available in the post-harvest soil as affected by lime and Zn application. Zn application had no influence on soil Mg after harvest at both stages of Zn times of application. Increasing lime, especially at high rates corresponding to pH 6.1 and 6.6 significantly increased Mg concentration in the post-harvest soil when Zn was applied at planting and not when Zn was applied at flowering. The amount of Mg present in the soil when Zn was applied at planting was also greater than when Zn was applied at flowering. The addition of the lime resulted in a significant increase of K, Ca and Mg. These results are similar to the results of Sultana *et al.* (2009), who recorded an increase in P, Ca and Mg due to the increasing rates of lime application. These results are also similar to the findings of Makgoba (2013), who found an increase in exchangeable basic cations, especially Ca and K, as a result of the addition of biochar which is a lime-based material. Makgoba (2013) stated that the increase of K, Ca and Mg are important and profitable for the plant, since their deficiency can limit crop growth and reduce yield.

Table 4.4: Mg concentration in the post-harvest soil for both application times (planting and flowering)

Stage	Zn levels	pH levels				Average
	(mg/kg)	5.1	5.6	6.1	6.6	
Planting	Zn1	0.31	0.29	0.33	0.37	0.325
	Zn2	0.28	0.29	0.34	0.31	0.305
	Zn3	0.31	0.29	0.32	0.35	0.3175
	Zn4	0.28	0.29	0.33	0.31	0.3025
	Zn5	0.31	0.32	0.31	0.33	0.3175
	Average	0.298	0.296	0.326	0.334	
Flowering	Zn1	0.26	0.29	0.31	0.25	0.2775
	Zn2	0.27	0.27	0.30	0.26	0.275
	Zn3	0.28	0.26	0.29	0.30	0.2825
	Zn4	0.24	0.23	0.29	0.28	0.26
	Zn5	0.26	0.24	0.28	0.30	0.27
	Average	0.262	0.258	0.294	0.278	

Significance between means was tested at $p < 0.05$

4.1.2 pH in the post-harvest soil

The liming material used was calcite ($CaCO_3$), which on dissolution, releases a large amount of Ca and Mg (Sultana *et al*., 2009). Therefore, the available Ca and Mg increased as lime application increased, even in the post-harvest soil. Figure 4.1 presents the soil pH in the post-harvest soils, which showed a similar trend of the availability of Ca and Mg in those soils.

The increase in application of lime led to a significant soil pH increase at the start of the experiment (Table 3.1). Yet, in the post-harvest soils, the soil analysis showed a decrease in soil pH for different treatments compared to the soils at the start of the experiment (Figure 4.1). This is probably due to the application of acidifying fertilizers such as elemental Sulphur (S), urea or ammonium (NH^{+4}) salts and the nutrient uptake by crops and root exudates. These reasons are listed as the causes of acidification by Goulding (2016). In the current experiment nitrogen was supplied as urea in sufficient amounts aiming at high grain yield and zinc fertilizer was supplied as zinc sulphate. Portmann (2012) found a reduction of pH in the high nitrogen treatments compared to the low nitrogen treatments and a reduction of about 0.2 units in the zinc treatments compared to the control soil.

However, the slight increase of pH in the control soil (pH5.1) are conceivably a result of the reduction reactions occurring over time (Sika, 2012). The results show a drastic reduction of soil pH when Zn is applied at flowering than at planting.

Given these points, the addition of lime to the soil at four different rates (0t/ha, 0.5t/ha, 1t/ha and 1.5t/ha) was shown to lead to a significant increase in soil pH. This increase in pH was mainly attributed to Ca and Mg, which therefore explains their significant increase in the soil as lime rate increased. These findings are confirmed by Kamaruzzaman, (2013) who reported that liming increases the availability of P, Ca and Mg. Nonetheless, the soil analysis carried out at the end of the experiment shows a reduction in pH in all the treatments. The interactions resulting from the effects of N application help to promote plant growth and to a lesser extent, change the pH of the root environment (Hafeez *et al.,* 2013). Portmann (2012) explained that the addition of mineral nitrogen fertilizer reduces the pH in the soil solution which results in a higher hydrogen ion activity leading to increased competition for uptake sites on the negatively charged soil minerals and organic matter particles between the positively charged hydrogen ions and the positively charged metals.

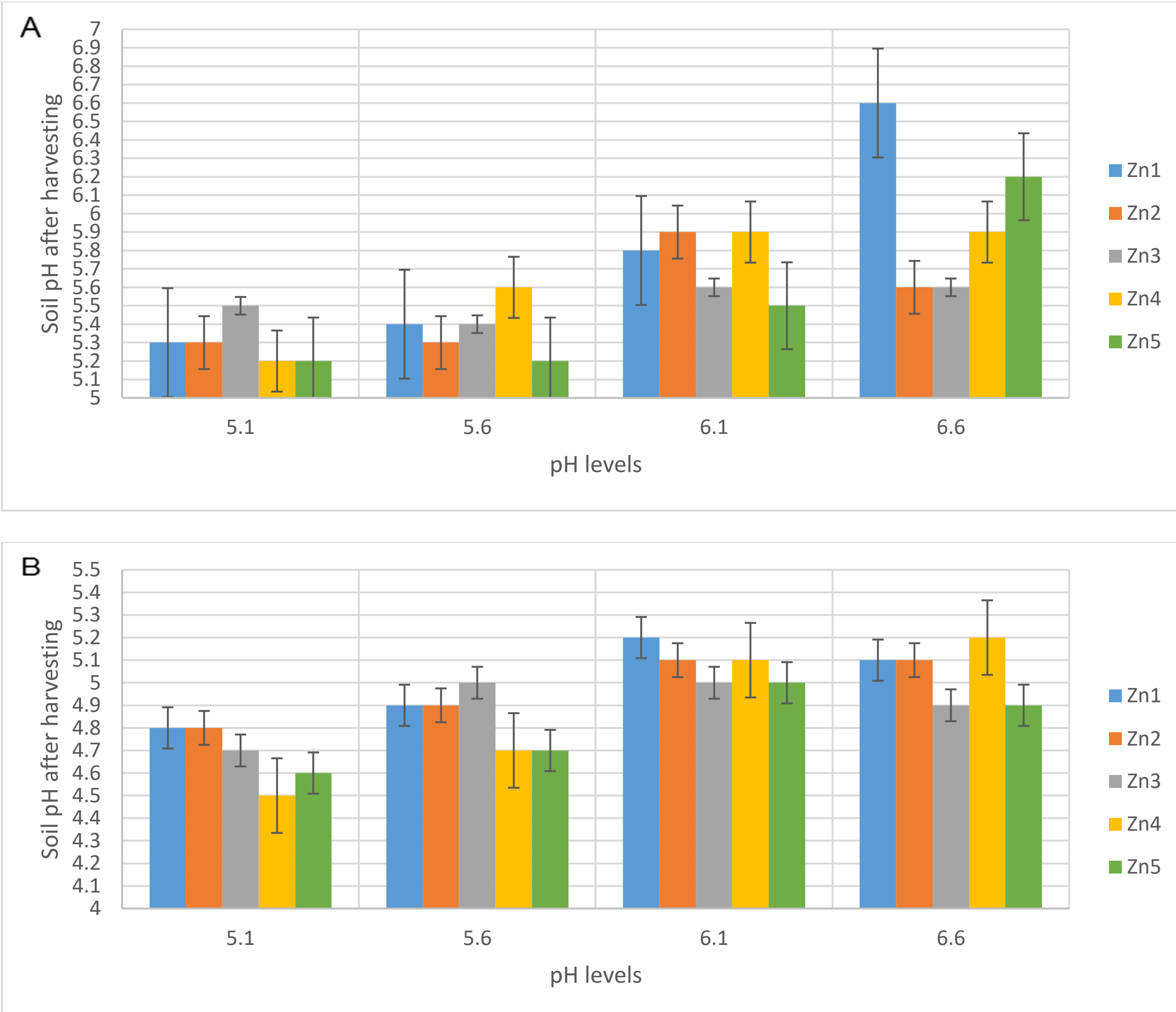


Figure 4.1: Soil pH of post-harvest soil as affected by lime and Zn applications (A) at planting; (B) at flowering. The overlap between two error bars shows there are no significant differences between the two treatments while no overlap shows significant differences.

4.1.3 Zinc in the post-harvest soil

Table 4.5 represents the amount of Zn available in the post-harvest soil as affected by lime and Zn application. There was no significant effect of Zn application at any stage on the amount of Zn present in the post-harvest soil. Table 4.5 shows that when Zn is applied at planting at the rates 3.5, 4.5 and 5.5 mg/ kg of soil (Zn1, Zn2 and Zn3 respectively), the amount of Zn in the post-harvest soil significantly increases as the pH increases from 5.6 to 6.6. These results imply that, when Zn is applied at planting at a rate lower or equal to 5.5 mg/kg, the higher the pH, the lower the availability or uptake of Zn by the plant.

Table 4.5: Zn concentration in the post-harvest soil for both application times (planting and flowering)

Stage	Zn levels (mg/kg)	pH levels 5.1	5.6	6.1	6.6	Average
Planting	Zn1	0.8	0.7	1.7	3.2	1.6
	Zn2	1.4	1	2	3.3	1.925
	Zn3	1.7	1.4	2.4	3	2.125
	Zn4	0.7	3.8	1.9	1.6	2
	Zn5	1.9	1.4	1.1	2.4	1.7
	Average	1.3	1.66	1.82	2.7	
Flowering	Zn1	0.9	1.1	1.1	0.7	0.95
	Zn2	0.7	0.9	0.8	1.5	0.975
	Zn3	1	1.1	0.9	1.1	1.025
	Zn4	0.6	1.9	1.4	3.2	1.775
	Zn5	1.3	1.7	0.9	1.9	1.45
	Average	0.9	1.34	1.02	1.68	

Significance between means was tested at $p < 0.05$

When Zn was applied at the rates 6.5 and 7.5 mg/ kg of soil (Zn4 and Zn5 respectively), the amount of Zn in the post-harvest soil significantly decreased as the pH increased from 5.6 to 6.6 (except for pH 6.6_Zn5). This result implies that the higher the pH, Zn need to be applied at a higher rate than 5.5 mg per kg of soil to ensure its availability and uptake to the plant. In addition, for Zn applied at planting, the amount of Zn in the post-harvest soil was lower in soil with pH 5.6 (corresponding to lime application rate of 0.5t/ha) than in soil with pH 5.1(control) for every Zn level except Zn4, probably because the amount of lime added was not significant to inhibit the uptake of Zn by the plant. There was no significant difference between the amount of Zn in the post-harvest soil when it was applied at flowering.

However, Table 4.5 shows that at each pH and Zn level, Zn in the soil is greater at planting compared to Zn application at flowering.

Moreover, for both times of application of Zn, the average Zn in the post-harvest soil increased as soil pH increased. In addition, Zn concentration in the soil without lime (with pH 5.1) at post-harvest, was the lowest at all Zn levels (except Zn5 at planting). This implies that Zn uptake by the plant was higher in the control soil (the soil without lime) than other soils (soils with lime). This is probably due to the higher carbonate contents in soils with high pH which also absorb Zn and hold it in an unexchangeable form and is one of the factors that contribute to the low availability of Zn at higher pH values (Hafeez *et al*., 2013). Degryse (2015) reported that zinc is more available in soils with pH below 5.5 than in soils with pH between 5.5 and 6.5 and declines above 6.5. Also, liming of acidic soils increases pH and the Zn fixing capacity, particularly in soils with high P levels (Hafeez *et al*., 2013). Therefore, caution needs to be taken while liming a soil because raising the pH over 6.50 (in water) results in over-liming and micronutrient deficiencies namely Zn, Cu, and B (Sika, 2012).

4.2 Influence of soil pH and Zn fertilizer on yield components and yield of wheat.

4.2.1 Influence of soil pH and Zn fertilizer on number of tillers at 52 Days After Planting

The results of the influence of soil pH and Zn fertilization on the number of tillers at 52 days after planting (DAP) are presented in figures 4.2 A and B. At both planting and flowering, the number of tillers 52 DAP was at least 4 tillers for all Zn levels at pH 6.1 except the value for Zn1 at flowering. At planting, the number of tillers 52 DAP when Zn5 was applied at pH6.1 was 9 tillers and was significantly the highest (Figure 4.2). While at flowering, only Zn2: 4.5mg/kg and Zn3: 5.5mg/kg applied at pH 6.1 were significantly high compared to other pH levels. These results revealed that Zn influence on the number of tillers 52 DAP was more pronounced at pH 6.1. Therefore, they reaffirm the role of Zn in biosynthesis of the natural auxin (IAA) that can account for the promotion of the shoot system elongation and dry matter production and reflected in enhancing wheat tillering (Alloway, 2008).

Regarding Zn application, when applied at planting, only Zn5: 7.5mg/kg significantly increased the number of tillers at pH6.1. While at flowering, Zn5 at pH 5.6 and Zn2: 4.5mg/kg, Zn3: 5.5mg/kg, Zn4: 6.5mg/kg at pH6.1 significantly increased the number of tillers 52 DAP.

However, at planting, only pH6.1 (Zn4 and Zn5) and pH6.6 (Zn4) values exceeded 4 tillers 52 DAP; but the highest number of tillers 52 DAP was 4 for all Zn and pH levels. While at flowering, the number of tillers observed 52 DAP on at least two Zn levels exceeded 4 tillers per plant at each pH level. It entails that the response of the number of tillers 52 DAP to Zn application was greater at flowering (4.18) than planting (3.80), but was not significant. Nevertheless, the optimum combinations of pH and Zn levels at planting is 6.1_Zn5 (9 tillers) (Figure A).

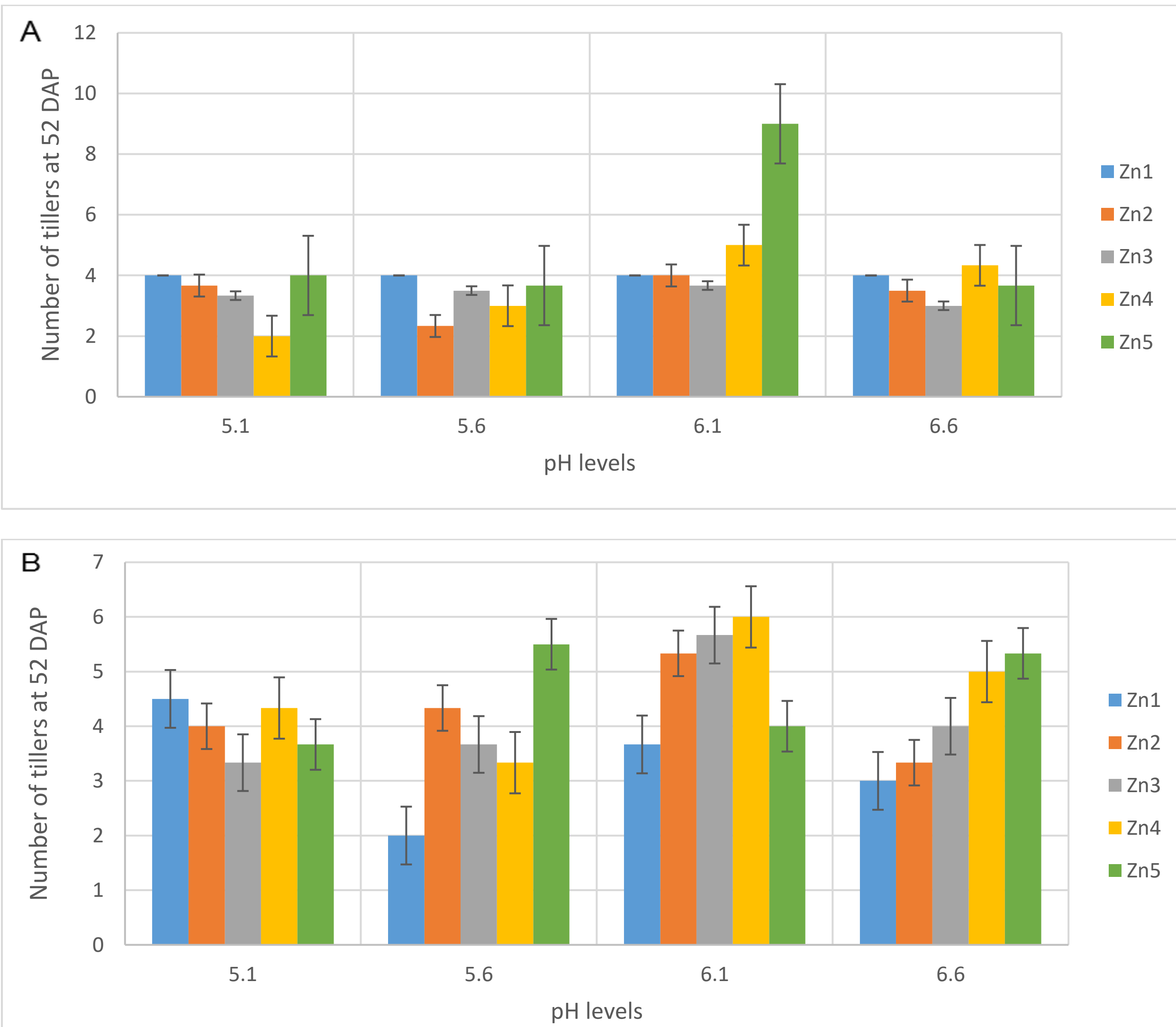


Figure 4.2: Effect of soil pH and zinc fertilizer on the number of tillers at 52 DAP (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.2.2 Influence of soil pH and Zn fertilizer on plant height

The results of the influence of soil pH and Zn fertilization on plant height are presented in Figures 4.3 A and B. At planting, the average plant height per pH level was 81.2, 83.2, 88.8 and 86.5 cm for pH 5.1, 5.6, 6.1 and 6.6 respectively (Figure 4.3 A). The results show that pH 6.1 significantly increased plant height when Zn was applied at planting. Similar results were reported by Van Zwieten *et al*., (2007) who found a 30-40 % increase in wheat height cultivated on an acidic soil. The increase was primarily attributed to the liming potential of biochar.

Contrary to the trend at planting, the average plant height per pH at flowering was 88.6, 84, 86.4 and 85.2 cm for pH 5.1, 5.6, 6.1 and 6.6 respectively (Figure 4.3 A). These results show that lime application had a negative effect on wheat plant height at flowering.

However, at planting, plant height was significantly greater for Zn1 and Zn2 than other Zn levels at pH 5.1; while at flowering, the plants had the greatest height (94 cm) for Zn4 at pH 6.6. This indicates that Zn applied at planting, and at a rate lower than 5.5 mg/kg (Zn3) has significant impact on plant height in the absence of lime. But at high pH level (pH6.6), Zn needs to be applied at 6.5mg/kg and at flowering to significantly increase plant height. Zn deficiency is known to be characterized by short internodes or stunted plants and reduced plant growth (Mengel & Kirkby, 2001). Zinc supplied in required amounts ensures plant growth, therefore increasing plant height.

In addition to the fact that the average plant height per pH level was greater at flowering than planting (except from pH6.6), the analysis of variance revealed that the average plant height was significantly greater at flowering (86.37cm) than planting (84.23cm). The optimum combination of pH and Zn levels at planting was 6.1_Zn5 (91cm) (Figure 4.3 A) and 6.6_Zn4 (94cm) at flowering (Figure 4.3 B).

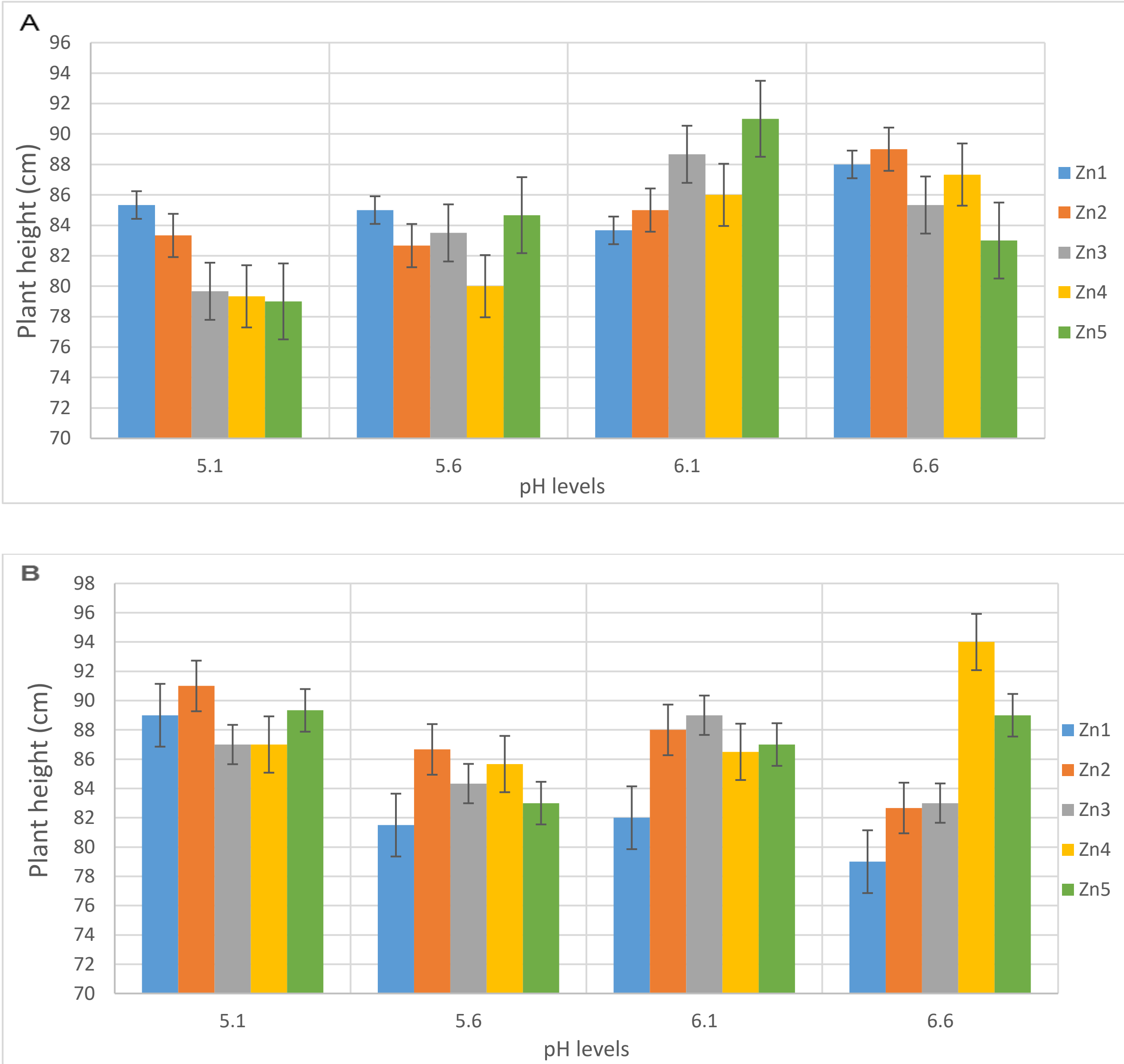


Figure 4.3: Effect of soil pH and zinc fertilizer on the plant height (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.2.3 Influence of soil pH and Zn fertilizer on total biomass

At maturity, plant heads, shoots and roots were dried and weighed to determine the total biomass. Figures 4.4 A and B depict the response of the total biomass to pH and Zn at different times of application.

The average total biomass per pH level at planting were 25.9, 22.06, 30.79 and 28.86 g for pH 5.1, 5.6, 6.1 and 6.6 respectively (Figure 4.4 A). These results show significantly high total biomass at pH 6.1 and 6.6. In addition, although there was no significant difference between the values, the highest total biomass for Zn2, Zn3 and Zn5 were attained at pH6.1 and for Zn1 and Zn4 at pH6.6. Sultana *et al.* (2009) in their study reported that lime had a positive effect on biomass. This might be explained by the increase of macronutrients like P, Ca, K, and Mg as a result of lime application.

As mentioned earlier, there was no significant difference between the total biomasses at any pH level although the total biomass showed an increase as Zn level increased at pH6.1. Zinc application had no effect on total biomass when applied at planting. These results are similar to those of Portmann, (2012) who found that plants grown on soils treated with zinc had significantly lighter heads, shoots and grain biomass in comparison to those grown on soils non- treated with zinc.

Moreover, the average total biomass per pH level at flowering were 29.83, 28.6, 29.75 and 25.49 g for pH 5.1, 5.6, 6.1 and 6.6 respectively (Figures 4.4B). These results show no significant differences ($p<0.05$) in total biomass between pH 5.1, 5.6 and 6.1, but a significant decrease at pH 6.6.

Zn1 showed a slight biomass increase at pH5.1, while Zn2 and Zn3 significantly increased biomass respectively at pH 5.6 and 6.1 and Zn4 and Zn5 significantly increased biomass at ph6.6. In fact, a simultaneous pH and Zn increase significantly increased biomass. This implies that the increasing soil pH requires an increase in Zn fertilizer, supplied at flowering to increase total biomass or dry matter in wheat.

The analysis of variance revealed that the average total biomass was greater at flowering (29.41g) than planting (26.59g), but the difference was not significant.

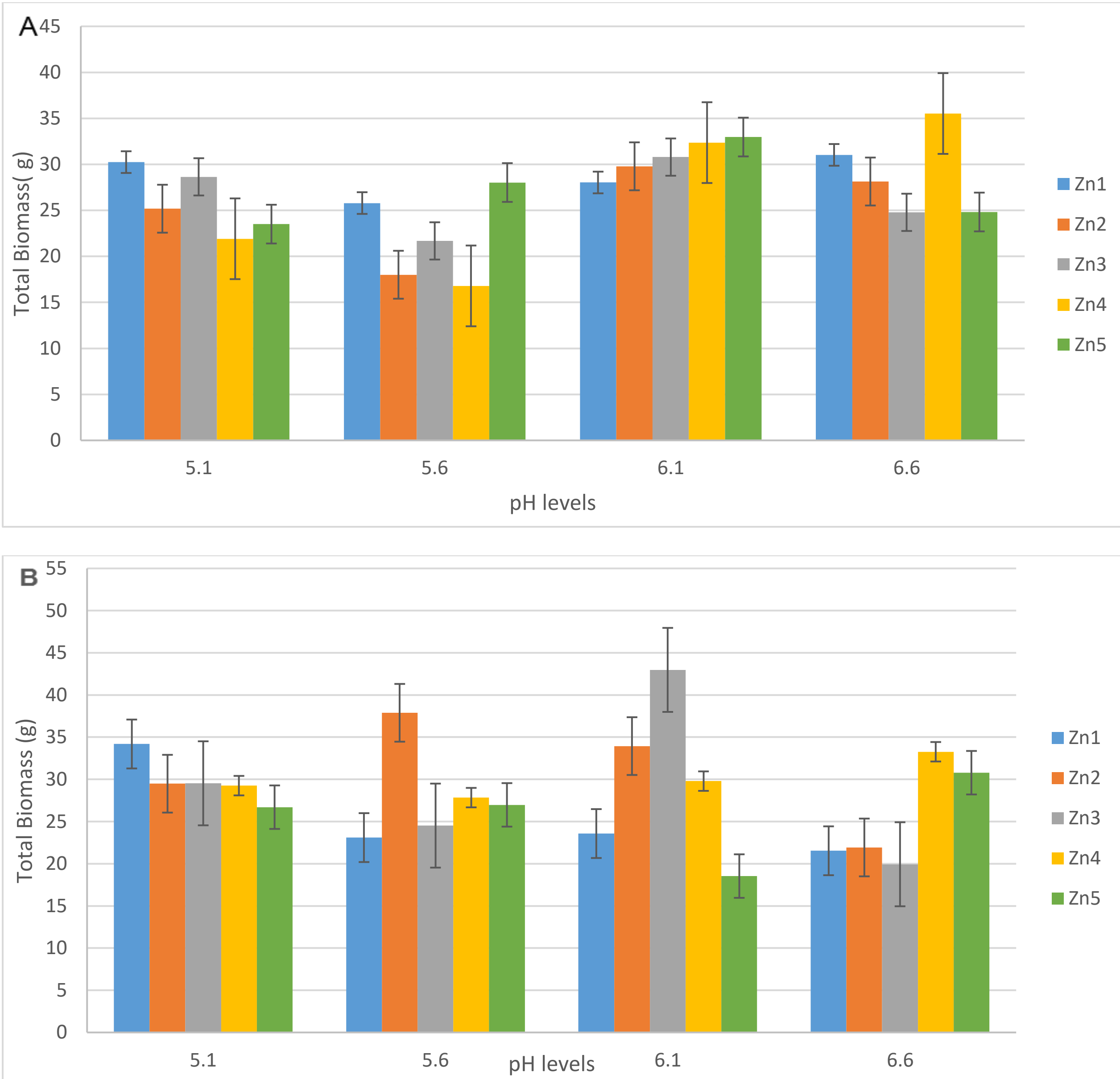


Figure 4.4: Effect of soil pH and zinc fertilizer on total biomass (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.2.4 Influence of soil pH and Zn fertilizer on the harvest index

The results of the influence of soil pH and Zn fertilization on the harvest index are presented in figures 4.5 A and B. At both planting and flowering, the harvest index, which represents the grain biomass as a percentage of the total biomass, show a decreasing trend as pH levels or lime application rates increased. At both planting and flowering, the average harvest index was about 0.3, 0.32, 0.27 and 0.23 for pH 5.1, 5.6, 6.1 and 6.6

respectively. In addition, Zn levels had their highest harvest index at pH 5.1 and 5.6 but only Zn4 (pH5.6) was significantly lower than other Zn4 values at planting. These results suggest that increasing lime application promotes the vegetative growth of the plant more than its reproductive growth.

However, the harvest index values for Zn4 and Zn5 at pH 5.1 and Zn5 at pH 6.1 at planting were significantly lower compared to other values within those pH levels. These findings are in close agreement with those of Portmann (2012) who found that the plants affected by zinc invested less of their biomass into the grain to the detriment of the rest of the plant.

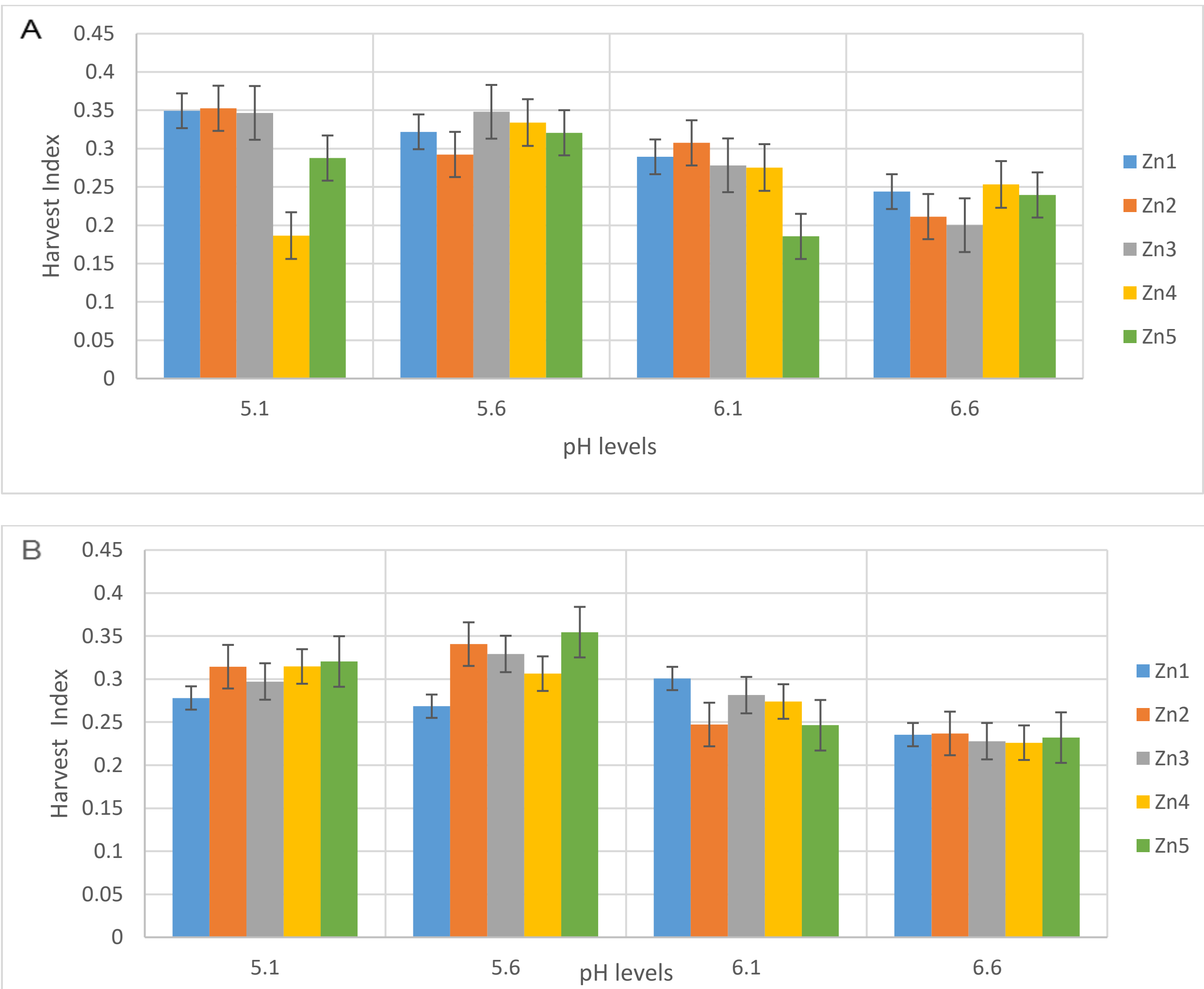


Figure 4.5: Effect of soil pH and zinc fertilizer on harvest index (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.2.5 Influence of soil pH and Zn fertilizer on Thousand Grains Weight

The results of the influence of soil pH and Zn fertilization on the weight of a thousand grains are presented in figures 4.6 A and B.

The average thousand grains weight per pH levels at planting were 3.04, 2.66, 3.06 and 2 g for pH 5.1, 5.6, 6.1 and 6.6 respectively (Figure 4.6 A). The results show that pH 6.1 led to significantly heavier grains when Zn was applied at planting, but was statistically similar with the thousand grains weight at pH 5.1.

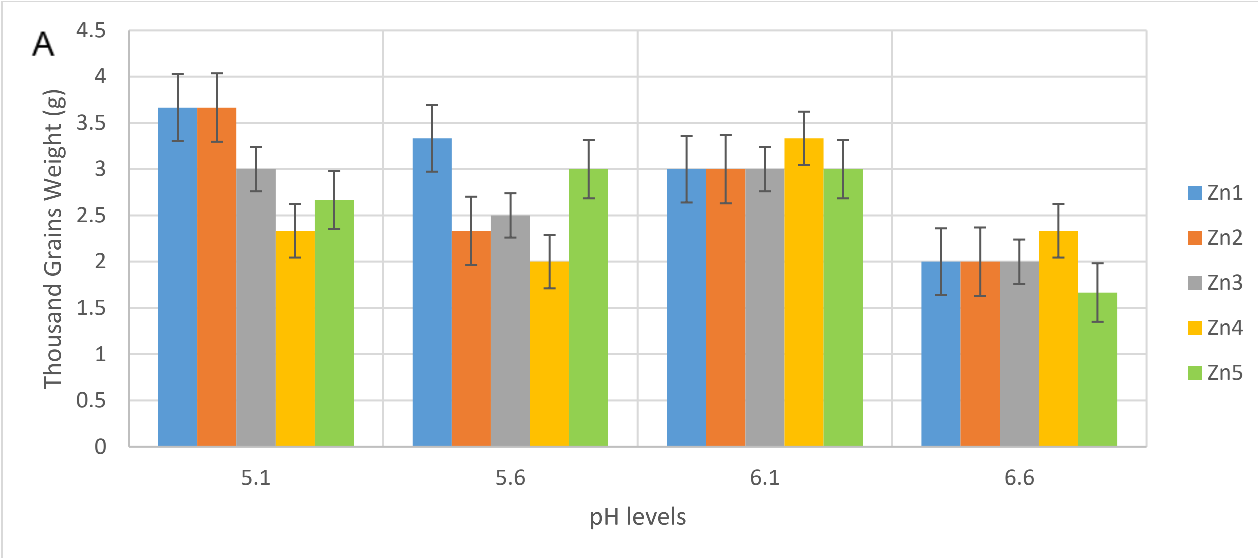
With Zn applied at flowering, the average thousand grains weight per pH level were 3.15, 2.92, 2.57 and 2.14g for pH 5.1, 5.6, 6.1 and 6.6 respectively (Figure 4.6 B). The analysis of variance revealed that the thousand grains weight significantly decreased as pH level increased.

When Zn was applied at planting and at pH 5.1, the thousand grains weight for Zn1 and Zn2 were significantly higher than Zn3, Zn4 and Zn5. Although Zn2, Zn3 and Zn5 showed a decrease in thousand grains weight at pH5.6, Zn application had no significant difference on the thousand grain weight at pH 5.6; 6.1 and 6.6.

The results revealed that when Zn is applied at flowering, thousand grains weight shows a decreasing trend as pH levels or lime application rates increased, even though this was not significant ($p<0.05$). At pH 5.1, the thousand grains weight was higher for Zn3, Zn4 and Zn5, than for Zn1 and Zn2 but the difference was not significant. At pH 5.6, thousand grains weight was significantly lower for Zn1 than other Zn levels. At pH 6.1 and 6.6, there was no significant difference between the average thousand grain weight at different Zn levels. In as much as Zn application had no significant effect on the thousand grains weight at any pH, Zn2 and Zn3 led to a significant reduction in thousand grains weight at pH 6.6 compared to other pH levels.

We can therefore conclude that Zn application had little to no effect on thousand grains weight, while soil pH or lime application especially at 1.5 ton/ha (pH6.6) negatively influenced thousand grains weight regardless of the time of application of Zn.

The analysis of variance revealed that there was no significant difference between thousand grains weight at planting (2.71g) and at flowering (2.68g).



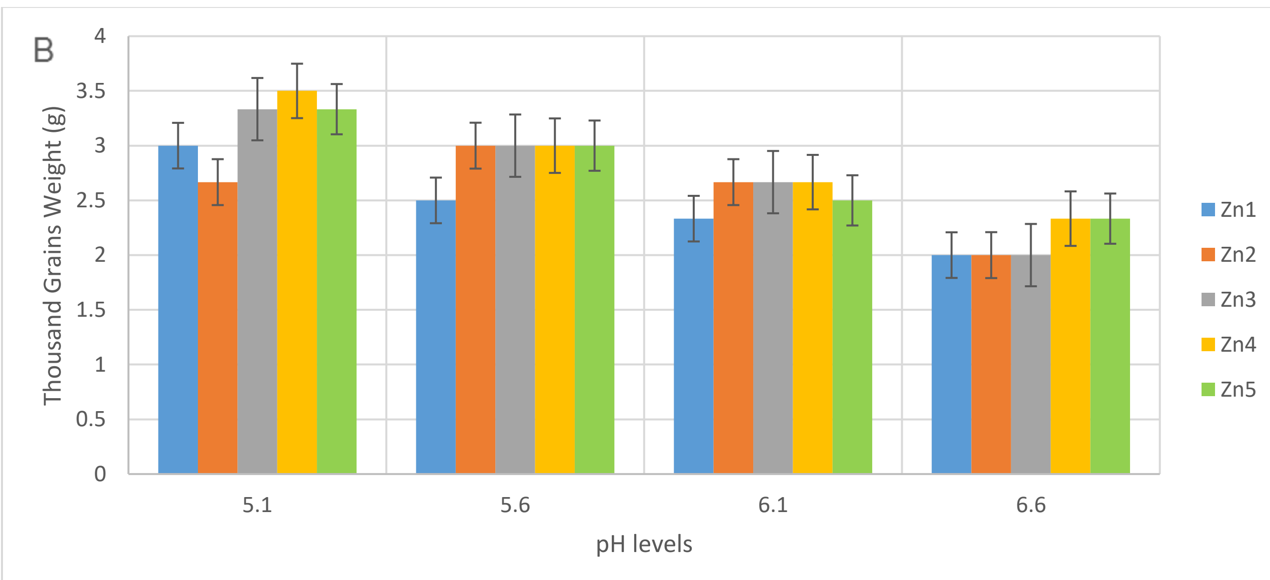


Figure 4.6: Effect of soil pH and zinc fertilizer on Thousand Grains Weight (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.2.6 Influence of soil pH and Zn fertilizer on wheat grain yield

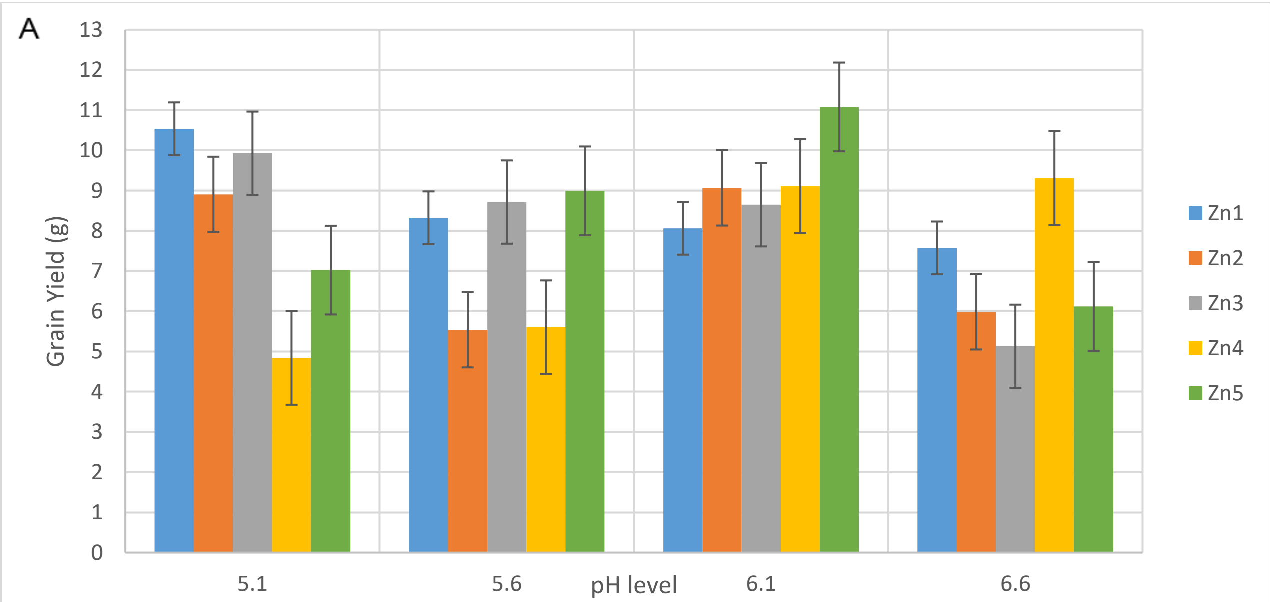
The results of the influence of soil pH and Zn fertilization on grain yield are presented on Figures 4.7 A and B. At planting, the average grain yield was 8.24, 7.43, 9.19 and 6.82g per plant for pH 5.1, 5.6, 6.1 and 6.6 respectively. These results show that pH6.1 or a lime application rate of 1t/ha significantly increased ($p<0.05$) wheat grain yield.

At flowering, the average grain yield was 9.55, 9.05, 8.16 and 5.65g per plant for pH 5.1, 5.6, 6.1 and 6.6 respectively. These results show a significant decrease in grain yield as lime application rates or pH levels increased. However, this effect was particularly more pronounced under pH6.1.

The present results show that at planting, there was no significant difference in grain yield as affected by Zn application, at any pH level. However, the low Zn levels 3.5, 4.5 and 5.5mg/kg soil (Zn1, Zn2 and Zn3) recorded greater grain yield per plant at pH 5.1 and a decrease in the grain yield as pH increased. While the grain yield values for the high Zn levels 6.5 and 7.5 mg/kg soil (Zn4 and Zn5) increased as pH levels increased up to pH6.1. This implies that in the presence of lime, Zn applied at levels lower than 5.5mg/kg soil has no or a negative effect on grain yield. Lime application therefore requires high Zn levels to affect grain yield positively.

At flowering, Zn1 showed a slight increase on grain yield at pH5.1, and Zn2, Zn3, and Zn4 significantly increased grain yield, respectively at pH 5.6, 6.1 and 6.6. This also implies that an increase in soil pH requires an increase in Zn fertilizer, supplied at flowering to increase wheat grain yield.

The analysis of variance revealed that the average grain yield was greater at flowering (8.16g) than planting (7.9g), but the difference was not significant.



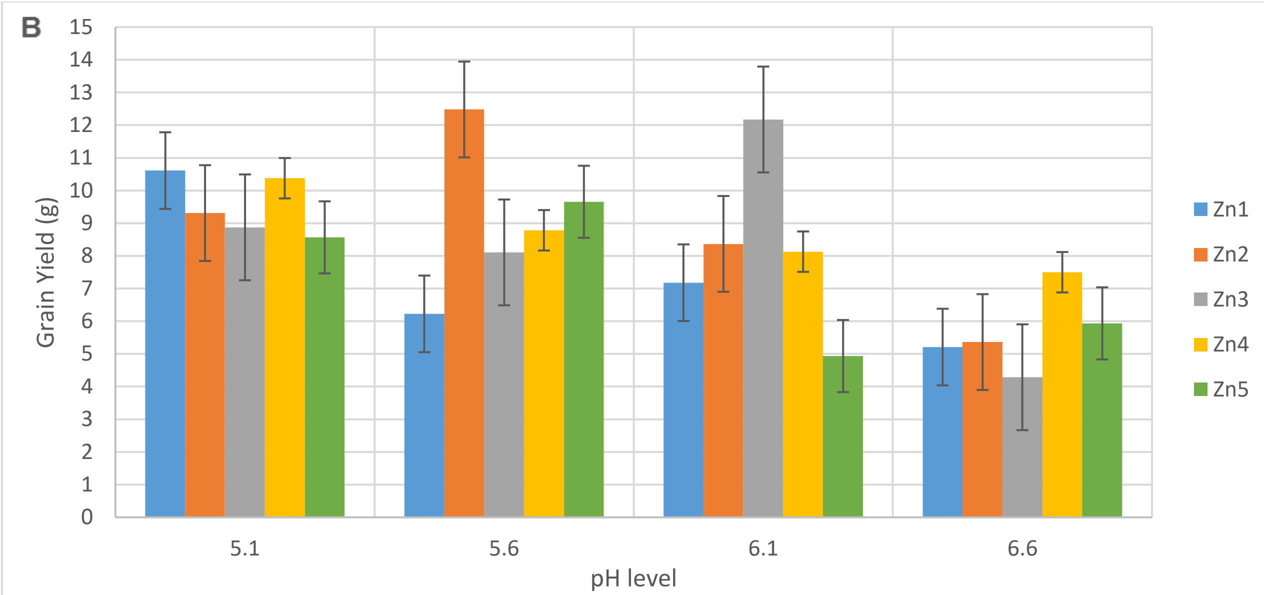


Figure 4.7: Effect of soil pH and zinc fertilizer on grain yield (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

As described, the grain yield and most yield components reacted similarly to lime and Zn applications. For Zn applied at planting, the lime rate 1t/ha (corresponding at pH 6.1), led to a significant increase in the number of tillers per plant, the plant height, the total biomass, thousand grains weight and the grains yield when Zn was applied at planting. These results show that the lime application rate at 1ton/ha or 6.1 at all Zn levels, is the optimum combination of pH levels regarding plant growth and grain yield when Zn is applied at planting. These results are closely similar to the findings of Kamaruzzaman et al, (2013) who reported that the application of 1.5 t/ha lime significantly increased most yield components and grain yield of wheat. The results confirm the finding of Fageria (2009) and Kamaruzzaman *et al.,* (2013) who stated that liming is a vital and prevailing practice to improve yield of crop on highly weathered acid soils. The increase in yield and yield components due to liming was also reported in other crops such as Indian spinach (Sarker *et al.,* 2014). Whereas, for Zn applied at flowering, the response of yield components and grain yield were adversely affected by lime as their performance in treatments with lime was less compared to the treatments with pH 5.1(no lime). This effect was significantly felt on the grain yield.

Moreover, Zn application had no significant effect on yield components and the grain yield at planting, except at pH 5.1 where Zn1, Zn2 and sometimes Zn3 led to better performance than Zn4 and Zn5. Whereas when applied at flowering, most yield components and grain yield were increased by Zn1(pH5.1), significantly by Zn2 (pH5.6), Zn3(pH 6.1) and Zn4(pH6.6). This trend was significant for total biomass and grain yield. This result suggested that the increase in lime application requires an increase in Zn fertilizer, supplied at flowering to positively affect growth parameters and grain yield of wheat. This might be explained by the interaction between N and Zn fertilizers on plant. Liming an acidic soil increases N availability in soil and in plant (Sultana et al., 2009), while increasing levels of Zn and N fertilizers (Alloway, 2004). This is supported by Portmann (2012), who stated that the enhanced plant growth caused by the higher nitrogen pool for the plants through fertilization increased the plant requirements for nutrients such as zinc, manganese and iron resulting in higher uptake. Zn application failed to influence yield components and grain yield at planting probably due to the inhibiting presence of P on Zn, which must had been used for plant growth before Zn was applied at flowering. This result is similar to the findings of Sharma et al, (1990) who stated that deficiency of zinc can occur after seeding of the crop in soils with high P contents, therefore zinc application during the crop growth may be necessary.

4.3 Influence of soil pH and Zn fertilizer on grain nutrient concentrations or nutritional quality

4.3.1 Grain Nitrogen

The results of the influence of soil pH and Zn fertilization on the accumulation of N in wheat grain are presented in Figures 4.8 A and B. When Zn was applied at planting, the average grain N concentration (1.8 mg) was significantly higher in the treatment without lime (pH5.1) than the treatments with lime (1.6mg), namely pH 5.6, 6.1 and 6.6. In addition, grain N concentration peaked for Zn levels (except Zn5) at pH5.1. Likewise, at flowering, the average grain N concentration (1.8 mg) was significantly higher in the treatment with lime (0.5t/ha or pH5.6) than the treatments pH 5.1, 6.1 and 6.6. These results show that at both planting and flowering, grain N concentration was significantly affected by low pH levels.

At planting, the application of Zn4: 6.5mg/kg significantly led to the highest grain N concentration at pH 5.1, while there was no significant difference in grain N concentrations at pH 5.6, 6.1 and 6.6. At flowering, grain N concentration was significantly high on application of Zn2: 4.5mg/kg at pH 5.6, and significantly decreased as Zn levels increased up to Zn5: 7.5mg/kg.

The current results are in disagreement with the findings of Saker *et al*, (2014) and Doddamani, (1975), who reported that the application of lime (CaCO3) increased soil pH and might have augmented the process of mineralization of nitrogen which in turn promoted the uptake of nitrogen. In a related report, Vitosh (1998) concluded that growing wheat below pH 6 results in the slow mineralization of nitrogen. Fageria and Zimmermann (1998) found that increasing soil pH increased the uptake of N in wheat. It is also well documented that the nitrification process is restricted in acidic soils (Harmsen & van Schevren, 1955). However, Alloway, (2008) reported that it is likely to find positive interactions between increasing levels of Zn and N. the previous author was also supported by Chauldry, (1972) who observed a strong response of the wheat plant to Zn application in the presence N fertilizer. In another report, Hafeez *et al,* (2013) found that Zinc concentrations progressively decreased with increasing Ca concentrations in solution. Therefore, in the context of the present study, the low grain N concentration recorded at high pH levels might be explained by the high amount of Ca supplied by CaCO3 (lime) that would have neutralized Zn concentrations, therefore reducing the positive interaction between Zn and N (Hafeez *et al,* 2013). In addition, Kaya *et al*. (2000) reported that Zn exerts a great influence on basic plant life processes such as (i) nitrogen metabolism - uptake of nitrogen and protein quality and (ii) photosynthesis - chlorophyll synthesis and carbon anhydrase activity.

However, grain N concentration showed a similar trend with the harvest index, which implies that the plants used a great portion of N to produce more biomass during the vegetative stage than they used in the production of grain when lime was applied at high rate. It explains the symptoms of N deficiency noticed at the reproductive phase of the plant growth during our experiment. Therefore, it is suggested that in future, wheat grown in lime-amended sandy soils should be closely monitored for N deficiency symptoms.

The analysis of variance of grain N concentration revealed no significant difference between the two times of application of Zn.

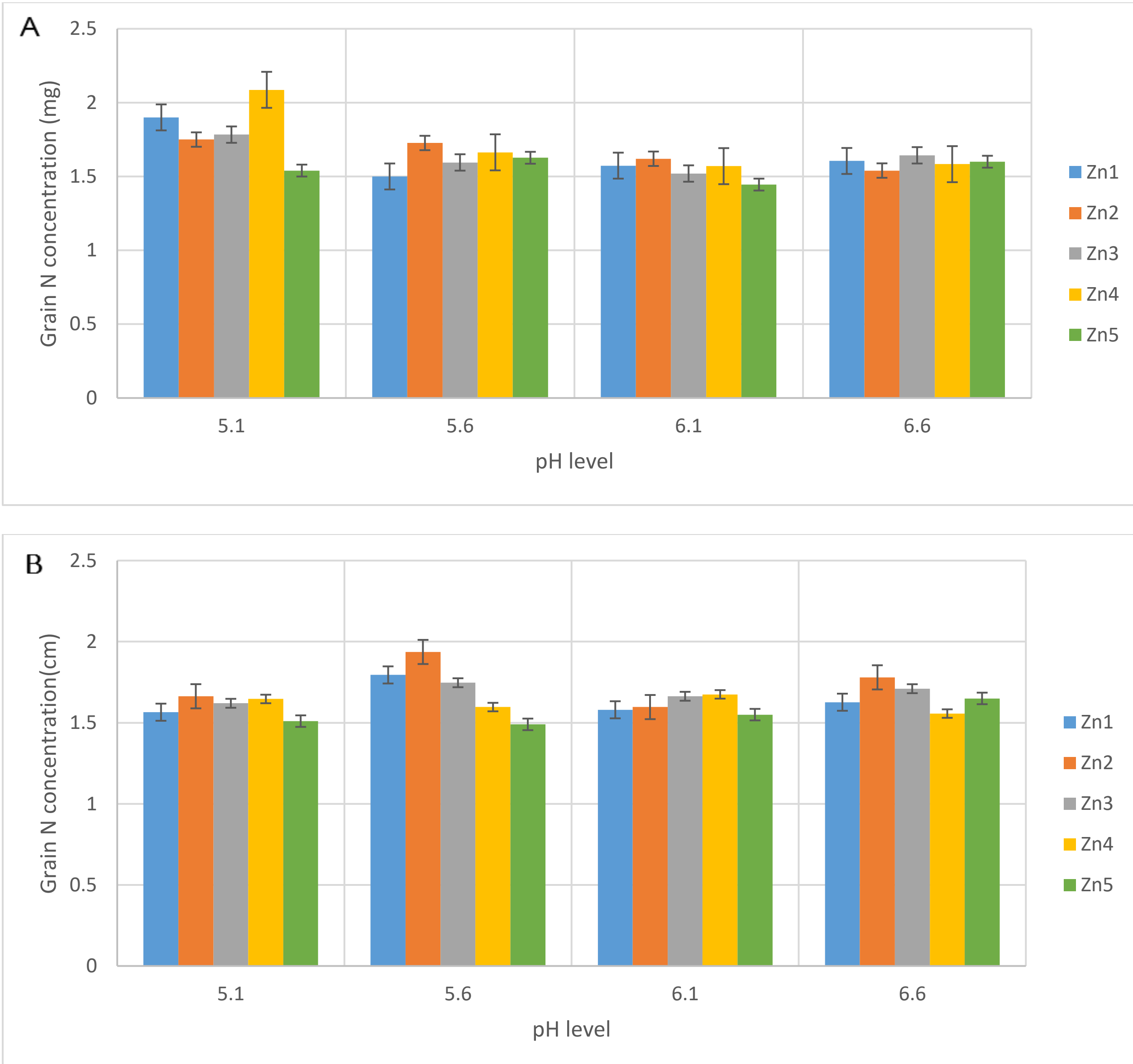
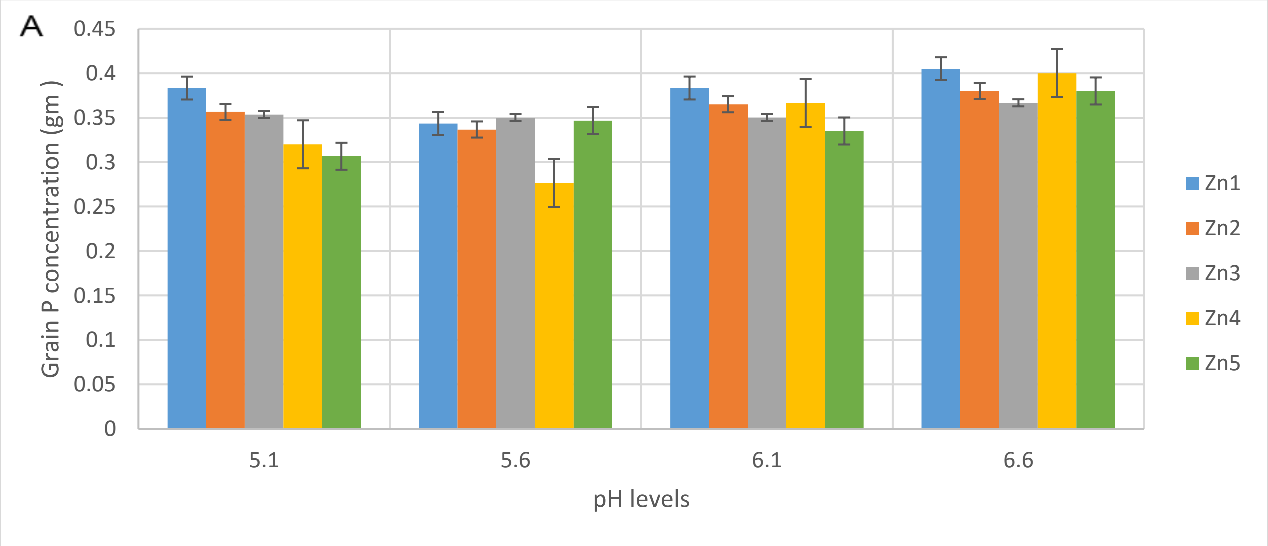


Figure 4.8: Effect of soil pH and zinc fertilizer on grain N concentration (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.3.2 Grain Phosphorus

The results of the influence of soil pH and Zn fertilization on the accumulation of P in wheat grain are presented in Figures 4.9 A and B. For both planting and flowering, the average grain P concentrations per pH level increased as lime application increased. In addition, grain P concentrations for all Zn levels reached their peak at pH 6.6. These results are in agreement with Vitosh (1998) who reported that growing wheat at pH below 6 results in reduced availability of P.

At planting there was a significant decrease of grain P concentrations from Zn1 to Zn3 at all pH levels except pH 5.6. These results are in support of Zeidan (2001), who indicated that Zn application significantly enhanced grain Zn concentration, while simultaneously reduced grain P concentration. At flowering, Zn application had no significant effect on grain P concentrations at any pH level.
Grain P concentration was expected to be high at pH 6.1 and 6.6 because according to Miller (2016), the optimum P availability to the plant is at pH 6.5. At planting, grain P concentration significantly decreased as Zn level increased at pH5.1 and also tended to decrease as Zn level increased up to Zn3 for lime treatments. It's probably due to the adverse interaction between P and Zn. Yang *et al.,* (2011) stated that this disorder in plant growth is associated with high levels of available P or with application of P to soil. This might probably be the case in the current study whereby optimum P rate was applied to target high yield.



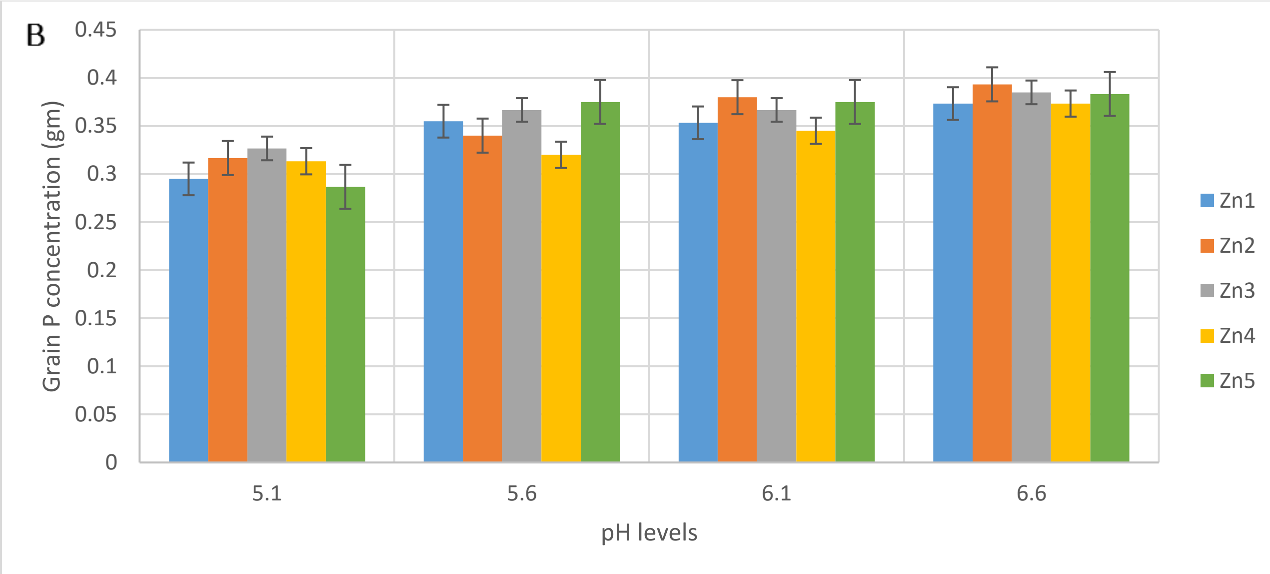
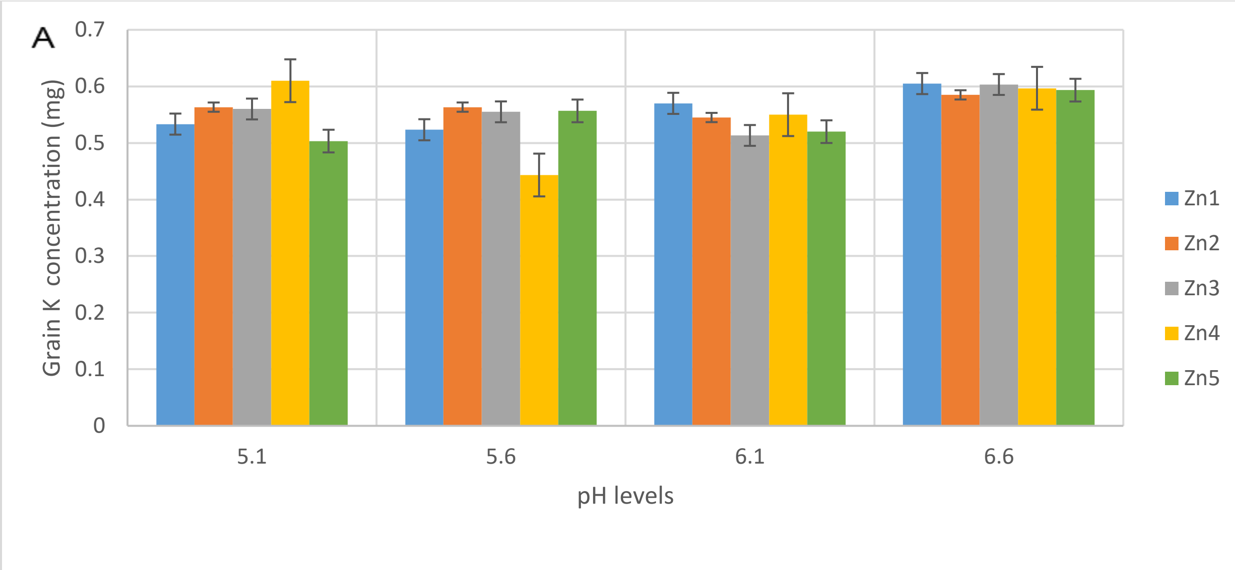


Figure 4.9: Effect of soil pH and zinc fertilizer on grain P concentration (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.3.3 Grain Potassium

The results of the influence of soil pH and Zn fertilization on the accumulation of K in wheat grain are presented in Figures 4.10 A and B. At both planting and flowering, the average grain K concentration at pH 6.6 was significantly higher than at other pH levels. Zn application affected grain K concentration at planting and pH 5.1 by significantly increasing grain K concentration as Zn level increased from 3.5 to 6.5mg/kg (Zn1 to Zn4) and at flowering by Zn2 and pH 6.1.



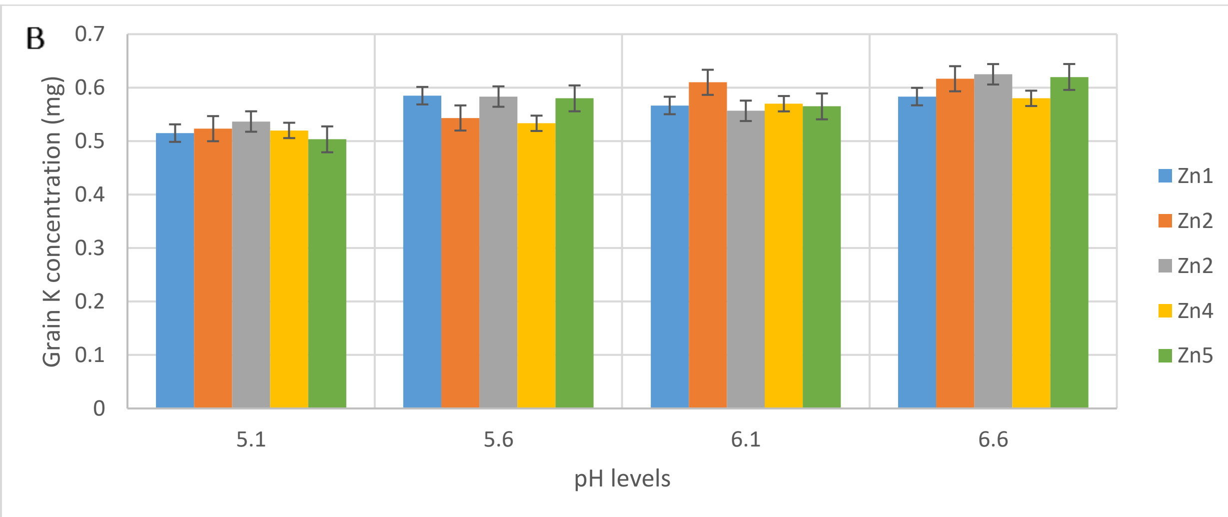


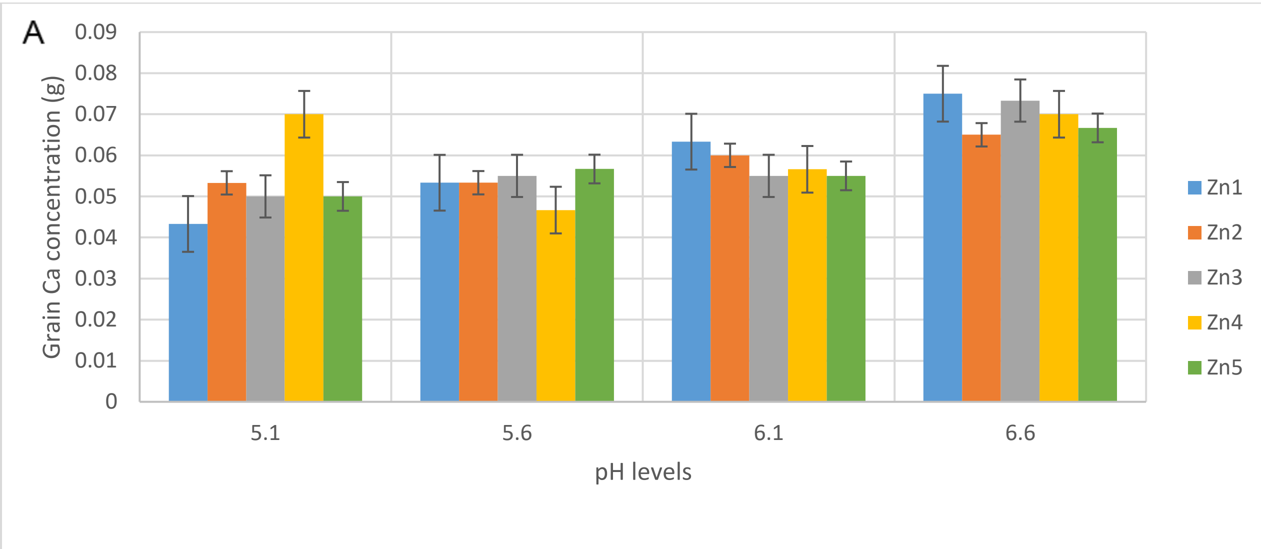
Figure 4.10: Effect of soil pH and zinc fertilizer on grain K concentration (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.3.4 Grain Calcium

The results of the influence of soil pH and Zn fertilization on the accumulation of Ca in wheat grain are presented in Figures 4.11 A and B. The average Ca concentrations in grain per pH level at planting were 0.053, 0.053, 0.058 and 0.07 g for pH 5.1, 5.6, 6.1 and 6.6 respectively (Figure 4.11 A). At flowering, the average Ca concentrations in grain per pH level were 0.046, 0.052, 0.058 and 0.08 g for pH 5.1, 5.6, 6.1 and 6.6 respectively

(Figure 4.11B). These results show that pH 6.6 led to high Ca concentrations in grain at both stages although the increase was not significant.

The results showed that Zn application had no significant effect on Ca concentrations in grain at any pH level, except at planting and pH 5.1, where grain Ca concentration significantly increased as Zn levels increased from 3.5 to 6.5mg/kg (Zn1 to Zn4). Grain Ca concentration for Zn2 and Zn3 were significantly identical. The combination 6.6(Zn4) was the optimum at both planting and flowering.



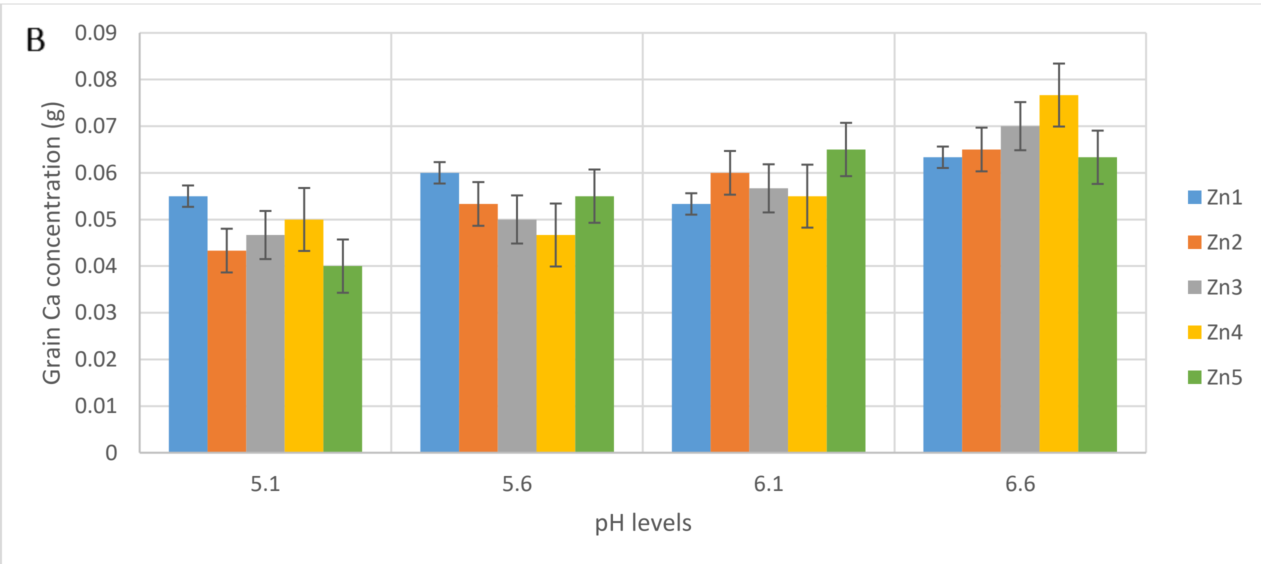


Figure 4.11: Effect of soil pH and zinc fertilizer on grain Ca concentration (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.3.5 Grain Magnesium

The results of the influence of soil pH and Zn fertilization on the accumulation of Mg in wheat grain are presented in Figures 4.12 A and B. The average Mg concentration in grain per pH level at planting were 0.102, 0.097, 0.104 and 0.115 g for pH 5.1, 5.6, 6.1 and 6.6 respectively (Figure 4.12 A). while at flowering, the average Mg concentration in grain per pH level were 0.087, 0.101, 0.107 and 0.112 g for pH 5.1, 5.6, 6.1 and 6.6 respectively (Figure 4.12B). The analysis of variance results show that pH 6.6 significantly increased Mg concentration in grain at both stages. The results show that Zn application did not significantly affect Mg concentrations in grain at any pH level, except at flowering and pH 5.1, where Zn3 significantly increased Mg concentration in grain.

The combination 6.6 (Zn4) is the optimum at planting and 6.6 (Zn4 or Zn5) at flowering.

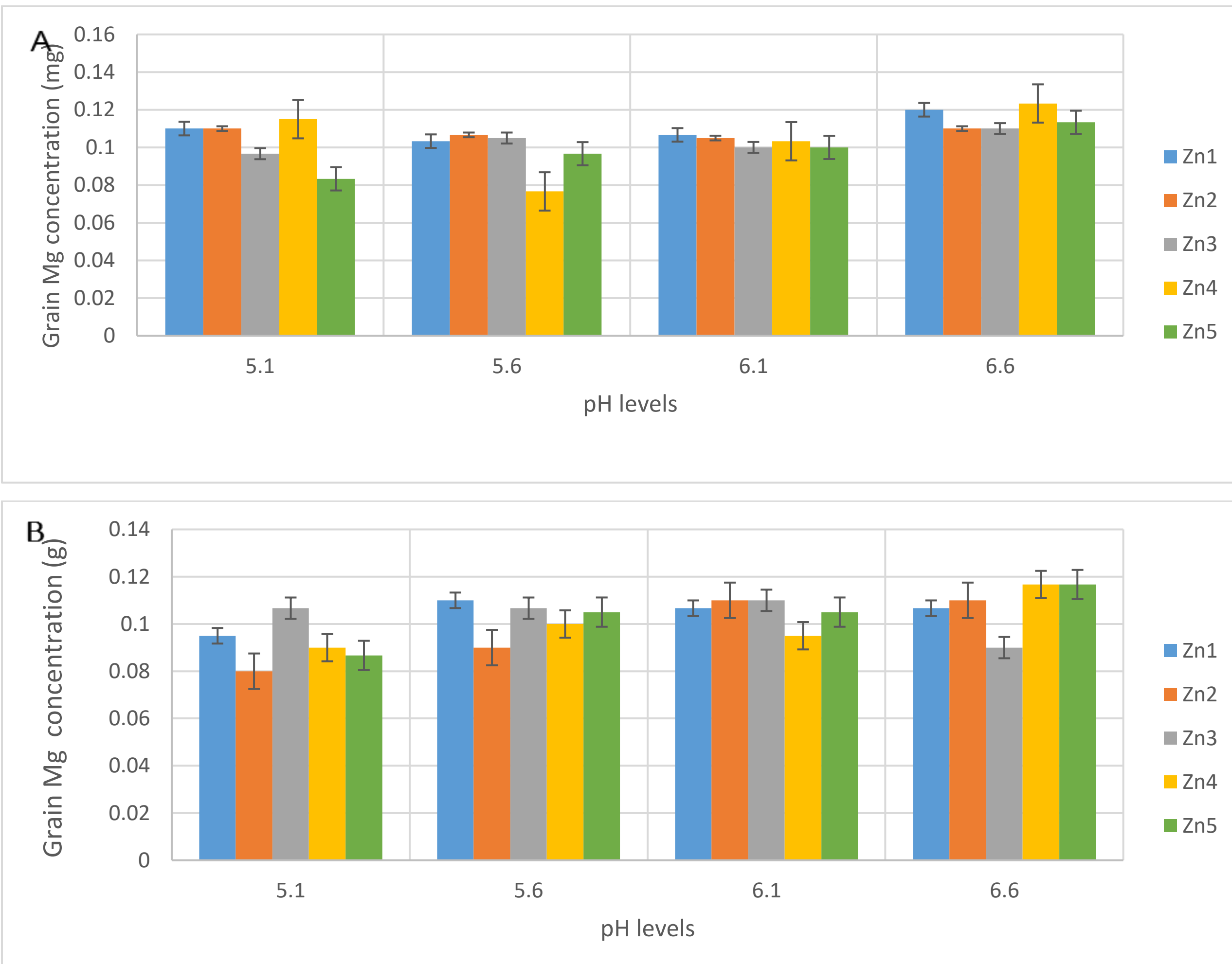


Figure 4.12: Effect of soil pH and zinc fertilizer on grain Mg concentration (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.3.6 Grain Sodium

The effect of soil pH and Zn fertilization on the accumulation of Na in wheat grain are presented in Figures 4.13 A and B. At planting, pH levels (lime) had no significant effect on grain Na concentration. At flowering, the average grain Na concentrations were 93.93, 129.77, 119.69 and 126.14mg at pH 5.1, 5.6, 6.1 and 6.6 respectively. The average grain Na concentrations showed no significant difference between the treatments of lime (pH5.6, 6.1 and 6.6) but were significantly higher than in the control (pH5.1).

At planting grain Na concentrations significantly decreased when Zn5 was applied at pH5.1. At pH 5.6, grain Na concentrations significantly increased as Zn levels increased from Zn1 to Zn3 and significantly decreased as Zn level increased from Zn3 to Zn5. At pH 6.1, grain Na concentrations significantly increased when Zn1 and Zn5 were applied. At pH 6.6, Zn application had no significant effect on grain Na concentration, while at flowering, grain Na concentration was significantly increased by Zn3 (pH6.1) and Zn5 (pH6.6).

Lime application significantly increased the concentrations of K, Ca, Na and Mg in grain wheat, when Zn was applied at both planting and flowering. These results confirm the findings of Sultana *et al*., (2009), who stated that liming an acid soil increases macronutrients availability in soil and to plant. In the other hand, Zn application had no effect on these macronutrients for the treatments with lime. However, the result of this shows that Zn fertilizer had no significant effect the grain concentration of these macronutrient cations (Ca, Mg, and K, except Na) at planting and also at flowering. This is justified by Alloway (2008) and Hafeez *et al.* (2013), who found that the macronutrient cations such as Ca, Mg, Na and K inhibit the absorption of zinc by plants from solution. But in the absence of lime (pH5.1), Zn4:6.5mg/kg significantly increased the macronutrients especially at planting. These cations need to be considered when interpreting the results of solution culture experiments involving zinc nutrition, however, in soil they seem to be less effective in the inhibition of zinc absorption compared to the effects of their salts on soil pH (Hafeez *et al.,* 2013).

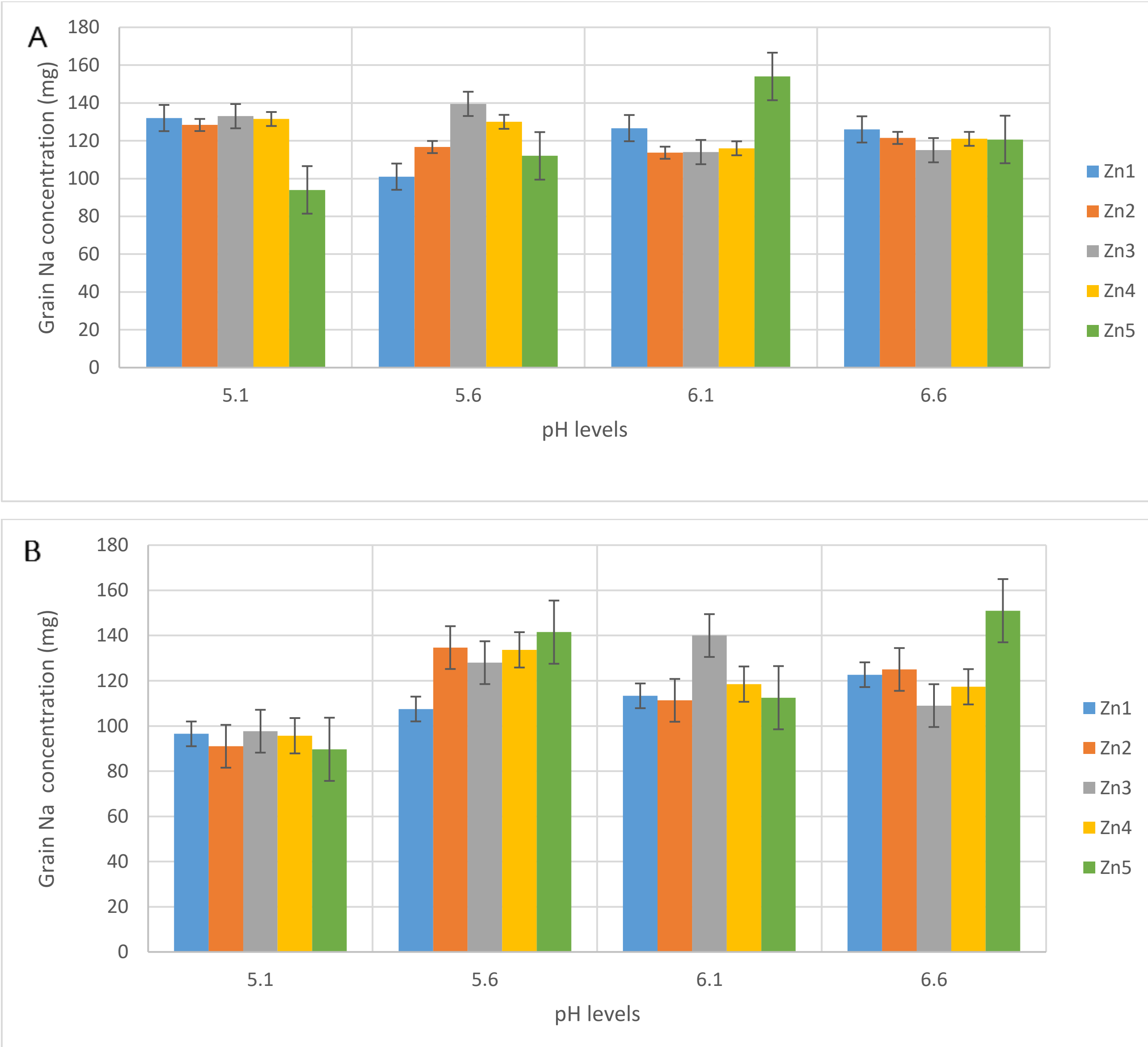
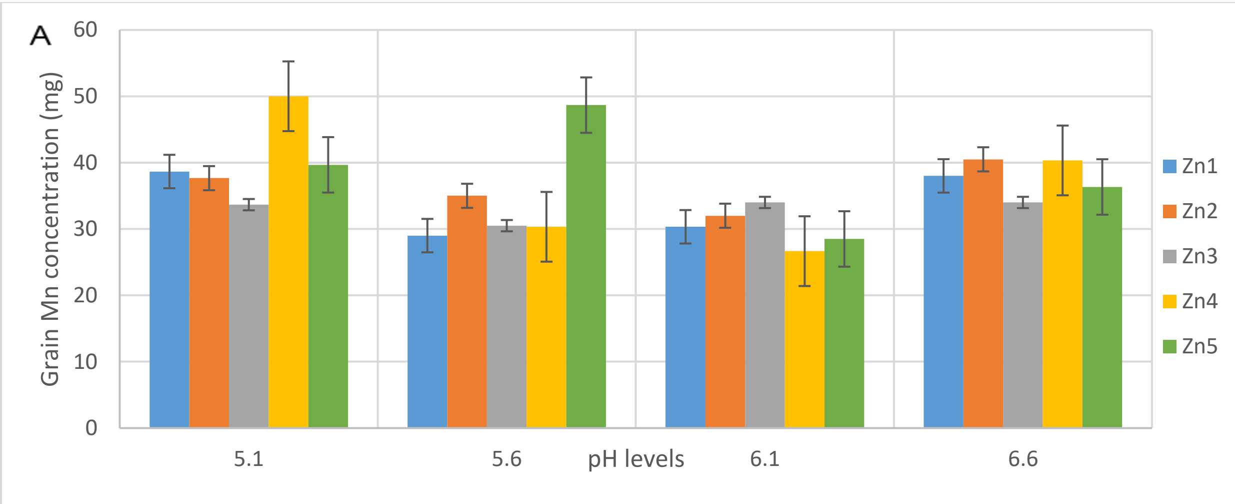


Figure 4.13: Effect of soil pH and zinc fertilizer on grain Na concentration (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.3.7 Grain Manganese

The results of the influence of soil pH and Zn fertilization on the accumulation of Mn in wheat grain are presented in Figures 4.14 A and B. At planting, the average grain Mn concentration was significantly greater at pH 5.1 than other pH levels. This result is similar to the findings of Vitosh (1998), who reported that growing wheat at pH below 6 increases the possibility of Mn toxicity.

At flowering, grain Na concentration was significantly increased by pH6.6. At planting, grain Mn concentration was significantly increased by Zn4 (5.1) and Zn5 (5.6) and they were significantly the highest of all Zn4 and Zn5 values.



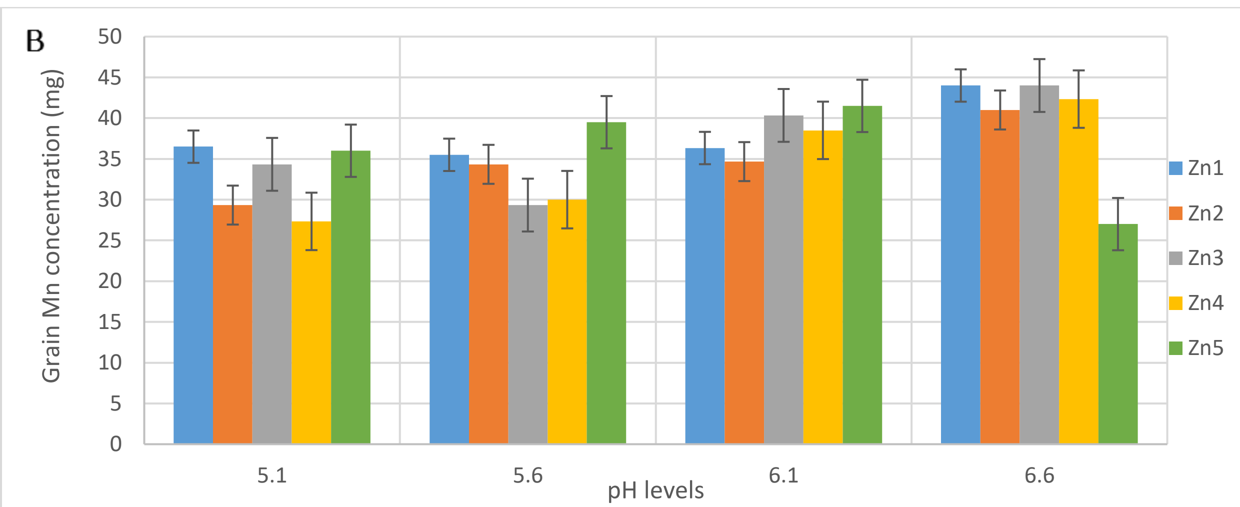


Figure 4.14: Effect of soil pH and zinc fertilizer on grain Mn concentration (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.3.8 Grain Iron

The effect of soil pH and Zn fertilization on the accumulation of Fe in wheat grain are presented in Figures 4.15 A and B. At both planting and flowering, pH 6.6 significantly led to high Fe concentration in grain. In addition, grain Fe concentration for all Zn values (except Zn2) significantly reached their peak at pH 6.6.

Grain Fe concentration was significantly increased by Zn4 (5.1) at planting and Zn2 (6.1) flowering. At both planting and flowering, the combination Zn1 (pH6.6) was the optimum.

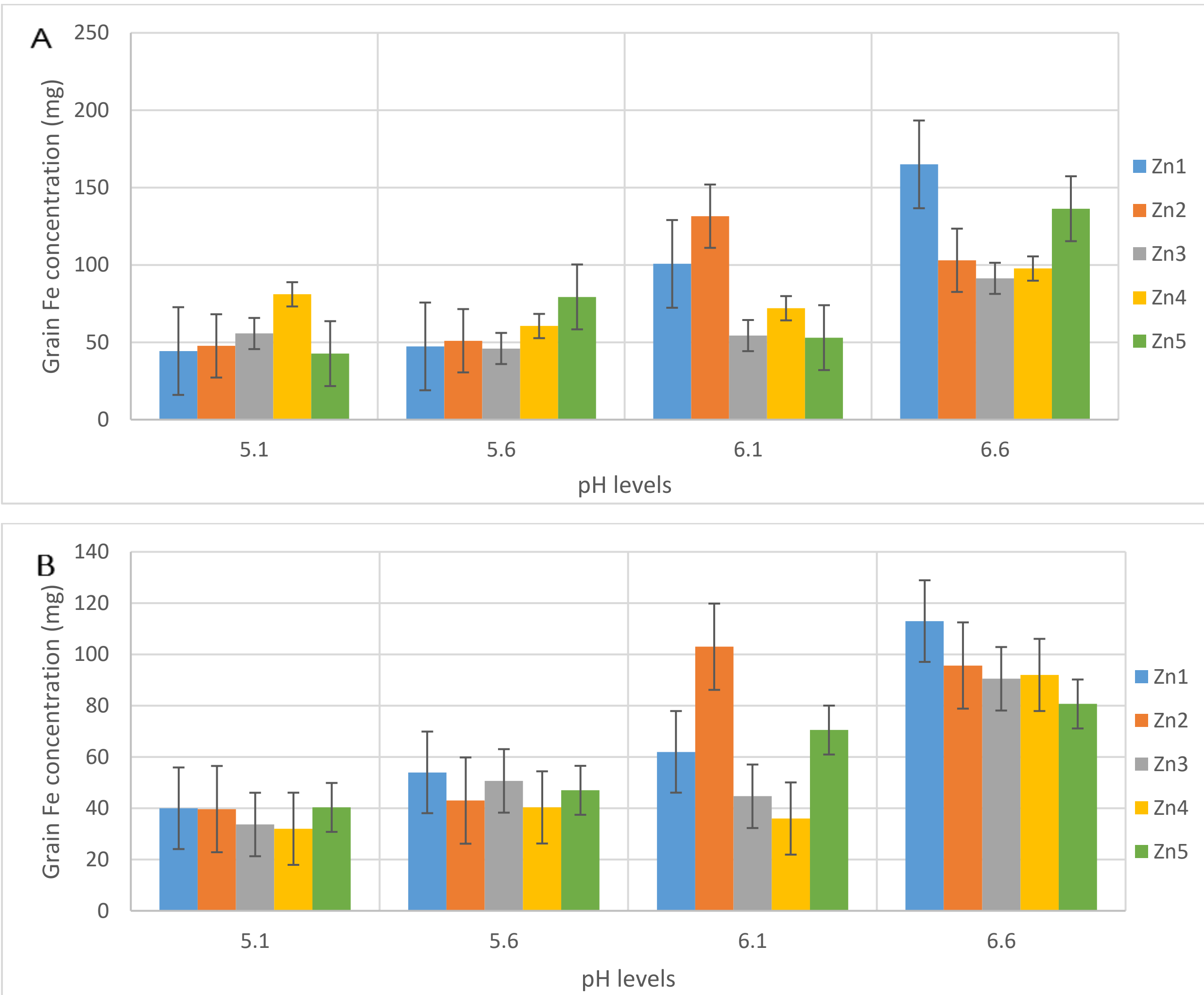


Figure 4.15: Effect of soil pH and zinc fertilizer on grain Fe concentration (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.3.9 Grain Copper

The results of the influence of soil pH and Zn fertilization on the accumulation of Cu in wheat grain are presented in Figures 4.16 A and B. pH 6.6 significantly led to high Cu concentration in grain at planting but not at flowering.

At planting, Zn1 generally affected grain Cu concentration but this was only significant at pH5.6. While at flowering Zn1 significantly affected grain Cu concentration at all pH levels except pH6.1 and Zn2 at pH6.1 and 6.6.

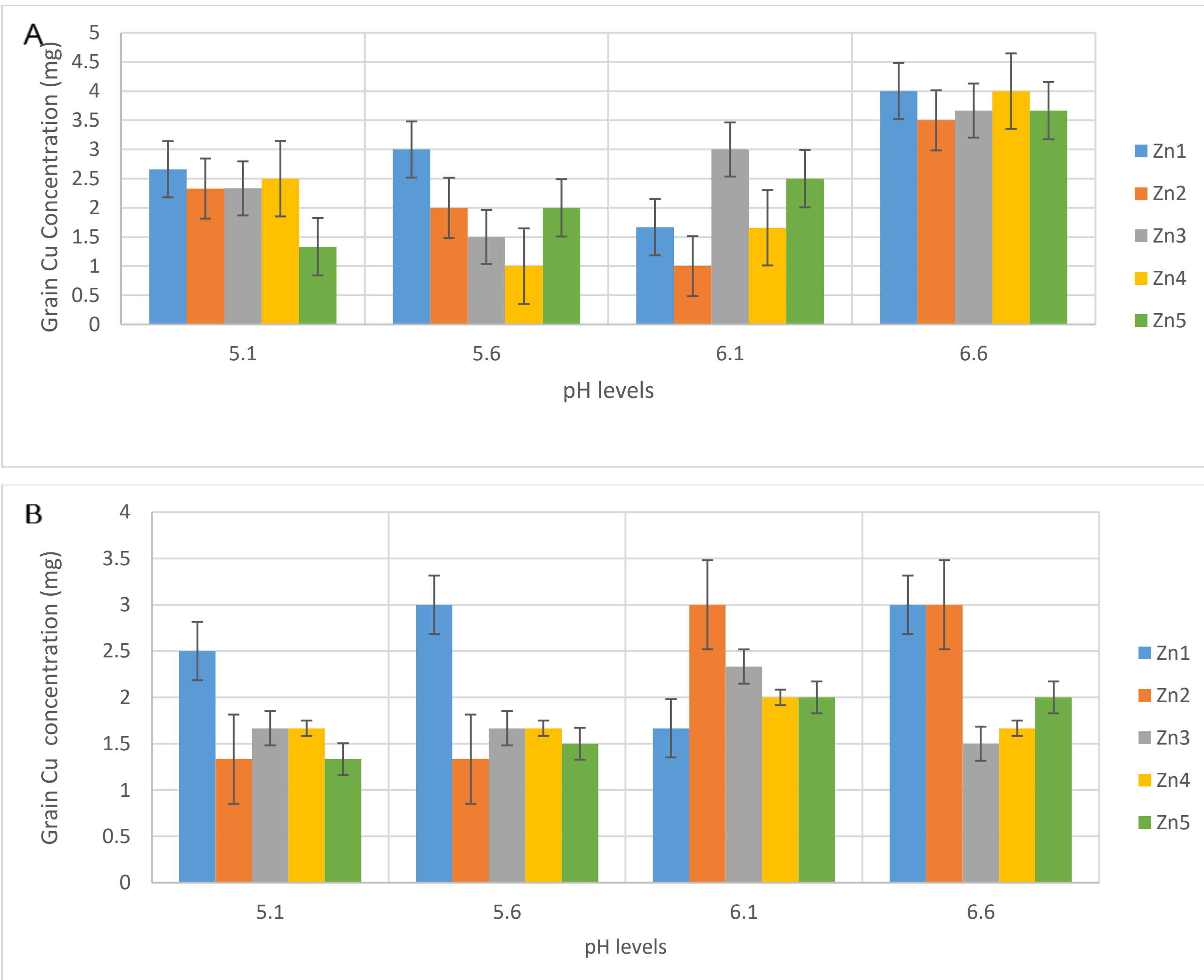


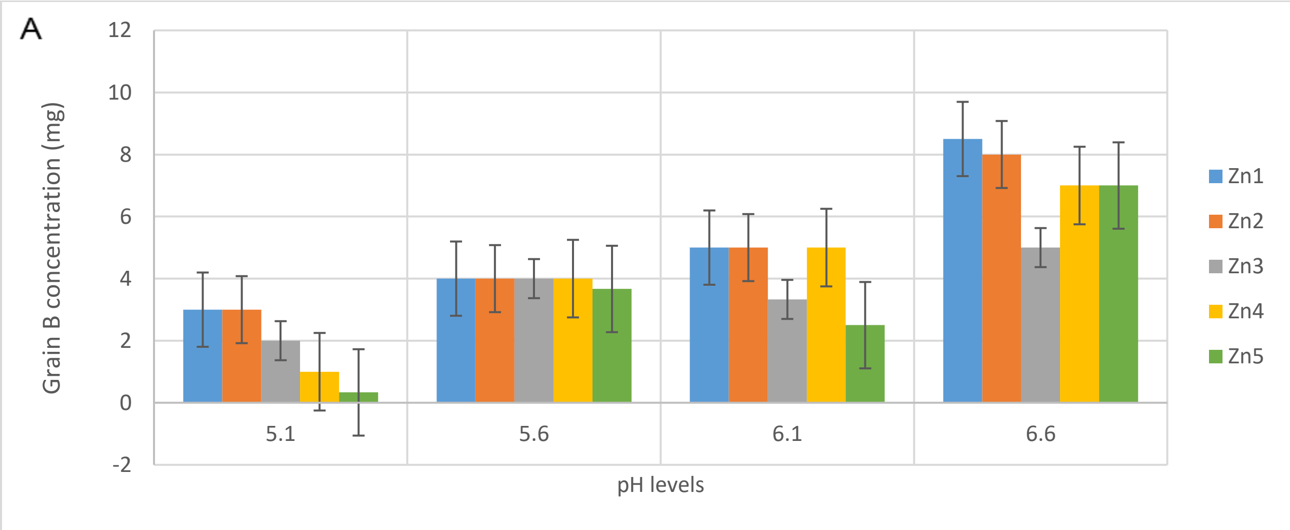
Figure 4.16: Effect of soil pH and zinc fertilizer on grain Cu concentration (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.3.10 Grain Boron

The effect of soil pH and Zn fertilization on the accumulation of B in wheat grain are presented in Figures 4.17 A and B. At both planting and flowering, pH 6.6 significantly led to high B concentration in grain. Grain B concentration values were greater for all Zn levels at pH 6.6. Zn application had no significant effect on grain B concentration at any stage. The combination Zn1 (pH6.6) was the optimum at both planting and flowering.

Regarding the concentration of micronutrients in the grain, lime application significantly increased the concentrations of Fe, Cu and B; but decreased Zn and Mn. These results are partly similar to the findings of Karan *et al.,* (2014) and Rathod *et al*., (2016), who reported that Fe, Cu, Zn and Mn decreased significantly following liming, whereas liming increases B availability. The increase of Fe and Cu at high rates of lime might have occurred as a result of the decrease in pH during the experiment; or another reason might be the competitive interaction between Zn, Cu and Fe, whereby the availability of one can lead to the reduction of the other (Fageria, 2009).

The results showed that Fe and Cu were affected by low Zn levels Zn1:3.5mg/kg and Zn2:4.5mg/kg. This result confirms the findings of Olsen (1972) who stated that the competitive interaction is strong at low Zn levels, and be corrected by modest Zn application. However, the in the absence of lime, Fe and Mn was significantly increased by Zn4:6.5mg/kg when applied at planting.



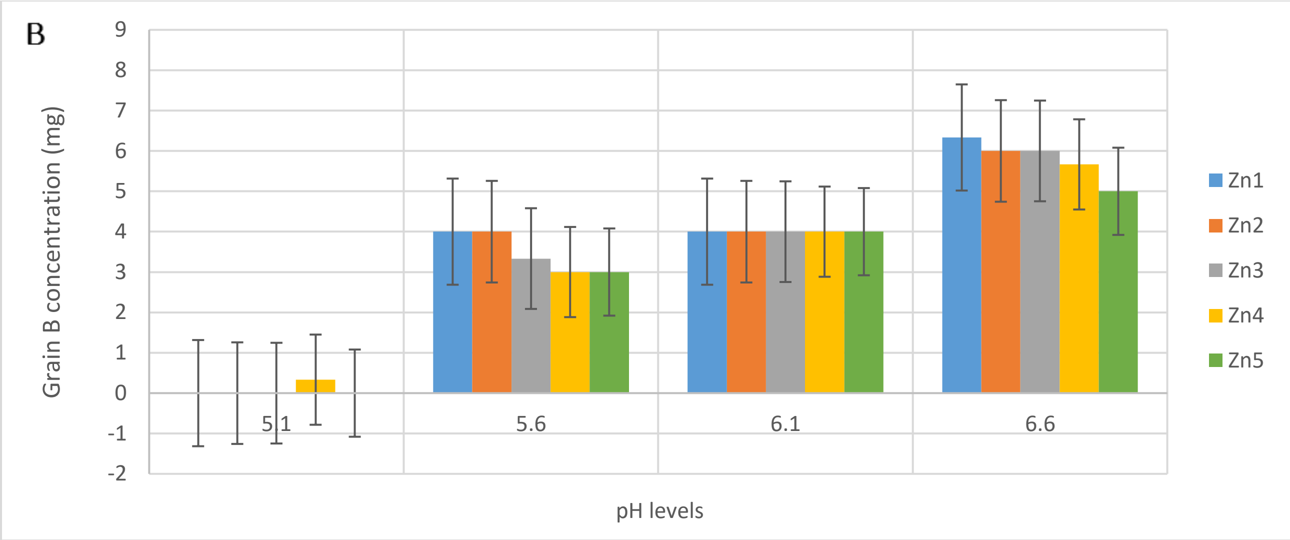


Figure 4.17: Effect of soil pH and zinc fertilizer on grain B concentration (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.3.11 Grain Zinc

The effect of soil pH and Zn fertilization on the accumulation of Zn in wheat grain are presented in Figures 4.18 A and B.

At planting, the average Zn concentration in grain per pH level were 53, 43.3, 35.9 and 48.8 mg for pH 5.1, 5.6, 6.1 and 6.6 respectively (Figure 4.18 A). While at flowering, the average Zn concentration in grain per pH level were 35.1, 42.4, 36.5 and 41.9 mg for pH 5.1, 5.6, 6.1 and 6.6 respectively (Figure 4.12B). The analysis of variance results showed that grain Zn concentration was significantly high in plants grown on pH 5.1 (treatment without lime) at planting, recording the peak of grain Zn concentration at most Zn levels. At flowering, pH 5.6 significantly led to high Zn concentration in grain. These results are similar to the findings of Fageria (2009), who found that Zn uptake decreased four times with increasing soil pH in the range of 4.6 to 6.8. He attributed the decrease to the low Zn content or low Zn-retaining capacity of the sandy soil. Furthermore, Linsay and Norwell (1969) stated that Zn availability is supposed to increase 100-fold for each unit in pH.

At planting, the application of Zn4: 6.5mg/kg significantly led to the highest grain Zn concentration (67 mg) at pH 5.1, while there was no significant difference in grain Zn concentrations at pH 5.6, 6.1 and 6.6. At flowering there was a significant decrease in grain Zn concentration (from 50.5 to 34mg) at pH 6.6 as Zn application increased from Zn3: 5.5mg/kg to Zn5: 7.5mg/kg.

Moreover, the average grain Zn concentration was significantly higher at planting (45mg) than flowering (39mg). In addition, for Zn applied at planting, grain Zn concentration was above 40mg/kg of grain for most Zn levels at all pH levels except (pH6.1) while at flowering only pH5.6 and pH 6.6 had grain Zn concentration above 40mg/kg of grain for most Zn levels. However, 40 mg kg^{-1} is the target concentration set by nutritionists for biofortification (Chen *et al.,* 2017). In addition, for a measurable impact on human health, agronomic biofortification should enhance grain zinc content from 35 to 45 mg kg^{-1} (Pfeiffer & McClafferty, 2007; Cakmak, 2002). Nonetheless the increases were not significant.

Our findings revealed that soil pH 5.1 (control) had greater grain Zn concentration for all Zn levels compared to other pH levels treatments that received lime when Zn is applied at planting, and the average grain Zn concentration at pH5.6 was greatest compared to other pH levels at flowering. Firstly, Hafeez *et al.,* (2013) stated that zinc availability is highly dependent on pH. When the pH is above 6, the availability of Zn is usually very low. In addition, the movement of Zn in limed soils is considerably lower than in acidic soils so that absorption of Zn by the crop may be low. Liming can thus reduce the Zn uptake (Shukla & Morris, 1967) and induce Zn deficiency (Viets, 1966). Lastly in a study reported by Curtin (2010), lime application was found to decrease grain Zn at all sites of study.

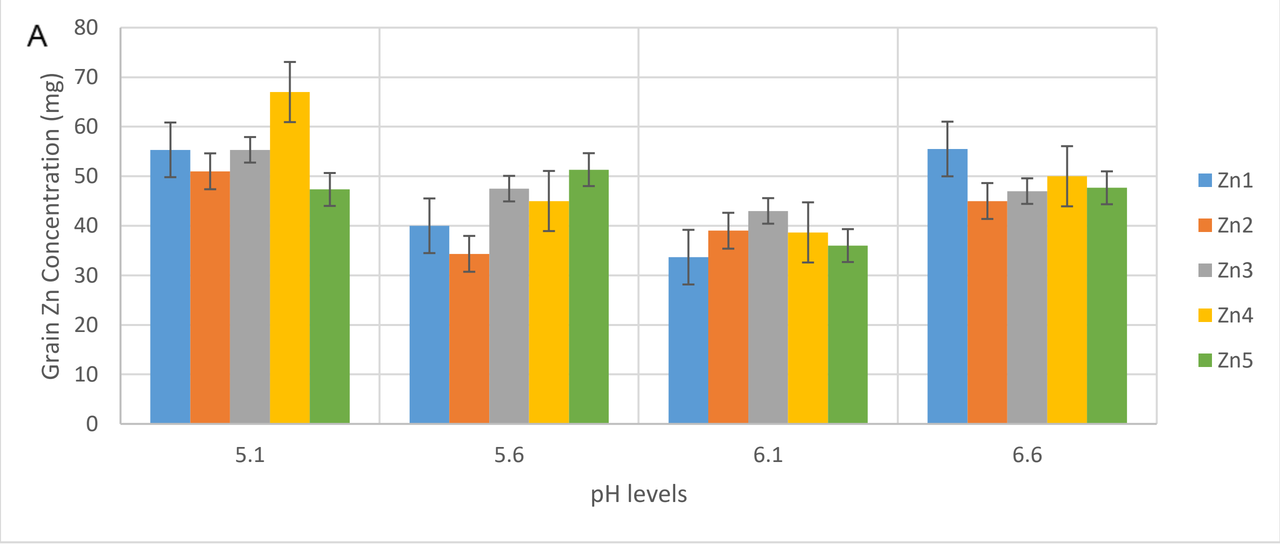
At planting, Zn application had no significant effect on grain Zn concentrations at any treatment with lime (pH 5.6, 6.1 and 6.6), while in the treatment without lime (pH 5.1), Zn application at Zn4: 6.5mg/kg significantly led to the highest grain Zn concentration (67 mg) at pH 5.1. These results are similar to those of Dolling et al. (1991), who found no interaction between lime and nutrient application to barley grain yield at nine sites over 2 years on acidic soils in Western Australia. At flowering there was a significant decrease in grain Zn concentration from 50.5 to 34mg at pH 6.6 as Zn application increased from Zn3: 5.5mg/kg to Zn5: 7.5mg/kg.

While grain Zn concentration was significantly greater for Zn applied at planting than at flowering. Sharma *et al.,* (1990) notified that sometimes, because the amount of Zn applied is less than that required or because of an abnormally low soil temperature, deficiency of Zn can appear during crop growth. In addition to that, a high-P soil can also cause Zn deficiency in crop after seeding. Therefore, under such conditions, it may appear important to apply Zn during crop growth. However, Mustafa *et al.,* (2011) found that a basal application of Zn recorded maximum paddy yield and number of tillers compared to Zn application at 75 and 60 days after transplanting respectively, who recorded the minimum values. Harsh, (2010) confirmed these findings, by stating that the best time of zinc addition is prior to sowing or transplanting of crops because maximum zinc absorption by plants takes place up to tillering or pre-flowering stages. These results are supported by Curtin (2010), who found that tissue concentrations were generally higher at mid-late tillering (Zadoks growth stage 22-29) than at ear emergence, confirming that uptake of these elements precedes biomass accumulation during the early part of the growing season Curtin (2010). These results are also in agreement with the findings of Flyman and Afolayan (2008) as well as Khader and Rama (1998), who found Zn concentration to increase with increasing age, further confirming a latter study by Lewu *et al.,* (2012) who found that Zn content of all the genotypes of *B. oleracea* were initially low and peaked at harvest stage. And in general, the mineral element concentrations were higher when Zn is supplied at planting than at flowering.

It is also important to note that grain Zn concentration and grain yield showed opposite trend towards Zn application at both stages and at all pH. For instance, compared to other pH levels, pH6.1 yielded relatively great grain yield while storing the smallest grain Zn concentration for most of Zn level and pH 6.6 had the smallest grain yield but stored great Zn concentration in grain. And although there was an increase in grain Zn concentration as Zn application level increased, this increase was neither significant nor turned into high grain yield. These might be explained by a possible Zn dilution effect in plant. Similar to previous studies by Kalayci *et al*., (1999) and Rengel *et al*., (1999), Aghili *et al.,* (2014) found a clear increase in grain zinc concentration after application of mineral zinc fertilizer. However, application of mineral zinc fertilizer neither translated into increased plant growth nor into higher grain yields; showing that zinc availability was not the primary plant growth-limiting factor in this soil. Also, in the United Kingdom, wheat (*Triticum aestivum*) yields have increased substantially over time but yield improvements have not been accompanied by increases in Zn uptake (McGrath et al., 2007). In addition, Waters *et al.,* (2009), Stomph *et al.,* (2011) and Liu *et al.,* (2014) found in their studies that increasing Zn supply seems to be an adequate solution for increasing grain Zn concentration in wheat plant. But they didn't state on the influence of Zn fertilizer on grain yield.

Moreover, the negative effect of high rate of lime on grain N concentration was ascribed to Zn availability at low pH levels, which promoted the positive interaction between Zn and N (Hafeez *et al.,* 2013 and Kaya *et al*., 2000). In addition, this interaction is confirmed by the fact the increase in Zn application from Zn1 to Zn4, at planting and pH5.1 led to an increase in the grain concentration of N and Zn, and to a decrease in both N and Zn concentrations for pH 5.6 at flowering.

The strong and positive correlations between Zn and Mn was also found by Murphy et al. (2008) who indicated high a correlation coefficient (r = 0.900) between Zn and Mn. However, the strong and positive correlations between Cu and P and between Ca and B at pH6.6 were ascribed to the high rate of lime application. Would it be in the absence of lime; these micronutrients would have behaved differently. Murphy *et al.,* (2008); Liu *et al.,* (2014); and Wojtkowiak *et al.*, (2017) found positive correlations among Zn, Fe, Cu and Mn concentrations or content.



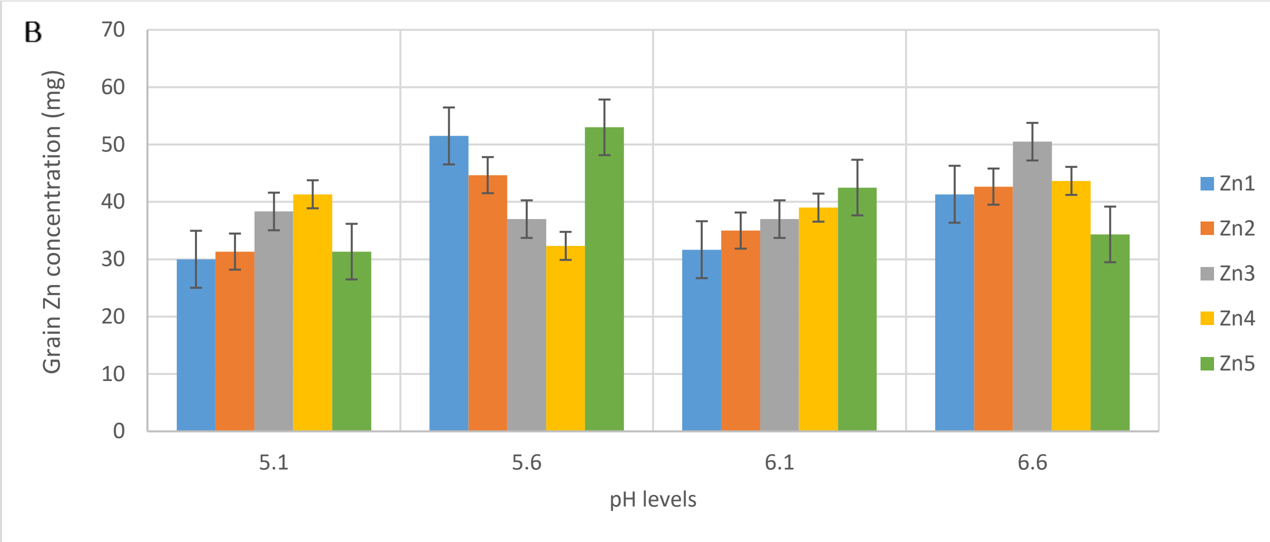


Figure 4.18: Effect of soil pH and zinc fertilizer on grain Zn concentration (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.3.12 Grain protein content

The results of the influence of soil pH and Zn fertilization on the accumulation of protein content in wheat grain are presented in Figures 4.19 A and B. Grain protein content is particularly important in defining wheat grain quality because a high protein content (>11 %) is a quality measure required by commercial bakeries to grade the bread produced to guarantee that it meets consumer standards (Killian & Burger, 2016). The grain protein content showed a similar trend as the grain concentration in response to lime and Zn application. Grain protein content was significantly increased by Zn4:6.5 mg/kg (pH5.1) at planting and Zn2:4.5 mg/kg (pH5.6) at flowering, which also reached a grain protein content of 11%. The result also indicates a progressive dip in grain protein content from Zn2 with increasing Zn application when it was applied at flowering.

Moreover, the protein content showed the same trend as grain N concentration regarding lime and Zn applications, because one is the direct function of the other. Fageria, (2009) reported that a plant deficient in Zn experiences reduction in protein synthesis, because Zn is closely involved in the N metabolism of plant.

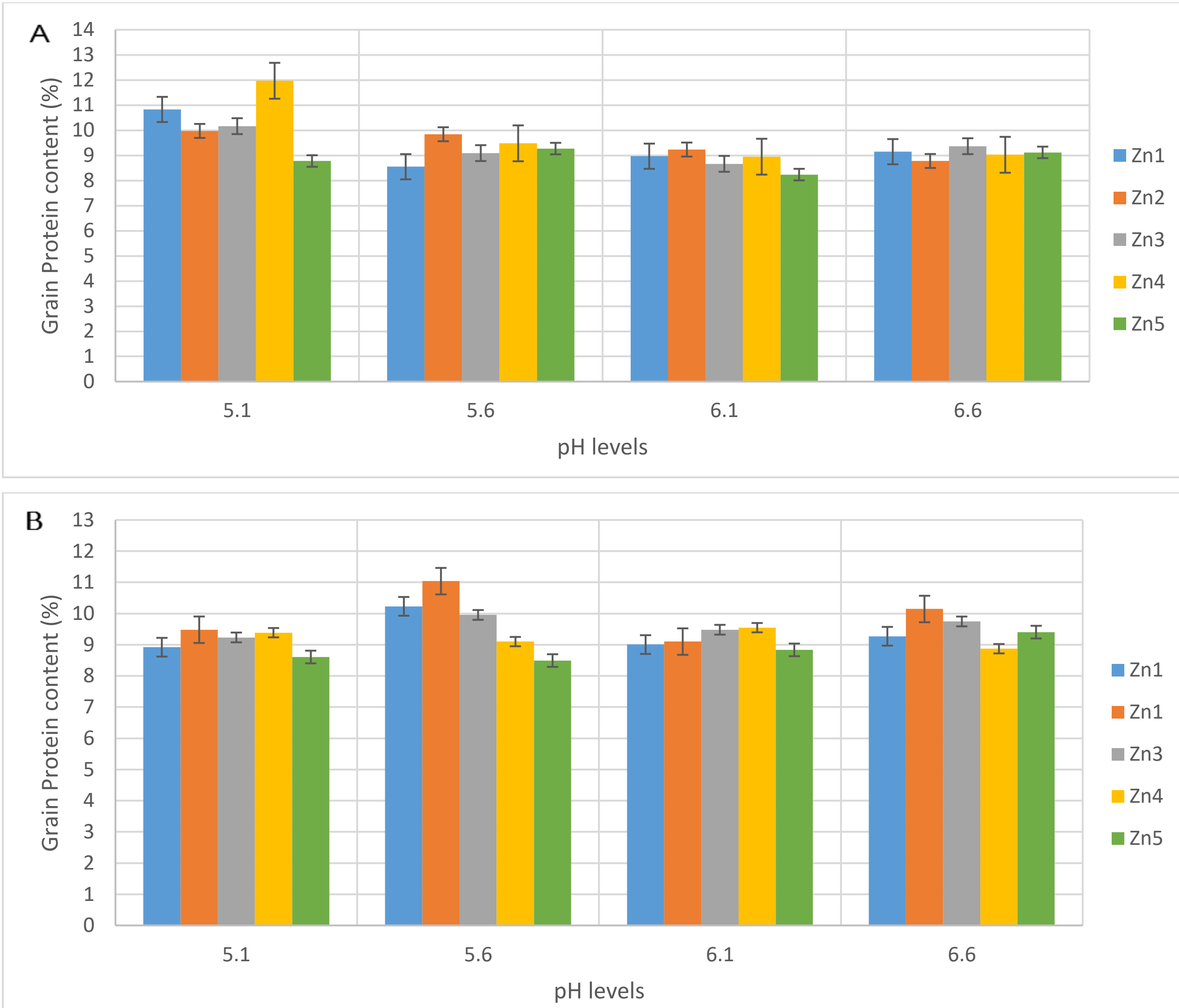


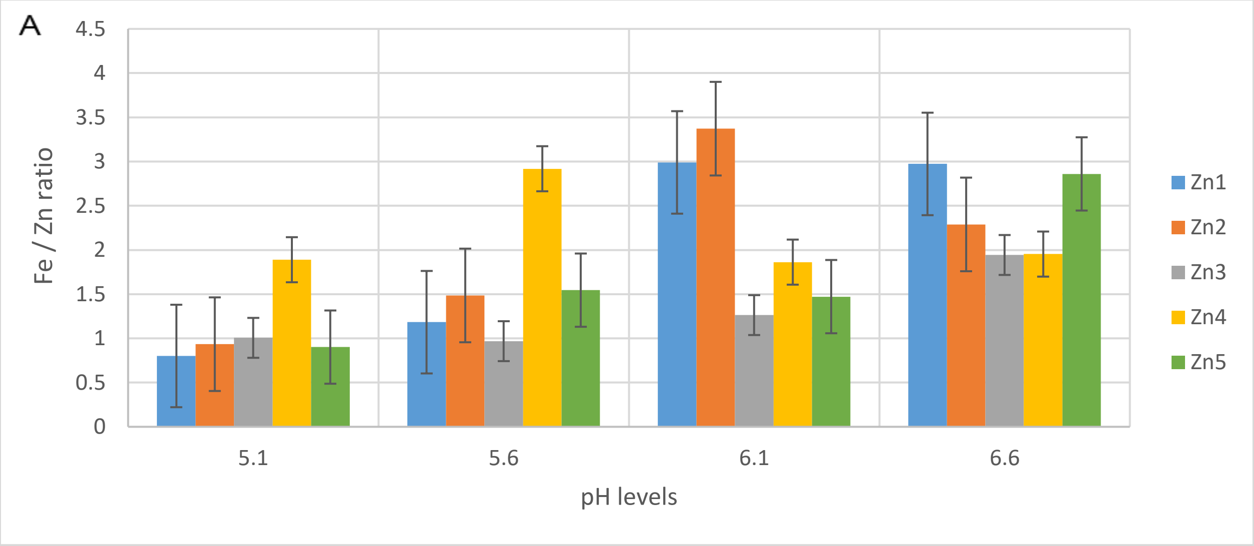
Figure 4.19: Effect of soil pH and zinc fertilizer on grain protein content (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

4.3.13 Iron-to-zinc ratio

The results of the influence of soil pH and Zn fertilization on the iron-to-zinc ratio in wheat grain are presented in Figures 4.20 A and B. Early reports indicate that the actual bioavailability of Fe and Zn could be affected by the interactions between these two micronutrients and an iron-to-zinc ratio above 2 has been documented to inhibit the absorption of zinc (Lewu *et al,* 2012).

The results of the present study showed that at both planting and flowering, iron-to-zinc ratio is below 2 at pH 5.1 and 5.6 for all Zn levels except Zn4 (pH5.6). While at pH 6.1, the iron-to-zinc ratio was above 2 for low Zn level (Zn1 and Zn2). At pH6.6, iron-to-zinc ratio was above 2 for all Zn levels except Zn3 and Zn4 at planting and only Zn3 when flowering. This implies that the availability of Zn in the grain was promoted at pH 5.1 and 5.6 at all Zn levels applied and at both planting and flowering, with an exception of Zn4 when applied at planting. While, only high Zn levels (Zn3, Zn4 and Zn5) at pH 6.1, and Zn3 and Zn4 at pH6.6 were able to increase Zn availability in grain.

These results indicate that iron-to-zinc ratio recorded for Zn3 was below 2 at all pH levels and both stages. This implies that Zn absorption in grain can be ensured when Zn applied is at 5.5mg/kg at any stage and at a pH between 5.1 and 6.6. The results showed that Zn was available in the grain at low pH and reduced when pH or lime application increased. Zinc availability is highly dependent on pH (Fageria, 2009 and Hafeez *et al.,* 2013). The figure 4.20 A and B revealed that Zn3:5.5mg/kg promoted Zn absorption in the grain at all pH levels and at both planting or flowering (for having the iron-to-zinc ratio below 2). In addition, grain Zn concentration was above 40mg/kg at all pH levels when Zn3 was applied at planting. According to Harsh (2010), Zinc deficiency in wheat can be best alleviated with the use of 11 kg Zn/ha (or 5.5 mg/kg of soil), which corresponds to Zn3 in our study.



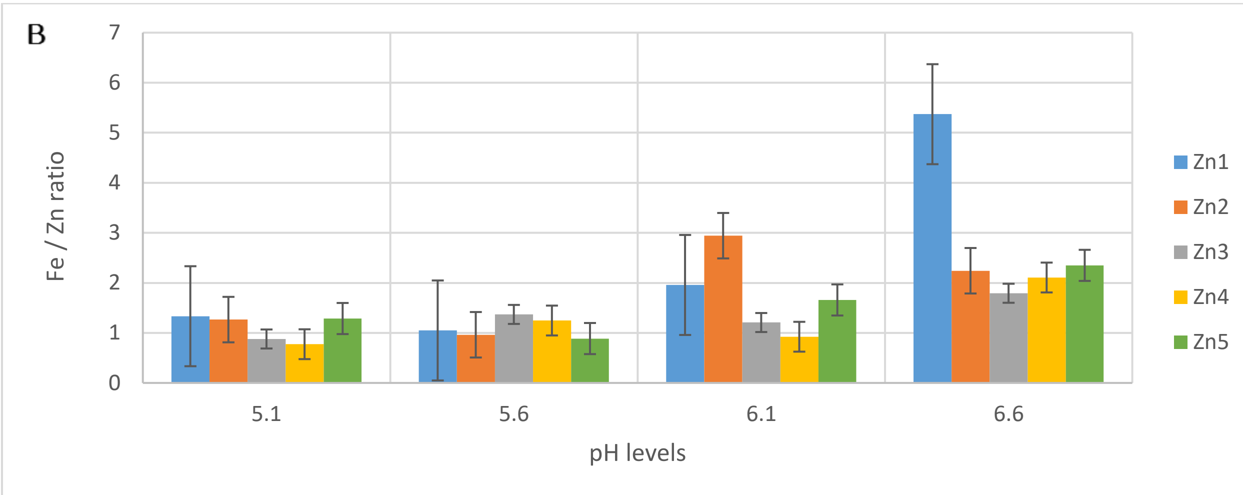


Figure 4.20: Effect of soil pH and zinc fertilizer on Fe / Zn ratio (A) at planting; (B) at flowering. The overlap between two error bars shows that there were no significant differences between the two treatments while no overlap shows that there were significant differences.

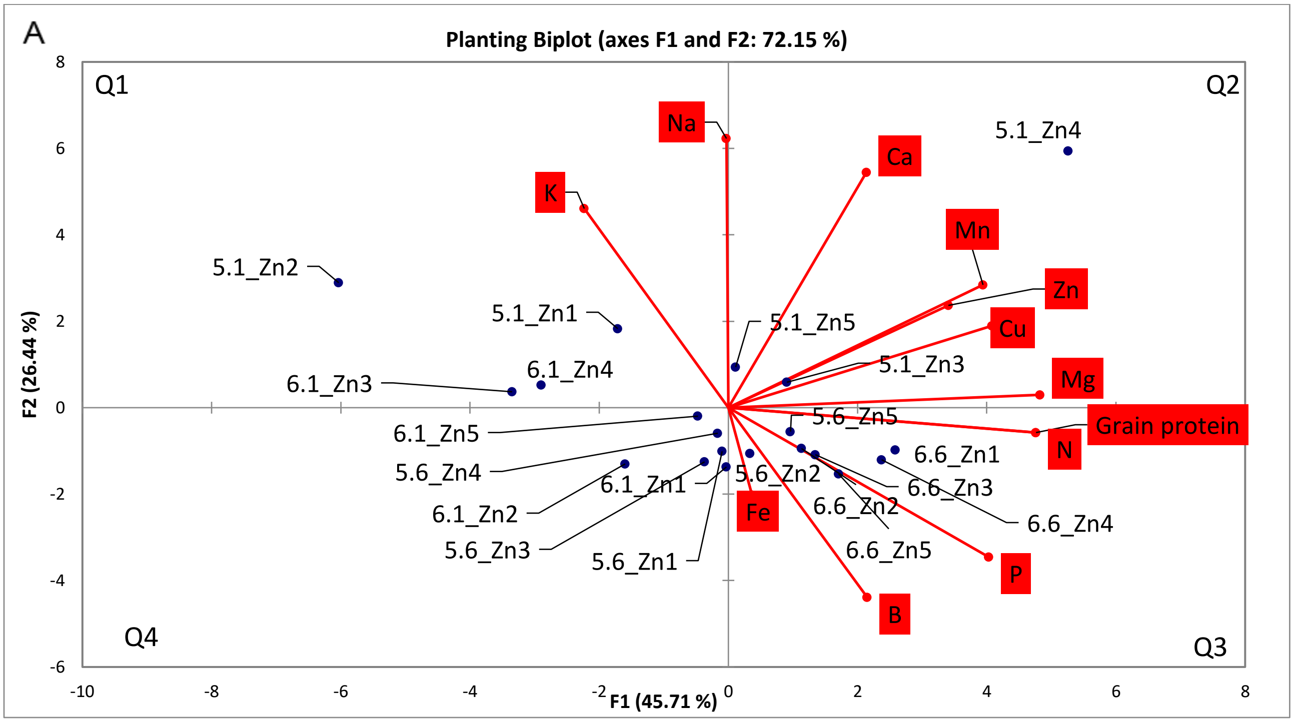
4.4 Relationship between pH levels, Zn levels and nutrient concentrations in grain.

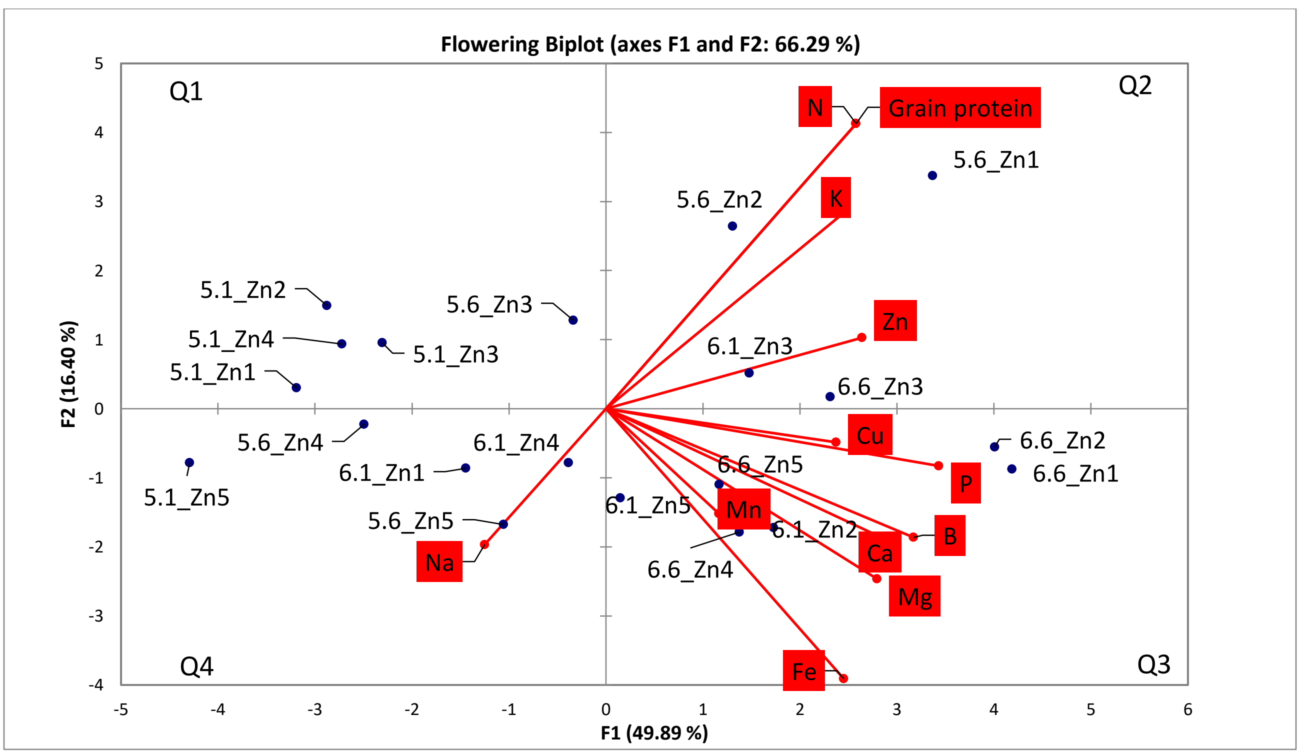
To investigate the relationship between nutrient concentrations in grain as affected by soil pH and Zn fertilizer, data of this study were subjected to Principal Component Analysis (PCA).

The influence of pH and zinc levels on nutrients is depicted in Figure 4.21 A and B below. Figure 4.21 A (Q2 and Q3) show that when Zn is applied at planting, the concentration of most elements in wheat grain – including Na, Ca, Mn, Zn, Cu, Mg and N – increased the highest under pH 5.1 at Zn4:6.5mg/kg soil; whilst P, B, and Fe are majorly increased under pH 6.6 at all Zn levels. There were also strong and positive correlations between Zn and Mn and between N and protein content while K and B were strongly and negatively correlated.

However, for zinc applied at flowering, there was no increase in elemental concentrations under pH 5.1 (see Figure 4.21 B, Q1). Under pH 5.6, Zn1 and Zn2 (3.5 and 4.5 mg/Kg of soil) increased the concentrations of N, K and Zn in wheat grain (see Fig 4.21 B, Q2). The concentrations of the Cu, P, Ca, Mg, Mn, Fe and B were increased under soil pH 6.6 (for most Zn levels) and under pH 6.1 (for majorly Zn2: 4.5 mg/kg and Zn5: 7.5 mg/kg of soil) (see Fig 4.21 B, Q3) and Na concentration under pH 5.6 especially at Zn5 (see Fig

4.21 B, Q4). Figure 4.21 B also revealed strong and positive correlations between Cu and P and between Ca and B while N and Na were strongly and negatively correlated.





Q=quadrant

Figure 4.21 (A): The PCA biplot for stage planting, (B): The PCA biplot for stage flowering.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In summary, yield and yield components were positively affected by lime application rate 1t/ha or pH 6.1 at all Zn levels when Zn was applied at planting. At flowering, liming negatively affected yield and yield components. However, Zn application had little to no effect on the yield and yield components when applied at planting, while at flowering, the simultaneous increase in lime and Zn levels significantly increased yield and yield components. Although Zn application at flowering was beneficial to the yield and yield components than when applied at planting, their performances were not statistically different, but Zn application at planting promoted plant growth than at flowering.

The concentrations of most nutrients including P, K, Ca, Na, Mg, Fe, Cu and B were significantly increased by pH6.6 followed by pH 6.1 (lime application rate 1.5t/ha and 1t/ha). Unlike the other nutrients, N, Zn and Mn decreased as lime rate increased. The application of Zn had no effect on most nutrients due to the presence of lime. However, at the absence of lime which is pH5.1, Zn4: 6.5mg/kg (corresponding to 13kg/ha) significantly increased the nutrients. In addition, Zn3: 5.5mg/kg (corresponding to 11kg/ha) which happens to be the recommended Zn application rate, promoted Zn absorption by grain in all treatments.

5.2 Recommendation

This study has shown that the application of lime at 1t/ha (pH6.1) has increased yield, yield components, while the application greater that rate considerably reduced the yield. It was probably due to the deficiency of N, caused by the over-liming effect. Lime application rate 1,5 t/ha increased most nutrients in the grain but induced N and Zn decrease. In fact, Zn action was inhibited by the lime. However, Zn application rate Zn3: 5.5mg/kg increased most nutrients in the grain. Therefore, we recommend lime application rate 1t/ha (pH6.1) and Zn application rate Zn3: 5.5mg/kg for wheat cultivation in sandy soil with timely amendment of N.

Numerous studies have endeavored to fight zinc deficiency in crops and have found Zn fertilization a reliable method to increase this mineral in crops. However, zinc fertilization under acidic soils has always been a delicate topic. In the case of our study, we conducted a pot experiment which might have resulted in severe root restriction and limited nutrient availability as well as root binding. And the soil material used was a mixture of top soil and sand which is less acidic than the soil alone. In addition, only one cultivar was tested. For these reasons we would recommend that further research be done under natural field conditions to confirm our findings.

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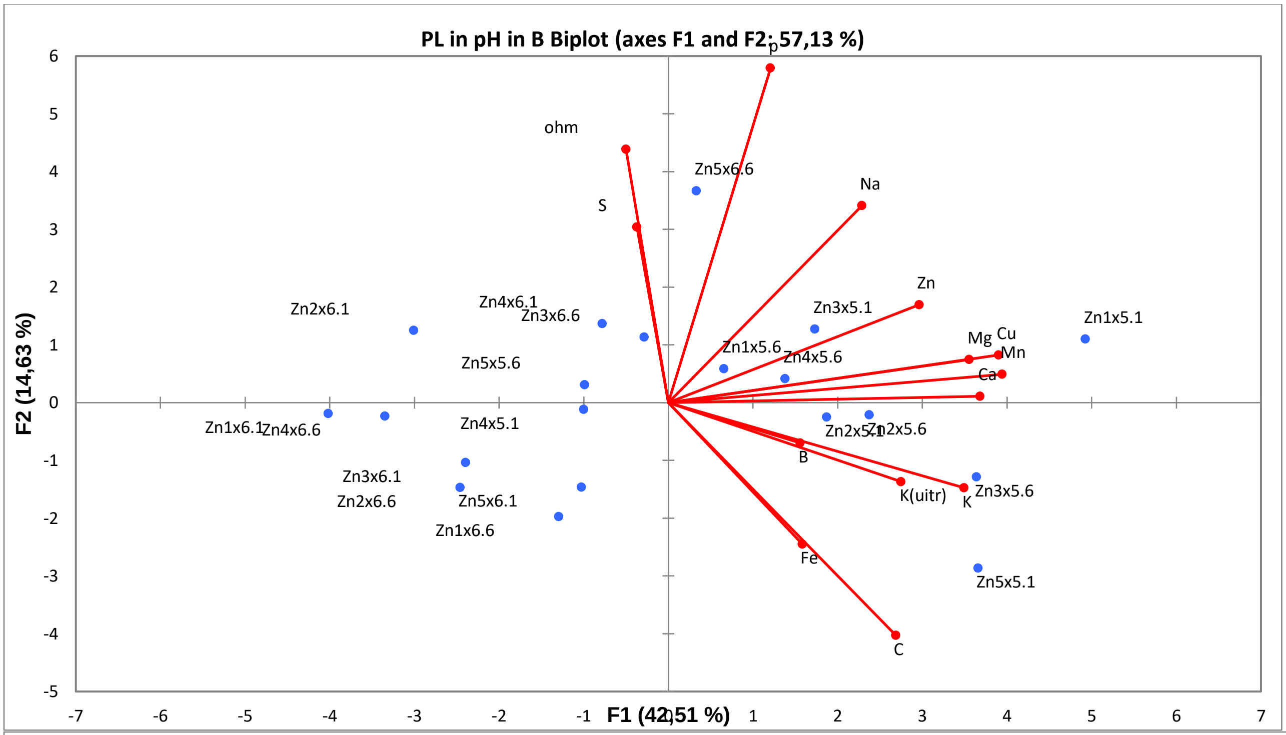
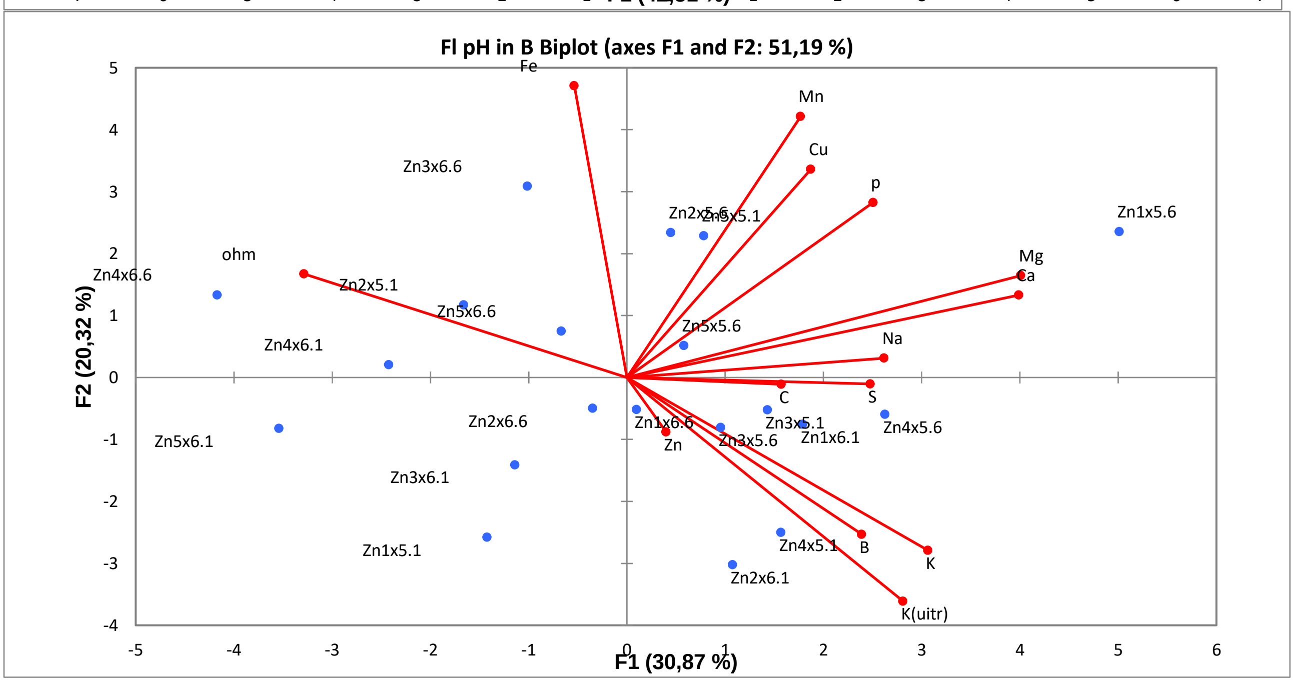
APPENDICES

Appendix 1: Chemical analysis of soil treatments at the end of experiment

Stage	pH Level	Zn	pH (KCl)	P (mg/kg)	K	Na (cmol/kg)	Ca	Mg	Cu (mg/kg)	Zn	Mn	B	Fe	C (%)	S (mg/kg)
Planting	**6.6**	**Zn1**	6.6	49	13	0.06	1.38	0.37	0.6	3.2	6.6	0.24	65	0.36	7.04
	6.6	**Zn2**	5.6	39	12	0.06	1.04	0.31	0.5	3.3	5.6	0.22	61	0.35	5.85
	6.6	**Zn3**	5.6	39	10	0.06	1.08	0.35	0.5	3	5.9	0.21	51	0.32	6.87
	6.6	**Zn4**	5.9	34	10	0.05	1.1	0.31	0.4	1.6	4.6	0.2	72	0.26	7.27
	6.6	**Zn5**	6.2	15	15	0.06	1.16	0.33	0.5	2.4	6.4	0.2	74	0.49	6.82
	6.1	**Zn1**	5.8	37	10	0.06	1.06	0.33	0.5	1.7	5.3	0.2	68	0.3	6.81
	6.1	**Zn2**	5.9	41	13	0.06	1.16	0.34	0.5	2	5.8	0.2	67	0.38	7.01
	6.1	**Zn3**	5.6	36	13	0.06	1.05	0.32	0.6	2.4	7.4	0.2	132	0.45	6
	6.1	**Zn4**	5.9	36	11	0.06	1.08	0.33	0.5	1.9	5.6	0.2	48	0.42	7.77
	6.1	**Zn5**	5.5	34	10	0.06	0.92	0.31	0.4	1.1	4.2	0.2	45	0.37	6.89
	5.6	**Zn1**	5.4	29	9	0.05	0.76	0.29	0.3	0.7	3.6	0.21	40	0.29	7.02
	5.6	**Zn2**	5.3	34	9	0.06	0.75	0.29	0.4	1	4	0.2	53	0.24	6.66
	5.6	**Zn3**	5.4	31	10	0.05	0.88	0.29	0.4	1.4	4	0.19	91	0.31	6.66
	5.6	**Zn4**	5.6	38	9	0.06	0.94	0.29	0.4	3.8	5.3	0.19	96	0.28	7.24
	5.6	**Zn5**	5.2	33	11	0.05	0.86	0.32	0.4	1.4	4.2	0.2	81	0.32	6.12
	5.1	**Zn1**	5.3	26	10	0.05	0.82	0.31	0.4	0.8	4	0.23	70	0.4	7.62
	5.1	**Zn2**	5.3	34	9	0.05	0.95	0.28	0.4	1.4	4.4	0.2	42	0.32	6.81
	5.1	**Zn3**	5.5	39	11	0.06	0.99	0.31	0.4	1.7	5	0.21	44	0.29	6.56
	5.1	**Zn4**	5.2	25	10	0.06	0.77	0.28	0.3	0.7	3	0.19	37	0.31	6.81
	5.1	**Zn5**	5.2	60	12	0.06	0.9	0.31	0.5	1.9	5.4	0.19	46	0.29	8.22
Flowering	**6.6**	**Zn1**	5.1	26	10	0.05	0.63	0.25	0.2	0.7	2.8	0.19	33	0.4	5.65
	6.6	**Zn2**	5.1	28	9	0.05	0.7	0.26	0.3	1.5	3	0.18	53	0.36	5.55
	6.6	**Zn3**	4.9	29	12	0.06	0.72	0.3	0.3	1.1	3.1	0.19	41	0.33	5.72
	6.6	**Zn4**	5.2	30	10	0.06	0.79	0.28	0.2	3.2	2.6	0.2	37	0.36	7.05
	6.6	**Zn5**	4.9	35	9	0.06	0.85	0.3	0.3	1.9	3.3	0.19	50	0.14	5.53
	6.1	**Zn1**	5.2	38	12	0.06	1.01	0.31	0.3	1.1	4	0.19	52	0.4	7.52
	6.1	**Zn2**	5.1	29	9	0.06	0.79	0.3	0.3	0.8	3.2	0.17	55	0.36	6.54
	6.1	**Zn3**	5	27	13	0.06	0.71	0.29	0.3	0.9	3	0.17	39	0.26	6.36
	6.1	**Zn4**	5.1	31	11	0.06	0.94	0.29	0.3	1.4	3	0.19	37	0.31	6.65
	6.1	**Zn5**	5	30	10	0.06	0.77	0.28	0.3	0.9	3	0.19	49	0.32	6.62
	5.6	**Zn1**	4.9	27	11	0.06	0.67	0.29	0.3	1.1	3.4	0.21	46	0.25	6.13
	5.6	**Zn2**	4.9	31	12	0.06	0.65	0.27	0.2	0.9	2.5	0.2	34	0.3	6.61
	5.6	**Zn3**	5	31	11	0.05	0.66	0.26	0.2	1.1	3.2	0.18	34	0.24	4.59
	5.6	**Zn4**	4.7	29	9	0.05	0.56	0.23	0.3	1.9	2.9	0.18	47	0.26	5.84
	5.6	**Zn5**	4.7	27	9	0.05	0.55	0.24	0.2	1.7	2.8	0.18	40	0.19	6.64
	5.1	**Zn1**	4.8	28	11	0.05	0.64	0.26	0.3	0.9	3.1	0.19	47	0.38	6.98
	5.1	**Zn2**	4.8	30	10	0.06	0.61	0.27	0.3	0.7	3	0.19	38	0.26	5.49
	5.1	**Zn3**	4.7	33	8	0.05	0.64	0.28	0.3	1	3.8	0.18	52	0.35	5.74
	5.1	**Zn4**	4.5	28	8	0.06	0.5	0.24	0.2	0.6	2.9	0.17	59	0.25	5.41
	5.1	**Zn5**	4.6	31	8	0.06	0.59	0.26	0.3	1.3	3.3	0.19	36	0.29	6.85

Appendix 2: Distribution of mineral element in the soil affected by the combination of lime application (pH level), zinc level and zinc time of application





Appendix 3a: Significance levels (Pr>F) of selected yield components of wheat as affected by main effect (pH level, zinc level and Stage) and the interactions between mains effects

Source	Probability > F				
	Nbr Tillers 52DAP	Nbr Tillers 80DAP	Plant height (cm)	Head weight/ plant (g)	Shoot weight/ plant (g)
pH	**0.0043**	**< 0.0001**	0.1082	**0.0414**	**0.0147**
Stage	0.1755	0.2415	**0.0122**	0.1863	0.0675
pHxStage	0.934	0.5338	**0.0034**	0.2304	0.2392
ZnTrt	0.1145	0.3218	0.6189	0.9426	0.9694
ZnxpH	0.6129	0.5431	0.0623	0.1525	0.0536
ZnxStage	0.2227	0.2373	0.0664	0.6031	0.6029
ZnxpHxStage	0.0608	0.0889	0.387	0.6337	0.1149

Numbers in bold are significant at 5%

Appendix 3b: Significance levels (Pr>F) of selected yield components of wheat as affected by main effect (pH level, zinc level and Stage) and the interactions between mains effects

Source	Probability > F					
	Nbr heads/plant	Nbr grains/plant	Biomass	1000 grains weight/plant	Harvest Index	Grain yield
pH	0.1291	0.3163	0.1107	**<.0001**	**<.0001**	**0.0031**
Stage	**0.0399**	0.4352	0.0634	0.9894	0.9034	0.5518
pHxStage	0.3657	**0.0035**	0.2031	0.075	0.9728	0.1622
ZnTrt	0.9873	0.7018	0.8241	0.9848	0.5897	0.9868
ZnxpH	0.2533	0.5104	**0.0464**	0.7307	0.1638	0.2681
ZnxStage	0.8806	0.2237	0.4208	0.1177	0.2942	0.3695
ZnxpHxStage	0.07	0.1207	0.2768	0.269	0.0523	0.1979

Numbers in bold are significant at 5%

Appendix 4: Significance levels (Pr>F) of the nutrient concentrations in the different parts of wheat as affected by main effects (pH level, Zinc level and Stage) and the interactions between mains effects

Source	Probability > F

Grain												
	N	P	K	Ca	Mg	Na	Mn	Cu	Grain Klity	Fe	Zn	B
pH	0.0947	**<.0001**	0.2818	0.5407	0.0996	0.9692	0.291	0.1809	0.0947	**<.0001**	**0.0171**	**<.0001**
Stage	0.8239	0.8619	0.62	0.2809	0.1378	0.1741	0.2764	**0.0119**	0.8239	0.0665	**0.0038**	**0.0007**
pHxStage	**0.0022**	0.2621	0.0476	0.2017	0.0347	**0.0491**	**0.0057**	0.15	0.0022	0.6635	**0.0155**	0.1301
ZnTrt	**0.0218**	0.3504	0.8436	0.5308	0.4932	0.5391	0.6795	0.7653	**0.0218**	0.2505	0.7012	**0.0069**
ZnxpH	0.2693	0.5714	0.297	0.7462	0.2166	0.6433	0.2276	0.2972	0.2693	0.1046	0.4477	0.7166
ZnxStage	0.3248	0.4843	0.4935	0.5296	0.3272	0.4453	0.186	0.5165	0.3248	0.323	0.6689	0.1787
ZnxpHxStage	0.4127	0.9615	0.4713	0.6834	0.3265	0.7074	0.2483	0.3781	0.4127	0.8608	0.6247	0.0773
Shoot												
pH	**<.0001**	**<.0001**	**0.0215**	**0.0075**	**<.0001**	**<.0001**	**0.0912**	**0.0086**	**<.0001**	**<.0001**	**0.0054**	0.2529
Stage	**0.0345**	**0.0257**	0.1792	0.8298	**0.0256**	0.3519	**0.0275**	0.2783	**0.0345**	0.967	**<.0001**	0.9991
pHxStage	**0.0058**	**0.0143**	**0.0002**	0.1737	0.0935	0.3949	0.4629	0.6422	**0.0058**	**0.0186**	**0.0002**	0.7149
ZnTrt	**0.0094**	**0.0001**	**0.0261**	0.364	0.6742	**0.0039**	0.0942	0.3846	**0.0094**	**0.0009**	0.1833	0.1484
ZnxpH	0.2452	0.0542	**0.0322**	0.6376	0.9335	0.0622	0.0788	**0.0242**	0.2452	0.3528	0.7053	0.4438
ZnxStage	0.1401	0.7572	0.5667	0.2431	0.4822	**0.0018**	0.3714	0.1395	0.1401	**0.0155**	**0.0083**	**0.0076**
ZnxpHxStage	**0.0328**	0.1855	0.1022	0.274	0.6615	0.1213	0.9266	0.2031	**0.0328**	0.115	0.6155	0.2428

Numbers in bold are significant at 5%