Wheat Response to Soil-Applied Micronutrients and Relationships Among Soil and Tissue Tests

by

Mosaed Abdullah Majrashi

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Major Professor
Dorivar Ruiz Diaz

Abstract

Optimum plant growth under field conditions requires adequate levels of essential nutrients. The objectives of this study were; i) to determine the effect of micronutrient fertilizer application on the concentration of macro and micronutrients in winter wheat plant tissue, and ii) investigate the relationship between soil test parameters and concentration of macro and micronutrients in plant tissue. The study was conducted at six locations in 2012 and 2013 in Kansas. The experimental design consisted of two treatments in a randomized complete block design with three replications. The treatments were applied in field-long strips approximately 364 meters (1,200 feet) long and a minimum of 12 meters (40 feet) wide. The treatments included a fertilized strip and a control strip. The study was initially established to evaluate micronutrients with no P, and K fertilizer applied. The fertilized strips included N, Zn, Mn, Cu (11.2 $kgha^{-1}$), and B (2.8 $kgha^{-1}$). Soil samples were collected at planting from points marked with flags located every 30 meters along each strip. Soil samples were collected at the 0 to 15-centimeter depth with 15-20 cores per sample from around each flag in about a five-meter radius. Tissue samples were also collected in a five-meter radius of each flag. Wheat flag leaves were collected at flowering with at least 30 leaves per sample. Soil samples were analyzed for pH, organic matter, soil test phosphorus, potassium, boron, copper, iron, manganese, and zinc. Tissue samples were also analyzed for nitrogen, phosphorus, potassium, sulfur, copper, iron, manganese, and zinc. A complete analysis was done for each location as well as across all study locations using the Proc Mixed procedure in SAS. The micronutrient fertilizer application did not significantly (at P-value level <0.05) influenced tissue N, P, and K but increased S, Zn and Cu tissue concentration across all locations. Manganese tissue concentration was not affected by the application of Mn fertilizer application. Soil test Cu, Fe, and Mn showed good correlation

with soil pH and soil test Zn with soil OM. However, only Cu and Mn in the wheat tissue show correlation to soil test for these nutrients. These results suggest that micronutrient concentration in the tissue is governed by multiple soil factors and only partially by DTPA extractable micronutrients. Results from this study also showed that tissue analysis could reflect fertilizer application and availability of micronutrients to the plant. However, there was significant variability in tissue analysis, likely affected by abiotic factors influencing plant nutrient uptake and concentration. While tissue analysis can help as diagnostic tool, producers should be aware of the limitations, and decisions on fertilizer recommendations cannot be based exclusively on tissue test.

Table of Contents

List of Figures

List of Tables

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Dedication

I would like to dedicate this thesis to my family members especially my father and mother. Thanks for all of the love and support.

Chapter 1- Introduction and thesis organization

The mineral soil and organic matter are the main sources of essential macro and micronutrient such as nitrogen (N), phosphorus (P), potassium (K), sulfur (S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and boron (B). Through mineral weathering and mineralization from organic matter, these nutrients are released into the soil solution and available for plant uptake (Harry and Benton, 1996). Organized and well-timed management actions are necessary to improve the nutrient availability and overall fertility of agricultural soils. Increasing crop yields should involve the economically feasible and efficient use of fertilizers, including organic wastes and crop residues (Smaling, 1993; Van, 1996).

Nutrients are taken up by the roots as cations and anions (Marschner, 1995). Many factors, such as soil moisture, pH, cation-exchange capacity, and fertilizer application, may affect the mineral forms present in the soil solution and therefore nutrient uptake by plant roots. A change in nutrient uptake will directly impact yield response (Asher, 1978; Marschner, 1995). Soil moisture, pH, organic matter and cation-exchange capacity are known to affect the availability of micronutrients such as Zn, Mn, Fe, and Cu (Jenne, 1968). According to Shuman (1998), soil pH has the most influence on micronutrient availability, and generally, lower soil pH results in higher micronutrient availability. This effect of soil pH is opposite for Molybdenum (Mo) which generally increase in availability at higher soil pH.

Nitrogen is often the most limiting nutrient for wheat production. Most of the N in soil is found in organic forms, and its mineralization depends on soil and climatic factors that constantly vary during the growing season (Fageria et al., 1991). Nitrogen loss is also a potential limitation in many soils and environments including the risk of leaching, denitrification, and immobilization by microorganisms. These potential losses further complicate the development of an effective soil test for available nitrogen (Dahnke and Vasey, 1973).

Phosphorus is another essential element and is typically the second-most limiting nutrient for crop production (Raghothama, 2005). Phosphorus plays a crucial role in energy transport and storage, nucleotides, phospholipids, and certain coenzymes. Stunted and delayed maturity are common symptoms of P deficiency in all plants, and tillering are typically reduced in sorghum and wheat. Root growth and nutrient uptake are also affected by P deficiency since energy cannot be easily transported. Phosphorus is highly mobile within the plant and will accumulate in young leaves, flowers, and seeds (Harry and Benton, 1996). However, mobility in the soil is limited and is consider an immobile nutrient in the soil.

Potassium does not form stable compounds in plants; instead, it is found as K^+ ions. One main function of K appears to be in maintaining ionic strength and ionic balance in the cells. Also, over 80 enzyme systems require K for activation. Potassium also plays a crucial role in plant-water relations through the maintenance of osmotic potential and regulation of stomata opening (Harry and Benton, 1996).

Approximately 90% of the sulfur (S) in plants can be found in the amino acids, cysteine, and methionine (Ravanel et al., 1998). Deficiency of S leads to yellowing, spindly, stunted, and chlorotic plants, similar to N deficiency. However, S is much less mobile than N in the plant, and early stages of deficiency tend to appear at the newest growth (Freney et al., 1978). Sulfur deficiency in wheat presents as yellowing of young tissue, stunting, and limited tillering. The distribution of S in the tissue of S-deficient plants can be affected by the nitrogen supply. Sulfur deficiency symptoms can occur either in young or old leaves (Robson and Pitman, 1983). The extent of remobilization and re-translocation from older leaves can be affected by the nitrogen supply.

Micronutrient deficiency had become a significant constraint for crop production in some soils and production systems. The deficiency may either be primary, due to low micronutrient levels in the soil, or secondary, caused by soil factors that reduce the availability of micronutrients to plants (Sharma and Chaudhary, 2007). Induced stress in plants leads to low crop yield and quality. Change in plant morphological structure, such as fewer xylem vessels of smaller size, infestations of diseases and pests, and reduced efficiency of fertilizer use are also some of the leading adverse effects of micronutrient deficiency (Malakouti, 2008). Kumar et al. (2009) reported that copper (Cu) and its interactions with other micronutrients, such as the Fe, Mn, Zn can affect the growth and yield of wheat. Excess Cu may also induce the deficiency of other micronutrients and adversely affect yield.

In recent years, the use of tissue analysis as a diagnostic tool has increased, and questions remain about its reliability for some micronutrients. Khan et al. (2006) reported that the application of mineral fertilizers was directly correlated with tissue analysis of Cu, Fe, Mn, and Zn, in the leaf, straw, and grains of wheat. Soleimani et al. (2006) found that application of Zn affected the Mn and Cu concentration of wheat grain. Arif et al. (2006) advocated for foliar application of nutrient solutions at tillering, jointing, and boot stages to increase yield and grain quality of wheat.

Iron is another essential micronutrient for plant growth, and deficiency for human nutrition is perhaps the most widespread nutrient deficiency in the world. Which is estimated to affect over 2 billion people (Stoltzfus and Dreyfuss, 1998). Zinc deficiency for human nutrition is also widespread, especially in sub-Saharan Africa and South Asia. It has been estimated to account for 800,000 deaths among children every year (Micronutrient Initiative, 2006). Therefore, there are concerns about low Fe and Zn content in the wheat grain in addition to any potential reduction in grain yield due to micronutrient deficiencies in the plant (Shewry, 2007).

Thesis organization

This thesis contains three chapters. The first chapter provides an overall introduction and thesis organization. Chapter 2 includes a complete manuscript with the title “Wheat response to micronutrients and relationships among soil and tissue tests”. And Chapter 3 provides overall conclusions and summary of this thesis.

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Chapter 2- Wheat response to soil-applied micronutrients and relationships among soil and tissue tests

Abstract

Plant growth in production fields requires adequate amounts of available nutrients, including macro and micronutrients. The objectives of this study were to; i) determine the effect of micronutrient fertilizer application on tissue nutrient concentration in winter wheat (*Triticum aestivum*), and ii) explore the relationship among soil test and tissue analysis for winter wheat. This study was conducted at six locations during the 2012-13 and 2013-14 wheat growing season in Kansas. The experimental design consisted of two treatments in a randomized complete block design with three replications. The treatments were applied in field-long strips of approximately 364 meters (1,200 feet) long and a minimum of 12 meters (40 feet) wide. The treatments included a fertilized strip and a control strip. The fertilized strips received nitrogen (N), zinc (Zn), manganese (Mn), copper (Cu) (11.2 $kgha^{-1}$), and boron (B) (2.8 $kgha^{-1}$). Soil samples were collected at planting from points marked with flags located every 30 meters along the center of each strip. Soil samples were collected at the 0 to 15-cm depth with 15-20 cores per sample from around each flag in about a five-meter radius. Tissue samples were also collected in a five-meter radius of the flags. Wheat flag leaves were collected at flowering (at least 30 leaves per sample). Soil samples were analyzed for pH, organic matter, soil test phosphorus (P), potassium (K), iron (Fe), Cu, Mn, and Zn. Tissue samples were also analyzed for total N, P, K, Cu, Fe, Mn, Zn and sulfur (S). The micronutrient fertilizer application did not significantly (at *P*-value level <0.05) influence tissue N, P, and K but increased S, Zn and Cu tissue concentration across all locations. Manganese tissue concentration was not affected by the application of Mn fertilizer application. Soil test Cu, Fe, and Mn showed good correlation with soil pH and soil test Zn with soil OM. However, only Cu and Mn in the wheat tissue showed correlation to soil test

for these nutrients. These results suggest that micronutrient concentration in the tissue is governed by multiple soil factors and only partially by DTPA extractable micronutrients.

Introduction

Most soils may provide sufficient levels of micronutrients that are needed in small amounts for yield and grain quality in wheat. However, some soils are deficient in essential micronutrients and can show a significant response to fertilizer application (Tandon, 1995). The macronutrients and micronutrients that are involved in critical plant metabolic processes include N, Cu, Mn, and Zn where the other micronutrients can improve yield by affecting the cell physiology (Adediran et al., 2001; Adediran et al., 2004). Deficiency of any of these nutrients can affect essential biochemical processes and limit crop productivity (Sing et al., 2013; Wojtkowiak and Stepien, 2015). According to Ahmadikhah et al. (2010), in many Asian countries, calcareous soils with low organic matter and imbalanced application of N, P, K fertilizers are resulting in micronutrient deficiency in wheat. Micronutrient deficiency may be due to a primary factor (low nutrient content of the soil) or may be caused by a secondary factor (soil factors that reduce the availability to plants) (Sharma and Chaudhary, 2007).

Factors that can impact the biochemical processes for plant growth can also affect micronutrient uptake (temperature, light, water) (Foth and Ellis, 1988; Jones and Olsen-Rutz, 2016; Bell and Dell, 2008; Sud et al., 1995). Plant availability of soil micronutrients can be affected by soil properties such as organic matter, pH, calcium carbonate content, and total micronutrients concentrations (Schuin et al., 2009).

Both availability and solubility of micronutrients in the soil is influenced by Soil pH and organic matter influence. While soil is the most referenced source of plants nutrients, their micronutrient uptake is impacted by the competition of major nutrient uptake due to either negative or positive interaction (Fageria, 2001). The availability of Mn, Fe, Cu, Zn, and B tend to decrease drastically (Essington, 2004) under the influence of elevated pH. Soil erosion over time in most of the agricultural soils have shown a reduction of soil organic matter, which is a major source of micronutrients. This reduction in soil organic matter might lower the availability of micronutrients in the soil. In Kansas, micronutrient deficiencies are not common in wheat (Widmar, 2013). However, it is possible to see a response from other additional available soil nutrients to the plant. Tissue nutrient analysis provides information about the nutrients content of the plant at a given point in time (Ritchey, 2011) and more often, serve as a better indicator of secondary and micronutrients than soil testing. In general, the nutrient sufficiency for wheat ranges (for various growth stages) are: Fe 30-200 mg kg^{-1}, Mn 20-150 mg kg^{-1}, Zn 15-70 mg kg^{-1}, Cu 5-25 mg kg^{-1}, and B 1.5-4.0 mg kg^{-1} (Jones, 1967). For example, copper uptake by wheat plants can be affected by the interaction between the Cu application and soil components in certain soil temperatures over the range 10-30 ^{o}C; results indicated that less copper was taken up by wheat plants that had been estimated by Brennan et al. (1984).

Another study from Li et al. (2007), reported the importance of organic matter as a contributor to the availability of micronutrients for the crop and to increase the concentration of Zn, Fe, and Mn in the soil. However, the study showed that organic matter had little influence on available Cu. Graham et al. (1999) found that zinc fertilizer application to a soil with a low zinc content at planting time can significantly increase the zinc concentration in the grain as well as yield in wheat. Some studies have shown increases in zinc concentration by the three times the original concentration with no fertilizer Zn application (Ranjbar and Bahmaniar, 2007; Yilmaz et al., 1997). Increased Zn and other micronutrients in the grain can play a crucial role in biofortification and improved human nutrition in some regions.

The purpose of this study were to; i) determine the effect of micronutrient fertilizer application on tissue nutrient concentration in winter wheat (*Triticum aestivum*); and ii) explore the relationship among soil test and tissue analysis for winter wheat.

Material and Methods

This study was conducted at six locations in Kansas USA during the 2012-13 and 2013-14 wheat growing seasons. Locations were established at the following counties 1- Ellis, 2- Jewell, 3- Saline, 4- Sherman, 5- Smith, and 6- Thomas. (Table 2.1). The experimental design consisted of two treatments in a randomized, complete block design with three replications. The treatments were applied in field-long strips of approximately 364 meters (1,200 feet) long and a minimum of 12 meters (40 feet) wide. The treatments included a fertilized strip and a control strip. The fertilized strips included N, Cu, Mn and Zn fertilizer at a rate of 11 kg ha^{-1}. All of the micronutrients were sulfate-based products. Nutrients were applied at all location as granular broadcast after wheat planting in the fall.

Soil samples were collected before fertilizer application from points marked with flags, located every 30 meters along the center of each strip. Soil samples were collected at the 0-15 cm depth. Fifteen to 20 cores per sample were collected from a five-meter radius around each flag. Tissue samples were also collected from an approximately five-meter radius of the flags. Wheat flag leaves were collected at flowering, with at least 30 leaves per sample.

Soil pH was analyzed using a 1:1 soil:water method and samples were analyzed for K using the ammonium acetate (1M, pH 7.0) method, as described by Warncke and Brown (1998). Soil samples for P were extracted with Mehlich-3 and analyzed colorimetrically (Frank et al., 1998). The Walkley-Black method was used to analyze the soil organic matter (Combs and Nathan, 1998). Soil samples were analyzed for Cu, Fe, Mn, and Zn using the

DTPA extraction and ICP Spectrometer (Whitney, 1998). Tissue samples were analyzed for total N, P, and K using sulfuric peroxide digestion as described by Linder and Harley (1942). Tissue samples were digested with nitric acid (HNO3) for S determination using inductively coupled plasma (ICP) (Munter and Grande, 1981). Tissue samples were analyzed for Cu, Fe, Mn and Zn using the perchloric digestion (Gieseking et al., 1935).

Statistical analysis was done with the PROC UNIVARIATE and the PROC MIXED procedures in SAS 9.4 (SAS Institute, Inc. 2014). The procedure of Tukey's Honest Significant Difference (HSD) was used at significantly at P-value <0.05.

RESULTS AND DISCUSSION

Descriptive statistics for soil test values

The mean values for soil pH ranged from 5.3 at the Jewell Co location to 7.3 at the Ellis Co location. Also, the Jewell Co location had the lowest minimum pH value 5.1, and the Thomas Co location had a maximum soil pH value of 7.9. The mean values for OM (%) in the soil ranged from 1.8 % at the Thomas Co location to 2.7 % at the Saline Co location. The Thomas Co location had the lowest minimum OM (1.4%), and the Saline Co location showed the maximum OM level of 3.6% (Table 2.1).

Across all study locations the mean value of soil test P and K were above the critical soil level 20 mg kg^{-1} for P and 130 mg kg^{-1} for K (Leikam, 2003), respectively, which ranged from 25.2 mg kg^{-1} to 87.6 mg kg^{-1} and from 202.5 mg kg^{-1} to 1058.2 mg kg^{-1}, respectively, (Table 2.1). The Saline Co location showed the lowest soil test P and K when compared to other locations. Mean soil test P was greater at the Ellis Co location with a maximum value of 127 mg kg^{-1}, this soil test P value may be the result of manure application or history of high fertilizer P application over time. The Sherman Co location had the greatest soil test K (1058 mg kg^{-1}) concentration when compared to other study locations. The Ellis Co location had the highest mean value of soil test B and also highest maximum values of 1.3 and 2.0 mg kg^{-1}, respectively (Table 2.2). The Jewell and Thomas Co locations had a lowest mean soil B content of 0.7 mg kg^{-1}. These two locations also had the highest and lowest mean value as well as the highest maximum and lowest minimum values for the soil test Cu and Fe (ranged from 0.8 to 1.3 and 11.2 to 67. mg kg^{-1}, respectively). The lowest minimum and highest maximum of value for Cu and Fe were 0.5 to 1.4 mg kg^{-1} and 3.7 to 91.4 mg kg^{-1}, respectively (Table 2.2). The Jewell Co location showed the highest mean and highest maximum value for soil test Mn of 64.4 mg kg^{-1} and 84.6 mg kg^{-1}, respectively. The lowest mean soil Mn was 32.7 mg kg^{-1} at the Ellis Co location, and the lowest minimum value was 10.7 mg kg^{-1} at in Thomas Co location. According to Jones, (1981) the critical range for tissue Zn is 0.2 to2.0 mg kg^{-1}, Fe is 2.5 to 5.0 mg kg^{-1}, Mn is 1.0 to 5.0 mg kg^{-1}, and B is 0.1 to 2.0 mg kg^{-1}, and for Cu is 0.53 mg kg^{-1} (Westerman, 1989). All locations had soil test levels above these critical values (Table 2.2). The Ellis Co location had the highest mean soil Zn concentration when compared to other study locations (Table 2.2).

Extractable DTPA soil micronutrients relationship with pH and OM

Micronutrient availability can be affected by soil pH and OM, the relationship of these soil parameters and DTPA extractable soil micronutrients are shown in Figures 2.2 and 2.3. Soil pH was associated with DTPA extractable Fe (R^2 = 0.93), Mn (R^2 = 0.68), and Cu (R^2 = 0.66). There was no apparent relationship between soil pH and DTPA Zn level for the locations included in this study. However, as shown in Figure 2.3, we did not find any association (or very weak association) between soil DTPA and OM for the above elements tested across all study locations. A study from Australia concluded that soil pH, clay content, and organic matter content together accounted for 87% of variation in Zn level in the soil (Brennan and Bolland, 2006).

Soil test values impact on tissue concentration

When evaluating the relationship between flag leaf tissue nutrient concentration and soil DTPA micronutrients, only Cu ($R^2 = 0.14$) and Mn ($R^2 = 0.07$) showed a slight association when compared to Fe and Zn (Figure 2.4). In contrast, a study from India reported a high and significant correlation between the nutrient status in soil and whole plant for N, P, K, Zn, and B (Biswas et al., 2015). Upward movement of micronutrient to the root surface in soils occurs predominantly via diffusion, and soil moisture plays an essential role in this process, for both Zn and Fe (Cakmak, 2008). The diffusion coefficient of Zn in the soil is inversely proportional to the soil moisture content (Rattan and Deb, 1981). This can have a significant effect on nutrient concentration in flag leaf samples collected from wheat plants at flowering in Kansas. During this growth stage, some plant stress due to low soil moisture can be a limiting factor and therefore affect tissue nutrient concentration. Earlier studies have also suggested that soil-water conditions significantly influence nutrient uptake and particularly micronutrient uptake **(**Bagci et al., 2007; Karim et al., 2012). Wang et al. (2014), showed that the grain Zn and tissue Zn concentration increased under irrigation mainly because of good water supply in the soil. This increased Zn accumulation in wheat was not observed for other micronutrients such as Fe, Mn, and Cu in the grain and tissue. High temperature and limited water availability affect nutrient uptake by the root (Wang et al., 2014).

Phosphorus in the soil can form metal complexes with iron (Fe), Al, and Ca leading to its precipitation and or adsorption (Igual et al., 2001; Gyaneshwar et al., 2002). Therefore in some cases, P fertilizers may not be available to plants and the P can easily get bound in the soil or become less soluble **(**Gyaneshwar et al., 2002). Our results from Ellis Co location may be a situation of negative interaction between soil P and Zn; this location showed the highest mean soil test P (87.6 mg kg^{-1}) while the soil test Zn was low (1.6 mg kg^{-1}). The high soil pH (7.3) compared to other study locations may also be a contributing factor (Table 1.1 and 1.2).

Fertilizer application and tissue nutrient concentration

Figure 2.5, shows N, P and K concentrations across all six locations as affected by the two treatments. Mean variations in tissue N across all study locations ranged from 1.5% to 3.5%. The Jewell, Saline, and Sherman Co locations had the highest tissue N when compared to other locations in the study. The tissue P concentration had less variation when compared to N concentration for the same study locations. In summary, fertilizer application did not significantly affect N, P and K in the wheat tissue across all locations (P>0.05). However, tissue S concentration was significantly affected (P< 0.0001). This result should be expected due to the source of micronutrient fertilizer (sulfate-based), providing significant levels of S applied in combination with micronutrients. The micronutrient fertilizer application significantly affected the tissue concentration of Cu and Zn (Figure 2.7). However, the tissue concentration of Fe and Mn were not affected by micronutrient fertilizer application across all the study locations (Figure 2.7). Previous studies showed a positive response from micronutrient application (Zn, Mn, Cu, and Fe) including grain yield, straw yield, 1000- grain weight, number of spikelet/grain spike^{-1}, and harvest index (Zeidan et al., 2010; Mekkei and El-Haggan, 2014). Previous studies also showed increased tissue and grain concentration of Zn, Mn, and Fe with the application of fertilizer (Zeidan et al., 2010). Additionally, the soil test of Zn was increased from 15 to 37 mg kg^{-1} with the application of 10 kg Zn ha^{-1}.

Macronutrient concentration in the tissue

The flag leaf tissue N concentration was not significantly affected (P>0.05) across all study locations, except for the Thomas Co location, as shown in (Table 2.3). The Thomas Co location had the lowest soil OM content and therefore is possible that small changes in the N cycle has a significant impact on N availability and uptake (Jetten, 2008).

The tissue K concentration was significantly affected by the fertilizer treatment ($P<0.05$) at three locations (Ellis, Saline and Smith Co). The micronutrients fertilizer application did influence the tissue concentration of S significantly ($P<0.05$) at the Saline, Ellis, Jewell, and Sherman Co locations where it increased S tissue concentration when compared to other study locations ($P>0.05$). The tissue P concentration was significantly affected by the fertilizer treatment ($P<0.05$) in Ellis, Jewell, and Smith Co locations. Previous studies in wheat has reported inconsistent results that affected P concentration in relation to increasing in N concentration in the plant (Ziadi et al., 2008). However other studies from Australia (Elliott et al., 1997b; Elliott et al., 1997c), reported that P concentration in wheat shoots usually declined as N plant status increased. Whereas, Ishaq et al. (2001) from Pakistan in a study on wheat reported no effect of increasing N plant status on P concentration. Other related studies in corn reported an increase in P concentration with increasing N concentration in the plant (Ziadi et al., 2008). This trend was similar for tissue N and P concentrations at some locations in our study (Table 2.3). Previous studies showed that the influence of the level of available Zn was secondary to that of P (Zou et al., 2012). Furthermore, increasing available P in the control soils increased yield and decreased grain Zn concentration to an extent consistent with a dilution effect (Zou et al., 2012). Addition of P to the soils lead to a negative P and Zn interaction due to a significant increase in grain yield while decreasing grain Zn concentration. Addition of Zn to the soils increased grain Zn concentration but failed to increase yield. Both, P and Zn proved effective in fulfilling both the goals: increasing yield and maintaining or increasing grain Zn concentration (Zou et al., 2012). However, in recent study authors reports that the accumulation of available soil P at levels above those required for optimal production may diminish cereal grain quality regarding Zn concentration and P/Zn ratio in low Zn soils (Sánchez-Rodríguez et al., 2017).

Micronutrient concentration in the tissue

Fertilizer application affected micronutrient tissue concentrations across all study locations as shown in Table 2.4 and Figure 2.7. The tissue Mn and Fe concentration were not significantly affected by the fertilizer treatment across all study locations (*P*>0.05) as shown in Figure 2.7. The fertilizer application affected Mn concentration only at the Jewell Co location where Fe concentration was also affected significantly (Table 2.4). In our study, we reported tissue Mn concentrations of 42 to 133 mg kg^{-1} for all study locations and across treatments. The sufficient soil Mn levels are considered at 1.0-5.0 mg kg^{-1} according to Jones (1981), and tissue Mn concentrations are optimum at the 20-150 mg kg^{-1} (Jones, 1967). In another study, Widmar (2013) reported the range of Mn concentrations from 97 to 104 mg kg^{-1} for wheat in Kansas.

In our study, we reported the Fe concentrations of 92 to 160 mg $kg^{-1}$for all study locations and across treatments. Jones et al. (1967), also reported an optimum range for plant Fe from 30 to 200 mg kg^{-1}; previous studies reported values of 90 to 101mg kg^{-1} in Kansas (Widmar, 2013). Soil Fe concentration from our study ranged from 11 to 67 mg kg^{-1}, which was higher than the reported optimum soil Fe content of 2.5 to 5.0 mg kg-1 by Jones et al. (1981).

The tissue Cu and Zn concentration were significantly affected by the fertilizer treatment across all study locations (*P*<0.05) (Figure 2.7). The Saline and Thomas Co locations showed a significant increase in tissue Cu concentration (*P*<0.05) from fertilizer application (Table 2.4). Across all the study locations, the tissue Cu concentration ranged from 3 to 6 mg kg^{-1} (at both significant Study locations). This range was similar to those found by Widmar (2013). Previous guidelines suggest an optimum range of 5 to 25 mg kg^{-1} for Cu in the plant (Jones et al., 1967). However, these published values for "optimum" tissue Cu concentration may be higher than typical values found in the field. Furthermore, a previous study indicated that plant response to Cu fertilizer was unlikely with soil Cu levels above 0.6 mg kg-1 (Franzen and McMullen, 1998). The soil Cu concentrations from our study ranged from 0.8 to 1.3 mg kg^{-1} (Table 2.2).

Tissue concentration of zinc increased significantly at the Jewell, Saline, Smith and Thomas Co locations with fertilizer application. A previous study by Zeindan et al. (2010) found that applications of Zn increased the tissue concentration over the control. The Zeindan et al. (2010) study had 0.13 mg kg^{-1} as averaged soil Zn test, which is considered below the critical range of 0.2 to 2.0 mg kg^{-1} (Jones, 1981). However, another study reported very small increased in tissue Zn concentration with the application of Zn fertilizer in wheat Zn when compared to the control, suggesting that the fertilizer source used for Zn can determine the plant availability particularly in the short term during the growing season (Widmar,2013). In this study, authors reported a range of 0.5 to 2.8 mg kg^{-1} which was to the values found in our study (0.5 to 1.5 mg kg^{-1}). The Zn tissue concentration from our study ranged from 8 to 27 mg kg^{-1} (Table 2.4) and similar to values reported by Widmar (2013) (18 to 22 mg kg^{-1}). However, one earlier study reported Zn concentrations of 5 to 25 mg kg^{-1} for flag leaf tissue in wheat (Jones et al., 1967).

The plant nitrogen status can exert positive effects on root's ability to uptake Zn and Fe. A previous study had shown positive correlations between grain Zn and protein concentrations when Zn fertilizer was applied in combination with N (Zeidan et al., 2010). The positive impact of improved plant N status on Zn and Fe concentration in plants is relevant and require further research. There are several steps during uptake and transport of Zn and Fe in plants which might be affected by plant N status. Nitrogen may influence the mobility and root uptake of Zn and Fe from soils by affecting the root growth and stimulating root exudation of organic compounds (Marschner, 1995; Paterson et al., 2006). Nitrogen status of plants may also create positive effects on root uptake of Zn and Fe. Recent studies showed a positive correlation between grain Zn and protein concentrations under high application rates of Zn and N (Cakmak et al., 2010). The previous study had shown that the tissue Zn concentration was increased by soil application of Zn fertilizer by 173 to 176% compared to control when soil test Zn was below 5 mg kg-1. However, tissue Zn concentration increased by only 12 to 112% when soil test Zn was above 5 mg kg-1.

CONCLUSION

Micronutrients fertilizer application did not significantly influence N, P and K tissue concentration, but increased S tissue concentration across all locations. The tissue P concentration was affected by the micronutrient fertilizer application when the soil pH was less than 6 and above 7. The Ellis study location had the highest soil P content (87.6 mg/kg), soil test Zn (1.6 mg/kg), and soil pH (7.3). Also, with micronutrient fertilizer application, we found a significant effect on tissue S, Cu, and Zn across all locations (at $P<0.05$). Tissue S concentration was significantly ($P<0.05$) impacted by the micronutrients fertilizer application and was the most consist response across different soils.

Extractable DTPA soil micronutrients were correlated with soil pH. However, the relationship with soil OM was poor suggesting that soil pH would be a more relevant soil parameter determining micronutrient availability in Kansas soils. Results from this study also showed that tissue analysis could reflect fertilizer application and availability of micronutrients to the plant. However, there was significant variability in tissue analysis, likely affected by abiotic factors influencing plant nutrient uptake and concentration. While tissue analysis can help as a diagnostic tool, producers should be aware of the limitations, and decisions on fertilizer recommendations cannot be based exclusively on tissue test.

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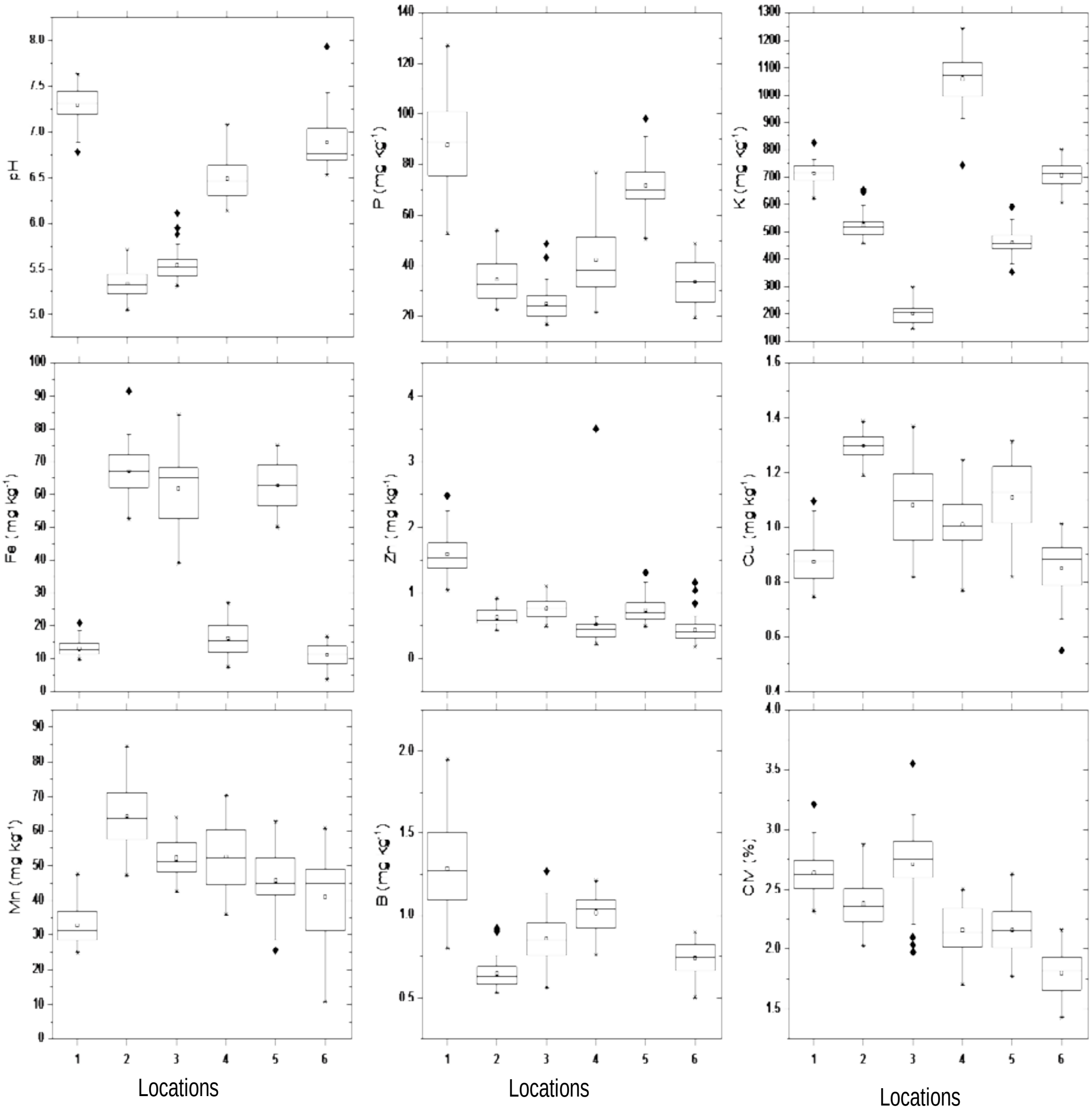

Figure 2.1 Boxplots of soil test values for pH, organic matter (OM), phosphorus (P), potassium (K), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) at all study locations (1, Ellis; 2, Jewell; 3, Saline; 4, Sherman; 5, Smith; 6, Thomas).

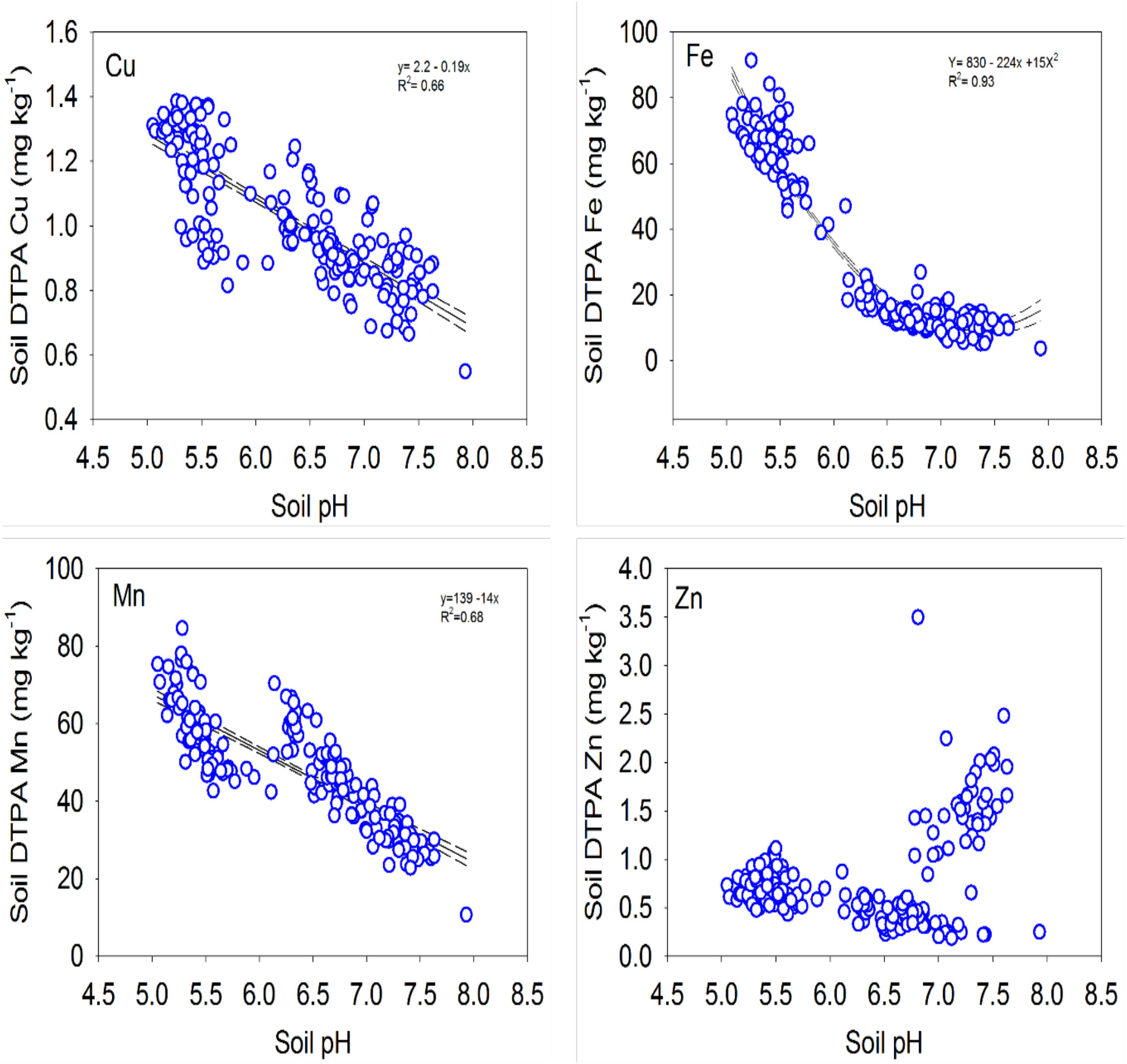

Figure 2.2 Soil pH relationship with soil DTPA copper (Cu), iron (Fe), manganese (Mn), and Zinc (Zn) across all the study locations

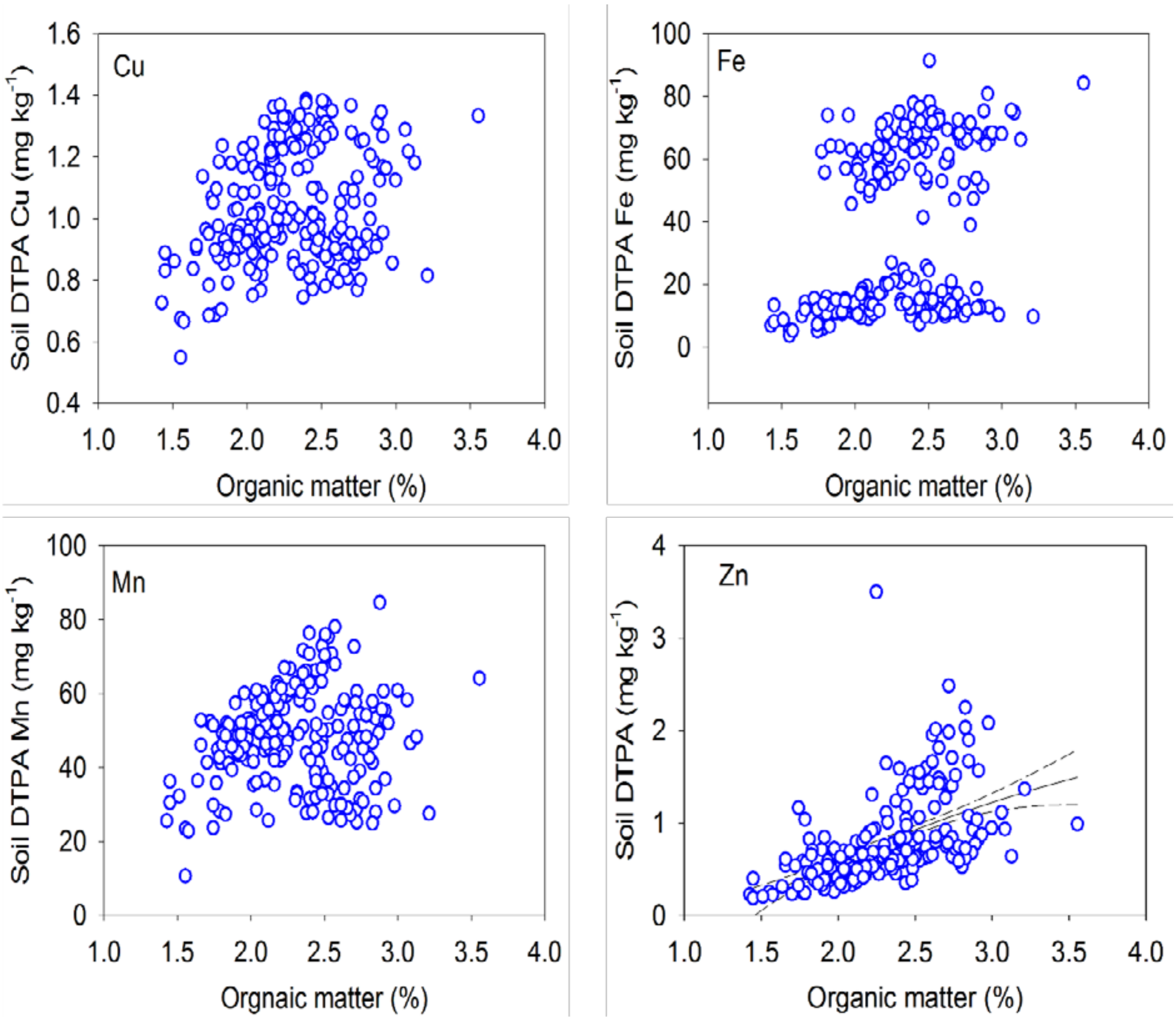

Figure 2.3 Soil organic matter relationship with soil DTPA copper (Cu), iron (Fe), manganese (Mn), and Zinc (Zn) across all the study locations

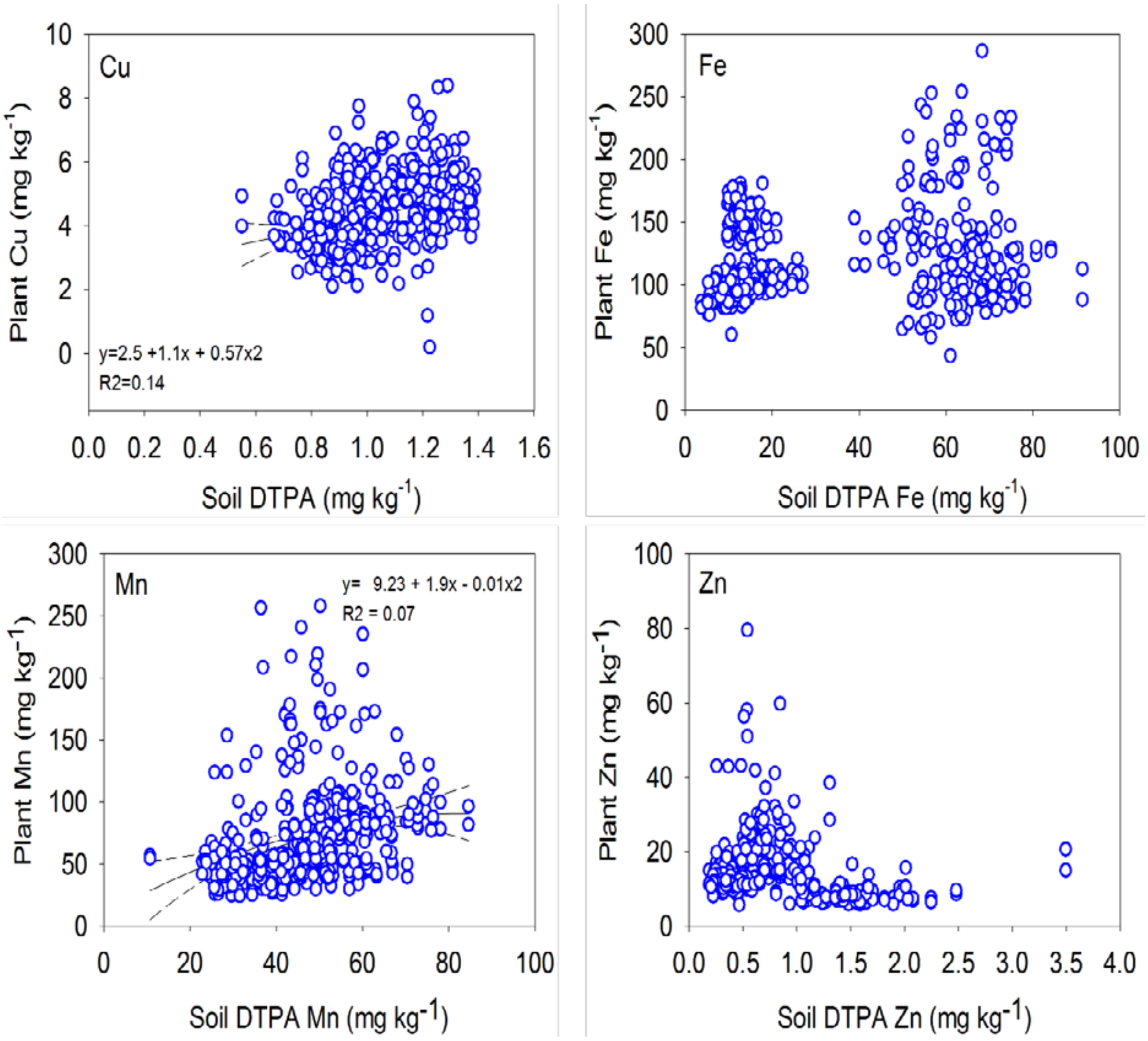

Figure 2.4 Plant tissue nutrient concentration relationship with soil DTPA copper (Cu), iron (Fe), manganese (Mn), and Zinc (Zn) across all the study locations

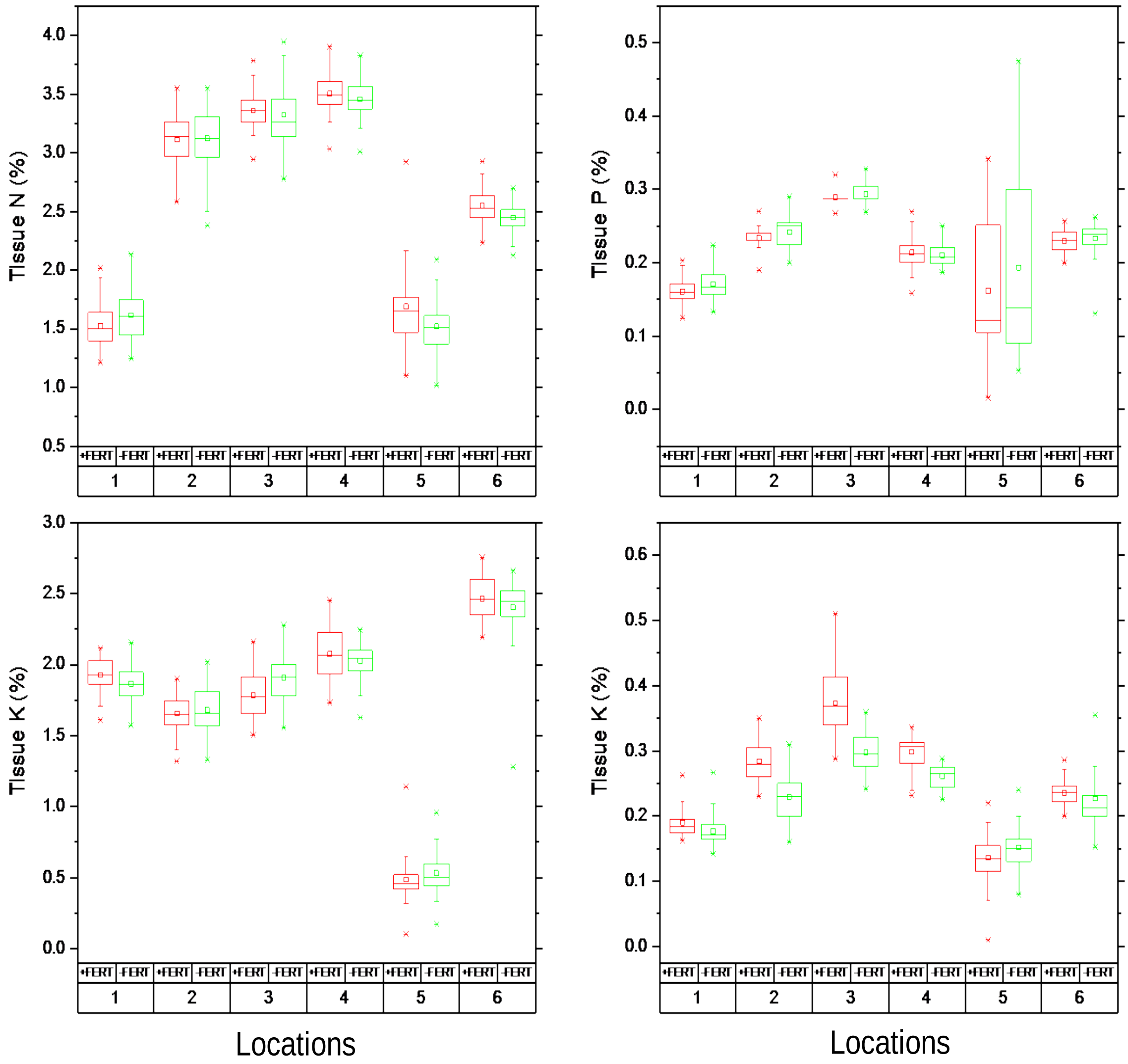

Figure 2.5 Boxplots of plants tissue concentration of nitrogen (N) phosphorus (P), potassium (K), and sulfur (S) as affected by fertilizer treatments at all the study locations (1, Ellis; 2, Jewell; 3, Saline; 4, Sherman; 5, Smith; 6, Thomas).

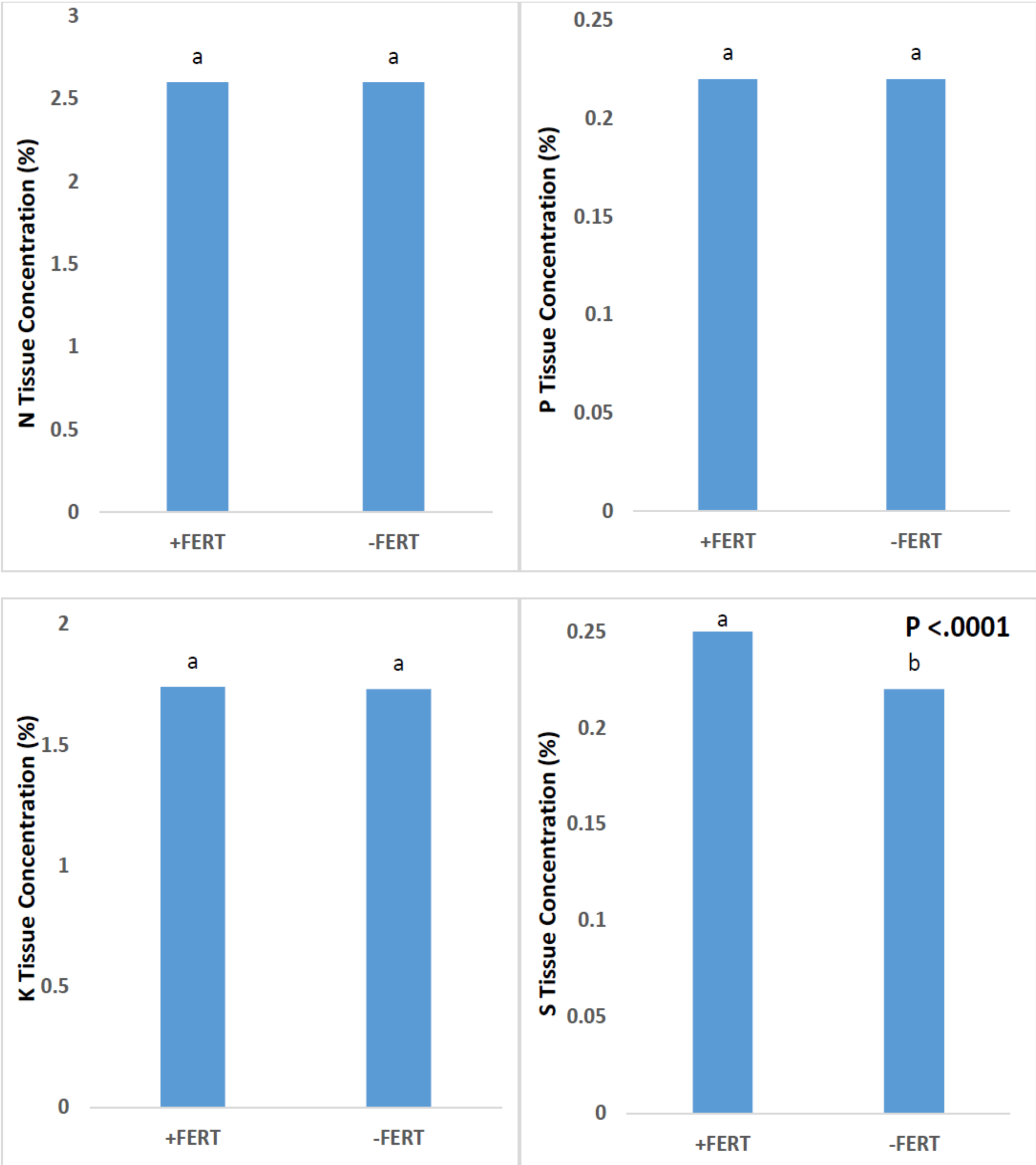

Figure 2.6 Wheat tissue concentrations of nitrogen (N), phosphorus (P), potassium (K), and sulfur (S) across all the study locations as affected by fertilizer treatment application.

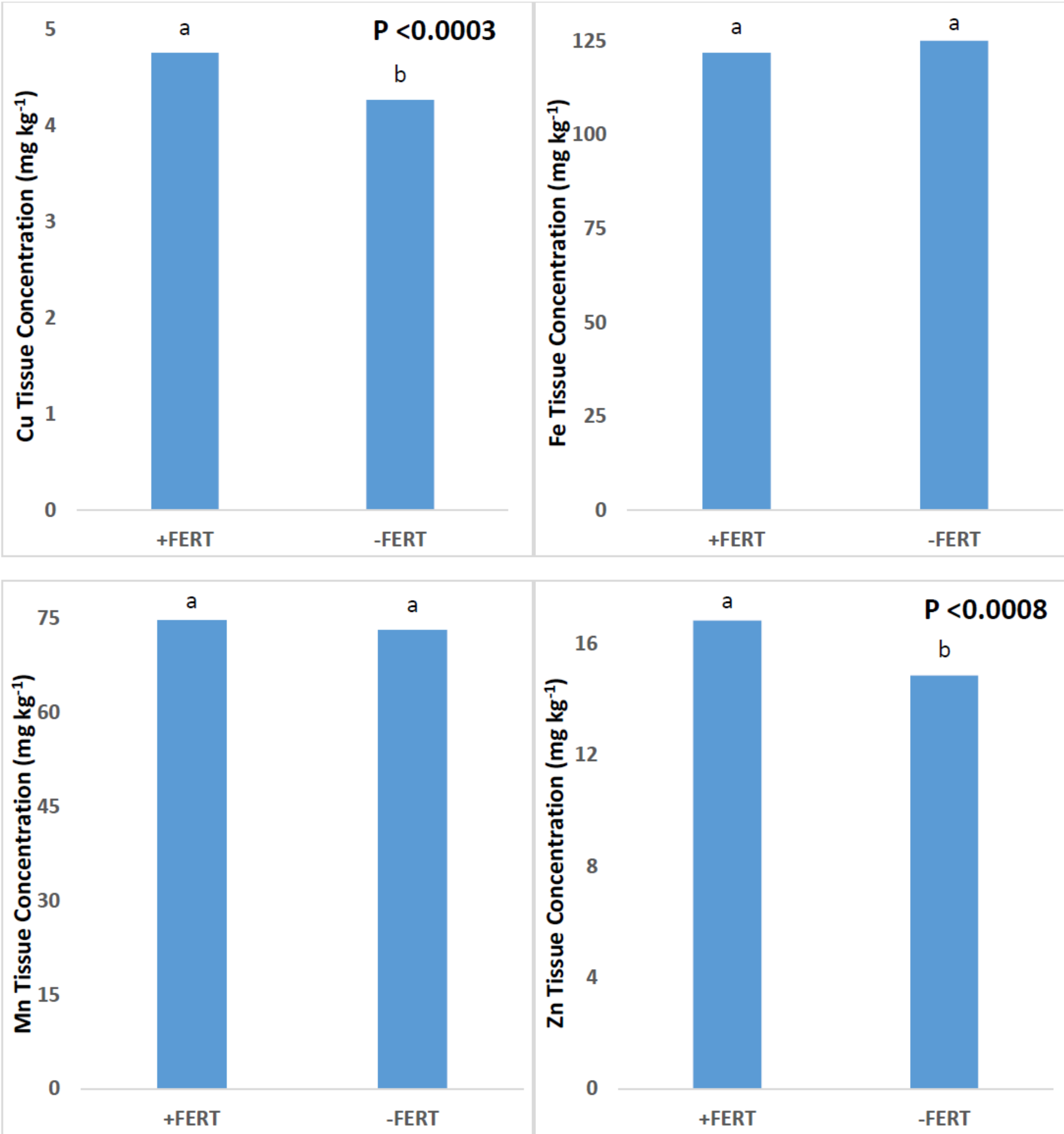

Figure 2.7 Wheat tissue concentrations of copper (Cu), iron(Fe), manganese (Mn), and zinc (Zn) across all the study locations as affected by the fertilizer treatment application.

Table 2.1 Descriptive statistics for soil test value of pH, organic matter, phosphorus, and potassium at all the study locations

Location	Mean	Standard Deviation	Minimum	Median	Maximum
		pH			
1- Ellis	7.3	0.2	6.8	7.3	7.6
2- Jewell	5.3	0.2	5.1	5.3	5.7
3- Saline	5.6	0.2	5.3	5.5	6.1
4- Sherman	6.5	0.2	6.1	6.5	7.1
5- Smith	--	--	--	--	--
6- Thomas	6.9	0.3	6.5	6.8	7.9
		Organic Matter (%)			
1- Ellis	2.6	0.2	2.3	2.6	3.2
2- Jewell	2.4	0.2	2.0	2.4	2.9
3- Saline	2.7	0.3	2.0	2.8	3.6
4- Sherman	2.2	0.2	1.7	2.1	2.5
5- Smith	2.2	0.2	1.8	2.2	2.6
6- Thomas	1.8	0.2	1.4	1.8	2.2
		Soil test P (mg kg^{-1})			
1- Ellis	87.6	18.4	52.7	88.8	127.0
2- Jewell	34.5	8.5	22.5	32.9	53.9
3- Saline	25.2	7.0	16.7	24.2	48.8
4- Sherman	42.3	14.7	21.5	38.1	76.7
5- Smith	71.8	10.0	50.6	69.8	98.1
6- Thomas	33.6	8.7	19.4	33.7	49.0
		Soil test K (mg kg^{-1})			
1- Ellis	714	46.1	621	715	826
2- Jewell	524	46.2	457	517	653
3- Saline	202	41.9	147	204	299
4- Sherman	1058	94.2	743	1069	1242
5- Smith	463	46.5	354	458	592
6- Thomas	707	47.1	604	713	802

Table 2.2 Descriptive statistics for soil test value of boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) at all the study locations

Location	Mean	Standard Deviation	Minimum	Median	Maximum
		Soil test B (mg kg^{-1})			
1- Ellis	1.3	0.3	0.8	1.3	2.0
2- Jewell	0.7	0.1	0.5	0.6	0.9
3- Saline	0.9	0.2	0.6	0.9	1.3
4- Sherman	1.0	0.1	0.8	1.0	1.2
5- Smith	--	--	--	--	--
6- Thomas	0.7	0.1	0.5	0.7	0.9
		Soil test Cu (mg kg^{-1})			
1- Ellis	0.9	0.1	0.7	0.9	1.1
2- Jewell	1.3	0.1	1.2	1.3	1.4
3- Saline	1.1	0.2	0.8	1.1	1.4
4- Sherman	1.0	0.1	0.8	1.0	1.2
5- Smith	1.1	0.1	0.8	1.1	1.3
6- Thomas	0.8	0.1	0.5	0.9	1.0
		Soil test Fe (mg kg^{-1})			
1- Ellis	13.1	2.6	9.7	12.8	20.9
2- Jewell	67.0	7.7	52.5	67.0	91.4
3- Saline	61.7	10.9	39.0	65.0	84.2
4- Sherman	16.3	5.1	7.4	15.5	27.0
5- Smith	62.7	7.1	50.0	62.7	75.0
6- Thomas	11.2	3.5	3.7	11.6	16.9
		Soil test Mn (mg kg^{-1})			
1- Ellis	32.7	5.5	24.9	31.5	47.6
2- Jewell	64.4	8.9	47.2	63.4	84.6
3- Saline	52.2	5.4	42.4	51.2	64.1
4- Sherman	52.6	9.2	35.8	52.4	70.4
5- Smith	45.7	9.1	25.7	45.0	62.9
6- Thomas	40.9	11.4	10.7	44.9	60.9
		Soil test Zn (mg kg^{-1})			
1- Ellis	1.6	0.3	1.0	1.5	2.5
2- Jewell	0.6	0.1	0.4	0.6	0.9
3- Saline	0.8	0.2	0.5	0.8	1.1
4- Sherman	0.5	0.5	0.2	0.5	3.5
5- Smith	0.7	0.2	0.5	0.7	1.3
6- Thomas	0.5	0.2	0.2	0.4	1.2

Table 2.3 Nitrogen, P, K and S concentration in plant tissue as affected by the fertilizer treatment application at each study location.

Nutrients	Treatments	Saline	Ellis	Jewell	Thomas	Sherman	Smith
		----------	----------	%	-----------	------------	----------
N	**+Fer.**	3.36a	1.52a	3.11a	2.55a	3.50a	1.56a
	-Fer.	3.33a	1.61a	3.12a	2.45b	3.46a	1.66a
P	**+Fer.**	0.29a	0.16b	0.23a	0.23a	0.21a	0.19a
	-Fer.	0.30a	0.17a	0.24b	0.23a	0.21a	0.16b
K	**+Fer.**	1.79b	1.93a	1.66a	2.46a	2.08a	0.55a
	-Fer.	1.91a	1.87b	1.68a	2.41a	2.03a	0.47b
S	**+Fer.**	0.37a	0.19a	0.28a	0.24a	0.30a	0.15a
	-Fer.	0.30b	0.17b	0.23b	0.23a	0.26b	0.14a

Means value with the same letter are not significantly different at $P<0.05$; NS= Not significant different.

Table 2.4 Micronutrients concentration in the tissue as affected by the fertilizer treatment application at each study location.

Nutrients In tissue	Treatments	Saline ----------	Ellis ----------	Jewell mg kg^{-1}	Thomas -----------	Sherman ------------	Smith ----------
Cu	**+Fer.**	6.09a	3.74a	5.09a	3.76a	5.20a	4.66a
	-Fer.	5.29b	3.61a	4.85a	3.22b	4.82a	3.82a
Fe	**+Fer.**	132.64a	151.96a	93.88b	92.33a	105.01a	155.28a
	-Fer.	132.64a	155.27a	100.27a	95.22a	106.59a	160.41a
Mn	**+Fer.**	85.56a	42.97a	89.28a	52.78a	44.84a	133.20a
	-Fer.	81.83a	43.00a	92.07a	49.59a	43.69a	129.17a
Zn	**+Fer.**	18.68a	8.38a	16.98a	12.64a	17.29a	27.03a
	-Fer.	16.33b	8.48a	14.49b	10.75b	17.83a	21.28b

Means value with the same letter are not significantly different at $P<0.05$; NS= Not significant different.

Overall conclusions and summary

The influence of the micronutrients fertilizer application on the tissue nutrient concetration of winter wheat was evaluated. In general, the micronutrients fertilizer application did not significantly (at *P*-value level <0.05) increased the macronutrients tissue concentration of N, P, and K but increased the S tissue concentration across all study locations. Tissue N concentration seems to be related to soil OM content but showed poor relation to micronutrient fertilizer application. The tissue P concentration was affected by the micronutrients fertilizer application when the soil pH was less than 6 and above 7. Such as the Ellis Co study location that had the highest soil P content (87.6 mg/kg), soil test Zn (1.6 mg/kg), and soil pH (7.3).

Micronutrients fertilizer application of Cu, Zn, and Mn, increased significantly (*P*<0.05) the tissue concentration of S, Zn, Cu, and Fe for some locations, but not for the tissue concentration of Mn. Evaluation across locations showed a significant increase in S, Zn, and Cu concentration (*P*<0.05) as affected by the fertilizer treatments. The tissue concentration of S was significantly (P<0.05) impacted by the micronutrients fertilizer application, particularly for locations with lower soil organic matter (OM). Results from this study showed that soil test properties (such as OM and pH) can influence tissue nutrient concentration across locations. Extractable DTPA soil micronutrients were correlated with soil pH. However, the relationship with soil OM was poor suggesting that soil pH would be a more relevant soil parameter determining micronutrient availability in Kansas soils.

Results from this study also showed that tissue analysis could reflect fertilizer application and availability of micronutrients to the plant. However, there was significant variability in tissue analysis, likely affected by abiotic factors influencing plant nutrient uptake and concentration. While tissue analysis can help as a diagnostic tool, producers should be aware of the limitations, and decisions on fertilizer recommendations cannot be based exclusively on tissue test.

Appendix

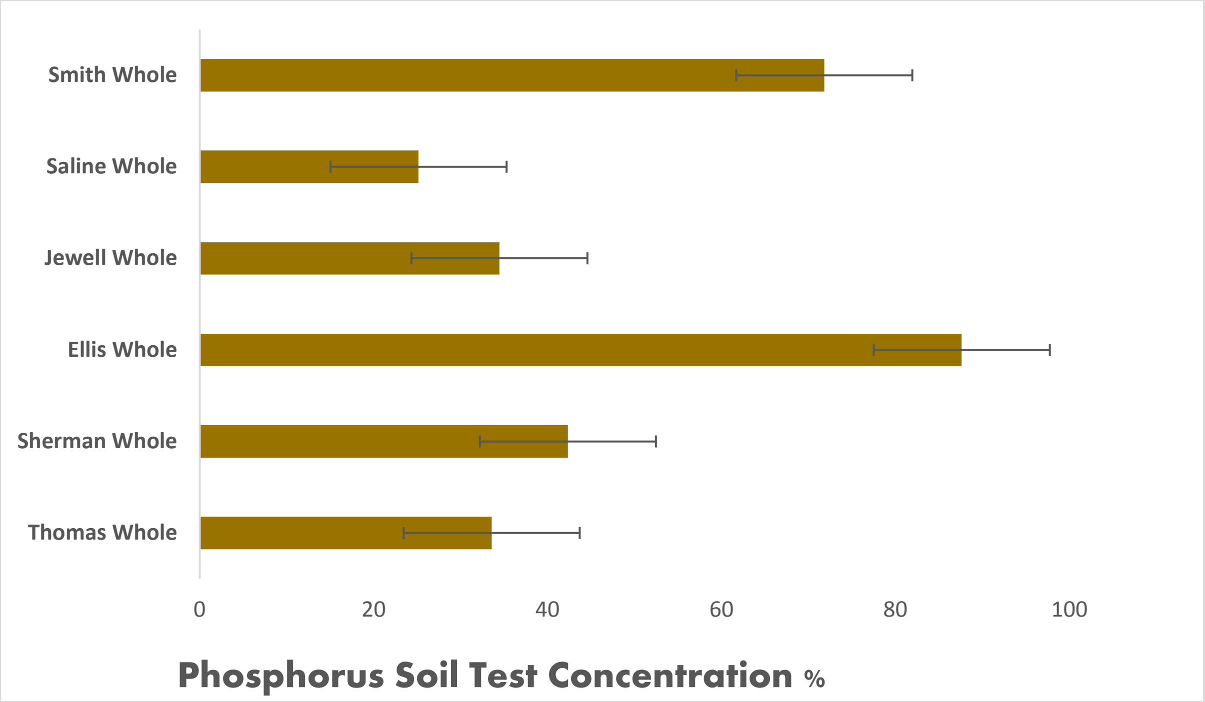

Figure 1: Phosphorus soil test for all the study locations.

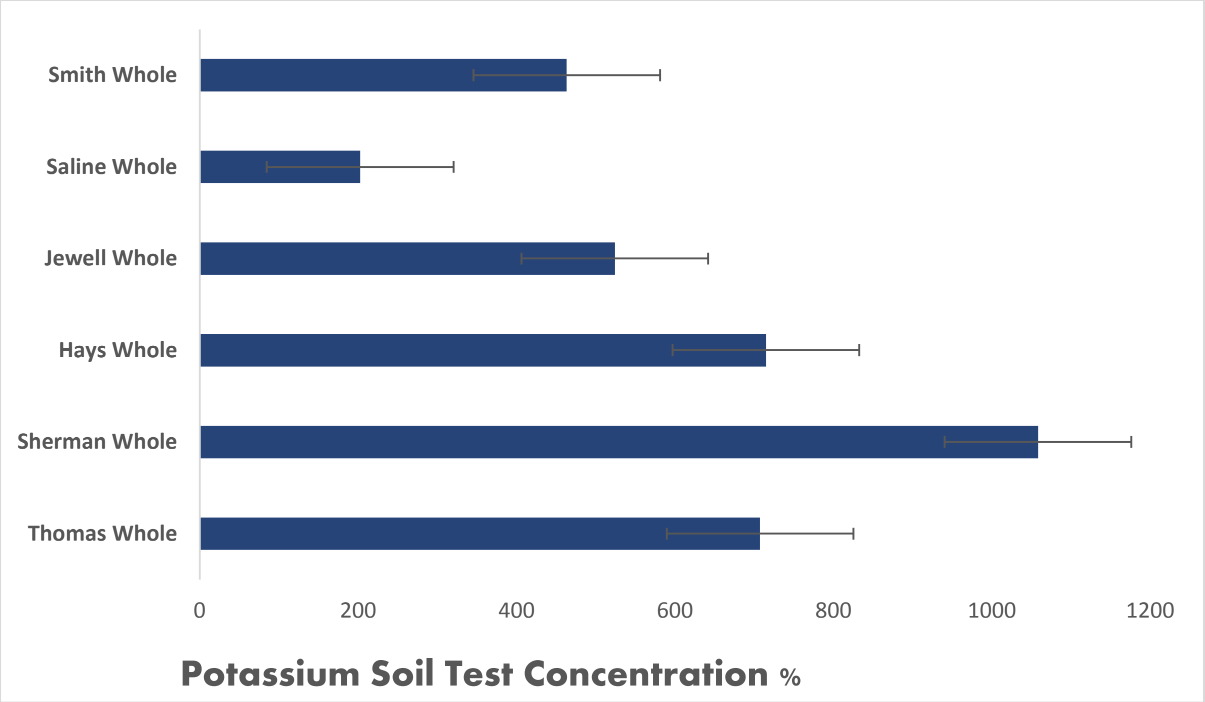

Figure 2: Potassium soil test for all the study locations

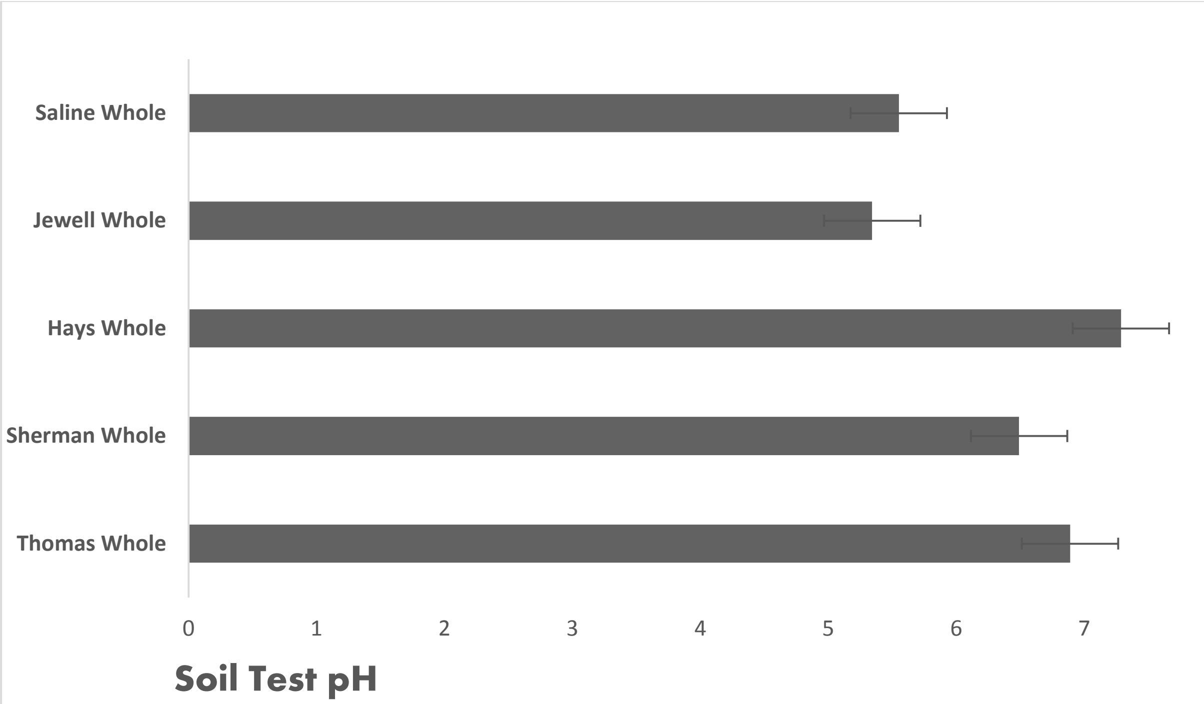

Figure 3: Soil pH for all the study locations.

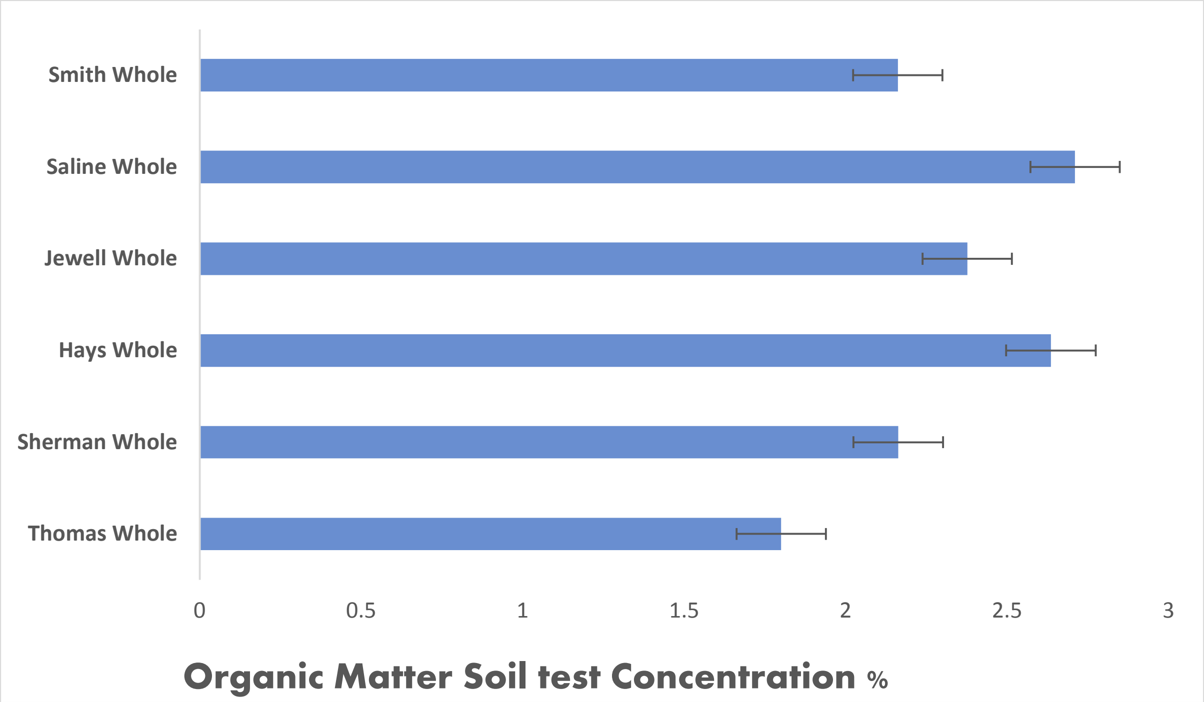

Figure 4: Organic matter for all the study locations.

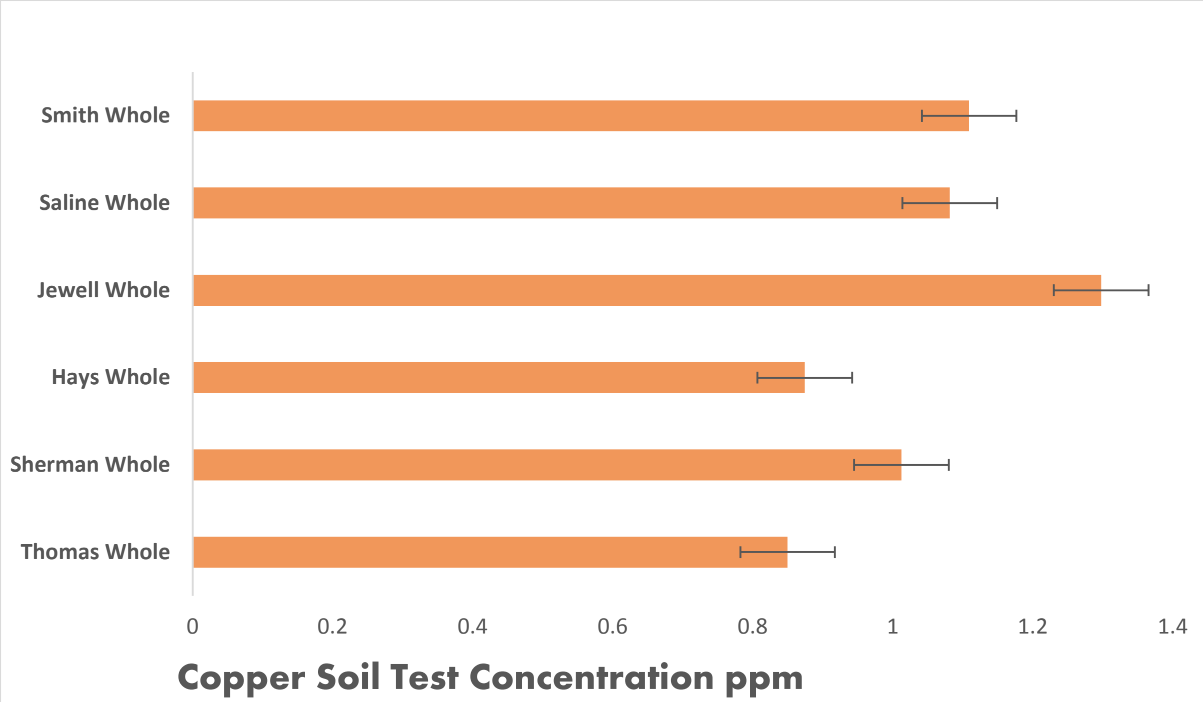

Figure 5: Copper soil test for all the study locations.

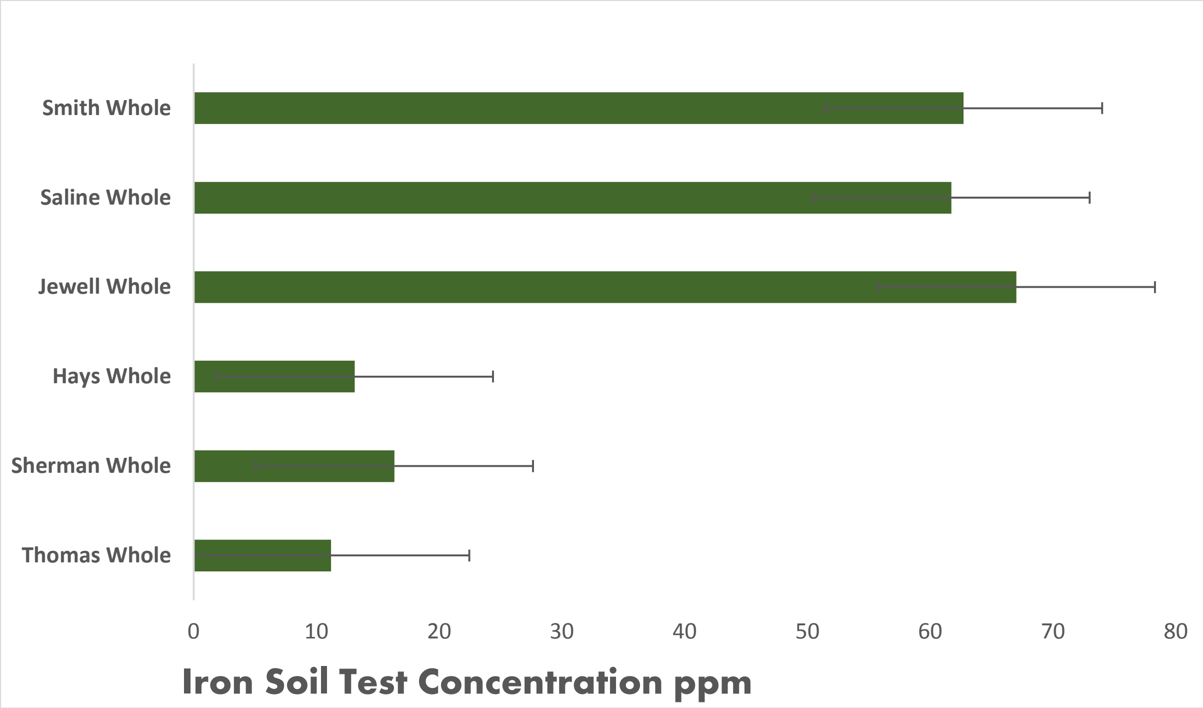

Figure 6: Iron soil test for all the study locations.

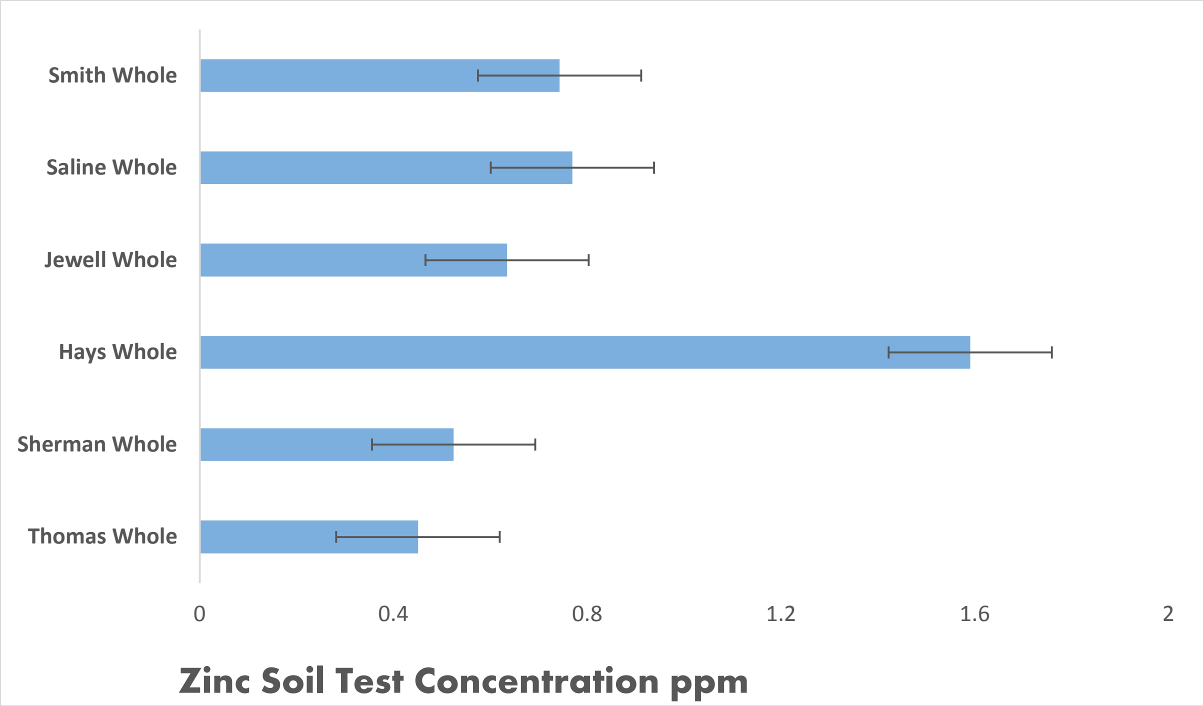

Figure 7: Zinc soil test for all the study locations.

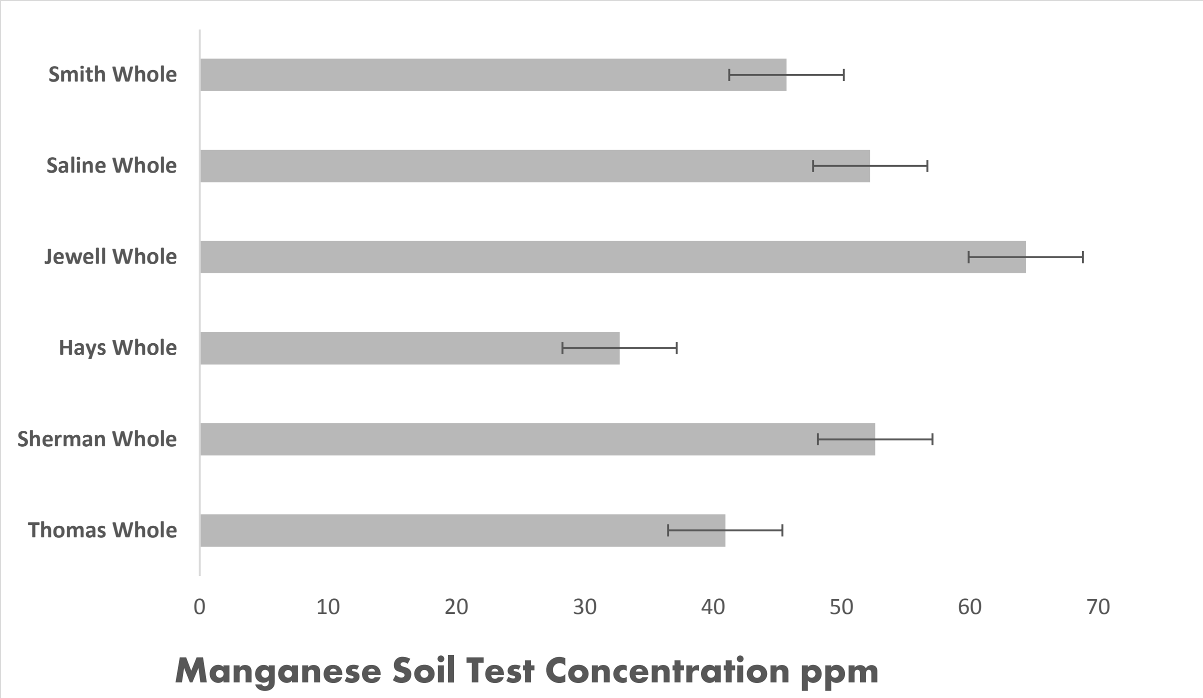

Figure 8: Manganese soil test for study locations.

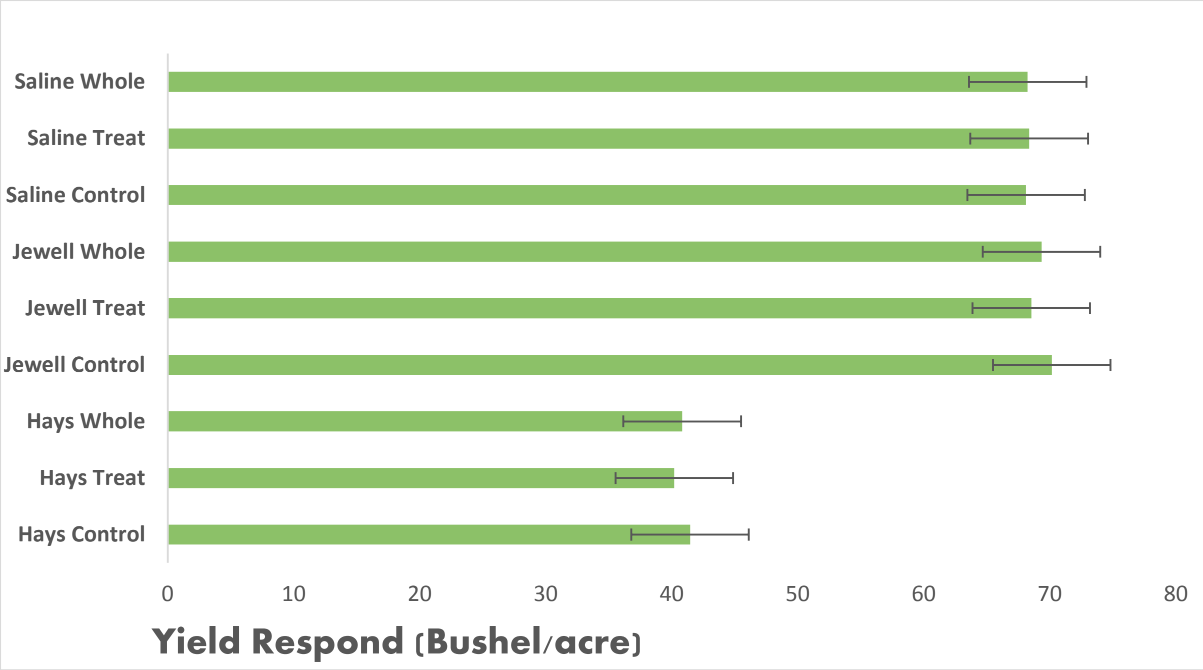

Figure 9: Yield response for some study locations.

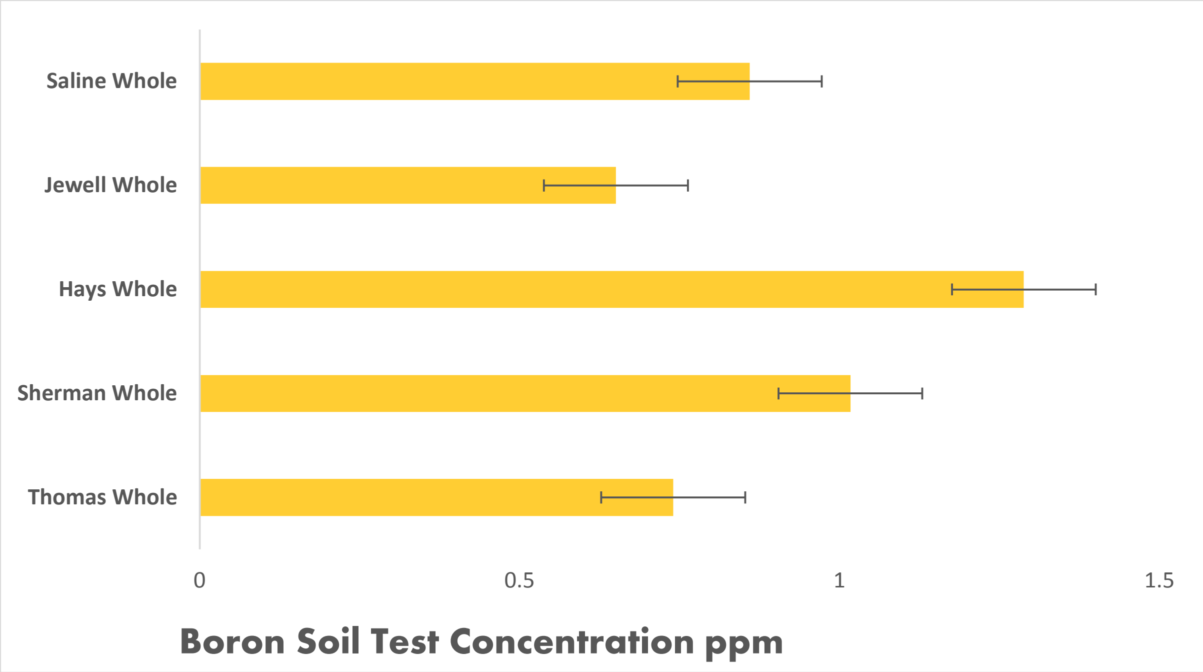

Figure 10: Boron soil test for all the study locations.