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Article

Antagonistic effect of copper and zinc in fertilization of spring wheat under low soil phosphorus conditions

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Abstract

Sound micronutrient management requires an understanding of nutrient interactions and transformation processes in soil–plant systems which can regulate bioavailability and plant uptake. A series of studies were conducted under controlled environment and field conditions to evaluate wheat response to Cu and Zn fertilization on P-deficient soils from western Canada. The grain and straw yields of wheat were reduced in two (Waskada and Tisdale) of three soils used in the controlled environment study, while yield was not affected at the Echo field site in 2016 when both Cu and Zn sulfate fertilizer were applied at 5 kg·ha^{-1} rates. Zinc concentration in soil and plant tissues was increased to apparent toxic levels with fertilizer addition in Waskada soils. An imbalance in tissue P:Zn concentration related to micronutrient fertilization was observed in Waskada and Tisdale soils. The availability of Cu and Zn in post-harvest soils was increased with increasing rate of these fertilizers' addition. Chemical and spectroscopic speciation using sequential extraction and X-ray absorption near edge structure, respectively, revealed that Cu and Zn were mostly speciated as carbonate phases, and complexation of these elements with carbonate and phyllosilicate minerals is likely the process controlling bioavailability in the soils.

Key words: copper, zinc, antagonism, speciation, spring wheat, biomass yield

Résumé

Pour bien gérer les oligoéléments, il faut comprendre les interactions des nutriments et les processus de transformation du système plante-sol qui en régulent la biodisponibilité ainsi que l'absorption par la plante. Les auteurs ont réalisé une série d'études en milieu contrôlé et sur le terrain pour évaluer la réaction du blé à des amendements de Cu et de Zn sur des sols carencés en P de l'Ouest canadien. Le rendement grainier et la quantité de paille du blé ont diminué sur deux des trois sols utilisés pour l'expérience dans des conditions contrôlées (Waskada et Tisdale), mais le rendement n'a pas été affecté sur le troisième (Echo), en 2016, après application de 5 kg de sulfate de Cu et de Zn par hectare, sur le terrain. Ajouter de l'engrais aux sols de Waskada a accru la concentration de zinc dans le sol et les tissus végétaux jusqu'à un taux apparemment toxique. Les auteurs ont relevé un déséquilibre de la concentration P:Zn dans les tissus végétaux après fertilisation des sols de Waskada et de Tisdale avec les deux oligoéléments. La quantité de Cu et de Zn disponible dans le sol après la récolte augmente avec le taux d'application des engrais. La spéciation chimique et la spéciation spectroscopique établies respectivement par extraction séquentielle et spectroscopie absorption des rayons X près de la structure du bord indiquent que le Cu et le Zn se retrouvent le plus souvent dans une phase carbonée et que le processus qui en régule la biodisponibilité dans le sol correspond sans doute à la formation de complexes par ces éléments et les carbonates ou les phyllosilicates. [Traduit par la Rédaction]

Mots-clés : cuivre, zinc, antagonisme, spéciation, blé de printemps, rendement de la biomasse

Introduction

A balanced supply of macro and micronutrients is essential for optimization of crop yields in a productive farming system. Most agricultural soils of Canadian prairies have limited available P, and P fertilization is widely recommended to promote yield (Ziadi et al. 2013). Conversely, deficiencies of Cu and Zn may also occasionally arise in cereals such as wheat in the cereal–pulse–oilseed rotations (e.g., wheat–pea–canola) commonly employed in Saskatchewan, under specific soil conditions. Soils of sandy textures are known to be more often deficient in micronutrients, while soils with high pH, carbonate, and organic matter content may also adsorb and restrict Cu and Zn availability (Kruger et al. 1985; Karamanos et al. 1986; Singh et al. 1987, 1988; Rahman et al. 2020; Rahman and Schoenau 2020). Based on a large number of field experiments, Karamanos et al. (2003*a*) summarized that Cu fertilization led to significant increases in grain yield of wheat in instances where the diethylene-triamine-pentacetic acid (DTPA) extractable Cu was less than 0.4 mg kg^{-1}. However, less predictability of response was observed with Zn fertilization of pulses (Maqsood et al. 2016; Anderson et al. 2018). While various studies (Flaten et al. 2003; Karamanos et al. 2003*a*; Goh and Karamanos 2006; Malhi and Karamanos 2006; Maqsood et al. 2016) have explored the crop yield response to Cu or Zn fertilization, fewer studies have focused on the interaction between these micronutrients, as well as other macronutrients that could be limiting for crop production.

Although Cu and Zn are required in much smaller amounts than macronutrients like P, the proper management of these nutrients is complicated by the complex nature of interactions among individual nutrients as well as the environment in soils and plants (Loneragan and Webb 1993; Alloway 2008). The interactions between Cu and Zn are often antagonistic because of their competitive adsorption onto soil components and for absorption sites on plant roots (Alloway 2008). Copper fertilization has been shown to alter Zn dynamics in soil or vice versa (Loneragan and Webb 1993; Luo and Rimmer 1995). Tani and Barrington (2005) reported that Cu fertilization had an antagonistic effect on Zn translocation and uptake in buckwheat plants. However, some studies have shown no antagonism or positive effects. Copper fertilization had no significant effect on Zn uptake in rice but did have a synergistic effect on Zn uptake in bean plants (Fageria 2002). Therefore, the complexity of interaction between Cu and Zn continues to pose a dilemma when micronutrient addition is needed to correct deficiency problems in diverse types of soils and cropping systems.

In addition to competitive effects among micronutrient elements, the reduction of availability of micronutrient metals may also occur through interactions with macronutrients. The best-known example is reduced availability of micronutrient metal induced by high content of reactive phosphate in the soil (Loneragan and Webb 1993). Earlier research (Singh et al. 1988; Loneragan and Webb 1993; Foth and Ellis 1997) reported that Cu and Zn availability was markedly reduced by high levels of soil P or high rate of P addition in soils. Singh et al. (1986) have confirmed that increased P availability induced Zn deficiency in wheat grown on western Canadian soils. The P-induced micronutrient deficiency was attributed to dilution effect of increased shoot growth obtained from P addition rather than reduced absorption of micronutrients by roots (Singh et al. 1988). Overall, induced deficiency seems to mostly occur in soil with P deficiency and marginal in available micronutrients, where crop growth benefits from P fertilization.

Despite the reported P-Zn and P-Cu antagonisms at high soil P concentration, there is limited information on P-Zn and P-Cu antagonism in low available P soils. Moreover, current nutrient management studies are geared toward balancing the quantities of essential nutrients added to soils to favor plant growth without inducing deficiency or toxicity. Our previous study (Rahman and Schoenau 2021) revealed significant yield reduction of wheat associated with the combined addition of Cu and Zn at a rate of 5 kg·ha^{-1} in a P-deficient soil. This study was conducted to reveal the nutrient transformation process related to antagonistic effects of micronutrient fertilization on wheat yield when Cu and Zn sulfate fertilizers were applied separately or combined at different rates. It was hypothesized that fertilization with Cu and Zn sulfate will reduce wheat yield in a P-deficient soil, associated with nutrient imbalances in plants and (or) aggravated P deficiency due to restricted P availability in the soil through formation of insoluble nutrient species. Both chemical and spectroscopic speciation techniques were used to elucidate the forms and potential complexation processes that may be occurring in several type of P-deficient soils amended with Cu and Zn fertilizers.

Materials and methods

Experimental set-up, management, sample collection, and processing

The study was set up as replicated trials conducted under controlled environment and field conditions. The surface layer (0–15 cm) of Levine (Gleyed Cumulic Regosol) and Waskada (Orthic Black Chernozem) series soils collected from Manitoba and Tisdale association (Dark Gray Chernozem) soil from Saskatchewan were used as growth media for a pot study in the controlled environment growth facilities at the University of Saskatchewan, whereas the field study site was conducted near Central Butte in south-central Saskatchewan, Canada, on a Brown Chernozem (Echo association) soil. The soil used for the studies was all categorized as P-deficient according to soil test (<10 mg P·kg^{-1} modified kelowna extractable P), and the soils were selected to represent diverse soil types and climatic conditions existing in the prairies (Table 1). The Waskada variety of hard red spring wheat (*Triticum aestivum*) was grown as a test crop. Eight treatments were evaluated in the study: T_1, Control (no micronutrient and no phosphorus); T_2, Control (no micronutrient, but phosphorus added at 20 kg P_2O_5·ha^{-1}); T_3, Cu at 2.5 kg·ha^{-1}; T_4, Zn at 2.5 kg·ha^{-1}; T_5, Cu + Zn at 2.5 kg·ha^{-1}; T_6, Cu at 5 kg·ha^{-1}; T_7, Zn at 5 kg·ha^{-1}; and T_8, Cu + Zn at 5 kg·ha^{-1}. The Cu and Zn fertilizers were added as Cu and Zn sulfate salts. Triple Super Phosphate fertilizer was used as a source of P, and sulfate salts of Cu and Zn were applied in soils. The blanket application of other fertilizers included urea at 200 kg N·ha^{-1} and potassium sulfate (0-0-44-17) at 20 kg S·ha^{-1} and 47 kg K_2O·ha^{-1}. The experiments were set up as standard completely randomized design (CRD) and randomized complete block design (RCBD) for the controlled environment and field trials, respectively ($n = 4$). This study was designed to further investigate the similar results we found under low phosphorus soil conditions in one of our previous study (Rahman and Schoenau 2021). The negative effect was observed only with P-limited soil condition, but there was no effect of Cu and Zn with P fertilization. The rate of Cu and Zn used in this study is often considered as recommended rate of application in deficient soils. Additionally, we observed an antagonistic effect with the rate of 5 kg·ha^{-1} of each micronutrients when both Cu and Zn were applied in soil. Therefore, the study was designed with different rates for single and combine application of these micronutrients.

The controlled environment experiment was set up using plastic containers each filled with 2 kg of field soil. The surface layer of field soil (0–15 cm) was collected and

Table 1. Physicochemical properties and fertility status of soils (0–15 cm) used in controlled environment (Levine, Waskada, and Tisdale) and field (Echo) studies.

Soil	Basic properties					Extractable nutrients (mg kg^{-1})		
	pH	EC	FC	OC	Sand	P	Cu	Zn
Levine	7.5	0.66	45.8	3.94	38	3.95	0.83	2.29
Waskada	6.7	0.13	18.2	1.57	76	2.65	0.47	11.3
Tisdale	6.7	0.32	32.9	3.81	10	5.81	1.00	1.43
Echo	7.1	0.21	25.8	1.64	46	4.59	0.85	0.37

Note: EC, electrical conductivity (mS·cm^{-1}); FC, moisture content at field capacity (%); OC, organic carbon (%); sand (%). Phosphorus was extracted using modified Kelowna and diethylene-triamine-pentacetic acid extraction was used for Cu and Zn.

homogenized for experimental use. The pots were filled with those soil without imposing any compaction. Based on the pot volume occupied and weight of soil added, it usually works out to a bulk density of ~1.0–1.1 g·cm^{-3} immediately following addition. Bulk densities of 1.0–1.2 g·cm^{-3} are typical of surface soils in the region. Prior to seeding, all fertilizers were mixed in a layer 2 cm of soil surface to simulate a broadcast and incorporated application. Initially, six wheat seeds were planted and, after germination, thinned to three plants to maintain a uniform plant population in each treatment replicate. The growth chamber environment was regulated at 18 hour photoperiod (day) with an average of 450 μmol·m^{-2}·s^{-1} photon flux density, while the temperature was 23 °C and 18 °C for day and night (6 hours), respectively. The soil moisture content was maintained at a regulated level by watering the trays on which the pots were placed. The watering schedule was maintained based on the moisture status of pot soils and the plants received constant amount of moisture by capillary absorption. Pot position was randomized every 2 days over the course of the study. In the field study at Central Butte, the individual experimental plot size was 1 m × 3 m with wheat grown in three rows per plot. All basal granular macronutrient fertilizers and a solution of Cu- and Zn-sulfate fertilizer was broadcast and incorporated to ensure uniform distribution in soil. Appropriate herbicides were sprayed for in-crop weed control. There was a gentle slope at the field site, which is difficult to differentiate by slope gradient percentage. However, the experimental blocking was done across the slope where the block I was located on knoll and block IV at low slope position. There was not much difference in slope gradient between block I and II. Interestingly, during the growing season, we observed that the crops grown on knoll position (block 1) showed some stress-related symptoms, which were similar to the pot study. Therefore, four subsamples were collected from each plot to understand the treatment differences within a block. The crop was harvested at maturity, dried at 40 °C to a constant weight, and then threshed mechanically using a rubber belt threshing machine to measure grain and straw biomass yields. A random subsample of grain and straw materials was ground using a stainless steel (chromium, nickel, and iron alloy) grinder to avoid Cu and Zn metal contamination. Soil samples were also collected at harvest, air-dried at 25 °C, and a random subsample was ground manually using a wooden roller pin and passed through a 2 mm sieve. The processed plant and soil samples were stored in plastic vials for laboratory analyses.

Extraction procedures and analyses

Measurements of pH and EC on soil extracts were made with a glass electrode using a soil-to-water ratio of 1:2 (Hendershot et al. 2007; Miller and Curtin 2007). The LECO-C632 carbon analyzer set (LECO© Corporation, St. Josesph, MI, USA) was used to quantify soil organic carbon (OC) in samples pretreated with HCl (Harris et al. 2001). The particle size analysis was performed using the modified pipette method described in Indorante et al. (1990). Moisture content at field capacity was measured by gravimetric method (Reynolds 1970). Soil-available nutrient extraction protocol include the modified Kelowna method for P (Qian et al. 1994) and 0.005 mol·L^{-1} DTPA extraction for Cu and Zn (Lindsay and Norvell 1978), respectively. Modified Kelowna is the most commonly used soil test method in western Canada for plant available P assessment. Generally, the Modified Kelowna extracting solution contains ammonium fluoride, ammonium acetate, and acetic acid, which were considered to perform better over a wide range of soil pH. For total digestible concentration, the plant and soil samples were digested using a microwave digestion system following the USEPA 3051 A method (USEPA 2007). The three-step modified BCR (Community Bureau of Reference) sequential extraction procedure was used for operationally defined speciation analysis of micronutrient metals in soil (Zemberyova et al. 2006). In brief, soil solution-carbonate-exchangeable fraction (F_1) was extracted first by 0.11 mol·L^{-1} acetic acid followed by oxyhydroxide fraction (F_2) and organic matter and sulphide-bound fraction (F_3) extraction using 0.5 mol·L^{-1} hydroxylamine hydrochloride, and hydrogen peroxide (8.8 mol·L^{-1}) treated 1.0 mol·L^{-1} ammonium acetate, respectively. The residual fraction was calculated by subtracting all these fractions from the total concentration. The concentrations of Cu and Zn in solutions were analyzed by flame atomic absorption spectrophotometer (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA), while a Technicon Autoanalyzer II (Technicon Industrial Systems, Tarrytown, NY, USA) was used for colorimetric method of P measurements. According to the instrument manufacturer, the optimum working range using the spectrophotometer is 0.03–10 μg Cu·mL^{-1} and 0.01–2 μg Zn·mL^{-1}. Therefore, the use of spectrophotometer was suitable to quantify extractable Cu and Zn of experimental soil.

A number of reference soil and plant materials, such as BCR-701, SRM-1515, SRM-1570a, SRM-1573a, and SRM 2709a, were used to validate the analytical results.

Spectroscopic speciation and data processing

The K-edge X-ray absorption near edge structure (XANES) spectra of Cu and Zn were collected on the hard X-ray micro analysis (HXMA) beamline (06ID-1) of the Canadian Light Source, using a Si (III) double crystal monochromator and a 32 element Ge detector. The beam energy was calibrated with a standard Cu or Zn foil to set the first inflection point of 8979 eV and 9569 eV for Cu and Zn measurements, respectively. Spectra were collected in fluorescence mode on solid-state samples at room temperature. Multiple scans were collected to improve the signal-to-noise ratio. Data were processed and analyzed by using Athena interface of Demeter 0.9.23 software (Ravel and Newville 2005). With an extensive and detailed library of standard spectra previously developed in our lab, the liner combination fitting (LCF) was used for species identification and quantification.

Statistical analysis

All statistical analyses were carried out using SAS 9.4 software (SAS Institute 2013). Prior to analyses, the data were tested for normality using PROC UNIVARIATE and homogeneity of variance was validated using Bartlett's test. The one-way analysis of variance (ANOVA) was performed using PROC MIXED model of SAS 9.4 to determine the significant difference among treatment means. Multitreatment comparisons were made using the Tukey's studentized range test at the probability level of $p \leq 0.05$ to establish statistical significance, where grouping was assigned by the pdmix800 SAS macro (Saxton 1998).

Results and discussion

Yield and nutrient concentration

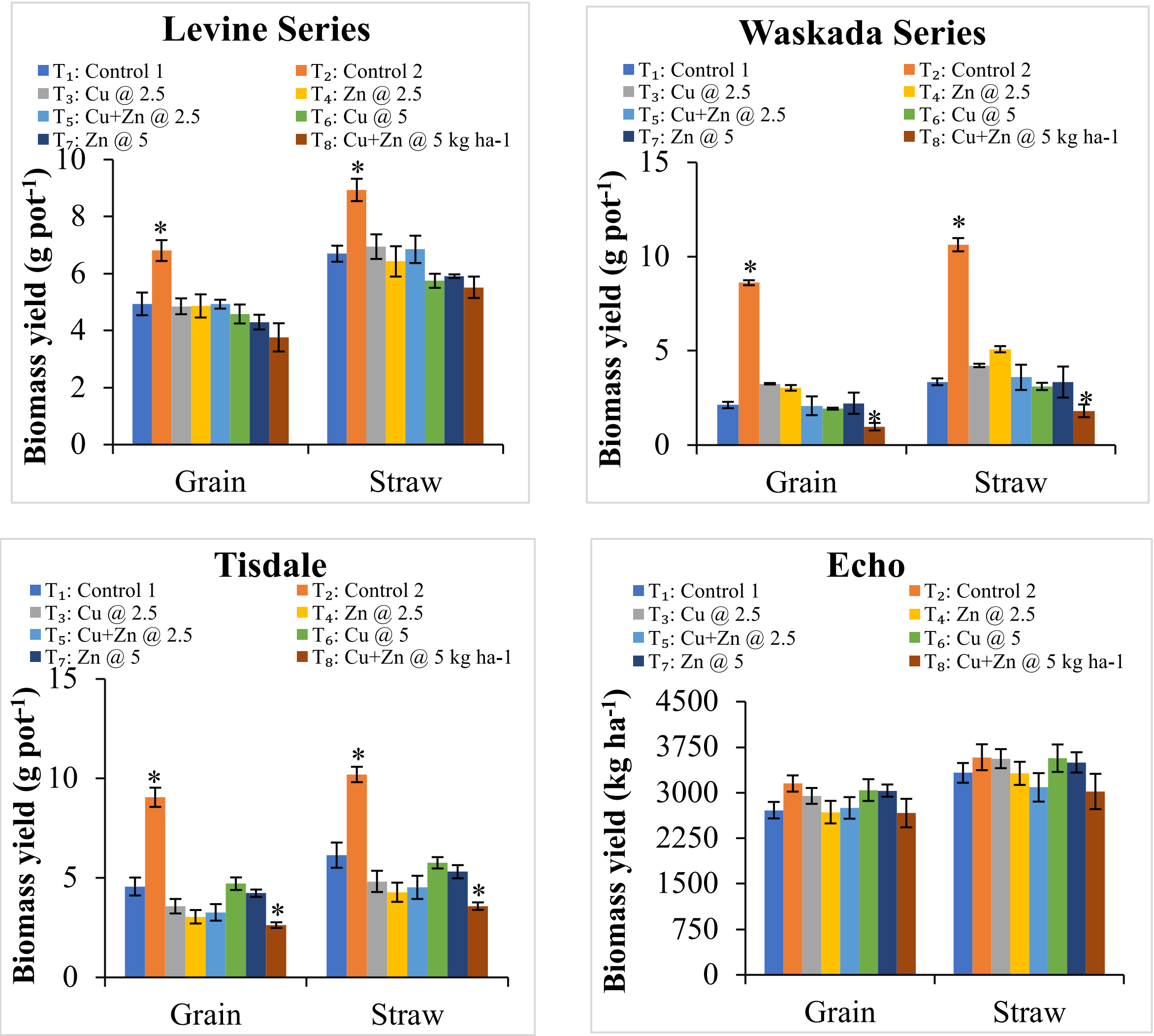
Balanced fertilization is vital for optimizing crop growth and yield. Recommended rate of P addition at 20 kg $P_2O_5 \cdot ha^{-1}$ increased grain and straw biomass yield of wheat grown in all three P-deficient soils used in the controlled environment study (Fig. 1). Much past research (Zentner et al. 1993; McKenzie et al. 2003; Grant et al. 2009; Malhi et al. 2015) has found that wheat responds strongly to starter P on most western Canadian soils that are inherently low in available P. Conversely, adding both Cu and Zn resulted in reduced yields compared to control treatment in two (Waskada and Tisdale) of the three soils under controlled environment conditions, while yield was also depressed at the field site in 2016 (Fig. 1, Table 2). Restricted crop growth was observed when each of these micronutrients were applied at the rate of 5 kg Cu or $Zn \cdot ha^{-1}$. The symptoms of micronutrient metal toxicity that included purplish-red color and chlorotic leaves were visually observed at early growth stages, which could be attributed to aggravated P deficiency, imbalance nutrient uptake, and (or) direct toxic effect of micronutrient excess (Lee et al. 1996; Yadav 2010; Silva et al. 2014).

The tissue analyses confirmed an elevated concentration of Zn in the wheat grown in amended Waskada soil (Tables 3 and 4). Similar results were obtained in a pot study by Takkar and Mann (1978) who reported that >60 ppm Zn in plant tissue was toxic for wheat production in India. High concentrations of Cu in plant tissue can also be associated with toxicity (Havlin et al. 2013). However, this is not always the case as elevated tissue Cu concentration of 9 ppm obtained from the addition of 50 kg $Cu \cdot ha^{-1}$ was not associated with phytotoxicity in barley and wheat grown at a field site in eastern Canada (Gupta and Kalra 2006). In the current study, plant Zn concentrations were more responsive to amendment than Cu concentrations. High level of micronutrient metals in plants causes direct toxic effects including inhibition of enzyme activities and damage to cell structures due to oxidative stress (Van Assche and Clijsters 1990; Yadav 2010).

The concentration of P in grain tissues was increased when recommended rate of P fertilizer added in Levine and Tisdale soils (Table 3). The growth and yield reduction from micronutrient fertilization of the wheat may be associated with imbalances of the P:Cu and P:Zn ratios in plant tissues. The straw Zn concentration was decreased 5.7 fold with P fertilizer addition compared to without P control treatment in the Tisdale soil. However, P fertilization did not appear to be a factor for wheat grown in marginally Cu-deficient (DTPA-extractable Cu $= 0.47$ $mg \cdot kg^{-1}$) Waskada soil in the controlled environment study or in Zn-deficient Echo (DTPA-extractable Zn $= 0.37$ $mg \cdot kg^{-1}$) soil in the field study. In these soils, the tissue concentration of Cu and Zn remained within the range indicating sufficient level. The critical deficiency concentration of Cu and Zn in wheat grain was reported to be less than 1.5 and 15 $mg \cdot kg^{-1}$, respectively, for wheat grown in soils of South Australia (Reuter and Robinson 1997).

Zinc fertilization was effective in increasing wheat Zn concentrations in wheat that was grown on the Echo association soil at the Central Butte field site. Increased concentrations of Zn and P were observed with combined application of Cu and Zn at 5 $kg \cdot ha^{-1}$ rates in Waskada soil. These results suggest that the interaction between P and Cu/Zn may not be always antagonistic and could be synergistic in P uptake process and (or) related to reduced biomass production. Along this line, Stanisławska-Glubiak and Korzeniowska (2005) reported that Zn fertilization enhanced P concentration in wheat under P-deficient soil conditions in Poland. However, they also found that P concentration was reduced with excess Zn application and might be related to Zn toxicity effects. The initial Zn content of Waskada soil was very high and additional Zn fertilization might have resulted in toxic effect in plant growth. For oil seed flax, Zn application did not consistently influence P uptake in western Canadian soil (Grant and Bailey 1993). Overall, in the current study, the reduced crop growth with limited soil P and higher levels of micronutrient metals arising from amendment with micronutrient fertilizer appear to result in imbalanced ratio of P:Cu (753) and P:Zn (36) in wheat grains (Waskada soil), which could have altered and impaired normal metabolic functions in wheat (Neue and Lantin 1994; Cakmak et al. 1997).

Fig. 1. Effect of Cu and Zn fertilization on grain and straw yield of wheat grown in four different soils with low available P. The experiments were conducted in controlled environment condition using three soils (Levine Series GLCU.R, Waskada Series O.BLC, and Tisdale association O.DGC) and at a field site in south central Saskatchewan (Echo B.SS). Treatments are T_1, Control 1 (no micronutrient and no phosphorus); T_2, Control 2 (no micronutrient, but phosphorus added at 20 kg $P_2O_5 \cdot ha^{-1}$); T_3, Cu at 2.5 $kg \cdot ha^{-1}$; T_4, Zn at 2.5 $kg \cdot ha^{-1}$; T_5, Cu + Zn at 2.5 $kg \cdot ha^{-1}$; T_6, Cu at 5 $kg \cdot ha^{-1}$; T_7, Zn at 5 $kg \cdot ha^{-1}$; and T_8, Cu + Zn at 5 $kg \cdot ha^{-1}$. Treatment columns with an asterisk (∗) for grain or straw yield were significantly different ($p < 0.05$) from Control 1 (T_1). Error bar represents standard error of mean. [Colour online]



Extractable available Cu, Zn, and P in post-harvest soil

The DTPA-extractable Cu and Zn were significantly increased with increasing rate of respective fertilizer addition in all soils (Table 4). However, the magnitude of the increase was greater in the pot trials compared to the field study as would be expected given greater dilution in field soil. Although similar treatments were evaluated in control environment pot study and under field condition, several factors such as rainfall and soil sampling were likely to be associated with more dilution in field soil. The limited rainfall throughout the growing season could have an effect on horizontal diffusion and the vertical soil sampling from 0 to 15 cm might have resulted in more dilution effect. It is recognized that soil placement of micronutrients can provide longer-term residual benefits beyond the season of application to succeeding crops (Karamanos et al. 2005; Goh and Karamanos 2006; Fageria et al. 2009). Singh et al. (1987) reported that single application of 10 kg $Zn \cdot ha^{-1}$ significantly enhanced residual Zn level in several Saskatchewan soils. In a similar study, Carsky and Reid (1990) found that a single broadcast and incorporation application of 8 lb $Zn \cdot acre^{-1}$ was effective in correcting the Zn deficiency problem up to 5 years for corn production in New York. However, single large applications of micronutrients are not without potential issues, as excess or unnecessary application of these elements could be toxic for some plant species (Fageria 2000, 2001). The fertilization rate of 51 mg $Cu \cdot kg^{-1}$ of soil (Fageria 2001) or 40 mg $Zn \cdot kg^{-1}$ of soil (Fageria 2000) was found to inhibit wheat growth and yield in Brazilian Oxisols. Initial DTPA-extractable Zn level of

Table 2. Effect of Cu and Zn fertilization on grain and straw yield of wheat grown along an increasing phosphorus fertility gradient moving downslope in a hummocky landscape near Central Butte, Saskatchewan.

Treatment	Block I (upper slope)	Block II (mid slope)	Block III (mid slope)	Block IV (low slope)	Average
			Grain yield (kg·ha^{-1})		
T$_1$	2374bcd	2997ab	2817a	2608ab	2699a
T$_2$	2692abcd	3757a	2950a	3409a	3202a
T$_3$	3128ab	2481b	3410a	2769ab	2947a
T$_4$	3513a	2285b	2870a	2043b	2678a
T$_5$	1967cd	3173ab	3118a	2740ab	2750a
T$_6$	2834abc	3103ab	3715a	2522ab	3044a
T$_7$	2927abc	2987ab	3074a	3250a	3060a
T$_8$	1734c	2223b	3638a	3063ab	2665a
p values	0.0003	0.0008	0.165	0.0077	0.180
SEM	245.6	222.3	268.6	227.5	170.8
			Straw yield (kg·ha^{-1})		
T$_1$	2895ab	3390abc	3801a	3220ab	3327a
T$_2$	2828ab	4303a	2780a	4395a	3577a
T$_3$	3492a	3217abc	4130a	3711ab	3637a
T$_4$	3987a	2691bc	3650a	2947b	3319a
T$_5$	1923b	3527abc	3311a	3590ab	3088a
T$_6$	3366a	3808ab	4275a	3188b	3659a
T$_7$	3449a	3623abc	3337a	3810ab	3555a
T$_8$	1876b	2277c	3692a	4100ab	2986a
p values	0.0004	0.0025	0.088	0.0309	0.215
SEM	306.5	297.2	330.2	280.1	216.6

Note: Treatments are T$_1$, Control (no micronutrient and no phosphorus); T$_2$, Control (no micronutrient, but phosphorus added at 20 kg P_2O_5·ha^{-1}); T$_3$, Cu at 2.5 kg·ha^{-1}; T$_4$, Zn at 2.5 kg·ha^{-1}; T$_5$, Cu + Zn at 2.5 kg·ha^{-1}; T$_6$, Cu at 5 kg·ha^{-1}; T$_7$, Zn at 5 kg·ha^{-1}; and T$_8$, Cu + Zn at 5 kg·ha^{-1}. Four subsamples were collected from each treatment and block. Treatment columns followed by same letter for grain or straw yield are not significantly different ($p > 0.05$). SEM, standard error of mean ($n = 4$).

Waskada soil (11.3 mg·kg^{-1}) was within the range of toxicity according to the findings of a pot study conducted by Takkar and Mann (1978) who reported that 7 ppm DTPA-extractable soil Zn was toxic for wheat grown in India. However, the toxicity of these micronutrient metals could be associated with a number of soil factors, including soil moisture, pH, clay minerals, organic matter, inorganic anions and cations, and chemical forms in soil (Alloway 1995; Mortvedt 2000; Havlin et al. 2013).

Chemical speciation of Cu and Zn

The adsorption and transformations that applied micronutrient metals undergo in soils affect bioavailability as well as the efficiency of fertilization. Chemical speciation results obtained from the sequential extractions of post-harvest soils are shown in Tables 5 and 6. The sequential extraction provided more or less detail information on relative distribution of applied Cu and Zn to operationally defined soil solution-carbonate-exchangeable fraction, oxyhydroxide fraction, and organic matter and sulphide-bound fractions. The amount of Cu and Zn in the soil solution-carbonate-exchangeable fraction constituted the smallest of all fractions in all soils. It appears that in these four soils, the major proportion of the micronutrients were associated with the organic-bound and residual fractions. The residual fraction is considered as chemically stable and biologically inactive (Alloway 1995; Mortvedt 2000). Similar speciation results were reported for agricultural soils of Canadian prairies (Liang et al. 1991*a*, 1991*b*; Qian et al. 2003; Maqsood et al. 2016; Anderson et al. 2018), which showed that the largest proportion of micronutrient elements were occluded with more stable organic and mineral-bound fractions.

The average total concentration of Cu and Zn significantly increased with fertilizer addition. However, the added micronutrients were preferentially speciated into labile and adsorbed forms, with more reactive species including the organic fraction and amorphous oxyhydroxides of Fe and Mn. Within a crop-growing season, the soil-applied micronutrients are unlikely to enter the crystalline lattice of primary and secondary minerals. Apart from the increased concentration in soil solution-carbonate-exchangeable fraction, the majority of Cu was distributed to oxyhydroxides and organic-bound species, and Zn was primarily associated with oxyhydroxide fractions. It is widely known that Cu has a stronger affinity for organic matter complexation than Zn (Alloway 1995; Mortvedt 2000). Copper is also known to form inner sphere complexes with organic matter (specific adsorption) due to the prevalence of reactive surface sites, whereas Zn adsorption typically occurs on the external surface of silicate clay minerals through weaker electrostatic interactions

Table 3. Effect of Cu and Zn fertilization on total concentration of Cu, Zn, and P in wheat tissue.

	Experiment and soil type											
	Controlled environment									Field		
	Levine Series			Waskada Series			Tisdale			Echo		
Treatments	Cu	Zn	P	Cu	Zn	P	Cu	Zn	P	Cu	Zn	P
Concentration in grain (mg·kg^{-1})												
T_1	5.13ab	51.2a	1654b	3.41ab	47.0cd	1795b	4.66b	47.3bc	1770bcd	4.77a	39.3c	4965a
T_2	4.79ab	48.1a	2495a	3.07b	39.6d	2165b	4.62b	35.6d	2527a	4.70a	38.3c	5192a
T_3	5.13ab	52.2a	1705b	4.07ab	74.9ab	2075b	5.51ab	46.9c	1782bc	4.84a	41.4bc	5064a
T_4	5.35a	53.1a	1791b	3.31ab	72.4ab	2035b	4.60b	48.7bc	1569cd	4.52a	46.1a	5154a
T_5	5.41a	54.3a	1676b	3.50ab	71.5ab	1935b	5.23ab	49.1bc	1528d	4.55a	45.0ab	4962a
T_6	5.57a	49.7a	1528b	4.02ab	60.2bc	1715b	5.99a	65.8a	1893b	4.73a	37.9c	4917a
T_7	5.16ab	55.0a	1621b	3.42ab	75.9ab	1987b	5.46ab	64.1a	1739bcd	4.70a	47.5a	4919a
T_8	4.31a	47.7a	1730b	4.25a	89.0a	3200a	5.05ab	51.7b	1601cd	4.80a	48.3a	5107a
p values	0.004	0.126	0.0002	0.014	<.0001	<.0001	0.0004	<.0001	<.0001	0.053	<.0001	0.214
SEM	0.196	1.61	114.5	0.239	4.03	135.6	0.203	1.06	54.1	0.075	0.996	64.7
Concentration in straw (mg·kg^{-1})												
T_1	2.88abc	27.9ab	112a	2.25ab	21.8cd	93.5b	4.14a	14.4c	99abc	2.29ab	9.30de	749a
T_2	2.95 abc	14.0d	103a	1.79b	8.0d	121.2b	3.90a	2.54d	111ab	2.25ab	8.25e	869a
T_3	3.09 abc	21.6bcd	103a	2.64a	61.8ab	103.2b	4.24a	14.9c	72bc	2.48a	11.3cde	852a
T_4	3.08 abc	28.1ab	114a	2.58ab	55.4abc	127.7b	3.30a	17.2bc	72bc	2.42a	17.2ab	937a
T_5	3.22ab	30.2ab	96a	2.65a	57.4abc	122.1b	5.25a	22.7bc	65c	2.19ab	16.5abc	845a
T_6	3.65a	24.4abc	79a	2.53ab	27.8bcd	65.7b	4.46a	27.7ab	114a	2.21ab	9.91de	800a
T_7	2.51bc	33.3a	82a	2.27ab	85.4a	121.8b	4.66a	35.7a	92abc	2.00b	13.7bcd	704a
T_8	2.13c	15.6cd	78a	2.49ab	77.2a	265.5a	5.04a	20.2bc	70bc	2.24ab	19.0a	812a
p values	0.005	<.0001	0.077	0.0404	<.0001	<.0001	0.2720	<.0001	0.0016	0.0002	<.0001	0.1749
SEM	0.290	2.20	9.38	0.181	7.57	18.2	0.526	1.96	7.87	0.067	1.21	57.2

Note: Experiments were conducted in controlled environment chamber using three soils (Levine Series GLCU.R, Waskada Series O.BLC, and Tisdale association O.DGC) and at a field site in south-central Saskatchewan (Echo B.SS). Treatments are T_1, Control (no micronutrient and no phosphorus); T_2, Control (no micronutrient, but phosphorus added at 20 kg P_2O_5·ha^{-1}); T_3, Cu at 2.5 kg·ha^{-1}; T_4, Zn at 2.5 kg·ha^{-1}; T_5, Cu + Zn at 2.5 kg·ha^{-1}; T_6, Cu at 5 kg·ha^{-1}; T_7, Zn at 5 kg·ha^{-1}; and T_8, Cu + Zn at 5 kg·ha^{-1}. Means in columns followed by same letter are not significantly different ($p > 0.05$). SEM, standard error of mean ($n = 4$).

Table 4. Soil diethylene-triamine-pentacetic acid extractable Cu, Zn, and modified Kelowna extractable P (mg·kg^{-1}) in soils collected post-harvest after wheat was grown in four different soils.

	Experiment and soil type (mg·kg^{-1})											
	Controlled environment									Field		
	Levine Series GLCU.R			Waskada Series O.BLC			Tisdale O.DGC			Echo B.SS		
Treatments	Cu	Zn	P	Cu	Zn	P	Cu	Zn	P	Cu	Zn	P
T_1	0.91c	1.99c	3.51a	0.65c	11.4cd	3.88a	0.90c	1.47c	5.06ab	0.86c	0.35c	4.62b
T_2	0.89c	1.96c	4.08a	0.50c	10.1d	4.14a	1.00c	1.30c	5.89a	0.80c	0.36c	5.74a
T_3	5.24b	1.94c	3.75a	4.75b	12.4c	2.01c	4.15b	1.37c	5.17ab	1.35b	0.34c	4.46b
T_4	0.89c	6.67b	3.46a	0.16c	15.3b	2.52bc	1.22c	4.50b	4.89ab	0.79c	1.24b	5.13ab
T_5	5.62b	5.98b	3.49a	3.61b	14.4b	3.30ab	4.74b	4.94b	4.61ab	1.41b	1.18b	4.39b
T_6	8.39a	1.88c	3.68a	7.56a	11.4cd	2.95abc	9.95a	1.41c	4.09b	1.85a	0.34c	4.82b
T_7	0.92c	9.69a	3.73a	0.48c	17.4a	2.49bc	1.06c	8.27a	4.97ab	0.87c	2.13a	5.07ab
T_8	7.64a	8.72a	4.28a	8.34a	17.2a	2.97abc	9.02a	7.80a	5.28ab	1.81a	2.00a	4.86b
p values	<.0001	<.0001	0.063	<.0001	<.0001	<.0001	<.0001	<.0001	0.034	<.0001	<.0001	<.0001
SEM	0.343	0.235	0.195	0.311	0.340	0.259	0.389	0.433	0.318	0.054	0.132	0.193

Note: Experiments were conducted in controlled environment chamber using three soils (Levine Series GLCU.R, Waskada Series O.BLC, and Tisdale association O.DGC) and at a field site in south-central Saskatchewan (Echo B.SS). Treatment evaluations include T_1, Control (no micronutrient and no phosphorus); T_2, Control (no micronutrient, but phosphorus added at 20 kg P_2O_5·ha^{-1}); T_3, Cu at 2.5 kg·ha^{-1}; T_4, Zn at 2.5 kg·ha^{-1}; T_5, Cu + Zn at 2.5 kg·ha^{-1}; T_6, Cu at 5 kg·ha^{-1}; T_7, Zn at 5 kg·ha^{-1}; and T_8, Cu + Zn at 5 kg·ha^{-1}. Means in columns followed by same letter for total nutrient concentration in plant tissue are not significantly different ($p > 0.05$). SEM, standard error of mean ($n = 4$).

Table 5. Chemical fractionation of Cu and Zn in three soils following growth of wheat under controlled environment conditions.

	Cu					Zn				
Treatments	SEC$^{\text{F1}}$	OXB$^{\text{F2}}$	OMB$^{\text{F3}}$	RES$^{\text{F4}}$	TOT$^{\text{F5}}$	SEC$^{\text{F1}}$	OXB$^{\text{F2}}$	OMB$^{\text{F3}}$	RES$^{\text{F4}}$	TOT$^{\text{F5}}$
——Levine Series (mg·kg^{-1} soil)——										
T_1	0.67c	0.59c	3.21c	5.57a	10.0c	1.22c	11.7d	12.7b	26.7a	52.3bc
T_2	0.73c	0.69c	3.34c	5.09a	9.86c	1.17c	12.3cd	12.4b	26.8a	52.7bc
T_3	2.36b	2.89b	5.10b	3.88ab	14.2b	1.24c	11.9d	12.4b	26.1ab	51.6c
T_4	0.82c	0.51c	3.07c	4.93ab	9.33c	2.88b	14.9b	12.6b	25.1ab	55.5ab
T_5	2.23b	2.27b	4.55b	5.09a	14.1b	2.73b	13.9bc	12.6b	25.1abc	54.3abc
T_6	4.15a	4.10a	7.28a	3.60ab	19.1a	1.34c	11.7d	12.1b	26.6a	51.7bc
T_7	0.78c	1.31c	2.84c	4.12ab	9.05c	5.80a	17.3a	13.9a	20.9c	57.9a
T_8	4.33a	4.25a	7.43a	2.23b	18.2a	5.00a	16.7a	12.8b	22.2bc	56.7a
p values	<.0001	<.0001	<.0001	0.0108	<.0001	0.0003	<.0001	0.0035	0.0496	0.0116
SEM	0.167	0.321	0.345	0.584	0.506	0.308	0.598	0.262	1.435	1.293
——Waskada Series (mg·kg^{-1} soil)——										
T_1	0.51c	0.49d	2.10c	3.43bc	6.53c	6.31c	10.2c	8.38a	10.3a	35.2b
T_2	0.46c	0.51d	2.38c	3.36bc	6.71c	6.09c	9.74c	7.99a	10.7a	34.6b
T_3	1.90b	2.21c	3.97b	2.81c	10.9b	6.03c	10.1c	8.47a	9.65a	34.3b
T_4	0.63c	0.40d	2.43c	3.73bc	7.19c	8.77b	12.0b	8.35a	9.46a	38.6a
T_5	1.61b	2.08c	3.60b	2.82c	10.1b	8.53b	11.9b	8.36a	9.22a	38.0a
T_6	3.88a	3.92a	4.98a	4.16ab	16.9a	6.65c	10.1c	8.16a	8.24a	33.1b
T_7	0.60c	0.46b	2.36c	3.19bc	6.61c	10.9a	13.9a	8.31a	7.20a	40.3a
T_8	3.57a	3.49d	4.82a	4.96a	16.8a	10.5a	13.5a	7.96a	7.69a	39.7a
p values	<.0001	<.0001	<.0001	0.021	<.0001	<.0001	<.0001	0.915	0.065	<.0001
SEM	0.159	0.138	0.178	0.417	0.383	0.379	0.338	0.314	0.834	0.800
——Tisdale association (mg·kg^{-1} soil) ——										
T_1	0.47c	0.51d	3.94c	10.4a	15.3c	0.87c	6.47c	10.0ab	39.2a	56.6c
T_2	0.50c	0.61d	4.22c	10.2a	15.6c	0.98c	6.49c	9.75b	39.2a	56.4c
T_3	2.73b	1.03c	5.58b	10.7a	20.0b	0.92c	6.55c	10.4ab	38.4a	56.3c
T_4	0.43c	0.60d	4.40c	10.0a	15.4c	2.86b	10.9b	10.1ab	36.6a	60.5ab
T_5	2.56b	1.20c	5.74b	9.68a	19.2b	2.34b	11.0b	11.0a	36.1a	60.4b
T_6	5.32a	2.64a	8.10a	7.69a	23.8a	0.91c	8.29c	10.8ab	36.1a	56.2c
T_7	0.44c	0.58d	3.90c	10.7a	15.6c	4.85a	14.3a	10.6ab	34.2a	64.0a
T_8	5.25a	2.08b	7.61a	8.71a	23.6a	4.53a	14.9a	10.9ab	33.2a	63.6ab
p values	<.0001	<.0001	<.0001	0.138	<.0001	<.0001	<.0001	0.017	0.083	<.0001
SEM	0.142	0.103	0.312	0.795	0.821	0.267	0.729	0.263	1.534	1.22

Note: Different fractions are SEC$^{\text{F1}}$, soil solution-carbonate-exchangeable fraction; OXB$^{\text{F2}}$, oxyhydroxide bound fraction; OMB$^{\text{F3}}$, organic-bound fraction; RES$^{\text{F4}}$, residual fraction; TOT$^{\text{F5}}$, total concentration in soil. Treatments are T_1, Control (no micronutrient and no phosphorus); T_2, Control (no micronutrient, but phosphorus added at 20 kg P_2O_5·ha^{-1}); T_3, Cu at 2.5 kg·ha^{-1}; T_4, Zn at 2.5 kg·ha^{-1}; T_5, Cu + Zn at 2.5 kg·ha^{-1}; T_6, Cu at 5 kg·ha^{-1}; T_7, Zn at 5 kg·ha^{-1}; and T_8, Cu + Zn at 5 kg·ha^{-1}. Means in a column followed by same letter for total nutrient concentration in plant tissue are not significantly different ($p > 0.05$). SEM, standard error of mean ($n = 4$).

(Schlegel et al. 2001; Trivedi et al. 2001; Refaey et al. 2014). Further, the specific adsorption is selective and less reversible than cation exchange or nonspecific adsorption (Bradl 2004). Overall, the majority of added Cu and Zn that was not taken up by the wheat appears to remain or distribute to forms that are considered readily available for plant utilization.

Spectroscopic speciation

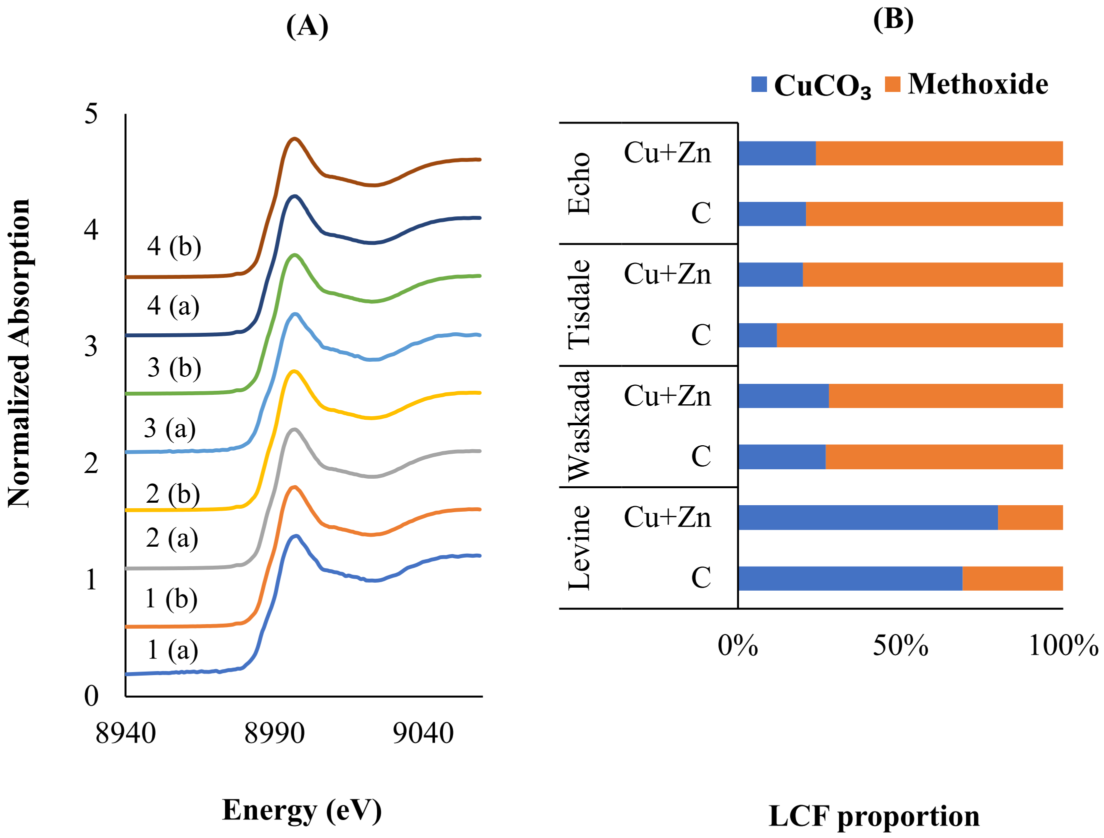
Soil-applied micronutrient metals have a strong tendency to be adsorbed onto the surfaces of mineral and organic matter (Manceau et al. 2000). Therefore, the physicochemical forms or speciation can regulate micronutrient mobility and bioavailability in the soil–plant system. Synchrotron-based K-edge XANES spectroscopy was used to probe the molecular nature of Cu and Zn species in the initial and post-harvest soils fertilized with both of these micronutrients at 5 kg·ha^{-1} rates. The linear combination fitting results revealed that Cu was predominantly associated with carbonate and methoxide phases, regardless of the different types of soils and crop growth environments. Methoxide is the conjugate base of methanol and considered as strong organic base. Copper methoxide is an organic salt usually formed by the

Table 6. Chemical fractionation of Cu and Zn in post-harvest soils (0–15 cm depth) collected in fall after wheat that was grown at a field site near Central Butte, Saskatchewan in 2016.

	Cu (mg·kg^{-1} soil)					Zn (mg·kg^{-1} soil)				
Treatments	SEC^{F_1}	OXB^{F_2}	OMB^{F_3}	RES^{F_4}	TOT^{F_5}	SEC^{F_1}	OXB^{F_2}	OMB^{F_3}	RES^{F_4}	TOT^{F_5}
T_1	0.41d	0.66d	3.90d	5.91ab	10.9c	0.34e	3.99c	8.25a	25.8a	38.4e
T_2	0.42d	0.74d	3.88d	5.65ab	10.7c	0.31e	3.90c	8.65a	25.3a	38.2e
T_3	0.81b	1.13b	4.93ab	5.29ab	12.2b	0.40e	3.87c	8.96a	25.1a	38.3e
T_4	0.37d	0.73d	4.20cd	5.68ab	11.0c	0.90c	5.40b	8.92a	25.7a	40.9bc
T_5	0.75c	0.99c	4.59bc	5.84ab	12.2b	0.72d	5.59b	8.52a	25.7a	40.6 cd
T_6	1.21a	2.10a	5.33a	5.11ab	13.8a	0.30e	4.06c	8.87a	25.7a	39.0de
T_7	0.40d	0.68d	3.97d	6.15a	11.2c	1.79a	7.31a	8.70a	25.1a	42.9a
T_8	1.19a	2.16a	5.17a	4.75ab	13.3a	1.63b	7.39a	8.51	24.9a	42.4ab
p values	<.0001	<.0001	<.0001	0.0013	<.0001	<.0001	<.0001	0.553	0.882	<.0001
SEM	0.028	0.060	0.184	0.459	0.461	0.061	0.707	0.646	1.805	2.94

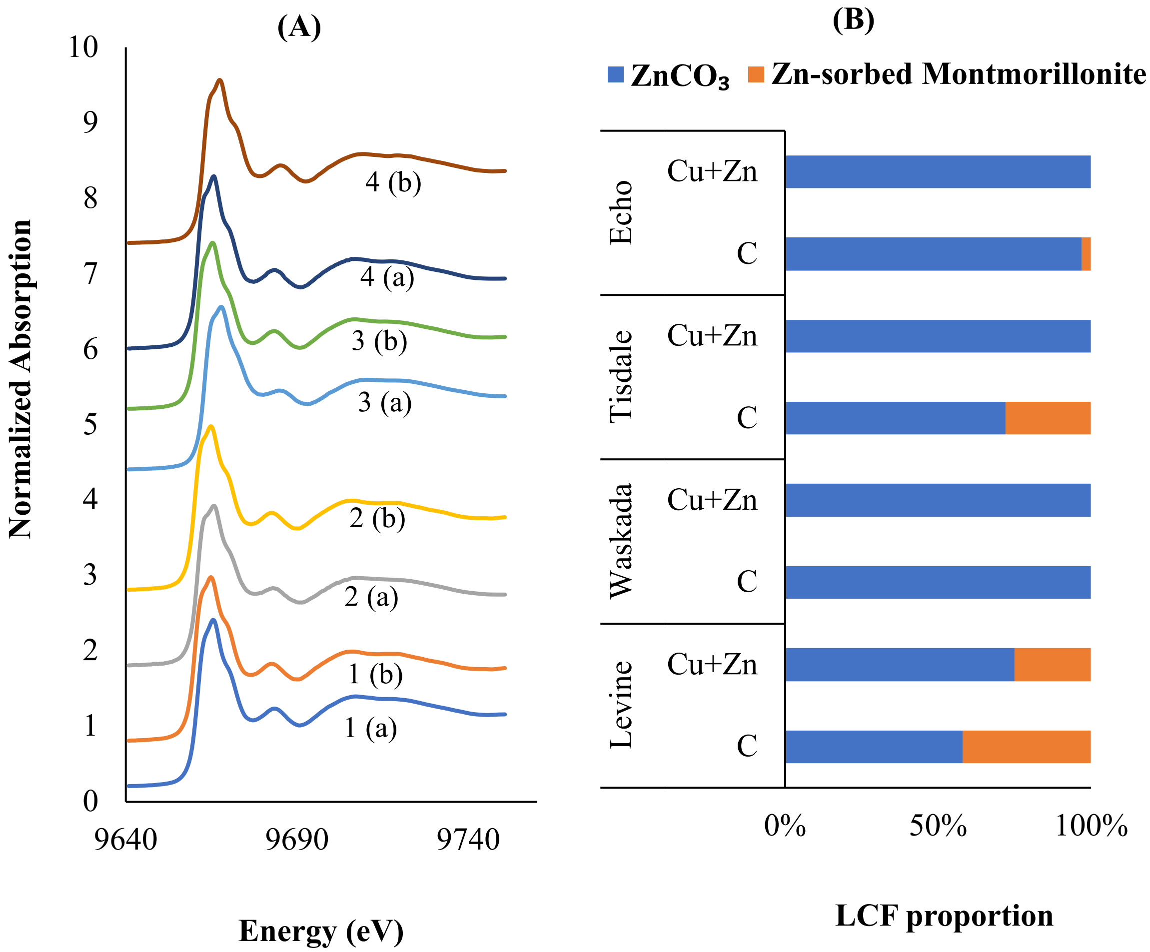
Note: Different fractions are SEC^{F_1}, soil solution-carbonate-exchangeable fraction; OXB^{F_2}, oxyhydroxide-bound fraction; OMB^{F_3}, organic-bound fraction; RES^{F_4}, residual fraction; TOT^{F_5}, total concentration in soil. Treatments are T_1, Control (no micronutrient and no phosphorus); T_2, Control (no micronutrient, but phosphorus added at 20 kg P_2O_5·ha^{-1}); T_3, Cu at 2.5 kg·ha^{-1}; T_4, Zn at 2.5 kg·ha^{-1}; T_5, Cu + Zn at 2.5 kg·ha^{-1}; T_6, Cu at 5 kg·ha^{-1}; T_7, Zn at 5 kg·ha^{-1}; and T_8, Cu + Zn at 5 kg·ha^{-1}. Means in a column followed by same letter for total nutrient concentration in plant tissue are not significantly different ($p > 0.05$). SEM, standard error of mean ($n = 4$).

Fig. 2. (A) Normalized Cu X-ray absorption near edge structure K-edge spectra of four P-deficient soils without (Control) and with (Cu + Zn at 5 kg·ha^{-1}) $CuSO_4$ + $ZnSO_4$ fertilizer amendments. The spectra of four different soils are labelled as: 1 = Levine, 2 = Waskada, 3 = Tisdale and 4 = Echo, where (a) = Control and (b) = Cu + Zn at 5 kg·ha^{-1}. (B) Results of linear combination fit, showing the relative proportions of Cu species among soil types and fertilization treatments. Four different soils are labelled in *Y* axis as C = control and Cu + Zn = amended with $CuSO_4$ + $ZnSO_4$ fertilizers. [Colour online]



deprotonation of methanol, and it can act as a nucleophile. The proportion of $CuCO_3$ was slightly increased in fertilizer-amended post-harvest soils (Fig. 2). Additionally, Zn was found to form several species including $ZnCO_3$ and Zn-sorbed montmorillonite in all studied soils. Zinc fertilization had similar effect on the proportional changes of $ZnCO_3$ species in studied soils according to XANES (Fig. 3). Inorganic Cu and Zn carbonates were used as the reference standard for

Fig. 3. (A) Normalized Zn X-ray absorption near edge structure K-edge spectra of four P-deficient soils without (C, Control) and with (Cu + Zn at 5 kg·ha^{-1}) $CuSO_4 + ZnSO_4$ fertilizer amendments. The spectra of four different soils are labelled as: 1 = Levine, 2 = Waskada, 3 = Tisdale, and 4 = Echo where (a) = Control and (b) = Cu + Zn at 5 kg·ha^{-1}. (B) Results of linear combination fit, showing the relative proportions of Zn species among soil types and fertilization treatments. Four different soils are labelled in *Y* axis as C = control and Cu + Zn = amended with $CuSO_4 + ZnSO_4$ fertilizers. [Colour online]



spectral fitting, and the speciation results are most likely associated with the geologic carbonate materials of soils such as limestone or lime-enriched glacial till.

Extended X-ray absorption fine-structure (EXAFS) spectroscopy revealed that Cu^{2+} and Zn^{2+} adsorbed in the Ca site of calcite structure formed mononuclear inner-sphere complexes at the carbonate mineral surfaces (Elzinga and Reeder 2002). Using XANES and EXAFS spectroscopy on contaminated agricultural soils, it was also found that Cu was mostly associated with soil organic matter, rather than carbonates or oxyhydroxide minerals (Boudesocque et al. 2007; Strawn and Baker 2008). Although Cu had a stronger preference for the dissolved OC, adsorption of Cu onto calcite surfaces occurred in the presence of dissolved humic acid (Lee et al. 2005). However, a decrease in Cu adsorption was observed with increasing concentrations of humic acid (Lee et al. 2005).

Earlier research of Zn sorption on mineral surfaces indicated that outer-sphere complexes were formed with montmorillonite (Schlegel et al. 2001), whereas both inner- and outer-sphere complexes were observed on ferrihydrite mineral surfaces (Trivedi et al. 2001). Typically, the inner-sphere complexation is a stable metal sequestration pathway in most soil environments (Sparks 2005). In addition to adsorbed phases, Zn can be precipitated as Zn-rich phyllosilicates, Zn-layered double hydroxides (Zn-LDH), and hydrozincite at the surfaces of phyllosilicate minerals depending on soil pH and total Zn content of soils (Jacquat et al. 2009). In general, the adsorption or complexation mechanism is favored by low Zn concentration (Janssen et al. 2003), while precipitation will occur with increased concentration due to the saturation of sorption sites (Jacquat et al. 2008). Overall, both chemical and spectroscopic speciation results indicate that carbonate associated is a dominant form of Cu and Zn in these soils, and carbonate-exchangeable forms are important reaction products arising from amendment with Cu and Zn fertilizers.

Conclusion

Phosphorus fertilization was effective in increasing wheat yield in all three soils used in the controlled environment study. Adding both Cu and Zn fertilizers at 5 kg·ha^{-1} rates resulted in significant yield reduction in Waskada and Tisdale soils. The DTPA-extractable Zn concentration of Waskada soil appeared to be high enough to create toxicity problems in wheat production, especially under P deficiency potentially due to an imbalance in P:Zn ratio. An imbalance between P and Zn concentration in plant tissues was observed in Tisdale soils, which might have caused significant disruption in the plant physiology. Tissue Zn concentration was consistently increased with fertilizer addition, especially in Zn-deficient Echo soil. Most of the added Cu and Zn fertilizer remained in the soil in plant available form post-harvest. Chemical and spectroscopic speciation revealed that Cu and Zn associated with carbonates along with phyllosilicate species are dominant reaction products of Cu and Zn sulfate fertilizers regulating exchangeability and bioavailability of micronutrient metals in agricultural soils. In addition, Cu was found to be complexed with oxyhydroxide minerals and organic matter, whereas Zn was adsorbed to oxyhydroxide minerals.

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Author contributions

N.R. conducted data collection, analysis, and wrote the manuscript. D.P. helped in XANES data collection and analyses, and J.S. supervised the project and review the manuscript.

Competing interests

The authors declare no conflict of interest.

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