

Original Research

Manganese and Zinc Foliar Applications Increase Nutrient Content and Mitigate Cadmium-Induced Growth Inhibition in Spring Wheat

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Abstract

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops, and its sustained production and nutritional security have attracted much attention. Cadmium, a heavy metal toxic to plants and humans, can enter wheat in various ways. Foliar fertilization can reduce the cadmium content in plants, but how different fertilizers reduce the cadmium toxicity of plants is still unclear. In this study, the effects of foliar application of fertilizers on spring wheat yield, cadmium accumulation, and trace element contents were studied by applying three kinds of fertilizers (multi-element compound fertilizer, manganese-zinc micro fertilizer, and foliar silicon fertilizer) to spring wheat planted in mildly and moderately cadmium polluted areas. The results showed that the yield of wheat when fertilized with multi-element compound fertilizer, manganese-zinc micro fertilizer, and silicon fertilizer increased by 17.1%, 15.7%, and 16.9%, respectively, compared with the control. In addition, all three foliar fertilizers reduced the cadmium content in wheat grains to below the standard value of food pollutants (≤ 0.1 mg/kg), and significantly reduced the transport of cadmium from glume to grain, among which manganese-zinc micro fertilizer had the best effect, with a cadmium reduction rate of 41.7%. At the same time, manganese-zinc micro fertilizer significantly increased the absorption of trace elements in wheat organs. These results indicated that the foliar application of manganese-zinc micro fertilizer could be an

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effective way to increase wheat yield, inhibit cadmium accumulation, and increase nutrient absorption in mildly to moderately cadmium-polluted areas.

Keywords: cadmium, spring wheat, foliar-fertilizer, bioconcentration factor, transport coefficients, trace elements

Introduction

Cadmium (Cd) is a heavy metal element with high toxicity and strong concealability. It is easily absorbed and enriched by crops, eventually harming human health through the food chain and web [1, 2]. Cd often enters the soil through atmospheric sedimentation, sewage discharge and fertilizer application. When Cd pollutes the soil, plants' productivity and biological efficiency are limited by the presence of toxic elements [3]. Reducing Cd content in spring wheat cultivated on moderately and lightly contaminated soils is therefore critical for human health, and efforts should be made to minimize Cd accumulation in grains. Agronomic methods, such as foliar fertilization, appear to be another effective option.

Wheat (*Triticum aestivum* L.) is one of the world's most important food crops and a major contributor of micronutrients to human diets [4]. In addition to its toxic effects, Cd may interfere with the uptake of micronutrients in wheat grains [5]. At present, trace element deficiency is still prevalent in the population of developing countries [6]. Therefore, attention should be paid to the content of trace elements in wheat grains while ensuring that Cd content in wheat grains is safe and edible.

Zn is an essential plant micronutrient involving biochemical functions and maintenance of biofilm [7]. Mn is a mineral element essential for plant growth and crop productivity, due to its active participation in many biological processes, such as activating enzymes, chlorophyll biosynthesis, and photosynthesis [8]. Previous studies have shown that Zn and Mn are Cd congeners, having similar geochemical and environmental characteristics. Foliar Zn and Mn can increase Zn, S, Cu, crude protein content, etc., in grain, and Zn-Mn fertilizer applications are effective in minimizing Cd concentration [9, 10]. It is not clear whether spraying trace elements to plant leaves can affect the change of Cd content in spring wheat grains.

We measured trace elements and Cd contents in wheat organs to investigate this.

The responses of Cd accumulation and trace elements in wheat organs to three different foliar fertilizers were studied. Our objectives were to explore: the content variations of trace elements and Cd after manganese-zinc foliar application.

Materials and Methods

Survey of the Study Area and Plant Culture

The study was conducted in mildly Cd polluted farmland near a mining area in Inner Mongolia, China. (41°6'N, 107°6'E). Heavy metals such as cadmium have been measured in the soil, and heavy metals transferred to wheat grains can have adverse effects on humans.

Wheat cultivar YL4 was used in the present study. It was a variety of wheat that the locals often grew. During the whole growth period, soil, water and fertilizer conditions were consistent with local conditions. Basic physical and chemical properties of local soil: pH 8.06, total nitrogen (TN) 2.87 g/kg, total phosphorus (TP) 0.50 g/kg, total potassium content (TK) 17.97 g/kg, soil organic carbon (SOC) 19.81 g/kg, alkaline-hydrolysis nitrogen (AN) 134.5 mg/kg, available phosphorus (AP) 47.2 mg/kg, available potassium (AK) 279 mg/kg, cadmium content 1.52 mg/kg. The soil is a Salic Calcic Solonetz (WRB classification).

A completely randomized block design was used in this study. The experiment was divided into four treatments: three foliar fertilization treatments and one control treatment. Each treatment was conducted in triplicate and randomly placed in the field. Each plot was 20 m². Three foliar fertilizations are multi-element compound fertilizer (M), manganese-zinc micro fertilizer (MZ, Mn+Zn≥10%), and foliar silicon fertilizer (Si, Si≥8.5%). The multi-element compound fertilizer (M) was developed by the Chinese Academy of Agricultural Sciences. It was a concentrated foliar fertilizer based on the principle of mutual promotion and antagonism between plant nutrient elements. The main components included silicon, iron, copper, and manganese, and the applied concentration was 1.4 ml/L. Manganese-zinc micro-fertilizer (MZ), which mainly consists of manganese and zinc, is a foliar fertilizer produced by Hunan Meixinlong, and the applied concentration is 5.7 g/L. The main component of foliar silicon fertilizer (Si) was silicon, and the applied concentration was 6 ml/L. The plots that received an equal amount of deionized water spray were used as the control group (CK). The wheat was sprayed once at the tillering and heading stages with a handheld electric sprayer. The tillering stage and booting stage were treated with 3.5L and 7L liquid per plot, respectively. The wheat leaves were uniformly covered with water droplets as the standard. All solutions were sprayed to each treatment in the late afternoon of a sunny day to avoid unnecessary loss. Agricultural practices were

performed according to local farmer habits. The spring wheat was sown on March 29 and harvested on July 18. The sowing capacity of spring wheat is 450 kg/ha. The amount of diammonium phosphate applied to the soil during sowing was 375 kg/ha (N-P₂O₅ is 18-46), and the amount of compound fertilizer applied was 225 kg/ha (N-P₂O₅-K₂O is 12-18-15). Other water and fertilizer conditions were consistent with local conditions throughout the growth period.

Determination of Yield Composition

Wheat maturity stage determination of yield, including plant height, ear length, number of grains per ear, 1000-grain weight, biological yield, and economic yield.

Determination of Cd Concentrations and Trace Elements

Before sowing, soil samples were collected by multi-point mixing method to determine soil's basic physical and chemical properties. At the maturity stage of wheat, wheat plants with a length of 30 cm, a width of 10 cm, and a depth of 50 cm were randomly dug in three ridges of each plot. The whole wheat plant and root-soil samples were collected. The soil attached to the roots of the wheat samples was removed with clean water, and then the whole wheat plant was cleaned with deionized water. The samples were oven-dried at 105°C for 30 min, and then at 75°C until reaching a constant weight. The samples were pulverized. Oven-dried samples were digested with a mixture of HNO₃ and HClO₄ (3:1, v/v) on an electric hot plate. The digested samples were maintained at constant volume with deionized water. Cd concentrations were determined by Inductively coupled plasma mass spectrometry (ICP-MS 7500c, Agilent, USA). The levels of iron, manganese, copper, and zinc were estimated by using Atomic Absorption Spectrophotometer (AAS).

Statistical Analysis

The bioconcentration factors (BCF) and the translocation factor (TF) from the lower parts to the upper parts of the plants were calculated:

$$BCF = C_i / C_{soil}$$

$$TF_{ij} = C_i / C_j$$

C_i, C_j, and C_{soil} were the Cd concentrations in i, j, and the soil, respectively; where i, j are the two plant parts being compared from the root, stem, leaf, and grain [11].

Statistical analysis of all data was performed using SAS 9.4 version, and all graphs were drawn using Microsoft Excel 2019. Differences between treatments were analyzed by one-way ANOVA for comparisons between groups. Differences were considered statistically significant when p-values < 0.05.

Results

Growth Status

Spraying different foliar fertilizers could significantly increase the economic yield of wheat, and manganese-zinc micro fertilizer and multi-element compound fertilizer also significantly increased the 1000-grain weight of wheat (Table 1). Compared with the control, the thousand-grain weight of manganese-zinc micro fertilizer and multi-element compound fertilizer treatment increased by 9.08% and 4.21% respectively. However, the economic yield of multi-element compound fertilizer treatment was significantly higher than that of manganese-zinc micro fertilizer treatment by 1.20% (p < 0.05).

Tissue Cd Concentrations

In the comparison of different organs, the content of Cd in wheat grains was less (0.12 mg/kg), but it was still beyond the range of agricultural product quality and safety standards (Table 2). Some safety measures of agronomic regulation should control the Cd content in wheat grain. As can be seen from Table 2, by spraying different types of fertilizers on the wheat leaf surface, it was found that compared with the control, the Cd content in wheat grain under the treatment of three kinds of fertilizers significantly reduced to the range of agricultural product quality and safety standards (≤ 0.1 mg/kg). It is worth noting that, unlike the variation

Table 1. Wheat growth status of different foliar fertilizer treatments.

Treatment	Plant Height (cm)	Spike Length (cm)	Grain Number (g)	Thousand-Grain Weight (g)	Biological Yield (t/ha)	Economic Yield (t/ha)
CK	57.63±1.12 a	8.13±0.06 a	28.63±1.63 a	41.07±0.81 b	9.92±0.09 a	3.60±0.04 b
M	59.77±2.12 a	8.27±0.49 a	32.93±1.12 a	42.80±2.20 ab	10.65±0.20 a	4.22±0.01 a
MZ	60.5±1.75 a	8.47±0.4 a	30.77±3.11 a	44.80±1.74 a	10.37±0.15 a	4.17±0.02 a
Si	59.63±0.9 a	8.57±0.55 a	29.17±4.96 a	41.67±1.29 b	11.00±0.13 a	4.21±0.03 a

Note: Values are mean±standard deviation (SD) (n = 3). CK: control; M: multi-element compound fertilizer; MZ: manganese zinc micro fertilizer; Si: foliar silicon fertilizer. Within each column, values followed by different letters indicate significant differences at the P < 0.05 level.

Table 2. Cd content in wheat organs of different treatments. (mg/kg).

Treatment	Root	Stem	Leaf	Glume	Grain
CK	0.64±0.064 b	0.56±0.009 a	0.59±0.065 a	0.46±0.064 ab	0.12±0.005 a
M	0.72±0.119 ab	0.63±0.133 a	0.44±0.032 b	0.40±0.021 b	0.08±0.006 c
MZ	0.91±0.100 a	0.59±0.141 a	0.38±0.016 b	0.41±0.046 b	0.07±0.007 d
Si	0.70±0.12 ab	0.59±0.216 a	0.60±0.056 a	0.52±0.04 a	0.09±0.002 b

Note: Values are mean±standard deviation (SD) (n = 3). CK: control; M: multi-element compound fertilizer; MZ: manganese zinc micro fertilizer; Si: foliar silicon fertilizer. Within each column, values followed by different letters indicate significant differences at the P<0.05 level.

trend of Cd content in wheat grains, Cd content in wheat roots showed an increasing trend. The effect of manganese-zinc micro fertilizer was the most significant. Compared with the control, the content of Cd in the root increased by 0.27 mg/kg. This may be because the fertilizer is applied directly to the wheat leaves, and the cadmium reduction effect mainly occurs in the wheat grains.

Cadmium Enrichment and Transport

The bioconcentration factor (BCF) reflects the ability of plants to absorb and accumulate heavy metal pollutants from soil. It assesses one of the important indicators of the safety of heavy metal contaminants for human edible agricultural products in the food chain. Fig. 1a) showed that the addition of different foliar fertilizers could increase the bioconcentration factor of wheat roots (The content of Cd in the soil during the harvest period was 1.43 mg/kg-1.67 mg/kg), among which manganese-zinc micro fertilizer (MZ) had the most significant effect (0.60 mg/kg). At the same time, the bioconcentration factor of the wheat stem showed a downward trend, but no significant effect was achieved ($p<0.05$). Different from the other two fertilizers, foliage-sprayed silicon fertilizer (Si) increased the bioconcentration factor of wheat leaves, which may be because Si was directly sprayed on the leaves, and a large amount of Cd was directly fixed in the leaf cells.

Unlike BCFs, TFs mean the ability of heavy metal pollutants to transport between organs in the plant. According to Fig. 1b), spraying of different leaf fertilizers did not significantly change the translocation factor between Root-Stem and Stem-Leaf, but had significant effects on Leaf-Glume and Glume-Grain ($p<0.05$). Under different foliar fertilizer treatments, the translocation ability between Leaf-Glume and Glume-Grain had opposite effects. Foliar fertilizer increased the translocation ability between Leaf-Glume, and manganese-zinc micro fertilizer (MZ) had the most significant effect (1.07). On the contrary, all foliar fertilizers effectively reduced the Cd translocation ability between Glume-Grain.

Trace Elements in Wheat

According to the changes of trace elements in wheat organs, it can be seen that the contents of Fe, Mn, Cu, and Zn in wheat roots can be increased by spraying different foliar fertilizers (Fig. 2). The content of Mn was significantly increased by 0.047 g/kg (M), 0.076 g/kg (MZ) and 0.041 g/kg (Si) compared with the control. However, foliar fertilizer spraying did not significantly change the trace elements in wheat stems (Fig. 3).

It can be found that the contents of Mn and Zn in wheat leaves are significantly increased, which may be due to the direct application of manganese-zinc micro fertilizer (MZ) on wheat leaves, leading to the absorption and accumulation of large amounts of Mn

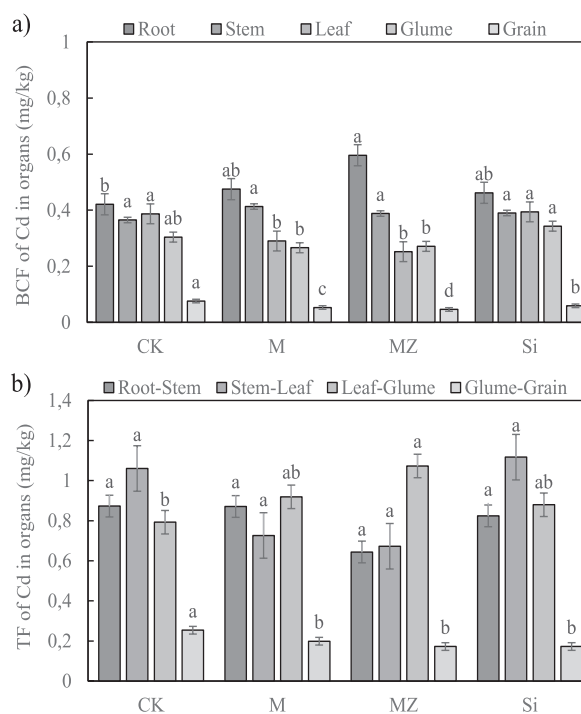


Fig. 1. Bio-concentration factors a) and transport factors b) in different organs of wheat under different treatments. Values are mean±standard deviation (SD) (n = 3). Different lowercase letters indicate significant differences in the same organ under different treatments ($p<0.05$).

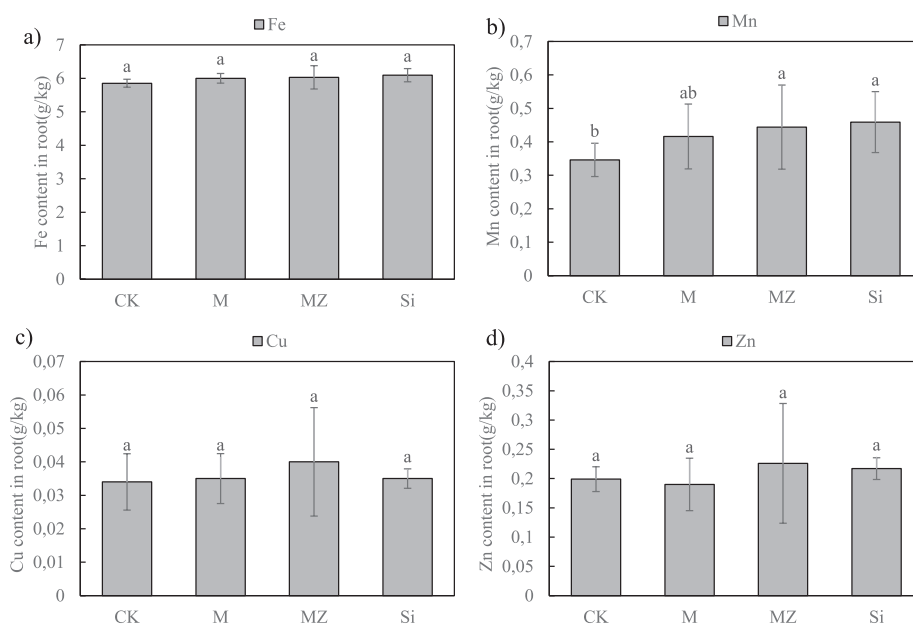


Fig. 2. Effects of different leaf surface fertilization treatments on trace elements: Fe a), Mn b), Cu c) and Zn d) in spring wheat roots. Different lowercase letters indicate significant differences between treatments ($P < 0.05$).

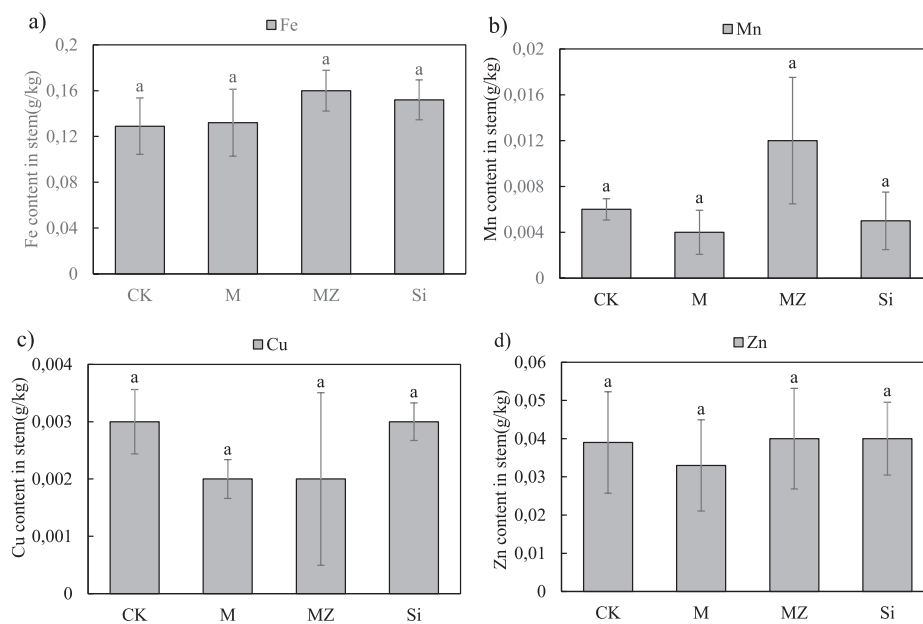


Fig. 3. Effects of different leaf surface fertilization treatments on trace elements: Fe a), Mn b), Cu c) and Zn d) in spring wheat stems. Different lowercase letters indicate significant differences between treatments ($P < 0.05$).

and Zn elements in wheat leaves. Similarly, the Cu content in wheat leaves increased significantly under multi-element compound fertilizer (M) application. In contrast to other treatments, foliage-sprayed silicon fertilizer (Si) reduced the contents of trace elements other than Fe (Fig. 4). It is obvious that the contents of various trace elements in wheat glumes and wheat leaves are consistent (Fig. 5).

According to the contents of trace elements in wheat grains, although Mn and Zn in wheat leaves and

glumes increased significantly under the treatment of manganese-zinc micro fertilizer (MZ), only Mn content in grains increased significantly (0.025 g/kg), while Zn content in grains did not change. However, the contents of Fe and Cu in the grains were slightly increased by applying multi-element compound fertilizer (M) and foliage-sprayed silicon fertilizer (Si) (Fig. 6).

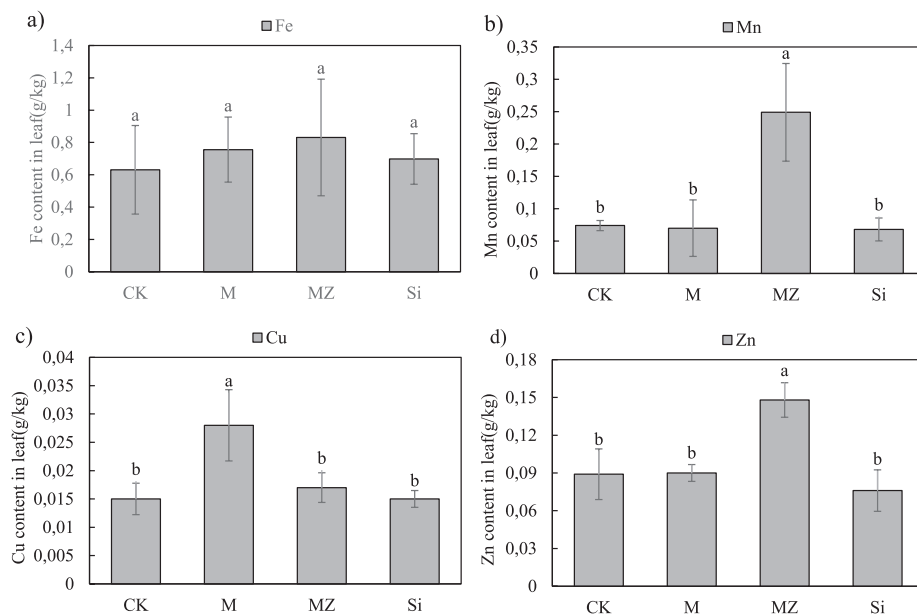


Fig. 4. Effects of different leaf surface fertilization treatments on trace elements: Fe a), Mn b), Cu c) and Zn d) in spring wheat leaves. Different lowercase letters indicate significant differences between treatments ($P < 0.05$).

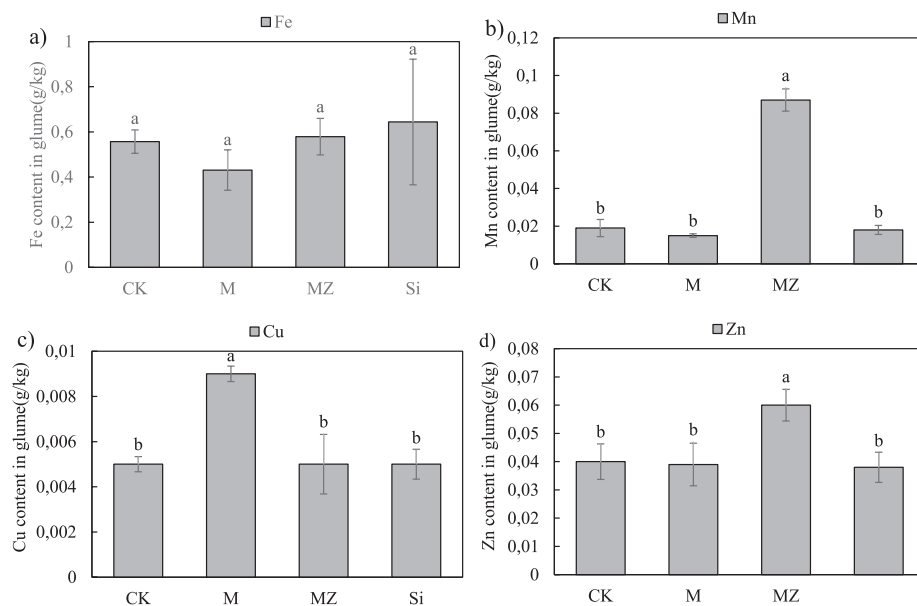


Fig. 5. Effects of different leaf surface fertilization treatments on trace elements: Fe a), Mn b), Cu c) and Zn d) in spring wheat glumes. Different lowercase letters indicate significant differences between treatments ($P < 0.05$).

Discussion

Growth Status

The growth and yield of wheat will be affected by many factors. Heavy metal stress will hinder crop growth and development and lead to crop yield decline [12-14]. The effects of wheat growth factors and the yield on heavy metal stress were also changed by foliar spraying with different fertilizers. Similar to previous

studies [15-17], foliar application of Mn, Zn, Fe, and other elements can significantly increase the number of panicles and 1000-grain weight of crops, ultimately increasing yield. In this study, foliar spraying using the Si element did not significantly increase plant height, spike number, and 1000-grain weight, but economic yield still increased significantly. This may be because Si mainly plays a role in preventing Cd in wheat, while its role in promoting wheat growth is not obvious, which is similar to the results of Hu et al. [18].

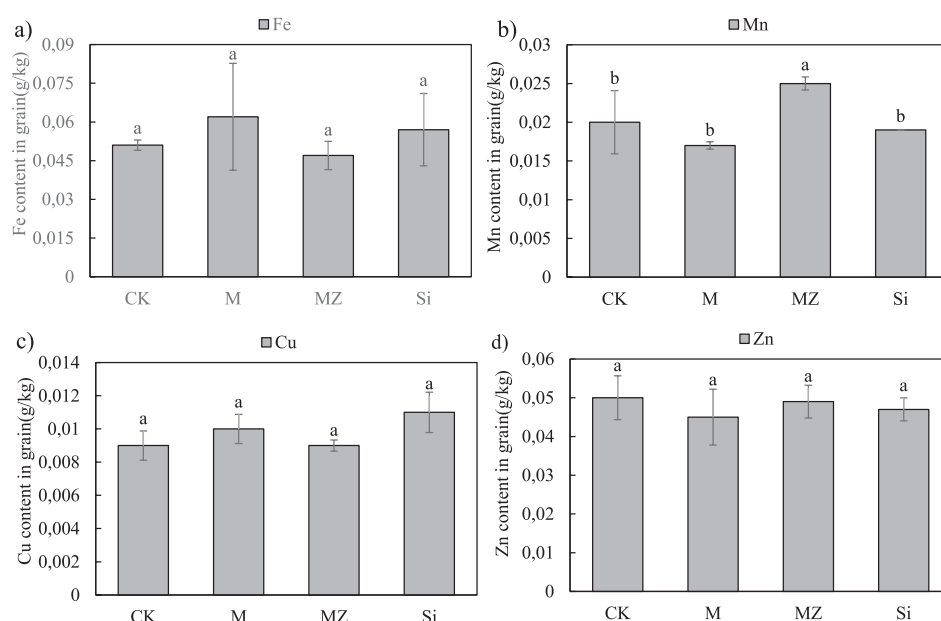


Fig. 6. Effects of different leaf surface fertilization treatments on trace elements: Fe a), Mn b), Cu c) and Zn d) in spring wheat grains. Different lowercase letters indicate significant differences between treatments ($P < 0.05$).

Mitigation of Cd toxicity in Wheat by Spraying Different Foliar Fertilizers

Numerous studies have shown that adding some essential nutrients can reduce heavy metal content in edible parts of crops [19-21]. In this study, three different foliar fertilizer spraying reduced the Cd content in wheat grains to the limited value of food pollutants. The bioconcentration factor of Cd in wheat leaves decreased significantly by spraying manganese-zinc micro fertilizer (MZ) (Fig. 1a), which was mainly composed of Mn^{2+} and Zn^{2+} , indicating that the adsorption of Cd^{2+} in wheat leaves decreased with the increase of Mn^{2+} and Zn^{2+} . In fact, as essential trace elements of crops, Mn and Zn have similar physical and chemical properties to Cd, and they compete for the same carrier proteins and ion channels (such as ZIP, HMAs, and NRAMPs) in crops, so Mn, Zn, and Cd often have antagonistic effects in crops [22-24]. In addition, some studies have shown that the detoxification effect of Mn and Zn on Cd is partly because Mn and Zn can improve crops' photosynthesis and antioxidant enzyme activities, thus alleviating heavy metals' stress on crops [25, 26].

Silicon is not an essential element for higher plants, but it increases crop tolerance to abiotic stresses such as pests, diseases, drought, cold, and heavy metals. Therefore, silicon is a beneficial element in the growth and development stages of crops [27]. However, the Si element is often used to reduce the absorption of heavy metals by plants by affecting crop absorption and transport [28-31]. The application of Si can increase the adsorption of Cd on the cell wall of crops [32]. After the spraying of leaf silicon fertilizer, the content of Cd in the other organs of wheat except the kernel part showed an increasing trend (Table 2), but in this experiment, only

the glume and root showed significant growth. Among them, the significant increase of Cd content in roots may be because Si enters the body of crops through foliar spraying and is partially transported to the roots under the action of a nutrient cycling system, thus stimulating the crop roots to produce metal-chelating secretions [33]. As a large amount of Cd in the glume is adsorbed on the cell wall, intracellular transport is reduced (Fig. 1b), significantly decreasing Cd content in crop grains.

Soil contaminated by heavy metals will have many adverse effects on crops, such as reducing the content of nutrient elements in crops, weakening photosynthesis, increasing the accumulation of heavy metals in crops, inducing oxidative damage, etc. [34-37], which will eventually lead to the decline of crop yield and quality. However, previous studies have found that applying multi-element compound fertilizer can effectively improve the ability of crops to resist the negative effects of abiotic stress [38-41]. In the present study, by spraying multi-element compound fertilizer on spring wheat leaf surface, it was found that compared with the control, the plant height, ear length, 1000-grain weight, and wheat yield increased, and the grain Cd content decreased by 34%. This may be because the addition of multi-element compound fertilizer, on the one hand, increased the increase of the aboveground biomass of wheat, thus diluting the content of Cd in grain [42]. On the other hand, it alleviates nutrient deficiency symptoms caused by the toxic effect of Cd in wheat [34, 43].

Changes of Elements in Wheat

Many papers reported that soil Cd pollution, on the one hand, accumulates a large amount of Cd in

crop seeds, thereby endangering human health [44, 45]. On the other hand, the presence of Cd also affects the absorption of nutrients by crops, especially iron, manganese, copper, zinc, and other elements with the same price as Cd, leading to the deficiency of some nutrients in crops, and eventually inhibiting the growth and development of crops [46-48]. As an important factor in regulating plant tolerance and resisting environmental pressure, micronutrients are especially important in the face of heavy metal pollution [49-50]. In this experiment, by spraying different foliar fertilizers, the exogenous addition of different nutrient elements had a good control effect on the content of Cd in wheat grains, but had no significant effect on the accumulation of Fe, Cu, Zn, and other nutrient elements in wheat grains. In contrast, the accumulation of Mn and Zn in wheat leaves was significantly increased by spraying manganese-zinc micro fertilizer (MZ) on the leaf surface, but only Mn was significantly increased in wheat stems and grains, which may be due to the low fluidity of zinc in leaves [51].

Conclusions

In this study, we studied the effects of different leaf fertilizers on Cd content in various organs of spring wheat. The results showed that different leaf fertilizers could reduce the Cd content of spring wheat grain to the standard range of food pollutants. Among them, leaf spraying manganese-zinc micro fertilizer (MZ) had the best effect on Cd reduction of spring wheat grain and effectively increased the nutrient content in grain and crop economic yield. These results indicate that manganese-zinc micro fertilizer (MZ) can effectively reduce grain Cd content in wheat, which may be related to the ion competition transport channel and biomass. In addition, according to the bioconcentration factor and transport coefficient of Cd among various wheat organs, leaf silicon application effectively fixed Cd in wheat leaves, significantly reducing the upward transfer of Cd and thus reduced the heavy metal content in grains. Silicon spraying may have produced metal chelation in plant leaves.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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