Chemosphere 205 (2018) 350–360

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Effects of $ZnSO_4$ and Zn-EDTA broadcast or banded to soil on Zn bioavailability in wheat (*Triticum aestivum* L.) and Zn fractions in soil

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HIGHLIGHTS

- Zn banded into soil had an unsatisfactory short-term effect on wheat Zn levels.
- Zn banded into soil produced high LOM-Zn levels.
- Zn banded into soil had a beneficial long-term effect on subsequent crops.
- Both Zn-EDTA and $ZnSO_4$ were fixed at a high rate in this calcareous soil.

ARTICLE INFO

Article history:
Received 26 December 2017
Received in revised form
26 February 2018
Accepted 18 April 2018
Available online 21 April 2018

Handling Editor: Patryk Oleszczuk

Keywords:
$ZnSO_4$
Zn-EDTA
Broadcast
Banded
Zn fractions
Triticum aestivum

ABSTRACT

Human Zn deficiency is prevalent in developing countries, and staple grains are commonly bio-fortified to increase their Zn contents. We measured Zn content, distribution, and bioavailability in calcareous soil and in wheat plants (*Triticum aestivum* L.) in Shaanxi Province, China, when either an organic Zn-ethylenediaminetetraacetate (Zn-EDTA) or an inorganic zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) Zn source was banded below the seedbed or broadcasted into soil. Compared with $ZnSO_4 \cdot 7H_2O$, Zn-EDTA fertilization produced higher Zn concentration and uptake in wheat plants. However, Zn bioavailability in grain remained low, with [phytate]/[Zn] ratio >15 and the resulting estimated dietary total absorbed zinc (TAZ) < 3 mg Zn/d. $ZnSO_4$ banded into soil had little short-term effect on grain Zn concentration but had a high residual effect and promoted the maintenance of a high concentration of the Zn fraction bound to loose organic matter (LOM-Zn) in rhizosphere soil. Both $ZnSO_4$ and Zn-EDTA were more efficient if uniformly mixed through the soil than if banded to soil. Both $ZnSO_4$ and Zn-EDTA had limited effects on Zn bioavailability in wheat plants due to the high rate of Zn fixation in this calcareous soil.

1. Introduction

Recent estimates have suggested that two billion people globally are at risk of Zn deficiency. Zn deficiency is a major public health problem in many countries, especially in regions where people rely on cereal-based foods with low Zn concentrations (Cakmak, 2008). Low Zn availability rather than inadequate Zn content in soil is the main cause of Zn deficiency in cereal crops, which is a globally widespread problem (Welch and Graham, 2004).

Bio-fortification of wheat plants is considered a promising strategy to improve Zn levels through agronomic practices (Cakmak, 2008). Agronomic intervention to Zn bio-fortify wheat grain involves fertilizer selection and management. Agronomic Zn bio-fortification practices, such as foliar application of $ZnSO_4$ with or without soil Zn fertilization of wheat plants, were shown to be effective in Zn deficient soils in seven developing countries (Zou et al., 2012). However, the foliar application of Zn fertilizer is associated with relatively high economic and labor costs and therefore has not been widely adopted by farmers (Wang et al., 2016). Additionally, solutions of foliar spray with high Zn concentrations can damage leaves (Zhao et al., 2014; Wang et al., 2015). In comparison, soil Zn fertilization is an effective short-term practice that is used globally to improve Zn bioavailability in soil, especially

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https://doi.org/10.1016/j.chemosphere.2018.04.115

in extremely Zn-deficient soil (i.e. < 0.1 mg/kg soil diethylenetriaminepentaacetic acid-Zn [DTPA-Zn]) (Gonzalez et al., 2007; Ortiz et al., 2009). The usefulness of a Zn fertilizer will depend on its stability and bioavailability in soil. However, 90% of the inorganic Zn fertilizer ($ZnSO_4 \cdot 7H_2O$) applied to a calcareous soil quickly becomes unavailable to plants due to the soil's physical and chemical properties, including high pH and high levels of carbonate (Alloway, 2009; Lu et al., 2012). Soil pH is an important factor in determining Zn availability, and Zn availability decreases as soil pH increases (Li and Shuman, 1996; Lu et al., 2012). At pH values below 7.7, Zn^{2+} predominates, but $ZnOH^+$ is predominant between pH 7.7 and 9.1 and $Zn(OH)_2$ is predominant above pH 9.1 (Alloway, 2008). Additionally, the presence of high levels of active carbonate induces the insolubilization of Zn^{2+} via either Zn adsorption by carbonates and the precipitation of Zn carbonate or the formation of insoluble calcium zincate (Rico et al., 1996). When applied Zn has low availability in soil, fertilization consequently has unsatisfactory effects on Zn levels in plants (Zhao et al., 2014). Accordingly, Zn fertilization should be managed with the aim of supplying appropriate Zn sources, minimizing Zn loss and increasing Zn use efficiency (Timsina and Conner, 2001).

Unlike inorganic Zn fertilizers, organic fertilizers such as Zn-EDTA, in which Zn^{2+} is surrounded by chelating ligands, can supply plants with a substantial amount of Zn that does not become bound to soil components (Gonzalez et al., 2007). At the same application rate, Zn-EDTA was generally three to five times more efficient than $ZnSO_4$ at improving plant Zn concentration (Gangloff et al., 2002; Karak et al., 2005). Additionally, when Zn-EDTA was applied at one-fifth of the $ZnSO_4$ application rate, grain Zn concentrations were 26%–64% higher than those in $ZnSO_4$ treatments (Zhao et al., 2016). Zn-EDTA has shown high efficiency in Zn biofortification of maize (Goos et al., 2000), navy bean (Gonzalez et al., 2007), rice (Karak et al., 2005), and wheat (Zhao et al., 2016) in cultivation. However, research into Zn-EDTA fertilization has mainly focused on greenhouse experiments, which do not directly reflect field-based outcomes. Although Zn-EDTA is expensive, its greater efficiency compared with inorganic Zn may enable Zn bio-fortification in plants with a lower dosage. Therefore, research into the efficiency of Zn-EDTA under field conditions is required.

The response of plants to soil Zn application is primarily affected by the distribution of available Zn, which is influenced by the application method (e.g., broadcast, in band, etc.) (Alloway, 2009). Traditional application of Zn fertilizer involves broadcasting or mixing into 0–20 cm surface soil. Planting pattern, tillage pattern and the low mobility of Zn make Zn concentrations spatially heterogeneous in soil (Zhao et al., 2014). Targeting $ZnSO_4$ to plant roots through banded application led to a higher grain Zn concentration in maize in greenhouse experiments (Goos et al., 2000; Zhang et al., 2013; Zhao et al., 2014). Additionally, banded application requires a smaller amount of Zn fertilizer compared with broadcasted application to achieve the same plant Zn level (Bickel and Killorn, 2007). Accordingly, banded application of Zn fertilizer could be employed to minimize losses and maximize Zn use efficiency.

Plant-available Zn includes the most labile Zn fractions. Measuring Zn fractions in soil can provide researchers with useful information on Zn mobility, availability, and reactivity in the soil (Gonzalez et al., 2007; Bacon and Davidson, 2008). Additionally, root activity significantly influences the biological and chemical properties of rhizosphere soil compared to bulk soil, and it affects the proportions of metal fractions and therefore metal bioavailability (Wang et al., 2002). However, most previous experiments were conducted in pots or hydroponic setups, which do not directly and truly reflect outcomes in the field. Additionally, in these studies, soil Zn fractions were only studied in a specific stage of wheat (at the harvest stage), which does not show the dynamic changes of Zn fractions during wheat growth stages. The growth of wheat roots may significantly affect the transformation of Zn fractions, especially in rhizosphere soil.

In this study, soil Zn fractions were studied throughout all wheat growth stages in a field experiment. We hypothesized that (i) Zn-EDTA applied to calcareous soil will effectively increase Zn availability by increasing the amount of exchangeable and organic matter-bound Zn and reducing the accumulation of Zn in less readily-available fractions, such as Zn associated with calcium carbonate, Fe and Mn oxides, and clay minerals, that (ii) compared to Zn fertilizer broadcasted to soil, Zn fertilizer banded to soil will effectively increase the amount of mobile Zn in calcareous soil and consequently improve plant Zn concentration, and that (iii) Zn availability will be higher in rhizosphere soil than in bulk soil.

2. Materials and methods

2.1. Experimental site and soil properties

This study was conducted during the 2011–2012 and 2012–2013 growing seasons in two neighboring plots at the same Experimental Farm (525 m above sea level, 34°17′56″N, 108°4′7″E) at Northwest A&F University, Yangling, Shaanxi Province, China. The climate at the site is semi-humid, with an average annual temperature of 13 °C and an average annual rainfall of 600 mm. The soil is classified as Eum-Orthic Anthrosol (a Udic Haplustalf in the U.S. soil taxonomy). Before planting wheat, soil samples (0–20 cm depth) were collected, air dried and crushed to pass a 2-mm sieve. The main properties of the topsoil are as follows: pH 8.2 (1:1 soil/water ratio), 13.8 g/kg organic matter, 3.65 mg/kg NH_4-N, 12.9 mg/kg NO_3-N, 10.9 mg/kg Olsen-P, 150 mg/kg available K, 75.2 g/kg $CaCO_3$ and 72.9 mg/kg total Zn. The DTPA-Zn concentration is 0.82 mg/kg, which exceeds the critical deficiency level for DTPA extractable Zn in soil of 0.5 mg/kg (Cakmak, 2008). Lu et al. (2012) indicated that soil is potentially Zn deficient at concentrations of 0.5–1.0 mg DTPA-Zn/kg.

2.2. Experimental design and planting

The experiment included two Zn fertilizer treatments: 20 kg Zn/ha as $ZnSO_4 \cdot 7H_2O$ and 4 kg Zn/ha as Zn-EDTA. The application levels were chosen based on these fertilizers' different efficiencies in supplying Zn to plants (Gangloff et al., 2002). Each of these treatments was further divided into two amendment methods: Zn fertilizer broadcasted to surface soil (0–20 cm depth) and Zn banded in the vicinity of the seedbed at a depth of 20 cm in soil. In addition, one treatment without Zn was prepared as a control. Each treatment had four replicates. The size of each plot was 2 by 3 m, with a 30-cm space between plots and 1 m between blocks.

Before planting, urea (100 kg N/ha), calcium superphosphate (120 kg P_2O_5/ha) and Zn fertilizers were applied to all plots on Oct. 29th, 2011, and after that the first wheat crop (*Triticum aestivum* L. Xiaoyan 22) was sown. The seeding rate was 100 kg/ha. The plots were managed according to local practices. The wheat was harvested on June 14th, 2012. The plots were moved to an adjoining area in the second year to avoid the effects of residual Zn fertilizers. The second wheat crop was sown on Oct. 29th, 2012 and harvested on June 14th, 2013.

2.3. Sample collection

In 2011–2012, soil samples were collected on days 60, 80, 100, 130, 160, and 190 after Zn fertilizer application. These sampling days corresponded to the seedling, tillering, regreening, jointing,