Development of ZnO Nanoparticles as an Efficient Zn Fertilizer: Using Synchrotron-Based Techniques and Laser Ablation to Examine Elemental Distribution in Wheat Grain

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BACKGROUND

- **Zinc (Zn) is an essential micronutrient for animals (including humans) and plants and plays a variety of roles in cell function and protein and enzyme synthesis.**
- **Almost half of the world's arable soils have low levels of plant-available Zn, resulting in widespread Zn deficiency in crops.**
- **Wheat (*Triticum aestivum*) is a major source of nutrition for humans that provides up to half of daily calorific intake. Thus, the consumption of wheat with low Zn concentrations in the grain can potentially have adverse impact on people's health, especially in many developing countries where wheat is a critical component of the diet.**
- **However, traditional Zn fertilizers are not always efficient, and in some soils, they may result in only low (or no) improvements in plant nutrition.**
- **Can we find a new fertilizer with low dosage and high efficiency that can replace traditional fertilizer?**

MATERIALS AND METHODS

- **The four treatments were control (no Zn fertilizer), $ZnSO_4$ sprayed at the stem elongation stage and early milk stage (Zn-2), ZnO-NPs sprayed at the stem elongation stage and early milk stage (ZnO-2), and ZnO-NPs sprayed at the stem elongation stage, booting stage, flowering stage, and early milk stage (ZnO-4). At each application, $ZnSO_4\cdot 7H_2O$ was applied at a rate of 4.2 $kg\cdot ha^{-1}$ (0.96 kg Zn/ha) using a solution (0.7%, w/v, i.e., 7 $g\cdot L^{-1}$) while the ZnO-NPs were applied a rate of 1.2 $kg\cdot ha^{-1}$ (0.96 kg Zn ha^{-1}) using a suspension (0.2%, w/v, i.e., 2 $g\cdot L^{-1}$).**
- **The wheat grain harvested in June 2018 was placed in liquid nitrogen for 30 min after washing with deionized water three times. Transverse sections (150 μm thick) were cut using a Lecia CM1950 cryomicrotome with Tissue Tek (Sakura Finetek USA, Torrance, CA, U.S.A.) embedding medium at -20 ℃. Then, a series of these sections were placed on Kapton tape before being freeze-dried at -53 ℃ and 0.140 bar pressure for 1 h (Telstar LyoQuest-85 Plus, Spain). Subsequently, we conducted a μ-XRF mapping of elemental distribution within the grain sections at beamline 4W1B of the Beijing Synchrotron Radiation Facility (BSRF), China.**
- **Sections of the wheat grains were also examined using LA-ICP−MS, with the preparation of the sections the same as described above. A quadrupole-based Agilent 7700X ICP mass spectrometer (Palo Alto, CA) coupled to the Coherent GeoLas 193 nm ArF excimer LA system (Santa Clara, CA) was used to determine the Zn concentration across linear transects of the grain sections.**

Table 1. Effects of Different Zn Treatments on Grain Yield and Bulk Element Concentration of Winter Wheat. [a,b]

Treatment	Grain yield ($kg\cdot ha^{-1}$)		Zn concentration ($mg\cdot kg^{-1}$)		Fe concentration ($mg\cdot kg^{-1}$)		Mn concentration ($mg\cdot kg^{-1}$)		Cu concentration ($mg\cdot kg^{-1}$)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	5400a	5380a	23.3d	17.9c	41.2a	35.8b	53.3a	43.0a	6.6a	5.7a
Zn-2	5160a	5420a	29.7c	28.4b	42.8a	32.0b	48.7a	45.8a	6.0a	5.1a
ZnO-2	5060a	5570a	33.2b	30.5b	44.5a	43.0a	50.8a	44.9a	6.3a	6.2a
ZnO-4	5280a	5450a	38.6a	40.2a	36.0b	32.9b	54.8a	51.5a	5.5a	5.9a

[a] The first harvest was in 2017 and the second in 2018. [b] Data are the average of the four replicates. Different letters indicate statistical differences between treatments ($P < 0.05$).

Table 2. Effects of Different Zn Treatments on the Concentrations of Various Elements in Wheat Grain.

Treatment	N ($g\cdot kg^{-1}$)		P ($g\cdot kg^{-1}$)		K ($g\cdot kg^{-1}$)		Starch (%)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	24.2a	26.8a	3.9a	3.6a	3.7a	3.9a	60.2a	67.0b
Zn-2	25.8a	27.5a	3.7a	3.3a	3.8a	3.6a	58.5a	66.4b
ZnO-2	25.3a	27.3a	3.7a	3.6a	3.8a	3.8a	58.6a	65.2a
ZnO-4	27.4a	29.4a	3.5a	3.7a	3.8a	3.5a	59.3b	65.6b

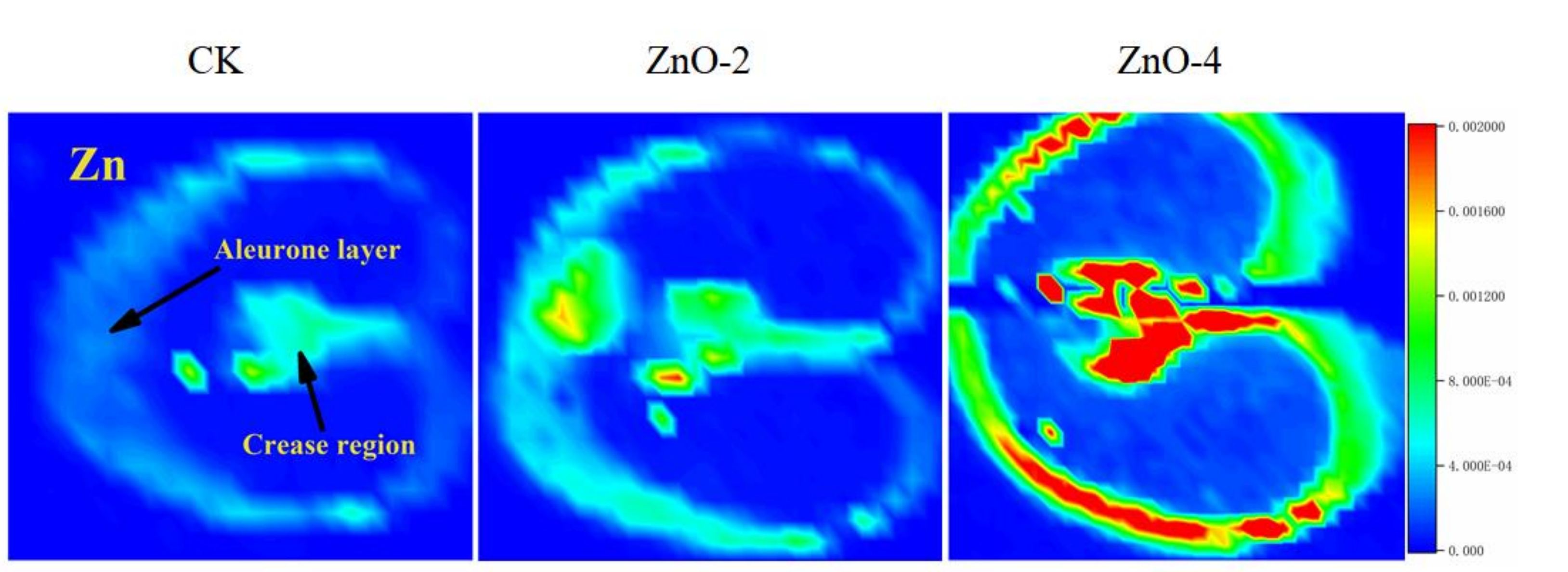

Figure 1. Distribution of Zn in transverse sections (150 µm thick) of wheat grains. The wheat was grown in one of three treatments, being control (no Zn added), ZnO-2 (ZnO-NPs sprayed twice), and ZnO-4 (ZnO-NPs sprayed four times).

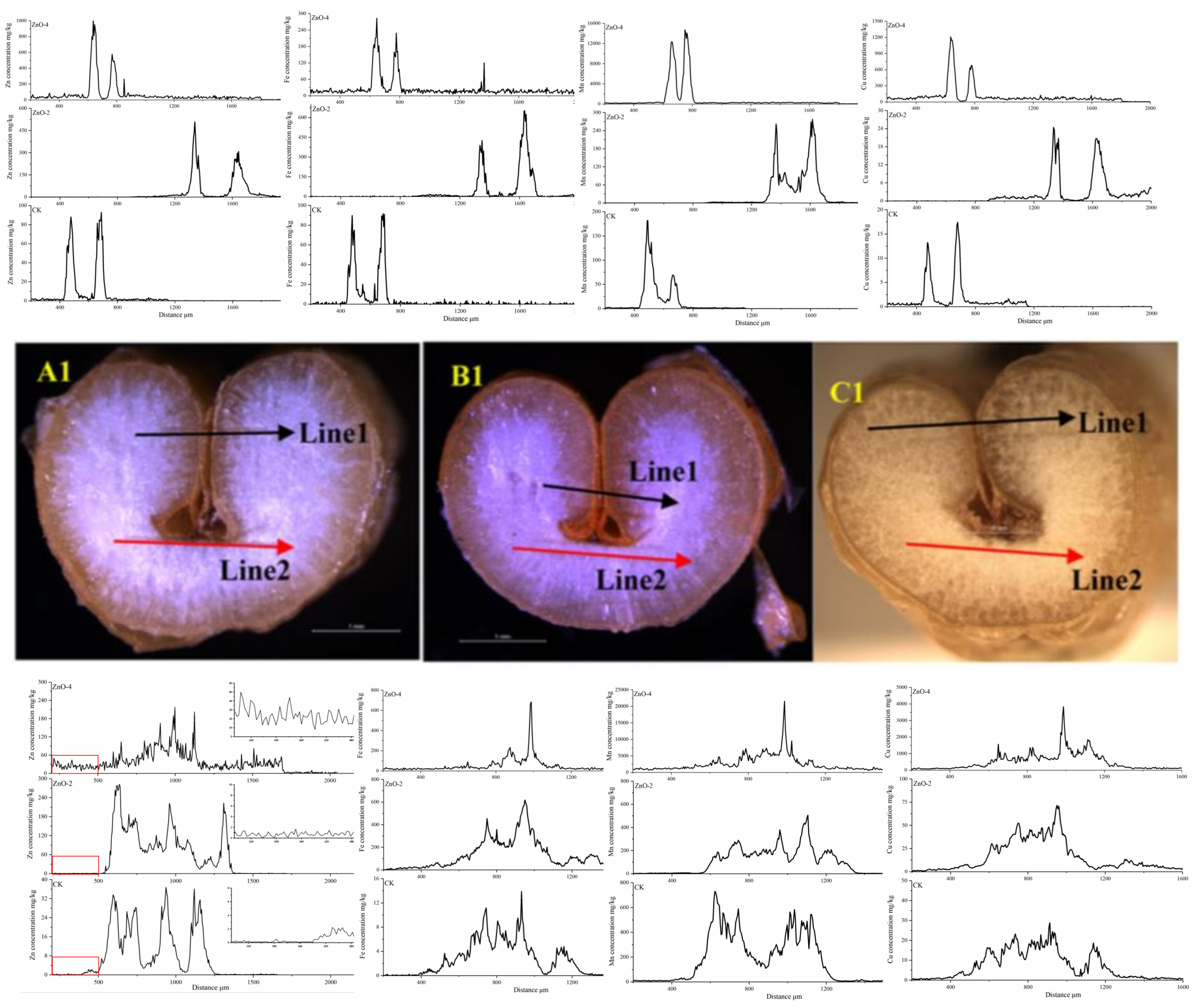

Figure 2. Zn concentration for one-dimensional (linear) transects across the wheat grain sections. Data are presented for three different treatments, being control (A1), ZnO-2 (B1), and ZnO-4 (C1). The top series of graphs show the data for line 1, and the bottom series of graphs show the data for line 2.

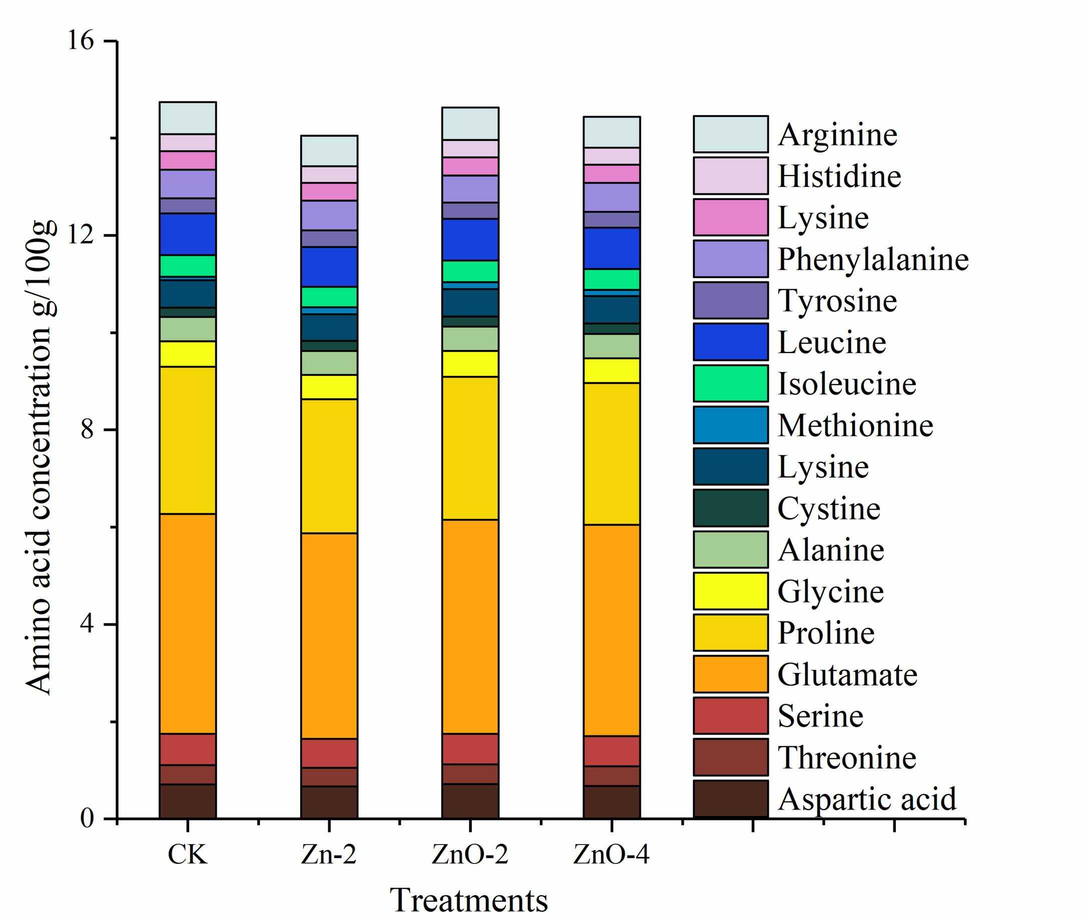

Figure 3. Effects of different Zn treatments on amino acids in the grain of wheat, being for control (no Zn added, CK), ZnO-2 (ZnO-NPs sprayed twice), and ZnO-4 (ZnO-NPs sprayed four times).

SUMMARY AND CONCULSIONS

- **Despite the soil containing low levels of plant-available Zn (see the Materials and Methods), the foliar application of neither ZnO-NPs nor $ZnSO_4$ resulted in significant increases in the wheat grain yield (Table 1).**
- **Across all three treatments, it was observed that the trace elements accumulated primarily in the aleurone layer and the crease region.**
- **Our field-based study has demonstrated that the foliar-application of ZnO-4 can increase the Zn concentration of the endosperm of wheat grain endosperm by ca. 30-fold compared to the control.**
- **For our field-based trial, the foliar application of neither ZnO-NPs nor $ZnSO_4$ resulted in significant changes in amino acid concentrations of the wheat grain.**
- **Overall, in a field study, we have shown that the application of ZnO-NPs is suitable for use as a foliar-applied fertilizer for increasing the Zn concentration in wheat grain.**

FUNDING

This work was supported by the National Natural Science Foundation of China (41571282), the Fundamental Research Funds for the Central Universities (2452015047), the Natural Key Technologies R&D Program (2015BAD23B04), and the Special Fund for Ago-scientific Research in the Public Interest (201503124).

References: Sun H, Du W, Peng Q, et al. Development of ZnO Nanoparticles as an Efficient Zn Fertilizer: Using Synchrotron-Based Techniques and Laser Ablation to Examine Elemental Distribution in Wheat Grain[J]. *Journal of Agricultural and Food Chemistry*, 2020, 68(18): 5068-5075.