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Exposure to Weathered and Fresh Nanoparticle and Ionic Zn in Soil Promotes Grain Yield and Modulates Nutrient Acquisition in Wheat (*Triticum aestivum* L.)

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ABSTRACT: This study evaluated weathered and fresh ZnO-nanoparticles and Zn-salt effects on nutrient acquisition and redistribution in wheat. Weathered and fresh ZnO-nanoparticles and Zn-salt significantly increased grain yield by 15% and 29%, respectively. Postharvest soil acidification indicated ZnO-nanoparticles dissolved during growth. Zn was significantly bioaccumulated from both Zn types, but with low root-to-shoot bioaccumulation efficiency: 24% and 20% for weathered nanoparticles and salt, and 48% and 30% for fresh nanoparticles and salt. Grain Zn content was increased 186% and 229% by weathered nanoparticles and salt, and 229% and 300% by fresh nanoparticles and salt. Shoot-to-grain translocation efficiency was high: 167% and 177% for weathered nanoparticles and salt, and 209% and 155% for fresh nanoparticles and salt. However, Zincon assay indicated grain Zn does not exist as ions. This study demonstrates that ZnO-nanoparticles and Zn-salt vary in their effects on nutrient acquisition in wheat, with relevance for biofortification of Zn for human nutrition.

KEYWORDS: *bioaccumulation, dissolution, residual zinc, transformation, translocation factor, ZnO nanoparticles*

■ INTRODUCTION

Zinc (Zn) is an essential element required to maintain healthy growth and development in both plants and animals. However, Zn scarcity is prevalent in soils in many parts of the globe, resulting in human Zn deficiency due to long-term consumption of staple diets from crops grown in Zn-deficient soils.[1−3] Accordingly, Zn enrichment of food sources globally has been a subject of long-standing interest. Numerous studies show increased yields and/or Zn content in seed or grain of crop plants when exposed to supplemental Zn.[4−9] Thus, agronomic fortification of crops with Zn through fertilization is one way to both enhance yield and enrich edible plant tissues with the nutrient.[2,3] The current major Zn fertilizers include bulk oxides, sulfate salts, and chelated Zn forms; however, nanoscale Zn oxide (ZnO) is increasingly becoming of interest.

Engineered nanoparticles are man-made structures having one or more dimensions in the nanoscale (≤100 nm). These materials are characterized by small size and large surface area, two properties that confer high reactivity and hence high functionality to nanoparticles. Accordingly, one such nanoparticle, ZnO, is being incorporated into different products to improve quality and performance, and this includes use as nanofertilizers and nanopesticides.[10−14] When plants are exposed to Zn of any form in the soil, they acquire the nutrient in their aerial tissues primarily in the ionic form,[15−18] which can be free or chelated; rarely has intact particulate ZnO been detected in shoot tissues.[19] This implies that bulk- and nanoscale ZnO undergo dissolution into ions in the soil, prior to accumulation by most plant species. The other potential fates of ZnO in the rhizosphere include aggregation and surface modification by plant- or soil-derived components and biomolecules,[16,20−22] all of which influence ZnO nanoparticles in different ways. Zn ions released into soil from particulate ZnO can be sorbed or chelated by a variety of compounds, including phosphate, organic acids, clay minerals, other metal oxides, as well as plant or microbially produced metal chelators. These processes may increase or decrease Zn absorption by the plant, dependent on the specific chemistry of the complex and likely on the plant species.[21−26] The so-called "nano-specific effects" are lost upon dissolution, as subsequent nanobioactivity proceeds similarly as dissolved Zn from salt or chelated sources. Still, in planta reduction of ionic Zn back to nanoparticulate Zn occurs,[27] indicating that dissolution is not a dead-end, but one end of a reversible chemical reaction.

It is likely due to nanoparticle dissolution that, despite differences in their pristine physical properties, ZnO nanoparticles and Zn salt (hence, ionic) do not consistently evoke different effects on plants when exposed to comparable levels.[5,8,10,28,29] Plants will be confronted with both new and

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Table 1. Effects of ZnO Nanoparticles and Zn Salt in Used and New Soil on Chlorophyll Production and Vegetative Growth of Wheat[a]

treatment		chlorophyll (SPAD)	tiller number	plant height (cm)	root dry weight (g)	shoot dry weight (g)
used soil	control	42 ± 3 d	37 ± 8 a	97 ± 5 b	9 ± 5 a	78 ± 3 a
	ZnO nano	52 ± 3 a	39 ± 5 a	102 ± 4 a	12 ± 3 a	81 ± 5 a
	Zn salt	50 ± 4 ab	40 ± 3 a	102 ± 3 a	10 ± 2 a	80 ± 3 a
new soil	control	46 ± 4 c	38 ± 3 a	101 ± 3 a	12 ± 4 a	81 ± 5 a
	ZnO nano	47 ± 4 bc	38 ± 6 a	101 ± 4 a	11 ± 2 a	79 ± 4 a
	Zn salt	48 ± 4 bc	40 ± 3 a	100 ± 2 ab	11 ± 3 a	82 ± 7 a

[a]Data are means and standard deviations, and different letters associated with numbers indicate significant differences among the treatments at $p < 0.05$ ($n = 4$).

weathered ZnO nanoparticles in soil. Also, aging of ZnO nanoparticles in soil may influence their bioavailability and bioactivity[29,30] as these materials transform into different species. Such effects might be distinct from those of fresh exposures. However, comparative studies on the response of plants to different Zn forms under weathered and fresh soil exposures are lacking. Such studies may not only shed light on the longer term impacts of Zn as crop fertilizers, but may also provide information on the differential effects of residual versus fresh Zn exposure in plants as nutrients or potential toxicants. Accordingly, the objective of this study was to evaluate the effects of weathered (residual) and fresh Zn from ZnO nanoparticles and Zn salt on wheat productivity and nutrient acquisition and redistribution within the plant under these soil conditions.

■ MATERIALS AND METHODS

Chemicals and Soils. Nanopowder of ZnO (particle size = 18 nm) was obtained from US Research Nanomaterials, Inc., Houston, TX, while Zn salt ($ZnSO_4 \cdot 7H_2O$) was from J.T. Baker, New Jersey. The soil used for the study is denoted as "Brownfield" and was collected from Plains, TX. Brownfield is a sandy loam with a pH of 6.87 and nitrogen (N), phosphorus (P), and potassium (K) levels of 4.0, 2.05, and 246 mg/kg, respectively. The level of bioavailable (DTPA-extractable) Zn fraction is 0.1 mg/kg, indicating a bioavailable Zn status that is well below the critical soil level of Zn for most crops, 0.5−1.0 mg/kg. Portions of this soil were previously cropped with sorghum exposed or not to 6 (+ 0.1) mg Zn/kg from ZnO nanoparticles and Zn salt, as well as NPK (100:50:75 mg/kg).[8] Under these Zn conditions, an average of 0.96 and 1.19 mg Zn was recovered in the above-ground biomass (shoot and grain) by each sorghum plant, from the ZnO nanoparticles and Zn salt treatments, respectively. Therefore, the theoretical soil residual Zn level from the ZnO nanoparticle treatment would be 5.98 mg/kg, and that of the Zn salt would be 5.95 mg/kg. Following harvest of the sorghum, the control, ZnO nanoparticles, and Zn salt treatment soils were cleaned of root materials and were weathered for 6 months to be reused for the current experiment. Eight kilograms of the Zn-weathered soil as well as a fresh batch of Brownfield soil not previously exposed to Zn were loaded onto pots after mixing with NPK at the rates of 200:75:200 mg/kg soil.

Plant Treatment and Growth Conditions. Three winter wheat seeds (*Triticum aestivum* var. Dyna-Gro 9522, obtained from University of Tennessee Knoxville) were sown per pot and were later thinned to one seed per pot after germination. Two weeks after sowing (i.e., 1 week postgermination), plants growing in the new soil were not treated (hence, control; denoted as "new soil") or were treated with ZnO nanoparticles or Zn salt at a rate of 6 mg Zn/kg soil. The Zn products were administered as dry nanopowder or salt crystals in subsurface applications in the vicinity of the roots. In contrast, plants in the used or weathered soil were not treated with new ZnO nanoparticles or Zn salt. Thus, on the basis of the levels of Zn uptake by the previous crop, the used soil treated with ZnO nanoparticles and Zn salt contained about 16% and 20% less Zn, respectively, than the fresh soil. In these used soil treatments, the pots that did not receive Zn in the preceding cropping event represented the control treatment (denoted as "used soil"). Treatments were randomly assigned using a block design consisting of four replicated plants per treatment. Watering and other greenhouse growth management practices were conducted as required. During growth, chlorophyll production and plant vegetative growth were determined. Chlorophyll measurements were taken 2 weeks after Zn treatment using a SPAD meter (Soil & Plant Analyzer Development; Konica Minolta). Late (preanthesis) tiller numbers were also recorded. At full maturity, end-point plant heights for the parent shoots were measured, plants were harvested and separated into root, shoot, and grain, and the biomass weight for each fraction was recorded.

Plant and Soil Analyses. Upon harvest, shoot, root, and grain samples were oven-dried at 60 °C, ground into powder, and acid-digested (3 mL of sulfuric acid + 1 mL of H_2O_2), before being subjected to heating for 1 h at 350 °C, cooling to room temperature, and equilibrating with dd-H_2O. Thereafter, the samples were subjected to analytical procedures to determine total nutrient contents: N and P by Skalar segmented flow analysis; and Zn, manganese (Mn), and iron (Fe) by inductively coupled plasma-optical electron spectroscopy (ICP-OES). Postharvest soil samples were collected per treatment to determine pH and residual nutrient levels for Zn, N, P, Mn, and Fe. For the nutrients, soils were ground and sieved, and soil nitrate-N and ammonium-N were extracted with KCl, while P was extracted by the Pi method. Bioavailable Zn, Mn, and Fe were extracted by DTPA (1:2 w/v [soil:DTPA solution]). All samples were shaken for 2 h and filtered, before being subjected to analytical procedures using the respective above instrumentations. In a separate assay, 2 g of the new soil treated with ZnO nanoparticles was collected from the plant rhizosphere at harvest, mixed in water, and centrifuged at 13 000 rpm. The supernatant was collected, and absorbance at 374 nm was measured using a Hach DR 5000 spectrophotometer to detect whether any intact ZnO nanoparticles were present.[15,31] Pristine ZnO nanoparticle suspension in water was used for comparison.

Determination of Free Zn (Zn Ion) in Wheat Grain. On the basis of the measurement of total Zn content in the grain, an attempt was made to determine whether the Zn existed in the free (ionic) state or was otherwise bound by components of the plant and, therefore, not readily bioavailable. To this end, Zincon (zincon monosodium salt; Alfa Aesar, Ward Hill, MA) was used. Zincon is a well-known chelator of free Zn in complex matrixes, and its complex with Zn is detectible using colorimetry; specifically, complexation of Zn by Zincon results in a color change from dark red to blue. This blue-colored complex has a maximum absorption at 620 nm. Use of Zincon has been applied in plant studies.[32,33] A Zincon solution (50 mg/L) was prepared in mild alkali (0.001 N NaOH). One gram of wheat grain from the control, ZnO nanoparticle, and Zn salt treatments from the new soil was ground into powder. Subsequently, 4 mL of Zincon solution was added onto the dry powder, and the mixture was shaken manually and incubated overnight. Subsequently, 1 mL of each of the suspensions was transferred to an Eppendorf tube and then centrifuged at 13 000 rpm for 10 min. To demonstrate the effectiveness of the Zincon assay to detect Zn ions under these conditions, a series of controls was established: (i) a newly prepared

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Zn sulfate solution (50 mg/L) was reacted with Zincon (1:1 v:v); (ii) 1 g of ground wheat grain from the control treatment was mixed with 2 mL of the Zn sulfate solution and incubated for 30 min, followed by centrifugation and addition of Zincon to the supernatant (1:1 v:v); and (iii) deionized water was mixed with Zincon (1:1 v:v). The color change to blue was observed for all preparations during 5 min, after which the absorbance of all experimental treatments and the quality controls were recorded using a spectrophotometer (Hach DR 5000) set at 620 nm.

Data Analysis. A two-way analysis of variance (ANOVA; OriginPro 2018) was used to determine significant differences in wheat responses to the treatments for each parameter evaluated. A Fisher LSD mean comparison was performed to further explore the differences with significant ($p < 0.05$) ANOVA.

■ RESULTS

Effects of ZnO Nanoparticles and Zn Salt on Chlorophyll Production and Vegetative Growth of Wheat. Leaf chlorophyll levels were significantly increased by ZnO nanoparticles and Zn salt in the used soil, with marginal increases by both Zn types in the new soil (Table 1). Tiller numbers were not significantly affected in all treatments, regardless of soil Zn history and Zn type (Table 1). Plant shoot growth responses to the Zn types mimicked those of chlorophyll; significant average height (cm) differences were observed in the Zn-treated plants in the used soil, relative to the control. In contrast, the shoot growth effects of the Zn types were not significant in the new soil (Table 1). Root and shoot biomass (dry weight basis; g) were insignificantly affected by Zn treatment, regardless of soil Zn history (Table 1).

Effects of ZnO Nanoparticles and Zn Salt on Grain Yield of Wheat. The control treatment in the used soil produced significantly greater ($p < 0.05$) grain yield than the control treatment in the new soil. However, grain yield was promoted by Zn treatment in both used and new soils (Figure 1). In the used soil, the residual ZnO nanopowder and Zn salt increased grain yield significantly ($p < 0.05$), by 15% and 14%, respectively, as compared to the control treatment. In the new soil, yield was also significantly increased, on average by 29%, by both ZnO nanoparticles and Zn salt, relative to the control. Comparing the average influence of Zn on grain yield in the old and new soils against each control (14.5% vs 29%), it can be seen that fresh Zn treatment was more effective in promoting yield than was residual Zn.

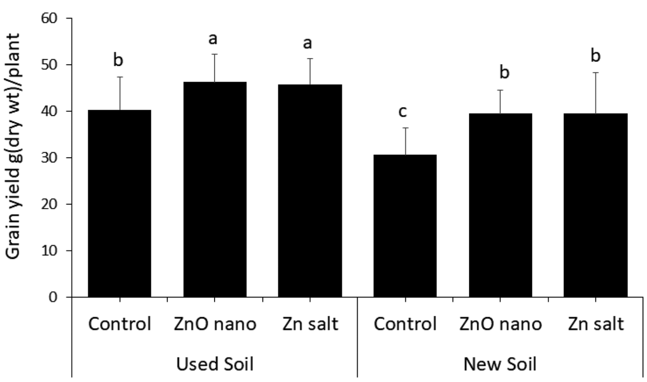

Figure 1. Effects of ZnO nanoparticles and Zn salt in used and new soils on grain yield of winter wheat. Values are means and standard deviations, and different letters on bars indicate statistically significant differences ($p < 0.05$) among the treatments ($n = 4$).

Postharvest Rhizosphere Levels of N, P, and Zn Vary On the Basis of Zn Aging and Type. Plant growth strongly lowered the initial pH of the soil from 6.87 to between 5.60 and 6.19 (Table 2). In addition, the overall postharvest soil chemical properties differed between the used and new soils, especially for pH, ammonium-N, P, and Zn levels, wherein pH reduction was significant between the used and fresh soils (Table 2). In the used soil, Zn treatment generally had a minimal effect on soil properties; no significant differences were observed among the treatments for pH and the respective nutrients, except for the expected increase in Zn content in the exposed soils. In the new soil, Zn treatment effects were more apparent: ZnO nanoparticles increased the residual nitrate-N, which was significant as compared to Zn salt; ZnO nanoparticles also slightly increased the residual soil ammonium-N; both Zn types showed a trend for decreasing P levels, significant for the Zn salt, as compared to the control; and both Zn types showed significantly more residual Zn than the control, more so for the Zn salt (Table 2).

Postharvest Zn from Fresh ZnO Nanoparticles' Exposure Does Not Exist as Nanoparticles in Wheat Rhizosphere. Given that the Zn salt treatment showed more residual Zn than the ZnO nanoparticle treatment, it was of interest to determine the fate of the fresh ZnO nanoparticles treatment after plant growth. Further analysis of the wheat rhizosphere in new soil samples collected after plant growth indicated that the ZnO nanoparticles dissolved in the soil. This is corroborated by the lack of absorbance peak at 374 nm, which would be indicative of the presence of nanosize ZnO as demonstrated in Figure 2 for the pristine ZnO nanoparticle suspensions.

Effects of ZnO Nanoparticles and Salt on Zn Acquisition by Wheat. In the control treatments, more Zn was associated with the plant root than with the shoot or grain; however, similar levels of Zn were partitioned in the shoot and grain in the new soil, while more Zn was partitioned in the shoot than in the grain in the used soil (Figure 3). Zn exposure in both the used and the new soils strongly increased Zn content in the plant (Figure 3). Averaged over all tissues and taking biomass into account (Table 1), as compared to the controls, about 143% and 269% more Zn was bioaccumulated in the plant from residual and fresh exposure to Zn salt, respectively, while 98% and 97% more Zn was present from residual and fresh exposure to ZnO nanoparticles. Accordingly, Zn salt exposure yielded more Zn in the root than did ZnO nanoparticles, although the effect was only statistically significant in the new soil (Figure 3). Zn fertilization did not improve shoot Zn concentration in the used soil, while shoot Zn content in the new soil was significantly increased by the nanoparticles; this effect was even more pronounced for the Zn salt. Grain Zn contents were higher in ZnO nanoparticle and Zn salt treatments than the control. Yet while grain Zn content was similar between the nanoparticle and salt treatments in the used soil, plants with Zn salt treatment contained significantly more Zn in the grain than those treated with nanoparticle ZnO in the new soil (Figure 3). Taking into account the grain biomass yield (Figure 1), grain Zn content was increased by 186% and 229% by residual ZnO nanoparticles and Zn salt, respectively, and by 229% and 300% by fresh ZnO nanoparticles and Zn salt, respectively. Thus, as compared to the respective controls, grain Zn content was increased more by Zn salt than by ZnO nanoparticles, regardless of soil.

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Table 2. pH and mg/kg Levels of Residual Nutrients of the Used and New Soils Exposed to ZnO Nanoparticles (Nano) and Zn Salt[a]

treatment		pH	NO_3^--N	NH_4^+-N	P	Zn
used soil	control	5.6 ± 0.2 b	1.3 ± 0.1 ab	5.2 ± 0.4 a	19.1 ± 4.7 a	0.1 ± 0.01 d
	ZnO nano	5.7 ± 0.1 b	1.5 ± 0.1 ab	5.5 ± 0.2 a	22.0 ± 1.9 a	0.4 ± 0.1 c
	Zn salt	5.6 ± 0.2 b	1.1 ± 0.3 b	5.0 ± 0.7 a	16.6 ± 6.4 ab	0.7 ± 0.03 c
new soil	control	6.02 ± 0.1 a	1.4 ± 0.1 ab	4.3 ± 0.3 b	17.4 ± 7.4 ab	0.1 ± 0.01 d
	ZnO nano	6.2 ± 0.3 a	1.8 ± 0.6 a	4.9 ± 0.1 ab	14.7 ± 3.9 b	2.0 ± 0.4 b
	Zn salt	6.2 ± 0.2 a	1.2 ± 0.4 b	4.3 ± 0.3 b	9.4 ± 2.4 c	4.3 ± 1.8 a

[a]Data are means and standard deviations, and different letters after numbers indicate significant differences among the treatments at $p < 0.05$ ($n = 4$).

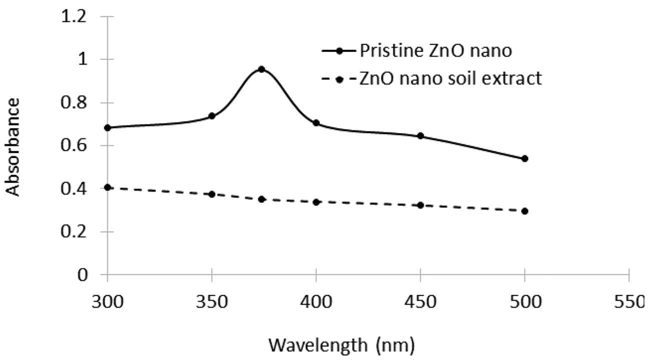

Figure 2. UV−vis spectra of soil extract from fresh ZnO nanoparticle treatment after plant harvest, as compared to pristine ZnO nanoparticle (nano) suspension in water.

A Zn mass balance for the new Zn exposure was determined to account for nutrient sources (originally in soil or added) and sinks (in postharvest soil and plant tissues). Table 3 shows the masses of Zn in soil and plant tissues per treatment. In the control treatment, the values in column 2 (total mass of Zn source) and column 8 (total mass of Zn sink) are not equal. The difference between these values is likely due to the fact that DTPA extraction captures only the bioavailable Zn in soil, rather than total Zn. In contrast to DTPA, the plant roots were able to extract and accumulate additional Zn from the pool of non-DTPA-extractable Zn; a full assessment of this fraction is only evident after total acid digestion of plant tissues. The data further show that more DTPA-extractable Zn was contained in the Zn salt-exposed soil than in the ZnO nanoparticle-exposed soil, postharvest (column 3); it also showed that more Zn was recovered by the salt-exposed plants than by the ZnO nanoparticle-exposed plants (column 7). Accordingly, the total combined Zn in the soil and plant sinks was greater in the Zn salt treatment (37.6 mg) than in the ZnO nanoparticle treatment (17.7 mg). However, these values were each below the corresponding Zn sources (48.8 mg; column 2). This implies that, in both cases, Zn was remaining in the soil (11.2 mg for Zn salt, and 31.1 mg for ZnO nanoparticles) that was neither extracted by DTPA nor by the plant.

Does Zn Translocated to Wheat Grain from Soil Exist as Ionic Zn? As shown in Figure 4, a blue color was formed with the mixture of Zn sulfate and Zincon solutions, whereas no color formation was observed when Zincon was mixed with deionized water, demonstrating the specificity of the assay for Zn. Similarly, no color change was observed with the treatment involving the addition of Zn sulfate to the ground wheat. With the wheat treatments, no blue color formation was observed, regardless of the Zn type to which the plants were exposed (Figure 4). This suggests that the Zn in the grain does not exist as free Zn ions in any significant amount.

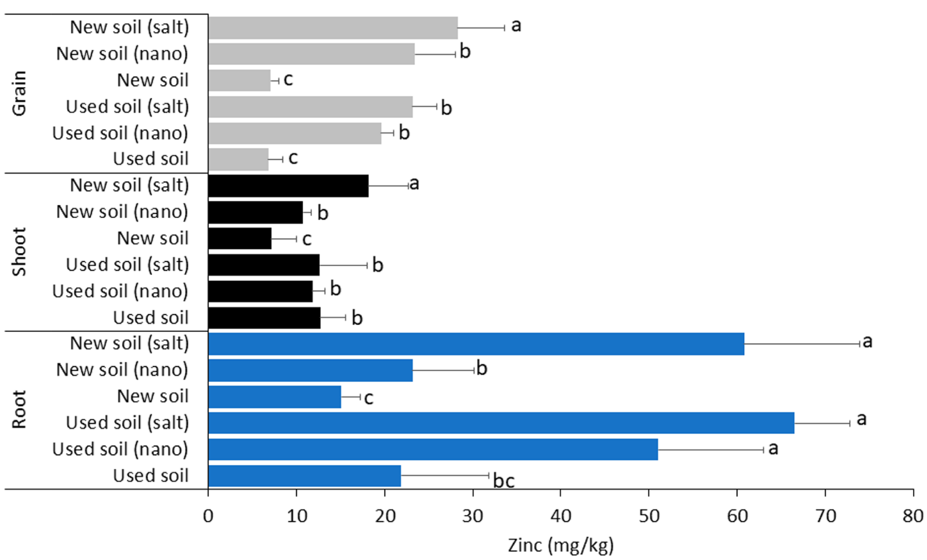

Figure 3. Effects of ZnO nanoparticles (nano) and Zn salt in used and new soils on zinc concentrations in root, shoot, and grain of winter wheat. Values are means and standard deviations, and different letters on bars indicate statistically significant differences ($p < 0.05$) among the treatments, separately analyzed for root, shoot, and grain ($n = 4$).

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Table 3. Mass Balance of Zn in Experimental and Postharvest Soils, and in Harvested Plant Tissues under Fresh ZnO Nanoparticles or Zn Salt Exposure

treatment	experimental soil Zn (mg/pot)	postharvest soil Zn (mg/pot)	mg/plant root Zn	mg/plant shoot Zn	mg/plant grain Zn	total plant Zn (mg)	total postharvest soil and plant Zn (mg/pot/plant)
control	0.8 (0.1 × 8)	0.56	0.19	0.57	0.21	0.97	1.53
ZnO nano	48.8[a] (0.8 + 48)	15.6	0.30	0.91	0.91	2.12	17.7
Zn salt	48.8 (0.8 + 48)	34.24	0.67	1.53	1.11	3.31	37.6

[a]6 mg Zn/kg soil × 8 kg soil = 48 mg Zn/pot.

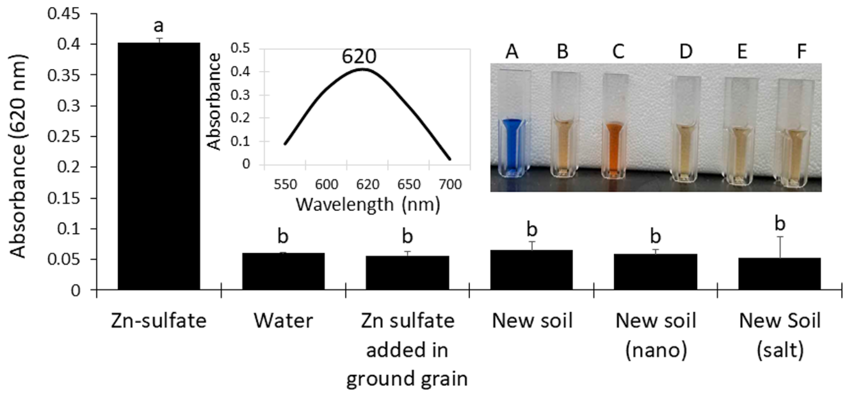

Figure 4. Detection of free Zn ions in grains of wheat exposed to ZnO nanoparticles (nano) and Zn salt in new soil. Data are means and standard deviations, and different letters associated with bars indicate significant differences among the treatments at $p < 0.05$ ($n = 3$). The left inset is the absorption spectrum of Zincon−Zn complex indicating absorption maximum at 620 nm. The right inset is a photograph of Zincon incubations with Zn-sulfate (A); supernatant from wheat grain mixed with Zn-sulfate solution (B); water (C); grain from new soil (D); grain from ZnO nanoparticle treatment in new soil (E); and grain from Zn salt treatment in new soil (F).

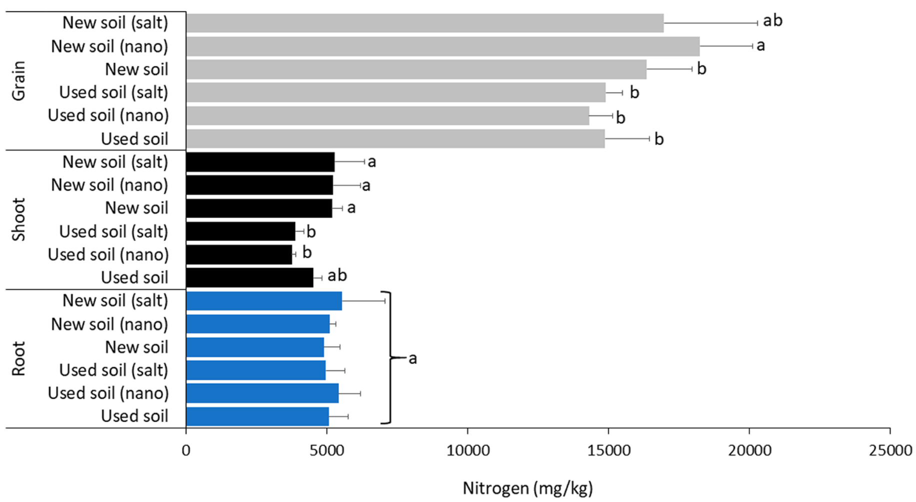

Figure 5. Effects of ZnO nanoparticles (nano) and Zn salt in used and new soils on nitrogen concentration in root, shoot, and grain of winter wheat. Values are means and standard deviations, and different letters on bars indicate statistically significant differences ($p < 0.05$) among the treatments, separately analyzed for root, shoot, and grain ($n = 4$).

Effects of ZnO Nanoparticles and Zn Salt on the Acquisition of Other Nutrients in Wheat. In the control plants, more N was accumulated in the grain than in the shoot or root, regardless of whether the soil was old or new (Figure 5). This suggests a preferential partitioning of N in the grain of wheat plants. Treatment with nanoparticle or ionic Zn did not dramatically alter N partitioning in the different plant parts (Figure 5). In the root, no effect of Zn was observed on N concentration in all treatments and regardless of the soil age. In the shoot, the plants differed in their N concentration on the basis of Zn aging in the soil. Residual ZnO nanoparticles and Zn salt tended to reduce shoot N, albeit insignificantly. In contrast, there was virtually no difference between the control and the fresh Zn treatments in the new soil (Figure 5). In the grain, differences in N concentration were also observed that were based on Zn soil aging: N translocation to grain was not influenced by Zn in the used soil, whereas in the new soil, grain N was significantly ($p < 0.05$) increased by ZnO nanoparticles, relative to the control, while a median effect was observed for the Zn salt.

As with N, there was a preferential partitioning of P in the grain, as compared to the root and shoot. Also, significantly

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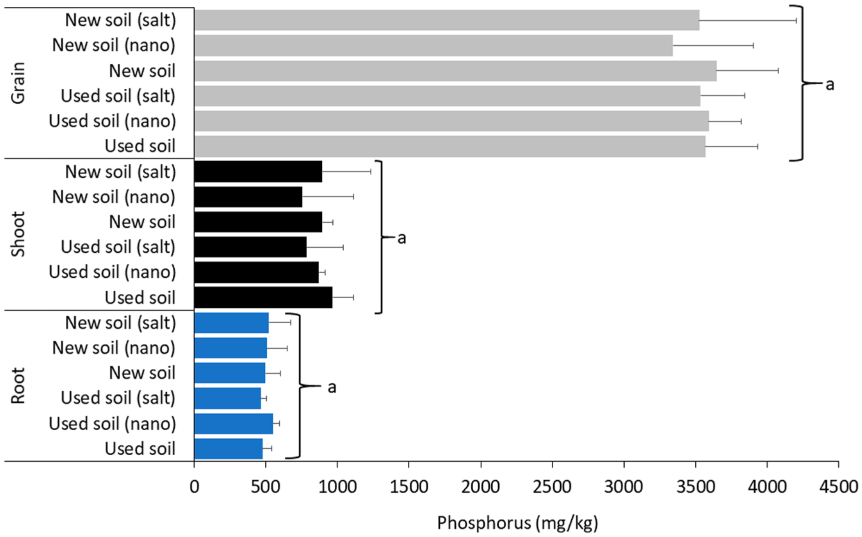

Figure 6. Effects of ZnO nanoparticles (nano) and Zn salt in used and new soils on phosphorus concentrations in root, shoot, and grain of winter wheat. Values are means and standard deviations, and different letters on bars indicate statistically significant differences ($p < 0.05$) among the treatments, separately analyzed for root, shoot, and grain ($n = 4$).

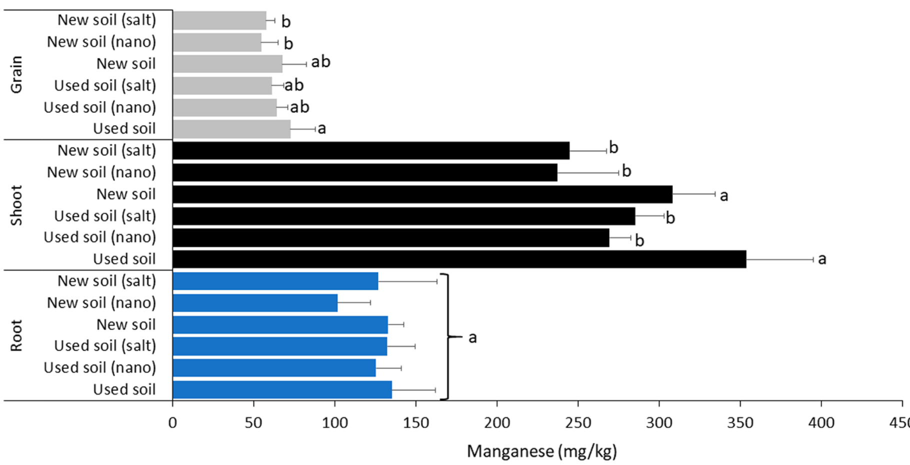

Figure 7. Effects of ZnO nanoparticles (nano) and Zn salt in used and new soils on manganese concentrations in root, shoot, and grain of winter wheat. Values are means and standard deviations, and different letters on bars indicate statistically significant differences ($p < 0.05$) among the treatments, separately analyzed for root, shoot, and grain ($n = 4$).

more P was detected in the shoot than in the root (Figure 6). These findings indicate high bioaccumulation potential of P in winter wheat, regardless of whether the soil was used or new. However, there was no significant effect of Zn treatment on P accumulation, although slight alterations in average P accumulation are noticeable in the shoot levels where the ZnO nanoparticle and Zn salt treatments reduced P contents by 10% and 18%, respectively, in the used soil. ZnO nanoparticle reduced P content in the new soil by 15%. The average grain P content was insignificantly reduced (8%) in the ZnO nanoparticle treatment relative to the control in the new soil.

Manganese accumulated in the plants was partitioned to a greater degree in the shoot, followed by the root, as compared to the grain (Figure 7). There was no significant effect of soil history or Zn type on Mn concentration in the root, except for a slight decrease in the Mn concentration of the ZnO nanoparticle treatment in the new soil. In the shoot, there were significant differences in Mn accumulation; both ZnO nanoparticles and Zn salt inhibited uptake, thereby lowering the shoot Mn content in both the new and the used soil. Translocation of Mn from the shoot to the grain was low; in each soil, the Zn forms showed only a slight but statistically insignificant reduction in Mn contents.

Nearly all of the Fe associated with the plant was partitioned in the root, with relatively insignificant amounts translocated to the shoot and grain (Figure 8). Zn fertilization did not significantly influence root Fe contents in both used and new soils. Shoot Fe contents of the treatments in the used soil indicated statistically insignificant depression of Fe translocation from the root by both Zn types, but only the nanoparticle depressed Fe levels in the new soil. This is in stark contrast to the effect of Zn salt, where significantly more Fe (92%) was accumulated by the plant. Grain Fe was largely unaffected by Zn treatment, although a trend of reduced Fe content in the presence of Zn in the used soil and an increase in Fe content in the presence of Zn salt in the new soil were evident.

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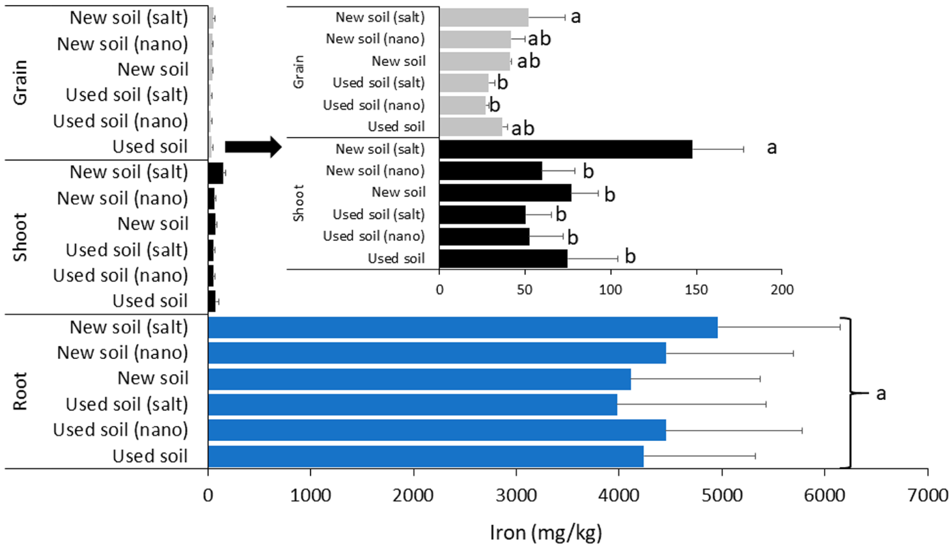

Figure 8. Effects of ZnO nanoparticles (nano) and Zn salt in used and new soils on iron concentrations in root, shoot, and grain of winter wheat. Values are means and standard deviations, and different letters on bars indicate statistically significant differences ($p < 0.05$) among the treatments, separately analyzed for root, shoot, and grain ($n = 4$).

■ DISCUSSION

This study reports the effects of exposure to residual and fresh Zn from ZnO nanoparticles and Zn salt on grain yield and nutrient acquisition in winter wheat. The residual or fresh Zn in the soils resulted in grain yield enhancement that was accompanied by minimal effect on the vegetative growth of wheat, except for modulation of chlorophyll content and shoot growth in the used soil. The promotion of chlorophyll production and yield by various doses of ZnO nanoparticles or Zn salt has been previously reported in grain crops.[4,5,7,8,34] Given these results, enhancement of wheat grain yield may not be particularly surprising with the fresh Zn exposure. It is, however, interesting that grain yield is enhanced by the residual ZnO nanoparticles and Zn salt, considering that substantial amounts of the bioavailable Zn were removed from the soil by the preceding crop, 16% and 20%, respectively.[8] A previous study with nanoparticle ZnO, bulk ZnO, and Zn salt evaluated growth and physiological effects of Zn type in soil previously cropped with bean and tomato and succeeded with beet and pea;[29] the authors reported that ZnO nano/bulk particles and Zn salt affected plant growth similarly. However, in contrast to the present study, the prior study did not involve growing the crops to full physiological maturity, nor was the effect in the aged soil compared to that in a newly amended soil. Nevertheless, the result from the previous study for biomass yield was similar to the present result, with respect to the effect of Zn type. We noted that a higher grain yield was obtained in the control used soil, as compared to the control new soil, which was also reflected in the respective Zn treatments. This is likely due to the residual contributions of NPK, and root exudate activity from the preceding fertilization in the used soil. Nevertheless, the findings demonstrate that Zn treatment has both residual and immediate effects on wheat productivity, regardless of whether the original Zn source was from a nanoparticulate or ionic source. However, considering the rate of grain increase between the residual (approximately 15%) and fresh (29%) Zn relative to their respective controls, fresh Zn application was clearly more effective. It should be noted that the starting Zn rate in the residual Zn soil was lower.

The present study enhances our understanding of the mechanistic aspects of ZnO nanoparticle interactions in plant—soil systems in that it demonstrates that the ZnO nanoparticles underwent transformation/dissolution in the soil, as indicated by the reduction in postharvest soil pH, loss of the characteristic spectral peak at 374 nm, and increased DTPA-extractable (hence, soluble) Zn. Dissolution of the nanoparticles then resulted in significant Zn uptake into the plant. The soil pH after plant harvest was lower than that before planting, indicating that substances of wheat plant origin, probably organic acids in root exudates or perhaps plant-associated microbial activity, were acidifying the soil and likely facilitating ZnO dissolution. Subsequently, the Zn ion, being a strong Lewis acid, contributes to acidifying the rhizosphere.[21,35] This hypothesis of dissolution in the soil is supported by the studies of García-Gómez et al.[29] and Watson et al.,[36] both showing that ZnO nanoparticles dissolved at higher rates in acidic soil. Loss of the ZnO nanoparticle spectral peak is characteristic of ZnO nanoparticle transformation when exposed in different environments; however, ZnO nanoparticles do not exclusively transform to dissolved Zn ions. The loss of the ZnO nanoparticle absorbance peak may also indicate homo- or heteroaggregation, or transformation to other Zn complexes with soil-borne compounds.[15−17,22,29,31] The process of ZnO nanoparticle transformation into ions or other structures is highly dependent on a range of soil properties.[22] Importantly, it is unclear at which point during plant growth were the ZnO nanoparticles lost to non-nanoscale structures. As demonstrated with the residual and fresh Zn treatments in this study, the nanoparticle transformation could be rapid enough to supply Zn to plants in the immediate term, while at the same time prolonged enough to provide the nutrient on a longer-term basis.[2,17,29] In the residual scenario, the remnant Zn from ZnO nanoparticles in soil would most certainly have undergone transformation, although likely in ways that are different from the fresh ZnO exposure based on longer weathering time and potential effects of prior sorghum growth and root exudation on soil pH and

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other soil characteristics. Yet, as was shown, the residual Zn was present in sufficient bioavailable amounts to yield significant Zn uptake into the plant tissue, particularly the grains.

The presence of more soil-residual and plant-associated Zn in the Zn salt than in the ZnO nanoparticle treatment is supported by a previous study with similar results.[35] However, nanoparticles are known to serve as reservoirs for continued ion release. So, where did the Zn ions dissolving from the ZnO nanoparticles go? It seems probable that while a fraction of the Zn accumulated in the plant, initially as free Zn ions, some of the intact nanoparticles would have been sorbed directly onto soil components[37] or perhaps homoaggregated.[21] Alternatively, some fraction would have dissolved and complexed with other soil components via heteroaggregation or root/microbial exudates.[17,21−23,37] It is important to remember that these processes are all chemical reactions that proceed in both directions, largely as a function of system conditions. In other words, a sorbed or complexed fraction may be retransformed over time, resulting in dynamic reactivity and bioavailability. It would seem that the higher amount of postharvest soil Zn in the Zn salt than the ZnO nanoparticle treatment in the fresh applications is due to the fact that the DTPA extraction used for detecting Zn is specific for ionic Zn. As such, most adsorbed or coprecipitated Zn from ZnO nanoparticles might have gone undetected during measurement. Hence, we see the differences in the Zn mass balance between the Zn source (48.8 mg) and the sinks, 37.6 and 17.7 mg, respectively, for Zn salt and ZnO nanoparticle exposures (Table 3). Clearly, the overall combined soil−plant Zn mass balance for the salt treatment is closer to the starting experimental Zn total than that of the nanoparticle treatment. This suggests two scenarios: (i) soils treated with Zn salt will contain more immediately plant-available Zn than soils exposed to ZnO nanoparticles; or (ii) soil exposed to ZnO nanoparticle will contain more residual and less extractable Zn fractions than those exposed to Zn salt. However, with aging, this hitherto unextractable Zn becomes potentially available for subsequent cropping, as demonstrated in this study with the used soil.

Another important finding from this study is that Zn grain content of the plants increased significantly when Zn was provided in fertilizers as ZnO nanoparticles or Zn salt in both residual and fresh exposures. However, more Zn was associated with the plant root than with shoot or grain. Munir et al.[34] conducted studies involving a higher Zn dose range, 25−100 mg/L, and also reported more Zn from ZnO nanoparticles to be present in the wheat root than shoot or grain. Taken together, our findings, which also agree with those of García-Gómez et al.,[35] indicate that uptake of added Zn from root to shoot is not a highly efficient process. However, it should be noted that a substantial portion of the Zn could be sorbed to the outer root surfaces, rather than internalized in the plant tissues, especially in the case of the nanoparticles.[15] Nevertheless, our data show subtle differences in the in planta Zn mobility between used and new soils, as well as between the controls and the different Zn types. These differences, based on the ratios of shoot versus root Zn, show higher root-to-shoot accumulation efficiency of the native Zn in the control plant (59%) (bioaccumulation factor; BAF = 0.59) as compared to that of residual Zn from ZnO nanoparticles (BAF = 0.24) and Zn salt (BAF = 0.20). In contrast, in the new soil, Zn from ZnO nanoparticle fertilization showed a 48% root-to-shoot accumulation efficiency (BAF = 0.48), while Zn from Zn salt had a BAF of 0.30, and the control soil had a BAF of 0.47. A high Zn BAF, 0.8−2.0, was previously reported for maize exposed to ZnO nanoparticles, although this was noted at extremely high exposure levels of 100−800 mg/kg.[20] In the present study, with the used soil, the higher BAF in the control versus the Zn treatments may be due to one of several factors: (i) Zn-starved plants responding to the low-availability Zn more efficiently; (ii) aging-enhanced precipitation of Zn ions (following dissolution, in the case of ZnO nanoparticles); or (iii) aggregation or sorption of the Zn in the soils at high rates.[20] That being said, this root-to-shoot bioaccumulation analysis clearly indicates that ZnO nanoparticles are more efficient than Zn salt in facilitating root-to-shoot Zn accumulation, even with a slightly lower starting soil Zn rate in the case of the used soil. However, a prior study in a previously cropped aged soil reported similar effects of ZnO nanoparticles and salt on shoot Zn content of succeeding crops, beet and pea,[29] although the levels for each Zn type were significantly greater than the control treatment only when the Zn dose was high, 20 and 225 mg/kg.

Still, it is noteworthy that, regardless of the soil's cropping history effects on availability and shoot accumulation, when Zn does get into the shoot from residual or new fertilization, there is efficient translocation to the grain as measured by the relevant tissue ratios (grain to shoot Zn). Among the treatments, subtle differences could be observed between the soil types as well as between Zn types. In the used soil, Zn shoot-to-grain translocation efficiencies of 0.53, 1.67, and 1.77 occurred for the control, ZnO nanoparticles, and Zn salt, respectively. In the new soil, Zn shoot-to-grain translocation efficiencies were 1.0, 2.1, and 1.5 for the control, ZnO nanoparticles, and Zn salt treatments, respectively. These translocation efficiencies indicate that residual Zn from the salt may be more effective than residual Zn from the nanoparticle form for fortifying wheat grains with the nutrient, while the reverse is the case for fresh Zn fertilization, although there is a slightly higher starting soil Zn rate for Zn salt in the used soil. Each of these scenarios supports the strong remobilization of Zn from shoot to grain that has been previously reported in wheat.[38] Also, Garnett and Graham[39] reported that 42% of the total above-ground Zn in the control (no Zn fertilization) wheat plant is distributed to the grain. This reported grain Zn rate is comparable to the grain Zn rate in this study in the used (35%) and new (50%) soils for the control plants. When compared to the average rates for the ZnO nanoparticles (65%) and Zn salt (63%), it is evident that providing Zn in fertilizers increases the rate of Zn-nutrient distribution to the grain by at least 48%.

Collectively, our findings add to the body of knowledge that agronomic fortification of cereal or grain crops with Zn through soil fertilization contributes to the nutritional quality of the edible plant material. However, a different but associated concern is whether the grain Zn exists in forms that allow sufficient Zn to be readily available for trophic transfer to higher order organisms (in this case, humans and animals). Data from Figure 4 indicate that the grain Zn may exist mostly in a form that is not readily bioavailable. Indeed, previous studies involving exposure of wheat and maize to ZnO nanoparticles and Zn ions show the nutrient to be present in the plant shoot and grain tissues mostly as Zn-phosphate, an insoluble compound with limited mobility.[9,15,16,18] The Zincon assay to detect free Zn in grain did not involve acid digestion of the tissue after grinding, as is done for ICP-OES to detect

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total grain Zn. Thus, little if any modification of the original state of the grain Zn would be expected. The formation of other complexes, such as Zn-cysteine, Zn-histidine, Zn-citrate, Zn-isocitrate, Zn-malate, and Zn-oxalate, upon exposure of crop plants to ZnO nanoparticles and Zn ions has also been reported and modeled.[17,21] However, it is likely that the wheat grain Zn in the current study exists as Zn-phosphate because these organic acid-Zn chelates are quite mobile and thus more likely to be exchangeable with Zincon. Notably, no color change was observed when soluble Zn from a Zn-sulfate solution was reacted with the ground wheat grain, suggesting that bioaccumulated Zn ions are rapidly complexed by one or more wheat components, and the stability of the formed complex appears to be stronger than that between Zn and Zincon. Assuming that the formation of Zn-phosphate occurred in the plant rhizosphere as previously suggested,[8] sufficient free Zn was still present to be accumulated as ions. Subsequently, these free Zn ions were likely complexed with P in the grain. Significant formation of such Zn-phosphate complexes in the soil would have strongly deterred Zn and/or P uptake even into the root, because it is highly insoluble. The high level of root Zn, the efficient shoot-to-grain Zn translocation, and the high level of grain P all point to the likelihood of a post-translocation formation of Zn-phosphate complexes in the grain, as previously shown.[9] Considering all of the findings from the Zincon assay, it can be concluded that despite Zn uptake from soil and high translocation (Figure 3), a significant amount of the grain Zn may exist in a form that is not readily bioavailable for human or animal adsorption.

Not surprisingly, more N and P, two highly plant mobile nutrients, were associated with above-ground tissues (shoot and grain) than with the root, similar to our previous finding with this crop.[40] Upon accumulation in the plant, these nutrients exhibited specific in planta partitioning or distribution preferences in grain, shoot, or root, and Zn treatment had little influence on these processes. However, Zn treatment differently regulated the actual acquisition of these nutrients from the soil by the crop, dependent on plant tissue, soil Zn history/aging, and Zn type. For shoot and root nutrient acquisition, in cases where nutrient acquisition was inhibited, this was not related to a nutrient dilution effect given that shoot and root biomasses were largely unaffected by Zn treatment. However, for grain where yield was improved by Zn treatment, the additional grain biomass could be contributing to some dilution in cases where nutrients levels were somewhat lower than in the controls, such as with P in new soil for the ZnO nanoparticle; Fe in used soil for both Zn types; and Mn in both soils for both Zn types. With N, efficient use by crop plants such as wheat is key to improved productivity, and critical to this is the degree to which plants are able to distribute N from soil via the roots to the grain.[41] Here, we show that N is efficiently distributed to the grain by winter wheat; approximately 62% of all N taken up into the root from a previously used or fresh soil was preferentially deposited in the grain. This finding agrees with a previous report on the partitioning of N in winter or spring wheat cultivars,[40,41] but disagrees with another report indicating the proportion of winter wheat grain N to be small relative to total above-ground N.[42] Ionic Zn is known to promote shoot N accumulation in wheat,[4,43] and the effect is dose-dependent.[4] In the present study, when ZnO nanoparticles or Zn salt was included in the fertilization, differences in grain N accumulation were evident, dependent on soil history and the type of Zn. In the used soil that received N in both the preceding and the current cropping years, Zn had little effect in modulating grain N accumulation. This contrasts with findings in the new soil that received only one (the current) N fertilization, where ZnO nanoparticles strongly increased grain N content. To the best of our knowledge, this is the first report of grain N stimulation in wheat upon exposure to ZnO nanoparticles. The Zn rate used in this study, 6 mg/kg (12 kg/ha), is within the Zn salt range that increased N accumulation in wheat.[4] In our previous studies with other crops, supplementation with ZnO nanoparticles or Zn salt, solely or in combination with other micronutrients (copper and boron), strongly increased grain N content in soybean or sorghum.[7,8] The ability of Zn to regulate N accumulation in plants is related to its role as a potential ammonification and/or nitrification inhibitor;[44,45] these are processes that slow N transformation to ammonia and/or nitrous oxide in the soil, reducing gaseous N losses. The fact that grain N accumulation correlated with grain yield in the case of ZnO nanoparticles in the new soil, together with the trend for higher postharvest nitrate-N and ammonium-N in the soil with ZnO nanoparticles, may indicate a "nano-specific" effect that is specific to fresh nano ZnO exposure.

In the case of P, 72% of the total P acquired by the plant was transferred to the grain. This is comparable to previous reports in which 73% and 87% of total P in wheat were present in the grain.[40,46] However, Peng and Li[47] indicated partitioning of P in wheat may be cultivar-dependent; wherein of four cultivars studied, two partitioned at least 84% of the total P in the grain, while the other two only transferred 25%. In the current study, plant exposure to Zn had no clear influence on the partitioning of P in the root, shoot, or grain, and there was very little effect of Zn on the accumulation of P in each of these tissues. The later result agrees with previous studies where Zn supply had little effect on both shoot and root P concentrations in wheat and maize.[48−50] However, total P content has been shown to increase in successive wheat crops due to prior Zn fertilization.[4] Nevertheless, a negative interaction is known to exist between Zn and P that may interfere with their respective uptake into plants.[51] This has also been observed with wheat, where Zn salt application reduced both shoot uptake and grain translocation of P.[46] In the current study, there was a statistically weak trend for ZnO nanoparticles and Zn salt to reduce shoot P content, and for ZnO nanoparticles to reduce grain P content. Apparently, the interaction between P and Zn is Zn dose- and, perhaps, crop-dependent, based on studies with varying nanoparticle and/or Zn salt doses showing decreased plant P content in different crops.[7,8,28] The lack of Zn treatment effect on soil P in the used soil, which was opposite to what was observed in the new soil, agreed with the report of Abbas et al.[4] where a range of Zn doses had no effect on the wheat postharvest soil P level. However, we have previously reported an increase in soil P for sorghum exposed to ZnO nanoparticles and Zn salt,[8] which could be related to the formation of immobile Zn-phosphate complexes with less leaching potential.[18,24] On the basis of these contrasting results, it remains unclear whether lowering of soil P by Zn is crop-specific, perhaps related to root exudate quantity and quality.

The effect of Zn fertilization on the accumulation of the micronutrients Mn and Fe was also evaluated. For Mn, preferential partitioning in the shoot may be linked to its role in photosynthesis[52] and/or its poor remobilization to the grain from the shoot.[39] Zn treatment did not influence any of these

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processes; however, Mn root-to-shoot uptake was inhibited by ZnO nanoparticles and Zn salt. This observation was not quite unexpected, having been demonstrated previously in winter wheat.[53] As discussed, divalent cations such as Zn and Mn utilize similar uptake pathways in the root, and may interact competitively, dependent on their respective ratios and nutritional requirements.[2] Here, with no Mn added in the treatments, Zn exposure allowed out-competition of Mn. Nevertheless, antagonism between Zn and Mn is not universal; data have shown that this interaction is Zn/Mn ratio-dependent and also cultivar- or species-dependent, given that shoot Mn levels increased under Zn treatment in other wheat cultivars,[4] as well as in soybean and sorghum.[7,8] Fe, being another critical micronutrient, was also evaluated in this study. Fe was poorly translocated to the wheat shoots from roots, even to a lesser extent than Zn. Notably, treatment with fresh Zn salt promoted Fe uptake and grain translocation. Such a positive interaction between Zn salt and Fe in wheat was previously reported and shown to be dose-dependent;[4] at higher Zn levels, Fe uptake into the shoot was inhibited in wheat and other crops.[4,26,53] Previously, we showed that grain Fe was also increased by ZnO and Zn salt in sorghum; however, a strong inhibition of Fe uptake into the shoot by Zn led to an overall negative effect of Zn on Fe accumulation in sorghum.[8] Viewed broadly, the inhibition of shoot Mn accumulation may impact shoot-specific Mn functions, such as photosynthesis. However, a concomitant increase in grain Fe and Zn will be beneficial to plants and humans from a nutritional perspective.

Collectively, this study has demonstrated that weathered Zn from ZnO nanoparticles or Zn salt at a low initial Zn exposure, 6 mg/kg, similarly promotes grain yield in wheat, albeit less so than fresh Zn application. The yield-enhancing effect of Zn occurred with the dissolution of ZnO nanoparticles in the wheat rhizosphere of fresh soil, allowing significant increases in grain Zn content. However, alternative nanoparticle transformation pathways such as aggregation seem likely, affecting the utility of DTPA Zn extraction in soil. Subtle differences between the Zn types were evident: more Zn was associated with the grain from Zn salt than that from ZnO nanoparticles, irrespective of soil history; however, residual Zn from Zn salt was more efficiently translocated to the grain than was residual Zn from ZnO nanoparticles. Conversely, freshly applied ZnO nanoparticles were a more efficient grain Zn source than freshly applied Zn salt. Nevertheless, despite large increases in grain Zn pool, Zn in the grain may exist in a nonfree state, most likely as Zn-phosphate, thus potentially reducing Zn bioavailability at higher trophic levels. The freshly applied ZnO nanoparticles increased grain N content, while freshly applied Zn salt improved shoot Fe accumulation. The modulation of nutrient levels in crop plants based on Zn type has broader implications for both plant and human nutrition, with specific ramifications related to the use of particular Zn types as fertilizers. Additional studies on the use of ZnO nanoparticles in different forms (coatings, sizes, morphologies) should be undertaken to explore potential pathways for nanoenabled agronomic fortification and increase understanding of the bioactive mechanism of functionalized ZnO nanoparticles in the soil.

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Notes

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REFERENCES

(1) Oliver, M. A.; Gregory, P. J. Soil, food security and human health: a review. *Euro. J. Soil Sci.* **2015**, *66*, 257−276.

(2) Dimkpa, C. O.; Bindraban, P. S. Micronutrients fortification for efficient agronomic production. *Agron. Sustain. Dev.* **2016**, *36*, 1−26.

(3) Cakmak, I.; Kutman, U. B. Agronomic biofortification of cereals with Zn: a review. *Euro. J. Soil Sci.* **2018**, *69*, 172−180.

(4) Abbas, G.; Khan, M. Q.; Jamil, M.; Tahir, M.; Hussain, F. Nutrient uptake, growth and yield of wheat (*Triticum aestivum*) as affected by zinc application rates. *Int. J. Agric. Biol.* **2009**, *11*, 389−396.

(5) Subbaiah, L. V.; Prasad, T. N. V. K. V.; Krishna, T. G.; Sudhakar, P.; Reddy, B. R.; Pradeep, T. Novel effects of nanoparticulate delivery of zinc on growth, productivity, and zinc biofortification in maize (*Zea mays L.*). *J. Agric. Food Chem.* **2016**, *64*, 3778−3788.

(6) Ciccolini, V.; Pellegrino, E.; Coccina, A.; Fiaschi, A. I.; Cerretani, D.; Sgherri, C.; Quartacci, M. F.; Ercoli, L. Biofortification with iron and zinc improves nutritional and nutraceutical properties of common wheat flour and bread. *J. Agric. Food Chem.* **2017**, *65*, 5443−5452.

(7) Dimkpa, C.; Bindraban, P.; Fugice, J.; Agyin-Birikorang, S.; Singh, U.; Hellums, D. Composite micronutrient nanoparticles and salts decrease drought stress in soybean. *Agron. Sustainable Dev.* **2017**, *37*, 5.

(8) Dimkpa, C. O.; White, J. C.; Elmer, W. H.; Gardea-Torresdey, J. Nanoparticle and ionic zn promote nutrient loading of sorghum grain under low NPK fertilization. *J. Agric. Food Chem.* **2017**, *65*, 8552−8559.

(9) Zhang, T.; Sun, H.; Lv, Z.; Cui, L.; Mao, H.; Kopittke, P. M. Using synchrotron-based approaches to examine the foliar application of $ZnSO_4$ and ZnO nanoparticles for field-grown winter wheat. *J. Agric. Food Chem.* **2018**, *66*, 2572−2579.

(10) Graham, J. H.; Johnson, E. G.; Myers, M. E.; Young, M.; Rajasekaran, P.; Das, S.; Santra, S. Potential of nano-formulated zinc oxide for control of Citrus Canker on grapefruit trees. *Plant Dis.* **2016**, *100*, 2442−2447.

(11) Monreal, C. M.; DeRosa, M.; Mallubhotla, S. C.; Bindraban, P. S.; Dimkpa, C. O. Nanotechnologies for increasing the crop use efficiency of fertilizer-micronutrients. *Biol. Fertil. Soils* **2016**, *52*, 423−437.

(12) Dimkpa, C.; Bindraban, P. Nanofertilizers: new products for the industry? *J. Agric. Food Chem.* **2018**, *66*, 6462−6473.

(13) Raliya, R.; Saharan, V.; Dimkpa, C.; Biswas, P. Nanofertilizer for precision and sustainable agriculture: current state and future perspectives. *J. Agric. Food Chem.* **2018**, *66*, 6487−6503.

(14) Elmer, W.; De La Torre-Roche, R.; Pagano, L.; Majumdar, S.; Zuverza-Mena, N.; Dimkpa, C.; Gardea-Torresdey, J.; White, J. C.

DOI: 10.1021/acs.jafc.8b03840
J. Agric. Food Chem. 2018, 66, 9645−9656

Effect of metalloid and metal oxide nanoparticles on Fusarium wilt of watermelon. *Plant Dis.* **2018**, *102*, 1394−1401.
(15) Dimkpa, C. O.; Latta, D. E.; McLean, J. E.; Britt, D. W.; Boyanov, M. I.; Anderson, A. J. Fate of CuO and ZnO nano and micro particles in the plant environment. *Environ. Sci. Technol.* **2013**, *47*, 4734−4742.
(16) Dimkpa, C. O.; McLean, J. E.; Britt, D. W.; Anderson, A. J. Antifungal activity of ZnO nanoparticles and their interactive effect with a biocontrol bacterium on growth antagonism of the plant pathogen *Fusarium graminearum*. *BioMetals* **2013**, *26*, 913−924.
(17) Wang, P.; Menzies, N. W.; Lombi, E.; McKenna, B. A.; Johannessen, B.; Glover, C. J.; Kappen, P.; Kopittke, P. M. Fate of ZnO nanoparticles in soils and cowpea (*Vigna Unguiculata*). *Environ. Sci. Technol.* **2013**, *47*, 13822−13830.
(18) Lv, J.; Zhang, S.; Luo, L.; Zhang, J.; Yang, K.; Christie, P. Accumulation, speciation, and uptake pathway of ZnO nanoparticles in maize. *Environ. Sci.: Nano* **2014**, *2*, 68−77.
(19) Bandyopadhyay, S.; Plascencia-Villa, G.; Mukherjee, A.; Rico, C. M.; José-Yacamán, M.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Comparative phytotoxicity of ZnO NPs, bulk ZnO, and ionic zinc onto the alfalfa plants symbiotically associated with *Sinorhizobium meliloti* in soil. *Sci. Total Environ.* **2015**, *515*, 60−69.
(20) Zhao, L.; Peralta-Videa, J. R.; Ren, M.; Varela-Ramirez, A.; Li, C.; Hernandez-Viezcas, J. A.; Aguilera, R. J.; Gardea-Torresdey, J. L. Transport of Zn in a sandy loam soil treated with ZnO NPs and uptake by corn plants: Electron microprobe and confocal microscopy studies. *Chem. Eng. J.* **2012**, *184*, 1−8.
(21) Martineau, N.; McLean, J. E.; Dimkpa, C. O.; Britt, D. W.; Anderson, A. J. Components from wheat roots modify the bioactivity of ZnO and CuO nanoparticles in a soil bacterium. *Environ. Pollut.* **2014**, *187*, 65−72.
(22) Dimkpa, C. O. Soil properties influence the response of terrestrial plants to metallic nanoparticles exposure. *Curr. Opin. Environ. Sci. Health* **2018**, *6*, 1−8.
(23) Sims, T. J. Soil effects on the distribution and plant availability of manganese, copper and zinc. *Soil Sci. Soc. Ame. J.* **1986**, *50*, 367−373.
(24) Lv, J.; Zhang, S.; Luo, L.; Han, W.; Zhang, J.; Yang, K.; Christie, P. Dissolution and microstructural transformation of ZnO nanoparticles under the influence of phosphate. *Environ. Sci. Technol.* **2012**, *46*, 7215−7221.
(25) Oburger, E.; Gruber, B.; Schindlegger, Y.; Schenkeveld, W. D. C.; Hann, S.; Kraemer, S. M.; Wenzel, W. W.; Puschenreiter, M. Root exudation of phytosiderophores from soil-grown wheat. *New Phytol.* **2014**, *203*, 1161−1174.
(26) Dimkpa, C. O.; Hansen, T.; Stewart, J.; McLean, J. E.; Britt, D. W.; Anderson, A. J. ZnO nanoparticles and root colonization by a beneficial pseudomonad influence metal responses in bean (*Phaseolus vulgaris*). *Nanotoxicology* **2015**, *9*, 271−278.
(27) Marchiol, L. Synthesis of metal nanoparticles in living plants. *Italian J. Agron* **2012**, *7*, 37.
(28) Watts-Williams, S. J.; Turney, T. W.; Patti, A. F.; Cavagnaro, T. R. Uptake of zinc and phosphorus by plants is affected by zinc fertiliser material and arbuscular mycorrhizas. *Plant Soil* **2014**, *376*, 165−175.
(29) García-Gómez, C.; García, S.; Obrador, A.; González, D.; Babin, M.; Fernández, M. D. Effects of aged ZnO NPs and soil type on Zn availability, accumulation and toxicity to pea and beet in a greenhouse experiment. *Ecotoxicol. Environ. Saf.* **2018**, *160*, 222−230.
(30) Romero-Freire, A.; Lofts, S.; Martín-Peinado, F. J.; van Gestel, C. A. Effects of aging and soil properties on zinc oxide nanoparticle availability and its ecotoxicological effects to the earthworm *Eisenia andrei*. *Environ. Toxicol. Chem.* **2017**, *36*, 137−146.
(31) Dimkpa, C. O.; McLean, J. E.; Latta, D. E.; Manangón, E.; Britt, D. W.; Johnson, W. P.; Boyanov, M. I.; Anderson, A. J. CuO and ZnO nanoparticles: phytotoxicity, metal speciation and induction of oxidative stress in sand-grown wheat. *J. Nanopart. Res.* **2012**, *14*, 1125.
(32) Macnair, M. R.; Smirnoff, N. Use of zincon to study uptake and accumulation of zinc by zinc tolerant and hyperaccumulating plants. *Commun. Soil Sci. Plant Anal.* **1999**, *30*, 1127−1136.
(33) De Martino, M. G.; Macarovscha, G. T.; Cadore, S. The use of Zincon for preconcentration and determination of zinc by flame atomic absorption spectrometry. *Anal. Methods* **2010**, *2*, 1258−1262.
(34) Munir, T.; Rizwan, M.; Kashif, M.; Shahzad, A.; Ali, S.; Amin, N.; Zahid, R.; Alam, M. F. E.; Imran, M. Effect of zinc oxide nanoparticles on the growth and Zn uptake in wheat (*Triticum aestivum* L.) by seed priming method. *Dig. J. Nanomat. Biostruct.* **2018**, *13*, 315−323.
(35) García-Gómez, C.; Babin, M.; Obrador, A.; Álvarez, J. M.; Fernández, M. D. Integrating ecotoxicity and chemical approaches to compare the effects of ZnO nanoparticles, ZnO bulk, and $ZnCl_2$ on plants and microorganisms in a natural soil. *Environ. Sci. Pollut. Res.* **2015**, *22*, 16803−16813.
(36) Watson, J.-L.; Fang, T.; Dimkpa, C. O.; Britt, D. W.; McLean, J. E.; Jacobson, A.; Anderson, A. J. The phytotoxicity of ZnO nanoparticles on wheat varies with soil properties. *BioMetals* **2015**, *28*, 101−112.
(37) Milani, N.; Hettiarachchi, G. M.; Kirby, J. K.; Beak, D. G.; Stacey, S. P.; McLaughlin, M. J. Fate of zinc oxide nanoparticles coated onto macronutrient fertilizers in an alkaline calcareous soil. *PLoS One* **2015**, *10*, e0126275.
(38) Pearson, J. N.; Rengel, Z. Distribution and remobilization of Zn and Mn during grain development in wheat. *J. Exp. Bot.* **1994**, *45*, 1829−1835.
(39) Garnett, T. P.; Graham, R. D. Distribution and remobilization of iron and copper in wheat. *Ann. Bot.* **2005**, *95*, 817−826.
(40) Dimkpa, C. O.; Singh, U.; Adisa, I. O.; Bindraban, P. S.; Elmer, W. H.; Gardea-Torresdey, J. L.; White, J. C. Effects of manganese nanoparticle exposure on nutrient acquisition in wheat (*Triticum aestivum* L.). *Agronomy* **2018**, *8*, 158.
(41) Anderson, A.; Johansson, E. Nitrogen partitioning in entire plants of different spring wheat cultivars. *J. Agron. Crop Sci.* **2006**, *192*, 121−131.
(42) Pask, A. J. D.; Sylvester-Bradley, R.; Jamieson, P. D.; Foulkes, M. J. Quantifying how winter wheat crops accumulate and use nitrogen reserves during growth. *Field Crops Res.* **2012**, *126*, 104−118.
(43) Manzoor, A.; Khattak, R. A.; Dost, M. Humic acid and micronutrient effects on wheat yield and nutrients uptake in salt affected soils. *Int. J. Agric Biol.* **2014**, *16*, 991−995.
(44) Daverey, A.; Chen, Y.-C.; Sung, S.; Lin, J.-G. Effect of zinc on anammox activity and performance of simultaneous partial nitrification, anammox and denitrification (SNAD) process. *Bioresour. Technol.* **2014**, *165*, 105−110.
(45) Khariri, R. B. A.; Yusop, M. K.; Musa, M. H.; Hussin, A. Laboratory evaluation of metal elements urease inhibitor and DMPP nitrification inhibitor on nitrogenous gas losses in selected rice soils. *Water, Air, Soil Pollut.* **2016**, *227*, 232.
(46) Verma, T. S.; Minhas, R. S. Zinc and phosphorus interaction in a wheat-maize cropping system. *Fert. Res.* **1987**, *13*, 77−86.
(47) Peng, Z.; Li, C. Transport and partitioning of phosphorus in wheat as affected by P withdrawal during flag-leaf expansion. *Plant Soil* **2005**, *268*, 1−11.
(48) Zhu, Y. G.; Smith, S. E.; Smith, F. A. Zinc (Zn)-phosphorus (P) interactions in two cultivars of spring wheat (*Triticum aestivum* L.) differing in P uptake efficiency. *Ann. Bot.* **2001**, *88*, 941−945.
(49) Sahrawat, K. L.; Rego, T. J.; Wani, S. P.; Pardhasaradhi, G. Sulfur, boron and zinc fertilization effects on grain and straw quality of maize and sorghum grown in semi-arid tropical region of India. *J. Plant Nutr.* **2008**, *31*, 1578−1584.
(50) Puga, A. P.; Prado, R. M.; Mattiuz, B.-H.; Vale, D. W. V.; Fonseca, I. M. Chemical composition of corn and sorghum grains cultivated in oxisol with different application methods and doses of zinc. *Cien. In. Agr.* **2013**, *40*, 97−108.
(51) Rietra, R. P. J. J.; Heinen, M.; Dimkpa, C. O.; Bindraban, P. S. Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. *Commun. Soil Sci. Plant Anal.* **2017**, *48*, 16.

(52) Pradhan, S.; Patra, P.; Das, S.; Chandra, S.; Mitra, S.; Dey, K. K. Photochemical modulation of biosafe manganese nanoparticles on *Vigna radiata*: a detailed molecular, biochemical, and biophysical study. *Environ. Sci. Technol.* **2013**, *47*, 13122−13131.
(53) Stewart, J.; Hansen, T.; McLean, J. E.; McManus, P.; Das, S.; Britt, D. W.; Anderson, A. J.; Dimkpa, C. O. Salts Affect the Interaction of ZnO or CuO Nanoparticles with Wheat. *Environ. Toxicol. Chem.* **2015**, *34*, 2116−2125.

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