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RESEARCH PAPER

Bioavailability of Zn in ZnO nanoparticle-spiked soil and the implications to maize plants

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Abstract Little is known about the relationships between Zn bioavailability in ZnO nanoparticle (NP)-spiked soil and the implications to crops. The present pot culture experiment studied Zn bioavailability in soil spiked with different doses of ZnO NPs, using the diethylenetriaminepentaacetic acid (DTPA) extraction method, as well as the toxicity and Zn accumulation in maize plants. Results showed that ZnO NPs exerted dose-dependent effects on maize growth and nutrition, photosynthetic pigments, and root activity (dehydrogenase), ranging from stimulatory (100–200 mg/kg) through to neutral (400 mg/kg) and toxic effect (800–3200 mg/kg). Both Zn concentration in shoots and roots correlated positively ($P < 0.01$) with ZnO NPs dose and soil DTPA-extractable Zn concentration. The BCF of Zn in shoots and roots ranged from 1.02 to 3.83 when ZnO NPs were added. In most cases, the toxic effects on plants elicited by ZnO NPs were overall similar to those caused by bulk ZnO and soluble Zn ($ZnSO_4$) at the same doses, irrespective of some significant differences suggesting a higher toxicity of ZnO NPs. Oxidative stress in plants via superoxide free radical production was induced by ZnO NPs at 800 mg/kg and above, and was more severe than the same doses of bulk ZnO and $ZnSO_4$. Although significantly lower compared to bulk ZnO and $ZnSO_4$, at least 16 % of the Zn from ZnO NPs was converted into DTPA-extractable (bioavailable) forms. The dissolved Zn^{2+} from ZnO NPs may make a dominant contribution to their phytotoxicity. Although low amounts of ZnO NPs exhibited some beneficial effects, the accumulation of Zn from ZnO NPs into maize tissues could pose potential health risks for both plants and human.

Keywords Nanoparticles · Bioavailability · Maize · Nanoparticle pollution · Phytotoxicity

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Introduction

ZnO nanoparticles (ZnO NPs) have a wurtzite crystal structure that leads to their unique optoelectric

properties (Wang 2004), and are widely used in various products including plastics, ceramics, glass, cement, rubber, lubricants, paints, pigments, foods (source of Zn nutrient), batteries, fire retardants, personal care products, etc. (Ma et al. 2013). They can enter natural ecosystems through direct application, biosolid application, accidental release, contaminated soil/sediments, or atmospheric fallout (Rico et al. 2011). Environmental levels of ZnO NPs are expected to increase because of their widespread application (Rico et al. 2011), and thus, their environmental behavior and fate, toxicity, and potential risk to ecosystems have attracted more attention (Klaine et al. 2008; Ma et al. 2013; Navarro et al. 2008).

Recent studies have shown that NPs, such as metal or metal oxide NPs, may lead to accumulation of themselves and/or the component metal in edible plants, and have detrimental or beneficial effects on the agronomic traits, yield, and productivity of crops (Gardea-Torresdey et al. 2014; Rico et al. 2011). Consequently, concerns are increasing over the effects of NPs in agricultural ecosystems and their subsequent health risks. Numerous studies have shown the toxic effects of ZnO NPs to many organisms, including bacteria, algae and plants, aquatic and terrestrial invertebrates, and vertebrates (Ma et al. 2013). ZnO NPs have been found to cause phytotoxicity in a limited number of crops, such as *Raphanus sativus*, *Brassica napus*, *Lactuca sativa*, *Zea mays* and *Cucumis sativus* (Lin and Xing 2007), *Cucurbita pepo* (Stampoulis et al. 2009), *Glycine max* (López-Moreno et al. 2010), *Allium cepa* (Kumari et al. 2011), and *Vicia faba* (Manzo et al. 2011). However, these studies were all conducted in hydroponic systems. Because most food crops are mainly grown in terrestrial environment in most agricultural countries, the effects of ZnO NPs in soil ecosystems deserve more attention. High doses of ZnO NPs generally produced negative effects on agricultural ecosystem, such as declines in soil quality (Du et al. 2011; Priester et al. 2012), reduced growth and biomass/yields of *Triticum aestivum* (Du et al. 2011), *Z. mays* (Zhao et al. 2013), and *G. max* (Yoon et al. 2014), and excess Zn accumulation in plant tissues of *C. sativus* (Kim et al. 2011) and *Pisum sativum* (Mukherjee et al. 2014), and even in seeds of *G. max* (Priester et al. 2012). Overall, ZnO NPs may adversely affect crop growth, yields, and quality, and further pose risks to environment and human health, but the underlying mechanisms are far from being well known.

The phytotoxicity of ZnO NPs generally varied with their dose and particle size, soil properties, and plant traits (Handy et al. 2008). Contradictory evidences have been obtained in several soil culture experiments. For example, 400 or 800 mg/kg ZnO NPs significantly reduced the root and shoot biomass production of *Z. mays* (Zhao et al. 2013); however, 500 mg/kg ZnO NPs slightly stimulated plant growth of *G. max* (Priester et al. 2012). In another study, soybean development was delayed by ZnO NPs, and those plants grew in soil added with 500 mg/kg ZnO NPs did not produce seeds (Yoon et al. 2014). Thus, it seems that the effects of ZnO NPs on crops are still inconclusive (Gardea-Torresdey et al. 2014). Moreover, to the authors' knowledge, little is know about relationships between Zn bioavailability in ZnO NP-spiked soil and the toxicity and Zn accumulation in plants.

Here, a soil microcosm experiment was conducted (1) to investigate the effects of different doses of ZnO NPs after entering soil on maize growth, nutrient acquisition, physiological responses, and Zn bioavailability, and (2) to compare the effects and Zn bioavailability in soil spiked with three forms of Zn (ZnO NPs, bulk ZnO, and $ZnSO_4$) at the same dose.

Materials and methods

Soil

The test soil was sampled from an experimental field (0–15 cm depth) at the Henan University of Science and Technology. To eliminate the uncertain influences of soil microbes on NPs behavior, the soil was autoclaved at 121 °C for 2 h after sifting through a 2-mm sieve, and then air-dried. The soil is classified as Aquic Ustochrepts (US soil taxonomy) and soil texture is loamy, with a pH (1:2.5 soil/water) 8.2, 2.08 % organic matter, 1.03 g/kg total N, 65.2 mg/kg alkali-hydrolyzable N, 1.82 g/kg total P, 9.02 mg/kg Olsen P, 19.2 g/kg total K, and 278.64 mg/kg 1 M NH_4OAc-extractable K, 48.46 mg/kg total Zn, and 0.47 mg/kg DTPA-extractable Zn.

Zno NPs, standards and reagents

ZnO NPs (mean particle size 90 ± 10 nm, purity 99.9 %) were purchased from Nanjing Aipurui

Nanometer Materials Co. Ltd., China. Bulk ZnO (particle size 0.5–1.5 μm, purity 99 %, AR) and $ZnSO_4$ powders (purity 99.5 %, AR) were all purchased from Tianjin Bodi Chemical CO. Ltd., China. The size and morphology of ZnO NPs and bulk ZnO were determined using transmission electronic microscopy (TEM) (JEM-2100, JEOL Ltd., Japan) with an accelerating voltage of 200 kV. TEM images of ZnO nanoparticles and bulk ZnO powders are shown in Fig. S1 (see supporting information). Standard plant materials [GBW-07603 (GSV-2) were purchased from China Standard Materials Research Center, Beijing, PR China. HNO_3 (65–68 %) and $HClO_4$ (70–72 %) were guaranteed reagent (Luoyang Haohua Chemical Reagents Co. Ltd., China) and other reagents were all analytical grade.

Experimental design and procedure

The powders of ZnO NPs, bulk ZnO, and $ZnSO_4{\cdot}7H_2O$ were used to directly mix with soil following the method described by Priester et al. (2012). Considering the uncertain release of NPs to environments (Keller and Lazareva 2014; Lazareva and Keller 2014), a wide range of ZnO NPs concentrations were designed as 100, 200, 400, 800, 1600, and 3200 mg/kg respectively. Each pot was filled with 2000 g air-dried ZnO NP-spiked soil. Additionally, the same amount of Zn as the 800 mg/kg ZnO NPs in its non-nanoparticles (bulk ZnO) and ionic form ($ZnSO_4{\cdot}7H_2O$) was used to compare the effects of different Zn forms. The control treatment received no ZnO NPs, bulk ZnO, or $ZnSO_4$. Soils were watered and maintained at about 70 % of water-holding capacity. Each treatment replicated three times.

Seeds of maize (*Zea mays* L. var. Zhengdan958) were surface-sterilized with 0.5 % NaClO solution and subsequently washed several times with distilled water and germinated at 28 °C (about 48 h) in dark until the radicles appeared. Six uniform germinated seeds were transplanted into each pot. The seedlings were arranged in a non-environment-controlled greenhouse with a day/night (14–15/9–10 h) temperature (25–35/18–24 °C) and irrigated with tap water to maintain about 70 % of field water-holding capacity. Soil moisture was monitored by weighing the pots every 3 days and adjusted as required with tap water.

Plant and soil analysis

After 8 weeks of growth, shoots and roots were harvested separately. The shoots were rinsed with deionized water for three times. The roots were removed by carefully breaking apart the soil and then rinsed with deionized water. Then their fresh weights were determined. A sub-sample of fresh root was taken to evaluate root activity. Fresh leaves (the third or fourth leaf from the top of the plant) were sampled to determine the contents of chlorophyll and ROS. Remaining shoots and roots were weighed after oven drying at 70 °C for 48 h. The ratio of dry weight to fresh weight of the remaining plant materials, and total fresh weight of shoots and roots were used to estimate total dry weights. The whole pot of soil was thoroughly mixed and then sampled for analysis of DTPA-extractable Zn.

Root activity was measured using triphenyl tetrazolium chloride (TTC) method. TTC can be reduced by dehydrogenase when it is added to a plant tissue, and so it has been widely used for studying the vitality of different plant tissues, including root activity (Clemensson-Lindell 1994). Reduction of TTC and formation of red triphenyl formazan (TTF) were quantified spectrophotometrically at 485 nm after acetic ester extraction. The root activity was defined as the product of TTF per hour and per gram fresh weight (FW) of the root. Chlorophyll (chl) and carotenoid were estimated by extracting fresh leaf materials in 80 % acetone, and the absorbances were recorded at 470, 646, and 663 nm. Chl a, chl b, and carotenoid concentrations were calculated as described by Arnon (Arnon 1949). The cleaned leaves were cleaned and cut, and 1.0 g was mixed and homogenized in dry-ice with 4 mL of phosphate buffer (50 mmol/L, pH 7.8) containing 1 % PVP (V/V) and a little amount of quartz sand using a pre-chilled pestle and mortar. The homogenate was transferred to centrifuge tubes and centrifuged at 4 °C for 15 min at 10,000×*g*. Superoxide free radical (O_2^-) production was assayed using the supernatant according to the method of Wang and Luo (1990). Briefly, the supernatant (1 mL) was added with 0.9 mL of 65 mM phosphate buffer (pH 7.8) and 0.1 mL of 10 mM hydroxylamine hydrochloride, and then incubated at 25 °C for 30 min. The incubated solution (1 mL) was added to 1 mL of 17 mM 3-aminobenzenesulfonic acid and 1 mL of 7 mM

1-naphthylamine, and then kept at 25 °C for 20 min. The absorbance was recorded at 530 nm. The O_2^- production rate was calculated based on a standard curve from the reaction equation of O_2^- with hydroxylamine. The O_2^- production rate was expressed as nmol/g FW.

Diethylenetriaminepentaacetic acid (DTPA) extraction provides a useful method to evaluate Zn bioavailability in calcareous soil (Bidwell and Dowdy 1987; Hooda and Alloway 1994), and this method was used to evaluate the bioavailability of Zn derived from ZnO NPs, bulk ZnO, or $ZnSO_4$. Briefly, 5 g (dry wt.) of soil sample in each pot was added into 100-mL plastic bottle with 50 mL of 0.005 M DTPA, 0.01 M $CaCl_2$, and 0.1 M TEA (triethanolamine) buffered at pH 7.3, and shook for 2 h at 25 °C (Lindsay and Norvell 1978). The extracts were centrifuged at 3000 rpm for 10 min, and supernatants were filtered with a Millipore 0.025-μM filter and then used for analysis.

The dried plant materials were wet-digested in a mixture of concentrated HNO_3 and $HClO_4$ (4:1, v/v). Then concentrations of Zn, K, and other microelements in plant tissues, as well as soil DTPA-extractable Zn concentrations, were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, Varian AA240, USA). The concentration of P in the digested solution was measured using vanadium–molybdenum yellow colorimetry. Subsamples of plant tissues were digested in a mixture of H_2SO_4 and H_2O_2, and then N concentrations were determined using Kjeldahl method (Lu 1999). For quality assurance of Zn, N, P, K, and other elements, blanks and standard plant materials [GBW-07603 (GSV-2)] and external certified standard were used. The recoveries were between 95.0 and 100.5 %.

Data analysis

Data (mean ± SD, $n = 3$) were subjected to a one-way ANOVA using SPSS 13.0 (SPSS, Inc., Chicago, IL, USA). Tukey's multiple-range test ($P < 0.05$) was used to compare the significance among all the different treatments. Pearson correlation coefficients were calculated to evaluate the strength of the relationship between ZnO NPs dose, soil DTPA-extractable Zn concentration, and other parameters.

According to Bose and Bhattacharyya (2008), translocation factor (TF) and bio-concentration factor (BCF) were calculated.

Translocation factor is the ratio of metal concentration in aerial parts and metal concentration in plant root,

$$\text{i.e.: TF} = C_{\text{shoot}}/C_{\text{root}},$$

where C_{shoot} = conc. in plant's aerial part and C_{root} = conc. in plant's root.

BCF is the ratio of concentration of trace element in plant tissue (mg/kg) at harvest and concentration of trace element in soil before plant growth.

Results

Plant biomass production

Compared with the control, the dry weights of plants did not change significantly from 100 to 400 mg/kg ZnO NPs, while decreased gradually from 800 to 3200 mg/kg (Fig. 1). At the highest dose, the dry weights of shoots and roots decreased by 67 and 59 %, respectively. Root/shoot ratio significantly increased at 1600 and 3200 mg/kg. Plant height increased at 100 and 200 mg/kg, did not change at 400 mg/kg, but decreased from 800 to 3200 mg/kg.

The plants treated with bulk ZnO and $ZnSO_4$ had similar dry weights to the controls, except for a significant but lower shoot dry weight in bulk ZnO-

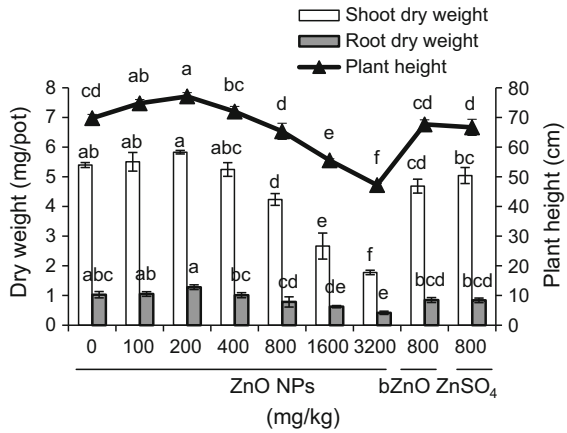

Fig. 1 Dry weight and plant height (mean ± SD, $n = 3$) of maize plants. bZnO represents bulk ZnO. *Different letters on the bars* indicate significant differences ($P < 0.05$)

treated plants (Fig. 1). Shoot dry weight of plants in 800 mg/kg ZnO NPs treatment was similar to those treated with bulk ZnO, but significantly lower than those treated with $ZnSO_4$. No significant differences were observed in root dry weight and plant height among ZnO NPs (800 mg/kg), bulk ZnO, and $ZnSO_4$.

Mineral nutrition of maize plants

Compared with the control, P uptake increased in shoots at ZnO NPs doses from 100 to 400 mg/kg, and in roots at 200 mg/kg, while decreased both in shoots and roots from 1600 to 3200 mg/kg (Table S1). The total uptake of most of these mineral elements in plants showed a similar parabolic trend, that is, it first increased and reached a peak at 200 mg/kg ZnO NPs and thereafter decreased gradually with ZnO NPs dose (Table S1). The lowest mineral uptake of plants was observed at 3200 mg/kg.

Bulk ZnO and $ZnSO_4$ produced no different effects on shoot and root P uptake, but showed a significant increase in shoot P uptake when compared to ZnO NPs at 800 mg/kg (Table S1). In most cases, ZnO NPs (800 mg/kg), bulk ZnO, and $ZnSO_4$ produced no different effects on shoot and root N, K, Ca, Mg, and Fe uptake.

Photosynthetic pigments, root activity, and O_2^- concentration

The concentrations of chl a, chl b, and carotenoid in leaves did not change significantly from 100 to 1600 mg/kg, but decreased markedly at 3200 mg/kg (Fig. 2). Root activity showed a similar trend: it did not change from 100 to 800 mg/kg and decreased from 1600 to 3200 mg/kg (Fig. 3). O_2^- showed an opposite trend with the lowest value at 200 mg/kg and the highest at 3200 mg/kg (Fig. 3). ZnO NPs (800 mg/kg), bulk ZnO, and $ZnSO_4$ produced no different effects on photosynthetic pigments and root activity; however, O_2^- concentration in ZnO NPs treatment was significantly higher than that in bulk ZnO and $ZnSO_4$ treatments.

Zn accumulation by maize plants

Concentration and uptake of Zn in maize roots and shoots showed an increasing trend as the ZnO NP dose increased (Fig. 4). Zn concentration varied from 438 to 2664 mg/kg in shoots and from 492 to 2668 mg/kg in roots, respectively, when soils were spiked with different doses of ZnO NPs (from 100 to 3200 mg NPs/kg soil). All treatments showed higher Zn concentration than those in the control plants (36 mg/kg in shoots and 114 mg/kg in roots, respectively); however, TF did not change markedly and BCF for shoots and roots decreased with the increasing ZnO NPs dose (Table 1).

Shoot Zn concentration in the plants treated with different Zn forms varied from ZnO NPs (800 mg/kg) > bulk ZnO > $ZnSO_4$, while root Zn concentration showed no significant difference. There

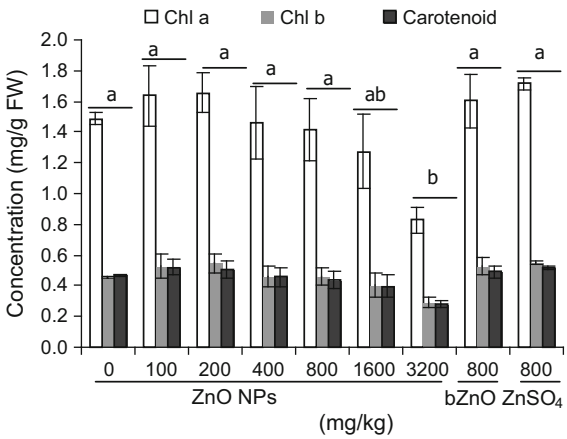

Fig. 2 Chl a, chl b, and carotenoid concentrations (mean ± SD, $n = 3$) of maize plants. *Different letters on the bars* indicate significant differences ($P < 0.05$)

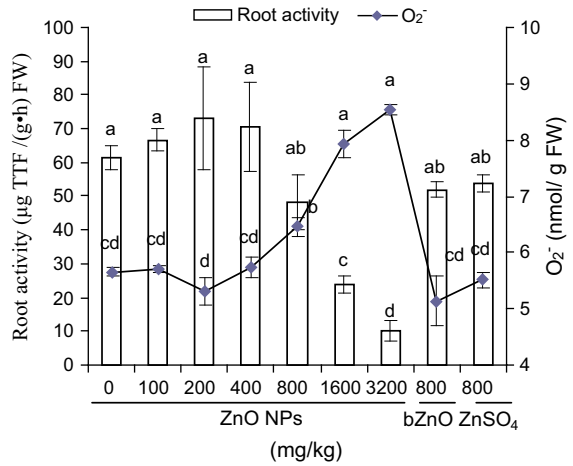

Fig. 3 O_2^- concentrations in leaves and root activity (mean ± SD, $n = 3$) of maize plants. *Different letters on the bars* indicate significant differences ($P < 0.05$)

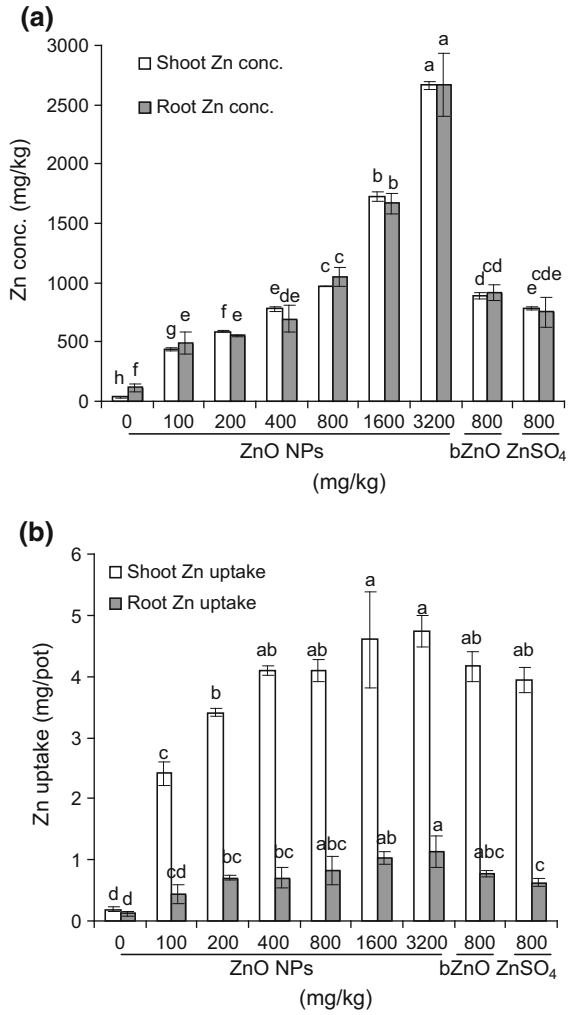

Fig. 4 Zn concentrations (**a**) and uptake (**b**) (mean ± SD, $n = 3$) in maize plants. *Different letters on the bars* indicate significant differences ($P < 0.05$)

were no significant differences in Zn uptake of the plants treated with ZnO NPs (800 mg/kg), bulk ZnO, or $ZnSO_4$ (Fig. 4). The plants treated with the three Zn compounds had similar TF and BCF of Zn in roots, while those treated with ZnO NPs (800 mg/kg) had higher BCF in shoots than those treated with $ZnSO_4$.

DTPA-extractable Zn concentration in soil

Soil DTPA-extractable Zn concentrations after harvest increased as ZnO NPs dose increased (Fig. 5). Soil DTPA-extractable Zn concentration was positively correlated with ZnO NPs dose (Table 2). As shown from the linear regression equation in Fig. 5, after plant harvest, about 16 % of the Zn from ZnO NPs was converted into DTPA-extractable forms in soil. At 800 mg/kg, soil DTPA-extractable Zn concentration varied significantly from ZnO NPs < bulk ZnO < $ZnSO_4$.

Correlation between ZnO NPs, soil DTPA-extractable Zn, and other parameters

Pearson correlation analysis revealed that both ZnO NPs dose and soil DTPA-extractable Zn concentration were correlated negatively with plant biomass production, plant height, P nutrition, photosynthetic pigments, and root activity, but positively with O_2^- concentrations in leaves, Zn concentrations in shoots and roots, and root Zn uptake (Table 2). Furthermore, both ZnO NPs dose and soil DTPA-extractable Zn concentration were correlated negatively with the uptake of P, N, K, Ca, Mg, and Fe in shoots and roots (data not shown).

Discussion

Our present results confirm a high Zn bioavailability in ZnO NP-spiked soil and to maize. The first evidence comes from Zn concentrations in maize plants, which have significant positive correlations with ZnO NPs dose, indicating the Zn in plants is at least partly from ZnO NPs. The Zn absorbed by plants is surely "bioavailable." The second evidence comes from the comparison with $ZnSO_4$. Undoubtedly, among the three compounds, the soluble Zn salt $ZnSO_4$ is highly bioavailable in soil, because it can release Zn^{2+}. Compared with bulk $ZnSO_4$, ZnO NPs produced similar plant Zn uptake or even higher shoot Zn concentration, indicating that the bioavailability of Zn released from ZnO NPs is similar to or higher than that from $ZnSO_4$. Another evidence is that soil DTPA-extractable Zn concentrations (this form often represents bioavailable nutrient fractions) correlate significantly with ZnO NPs dose and Zn concentrations in plants, indicating ZnO NPs indeed released Zn^{2+} or other exchangeable forms into soil.

The bioavailability of Zn derived from ZnO NPs can also explain their beneficial, neutral, or toxic effects at different doses. Zn is an essential micronutrient for plants but toxic when in excess. At low doses, ZnO NPs may serve as a zinc fertilizer and supply

Table 1 TF of Zn from root to shoot (S/R) and BCF of Zn in shoots and roots

	Nominal dose (Zn calculated) (mg/kg)	TF	BCF in shoots	BCF in roots
ZnO NPs 0	0 (0)	0.33 (0.07)b	0.74 (0.10)g	2.36 (0.40)bc
100	100 (80)	0.91 (0.09)a	3.41 (0.04)a	3.83 (0.39)a
200	200 (160)	1.06 (0.00)a	2.80 (0.02)b	2.65 (0.01)b
400	440 (320)	1.15 (0.10)a	2.12 (0.03)c	1.88 (0.18)bcd
800	800 (640)	0.93 (0.04)a	1.40 (0.00)d	1.52 (0.06)cd
1600	1600 (1280)	1.04 (0.03)a	1.30 (0.02)de	1.25 (0.04)d
3200	3200 (2560)	1.00 (0.05)a	1.02 (0.01)f	1.02 (0.06)d
Bulk ZnO 800	800 (640)	0.97 (0.03)a	1.29 (0.02)de	1.33 (0.06)d
$ZnSO_4$ 800	800 (640)	1.06 (0.11)a	1.14 (0.01)ef	1.09 (0.11)d

Zn calculated is based on the spiked doses and the percentage of Zn in the compounds. It is important to note that the $ZnSO_4$ added had as same amount Zn as ZnO NPs (800 mg/kg). Its nominal dose was noted as 800 mg/kg just for comparison, which is not the actual dose. BCF was calculated based on the total Zn concentrations in soil which included the sum of the natural and the spiked Zn. Different letters following means (±SE) in the same column indicate significant differences ($P < 0.05$)

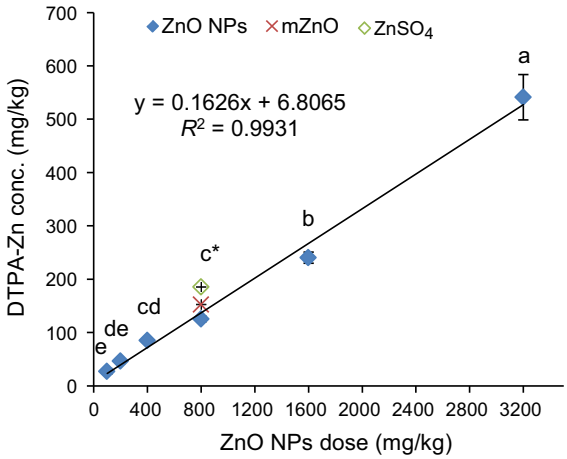

Fig. 5 Soil DTPA-extractable Zn concentration (mean ± SD, $n = 3$) after harvest. *Different letters on the bars* indicate significant differences among ZnO NPs doses ($P < 0.05$), and the *asterisk* indicates a significant difference among ZnO NPs (800 mg/kg), bulk ZnO, and $ZnSO_4$

Zn^{2+} for plant growth, but at high doses, they will be toxic because they release excess amount exceeding plant requirement. As a result, the effects of ZnO NPs varied closely with their dose and the DTPA-extractable Zn concentrations in soil. Also, soil DTPA-extractable Zn correlated negatively with maize growth, and nutritional and physiological parameters, but positively with tissues Zn concentrations. Thus, it can be speculated that dissolution of ZnO NPs to Zn^{2+} dominates their toxicity to maize.

Table 2 Pearson correlation coefficients between ZnO NPs dose, soil DTPA-extractable Zn concentration, and other parameters

	ZnO NPs dose	Soil DTPA-Zn
Shoot dry weight	−0.958**	−0.934**
Root dry weight	−0.909**	−0.884**
Plant height	−0.950**	−0.928**
Shoot P conc.	−0.842*	−0.844*
Root P conc.	−0.927**	−0.909**
Chl a conc.	−0.966**	−0.966**
Chl b conc.	−0.907**	−0.904**
Carotenoid conc.	−0.969**	−0.966**
Root activity	−0.950**	−0.911**
O_2^- conc. in leaves	0.888**	0.928**
Shoot Zn conc.	0.977**	0.976**
Root Zn conc.	0.983**	0.982**
Shoot Zn uptake	0.638ns	0.624ns
Root Zn uptake	0.788*	0.780*
Soil DTPA-Zn conc.	0.997**	–

Significant levels * $P < 0.05$; ** $P < 0.01$; *ns* non-significant effect

Unfortunately, so far it is still uncertain whether ZnO NPs phytotoxicity is due to the ZnO NPs themselves, dissolution to Zn^{2+}, or some combination thereof. Compared with bulk ZnO and $ZnSO_4$, ZnO NPs produced lower DTPA-Zn concentration in soil, but a higher toxicity and higher shoot Zn concentration

(Figs. 1, 4, 5). If Zn^{2+} release is the sole toxicity pathway, ZnO NPs must be less toxic than bulk ZnO and $ZnSO_4$. So, it can be inferred that the dissolved Zn^{2+} cannot solely account for the observed toxicity of ZnO NPs, and there must be some toxicity relative to nanospecific properties. The lower phytotoxicity of $ZnSO_4$ is perhaps partly due to nutritional effects of the concomitant SO_4^{2-}. ZnO NPs have smaller particle size than bulk ZnO NPs and may migrate further and dissolve more easily, and then they and their derivatives may exert adverse impacts on soil structure and properties, and seed germination and root elongation, as well as the seedling growth and mineral nutrient uptake. Under soil culture conditions, both ZnO NPs and Zn salt ($ZnCl_2$) (500 Zn mg/kg) had no phytotoxicity to *Vigan unguiculata* and produced similar biomass and tissues concentration (Wang et al. 2013), while ZnO NPs induced more toxicity/stress compared to bulk ZnO for *P. sativum* plants (Mukherjee et al. 2014). Our results seem to be partly consistent with the latter.

Moreover, it is still unclear whether the Zn accumulated in maize plants is in forms of NPs themselves, or Zn^{2+} released from them, or both, because the parameters determined correlating with ZnO NPs also correlated significantly with soil DTPA-extractable Zn. Several studies show that ZnO NPs were not observed within roots of plants exposed to ZnO NPs (De La Rosa et al. 2011; Hernandez-Viezcas et al. 2011, 2013; López-Moreno et al. 2010). In pure kaolin suspensions, ZnO NPs rapidly dissociate to Zn^{2+} within 1 day of reaction (Scheckel et al. 2010). In another study, ZnO NPs were found to dissolve rapidly following their entry into the soil, and could not be detected after incubation for 1 h in soil; the speciation of Zn was similar in shoot tissues of *V. unguiculata* for both $ZnCl_2$ and ZnO NPs, and no roots to shoots translocation of ZnO NPs was observed (Wang et al. 2013). The free Zn ion (Zn^{2+}) is generally considered as the main bioavailable species preferentially absorbed by plants (Kalis et al. 2007). The DTPA-extractable Zn mainly consists of water soluble and exchangeable zinc ions, as well as the adsorbed and organically bound fractions (Lindsay and Norvell 1978). Therefore, to say the least, even though ZnO NPs and Zn^{2+} coexist in soil, plants would preferentially take up Zn^{2+}, but not NPs. Based on Figs. 4 and 5, total DTPA-Zn in soil could provide enough Zn sources required for plants. Indeed, Zn NPs have been observed within plant tissues (Priester et al. 2012; Zhao et al. 2012). However, it is still unclear that these nano-sized Zn NPs in plants are directly from the ZnO NPs, or synthesized by plants themselves using the Zn^{2+} released from ZnO NPs, because plants can also synthesize metal or metal oxide NPs within their tissues (Iravani 2011; Qu et al. 2011). In our study, the ZnO NPs have a particle size of 90 nm, and they are unlikely to be absorbed directly by plants. Thus, it could be inferred that the Zn accumulated in plants mainly comes from the Zn ions derived from ZnO NPs, but not the ZnO NPs themselves.

Usually, biomass production and plant height are useful indicators of plant health. Different, even conflicting ZnO NPs effects on plant biomass have been reported under soil culture condition, varying with ZnO NPs dose, soil condition, and plant species (Du et al. 2011; Kim et al. 2011; Lee et al. 2012; Manzo et al. 2011; Priester et al. 2012; Zhao et al. 2013). ZnO NPs with a larger particle size may have a lower solubility and thus a lower phytotoxicity. Also, organic matter can directly or indirectly improve plant growth through releasing nutrients to plants (serve as fertilizers), and influencing the bioavailability and toxicity of ZnO NPs to plants (Aiken et al. 2011). In our present study, the particle size of ZnO NPs we used was about 90 nm, and the organic matter content was up to 2 %, which may partly explain the differences of our findings with others.

ZnO NPs have been found to alter the nutritional value of soil cultivated *G. max* plants (Peralta-Videa et al. 2014). Less P uptake was also observed in ZnO NP-treated plants compared to those treated with bulk ZnO and $ZnSO_4$. All the three compounds can release Zn^{2+} and decrease the phytoavailability of P via forming phosphate precipitation such as $Zn_3(PO_4)_2$, but soil pH also influences the availability of P and other micronutrients. Soil pH after harvest was 7.9, 7.9, and 7.6 for ZnO NPs, bulk ZnO, and $ZnSO_4$ treatment, respectively (data not shown). Dissolution of ZnO generally produces OH^- and results in higher soil pH, while $ZnSO_4$ often leads to a little lower soil pH. This may explain a higher P (and higher biomass) in $ZnSO_4$-treated plants. Furthermore, ZnO NPs are small enough to fit into smaller spaces between soil particles and might therefore migrate further than bulk ZnO particles, and thus display a more pronounced effect on P availability. Because of their smaller particle size, ZnO NPs in soil may display different

behaviors and deserve more attention compared with larger particles, such as aggregation, transport and deposition, sorption and desorption, and stabilization and dissolution, which potentially affect soil properties and plant growth.

Numerous studies have shown that ZnO NPs can induce oxidative stress and change activity of antioxidant enzymes (Hernandez-Viezcas et al. 2011; Kim et al. 2012; Mukherjee et al. 2014; Zhao et al. 2013). Increased O_2^- in leaves induced by higher ZnO NPs doses confirms that the toxicity of ZnO NPs is partly due to the production of ROS. Higher Zn levels suppress leaf chlorophyll synthesis and root activity (Yang et al. 2011). Excess Zn^{2+} may replace the central Mg^{2+} of chlorophyll and such substitution will lead to impairment of photosynthesis. ZnO NPs may interfere with the synthesis of photosynthetic pigments. Meanwhile, ZnO NPs (800 mg/kg) and $ZnSO_4$ produce similar photosynthetic pigments and root activity, indicating that ZnO NP-induced toxicity may be mainly due to the dissolved Zn^{2+}.

The TFs of Zn under all ZnO NP treatments (0.91–1.15) were similar to the results obtained using soil culture (0.78–0.91) (Zhao et al. 2013), but significantly higher than those reported in a previous study using a hydroponic culture system (0.02–0.01) (Lin and Xing 2008). The possible reason is that ZnO NPs dissolved rapidly in soil, but only released less Zn^{2+} (lower than 8 mg/L) in solution (Lin and Xing 2008), while ZnO NPs are not easily transported from root to shoot. The plants treated with ZnO NPs, bulk ZnO, and $ZnSO_4$ all had similar TF of Zn, which may be explained by that the Zn fraction absorbed and translocated by all the plants is probably the same form (i.e., Zn^{2+}). Comparing with the control (0.33), all Zn treatments increased TF. In the control treatment, soil bioavailable Zn was inadequate (0.47 mg/kg), Zn may not be easily transported from roots to shoots, but ZnO NPs added to soil released more bioavailable Zn, and thereby, the transport to shoots was enhanced.

The expected increase in concentrations of ZnO NPs in biosolid-treated soils is about 2 μg/kg per year (Gottschalk et al. 2009). If the ZnO NPs input to agricultural fields keeps so low in future, their detrimental effects in soil are negligible in the near future. But ZnO NPs and/or the dissolved Zn can accumulate in soil, and if their concentrations reach a level as studied (>800 mg/kg), the negative outcomes are possible. Even at low doses, their impacts on other components of terrestrial ecosystems also need further evaluation. Moreover, although the state of Zn in plant tissues remains unknown, the accumulation of Zn from ZnO NPs into crop plants may have important health implications for both plants and human. Anyway, considering potential implications of ZnO NPs to soil fertility and food quality (Priester et al. 2012), and soil microorganisms (Dinesh et al. 2012; Ge et al. 2014), both their beneficial and adverse effects in terrestrial ecosystems need much more concerns.

Conclusion

Here, we firstly associated Zn bioavailability in ZnO NP-spiked soil with the toxicity and Zn accumulation in crops. ZnO NPs released a large amount of DTPA-extractable Zn in soil, which was as highly bioavailable as that from bulk ZnO and soluble Zn salt. ZnO NPs exerted dose-dependent effects on maize plants ranging from stimulatory through to neutral and toxic effect, when their dose increased from 100 to 3200 mg/kg. Both Zn concentrations in shoots and roots correlated positively with ZnO NPs dose and soil DTPA-extractable Zn concentration. High amounts of ZnO NPs also induced oxidative stress to plants via ROS production. The dominant phytotoxicity could be attributed to the dissolved Zn^{2+} from ZnO NPs. Although the state of Zn in plant tissues remains unknown, the accumulation of Zn from ZnO NPs into maize tissues may pose potential health risks for both plants and human.

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