

TECHNICAL REPORT

Plant and Environment Interaction

Comparative study on changes in Cd accumulation and ionome between rice and spinach: Impact of zinc ion activity

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Abstract

Excessive Cd accumulation in rice grain has caused chronic Cd diseases in humans. In most crops, 100 times more Zn than Cd strongly inhibits Cd uptake and translocation. However, this response is not found for rice (*Oryza sativa* L.), which was found to have an unusual Cd uptake pattern compared with other crops, such as spinach (*Spinacia oleracea* L.). Moreover, studies on shared transporters between Zn and Cd using normal solution experiments with traditional high concentrations of metal ions may result in irrelevant interactions. Therefore, we developed ethyleneglycoltetraacetate-buffered nutrient solutions in this work. Rice and spinach seedlings were grown under calibrated low Cd²⁺ activity and low to phytotoxic Zn²⁺ activity levels while buffering other micronutrient cations at sufficient levels. Results showed that as rice grew with pZn²⁺ = 8.1–5.4, root Cd and shoot Ni decreased significantly and gradually. However, shoot Cd and Mn in rice decreased slightly with the increase of solution Zn²⁺ from deficiency to sufficiency and then increased at toxic Zn²⁺ solution (pZn²⁺ = 5.4). The shoot/root ratios of Cd in rice under toxic pZn²⁺ (5.6 and 5.4 pZn²⁺ activity) were significantly increased ($p < .05$). It could be concluded that rice absorption of Cd is not inhibited by co-contaminating (toxic) Zn. For spinach, with Zn varying from pZn²⁺ = 8.1–5.7, both shoot and root Cd substantially decreased, as did shoot Ni. This work revealed that, to understand food chain Cd risks, one needs to consider the inhibitory role of Zn in limiting Cd absorption in all crops studied except rice.

1 | INTRODUCTION

Rice (*Oryza sativa* L.) and spinach (*Spinacia oleracea* L.) represent typical grass and leafy crops, respectively, that can accumulate excessive Cd from the plant available Cd

pool in Cd-contaminated or Cd-mineralized soils and potentially harm human health after decades of high Cd ingestion (Chaney, 2015). Rice is the crop most important in human Cd disease because rice grain reaches high levels of Cd without having increased Zn levels, very unlike other crop species. Furthermore, Cd in rice grain has high bioavailability (Reeves & Chaney, 2008). Cadmium contamination in crops and

Abbreviations: DW, dry weight; EGTA, ethyleneglycoltetraacetate; NRAMP5, natural resistance-associated macrophage protein 5.

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transfer to the human food chain is thus an increasingly important food safety issue (Chaney, 2012, 2015).

Soil factors that significantly interact with Cd accumulation in most species include pH, Cd, Cd/Zn ratio, chloride, and levels of metal sorbents such as hydrous Fe and Mn oxides and organic matter (Chaney, 2010). Because Zn levels are usually ≥ 100 -fold higher than Cd in Cd-contaminated soils, for nearly all crops, soil Zn provides protection against excessive Cd transfer to foods by its potential to induce Zn phytotoxicity if soils are acidified and by Zn inhibition of Cd uptake and translocation in plants (Chaney, 2010). The interaction between Zn and Cd in their uptake and translocation has been widely studied. Among dicots, for example, spinach and lettuce (*Lactuca sativa* L.) accumulated higher levels of Zn and Cd especially when soluble Zn and Cd salts were added to soils. However, when 100 times more Zn than Cd is added to soils, Cd accumulation by these species is not unusual, and the addition of Zn can significantly reduce spinach accumulation of Cd (Chaney, 2010; Paul & Chaney, 2017). Antagonistic interaction between Cd and Zn was also reported from hydroponic experiments with rice (e.g., Honma & Hirata, 1978), wheat (*Triticum aestivum* L.) (Hart et al., 2005), and lettuce and spinach (McKenna et al., 1993). In recent years, radiation-mutated rice was examined to find rice with little or no Cd accumulation in grain when grown in a moderately Cd- and Zn-contaminated paddy soil, which is a soil typical of the rice soils contaminated by Zn mine and smelter emissions (>100 times more Zn than Cd) (Ishikawa et al., 2012).

The similarity in characteristics of divalent cations Ca, Mg, Fe, Cu, Ni, Mn, and Zn allows them to compete with each other in soil binding, root uptake, translocation, and subsequent utilization in the plant system. Parker (1997) reported that shoot concentrations of Mg, Cu, Fe, and Mn were often increased in strongly Zn-deficient plants for most crops he studied, including maize (*Zea mays* L.), wheat, tall wheatgrass [*Thinopyrum ponticum* (Podp.) Z.-W. Liu & R.-C. Wang], alfalfa (*Medicago sativa* L.), soybean [*Glycine max* (L.) Merr.], and tomato (*Solanum lycopersicum* L.). Zinc deficiency also led to phosphate toxicity in crops (Parker, 1997), at least in nutrient solutions with traditional high phosphate levels, although high Zn concentrations might induce Fe-deficiency chlorosis (Chaney, 1993) in most species. Further, increased Zn supply inhibits uptake or translocation of Ni to plant shoots (Nishida et al., 2015).

The sharing of uptake transporters among Zn, Mn, Ni, and Cd in plants has been reported. It was suggested in *Arabidopsis* that ZIP family AtZIP1 and AtZIP2 might be root Zn and Mn transporters and play a role in Mn (and possibly Zn) translocation from the root to the shoot (Milner et al., 2013). In rice, OsHMA2 transported Cd and Zn into the xylem of rice after absorption by different rhizodermal transporters (NRAMP5, ZIP3) (Nocito et al., 2011; Satoh-Nagasawa et al., 2013; Takahashi et al., 2012).

Core Ideas

- Shoot Cd and Mn in rice were the lowest under sufficient external Zn^{2+} .
- Shoot Cd and Mn in rice were elevated under toxic external Zn^{2+} .
- Both shoot and root Cd and Mn in spinach decreased with increasing external Zn^{2+} .
- Shoot Ni in both rice and spinach decreased with increasing external Zn^{2+} .

OsHMA3 is a tonoplast-localized transporter for Cd and Zn in the roots of all studied species, including rice (Kumagai et al., 2014), and overexpression of *HMA3* in rice roots very strongly limits Cd transport to shoots (Ueno et al., 2010). The Mn transporter *OsNRAMP5* was the only transporter proved to be involved in the uptake of Cd from the soil in rice (Ishikawa et al., 2012; Ishimaru et al., 2012; Sasaki et al., 2012; Yang et al., 2014). *OsNRAMP5* was expressed in the root epidermis, exodermis, and outer layers of the cortex (Ishimaru et al., 2012). The expression of *OsNRAMP5* was not affected by Mn and Fe deficiency but might decrease in the presence of Cd (Ishimaru et al., 2012; Yang et al., 2014).

Chelator-buffered solution methods are helpful to examine the effects of the interaction of ions over genetic, protein, and phenotypic levels (Parker et al., 1995). Besides, the concentrations of ions such as Cd^{2+} , Cu^{2+} , and Zn^{2+} in the real soil solution are usually as low as single digits of $\mu g L^{-1}$ (Kim & Owens, 2009). Studies on shared transporters between Zn/Mn and Zn/Cd using normal solution experiments with traditional high concentrations of metal ions may result in irrelevant interactions. For example, Cohen et al. (1998) reported that IRT1 was involved in uptake of both Cd and Zn, but their later study with solutions containing soil solution activities of Cd and Zn found that IRT1 did not play a role in root Zn or Cd uptake at these physiologically relevant soil Zn activities. Thus, studies on the interactions among trace element cations in plants need to be stressed at genomic, ionic, and proteomic levels combined with the application of the chelator-buffered solution method to supply soil solution relevant activity of test cations. By using a computer program such as Geochem-PC, one can calculate the free ion activities of elements for a fixed solution composition under specific pH conditions. To add power to this metal ion buffering method, the chemical activity of Cd and Zn is relatively constant across the normal pH range of root environments (pH 5–8) in the field, as reported by Wang et al. (2018). The selection of a proper chelator is critical for specific experimental goals. Technically, chelator buffering could provide a solution with either deficiency, sufficiency, or toxicity of common

nutrient and toxic microelement cations such as Cu, Fe, Zn, Mn, Ni, Co, and Cd (Parker et al., 1995). Parker (1997) used the HEDTA-buffering solution to study the effects of Zn deficiency on crops. Kukier & Chaney (2002) studied the uptake of Zn and Cd by rice plants using ethyleneglycoltetraacetate (EGTA) and Ferrozine (a ferrous chelator; Thermo Scientific) to buffer Fe^{2+} . Thus, this buffered system was useful for the study of low levels of Zn, but the high Zn levels needed to test phytotoxicity of Zn interfered with the stability of the Ferrozine Fe^{2+} chelate.

It was reported that 60% of the final grain Cd concentration was remobilized from that accumulated by the plant prior to flowering and that the other 40% came from direct uptake during grain maturation (Rodda et al., 2011). The uptake and accumulation of Cd by rice seedlings can be thus used to infer the characteristics of Cd accumulation in rice grain. Therefore, seedlings harvested 4 wk after transplanting to treatments in the present work were used. We investigated the effects of Zn^{2+} activity from deficiency to toxicity on the ionome homeostasis of rice and spinach, with a focus on Cd accumulation. We aimed to reveal the differing interaction between Zn and Cd in these contrasting plant species and to provide the basis for reducing plant Cd by manipulating Zn supply at levels commonly found in co-contaminated soils.

2 | MATERIALS AND METHODS

2.1 | Solution composition

Ethyleneglycoltetraacetate binds Cd strongly but Zn weakly. In addition, EGTA buffering makes it possible to supply adequate Fe for both species while controlling phytoavailable Cd, Zn, Ni, Cu, and Mn at desired levels. Preliminary experiments defined the buffered activities needed to attain both deficient and phytotoxic levels of Zn and usual concentrations of Cd, Zn, Ni, P, and Mn in rice and spinach, and then the interactions reported in this paper were tested.

Experiments for both spinach and rice were designed with seven levels of Zn^{2+} in the nutrient solutions. Zinc was added as Zn-EGTA. For rice, Zn concentrations included 0.1, 0.32, 1, 3.2, 10, 32, and 56 $\mu\text{mol L}^{-1}$, corresponding to free Zn ion activity (pZn^{2+}) of 8.1, 7.6, 7.1, 6.6, 6.1, 5.6, and 5.4. As reported, spinach has a strong tendency to accumulate Zn (Boawn & Rasmussen, 1971); thus, the highest level of Zn was dropped, and an intermediate level was inserted as 0.1, 0.32, 1, 3.2, 5.62, 10, and 32 $\mu\text{mol L}^{-1}$, with corresponding pZn^{2+} activities of 8.1, 7.6, 7.1, 6.6, 6.4, 6.2, and 5.7. Cadmium concentration for rice was 3.2 $\mu\text{mol L}^{-1}$ ($\text{pCd}^{2+} = 10.4$) and for spinach was 5.0 $\mu\text{mol L}^{-1}$ ($\text{pCd}^{2+} = 10.2$) because rice accumulated Cd more strongly than spinach at low Zn supply. All treatments had Mn of 1 $\mu\text{mol L}^{-1}$ (about $\text{pMn}^{2+} = 6.7$). Phosphate concentration was

0.1 mmol L^{-1} for rice and 0.2 mmol L^{-1} for spinach. The concentration of Ni in solutions was 0.1 $\mu\text{mol L}^{-1}$ for rice, whereas 1 $\mu\text{mol L}^{-1}$ was used for spinach because rice is more efficient at absorbing Ni than spinach. Half-strength Hoagland solution, except for the adjusted concentration of P, was used as a basal solution. Ethyleneglycoltetraacetate was added at 100 $\mu\text{mol L}^{-1}$ in excess of the sum of Mn, Zn, Fe, Ni, Cu, and Cd to buffer microelement cation activities (Supplemental Table S1). Solution pH was adjusted to 6.2 by $\text{KOH}-2\text{-[N-Morpholino]ethanesulfonic acid}$. Micronutrient concentrations in the solution are shown in Supplemental Table S1. Total Zn and Cd activity and free ion Zn and Cd activity were calculated using Geochem-PC (Parker et al., 1995), with calculated activities shown in Supplemental Table S2. With EGTA buffering, part of the added Fe^{3+} precipitates at equilibrium.

2.2 | Plant growth

Rice ('Presidio') and spinach ('Bloomsdale Long Standing') seeds were germinated in standard germination paper saturated with half-strength Hoagland macronutrient solution in the dark at 25 °C. Ten days later, after the emergence of the first two real leaves, seedlings were transplanted to 1.25-L beakers. Each beaker held one bundle of seedlings (four seedlings per bundle for rice and three seedlings per bundle for spinach) supported by polyurethane foam in a black Plexiglass cover. Beakers were wrapped with black polyethylene to prevent light exposure to roots and solution. Rice and spinach seedlings were grown in a growth chamber with the temperature kept at 28/20 °C day/night and 20/15 °C day/night, respectively. Relative humidity was kept at 70/90% day/night, and the light was 300 μmol photosynthetically active radiation $\text{m}^{-2} \text{s}^{-1}$ with a 16/8 h light/dark cycle. Solutions were continuously aerated, and deionized water was added daily to maintain the solution volume of each beaker. Solutions were replaced once a week. Seedlings were harvested 4 wk after transplanting to treatments.

Roots and shoots were harvested separately. Roots were rinsed using half-strength Hoagland macronutrient solution three times and blotted with paper towels. Plant tissues were dried in a forced draft oven at 70 °C for 3 d and ground in preparation for chemical analysis.

2.3 | Chemical analysis

Dried plant tissues (<5 g) were weighed into 100-ml beakers and dry ashed in a muffle furnace at 450 °C for 16 h (Kukier & Chaney, 2002). Ashed samples were digested with 2 ml concentrated HNO_3 on a hotplate until the acid evaporated. The samples were then refluxed with 10 ml 3 mol L^{-1} HCl for

TABLE 1 Analysis of variance for biomass and element concentrations among different Zn treatments in rice and spinach

Parameters	Rice		Spinach	
	Shoot	Root	Shoot	Root
Biomass	<.001**	<.001**	.407	.429
Ca	<.001**	.021*	.276	.113
Cd	.024*	<.001**	<.001**	<.001**
Cu	<.001**	.58	.915	.457
Fe	.067	.095	<.001**	.635
K	.002**	.089	.414	.026*
Mg	<.001**	.071	.256	.121
Mn	.007**	.434	<.001**	<.001**
Ni	<.001**	.363	<.001**	<.001**
P	<.001**	.025*	.376	.149
Zn	<.001**	<.001**	<.001**	<.001**

*Significant at the .05 probability level.

**Significant at the .01 probability level.

2 h. Digested samples were filtered using Whatman 40 filter paper and diluted with 0.1 mol L⁻¹ HCl to 25 ml. Elements Ca, Fe, K, Mg, P, Cd, Cu, Mn, Ni, and Zn were determined by inductively coupled plasma spectrometry with yttrium internal standardization. Two samples of the National Institute of Standards and Technology standard reference material 1570 (spinach), two blanks, and two random duplicate samples were digested for every 20 unique samples in every batch of samples.

2.4 | Data handling

Free cation activities were calculated using Geochem-PC Version 2 with corrected stability constants (Parker et al., 1995). Analysis of variance and multiple comparison (LSD) of element concentration among treatments within plant species were conducted using PASW statistics 18.0. Graphs were made using SigmaPlot 12.0. Zinc concentrations in roots and shoots were normalized by logarithmic transformation.

3 | RESULTS

3.1 | Effects of Zn activity on biomass and Zn accumulation in shoots and roots

Root and shoot Zn in rice and spinach varied significantly and substantially over the range of tested pZn²⁺ (ANOVA in Table 1). Rice root and shoot biomass was also affected significantly, whereas an insignificant effect on yield was found for spinach (Table 1; Supplemental Figure S1). As shown in Supplemental Figure S1a, when pZn²⁺ activity ranged from

8.1 to 5.4 (deficient to toxic), the biomass of rice shoot and root increased when the deficiency was corrected and then decreased when phytotoxicity occurred. The highest biomass was observed at pZn²⁺ = 6.6. The shoot biomass decreased by 20–30% and root biomass by 10–20% compared with the highest yield when pZn²⁺ was decreased to 8.1 (deficient). When Zn²⁺ was increased to pZn²⁺ = 5.4, there was once again a decrease in shoot (23–30%) and root biomass (33–44%).

Shoot Zn in rice in the treatment corresponded with the change of free Zn²⁺ activity. With Zn²⁺ increased from pZn²⁺ 8.1 to pZn²⁺ 5.6 and 5.4, shoot Zn demonstrated Zn deficiency at 7.9 mg kg⁻¹ dry weight (DW) and increasing up to Zn phytotoxicity at 948 and 1,460 mg kg⁻¹ DW, respectively (Figure 1a). Root Zn was comparable to that in shoots until the higher levels. The lowest shoot Zn in spinach was 48 mg kg⁻¹ DW (Figure 1b), with the highest spinach shoot Zn (for the treatment of pZn²⁺ = 5.7) of 441 mg kg⁻¹ DW. Although external pZn²⁺ ranged from 8.1 to 5.7 (about a 300-fold increase), shoot Zn in spinach only varied from 48.0 to 441 mg kg⁻¹ DW (<10-fold). Root Zn in spinach was at almost the same level as shoot Zn.

Chaney (1993) summarized the literature showing that crop yields are reduced when leaves contain about 300–1,000 mg kg⁻¹ DW and that 500 mg kg⁻¹ DW in leaves identifies Zn phytotoxicity in most crops. Pahlsson (1989) suggested that 200–300 mg kg⁻¹ DW was critical for most plant species. Parker suggested that shoot Zn <20 mg kg⁻¹ DW was demonstrative of Zn deficiency. Thus, based on yield and tissue Zn, a deficiency of critical free Zn²⁺ was about 8 nmol L⁻¹ (pZn²⁺ = 8.1), which is comparable to Parker's estimation of 10 nmol L⁻¹ for alfalfa and soybean but slightly lower than wheat and tall wheatgrass that were 15 and 13 nmol L⁻¹, respectively (Parker, 1997). The toxic critical free Zn²⁺ activity for rice in this work was about 5.6 pZn²⁺, which is consistent with the work by Green et al. (2017). However, no apparent change was observed in the phenotype of rice at the whole tested Zn²⁺ activity range except for slightly drooping of leaves at deficiency (pZn²⁺ = 8.1) (Supplemental Figure S2). For spinach, no deficiency was found even at the lowest Zn²⁺ activity tested, whereas marginal toxicity was observed at the highest Zn²⁺ level (pZn²⁺ = 5.7) based on yield and tissue Zn. The phenotype of spinach at the whole tested Zn²⁺ activity range did not show apparent difference (pictures not shown).

3.2 | Effects of Zn activity on Cd accumulation in shoots and roots

The increase in solution Zn²⁺ significantly affected Cd accumulation both in spinach and rice (Table 1). As shown in Figure 2a, Cd in roots of rice was about 1.5- to 8.5-fold greater than in shoots, depending on the different solution pZn²⁺

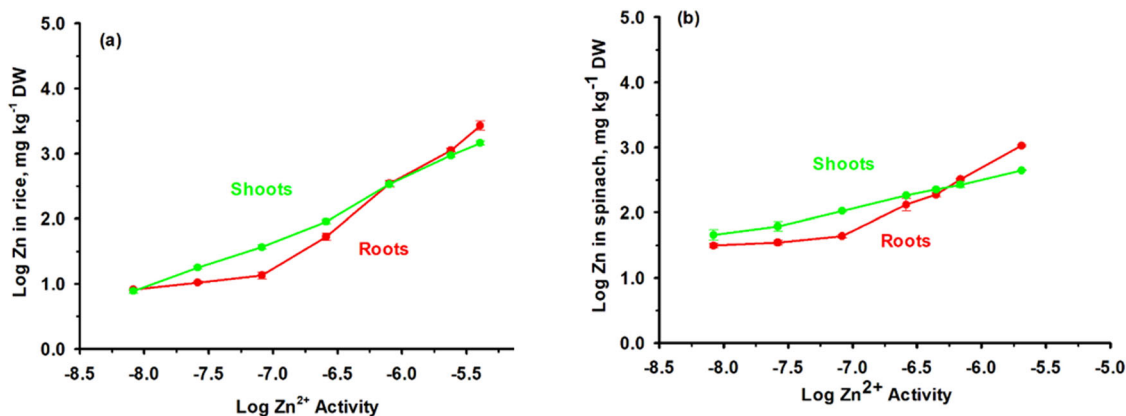


FIGURE 1 Effect of solution pZn^{2+} treatments on Zn in (a) rice and (b) spinach shoots and roots. DW, dry weight

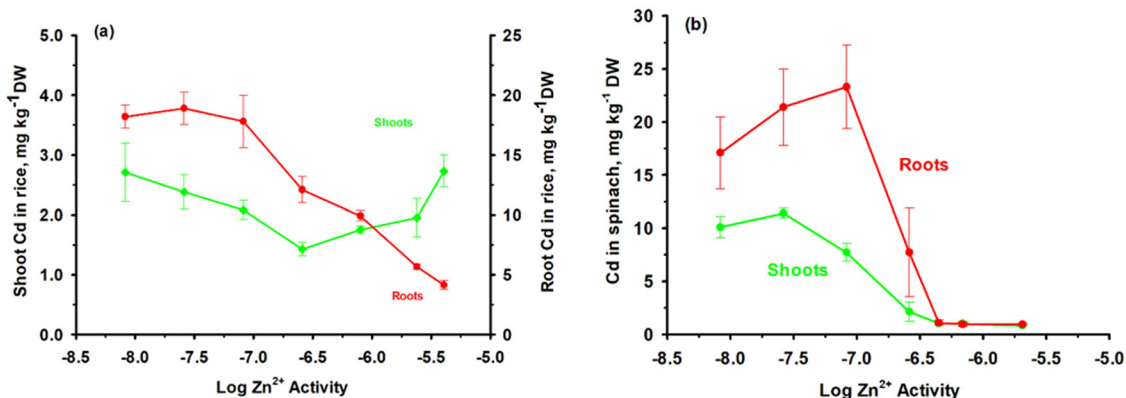


FIGURE 2 Effect of solution pZn^{2+} treatments on Cd in (a) rice and (b) spinach shoots and roots, DW, dry weight

levels. Root Cd in rice decreased significantly and gradually (from 18.2 to 4.1 $mg\ kg^{-1}\ DW$) with the increase of solution Zn^{2+} from deficiency to toxicity. The mean Cd in shoots decreased slightly from 2.7 to 1.4 $mg\ kg^{-1}\ DW$ with the increase of solution Zn from deficiency to sufficiency (pZn^{2+} from 8.1 to 6.6) and then increased to 2.7 $mg\ kg^{-1}\ DW$ at the phytotoxic Zn activities in solution (pZn^{2+} 5.4). The shoot/root ratios of Cd under 5.6 and 5.4 pZn^{2+} levels (average, 0.343 and 0.659, respectively) were significantly higher compared with other treatments with deficiency and sufficiency (ranging from 0.117 to 0.176 on average; $p < .05$). The results were consistent with Kukier & Chaney (2002), who reported that Cd translocation from roots to shoots in rice was increased at higher Zn^{2+} activity rather than inhibited by Zn.

Compared with rice, Cd concentration both in root and shoot of spinach decreased remarkably with increasing Zn^{2+} supply (Figure 2b). As solution Zn^{2+} was increased from 7.1 to 6.4, shoot Cd decreased significantly from 7.72 to 1.02 $mg\ kg^{-1}\ DW$, whereas root Cd declined from 23.3 to 1.09 $mg\ kg^{-1}\ DW$. Because external Zn^{2+} increased from pZn^{2+} 6.4 to 5.7, both shoot and root Cd remained at $\sim 1\ mg\ kg^{-1}\ DW$. Throughout the tested external Zn^{2+} range, shoot Cd

decreased from 10.1 to 0.80 $mg\ kg^{-1}\ DW$, whereas root Cd decreased from 17.1 to 0.94 $mg\ kg^{-1}\ DW$.

OsZIP1 and *OsZIP3* are important for Zn uptake by rice from soils (Bashir et al., 2012). *OsZIP3* was found broadly expressed under all conditions, whereas *OsZIP1* was mainly expressed in roots and induced by Zn deficiency (Chen et al., 2008). It was also found that *OsZIP1*-expressing cells accumulated more Cd than the empty vector (the control) in yeast cells or those expressing *OsZIP3* (Ramesh et al., 2003). The higher Cd in roots of rice under the deficiency of Zn in this work may be due to the inducing of *OsZIP1* in the epidermis and stele of roots, which would have increased the uptake of Cd, or the lack of Zn to compete with Cd on this transporter could have allowed the higher Cd accumulation at deficient Zn. Although there are no reported studies of ZIP transporters in spinach, the increase of Cd accumulation in roots of spinach under Zn deficiency like that in rice might be attributed to ZIP transporters. All those hypotheses above are open to further study.

The rice Mn transporter *OsNRAMP5* is the only transporter proved to be involved in the uptake of Cd by rice under field conditions (Ishikawa et al., 2012; Ishimaru et al., 2012; Yang et al., 2014). *OsNRAMP5* was found expressed in the root

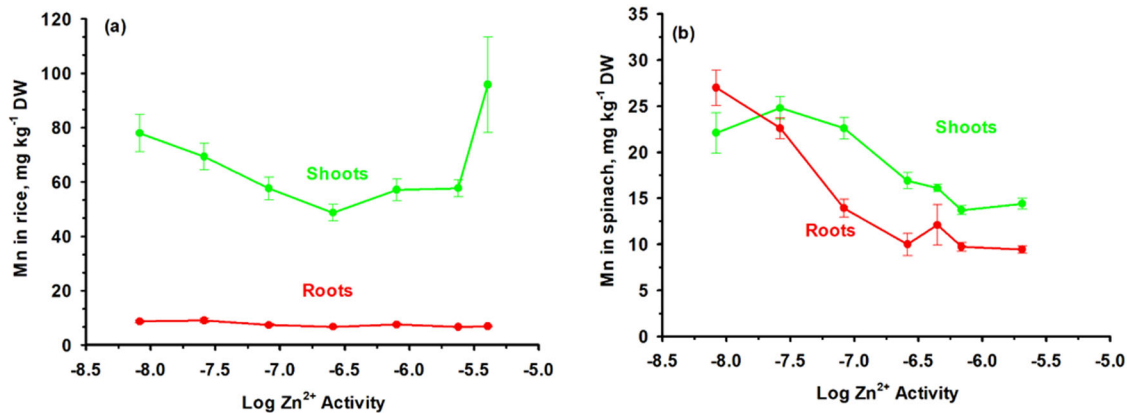


FIGURE 3 Effect of solution pZn^{2+} treatments on Mn in (a) rice and (b) spinach. DW, dry weight

epidermis, exodermis, and outer layers of the cortex (Ishimaru et al., 2012). The expression of *OsNRMP5* was not affected by Mn and Fe deficiency but might have been decreased in the presence of increased Cd (Ishimaru et al., 2012; Yang et al., 2014). However, there is still no evidence of the effects of Zn deficiency or toxicity on the expression of *OsNRMP5*.

3.3 | Effects of Zn on Mn, Ni, and Fe in shoots and roots of rice and spinach

Shoot Mn in rice and shoot and root Mn in spinach were affected significantly by varied solution Zn^{2+} (Table 1). As shown in Figure 3a, Mn in shoots of rice was significantly higher at both low and high levels of Zn (pZn^{2+} 8.1 and 5.4, respectively) than under Zn-sufficient levels. However, as shown in Supplemental Figure S3A, total Mn concentrations in rice shoots decreased gradually and significantly ($p < .05$) with the increase of solution Zn^{2+} from deficiency to toxicity. Therefore, foliar Mn in rice increased in concentration at both pZn^{2+} 8.1 and 5.4 with decreasing shoot yield, which might cause the translocated Mn to be more concentrated.

As for spinach, both shoot and root Mn decreased about twofold with the increase of solution Zn^{2+} (Figure 3b). Unlike rice, an insignificant effect on yield was found for spinach by Zn treatment (Table 1; Supplemental Figure S1b). Therefore, the decrease of Mn in spinach was caused by the interaction between Zn and Mn. Such an interaction between Zn and Mn was inconsistent from crop to crop and among studies. Parker's study, also done in chelator-buffered nutrient solutions, showed that shoot Mn was often increased for crops such as wheat, tall wheatgrass, soybean, and tomato under strong Zn deficiency (Parker, 1997). In soils, it was reported that tissue Mn of 20 cultivars of soybean significantly increased with the Zn addition both at pH 5.5 and 6.5 in never-dried Sassafras soil (White et al., 1979) (air drying soils causes an increase in soluble Mn^{2+} by reduction of Mn

oxides by organic matter). However, another study observed that Zn addition to Sassafras soil decreased shoot Mn in soybean at pH 5.5 and increased shoot Mn at pH 6.3, whereas shoot Mn of soybean both at pH 5.5 and 6.3 in Pocomoke soil did not change significantly with the addition of Zn (White & Chaney, 1980). Similar to Zn effects on Cd, our solution experiment showed shoot Mn in rice changed in a U-shape trend with solution Zn^{2+} increase from deficient to adequate to phytotoxic levels, whereas for spinach, shoot Mn decreased continuously with increasing Zn^{2+} . Singh & Steenberg (1974, 1975) found elevated Mn in barley (*Hordeum vulgare* L.) with the addition of Zn in Zn polluted soil, whereas under Zn-deficient and normal agricultural soils, application of Zn reduced the concentration of Mn in maize and barley plants. Thus, the responses of rice shoot Mn to external Zn^{2+} were similar to those of barley and maize in Singh and Steenberg's early work.

Nickel is an essential micronutrient in plants and plays a critical role in several plant physiological processes. Without an adequate supply of Ni, plants cannot complete their life cycle. It was reported that small quantities of Ni (0.01–5 mg kg⁻¹ DW) were essential for many studied plant species (Joseph, 1977; Polacco & Brown et al., 1987). In the case of pecan [*Carya illinoensis* (Wangenh.) K. Koch], Ni deficiency occurred in the field, especially in soils with repeated Zn applications from foliar Zn sprays (Boawn & Rasmussen, 1971; Wood et al., 2006). Nickel in shoots of rice and shoot and root of spinach was significantly reduced by the increase of solution Zn^{2+} (Table 1). As shown in Figure 4a, Ni in shoots of rice decreased significantly from 1.88 to 0.57 mg kg⁻¹ DW with the increase of Zn^{2+} activity. The shoot Ni at toxic Zn level (pZn^{2+} 5.4) was only about 30% of that at deficient Zn level (pZn^{2+} 8.1). An external increase of Zn did not affect root Ni concentration in rice (Figure 4a).

Nickel in spinach roots and shoots was reduced continuously with increasing solution Zn^{2+} activity. Translocation of Ni from roots to shoots is lower (shoot/root ratio,

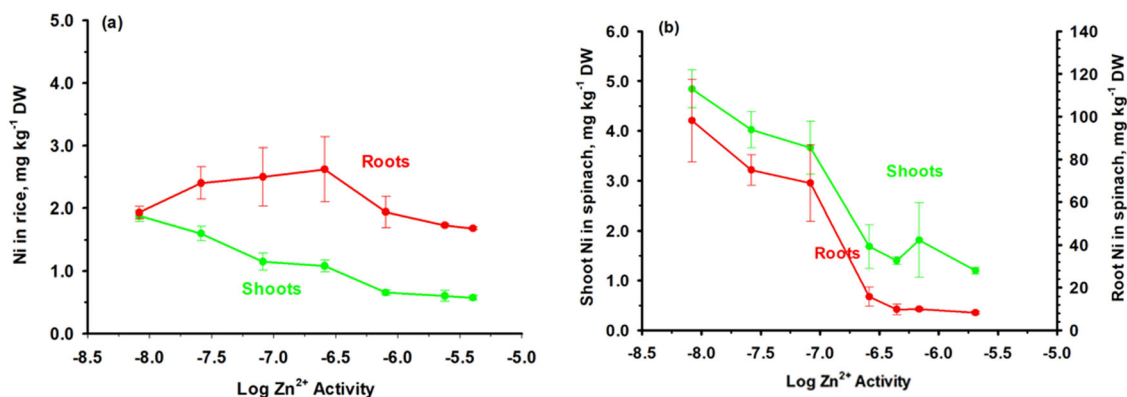


FIGURE 4 Effect of solution pZn^{2+} treatments on Ni in (a) rice and (b) spinach. DW, dry weight

0.045–0.268; average, 0.102) than for Mn (shoot/root ratio, 0.684–2.09; average, 1.39), Cd (shoot/root ratio, 0.189–4.14; average, 0.845), or Zn (shoot/root ratio, 0.389–5.40; average, 1.60), perhaps because Ni can be chelated and remain in root cells or vacuoles.

It was reported that in a crop where Ni deficiency has occurred in the field, high soil Zn is part of the causal factors of Ni deficiency (Wood et al., 2006). However, the interaction did not reduce shoot Ni to deficient levels in this work. It could be also concluded that antagonisms occurred in spinach between Zn and trace elements such as Mn, Cd, and Zn.

Shoot Fe in spinach changed significantly with the increase of solution Zn^{2+} ($p < .001$) (Table 1). Concentrations of Fe in rice roots and shoots were not significantly changed by increasing Zn^{2+} (Supplemental Figure S3a), whereas low levels of pZn^{2+} from 8.1 to 7.1 significantly increased shoot Fe in spinach (Supplemental Figure S3b). The change in Fe in spinach roots was insignificant. Shoot Fe in many crops is increased in Zn deficiency treatments (Parker, 1997), whereas high Zn concentrations might induce Fe-deficiency chlorosis (Chaney, 1993). White et al. (1979) also found that foliar Fe in soybean at both pH 5.5 and 6.5 decreased with the increase of soil Zn to phytotoxic Zn levels.

4 | CONCLUSIONS

Effects of Zn activity on the accumulation of Cd and other mineral elements in rice and spinach were studied in this work using novel EGTA-buffered solutions to more nearly mimic soil solution levels. Results showed significant changes in Cd accumulation and nutrition imbalance in rice and spinach under Zn deficiency and toxicity.

1. Rice showed Zn deficiency at $pZn^{2+} = 8.1$ and marginal deficiency at $pZn^{2+} = 7.6$. Phytotoxicity was observed at $pZn^{2+} = 5.6$ and 5.4. For spinach, no deficiency was found

even at the lowest level tested ($pZn^{2+} = 8.1$), and marginal toxicity was observed at $pZn^{2+} = 5.7$.

- Both Mn and Cd in rice shoot slightly decreased with increasing Zn^{2+} from deficiency to sufficiency but then increased with increasing Zn^{2+} from sufficiency to toxicity, whereas rice root Cd decreased gradually with the increase of external Zn from deficiency to toxicity.
- Spinach shoot and root Cd increased slightly but significantly when external pZn^{2+} increased from $pZn^{2+} 8.1$ to 7.6 and 7.1, respectively. However, with further increase of Zn to levels similar to the >100-fold higher Zn than Cd in most soil solutions, root and shoot Cd were remarkably reduced. These findings agree with Zn and Cd competition for transport on ZIP1 as reported. As for rice, the decreasing accumulation of Mn, Zn, and Cd under sufficient external Zn^{2+} appears to be explained by the competition for *OsNRAMP5* among those elements in rice, in agreement with recent genetic studies and the development of *OsNRAMP5*-null mutant rice.
- Nutrient solutions buffered using EGTA offer a tool to grow rice with controlled microelement levels. The EGTA-buffered system clearly showed the remarkable difference between Cd accumulation in rice and spinach and the strong effect of Zn^{2+} on Cd^{2+} accumulation in spinach and the more complex effect of Zn on rice Cd. These findings correct confusion regarding how rice grown on Cd-contaminated soil causes human Cd disease even when Zn is 100-fold higher than Cd.

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AUTHOR CONTRIBUTIONS

Meie Wang: Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Validation; Writing—original draft; Writing—review & editing. Wankai Ma: Formal analysis; Visualization; Writing—review &

editing. Rufus L Chaney: Conceptualization; Data curation; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Writing–review & editing. Carrie E Green: Formal analysis; Methodology; Validation; Writing–review & editing. Weiping Chen: Funding acquisition; Project administration.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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SUPPORTING INFORMATION

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