

## TECHNICAL REPORT

## Plant and Environment Interaction

Effects of Mn<sup>2+</sup> on Cd accumulation and ionome in rice and spinachMeie Wang<sup>1,2</sup> | Wankai Ma<sup>1,3</sup>  | Rufus L. Chaney<sup>2</sup> | Carrie E. Green<sup>2</sup> | Weiping Chen<sup>1</sup>

<sup>1</sup>State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

<sup>2</sup>USDA-ARS, Crop Systems and Global Change Lab., Beltsville, MD 20705, USA

<sup>3</sup>College of Resources and Environment, Univ. of Chinese Academy of Sciences, Beijing 100049, China

## Correspondence

Meie Wang or Rufus L. Chaney, State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China.  
Email: [mewang@rcees.ac.cn](mailto:mewang@rcees.ac.cn); [rufuschaney@verizon.net](mailto:rufuschaney@verizon.net)

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## Abstract

Health risks caused by food containing Cd is a concern worldwide. Interaction between Mn and Cd has been widely studied in normal hydroponic solution with high ion activities (e.g., the study on sharing of transporter Natural Resistance–Associated Macrophage Protein 5 between Mn and Cd in rice [*Oryza sativa* L.]). However, interaction of Mn and Cd in crops like rice and spinach (*Spinacia oleracea* L.) at field ion activity level is still unknown. Thus, an ethyleneglycoltetraacetate-buffered solution experiment was conducted to explore the effect of Mn on the uptake and accumulation of Cd and other mineral elements in rice and spinach. In rice, antagonism of Mn and Cd was only observed in roots at deficient and toxic levels of external Mn<sup>2+</sup>. Compared with those at Mn<sup>2+</sup> sufficiency (pMn<sup>2+</sup> 6.7–5.3), average root Cd levels were elevated significantly by 1.85–3.05 times at Mn<sup>2+</sup> deficiency (pMn<sup>2+</sup> 8.2) but decreased by 1.57–2.59 times at Mn<sup>2+</sup> toxicity (pMn<sup>2+</sup> 4.8). The antagonism between Mn and K/Mg in rice shoots might be caused by their common role in physiological processes in plants. Antagonism of Mn/Ni in spinach in this work was consistent with their shared transporters in dicots. Results about the antagonism of root Cd/Mn at Mn<sup>2+</sup> deficiency suggest that sufficiently available Mn<sup>2+</sup> is significant to reduce Cd uptake in rice under field levels of ion activity, but it was not for spinach because the change of tissue Cd was insignificant with the increase of Mn<sup>2+</sup> activity from deficiency to toxicity.

## 1 | INTRODUCTION

Cadmium in crops is a food issue worldwide, especially in Asian countries where rice (*Oryza sativa* L.) is the staple food. About 60–70% of rice grain samples exceeding the Chinese food standard of 0.2 mg kg<sup>-1</sup> dry weight (dw) have been reported in southern China (Du et al., 2013; Wang et al., 2018). Among dicots, spinach (*Spinacia oleracea* L.)

is known for higher accumulation of soil Cd. Uptake and accumulation of Cd by rice and spinach has been extensively reported.

Manganese is an essential micronutrient in plants. It plays an important role in photosynthesis, ATP synthesis, biosynthesis of chlorophyll, and assimilation of nitrate and is a cofactor of various enzymes (Millaleo et al., 2010; Peng et al., 2008; Rahman et al., 2016). However, both excess and deficiency of Mn depress leaf photosynthetic capacity in plants and thus decrease plant yield. Only Mn<sup>2+</sup> is available for plants in soils, whereas Mn<sup>3+</sup> and Mn<sup>4+</sup> are slightly

**Abbreviations:** dw, dry weight; EGTA, ethyleneglycoltetraacetate; PCA, principal component analysis.

soluble. Manganese excess takes place often in low-pH conditions (e.g., acid soils) as well as in reducing environments (e.g., waterlogged soils or applications of organic material) (Hue & Mai, 2002), whereas Mn deficiency occurs in calcareous soils with high pH (Jhanji & Sadana, 2014). Upland rice in Brazil and rice and wheat (*Triticum aestivum* L.) grown in a wheat–rice cropping system in Punjab, India, reported Mn deficiency due to calcareous soils or elevated pH by liming (Fageria et al., 2008; Jhanji & Sadana, 2014).

Manganese has been found to alleviate Cd toxicity in rice (Rahman et al., 2016; Sebastian & Prasad, 2015), maize (*Zea mays* L.) (Pal'ove-Balang et al., 2006), and Mn hyperaccumulator pokeweed (*Phytolacca americana* L.) (Peng et al., 2008). In their studies, reducing Cd uptake by plants and the further translocation from roots to shoots was one of the key roles for Mn in improving Cd tolerance. Supplemental Mn also plays an important role in alleviating Cd stress by improving the antioxidant defense and glyoxalase systems in rice (Rahman et al., 2016; Sebastian & Prasad, 2015). Meanwhile, addition of Mn efficiently decreased Cd accumulation in crops, including pea (*Pisum sativum* L.) (Hernandez et al., 1998), maize (Pal'ove-Balang et al., 2006), white lupin (*Lupinus albus* L.) (Zornoza et al., 2010), and hyperaccumulator *Phytolacca* (Liu et al., 2013; Peng et al., 2008). Antagonism was also observed between Cd and Mn in rice in a hydroponic solution experiment with the increase of external Mn from 0.08 to 800  $\mu\text{mol L}^{-1}$  (Yang et al., 2014). A field investigation in Hunan Province, China, revealed that a large proportion of rice samples with Cd concentration  $<0.2 \text{ mg kg}^{-1}$  was grown in paddy soil having total Mn concentration  $>300 \text{ mg kg}^{-1}$  (Wang et al., 2018).

Antagonistic accumulation in plants occurs between two ions due to competition for common transporters. Manganese has been reported to share transporters with other cations, such as OsNramp5 for  $\text{Mn}^{2+}$  and  $\text{Cd}^{2+}$ ; AtIRT1 for  $\text{Mn}^{2+}$  and  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Cd}^{2+}$  under Fe deficiency conditions; AtCAX2 for  $\text{Mn}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Cd}^{2+}$ ; and AtNramp3 for  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Cd}^{2+}$  under Fe deficiency conditions (Millaleo et al., 2010; Mills et al., 2008; Pittman, 2005; Sasaki et al., 2012). Rice is the only crop species that has been reported to absorb Cd specifically using Mn transporter NRAMP5 in roots (Ishikawa et al., 2012; Ishimaru et al., 2012; Sasaki et al., 2012; Yang et al., 2014), although TaNramp5A and TaNramp5D in wheat or ZmNramp5 in maize were also reported to be related to Cd uptake (Sui et al., 2018). However, most of these studies regarding interactions between Mn and Cd at physiological and genomics levels were conducted using hydroponic solution experiments with high ion concentrations. Few studies have examined ionomics levels as low as those in the field. Neither scenario has been reported for spinach, the representative dicots of Cd accumulator. Studies on interactions among different ions using normal solution experiments with traditional high concentrations of metal ions

### Core Ideas

- Antagonism of Mn/Cd in rice roots occurs at  $\text{Mn}^{2+}$  deficiency and toxicity.
- Antagonism of Mn/Mg (K) in rice shoots might be caused by their common physiological function.
- Antagonism of Mn/Ni in spinach corresponded to their common transporters in dicots.
- Sufficient  $\text{Mn}^{2+}$  is critical to reduce Cd in rice at field level of ion activity.

may result in irrelevant interactions. For example, Cohen et al. (2004) reported that IRT1 did not play a role in root Zn or Cd uptake in physiologically relevant soil Zn activities, although its significance in Zn and Cd uptake was demonstrated when these metals were present at elevated concentrations (Cohen et al., 1998).

Thus, the effects of different levels of external  $\text{Mn}^{2+}$  activity ranging from deficiency to toxicity on accumulation of Cd and other nutrient elements in rice and spinach were studied using chelator-buffered solution (pH 6.2). Chelator-buffered solution methods are helpful to examine effects of interaction of ions over genetic, protein, and phenotypic levels. Addition of chelators in excess of divalent metal ions reduced excessive uptake of divalent metals by plants and maintained constant free ion activity despite of the uptake by plants (Chaney, 1988). By using a computer program such as Geochem-PC, one can calculate free ion activities of elements for a fixed solution composition under specific pH conditions. Chelator-buffered nutrient solutions have been shown to be a practical way to study interactions among elements at activity levels of soil solution and obtain foliar levels similar to field-grown plants (Parker et al., 1995).

The goals of the work were to find out how and to what degree  $\text{Mn}^{2+}$  affects the uptake of Cd and the ionome (e.g., Ca, K, Mg, Zn, Ni, and Fe) in rice and spinach while keeping their biomass at a normal level and thus to provide a scientific basis for controlling Cd accumulation in crops and vegetables by controlling the Mn supply in soils.

## 2 | MATERIALS AND METHODS

### 2.1 | Chelator-buffered nutrient solution

Preliminary experiments defined that ethyleneglycoltetraacetate (EGTA) was suitable to buffer activities needed to attain both deficient and phytotoxic levels of Mn and concentrations of Cd, Zn, Ni, and Mn in rice and spinach. Experiments for both spinach and rice were designed with eight levels of Mn

in the nutrient solutions. Manganese was added as  $\text{MnCl}_2$ . For both rice and spinach, Mn concentrations included 0.032, 0.1, 0.316, 1, 3.16, 10, 31.60, and 100  $\mu\text{mol L}^{-1}$ , corresponding to free Mn ion activity ( $\text{pMn}^{2+}$ ), including 8.2, 7.7, 7.2, 6.7, 6.2, 5.7, 5.3, and 4.8. Cadmium concentrations were 3.16  $\mu\text{mol L}^{-1}$  ( $\text{pCd}^{2+} = 10.4$ ) for rice and 5.0  $\mu\text{mol L}^{-1}$  ( $\text{pCd}^{2+} = 10.2$ ) for spinach. Thus, the  $\text{Cd}^{2+}$  activities in solution were close to field level (Ivezic et al., 2012). All treatments had Zn of 1  $\mu\text{mol L}^{-1}$  ( $\text{pZn}^{2+} = \sim 7.1$ ). Compared with spinach, rice is more efficient at using P and Ni. Therefore, P concentration was 0.1  $\text{mmol L}^{-1}$  for rice and 0.2  $\text{mmol L}^{-1}$  for spinach. Concentrations of Ni in solutions were 0.1  $\mu\text{mol L}^{-1}$  for rice and 1  $\mu\text{mol L}^{-1}$  for spinach. Half-strength Hoagland solution with an adjusted concentration of P was used as a basal solution. The EGTA was added at a concentration of 100  $\mu\text{mol L}^{-1}$  in excess of the sum of Mn, Zn, Fe, Ni, Cu, and Cd ions to buffer microelement cation activities. Micronutrient concentrations in the solution are shown in Supplemental Tables S1 and S2.

## 2.2 | Rice and spinach planting

Rice ('Presidio') and spinach ('Bloomsdale long standing') seeds were germinated in standard germination paper saturated with half-strength Hoagland solution in the dark at 25 °C. About 10 d after 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) and were supported by polyurethane foam. Rice and spinach seedlings were grown in growth chambers with 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 light was 18/8 LUX light/dark. Aeration was applied to the solutions continuously, and deionized water was added daily to maintain the solution volume of each beaker. Solutions were replaced once a week. Seedlings were harvested after 4 wk.

Roots and shoots were harvested separately. To remove ions adsorbed on the root surface and avoid the leaking of ions from root cells, roots were rinsed three times using half-strength Hoagland solution and blotted with paper towels. Plant tissues were dried in an oven at 70 °C for 3 d before chemical analysis.

## 2.3 | Cd and other element analysis

As reported by Kukier and Chaney (2002), dried plant tissues (<5 g) were put into 100-ml beakers and ashed in a muffle furnace at 450 °C for 16 h. Ashed samples were digested with 2 ml concentrated  $\text{HNO}_3$  on hotplate until the acid evaporated to dryness. Then, the sample was refluxed with 10 ml of 3 mol  $\text{L}^{-1}$  HCl for 2 h. Digested samples were filtered using What-

**TABLE 1** The *p* values from one-way ANOVA for elements between different treatments in rice and spinach

Parameters	Rice		Spinach	
	Root	Shoot	Root	Shoot
Biomass	.101	.25	.181	.094
Ca	.124	.841	.14	.217
Cd	.000**	.745	.198	.160
Cu	.547	.836	.753	.012*
Fe	.776	.886	.444	.978
K	.883	.000**	.056	.007**
Mg	.135	.000**	.442	.001**
Mn	.000**	.000**	.000**	.000**
Ni			.002**	.192
P	.882	.522	.084	.005**
Zn	.693	.153	.778	.000**

\*Significant at the .05 probability level. \*\*Significant at the .01 probability level.

man 40 filter paper and diluted with 0.1 mol  $\text{L}^{-1}$  HCl to 25 ml. Calcium, Fe, K, Mg, P, Cd, Cu, Mn, Ni, and Zn were determined by inductively coupled plasma spectrometry using Y as an internal standard. For quality control, a National Bureau of Standards Standard Reference Material (No. 1570a spinach) was included. Recovery of Ca, Fe, K, Mg, P, Cd, Cu, Mn, Ni, and Zn ranged from 80 to 98%.

## 2.4 | Data analysis

Cation free activity was calculated using Geochem-PC Version 2 with corrected stability constants (Parker et al., 1995). Analysis of variance was conducted to reveal differences in biomass weight and mineral element concentration of rice and spinach among treatments with different external  $\text{Mn}^{2+}$  activities using SPSS-PASW statistics 18. Selected parameters were further demonstrated in plots at the whole tested range of external  $\text{Mn}^{2+}$  activity using SigmaPlot 12.0. Manganese concentrations in roots and shoots were normalized by logarithm. Multiple comparison (LSD) was conducted between treatments. Principal component analysis (PCA) was conducted to demonstrate correlations among tested mineral elements in roots and shoots of plants using R3.0.2 software.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Statistical analysis of tested elements and biomass in rice and spinach among different treatments

Significant differences in root Cd and shoot K and Mg in rice were observed among different  $\text{Mn}^{2+}$  treatments (Table 1). For spinach, significant differences were found in root Ni and

**TABLE 2** Factor transformation matrix in principal component analysis for rice and spinach roots and shoots

	Rice root			Rice shoot		Spinach root			Spinach shoot		
	PC1	PC2	PC3	PC1	PC2	PC1	PC2	PC3	PC1	PC2	PC3
Ca				0.670	0.217	0.102	-0.924	-0.088	-0.249	0.770	-0.062
Cd	-0.086	0.955	-0.086	0.849	-0.127	-0.851	0.239	0.140	-0.632	0.684	-0.018
Cu	-0.668	-0.041	0.005	0.934	0.061	0.133	-0.795	0.064	-0.729	0.339	-0.059
Fe	0.876	-0.114	0.141	0.952	-0.101	0.877	-0.377	0.016	0.441	0.633	0.159
K	0.669	0.626	0.244	0.015	0.942	0.621	0.721	0.155	0.935	0.094	-0.111
Mg	0.065	0.004	0.982	0.291	0.885	-0.671	0.593	0.255	0.899	0.148	0.326
Mn	0.626	-0.488	-0.223	0.130	-0.683	-0.024	0.183	-0.917	-0.157	0.317	-0.833
Ni						-0.119	0.394	0.826	-0.015	0.305	0.856
P	0.972	0.023	0.053	0.948	-0.030	0.948	0.124	0.122	0.949	-0.135	0.145
Zn	0.948	-0.042	0.040	0.675	0.359	0.785	0.090	-0.084	0.758	-0.285	0.010

for shoot Cu, K, Mg, P, and Zn. Both rice and spinach had significant differences of Mn in root and shoot. Insignificance was observed in biomass of roots and shoots for both rice and spinach. Data about Ni in rice roots and shoots and Ca in rice roots are not shown because a part of the rice root and shoot samples had Ni and Cd concentrations below the detection limit (Ni <0.875 mg L<sup>-1</sup>; Ca <312 mg L<sup>-1</sup>).

Principal component analysis was conducted to analyze correlations among tested elements in roots and shoots of rice and spinach, respectively (Supplemental Table S3; Table 2). The Kaiser–Meyer–Olkin measure of sampling adequacy for both rice and spinach roots and shoots was >0.5 and had significance at  $p = .001$ , which suggested that those tested tissue elements were well detected by PCA (Supplemental Table S3). Three components for rice roots (PC1–PC3), spinach roots, and spinach shoots were extracted, representing 82.8, 83.2, and 78.7% of variance, respectively, whereas two components for rice shoots were extracted (PC1 and PC2), representing 75.0% of variance (Supplemental Table S3).

The factor transformation matrix in PCA is shown in Table 2. For rice roots, Fe, K, Mn, P, and Zn were in PC1, and Cd and Mg were included solely in PC2 and PC3, respectively. For rice shoots, Ca, Cd, Cu, Fe, P, and Zn were in PC1, whereas K, Mg, and Mn were in PC2, with Mn negatively correlating to K and Mg. For spinach roots, Cd, Fe, Mg, P, and Zn were in PC1; Ca, Cu, and K were in PC2; and Mn and Ni were in PC3. For spinach shoots, Cu, K, Mg, P, and Zn were in PC1; Ca and Cd were in PC2; and Mn and Ni were in PC3.

As mentioned above, Mn was negatively correlated with tissue Mg and K in rice shoots (Table 2). An increase of major elements in response to the deficiency of some essential element widely occurs in plants. For example, Mg increases in shoots of many crops (e.g., wheat, alfalfa [*Medicago sativa* L.], soybean [*Glycine max* (L.) Merr.], tomato [*Solanum lycopersicum* L.], and tall wheatgrass [*Thinopyrum ponticum* (Podp.) Z.-W. Liu & R.-C. Wang]) under Zn deficiency (Parker, 1997). That might be because Mg associates

with chlorophyll and thus would be accumulated in large amounts to compensate for the decrease of photosynthesis due to chlorophyll loss induced by nutrient (e.g., Mn) deficiency (Peng et al., 2008). On the other hand, an association between Mn toxicity and the decrease of Ca and K concentration in barley (*Hordeum vulgare* L.) was also observed (Alam et al., 2005, 2006).

Results from this study demonstrate that P and Zn were always in the same group both in rice and spinach shoots and roots and positively correlated (Table 2). However, Parker (1997) found a negative relationship between Zn and P in that Zn deficiency increased P in tissues to a toxic level (>15 g kg<sup>-1</sup> dw).

### 3.2 | Mn accumulation in shoots and roots of rice and spinach

Both shoot and root Mn of rice and spinach decreased significantly with the decrease of Mn<sup>2+</sup> activity in solutions (Figure 1). Normal Mn concentration in leaves varies greatly between species (Millaleo et al., 2010). Snyder et al. (1990) reported that rice seedlings containing <20 mg kg<sup>-1</sup> dw tissue Mn, indicating that they were affected by Mn deficiency. The average concentration of rice shoot Mn was 8.35, 11.7, and 15.4 mg kg<sup>-1</sup> dw for the treatments of pMn<sup>2+</sup> 8.2, 7.7, and 7.2, respectively, showing deficiency in Mn. Root Mn in rice was lower than shoot Mn (Figure 1a).

For normal plants, shoot Mn <15 mg kg<sup>-1</sup> dw is deficient. Spinach shoots treated with external Mn<sup>2+</sup> activity (pMn<sup>2+</sup> 8.2, 7.7, and 7.2) had average tissue Mn concentrations of 5.53–5.83 mg kg<sup>-1</sup> dw, indicating Mn deficiency at all levels (Figure 1b). Unlike rice, spinach roots had similar concentrations of Mn as shoots at pMn<sup>2+</sup> 8.2–6.2 and much higher concentrations than shoots at pMn<sup>2+</sup> 5.7–4.8.

Rice shoot Mn was ~3,096 mg kg<sup>-1</sup> dw at the highest Mn treatment (pMn<sup>2+</sup> 4.8) (Figure 1a). Normal Mn concentration

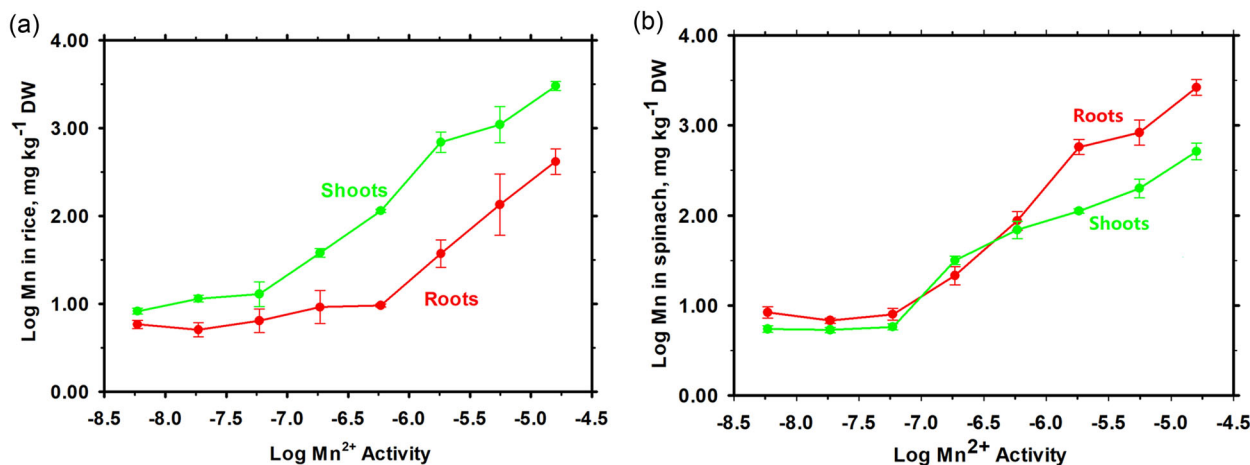


FIGURE 1 Changes of Mn in shoots and roots in (a) rice and (b) spinach with the increase of external  $Mn^{2+}$  activity

in plant leaves ranges from 30 to 500  $mg\ kg^{-1}\ dw$  (Clarkson, 1988). However, rice was reported to accumulate 5–10 times more Mn in leaves than other grasses (Foy et al., 1978). Shoot Mn at concentrations  $>2,000\ mg\ kg^{-1}\ dw$  was reported to be phytotoxic to rice (Lidon & Teixeira, 2000). In spinach, the highest Mn concentration in shoots was  $\sim 570\ mg\ kg^{-1}\ dw$  (Figure 1b). Field investigation for mineral composition of spinach showed the range of Mn in spinach shoots was 31–979  $mg\ kg^{-1}\ dw$  (Bhattacharjee et al., 1998). This result means no or marginal toxicity to spinach even at external  $pMn^{2+}\ 4.8$ .

The change of phenotype of rice and spinach at the whole range of external  $Mn^{2+}$  activity is shown in Supplemental Figure S1. Rice shoots at  $pMn^{2+}\ 4.80$  were the shortest and showed chlorotic and brown spots in leaves (Supplemental Figure S1A,C). Necrotic brown spots and chlorotic leaves were frequently observed symptoms for Mn toxicity (Wissemeier & Horst, 1991). No apparent change in phenotype was found for spinach among the eight treatments (Supplemental Figure S2B).

Manganese deficiency was observed for both rice and spinach at  $pMn^{2+}\ 8.2$ , 7.7, and 7.2, whereas only rice showed toxicity of Mn at  $pMn^{2+}\ 4.8$ , although insignificant changes in biomass were observed. Our results suggest that, compared with rice, spinach was more able to block accumulating high levels of Mn when both were at the same high external  $Mn^{2+}$  activity, which protects spinach from Mn toxicity.

### 3.3 | Changes of Cd accumulation in rice and spinach with the increase of external $Mn^{2+}$ activity

Significant differences in root Cd concentrations of rice were found among treatments with different external  $Mn^{2+}$  activities ( $p < .01$ ) (Table 1). Root Cd concentrations decreased

significantly and gradually with external  $Mn^{2+}$  activity increasing from deficiency to toxicity (Figure 2a), although an insignificant correlation between Cd and Mn in rice roots was observed by PCA (Table 2). When compared with those of treatments with sufficient external  $Mn^{2+}$  ( $pMn^{2+}\ 6.7$ – $5.3$ ), root Cd in deficient  $Mn^{2+}$  treatment ( $pMn^{2+}\ 8.2$ ) increased by almost three times, whereas it decreased by about two times in the toxic  $Mn^{2+}$  ( $pMn^{2+}\ 4.8$ ) treatment. Rahman et al. (2016) found the addition of Mn decreased Cd accumulation even more in the shoots compared with the roots and that addition of Cd also decreased Mn uptake both in roots and shoots, although in their work the addition of external Mn and Cd was as high as  $0.3\ mmol\ L^{-1}$ , which was much higher than the concentrations in our work. Field investigation in a mining area contaminated with Pb and Cd found that rice cultivars with higher concentration of Mn had low concentrations of Cd in brown rice (Li et al., 2012). Yang et al. (2014) suggested the antagonism of root Cd/Mn could be explained by the competition for transport *OsNRAMP5* between Cd and Mn, based on the result that the antagonism of root Cd/Mn was observed only for the wild type and not for the *OsNRAMP5* knock-out mutant. It was also reported that addition of Mn, Cd, and Cu did not affect the expression of *OsNRAMP5* in rice (Sasaki et al., 2012). However, our previous work conducted in chelator-buffered solution found that the expression of *OsNRAMP5* in rice roots increased significantly at Mn phytotoxicity compared with that in Mn deficiency (Cai et al., 2020). Both Yang et al. (2014) and our present work demonstrated that Mn/Cd antagonism occurred at Mn toxic level. Therefore, it is hard to assert that the antagonism is caused by the competition of *OsNRAMP5* at the genomic level because the increase of transporter expression and the antagonism occurred simultaneously. On the other hand, Yang et al. (2014) suggested that the inactivation of *OsRAMP5* protein could be a mechanism to limit excessive Cd absorption in roots at high external Mn. It was reported that Mn deficiency

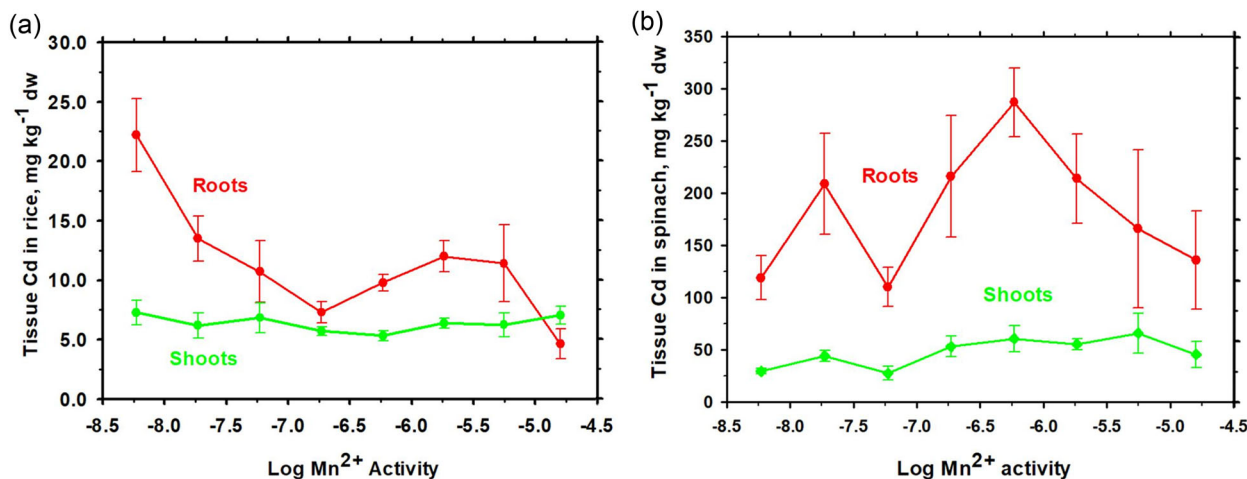


FIGURE 2 Changes of shoot and root Cd in (a) rice and (b) spinach with the increase of Mn<sup>2+</sup> activity

would downregulate the transcript level of *IRT1* and *HAM3* in *Arabidopsis* (Yang et al., 2008), and *OsHAM3* was widely acknowledged to sequester Cd in roots in rice (Sasaki et al., 2014; Satoh-Nagasawa et al., 2013), which may lead to an increase of root Cd in rice. Thus, the increase of root Cd in rice at Mn deficiency may have contributed to the downregulation of *HAM3*. It is also widely acknowledged that heavy metal ATPase (*OsHMA2*) contributes to the transportation of Cd from root to shoot in rice, although little evidence has suggested that *OsHMA2* is involved in Mn transportation in rice. Thus, a proteogenomic approach coupled with the chelator-buffered solution method may clarify the competition of *OsRAMP5* transporter among Mn/Cd in future research.

Unlike rice, spinach did not show significant differences in root and shoot Cd between treatments, with external Mn<sup>2+</sup> activity ranging from deficiency to toxicity (Table 1; Supplemental Table 4S). Root Cd in spinach was about one order of magnitude higher than shoot Cd (Figure 2b). Both root and shoot Cd changed irregularly with the increase of external Mn<sup>2+</sup> activity and had a great standard deviation for each treatment.

### 3.4 | Effects of Mn on mineral elements Ni, Zn, Fe, and Cu accumulation in rice and spinach

Nickel was the only element belonging to the same group as Mn (PC3) and was negatively correlated with Mn both in roots and shoots of spinach (Table 2). Moreover, root Ni in spinach decreased significantly with the increase of external Mn<sup>2+</sup> activities (Supplemental Figure S2A). Nickel uptake in spinach has been proved to be internalization limited (Degryse & Smolders, 2012; Degryse et al., 2012). Some YSL fam-

ily transporters, as well as *IRT1* and *ZIP10*, were reported to transport Ni in hyperaccumulator plant *Thlaspi caerulescens* (Halimaa et al., 2014; Mizuno et al., 2005; Sharma et al., 2022). Meanwhile, *AtIRT1* and *AtYSL* have been reported to be the main Mn<sup>2+</sup> uptake transporters in the plasma membrane in plants (Conte et al., 2013; Ducic & Polle, 2005; Pittman, 2005). Therefore, it is plausible that Ni shared a common uptake transporter with Mn in spinach.

Both shoot and root Zn in rice decreased about 40% with the external Mn<sup>2+</sup> activity changing from deficiency to sufficiency and elevated slightly at toxic Mn<sup>2+</sup> activities (Supplemental Figure S2B). In spinach, Zn in roots and shoots decreased significantly when external Mn<sup>2+</sup> activity increased from deficiency (pMn<sup>2+</sup> = 8.2) to sufficiency (pMn<sup>2+</sup> = 6.7–5.3) (Supplemental Figure S2C). Generally, Fe and Cu in rice and spinach did not change significantly, except for shoot Cu in spinach, which increased significantly at pMn<sup>2+</sup> 5.3 (data not shown).

## 4 | CONCLUSIONS

Effects of external Mn<sup>2+</sup> activity at a range from deficiency to excess on Cd and mineral elements accumulation in rice and spinach were studied using chelator-buffered solutions. It could be concluded that

1. Rice accumulated much higher concentrations of Mn up to toxic level in shoot compared with spinach and showed toxic symptom at Mn<sup>2+</sup> toxic levels. It could be concluded that spinach was more able to block accumulating high levels of Mn compared with rice.
2. Competition between Mn and Cd for the transporter *OsNRAMP5* was not asserted based on our work and previous studies. A proteogenomic approach coupled with

chelator-solution method may clarify the mechanism of antagonisms among elements in the future research.

3. Antagonism of Mn/Mg (K) in rice shoots was observed, which might be caused by their common physiological functions, such as photosynthesis.
4. Similarly, the antagonism between Mn and Ni in spinach can be caused by their shared common transporters in dicots as reported, although it needs to be further proved.

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## AUTHOR CONTRIBUTIONS

Meie Wang: Conceptualization; Writing – original draft. Wankai Ma: Resources; Writing – review & editing. Rufus L. Chaney: Supervision; Validation; Conceptualization. Carrie E Green: Data curation; Formal analysis. Weiping Chen: Project administration.

## CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

## ORCID

Wankai Ma  <https://orcid.org/0000-0001-7509-1304>

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## SUPPORTING INFORMATION

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