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Effects of sulfate on cadmium uptake in wheat grown in paddy soil – pot experiment

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Abstract: Rice-wheat rotation is common in China. Cadmium (Cd) and sulfur (S) are added to rice fields through various activities. The sulfur amendment has been recommended to control the uptake of Cd in rice. However, the effect of S on Cd uptake in winter wheat cultivated in paddy soil is rarely reported. A greenhouse pot experiment including two Cd levels (0, 10 mg Cd/kg, as $CdCl_2$) combined with three S rates (0, 30, 60 mg S/kg, as Na_2SO_4) was performed to investigate the effect of S application on uptake and allocation of Cd in wheat cultivated in paddy soil. Cadmium concentrations in wheat grain significantly ($P < 0.05$) increased by 37% at 30 mg S/kg, and the percentage of Cd allocation to grain significantly ($P < 0.05$) increased by 7% at 60 mg S/kg compared with non-S addition treatment when wheat was grown in Cd-added soil. For the low Cd soil, a similar trend was seen, but Cd increases were insignificant for grain while significant ($P < 0.05$) for root at 60 mg S/kg. In conclusion, S fertiliser may promote Cd accumulation in wheat grain and should be considered when it is used for wheat in paddy soils.

Keywords: cadmium toxicity; *Triticum aestivum* L.; plant uptake; translocation; distribution

Both cadmium (Cd) and sulfur (S) are added to rice fields through various activities (Chen et al. 2015, Gao et al. 2018). One-third of paddy soils in the main rice-growing area in China is contaminated with Cd (Mu et al. 2019). The S content of paddy soil is usually higher than that of dryland soil with the same parent material (Cao et al. 2011), due to the slow decomposition of organic matter including S in the anaerobic environment (Vityakon et al. 2000).

The annual paddy rice-winter wheat rotation covers around 10% of the total rice planting area in China (Frolking et al. 2002). Application of S has been recommended for reducing Cd accumulation in rice (Fan et al. 2010), while its effect on grains of wheat grown in paddy soils is still not clear.

The present results about S fertilising effects on Cd uptake in plants are conflicting. For instance, Khan et al. (2015) revealed that sulfate application could enhance Cd tolerance by promoting S assimilation in wheat. Complexation of free Cd^{2+} by phytochelatins synthesized from S-containing glutathione, and deposition of Cd-phytochelatins into vacuoles is one of the mechanisms in response to Cd stress in wheat (Khan et al. 2007). Similar results were also found in maize (Adhikari et al. 2018). On the contrary, McLaughlin et al. (1998) demonstrated that sulfate

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application significantly increased Cd solubility in soil, which resulted in increasing concentrations of Cd in shoots of Swiss chard. In addition, Cd uptake in spring wheat increased in a pot experiment with K_2SO_4 supply (Zhao et al. 2003). In all, we aim at investigating whether S application affects Cd accumulation in wheat grain, as well as Cd distribution in wheat plant tissues.

MATERIAL AND METHODS

Soil used. The topsoil (0–20 cm) in a rice field was collected in September 2016 from Anqing city, Anhui province, China, where wheat-rice rotation is popular. The moist soil was air-dried, thoroughly mixed, and sieved through a 2-mm mesh. The main physicochemical properties are as follows: pH_{H_2O} 5.92; pH_{KCl} 5.42; total and available Cd contents were 0.35 mg/kg and 0.20 mg/kg; total and available S was 251 mg/kg and 15.5 mg/kg; soil texture was silt loam.

Experimental design. A greenhouse pot experiment was performed. There were six treatments, including two Cd rates (0, 10 mg Cd/kg, as $CdCl_2$) combined with three S levels (0, 30, 60 mg S/kg, as Na_2SO_4). Each treatment had four replicates. In total, 4 kg air-dried soil was placed in each polyethylene pot (20 cm diameter at the top, 18 cm diameter at the bottom, 20 cm height). All pots received the basic nutrients: 200 mg N/kg urea, 150 mg P/kg (KH_2PO_4), and 189 mg K/kg (KH_2PO_4). Cadmium, S, and the fertilisers were added to the soil in the form of solids and mixed thoroughly by a mixer machine (YG-5KG, Jinjie, Shenzhen, China). Each pot was performed individually.

Plant cultivation and sampling. Soil moisture content was controlled at 20–22% (*v/v*) during the entire experiment, and monitored continuously by a combined soil moisture sensor-meter (TR-6, Shunkeda, Beijing, China). In each pot, 12 seeds of the wheat cultivar Huaimai33 (*Triticum aestivum* L., Chinese Academy of Agriculture Sciences) were sowed and thinned to 4 plants two weeks later. The wheat grew at temperatures ranging between 0°C and 20°C, and relative humidity of 30% to 50%. All pots were arranged randomly. At maturity, 203 days after sowing, the whole plant was removed from the soil, and divided into 6 parts: root, stem, old leaves, top 3 leaves, husk, and grain. Soils on the root surface were collected as rhizosphere soils by shaking; the other soils in the pot were homogenized thoroughly and referred to as bulk soils (Wieland et al. 2001). All plant parts were rinsed with deionized water and dried to constant weight at 70°C for 48 h, and dry weights (DWs) recorded.

Analysis of plant samples. Dried plant samples were finely grinded into powder with a stainless steel miller (FW80, Yongguangming, Beijing, China), except grain, which was grinded by hand with an agate mortar. Plant samples were digested according to Matusiewicz et al. (1989) with mixed acid (0.2 g plant + 8 mL HNO_3 + 3 mL H_2O_2) in a microwave oven (Mar 6, CEM Corporation, North Carolina, USA). Cadmium concentrations in the digestate were determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, ELAN DRC-e, Waltham, USA), and S concentrations by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer, Optima 5300 DV, Waltham, USA). A reagent blank and standard materials (Celery GBW10048 (GSB-26), National Certified Reference Materials) were included to verify the accuracy and precision of the analysis. The recovery of Cd and S were 90–110% and 80–91%, respectively.

Analysis of soil samples. After harvesting, soils were extracted by diethylenetriamine pentaacetic acid (DTPA: 0.05 mol/L DTPA, 0.1 mol/L triethanolamine and 0.01 mol/L $CaCl_2$ at pH 7.3) at a soil:solution ratio of 1:2 (g/mL) as plant available Cd, and by 0.01 mol/L $Ca(H_2PO_4)_2$ at a soil:solution ratio of 1:5 (g/mL) as plant available S (Lu 1999). Cadmium and S in the extracts were determined by ICP-MS and ICP-OES, respectively. Soil pH was determined in a 1:2.5 suspension of soil and water or 1 mol/L KCl. Soil total Cd was determined by ICP-OES after digestion by HCl-HNO_3-HF (Lu 1999), and total S by ICP-OES after digestion by $Mg(NO_3)_2$ (Lu 1999).

Data analysis. Cadmium transfer factors were the ratios of Cd concentrations in aboveground tissues (grain, husk, top 3 leaves, old leaves, stem) to that in roots (Boussen et al. 2013). Analysis of variance followed by the least significant difference (*LSD*) test and Pearson correlation analysis was performed using Window-based SPSS 25 (SPSS Inc., Armonk, USA).

RESULTS

Wheat plant growth. Cadmium addition significantly ($P < 0.05$) decreased number of spikes and grain DWs by 8% and 15% at zero mg S/kg level, as well as root DWs by 29% at 30 mg S/kg level, husk DWs and straw DWs by 23% and 22% at 60 mg S/kg level (Table 1).

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The effects of S application on wheat growth were related to Cd levels and differed among different plant tissues (Table 1). Compared with non-S addition treatment, application of 30 mg S/kg significantly ($P < 0.05$) increased straw DWs and root DWs by 21% and 8% in −Cd treatments. In +Cd treatments, plant height and grain DWs significantly ($P < 0.05$) decreased by 7% and 9% at 30 mg S/kg, as well as straw DWs by 21% at 60 mg S/kg compared with non-S addition treatment. On the contrary, in +Cd treatments, DWs of root, straw, and husk decreased linearly with the increasing rate of S application. Consequently, total plant DWs presented no significant difference among S levels in −Cd treatments, but significantly ($P < 0.05$) decreased linearly in +Cd treatments, i.e., by 7.6% and 13% at 30 and 60 mg S/kg levels, respectively.

Uptake, distribution, and transfer of cadmium in wheat. The concentrations of Cd in all plant tissues were significantly ($P < 0.001$) higher in +Cd treatments than in −Cd treatments irrespective of S application. For instance, the grain Cd concentrations in +Cd treatments were 28, 34 and 23 times higher than in −Cd treatments at 0, 30, and 60 mg S/kg levels, respectively. The corresponding values for roots were 55, 46, and 22 times higher (Table 4).

There were interaction effects ($P < 0.001$) of S and Cd applications on Cd concentrations in wheat grains and stems. In −Cd treatments, the grain Cd concentrations tended to increase with the increase of S levels, and exceeded the acceptable value of Cd (0.1 mg/kg) set in the maximum safe contaminant concentration standard in food of China (GB 2762-2017) by 50, 130 and 170% at 0, 30, 60 mg S/kg levels, respectively. However, in +Cd treatments, concentrations of Cd in grains and stems reached their peaks at the 30 mg S/kg level, and grain Cd concentrations exceeded the acceptable value (GB 2762-2017) 57–78 times. In +Cd treatments, there was no significant difference in Cd concentrations in other tissues (husk, leaves, roots) among S levels. However, for the −Cd treatment, S application significantly ($P < 0.05$) decreased Cd concentrations in leaves and increased that in roots. In +Cd treatments, the sequence of Cd concentrations in plant tissues was: root > stem ≈ old leaves ≈ husk > top 3 leaves > grain; a similar sequence was seen for the −Cd series, but differences were smaller (Table 4).

With the application of S at 0, 30, 60 mg/kg levels, the mean of total Cd uptake by the whole plant was 23.7, 21.5, and 20.9 µg/pot in −Cd treatments, and 620, 615, 489 µg/pot in +Cd treatments, respectively. Cadmium was predominately distributed in roots and straws, which accounted for 29% and 44%, respectively, followed by grains (15%) and husks (11%). Sulfur application tended to increase Cd allocation in grains and husks either in −Cd treatments or in +Cd treatments (Figure 1). In addition, S application significantly ($P < 0.05$) decreased Cd transfer factors

Table 1. Effect of sulfur (S) application on plant heights, number of spikes, dry weights of grain, husk, straw, root and total plant of wheat grown in paddy soil with and without the addition of 10 mg Cd/kg

S level (mg/kg)	Plant heights (cm)		Number of spikes (/pot)		Grain (g/pot)		Husk (g/pot)		Straw[a] (g/pot)		Root (g/pot)		Total plant (g/pot)	
	−Cd	+Cd	−Cd	+Cd	−Cd	+Cd	−Cd	+Cd	−Cd	+Cd	−Cd	+Cd	−Cd	+Cd
0	59 ± 2^{a}	61 ± 1^{b}	12 ± 1^{a}	11 ± 1^{a}	13.0 ± 1.5^{a}	11.0 ± 0.7^{ab}	6.51 ± 0.55^{a}	5.86 ± 0.89^{a}	17.0 ± 1.0^{a}	18.3 ± 1.1^{b}	5.93 ± 1.17^{a}	5.84 ± 1.73^{a}	42.4 ± 1.7^{a}	41.0 ± 3.0^{b}
30	60 ± 3^{a}	57 ± 1^{a}	12 ± 1^{a}	10 ± 1^{a}	11.8 ± 1.6^{a}	10.6 ± 0.5^{a}	6.50 ± 1.06^{a}	5.49 ± 0.73^{a}	20.5 ± 2.4^{b}	17.3 ± 1.2^{b}	6.39 ± 0.87^{b}	4.51 ± 0.97^{a}	45.2 ± 5.0^{a}	37.9 ± 2.3^{ab}
60	61 ± 3^{a}	59 ± 4^{ab}	12 ± 2^{a}	10 ± 1^{a}	12.2 ± 0.6^{a}	12.0 ± 1.1^{b}	6.49 ± 0.57^{a}	5.00 ± 0.49^{a}	18.7 ± 2.1^{ab}	14.5 ± 1.4^{a}	4.33 ± 0.66^{a}	4.18 ± 0.57^{a}	41.7 ± 2.3^{a}	35.7 ± 0.8^{a}
Analysis of variance for Cd levels														
0	ns		*		*		ns		ns		ns		ns	
30	ns		ns		ns		ns		ns		*		*	
60	ns		ns		ns		**		*		ns		**	
S × Cd	ns		ns		ns		ns		**		ns		ns	

[a]Straw = top 3 leaves + old leaves + stem. Values are mean ± standard deviations. Values followed by different lower case letters within a column indicate significances at $P < 0.05$ (least significant difference) for S application levels. **$P < 0.01$, *$P < 0.05$ indicate the difference between −Cd and +Cd treatments for the same S level; ns – non-significant difference

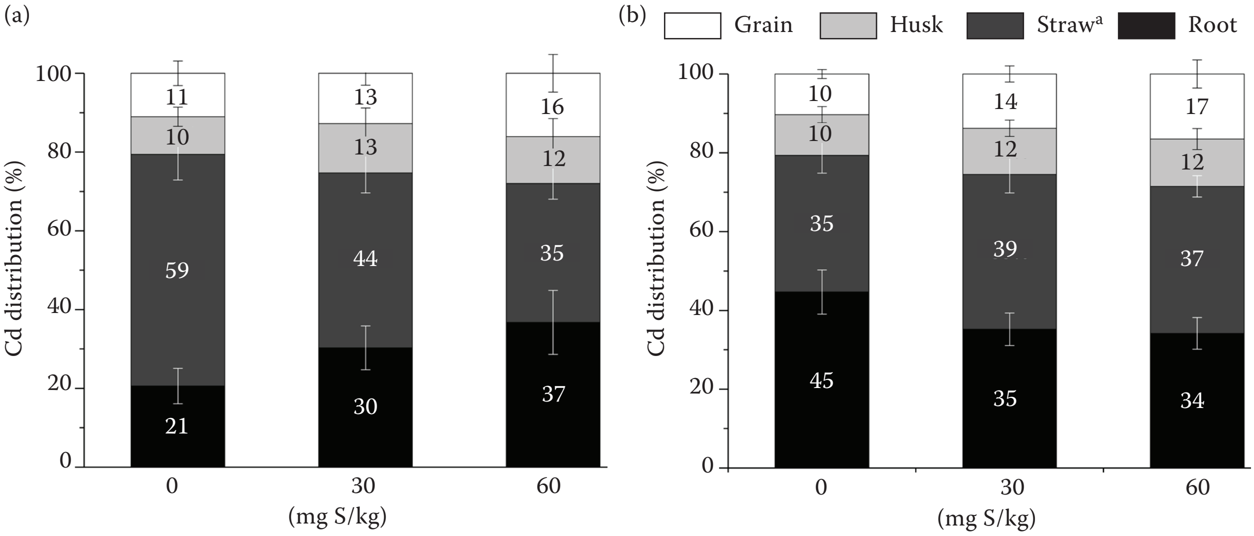

Figure 1. Effect of sulfur (S) application on the ratio of cadmium (Cd) distribution in plant tissues of wheat grown in paddy soil (a) without and (b) with addition of 10 mg Cd/kg. [a]Straw = top 3 leaves + old leaves + stem. Values demonstrate the mean ratio of Cd distribution in plant tissues. Error bars present standard deviations (n = 4)

of aboveground tissues with an exception for grain in –Cd treatments compared with non-S addition treatment, while tended to increase Cd transfer factors of aboveground tissues in +Cd treatments (Table 2).

Plant available Cd and S in soils. Generally, DTPA-extractable Cd in soils significantly ($P < 0.001$) increased by Cd addition, and it was slightly higher in rhizosphere soils than in bulk soils (Table 3). In addition, $Ca(H_2PO_4)_2$-extractable S in soil increased linearly ($P < 0.001$) with the increase of S application irrespective of Cd addition (Table 3).

DISCUSSION

In the present study, S and Cd application positively influenced Cd accumulation in wheat grains. In –Cd treatments, concentrations, and allocations of Cd in grains and roots tended to increase with S application (Table 4, Figure 1), as also seen from the decreasing Cd transfer factors from roots to aboveground tissues with increasing S application (Table 3). The results indicate that by wheat was cultivation in low Cd-contaminated paddy soils, S application

Table 2. Effect of sulfur (S) application on cadmium (Cd) transfer factors in above-ground tissues of wheat grown in paddy soil with and without the addition of 10 mg Cd/kg

S level (mg/kg)	Grain		Husk		Top 3 leaves		Old leaves		Stem	
	–Cd	+Cd	–Cd	+Cd	–Cd	+Cd	–Cd	+Cd	–Cd	+Cd
0	0.25 ± 0.09^a	0.13 ± 0.07^a	0.44 ± 0.16^b	0.23 ± 0.07^a	0.82 ± 0.36^b	0.13 ± 0.05^a	1.59 ± 0.86^b	0.29 ± 0.14^a	0.70 ± 0.20^b	0.25 ± 0.09^a
30	0.24 ± 0.09^a	0.17 ± 0.04^a	0.41 ± 0.14^{ab}	0.27 ± 0.06^a	0.28 ± 0.13^a	0.14 ± 0.03^a	0.52 ± 0.19^a	0.25 ± 0.07^a	0.50 ± 0.25^{ab}	0.34 ± 0.10^a
60	0.16 ± 0.05^a	0.17 ± 0.05^a	0.22 ± 0.08^a	0.30 ± 0.08^a	0.15 ± 0.06^a	0.19 ± 0.08^a	0.15 ± 0.04^a	0.32 ± 0.03^a	0.29 ± 0.06^a	0.33 ± 0.07^a
Analysis of variance for Cd levels										
0	ns		ns		**		*		**	
30	ns		ns		ns		*		ns	
60	ns		ns		ns		***		ns	
S × Cd	ns		*		***		**		*	

Values are mean ± standard deviations. Values followed by different lower case letters within a column indicate significances at $P < 0.05$ (least significant difference) for S application levels. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$ indicate difference between –Cd and +Cd treatments at for the same S level; ns – non-significant difference

Table 3. Effect of sulfur (S) application on DTPA-extractable cadmium (Cd) and $Ca(H_2PO_4)_2$-extractable S (mg/kg) in the rhizosphere and bulk soils of wheat grown in paddy soil with and without the addition of 10 mg Cd/kg

	S level (mg/kg)	DTPA-extractable Cd		$Ca(H_2PO_4)_2$-S	
		−Cd	+Cd	−Cd	+Cd
Rhizosphere soils	0	0.10 ± 0.04	7.61 ± 0.35	20.6 ± 3.3	19.7 ± 5.8
	30	0.12 ± 0.02	8.03 ± 0.68	47.9 ± 22.8	39.9 ± 1.9
	60	0.15 ± 0.09	8.51 ± 0.35	74.5 ± 21.8	53.8 ± 18.8
Bulk soils	0	0.12 ± 0.03	7.66 ± 0.50	21.0 ± 2.7	17.7 ± 0.7
	30	0.12 ± 0.06	7.17 ± 0.71	44.0 ± 18.3	29.6 ± 1.6
	60	0.12 ± 0.07	7.09 ± 0.77	65.5 ± 9.8	80.4 ± 20.3

Analysis of variance

	DTPA-extractable Cd	$Ca(H_2PO_4)_2$-S
S	ns	***
Cd	***	ns
Distance (D)	**	ns
S × Cd	ns	ns
S × D	*	ns
Cd × D	**	ns
S × Cd × D	ns	ns

Values are mean ± standard deviations. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns – non-significant difference

reduced Cd translocation to aboveground tissues and thus caused higher root Cd contents. This agrees with the results reported by Khan et al. (2015) and Adhikari et al. (2018), who demonstrated that the production of glutathione and phytochelatins was stimulated by increasing S supply. The phytochelatins in wheat roots may restrain Cd and limit its translocation from root to shoot (Khan et al. 2007). The tendency of S facilitated Cd uptake and accumulation in roots is also consistent with results observed by McLaughlin et al. (1998), for Swiss chard. The higher Cd uptake might be due to the increase of Cd phytoavailability in rhizosphere soil when sulfate is added (Table 3). Cadmium solubility in soil solution was observed to increase due to Cd-sulfate complexation by sulfate addition (McLaughlin et al. 1998). Furthermore, Cd uptake by *Zea mays* L. was

Table 4. Effect of sulfur (S) application on concentrations (mg/kg) of cadmium (Cd) in tissues of wheat grown in paddy soil with and without the addition of 10 mg Cd/kg

S level (mg/kg)	Grain		Husk		Top 3 leaves		Old leaves		Stem		Root	
	−Cd	+Cd	−Cd	+Cd	−Cd	+Cd	−Cd	+Cd	−Cd	+Cd	−Cd	+Cd
0	0.20 ± 0.05^{a}	5.78 ± 0.30^{a}	0.34 ± 0.08^{a}	10.9 ± 1.6^{a}	0.64 ± 0.21^{b}	6.16 ± 0.46^{a}	1.21 ± 0.39^{b}	13.2 ± 1.4^{a}	0.56 ± 0.17^{a}	11.5 ± 1.1^{a}	0.91 ± 0.58^{a}	51.2 ± 17.1^{a}
30	0.23 ± 0.06^{a}	7.94 ± 0.89^{b}	0.40 ± 0.08^{a}	13.0 ± 2.1^{a}	0.26 ± 0.07^{a}	6.69 ± 1.80^{a}	0.53 ± 0.24^{a}	11.9 ± 2.3^{a}	0.47 ± 0.10^{a}	16.1 ± 2.1^{b}	1.05 ± 0.35^{a}	49.2 ± 11.4^{a}
60	0.27 ± 0.08^{a}	6.55 ± 0.47^{a}	0.37 ± 0.10^{a}	11.5 ± 0.7^{a}	0.26 ± 0.10^{a}	7.38 ± 1.23^{a}	0.26 ± 0.06^{a}	13.3 ± 3.9^{a}	0.50 ± 0.08^{a}	12.9 ± 1.9^{a}	1.76 ± 0.25^{b}	40.9 ± 11.3^{a}

Analysis of variance for Cd levels

	Grain	Husk	Top 3 leaves	Old leaves	Stem	Root
0	***	***	***	***	***	***
30	***	***	***	***	***	***
60	***	***	***	***	***	***
S × Cd	***	ns	ns	ns	**	ns

Values are mean ± standard deviations. Values followed by different lower case letters within a column indicate significances at $P < 0.05$ (least significant difference) for S application levels. ***$P < 0.001$, **$P < 0.01$ indicate the difference between −Cd and +Cd treatments at for the same S levels; ns – non-significant difference

Table 5. The Pearson correlation coefficients between concentrations of plant-available cadmium (Cd) in soils and concentrations of Cd in plant tissues and among itself

		Concentrations of Cd in wheat plant tissues					
		grain	husk	top 3 leaves	old leaves	stem	root
Concentrations of Cd in wheat plant tissues	husk	0.979*					
	top 3 leaves	0.945	0.929				
	old leaves	0.929	0.947	0.910			
	stem	0.970	0.961	0.955	0.914		
	root	0.895	0.948	0.964	0.926	0.922	
Concentrations of DTPA-Cd in soils	rhizosphere	0.981	0.978	0.959	0.960	0.963	0.914
	bulk soils	0.960	0.966	0.948	0.971	0.956	0.927

*All of the coefficients are significant at $P < 0.01$

enhanced by adding sulfate to the nutrient solution due to $CdSO_4$ complex (López-Chuken and Young 2010).

An opposite pattern was seen in +Cd treatments, where concentrations of Cd decreased in roots while Cd allocation in grains increased with increasing S application (Table 4, Figure 1). The results indicate that, when wheat was cultivated in highly contaminated paddy soil, S application tended to promote Cd transfer from root to aboveground tissues. This agrees with results reported by Zhao et al. (2003), who revealed that Cd concentrations of two spring wheat cultivars increased in shoots but tended to decrease in roots with the increase of K_2SO_4 fertiliser. There may be two reasons. Firstly, under high Cd stress, active oxygen species, e.g., superoxide anion (O_2^-), hydroxyl radicals ($^{\cdot}$OH), and H_2O_2 are formed in wheat plant tissues (Ranieri et al. 2005, Khan et al. 2007). The Cd-induced active oxygen species can harm cell membranes and increase their permeability (Astolfi et al. 2005, Lin et al. 2007). Consequently, small-molecular-weight Cd-S compounds, such as Cd-cysteine and Cd-glutathione, can easily load into phloem tissues from xylem, and finally, deposit in grains. Cadmium-phytochelatins in the root cell vacuoles may release and decompose again due to the solubilisation of vacuole membranes. As a result, the limiting effects of S application present in –Cd treatments disappeared under high Cd stress. Secondly, the decrease of Cd allocation in roots with increasing S application (Figure 1) may be attributed to a decrease of root DWs by S application in +Cd treatments (Table 1).

Irrespective of S levels, Cd uptake by wheat was significantly ($P < 0.001$) higher in +Cd treatments than in –Cd treatments (Table 4). Besides, significant positive correlations ($P < 0.01$) existed between DTPA-Cd in rhizosphere/bulk soils and Cd concentrations in plant tissues, as well as among tissues itself (Table 5). The results indicate that plant available Cd in paddy soils is the main source of Cd in wheat grains, which agree with results reported by Kikuchi et al. (2009). Our study clearly shows that S application could increase the accumulation of Cd in the grain of wheat, and hence, S fertilisation should be considered for winter wheat in Cd-polluted paddy soil. Some measures to reduce the bioavailability of Cd in the soil are recommended. For example, liming decreases the bioavailability of Cd in Cd-contaminated paddy soil (Zhu et al. 2016).

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