

# Effect of coated urea on cadmium accumulation in *Oryza sativa* L. grown in contaminated soil

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**Abstract** Experiments were conducted to determine the effects of three types of coated urea on the accumulation of cadmium (Cd) in rice (*Oryza sativa* L.) grown in contaminated soil. Pot-culture experiments were conducted in a greenhouse from July to November 2012 on the rice cultivar “Hua Hang Si Miao” in Guangzhou (China). The experimental design was completely randomized with four treatments and three replications. The treatments were control (CK) (N 0 mg/kg), prilled urea (PU) (N 200 mg/kg), polymer-coated urea (PCU) (N 200 mg/kg), and sulfur-coated urea (SCU) (N 200 mg/kg). Our results indicated that applications of PCU and SCU slightly increased the dry weight of rice grains. The application of SCU significantly decreased the CaCl<sub>2</sub> and toxicity characteristic leaching procedure (TCLP)-extractable Cd concentrations by 15.4 and 56.1 %, respectively. Sequential extractions showed that PCU and SCU applications led to a significant decrease in Cd in the exchangeable fraction and an increase in the

bound iron (Fe) and manganese (Mn) oxides fractions. Cd concentrations in grains treated with PCU were reduced by 11.7 %, whereas SCU significantly reduced Cd concentrations by 29.1 %. SCU reduced Cd transfer from the straws to the grain. Our results demonstrated that PCU and SCU may be effective in mitigating Cd accumulation in rice grown in acidic Cd-contaminated soil, especially in plants receiving SCU.

**Keywords** Contaminated soil · Coated urea · Heavy metal · Availability · Rice

## Introduction

Cadmium (Cd) is one of the most toxic heavy metals and is distributed in soil by natural processes and anthropogenic activities. Cd contamination in croplands has a great impact on human health, increasing dietary risks through its transfer from soil to crops, causing toxic Cd accumulation in human organs (Chaney et al. 2004). Rice (*Oryza sativa* L.) is a major staple crop widely cultivated in Asia and has a high Cd uptake in its grain (Gong and Pan 2006; Reeves and Chaney 2008; Römkens et al. 2009). Thus, Cd uptake by rice plants plays an important role in the transfer of this toxin through the food chain, posing significant health risks to humans (Liu et al. 2003; Zhao et al. 2015).

Nitrogen (N) is an important nutrient affecting rice production, and the Cd concentration in rice is correlated with the type and amount of N fertilizer used. Rice plants treated with nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium

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( $\text{NH}_4^+$ ) ions had the highest and lowest Cd concentrations in plant tissues, respectively (Jalloh et al. 2009). At identical nitrogen levels, ammonium chloride ( $\text{NH}_4\text{Cl}$ ) significantly increased the Cd concentration in rice grain concentration compared to urea [ $\text{CO}(\text{NH}_2)_2$ ], ammonium sulfate [ $(\text{NH}_4)_2\text{SO}_4$ ], and ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ). In addition, there were no significant differences in Cd concentrations in rice grains treated with urea, ammonium sulfate, and ammonium nitrate (Jia et al. 2010). The optimal rate of urea ( $0.2 \text{ g N kg}^{-1}$ ) significantly lowered Cd concentrations in rice grain, whereas the application of either zero N or high urea ( $0.4 \text{ g N kg}^{-1}$ ) significantly increased the Cd concentration (Jia et al. 2010). Increasing the N content also increased the Cd concentration in rice; significant differences in Cd concentrations occurred between medium ( $150 \text{ kg hm}^{-2}$ ) and high ( $225 \text{ kg hm}^{-2}$ ) nitrogen levels. To produce high-quality rice, a proper quantity of N fertilizer should be applied, and the rate should not exceed  $150 \text{ kg hm}^{-2}$  (Teng et al. 2011). Therefore, appropriate N management is important for effectively reducing Cd concentrations and increase rice yields.

Controlled release of N fertilizer reduces ammonia volatilization, leaching, and denitrification to suppress N loss and has become a hotspot of modern agricultural and environmental scientific research (Granta et al. 2012; Jantalia et al. 2012). However, there is little information regarding the growth response and Cd uptake in rice plants grown in soil contaminated with Cd using different controlled release N fertilizers. The objective of this study was to investigate these effects under flooding conditions in greenhouse pot-culture experiments.

## Materials and methods

### Experimental materials

Soil was collected from the horizon layer (0–20 cm) of a paddy field in Shangba Village, Xinjiang, located 6 km from the Dabao Mountain mining area in Shaoguan, Guangdong, China. The soil was air-dried, crushed, and sieved with 2-mm mesh. Soil properties are listed in Table 1.

Analytical grade prilled urea (PU; 46-0-0), potash, and phosphate fertilizers were used in the experiments. Polymer-coated urea (PCU; 42-0-0; including 8 % resin in total weight) and sulfur-coated urea (SCU; 34-0-0;

including 18 % sulfur and 1 % paraffin in total weight), with a controlled-release time of approximately 3 months, were obtained from Shandong Kingenta Ecological Engineering Group Co. Ltd. (Shandong, China).

Seeds of rice cultivar “Hua Hang Si Miao” were obtained from the College of Agriculture, South China Agricultural University (SCAU) (Guangzhou, China).

### Pot-culture experiment

Cylindrical plastic pots (26.9 cm in height, 18.4 cm bottom diameter, and 26.5 cm top diameter) were used in experiments conducted in a greenhouse at SCAU from July 19 to November 16, 2012. All pots were arranged in a completely randomized design with three replications and four treatments: control (CK) (N 0 mg/kg), PU (N 200 mg/kg), PCU (N 200 mg/kg), and SCU (N 200 mg/kg). In total, 5.0 kg of dried soil was placed in each pot. To each pot was applied potassium dihydrogen phosphate (1.438 g/pot) and potassium chloride (0.798 g/pot) equivalent to 65.5 and 166 mg/kg of P and K, respectively. Fertilizers were added to the soil as solid granules and were mixed thoroughly before watering-in with tap water to form a 3-cm layer above the soil surface. Pots were incubated for 2 days following fertilizer application.

Rice seeds were surface sterilized with 0.1 %  $\text{H}_2\text{O}_2$  for 30 min, rinsed thoroughly with tap water, soaked overnight in deionized water at room temperature, and germinated in moist sand in a greenhouse. At the three-leaf stage, seedlings were selected for uniformity and transplanted into the pots with six seedlings per pot. Tap water was added daily to maintain 3-cm submergence throughout the experimental period.

### Harvest and sampling

Rice shoots were harvested 120 days after transplanting. After harvesting, rice shoots were divided into two parts: straws and grains. Harvested plants were rinsed thoroughly with tap water followed by deionized water and oven-dried at  $105 \text{ }^\circ\text{C}$  for 30 min and then at  $60 \text{ }^\circ\text{C}$  until their weight remained constant, and the dry weights yield was recorded. After weighing, the grain samples were shelled and separated into grains (i.e., seeds) and husks (i.e., seed coverings). Dried tissue samples were ground with an electric steel mill, passed

**Table 1** Soil characteristics

Soil type	pH (H <sub>2</sub> O)	Organic matter (g/kg)	Total N (g/kg)	NH <sub>4</sub> <sup>+</sup> -N (mg/kg)	NO <sub>3</sub> <sup>-</sup> -N (mg/kg)	Olsen-P (mg/kg)	Water-soluble SO <sub>4</sub> <sup>2-</sup> (mg/kg)	CaCl <sub>2</sub> -extractable Cd (mg/kg)	TCLP-extractable Cd (mg/kg)	Total Cd (mg/kg)
Paddy soil	3.98	9.65	0.65	10.19	10.66	4.67	66.36	0.053	0026	0.652

through a 1-mm sieve, and homogenized prior to chemical analysis.

Soil from each pot was collected and air-dried at room temperature, treated to remove plant debris, ground with a wood grinder, and passed through a 1-mm nylon sieve. The sieved soil samples were collected, and most of them were stored in plastic bags to measure pH, ammonium N, nitrate N, water soluble sulfur (S), available phosphorus (P), and extractable Cd concentrations. The remaining parts were ground again, passed through a 0.149-mm nylon sieve, and stored in plastic bags until their Cd concentration was measured.

#### Sample analysis

This study was conducted under the condition of flooding with water. The soil was in a flooded condition when the rice was harvested; therefore, the moisture content was very high in the soil samples. Air-dried soil was used to assay NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> N concentrations to avoid the effect of high water content on the results and because previous researchers have used the same method (Rahmatullah et al. 2006; Barth et al. 2008).

Soil pH, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, total N, water SO<sub>4</sub><sup>2-</sup>, available P, and organic carbon were analyzed by method given by Lu (2000). Soil pH was measured using a pH meter (PHJB-260, Shanghai Jingmi Kexue Instrument Factory, Shanghai, China) in a 1:2.5 suspension of soil to water. Fifty milliliters of 2 M KCl solution was added to 5 g of dry soil and shaken for 1 h and then filtered; NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations in the liquid were determined using the indophenol blue method and ultraviolet spectrophotometry, respectively (DSH-UV755B UV-Vis Spectrophotometer; Guangzhou SH Biological Technology Co. Ltd., Guangzhou, China). Total N concentration of soil was determined using the Kjeldahl method with modifications. Soil SO<sub>4</sub><sup>2-</sup> concentrations in water were determined using the spectroscopic barium turbidity method after extraction with deionized water (DSH-UV755B UV-Vis Spectrophotometer; Guangzhou SH Biological Technology Co. Ltd.,

Guangzhou, China). Available P was extracted with 0.5 M NaHCO<sub>3</sub> at a 1:20 soil to solution ratio (*w:v*), shaken for 30 min, and then filtered. The quantity of available P was determined using Mo-Sb-Vc coloration reagents and the colorimetric method (DSH-UV755B UV-Vis Spectrophotometer; Guangzhou SH Biological Technology Co. Ltd., Guangzhou, China). Soil organic carbon was determined using the potassium dichromate oxidation (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O) spectrophotometric method (DSH-UV755B UV-Vis Spectrophotometer; Guangzhou SH Biological Technology Co. Ltd., Guangzhou, China). Available Cd was extracted with 0.1 M CaCl<sub>2</sub> (pH = 7.0) in 1:5 soil to solution ratio (*w:v*), shaken for 1 h, and then filtered (Xiao et al. 2008). Cd concentration was determined using a flame atomic absorption spectrophotometer (FAAS) (Z-2300; Hitachi Ltd., Tokyo, Japan). Toxicity characteristic leaching procedure (TCLP)-extractable Cd in soil was extracted using TCLP reagent (pH = 4.93) in a 1:20 soil to solution ratio (*w:v*), shaken for 18 h, and then filtered (Sun et al. 2005). Cd concentrations were determined using a graphite furnace atomic absorption spectrophotometer (GFAAS) (Z-2700; Hitachi Ltd., Tokyo, Japan). Total Cd in soil was digested with an acid mixture composed of hydrochloric (HCl), nitric (HNO<sub>3</sub>), and perchloric acids (HClO<sub>4</sub>) (*v:v:v<sub>4</sub>* = 15:5:1), and the total Cd was then determined by FAAS. The geochemical form of soil Cd was assessed using the method of Tessier et al. (1979). Cd concentrations in plant tissues were determined by FAAS after digesting the plant material in a heated mixture of concentrated HNO<sub>3</sub> and HClO<sub>4</sub> (*v:v* = 20:3) (Lu 2000).

#### Data analysis

All data were analyzed in SPSS version 16.0 (SPSS Corporation. Released 2008) using the ANOVA at a significance level of *p* < 0.05. Duncan's test was used to detect significant differences between treatment means. Bivariate correlation was used in this study,

and the Pearson correlation coefficients (at  $p < 0.05$  level) were measured simultaneously.

## Results

### Rice biomass

Three treatments (PU, PCU, and SCU) significantly increased the dry weight of rice straws and grains (Fig. 1). The dry weight of grains was smaller when treated with PU than with PCU and SCU, whereas the dry weight of straws significantly increased.

### Cd concentrations in rice straws and grains

The PU treatment significantly reduced Cd concentrations in grains ( $p < 0.05$ ) (Fig. 1). The PCU treatment significantly reduced the Cd concentrations in grains, but increased the Cd concentrations in straws ( $p < 0.05$ ) (Fig. 1). Cd concentrations in straws significantly increased in the SCU treatment, whereas the Cd concentrations in grains significantly decreased ( $p < 0.05$ ) (Fig. 1). Compared with PU, there was no significant difference in Cd concentrations between straws and grains in the PCU treatment (Fig. 1). As compared to PU, Cd concentrations in straws significantly increased in the SCU treatment and significantly decreased in grains ( $p < 0.05$ ) (Fig. 1). Furthermore, in the control and PU treatments, Cd concentrations in grains exceeded the National Food Safety Standard – Maximum Levels of Contaminants in Food (ML) (the ML of Cd is  $0.2 \text{ mg kg}^{-1}$ ) (Ministry of Health of the

People's Republic of China's 2012). Grains treated with PCU and SCU met the safety standards.

Soil pH, water-soluble  $\text{SO}_4^{2-}$ ,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$

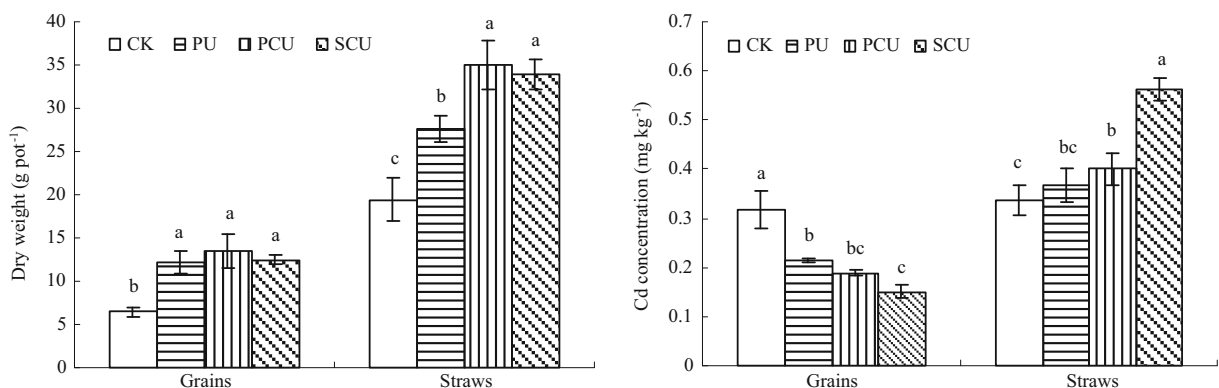
Soil pH values of the PU, PCU, and SCU treatments at harvest were not significantly different from the CK treatment. Soil pH values were significantly higher in the PCU treatment than in the SCU treatment (Table 2). The addition of PU and PCU significantly increased water-soluble  $\text{SO}_4^{2-}$  concentrations in soil (Table 2). Compared with PU, water-soluble  $\text{SO}_4^{2-}$  concentrations in soil significantly decreased following treatment with SCU. Ammonium ion concentrations in soil significantly increased after the addition of different types of coated ureas, and  $\text{NH}_4^+$  concentrations in PU and PCU were higher than that in the SCU treatment. Nitrate ion concentrations were significantly higher in the PU treatment than in the CK treatment (Table 2).

### Soil Cd mobility

Treatment with PU significantly increased soil  $\text{CaCl}_2$  and TCLP-extractable Cd, whereas treatment with SCU significantly reduced soil TCLP-extractable Cd. Compared to the PU treatment, significant reductions of  $\text{CaCl}_2$  and TCLP-extractable Cd were observed in the SCU treatment (Fig. 2).

### Sequential chemical extraction of soil Cd

Most Cd was present in the residual fraction (72.46–74.88 % of the total concentration) (Fig. 3). Fraction distributions of Cd were significantly different among



**Fig. 1** Rice biomass and Cd concentrations of grains and straws. Data are presented as the mean  $\pm$  standard error. Mean values followed by the same letter are not significantly different (Duncan's multiple range test at  $p = 0.05$ )

**Table 2** Soil pH, water-soluble  $\text{SO}_4^{2-}$ ,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$  values at harvest under different coated urea treatments

Treatment	pH	Water-soluble $\text{SO}_4^{2-}$ (mg/kg)	$\text{NH}_4^+$ -N (mg/kg)	$\text{NO}_3^-$ -N (mg/kg)
CK	3.73 ± 0.03 ab	153.3 ± 27.9 c	11.70 ± 2.45 c	14.51 ± 3.11 b
PU	3.77 ± 0.07 ab	209.6 ± 6.0 a	59.44 ± 7.96 a	22.04 ± 5.67 a
PCU	3.81 ± 0.07 a	185.7 ± 3.2 ab	62.09 ± 10.75 a	16.51 ± 2.32 ab
SCU	3.69 ± 0.02 b	175.8 ± 8.4 bc	29.29 ± 4.91 b	16.17 ± 0.46 ab

Values are mean ± standard deviations. Values followed by different letter(s) within a column are significantly different at  $p = 0.05$  (Duncan’s multiple range test)

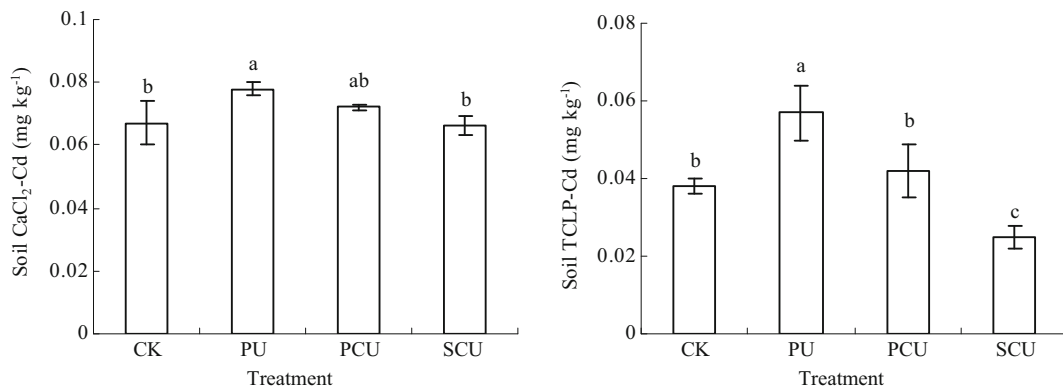
treatments, including the exchangeable fraction and carbonate-bound and iron (Fe) and manganese (Mn) oxide-bound fractions ( $p < 0.05$ ). The percentage of Cd in the exchangeable fraction significantly decreased from 10.06 in the CK treatment to 7.92 in the SCU treatment. The carbonate-bound Cd percentage significantly increased from 1.94 in the CK treatment to 3.66, 3.13, and 4.21 in the PU, PCU, and SCU treatments, respectively, representing a decrease in bioavailability. The Cd concentration in the Fe and Mn oxide-bound fractions significantly decreased from  $48.0 \mu\text{g kg}^{-1}$  in the CK treatment to  $40.0 \mu\text{g kg}^{-1}$  in the PU treatment. The Cd concentration in the exchangeable fraction significantly decreased from  $72.0 \mu\text{g kg}^{-1}$  in the PU treatment to  $49.0 \mu\text{g kg}^{-1}$  in the SCU treatment (Fig. 3). The observed differences in Cd distribution between the fractions in PU- and SCU-treated soils highlight the implications for the mobility and/or bioavailability of Cd when S is applied to the soil.

There was a significant and positive correlation between  $\text{CaCl}_2$ -extractable and TCLP-extractable Cd in soil ( $r = 0.733, p < 0.05$ ). There was a significant and negative correlation between TCLP-extractable Cd and Cd in straw ( $r = -0.616, p < 0.05$ ), but there was no

significant correlation between Cd in grain and TCLP-extractable Cd in soil. Therefore, S-induced reductions of TCLP-extractable Cd in soil did not explain the effect of the S supply on Cd uptake by grains. However, there was a significant negative correlation between Cd in grains and straws ( $r = -0.729, p < 0.05$ ).

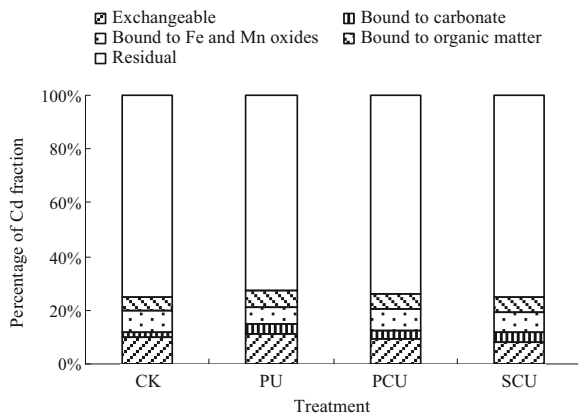
**Discussion**

The application of SCU significantly decreased exchangeable and TCLP-extractable Cd concentrations in soil (Figs. 2 and 3). The application of S resulted in CdS formation and precipitation due to soil that have been waterlogged for 160 days, which subsequently decreased  $\text{Cd}^{2+}$  availability (Chen and Zheng 1996; Yi et al. 1996; Kashem and Singh 2001). Dissolved Cd decreased during sulfate reduction, and Cd was transformed into a poorly soluble phase identified as Cd-sulfide using Cd K-edge X-ray absorption spectroscopy (XAS) (Fulda et al. 2013). The greater negative charge imparted to soil surfaces through  $\text{SO}_4^{2-}$  adsorption may increase Cd retention and reduce solution concentrations of Cd (McLaughlin et al. 1998). In our experiment,



**Fig. 2** Level of  $\text{CaCl}_2$ -extractable Cd and TCLP-extractable Cd in soil at harvest under different coated urea treatments. Data are presented as the mean ± standard error. Mean values followed by the same letter are not significantly different (Duncan’s multiple range test at  $p = 0.05$ )





**Fig. 3** Sequential chemical extraction of Cd in soil

the water-soluble  $\text{SO}_4^{2-}$  concentration of soil decreased significantly following treatment with SCU, and there was a positive correlation between TCLP-Cd and water-soluble  $\text{SO}_4^{2-}$  ( $r = 0.558$ ).

As compared to PU, a significant reduction in TCLP-extractable Cd was observed in the PCU treatment (Fig. 2). The main component of PCU is polyacrylates, forming lightly cross-linked networks. This highly water-absorptive resin is composed of water and the electrolyte ions, and its meshes can mechanically absorb heavy metal ions, molecules, and particulates. In addition, the surface of polyacrylate moleculars and associated chain breakage releases negatively charged carboxyl groups, increasing the negative charge in the soil, enhancing cation exchange capacity, and adsorbing heavy metal ions (Qu and Yi 2006).

Treatment with SCU significantly decreased Cd concentrations in grains as compared with PU (Fig. 1). Hu et al. (2011) and Wang et al. (2015) also found that S in particular reduced Cd concentration in rice grains. In addition, the S supply had different effects on Cd accumulation in different parts of rice plants (An et al. 2004; Zhang et al. 2013). An et al. (2004) observed that increasing the S supply (2, 6, and 12 mmol  $\text{SO}_4^{2-} \text{L}^{-1}$ ) considerably enhanced Cd accumulation in rice leaves due to sulfate-induced increases of non-protein thiol content in seedlings, but decreased Cd accumulation in rice stems. The S supply reduced Cd uptake (20.4 %) in rice roots and increased in shoots (13.0 %) after 20 days (Zhang et al. 2013). The present study showed that treatment with SCU significantly reduced the Cd concentration in grains, but increased the Cd concentration in straws (Fig. 1). In Cd-treated rice, the addition of S significantly decreased Cd concentrations in grains, but

tended to increase Cd concentrations in roots and leaves (Fan et al. 2010). The S supply had an important influence on Cd accumulation in different parts of rice plants.

Cd uptake and accumulation in grains decreased due to the S supply. This result may be regulated by the following mechanism. Firstly, the S supply decreased Cd mobility and/or availability as discussed above (Chen and Zheng 1996; Yi et al. 1996; Kashem and Singh 2001; Fulda et al. 2013). Secondly, adding S significantly increased Cd distribution percentage in cell walls of straw cells and decreased the Cd distribution percentage in the soluble fraction (Pan et al. 2015). The subcellular distribution of Cd with the addition of S may be one of the important mechanisms regulating exogenous S-Cd transport from straws to grains. Thirdly, the S supply increased glutathione in rice leaves (Fan et al. 2010). These results indicate that S could prevent Cd transfer from straws to grains in a certain extent, similar to the results of Hu et al. (2011) and Wang et al. (2015). This suggests that straws may be the main storage organs or filters of Cd in rice. Taking specific retention measures leads to Cd precipitating in straws, effectively reducing the transfer and accumulation of Cd in rice grains (Table 3).

## Conclusions

The application of PCU and SCU effectively reduced the mobility and bioavailability of Cd in Cd-contaminated acidic soil and greatly reduced Cd concentrations in rice grains, especially in plants receiving the SCU treatment. Application of SCU significantly decreased Cd concentration in rice grains by decreasing Cd availability and transferring from straws to grains. Future research must focus on the effects of three types of coated urea on the accumulation of Cd in rice grown

**Table 3** Correlations among soil Cd concentrations in straws and grains and  $\text{CaCl}_2$ -extractable Cd and TCLP-extractable Cd

	$\text{CaCl}_2$ -Cd	TCLP-Cd	Cd in straw	Cd in grain
$\text{CaCl}_2$ -Cd	1	0.733*	-0.439	0.003
TCLP-Cd		1	-0.616*	0.212
Cd in straw			1	-0.729*
Cd in brown rice				1

\*significant at  $p < 0.01$

in contaminated soil in a range of soil types and climatic conditions.

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