



# Sulfur application combined with water management enhances phytoextraction rate and decreases rice cadmium uptake in a *Sedum plumbizincicola* - *Oryza sativa* rotation

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## Abstract

**Background and aims** Soil cadmium (Cd) contamination threatens food safety and human health. Increasing Cd phytoextraction efficiency by hyperaccumulators and growing safe products during remediation remain challenges.

**Methods** A pot experiment was conducted to explore the effects of different rates of sulfur (S) associated with alternating drying and wetting on a *Sedum plumbizincicola*-*Oryza sativa* rotation in Cd-contaminated neutral and calcareous soils.

**Results** The oxidation of added S under aerobic conditions significantly decreased soil solution pH and increased soluble sulfate ( $\text{SO}_4^{2-}$ ), Cd and iron (Fe) concentrations in both soils. During the rice growing season the soil solution redox (Eh) decreased to  $< -200$  mV and the solution pH increased to neutral during the first few days of flooding. Soluble  $\text{SO}_4^{2-}$  and Cd in the S treatments decreased significantly with increasing duration of flooding. Sulfur addition promoted shoot Cd concentrations of *S. plumbizincicola* by 1.7–5.5 times on

calcareous soil and 1.7–2.3 times on neutral soil compared to the controls. Rice yields increased but Cd concentrations decreased at suitable S addition rates.

**Conclusions** Appropriate sulfur amendment combined with water management can be a feasible strategy to enhance the Cd remediation efficiency of the hyperaccumulator and reduce the accumulation of Cd in the rice grains in this rotation.

**Keywords** Cadmium contamination · *Oryza sativa* · Phytoextraction · *Sedum plumbizincicola* · Sulfur · Water management

## Introduction

Elevated cadmium (Cd) concentrations in Chinese arable soils due to industrial and agricultural activities represent an important threat to human and animal health (Teng et al. 2014; Chen et al. 2015; Xue et al. 2017). Many Cd-polluted soils urgently require remediation. A range of options have been proposed including the identification of the contaminant sources in agricultural systems, minimization of contaminant inputs, reduction of the phytoavailability of potentially toxic elements in soils by liming or the application of other metal immobilizing materials, selection and breeding of crop cultivars with low metal accumulation ability, adoption of appropriate water and fertilizer management practices, bioremediation, and changes in land use to grow non-food crops (Zhao et al. 2015). One technique, phytoextraction using hyperaccumulators, is an in-situ,

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environmental friendly and low-cost approach to the remediation of metal contaminated agricultural soils (Van der Ent et al. 2013). Rotation or intercropping of hyperaccumulators with crops is now widely used to remove metals from contaminated soils and continue agricultural production (Yang et al. 2017; Zhan et al. 2016; Deng et al. 2016). However, technologies for increasing metal phytoextraction efficiency and minimizing remediation time need to be developed (Li et al. 2018). Growing safe products during the period of remediation by hyperaccumulators remains a major challenge (Wan et al. 2016; Shen et al. 2010).

Synthetic or natural metal chelating agents such as EDTA (ethylene diamine tetraacetic acid), EDDS (ethylene diamine disuccinic acid), and citric acid are often used to increase phytoextraction efficiency (Anwar et al. 2017; Freitas et al. 2013; Attinti et al. 2017). Acidifying compounds such as elemental sulfur (S) have been proposed to enhance metal uptake by phytoextraction crops grown in polluted soils (Kayser et al. 2000; Wang et al. 2006; Iqbal et al. 2012). Sulfur is an essential element to all living organisms because it forms part of important life sustaining molecules such as cysteine, thioredoxin metallothionein, enzymes and vitamins (Hell 1997; Saito 2000). These compounds can increase plant tolerance to metals through complexation and/or sequestration into vacuoles (de Oliveira et al. 2014). It is well known that sulfur changes its valence state due to moisture-driven soil oxidation status. In aerobic conditions S application can decrease soil pH because the oxidation of S to sulfate ( $\text{SO}_4^{2-}$ ) by sulfur-oxidizing bacteria or chemical process releases protons ( $\text{H}^+$ ) (Cui et al. 2004). After sulfur application the soil pH decreases significantly, thus increasing the solubility of soil Cd and plant Cd accumulation (Wang et al. 2007). In anaerobic environments,  $\text{HS}^-$  and  $\text{S}^{2-}$  produced by sulfate reduction can precipitate with thiophilic elements (Cd, Cu, Fe) or co-precipitate with FeS (Khaokaew et al. 2011; Hu et al. 2013; Fulda et al. 2013; Hashimoto et al. 2016). However, there is little information on the effects of S on Cd solubility in drying-wetting rotation systems.

*Sedum plumbizincicola*, a native Cd hyperaccumulator found in east China, has shown a considerable capacity to extract Cd from soils (Wu et al. 2013; Hu et al. 2015; Li et al. 2014b). Several modes have been developed using this plant for remediation practice, for example rotation with rice and intercropping with maize or aubergine (Deng et al. 2016). A recent study shows that the optimum pH for

phytoextraction with *S. plumbizincicola* was ~5.5 because of high Cd bioavailability (Wu et al. 2018). However, the bioavailability of Cd in neutral and calcareous soils is usually poor, and phytoextraction takes longer times (Li et al. 2014a, 2016; Wu et al. 2018). It is therefore necessary to develop techniques to increase the phytoextraction efficiency of *S. plumbizincicola* and to inhibit Cd accumulation in associated rice.

In the present study a pot experiment was conducted to explore the effects of different addition rates of S associated with drying-wetting rotation between *S. plumbizincicola* and rice in Cd-contaminated neutral and calcareous soils. We hypothesized that S oxidation in aerobic conditions decreases soil pH and increases Cd bioavailability and thus increases Cd phytoextraction by *S. plumbizincicola*. Furthermore, sulfate reduction in flooded conditions can precipitate Cd and restrict Cd accumulation by rice. The aim was to develop a rotation system that would optimize phytoextraction and produce safe rice on Cd-contaminated agricultural soils.

## Materials and methods

### Soil characterization

Two test soils were collected from the top 15 cm of the soil profile of agricultural fields. One is classified as a Calcaric Purpli-Orthic Primosols from Lanping County, Yunnan Province (designated L-soil) with a pH of 8.0 and a total Cd concentration of 4.44 mg kg<sup>-1</sup>. The other is classified as a Typic Hapli-Stagnic Anthrosols from Taicang City, Jiangsu Province (designated T-soil) with pH 6.5 and total Cd 1.33 mg kg<sup>-1</sup>. Selected physico-chemical properties of the test soils are listed in Table 1.

### Design of pot experiment

A glasshouse pot experiment was conducted with two consecutive crops comprising *S. plumbizincicola* (days 0 to 90) followed by rice (days 120 to 210). There were five rates of S addition, namely controls (L0 and T0), 0.5 g kg<sup>-1</sup> (L0.5 and T0.5), 1.0 g kg<sup>-1</sup> (L1 and T1), 2.0 g kg<sup>-1</sup> (L2 and T2) and 4.0 g kg<sup>-1</sup> (L4 and T4). Sulfur was added to the soils before *S. plumbizincicola* was transplanted. Four cuttings of *S. plumbizincicola* were transplanted into each 15-cm-diameter plastic pot containing 1.5 kg (oven dry basis) of 2-mm sieved air dry soil. There were four replicate pots of each treatment

**Table 1** Selected physicochemical properties of the two test soils

Soil	L-Soil	T-Soil
pH	8.0	6.5
Organic matter (g kg <sup>-1</sup> )	56.8	51.5
Clay (%)	8.64	12.8
Available N (mg kg <sup>-1</sup> )	167	134
Available P (mg kg <sup>-1</sup> )	116	77.5
Available K (mg kg <sup>-1</sup> )	203	170
Available S (mg kg <sup>-1</sup> )	23.2	40.4
Total Cd (mg kg <sup>-1</sup> )	4.44	1.33
Total Zn (mg kg <sup>-1</sup> )	331	137
Total Fe (g kg <sup>-1</sup> )	18.8	25.2
Total Mn (g kg <sup>-1</sup> )	0.37	0.32

giving a total of 40 pots. Fertilizers were applied at rates of 0.3 g CO(NH<sub>2</sub>)<sub>2</sub> and 0.3 g KH<sub>2</sub>PO<sub>4</sub> kg<sup>-1</sup> soil 30 days after *S. plumbizincicola* was transplanted. Deionized water was added daily to maintain soil moisture at about 60% of soil water holding capacity (WHC) during the *S. plumbizincicola* growth period. Shoots of the hyperaccumulator were harvested after growth for 90 days. The remaining soil was used for the subsequent rice crop (*Oryza sativa* cv. Xiangzaoxian 24) which grew from days 120 to 210. The soil in each pot was mixed with basal fertilizer composed of 0.4 g CO(NH<sub>2</sub>)<sub>2</sub> kg<sup>-1</sup> and 0.4 g KH<sub>2</sub>PO<sub>4</sub> kg<sup>-1</sup>. Then 0.6 g CO(NH<sub>2</sub>)<sub>2</sub> and 0.6 g KH<sub>2</sub>PO<sub>4</sub> kg<sup>-1</sup> were top dressed at the tillering and grain filling stages, respectively. A water layer about 4 cm deep above the soil surface was maintained during the rice growing period. The pots were fully randomized and then re-randomized once a month.

One Rhizon soil moisture sampler (Rhizon-MOM, Wageningen Research Products, Wageningen, The Netherlands) was installed in the middle of the soil in each pot at an angle of 45° during the growth of both plant species. Soil solution samples of ~15 ml were extracted from each pot on days 1, 10, 20, 30, 50, 70, 90, 121, 125, 150, 175 and 200. The soil water content was adjusted to 80% of the WHC with deionized water 12 h before sampling during the *S. plumbizincicola* growing period.

At harvest, *S. plumbizincicola* shoots and rice grains were collected and washed with running tap water and rinsed twice with deionized water, oven dried at 75 °C, weighed, and ground. Fresh soil samples were collected at the same time and immediately used for the determination of soil extractable Cd.

## Chemical analysis

Soil pH, organic matter content, texture, and available nitrogen, available phosphorus and available potassium contents were determined following the methods of Lu (2000). Soil available sulfur was extracted with Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> and determined by ion chromatography (ICS3000, Thermo Dionex, Sunnyvale, CA). Soil total Cd, Zn, Fe and Mn concentrations were determined by digestion of 0.20-g samples with 5 mL HCl and 5 mL HNO<sub>3</sub>. Available Cd in fresh soil samples was extracted with 0.01 M CaCl<sub>2</sub> at a soil:water ratio of 1:10 (w/v). Plant samples (0.20 g) were digested with 6 mL HNO<sub>3</sub> and 2 mL H<sub>2</sub>O<sub>2</sub>. Metal concentrations in the digest solutions and extract solutions were determined by inductively coupled plasma-mass spectrometry (ICP-MS, Ultramass, Varian, Palo Alto, CA). Plant S concentrations were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin Elmer Optima 8000, Waltham, MA). Replicates, blanks and a certified reference material (GBW07406) were included for analytical quality control.

The pH and Eh values of soil solutions were determined immediately after sampling. Soil solutions were acidified (1% (v/v) of concentrated HNO<sub>3</sub>) and stored at -20 °C for metal and SO<sub>4</sub><sup>2-</sup> determination (Fulda et al. 2013). Metal concentrations in solutions were determined by ICP-MS or ICP-OES and SO<sub>4</sub><sup>2-</sup> concentration was determined by ion chromatography as described above.

## Statistical analysis

All data are expressed as mean ± standard error (SE). The data were tested by one-way analysis of variance and mean values were compared by least significant difference (LSD) at the 5% protection level. Data processing was conducted using Microsoft Excel 2013 and the SPSS 21.0 for Windows software package (SPSS Inc., Chicago, IL).

## Results

Soil solution pH, Eh and dissolved SO<sub>4</sub><sup>2-</sup> at different plant growth stages

The dynamics of pH, Eh, SO<sub>4</sub><sup>2-</sup>, Cd, Fe, and Mn in the soil solution during aerobic (the first 90 days, *S. plumbizincicola*) and anaerobic (days 120–210, rice)

culture under the different rates of S amendment are shown in Fig. 1. In the L-soil control treatment solution pH was maintained between 6.6 and 7.6 in the early part of the aerobic stage but showed a decreasing trend after 30 d. Soil solution pH increased and then remained between 7.5 and 8.2 during the flooded stage and similar trends occurred in the T-soil. Sulfur treatments led to lower pH than in the control in both soils. A rapid decrease in soil solution pH occurred during the first 30 days. On day 30 the solution pH in L2 (5.68), L4 (5.03), T2 (3.78) and T4 (3.23) decreased by 1.82, 2.47, 2.62 and 3.17 units compared with the controls L0 (7.50) and T0 (6.40), respectively. On day 90 the solution pH of L2 (4.63), L4 (3.98), T2 (3.19) and T4 (2.77) decreased by 1.95, 2.60, 2.09 and 2.81 units compared with the controls L0 (6.58) and T0 (5.58), respectively. During the growth of the rice the soil solution pH changed from acid to neutral with the exception of treatments L4, T2 and T4.

Soil solution Eh of all treatments was between 200 mV and 400 mV during the growth of *S. plumbizincicola* and decreased to  $-200$  mV five days after flooding and then remained between  $-200$  mV and  $-300$  mV until the rice harvest. Dissolved  $\text{SO}_4^{2-}$  in the controls was maintained at  $50.8\text{--}103$   $\text{mg L}^{-1}$  in the L-soil and  $83.1\text{--}189$   $\text{mg L}^{-1}$  in the T-soil during the *S. plumbizincicola* growth period. Dissolved  $\text{SO}_4^{2-}$  in the S treatments increased significantly within the first 30 days in both soils. Dissolved  $\text{SO}_4^{2-}$  reached a maximum of  $1151$   $\text{mg L}^{-1}$  after 50 days in the T4 treatment. On day 90 the concentrations of  $\text{SO}_4^{2-}$  were 461 and 646  $\text{mg L}^{-1}$  in treatments L2 and L4, respectively, values 4.48 and 6.27 times that of the control L0 ( $103$   $\text{mg L}^{-1}$ ). The  $\text{SO}_4^{2-}$  of treatments T2 and T4 were 445 and 876  $\text{mg L}^{-1}$ , respectively, 2.35 times and 4.63 times that of the control T0 ( $189$   $\text{mg L}^{-1}$ ). After one day of flooding (day 121) the soil solution  $\text{SO}_4^{2-}$  concentrations in all treatments increased above those on day 90. During flooded rice growth, dissolved  $\text{SO}_4^{2-}$  declined in general. After 200 days the concentrations of  $\text{SO}_4^{2-}$  in treatments L0 and L4 were 0.47 and 360  $\text{mg L}^{-1}$ , respectively, and the  $\text{SO}_4^{2-}$  concentrations in treatments T0 and T4 were 0.67 and 154  $\text{mg L}^{-1}$ , respectively.

Soil solution ion concentrations and  $\text{CaCl}_2$ -extractable Cd concentrations at different growth stages

During the growth of *S. plumbizincicola* the solution Cd in both soils increased with increasing S addition

rate from 0 to 30 days. Solution Cd declined slightly from day 30 to day 90 in the controls and some of the S treatments. On day 90 the solution Cd concentrations in L2 and L4 were 21.7 and 184  $\mu\text{g L}^{-1}$ , respectively, values 9.0 and 76.3 times that of L0 ( $2.41$   $\mu\text{g L}^{-1}$ ). In T-soil the  $0.5$   $\text{g kg}^{-1}$  S treatment (L0.5) increased the solution Cd concentration to  $7.4\text{--}24.8$   $\mu\text{g L}^{-1}$ , and higher S application led to higher solution Cd concentrations. During flooding (days 120 to 210) the soil solution Cd showed a maximum value after 5 days of flooding (on day 125). After flooding for 30 days (day 150) the concentration rapidly decreased to  $\sim 0.1$   $\mu\text{g L}^{-1}$  and was maintained until the harvest in the control and lower S treatments, e.g.  $0.5\text{--}2$   $\text{g kg}^{-1}$  in L-soil and  $0.5$   $\text{g kg}^{-1}$  in T-soil. The high S treatments also reached minimum concentrations but did so over a longer time period.

Soil  $\text{CaCl}_2$ -extractable Cd concentrations in both soils were determined at the *S. plumbizincicola* and rice harvests (Table 2). They increased with increasing S application rate at the *S. plumbizincicola* harvest. Soil  $\text{CaCl}_2$ -extractable Cd concentrations decreased substantially after flooding compared to aerobic conditions. Furthermore, the S treatments at  $0.5\text{--}2$   $\text{g kg}^{-1}$  in L-soil and  $0.5\text{--}1$   $\text{g kg}^{-1}$  in T-soil led to significantly lower extractable Cd than in the controls. However, S application at  $4$   $\text{g kg}^{-1}$  in L-soil and  $2\text{--}4$   $\text{g kg}^{-1}$  in T-soil produced significantly higher extractable Cd than in the controls.

The dynamic changes in soil solution Fe and Mn followed similar trends. From 0 to 90 days Fe and Mn increased with plant growth time and S rate. Fe showed a decrease after flooding for 5 days (on day 125) and then increased rapidly. Sulfur treatments showed higher solution Fe and Mn concentrations during the rice growing period compared to the controls.

#### Plant biomass and elemental accumulation

Compared with the controls the growth of *S. plumbizincicola* was significantly affected when the S rate was  $\geq 2$   $\text{g kg}^{-1}$  in L-soil and  $\geq 1$   $\text{g kg}^{-1}$  in T-soil (Table 3). The *S. plumbizincicola* shoot Cd concentrations in the S treatments were significantly higher than in the controls in both soils. For example, the shoot Cd concentrations in L2 and T0.5 were 106 and 96.8  $\text{mg kg}^{-1}$ , respectively, values 4.16 and 1.67 times those of L0 and T0. Soil Cd removal rate increased after

**Table 2** Soil CaCl<sub>2</sub>-extractable Cd at the harvests of *S. plumbizincicola* and rice ( $\mu\text{g kg}^{-1}$ )

S rate (g kg <sup>-1</sup> )	<i>S. plumbizincicola</i>		Rice	
	L-soil	T-soil	L-soil	T-soil
0.0	34.1 ± 0.8e	44.8 ± 1.5e	1.19 ± 0.11b	12.7 ± 2.6c
0.5	41.6 ± 3.0d	83.6 ± 7.6d	0.27 ± 0.03c	2.48 ± 0.67d
1.0	65.8 ± 1.5c	151 ± 4c	0.24 ± 0.03c	5.86 ± 1.72cd
2.0	164 ± 7b	296 ± 37b	0.22 ± 0.01c	120 ± 11b
4.0	920 ± 28a	772 ± 54a	11.7 ± 1.2a	228 ± 38a

Data are mean values ± SE ( $n = 4$ ); means followed by the same letter are not significantly different at  $P < 0.05$

S treatment. For example, the soil Cd removal rates in L2 and T0.5 treatments were 14.6 and 44.7%, respectively, values significantly higher than in L0 (4.03%) and T0 (32.0%). Sulfur treatments significantly increased brown rice yields in L4, T1 and T2 treatments compared to the controls (Table 4). Brown rice Cd concentrations at 0.5–2 g kg<sup>-1</sup> S treatments in both soils were significantly lower than the controls. Sulfur treatments also significantly increased S concentrations in *S. plumbizincicola* shoots and brown rice (Tables 3 and 4).

## Discussion

Dynamics of soil chemical processes affected by sulfur addition and water management

Our experiment indicates that S addition combined with alternating drying and wetting of the soil significantly affected soil chemical processes in the *S. plumbizincicola* and rice rotation system. The

behaviour of S is closely linked to paddy soil redox conditions because of the transformation of inorganic species between valence -2 and valence +6 and oxidation of organic S compounds. In aerobic conditions with *S. plumbizincicola* a dramatic increase in soil soluble sulfate indicates the oxidation of sulfur which was very pronounced for the first 30 days (Fig. 1). This was the period of rapid sulfur oxidation (Wen et al. 2001; Wang et al. 2007). The sulfur oxidation process under aerobic conditions resulted in a decrease in soil pH. Generally, soil pH determines the processes of dissolution-precipitation and adsorption-desorption of metals in soils. The decrease in soil solution pH therefore further increased the soil solution Cd, Mn and Fe concentrations (Fig. 1). These effects depended on both the sulfur addition rate and soil properties. In general, the degree of change is proportional to the sulfur rate. However, similar amounts of S had significantly different effects in the two soils. For example, on day 30 the soil solution pH values in treatments L4 and T4 were 5.03 and 3.23, respectively. This is because of the different properties

**Table 3** Effects of sulfur on biomass and elemental accumulation by *S. plumbizincicola* shoots

S rate (g kg <sup>-1</sup> )	L-soil				T-soil			
	Dry biomass (g pot <sup>-1</sup> )	[Cd] (mg kg <sup>-1</sup> )	[S] (g kg <sup>-1</sup> )	Soil Cd removal rate (%)	Dry biomass (g pot <sup>-1</sup> )	[Cd] (mg kg <sup>-1</sup> )	[S] (g kg <sup>-1</sup> )	Soil Cd removal rate (%)
0.0	10.3 ± 1.2a	25.5 ± 2.6d	1.83 ± 0.15d	4.03 ± 0.60c	11.1 ± 1.1a	58.1 ± 2.1d	1.46 ± 0.11b	32.0 ± 2.0b
0.5	8.79 ± 0.99ab	44.3 ± 4.5c	2.93 ± 0.22c	6.44 ± 0.09b	9.45 ± 1.14ab	96.8 ± 9.2c	1.74 ± 0.11b	44.7 ± 5.0a
1.0	8.49 ± 0.38ab	54.7 ± 4.2c	3.24 ± 0.28bc	7.15 ± 0.78b	7.90 ± 0.40b	115 ± 7bc	1.93 ± 0.13b	44.9 ± 1.3a
2.0	8.95 ± 0.77ab	106 ± 4b	3.80 ± 0.37b	14.6 ± 1.3a	4.78 ± 0.11c	155 ± 9a	2.97 ± 0.16a	36.7 ± 1.2ab
4.0	7.43 ± 0.57b	139 ± 2a	6.21 ± 0.26a	15.8 ± 1.3a	2.86 ± 0.16c	135 ± 8ab	3.02 ± 0.31a	19.3 ± 1.9c

Data are mean values ± SE ( $n = 4$ ); means followed by the same letter are not significantly different at  $P < 0.05$

Soil Cd removal rate (%) =  $(M_1 \times C_1 \times 10^{-3}) / (M_2 \times C_2)$ .  $M_1$  = the dry biomass of *S. plumbizincicola* shoots (g pot<sup>-1</sup>);  $M_2$  = the soil mass of a pot (1.5 kg);  $C_1$  = Cd concentration in *S. plumbizincicola* shoots (mg kg<sup>-1</sup>);  $C_2$  = initial soil Cd concentration, e.g. 4.33 mg kg<sup>-1</sup> and 1.33 mg kg<sup>-1</sup> in L-soil and T-soil, respectively

**Table 4** Effects of sulfur amendment on yield and elemental concentrations in brown rice

S rate (g kg <sup>-1</sup> )	L-soil			T-soil		
	Yield (g pot <sup>-1</sup> )	[Cd] (μg kg <sup>-1</sup> )	[S] (g kg <sup>-1</sup> )	Yield (g pot <sup>-1</sup> )	[Cd] (μg kg <sup>-1</sup> )	[S] (g kg <sup>-1</sup> )
0.0	11.2 ± 1.2b	40.9 ± 6.0a	0.62 ± 0.04d	18.8 ± 0.6b	144 ± 12b	1.17 ± 0.07b
0.5	12.2 ± 1.3ab	19.8 ± 1.9b	1.22 ± 0.08bc	19.7 ± 0.7b	40.4 ± 6.5d	1.30 ± 0.12b
1.0	12.0 ± 0.5ab	16.1 ± 3.0b	1.10 ± 0.07c	23.0 ± 1.5a	44.3 ± 7.0d	1.18 ± 0.07b
2.0	13.6 ± 0.7ab	20.1 ± 2.4b	1.43 ± 0.09b	25.4 ± 1.2a	94.4 ± 9.0c	1.80 ± 0.19a
4.0	14.6 ± 0.7a	28.8 ± 4.9ab	2.29 ± 0.15a	8.42 ± 0.85c	179 ± 12a	2.02 ± 0.10a

Data are mean values ± SE (n = 4); means followed by the same letter are not significantly different at  $P < 0.05$

of the two soils including the initial pH and clay concentrations (Table 1). It is notable that soil solution pH and solution Cd decreased slightly in some treatments from 30 to 90 days and this can be explained by rhizosphere acidification and Cd accumulation by *S. plumbizincicola* (Sun et al. 2019).

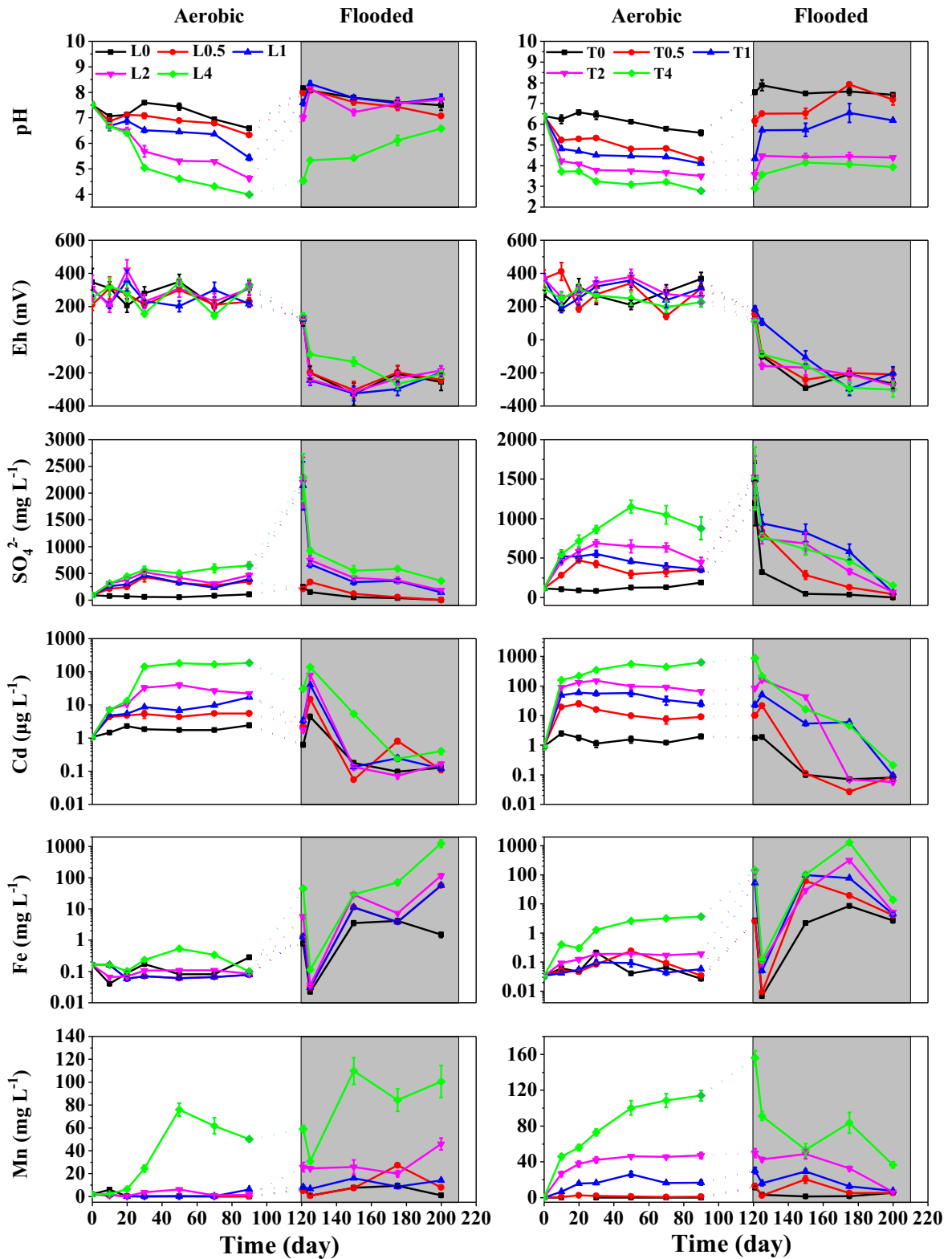
Upon flooding, O<sub>2</sub> in the soil is depleted quickly and this is followed by reduction of nitrate, manganese oxides and then iron oxides/hydroxides. After the reduction of Fe, sulfate is reduced to sulfide mainly due to microbial activity (Borch et al. 2010; Hashimoto et al. 2016). One day after flooding (day 121) in the current study the concentration of soil solution SO<sub>4</sub><sup>2-</sup> in all treatments increased compared to day 90. This was due to the competitive effects of anions e.g. CO<sub>2</sub>, acidic carbonates and organic acids resulting from the decomposition of organic matter (Ding and Xu 2011). Subsequently, soil solution SO<sub>4</sub><sup>2-</sup> showed an overall downward trend (Fig. 1) which indicates reduction of SO<sub>4</sub><sup>2-</sup> (Burton et al. 2013, 2014). When flooding was prolonged the soil solution Eh decreased from -200 mV to -300 mV and soil SO<sub>4</sub><sup>2-</sup> was reduced to generate reducing substances such as HS<sup>-</sup>/S<sup>2-</sup> which formed sulfide precipitates with various trace metals, e.g. Cu, Cd, Fe, and Zn (Fulda et al. 2013; Hashimoto et al. 2016). On the other hand, the reduction reaction consumes protons, thus the soil pH of all treatments changed to neutral (Burton et al. 2013). However, the pH in treatment T4 remained low, possibly because the sulfur rate exceeded the buffering capacity of the soil.

It is notable that at day 125 (five days after flooding) the soil solution Cd concentration increased but Fe and Mn concentrations decreased (Fig. 1). One possible explanation is that Fe/Mn oxides underwent a reduction and dissolution process, releasing Cd<sup>2+</sup> (Kocar et al. 2010; Stroud et al. 2011). The soil solution Fe concentration is higher than that of Cd and therefore Fe readily combines

with S<sup>2-</sup> to form FeS precipitate. This resulted in a temporary decline in solution Fe concentration. After prolonged submergence the continuous reduction of Fe/Mn oxides provided soluble metals while soil solution Cd directly formed CdS or replaced Fe in FeS (Dituro et al. 1990). Moreover, the continuous flooding within 30 days in appropriate S treatments eliminated elevated soluble Cd resulting from S oxidation, and maintained soluble Cd at a low level (~0.1 μg L<sup>-1</sup>) until the rice harvest. This is consistent with previous studies (de Livera et al. 2011; Fan et al. 2010). It has been reported that moderate sulfur-containing substances in flooded soils change the diversity of the bacterial community and this accelerates the reduction of sulfate and facilitates the precipitation of metal sulfides (Wu et al. 2019; Sebastian and Prasad 2014; Kelly and Wood 2000). The 0.5–2 g kg<sup>-1</sup> S treatments in L-soil and 0.5–1 g kg<sup>-1</sup> S treatments in T-soil showed significantly lower extractable Cd concentrations than the controls (Table 2). More Cd would be expected to form CdS precipitate which would be stable and non-extractable by weak extracting agents such as CaCl<sub>2</sub> (Furuya et al. 2016). However, the excessive S treatments, e.g. L4, T2 and T4, took a long time to reduce soluble Cd to the minimum level, and the concentration of CaCl<sub>2</sub>-extractable Cd after the rice harvest was also maintained at a high level (Fig. 1, Table 2). One possible explanation is the inhibition of Cd precipitation under extremely low soil pH conditions.

Effects of sulfur application and water management on phytoextraction and accumulation of Cd by *S. plumbizincicola* and rice

Sulfur addition and water management also affected plant growth and metal accumulation. Appropriate S



**Fig. 1** Dynamics of pH, Eh, SO<sub>4</sub><sup>2-</sup>, Cd, Fe, and Mn in the soil solution during aerobic (0–90 days, *S. plumbizincicola*) and flooded (120–210 days, rice) culture under different application rates of S amendment to calcareous L-soil (left) and neutral T-soil (right)

treatment had no significant negative effect on *S. plumbizincicola* biomass but significantly increased the shoot Cd concentrations because of the increase in soil Cd bioavailability both in neutral and calcareous soils. For example, shoot Cd concentrations in the S treatment in L-soil were 1.74–5.45 times and in T-soil were 1.67–2.67 times higher than in the controls. Moreover, the soil Cd removal rate in L2 and T0.5 reached 14.6 and 44.7%, respectively, values significantly higher than in L0 (4.03%) and T0 (32.0%). The increase in Cd removal rate with S treatment will markedly shorten the duration of remediation. For example, we assume that the Cd removal rate by *S. plumbizincicola* is consistent in subsequent remediation, costing three crops in T0.5 but four crops in T0 to decrease the soil Cd ( $1.33 \text{ mg kg}^{-1}$ , pH 6.5) to below the Chinese risk screening value ( $0.4 \text{ mg kg}^{-1}$ ,  $5.5 < \text{pH} \leq 6.5$ , GB15618–2018). Similarly, the cost is around 42 crops in L0 but only 11 crops in L2 to decrease soil Cd ( $4.44 \text{ mg kg}^{-1}$ , pH 8.0) to below the Chinese risk screening value ( $0.8 \text{ mg kg}^{-1}$ , pH > 7.5, GB15618–2018). Previous studies also report that phytoextraction efficiency in S treatments was enhanced by 50% during microbial S oxidation in the rhizosphere of willow (Iqbal et al. 2012). The addition of S had positive effects on the accumulation of Cd and Zn in *Sedum alfredii* because of a “sulfur-induced Cd requirement” (Li et al. 2008). In our study the S treatments at  $2 \text{ g kg}^{-1}$  in L-soil and  $0.5 \text{ g kg}^{-1}$  in T-soil produced relatively high Cd removal rates by *S. plumbizincicola* (Table 3). Further increase in S treatment did not promote Cd removal or even decrease Cd removal because of the toxic effects on plant growth. Excessive S over-acidifying the soil during the first few weeks might inhibit new root development and induce aluminium toxicity (Rao et al. 2016).

Furthermore, a novel finding was that appropriate S treatments significantly increased yields and reduced total Cd concentrations of brown rice from both test soils. There are several possible contributory factors. Firstly, as discussed above the continuous flooding and reduction of  $\text{SO}_4^{2-}$  maintained soil soluble Cd at a very low level during the intermediate and later growth stages of rice (Fig. 1 and Table 2). Secondly, S might enhance the formation of iron plaque in the rhizosphere which would suppress Cd accumulation by rice (Hu et al. 2007). Thirdly, S treatment increased rice S concentrations and led to the formation of glutathione and chlorophyll that alleviated Cd stress (Sarwar et al. 2010; Dixit et al. 2015). This type of defence mechanism

involving Cd-induced photosynthetic inhibition appears to be more effective when the soil is treated with the optimum rate of S rather than excessive S (Masood et al. 2012). However, it is important to note that excessive levels of S in T-soil increased rice Cd accumulation and thus increased the Cd risk (Table 4).

Taking into account the effects of S addition on soil chemical processes, plant growth and Cd uptake and potential environmental risk, S addition at  $2 \text{ g kg}^{-1}$  to the calcareous soil (L-soil) and  $0.5 \text{ g kg}^{-1}$  to the neutral soil (T-soil) are the optimum treatments to simultaneously increase the soil Cd removal rate by *S. plumbizincicola* and depress the Cd concentration in the brown rice. Excessive levels of S should be avoided because of growth inhibition of *S. plumbizincicola* and increased Cd uptake by rice. This may also increase the potential risk of Cd leaching and induce S toxicity to the plants.

#### Strategy for phytoextraction and safe rice production in Cd-contaminated soils

This study indicates a new strategy for phytoextraction together with the safe production of food in Cd-contaminated soils. This involves the rotation of the Cd hyperaccumulator *S. plumbizincicola* with a low Cd accumulating cultivar of rice. Appropriate application rates of S are recommended during the *S. plumbizincicola* growing season especially in neutral and calcareous soils to activate soil Cd through S oxidation to increase Cd phytoextraction and shorten the duration of the remediation. Then, during the rice season, flooding should be maintained to promote the reduction of sulfate and the formation of CdS precipitate to ensure low Cd availability and thus minimize the risk of Cd contamination in the rice. In the next rotation, there is no need to add S again. Soil pH will decline in oxidised conditions and the re-oxidation of sulfide compounds will release Cd again (Fulda et al. 2013; Furuya et al. 2016) which is beneficial for phytoextraction by *S. plumbizincicola*. Soil total Cd concentrations can be removed gradually with safe agricultural production until the soil remediation goal is finally achieved. In addition, it is notable that Cd release with S oxidation is a rapid process compared to plant uptake, and this may increase the Cd leaching risk in field practice. The future development of slow release S fertilizers and relevant agronomic measures, e.g. low dosage with suitable frequency, may help to overcome this limitation (Mann et al. 2019).



## Conclusions

Sulfur addition combined with water management significantly affected soil chemical processes and plant Cd uptake in neutral and calcareous soils. The oxidation of applied S in aerobic conditions significantly acidified the soil and increased soluble  $\text{SO}_4^{2-}$ , Cd and Fe concentrations and promoted Cd phytoextraction by *S. plumbizincicola*. In the rice season continuous flooding decreased soil Eh and increased pH. Appropriate rates of S application induced the reduction of  $\text{SO}_4^{2-}$  and facilitated the stabilization of Cd. This rapidly eliminated elevated soluble Cd resulting from S oxidation in the former season and also maintained available Cd at lower levels than in the controls until the rice harvest. This increased rice yields and reduced rice Cd uptake. Therefore, appropriate sulfur amendment associated with alternating drying and wetting of the soil can enhance soil Cd remediation efficiency by the hyperaccumulator and control Cd accumulation in rice grains in the rotation system. This study thus proposes a new strategy for phytoextraction and continued safe production of rice in Cd-contaminated neutral and calcareous soils.

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