Influence of Rapeseed Cake on Heavy Metal Uptake by a Subsequent Rice Crop After Phytoextraction Using *Sedum plumbizincicola*

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A glasshouse pot experiment was conducted to study the effects of phytoextraction by *Sedum plumbizincicola* and application of rapeseed cake (RSC) on heavy metal accumulation by a subsequent rice (*Oryza sativa L.*) crop in a contaminated paddy soil collected from east China. After phytoextraction by *S. plumbizincicola* the soil and brown rice Cd concentrations effectively declined. After phytoextraction, RSC application reduced brown rice Cd concentrations in the subsequent rice crop to 0.23–0.28 mg kg$^{-1}$, almost down to the standard limit (0.2 mg kg$^{-1}$). After phytoextraction and then application of RSC, the soil solution pH, dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) concentrations increased during early stages of rice growth resulting directly and indirectly in lowering the bioavailability of the heavy metals. Thus the grain yield of the subsequent rice crop increased and the heavy metals in the brown rice declined significantly. In this contaminated acid soil, growing the hyperaccumulator *S. plumbizincicola* and rice in rotation together with RSC application may therefore be regarded as a viable strategy for safe grain production and bioremediation.

Keywords: cadmium, hyperaccumulator, soil remediation, rapeseed cake, brown rice

**Introduction**

Heavy metal contamination is a major indicator of soil degradation. The incidence of heavy metal contamination has increased in regions with substantial industrial development and agricultural intensification, including the Yangtze River Delta and the Pearl River Delta in east and south China (Luo 2009). In these regions heavy metal contamination of vegetables and cereal crops has threatened the quality and safety of the human food chain (Zhao et al. 2010; Xiao et al. 2010). Effects of heavy metals on environmental and human health and their potential implications for international trade are becoming increasingly important (Park et al. 2011). It is therefore essential to develop remediation technologies for agricultural soils contaminated with heavy metals to ensure that the metal concentrations in food products comply with regulatory standards.

Bioremediation has been developed as an alternative to traditional soil remediation technique and is being used as an effective approach for mitigating heavy metal contamination in soils. It is a natural process to modify the bioavailability and plant uptake of heavy metals. Studies have indicated that the bioavailability of heavy metals can be influenced by soil pH, organic matter, and rhizosphere properties (Akhtar and Malik 2000; Barančíková et al. 2004; Basta et al. 2005; Kukier et al. 2004). Organic amendments are often used as soil conditioners to reduce the bioavailability of heavy metals by altering the physicochemical properties of contaminated soils (Bolan et al. 2003; Bolan and Duraisamy 2003; Dhillon et al. 2010). Thus, there may be some scope to enhance bioremediation by the addition of organic amendments (Park et al. 2011). Several studies have indicated that organic amendments increase grain yields and decrease heavy metal concentrations in grain significantly in contaminated paddy soils through their effects on lowering the bioavailability of heavy metals by increasing soil pH and transforming heavy metals from soluble and exchangeable fractions to other (less available) fractions (Li et al. 2008; Li et al. 2009; Walker et al. 2003). Although heavy metal bioavailability may be decreased by adding amendments, the...
metals remain in the soil and continue to represent a potential hazard, especially in highly contaminated sites.

Phytoextraction using metal hyperaccumulating plant species has been proposed as a potential remediation technique for metal-polluted soils. *Sedum plumbizincicola* is a Zn and Cd hyperaccumulator and it has the potential to phytoextract Zn and Cd from severely contaminated soils (Wu et al. 2012; Liu et al. 2011; Jiang et al. 2010; Wu et al. 2008), but phytoextraction by this plant species might activate the heavy metals sorbed by the soil matrix. Zhao et al. (2011) found that heavy metal concentrations in the shoots of wheat (*Triticum aestivum* L.) intercropped with *S. plumbizincicola* were higher than in monoculture. Another study also showed that rotation with *S. plumbizincicola* increased the Zn and Cd concentrations in rice (Shen et al. 2010). It may be concluded that phytoextraction activates the heavy metals in contaminated soils, thereby increasing the risk to the food chain in the subsequent crop plant in the rotation. Appropriate management practices must be implemented to minimize the bioavailability of soil heavy metals during the crop growth season.

Our previous studies showed that application of rapeseed cake as a waste product and also a slow-release fertilizer increased rice grain yields and significantly decreased the heavy metal concentrations in brown rice and straw (Zhou et al. 2008). In present study *S. plumbizincicola* was grown to lower the total heavy metal concentration and then rapeseed cake was applied to immobilize soil heavy metals during the rice growth season. The purpose was to determine the regulatory effects of phytoextraction by *S. plumbizincicola* and application of rapeseed cake (RSC) on heavy metal accumulation in a subsequent rice crop in a contaminated paddy soil and to investigate the mechanisms by which RSC may immobilize heavy metals in the soil-rice system so as to accomplish concurrent metal remediation and safe grain production.

**Materials and Methods**

**Soil Collection and Phytoextraction**

The experimental soil was collected from the top 15 cm of the profile in an agricultural field adjacent to an electronic waste dismantling site at Taizhou, Zhejiang province, east China. The name of the tested soil in the Chinese Soil Taxonomy is ‘Typic Fe-leachi-Stagnic Anthrosols’ and in the World Reference Base for Soil Resources (WRB) it is ‘Hydragric Anthrosols’. The soil had a pH (in water) of 4.52, an organic matter content of 36.2 g kg$^{-1}$, available phosphorus and NH$_4$OAc-extractable potassium concentrations of 7.79 and 107 mg kg$^{-1}$, and total nitrogen, phosphorus and potassium concentrations of 2.03, 0.78, and 17.6 g kg$^{-1}$. The total Cd, Cu and Zn concentrations were 11.5, 323, and 155 mg kg$^{-1}$, respectively. The total Cd and Cu concentrations of this soil significantly exceeded the environmental quality standards for agricultural soils (GB 15618-1995; Cd 0.3 and Cu 50 mg kg$^{-1}$) issued by the State Environmental Protection Administration of China. Half of the soil had previously been phytoextracted for two cycles with *S. plumbizincicola* from October 2009 to May 2011 before transplanting the rice seedlings. The soil had been phytoextracted in pots and 5 plants per pot. Phytoextraction had lowered the total Cd, Cu, and Zn and the pH of the soil to 2.04, 319, 139 mg kg$^{-1}$ and 4.15, respectively.

**Pot Experiment**

The soil samples were air-dried and passed through a 2-mm nylon sieve prior to the glasshouse pot experiment. The diameter and height of the pot was 21.7 cm and 24.5 cm respectively, pots were filled with 6 kg of soil. The experiment comprised three treatments, namely a control to which chemical fertilizer was applied, a treatment in which rapeseed cake was added at a rate of 3 g kg$^{-1}$ (RSC1), and RSC added at 6 g kg$^{-1}$ (RSC2). These treatments were set up using both phytoextracted and non-extracted soil (as described above), with four replicate pots per treatment. In addition, a soil culture pot experiment which also comprised three treatments and four replicate pots per treatment was added to provide information on the dynamics of soil solution heavy metal concentrations, giving a total of 36 pots arranged in a fully randomized design.

Rice was planted between June and October 2011. The chemical fertilizers used in control pots were urea, monopotassium phosphate and potassium chloride, giving N, P, and K application rates of 0.36, 0.18, and 0.27 g kg$^{-1}$. The N, P, and K concentrations in all the treatments were made equivalent to the control by adjustment with additional chemical fertilizers where necessary. Total N, P, and K concentrations of the RSC were 60.5, 10.2, and 8.52 g kg$^{-1}$, and total Cd, Cu and Zn were 0.067, 8.01, and 74.1 mg kg$^{-1}$, respectively. Soil (6 kg, oven dry basis) was mixed with fertilizers and/or RSC (filtered with a 0.4 mm sieve and fully decomposed) and transferred into the pots. One soil moisture sampler (Rhizon SMS, Rhizosphere Research Products, the Netherlands) was installed in the soil in each pot on June 9. The soil moisture content was maintained at 60% of the water holding capacity (WHC). Three weeks later seedlings of a low-cadmium accumulating rice cultivar Zhongzheyou 1 (ZZY1) were transplanted in the pots.

The seeds were surface-sterilized in carbendazim solution (Renukdas et al. 2010) and soaked for 48 h in deionized water for germination in an incubator at 30 ± 0.2°C before sowing and sown in plastic plates with clean soil on June 5, 2011. Three seedlings were transplanted in each pot on June 30 and placed in a greenhouse with a natural day and night regime and no supplementary illumination. All pots were irrigated with deionized water for submerged cultivation except during the soil drying period at late tillering stage from August 10 to 15. Additional urea was applied as topdressing at a rate of 0.12 g kg$^{-1}$ N to all treatments on August 23. The watering was continued until October 23 and the rice was harvested one week later.

Soil solution was collected at different plant growth stages, namely pre-tillering (July 10), tillering (August 3), booting (August 29), heading (September 20), grain filling (October 5) and ripening (October 20). Soil solution was collected after 10, 20, 30, 40, and 70 days for the soil culture experiment.

**Chemical Analysis**

Soil pH and organic matter content, total N, P, and K, available P and K, and total Cd, Cu, and Zn of the contaminated
soil were determined. Soil pH was determined as described by Luo and Christie (1997) using a glass pH electrode with a water-solid ratio of 2.5:1. Organic matter content was determined by the potassium dichromate oxidation method. Total N was determined by Kjeldahl digestion and distillation, total P by the molybdenum blue method and total K by flame photometry. Available P was determined by the molybdenum blue method and total K by flame photometry after extraction with 1 mol L−1 NH₄OAc. Soil total heavy metal concentrations were determined using atomic absorption spectrophotometry (Varian SpectrAA 220FS, Varian 220Z, varian, Palo Alto, CA) after digestion of ~0.2 g samples with 10 ml of HCl-HNO₃ (4:1, v/v). A certified reference soil (GBW07406, provided by the Institute of Geophysical and Geochemical Exploration, Langfang, Hebei Province, China) was used for quality control.

The total N, P, and K and total Cd, Cu, and Zn of the rapeseed cake were also determined. Total N was determined using an elemental analyser (Vario MAX CNS, Elementar, Hanau, Germany). Total P was determined by H₂SO₄/HClO₄ digestion and analysed by the molybdenum blue method and total K by flame photometry after extraction by aqua-regia digestion. Rapeseed cake samples (~0.5 g) were digested with 10 ml of HNO₃-HClO₄ (3:2, v/v), and then the total concentrations of Cd, Cu, and Zn were also determined by AAS. A certified reference material (GBW07603, provided by the Institute of Geophysical and Geochemical Exploration, Langfang, Hebei Province, China) was used for quality control.

After harvest each rice plant was divided into grain, straw and roots and washed with tap water and then deionized water. Before oven drying at 70°C and weighing, the roots were immersed in 20 mmol L⁻¹ Na₂EDTA solution for 20 min and then washed with deionized water to remove the heavy metal ions on their surfaces. Grains were shelled into husk and brown rice fractions. All plant parts were ground and digested for determination of heavy metal concentrations. The procedures were the same as for the determination of total Cd, Cu, and Zn in RSC. In addition, soil from all the pots was sampled and passed through a 2-mm sieve. Soil extractable Cd, Cu, and Zn were extracted with 0.01 mol L⁻¹ CaCl₂ and determined by AAS.

The soil solution samples were subjected to measurement of pH, heavy metal concentrations, N concentrations and dissolved organic carbon. In addition, total heavy metal concentrations (Mₚ) and the labile fractions (Mₐ) were measured in the soil culture experiment as described by Luo and Christie (1997). The pH value was measured with a glass electrode. Soil solution (8 ml) was adjusted by adding two drops of nitric acid to maintain the soil solution pH <2 for determination of heavy metals (both Mₚ and Mₐ) by inductively coupled plasma—atomic emission spectroscopy (IRIS Intrepid, Thermo Electron, Franklin, MA) and ICP-mass spectrometry (Thermo X7). An 8-ml aliquot of soil solution was used for the analysis of ammonium-N, nitrate-N, and total N with a continuous flow analyser (San++, Skalar, the Netherlands). The dissolved organic nitrogen (DON) concentration was determined by the subtraction of the inorganic N concentrations from total N. An 8-ml aliquot of soil solution was used to determine dissolved organic carbon (DOC) using a total organic carbon analyser (Multi N/C 3000, Analytik Jena, Germany).

### Statistical Analysis

The data were subjected to T-test and one-way analysis of variance using the SPSS 13.0 for Windows statistical software package. Duncan’s multiple range test was used to detect significant differences among treatment means at the 5% level.

### Results

#### Rice Biomass and Heavy Metal Uptake

The effects of phytoextraction by *S. plumbizincicola* and application of rapeseed cake on the biomass composition and allocation of the subsequent rice crop are shown in Table 1. The grain yield and total biomass increased after phytoextraction (T-test, p < 0.05). The grain yield increased by 8.4–14.5% after RSC application to the soil after phytoextraction. All results show that in this high cadmium contaminated soil application of RSC increased plant biomass and grain yields compared with the control.

Heavy metal concentrations in different plant parts are shown in Table 2. After phytoextraction by *S. plumbizincicola* the heavy metal concentrations for control in brown rice decreased, Cd by 33.3% and Zn by 9.6%. The Cu concentrations in all plant parts were significantly higher than with soil not subjected to phytoextraction. However, Cu concentrations in brown rice, husk and straw declined significantly after RSC was added to the soil after phytoextraction. Without phytoextraction and RSC application the Cd

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**Table 1.** Effect of phytoextraction by *S. plumbizincicola* and application of rapeseed cake on the biomass and components of the subsequent rice crop (g pot⁻¹)

| Treatment  | Non-phytoexisted                      | After phytoextraction                      |
|------------|---------------------------------------|-------------------------------------------|
|            | Grain | Straw | Roots | Grain | Straw | Roots |
| Control    | 29.1 ± 1.5b | 39.3 ± 3.1a | 9.48 ± 0.47a | 35.9 ± 1.4b | 41.8 ± 1.8b | 11.9 ± 1.0a |
| RSC1       | 35.1 ± 2.0a | 34.1 ± 3.1a | 9.65 ± 1.61a | 38.9 ± 1.8ab | 45.6 ± 0.9a | 13.9 ± 2.4a |
| RSC2       | 31.9 ± 1.9ab | 37.0 ± 1.7a | 10.6 ± 1.0a | 41.1 ± 1.7a | 42.0 ± 1.6b | 11.8 ± 0.5a |

Values are means ± SD; means followed by the same small letter in each column are not significantly different at P < 0.05; RSC1 and RSC2, low and high application rates of rapeseed cake. The same abbreviations are used below.
concentrations in brown rice exceeded the standard limit (0.2 mg kg\(^{-1}\)) by 2.4- to 3.4-fold and the Cd concentrations also exceeded the 10 mg kg\(^{-1}\) standard limit (NY 861–2004) issued by the Ministry of Agriculture, China. After phytoextraction, RSC application reduced Cd concentrations in brown rice to 0.23–0.28 mg kg\(^{-1}\) compared to pre-extraction, RSC application reduced Cd concentrations in soil to 0.746, rCd = −0.870; for non-remediated soil, n = 9, rZn = −0.746, rCd = −0.870; for non-remediated soil, n = 9, rZn = −0.802, rCd = −0.837), indicating that after the increase in soil pH with RSC application the concentrations of the soil extractable heavy metals declined. Growing the hyperaccumulator lowered the soil pH but RSC application had the opposite effect. After the rice growth season concentrations of extractable Cd and Zn decreased but extractable Cu increased in phytoextracted soil.

### Soil pH and CaCl\(_2\)-extractable Heavy Metals

After phytoextraction the soil pH for control was 0.4 units lower than that of non-phytoextracted soil (Table 3) and concentrations of extractable Cd and Zn declined by 66.2 and 39.6% respectively. However, the concentration of extractable Cu was 3.2 times higher than that of non-remediated soil. Extractable Cu in non-remediated soil were lowered by the application of RSC. Concentrations of soil extractable Zn and Cd in both remediated and non-remediated soil were negatively correlated with soil pH (for remediated soil, n = 9, rZn = −0.746, rCd = −0.870; for non-remediated soil, n = 9, rZn = −0.802, rCd = −0.837), indicating that after the increase in soil pH with RSC application the concentrations of the soil extractable heavy metals declined. Growing the hyperaccumulator lowered the soil pH but RSC application had the opposite effect. After the rice growth season concentrations of extractable Cd and Zn decreased but extractable Cu increased in phytoextracted soil.

### Soil Solution Heavy Metal Concentrations At Different Growth Stages

As shown in Figure 1, growth of the hyperaccumulator significantly lowered the soil solution pH over the whole growing season, with the change varying from 0.7 to 3.2 units. Application of RSC increased soil solution pH, especially at the pre-tillering and tillering stages, with the pH increasing by up to 1.9 units in non-remediated soil and 3.2 units in remediated soil.

![Figure 1. Effect of phytoextraction by *S. plumbizincicola* and application of rapeseed cake on soil solution pH at different growth stages of the subsequent rice crop. PT, pre-tillering; TS, tillering stage; BS, booting stage; HS, heading stage; GF, grain filling; and RS, ripening stage. N, Non-phytoextracted by *S. plumbizincicola*; A, after phytoextraction by *S. plumbizincicola*. RSC1, low rate and RSC2, high rate of rapeseed cake. The same abbreviations are used below.](image-url)
Table 3. Effect of phytoextraction by *S. plumbizincicola* and application of rapeseed cake on soil pH and concentrations of CaCl₂-extractable heavy metals at the ripening stage

| Treatment | pH | Cd (mg kg⁻¹) | Cu (mg kg⁻¹) | Zn (mg kg⁻¹) | pH | Cd (mg kg⁻¹) | Cu (mg kg⁻¹) | Zn (mg kg⁻¹) |
|-----------|----|--------------|--------------|--------------|----|--------------|--------------|--------------|
| Control   | 4.76±0.04 b | 4.05±0.26 a | 8.88±1.16 a  | 11.1±0.5 a   | 4.37±0.04 b | 1.37±0.05 a | 28.2±1.9 a   | 6.69±0.19 b  |
| RSC1      | 4.78±0.03 ab| 3.92±0.20 a | 7.35±0.10 ab | 10.3±0.8 a   | 4.48±0.06 a | 1.11±0.05 b | 23.4±0.3 b   | 6.65±0.07 b  |
| RSC2      | 4.85±0.03 a | 3.63±0.34 a | 5.68±0.77 b  | 9.57±0.70 a  | 4.34±0.03 b | 1.38±0.07 a | 22.2±0.2 b   | 6.98±0.03 a  |

was notably higher than in non-remediated soil, and RSC application resulted in a marked decline. Thus, phytoextraction increased the solubility of the heavy metals in the soil solution, especially at the early growth stages of the rice crop. RSC application effectively lowered soil solution heavy metal concentrations.

**Fig. 2.** Effect of phytoextraction by *S. plumbizincicola* and application of rapeseed cake on heavy metal concentrations in soil solutions at different growth stages of the subsequent rice crop.

**Dissolved Organic C and N in the Soil Solution at Different Rice Growth Stages**

As shown in Figure 3, at pre-tillering the DOC concentration in remediated soil was 3.8 times higher than that in non-remediated soil and from tillering to ripening the DOC concentration in remediated soil declined by 48.1–57.2% compared with the control. After application of RSC to non-remediated soil the DOC concentration in the soil solution at the three growth stages from pre-tillering to tillering were 6.6 to 9.2, 1.9 to 2.2, and 2.2 to 2.7 times the control but the DOC concentrations declined at the three growth stages from heading to ripening. After RSC application to remediated soil the soil solution DOC concentration was significantly higher than that in the control over the whole growing season. This difference was especially marked between tillering and heading stages and peaked at the booting stage with a DOC concentration 3.0 to 5.4 times that of the control. It may be concluded that RSC application greatly increased soil solution DOC concentrations from pre-tillering to booting.

DON concentrations increased initially from pre-tillering to the booting stage and then decreased with the sole exception of the control remediated soil (Figure 4). DON concentrations reached a minimum at heading and then increased at grain filling. At pre-tillering and tillering the DON concentrations in the soil solution of remediated soil were 3.4 and 2.0 times the non-remediated soil values. After RSC application to non-remediated soil the DON concentrations in the soil solution at pre-tillering, tillering, and booting increased markedly. In the remediated soil RSC application increased the DON concentration in the soil solution in contrast to the control during the whole growing season.

**Fig. 3.** Effect of phytoextraction by *S. plumbizincicola* and application of rapeseed cake on concentration of dissolved organic carbon in the soil solution at different growth stages of the subsequent rice crop.
Decrease Cadmium in Rice After Phytoextraction

Total Dissolved and Labile Fractions of Heavy Metals in the Soil Solution (Soil Culture Experiment)

As shown in Figures 5 and 6, RSC application increased the total dissolved concentrations of Cd and Cu significantly at the start of the experiment but their concentrations declined markedly as the experiment proceeded. The trend in total Zn differed somewhat and RSC application increased total Zn significantly throughout the experiment.

RSC application increased the labile fractions of Cd, Cu, and Zn significantly over the first 10 days and then from 20 to 70 days the concentrations declined markedly until there were no differences among the treatments.

Discussion

After phytoextraction by S. plumbizincicola in the present study the Cd concentration in contaminated soil declined, the grain yield increased and the Cd concentration in the brown rice dropped significantly. Although the heavy metal concentrations in the soil decreased after phytoextraction, at the early growth stages (from pre-tillering to booting) of the rice crop the soil solution heavy metal concentrations increased (Figure 2). The water-soluble and exchangeable fractions of heavy metals are the main sources of the metals absorbed by plants, however a certain amount of heavy metal accumulation by hyperaccumulator plants is derived from potentially bioavailable heavy metals (Al-Najar et al. 2003; McGrath et al. 1997; Whiting et al. 2001). Thus hyperaccumulators can activate unavailable heavy metals to resupply depleted metals from the non-mobile fractions (Long et al. 2008; Liu et al. 2011; Li et al. 2011). The soil heavy metals activated by phytoextraction influenced heavy metal bioavailability to the subsequently planted rice crop.

Although S. plumbizincicola is a Zn and Cd hyperaccumulator it played no role in the assimilation and transfer of Cu. Most of the activated Cu was retained in the soil, consequently Cu concentrations in the soil solution increased markedly and Cu concentrations and accumulation increased in the rice crop (Table 2). The bioavailability of heavy metals plays a key role in the transfer of heavy metals from soils to plants. Phytoextraction by S. plumbizincicola enhanced the bioavailability of soil heavy metals. Similarly, Zhao et al. (2011) and Shen et al. (2010) reported that intercropping with S. plumbizincicola increased the accumulation of heavy metals in crop plants and rotation with the hyperaccumulator will increase heavy metal concentrations in the edible parts of the succeeding crop.

Application of organic amendments can lower soil heavy metal bioavailability (Park et al. 2011). The in-situ immobilization technique can be considered as part of integrated techniques for risk management. In our study the application of RSC resulted in a significant decline in heavy metal concentrations in the soil solution from the pre-tillering stage to booting (Figure 2) The results of the soil culture experiment showed that RSC application increased heavy metal concentrations in the soil solution in the short term in the absence of plants, presumably indicating metal adsorption onto soil solid components. Application of RSC did not increase plant biomass from pre-tillering to booting and there was a significant decline in heavy metal concentrations in the soil solution.
Huang et al. (2008) reported that the sensitive stages when heavy metals affect the growth of rice plants are the tillering and booting stages. Application of RSC can lower heavy metal concentrations in the soil solution at these sensitive stages and this may increase the biomass of rice plants and increase the grain yield. The soil solution heavy metal concentrations can be considered to represent the concentrations of plant available heavy metals in the soil (McGrath et al. 1997; Luo et al. 2000). Heavy metal accumulation by the rice plants was then lowered and the increase in grain yield resulted in a dilution effect as plant biomass increased significantly compared with the earlier growth stages so that the heavy metal concentrations in the brown rice decreased significantly.

The decomposed products of organic amendments affect the partitioning of heavy metals between the soil solid and solution phases. Transformation of the exchangeable metal fraction to organic-, Fe-Mn oxide-, and carbonate-bound fractions has often been reported after application of organic amendments to contaminated soils. This lowers the bioavailability and toxicity of the heavy metals. Finally, the heavy metals assimilated by crop plants decrease (Park et al. 2011). In our study the application of RSC to the soil after phytoextraction significantly lowered the concentration of extractable Cu. Application of organic materials increases the concentration of soil DOM (McDowell 2003). The soil DOM is the most active and important organic matter fraction which acts reciprocally with the heavy metals, thereby playing a key role in the control of their bioavailability (Temminghoff et al. 1997; Temminghoff et al. 2008). More importantly, the DOM produces more heavy metal binding sites and this can impact the activity of the soil heavy metals. The pH of the soil solution is also impacted by the application of organic materials and the solubility of heavy metals can change. RSC application to remediated soil can increase soil solution pH at pre-tillering and tillering and the concentration of DOC will increase significantly. As a result, the Cu concentration in the soil solution decreases markedly. From pre-tillering to heading the soil solution pH and the DOC concentration were positively correlated ($r = 0.71, P < 0.01$). The coordination capacity between DOM and Cu is higher than that between DOM and Zn or Cd, and the binding rate increases with increasing soil solution pH (Park et al. 2011; Temminghoff et al. 1997). Thus, the activated Cu is coordinated and immobilized, and the Cu concentration in the soil solution declines. A balance of DOM distribution between the solid and liquid phases is maintained in the soil solution. The DOM is adsorbed intensely by the soil minerals resulting in a lowering of DOM concentration in the soil solution (Kalbitz 2000). The mechanism by which the increase in DOM results in a lower concentration of heavy metal in the soil solution could be postulated as a weakening of the inhibition of DOM absorption of heavy metal by soil as a result of the strong adsorption of DOM by soil. Wang et al. (2000) demonstrated that the total soluble Cd changed in reverse of DOC and pH. It can be concluded that after application of RSC to the remediated soil the DOC concentration in the soil solution from pre-tillering to booting increased significantly, soil solution pH increased, and Cd and Cu concentrations in the soil solution declined, thus bioavailability declined, then the Cd and Cu concentrations in the brown rice finally declined.

RSC contains large amounts of nitrogen compounds and the decomposition products of RSC can significantly increase DON in the soil solution at the early growing stages (Figure 4). DON is the most active form of soil nitrogen (Nasholm et al. 1998; Perakis and Hedin 2002), is interchangeable with other components of the soil N, and is readily decomposed by microorganisms. Because it is highly bioavailable, DON is supposed to be an important source of soil nutrients (Zhang et al. 2012). Nitrogen metabolism is hyperactive before the heading stage in rice plants, so an increase in soil solution DON at early growth stages is conducive to N accumulation in rice plants. As a result, plant growth is promoted and plant biomass increases as DON increases. The improvement in the nutrition of the rice plants can enhance their resistance to heavy metal stress and then indirectly lower the bioavailability of heavy metals.

Conclusions

Phytoextraction by Sedum plumbizincicola activated the heavy metals in the soil matrix. RSC application significantly lowered the Cd and Cu concentrations in the soil solution at
early stages of subsequent rice growth (from pre-tillering to booting stage) then grain yield increased and the Cd and Cu concentrations in the brown rice were diluted and lowered. Thus, after phytoextraction and then application of RSC, the simultaneous phytoextraction of the soil and production of safe foodstuffs on the contaminated soil were accomplished.

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