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ABSTRACT

Human activities have resulted in As and heavy metals accumulation in paddy soils in China. Phytoremediation has been suggested as an effective and low-cost method to clean up contaminated soils. A combined soil-sand pot experiment was conducted to investigate the influence of red mud (RM) supply on iron plaque formation and As and heavy metal accumulation in two wetland plant species (Cyperus alternifolius Rottb., Echinodorus amazonicus Rataj), using As and heavy metals polluted paddy soil combined with three rates of RM application (0, 2%, 5%). The results showed that RM supply significantly decreased As and heavy metals accumulation in shoots of the two plants due to the decrease of As and heavy metal availability and the enhancement of the formation of iron plaque on the root surface and in the rhizosphere. Both wetland plants supplied with RM tended to have more Fe plaque, higher As and heavy metals on roots and in their rhizospheres, and were more tolerant of As and heavy metal toxicity. The results suggest that RM-induced enhancement of the formation of iron plaque on the root surface and in the rhizosphere of wetland plants may be significant for remediation of soils contaminated with As and heavy metals.

KEYWORDS
arsenic; heavy metals; iron plaque; red mud; wetland plant

Introduction

Contamination of soils by As and heavy metals is a worldwide problem that affects a large number of sites. The accumulation of As and heavy metals in soil is an important issue because of the adverse effects they may have on food quality, soil health, and the environment (Gray *et al.* 2006; Zhuang *et al.* 2009; Liu *et al.* 2013a). For example, As is the second most common inorganic contaminant after Pb at Superfund sites in the USA, being present at 41% of the sites (USEPA 1997). Perhaps the most important of the anthropogenic sources of As is associated with mining and smelting activities. Arsenic is usually associated with gold (Au), copper (Cu), and lead (Pb) ores, and the mining and smelting of these ores can lead to complex situations with pollution by both As and metals.

As public awareness of the adverse effects of As and heavy metals on animal and human nutrition has grown, interest in developing guidelines and remediation technologies for mitigating As- and heavy metal contaminated ecosystems has also increased. In China, as most contaminated soils are located in agricultural areas, one may reasonably assume that remediated soils would be reusable in most cases (Zhuang *et al.* 2009). Thus, innovative, low-cost, low-input technologies are needed for the remediation of contaminated agricultural soils and community acceptance.

Recently, wetland plants have been shown to play important roles in constructed wetlands to remove As and heavy metals from polluted sediments and wastewater (Ye *et al.* 2001; Lizama *et al.* 2009). Wetland plant oxygenates its rhizosphere, resulting in the formation of an iron (Fe) oxyhydroxide plaque (Armstrong 1964). The main forms of iron plaque are ferric hydroxides, goethite, and lepidocrocite (Chen *et al.* 1980). Due to the high adsorption capacity of functional groups on iron hydroxides, Fe plaque could influence plant uptake of As and heavy metals and the effect of Fe plaque on As and metal uptake may depend on the amount of Fe plaque on the root surface (Otte *et al.* 1989; Zhang *et al.* 1998; Hu *et al.* 2007; Liu *et al.* 2013b). Thus, Fe plaque may be a barrier or a buffer to As and the metals (Taylor and Crowder 1983; Liu *et al.* 2004). The formation of Fe plaque on wetland plant roots is known to be influenced by Fe^{2+}/Mn^{2+} availability, redox potential and radial oxygen loss rate (ROL) of root (Weiss *et al.* 2003).

Another promising technology is the in situ stabilization of As and heavy metals in soil by the addition of Fe-rich amendments (Lee *et al.* 2011). Oxy-hydroxides of Fe have been identified as primary sinks for As in soils (Lombi *et al.* 2004). Boisson *et al.* (1999) reported that application of steel shot to a contaminated soil was effective in decreasing the mobility of As. Lombi *et al.* (2004) showed that the application of red mud

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Table 1. Physico-chemical properties of the paddy soil and red mud used for the experiment.

Parameter	Soil	Red mud
pH	4.57	11.1
SSA N_2-BET ($m^2\ g^{-1}$)	–	12.23
CEC (cmol(+) kg^{-1})	21	13.2
Organic matter (g kg^{-1})	44.3	-
Total N (g kg^{-1})	2.59	-
Total P (g kg^{-1})	0.337	-
Total Pb (mg kg^{-1})	632	62
Zn (mg kg^{-1})	217	94
Cd (mg kg^{-1})	1.96	< 0.01
As (mg kg^{-1})	68.16	9.31
Fe (g kg^{-1})	70.59	210
Mn (mg kg^{-1})	65.37	-
Al (g kg^{-1})	–	120

Note: - means not determined.

(RM), a by-product of aluminium (Al) manufacturing and rich in iron oxy-hydroxides, was simultaneously effective in decreasing the mobility of As and Cu. Similarly, application of RM to As and heavy metal contaminated soil was shown to decrease the availability and plant uptake of As, Cd, Pb, and Zn (Lee *et al.* 2011). Therefore, the use of Fe-rich amendments could be appropriate to treat soils contaminated with both As and heavy metals.

However, technologies to simultaneously treat As and metals contaminants are far from complete. In addition, several studies have investigated the ability of Fe-rich amendments or wetland plants to plantation reduce As and heavy metal mobility in contaminated soils, the influence of Fe-rich amendments per se on Fe plaque on root and Fe oxidation in rhizosphere of wetland plants remains poorly investigated. In this study, we assessed the risk reduction caused by the use of RM, combined with two wetland plant species (*Cyperus alternifolius* Rottb. and *Echinodorus amazonicus* Rataj) with different As and heavy metal accumulation, based on the results obtained from our earlier experiments (Yang *et al.* 2010; Li *et al.* 2011), to remediate soil contaminated with As and heavy metals.

Materials and methods

Soil properties and preparation

The soil used in the pot trial was collected from an abandoned hydragric paddy field (0–20 cm) in Huanjiang city, Guangxi Province, China. The soil was thoroughly mixed, air-dried and grounded to < 2 mm. The physical and chemical properties of the soil were analyzed and presented as Table 1. The soil used in the pot trail had a pH (1:5 soil:water suspension) of 4.57 and contained 44.3 g kg^{-1} organic matter, 2.59 g kg^{-1} total N and 0.337 g kg^{-1} total P as measured by the standard methods given in Jackson (1958). Total Pb, Zn, Cd, As, Fe, and Mn concentrations of the soil were 632 mg kg^{-1}, 217 mg kg^{-1}, 1.96 mg kg^{-1}, 68.16 mg kg^{-1}, 70.59 g kg^{-1} and 65.37 mg kg^{-1}, respectively, measured according to Jackson (1958). The cation exchange capacity (CEC) of the soil, determined with the $BaCl_2$-triethanolamine method, was 21 cmol(+) kg^{-1}. The sources of As and heavy metals contamination were mining.

Soil amendment

A fresh RM sample was collected from the Shandong Aluminum Limited Company (Zibo City, Shandong Province, China), which was the residue of the Bayer Process for extracting alumina from bauxite. The RM sample was dried overnight at 105 °C (Luo *et al.* 2011), finely ground and sieved to <0.075 mm. The physical and chemical properties of the RM were presented as Table 1. The RM collected had a pH (1:5 soil: water suspension) of 11.1 and contained 210 g kg^{-1} total Fe, 120 g kg^{-1} total Al, 62 mg kg^{-1} total Pb, 94 mg kg^{-1} total Zn and 9.31 mg kg^{-1} total As measured by the standard methods given in Jackson (1958) (Table 1). Cadmium concentration in RM was below the detection limit (BDL = 0.01 mg kg^{-1}). The cation exchange capacity (CEC) of RM, determined with the $BaCl_2$-triethanolamine method, was 13.2 cmol(+) kg^{-1}. The specific surface area, determined by the BET/N_2-adsorption method (Sorptomatic CarloErba), was 12.23 $m^2\ g^{-1}$ for RM. X-ray diffraction (XRD) analysis was performed using a PANAnalytical X'Pert Pro X-ray diffractometer equipped with a Cu source (Cu Kα).

Pot experiment

Sand is a common substrate used in constructed wetlands (Bubba *et al.* 2003) and so a rhizobag soil sand combination incubation pot experiment was designed for the present study. Rhizobags, made of nylon netting with a mesh size of 40 μm, were 5 cm in diameter and 8 cm height filled with 50 g of sand. The sand was collected from Shahe, Beijing, PR China and was not contaminated with heavy metals. Before use, the sand was washed, air-dried and sieved (< 2 mm). The sand-filled rhizobags were placed in the center of each soil pot (10 cm diameter × 12 cm height). *C. alternifolius* and *E. amazonicus*, two common wetland plant species in Southern China, were transplanted into sand at the beginning of the pot experiment and the sand is here referred to as 'rhizosphere' material at the end of the study as it was totally permeated by roots. The rest of the pot outside the rhizobag was filled with 0.45 kg of air-dried paddy soil with an inundation depth of c. 3–4 cm; this soil is here referred to as 'non-rhizosphere' (the comparable soil zone). This design successfully prevented roots and even root hairs from entering the adjacent non-rhizosphere soil zone, whilst permitting the transfer of microfauna and root exudates between the two compartments. This meant that although the soil was used to enclose the outside of the rhizobag, the rhizosphere was confined to the sand compartment and effectively separated from the non-rhizosphere soil compartment.

Two wetland plant species (*C. alternifolius* and *E. amazonicus*) were used for the pot trial. Tillers of *C. alternifolius* and *E. amazonicus* were all collected from "clean," i.e., no As and heavy metal contamination) sites. Cuttings of the two vegetative species were rooted in sand and watered with 20% Hoagland solution (Hoagland and Arnon 1938). In order to minimize carry-over effects resulting from growth in the field, a minimum of three successive vegetative generations, i.e., ramets were removed from the parents with at least three vegetative generations before use. Considering the different growth rates of the species studied, new uniform tillers (about 10 cm in

Table 2. Effect of RM application on plant height (cm) and biomass (g plant^{-1}) of the two plants exposed to polluted paddy soils.

Species	RM levels	Plant height	Root weight	Shoot weight
C. alternifolius	0	69.2 ± 1.46 b	2.45 ± 0.13 b	11.82 ± 0.55 c
	2%	72.3 ± 0.21 ab	3.28 ± 0.27 a	14.59 ± 0.91 b
	5%	73.8 ± 1.21 a	3.69 ± 0.21 a	17.99 ± 0.35 a
E. amazonicus	0	24.6 ± 0.28 b	0.65 ± 0.04 b	1.01 ± 0.08 b
	2%	27.9 ± 0.94 b	0.83 ± 0.04 a	1.26 ± 0.02 a
	5%	30.8 ± 1.44 a	0.73 ± 0.02 ab	1.44 ± 0.06 a

Note: Different letters within the same column of height and biomass of the two plants indicate significant variation between RM treatments at $P < 0.05$.

height) from propagation were selected. For each species, there were three treatments with three levels of RM (0, 2%, 5%). Three replicates were prepared for each plant species and each treatment. For each treatment, soil in the pots was kept flooded with about 2 cm water. All plants were grown in a light- and temperature-controlled greenhouse at a day (16 h, 22–28°C)/night (8 h, 16–22°C) regime. Rhizobag pots were arranged in a randomized design and their positions in the glasshouse were rotated regularly to ensure uniform growing conditions. At the end of 180 days, plants in each rhizobag pot were carefully removed from the sand, and the rhizosphere and non-rhizosphere materials separated. Plants were manually separated into roots and shoots, thoroughly rinsed with deionized water and used for the determination of height, biomass, concentrations of As, Pb, Zn, Cd, Fe, and Mn in root and shoot tissues and on root surfaces.

At harvest, Fe plaque on fresh root surfaces or sand (the rhizosphere material) was extracted using dithionitecitrate-bicarbonate (DCB solution containing 0.3 M sodium citrate, 1.0 M sodium hydrogen carbonate, with the addition of 1.5 g sodium dithionite) (Taylor and Crowder 1983; Otte *et al.* 1989). Roots or sand were immersed in 22.5 mL DCB solution and agitated for 3 h at room temperature. The extract was then filtered with quantitative filter papers, rinsed three times with deionized water that finally was added to the DCB extract. The resulting solution was made up to 100 mL with deionized water. After extraction with DCB, roots and shoots were oven-dried to constant weight at 60°C for chemical analysis. Available Pb, Zn and Cd in non-rhizosphere soils were extracted by diethylenetriamine pentaacetic acid (DTPA: 0.005 M DTPA, 0.1 M triethanolamine and 0.01 M $CaCl_2$ at pH 7.3) in a soil: solution ration 1:2 (V/V) (Lindsay and Norvell 1978). Available As in non-rhizosphere soils were extracted by sodium hydrogen carbonate ($NaHCO_3$: 0.05 M $NaHCO_3$) in a soil: solution ration 1:10 (V/V) (Bandyopadhyay *et al.* 2004).

Chemical analysis

Oven-dried plant shoot or root samples were ground using a Retsch grinder (Type: 2 mm, Retsch Company, made in Germany), and As, Cd, Pb and Zn in plant tissue was extracted by digesting of the sample Oven-dried plant tissues were digested in a mixture of $HNO_3/HCLO_4$ (85/15, V/V) and total concentrations of Cd, Pb, Zn, Fe, and Mn and DTPA-extractable Pb, Zn, and Cd were determined by inductively coupled plasma mass spectrometry (ICP-MS, Elan 5000, Perkin Elmer, USA). Total concentration of As was determined by liquid chromatography coupled to atomic fluorescence spectroscopy (LC-AFS, AFS-9130, Titan Instrument, Beijing, China). The temperature of digestion was controlled under 110°C to avoid As volatilization (Cai *et al.* 2000). Blank and bush leaf material (BGW-07603) (China Standard Materials Research Center, Beijing, PR China) were used for quality control. The recovery rates of As, Fe, Mn, Pb, Zn, and Cd were 90 ± 10%.

Statistical analysis

Data on plant performances were tested for their normality and variance prior to a one-way analysis of variance (ANOVA), as no data transformation was needed. If the differences among different amendment treatments for each plant species were significant at 5% level, the least significant difference (LSD) was calculated as the post hoc test to determine where differences lay. All statistical analyses were performed using the SPSS 11.0 statistical package.

Results

Plant growth

The growth (height, roots, and shoots) of both wetland plants was significantly ($P < 0.05$) increased in RM-amended soils compared to in untreated paddy soil (Table 2). In particular, the above ground biomass of *C. alternifolius* and *E. amazonicus* grown in the soil amended with 5% RM increased, respectively, by a factor of 0.52 and 0.51 compared to the control plants.

Table 3. Effect of RM application on As and heavy metal concentrations (mg kg^{-1} DW) in the shoot and root of the wetland plants exposed to polluted paddy soils.

		Root				Shoot			
Species	RM levels	Pb	Zn	Cd	As	Pb	Zn	Cd	As
C. alternifolius	0	42.21±4.82 a	14.7 ± 0.29 a	0.233 ± 0.015 a	70.6 ± 2.4 a	9.93 ± 0.70 a	29.95 ± 2.15 a	0.078 ± 0.005 a	3.59 ± 0.14 a
	2%	17.98 ± 3.96 b	10.75 ± 0.37 b	0.085 ± 0.005 b	52.7 ± 2.9 b	5.37 ± 0.57 b	23.29 ± 0.79 b	0.044 ± 0.004 b	2.59 ± 0.09 b
	5%	10.29 ± 0.33 b	8.47 ± 0.13 c	0.016 ± 0.008 b	43.6±1.5 c	2.82 ± 0.41 c	19.79 ± 0.76 b	0.015 ± 0.004 c	1.89 ± 0.12 c
E. amazonicus	0	284.93 ± 7.71a	108.47 ± 9.77 a	6.63 ± 0.45 a	87.7 ± 3.3 a	8.58 ± 0.20 a	48.0 ± 1.83 a	0.445 ± 0.043 a	5.39 ± 0.12 a
	2%	30.91 ± 3.22 b	71.79 ± 3.09 b	2.46 ± 0.23 b	78.3 ± 3.8 a	6.66±0.03 b	41.37 ± 1.26 a	0.176 ± 0.022 b	3.09 ± 0.16 c
	5%	18.51 ± 0.63 b	39.26 ± 2.59 c	1.30 ± 0.17 c	50.0 ± 4.6 b	4.50 ± 0.25 c	23.16 ± 2.87 b	0.093 ± 0.013 b	4.19 ± 0.09 b

Note: Different letters within the same column of heavy metal in plant tissues of the two plants indicate significant variation between RM treatments at $P < 0.05$.

Table 4. Effect of RM application on DCB-extractable Fe, Mn, As, and heavy metal concentrations (mg kg^{-1}) in iron plaque on the root surface of the wetland plants exposed to polluted paddy soils.

		Root surface					
Species	RM levels	Fe	Mn	Pb	Zn	Cd	As
C. alternifolius	0	2116 ± 186 c	46.79 ± 1.95 a	8.38 ± 0.23 c	4.36 ± 0.19 c	0.153 ± 0.015 c	15.1 ± 0.26 c
	2%	3479 ± 46 b	73.73 ± 1.27 b	10.59 ± 0.703 b	7.05 ± 0.44 b	0.237 ± 0.024 b	19.2 ± 0.52 b
	5%	4936 ± 176 a	82.52 ± 0.57 c	13.4 ± 0.401 a	12.18 ± 0.61 a	0.373 ± 0.020 a	28.0 ± 0.66 a
E. amazonicus	0	477 ± 101 b	17.27 ± 3.07 c	2.55 ± 0.095 b	1.93 ± 0.11 c	0.103 ± 0.012 c	12.1 ± 0.26 c
	2%	712 ± 55 b	32.37 ± 4.06 b	4.57 ± 0.16 b	3.75 ± 0.29 b	0.17 ± 0.036 b	15.4 ± 0.44 b
	5%	1868 ± 257 a	49.97 ± 2.05 a	7.12 ± 1.03 a	6.87 ± 0.53 a	0.243 ± 0.025 a	22.4 ± 0.89 a

Note: Different letters within the same column of metals on root surface of the two plants indicate significant variation between RM treatments at $P < 0.05$.

As and heavy metal concentrations in plants

The application of RM significantly ($P < 0.05$) decreased the concentrations of As and heavy metals in roots and shoots of *C. alternifolius* and *E. amazonicus* compared to the control soil (Table 3), except that for Zn and As concentrations in the roots of *E. amazonicus* grown in the soil amended 2% RM. Arsenic and heavy metals in the two plants followed the order of root tissues > shoot tissues.

Concentrations of Fe, Mn, As, and heavy metal on root surface and rhizosphere sand

The application of RM significantly ($P < 0.05$) increased the concentrations of Fe, Mn, As and heavy metals on root surfaces and in rhizospheres of *C. alternifolius* and *E. amazonicus* compared to the control soil (Tables 4 and 5). Iron concentrations were also higher than Mn, As, or heavy metals on both root and sand surfaces.

Concentrations of $NaHCO_3$-extractable as and DTPA-extractable heavy metals and pH in the non-rhizosphere soil

The application of RM significantly ($P < 0.05$) decreased the concentrations of $NaHCO_3$-extractable As and DTPA-extractable heavy metals in the non-rhizosphere soils (Table 6). There was most noticeable in the 5% red mud amendment, which decreased the proportion of extractable Pb, Zn, and Cd to approximately 25%, 35%, and 84%, respectively, of the total metal content in the non-rhizosphere soils.

Following the application of RM, there was a significant ($P < 0.05$) increase in soil pH from 4.57 to approximately 5.16 and 5.64 in the 2% and 5% RM amended non-rhizosphere soils, respectively (Table 6).

Correlations between as, heavy metal, and Fe and Mn on roots and in the rhizosphere

Positive correlations were observed between As, Pb, Zn, Cd concentrations and Fe or Mn concentration on root surfaces, as well as between As, Pb, Zn, Cd, and Fe or Mn concentration on sand surfaces (Figs. 1 and 2).

Discussion

The application of soil amendments that can immobilize As and/or heavy metals in situ may provide a cost-effective and sustainable solution for remediation of contaminated soils (Mench *et al.* 2000).

Many studies have shown that addition of RM amendment could significantly reduce As and heavy metals availability (Lombi *et al.* 2004; Lee *et al.* 2011; Garau *et al.* 2011). Lee *et al.* (2011) indicated that the application of 5% red mud significantly reduced As, Cd, Pb and Zn extracted by 0.1 M $Ca(NO_3)_2$ by up to 29%, 98%, 99%, and 99%, respectively. In the present study, the application of red mud also significantly decreased $NaHCO_3$-extractable As and DTPA-extractable heavy metal concentrations (Table 6), which represent As and heavy metals that are sorbed onto soil solid phase and able to be desorbed into solution to replenish the soluble As and heavy metals pool (McLaughlin *et al.* 2000).

Table 5. Effect of RM application on DCB-extractable Fe, Mn, As, and heavy metal concentrations (mg kg^{-1}) in iron plaque on the rhizosphere of the wetland plants exposed to polluted paddy soils.

		Rhizosphere					
Species	RM levels	Fe	Mn	Pb	Zn	Cd	As
C. alternifolius	0	549 ± 9.8 c	14.5 ± 0.53 c	1.24 ± 0.07 c	0.257 ± 0.015 c	0.115 ± 0.006 c	3.87 ± 0.19 c
	2%	621 ± 8.5 b	17.4 ± 0.53 b	1.53 ± 0.05 b	0.503 ± 0.024 b	0.162 ± 0.004 b	5.87 ± 0.29 b
	5%	782 ± 4.5 a	21.2 ± 0.71a	2.63 ± 0.07 a	0.707 ± 0.082 a	0.257 ± 0.018 a	7.73 ± 0.38 a
E. amazonicus	0	473 ± 15 b	6.82 ± 0.18 c	1.01 ± 0.04 b	0.181 ± 0.005 b	0.060 ± 0.006 b	2.13 ± 0.22 c
	2%	560 ± 7 a	7.66 ± 0.24 b	1.09 ± 0.009 b	0.257 ± 0.023 b	0.083 ± 0.003 b	3.67 ± 0.12 b
	5%	587 ± 4 a	10.45 ± 1.07 a	1.20 ± 0.027 a	0.547 ± 0.049 a	0.14 ± 0.023 a	5.40 ± 0.23 a

Note: Different letters within the same column of metals in the rhizosphere of the two plants indicate significant variation between RM treatments at $P < 0.05$.

Table 6. Effect of RM application on $NaHCO_3$-extractable As concentrations, DTPA-extractable Pb, Zn, and Cd concentrations (mg kg^{-1}) and pH in the non-rhizosphere of wetland plants exposed to polluted paddy soils.

		$NaHCO_3$ (mg kg^{-1})	DTPA (mg kg^{-1})			
Species	RM levels	As	Pb	Zn	Cd	pH
C. alternifolius	0	1.80 ± 0.012 a	307 ± 4.9 a	4.29 ± 0.05 a	0.36 ± 0.025 a	4.82 ± 0.07 c
	2%	1.25 ± 0.087 c	263 ± 3.7 b	3.68 ± 0.16 b	0.083 ± 0.009 b	5.17 ± 0.05 b
	5%	1.48 ± 0.057 b	230 ± 1.7 c	2.79 ± 0.16 c	0.059 ± 0.001 b	5.81 ± 0.14 a
E. amazonicus	0	1.72 ± 0.024 a	287 ± 8.9 a	4.67 ± 0.12 a	0.30 ± 0.045 a	4.68 ± 0.05 c
	2%	1.28 ± 0.055 c	258 ± 3.7 b	3.91 ± 0.07 b	0.086 ± 0.005 b	5.14 ± 0.12 b
	5%	1.53 ± 0.061 b	238 ± 1.4 b	3.47 ± 0.08 c	0.065 ± 0.002 b	5.47 ± 0.04 a

Note: Different letters within the same column of available metals in the non-rhizosphere of the two plants indicate significant variation between RM treatments at $P < 0.05$.

The biomass of experimental plants can serve as an indicator of heavy metal toxicity (Deng *et al*. 2006). The present study clearly showed that dry weights of the two tested wetland species were significantly increased in amended treatments compared to the control, may be due to a better heavy metals sorbing capacities of RM or to their ability to change soil pH, thus alleviating heavy metal toxicity and promoting plant growth (Castaldi *et al*. 2009).

Concentrations of As and heavy metal in shoots and roots also showed remarkable differences between the two wetland plants grown in the paddy soil without amendment treatment (Table 3) also demonstrating different abilities in As and heavy metal transport and accumulation in the two wetland plant species (Yang *et al*. 2010; Li *et al*. 2011). Concentrations of As and heavy metal in roots were higher than those in shoot tissues ($P < 0.05$). The reason for concentrations of As and heavy metals in the shoots of two wetland plants were maintained at a relative low level compared to those in the root tissues might be due to the operation of an exclusion mechanism (Baker 1981). The notably low Pb concentrations in shoots of *C. alternifolius* and *E. amazonicus* (8.58–9.93 mg kg^{-1}) also explains why plants grown in the 632 mg Pb kg^{-1} treatment did not show any visible Pb toxicity symptoms. However, even *E. amazonicus*, having the highest accumulation of Pb in roots (up to 284 mg Pb kg^{-1}), still did not show any severe symptoms of Pb toxicity. These findings indicate that internal mechanisms of Pb detoxification may also exist in some wetland plants, such as *E. amazonicus,* in addition to the exclusion mechanism. Recently, Yang and Ye (2015) indicated that the antioxidant system of wetland plants may play an important role in alleviating Pb toxicity. Baker (1981) also suggested that internal (genetically-determined) tolerance mechanisms can exist in plants when grown in heavy metal-contaminated soils.

Concentrations of As and heavy metals in shoots of both wetland plants grown in the paddy soil decreased with increasing rates of red mud application (Table 3). For example, by 6 months, the application of 5% RM had reduced As, Pb, Zn, and Cd concentrations in shoot of *E. amazonicus* from 5.39, 8.58, 48, and 0.445 to 4.19, 4.5, 23.16, and 0.093 mg kg^{-1} compared to the control, respectively. This result strongly suggested that the application of red mud significantly decreased As and heavy metals uptake by wetland plants. There are three principal mechanisms controlling the effect of red mud on As and heavy metal uptake into wetland plant. Firstly, an increase in soil pH caused by the application of red mud (Table 6). This increase in soil pH has been shown in other studies where red mud has been used as a soil amendment (Lombi *et al*. 2002; Friesl *et al*. 2004) and was a result of the alkaline nature of red mud which has a pH of greater than 10. An increase in soil pH results in a corresponding increase in the net negative charge of variably charged colloids in soils such as clays, organic matter and Fe and Al oxides. This can result in an increase in heavy metal sorption and a decrease in desorption and hence reduction in soluble metal concentrations in soils (Gray *et al*. 1998). Secondly, the large content of Fe and Al oxides in red mud introduces new sorptive surfaces which may immobilize As and heavy metals in soils, through specific or chemisorption. The great reduction in extractable As and heavy metals was likely due to their sorption via inner sphere complexation on the reactive surface of newly formed Fe oxides. For example, several studies (Lee *et al*. 2011; Garau *et al*. 2011) have used chemical sequential fractionation to show that amending contaminated soils with red mud results in a redistribution of As and heavy metals from soluble and exchangeable pools to the Fe, Al and Mn oxide and residual fractions. In addition, Luo *et al*. (2011) investigated the sorption mechanism of Cd on red mud using X-ray absorption near edge structure (XANES) spectroscopy and supplied evidence of the formation of inner-sphere complexes of Cd similar to XCdOH (X represents surface groups on red mud) on the red mud surfaces although outer-sphere complexes of Cd were the primary species. Once As and heavy metals are specifically sorbed onto these oxide surfaces, potentially they may become irreversibly fixed as a result of several mechanisms, including adsorption processes and co-precipitation (Kumpiene *et al*. 2008). Though some studies showed that the influence of alkaline materials could increase As mobility in soil (Mench *et al*. 2006; Kumpiene *et al*. 2008), there was also evidence that specific sorption of metals by Fe and Al oxides in red mud was more stable than simple pH moderated sorption (Lombi *et al*. 2003). Recently, by two years after red mud addition, Garau *et al*. (2011) showed that red mud addition caused a pH increase, a striking decrease of As and heavy metal (Pb, Zn, and Cd) mobility in the red mud-soil compared to the contaminated soil.

Thirdly, the application of red mud increased the formation of iron plaque on the root surface and in the rihzosphere (Tables 4 and 5). Root surfaces and rhizosphere material (sand) of the two wetland species appeared more reddish with the application of red mud. Present results indicated that DCB-extractable Fe/Mn on iron plaque of roots and in rhizosphere

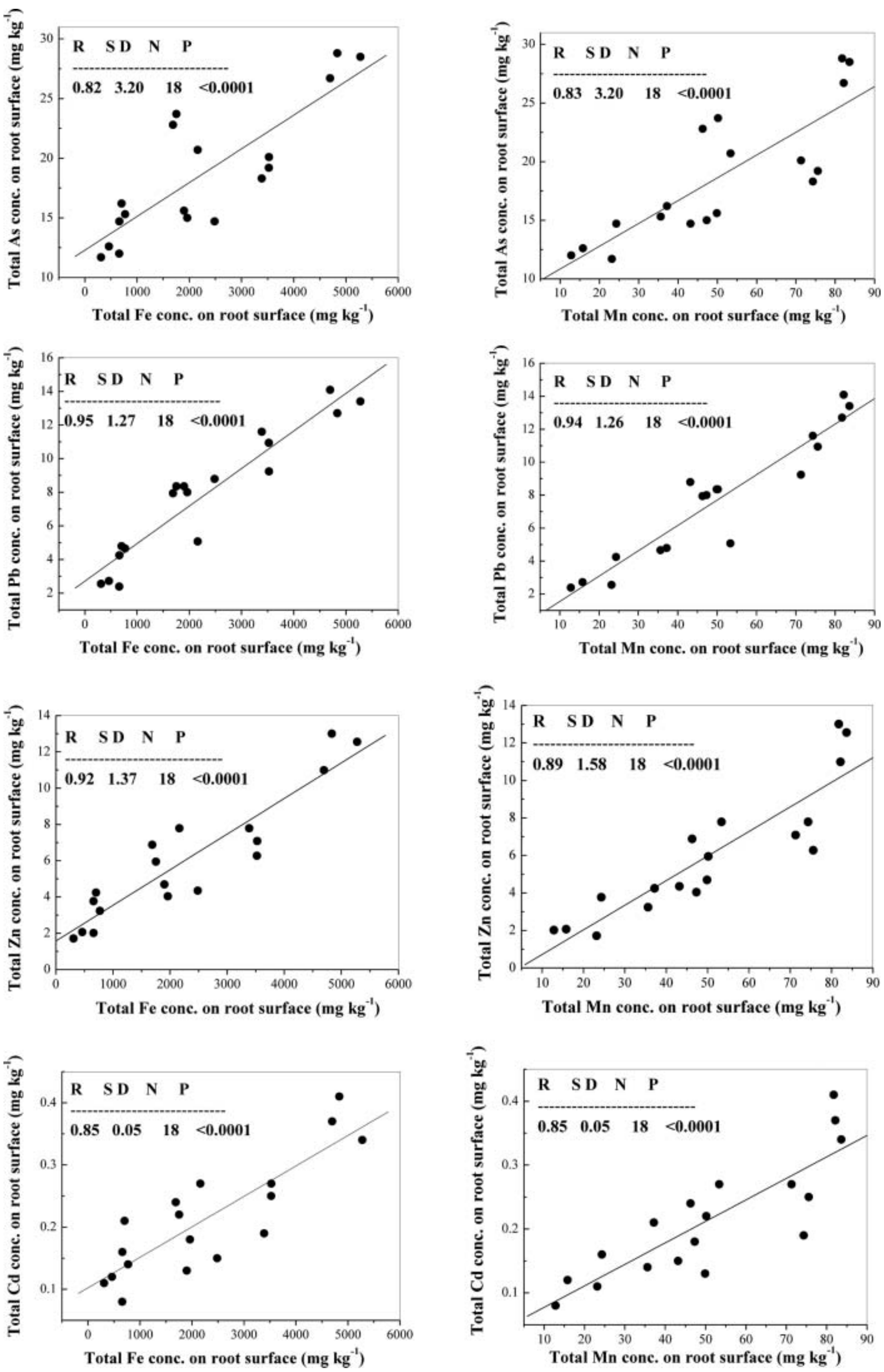

Figure 1. Correlations between total Fe (left panels) or Mn (right panels) concentrations and total As, Pb, Zn, and Cd concentrations on root surfaces of the two wetland plants exposed to the control soil and RM-amended soils.

of the studied wetland plants was significantly increased with the increasing RM application (Tables 4 and 5), which strongly suggested that red mud addition could induce the formation of iron-manganese oxides plaque on root surface and on the rhizosphere sand. Red mud-induced enhancement of plaque formation is probably due to an increase in concentrations of Fe^{2+} in soil with red mud addition. Our results showed that the concentrations of Fe were higher than those of Mn both on root surfaces and in the rhizospheres (Table 5). Similar results have been reported by Ye *et al*. (2001) and Hu *et al*. (2007). It is possible that Fe oxides and hydroxides may precipitate at lower redox potentials than Mn oxides at any pH values, or at a lower pH than Mn under fixed Eh conditions (St-Cyr and Crowder 1990).

Our study showed that the concentrations of As, Pb, Zn, and Cd on root surfaces were significantly correlated to Fe ($P < 0.05$) and Mn ($P < 0.05$) concentrations on root surfaces when the two wetland species were grown in the paddy soils amended with red muds (Fig. 1). Similarly, a positive correlation was also observed between As, Pb, Zn, and Cd in rhizosphere and Fe ($P < 0.05$) or Mn ($P < 0.05$) concentrations in rhizosphere in Fig. 2. These results suggested that the

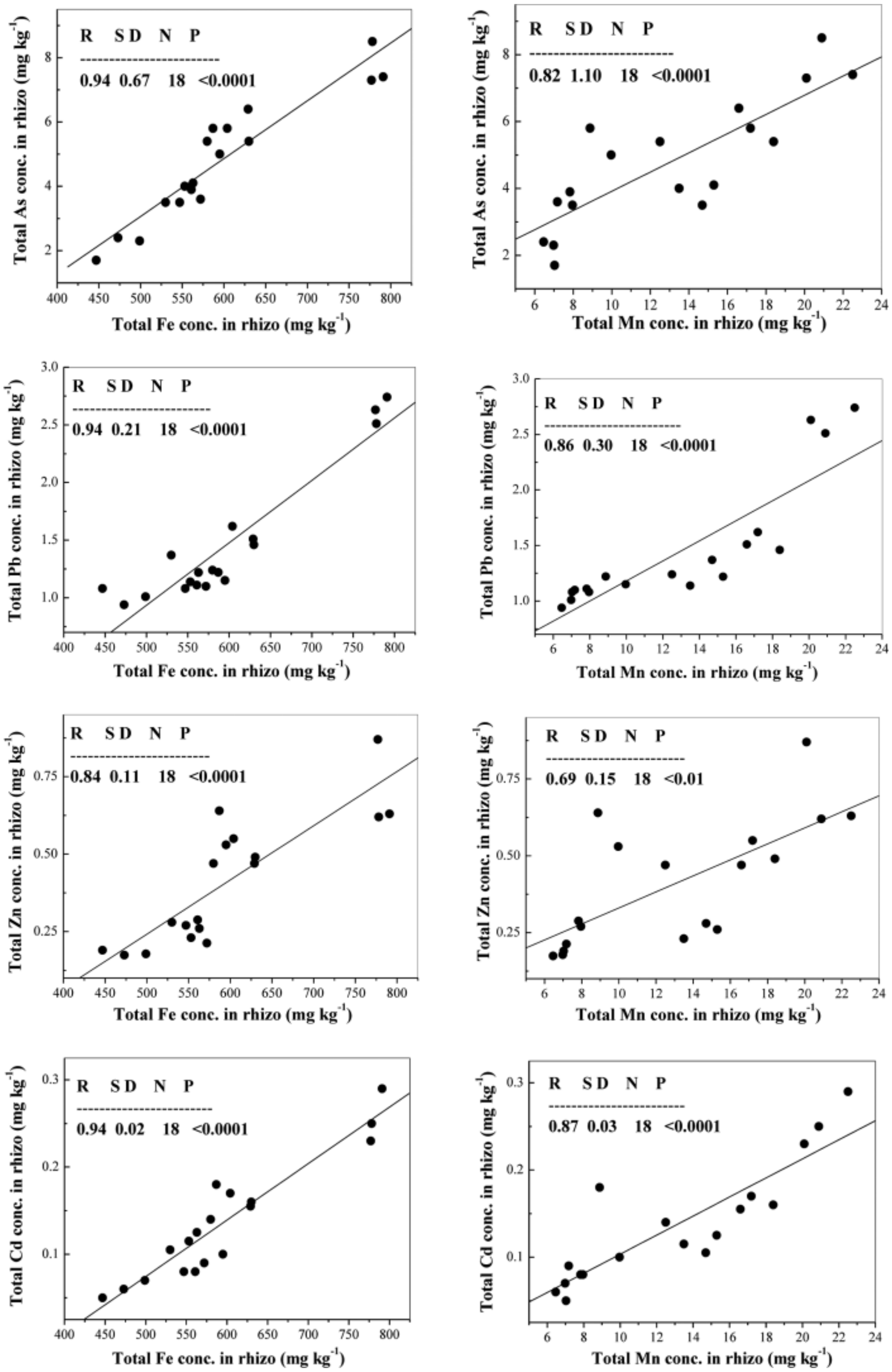

Figure 2. Correlations between total Fe (left panels) or Mn concentrations (right panels) and total As, Pb, Zn, and Cd concentrations on sand surfaces (rhizosphere) of the two wetland plants exposed to the control soil and RM-amended soils.

enhancement in the formation of Fe and Mn plaques on wetland plants can increase the As, Pb, Cd, and Zn adsorption on root surfaces and in rhizosphere. Compared to terrestrial plants, wetland plants could release more oxygen to rhizosphere and tend to form more iron plaque because of oxidizing Fe(II) to Fe(III) on their root surfaces and in rhizosphere and prevent toxic oxidative zone (Taylor and Crowder 1983). Due to the fact that concentrations of As, Pb, Cd, and Zn on root surfaces and in rhizosphere were positively correlated to Fe and Mn as indicated above, the enhancement in the formation of Fe plaque by the red mud addition would increase the concentrations of As, Cd, Zn, Fe, and Mn on root surfaces and in rhizosphere. Therefore, more As and heavy metal deposited in Fe plaque may result in less As and heavy metal transfer from root to shoot. An increase of As and metal adsorption on root surfaces due to Fe plaque has also been reported in other wetland plants, such as *Aster tripolium, Typha latifolia,* and *Juncus effusus* (Otte *et al.* 1989; Ye *et al.* 2001; Greipsson 1995; Li *et al.* 2011; Wang *et al.* 2011; Yang *et al.* 2014). In addition, our present results also indicated that the roots may act as a major barrier for As and heavy metal uptake and translocation (Table 3), as it was also reported that most of the absorbed As and heavy metal could accumulate in the outer cortex of roots of wetland plants (Ye *et al.* 1998; Vesk *et al.* 1999). Furthermore, at the cell membrane level, iron plaque may also act as an effective Fe reservoir to increase Fe concentrations in active cells and then ameliorate As and metal toxicity (Ye *et al.* 1998; Li *et al.* 2011).

Our results indicated that the overall effect of RM on As and heavy metal uptake into wetland plant may have been caused by (1) an increase in soil pH by RM addition; (2) the increased amount of Fe and Al oxides in RM; (3) the enhanced formation of Fe plaque by the RM addition. The high content of Fe and Al oxides in RM and the enhancement of Fe plaque were the two main processes. In addition, changes of pH and redox (Eh) in rhizosphere soils caused by root activities of wetland plants may be temporal and spatial, thus more in-depth studies on the effect of RM on As and heavy metals availability are clearly needed both in non-rhizosphere soils and bulk soils in the future.

Conclusion

These results obtained from the current study demonstrated that the application of red mud could significantly decrease As and heavy metal uptake by wetland plants. Together with the large decrease in extractable As and metal concentrations and the increase in soil pH following the application of red mud, the principal mechanism might be the red mud-induced enhancement of the formation of iron plaque on root surface and in the rhizosphere. The concentrations of Fe and Mn in iron plaque of root surface and in the rhizosphere increased with an increase in red mud application. It is envisaged that red mud-induced enhancement of the formation of iron plaque on the root surface and in the rhizosphere of wetland plants may be significant for remediation of soils contaminated with As and heavy metals.

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