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RESEARCH ARTICLE

Effects of alkaline and bioorganic amendments on cadmium, lead, zinc, and nutrient accumulation in brown rice and grain yield in acidic paddy fields contaminated with a mixture of heavy metals

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Abstract Paddy soils and rice (*Oryza sativa* L.) contaminated by mixed heavy metals have given rise to great concern. Field experiments were conducted over two cultivation seasons to study the effects of steel slag (SS), fly ash (FA), limestone (LS), bioorganic fertilizer (BF), and the combination of SS and BF (SSBF) on rice grain yield, Cd, Pb, and Zn and nutrient accumulation in brown rice, bioavailability of Cd, Pb, and Zn in soil as well as soil properties (pH and catalase), at two acidic paddy fields contaminated with mixed heavy metals (Cd, Pb, and Zn). Compared to the controls, SS, LS, and SSBF at both low and high additions significantly elevated soil pH over both cultivation seasons. The high treatments of SS and SSBF markedly increased grain yields, the accumulation of P and Ca in brown rice and soil catalase activities in the first cultivation season. The most striking result was from SS application (4.0 t ha^{-1}) that consistently and significantly reduced the soil bioavailability of Cd, Pb, and Zn by 38.5–91.2 % and the concentrations of Cd and Pb in brown rice by 20.9–50.9 % in the two soils over both cultivation seasons. LS addition (4.0 t ha^{-1}) also markedly reduced the bioavailable Cd, Pb, and Zn in soil and the Cd concentrations in brown rice. BF remobilized soil Cd and Pb leading to more accumulation of these metals in brown rice. The results showed that steel slag was most effective in the remediation of acidic paddy soils contaminated with mixed heavy metals.

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Introduction

Agricultural soil contaminated by heavy metals is of great environmental concern. Mining activities can result in excessive accumulation of heavy metals [e.g. cadmium (Cd), lead (Pb), zinc (Zn)] and acidification in farming soils (Zhuang et al. 2009; Li et al. 2014). Heavy metals in soils become highly bioavailable at low pH and affect soil properties, especially biological characteristics including the diversity of microorganisms and enzyme activities (Mohammed et al., 2011). Heavy metals also reduce crop growth and nutrient uptake and frequently accumulate in cereals, leading to potential hazards to human health (Mohammed et al. 2011). Paddy rice (*Oryza sativa* L.) is one of the most important cereal crops in the world, especially in Asian countries (Kögel-Knabner et al. 2010). However, an increasing number of paddy fields in some Asian countries are subjected to contamination by heavy metals because of mining activities (Li et al. 2012; Liu et al. 2012). Due to a serious shortage of arable land and the demand for an ever-expanding population, crops are still commonly grown on contaminated land. Consequently, the remediation of heavy metal-contaminated agricultural soils is urgently needed for safe food production and to protect human health in these areas.

Remediation techniques for heavy metal-contaminated agricultural soils include physical, chemical, and biological approaches. In situ chemical stabilization by adding amendments to soils has been considered as a cost-effective and environmentally compatible technology (Hseu et al. 2010). Soil amendments with addition of alkaline and organic materials, such as limestone, fly ash, lime, biochar, green manure, and composts, have been employed in recent years to stabilize heavy metals in soil, as they can increase soil pH, enhance metal-organic matter interactions, and/or the adsorption and precipitation of metals, leading to the decrease of metal bioavailability (Houben et al. 2012; Tang et al. 2015; Yang et al. 2016). Among the alkaline amendments, limestone and fly ash have been studied extensively for the remediation of contaminated soils, but most studies with them have been limited to laboratory or glasshouse conditions. Steel slags, consisting of variable proportions of calcium oxide, iron oxides, and phosphorus compounds, are a low cost and alkaline industrial byproduct from steel mills (Navarro et al. 2010). They have been applied in wastewater treatments as an absorbent to remove heavy metals (e.g., Cd and Pb) (Liu et al. 2009; Saki et al. 2013), and their addition can also decrease the available Cd in contaminated soils (Gu et al. 2011; Zhou et al. 2012). Nevertheless, the effects of steel slag on soil biological properties, mixed heavy metals, and nutrient uptake by crop plants have seldom been investigated under field conditions.

In addition to alkaline materials, organic fertilizers have been investigated as potential soil amendments. A bioorganic fertilizer is a combination of organic fertilizers and microorganisms. They have generally been applied to improve soil fertility, garden plant growth, and biocontrol of *Fusarium* wilt (Qiu et al. 2012; Wu et al. 2015). This amendment has been shown to influence the bioavailability of heavy metals in orchard soils (Wang et al. 2009). However, the effects of a bioorganic fertilizer on the availability of heavy metals in paddy soils and their uptake by rice plants are unknown. The combined use of alkaline materials such as steel slag and a bioorganic fertilizer on crop growth and heavy metal uptake by plants has also not been examined before although these two amendments may be complementary.

Most previous studies in this area have been limited to laboratory or glasshouse conditions and focused on a single toxic metal, and the results might therefore not be directly applicable to the field environment. A recent field study by Zheng et al. (2015) found that the use of bean stalk compost dramatically reduced soil Cd availability and its concentrations in rice in a paddy field over a cultivation season. However, the amendment efficiency can change with time (Bolan et al. 2014). It is thus important to assess the remedial effects of limestone, fly ash, and other amendments under field conditions in soils with a mixture of heavy metals and over consecutive cultivation seasons. Their effects on the biological properties of the soil, crop yield and the uptake of nutrients by crops, especially rice, should also be investigated under field conditions. The present study was therefore designed to investigate the effects of different amendments on soil biochemical properties, bioavailability of heavy metals in soils, and their concentrations in brown rice, as well as rice grain yield and nutrient uptake.

In the present work described here, two acidic paddy field sites contaminated with mixed heavy metals were studied over two rice cultivation seasons. The two paddy fields, namely Tongxi (TX) and Shangba (SB), were in proximity to traditional metal mines in Guangdong Province, southern China. They had different levels of Cd, Pb, and Zn contamination according to a previous field reconnaissance in 2012. Rice yields at TX were lower than those at SB. Based upon these findings, the effects of steel slag, bioorganic fertilizer, and their combination on rice grain yield, Cd, Pb, and Zn and nutrient accumulation in brown rice were tested. Additionally, the bioavailability of Cd, Pb, and Zn in soil and soil properties (pH and catalase) were investigated at TX. The SB trial was focused on a comparison of the effects of three alkaline amendments (steel slag, fly ash, and limestone) on the properties of rice and the acidic paddy soil.

Materials and methods

Experimental sites and materials

Seeds of rice (*O. sativa* cv. Tianyou 122), supplied as a pure line by the Rice Research Institute of Guangdong Academy of Agricultural Sciences, were grown at two paddy field sites, TX (Tongxi) (23°51′N; 113°39′E) and SB (Shangba) (24°27′N, 113°48′E). The former is in Tongxi village around the Tongxi mine, Qingyuan City. This area has a humid subtropical climate with an annual average temperature of 21.2 °C and rainfall of 2206 mm. The latter site is in Shangba village close to Dabaoshan mine, Shaoguan City. This area also has a humid subtropical climate with an annual average temperature of 20.3 °C and rainfall of 1762 mm. Both Tongxi and Dabaoshan mines are multi-metal mines commencing operations in the 1960s and are now still in full operation, releasing large volumes of acid mine drainage into local water channels and rivers. Soils at TX and SB have been subject to acidification and contamination by a mixture of heavy metals (Cd, Pb, and Zn), due to the use of the river water for agricultural irrigation.

Steel slag (SS), fly ash (FA), and limestone (LS) used in the study were obtained from the Shaoguan Steel Group Company, Huangpu Power Plant in Guangzhou and Yingde Lime Plant, respectively. The bioorganic fertilizer (BF) used, a mixture of amino acid fertilizer, cow manure composts, and *Bacillus subtilis*, was produced by Tianran Biological Engineering Technology Co. Ltd. in Fogang. The concentration of *B. subtilis* in BF, determined in Guangdong Detection Center of Microbiology, was *c*.1 × 10^8 CFU g^{-1}. These companies and plants are located in Guangdong Province, southern China. The main properties of the two soils and the four amendments are shown in Table 1.

Experimental design

The two paddy field sites (TX and SB) were prepared according to usual agricultural practices. At each, a total of 28 experimental plots was established, each with an area of 10 m^2 (4 × 2.5). The plots, with 20 cm uniform spacing between each and 30 cm ridges between adjacent plots, were divided into seven treatments, each with four replicates. The treatments at TX and SB are shown in Table S1. There were two levels of addition (low and high dosage) per amendment. At TX, seven treatments were made including a control (no amendment), SS1 (2.0 t ha^{-1} SS), SS2 (4.0 t ha^{-1} SS), BF1 (4.5 t ha^{-1} BF), BF2 (9.0 t ha^{-1} BF), SSBF1 (the combination of SS1 and BF1), and SSBF2 (the combination of SS2 and BF2). At SB, the seven treatments were control (no amendment), SS1 (2.0 t ha^{-1} SS), SS2 (4.0 t ha^{-1} SS), FA1 (3.0 t ha^{-1} FA), FA2 (6.0 t ha^{-1} FA), LS1 (2.0 t ha^{-1} LS), and LS2 (4.0 t ha^{-1} LS).

The amendments were added on a dry weight basis at the beginning of the experiment and mixed thoroughly with the soils by tilling to a depth of 15 cm prior to transplanting 20-day-old rice seedlings which had first been grown in a separate seedbed at an uncontaminated field. Early season rice seedlings were transplanted on 8th of April and harvested after 90 days, and late season rice seedlings were transplanted on 20th of July and harvested after 100 days, in 2013–2014. All control and treatment plots followed the same scheme of agricultural managements, such as conventional fertilization, irrigation with clean water, and manual weeding. For fertilization, the common practice was followed among farmers in Guangdong Province. Applied chemical fertilizer was a type of bulk-blended fertilizer (N:P:K = 24:6:18, GB21633-2008, Nongbao Fertilizer Co., Ltd., Qingxin, China). Fertilizers were applied to the rice crops at a rate of 240, 180, and 180 kg ha^{-1} in each one of the three phases: before transplantation, at the tillering stage, and at the panicle-formation stage, respectively.

Table 1 Properties of the rice paddy soils (mean ± SE, *n* = 5) and amendments (mean ± SE, *n* = 4)

	Soils		Amendments			
Property	Tongxi soil	Shangba soil	Steel slag	Fly ash	Limestone	Bioorganic fertilizer
pH (1:2.5 *w*/*v* water)	4.30 ± 0.04b	4.65 ± 0.03a	11.8 ± 0.14	10.0 ± 0.13	12.0 ± 0.13	7.65 ± 0.02
Total Cd (mg kg^{-1})	4.02 ± 0.04a	1.83 ± 0.03b	0.06 ± 0.002	0.10 ± 0.003	0.09 ± 0.005	0.10 ± 0.003
Total Pb (mg kg^{-1})	107 ± 2.0b	269 ± 19a	23.3 ± 0.1	29.6 ± 0.6	3.95 ± 0.10	9.82 ± 0.17
Total Zn (mg kg^{-1})	720 ± 15a	402 ± 17b	80.8 ± 3.5	39.2 ± 1.0	3.83 ± 0.01	77.0 ± 3.9
Total P (g kg^{-1})	0.64 ± 0.02a	0.61 ± 0.02a	6.78 ± 0.1	1.63 ± 0.02	0.08 ± 0.003	5.46 ± 0.10
Total K (g kg^{-1})	0.51 ± 0.03b	0.80 ± 0.03a	0.09 ± 0.003	0.57 ± 0.05	0.20 ± 0.01	3.47 ± 0.06
Total Ca (g kg^{-1})	1.30 ± 0.04a	1.12 ± 0.02b	216 ± 9.0	69.0 ± 1.5	380 ± 7.0	57.0 ± 1.8
Total Fe (g kg^{-1})	11.8 ± 0.3b	39.1 ± 0.37a	82.0 ± 1.0	28.0 ± 0.4	1.30 ± 0.04	3.60 ± 0.07
Total Mn (g kg^{-1})	0.16 ± 0.01a	0.18 ± 0.01a	1.90 ± 0.04	0.56 ± 0.02	0.11 ± 0.003	0.31 ± 0.003

Different letters in mean values indicate significant differences ($P < 0.05$) between the two soils

Grain yield, sampling and analysis

The yields of rice grain were determined at maturity by manual harvesting. Grains were collected from each plot and oven-dried at 50 °C to constant weight. Dried grains were collected randomly from each plot collection and analyzed for the concentrations of heavy metals (Cd, Pb, and Zn) and nutrient elements (P, K, Ca, Fe, and Mn) following the methods described in Li et al. (2012).

At harvest, soil samples were also collected randomly from the top layer (0–15 cm) of each plot. Soil pH was measured using a portable pH meter (pH 510, Eutech Instruments, Singapore) in a 1:2.5 soil:deionized water slurry (Li et al. 2012). Soil catalase (CAT, EC 1.11.1.6) activity was determined according to the method of Yang et al. (2011). Bioavailable soil Cd, Pb, and Zn fractions were extracted using 0.01 M $CaCl_2$ following the published procedure of Houba et al. (2000), as this extractant was reported to be the best performing for assessing the bioavailability of some heavy metals (such as Cd and Zn) in soils (Menzies et al. 2007; Römkens et al. 2009). Concentrations of Cd in the extracts were determined by atomic absorption spectrophotometry (AAS; Hitachi Z-5300, Japan), and Pb and Zn were measured by inductively-coupled plasma optical emission spectrometry (ICP-OES; Optima 2000 DV, Perkin Elmer, USA). Blanks and standard plant (rice powder, GBW-10045) and soil (GBW-07435) reference materials (China Standard Materials Research Center, Beijing, China) were used for quality control. The mean recovery rates for Cd, Pb, Zn, P, K, Ca, Fe, and Mn were 91, 106, 93, 94, 91, 98, 93, and 94 %, respectively.

Statistical analysis

Treatment effects were examined using a one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test at $P < 0.05$ level. Pearson's correlation coefficients were calculated to determine the relationships between parameters. All statistical analyses were performed using SPSS (version 18.0) for Windows.

Results

Characteristics of soils and amendments

The soil chemical properties at the TX and SB paddy fields, with the exception of total concentrations of P and Mn, were significantly different at $P < 0.05$ (Table 1). TX and SB soils were strongly acidic with pH values of 4.3 and 4.65, respectively. The total concentrations of Cd, Pb, and Zn in TX and SB soils were 13.4 times and 6.1 times, 1.3 times and 3.4 times, 3.6 times and 2.0 times, respectively, above Farmland Environmental Quality Evaluation Standards for edible agricultural products in China (HJ332-2006, enacted by the Ministry of Environmental Protection of the People's Republic of China) (Cd 0.3 mg kg^{-1}, Pb 80 mg kg^{-1}, and Zn 200 mg kg^{-1}; pH < 6.5).

The relevant properties of the four amendments are also shown in Table 1. The pH values for SS, FA, LS, and BF were 11.8, 10.0, 12.0 and 7.65, respectively, and their Cd, Pb, and Zn concentrations were below the safety standard listed in GB 8173-87 and NY 884-2012 of China, and are thus deemed safe to be used on agricultural lands. The nutrient element contents between amendments varied markedly.

Concentrations of Cd, Pb, and Zn in brown rice

The concentrations of Cd, Pb, and Zn in brown rice with and without the amendments in two cultivation seasons at TX and SB are summarized in Table 2. The most striking result was from treatment SS2 (4.0 t ha^{-1}) that consistently and significantly ($P < 0.05$) decreased Cd and Pb concentrations in brown rice (<0.2 mg kg^{-1}, the limit of the Food Quality Standard GB2762-2012 of China and the Food Quality Standard of the European Union). It also significantly ($P < 0.05$) decreased Zn concentrations in the first cultivation season, at both paddy fields. SS1 treatments (2.0 t ha^{-1}) markedly ($P < 0.05$) decreased Cd concentrations in brown rice by 19.3–33.6 % in the first cultivation season at both paddy fields.

At the TX site, BF treatments (BF1, 4.5 t ha^{-1} and BF2, 9.0 t ha^{-1}) did not significantly influence the concentrations of Cd, Pb, and Zn in brown rice in both cultivation seasons, with the exception of the reduced Cd by treatment BF2 in the first season. Nevertheless, BF treatments caused higher concentrations of Cd and Pb in brown rice in the second cultivation season compared to the controls (Table 2). Treatment SSBF2 (the combination of 4.0 t ha^{-1} SS and 9.0 t ha^{-1} BF) markedly ($P < 0.05$) decreased Cd concentrations by 47.4 and 21.8 % in the first and second cultivation seasons, respectively.

At SB site, treatment LS2 (4.0 t ha^{-1}) significantly ($P < 0.05$) reduced Cd concentrations by 48.3 and 31.5 % in brown rice in the first and second cultivation seasons, respectively, and also reduced the concentrations of Pb and Zn by 19.0–27.5 % in the first cultivation season (Table 2). Only FA2 treatment significantly ($P < 0.05$) increased the Cd concentration in brown rice in the first cultivation season.

Rice grain yield and nutrient uptake

Compared to the controls, significant ($P < 0.05$) increases in grain yields were observed in SS2 and SSBF2 treatments in both cultivation seasons at the TX site, and the increase also occurred in SS2 treatments in the first cultivation season at the SB site (Tables 3 and 4). It is clear that treatment SS2 consistently enhanced grain yields at both sites. The higher dosages of these amendments resulted in greater yields although yield

Table 2 Concentrations of Cd, Pb, and Zn (mg kg^{-1}, mean ± SE, n = 4) in brown rice with and without amendments in two cultivation seasons at TX (Tongxi) and SB (Shangba) paddy fields

Field	Treatment	Cd	Pb	Zn	Cd	Pb	Zn
		2013 early rice season			2013 late rice season		
TX	Control	0.29 ± 0.02a	0.19 ± 0.015a	33.1 ± 1.7ab	0.31 ± 0.02ab	0.16 ± 0.009ab	32.4 ± 3.4ab
	SS1	0.19 ± 0.02cd	0.16 ± 0.016ab	30.1 ± 2.0bc	0.25 ± 0.02bcd	0.15 ± 0.009bc	30.2 ± 2.4ab
	SS2	0.16 ± 0.01d	0.13 ± 0.009b	26.7 ± 1.5c	0.19 ± 0.01d	0.12 ± 0.007c	27.5 ± 2.0b
	BF1	0.27 ± 0.04ab	0.18 ± 0.010a	33.9 ± 3.2ab	0.33 ± 0.02a	0.17 ± 0.010ab	33.8 ± 2.8ab
	BF2	0.21 ± 0.01bcd	0.17 ± 0.016ab	37.5 ± 1.8a	0.33 ± 0.04a	0.18 ± 0.019a	35.4 ± 2.0a
	SSBF1	0.23 ± 0.02 ac	0.15 ± 0.017ab	31.6 ± 3.5 ac	0.27 ± 0.01 ac	0.16 ± 0.017ab	32.1 ± 2.4ab
	SSBF2	0.15 ± 0.01d	0.13 ± 0.008b	29.6 ± 1.8bc	0.24 ± 0.02cd	0.15 ± 0.011 ac	30.5 ± 1.9ab
		2013 late rice season			2014 early rice season		
SB	Control	0.29 ± 0.02a	0.21 ± 0.009a	23.7 ± 1.1a	0.27 ± 0.01a	0.22 ± 0.012a	24.3 ± 1.6a
	SS1	0.23 ± 0.02b	0.18 ± 0.011ab	20.7 ± 1.8ab	0.24 ± 0.03ab	0.19 ± 0.013ab	23.1 ± 2.3a
	SS2	0.14 ± 0.01c	0.16 ± 0.008b	18.8 ± 1.0b	0.17 ± 0.01b	0.17 ± 0.009b	20.5 ± 2.0a
	FA1	0.25 ± 0.01ab	0.20 ± 0.016ab	22.9 ± 1.6ab	0.25 ± 0.03a	0.22 ± 0.013ab	23.9 ± 2.0a
	FA2	0.22 ± 0.02b	0.19 ± 0.018ab	20.5 ± 1.9ab	0.21 ± 0.02ab	0.20 ± 0.020ab	23.6 ± 2.5a
	LS1	0.23 ± 0.01b	0.18 ± 0.020ab	20.5 ± 1.8ab	0.26 ± 0.02a	0.20 ± 0.015ab	22.8 ± 1.3a
	LS2	0.15 ± 0.01c	0.17 ± 0.007b	17.2 ± 1.2b	0.19 ± 0.02b	0.18 ± 0.013ab	20.9 ± 1.6a

SS steel slag, *BF* bioorganic fertilizer, *SSBF* combination of steel slag and bioorganic fertilizer, *FA* fly ash, *LS* limestone.
Different letters within the same column and the same season indicate significant differences (P < 0.05) between the treatments

increases were less in the second than in the first cultivation season (Tables 3 and 4).

In the first cultivation season, treatment SS2 significantly (P < 0.05) increased the accumulation of P and Ca in brown rice at both sites, and BF2 also significantly (P < 0.05) increased the accumulation of P, K, and Mn. Significantly more P, K, Ca, Fe, and Mn were accumulated in plots receiving SSBF2 treatment in the first cultivation season. Conversely, LS treatments slightly reduced the accumulation of P, Fe, and Mn in the brown rice (Tables 3 and 4).

Table 3 Grain yield and nutrient uptake by brown rice (mean ± SE, n = 4) with and without amendments in two rice cultivation seasons at TX (Tongxi) paddy field

Cultivation season in TX field	Treatment	Grain yield (kg ha^{-1})	Nutrient uptake by brown rice				
			P (kg ha^{-1})	K (kg ha^{-1})	Ca (kg ha^{-1})	Fe (g ha^{-1})	Mn (g ha^{-1})
2013 early season	Control	5400 ± 163c	10.6 ± 0.3d	3.43 ± 0.1c	0.65 ± 0.06c	70.9 ± 5.4b	120 ± 5d
	SS1	5882 ± 257bc	12.4 ± 0.8cd	3.62 ± 0.1bc	0.76 ± 0.07bc	81.5 ± 7.8ab	140 ± 8cd
	SS2	6463 ± 235ab	14.4 ± 1.1ac	3.85 ± 0.3bc	0.90 ± 0.09ab	90.4 ± 2.9ab	163 ± 10ac
	BF1	6095 ± 280ac	13.1 ± 1.1bcd	4.33 ± 0.8bc	0.76 ± 0.08bc	82.5 ± 8.9ab	159 ± 11bc
	BF2	6549 ± 529ab	15.4 ± 0.7ab	5.40 ± 0.1a	0.87 ± 0.10ac	90.5 ± 4.7ab	182 ± 7ab
	SSBF1	6237 ± 487ab	13.7 ± 0.7bc	4.08 ± 0.2bc	0.83 ± 0.06ac	90.8 ± 8.1ab	174 ± 12ac
	SSBF2	6747 ± 481a	16.6 ± 1.2a	4.61 ± 0.3ab	1.03 ± 0.11a	99.8 ± 11.8a	196 ± 23a
2013 late season	Control	4975 ± 261c	9.34 ± 0.5c	3.01 ± 0.2b	0.63 ± 0.07a	61.2 ± 6.6b	116 ± 8b
	SS1	5110 ± 405bc	9.70 ± 1.0bc	3.06 ± 0.4b	0.67 ± 0.06a	63.7 ± 6.2ab	125 ± 17ab
	SS2	5695 ± 189ab	11.5 ± 0.7ac	3.28 ± 0.1b	0.75 ± 0.07a	72.7 ± 5.8ab	140 ± 13ab
	BF1	5403 ± 191ac	10.6 ± 1.2ac	3.40 ± 0.2ab	0.69 ± 0.08a	69.6 ± 6.3ab	135 ± 19ab
	BF2	5651 ± 215ac	11.9 ± 0.5ab	3.99 ± 0.3a	0.73 ± 0.08a	76.0 ± 5.6ab	143 ± 12ab
	SSBF1	5561 ± 225ac	11.8 ± 0.8ab	3.30 ± 0.2b	0.73 ± 0.11a	71.1 ± 9.3ab	156 ± 20ab
	SSBF2	5853 ± 291a	12.6 ± 0.4a	3.53 ± 0.1ab	0.83 ± 0.08a	80.2 ± 3.3a	166 ± 16a

Different letters within the same column and the same season indicate significant differences (P < 0.05) between the treatments
SS steel slag, *BF* bioorganic fertilizer, *SSBF* combination of steel slag and bioorganic fertilizer

Table 4 Grain yield and nutrient uptake by brown rice (mean ± SE, n = 4) with and without amendments in two rice cultivation seasons at SB (Shangba) paddy field

Cultivation season in SB field	Treatment	Grain yield (kg ha^{-1})	Nutrient uptake by brown rice				
			P (kg ha^{-1})	K (kg ha^{-1})	Ca (kg ha^{-1})	Fe (g ha^{-1})	Mn (g ha^{-1})
2013 late season	Control	5144 ± 194b	8.27 ± 0.6cd	3.39 ± 0.41a	0.65 ± 0.08b	63.3 ± 6.1a	170 ± 10a
	SS1	5578 ± 214ab	9.92 ± 0.8ac	3.45 ± 0.26a	0.73 ± 0.03ab	71.7 ± 6.2a	190 ± 16a
	SS2	6020 ± 143a	11.6 ± 0.4a	3.64 ± 0.29a	0.86 ± 0.08a	79.4 ± 10.4a	212 ± 15a
	FA1	5345 ± 188b	8.86 ± 0.4bcd	3.60 ± 0.29a	0.70 ± 0.06ab	65.6 ± 5.5a	187 ± 27a
	FA2	5578 ± 212ab	10.1 ± 0.8ab	3.87 ± 0.45a	0.77 ± 0.03ab	70.9 ± 7.5a	199 ± 19a
	LS1	5361 ± 169b	8.30 ± 0.6cd	3.43 ± 0.31a	0.75 ± 0.07ab	61.6 ± 7.6a	174 ± 13a
	LS2	5436 ± 178b	7.88 ± 0.3d	3.43 ± 0.30a	0.83 ± 0.07ab	58.7 ± 6.5a	166 ± 15a
2014 early season	Control	5620 ± 178a	9.62 ± 0.2b	4.01 ± 0.14a	0.66 ± 0.06a	73.7 ± 6.5a	195 ± 6a
	SS1	5753 ± 311a	11.3 ± 1.0ab	4.08 ± 0.40a	0.70 ± 0.09a	78.0 ± 9.0a	203 ± 20a
	SS2	6378 ± 276a	13.0 ± 0.7a	4.30 ± 0.31a	0.84 ± 0.10a	88.1 ± 8.9a	228 ± 22a
	FA1	5698 ± 225a	10.1 ± 0.4b	4.15 ± 0.32a	0.68 ± 0.07a	75.2 ± 8.0a	205 ± 18a
	FA2	5855 ± 471a	10.7 ± 0.5b	4.35 ± 0.35a	0.73 ± 0.12a	82.6 ± 14.5a	213 ± 21a
	LS1	5823 ± 319a	9.52 ± 0.3b	3.99 ± 0.47a	0.74 ± 0.08a	73.5 ± 6.2a	192 ± 9a
	LS2	5878 ± 377a	9.61 ± 1.3b	3.84 ± 0.47a	0.79 ± 0.04a	68.9 ± 7.5a	191 ± 14a

Different letters within the same column and the same season indicate significant differences ($P < 0.05$) between the treatments

SS steel slag, *FA* fly ash, *LS* limestone

Changes in Cd, Pb, and Zn bioavailability in soil

The changes in bioavailable Cd, Pb, and Zn concentrations in the soils with amendments in the two cultivation seasons at TX and SB sites are shown in Table 5. The most striking result was from SS2 treatment which significantly ($P < 0.05$) decreased bioavailable soil Cd, Pb, and Zn concentrations. Treatment SS1 also markedly ($P < 0.05$) decreased the bioavailability of Cd and Pb in the first cultivation season at both paddy fields.

At site TX, the effects of BF treatments on the concentrations of Cd, Pb, and Zn in brown rice were mostly non-significant in both cultivation seasons. However, BF treatments remobilized soil Cd and Pb leading to a higher bioavailability of these metals in the second cultivation season compared to the controls (Table 5). The combined use of SS and BF, SSBF1 and SSBF2 treatments, strongly ($P < 0.05$) decreased bioavailable Cd, Pb, and Zn concentrations in the first cultivation season, respectively, but the decreased effects were mostly non-significant in the second season.

At SB site, LS1 and LS2 treatments significantly ($P < 0.05$) decreased bioavailable Cd, Pb, and Zn concentrations in the first cultivation season, but the decreases were less in the second season (Table 5). The Pb bioavailability was significantly ($P < 0.05$) decreased by FA2 treatment in the first cultivation season at SB.

Significant positive correlations ($P < 0.05$) were found between bioavailable Cd, Pb, and Zn concentrations in the soils and their respective concentrations in brown rice in both cultivation seasons at both TX and SB (Table 5).

Changes in pH and catalase activities in soil

Both high and low amendments with SS, SSBF, and LS as well as FA2 all significantly ($P < 0.05$) increased soil pH, and the increases in pH were more in treatments with high than low additions (Table 6). In the first cultivation season, significant ($P < 0.05$) increases in soil catalase activities were observed in SS, BF2, and SSBF treatments at site TX, and the increases also occurred in SS2 treatment at the SB site (Table 5). These findings clearly show that the application of SS greatly improved soil pH and catalase activities at both paddy fields. The higher dosage of the amendment, the greater the increase although the increment was less in the second season compared to that in the first cultivation.

Discussion

The two experimental paddy soils studied were from the vicinity of the two traditional metal mines. They were seriously acidified and contaminated with different levels of mixed heavy metals (Cd, Pb, and Zn), notably Cd (Table 1). It is well known that heavy metals in soil with a low pH are highly bioavailable and easily transferred into plants (Sarwar et al. 2010). Leaching, adsorption, or precipitation of Cd, Pb, or Zn

Table 5 Bioavailable concentrations of Cd, Pb, and Zn (mg kg^{-1}, mean ± SE, n = 4) in soils with and without amendments in two rice cultivation seasons at TX (Tongxi) and SB (Shangba) paddy fields, and their correlations with the respective concentrations in brown rice (Pearson correlation coefficients with R values and significance)

Field	Treatment	Cd	Pb	Zn	Cd	Pb	Zn
		2013 early rice season			2013 late rice season		
TX	Control	1.02 ± 0.09a	0.57 ± 0.04a	124 ± 8.4ab	1.18 ± 0.09ab	0.39 ± 0.03a	112 ± 8.1b
	SS1	0.77 ± 0.08b	0.14 ± 0.01c	102 ± 7.9bc	1.00 ± 0.12bcd	0.14 ± 0.02c	103 ± 7.6b
	SS2	0.22 ± 0.02d	0.05 ± 0.01d	56.9 ± 3.7d	0.69 ± 0.04de	0.12 ± 0.01c	69.0 ± 5.2c
	BF1	1.00 ± 0.12a	0.53 ± 0.05a	137 ± 13.1a	1.29 ± 0.08a	0.39 ± 0.02a	120 ± 10.7ab
	BF2	0.89 ± 0.06ab	0.42 ± 0.05b	149 ± 16.6a	1.33 ± 0.12a	0.43 ± 0.04a	140 ± 10.9a
	SSBF1	0.55 ± 0.04c	0.11 ± 0.01cd	93.9 ± 3.8c	1.08 ± 0.10ac	0.23 ± 0.03b	108 ± 7.9b
	SSBF2	0.17 ± 0.01d	0.03 ± 0.003d	78.4 ± 5.0cd	0.83 ± 0.06ce	0.16 ± 0.03bc	99.7 ± 7.3b
R value (n = 28)		0.56**	0.68**	0.65**	0.79**	0.59*	0.65**
		2013 late rice season			2014 early rice season		
SB	Control	0.79 ± 0.06a	0.58 ± 0.03a	34.5 ± 2.0a	0.70 ± 0.05a	0.58 ± 0.06a	40.9 ± 2.8a
	SS1	0.31 ± 0.04b	0.16 ± 0.03cd	15.4 ± 1.5b	0.52 ± 0.06ab	0.32 ± 0.05c	24.4 ± 2.3b
	SS2	0.09 ± 0.01c	0.07 ± 0.01d	4.25 ± 0.4c	0.24 ± 0.02c	0.16 ± 0.02d	14.8 ± 2.4c
	FA1	0.70 ± 0.08a	0.54 ± 0.06ab	30.9 ± 2.7a	0.69 ± 0.06a	0.57 ± 0.04a	38.3 ± 2.5a
	FA2	0.68 ± 0.07a	0.45 ± 0.05b	29.3 ± 2.8a	0.63 ± 0.07a	0.54 ± 0.05ab	34.8 ± 2.9a
	LS1	0.44 ± 0.08b	0.19 ± 0.02c	14.9 ± 1.5b	0.61 ± 0.06a	0.43 ± 0.05bc	36.7 ± 3.2a
	LS2	0.12 ± 0.01c	0.08 ± 0.001d	2.68 ± 0.4c	0.40 ± 0.09bc	0.18 ± 0.02d	15.0 ± 1.1c
R value (n = 28)		0.70**	0.59**	0.61**	0.69*	0.46*	0.40*

Different letters within the same column and the same season indicate significant differences (P < 0.05) between the treatments

SS steel slag, *BF* bioorganic fertilizer, *SSBF* combination of steel slag and bioorganic fertilizer, *FA* fly ash, *LS* limestone

* Significant (P < 0.05)

** Significant (P < 0.01)

in soils is strongly soil pH-dependent; bioavailability is lower at higher pH (Kumpiene et al. 2008; Zeng et al. 2011). A reduction in bioavailability is the key to reducing metal transfers along food chain via plant uptake (Bolan et al. 2014). Therefore, how to increase soil pH and reduce heavy metal bioavailability is the key for the remediation of acidic contaminated soils. This study found that the application of alkaline materials, especially SS (4.0 t ha^{-1}) and LS (4.0 t ha^{-1}), predictably increased soil pH and decreased soil Cd, Pb, and Zn bioavailability, and their subsequent concentrations in brown rice (Tables 2, 5, and 6). The bioavailable Cd, Pb, and Zn concentrations in the paddy soils were significantly positively related with their concentrations in the brown rice (Table 5). The increases in soil pH by the amendments could be primarily due to the hydration of calcium carbonate in LS (Li et al. 2008) and calcium oxide or other metallic oxides in SS or FA (Gu et al. 2011). The amendments could also reduce the amounts of bioavailable heavy metals, as exchangeable metals can be bound to the carbonate induced by LS or the Fe-Mn oxides in SS.

The bioorganic fertilizer (BF) used in the study contains organic fertilizer and an amount of *B. subtilis*. Jiang et al. (2009) found that the simultaneous application of a bio-fertilizer and *B. subtilis* exhibited a synergetic effect in immobilizing soil Cd under laboratory incubation for 90 days. However, the *B. subtilis* did not show any immobilizing effect on Cd when applied singly into the soil but showed an enhanced bioaccumulation potential for Cd. The immobilization effects of BF application could therefore be due mainly to the organic matter present. Zeng et al. (2011) and many other authors have reported that organic matter was one of the most important contributors to the ability of soils for retaining heavy metals. Similarly, our results also demonstrated that BF application to some extent reduced soil Cd and Pb bioavailability in the first cultivation season (90 days), leading to decreases in their concentrations in brown rice. However, their values increased in the second cultivation season (after 110 days from the end of the first cultivation season) (Tables 2 and 5). Park et al. (2011) reported that organic fertilizers had an initial effective adsorption or complexation capacity, which immobilized Cd and Pb in the soils, but the bound metals could be reactivated and remobilized into soils as the organic fertilizers degraded over time. Additionally, soil characteristics have large time and spatial variability under field conditions, due to the strong dependence of soil processes on the weather (Körschens et al. 2006), possibly leading to

Table 6 pH and catalase activities [mg H_2O_2 (g·20 min)$^{-1}$] (mean ± SE, $n = 4$) in soils with and without amendments for two rice cultivation seasons in TX (Tongxi) and SB (Shangba) paddy fields

Field	Treatment	pH	Catalase	pH	Catalase
		2013 early rice season		2013 late rice season	
TX	Control	4.26 ± 0.02d	2.14 ± 0.11d	4.39 ± 0.08d	1.84 ± 0.06a
	SS1	4.75 ± 0.03bc	2.76 ± 0.20c	4.95 ± 0.01c	1.87 ± 0.10a
	SS2	5.78 ± 0.31a	2.82 ± 0.14bc	5.40 ± 0.09a	1.92 ± 0.13a
	BF1	4.46 ± 0.06cd	2.61 ± 0.22cd	4.63 ± 0.10d	1.91 ± 0.12a
	BF2	4.49 ± 0.06cd	3.37 ± 0.18ab	4.64 ± 0.08d	2.04 ± 0.20a
	SSBF1	5.14 ± 0.15b	3.07 ± 0.15ac	5.18 ± 0.04b	1.98 ± 0.13a
	SSBF2	6.07 ± 0.15a	3.52 ± 0.32a	5.56 ± 0.02a	2.08 ± 0.11a
		2013 late rice season		2014 early rice season	
SB	Control	4.82 ± 0.02e	1.91 ± 0.06b	4.64 ± 0.09c	2.35 ± 0.19a
	SS1	5.38 ± 0.15bd	2.35 ± 0.22ab	5.03 ± 0.06b	2.38 ± 0.25a
	SS2	6.31 ± 0.11a	2.57 ± 0.42a	5.48 ± 0.10a	2.71 ± 0.17a
	FA1	5.17 ± 0.01cde	2.08 ± 0.16ab	4.87 ± 0.02bc	2.43 ± 0.17a
	FA2	5.58 ± 0.10bc	2.17 ± 0.12ab	5.07 ± 0.05b	2.43 ± 0.22a
	LS1	5.66 ± 0.28b	2.24 ± 0.18ab	5.09 ± 0.08b	2.40 ± 0.19a
	LS2	6.58 ± 0.22a	2.45 ± 0.16ab	5.65 ± 0.09a	2.56 ± 0.23a

Different letters within the same column and the same season indicate significant differences ($P < 0.05$) between the treatments

SS steel slag, *BF* bioorganic fertilizer, *SSBF* combination of steel slag and bioorganic fertilizer, *FA* fly ash, *LS* limestone.

less effective immobilization of heavy metals under field conditions. In contrast to Cd and Pb, Zn concentrations in BF-amended brown rice increased in both cultivation seasons, which might be related to the difference in the bioavailability between metallic elements under the influence of BF. Tang et al. (2015) found that the use of organic fertilizers (peat and pig manure) in low and high amounts reduced available Pb but increased available Zn in vegetable soils in a pot experiment. In the present study, the combined use of SS and BF to some extent reduced soil Cd, Pb, and Zn bioavailability and their concentrations in brown rice in both cultivation seasons, although the effects of the combined amendments were much less than that of SS application in the second cultivation season (Tables 2 and 5). It may be explained by the fact that the simultaneous use of SS and BF did not maintain a synergetic effect in immobilizing soil heavy metals, possibly because of the reactivating effects of BF on heavy metal mobility in soil over time. Kim et al. (2012) also showed that pH change induced by alkaline materials, such as SS tested in this study, was more efficient in immobilizing Cd, Pb, and Zn in soils than organic fertilizers.

Apart from the decreases of heavy metals in rice grain, attention should also be paid to rice yield when amendments are applied. It is difficult to establish plant growth in contaminated soils, due to metal toxicity and the infertile or acidic conditions (Park et al. 2011). In our study, the application of SS (4.0 t ha^{-1}), BF (9.0 t ha^{-1}), and their combination significantly increased the grain yields and rice nutrient uptake, whereas FA and LS treatments only slightly improved grain yields (Tables 3 and 4). The different performances between amendments could be explained for the following reasons. Firstly, amendments increased soil pH and changed the seriously acidic soil environment to a more neutral one for crop growth but the pH enhancement effects differed between amendments. Secondly, the composition and amounts of nutrients essential for rice growth and development were different between amendments. SS is composed principally of calcium, phosphorus, and iron compounds (Navarro et al. 2010), whereas BF contains high contents of organic matter and nutrient elements. Thirdly, the stimulatory effects of amendments on soil catalase activity, a parameter related to the metabolic activity of aerobic organisms and a useful indicator of soil quality (Karaca et al. 2011). The enhancement effects of SS, BF, and SSBF were more than for the other amendments (Table 6).

The dosage of amendments used is one of the key factors controlling remediation efficiency (Kumpiene et al. 2008). The effect of an amendment on rice plants and paddy soils varied with its dosages. Li et al. (2008) and Chen et al. (2016) found that higher additions led to a higher soil pH and lower metal availabilities. Similarly, the remediation effects in this study were substantially better under the higher than the lower dosages except for BF (Tables 2, 3, 4, 5, 6). However, the remediation effects declined from the first cultivation season to the second. This may be attributed to the decline of the amendment efficiency through natural weathering processes and the breakdown of complexes (Bolan et al., 2014) or the decrease of beneficial elements released by amendments during the progress of the field experiments.

In summary, our study has shown that steel slag, an industrial residue, achieved better remediation effects to reduce the soil bioavailability of Cd, Pb, and Zn and their concentrations in brown rice at two acidic paddy fields contaminated with mixed heavy metals (Cd, Pb, and Zn), compared to the alkaline amendments (fly ash and limestone), bioorganic fertilizer, and its combination with steel slag. Steel slag application consistently increased soil pH, soil catalase activity, rice yield, and nutrient uptake at the two paddy fields with significantly different soil properties, including pH, concentrations of heavy metals, organic matter, and nutrients. It is clear that amendment with steel slag could be a feasible and an ecologically friendly remediation treatment for acidic paddy soils contaminated with mixed heavy metals as it can steadily improve crop growth and reduce heavy metal accumulation in brown rice.

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