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Guilan Duan ^a, Hongmei Zhang ^b, Yunxia Liu ^a, Yan Jia ^a, Ying Hu ^a & Wangda Cheng ^b

^a Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Haidian District, 100085 Beijing, China

^b Jiaxing Academy of Agricultural Sciences, Jiaxing 314016, China

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ORIGINAL ARTICLE

Long-term fertilization with pig-biogas residues results in heavy metal accumulation in paddy field and rice grains in Jiaxing of China

Guilan DUAN¹, Hongmei ZHANG², Yunxia LIU¹, Yan JIA¹, Ying HU¹ and Wangda CHENG²¹Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Haidian District, 100085 Beijing, China and ²Jiaying Academy of Agricultural Sciences, Jiaying 314016, China

Abstract

Pig-biogas residue is widely used as organic fertilizer for rice (*Oryza sativa* L.) in China. To evaluate the risk of heavy metal contamination in paddy soil and rice grains caused by long-term pig-biogas residue fertilization, this study was conducted in paddy fields which had been fertilized continuously with pig-biogas residue for 8 years. We found that pig-biogas residues contained high concentrations of arsenic (As), cadmium (Cd), copper (Cu) and zinc (Zn). As a result, the concentrations of these four metals in paddy soils and rice grains sampled from biogas residue fertilized fields were significantly higher than those from control fields. In addition, in biogas residue fertilized fields, the concentrations of these four metals rapidly increased in the tillage layer compared with those in deep soil layers, and biological availability was significantly higher than in control fields. Moreover, we found that the accumulation of these four metals in paddy soils and rice grains increased in the second year compared with the first year. To reduce heavy metal accumulation in rice grains, we screened for rice genotype among 20 local rice genotypes. We found obvious genotypic differences in grains' accumulation of heavy metals, and identified some rice genotypes which had low accumulation of multiple heavy metals. These low-accumulating genotypes predicate the possibility to reduce heavy metal accumulation in rice grains grown in pig-biogas residue fertilized fields.

Key words: biogas residue, genotype, heavy metal, rice.

INTRODUCTION

Huge pork demand drives large-scale pork production. China is one of the largest pork producers in the world. In 2009, Chinese pork production was at 48.5 million tons, accounting for 48.2% of the global pork production (Chinese Agricultural Products Processing 2009). Intensive pig farms generate massive amounts of pig manure. In China, the annual animal manure output was over 3 billion tons (Huang *et al.* 2008).

Environmentally friendly disposal of this massive amount of pig manure is a big challenge for areas with intensive pig farming. Generating green energy from animal manure, using thermal treatment, has been recognized as a cost-efficient and eco-friendly means of animal manure disposal, and has been advocated in pig farms world-wide (Møller *et al.* 2004). In China, with the progress of China's 2003–2010 National Rural Biogas Construction Plan, 2200 biogas engineering projects were established, producing more than 100 million tons of biogas residues a year. Animal manure is a valuable (or concentrated) source of plant nutrients, such as nitrogen (N), phosphorus (P) and potassium (K), most of which are retained in the pig-biogas residues (PBR) after the extraction of the energy. Therefore, PBR is widely applied as a soil amendment and nutrient source for

Correspondence: Wangda CHENG, Jiaying Academy of Agricultural Sciences, Jiaying, China. Tel: 8657383779313. Fax: 8657383770001. Email: chwd228@163.com

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crops and vegetables (Cheung and Wong 1983; Dahiya and Vasudevana 1986).

In the main rice-producing regions of China, such as Zhejiang, Jiangsu and Hunan provinces, biogas slurry is mainly used as organic fertilizer for paddy rice (*Oryza sativa* L.). Annually, about 30 t ha⁻¹ of biogas residues (including solid and liquid residues) are applied to paddy fields. Biogas slurry is not only a source of nutrients, but also serves as an excellent soil conditioner which improves texture and aeration, enhances moisture-holding and buffering capacity, and stimulates the growth of microorganisms (Pimentel *et al.* 1995; Wagner 1999).

Although pig manure or PBR application to agricultural land is accepted as good fertilizer for crops and environmentally-friendly disposal of waste, the presence of heavy metal(s) (HMs) in PBR is of great concern (Wong and Cheung 1995; Moolenaar and Lexmond 1999). To shorten the duration of breeding and to control diseases, HMs are usually given to livestock and poultry as feed additives (Nicholson *et al.* 1999; Dach and Starman 2005; Li *et al.* 2007). HMs ingested by animals are mostly excreted with feces and urine, such as 92–96% of zinc (Zn), and 72–80% of copper (Cu). Therefore, high concentrations of HMs were present in pig manure and PBR (Poulsen 1998; Gräber *et al.* 2005). Cu and Zn are the most widely used HMs for pig diets as feed additives. Accordingly, high concentrations of Cu and Zn in pig manure and agricultural soils have been reported in areas with intensive pig production (Cang *et al.* 2004; Mishima *et al.* 2005; Ogiyama *et al.* 2005, 2006; Li *et al.* 2007; Zielińska *et al.* 2009). For example, Cu concentrations were reported to be 6.86–395.19 mg kg⁻¹ in pig feed sampled from Beijing and Fuxin cities (Li *et al.* 2007), and 1726.3 mg kg⁻¹ in pig manure sampled from Jiangsu province (Cang *et al.* 2004); also similarly high concentrations of Cu and Zn in pig feed and manure have been reported in some other countries (Nicholson *et al.* 1999).

Arsenic (As) is also used as a feed additive, for swine disease control, weight improvement, and meat coloring (Wiersma *et al.* 1986; Dach and Starman 2005). Although the use of As as an animal feed additive has been abandoned in European countries because of its toxicity, it is still used as an additive in some other countries, such as the USA and China (Li and Chen 2005). The concentrations of As in pig feed and manure samples from Beijing were reported to be 0.15 to 37.8 mg kg⁻¹ and 0.42 to 119.0 mg kg⁻¹, respectively, and annually about 13 t of As was introduced to Beijing farmlands through pig manure (Li and Chen 2005), and about 16 t was loaded into English and Welsh farmlands through animal manures, including pig manure (Nicholson *et al.* 2003). Cadmium (Cd) is usually present in Zn and Cu ores, so when Cu or Zn are used as pig feed additives, Cd will

inevitably also be added. Cd is also a trace element in phosphate ore which is mixed in feed to make pigs retain water and gain weight quickly. In addition to Cu, Zn, As and Cd, other HMs, such as lead (Pb) and chromium (Cr) can also be found in high concentrations in some animal manures (Wiersma *et al.* 1986; Moolenaar and Lexmond 1999; Dach and Starman 2005).

HM contamination of agricultural soil caused by animal manure fertilization has been widely reported (Nicholson *et al.* 1999, 2003; Cang *et al.* 2004; Mishima *et al.* 2004; Dach and Starman 2005; Li *et al.* 2007). However, with the popularization of biogas projects, less and less animal manure is used directly as a fertilizer for crops and vegetables, while PBR is replacing animal manure used as fertilizer. Nevertheless, so far it is not clear how the HMs from animal manure are retained in the PBR after the extraction of energy through thermal treatment, as well as the biological availability of HMs in PBR, and what risk PBR fertilization poses to the food chain. In addition, it has been well documented that there are significant genetic variations of HM accumulation in rice grains, including As, Cd, Cu, Zn, etc. (Cheng *et al.* 2006; Zeng *et al.* 2008; Norton *et al.* 2009). Selecting and breeding rice varieties with low HM accumulation in grains could be the most feasible and effective approach to reduce HM levels in the diets produced in HM-contaminated regions. Therefore, in the present study, a two-year field trial was conducted with 20 rice genotypes in paddy fields which had been fertilized continuously with PBR for over 8 y. The overall objectives of this study were to (1) determine the content of HMs in PBR; (2) examine the effects of PBR fertilization on paddy soil HM accumulation; (3) investigate the risk of rice HM contamination from PBR fertilization; (4) identify rice genotypes with low HM accumulation.

MATERIALS AND METHODS

Field description

The fields (N 30°04.007', E 120°54.348') are located in Jiaxing city, Zhejiang province, China. Jiaxing city is a big pork production base of China, providing pork for the adjacent cities with large populations, such as Shanghai and Hangzhou. In addition, Jiaxing is also an important rice production base of China. Therefore, in Jiaxing city, most paddy fields have been fertilized with PBR for more than 5 y. To investigate the long-term effects of PBR fertilization on HM accumulation in soil and rice, the field trial was conducted in three traditional paddy fields which had been continuously fertilized with pig manures or PBR for more than 8 y. Three other fields near these fields which had never been fertilized with pig manure or PBR were selected as a control.

Plant growth and fertilization

For the two-year accumulation experiment, rice (*Oryza sativa* L.) cultivar Xiushui128 was used (Supplementary Table 1). For the genotype screening experiment, 20 local japonica rice genotypes, which were previously shown to have different HM accumulation but similar maturity dates, were collected from the Jiaxing Academy of Agricultural Science. After germination, the rice seeds were sown on a control field (no PBR fertilization). About 25 d later, rice seedlings with six leaves were transplanted to the different experimental fields. At each field, 20 rice genotypes were grown randomly, and every genotype was planted in five rows, and each row was 2 m with 10 hills. One hill contained one seedling, and the space between rows was 20 cm.

For the test fields, solid PBR was used as basal fertilizer (15 t ha^{-1}), applied 15 d before transplanting; liquid biogas slurry was used as tillering and panicle fertilizer (7.5 t ha^{-1} per time). For the control fields, N application was 202 kg N ha^{-1} as urea, P application was $31.5 \text{ kg phosphorus pentoxide (P}_2\text{O}_5\text{) ha}^{-1}$ as calcium superphosphate, and K application was $180 \text{ kg potassium oxide (K}_2\text{O) ha}^{-1}$ as potassium chloride. Forty percent of total N, and 100% of P and K were used as basal fertilizers, applied 2d before transplanting. The remaining 60% of total N was averagely top-dressed 3 times at three different growth stages: early-tillering, mid-tillering and early spike. Other field management measures were followed according to the standard local practices, and were identical on test and control fields.

Sample preparation

PBR was mixed thoroughly and sampled randomly before fertilization, and 2 kg solid or 50 mL liquid PBR were bulked from 5 subsamples which were taken from different areas of the biogas residue pool. For solid residues, samples were air-dried in the shade, then ground and passed through a 2-mm nylon sieve. For liquid residues, samples were stored at 4°C until digestion after removing visible solid materials.

Plant samples were harvested after rice grains matured. Each grain sample contained 50 panicles, taken from 40–50 hills in the same field. Shoot samples were taken from the same hills as grains, about 10 cm above the soil. Grains were threshed manually from the panicles, air-dried, then separated into husks and brown rice using a chaff-removing machine (JLGJ45, Taizhou Food Instrument Factory, Taizhou, China). The brown rice was further polished. The shoots were oven-dried at 65°C until a constant weight was reached. All the dried plant samples were powdered using a mill (JNMJ3, Taizhou Food Instrument Factory, Taizhou, China), and stored at room temperature until digestion.

Soil samples were taken after the rice plants were harvested. From each field, three final composite soil samples were taken from three different fields. The composite samples were the mixture of five subsamples which were taken from the same field, containing 1 kg soil obtained from a depth of 0–20 cm. After transport to the laboratory, the soil samples were air-dried and crushed to pass through a 2-mm nylon sieve (10-mesh). The homogenized samples were then ground with an agate grinder to pass through a $150\text{-}\mu\text{m}$ nylon sieve (100-mesh).

For the investigation of HM vertical distribution in the soil profile, samples were collected at various soil depths (from 0–80 cm) after rice was harvested in the second year. Soil depth in the profile was measured by digging a trench ($1 \text{ m} \times 1 \text{ m}$). At each PBR-fertilized field, 3 independent trenches were dug, and from each trench, subsamples were taken from different soil depth (<10, 10–20, 20–30, 30–40, 40–50, 50–60, 60–70 and 70–80 cm). After sampling, soil subsamples from the same field and the same depth of three different trenches were pooled into one composite sample, and three independent fields were three replicates.

HM analysis

For plant and solid residue digestion, 0.2 g of rice grains or husks, 0.1 g of rice straw or residue samples were weighed into 50-mL polypropylene digestion tubes; 2 mL of nitric acid was added and the mixture was allowed to sit overnight at room temperature. For liquid PBR digestion, 5 mL of residue solution was shaken at 200 rpm overnight, and then loaded into 50-mL polypropylene digestion tubes with 5 mL of 2% nitric acid. The samples were then digested in a microwave oven (MARS, Matthew Inc., USA) with a 3-stage temperature ramping program: 55°C for 10 min, then to 75°C for 10 min, and finally 95°C for 30 min. For soil digestion, 0.2-g soil samples were weighed into quartz glass tubes, and 2.5 mL of nitric acid was added and allowed to sit overnight at room temperature. Samples were digested on the block digester at 100°C for 1 h, then at 120°C for 1 h, and finally at 140°C until the extracts were clear. For quality control, certified reference materials (10010 Chinese rice flour, GBW 07405 and 07401 Chinese soils) were used; spikes and blanks were also included.

After digestion, samples were cooled to room temperature, then filtered with Whatman No. 42 filter paper. Subsequently, the solutions were diluted to 50 mL with Millipore ultrapure water, and indium was added to a concentration of $10 \mu\text{g L}^{-1}$ as an internal standard. HM concentrations in the solutions were analyzed by ICP-MS (Agilent Technologies 7500).

Table 1 Heavy metal concentrations in soils and biogas residues

Soil & residue	Year	Arsenic	Cadmium	Lead	Chromium	Nickel	Copper	Zinc
PBR Soil	First	8.9 ± 0.4*	0.31 ± 0.03* [#]	15.9 ± 2.8	64.1 ± 4.9	47.1 ± 4.1	64.0 ± 5.8* [#]	182.6 ± 10.3* [#]
	Second	10.1 ± 0.5*	0.44 ± 0.09* [#]	16.7 ± 3.2	72.2 ± 8.9	36.9 ± 5.0	85.5 ± 6.7* [#]	224.1 ± 18.4* [#]
Control Soil	First	5.3 ± 0.5	0.23 ± 0.03	11.8 ± 2.1	54.7 ± 5.2	49.6 ± 5.8	19.9 ± 3.1	68.2 ± 6.7
	Second	6.5 ± 0.4	0.22 ± 0.05	12.0 ± 2.3	58.6 ± 6.8	40.9 ± 2.8	17.8 ± 4.2	72.4 ± 5.9
Manure	First	31.6 ± 3.7	1.22 ± 0.08	6.5 ± 2.0	12.1 ± 1.8	9.8 ± 1.2	1121.0 ± 231.5	1375.7 ± 279.5
	Second	21.6 ± 3.1	0.43 ± 0.05	1.7 ± 0.3	4.8 ± 1.0	9.4 ± 1.6	1105.4 ± 179.2	1475.1 ± 412.3
Solid PBR	First	20.7 ± 2.8	9.43 ± 1.52	8.1 ± 1.2	20.0 ± 2.9	28.8 ± 5.4	1143.0 ± 301.3	3460.1 ± 279.4
	Second	29.0 ± 4.1	9.94 ± 2.05	10.6 ± 0.9	25.6 ± 2.5	21.1 ± 4.3	1373.4 ± 198.7	3844.5 ± 301.5
Liquid PBR	First	0.7 ± 0.1	0.14 ± 0.02	0.3 ± 0.1	0.4 ± 0.1	1.1 ± 0.2	143.6 ± 20.8	249.9 ± 13.7
	Second	0.6 ± 0.1	0.12 ± 0.04	0.3 ± 0.1	0.3 ± 0.0	1.2 ± 0.1	176.8 ± 32.6	231.9 ± 21.0
Standards [†]		30	0.3	250	250	40	50	200
Background [‡]		15.0	0.2	35.0	90.0	40.0	35.0	100.0

Notes: Values given in mg kg⁻¹.Data are means ± SD, *n* = 4.[†]Chinese Soil Environmental Quality Standard, Grade II (Chinese Environmental Quality Standard for Soils 1995);[‡]Background concentrations of heavy metals in the Chinese soil, Chinese Soil Environmental Quality Standard, Grade I (Chinese Environmental Quality Standard for Soils 1995);*Indicates value significantly higher than control (*P* < 0.05);[#]Indicates value significantly higher than background (*P* < 0.05);

PBR, Pig-biogas residues.

Statistical analysis

Data were analyzed by one-way ANOVA using SPSS16.0. Curve fitting was performed using Sigma Plot 10.0.

RESULTS

HM accumulation in PBR and PBR-fertilized paddy soil

In pig manure, the concentrations of As, Cd, Cu and Zn were remarkably high, about 21, 0.35, 1105 and 1475 mg kg⁻¹ respectively. However, in solid PBR, the concentrations of these four metals were even higher than those in the pig manure (Table 1). Cd concentration in solid PBR was about 10 mg kg⁻¹, which is about 17 times higher than fertilizer standard (Standards for Contaminants in Imported Organic Fertilizers 1987); the concentrations of Cu and Zn were over 1000 and 3000 mg kg⁻¹ respectively, which were about 13 and 18 times higher than the soil quality standard respectively. The As concentration in PBR was similar to that in pig manure. In the liquid PBR, the concentrations of the observed metals were much lower than in solid PBR and pig manure (Table 1). These results suggested that the PBR contained high concentration of HMs, which were even higher than those in pig manure.

In the soil sampled from the PBR-fertilized field, the HM concentrations were also significantly high, with 10, 0.3, 80 and 200 mg kg⁻¹ of As, Cd, Cu and Zn respectively, which were significantly higher than those from the control fields (Table 1). In addition, the concentrations of these four HMs in the paddy soil

increased in the second year compared with the first year, by 14, 42, 34 and 23% for As, Cd, Cu and Zn, respectively (Table 1). Although the concentrations of all these HMs in the soil were still within the Chinese Soil Environmental Quality Standard, Grade II (Chinese Environmental Quality Standard for Soils 1995), Cu, Zn and Cd in the PBR-fertilized field were significantly higher than the background of local soil. The concentrations of Cr, Pb and nickel (Ni) in soils from PBR-fertilized fields were similar to those from the control fields. These data indicated that PBR fertilization resulted in As, Cd, Cu and Zn accumulation in paddy soil.

To investigate the effects of PBR fertilization on soil HM accumulation in soil profile, the vertical distribution of HMs among soil depths was analyzed (Fig. 1). The concentrations of As, Cd, Cu and Zn increased rapidly in the soil of the tillage layer. From 40 cm in depth to the surface soil, the concentrations of As, Cd, Cu and Zn increased 2.6, 2.3, 3.2 and 1.8 times respectively, while the concentrations of Cr, Pb and Ni did not change significantly over the soil depth. In addition, the concentrations of all the tested HMs did not increase in the topsoil of the control fields, including As, Cd, Cu and Zn (data not shown). This result further confirmed that PBR fertilization introduced As, Cd, Cu and Zn to the paddy soil.

PBR fertilization results in significantly higher concentration of HMs in rice plants

Rice straw and husks are usually used as feed for livestock and poultry. HM concentrations in straw and

Figure 1 Vertical distribution of heavy metals in soil fertilized with pig-biogas residues. Soil samples were taken from different soil depth (<10, 10–20, 20–30, 30–40, 40–50, 50–60, 60–70 and 70–80 cm). Data are the mean of three composite samples which were taken from 3 independent fields. The concentrations of Cd are given in $\mu\text{g kg}^{-1}$, the other metals in mg kg^{-1} . As, arsenic; Cd, cadmium; Pb, lead; Cr, chromium; Ni, nickel; Cu, copper; Zn, zinc.

husks were thus analyzed in this study (Table 2). Data showed that the contents of toxic elements, such as As, Cd, Pb and Cr, were lower than the livestock feed standards, such as Chinese Feed Hygienic Standard (2001), even in the samples fertilized with PBR. However, compared with the samples collected from the control fields, PBR fertilization increased the accumulation of As, Cd, Cu and Zn in straw by 1.9, 3.8, 2.2 and 5-fold, and in husks by 2.7, 2.4, 2.5 and 1.5-fold respectively. However, PBR fertilization did not significantly affect the accumulation of Cr, Pb and Ni in rice straw or husk. Moreover, from the PBR-fertilized field, the concentrations of As, Cd, Cu and Zn in straw and husk increased significantly in the second year compared to the first year, in rice straw by 38%, 23%, 23% and 39% respectively, and in husks by 10%, 29%, 17% and 16%, respectively (Table 2). However, in the control fields, the accumulation of all these metals did not exhibit a significant difference between the two years. These results demonstrated that PBR fertilization significantly increased the accumulation of As, Cd, Cu and Zn in rice straw and rice husk.

Polished rice grain is the staple food for a large portion of the world's population. In the polished rice harvested from the PBR-fertilized field, the contents of toxic elements, such as As, Cd, Pb and Cr, were below the allowable levels of the Chinese Food Safety Standards (2005). Although the contents of the toxic elements were within the standards, the concentrations of some metals were significantly higher than those from the control

fields, such as Cd, Cu and Zn, which were about 4, 3 and 2-fold higher, respectively, than those from control fields. For the other metals, such as As, Cr, Pb and Ni, the accumulations in polished rice from the PBR-fertilized field were similar to those from the control fields (Table 2). In contrast to straw and husks, the accumulation of all these metals in the polished rice harvested in the second year was not significantly higher than that in the first year (Table 2). These results demonstrated that in the grains grown in PBR-fertilized field, the concentrations of the toxic elements are currently lower than the standards of food quality, while the concentrations of Cd, Cu and Zn were significantly higher than those from the control fields, posing potential risk to food safety in the future.

HM translocation factor (TF) increased in the PBR-fertilized paddy soil

PBR fertilization results in massive HM input into paddy soil. To analyze the biological availability of these HMs from the soil to rice plants, the activity of HM translocation from soil to plants was compared among these fields. The translocation factor (TF) was determined as the ratio of straw concentration to soil concentration for each HM (Fig. 2). As, Cd and Zn had high translocation activities from soil to rice plants, while Cr and Ni had relatively low activities. In addition, the translocation activities of As, Cd and Zn in the PBR-fertilized field were significantly higher than those in the control fields, while the TF of Cu was significantly lower. This result indicated that the HMs in paddy soil derived from PBR fertilization were available to be taken up by rice plants.

Genotypic differences in HM accumulation in the polished rice grains

To investigate the genotypic differences of rice grain HM accumulation, 20 rice genotypes were grown in both PBR-fertilized and control fields. For each metal, the mean concentrations of As, Cd, Cu and Zn in polished grains from the PBR-fertilized field were significantly higher than those from control fields, while the absolute difference and coefficient of variation were similar among these three fields (Table 3). The difference between maximum and minimum ranged from 2.66 to 5.03-fold for different metals, and the coefficient of variation (CV%) ranged from 21.29 to 51.71 (Table 3). Among these HMs, except Cu, the absolute difference of all the other tested metals was higher than 3-fold, and Cd had the largest absolute difference (5.03-fold) and coefficient of variation (51.71). This result indicated that HM accumulation in polished rice grains exhibited

Table 2 Heavy metal concentrations in rice (*Oryza sativa* L.) tissues

Tissues	Fields	Year	Arsenic	Cadmium	Lead	Chromium	Nickel	Copper	Zinc
Polished rice	PBR	First	99.1 ± 4.6	32.0 ± 2.9*	74.4 ± 5.4	343.4 ± 21.5	84.5 ± 5.4	3.4 ± 0.2*	21.3 ± 1.9*
		Second	93.8 ± 6.1	35.3 ± 2.4*	77.7 ± 6.7	315.6 ± 29.6	102.3 ± 11.3	3.8 ± 0.3*	25.8 ± 1.6*
	Control	First	108.4 ± 9.2	7.3 ± 0.8	88.5 ± 8.6	393.2 ± 33.0	92.9 ± 20.1	1.7 ± 0.1	15.1 ± 2.1
		Second	96.4 ± 10.3	8.7 ± 1.1	78.5 ± 5.8	331.3 ± 18.3	84.7 ± 10.2	1.2 ± 0.1	16.9 ± 2.0
Husk	PBR	First	1157.0 ± 101.4*	42.8 ± 2.1*	1262.5 ± 101.5	2814.1 ± 100.2	670.6 ± 45.7	7.6 ± 0.5*	38.0 ± 2.9*
		Second	1267.2 ± 72.5*	55.4 ± 2.4*	1424.2 ± 89.3	2371.9 ± 211.4	895.5 ± 96.5	8.8 ± 0.8*	44.0 ± 3.7*
	Control	First	568.6 ± 32.1	27.1 ± 3.1	1233.4 ± 121.0	4142.7 ± 130.9	735.2 ± 53.5	3.0 ± 0.2	25.6 ± 1.8
		Second	465.2 ± 22.8	25.9 ± 3.5	1493.3 ± 95.1	3062.1 ± 301.2	827.4 ± 46.9	3.6 ± 0.3	29.4 ± 1.0
Straw	PBR	First	6079.4 ± 438.4*	171.5 ± 18.0*	3037.2 ± 203.1	3972.6 ± 189.9	653.0 ± 55.3	8.4 ± 0.8*	104.3 ± 9.9*
		Second	8390.5 ± 527.5*	211.1 ± 28.9*	3177.0 ± 198.2	3589.5 ± 213.6	800.3 ± 69.2	10.3 ± 0.6*	145.1 ± 12.4*
	Control	First	3037.2 ± 202.1	55.0 ± 5.0	3091.6 ± 200.2	4175.8 ± 322.0	839.1 ± 39.8	4.0 ± 0.2	35.7 ± 4.9
		Second	4285.1 ± 297.4	54.2 ± 3.1	2488.1 ± 176.4	4386.0 ± 277.3	858.4 ± 78.3	4.8 ± 0.1	29.0 ± 2.3
Standard [†]			150	200	200				

Notes: The concentrations of arsenic, cadmium, lead, chromium and nickel are given in μgkg^{-1} , copper and zinc in mgkg^{-1} .

Data are means $\pm$ SD, $n = 3$.

[†]Chinese Food Hygiene Standards (2005), for arsenic, the standard is the amount of inorganic arsenic;

*indicates value significantly higher than control ($P < 0.05$). PBR, pig-biogas residues.

significant genotypic difference, which provides the possibility of selecting rice varieties with low HM accumulation for PBR-fertilized regions.

To select the rice genotypes with low HM accumulation, HM accumulation in polished rice grains were compared among these twenty genotypes. The absolute concentrations of different HMs in polished rice grains varied significantly, following the trend $\text{Cd} < \text{Pb} < \text{Ni} < \text{As} < \text{Cr} < \text{Cu} < \text{Zn}$, and the concentration of Zn was about thousand times higher than that of Cd (Table 3). Therefore, to compare accumulation of all these HMs among rice genotypes, the relative concentrations were calculated, i.e., the concentration of all the metals in the polished grains of Genotype 1 (G1) were set as 1, and the concentrations of the other genotypes were expressed as a ratio relative to the concentration of G1 (Fig. 3). For most of the rice genotypes, the accumulation ability was not consistent for different HMs, e.g., Genotype 8, which had high concentrations of Cd and Ni, but low As, Pb, and Zn. However, there were also some genotypes which showed consistently low or high concentrations of most of the HMs. Therefore, for the multiple HM co-existing PBR-fertilized fields, rice genotypes should be selected for low accumulation of multiple HMs. From this study, G2, G3, G4, G9 and G17 could be classified as low HM-accumulating genotypes, since the relative concentrations of most these metals were lower than 1; while G10, G11, G13, G14, and G16 can be classified as high HM-accumulating genotypes, since the relative concentrations of most of these metals were higher than 1, with especially high accumulation of Cd and Ni. Therefore, selecting low HM-accumulating rice genotypes could be a prospective strategy for reducing HM levels in the diets for the PBR-fertilization regions. In addition, those high- and low-accumulation genotypes could be useful in molecular marker-assisted breeding of low HM-accumulating crops.

DISCUSSION

Animal manure and PBR fertilization saves a great deal of chemical fertilizers which, in addition to their high cost, are already contributing to environmental pollution (Pimentel *et al.* 1995; Bu *et al.* 2011). Therefore, biogas slurry fertilization has been promoted for sustainable agriculture (Wagner 1999; Smith *et al.* 2007). However, the high concentration of HMs in PBR is also of great concern. Excessive HM concentrations have been widely reported in animal manures (Cang *et al.* 2004; Li and Chen 2005; Li *et al.* 2007; Kuligowski *et al.* 2008). In this study, high concentrations of As, Cd, Cu and Zn were also found in pig manures (Table 1). Moreover, the concentrations of these metals in solid PBR were even

Figure 2 Heavy metals (HMs) translocation factors from soil to rice straw. The heavy metal translocation factors were determined as the ratio of straw concentration to soil concentration of each HM. Data are means $\pm$ SE ($n = 3$). As, arsenic; Cd, cadmium; Pb, lead; Cr, chromium; Ni, nickel; Cu, copper; Zn, zinc; PBR, pig-biogas residues.

Table 3 Difference of heavy metal concentrations in polished grain among 20 rice (*Oryza sativa* L.) genotypes

Difference	Fields	Arsenic	Cadmium	Lead	Chromium	Nickel	Copper	Zinc
Mean	PBR	99.1	32.0	74.4	343.4	84.5	3.4	21.4
	Control	108.4	7.3	88.5	393.2	93.0	1.7	15.1
Max	PBR	203.4	76.6	116.4	511.1	179.5	6.0	39.7
	Control	219.4	25.7	181.1	602.4	184.2	2.5	19.5
Min	PBR	57.4	15.2	37.0	132.4	46.4	2.3	12.9
	Control	60.0	4.8	43.1	206.0	41.1	1.0	7.5
Max/Min	PBR	3.5	5.0	3.2	3.9	3.9	2.7	3.1
	Control	3.7	5.3	4.2	2.9	4.5	2.5	2.6
CV%	PBR	38.6	51.7	21.3	24.9	41.9	25.5	30.4
	Control	38.8	52.6	43.8	27.2	42.8	32.3	25.9

Notes: The concentrations of arsenic, cadmium, lead, chromium and nickel are given in $\mu\text{g kg}^{-1}$, copper and zinc in mg kg^{-1} .

Data are means, $n = 3$.

PBR, pig-biogas residues.

significantly higher than those in pig manure (as shown in Table 1), or those reported in pig manures from other areas (Hsua and Lob 2000; Cang *et al.* 2004; Li and Chen 2005). This may be caused by the fermentation process which degrades organic materials to gas, while the HMs are then concentrated in the remaining solids (Kuligowski *et al.* 2008). In the liquid biogas slurry, HM concentrations were found to be much lower than those in the solid residue, which may be attributed to the dilution of the liquid slurry by pigsty washing water.

HMs in the PBR are loaded into paddy soil through fertilization, and eventually are taken up by rice plants. In this study, the HM levels in the PBR-fertilized soil were found to be within the Chinese soil quality standards (Chinese Environmental Quality Standard for Soils 1995) (Table 1); the toxic element concentrations in straw and husks of PBR-fertilized rice were below the

limits of the Chinese Feed Hygienic Standard (2001) and in the polished rice also meet the Chinese Food Safety Standards (2005) (Table 2). There are many other studies also reporting that biogas slurry fertilization for vegetables, wheat (*Triticum aestivum* L.) or maize (*Zea mays* L.) did not result in excessive HM levels in food (Cheung and Wong 1983; Brus and Jansen 2004; Berenguer *et al.* 2008; Islam *et al.* 2010). However, the results of these studies, including the present one, do not imply that HMs in the PBR can be ignored during long-term PBR application. We found that the long-term PBR-fertilized soil exhibited significantly higher As, Cd, Cu and Zn concentrations than the chemically fertilized control fields and local background (Table 1); this difference indicated the PBR fertilization contributed these metals to the paddy soil. In addition, the accumulation of these four metals increased rapidly in the soil of

Figure 3 Relative concentration of heavy metals (HMs) in polished rice (*Oryza sativa* L.) of different rice genotypes. G1 to G20 represent 20 rice genotypes used in this study. The concentrations of HMs in the polished rice of G1 were set as 1, and the concentrations of the other genotypes were expressed as a ratio relative to the concentration of G1. Data are the mean concentration from three fields. As, arsenic; Cd, cadmium; Pb, lead; Cr, chromium; Ni, nickel; Cu, copper; Zn, zinc.

the tillage layer, and the topsoil had the highest concentration (Fig. 1). These results further confirmed that As, Cd, Cu and Zn were loaded into the paddy soil from PBR fertilizer. Furthermore, As, Cd and Zn in the PBR-fertilized soil are more available and more efficiently transported to plant tissues, which had higher TFs than those in the control fields (Fig. 2). Owing to high accumulation and high bio-availability, the HMs accumulated to significantly higher levels in rice straw and grains from the PBR-fertilized field than those from the chemically fertilized fields (Table 2). Moreover, comparing the accumulation levels between two years, the HMs in both soil and rice plants increased significantly in the second year, especially for As, Cd, Cu and Zn. The observation that regular PBR fertilization resulted in increasing levels of HMs in soils and crops over time was also reported in some other areas (Brus and Jansen 2004; Hough *et al.* 2004). Therefore, increasing HM accumulation in soil and crops caused by long-term PBR fertilization should be an important issue which needs very careful consideration.

Rice has significant genotypic differences in HM uptake and accumulation (Liu *et al.* 2005; Cheng *et al.* 2006; Zeng *et al.* 2008; Norton *et al.* 2009). The genotypic differences in HM accumulation make it possible to screen and/or breed cultivars with low metal accumulation in rice grains, which could be the best potential strategy for harvesting safe rice from slightly and moderately HM-contaminated paddy fields. As

discussed above, PBR fertilization can cause slight to moderate HM contamination in paddy soil. In the present study, 20 major local rice genotypes were screened in long-term PBR-fertilized paddy fields. As expected, there were obvious genotypic differences in grain accumulation of HMs, such as As, Cd, Cu and Zn (Table 3). Among these genotypes, some had already been found to have low HM accumulation properties; for instance, G4 was found to have low accumulation of Cr (Cheng *et al.* 2006; Zeng *et al.* 2008). In the present study, G4 was found to have low accumulation of multiple HMs (Fig. 3). In addition, the genotypic differences examined among different fields were consistent regardless of the fertilization condition (Table 3). This consistent genotypic difference suggested that the difference in HM accumulation in rice grains is likely to be controlled by rice genetic variation. Therefore, for the PBR-fertilized paddy field, selecting rice cultivars with low accumulation of multiple HMs, such as the genotypes of G2, G3, G4, G9 and G17, could be the best potential strategy for reducing HM in rice grains.

CONCLUSION

This study demonstrated that long-term PBR fertilization poses risks of As, Cd, Cu and Zn contamination to paddy soil and rice grains, and identified some rice genotypes with low accumulation properties for multiple HMs. Selecting low HM-accumulating rice genotypes,

such G2, G3, G4, G9 and G17, could be a potential strategy for reducing the HM accumulation in rice grains grown in PBR fertilization regions.

SUPPLEMENTARY MATERIAL

The supplementary material for this article is available online at <http://dx.doi.org/10.1080/00380768.2012.726597>.

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