#### **RESEARCH ARTICLE**



# Bio-organic stabilizing agent shows promising prospect for the stabilization of cadmium in contaminated farmland soil

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#### Abstract

In situ immobilization of cadmium (Cd) has been considered as a cost-effective and non-disruptive remediation technique for Cdcontaminated soils. In this study, several immobilization approaches were compared in a Cd-contaminated agricultural farmland. The soil was treated with different combinations of the immobilizing agents such as biochar (C), rice straw (RS), lime (L), and engineered bacteria *P. putida* X4/pIME (B). The plant yield and Cd uptake of lettuce as well as soil Cd fractionations were measured. The Cd content in lettuce leaves and roots decreased by 46.8~67.2% and 36.8~60.2%, respectively. Among the five treatments, combined rice straw, lime, and engineered bacteria treatment showed the lowest Cd concentration in lettuce leaves (0.14 mg/kg) and the highest plant yield (21.5 t/ha). The alleviating effects are assigned to the significant transformation of water soluble and exchangeable Cd to humic substance bound, strong organic bound and residual Cd in the soils. This study suggests that this bio-organic stabilizing agent is more cost-effective than some other immobilization agents reported previously, and shows a great application prospect in improving agriculture production of heavy metal-polluted agricultural soils.

Keywords In situ immobilization  $\cdot$  Cd  $\cdot$  Rice straw  $\cdot$  Biochar  $\cdot$  Lime  $\cdot$  Bacteria *P. putida* X4/pIME  $\cdot$  Cost-effective

## Introduction

Increasing accumulation of heavy metals and metalloids has been reported in agricultural soils due to the application of bio-solids, industrial effluents (Alvarez and García 2007), and phosphate fertilizers (Huang et al. 2007; Bolan et al. 2014). This has been a worldwide problem especially in developing countries like China. The latest survey on soil pollution by the Ministry of Environmental Protection and Ministry of Land & Resources of China shows that Cd-contaminated agricultural soils amount to 7.0% of the total farmlands in the country (MEP and Ministry of Land and Resources 2014). Contamination of heavy metals in soils may lead to the excess uptake and accumulations by crops and ultimately impact food safety and human health. It is therefore crucial and imperative to remediate heavy metal-contaminated soils. In situ immobilization has been regarded as the most effective and practical strategy for the remediation of heavy metalcontaminated farmlands (Basta and McGowen 2004; Li et al. 2016; Sun et al. 2013). This approach relies on the addition of soil amendments to reduce the activity and bioavailability of heavy metals via sorption, precipitation, complexation, ion-exchange, and redox processes (Porter et al. 2004; Mohamed et al. 2010).

A number of organic and inorganic materials have shown to be effective in the immobilization of various heavy metals in soils (Liu et al. 2009; Chen et al. 2010; Cambier et al. 2019). The addition of 6% rice straw to the contaminated soil (5 mg/kg Cd) increased soil pH by 0.4 units, and soluble Cd concentration was decreased from 2.25 to 1.69  $\mu$ g/L (Cui et al. 2008). Biochar has been used in many experiments for the immobilization of toxic trace elements in soils (O'Connor et al. 2018). In a field trial, the application of biochar at 40 t/ ha in a Cd-polluted paddy soil (5 mg/kg) reduced the Cd concentration in rice grain from 0.72 to 0.24 mg/kg (Bian et al. 2014). The application of 40 t/ha wheat straw biochar to the Cd-contaminated soil (0.90 mg/kg) decreased the content of Cd in rice grain from 1.17 to 0.46 mg/kg (Chen et al.

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2016). Several inorganic materials have also been utilized in the stabilization of Cd for contaminated soils. Lee et al. (2011) demonstrated that the addition of 5% lime in a soil containing 2.45 mg/kg Cd decreased the content of Cd in lettuce shoots from 4.24 to 1.29 mg/kg and soil pH was raised from 4.58 to 8.10. The combined addition of limestone and sepiolite at the rates of 2, 4, and 8 g/kg to a Cd-contaminated soil (3.03 mg/kg) decreased the Cd content in brown rice by 56.1–66.8%, and exchangeable Cd concentration was decreased by 88.3–98.9% (Wu et al. 2016).

Microbes are rich in surface charges and functional groups and may have great potential in taking up and sorbing heavy metals, especially in the biosorption and precipitation of Cd (Fang et al. 2009; Wu et al. 2010; Wang et al. 2016). The inoculation of Cd-polluted soil with Ralstonia eutropha MTB enhanced the biomass production of tobacco (Nicotiana bentaminana) with the immobilization of approximately 70% of bioavailable Cd in soil (Valls et al. 2000). Increased binding capacity for Cd<sup>2+</sup> from 3.3mg/g to 12.6 mg/g dry weight was observed in Pseudomonus putida X4 cells with surface-expressed 4mMT $\alpha$ -EGFP (He et al. 2011). By using this EGFP tagged P. putida X4 ( $10^7$  CFU/g) in a contaminated soil with Cd concentrations from 0 to 10 mg/kg, Xu et al. (2012) observed decreases of Cd uptake by 10.0~62.0% and 8.1~60.1% in the shoots and roots of pak choi, respectively.

In previous studies with regard to the in situ remediation of Cd-contaminated soils, the stabilizing agents used were mainly single materials. Limited work has focused on composite materials, especially the materials containing microorganisms. In addition, the application cost of these stabilizing agents has rarely been taken into account, which limits their utilization and popularization on vast contaminated farmlands. The objective of the present study was to find practical and cost-effective composite agents to reduce the entry of metals into the food chain. In this work, we evaluated the combination of straw, calcium carbonate, and bacteria P. putida X4/pIME in the immobilization of Cd in a polluted farmland near the Cu mining area in central China and its application potentials in remediation practices. Lettuce was used as the indicator plant to assess the uptake and accumulation of Cd. Sequential extraction was employed to determine the chemical forms of Cd after soil treatments.

## Materials and methods

## **Field plot experiment**

Field plot experiment was set up at a contaminated farmland near the mining area of Daye, Hubei province (N: 30° 0', E:

114° 55'). The mean annual temperature of Daye is 16.3 °C. The total annual precipitation is 1386 mm. According to USDA classification, the soil is an Alfisol with the texture of silty clay loam. Soil pH, OM, and total Cd are 4.95, 40.10 g/kg, and 2.01 mg/kg, respectively. The main clay minerals in this soil are illite (28.6%), kaolinite (63.6%), and 1.4-nm intergrade minerals (7.8%).

Rice straw (RS), lime (L), and bacteria P. putida X4/pIME (B) were used as immobilizing agents in this field experiment. In our previous experiments, the combined application of RS (23.2 t/ha), B ( $1.5 \times 10^{17}$  CFU/ha), and L (2 t/ha) in a soil containing 5.33 mg/kg Cd decreased significantly the content of Cd in lettuce shoots as compared to the treatments of R and RS + B, respectively (unpublished data). Therefore, we included the RS + L + B treatment in this experiment. Biochar was selected as another organic material for comparison. Five treatments (Control, C, C + L, C + L + B, RS + L + B) were designed with the amounts of amendments used were 23.2 t/ ha (RS), 10 t/ha (C), 4 t/ha (L), and  $5 \times 10^8$  CFU/g soil (B). The amount of rice straw used in this experiment refers to our previous experiment (Mohamed et al. 2010), and the amount of biochar added was based on the amount of organic carbon in rice straw. The plots were arranged in a completely randomized design with four replicates per treatment, and the area of each plot was 5 m<sup>2</sup> (2 m  $\times$  2.5 m); those materials were evenly mixed into the topsoil (0-20 cm). Rice straw was obtained from the farm of Huazhong Agricultural University, Hubei province, China. It was dried in sunlight, and then cut into small pieces (4-5 cm). The lime was industrial grade reagent and bought from Tianjin BoDi Chemical Industry Company. Bacteria P. putida X4/pIME (Pseudomonus putida X4 cells with surface-expressed 4mMT $\alpha$ -EGFP) was constructed in our lab. Biochar made from bamboo was provided by Zhejiang Academy of Agricultural Sciences, China. Some relevant properties of the materials are given in Table 1.

### Sampling and soil and plant analysis

Lettuce (*Lactuca sativa*, a bolting-resistant and heat-resistant variety comes originally from Italy), was sowed 1 month after the application of various materials. The total amount of 250 kg N/ha as urea was applied. The basal fertilizer was broadcasted 1 month prior to sowing at the rates of 30% total N. Four and 6 weeks after sowing, 50% and 20% of total N were applied respectively. The plants were harvested after 3 months growth and the total above ground biomass (fresh weight) was measured. The soil and lettuce samples were collected for analysis.

Soil samples were air dried and ground to pass through the 2-mm nylon sieve. The samples were used for the measurement of pH, soil organic matter, available phosphate and potassium, total nitrogen, and Cd contents. The vegetable plants were washed with tap water and deionized water, then Table 1Some properties of thematerials used

	Total C (g/kg)	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	Total Cd (mg/kg)	pН
Rice straw	380.20	7.70	2.70	9.09	0.10	_
Biochar	889.00	6.90	1.10	5.60	0.02	9.80
Lime	ND	ND	ND	0.05	ND	11.2

ND, not determined

separated into roots and leaves, over dried at 80 °C to constant weight, and milled into powder for measurement of heavy metal content.

The soil pH was measured using a 1:2.5 (w/v) soil/water ratio. Soil organic carbon (SOC) was analyzed by potassium dichromate oxidation method (Bao 2000). Soil samples were digested by  $H_2SO_4$  with mixed catalyst ( $K_2SO_4$ :CuSO<sub>4</sub>:Se = 100:10:1), and total nitrogen (N) concentration was determined by Kjeldahl's azotometer (FIA-star 5000, FOSS Tecator, Sweden). Available P and K were assayed by NaHCO<sub>3</sub> and NH<sub>4</sub>OAc extraction methods respectively (Bao 2000). The dried plant and soil samples were digested with HNO<sub>3</sub>-HClO<sub>4</sub> (4:1 in volume). The fractionation of Cd in the soil was performed using a modified Tessier sequential extraction procedure (Tessier et al. 1979; Tang et al. 2006; Shi et al. 2018) which is shown briefly in Table 2. The activity or mobility of heavy metals in soil was evaluated by the mobility factor (MF) which was calculated as the percentage of the sum of water soluble, exchangeable, and specifically adsorbed fractions to the total amount of heavy metal species (Sablu and Krekelig 1998; Narwal et al. 1999; Kabala and Singh 2001). The concentrations of Cd in plant digestions and soil extracts were analyzed by atomic absorption spectrophotometer (AAS, Varian AA240FS, Australia).

#### **Statistical analysis**

All statistical analyses were conducted using the SPSS 20 program. One-way variance analysis (ANOVA) was carried out to compare the means of different treatments. The correlations between soil variables and heavy metal availability

were also assessed. The corresponding figures were drawn with Microsoft Office Excel 2013, OriginPro 9.0.

## Results

#### Soil chemical properties

As shown in Table 3, soil pH was increased significantly by various amendments. The increments of soil pH were in the range of 1.24~2.25 units for all the treatments with introduced materials. In particular, the largest increase in soil pH was from 4.95 to 7.07 which was observed in the treatment of combined addition of biochar, engineered bacteria, and lime. Soil organic matter was enhanced by 24.5~28.2% for all the amendments, and the largest increase was found in the RS + L + B treatment, from 40.10 to 51.43 g/kg. Soil available phosphorus and potassium were markedly enhanced by 96.2% and 2.5 times after the addition of RS + L+ B as compared with control. No significant differences were found for soil available P and K among all the amendments although the total amounts of P and K for rice straw added were larger than that of biochar. This is presumably due to the different availability of P and K in rice straw and biochar (Ngo et al. 2013; Uchimiya and Hiradate 2014; Al-Wabel et al. 2018)

#### Fractionation of soil cadmium

The sequential extraction on the distribution of Cd in untreated and amended soils is presented in Fig. 1. Water soluble Cd

 Table 2
 Metal concentrations in different forms extracted by Tessier improvement procedures

Fractionation	Extraction agent	Soil/solution (w/v)	Equilibrium conditions
Water soluble	Water without carbon dioxide $pH = 7$	1:10	25 °C, 2 h
Exchangeable	1 M MgCl <sub>2</sub> pH = 7	1:10	25 °C, 2 h
Specifically adsorbed	1 M NaOAc $pH = 5$	1:10	25 °C, 5 h
Humic substance bound	$1 \text{ M Na}_4 P_2 O_7 \cdot 10 H_2 O$ pH = 10	1:20	25 °C, 3 h
Fe-Mn oxides bound	0.25 M NH <sub>2</sub> OH-HCl	1:20	25 °C, 6 h
Strong organic bound	0.02 M HNO <sub>3</sub> , $H_2O_2$ , 3.2 M NH <sub>4</sub> OAc-HNO <sub>3</sub> , water	1:20	83 °C, 2.67 h/ 25 °C, 10 h
Residual	HNO <sub>3</sub> -HCl-HClO <sub>4</sub>	1:5	Digestion

**Table 3**Some basic properties ofsoils on field experiment

	рН	OM <sup>a</sup> (g/kg)	Total N (g/kg)	Available P (mg/kg)	Available K (mg/kg)
Control	4.95 d	40.10 b	1.72 a	5.28 b	81.19 d
С	5.11 d	49.92 a	2.19 a	10.37 a	116.14 bc
C+L	6.85 a	50.85 a	2.09 a	9.57 a	131.45 b
C + L + B	7.20 a	50.77 a	2.07 a	8.26 a	119.16 bc
RS + L + B	6.19 b	51.43 a	2.25 a	10.36 a	205.82 a

<sup>a</sup> Organic matter (control = no amendments, *RS* rice straw, *C* biochar, *B* engineered bacteria, L = lime. Different letters are significantly different at p < 0.05)

was decreased by 83.1% for RS + L + B treatment, and the reduction of water soluble Cd was in the range of 70.2~84.8% for other four treatments. The exchangeable Cd was decreased by 52.1% for RS + L + B treatment, and less reduction was observed in biochar treatments (29.3~43.9%). On the other hand, the humic substance-bound Cd was increased by 113~118% for the two biochar combination treatments. The RS + L + B enhanced the humic substance-bound Cd more remarkably by 165%. Similarly, the RS + L + B promoted the Fe-Mn oxide–bound Cd by 51.7%. At the same time, strong organic-bound Cd was increased separately by 197~258% while residual Cd was increased by 17.0~18.5% for all the treatments.

The MF of Cd for the control was 40.75% and those for the four biochar treatments ranged from 29.83 to 24.92%. The lowest MF value (22.32%) was observed in RS + L + B-amended soil (Table 4) indicating that larger amounts of cadmium were transferred from the exchangeable fraction to the immobile species. The results suggest that the combined additions of rice straw, lime, and bacteria *P. putida* X4/pIME performed better than the other treatments in the conversion of mobile Cd fractions.

The correlation analysis indicates that soil pH is negatively correlated with water soluble (r = -0.418, p < 0.05) and exchangeable Cd (r = -0.527, p < 0.01), while positively correlated with specifically adsorbed (r = 0.550, p < 0.01) and



Fig. 1 Soil Cd concentrations in different forms. a Water soluble Cd. b Exchangeable Cd. c Specifically adsorbed Cd. d Humus-bound Cd. e Fe-Mn oxides-bound Cd. f Strong organic-bound Cd. g Residual Cd.

 Table 4
 Distribution of cadmium fractions in different amendment addition soils

Treatment	Fraction (%)							
	Water soluble	Exchangeable	Specifically adsorbed	Humic substance bound	Fe-Mn oxides bound	Strong organic bound	Residual	(%)
Control	0.25a	36.59a	3.91a	3.74c	4.96b	0.32b	50.23b	40.75a
С	0.11b	25.82b	3.90a	4.82c	5.62ab	1.12a	58.61a	29.83b
C+L	0.08c	22.24c	4.17a	5.12c	6.48ab	1.16a	60.75a	26.49c
C + L + B	0.07c	20.99cd	4.17a	8.13b	6.39ab	1.05a	59.20a	25.23c
RS + L + B	0.04d	17.49d	4.79a	9.81a	7.51a	0.94a	59.42a	22.32d

The numbers represent the percentages of each fraction

Control no amendments, RS rice straw, C, biochar, B, engineered bacteria, L, lime. Different letters are significantly different at p < 0.05

humic substance-bound Cd (r = 0.449, p < 0.05) (Table 5). Likewise, soil organic matter concentration is negatively correlated with water soluble (r = -0.786, p < 0.01) and exchangeable Cd (r = -0.604, p < 0.01), while positively correlated with humic substance-bound (r = 0.546, p < 0.05), strong organic-bound (r = 0.741, p < 0.01), and residual Cd (r = 0.673, p < 0.01) (Table 5). These results suggest that the effect of amendments on the distribution of soil Cd is governed by soil pH and organic matter.

## Plant yield and cadmium uptake

Figure 2 shows that all the amendments had significant influences on the fresh weight of lettuce. The yields were increased by 77.1~83.0% for the C, C + L + B, and C + L + B treatments. More marked increase of lettuce biomass (1.26 times) was detected for the RS + L + B treatment. The significant improvements for plant yields in all amendments may be assigned to the higher contents of available phosphorus and potassium in these soils. This is further supported by the higher concentration of soil available K (1.56–1.77 times) in RS + L + B than in biochar treatment.

The Cd concentration of lettuce roots and leaves for the control was 1.06 mg/kg and 0.50 mg/kg, respectively. (Fig. 3). Biochar treatment decreased notably the content of Cd in lettuce leaves by 41.5%. More significantly, the decrements of Cd for C + L, C + L + B, and RS + L + B treatments

were in the range of  $63.9 \sim 71.1\%$ . Accordingly, the Cd content in lettuce roots for all treatments was declined by  $35.8 \sim 60.2\%$ . It is important to note that the Cd levels in the edible parts of lettuce for C + L, C + L + B, and RS + L + B treatments were lower than the maximum permissible concentration for Cd in leafy vegetables (0.2 mg/kg) according to the National Food Hygiene Standard of China (NFHSC, GB 2762-2017).

Regression analysis shows that the Cd contents in lettuce are significantly and positively correlated with the concentrations of water soluble (r = 0.992, p < 0.01) and exchangeable Cd in soils (r = 0.986, p < 0.01). Conversely, the Cd contents in lettuce leaves are negatively correlated with Fe-Mn oxide– bound Cd (r = -0.842, p < 0.01), strong organic-bound (r = -0.816, p < 0.01), and residual Cd (r = -0.935, p < 0.01) (Table 5). It is therefore evident that the transformation of water soluble and exchangeable Cd to organic-bound and residual Cd in soils may lead to lesser uptake of Cd by the lettuce.

# **Discussion and conclusions**

This in situ stabilization showed that the Cd contents in lettuce (0.14 mg/kg) for the rice straw combined with bacteria and lime treatment was lower than the maximum permissible concentration for Cd in leafy vegetables (0.2 mg/kg). The concentration of Cd in this soil is 2.01 mg/kg which falls in the

Table 5 Linear correlation analysis between soil pH, soil organic matters, cadmium contents in the lettuce, and cadmium fractions in the soil

Soil		Cd in leaves	Cd in roots	Water soluble	Exchangeable	Specifically adsorbed	Humic substance bound	Fe-Mn oxides bound	Strong organic bound	Residual
Pearson Correlation	Cadmium content in the leaves	1.000	0.991**	0.992**	0.986**	-0.494	-0.776	$-0.842^{*}$	-0.816*	-0.935**
	Soil pH	$-0.619^{**}$	$-0.643^{**}$	$-0.418^{*}$	$-0.527^{**}$	$0.550^{**}$	$0.449^{*}$	0.191	0.402	-0.022
	Soil organic matter	-0.714**	-0.801**	-0.786**	-0.604**	- 0.009	0.546*	0.286	0.741**	0.673**

(\**p* < 0.05; \*\**p* < 0.01)

**Fig. 2** Lettuce yield in the field experiment (Control, no amendments; RS, rice straw; C, biochar; B, engineered bacteria; L, lime. Different letters are significantly different at p < 0.05)



moderate range of Cd pollution. We utilize the combination of rice straw with bacteria and lime as stabilizing agent for soil Cd remediation. This agent was proved to be effective for the mitigation of cadmium uptake by plant in moderately contaminated soils. Li et al. (2014) reported that the Cd content of spinach was decreased from 0.75 to 0.41 and 0.47 mg/kg with the addition of rape and corn straw in a soil containing 1.5 mg/kg Cd; this result was obtained by a field plot experiment and the size of each plot was 4 m<sup>2</sup>. Recently, Zhang et al. (2017) addressed that the uptake of Cd by lettuce was reduced from 0.49 to 0.32 mg/kg following amendment of biochar (at 20 t/ha) to a Cd-contaminated soil (3.3 mg/kg); the field experiment was conducted in two typical vegetable greenhouses. Both of these studies demonstrated the ameliorating effect of the organic materials on soil Cd pollution. The maximum permissible concentration for Cd in leafy vegetables is 0.2 mg/kg. Therefore, our bioorganic agent has great potential in decreasing the bioavailability of soil Cd, which can meet the requirement of food safety ultimately. Although the C + L and C + L + B treatments produced the similar effect of Cd uptake by plants compared with RS + L + B treatment,



well as enhanced productivity for plants by the application of RS + L+ B. The restrained Cd uptake by lettuce could be assigned to the redistribution of Cd in soil fractions. The application of rice straw combined with bacteria and lime significantly decreased the water soluble and exchangeable Cd by 83.8% and 52.1%, and increased the humic substance bound and residual fractions by 165% and 18.3%, thus reduced the mobility and bioavailability of soil Cd. The immobilization of Cd by this treatment could be mainly explained by the increase of soil organic matter content and soil pH. The RS + L + B treatment significantly enhanced soil pH by 1.24 units and soil organic matter content by 11.33 g/kg (Table 3); both are significantly correlated with the depression of soil water soluble and exchangeable Cd (p < 0.01) (Table 5).

more marked increase of lettuce yield was observed for RS + L + B. This shows the depressed Cd bioavailability in soils as

Beside the immobilization efficiency, the cost of in situ stabilization techniques is also vital for their applications in farmlands at large scale. Although quite a number of studies have been devoted to biochar for the remediation of heavy



metal-polluted soils over the past years, the high cost has limited its application in agricultural fields. According to the current market quotation, the input for C + L and C + L + B is about 8000 \$/ha and 8600 \$/ha (biochar 765 \$/t, lime 78 \$/t, bacteria 4  $10^{15}$  CFU), respectively. By contrast, the RS + L+B would cost only about 2700 \$/ha (rice straw 76 \$/t). This is relatively much cheaper than the biochar treatment or some other immobilization agents reported previously. For example, the cost for manure amendment was around 8000 \$/ha regarding the remediation of Cu-contaminated soil (Pérez-Esteban et al. 2012). The in situ remediation of multiple metals in a polluted field by zero Fe was estimated to be as high as 24,000 €/ha (Hanauer et al. 2011). The cost of our combination agent corresponds to 2700 \$/ha which has large economic potential to be used and popularized by farmers. Therefore, the combined use of rice straw with lime and bacteria P. putida X4/pIME is more cost-effective for the in situ remediation of heavy metal-contaminated soils. In addition to the immobilization of heavy metals, this agent significantly improved soil quality and soil fertility. With the application of this stabilizing agent, soil organic matter content was increased by 28.3% while available phosphorus and potassium were increased by 96.2% and 153.5%, thereby induced the increment of lettuce yield (Table 3, Fig. 2).

Recent nationwide surveys show that 19.4% of the examined agricultural soil samples exceed China's soil environmental quality standard, mainly (82.4%) with toxic metals and metalloids (Zhao et al. 2014). Among the toxic metals and metalloid-contaminated agricultural soils, medium and mildly contaminated soil accounts for the majority (94.3%). Thus, this bio-organic agent shows a huge application prospect for the remediation of heavy metal-polluted agricultural soils in China. Furthermore, to make it easier for farmers to use this technology, we could consider to produce an organic fertilizer using rice straw, lime, and bacteria P. putida X4/ pIME as the major ingredients. It is obvious that this fertilizer could not only remediate Cd pollution, but also provide soil nutrients. It is supposed that the application of this fertilizer in heavy metal-contaminated soils will largely reduce the cost and increase the economic effect for the farmers.

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