

Influence of Biochar on Microbial Activities of Heavy Metals Contaminated Paddy Fields

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Biochar (BC) amendments might decrease the bioavailability of metals in soils that are contaminated with heavy metals. In general, soil microbial communities are sensitive to changes in soil property changes. Microbial communities were tested in a Cd- and Pb-polluted paddy field in southern China. BC was applied as a basal soil amendment before rice transplantation in 2009. The BC was applied at rates of 0, 10, 20, and 40 tons per hectare. Soil heavy metal fractions with sequential extraction procedure, soil microorganisms, and enzymes were monitored in 2011. The soil pH and soil organic carbon (SOC) were significantly increased by 2% to 5% and 16% to 51% under BC amendment, respectively. Compared to the non-BC treatment, the cadmium (Cd) and lead (Pb) acid-soluble fraction concentrations were significantly decreased by 15.3% to 26.7% and 18.2% to 30.9%. The Cd and Pb reducible fraction were decreased by 13.5% to 25.6% and 21.9% to 23.53%. The Cd and Pb oxidizable fraction by 15.4% to 69.2% and 22.7% to 29.3% with BC application, respectively. The populations of actinomycetes and fungi were increased by 19.0% to 38.5% and 3.7 to 9.3 times, respectively. Meanwhile, BC significantly increased the cellulose, urine enzyme, neutral phosphatase, and sucrase activities by 117.4% to 178.3%, 31.1% to 37.6%, 29.7% to 193.8%, and 36.5% to 328.6%, respectively. BC amendment offers a basic option to reduce Cd and Pb bioavailability and change the fractions. The BC also increases microorganism quantity and soil enzyme activity.

Keywords: Biochar (BC); Heavy metals; Rice paddy; Soil amendment; Soil microorganisms; Soil enzyme

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INTRODUCTION

Heavy metals of anthropogenic origin degrade soil quality and detrimentally affect many environments (Recatalá *et al.* 2010). The introduced metals have adversely impacted the ecology of microbial communities in soil. Such contamination prompts public health concerns that humans and livestock might inadvertently be exposed to potentially harmful heavy metal elements via staples harvested from contaminated soils (Chaney *et al.* 2004). In China, combatting cadmium contamination of rice harvested from industrial waste-tainted paddy fields has become a national priority.

Biochar, a by-product of biomass pyrolysis to generate energy, has shown promise in remediating heavy metal-contaminated soils and might reduce the potential of

cadmium transfer up the food chain (Lehmann *et al.* 2006; Lee *et al.* 2010). Direct benefits of biochar amendment may include: increasing pH, organic matter content, and moisture retention of soils; enhancing the soils' ability to adsorb heavy metals; improving soil structure and nutrient retention; and reducing N₂O and CH₄ emissions (Lehmann and Joseph 2009; Cao *et al.* 2009; Atkinson *et al.* 2010; Sohi *et al.* 2010). Cui *et al.* (2011, 2012) reported that crops responded to biochar application, and heavy metals contents of harvested plant tissues and grains were significantly reduced. Gomez-Eyles *et al.* (2011) observed significant reductions in available Cd and Cu and increases in pH two months after soils received the biochar treatments. Following amendment at rates of 4% and 8%, the biochar reduced plant-available Cu, Ni, Zn, and Pb of the affected soils. When the mobile fractions of soil Cu, Ni, Zn, Cd, and Pb were lowered, the risk of metals leaching in agricultural soils was retarded (Méndez *et al.* 2012). Through biochar (producing by straw, husk) was applied at rate of 5%, Cd, Zn, and Pb concentrations of rice shoots harvested at the affected soils decreased by up to 98%, 83%, and 72%, respectively, compared to that of the control in a pot experiment (Méndez *et al.* 2012).

Microbes (bacteria, actinomycetes, and fungi) are ubiquitous and integral parts of soils; they play significant roles in the recycling of soil-borne C, N, P, S, and metallic elements (Yang *et al.* 2007), making them available to plants (Rajkumar *et al.* 2012). Heavy metals in bioavailable forms adversely affect soil microbes by reducing their populations and changing the community structure and diversity (Renella *et al.* 2005). The adverse impacts are reflected in terms of decreased soil enzyme activities (Belyaeva *et al.* 2005) and interferences in plant-soil-metals association (Wang *et al.* 2008). Kandeler *et al.* (1996) noted that heavy metals reduce functional diversity of the affected soil microbial communities. Soil microbes by extension would reflect the overall health of the heavy metal-contaminated soils (Kennedy and Smith 1995). Activities of soil enzymes such as invertase, urease, and acid phosphatase become metrics of the soil quality (Chen *et al.* 2012). Ormsby *et al.* (2012) found that soil enzyme activity was reduced significantly in all fractions subjected to heavy metal pollution in the following order: arylsulfatase > phosphatase > urease > xylanase. The finding is corroborated by comparable effects on cellulose, invertase, sucrose, *etc.* (Lee *et al.* 2003). Soil enzyme activities are realistic, sensitive measurements of changes in the structure and diversity of the microbial community in metal-polluted soils (Kandeler *et al.* 2000).

If biochar is able to reduce metal uptake of plants, will the biochar amendments protect the microbial communities in heavy metal-polluted soils from harm (Wang *et al.* 2008)? We hypothesized that the biochar amendments in soil would enhance the health of soil microbial communities in Cd and Pb-polluted rice paddies. Our objectives were to illustrate changes of bioavailable Cd and Pb of the contaminated soils and show the corresponding changes in activities of cellulase, neutral phosphatase, urease, and arylsulfatase in relation to the biochar amendments.

EXPERIMENTAL

Site Description

The experiment was set up at a production field (31°24.434'N and 119°41.605'E) where atmospheric fallouts and effluent discharges of an iron smelter had contaminated the soils in the 1970s. Cadmium and lead were the primary pollutants. The local climate

was humid subtropical with a mean annual temperature of 22 °C and annual precipitation of 1,100 mm.

The paddy soil was characterized as ferric-accumulic stagnic anthrosols. The summer rice (*Oryza sativa* L.) and winter wheat (*Triticum aestivum*) rotation has been practiced at this location for a long time, and the heavy metals pollutants were well acclimated in the soils.

Experiment Design

The experiment followed a randomized complete block layout with three replicates for each treatment, and each plot measured 4 m x 5 m. The treatment included application of biochar at levels of 0(Control), 10, 20, and 40 metric tons per hectare in May, 2009. These biochar amendments were applied only at the beginning of the three-year study.

The biochar stock was a local product made from wheat straw pyrolyzed at 450 °C and then ground to pass through a 2-mm sieve. Upon application, the surface-deposited biochar was plowed-in and mixed thoroughly with the upper 20 cm soil. Before sowing, basal fertilizers, N, P₂O₅, and K₂O were applied at 125, 120, and 125 kg per hectare in the forms of urea, calcium biphosphate, and KCl, respectively. Wheat (Zhenmai-5) was planted by direct seeding, and rice (Wugeng-13) seedlings were transplanted. Properties of the biochar and receiving soil were noticeably different, especially in terms of pH, carbon content, and Cd and Pb levels (Table 1).

Table 1. Chemical Properties of the 0-15 cm Depth Topsoil of the Paddy Soil and Biochar Stock

Material	pH (H ₂ O)	Organic C (g kg ⁻¹)	Total N (g kg ⁻¹)	Total P (g kg ⁻¹)	Total K (g kg ⁻¹)	CEC (cmol kg ⁻¹)	Total Cd (mg kg ⁻¹)	Total Pb (mg kg ⁻¹)
Topsoil	6.07	20.71	3.19	0.82	11.4	18.05	21.84	603.31
BC	10.35	467.2	5.90	14.43	11.5	21.70	0.03	12.91

Soil Sampling and Analysis

The 0 to 15 cm depth soils were sampled after rice harvested in October, 2010. Three undisturbed cores were obtained from each plot to make one composite sample with soil collector tool of Eijkelkamp (Netherlands). Each soil sample was cleared of plant debris, air-dried at room temperature, and ground to pass through a sieve of 2 mm openings. A subsample of the soils was ground to pass through a sieve of 0.15 mm openings for Cd and Pb determinations. Soils were analyzed according to procedures described in Lu (2000).

Sequential extraction was performed using the modified four-stage procedure recommended by the European Community Bureau of Reference (BCR) (Žemberyová *et al.* 2006).

The total numbers of culturable heterotrophic bacteria and Colony Forming Units (CFU) of fungi and actinomycetes were determined by serial dilution and plating on selective media (Olsen and Bakken 1987; Davis *et al.* 2005).

Soil neutral phosphatase activity was measured spectrophotometrically by the disodium phenyl phosphate method of Wang *et al.* (2007). The assay for soil cellulose was adapted from Kandeler and Gerber (1988). The assay for soil urine enzyme was adapted from Guan (1986). The assay for soil sucrase was adapted from Schinner *et al.* (1996).

Infrared radiation spectra of biochar was detected by Fourier Transform Infrared Spectrometer (Nicolet 670, USA).

Data Processing and Statistics

All data were reported as mean \pm standard deviation. Differences between the treatments were examined using a two-way analysis of variance. All analyses were carried out using SPSS, version 18.0.

RESULTS

Soil pH and Organic Carbon

Due to the alkaline nature of biochar, amendments significantly increased the pH of soils amended with 10, 20, and 40 ton per hectare by 0.11, 0.27, and 0.29 pH units, respectively, over that of the control treatment (Table 1). The biochar-induced pH changes were significant, roughly equivalent to 0.01 pH unit rises for 1 ton per hectare biochar up to 20 ton per hectares, beyond which the effect of biochar became less noticeable. Meanwhile, soil organic carbon content (SOC) increased by 16.2%, 33.1%, and 51.0%, respectively, over that of the experimental control.

Table 2. pH and Organic Carbon Content of Biochar Amended Paddy Soil (n=3, mean \pm SD)

Biochar (t ha ⁻¹)	pH	SOC (g kg ⁻¹)
Control	6.18 \pm 0.04c	21.99 \pm 0.70d
10	6.29 \pm 0.13bc	25.55 \pm 3.51c
20	6.45 \pm 0.06ab	29.28 \pm 0.51b
40	6.47 \pm 0.07a	33.20 \pm 0.54a

Different lower case letters represent significant difference between the treatments.

Cd and Pb in Soils

Cd and Pb in soils were sequentially fractionated into acid soluble, reducible, oxidizable, and residual fractions. The overall mass recovery of the fractionation procedure was >90%.

Outcomes of the experimental control revealed that Cd was distributed primarily in the acid-soluble and reducible fractions (Table 3). In other words, the Cd derived from the pollution had primarily acclimated into the readily soluble mineral forms and into the oxidized state. Less than 10% of the soil-borne Cd were minerals in reduced forms such as sulfites or were bonded with the soil matrices. Lead was deposited overwhelmingly in the reducible fraction of the soil, and the acid-soluble Pb was the second largest fraction. The acid-soluble fraction was characterized by Cd and Pb minerals in carbonate forms, and these accounted for 50% and 10% of the total metals in polluted soils, respectively. The reducible fraction was characterized by Cd and Pb in association with the Fe-Mn minerals and accounted for 40% and 75% of the total metals in the polluted soils, respectively.

The biochar treatments resulted in distinctively different Cd distribution patterns. In proportion to application rates, Cd in acid-soluble and reducible fractions shifted to the residual fraction (Table 3). The shifts were clearly noticeable and were significantly higher than that of the experimental control. The Cd in acid-soluble and reducible

fraction was reduced by 15.3%, 17.1%, and 26.7% and 18.8%, 13.5%, and 25.6%, respectively, over their respective experimental control with 10, 20, and 40 tons per hectares biochar treatments. The Cd contents of the oxidizable fraction were not significantly changed by the biochar treatments. Accordingly, the amount of Cd in the residual fraction proportionally increased. As the biochar amendment did not involve any reductive or oxidative reaction, it appeared that biochar possessed properties to shift a portion of the Cd from the acid soluble and reducible chemical forms in soils to be tightly bonded to the chemical matrices of biochar. Despite the biochar-induced reductions, the acid soluble and reducible forms remained the primary chemical species of Cd in the soil.

Biochar treatments again reduced the Pb in acid soluble and reducible fractions, while Pb in the oxidizable fraction remained unchanged, and the residual fraction proportionally increased (Table 3). Apparently, the same chemical mechanisms shifted the distributions of Cd and Pb in the biochar amended soils. Pb in the acid-soluble fraction was significantly decreased by 24.7%, 18.2%, and 30.9% over that of the experimental control for biochar amendments of 10, 20, and 40 tons per hectare, respectively. Pb of reducible fractions was reduced by 21.9%, 23.53%, and 22.9%, respectively. The changes had consequences on the ability of plants to absorb Cd and Pb (Cui *et al.* 2011).

Table 3. Cd and Pb in Fractions of Biochar Amended Paddy Soil (n=3, mean \pm SD, mg kg⁻¹)

Biochar (t ha ⁻¹)	Cd				Pb			
	Acid soluble	Reducible	Oxidizable	Residual	Acid soluble	Reducible	Oxidizable	Residual
Control	14.60 \pm 0.85 a	10.35 \pm 0.70 a	0.22 \pm 0.03 a	0.92 \pm 0.16 c	70.64 \pm 5.11 a	538.72 \pm 21.54 a	27.60 \pm 2.28 a	45.73 \pm 4.73 b
10	12.36 \pm 0.42 b	8.40 \pm 1.23 ab	0.07 \pm 0.12 a	3.00 \pm 0.51 b	53.17 \pm 0.94 bc	420.65 \pm 25.50 b	18.95 \pm 1.44 b	46.88 \pm 6.76 b
20	12.11 \pm 0.77 b	8.95 \pm 1.35 ab	0.18 \pm 0.14 a	3.74 \pm 0.48 b	57.80 \pm 3.51 b	482.85 \pm 81.72 ab	23.53 \pm 4.76 ab	50.00 \pm 10.36 b
40	10.71 \pm 1.88 b	7.69 \pm 1.72 b	0.12 \pm 0.13 a	5.48 \pm 0.98 a	48.79 \pm 1.02 c	415.43 \pm 52.72 b	23.02 \pm 2.64 ab	64.68 \pm 6.20 a

Different lower case letters represent significant differences between the treatments.

Soil Microorganism and Enzyme Activity

The biochar amendments up to 40 tons per hectare did not significantly affect the bacterial population of the Cd and Pb-polluted soils (Table 4).

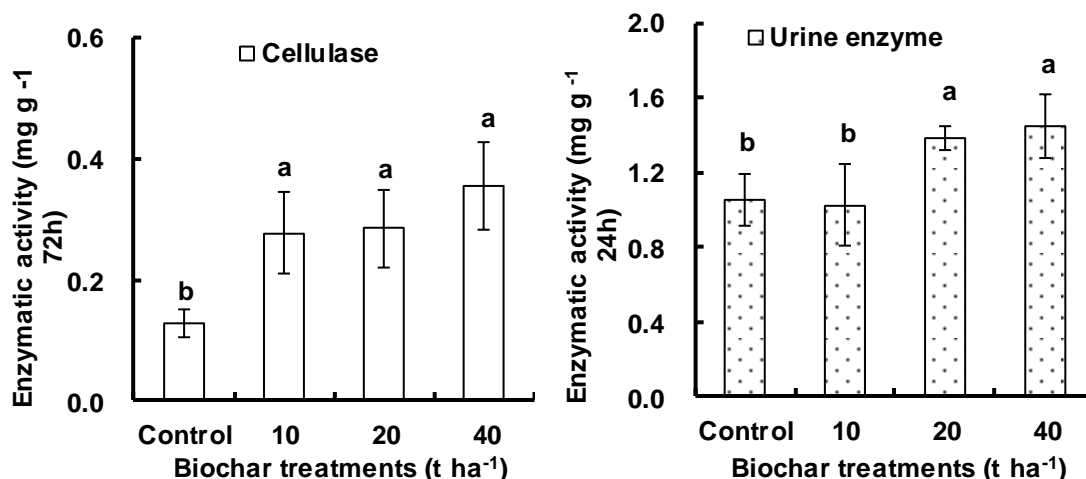
Table 4. Populations of Microorganisms with BC Application in 2011(n=3, mean \pm SD)

Treatments	Bacteria (10^7 CFU g^{-1})	Fungi (10^5 CFU g^{-1})	Actinomycetes (10^6 CFU g^{-1})
Control	2.06 \pm 0.94a	0.52 \pm 0.52c	1.76 \pm 0.59b
10	1.83 \pm 0.17a	2.47 \pm 2.12bc	2.12 \pm 0.62ab
20	2.81 \pm 2.51a	2.91 \pm 1.29b	2.10 \pm 0.41ab
40	2.48 \pm 1.74a	5.37 \pm 3.37a	2.44 \pm 0.18a

Different lower case letters represent significant differences between the treatments.

The fungi and actinomycete populations of the polluted soils were significantly affected. Populations of actinomycetes in treated soils increased by 19.9%, 19.0%, and 38.5%, respectively, and populations of fungi increased by 370%, 460%, and 930%, respectively over the respective experimental controls under 10, 20, and 40 tons per hectare. It appeared that heavy metals were not harmful to the microorganisms in the polluted soils. The soil enzyme activities were assessed to determine if metals inhibit microbial functions.

Compared to the non-BC treatment, cellulase significantly increased by 117.4%, 123.2%, and 178.3%, urine enzyme by -2.6%, 31.1%, and 37.6%, neutral phosphatase by 29.7%, 73.2%, and 193.8%, and sucrose by 36.5%, 254.0%, and 328.6% in 2011 at rates of 10, 20, and 40 tons per hectare under BC application, respectively (Fig. 1).



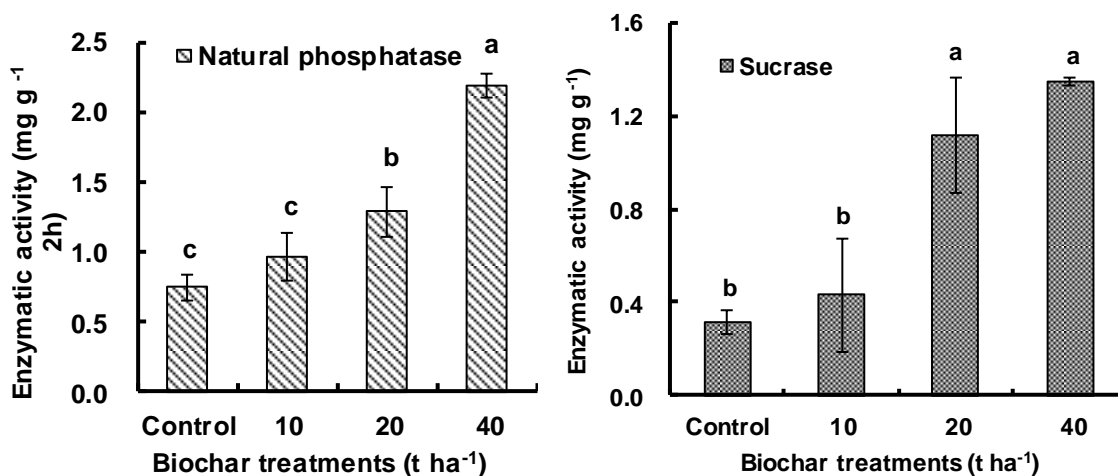


Fig. 1. Enzyme Activity with BC Application in 2011 (n=3, mean \pm SD)

DISCUSSION

The Cd and Pb-polluted soils were ideal for testing how biochar amendments mitigated the harmful effects of heavy metals on soil microbial populations and their metabolism. The experiment was carried out in a production-scale paddy field to test the hypothesis that biochar amendments would enhance microbial populations and metabolisms in Cd and Pb-polluted soils. The soil was contaminated by effluent discharges in the 1970s and has been cultivated without interruptions. In over 30 years, the anthropogenic metals and the inherent microbial ecosystems were all acclimated to the new status quo in the soils. The conditions of this soil were ideal for testing the hypothesis.

Biochar treatments shifted Cd and Pb in polluted soils from readily bioavailable forms to less active forms and added organic carbon into receiving soils. For biochar to enhance microbial populations and metabolisms in the Cd and Pb-polluted soils, the treatments needed to reduce the bioavailability of Cd and Pb in the receiving soils. Cui *et al.* (2011) reported that concentrations of CaCl₂-extracted Cd and Pb in the polluted soils could vary by a wide margin, indicative of transformations that have taken place upon biochar treatments. In practice, soil-borne biochar reduced Cd phytoavailability (Fellet *et al.* 2011). In a more precise manner, our results showed that the biochar amendments caused the Cd and Pb in the acid-soluble sulfate and carbonate precipitates and on surfaces of the reducible Fe-Mn oxides and hydroxide forms to migrate toward the lattices of clay minerals and biochar (Table 2). Biochar accelerated the processes that the metals' bioavailability reduced. The amendments would also be beneficial to microorganisms and microbial metabolisms.

The most direct impacts of biochar treatment in the receiving soils were in pH modulation and increasing stable organic matter levels (Sauve *et al.* 2000). Evidently, the concentrations of acid soluble Cd and Pb of the polluted soils decreased in proportion to the decreases in the receiving soils' pH and SOC contents (Fig. 3). In this regard, biochar resembled other soil amendments, such as active carbon (Pyrzyńska and Bystrzejewski 2010), red mud (Gray *et al.* 2006), cyclonic ashes (Ruttens *et al.* 2010), and calcium magnesium phosphate (Zhang *et al.* 2009), that were capable of reducing metals'

activities through increased soil pH and improved metal ion occlusion. Biochars contain alkaline and macro-organic carbon-based materials (Fig. 2) possessing $-NH$ (at 1628.25 cm^{-1}), $-OH$ (at 3443.78 cm^{-1}), $-PO_4$ (at 1089.20 cm^{-1}), and $-C-Cl$ (at 769.23 cm^{-1}) surface functional groups that are able to form complexes with Cd and Pb (Yuan *et al.* 2011).

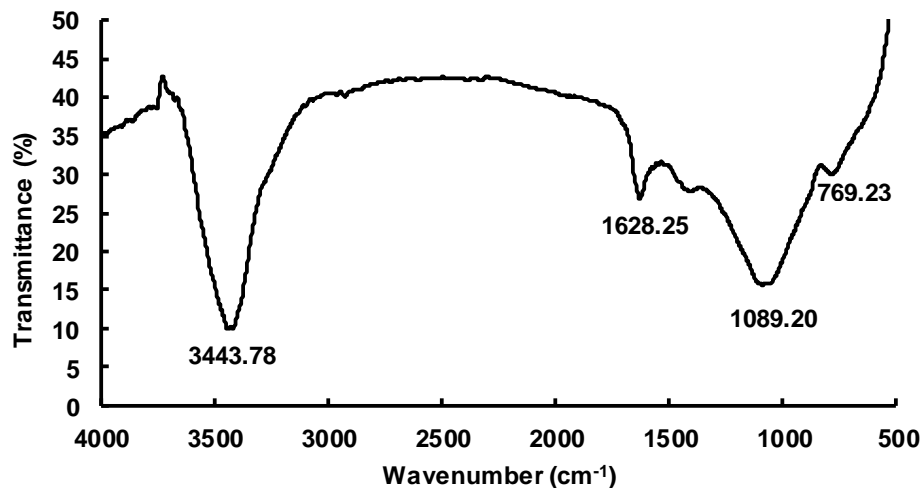


Fig. 2. Infrared radiation spectra of biochar

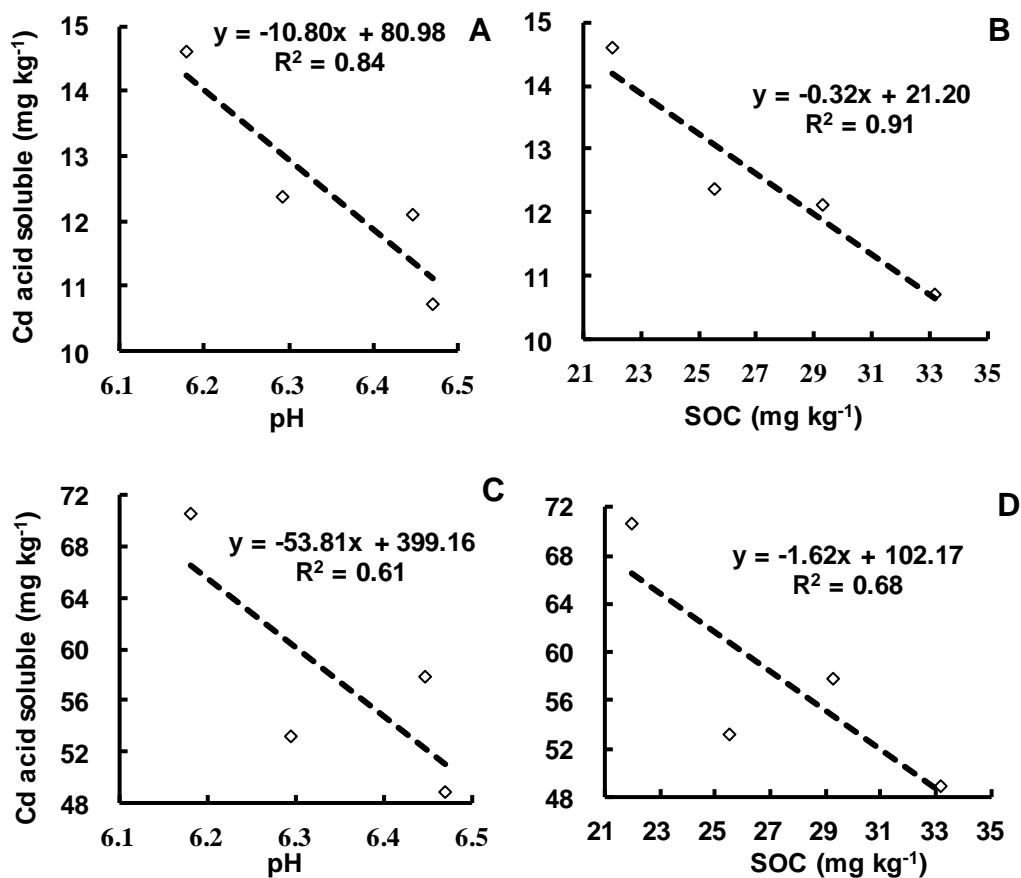


Fig. 3. Correlation of acid-soluble Cd and Pb fractions with soil pH and SOC

Principal Component Analysis (PCA) has been applied to indicate the effects of biochar amendment on heavy metals pollution, and the results of PCA are listed in Table 5 and Table 6. According to the results, the elements (Table 6) could be grouped into a two-component model, which accounted for 95.9% of all the data variation. Nearly all the elements were associated and showed high values in the first component (PC1), while the different fraction of the Oxidizable Cd, Residual Cd, and Oxidizable Pb were grouped into the second component (PC2). The PCA results imply that biochar amendment led to a change in soil properties, heavy metals fractions, soil enzymes, and microbial community of the slightly acid paddy soil and they affected each other in soil (Chen *et al.* 2013).

Table 5. Initial Eigenvalues and Extraction Sums of Squared Loadings of Principal Component (PCs) for Paddy Soil with Biochar Application

PCs	Initial Eigen values			Extraction Sums of Squared Loadings		
	Total	Variance (%)	Cumulative (%)	Total	Variance (%)	Cumulative (%)
PC1	12.45	73.22	73.22	12.45	73.22	73.22
PC2	3.86	22.68	95.90	3.86	22.68	95.90
PC3	0.70	4.10	100			

Table 6. Component Matrices of Principal Component (PCs)

Elements	Component		Elements	Component	
	PC1	PC2		PC1	PC2
SOC	0.969	0.249	pH	0.919	0.289
Acid soluble Cd	-0.998	0.057	Cellulase	0.989	-0.117
Reducible Cd	-0.951	0.298	Urine enzyme	0.794	0.599
Oxidizable Cd	-0.583	0.81	Neutral phosphatase	0.906	0.294
Residual Cd	-0.597	-0.704	Sucrase	0.873	0.481
Acid soluble Pb	-0.941	0.338	Fungi	0.984	0.075
Reducible Pb	-0.836	0.537	Actinomycetes	0.987	-0.063
Oxidizable Pb	-0.538	0.805	Bacteria	0.486	0.757

With reductions of Cd and Pb bioavailability, the biochar-amended soils supported greater populations of fungi and actinomycetes. Soil microorganisms and their metabolisms are sensitive indicators of soil fertility and environmental changes (Wang *et al.* 2007). Bacterial, fungal, and actinomycetes populations of soils are functions of the soils' organic carbon contents (Entry *et al.* 2008); a pH at the upper end of the normal range often has been observed to encourage microbial growth (Silva and Nahas 2002). However, heavy metals might alter the population size, diversity, and activities of microbes in the receiving soils (Rajapaksha *et al.* 2004). In this experiment, bacteria appeared to be less susceptible to heavy metals in the soils (Table 3; Plassart *et al.* 2008). The fungal and actinomycetes populations, however, responded to the biochar treatments and significantly increased over those of the experimental control. Again, it was the soil pH modulation and the addition of organic carbons that encouraged growth. The fungal

and actinomycetes populations of the polluted soils were inversely correlated to concentrations of acid soluble Cd and Pb, which suggests this was a function of the biochar treatments (Fig. 4.).

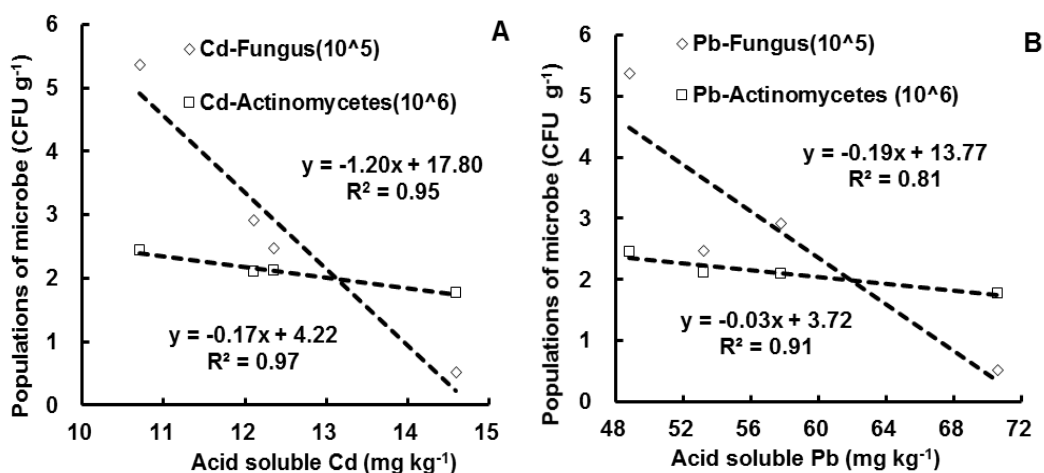


Fig. 4. Correlation of acid-soluble Cd and Pb fractions with fungal and actinomycetes populations

Soil enzyme activities of the Cd and Pb-polluted soils were enhanced by biochar. Modulating the pH and adding organic carbon into the receiving soils were primarily responsible for the enhancement. Enzyme activities can be considered as measurements of soil health because they respond to environmental stresses such as pollution (Lee *et al.* 2009). Heavy metals, when present in the soils, reduce the enzyme activities (Renella *et al.* 2005; Kizilkaya *et al.* 2004) and retard soil fertility (Sivakumar *et al.* 2012). As the biochar amendments increased, the acid-soluble and reducible Cd and Pb of the polluted soils decreased proportionally, and cellulase activities of the soils increased accordingly. The activities of neutral phosphatase, urease, and sucrase in the polluted soils also showed considerable improvements according to the biochar treatments.

CONCLUSIONS

Biochar amendments improved the health of microbial communities and metabolisms of heavy metal-polluted paddy soils via modulating pH and adding organic carbon into the treated soils. Biochar has a great potential to ameliorate soils contaminated with the heavy metals and improve the soil ecosystem. Long-term effects on soil health and potential offsetting effects deserve further field monitoring studies.

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